ASSISTED REPRODUCTIVE TECHNOLOGY

Monday, September 27, 1999

P-001

The Coflare Protocol in the Poor Responder: The Initial Flare Response is Predictive of Stimulation but not Outcome. S. D. Spandorfer, L. Kump, J. Navarro, Z. Rosenwaks. Department of Obstetrics/Gynecology. The New York Hospital - Cornell University Medical Center, New York, NY.

Objective: Poor responders to conventional IVF stimulations historically have a diminished prognosis. Included in this group are patients with borderline or elevated FSH levels. In this study we review our experience with a day 2 start, coflare protocol.

Design: Retrospective study.

Materials and Methods: 564 cycles were initiated over a 2.5 year time frame in patients that had either elevated FSH levels or a previous poor response to conventional leuprolide down-regulated stimulation. These patients were treated with our coflare protocol (1.0 mg of Lupron administered on day 2 and decreased on day 5 to 0.5 mg; gonadotropins initiated on day 3). For this study, we analyzed the initial flare response and the outcome for these patients.

Results: 564 cycles were initiated. The patients mean age was 39.4 ± 3.7 years. 20.2% (114/564) did not start because of an elevated FSH, elevated estradiol or a large baseline ovarian cyst (greater than 2cm). Of the 450 cycles that started, the outcomes were as follows: 24% (108/450) cancellation, 20.4% (92/450) clinical pregnancy per initiated cycle, 26.9% (92/342) clinical pregnancy per retrieval. We then analyzed the outcome based on the estradiol flare response (E_2 on day 3/E_2 on day 2) and compared the patients that doubled to those whose E_2 response was less than doubled. Not all patients had day 3 bloodwork.

	E_2 flare ≥ doubled (n=235)	E_2 flare < doubled (n=146)	*P* value
Cancelled cycles	13.6%	35.6%	<0.01
Mean age	39.2 ± 3.6	39.5 ± 3.4	NS
Peak estradiol	1543 ± 853	1011 ± 471	<0.001
Mature oocytes	5.8 ± 3.8	3.9 ± 2.3	<0.001
# ET	3.2 ± 1.5	2.4 ± 1.5	0.0002
Clinical Pregnancy/ retrieval	25.6%	23.4%	NS
Clinical Pregnancy/ transfer	26.9%	26.5%	NS

Conclusion: We have demonstrated an overall 26.9% clinical pregnancy rate per retrieval in these "poor responder" patients. While the initial flare response (as indicated by a doubling of the estradiol by the second day of stimulation) was indicative of a better stimulation, no difference in pregnancy outcome was seen.

P-002

Endometrial Pattern on the Day of Oocyte Retrieval is More Predictive of Implantation Success than the Pattern or Thickness on the Day of HCG Administration. F. I. Sharara, J. Lim, H. D. McClamrock. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD.

Objective: Multiple studies have confirmed a lower implantation (IR) and pregnancy rate (PR) in women who exhibit a homogeneous pattern (pattern II) of the endometrium compared to a triple-line pattern (pattern I) on the day of HCG administration. However, no data is available to evaluate if patients alter their endometrial thickness and pattern between the day of HCG administration (D_{HCG}) and the day of oocyte retrieval (D_{RET}), and whether these changes adversely affect endometrial receptivity.

Design: Prospective, observational.

Materials and Methods: 86 women (mean age 32.9 ± 3.8 years, range 24–40) undergoing 103 IVF/ET cycles. Endometrial pattern and thickness were measured on D_{HCG} and on D_{RET}.

Results: Endometrial thickness was not different between the D_{HCG} and the D_{RET} ($P = 0.09$). Pattern II on the D_{HCG} was noted in 7 cycles (6.8%), compared to 96 cycles with pattern I (93.2%). However, 20 cycles (19.4%) had pattern II on D_{RET}. No patients altered their endometrial pattern from II to I between the D_{HCG} and the D_{RET}. The ongoing PR for women with pattern II on D_{HCG} was 42.8% (3/7), compared to 43.8% (42/96) in women with pattern I ($P = 0.72$). The ongoing PR for women with pattern II on D_{RET} declined to 25.0% (5/20), compared to 47.0% (39/83) in women with pattern I on D_{RET} ($P = 0.12$). The ongoing IR was 13.0% (3/23) in pattern II group compared to 20.8% (76/365) in pattern I group on D_{HCG} ($P = 0.53$). However, a significant decrease in the ongoing IR to 9.9% (7/71) was noted in pattern II as compared to 23.3% (71/305) in the pattern I group on D_{RET} ($P = 0.019$). There was no difference in age, FSH, peak E_2, P_4 on day of HCG, # oocytes, # ET, or endometrial thickness between pregnant and non pregnant patients, nor between patients with patterns I and II on either days. There was a significant correlation between age and endometrial thickness on the D_{HCG} ($P = 0.024$), but not between peak E_2 and endometrial thickness ($P = 0.40$, power 62%).

Conclusions: This is the first study assessing the endometrium in the same cycle on the day of HCG administration and the day of oocyte retrieval. While endometrial thickness did not change significantly, the endometrial pattern was altered to a less receptive endometrium on the day of oocyte retrieval in an additional 12.6% of the cycles. Compared with the published literature evaluating the pattern on the day of HCG, the endometrial pattern on the day of oocyte retrieval is more predictive of implantation success. Endometrial pattern, rather than thickness, is a critical factor in IVF success.

P-003

Fasting Serum Insulin as a Determinant of Baseline Serum Levels of SHBG and Free Testosterone (FT), and of Ovarian Response to Gonadotropins (hMG), in Non-PCOS Classified In Vitro Fertilization (IVF) Patients. [1]B. A. Stone, [1]G. M. Hubbard, [2]J. M. Vargyas, [2]G. E. Ringler, [2]A. L. Stein, [1,2]R. P. Marrs. [1]Reproductive Technology Laboratories & [2]California Fertility Associates, Santa Monica, CA.

Objective: In PCOS, insulin resistance induces hyperinsulinemia, androgen excess, lower serum SHBG levels, and compromized ovulatory function. This study examines insulin levels in non-PCOS classified patients, and their inter-relationships with SHBG, FT, and ovarian response to hMG.

Design: Analysis of sera drawn from 123 patients for hormonal screening in a fertility center.

Materials and Methods: Day 2–3 fasting sera are routinely assayed for estradiol (E2), FSH and prolactin. In this study, levels of insulin, SHBG and FT were also measured. Data were analysed by linear regression, and by ANOVA after grouping of patients by insulin level. For those patients who proceeded to hMG stimulation and IVF, dosages of, and responses to, hMG were also analysed.

Results: The average (±SE) level of insulin in patient sera was 10.6(±1.0) μU/mL, higher than established population values for this assay (7.1 μU/mL), but lower than reported average values for PCOS (near 25 μU/mL). Average (±SE) values for the other analytes were: E2 (78 ± 7 pg/mL), FSH (11.8 ± 1.4 mIU/mL), prolactin (17.5 ± 1.4 ng/mL), SHBG (57 ± 4 nmol/L) and FT (0.85 ± 0.05 pg/mL). Consistent with the reported findings with PCOS patients, levels of insulin and of SHBG were inversely correlated ($P=0.0008$). Levels of SHBG and of FT were also inversely correlated ($P=0.007$), supporting a direct correlation between insulin and FT levels in our IVF patient population ($P=0.026$). Baseline serum insulin levels were not significantly correlated with any other measured analyte in serum. Neither numbers of days of hMG stimulation (near 9.5), nor total hMG dosages during COH (near 32 ampules), differed significantly between subgroups of patients with low (<2.2 μU/mL), high (>20 μU/mL) or normal serum insulin levels. However, total numbers of oocytes retrieved from patients with normal insulin levels (13.1 ± 0.7) were significantly higher than respective values for the patients with low (6.7 ± 1.2) or high (5.0 ± 0.7) fasting insulin values ($P<0.001$; ANOVA).

Conclusion: The findings establish that insulin is a potentially important regulator of ovarian steroid metabolism and folliculogenesis in non-PCOS classified patients. While mechanisms have not yet been elucidated, high serum insulin can clearly induce a hyperandrogenic intrafollicular milieu, and low levels may suppress steroidogenesis through low hepatic LDL cholesterol secretion.

P-004

Ultrasonographic Appearance of Polycystic Ovaries is Associated with Exaggerated Ovarian Response in Women Undergoing IVF Treatment. R. Bonzayen, W. M. Buckett, R. Hemmings, F. Bissonnette, S. L. Tan. Reproductive Center, Department of Obstetrics and Gynaecology, Royal Victoria Hospital, McGill University, Montreal, Canada.

Objectives: Infertile women with PCOS undergoing assisted reproductive techniques have a higher risk of over responding to ovulation induction and of developing ovarian hyperstimulation syndrome (OHSS). However, it is now well accepted that PCOS is a heterogenous clinical condition varying from the classical clinical phenotype including menstrual irregularities, clinical or biochemical hyperandrogenism to ultrasound diagnosed PCO in completely asymptomatic volunteers. Therefore, the objective of the study was to retrospectively compare the effects of ovarian stimulation on women undergoing IVF-ET treatment with classical PCOS and with only ultrasound appearing ovaries without clinical or biochemical markers of PCOS.

Design: Retrospective analyses of ovarian response, presence and severity of OHSS and rate of multiple pregnancies in couples undergoing IVF-ET treatment were assessed in the two groups.

Materials and Methods: The study included women undergoing IVF-ET with either PCO diagnosed ultrasonically or clinically. All information concerning clinical characteristics of patients, ovarian ultrasound assessment prior to treatment, nature of treatment and results of the assisted reproduction treatment in term of rate of OHSS and multiple pregnancies were collected retrospectively from the patient records and the embryology laboratory IVF register.

Results: 86 patients underwent 133 cycles of IVF-ET and fulfilled the inclusion criteria. Patients were classified into two groups. The first group included patients having the presence of polycystic ovaries based on ultrasound without clinical criteria (65 patients undergoing 81 cycles) and the second group, patients having polycystic ovaries syndrome based on clinical diagnosis (31 patients undergoing 52 cycles). The length of stimulation, the number of units of gonadotropins for stimulation and the mean number of eggs collected were statistically not different. Complications of treatment including OHSS was 4% in the PCOe group and 6% in the PCOS group ($P<0.05$) and rate of multiple pregnancies (determined by more than one gestationnal sac on ultrasound) were 43% and 35% respectively in the two groups ($P<0.05$).

Conclusions: Infertile women with ultrasonically PCO only ovaries and with typical clinical PCO syndrome have similar response to IVF-ET treatment. It is now clear that the ultrasonographic findings of polycystic ovaries identifies a group of patients who have the same risk of OHSS and multiple pregnancies than the PCOS patients. The identification of this high risk group is essential to avoid those serious complications during IVF-ET.

P-005

Impact of an "Egg Factor" on Pregnancy Rates Following In Vitro Fertilization. A. M. Dlugi, P. Kaplan, J. E. Osheroff, D. R. Slawny, M. R. Grelio. The Center for Reproductive Endocrinology, Atlantic Health System, Morristown, NJ.

Objective: To determine the impact of an egg factor on IVF fertilization, pregnancy and implantation rates.

Design: Clinical pregnancy rate per transfer, standard and ICSI fertilization rates and implantation rates were determined for all women under the age of 40 undergoing an IVF cycle between September 1, 1997 and December 31, 1988. An egg factor was defined by the presence of dysmorphic features of the oocyte including granular ooplasm, inclusion bodies, perivitteline debri (cytoplasmic factors), or abnormalities of the zona pellucida (zona factor).

Materials and Methods: All patients underwent standard ovarian stimulation following GnRH agonist down-regulation. Following oocyte retrieval, oocytes and embryos were cultured in human tubal fluid (HTF) with human serum albumin. Embryos with a zona factor underwent assisted hatching 4 hours prior to embryo transfer. The data was analysed using the Student's-T test and Chi square analysis.

Results:

Category	Average Age	Retrievals	Transfers	# Pregnant	# Clinical Pregnant
Non-egg factor	34.1	62	61	48	45
With male factor	33.2	12	12	11	11
Zona factor	33.5	16	15	11	11
With male factor	33.5	12	10	5	5
Cytoplasmic factors	33	25	23	5	4
With male factor	32.4	18	16	4	3

Category	Clinical Pregnancy/ Transfer (%)	Standard Fert Rate (%)	ICSI Fert Rate (%)	Implant Rate (%)
Non-egg factor	73.8[1]	75.2	76.7	43.3
With male factor	91.7[2,3]	83.6[6]	77.6	32.8
Zona factor	73.3[4]	58.5	67.6	40.4
With male factor	50[2,5]	25.5[6]	64.7	27.3
Cytoplasmic factors	17.4[1,4]	63.5	65.3	27.8
With male factor	18.8[3,5]	78.9	63.6	21.4

1,2,3,4,5,6: $P<0.05$.

Conclusions: 1) The presence of cytoplasmic factors (both with and without a male factor) resulted in the significantly lowest clinical pregnancy rates compared to all the other groups. 2) The presence of a male factor resulted in a significantly decreased standard fertilization and clinical pregnancy rate in the group with a zona factor compared to the non-egg factor group.

P-006

Randomized, Prospective Trial Comparing the Addition of Growth Hormone for the Ovulation of Induction of "Poor Responders" in IVF. J. S. Rinehart. Center for Human Reproduction, Chicago, IL.

Objective: The efficacy of IVF for the treatment of the "poor responder" patient has yet to be definitively established. Consequently, many variations have been proposed for the ovulation induction of these patients for IVF. One set of protocols that has been proposed include growth hormone during the gonadotropin stimulation. The purpose of this study was to determine if the addition growth hormone during gonadotropin stimulation improved the pregnancy rate for "poor responders" during an IVF cycle.

Design: Prospective, alternating randomization.

Methods: Patients, who had an IVF cycle cancelled for "poor response," were offered the option of participating in the study. A "poor response" was defined as fewer than four follicles > 18mm in diameter or an E2 of < 500 pg/ml on the day of HCG. The stimulation protocol used initiated leuprolide acetate on day 21 of a menstrual cycle. After menses and confirmation of down-regulation, gonadotropins were initiated at 450 IU FSH per day. The growth hormone group added 4IUGH per day during the gonadotropin stimulation. IVF was performed in the customary way using US guided retrieval and transfer of up to four embryos on day three after retrieval.

Results:

	N	Age	Peak E2	% Cancel	% With transfer	% Pregnant
NO GH	14	40.1	505	10/14=71%	3/14=21.4%	0/14=0%
GH	26	42.7	1146*	6/26=23%*	11/26=42.3%	2/26=7.7%

* = $P<0.05$.

Of the patients who had consented to participate in this study, 12/26 patients who were randomized to the arm without growth hormone chose not to continue treatment after they learned they had been randomized to the arm without growth hormone. One of these patients conceived spontaneously while awaiting matching for donor oocytes.

Conclusions:

1. The addition of growth hormone resulted in a higher estradiol on the day of HCG and fewer cancelled cycles.

2. Based upon cycle initiation, there was no difference in pregnancy rates.

3. Growth hormone does not significantly increase the pregnancy rate for patients who demonstrate a "poor response" to gonadotropin stimulation on previous IVF cycles.

P-007

Fluctuating FSH is Predictive of Poor Ovarian Response. R. Bouzayen, W. M. Buckett, F. Bissonnette, R. Hemmings, S. L. Tan. Reproductive Center, Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada.

Objectives: Early follicular FSH elevation between day 2 and day 5 is the cardinal feature of declining ovarian reserve, and the prognosis for conception in those patients is low. However, large swings of FSH production in early follicular phase has been reported to occur and occasional periods of normal FSH can be found in patients with a previous high FSH. The objective of the study was to assess the effects of fluctuating FSH on ovarian stimulation and rate of conception of women undergoing IVF-ET treatment during a normal early follicular FSH value despite previous high level of FSH(>15 mUI/ml).

Design: Retrospective analyses of ovarian response and pregnancy rate in couples undergoing IVF-ET treatment with a normal level of early follicular FSH despite a previously high FSH.

Materials and Methods: Patients were selected from June 1996 to March 1998 and all information concerning nature of treatment and outcome were retrospectively collected and analyzed from the patients records and from the embryology laboratory IVF register. Follicular recruitment, number of oocytes retrieved during collection, rate of fertilization and number of embryos obtained as well as rate of biochemical and clinical pregnancies were studied.

Results: A total of 20 couples undergoing 24 IVF-ET cycles were included in the study. Mean age was 36.6 years, infertility length was 3.7 years, 50% of patients had a prior pregnancy and 25% had a prior live birth, median FSH level prior to the IVF cycle was 9.4mUI/ml. The mean number of oocytes collected per cycles were 5.2, the number of fertilized eggs per cycle was 4.5 and a mean of 2 embryos were transferred per cycle. 37.5% of the patients had no embryo transfer because 2.4% had no follicular recruitment, 12.5% had no oocytes retrieved and 25% had no eggs fertilized.

Rate of biochemical pregnancies was 37.5%, clinical pregnancies (fetal heart beat on ultrasound) were 25% and the rate of live birth was 12.5%.

Conclusions: Variability in FSH from cycle to cycle permit to elect patients responding badly even if the FSH the day of stimulation is low and previous FSH is high. In assisted reproduction, these patients do not respond efficiently (37.5% of patients had no embryo transfer). The chance of live birth is decreased (12.5%) and the rate of miscarriage increased (33%).

P-008

Prognostic factors in Intracytoplasmic Sperm Injection (ICSI): Indications and Influence of Age. G. Van Thillo[1], A. Piazza[1], G. Speranza[1], R. Quintana[1], L. Kopcow[1], M. Vilela[1], G. Marconi[1], E. Young[1]. 1. Instituto de Ginecología y Fertilidad, (IFER), Buenos Aires, Argentina.

Objective: ICSI was initially used to treat couples with severe male factor infertility. Later the spectrum of indications became broader and is still under continuous revision. The aim of this study is to evaluate in terms of pregnancy and implantation rates, the results of ICSI in couples with male factor (MF) infertility versus other infertility diagnosis (non-MF), and the influence of female's age.

Design: Pregnancy rates (PR) per patient, per cycle and implantation rates (IR) were retrospectively analyzed in patients undergoing ICSI and reaching embryo transfer, for male factor infertility vs. different infertility diagnosis. Female's age was also considered as a variable.

Material and Methods: Ninety seven couples treated by ICSI who reached embryo transfer (ET) stage, between July and December 1997, were retrospectively reviewed from the computer data base at our Institute. Patients were initially divided into two groups: those in which the only indication was male factor infertility (n=56), and a second group treated for other indications (n=41). Both groups were subdivided into a younger group (women's age less than 37 years) (n=69) and an older group (37 or more years old) (n=28) at the time of treatment. Mean number of embryos replaced per transfer was 3.4. Pregnancy was considered as the visualization of an intrauterine gestational sac.

Results: Considering the indication for ICSI, the MF group had a PR per patient of 35.7% vs. 14.6% ($P=0.03$) for the non-MF group. Pregnancy rates per cycle were 30.7% vs. 12% ($P=0.031$); and implantation rates were 11.1 vs. 4.4%, for MF and non-MF groups respectively. Considering female's age, in younger patients with MF vs. non-MF infertility, the PR per patient, per cycle and IR were 44.4% vs. 15.5% ($P=0.019$), 36.6% vs. 12.1% ($P=0.018$) and 13.6% vs. 4.5% ($P=0.015$). In the older group, these differences showed a tendency in favor of MF, not being statistically significant.

Conclusion: According to these results ICSI has a better outcome for male factor infertility. In older patients, although a tendency in favor of male factor is shown, other factors may influence the outcome. A prospective study with a bigger sample of older patients should be undertaken to determine ICSI influence in this group.

P-009

Heterotopic Pregnancy After Ovulation Induction and Assisted Reproduction Techniques: Early Detection and Treatment. L. M. Augé, I. de Zúñiga, G. Marconi, R. Quintana, A. Kenny, E. Lombardi, G. Speranza, P. J. Buzzi, E. Young. Instituto de Ginecología y Reproducción (IFER), Buenos Aires, Argentina.

Objective: The aim of this report is to discuss diagnosis, risk factors and treatment of heterotopic pregnancies (HP). Simultaneous intrauterine and extrauterine gestation was a relative rare condition, but has become a more common complication of assisted reproduction techniques. However, diagnosis is often delayed because of its rarity and difficulty.

Design: Retrospective study.

Materials and Methods: Six cases of heterotopic pregnancy diagnosed at our institution are reviewed. Five of these gestations took place after different assisted reproductive techniques (2 GIFT, 2 FIV ET, 1 TET) and 1 following ovulation induction. The mean age was 30.3 years (range 28–33). A history of previous PID was recorded in 3 cases, endometriosis in 2 cases and anovulation in 1 case. All patients's follow up consisted in assessment of serum beta hCG, clinical examination and first transvaginal ultrasound at 5.5 weeks of gestation.

Results: In 66% of cases the heterotopic pregnancy was asymptomatic up to 6th week, while 1 patient presented minor vaginal bleeding and the other mild pelvic pain. In 1 patient the diagnosis was made incidentally during a sonographic exam for routine follow up after ART. The other 5 patients consulted between 6th and 8th week of gestation for acute abdominal pain and for hipovolemic shock, presenting pathological parauterine images by ultrasound scan in 3 cases. Salpingectomy was the treatment used for removal of the EP, carried out by laparotomy in 4 cases and by laparoscopy in 2. Four patients had an uneventful pregnancy with 3 live births and 1 still birth. Two miscarried within 2 weeks of surgery.

Conclusions: 1) A higher rate of pelvic pathology and exposure to multiple embryo-gamete transfer is a predisposing factor for ectopic pregnancy (EP) and therefore for heterotopic pregnancy. 2) Early diagnosis of HP is delayed with regard to EP because serum values of beta hCG are similar to those of an orthotopic pregnancy as subnormal hormone production by ectopic gestations is masked by higher placental production. 3) Furthermore, early detection of an intrauterine pregnancy and low sensitivity of sonographic identification in already enlarged ovaries due to ovulation induction do not encourage search for an EP in asymtomatic patients. However, careful sonographic assessment of the whole pelvis is critical and can depict an adnexal mass. 4) Laparoscopy is the method of choice to diagnose and treat the extrauterine component of HP, with laparotomy reserved for hemodinamically unstable cases.

P-010

A Study to Determine if Certain Sonographic Uterine Parameters are Associated with Multiple Gestation. C. Dietterich, J. H. Check, J. K. Choe, A. Nazari. UMDNJ, Robert Wood Johnson Medical School, Department of Obstetrics/Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ.

Objectives: To determine if lower uterine artery vascular impedance as measured through color Doppler sonography or increased endometrial thickness is associated with a greater likelihood of multiple gestation.

Materials and Methods: Color Doppler parameters of pulsatility index (PI), resistance index (RI) and endometrial thickness was measured in oocyte/retrieval cycles on days of human chorionic gonadotropin (hCG) injection, oocyte retrieval, and mid-luteal phase in cycles where at least 3 embryos were transferred. Comparisons of these parameters were made in patients with single versus multiple gestations.

Results:

	Single gestation	Multiple gestations	
PI			
Day of hCG	2.63 ± .52	2.51 ± .52	
Day of retrieval	2.77 ± .58	2.67 ± .54	P=NS
Luteal phase	2.35 ± .59	2.26 ± .52	
RI			
Day of hCG	.88 ± .04	.88 ± .05	
Day of retrieval	.87 ± .04	.86 ± .04	P=NS
Luteal phase	.85 ± .06	.84 ± .04	
Endometrial thickness			
Day of hCG	11.18 ± 2.28	11.47 ± 2.19	
Day of retrieval	11.37 ± 2.12	11.47 ± 2.33	P=NS
Luteal phase	11.43 ± 2.20	11.93 ± 2.33	

Conclusions: There was no association between uterine environment as measured by vascular impedance and endometrial thickness and number of embryos implanted. Thus, a more ideal uterine environment, at least as determined by these sonographic parameters, does not seem to facilitate multiple embryo implantations.

P-011

How Viable is a 6-Day Blastocyst? H. G. Yoon, S. H. Yoon, S. W. Lee, J. H. Moon, S. J. Park, S. P. Park, Y. M. Lee, J. H. Jung, J. H. Lim. Maria Infertility Clinic, Seoul, Korea.

Objective: To investigate whether 6-day blastocysts developed in vitro to the expanded or the hatching/hatched stage are viable compared with 5-day blastocysts which have reached the expanding or the expanded stage.

Design: This study was carried out through patients with 2PN ≥ 8 or "Good" embryos on day 2 postinsemination ≥3. Allocation of embryo transfer (ET) on day 5 or day 6 depended on the weekday of ovum pick-up.

Materials and Methods: All zygotes derived from IVF or ICSI were co-cultured with cumulus cells in 10 μl YS medium containing 20% hFF until day 6 postinsemination. 156 ET were performed on day 5 and 139 ET were performed on day 6. The number of embryos transferred was a maximum of three. One or two embryos were transferred whenever blastocysts with a clear ICM or hatching/hatched stage were generated. After embryo transfer, surplus blastocysts were cultured until day 6 and cryopreserved.

Results: The results obtained in this study were as follows:

	Blastocyst Transfer			
	On 5-day		On 6-day	
Cycles	156	156	139	139
Age		34.4 ± 3.2		33.2 ± 3.9
Zygotes	1585	10.2 ± 4.3	1387	10.0 ± 4.6
Blastocysts	833	52.6%	757	54.6%
Transferred Blastocysts	406	2.6 ± 0.5	308	2.2 ± 0.5
Freezing Blastocysts	427	2.7 ± 3.1	449	3.2 ± 3.3
Clinical Pregnancy	71	45.5%	62	44.6%
Implantation	104	25.6%	101	32.8%
Ongoing Pregnancy	61	39.1%	59	42.4%

Conclusion: Although the clinical and the ongoing pregnancy rate in 6-day blastocyst transfer were similar to those in 5-day transfer, the implantation rate of 6-day transfer appeared higher than that of 5-day transfer. It was confirmed that 6-day blastocysts developed to the expanded or the hatching/hatched stage are as viable as ever and a more viable blastocyst could be selected on day 6. Therefore, we suggest that blastocysts reaching late on day 6 be used for a selection of the best blastocysts in human IVF-ET program.

P-012

Day-Three Embryo Morphology Is a Poor Predictor of Blastocyst Quality in Extended Culture. J. Graham, S. Greenhouse, M. Levy, P. Morton, G. Mottla, A. Sagoskin, R. Stillman, C. Sweitzer, E. Widra, M. Tucker. Shady Grove Fertility Centers, Rockville, Maryland.

Objective: To compare embryo morphology on day-3 of development with morphology at the blastocyst-stage for transfer (ET) and cryopreservation (CRYO). The value of the predictive abilities of selection at the earlier stage was evaluated in terms of eventual assessment and utilization on day-5/6.

Design: Analysis of traditional parameters for selection of embryos for fresh ET and CRYO on day-3 of development were superceded by extended culture and selection of blastocysts 2 or 3 days later. Efficiency of embryo selection for potential viability at both stages of development was compared by correlating individual embryo quality assessment as a proportion of assumed good quality embryos in both circumstances and ultimate clinical outcome.

Materials and Methods: In 101 IVF cycles where women (average age 33.1 yrs) had sufficient embryos (range: 5–25) to justify some degree of embryo selection, extended in vitro culture was undertaken for an average of 5.4 days before use.

Results: From 1263 cleaving embryos 559 were judged to be of a standard suitable for use on day-3 by traditional parameters of selection. Day-3 use would have dictated that 355 embryos would have been transferred (average 3.5) and the rest frozen (44% utilization rate - 559/1263). Instead 234 blastocysts were transferred (average 2.3) and 237 frozen, yielding a blastocyst utilization rate of 37% (471/1263). While embryo usage merely fell by 15.7% (471 down from 559) with extended culture, only 47.5% (265/559) of day-3 embryos considered suitable for ET and CRYO on day-3 would actually be chosen for ET and CRYO as blastocysts. Conversely, 206 day-3 embryos (16.3%; 206/1263) that would not have been selected as potential candidates for ET or CRYO, ultimately formed blastocysts that were used for ET and CRYO. Current comparison of clinical pregnancy rates arising from embryo selection on day-3 (30.5%) or day-5/6 (45%) indicates a distinct improvement in efficiency of IVF-ET therapy through extended pre-implantation culture.

Conclusion: Assuming that routine blastocyst culture increases discrimination of potential embryonic viability with minimal deleterious impact, it can be seen that traditional criteria of early cleavage stage embryo selection are relatively inefficient. Consequently, extended embryo culture can be considered an effective means to increase overall clinical outcomes.

P-013

Culturing of Cryopreserved-Thawed Pronuclear Stage Embryos Before Transfer in ICSI Patients: Is There Any Advantage? S. Al-Hasani, N. Nikolettos, L. C. Demirel, B. Schöpper, R. Sturm, K. Diedrich. Department of Obstetrics and Gynecology, Medical University of Lübeck, Germany.

Objectives: Cryopreservation of human embryos has become an integrated part of assisted reproductive technologies, allowing the transfer of a limited number of embryos in the collection cycle and maximizing the overall pregnancy rate from a given treatment cycle. Human embryos can be cryopreserved at the pronucleate cell stage, at multicellular cleavage stage or at the blastocyst stage.

Design: We investigated whether in vitro culture of pronucleate stage embryos can improve the results per transferred embryo. We compared the effect on implantation rate and pregnancy rate of pronuclear stage oocytes obtained after ICSI and transferred immediately (2–3 hours) after thawing or after a 24 hours culture period.

Material and Methods: The study population consisted of patients who underwent ICSI and who in addition to having a fresh embryo transfer had supernumerary embryos which were cryopreserved. The surplus embryos were cryopreserved in pronuclear stage using 1,2 propanediol (PROH) and sucrose as cryoprotectant. After thawing, all viable embryos were trans-

ferred to the patients in a stimulated cycle. The stimulation protocols in the transfer cycles were clomiphene citrate, gonadotropins (HMG or rec FSH), GnRH agonists with oestrogen/progesterone or oestrogen/progesterone alone. In 298 thawing cycles 727 embryos transferred to the patients on the day of thawing. In 227 thawing cycles 559 embryos transferred to the patients after a 24 hours in-vitro culture period.

Results: The mean number of embryos per transfer was 2.44 in the thawing cycles with immediate transfer and 2.46 in the thawing cycles with the transfer performed after 24 hours of culturing (P=0.7009). The pregnancy rate per transfer was 8.7% and the clinical pregnancy rate per transfer was 6.7% in cycles with immediate transfer. The pregnancy rate per transfer in thawing cycles with transfer after overnight culture was 14.5% and the clinical pregnancy rate was 10.5% (statistically significant, P=0.0367). The implantation rate was also superior in thawing cycles with the transfer performed 24 hours later (statistically significant, P=0.0480).

Conclusions: Cryopreserved pronuclear stage oocytes resulting from ICSI when they were cultured for a 24 hours period after thawing can significantly improve the pregnancy rate and the implantation rate per transferred embryo and maximize so the overall pregnancy rate per oocyte retrieval.

P-014

Effectiveness of "Swim-Up Technique" to Recover Functionally Intact Spermatozoa from Cryopreserved Specimens for Assisted Reproduction. [1,2]S. C. Esteves, [2]R. K. Sharma, [2]A. J. Thomas, Jr., [2]A. Agarwal. [1]ANDROFERT-Andrology & Human Reproduction Clinic, Campinas, SP, Brazil and [2]Center for Advanced Research in Human Reproduction and Infertility, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH.

Objectives: The freeze-thaw process extensively damages human spermatozoa by decreasing motility and the percentage of intact acrosomes, and by elevating reactive oxygen species levels. Removal of defective and dead sperm, debris, leukocytes and seminal plasma is essential to optimize the fertilizing potential of cryopreserved spermatozoa. We studied if the "swim-up technique" can select the most functional sperm population from cryopreserved specimens as effectively as it does from fresh ones.

Design: Percentage sperm recovery (%REC), motion characteristics and acrosome integrity were assessed after sperm selection by "swim-up" in fresh and cryo-thawed specimens.

Materials and Methods: IRB approval was obtained for this study. Semen specimens from 15 proven fertile donors were divided into two equal aliquots: the first aliquot was treated by "swim-up" (fresh) and the second one was cryopreserved using the liquid nitrogen vapor method, and then treated by "swim-up" after thawing (frozen). Percentage recovery of motile spermatozoa (%REC), percentage sperm motility and motion characteristics using a computer-assisted semen analyzer (CASA), and acrosome integrity assessed by fluorescein iso-thiocyanate conjugated peanut lectin in conjunction with a viability staining Hoescht-33258 were evaluated in fresh and frozen specimens. The Wilcoxon rank sum test was used to detect differences in %REC, sperm motion characteristics and acrosome scores.

Results: The %REC after swim-up in fresh and frozen specimens were 13.9 (25%–75% interquartile range: 9.7–29.4) and 22.0 (25%–75% interquartile range: 11.0–43.0), respectively (P = 0.51). Swim-up treatment selected a sperm population with better motion characteristics, percent motility and viability in fresh than in frozen specimens (P <0.01). The frequency of acrosome intact spermatozoa after swim-up treatment was higher in fresh than in frozen specimens (P <0.001).

Conclusions: 1) Swim-up provides similar yields of spermatozoa from cryopreserved specimens of normozoospermic individuals compared to fresh ones. This finding may help in the prediction of the number of spermatozoa to be recovered from cryopreserved specimens after swim-up, thus allowing a better decision regarding the type of ART to be used; 2) The overall sperm quality after swim-up preparation is lower in frozen than in fresh semen, and it seems to be related to the osmotic effects and sublethal damage during the freeze-thaw process rather than the inability of the washing technique to select functional spermatozoa. This work was supported by a research grant from The Cleveland Clinic Foundation.

P-015

Modeling IUI Outcomes Using a Neural Network: A Large-Scale Retrospective Analysis. [1]B. N. Hendin, [1]A. Agarwal, [3]D. R. Nelson, [2]T. Falcone, [4]E. Onel, [4]K. Liu, [5]R. Golden, [1]A. J. Thomas, Jr., [4]C. Niederberger. Center for Advanced Research in Human Reproduction & Infertility, Departments of Urology, Gynecology-Obstetrics, and Biostatistics & Epidemiology, The Cleveland Clinic Foundation, Cleveland, OH; Department of [4]Urology, University of Illinois at Chicago, Chicago, IL; [5]Department of Psychology, University of Texas, Dallas, TX.

Objectives: Intrauterine insemination (IUI) is often the first line of assisted reproductive therapy offered to infertile couples. In the interests of both accurate patient counselling and cost-effective therapeutic management, it is important to predict the likelihood that a given treatment will succeed. Neural computation offers a robust, non-linear method of data modeling which has gained widespread use in a variety of applications. We modeled a large database of IUI outcomes using neural computational techniques, and compared to linear statistical tools including logistic regression and discriminant function analysis in order to determine whether the neural network offers an advantage in IUI outcome prediction.

Design: Retrospective modeling of an IUI outcomes database with neural computation, logistic regression, linear (LDFA) and quadratic (QDFA) discriminant function analysis.

Materials and Methods: Data regarding 1728 IUI treatment cycles among 533 women using partner's sperm were reviewed. Pre-treatment clinical female patient characteristics, the quality of the individual semen specimens used in IUI treatment cycles, and the manner of hormonal ovulatory management for each IUI cycle were assessed. Primary outcome measures were clinical pregnancy and live delivery. A neural network was constructed and compared to LDFA, QDFA, and logistic regression analysis.

Results: Modeling results are described in the table. Classification accuracy is defined simply as the correct number of predictions divided by the total number of outcomes. ROC area (receiver operator characteristic curve area) combines sensitivity and specificity for all possible thresholds.

	Neural Network	Logistic Regression	LDFA	QDFA
Pregnancy				
Classification Accuracy	89.8%	91.0%	49.9%	49.9%
ROC Area	0.826	0.742	0.500	0.500
Live Delivery				
Classification Accuracy	89.8%	93.5%	52.9%	52.9%
ROC Area	0.816	0.335	0.500	0.500

Conclusions: While the crude assessment parameter classification accuracy was similar with both logistic regression and neural computation (approximately 9 of 10 predictions correct) neural computation was superior in ROC analysis. Both LDFA and QDFA completely failed to model the IUI dataset. The neural network recognizes unique patterns and analyzes nonlinear trends, providing an overall improved sensitivity and specificity for predicting IUI outcomes. We thus present a neural computational model which accurately predicts IUI outcomes as a cognitive aid to physicians performing these procedures.

P-016

Outcome Analysis of Intracytoplasmic Injection of Testicular Sperm from Patients with Obstructive Versus Nonobstructive Azoospermia. [1]R. G. Lucena, [2]A. Spitz, [2]R. E. Brannigan, [2]F. Orejuela, [2]E. D. Kim, [2]M. Crone, [2]P. Cisneros, [2]S. Carson, [2]Peter Casson, [2]D. J. Lamb, [2]L. I. Lipshultz. [1]University of Campinas, Sao Paulo, Brazil and [2]Scott Department of Urology, Baylor College of Medicine, Houston, TX.

Objectives: To compare the fertilization and pregnancy outcomes of testicular sperm extraction (TESE)/intracytoplasmic sperm injection (ICSI) in patients with obstructive azoospermia (OA) versus non-obstructive azoospermia (NOA) (impaired spermatogenesis).

Design: A retrospective analysis of 26 patients who underwent TESE/ICSI for either unreconstructable OA or NOA.

Materials and Methods: Between June, 1994 and July, 1998, 26 consecutive patients underwent TESE/ICSI by a single ICSI team at three different IVF centers (Baylor Division of Assisted Reproduction Technology, Ob-Gyn Associates at the Columbia Women's Hospital, and Gramercy Clinic). 13 of these patients were diagnosed with NOA and 13 with unreconstructable OA. Histological findings in the patients with NOA varied from severe

hypospermatogenesis to maturation arrest. Histological findings in the patients with unreconstructable OA revealed normal spermatogenesis with some focal tubular sclerosis. The outcome of a total of 13 cycles (124 oocytes) using sperm from men diagnosed with OA followed by ICSI/IVF was compared with the outcome of a total of 13 cycles (146 oocytes) using sperm from men diagnosed with NOA. Pregnancy was determined by fetal ultrasound.

Results: The 2PN fertilization rate from TESE/ICSI in patients with OA was 54% (66/124 oocytes) which was significantly higher ($p<0.05$) than the fertilization rate from TESE/ICSI in patients with NOA, 39.7% (58/146 oocytes). However, the clinical pregnancy rate was 30.8% (4/13 cycles) for each group.

Conclusions: While fertilization and pregnancy rates are predictably high in patients with OA who undergo TESE/ICSI, similar pregnancy outcomes can be obtained in some of the most severe male factor patients with NOA who undergo TESE/ICSI, regardless of testicular histopathology.

P-017

Gestational Surrogacy: It is Time to Include it as Part of ART. J. Batzofin, J. Nelson, J. Wilcox, D. Potter, R. Rogoff, G. Norbryhn, C. Hatkoff, M. Feinman. Huntington Reproductive Center, Pasadena, CA.

Objectives: To address the issue of gestational surrogacy with respect to clinic specific reporting of outcomes data, in the hopes of creating a specific subcategory for this established, effective treatment modality. Currently, the data for such treatments are excluded from SART reporting, providing in our belief, a disservice both to patients who might benefit from these treatments, as well as the clinics which provide these services.

Design: A prospective non-randomized clinical trial.

Materials and Methods: We report here as we have intermittently in the past, on the efficacy of this treatment modality. Between 1989 and 1998, 350 prospective mothers (PM) aged 26–57 (39.4±7.9) years old, have participated in the IVF-surrogacy program. The clinical categories were as follows:

Uterine malformation including DES	n = 47	(13.4%)
Uterine synechiae	n = 22	(6.3%)
Absent uterus	n = 111	(31.7%)
Medical contraindication to pregnancy (e.g. lupus, renal, cardiac)	n = 74	(21.1%)
Repeated failed IVF/unexplained infertility	n = 96	(27.4%)
Total	350	(100%)

312 surrogate mothers have been obtained from 2 sources: a) agency recruiting (n=247=79%); b) personal contacts of PMs (n=65=21%). In both cases, all parties have undergone thorough counseling and rigorous medical-psychological screening before entering the program. PMs have undergone 526 cycles of COH resulting in 484 egg retrievals. 312 surrogate moms aged 31±4 have received 3.5±1.3 embryos per transfer in 484 trials. Of these 484 trials, 98 (20%) occurred in 1998 alone.

Results: Pregnancies have been established in 212 of 484 transfers (43.8%) per transfer with ongoing/delivered rates of 172 of 484 (35.6%). The 1998 data was 37/98 (38%) clinical pregnancy per transfer and ongoing/delivered rate of 28/98 (29%). The outcomes of the first 100 babies born have been previously reported (45 singletons, 23 sets of twins and 3 sets of triplets). When our clinic has attempted to include these data with our clinic specific outcomes report to SART, we have been informed that there is no specific category for surrogacy "because so few clinics are involved in surrogacy treatments". Therefore, these cycles (98 cycles in 1998 or 98 of 664 fresh transfer cycles i.e., 14.7% of our fresh embryo treatment cycles) need to be excluded from our reporting data.

Conclusion: IVF surrogacy is an established, viable treatment alternative for a subgroup of deserving couples. There are many useful intents of the clinic specific reporting system overseen by SART. By excluding these data, a major disservice occurs to couples performing their due diligence, as well as clinics performing these treatments. We therefore appeal to have gestational surrogacy recognized as "mainstream ART" with appropriate and separate reporting of outcomes.

P-018

Identification and Costs of Attrition Factors for Potential Oocyte Donors. R. Sachdev, J. J. Amato, R. M. Shelden, E. Kemmann, D. B. Seifer. Department of Obstetrics, Gynecology and Reproductive Sciences, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

Objective: Recruitment for oocyte donation is a labor intensive and costly endeavor. Extensive physician/nursing time and multiple tests are required during the recruitment process. The donor screening protocol on those meeting entry criteria (ages 21–34) at our center is as follows: visit with the physician for evaluation and testing for HIV, hepatitis, CMV, HTLV, cystic fibrosis, prenatal screen, gonorrhea and chlamydia, and psychological evaluation. Menstrual cycle day 3 FSH and E2 are obtained to assess for ovarian reserve. The total costs for the screening process per approved donor includes costs incurred for those not completing the screening process for reasons of rejection or loss of interest (LOI). In the latter group, some women stopped the work-up at various stages while others completed the entire approval process before "changing their mind" and deciding against participation. The purpose of this study was to determine the major attrition factors and their associated costs in the oocyte donation recruitment process.

Design: Retrospective analysis of all applicants to the oocyte donation program at an academic institution.

Materials and Methods: Potential applicants were provided with a genetics/personal medical history questionnaire and a description of the oocyte donation protocol. Upon completion they were entered into the study (n=91) and grouped into the following categories: approved (A), withdraw due to LOI and rejection for diminished ovarian reserve (DOR), personal medical (M), genetics (G) or psychological (P) reasons. Percentages in each group were obtained by using the overall total patient number, n=91 (%T) and also by eliminating LOI group, n=36. These calculations were made to determine percentages in the groups that were actually interested in proceeding with oocyte donation (T-LOI). FSH >7mIU/ml (Immulite, DPC) and/or E2>50pg/ml were consistent with DOR.

Results: The mean age in the DOR and A groups was 27.6 and 27.4 years respectively. Costs incurred in the rejected G and M groups were 1 hour of physician time ($250/hr) to review the questionnaire with the candidate. Significant costs were incurred in the P group since psychological evaluation is performed at the end of the screening process. The total cost per approved donor was $2948. Of the 36 women in LOI group, 10 completed the screening process (27.8%).

	G	P	M	DOR	LOI	A	Total
#	3	5	6	14	36	27	91
%T	3.3	5.5	6.6	15.4	39.6	29.7	
%(T-LOI)	5.5	9.1	10.9	25.5	–	49.1	
costs ($\$\times10^3$)	0.5	9.0	1.5	0.5	19.5	48.6	79.6

Conclusions: 1) 29.7% of all applicants are eventually accepted as donors. 2) A major rejection criteria in those interested in becoming oocyte donors was DOR (25.5%). This was unexpected since these women were from a "fertile age group". The high rejection rate due to DOR suggests that this should be part of the initial screening step. 3) A significant percent of women lost interest in proceeding (39.6%) and extensive financial costs were incurred ($19,500). Identifying reasons for LOI prior to initiating the screening process may significantly decrease overall costs.

P-019

ICSI Causes Deleterious Effects on the Outcome of Subsequent Frozen Embryo Transfers of Donor Oocyte Cycles. M. A. Cohen, S. R. Lindheim, M. V. Sauer. Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, NY.

Objectives: To evaluate whether or not ICSI has any effect on the outcome of subsequent embryo implantation in frozen/thawed donor oocyte cycles.

Design: Retrospective review of a university based practice.

Materials and Methods: From 6/97 to 11/98 women with donor oocyte FET cycles with ICSI (n=24) were compared to those without ICSI (n=62). All oocyte donors were under 35 years old and underwent GnRH-agonist downregulation followed by controlled ovarian hyperstimulation. All recip-

ients were synchronized to the donors with GnRH-agonist downregulation followed by estrogen and progesterone supplementation. Recipient ages were similar in the two groups (42.7 ± 0.8 years no ICSI vs. 42.3 ± 1.7 years (SEM) ICSI). 156 donor oocyte cycles were performed with conventional IVF compared to 113 with ICSI. The ICSI group had significantly poorer sperm count, motility, and morphology. The percentage of ICSI cycles resulting in cryopreserved embryos was significantly lower (37.2%, 42/113) compared to conventional IVF (62.2%, 97/156) (p-value < 0.05). Only embryos with a morphology grade of 4 or greater in a 1–5 scale were cryopreserved. Outcome measures included implantation, pregnancy, and miscarriage rates, and # of embryos transferred. Statistical analysis was done with the SPSS statistical package.

Results: The results of subsequent FET cycles are tallied below:

	No ICSI (n = 62)	ICSI (n = 24)	p-value
# embryos transferred/FET cycle	3.65 ± 0.14 (SEM)	3.75 ± 0.21 (SEM)	NS
Implantation rate/embryo	26.1% (59/226)	11.1% (10/90)	<0.01
Pregnancy rate/FET cycle	53.2% (33/62)	37.5% (9/24)	NS
Miscarriage rate/FET cycle	24.2% (8/33)	55.5% (5/9)	NS
Ongoing pregnancy rate/FET cycle	40.3% (25/62)	16.6% (4/24)	<0.05

NS = not significant SEM = standard error of the mean.

Conclusion: Our data reveal a significant detriment of ICSI on cryopreserved embryos as evidenced by a significantly lower implantation rate and a lower ongoing pregnancy rate. We believe the ICSI procedure causes some damage to the embryo that is exacerbated by the freeze/thaw process.

P-020

The Predictive Value of Day 3 Embryo Stage on Blastocyst Formation. J. D. Wininger, A. E. Jones, G. Wright, S. E. Smith, W. W. Brockman, J. Johnson-Ward, J. B. Massey. Reproductive Biology Associates, Atlanta, GA.

Objective: To determine the effectiveness of using embryo cell number on day 3 of embryo culture to predict which patients may benefit from extended embryo culture and blastocyst transfer.

Design: Retrospective analysis of all embryos cultured until day 6. A comparison of pregnancy and implantation rates on day 3 and blastocyst stage embryo transfers.

Materials and Methods: Oocytes and embryos were cultured in P1 (Irvine Scientific) until 72 hours post retrieval. On the morning of day 3, embryos that were not transferred were placed into blastocyst culture medium (Irvine Scientific) and cultured for 2 or 3 more days. Embryos cultured in blastocyst medium were grouped according to cell number. Patients with 3 or more 8-cell embryos on day 3 were given the option of continued embryo culture and embryo transfer at the blastocyst stage.

Results: Ongoing culture of 728 cleavage stage embryos yielded a total of 386 blastocysts (53% of embryos). Of these blastocysts 271 were deemed to be of sufficient quality to be either frozen or replaced to the uterus (37% of embryos). Day 3 embryos at 8, 7, 6, 5, and less than 4 cell had blastocyst development rates of 59%, 49.5%, 35%, 17.5%, and 7%, respectively. Pregnancy and implantation rates were as grouped as follows: Group A blastocyst transfer. Group B day 3 transfer patients who did not have 3–8-cell embryos. Group C day 3 transfer patients who had at least 3–8-cell embryos but declined to culture on to the blastocyst stage.

Group	Average # embryos replaced	Number of pregnancies (+βhCG)	Implantation rate
A	2.2	38/60 (63.3%)	15/45 (34%)
B	3.2	68/161 (42%)	53/412 (12.8%)
C	3.3	28/45 (62%)	38/132 (28%)

Conclusion: Culture of embryos to blastocyst allows for effective selection of embryos for uterine transfer with significantly higher implantation rates when compared to day 3 transfer (p=0.0097). Implantation rates in the day 3 and day 5/6 transfer groups were 17% (91/544) and 34% (15/45), respectively. A concern of assisted reproductive technology is that of multiple pregnancies. Transferring fewer embryos with a higher implantation potential can be a useful tool in lowering the multiple pregnancy rate whilst maintaining an acceptable pregnancy rate. An arbitrary selection criterion of 3–8-cell embryos on day 3 can be used to select those patients who may benefit most from a blastocyst transfer of fewer embryos.

P-021

Embryo Quality as a Predictor of the Risk of Multiple Gestation in In Vitro Fertilization (IVF). J. E. Osheroff, P. Kaplan, A. M. Dlugi. Center for Reproductive Endocrinology, Atlantic Health System, Morristown, NJ.

Objective: A major concern for both physicians and patients pursuing IVF is the potential for multiple pregnancies. It is the challenge of the practitioner to determine the optimal number of embryos for transfer to maximize the chance for pregnancy while minimizing the risk of multiples. It is logical that this decision should be based on the quality of the embryos for transfer; however no standard criteria have been set forth which may guide the physician in determining the number of embryos for transfer based on embryo quality. We attempted to determine if the empiric risk of a multiple gestation could be differentiated based on the quality of embryos transferred. We further investigated whether the probability of obtaining no pregnancy (P_0), a singleton pregnancy (P_1) or a multiple pregnancy (P_{mult}) based on embryo quality agreed with those values predicted by the binomial distribution, a mathematical model recently proposed in the literature to approximate the probability of outcomes in a given IVF cycle. To date, this model has not been validated in the literature with empiric data.

Design: Retrospective chart review of pregnancy outcomes in an IVF center for one year (1998). 2 groups were analyzed: those less than age 40 with at least 2 embryos of 8 cell grade 1 quality at the time of embryo transfer (group 1) and all others less than age 40 (group 2). The empiric rates of no pregnancy, one pregnancy and a multiple pregnancy were determined for each group and compared to those predicted by the binomial distribution. Pregnancy rates are defined as clinical pregnancy per embryo transfer. The average number of embryos transferred was the same for both groups (group 1=4.3, group 2=4.0, p>0.05).

Results: Group 1 contained 26 patients with an average implantation rate of 39% and an overall clinical pregnancy per transfer rate of 81%. Group 2 contained 49 patients with an average implantation rate of 20% and an overall clinical pregnancy per transfer rate of 51%. The comparison between expected and actual outcomes was as follows:

	Group 1		Group 2	
	Predicted	Actual	Predicted	Actual
P_0	12%	19%[1]	40%	49%[1]
P_1	29%	27%	33%	41%
P_{mult}	59%	54%[2]	19%	18%[2]

p>.05 for predicted vs actual values in all categories. [1,2] p<.05.

Conclusions: We have shown that the probabilities of obtaining a multiple pregnancy (P_{mult}) in an IVF cycle varies with the quality of embryos transferred and that the rates of no pregnancy, one pregnancy and multiple pregnancy is correctly predicted by the mathematical model of binomial distribution. Specifically, we have identified a group of patients with a high implantation rate (those less than age 40 with at least 2 embryos of 8 cell grade 1 quality at the time of embryo transfer) in whom the number of embryos transferred can potentially be limited while optimizing the cycle outcome.

P-022

Gamete IntraFallopian Transfer of Ova Immediately after Intra Cytoplasmic Sperm Injection (ICSI) vs Pronuclear Stage Tubal Transfer After ICSI as a Treatment for Severe Male Factor Infertility. [1,2]H. S. Kashaf, M. D. [2]M. Aghahosseini, M. D. [2]A. Aleyaseen, M. D. [2]M. Vahid Dastjerdi, M. D. [1,2]H. Saidi, PHD [1]N. Ghalavand, M. D. [1]G. H. Amini, M. D. [1]D. Etemadi, M. D. [1]Sh. Mohajeri, M. D. [2]A. Khademi, M. D. [1]S. S. Kashaf, M. D. 1-Navid's Institute of Infertility 2-Department of Infertility and Endocrinology Shariati Hospital Tehran University, Tehran-Iran.

Objective: Gift was originally developed as a therapeutic alternative for patients with at least one functional Fallopian tube. In most investigators experience its efficacy has proven superior to IVF in couples with most categories of infertility. Unfortunately success rates with Gift in cases of significant male factor infertility are lower than with other etiologies. In 1998 ASRM meeting we have described the efficacy of Gamete Intrafallopian Transfer of ova immediately following ICSI in couples exhibiting severe male factor infertility. This technique is superior to PROST after ICSI because that doubles the surgical exposure, doubles the cost, doubles the invasiveness yet it's effect on pregnancy rate is unknown. To address these questions we compare the clinical and ongoing pregnancy rate obtained after Gamete Intrafallopian Transfer of Ova immediately after ICSI and PROST after ICSI.

Design: Prospective Randomized Trial.

Materials and Methods: From Jan. 1997 to December 1998, 300 patients undergoing treatment with non-tubal infertility and severe male factor infertility were randomly allocated to either Gamete Intrafallopian Transfer of Ova immediately after ICSI or PROST after ICSI. After signing IRB consent all patients had been stimulated with LHRH Agonist Buserline (Suprefact) HMG combination in the form of long protocol. Both group underwent Identical Gift procedures and a minimum of four ova immediately after ICSI or pronuclear stage Zygote was transferred. The groups were matched for age and severity of male factor infertility.

Result: There was no significant difference of clinical and ongoing pregnancy rate per retrieval between the two groups. Clinical pregnancy rate after Gamete Intrafallopian Transfer of Ova immediately after ICSI 51.4% (72/140) and for PROST after ICSI 53.1% (85/160) ongoing pregnancy rates after Gamete Intrafallopian Transfer of Ova immediately after ICSI 41.5% (58/140) and for PROST after ICSI 43.5% (61/140) there was no difference in the Incidence of Ectopic or multiple gestation and abortion rates between the two groups.

Conclusion: Excellent pregnancy rates were obtained with both techniques but double surgical procedure, double cost, and double invasiveness in PROST after ICSI encourage Gamete Intrafallopian transfer of Ova immediately after ICSI as a treatment of choice for patients with at least one functional Fallopian tube and with severe male factor infertility.

P-023

Standardization of In Vitro Maturation Treatment for Patients with Polycystic Ovary (PCO) and Polycystic Ovarian Syndrome (PCOS).
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Objectives: Recovery of immature oocytes followed by in vitro maturation (IVM) of the oocytes could be developed as a new method for the treatment of patients with infertility due to PCO and PCOS. However, at present the data available on IVM treatment for patients are limited. To determine the IVM treatment for anovulatory patients with PCO and PCOS human chorionic gonadotropin (hCG) were given before immature oocyte retrieval.

Design: Maturation rate of immature oocytes, fertilization rate and ongoing pregnancies were determined following priming with or without hCG before immature oocyte retrieval.

Materials and Methods: The study was approved by IRB of the hospital. All patients were under 40 year-old and had failed conceive after at least 6 cycles of ovulation induction combined with intrauterus insemination. Three patients had also failed to achieve pregnancy following 3 cycles of conventional IVF treatment. Following the induction of a withdrawal bleed, an ultrasound scan was performed on day 2–4 of the menses in order to exclude any functional cyst, and repeated on day 7–9 in order to exclude the development of dominant follicle. Immature oocyte retrieval was then scheduled on day 10–14 and the patients randomly allocated to receive or not to receive 10,000 IU hCG 36 h prior to the retrieval. Immature oocytes were cultured in TC-199 medium supplemented with 20% FBS, 100 mIU/mL FSH and LH for 24 to 48 h and checked 12 h intervals. Matured oocytes were fertilized by ICSI. Embryos were transferred on day 2 or 3 following ICSI. Following immature oocyte retrieval all patients were given estradiol, while progesterone was started from the day of ICSI. Immediately prior to embryo transfer the endometrium and uterine blood flow parameters were assessed by ultrasound.

Results: A total of 12 patients performed 15 cycles of IVM treatment. Priming with hCG was given in 8 cycles, and without hCG was given 7 cycles. The mean number of immature oocytes was higher ($P<0.05$) in the group with hCG (7.25 ± 3.15) than the group without hCG (3.71 ± 2.19). Maturation and fertilization rates were 91.4% (53/58) vs. 88.5% (23/26) and 86.8% (46/53) vs. 78.3% (18/23) in two groups, respectively. The mean number of ET was 3.0 ± 0.87 vs. 2.0 ± 1.07 in the groups. Five (3 from with hCG group and 2 from without hCG group) pregnancies (33.3%) were ensured by blood test and by ultrasound scan.

Conclusions: Priming with hCG before immature oocyte retrieval increases the oocyte retrieval rate and improves maturational and developmental competence of the oocytes, thereby potentially improves the pregnancy rate of patients with PCO and PCOS. Following this standardization, the infertile women with PCO and PCOS are offered IVM treatment routinely.

P-024

Moderately Elevated Day 3 FSH Has Limited Predictive Value, Especially In Younger Women
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Objective: Cycle day 3 FSH levels are valuable predictors of fertility potential. The use of this screening test in young women and the interpretation of a moderately elevated FSH is still controversial. In this study we attempt to determine the predictive ability of a moderately elevated FSH value (10–11.4 mIU/mL, 2nd international standard) in different age groups, and to determine its predictive value in achieving pregnancy through IVF-ET, independent of follicular response to gonadotropins.

Design: Retrospective analysis of the association of cycle day 3 FSH levels with pregnancy outcome in women undergoing IVF using logistic regression to control for response to gonadotropins.

Materials and Methods: We reviewed 315 initiated IVF cycles and included 293 cycles (94%) in which records were complete. Age and FSH was stratified into the categories <35, 35–39, and ≥40 and <10, 10–11.4, and >11.4 respectively. The FSH value used was the highest value recorded in the 9 months preceding and including the initiation of the patient's first IVF cycle. Predictive values were calculated and compared between groups before and after controlling for ovarian response.

Results: Total and ongoing pregnancy rates stratified by age and FSH (total/ongoing per initiated cycle):

FSH	<35	35–39	≥40
<10	48.6%/36.9%	34.1%/29.7%	20.9%/11.6%
10–11.4	54.6%/36.4%	18.2%/18.2%	33.3%/33.3%
>11.4	22.2%/0%	12.5%/0%	14.3%/0%

Overall an FSH > 11.4 was 100% predictive of failure to achieve an ongoing pregnancy. However, a moderately elevated FSH (10–11.4) was inaccurate in predicting IVF failure 28% of the time (predictive value 72%). It failed to correctly predict IVF failure in 39.4% of women < 35 and in 18.8% of women 35–39. The mean number of embryos transferred, oocytes retrieved, estradiol level on the day of hCG administration and total dose of gonadotropins administered were all significantly associated with age, FSH value, and IVF success. When controlling for these variables using logistic regression, a high FSH value (>11.4) significantly predicted IVF failure, independent of response to gonadotropins, in women younger than 40 ($P = 0.02$) but not for women older than 40 ($P = 0.72$). Most importantly though, an FSH value of 10–11.4 was not an independent predictor of outcome at any age.

Conclusions: Even though a high FSH (>11.4) independently predicts IVF failure in younger women, a moderately elevated FSH (10–11.4) has no significant predictive ability independent of poor response to gonadotropins. We propose, that an intrinsic oocyte problem in these women can be clinically overcome by increasing oocyte numbers through improvement in ovarian stimulation.

P-025

Relationship Between Lead Follicular Size and Oocyte Maturity in the Cohort at the Time of hCG Administration R. Pyrzak, R. D. Dickey, S. Sartor, P. Y. Lu, S. N. Taylor, P. H. Rye. Fertility Institute of New Orleans, New Orleans, LA.

Objective: Maturity of oocyte at the time of retrieval influences fertilization rate and embryo quality. In this study we evaluated oocyte maturity by the size of lead follicle in the cohort on the day of hCG administration.

Design: Retrospective analysis of 238 ovarian stimulation cycles in patients undergoing IVF-ET.

Material and Methods: Superovulation was achieved by down regulation with GnRHa (Nafarelin nasal spray, Synarel) followed by ovarian stimulation with hMG or FSH. On the day of hCG administration (10,000 IU) the mean diameter of each follicle in the cohort was measured by ultrasound. Morphology of the cumulus-oocyte-complex was assessed for oocyte maturity immediately after retrieval. Patients' cycles were divided into four groups according to the mean size of the lead follicles, from both ovaries, on the day of hCG administration. Data are presented as means, differences among the groups were analyzed using *t*-test and chi square test. $P < 0.05$ was considered significant.

Results: There were no significant differences in mean age and years of infertility among the groups. There were significantly ($p<0.01$) higher number of oocytes in Group 1 than in Group 2 and highly significant ($p<0.0001$) from Group 3 and 4. The percent of mature oocytes were 71.9, 77.4, 77.8, 79.2, and the fertilization rate were 96%, 86%, 89%, 86% in Group 1, 2, 3, and 4.

Lead follicle size mm (n) Follicle Size	15–16 (24) Group 1	17–18 (36) Group 2	19–20 (70) Group 3	>21 (108) Group 4
9–10	5.9	3.6	3.3	2.4
11–12	7.0	3.7	3.0	2.6
13–14	5.9	4.5	3.2	2.4
15–16	2.7	3.1	2.6	2.0
17–18	0	2.3	2.3	2.0
19–20	0	0	1.7	1.5
>21	0	0	0	2.0
Total Follicles	21.6	17.2	16.1	15.0
Total oocytes	20.9	15.6	12.9	11.2
% recovery (≥9 mm)	96.8	90.7	80.1	74.2
Mature oocytes	15.0	12.1	10.0	8.9

Conclusion: Follicle size is a poor indicator of oocyte maturity. Sufficient numbers of mature oocytes can be retrieved from patients with smaller lead follicles. Follicles of 9 mm and smaller may produce mature and normal oocytes. Mature oocytes were retrieved from follicles 9–10 mm when lead follicle size were 15–16 mm. In addition the cohort with larger follicles may result in a decrease in number of mature oocytes, which may reduce the reproductive potential.

P-026

Basal Ultrasonographic Grading of Ovaries Seems to Predict Ovarian Reserve Better than Basal Inhibin B, FSH/LH Ratio and E_2 in IVF Population O. Sarlak, K. Ozgur, C. Sonmez, M. Uner, O. Erman. Akdeniz University Medical School, IVF Unit, Department of Obstetrics and Gynecology, Antalya, Turkey.

Objectives: The prediction of ovarian reserve is a vital information to determine the number of ampules used in high as well as low responder patients in IVF practice. We have recently introduced a basal ultrasonographic grading system based on mainly to follicle number. Our aim was to determine the best predictor of ovarian reserve in IVF population using the parameters that are used in current clinical practice.

Design: Prospective cohort study.

Material Method: Sixty patients have been selected between March 1998–January 1999. The ovarian grading at day 3 was done as follows; grade 1: only stroma and no follicles that are visible by ultrasound; grade 2: less than 4 follicles were visible; grade 3: more than 4 follicles but scattered both central and peripherally; grade 4: PCO-like ovaries with both central and peripheral follicles less than 12; grade 5: classical PCO type ovaries with more than 12 follicles aligned peripherally. All hormones were determined at day 3. Statistical analysis was performed using multiple linear regression analysis test to determine the best predictor for the number of mature oocytes that were collected.

Results: Two independent variables have been found to be significant to predict the number of mature oocytes ($r^2=0.29$). The first one was ovarian grade which revealed the best prediction for mature oocyte number ($B=4.7002$, $p=0.0026$) and the second one was day 3 E_2 ($B=-0.0714$, $p=0.0488$). However day 3 FSH, FSH/LH as well as inhibin B levels did not show any significance.

Conclusion: In our study we suggest that ovarian grading is the best predictor of ovarian response. We believe that this information will help the clinicians to adjust the gonadotropin dose in IVF patients.

P-027

Effect of ICSI on Embryo Development and Pregnancy Rates in Cases Undergoing *In Vitro* Fertilization and Day 5 Transfer. K. Zeitoun, J. O'Neil, D. Minjarez, G. Attia, B. R. Carr, W. Byrd, D. Smith. Department of Obstetrics and Gynecology, UT Southwestern, Dallas, Texas.

Objectives: Two techniques becoming increasingly important in *in vitro* fertilization and embryo transfer (IVF-ET) are ICSI and blastocyst stage (day 5) transfers. There is data suggestive of an adverse effect of ICSI on blastocyst development, but no data is available on the effect of ICSI on pregnancy rates with day 5 transfers. The objective of this study was to determine the effect of ICSI on embryo development (blastocyst) and pregnancy rates after day 5 transfers.

Design: Retrospective study in a university based IVF-ET program.

Patients and Methods: All patients undergoing blastocyst (day 5) transfers in 1998 ($n=43$ patients). Blastocyst transfer was performed in 28 cases after conventional (IVF) and 15 cases who underwent ICSI for male factor infertility. There was no significant difference in the age, number of oocytes fertilized or number of blastocysts transferred between both groups. Patients were stimulated by gonadotropins after luteal phase down regulation with GnRH agonists. Retrieval was performed after meeting standard criteria. Cases with 6 or more embryos on day 2 were selected for blastocyst (day 5) transfer. Embryo development to the morula and blastocysts stage and ongoing pregnancy rate were used as outcome measures for this study. Student t-test and chi square were used for parametric and nonparametric data analysis respectively.

Results: In the group undergoing conventional fertilization ($n=28$) and ICSI ($n=15$) the mean ± SEM % of fertilized oocytes reaching the blastocyst stage was 60 ± 4.7% and 47 ± 4.7% respectively. This was significantly higher in the conventional IVF group $p<0.025$. The ongoing pregnancy rates were however, not significantly different between both groups $P=0.8$. The ongoing pregnancy rate in the conventional IVF group was 54% and in the ICSI group 40%.

Conclusion: There seems to be an adverse effect of ICSI on embryo development due either to disruption of the egg during micromanipulation (zona or plasma membrane) or due to inherent defects in gametes (sperm or egg). This did not translate into decreased pregnancy rates in our group of patients. Actually day 5 transfers in cases undergoing ICSI might help exclude embryos of less quality (chromosomal defect) and thus improve implantation rates in this group.

P-028

Rate of Blastocyst Formation From Day Three Multi-Cell Embryos. [1]M. Langley, [1]D. Marek, [2]D. K. Gardner, [1]N. Confer, [1]L. Cram, [1]L. Underwood, [1]K. M. Doody, [1]K. J. Doody. [1]Center for Assisted Reproduction, Bedford, TX and [2]Colorado Center for Reproductive Medicine, Englewood, CO.

Objective: Embryonic cleavage rate during the first 72 hours following insemination is widely held to be an important indicator of embryo "quality". Previous studies suggest that rapidly cleaving embryos have an increased implantation rate. This study was undertaken to evaluate the association of blastomere number at 72 hours post insemination with subsequent rates of blastocyst formation.

Design: A retrospective analysis of embryo development resulting from

IVF and ICSI cases occurring through January 1, 1998 through December 31, 1998.

Materials and Methods: Patients undergoing IVF were selected for blastocyst culture regardless of the number of Day 3 eight cell embryos. Pronuclear embryos were cultured in 100 μl micro-drops S-1 Media (IVF Science) in groups of two under oil for 48 hours followed by culture in 100 μl micro-drops S-2 Media for 48 to 72 hours to blastocyst stage (Day 5 or Day 6).

Results:

Age	Day 3 cell stage:	2 cell	3 cell	4 cell	5 cell
<35	Total:	44	76	262	207
	Blast Day 5 (%):	4/9.1	4/5.3	56/21.4	51/24.6
	Blast Day 6 (%):	1/2.3	7/9.2	37/14.1	20/9.7
35–39	Total:	24	33	170	90
	Blast Day 5 (%):	0/0	0/0	18/10.6	25/27.8
	Blast Day 6 (%):	0/0	1/3.0	24/14.1	7/7.8
>39	Total:	8	5	35	18
	Blast Day 5 (%):	0/0	0/0	3/8.6	6/33.3
	Blast Day 6 (%):	0/0	0/0	6/17.1	3/16.7
All	Total:	76	114	467	315
Ages	Total Blast (%):	5/6.6	12/10.5	144/30.8	112/35.6

Age	6 cell	7 cell	8 cell	9 cell	10+ cell
<35	266	266	395	55	67
	120/45.1	145/54.5	270/68.4	34/61.8	45/67.2
	34/12.8	33/12.4	32/8.1	10/18.2	3/4.5
35–39	107	88	136	15	20
	37/34.6	45/51.1	86/63.2	11/73.3	13/65.0
	9/8.4	12/13.6	8/5.9	0/0.0	2/10.0
>39	21	13	46	3	2
	6/28.6	6/46.2	29/63.0	3/100	0/0
	2/9.5	1/7.7	1/2.2	0/0	1/50
All	394	367	577	73	89
Ages	208/52.8	242/65.9	426/73.8	58/79.5	64/71.9

Results include donor oocytes in age <35 category.

Conclusion: Although maximal blastulation rates were observed in embryos that had progressed to at least 8 cells by 72 hours post insemination, a significant number of embryos with initially retarded development were able to progress to blastocyst by 120–144 hours. The ultimate viability of these embryos remains to be quantified.

P-029

Validity of 17 OH Progesterone (17oh-P) Monitoring Pre hCG in IVF Cycles. [1,2]D. C. Daly, [1]C. L. Daly, [1]D. Mayo. [1]Grand Rapids Fertility, Grand Rapids, Mi. [2]Department of Obstetrics/Gynecology, Spectrum Health East, Grand Rapids, Mi and Michigan State College of Human Medicine, East Lansing, Mi.

Objective: Assess whether monitoring 17oh-P during IVF inductions is valid based on cycle outcome. Assess whether our SART pregnancy rate was predicted by 17oh-P pattern variation.

Design: Retrospective analysis of a standardized monitoring protocol using ultrasound (US), estradiol (E2), and 17oh-P for patients undergoing IVF from 1/1/95 to 12/31/98.

Materials and Methods: 17oh-P replaced progesterone during 1994 in our monitoring protocol. The rise in 17oh-P from day 4 of FSH/hMG to the day of a 16–17mm lead follicle (fol) was used in combination with US and E2 monitoring in making patient decisions. Four patterns were defined: type 1 >9 fol, rise 17oh-P >1.5ng/ml, type 2 >9 fol, rise 17oh-P <1.6 ng/ml, type 3 <10 fol, rise 17oh-P >0.15 ng/ml/fol, type 4 <10 fol, rise 17oh-P <0.16ng/ml/fol or >8 fol a rise 17oh-P <1.0 ng/ml. Cancellation was suggested for Type 4 cycles. Pregnancy, delivery, miscarriage, and implantation rate were assessed by chi square analysis. Using the 4 year delivery rate for each type induction and the number of each type a predicted pregnancy rate was generated and compared to the actual delivery rate for each year.

Results: Type 3 and 4 cycles tended to occur in older patients otherwise there was no historical or demographic differences based on induction type.

Results are in the tables:

	cycles	cancel	trans #	pregnancy	delivery	abortion	implantation
type 1	120	0	3.95	66 (55%)	58 (48%)	8 (12%)	25.3%
type 2	50	2	4.65	17 (35%)	9 (19%)	8 (44%)	12.5%
type 3	36	3	3.70	17 (51.5%)	12 (36%)	5 (29%)	20.5%
type 4	62	22	3.00	2 (5%)	1 (2.5%)	1 (50%)	1.5%
			p<.05	p<.001	p<.001	p<.05	p<.001

The overall delivery rate was 32.3%. The actual and predicted delivery rates for each year were: 1995 32.3% and 30.6%, 1996 22.4% and 25.5%, 1997 30.1% and 32.4%, 1998 38.9% and 35.1%. 70% of the variation in our yearly delivery data is accounted for by patient variation.

Conclusions: 17oh-P is predictive of cycle outcome. Type 4 cycles even extended 1–2 days had poor results, cancellation is recommended. Transfer in type 1 and 3 cycles should not exceed 4 embryos. In type 2 cycles 5 or 6 embryos may be OK. The data suggests the majority of variation in our year to year delivery rate was do to the patient population variability.

P-030

Dealing With Poor Response to IVF Stimulation: Do Not Cancel; Rescue the Cycle. K. T. Laremont, E. Katz, R. A. Yazigi. The GBMC Fertility Center, Baltimore, MD.

Objectives: To evaluate the outcome of interruption followed by resumption of gonadotropin stimulation in lieu of cancellation, in IVF cycles with poor initial response.

Design: Retrospective study.

Materials and Subjects: The IVF stimulation records of 32 patients with poor initial response to gonadotropin stimulation between January 1996 and December 1998 were reviewed. After initial gonadotropin stimulation all patients were subjected to a temporary interruption of treatment due to poor response, followed by resumption of therapy that same cycle (cycle rescue) until hCG administration. Leuprolide acetate was initially used either in a long or a flare protocol, but was not administered during the rescue stage.

Results: Mean patient age: 37.3 yrs; Retrievals: 23; Transfers: 21; Mean peak E2 at time of hCG: 967.35 IU; Mean number of stimulation days: 10.9; Mean number of interruption days: 3.34 Mean number of ampules consumed: 55.5; Mean number of embryos transferred: 3.45; Implantation rate: 9.9%; Pregnancies/retrieval: 17.4%; Pregnancies/transfer: 19% (singleton:1; twins:1; quadruplets:1; ectopic:1). Two chemical pregnancies occurred in addition. Eight cycles were canceled for persistent poor response (25%), and 1 cycle was canceled for personal reasons (3.1%).

Conclusion: Gonadotropin therapy interruption can effectively rescue an IVF cycle in poor responders who would have otherwise faced cycle cancellation. Cost savings may result and pregnancies do ensue. Results compare favorably with pregnancies/retrieval (20%), pregnancies/transfer (21.1%) in poor responders who did not meet criteria for cancellation.

P-031

Comparison of Viability Between Cryopreserved Fresh Human Testicular Spermatozoa and Testicular Spermatozoa That Were Cryopreserved After In Vitro Culture for 3 Days. J. Liu, X. Z. Zheng, T. A. Baramki, R. A. Yazigi, G. Compton, E. Katz. The Greater Baltimore Medical Center Fertility Center, The Greater Baltimore Medical Center, Baltimore, MD.

Objective: The combination of testicular biopsy and intracytoplasmic sperm injection is an efficient way for the treatment of infertile patients with azoospermia. Cryopreservation of testicular spermatozoa can avoid repeated testicular biopsy for patients who need more treatment cycles. It is known that the motility of live testicular sperm can be improved after they are cultured in vitro for a few days. In this study we wanted to determine whether human testicular spermatozoa should be frozen on the day of biopsy (fresh) or after they are cultured for 3 days. A single sperm freezing technique was used in this study. A few motile testicular spermatozoa were injected into an empty zona pellucida and then cryopreserved. In this way, the viability of testicular spermatozoa after cryopreservation could be precisely examined.

Design: From each sample, some of the fresh motile testicular spermatozoa were frozen 2 hours after biopsy (group 1); some of the testicular spermatozoa were first cultured in vitro for 72 h and then cryopreserved (group 2). Viability of frozen-thawed testicular spermatozoa was compared between group 1 and 2.

Materials and Methods: To prepare the sperm suspension, after biopsy, testicular tissue was minced and transferred into a tube containing modified human tube fluid medium (H-HTF) with 3% synthesis serum substitute (SSS). After removal of debris, the tube was centrifuged for 5 min at 300 g. The supernatant was removed and the pellet was resuspended in about 0.1 mL H-HTF. Culture of testicular sperm was carried out in H-HTF with 3% SSS (5 μL sperm suspension was added in a 40 μL droplet of HTF with 3% SSS). Empty zona pellucida was obtained from mouse eggs in which the ooplasm was completely aspirated by using an injection needle. Single motile sperm was selected and injected into the empty zona. The zona with testicular sperm was transferred into a H-HTF containing 8% glycerol and 3% SSS and kept at room temperature for 10 min. The zona with sperm was then loaded into a 0.25 mL plastic straw. The straws were exposed to liquid nitrogen vapor for 2 hours and then plunged into liquid nitrogen. For thawing, the straws were taken out of liquid nitrogen and put in a 37°C waterbath for 30 sec. The content of the straw was transferred into H-HTF with 3% SSS. The viability of the sperm was determined if the spermatozoa were motile or the non-motile spermatozoa showed viability using Eosin staining. The results were analyzed using χ^2 test. Probabilities <5% were considered significant.

Results: Testicular spermatozoa were obtained from 6 azoospermic patients; four had obstructive azoospermia and two had non-obstructive azoospermia. All frozen empty zona and spermatozoa were recovered after thawing. The rates of viable spermatozoa after cryopreservation were 64% (176/276) in group 1 and 59% (150/255) in group 2, respectively. There was no statistically significant difference in the viability of spermatozoa after cryopreservation between group 1 and 2 ($P > 0.05$, χ^2 test).

Conclusion: Human testicular spermatozoa can be cryopreserved either 2 h after biopsy (fresh) or after culture for 3 days. The single sperm freezing procedure is an efficient method to recover frozen testicular sperm and to study the outcome of cryopreservation.

P-032

In a Highly Successful IVF Program, Progesterone Administered from Single Daily IM Injections or Vaginal Progesterone Gel Applications Is Equally Effective at Providing Luteal Support. W. B. Schoolcraft, J. S. Hesla, M. Gee, D. K. Gardner. Center for Reproductive Medicine, Englewood, CO.

Objective: It is widely accepted that luteal support must be provided in IVF-ET cycles. Until now daily IM injections were used which cause pain and potential complications. Recently, progesterone supplementation from a bioadhesive vaginal gel has become available. The current trial looked at the efficacy of luteal support from daily administration of the vaginal gel in an IVF program with high pregnancy rates.

Design: Open trial comparing the impact of 2 types of luteal support on pregnancy rates.

Materials and Methods: Forty three infertile women undergoing IVF elected to receive luteal support from progesterone gel, Crinone 8% (Wyeth-Ayerst Laboratories, Radnor PA) administered once daily. This gel provides sustained release of progesterone through bioadhesive characteristics linked to its polycarbophil base. Forty six other women undertaking concurrent IVF cycles and receiving luteal support from daily IM injections (50 mg) served as controls. Pregnancy rates (PR) were evaluated approximately 2 weeks after the procedure by hCG (Chem PR), 2 to 4 weeks later by ultrasounds (Clin PR) and at 20 weeks (Ongoing PR).

Results: As illustrated, all parameters including demographic characteristics, number of embryo transferred (EMB), incidence of assisted hatching (AH) and blastocyst transfers (Blast), chemical, clinical and ongoing PR were similar for both groups of IVF patients, except for the incidence of ICSI which was higher in the IM group. Luteal support was initiated 2 ± 0.2 and 2 ± 0.3 days (mean ± SD) after oocyte retrieval in women receiving luteal support from IM or vaginal progesterone, respectively.

	Age mean (SD)	EMB mean (SD)	ICSI n (%)	Blast n (%)
IM (n = 46)	35 (4.5)	3 (1.2)	20 (44)	9 (20)
Vag (n = 43)	37 (4.6)	3 (1.2)	11 (26)	12 (28)

	AH n (%)	Chem PR (%)	Clin PR (%)	Ongoing PR (%)
IM (n = 46)	33 (72)	34 (73.9)	28 (60.9)	26 (56.5)
Vag (n = 43)	28 (65)	32 (74.4)	25 (58.1)	24 (55.8)

Conclusion: Our results showed that progesterone from progesterone gel or IM injection are equally effective at providing luteal support in IVF-ET as expressed by similar pregnancy rates in concomitant parallel groups. Because our program routinely achieves pregnancy rates in excess of 50%, this trial provides the ultimate proof of efficacy for luteal support from vaginal progesterone gel. This represents an appreciable improvement as it frees IVF patients from the need to use painful daily IM injections.

P-033

Blastocyst Culture and Transfer: Analysis of One Year's Experience. [1]W. B. Schoolcraft, [2]D. R. Meldrum, [2]M. Hamilton, [1]J. Stevens, [1]T. Schlenker, [1]L. Wagley, [1]S. Guadagnoli, [1]J. Johnson. [1]Colorado Center for Reproductive Medicine, Englewood, CO and [2]Center for Advanced Reproductive Care, Redondo Beach, CA.

Objective: Blastocyst culture and transfer utilizing chemically defined, serum-free, co-culture free, media has been successfully accomplished in several centers. To date, reports have focussed on relatively few patients. We review one year's experience in two IVF units with blastocyst stage transfer to determine if the initial high blastocyst development and implantation rates prevail in a large multicenter experience.

Design: Retrospective review of blastocyst transfer in two IVF clinics.

Materials and Methods: Patients with 10 or more follicles at the time of hCG were offered blastocyst culture and transfer. Insemination was performed in an overnight incubation of Ham's F-10, followed by culture of pronucleate embryos in medium G 1.2 for 48 h. On day 3 all embryos put through several washes of medium G 2.2 before being placed into culture for a further 48 to 72 h. Blastocyst development was scored on the morning of day 5 and transfers took place around noon. In four cases blastocyst formation and transfer took place on day 6. Expanded blastocysts that were not transferred were frozen.

Results: One hundred and fifty two patients underwent blastocyst culture, of which 3 (<2%), failed to have any blastocysts for transfer, and 2 patients had all their blastocysts frozen. The mean blastocyst development was 48%, with 87% of all blastocysts forming by day 5. The mean number of blastocysts transferred was 2.2 resulting in an implantation rate of 45% and a pregnancy rate of 61% per oocyte retrieval. The percentage of patients with twins was 40%. Seventy percent of patients had blastocysts frozen, with a mean of 3.9 blastocysts per patient. In this patient group of good responders to gonadotropins, blastocyst development and implantation rate were not affected by FSH levels or ICSI. Although blastocyst development was not affected by previous IVF failure, implantation (34%) and pregnancy (50%) rates were significantly lower than for patients undertaking their first IVF cycle (54%, $P < 0.01$; and 68%, $P < 0.05$, respectively). Stimulation protocol utilized had no effect on blastocyst development, but did have a significant effect on implantation rate and pregnancy outcome. Patients who received pure FSH either in the form of recombinant or urinary-derived had significantly reduced implantation (40%) and pregnancy (55%) rates compared to those patients who received FSH and LH together (60%, $P < 0.01$; 73%, $P < 0.05$ respectively).

Conclusions: Blastocyst culture and transfer is an effective means of treating patients who respond well to gonadotropins. High pregnancy rates can be established with low numbers of embryos transferred. Patients failing to achieve embryo transfer were rare. Controlled ovarian hyperstimulation protocols, by influencing oocyte competence, appear to have a significant impact on blastocyst implantation and resultant pregnancy rates.

P-034

The Impact of Blastocyst Transfer on the Outcome of Oocyte Donation. W. B. Schoolcraft, J. Stevens, T. Schlenker, L. Wagley, S. Guadagnoli, J. Hesla, D. K. Gardner. Colorado Center for Reproductive Medicine, Englewood, CO.

Objective: Oocyte donation represents an effective treatment for patients with impaired or absent oocyte function. Indeed, by utilizing a source of oocytes with optimal quality, IVF efficiency can be maximized. Resulting high success rates are often associated with unwanted multiple gestations. One year's experience of oocyte donation in our center was reviewed and the efficacy of early cleavage stage embryo transfer compared to blastocyst stage transfer.

Design: Retrospective review of oocyte donation outcome comparing cleavage stage to blastocyst transfer.

Materials and Methods: Between January and December 1998, 108 consecutive cycles of oocyte donation were analyzed. No cases performed in this calendar year were excluded from analysis. Recipients were prepared with GnRH-a down regulation followed by sequential transdermal estrogen and intramuscular progesterone beginning the day before oocyte retrieval. Cleavage stage transfers were performed on day two or three (4- to 8-cell stage). Embryo culture utilized Ham's F-10 with 15% fetal cord serum. Blastocyst transfers were performed on day five after embryo development in the serum-free chemically defined sequential culture system G1.2 and G2.2. In the majority of patients, two blastocysts were transferred on day five, compared to three or four embryos at the early cleavage stage.

Results: The mean recipient age for cleavage stage (39.8 ± 0.5; $n = 76$) and blastocyst transfers (42.6 ± 0.8; $n = 32$) was significantly different ($P < 0.01$). The mean number of embryos transferred was significantly higher ($P < 0.001$) on day 2 and 3 (3.2 ± 0.06) than on day 5 (2.0 ± 0.08). Percentage fetal sac and fetal heart development for cleavage stage transfers was 46 and 41% respectively. These values were significantly below ($P < 0.01$) those obtained for blastocyst transfers, 65 and 62%. There were no differences in resultant pregnancy rates (82% for cleavage stage embryos and 91% for blastocysts). Although there were no differences in the twinning rate between either day of transfer, there were no high order multiple gestations in the blastocyst group.

Conclusions: Oocyte donation may represent a model to assess the maximum efficiency of human in vitro fertilization. Although extremely high pregnancy and implantation rates were observed in this series of patients, multiple gestation rates were also high. The transfer of a maximum of two day-five (blastocyst stage) embryos eliminated the untoward outcome of triplet gestations. Blastocyst culture and transfer may therefore represent the way to maximize donated oocytes between different recipients.

P-035

Deferred Embryo Transfer to Reduce the Incidence of Ovarian Hyperstimulation Syndrome (OHSS) in In Vitro Fertilization (IVF). [1]G. Halverson, [1]P. Mehring, [1]K. Daly, [1]D. Kuklinski, [2]M. Roesler, [2]C. Gunnarson. [1]WomenCare, Waukesha, WI, [2]Waukesha Memorial Hospital, Waukesha, WI.

Objective: OHSS is a serious complication of gonadotropin therapy for in vitro fertilization and may be categorized as mild, moderate or severe. This study reviews the various approaches to limit severe OHSS as well as the frequency of pregnancy in a subpopulation of patients for whom fresh embryo transfer is deferred and all embryos are cryopreserved for use in future cycles.

Design: Retrospective analysis of 20 IVF cycles where fresh embryo transfer was deferred for fear of OHSS.

Materials and Methods: Between 1990 and 1998, 20 of 298 cycles were judged to be at risk for OHSS. All patients were stimulated with mid luteal phase GnRH-a/hMG and/or FSH. Stimulations were monitored by serial serum estradiol (E2) levels and follicular ultrasound measurements. All ova retrievals were performed by ultrasound guided needle aspirations. Efforts to limit the risk of OHSS through manipulation of the hCG dose and administration of intravenous albumin is reviewed. The pregnancy rate by transfer of thawed cryopreserved embryos in subsequent cycles is also presented.

Results: A single patient suffered from moderate OHSS involving ascites, and was managed at home. A single patient suffered severe OHSS requiring hospitalization. Table 1.

No. of Pts	Mean Pt Age	Mean E2 Day of hCG pg/ml	No. of Pts HCG Dose 5,000/10,000	No. of Pts given IV Albumin	Ongoing Pregnancy Rate
20	31.9	4664	11/9	13/20 (65%)	10/20 (50%)

Conclusions: The strategy of deferring fresh embryo transfer and employing the use of reduced hCG dose and albumin may offer an advantage in limiting the occurrence of OHSS. In addition to recognizing "safety first" this approach maintains an acceptable pregnancy rate.

P-036

Impact of Reducing the Number of Embryos Transferred From Three To Two in Women Under the Age of 35 Who Produced Three or More High Quality Embryos. S. J. Phillips, N. Dean, W. M. Buckett, R. Hemmings, F. Bissonnette, M. M. Biljan, S. L. Tan. McGill Reproductive Center, Royal Victoria Hospital, McGill University, Montreal, Canada.

Objectives: Transfer of multiple embryos in IVF has resulted in an increase in multiple pregnancy rates, which is associated with an increased risk of obstetric and neonatal morbidity and mortality. However, the fear of compromising pregnancy rates has led to a reluctance to reduce the number of embryos transferred. In this study we have investigated the effect of reducing the number of embryos transferred on pregnancy rates and the incidence of multiple pregnancy for women under 35 years of age.

Design: Prospective observational study with historical controls.

Materials and Methods: The results of IVF in women under the age of 35 years, who had an embryo transfer, over two time periods (December 1 1996 to November 30 1997 and December 1 1997 to November 30 1998) were compared. In the first period, patients were encouraged to have two embryos transferred if they had three or more good quality embryos (Grade 1 or 2) but they were permitted to have three embryos transferred if they desired. In the second period, all patients who had three or more good quality embryos were only permitted to have a maximum of two embryos transferred. The remaining patients were allowed to have up to three embryos transferred.

Results: One hundred and twenty one patients were treated in period 1 compared with 187 patients in period 2. There was no difference in age (Median difference (MD) = 0.1 95% Confidence Interval (CI) = −0.3 − 0.6) or cause of infertility ($p = 0.77$) between the two groups. In period 1 significantly more embryos were transferred (median = 3 (range 1–3) vs median = 2 (range 1–3) $p < 0.0001$ MD = 1 95% CI = 0–1). The Cumulative Embryo Score (CES) per embryo transferred (MD = −0.2 95% CI = −1.3–0.7), implantation rate (22.4% vs 24.6% Odds Ratio (OR) = 0.9 95% CI = −0.6–1.3), and ultimately pregnancy rate (37.2% vs 41.7% OR = 0.8 95% CI = 0.5–1.4) were not different between the two groups. However, while in period 1 the multiple pregnancy rate was 58.8%, the rate in period 2 was 31.3% ($p = 0.017$ OR = 3.0 95% CI = 1.2–7.9). Finally, patients in period 1 were ten times more likely to conceive triplets (17.6% vs 1.5% $p = 0.01$ OR = 10.0 95% CI = 1.5–321.8).

Conclusions: The results of this study show that the pregnancy rate for women under the age of 35 can be maintained, with a significant reduction in multiple pregnancy rate, by reducing the number of embryos transferred to two, if there are three or more high quality embryos available.

P-037

Withdrawn

P-038

Supernumerary Blastocysts Are A Valid Indicator for Improved Pregnancy Outcome with Early Transfers. R. A. Kaufmann, E. J. Servy, F. Gilchrist, C. Daley, Y. J. Menezo. Augusta Reproductive Biology Associates, Augusta, GA.

Objectives: Blastocysts are now becoming more commonplace and providing improved implantation rates. However, one limitation is the potential

lack of embryos for transfer. Approximately 40% of patients, where embryos are allowed to develop in culture, will not have blastocysts. In our program we transfer embryos on day 2 or 3, then allow the remainder to develop to the blastocyst stage. In this study we compared the pregnancy potential in patients with early stage transfer when blastocysts were or were not available for cryopreservation.

Design: Retrospective study of 300 IVF cycles to assess whether there is a difference in outcome in cycles with or without supernumerary blastocysts available for cryopreservation.

Materials and Methods: IVF patients undergoing controlled ovarian hyperstimulation from 1994 to 1999 were studied. Patients had embryo transfers on day 2 or 3 and it was determined whether or not embryos reached the blastocyst stage for cryopreservation. Data were analyzed by chi square analysis.

Results: There were 108 cycles that had an early transfer and blastocysts to cryopreserve. This group resulted in 38 on-going pregnancies for a 35% pregnancy rate. The other group consisted of 192 cycles, which did not have supernumerary embryos reaching the blastocyst stage. Forty four pregnancies resulted for a rate of 23%. There was a statistical significance between these two groups ($P < 0.025$).

Conclusions: Patients with fresh early stage transfers who obtained one or more blastocysts had a greater potential to become pregnant, than the patients whose supernumerary embryos did not reach the blastocyst stage. This may provide valuable information in counseling couples for subsequent cycles.

P-039

Does the Aspiration of Cervical Mucus Prior to Embryo Transfer in Women Undergoing In Vitro Fertilization and Embryo Transfer Improve Pregnancy Rates? [1]D. Soroka, [2]G. Wells [1,3]D. D. Kotarba. Ottawa Hospital, Ottawa, ON. [1]Department of Obstetrics and Gynecology. [2]Department of Clinical Epidemiology, [3]Division of Reproductive Medicine.

Objective: In Vitro Fertilization and Embryo Transfer (IVF-ET) is constantly evolving to improve pregnancy rates. Mechanical factors, such as the cervical mucus, may hinder the successful passage of embryos into the uterine cavity at ET. Nabi et al found that aspiration of cervical mucus prior to embryo transfer reduced the rate of embryos retained in the transfer catheter. Mansour et al reported that they were able to decrease the ejection of dye from the external cervical os from 57% to 23% by aspirating the cervical mucus prior to the introduction of dye. Our study was designed to determine if the aspiration of the cervical mucus prior to embryo transfer improves pregnancy rates in women undergoing IVF-ET.

Study Design: A prospective randomized controlled trial.

Materials and Methods: Block randomization was used to assign patients to a treatment and control group. Cervical mucus was aspirated using a 14-guage angiocatheter attached to a tuberculin syringe (treatment group). Cervical mucus was not aspirated in the control group. Entrance into the study followed a standard GnRHa-HMG long protocol stimulation and immediately preceded a standard day-3 embryo transfer. The outcome measured was the clinical pregnancy rate per embryo transfer (positive βhCG and visualization of a sac on ultrasound at 7 weeks gestation). A sample size of 468 will be required to show a 30% increase in the pregnancy rate. The interim analysis was conducted after 96 patients completed the study. The O'Brien-Flemming group sequential procedure was used for the interim analysis. A chi-square test was used to compare the clinical pregnancy rate between the treatment group and control group.

Results: The clinical pregnancy rate was 19 of 47 (40%) in the treatment group and 22 or 49 (45%) in the control group. This was not statistical significant ($p = 0.66$). The relative risk was 1.09 (CI 0.74–1.62).

Conclusion: To date there is no difference in the pregnancy rate in the control and treatment groups. This may be a function of the small sample size in our interim analysis. The wide confidence interval supports this conclusion. Upon completion of the study, the analysis will include logistic regression to exclude the effect of patient's age, diagnosis, embryo number and quality. The initial power calculation was based on a pregnancy rate of 30% per ET. Thus far the pregnancy rates are 40% (treatment group) and 45% (control group). Thus, at the completion of the study we should have sufficient power to conclude if the aspiration of cervical mucus prior to ET using and angiocatheter and tuberculin syringe has an impact on the pregnancy rate in IVF-ET.

P-040

Diagnostic Hysteroscopy: a Useful Tool for Evaluation of Uterine Cavity of Patients Undergoing IVF-ET. G. Ragusa, A. Vucetich, P. Antonazzo, G. Di Nola, C. Lanzani, C. Bruna, F. P. G. Leone, A. Bulfoni, P. E. Levi-Setti.* Department of Obstetrics and Gynecology, S. Paolo Hospital ISBM, University of Milan and *Reproductive Unit Humanitas Institute Rozzano, Italy.

Objective: To evaluate importance, diagnostic sensibility, good tolerability and low costs of diagnostic hysteroscopy in a IVF program.

Design: Retrospective, observational study.

Patients and Methods: 506 infertile patients undergoing IVF-ET were submitted to office hysteroscopy since January 1992 to September 1998 before first IVF-ET attempt in our center. Median age, years of infertility and infertility characteristics were similar either for patients with normal cavity than for those with uterine defects. Hysterosalpingograms of patients were normal except than for uterine malformations and had been performed in different centers in different periods. All procedures occurred in the proliferative phase of the cycle.

Results: In 90 (17.8%) patients an uterine defect for which was necessary an operative hysteroscopy was found and all these patients were operated under general anesthesia with a 10 mm rigid resectoscope within two months since the diagnosis. Only patients operated for uterine malformations, myomas and adhesions were submitted to a second look hysteroscopy and in two cases (1 septum and 1 adhesion post myoma resection) a second operation was necessary. No major complications occurred during either diagnostic and operative procedures. The table shows uterine findings of all patients, pregnancy rate per patient and obstetric outcome of the 459 patients who underwent almost one complete IVF-ET cycle.

Patients	Regular cavity	Uterine defects	p
506 Enrolled	416 (82.2%)	90 (17.8%) Polyps 49 (54%), Adhesions 18 (20%), Uterine malformations 14 (15.5%), Submucous myoma 8 (8.9%), Endometrial cancer 1 (1.1%)	
459 Transferred	378 (82.3%)	81 (17.7%)	
Pregnancies	141 (37.3%)	27 (33.3%)	n.s.
Miscarriages	37 (26.4%)	9 (33.3%)	n.s.
Ectopic	2 (1.4%)	1 (3.7%)	n.s.

Conclusion: Infertile patients undergoing IVF-ET very often sustained previous PID, multiple cycles of ovulation induction or IVF failure, and endometrial cavity can suffer of inflammatory disease or hormonal hyperstimulation that change the basal situation since first diagnostic examinations. Hysteroscopy is an excellent tool for evaluating uterine cavity of infertile patients. Implantation rate still remains low even when "good" embryos are replaced so it becomes very important to transfer embryos in the best uterine environment. In our department since 1992 all patients undergoing IVF-ET program have been submitted to diagnostic hysteroscopy before first IVF-ET attempt in our center. This decision was taken on the base of literature reported high percentage of uterine pathology in infertile women and on the base of a previous study carried out on 407 patients affected by infertility and multiple pregnancy loss in which 116 (28.5%) uterine defects were found. In that study, if little polyps (<5 mm) and thin adhesions removed with curettage or telescope in the same office procedure were not defined pathologic at hystological examination, the cavity was considered normal. Our findings suggest that this diagnostic, low cost and well tolerated procedure permit to detect in about 20% of infertile patients intrauterine defects that, restored before the IVF-ET program, lead to comparable pregnancy rate and obstetric outcome both in treated and untreated patients. In this way, our study group obtained a sensitive reduction of costs in term of fertility drugs, materials, staff and iatrogenic risks avoiding to perform cycles in patients with uterine diseases and reduced chances of pregnancy.

P-041

The Utilization of ICSI For Male Factor Infertility Does Not Affect Fresh Blastocyst Transfer Pregnancy Rates. K. A. Miller, G. W. Patton. Southeastern Fertility Center, Mt. Pleasant, SC.

Objectives: To analyze the influence of ICSI upon blastocyst development and fresh blastocyst transfer pregnancy rates since several centers have reported the negative effect of male factor infertility on blastocyst development and fresh blastocyst transfer pregnancy rates (Jany and Menezo, 1994; Trounson et al. 1998).

Design: A cohort study of fresh blastocyst transfers performed at a private IVF center.

Materials and Methods: 82 consecutive patients underwent IVF with subsequent embryo transfer (ET) and patients with > 5 fertilized eggs could elect to have a fresh blastocyst ET. 24 patients failed to meet criteria and underwent a day 3 ET. Oocytes were fertilized by ICSI or conventionally (CF). Oocytes and embryos in cohorts of 4 were cultured under oil in 100 uL microdroplets of P1 media (Irvine Scientific) +10% Synthetic Serum Substitute (SSS, Irvine Scientific) in 5 CO2/air until day 3. On the evening of day 3, all embryos were transferred to microdroplets of blastocyst media (Irvine Scientific) + 10% SSS in 5% CO2/air. On the morning of day 5, the two most expanded blastocysts were selected for transfer using a soft Frydman or Miller-Patton TDT (Fertility Technologies). On Day 6, expanded blastocyst with a well-defined inner cell mass were cryopreserved by the two step glycerol method of Menezo. Comparisons were made between the ICSI and CF blastocyst cycles for age, follicles, oocytes, fertilization rates (FR), cleavage rates (CR), % 8 cells on day 3 (8CD3), % blastocyst development from 8 cells (BD/8C), clinical pregnancy rates (CPR), and implantation rates (IR). FR, CR, %8CD3, %BD/8C, CPR, and IR were analyzed by Chi-Square with Yates Correction. All other parameters were analyzed by t-test and presented as mean values.

Results: No parameters were significant for reduced pregnancy rates in fresh blastocyst transfer cycles.

Transfer	Cycles	Age	Follicles	Oocytes	%FR
Day 3	24	31	12.5	9	54
Blast	58	31	23	17	72
ICSI	22	32	25	19	76
Conv	36	31	21	16	69

Transfer	%CR	%8CD3	%BD/8C	CPR	IR
Day 3	99	83		46%	26%
Blast	95	76	61	57%	43%
ICSI	94	75	53	73%	52%
Conv	96	77	66	47%	37%

Conclusions: High blastocyst development and pregnancy rates were achieved with both ICSI and CF fresh blastocysts ET. Due to the success of the day 3 and day 5 ET, we are adopting fresh blastocyst transfer as a routine procedure at our center.

P-042

Preliminary Experience with Cytoplasmic Transfer in an IVF Program. D. Levran, H. Nahum, J. Farhi, O. Kleiner, M. Glezerman, H. Zakut, A. Weissman. IVF Unit, Department of Obstetrics and Gynecology, Wolfson Medical Center, Holon, and Sackler Faculty of Medicine, Tel Aviv University, Israel.

Recently, there has been a growing interest in the role of the egg cytoplasm in oocyte maturation and fertilization. These processes are initiated and governed by fine signaling mechanisms, in which the cytoplasm plays a crucial role. Failure or inadequate response to these fine cytoplasmic signals may interfere with egg function. The concept of cytoplasmic transfer (CT) evolved based on this recognition. The purpose of the present study was to investigate the role of CT from healthy into presumably defective oocytes in three different entities: oocyte maturation arrest (OMA), heavily fragmented embryos in repeated cycles (HFE) and repeated implantation failure (RIF).

Design: Oocyte maturation and fertilization were determined following CT in a series of cases with presumably defective oocytes.

Materials and Methods: Full IRB approval was obtained, as well as written informed consent from cytoplasm donors and recipients. OMA was defined as complete and repeated arrest at the stage of GV (1 patient, 1 cycle in the present study), M1 (2 patients, 4 cycles), or M2 (1 patient, 2 cycles). HFE was defined as >50% fragmentation in >50% of embryos in 2 consecutive cycles (3 patients, 3 cycles in the present study). RIF was defined as failure to conceive in a minimum of 8 previous IVF-ET attempts in patients with longstanding infertility (>14 years) (5 patients, 6 cycles). Cytoplasmatic transfer was performed on the day of OPU. 10–20% of the cytoplasmic content was transferred from a donated oocyte into the recipient eggs. This was followed by ICSI in all oocytes. In the HFE and RIF patients some M2 oocytes were treated by ICSI alone and served as control. All oocytes were evaluated for maturation and fertilization.

Results: In the OMA group, 42 oocytes underwent CT. Of these, 48% progressed for the first time ever into the next stage of maturity. However, they did not progress beyond this stage, in which they all arrested. In the HFE group, 10 oocytes were treated and 12 served as controls. No improvement in embryo quality could be noticed. In the RIF group, (27 oocytes treated, 14 controls), embryo quality was comparable and implantation failed to occur. None of the study patients conceived.

Conclusions: Our preliminary experience suggests that CT may promote maturation in OMA. However, it was not found sufficient for the achievement of complete maturation and cleavage. No effect on embryo morphology (HFE group) or implantation (RIF group) was observed. Further studies on the physiology and pathophysiology of oocyte function, focusing on cytoplasmic events, should be conducted in order to help clarify the role of CT in the treatment of infertility.

P-043

Comparison of Ongoing Pregnancy Rates Utilizing the Wallace Catheter With and Without Stylet and/or Tenaculum for Multiple Attempt Embryo Transfer. M. Langley, D. Marek, L. Cram, L. Underwood, J. Patton, C. Auger, C. Robertson, K. M. Doody, K. J. Doody. Center for Assisted Reproduction, Bedford, TX.

Objective: The objective of this study was to compare ongoing pregnancy rates resulting from fresh embryo transfers when utilizing the Edward-Wallace catheter alone or in conjunction with a tenaculum and/or stylet for multiple attempt embryo transfer.

Design: A retrospective analysis of fresh embryo transfer results from January 1, 1998 through December 31, 1998.

Materials and Methods: Patients (n=419) underwent fresh embryo transfers. Mock or practice embryo transfers were not performed. A total of 356 (84.7%) of embryo transfers were performed on Day 5 or Day 6, the remaining 63 (14.3%) were performed on Day 3. Embryos were transferred with approximately 15–20 μl of media. An initial embryo transfer was attempted using the Edwards-Wallace Catheter (W, Wallace) alone. If the initial transfer attempt failed, repeated attempts followed utilizing either the Wallace Catheter only, Wallace Catheter with Tenaculum (W+T), Wallace Catheter with malleable stylet (W+S, Wallace) or Wallace Catheter with both tenaculum and stylet (W+T+S). All embryo transfers were performed with an abdominal ultrasound (5 MHz) to aid intrauterine placement of the embryo transfer catheter. Transfers were performed with a full bladder unless the uterine position was retroverted.

Results:

	W	W+T
Total Number Transfer (n=419):	379	16
Total/Average Number Transfer:	940/2.5	40/2.5
Pregnancies (+hCG)/Ongoing:	224/174	9/7
BC/SAB/Ectopic:	21/26/3	2/0/0
Pregnancy Rate per Transfer/Ongoing (%):	59.1%/45.9%	56.3%/43.8%
Age (Mean ± SD):	34.9 ± 4.9	33.7 ± 4.8

	W+S	W+T+S
Total Number Transfer (n=419):	6	18
Total/Average Number Transfer:	16/2.7	45/2.5
Pregnancies (+hCG)/Ongoing:	4/4	7/5
BC/SAB/Ectopic:	2/0/0	2/0/0
Pregnancy Rate per Transfer/Ongoing (%):	66.7%/66.7%	38.8%/27.8%
Age (Mean ± SD):	34.5 ± 3.4	33.1 ± 6.1

Conclusion: Based on this investigation, 90.5% transfers are successful using the Wallace Catheter alone with an ongoing pregnancy rate of 45.9%. Subsequent attempts that utilize the tenaculum or stylet produce an ongoing pregnancy rate of 43.8% and 66.7% respectively. Ongoing pregnancy rates using a Wallace with stylet and/or tenaculum are not statistically different (chi-square, $p > 0.1$) to pregnancy rates when a Wallace Catheter is used exclusively. For the most difficult transfers where a Wallace, tenaculum, and stylet are used in multiple attempts there appears to be a trend to a lower ongoing pregnancy rate (27.8%).

P-044

Double (Consecutive) Transfer of Early Embryos and Blastocyst(s) Does Not Improve Either Pregnancy or Implantation Rates. J. Ashkenazi, R. Yoeli, I. Bar-Hava, R. Bardin, R. Orvieto, D. Feldberg, I. Voliovich, M. Shelef, A. Schwartz, Z. Ben-Rafael. Department of Obstetrics and Gynecology, Rabin Medical Center, Golda Campus, Petah Tikva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Objectives: Questions have recently been raised regarding the beneficial role of blastocyst transfer. To avoid the possible failure of blastocyst growth and cancellation of the embryo transfer procedure, our unit has adopted a consecutive transfer approach of early embryos and blastocyst(s). The aim of this study was to evaluate the results of this method.

Design: Case-control study.

Materials and Methods: During the study period all consecutive women with more than 5 embryos were offered the option of double transfer, i.e., standard transfer of embryos on day 2 or 3, and a second transfer of a blastocyst(s). The total number of embryos transferred was consistent with our unit's policy, based on the women's age and cycle number. The results of three groups were compared: 1: Women who underwent a double transfer; 2: Women who had more than 3 high quality embryos available for early transfer and underwent an early transfer of only 2 embryos, and a second transfer of one blastocyst; 3: Women who received 3 high quality embryos in a single early transfer (controls).

Results: In all, 275 women were included in the study, of whom 136 underwent a double transfer. No differences were detected among the 3 groups in either pregnancy or implantation rates.

	Group 1 (n=136)	Group 2 (n=29/136)	Group 3 (n=139)
Age	31.1 ± 4.9*	29.5 ± 2.9*	32.2 ± 5.6*
No. of early embryos transferred	2.5 ± 0.8	2.0	3.0
No. of blastocysts transferred	1.3 ± 0.7	1.0	0
Clinical pregnancy rate (%)	50 (36.8%)*	12 (41.4%)*	52 (37.4%)*
Implantation rate	14.6%*	19.9%*	19.8%*

Figures are provided as mean±SD. * No significant difference.

Conclusion: The double (consecutive) transfer of early embryos and blastocyst(s) does not improve either pregnancy or implantation rates.

P-045

An Evaluation of the Outcome of GIFT Treatment in Women Aged 40–45 Years; Transferring a Flexible Number of Oocytes. A. Gorgy, C. Ayres, S. Beski, D. Menon, B. Podsiadly, I. L. Craft. London Fertility Centre, London, United Kingdom.

Objectives: The Human Fertilization and Embryology Authority (HFEA) guidelines stipulate to transfer a maximum of three IVF embryos in all patients. Women aged 40 years and above have been discriminated against as they might need more embryos to achieve pregnancy. Patients of this age group may respond differently to ovarian stimulation. Those who produce >3 oocyte might benefit from GIFT transferring a flexible number of oocytes. The technique does not come under HFEA jurisdiction unless donated gametes are used.

Design: A retrospective study of 28 GIFT cycles for 25 patients aged 40–45 years between April 1997 and December 1998. Pregnancy and live birth/on-going pregnancy rates were assessed in relation to the number of oocytes retrieved and transferred. The outcome was compared to the outcome in the patients' latest IVF and the national and our figures following IVF treatment for the same age group.

Materials and Methods: 5 patients had proven fertilization by a previous natural pregnancy and 14 in 31 previous IVF cycles with 2 live births (6.5%). Following routine ovarian stimulation, between 3 and 13 oocytes were replaced into one patent Fallopian tube. The study included 3 groups; (I): in 10 cycles 3–5 oocytes were recovered and replaced, (II): in 9 cycles 6–10 oocytes were recovered and a mean of 8 oocytes replaced and (III): in 9 cycles 11–26 oocytes were recovered and a mean of 10.8 oocytes were replaced.

Results: Eight patients had a positive pregnancy test (29%); two biochemical and 6 clinical pregnancies (21%). One singleton pregnancy ended in miscarriage (17%). Three patients gave birth to singleton babies, one patient gave birth to twins and one patient was in her ongoing twin pregnancy. Live birth/ongoing pregnancy rate was 18% per GIFT procedure. There were no triplets. Twin pregnancy rate was 7% per GIFT procedure and 33% per clinical pregnancy. Group (I): one clinical pregnancy (10%) resulted in one live birth (10%). Group (II): two clinical pregnancies (22.2%) resulted in one twin life birth and an on-going twin pregnancy (22.2%), Group (III): three clinical pregnancies (33.3%) resulted in one miscarriage and two singleton life births (22.2%).

Conclusion: 1) GIFT treatment transferring more than three oocytes (if possible) in patients aged 40–45 years improves the pregnancy (21%) and live birth/on-going pregnancy (18%) per treatment cycle. The HFEA's national IVF data for 1997 reports a 5.5% live birth rate for women aged 40–44 years and our own rate was 5.1%. 2) The risk of a high order multiple pregnancy more than twins is remote in this age group due to naturally low fertility potential. 3) Retrieving and replacing up to 5 oocytes in GIFT might not improve the outcome over transferring 3 embryos in IVF. The outcome significantly improves if ≥6 oocytes are replaced (28% vs 10% clinical pregnancy). 4) A larger study is needed to determine whether the improved outcome of GIFT transferring ≥6 oocytes in this age group was due to the high number of oocytes produced, the high number of oocytes replaced or intrinsic factors related to the GIFT procedure itself. 5) We agree with the HFEA guidelines that a maximum of 3 oocytes or embryos should be replaced in oocyte donation cycles.

P-046

Twin Pregnancies After IVF Compared to Ovulation Induction and Spontaneous Twin Pregnancies-Obstetrical Complications. [1]E. Lunenfeld, [1]E. Maman, [2]A. Levy, [2]H. Vardi, [1]G. Potashnik. [1]Fertility and IVF Unit Department Obstetrics/Gynecology, [2]Department of Epidemiology, Soroka University Medical Center and Ben Gurion University of the Negev, Beer Sheva, Israel.

Objectives: To compare obstetrical characteristics of twin pregnancies conceived by IVF and ovulation induction to those of spontaneous conceptions.

Design: Case control study.

Material and Methods: All twin pregnancies that delivered in our tertiary center during the period of 1989–1996 were evaluated. Those included: IVF (n=78), ovulation induction (n=81) and spontaneous twin deliveries (n=434).

Results: Patients in the IVF group tend to deliver prematurely compared to spontaneous conception group (OR=1.84, 95% CI=1.08–3.15, P=0.02). Using multivariate analysis adjusted for maternal age, gestational age, and parity, IVF and ovulation induction twin pregnancies had significantly higher risk to deliver via cesarean section (OR=1.97, 95% CI=1.26–3.08, P=0.02, OR=1.57, 95% CI=1.08–2.28, P=0.02 respectively) compared to spontaneous twin conceptions. Ovulation induction twin pregnancies had significantly higher risk to develop severe pregnancy induced hypertension (OR=3.65, 95% CI=1.44–9.23, P=0.006) compared to spontaneous twin pregnancies.

Conclusions: Although all twin pregnancies are at increased risk for pregnancy, delivery and neonatal complications it seems from this study that: IVF twin pregnancies are at increased risk for premature deliveries and ovulation induction twin pregnancies are at increased risk to develop severe pregnancy induced hypertension compared to spontaneous twin pregnancies. Both groups are at increased risk to deliver via cesarean section irrespective of gestational age maternal age and parity.

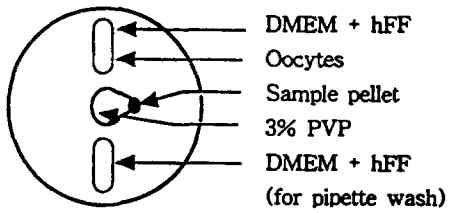
P-047

A New Method of Sperm Preparation at Testicular Sperm Extraction—Intracytoplasmic Sperm Injection (TESE-ICSI) Cycle: Simple, Effective and Rapid Method. [1]K. S. Park, [1]S. S. Chun, [1]T. H. Lee, [2]H. B. Song. [1]Department of Obstetrics and Gynecology, Kyungpook National University Hospital, Taegu and [2]Department of Animal Science, Taegu University, Kyungbuk, Korea.

Objectives: The recovery of spermatozoa from TESE samples for the use of ICSI procedure is very difficult. The aim of this study was to attempt to recover the spermatozoa easily from TESE samples using a 3% polyvinylpyrrolidone (PVP) droplet.

FIGURE 1

Arrangement of droplets for recovering spermatozoa from TESE samples.



Fertil Steril ©

FIGURE 2

Expansion of pellet (RBC and spermatozoa) in a 3% PVP droplet.

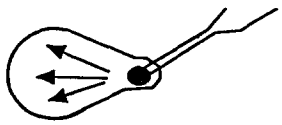


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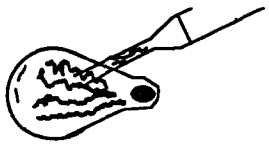
FIGURE 3

Discarding of RBC and cell debris with a pasteur pipette.



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Design: A prospective randomized study.

Materials and Methods: In TESE samples, excess tissue and viscous mass were removed and centrifuged (for 5 min at 1500 rpm). The pellet was suspended with 10 μl human follicular fluid (hFF). Thawed samples were diluted with 5 ml Ham's F-10 contained 10% hFF and centrifuged. Spermatozoa were recovered from the bottom of the 3% PVP droplet. Methods of arrangement of droplets (figure 1), expansion of pellet (figure 2) and discarding of red blood cells (RBC) and cell debris (figure 3) are shown on the figures.

Results: Results are presented in the following table.

	TESE-ICSI		
	Fresh	Frozen-thawed	Total
No. of TESE-ICSI cycles	5	3	8
Spermatozoa recovery rate (%)	5 (100)	3 (100)	8 (100)
No. of oocytes injected (/ cycle)	43 (8.6)	27 (9.0)	70 (8.8)
Fertilization rate (2PN, %)	29 (67.4)	11 (40.7)	40 (57.1)
No. of fertilization failure cycle (%)	0	1 (33.3)	1 (12.5)
No. of embryos cultured	29	11	40
Cleavage rate (%)	29 (100)	9 (81.8)	38 (95)
No. of ET cycles	5	2	7
No. of embryos transferred (/ cycle)	16 (3.2)	9 (4.5)	25 (3.6)
Pregnancy rate (%)	1 (20.0)	1 (50.0)	2 (28.6)

Conclusions: This new sperm preparation method is very simple, easy, effective and quick recovering spermatozoa from TESE samples for ICSI.

P-048

ICSI Using Surgically Retrieved Spermatozoa: Analysis of 1000 Consecutive Cycles. R. T. Mansour, I. Fahmy, M. Aboulghar, G. I. Serour, A. Kamal, N. Tawab. The Egyptian IVF-ET Center, Cairo, Egypt.

Objectives: Intracytoplasmic sperm injection using surgically retrieved spermatozoa has become a standard treatment for azoospermic men. In this study we analyze 1000 consecutive cycles concerning the indications, method of sperm retrieval, and outcome.

Design: Reterospective analysis of 1000 consecutive cycles that were done from September 1994 till December 1998.

Materials and Methods: For 818 azoospermic patients, 912 surgical sperm retrieval cycles and 88 cryo-thawed cycles were performed. Open testicular biopsies were done for 768 cases, while epididymal aspiration was done for 144 cases (19 MESA—25 PESA).

Results: The indications for surgical sperm retrieval were as follows: a) Obstructive azoospermia 448 cycles (175 congenital and 273 acquired), b) Non obstructive azoospermia 488 cycles, c) Total immotile spermatozoa 15 cycles (8 necrospermia, 3 immotile cilia syndrome and 4 short tail syndrome), d) Aspermia 49 cycles (21 failure of semen collection, 13 retrograde ejaculation, 15 anejaculation). Successful sperm retrieval was achieved in all cases (100%) of obstructive azoospermia, aspermia and total immotile spermatozoa. In non obstructive cases, spermatozoa were retrieved in 309 cycles, (63.3%), spermatids were used in 31 cycles (6.4%), and in 148 cycles (30.3%) oocyte injection was cancelled due to failure of finding neither spermatozoa nor spermatids.

	Total	obst. Azo	Non Obst. Azo	aspermia	necro-spermia
Cycles	851	448	308	49	15
Fertilization rate	50.3%	54.4%	45.5%	51.6%	41.7%
Pregnancy rate	28.1%	34.4%	22.4%	22.5%	20%

When we excluded cycles which the female age was ≥40 years old and/or cases in which Clomid was used for ovulation induction. The pregnancy rate was 39% in obstructive cases and 24.5% in non obstructive cases. The FR and PR in obstructive cases were significantly higher than all other indications. 155 pregnancies could be followed up and the rest were lost to follow up. There were 44 abortions (28.4%), one ectopic pregnancy, 56 deliveries,

and 54 ongoing pregnancies. The deliveries were 39 singletons (24 F&15 M) 11 twins (13 F and 9 M), 3 triplets (4 F and 5 M) and 3 stillbirths.

Conclusions: ICSI using surgically retrieved spermatozoa is a successful line of treatment for azoospermic men previously considered absolutely infertile. In about two thirds of non obstructive cases, there is a chance to find spermatozoa and achieve a pregnancy rate of 24%. The F.R. and P.R in obstructive cases are significantly higher than non obstructive cases.

P-049

Postponing Embryo Transfer Until Blastocyst Formation Does Not Delay Uterine Implantation. B. S. Shapiro, D. C. Harris, K. S. Richter. Fertility Center of Las Vegas, Las Vegas, NV.

Objective: The presence of human chorionic gonadotropin (hCG) in maternal serum is indicative of embryo implantation and pregnancy. Titers of hCG were used as indicators to investigate whether postponement of embryo transfer (ET) following in vitro fertilization (IVF), from 72 hours post-retrieval until the development of blastocysts on day 5 or 6 post retrieval, could result in a delay in the time of uterine implantation.

Design: A retrospective clinical study of IVF patients at a private ART center who became pregnant from either day 3 or blastocyst transfer.

Materials and Methods: HCG levels were reviewed from days 10, 12, 15, and 17 after oocyte retrieval in those patients with one (n = 47) or two (n = 38) fetal hearts subsequently detected by transvaginal ultrasound at 6 to 8 weeks gestation. Values for patients receiving 72-hour embryo ET were compared to those patients receiving blastocyst ET for all patients having only one fetal heart rate (FHR), and separately for all patients having two FHR's, using unpaired t-tests.

Results: There were no significant differences in hCG titers measured on the same day after oocyte retrieval between transfer types. Titers of hCG among patients having one FHR averaged 7.0 mIU/ml for day 3 ET and 7.7 mIU/ml for blastocyst ET (day 10, p = 0.64), 32.7 versus 41.1 (day 12, p = 0.23), 142.6 versus 159.6 (day 15, p = 0.54), and 303.3 versus 379.6 (day 17, p = 0.21). Titers of hCG among patients having two FHR's were 10.9 mIU/ml for day 3 ET and 14.3 mIU/ml for blastocyst ET (day 10, p = 0.15), 49.6 versus 66.1 (day 12, p = 0.07), 213.6 versus 258.3 (day 15, p = 0.27), and 470.7 versus 578.8 (day 17, p = 0.31).

Conclusions: No significant differences were observed in the hCG titers of pregnant patients with equal numbers of subsequently identified gestations following day 3 ET or blastocyst ET. The lack of observed discrepancy in hCG titers between blastocyst transfer and day 3 transfer suggests that the postponement of ET until the development of the blastocyst stage does not delay implantation. Embryos appear to implant at the same time regardless of their age at the time of transfer.

P-050

Intracytoplasmic Sperm Injection (ICSI)-Derived Pregnancies are Characterized by a Lower Clinical Abortion Rate than In-Vitro Fertilization (IVF)-Derived Pregnancies. I. Bar-Hava, B. Messing, J. Ashkenazi, R. Yoeli, R. Orvieto, R. Bardin, A. Ferber, H. Krissi, Z. Zahavi, D. Feldberg, I. Voliovich, M. Shelef, A. Schwartz, Z. Ben-Rafael. Department of Obstetrics and Gynecology, Rabin Medical Center, Golda Campus, Petah Tikva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Objective: To compare pregnancy course between IVF and ICSI-derived pregnancies.

Design: Case-control study.

Materials and Methods: Two hundred patients who conceived in 1996–1997, 100 with ICSI and 100 with IVF were evaluated. Data were retrieved from our computerized database prospectively created. In addition, all patients were interviewed by telephone, and the interviewing physician completed a detailed questionnaire. Findings for the IVF and ICSI pregnancies were compared. Main outcome measures included: maternal age, implantation rate, early pregnancy complications, clinical abortion rate, multiple pregnancy delivery rate, gestational age at delivery, mode of delivery and birth weight.

Results: Two hundred pregnancies were analyzed. In all, 238 children were born, including 104 singleton infants (45 IVF, 59 ICSI), 49 twin pairs (28 IVF, 21 ICSI), and 12 triplet sets (3 IVF, 9 ICSI). Statistically significant differences between the ICSI and IVF groups were noted for maternal age (31.3±4.4 vs. 33.4±4.8, respectively, $p<0.005$) and clinical abortion rate (11% vs. 24%, respectively, $p<0.05$).

Conclusion: ICSI-derived pregnancies are characterized by a lower clinical abortion rate than IVF-derived pregnancies. This finding is probably related mainly to the lack of other fertility problems in the majority of these women.

P-051

Singleton Assisted Reproduction Technology (ART) Derived Pregnancies are at Increased Risk of Preterm Delivery. [1]I. Bar-Hava, [1]T. Perri, [2]P. Merlob, [1]J. Ashcenazi, [1]R. Orvieto, [1]D. Feldberg, [1]M. Hod, [1]J. Bar, [1]Y. Peled, [1]B. Messing, [1]Y. Barzel, [1]Z. Ben-Rafael. Departments of [1]Obstetrics and Gynecology and [2]Neonatology, Rabin Medical Center, Petah Tikva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Objectives: The estimated singleton preterm delivery rate in the general population is about 7%. Various risk factors are known to be associated with preterm delivery. It is questionable whether singleton ART derived pregnancies are at an increased risk of preterm delivery. The purpose of this study was to evaluate the incidence of singleton preterm labor among our assisted reproduction technology (ART) derived pregnancies and to compare it to that of our general obstetrics population.

Design: Case control study.

Materials and Methods: Preterm singleton deliveries (both IVF and ICSI) in the years 1996–1997 were compiled from our computerized database. The control group was comprised of the singleton live-born general population at our center in 1996.

Results: A significantly higher preterm singleton delivery rate was detected for our ART derived pregnancy population than in the control group [28.3% (44/155) vs. 7.3% (185/2546) respectively, $p<0.0005$]. Singleton preterm delivery rates in our IVF and ICSI populations were 26.5% (22/83) and 30.5% (22/72) respectively, not significantly different.

Conclusions: ART singleton derived pregnancies are at an increased risk of preterm delivery. This may be attributed to various infertility co-factors such as uterine malformations, previous operative procedures that involved cervical dilatation and a history of pelvic infection.

P-052

Reduced Membrane Definition of Blastomeres on Day 3 Identifies Embryos Resulting in Higher Ongoing Pregnancy Rates After Transfer on Day 5. N. A. Cekleniak, K. V. Jackson, A. Nurredin, S. Shen, M. J. de los Santos, M. Pillar, C. M. Balint, P. M. Griffin, C. Racowsky. Department of Obstetrics/Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objectives: Reduced definition of blastomere membranes (RMD) on Day 3 may reflect early tight junction formation, and therefore signify enhanced developmental potential. We evaluated the predictive value of RMD as a marker for embryos of superior quality.

Design: Clinical outcomes from Day 5 (D5) cycles were retrospectively analyzed after stratification of Day 3 (D3) RMD embryos.

Materials and Methods: Embryos from 168 D5 IVF or ICSI cycles were cultured individually in 25 μl microdrops from D1-3 in IVF-500 and from D3-5 in S2 (Scandinavian IVF). All embryos wre evaluated for RMD on D3 and the most developmentally advanced were transferred on D5, regardless of RMD status. Cycles were stratified according to the % transferred embryos that exhibited RMD on D3 (0%, n=111; 33.3–66.7%, n=44; 100%, n=13). Further analyses included cycle stratification to 1) either no RMD embryos in the entire cohort (n=59) or at least one RMD embryo (n=109); and 2) clinical outcome. The proportion of embryos with and without RMD were compared. Data were analyzed by Chi-Square with Fisher's Exact test and the Mann Whitney U test where applicable, with $P<0.05$ considered statistically significant.

Results: Cycle strata did not differ with respect to age, attempt number, number of embryos transferred or the proportion of embryos transferred that were expanded blastocysts. However, the proportion of ongoing pregnancies was significantly higher in those cycles in which all transferred embryos exhibited RMD as compared with those in which no RMD embryos

were transferred (69.2% vs 35.1%; $p<0.05$). Compared with cycles having no RMD embryos, those with at least one RMD embryo exhibited a significantly higher ongoing pregnancy rate (27.1% vs 47.7%; $p<0.05$), and a significantly lower incidence of complete cleavage arrest (11.9% vs 2.8%; $p<0.05$). Furthermore, when all embryos were stratified by clinical outcome, ongoing pregnancies were associated with a significantly increased number of RMD embryos in the entire cohort as compared with all other outcomes.

Clinical Outcome	# RMD Embs/ Total Embs	% RMD EMbs/Total	P-value (vs Ongoing)
Cleavage arrest	4/98	4.1	$p<0.00001$
Not pregnant	145/945	15.3	$p<0.001$
Spontaneous Abortion	20/181	11.0	$p<0.05$
Ongoing pregnancy (+FHs)	197/898	21.9	

Conclusions: This analysis provides evidence for RMD as a new efficacious marker for assessing embryo quality. The data are consistent with the future application of RMD for identifying those embryos with maximum potential for establishing an ongoing pregnancy. Implementation of this factor in embryo assessment may lead to significantly increased success rates with IVF.

P-053

Method of Leuprolide Acetate Administration Influences Ovarian Response to Exogenous Gonadotropin Stimulation. F. Licciardi, A. S. Berkeley, N. Noyes, N. McGoff, D. Fantini, and J. Grifo. Program for In Vitro Fertilization, Reproductive Surgery and Infertility. New University School of Medicine, New, York, NY 10016

Objective: At our IVF center, two forms of leuprolide acetate (LA) are administered for luteal down regulation: standard IVF patients receive LA subcutaneously (s.c.) and oocyte donors receive LA depot. We compared the ovarian response and pregnancy rates in 138 standard IVF patients under the age of 30 and 153 oocyte donors.

Design: Retrospective analysis of patients in a university-hospital-based IVF clinic from 9/1/95 through 12/31/97.

Methods: The oocyte donors were given depot LA 3.75 mg on day 21 of their cycle. Stimulation was performed starting with 225–300 IU of gonadotropins and tapered until the day of hCG. Control group patients underwent similar protocols, but received 0.2 cc of s.c. LA daily pre-stimulation, and reduced to 0.1 cc until the day of hCG. Embryos were graded on a scale of 1–4, 1 being the best. Endocrine assays were performed using the Immulyte system.

Results:	donors(depot)	controls(s.c.)
age	26.0±0.34	28.2±0.16*
days of gonadotropins	10.7±0.14	9.88±0.12*
total amps of gonadotropins	35.2±0.73	27.1±10.76*
estradiol day of hCG(pg/ml)	1710±72.9	1997±89.3*
egg number	20.7±0.79	15.0±0.69*
embryo number	14.5±0.66	10.2±0.55*
highest quality embryo	1.47±0.03	1.51±0.0
picogram of E2 per egg	82.6	133.1*
implantation rate per embryo	36.9%	31.5% *$p<0.05$

Conclusions: Despite lower egg numbers in the s.c. group, the E2 level on the day of hCG was significantly higher than the depot group. The depot group also required significantly more time of stimulation and a higher dose of gonadotropins. Differences in the method of LA administration may account for differences in ovarian response to stimulation between the two groups. A lower E2 to egg ratio in the depot group may be related to differences in pituitary down regulation, or may be a result of differing effects on local ovarian factors. These may include lower androgen levels, or other substances involved in E2 formation. Depot LA may have an effect on the ovarian GnRH receptor. Such a restriction in E2 production does not seem to have an effect on egg development or implantation.

P-054

Human Immunodeficiency Virus Type 1 (HIV1) in Semen, Various Fractions of Processed Semen and the Peripheral Blood of Asymptomatic Infected Men with CD4+ Count of ≥200 Receiving Combination Antiretroviral Therapy. [1]M. Morshedi, [2]J. Luka, [3]E Oldfield, [2]R. Moriarty, [3]J. Gourly, [1]S. Oehninger and [1]S. Muasher. [1]Departments of Obstetrics and Gynecology [2]Pathology & [3]Medicine, Eastern Virginia Medical School, Norfolk, VA.

Objectives: 1) to assess the relation between viral DNA/RNA in peripheral blood and in various semen fractions in asymptomatic HIV infected men, and 2) to investigate the relative safety of using processed semen samples from these men in assisted reproduction.

Design: Prospective ongoing blind analysis of plasma and fractionated semen at an academic tertiary care center.

Materials and Methods: In the first phase of the study, a total of 22 peripheral blood and 22 semen samples from 10 HIV+ men were evaluated for the presence of virion-associated RNA (HIV RNA) and the cell-associated HIV-1 proviral DNA using reverse transcriptase polymerase chain reaction assay (RT-PCR) and PCR methods, respectively. All men had a CD4+ count of ≥200 and were receiving combination antiretroviral therapies. Plasma samples were obtained on the day of semen testing. Ejaculates were tested after liquefaction (semen) and following fractionation to seminal plasma, motile sperm, and a mixture of immotile/round cell portions using ISolate™. The motile sperm fraction was washed 2× before testing. Semen samples were processed within 1 hour after collection and were maintained at appropriate conditions to preserve the viral integrity. Kappa coefficient of agreement was used for comparison.

Results: Viral RNA: Overall, 50% (11/22) of the semen and 64% (14/22) of plasma samples evaluated showed detectable levels of viral RNA ranging from 100 to 143200 copies/mL for semen and 100 to 52900 for plasma. No agreement was observed between plasma and semen samples in regard to the viral load (kappa=0). Of the 11 positive semen samples, only 7 (64%) showed detectable viral loads in the corresponding plasma samples. On the other hand, of the 14 plasma samples with detectable viral loads, 7 (50%) had positive semen. Sixty four percent (14/22) of plasma samples showed detectable levels of viral RNA ranging from 100 to 52900 viral copies/mL. Nevertheless, only 50% (7/14) of these plasma samples had corresponding positive semen. Viral DNA: Only 2 of the 22 semen (9%) and 6 of the 22 plasma samples (27%) showed detectable levels of viral DNA. Their respective plasma and semen samples were negative for DNA. ISolate-Processed Motile Fraction: Following processing, the motile fraction of only 1 of the 11 RNA positive semen samples (9%) showed detectable viral load (≥100 copies/mL). Corresponding plasma in this particular sample had no detectable viral load. Semen Parameters and the Motile Sperm Recovery Rate: within normal range for all samples.

Conclusion: No relationship between the viral load in semen and in plasma was observed. Patients receiving antiretroviral therapy showed variable degrees of viral load in their plasma and semen. ISolate-double wash processing reduced the viral load to an undetectable level in 91% of positive semen samples. With the rapid turn around time for PCR results and the increase in sensitivity of the assay, it is possible to split the samples intended for assisted reproduction techniques and use one portion in the viral load/DNA are reported to be below the detection level.

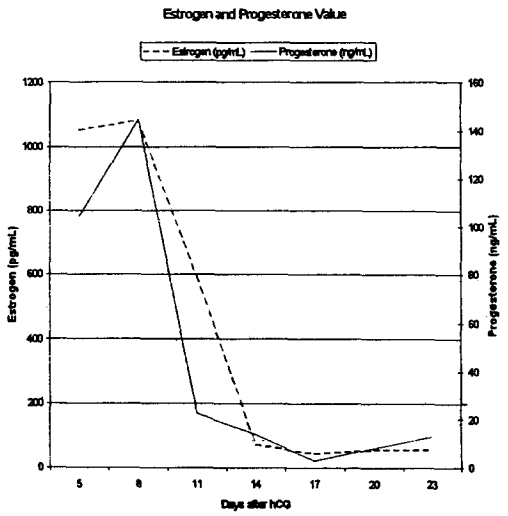
P-055

Early Drop in Serum Estrogen and Progesterone Levels May Explain Early Bleeding After Stimulated ART in Vaginal Progesterone Replacement Cycles. K. E. Jobanputra, J. A. Schnorr, S. E. Brown, R. A. Denoncourt, W. E. Gibbons, J. P. Toner. Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA.

Objectives: Analysis of serum estrogen and progesterone levels, endometrial stripe thickness and bleeding patterns while on Crinone 8% (90 mg once daily)* for progesterone replacement after gonadotropin ovarian stimulation.

Design: Prospective cohort study.

Materials and Methods: Serum estrogen, progesterone levels and bleeding patterns were analyzed on day 2, 5, 8, 11, 14, 17, 20, and 23 after hCG administration in 4 gonadotropin stimulated cycles. The study involved egg



donor patients between November 1998 and December 1998 at the Jones Institute for Reproductive Medicine.

Results: Average serum estrogen levels peaked 8 days after hCG at a level of 1081 pg/mL and fell to 73 pg/mL 14 days after hCG and remained low. Average serum progesterone levels also peaked 8 days after hCG at 144.72 ng/mL and fell to 22.62 ng/mL 11 days after hCG. Half of the patients began experiencing light vaginal bleeding 14 days after hCG and all patients experienced vaginal bleeding by 17 days after hCG.

Conclusions: Use of the vaginal route for progesterone replacement in gonadotropin stimulated cycles was associated with low progesterone levels 11 days after hCG and low estrogen levels 14 days after hCG. Vaginal bleeding started as early as 14 days after hCG and all patients were bleeding by 17 days after hCG. More patients are being studied.

P-056

Impact of Intracytoplasmic Sperm Injection on Embryo Cryopreservation and Clinical Outcome. J. A. Schnorr, S. E. Brown, J. P. Toner, S. O. Oehninger, J. F. Mayer, S. J. Muasher, S. L. Lanzendorf. Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA.

Objectives: To determine the impact of intracytoplasmic sperm injection (ICSI) on embryo cyropreservation survival and clinical outcome.

Design: Retrospective cohort study.

Materials and Methods: Comparison of embryo survival, implantation rates and pregnancy rates between cryopreserved embryos derived from ICSI and standard in-vitro fertilization (IVF) procedures. The study included patients whose excess zygotes and embryos were cryopreserved between January 1995 and April 1998 at the Jones Institute for Reproductive Medicine, Norfolk, VA.

Results: A total of 384 cycles were analyzed during which 1973 embryos were cryopreserved. This included 253 IVF cycles with 1283 embryos and 131 ICSI cycles involving 690 embryos. There was no significant difference between maternal age at cryopreservation and the proportion of embryos cryopreserved at the pre-zygote (PZ) and pre-embryo (PE) stage between the two groups. The IVF group had higher cryopreservation survival rates of 73.57% compared with the ICSI survival rate of 69.27% ($p=0.042$). Embryos preserved in the PE stage also demonstrated higher survival rates in the IVF group compared to the ICSI group, 71.16% and 62.22% ($p=0.019$) respectively. Embryos preserved in the PZ stage had similar survival rates, 76.01% for IVF and 73.30% for ICSI. The IVF group had 3.44 embryos transferred per cycle compared with 3.31 embryos per cycle in the ICSI group. There was no significant difference in IVF versus ICSI implantation rates (9.51% and 8.98%), pre-clinical pregnancy loss rates (4% and 1.57%) and miscarriage rates (4.41% and 1.57%). There was also no significant difference in delivery rate (22.89% and 22.83%).

Conclusions: ICSI negatively impacts embryo cryopreservation survival rates for embryos cryopreserved in the PE stage. ICSI does not have an adverse impact on implantation or pregnancy outcomes.

P-057

Midfollicular Serum LH Concentrations Do Not Predict Pregnancy Outcome in Controlled Ovarian Hyperstimulation and IVF. L. M. Figueira, N. P. Vlahos, J. Y. Phelps, H. A., Zacur, J. E. Garcia. Department of gynecology and Obstetrics. The Johns Hopkins School of Medicine, Baltimore, MD 21287-1247 USA.

Objective: In a menstrual cycle, the two-cell theory justifies the role of LH in ovarian folliculogenesis and estradiol (E2) production. Recently however the use of highly purified or recombinant FSH alone has been found adequate in controlled ovarian hyperstimulation (COH) for IVF. To address the role of LH, we examined the relationship between midfollicular luteinizing hormone (LH) concentrations and ovulation induction profiles in women undergoing controlled ovarian hyperstimulation (COH) with purified FSH.

Design: Retrospective chart review from a University based tertiary Fertility center.

Material and Methods: Between January 1st and December 31st 1998, all charts of women undergoing COH for IVF utilizing the flare protocol were retrospectively analyzed. Criteria to enter the study included, (1) initiation of leuprolide acetate on day 2 of the menstrual cycle, (2) ovarian stimulation starting on day 5 with purified FSH, (Fertinex), (3) availability of serum LH and E_2 results on day 9 of the cycle, and peak E_2 levels before oocyte retrieval. Outcome included midfollicular and peak E2 levels, number of MII oocytes, and pregnancy rate. ROC curve was used to determine the day 9 LH cutoff value. Chi-square analysis was used to compare pregnancy rates. Student's t-test was used to compare mean number of MII oocytes, midfollicular and peak estradiol concentrations. A P value of <0.05 was defined as statistically significant.

Results: Women with midfollicular serum LH concentrations of less than 3.0 mIU/ml had significantly lower midfollicular (388.42 versus 809.34 pg/ml) and peak serum E_2 concentrations (2837.53 versus 3679.68) as compared to women with LH concentrations equal to or more than 3.0 mIU/ml. However, mean number of MII oocytes and pregnancy rates in the two groups were not statistically different (12.22% and 15.38% versus 13.21% and 22.73% respectively). Age distribution was similar in the two groups.

Conclusion(s): Although low midfollicular serum LH concentrations are associated with significantly reduced ovarian E_2 production (low midfollicular and peak E_2 concentrations), quality of oocytes and pregnancy outcome were not affected by those low levels. Nevertheless, this could change in the future if the blastocyst formation rate is considered as another parameter.

P-058

Bacteria in the Vagina and at the Transfer Catheter Tip Influence IVF/ET Live Birth Rates. D. E. Moore, K. J. Agnew, N. A. Klein, V. Y. Fujimoto, V. L. Baker, M. R. Soules, and D. A. Eschenbach. Department of Obstetrics and Gynecology, University of Washington School of Medicine, Seattle, WA.

Objectives: Recovery of bacteria at the transfer catheter tip following IVF/ET transfer has been associated with a 50% reduction in pregnancy rates (Egbase, et al. 1996; Franchin, et al. 1998). However, we know of no study that has reported the effect of prophylactic antibiotics on the vaginal microbial flora or the effect of individual bacterial isolates on pregnancy rates in the IVF/ET setting, which we proposed to address.

Design: The effect of prophylactic antibiotics on vaginal bacteria and the impact of individual bacteria on live birth rates were determined. The Chi-square and the Fisher Exact Probability tests for significance were used.

Materials and Methods: Vaginal cultures were obtained for aerobic and anaerobic cultures both at the time of the sonographic egg recovery and embryo transfer. Following embryo transfer, the transfer catheter tip also

was cultured. Oral doxycycline 100 mg twice per day was given prophylactically for five days starting the day before the embryo transfer.

Results: 91 women had cultures of all three sites. Recovery of viridans streptococci from the vagina ($p=0.05$) and from the transfer catheter tip ($p=0.02$) was associated with a reduced live birth rate. In contrast, recovery of hydrogen peroxidase producing lactobacilli from the vagina ($p=0.05$) and from the transfer catheter tip ($p=0.01$) was associated with a significantly increased live birth rate. The use of doxycycline had no substantial impact on the recovery of the 18 individual bacteria examined in the vagina before and after doxycycline administration (data not shown).

LIVE BIRTH RATES PER EMBRYO TRANSFER

	VAGINA		TRANSFER CATHETER TIP	
Microorganism	Isolated	Not Isolated	Isolated	Not Isolated
Viridans streptococci	15/65=23%*	12/26=46%	1/17=6%**	26/74=35%
Bacterial Vaginosis	10/47=21%	17/44=39%	N/A	N/A
Lactobaccilli $H_2O_2^+$	15/35=43%*	12/56=21%	7/10=70%**	20/81=25%

* $p=0.05$; ** ≤ 0.02.

Conclusion: In summary, the recovery of viridans streptococci from the transfer catheter tip had a negative impact and peroxidase-producing lactobacilli a positive impact on live birth rates following IVF/ET. Whether the type of prophylactic antibiotics or a mechanical process could influence these relationships favorably remains to be studied.

P-059

Effects of Ovarian Hyperstimulation on Oocyte Quality in Terms of the Incidence of Apoptotic Granulosa Cells. T. Kaneko, H. Saito, M. Yoshida, T. Takahashi, N. Ohta, M. M. Ito, T. Saito, K. Nakahar, and M. Hiro. Department of Obstetrics and Gynecology, Yamagata University School of Medicine, Yamagata, Japan.

Objectives: Various stimulation protocols have been employed for IVF including natural cycle, hMG+hCG and GnRH analogue (GnRHa)+hMG+hCG. Each stimulation protocol has its own benefits. However it has not been examined that what effects are produced on the quality of oocyte during these stimulation protocols. Meanwhile the incidence of apoptotic granulosa cells has been reported to be a very sensitive indicator to estimate the quality of oocytes in an IVF program. The aim of the present study was to determine which ovarian hyperstimulation makes oocytes good quality among three different ovarian hyperstimulation protocols undergone in the same patients.

Design: The incidence of apoptotic granulosa cells, the endometrial thickness, the number of retrieved oocytes, the number of mature oocytes and hormones concentration in serum were determined.

Materials and Methods: Ten women underwent three different ovulation hyperstimulations {(A) Natural cycle, (B) GnRHa + hMG + hCG, (C) hMG + hCG} for IVF. The mural and cumulus granulosa cells were dispersed by hyaluronidase, placed on a slidegrass and fixed by neutral buffered formalin. The slidegrasses were stained with Hoechst 33258. The apoptotic granulosa cells were counted among 1,000 granulosa cells at random.

Results: The number of oocyte retrieved from group A was significantly lower than those of group B and C (A: 0.8 ± 0.3,B:5.8 ± 1.4, C:4.2 ± 1.2). The number of mature oocytes in group A was also significantly lower than those in group B and C (A: 0.6 ± 0.2, B:3.6 ± 0.7, C3.4 ± 0.8). The incidence of apoptotic cumulus granulosa cells in group B is significantly higher than those of group A and C (A: 0.9 ± 0.2, B:1.6 ± 0.3, C:0.4 ± 0.2). The incidence of apoptotic mural granulosa cells has same tendency but no significantly difference that of cumulus granulosa cells (A:1.2 ± 0.3, B:2.5 ± 0.5, C:1.0 ± 0.4).

Conclusion: HMG+ hCG protocol decreases the incidence of apoptotic granulosa cells and hMG+hCG is the most effective ovarian hyperstimulation protocol among three stimulations when we consider oocyte quality. GnRHa has a benefit in restraining the premature LH surge, but GnRHa reduces the quality of oocytes through increment of the incidence of apoptotic granulosa cells. Thus we should pay more attentions to the dose and frequency of administration of GnRHa during ovarian hyperstimulation for IVF.

P-060

Free Radical Scavengers and Implantation Rate in an ICSI Program. [1]L. Diotallevi, [2]L. Buffo, [1]V. Polli, [1]L. Fabbro, [2]M. Dusi, [3]C. Bulletti. [1]Department of Obstetrics, Gynecology and Phisiophatology of Reproduction, Rimini Hospital, Rimini, I [2]Centro Medico Palladio, Vicenza, I [3]1rst Department of Obstetrics and Gynecology, University of Bologna, Bologna, I.

Objectives: Reactive oxygen species (ROS) may be detrimental for embryo development. The addition of free radical scavengers to the culture medium could improved embryo quality and lead to higher implantation rate. Studies on mouse embryos have shown an improvement of the embryo development by adding superoxide dismutase (SOD), a free radical scavenger to the culture medium: the 2-cell block was reduced and the rate of blastocyst formation increased. The aim of this study was to investigate the performance of SOD (superoxide dismutase from bovine erytrocyte, SIGMA) supplementation in the culture of human embryos.

Design: Quality of the embryo, pregnancy rate and implantation rate were determined after culture in medium supplemented with or without SOD.

Materials and Methods: The study was conducted in two parts. In the first part we investigated the effect of SOD supplementation on the fertilization and development rate after ICSI. In 34 ICSI cycles, each patient, after sperm injection, had part of the oocytes cultured in HTF supplemented with 1% HSA and 500 IU/ml of SOD, and part in HTF + 1% HSA without SOD supplementation. In the second part of the study 24 ICSI and 16 TESE (non obstructive azoospermia) patients had all the oocytes allocated in culture with SOD, 25 ICSI and 14 TESE patients without SOD supplementation to investigate the effect on pregnancy and implantation rate.

Results: In the first study no statistically significant differences were found between the two culture systems. The rate of 2 PN (68% vs 64%) and of cleavage (84% vs 87) were similar in the two groups. All the patients were transferred with embryos deriving from both culture system. In 10 cycles a pregnancy was achieved (29.4%); 7 pregnancies progressed to term (20.5%). The ongoing pregnancy rate did not differ from our routine. No differences were found in the pregnancy rate between SOD supplemented ICSI cycles and SOD not supplemented cycles (28% vs 33%). Higher pregnancy rate (50% vs 21%) and implantation rate (23% vs 8%) were found in SOD supplemented TESE cycles compared to the not supplemented.

Conclusions: Antioxidant supplementation of the culture medium seems to improved the quality of the embryos obtained with testicular spermatozoa suggesting an higher sensitivity to oxidative stress. Studies are in course to evaluate the effect of SOD during sperm preparation.

P-061

Comparative Study of Recombinant Follicle Stimulant Hormone (rec FSH) and Highly Purified fsh (hp FSH) Plus Menotropins (hMG) in Intracytoplasic Sperm Injection (ICSI) Program. V. Batiza, R. Sants, P. Galache, S. Hernández, D. Montoya, M. Ruy Sánchez, J. F. Vélez, A. Hernández. Instituto para el Estudio de la Concepción Humana (IECH). Monterrey, Nuevo León. México.

Objectives: To compare the results of the use of recFSH over menotropins in our ICSI program.

Design: Prospective, randomized and comparative clinical study.

Materials and Methods: All the patients of our ICSI program from March 1997 until December 1998 were included. The patients were alleatory assigned to one of two groups according to the medication used in the ovarian stimulation. Group I: The recFSH (Gonal-F, Serono laboratories Inc.), and group II: the hp FSH (Fertinorm, Serono laboratories Inc.) plus hMG (Pergonal, Serono laboratories Inc.). The initial dose was calculated according to age, hormonal profile, follicular charge according to vaginal ultrasound and the previous history of response to gonadotrophins.

Results: Group I: Mean age: 29.8+4.4 years; mean No. Amps/pat: 34.19+8.8; mean serum estradiol on day 11: 3020.2+2496.2 pg/ml; retrieval day: 13.2+0.9. The number of eggs obtained per patient was 13.57+6.27, for a retrieval rate of 70.25%. Egg maturity rate: 82.1%; Fertilization rate: 71.93%; Mean number of embryos transferred: 3.18+0.75; Mean number of frozen embryos/pat: 4.14+2.92.

Group II: Mean age: 30.8+3.5 years; mean No. FSHhp Amps/pat: 24.18+15.1 plus mean No. hMG amps/pat: 22.2+11.6, which made a total No. amps/pat of 46.4 amps/pat in this group; mean serum estradiol on day 11: 1868.6+1076.1 pg/ml; retrieval day: 13.2+1.3; were obtained 10.9+6.8 eggs/pat, for a retrieval rate of 74.3%. Egg maturity rate: 86.9%; fertilization rate: 68.65%; Mean No. frozen embryos: 1.36+2.34; Mean No. Embryos/pat: 2.14+0.38. There was no significant statistical difference between age, retrieval day or number of embryos transferred. There was significant statistical difference between the total no. of recFSH ampules and FSHhp+hMG ampoules, estradiol levels on day 11, and frozen embryos between groups, being better in group I. In group II, the egg maturity rate was significantly better than in group I.

Conclusions: In the ICSI program in our institution, a better efficacy and efficiency were observed with the use of recFSH versus FSHhp+hMG. There are still a large number of pregnancies on-going, so we should wait to their resolution to have more accurate conclusions. In addition, there was no significant statistical difference between the complication rate as Severe Ovarian Hyperstimulation Syndrome (SOHS) and multiple pregnancy.

P-062

The Effect of Day 3 Embryo Quality on Blastocyst Formation and Quality. B. Balaban, A. Isiklar, S. Aksoy, C. Alatas, R. Mercan, A. Nuhoglu, and B. Urman. Assisted Reproduction and Fertility Unit, American Hospital of Istanbul, Turkey.

Objectives: Progression of cleavage stage embryos to the blastocyst stage related to their morphologic characteristics is not well defined. Bolton and coworkers (J In Vitro Fert Embryo Trans 1989) demonstrated that gross morphology alone could only predict 23% of embryos surviving continued culture. Similarly Dokras (Hum Reprod 1993) showed a poor correlation between the morphological grade of embryos on day 2 and their in vitro developmental potential to the blastocyst stage. The aim of this study was to determine the progression of cleavage stage embryos to the blastocyst stage related to their morphologic characteristics three days after insemination.

Design: Prospective case series.

Materials and Methods: A total of 2956 cleavage stage embryos obtained from 280 patients were observed under culture with the aim to transfer at the blastocyst stage. Embryo quality on the third day after insemination was determined according to the morphologic features including blastomere number, evenness of the blastomeres and the presence and extent of fragmentation. Cleavage stage embryos were graded from 1 to 4 with grade 1 representing best quality embryos. Blastocyst grading was performed according to Dokras and coworkers as they demonstrated a correlation between the morphologic grade of a blastocyst and its developmental potential as assessed by the amount of hCG secreted in vitro and the total number of cell nuclei (Hum Reprod 1993).

Results: Of the 2956 cleavage stage embryos on day 3, 1892 (64%) were G1 or G2 and 1064 (35.9%) were G3 or G4. Of the G1+G2 embryos 1112 (58.7%) progressed to the blastocyst stage, whereas only 260 (24.4%) of the G3+G4 embryos progressed to the blastocyst stage ($P<0.05$). Of all the blastocysts obtained 81% originated from G1+G2 and only 19% originated from G3+G4 embryos. The rate of progression to the blastocyst stage and the resulting blastocyst quality from day 3 embryos are shown in the table.

	No blastocyst	Blastocyst G1
G1 embryo on day 3 (n=910)	306 (33.6%)	211 (23.2%)
G2 embryo on day 3 (n=982)	474 (48.2%)	173 (17.6%)
G3 embryo on day 3 (n=569)	427 (75%)	49 (8.6%)
G4 embryo on day 3 (n=495)	381 (76.9%)	35 (7.1%)

	Blastocyst G2	Blastocyst G3
G1 embryo on day 3 (n=910)	242 (26.6%)	151 (16.6%)
G2 embryo on day 3 (n=982)	192 (19.5%)	143 (14.6%)
G3 embryo on day 3 (n=569)	54 (9.5%)	39 (6.9%)
G4 embryo on day 3 (n=495)	47 (9.5%)	32 (6.5%)

Conclusions: The rate of progression to the blastocyst stage and blastocyst grade appears to be clearly related to the embryo morphology and day 3.

P-063

Quality and the Developmental Stage of Blastocysts Predict Pregnancy and Implantation Rates in Assisted Reproduction. B. Balaban, A. Isiklar, C. Alatas, S. Aksoy, R. Mercan, A. Nuhoglu, and B. Urman. Assisted Reproduction and Fertility Unit, American Hospital of Istanbul, Turkey.

Objectives: While the implantation potential of cleavage stage embryos according to their morphology is clearly defined, there is lack of data regarding blastocysts in this respect. The aim of this study was to determine the implantation potential of human blastocysts in relation to their morphologic features.

Design: Prospective case series

Material and Methods: In 280 women with 4 or more cleavage stage embryos on day 2 transfer was performed at the blastocyst stage. Blastocyst culture was performed in sequential media (S1+S2; Scandinavian Science). Embryo transfer was undertaken on day 5 or 6 according to the developmental stage of the blastocyst. Patients were subdivided into three groups: Group 1: At least one grade 1 blastocyst available for transfer, Group 2: at least one Grade 2 blastocyst available for transfer, Group 3: Only grade 3 blastocysts available for transfer. Blastocyst grading was according to Dokras et al Hum Reprod 1993).

Results: The groups were comparable regarding mean female age, duration of infertility, and indications for IVF or ICSI. Mean number of blastocysts transferred was similar in groups 1 and 2. More blastocysts were transferred in Group.

	Group 1 >1 G1 blastocyst txf	Group 2 >1 G2 blastocyst txf	Group 3 All G3 blastocysts txf
Number of ET cycles	79	91	58
No oocytes retrieved	1114	1284	914
2PN fertilization	649 (58.2%)	732 (69.5%)	542 (59.3%)
Cleavage rate	636 (98%)	710 (97%)	537 (99%)
G1+G2 embryos on D3	439 (69%)	455 (64.1%)	349 (65%)
G3+G4 embryos on D3	197 (31%)	255 (35.9%)	188 (35%)
Blastocyst formation (%)	299 (47%)	313 (44%)	241 (44.8%)
Blastocysts transferred	3.2	3.4	4.1
Clinical pregnancy/ET	42/79 (53.1%)	44/91 (48.3%)	4/58 (6.9%)
Implantation rate/embryo	114/259 (44%)	109/313 (34.8%)	10/241 (4.1%)
Multiple pregnancy rate	31/42 (73.8%)	29/44 (65.9%)	2/4 (50%)

Discussion: This study showed a clear relationship between implantation and pregnancy rates and blastocyst grading. High implantation and pregnancy rates are to be expected when at least one of the blastocysts transferred is a G1 or G2.

P-064

Obstetric Outcome of Singleton Pregnancies Achieved By In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI). [1]H. Yarah, [1]A. Demirol, [1]O. Bükülmez, [1]T. Gürgan. Hacettepe University, School of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey.

Objective: The objective of this study is to determine whether singleton pregnancies achieved by IVF and ICSI are associated with increased risk for adverse pregnancy outcome.

Design: Retrospective case-control study. Consecutive 100 IVF, 76 ICSI and 528 control singleton pregnancies were included.

Materials and Methods: All singleton pregnancies achieved by IVF and ICSI at Hacettepe University Hospital between July 1991 and December 1998 were retrospectively analyzed. Age-matched, consecutive, primiparous singleton pregnancies with ≥2 antenatal visits delivered between the same time period were selected as controls with a cases/controls ratio of 1/3. One-way ANOVA post-hoc Bonferroni, Chi-square and Kruskal-Wallis tests were utilized where appropriate. The severe perinatal complications were defined as the presence of one or more of the following: Delivery ≤32 weeks, birthweight ≤1500 grams and fetal or neonatal exitus.

Results: The results are summarized in Table 1.

Table 1. The characteristics and outcome among IVF, ICSI and control singleton pregnancies

Parameter	IVF (n=100)	ICSI (n=76)
Age (y)	32.3±4.3	32.8±3.9
Delivery week	36.9±3.5*	37.7±3.1
Birthweight (g)	2939±725†	3054±657
Pregnancy induced hypertension (%)	2 (2)	2 (2.6)
Preterm premature membrane rupture (%)	5 (5)	1 (1.3)
Preterm labor (%)	18 (18)	4 (5.3)‡
Karyotypic/structural anomaly (%)	1 (1)	1 (1.3)
Fetal/neonatal exitus (%)	1 (1)	1 (1.3)
Delivery≤32 weeks (%)	8 (8)	2 (2.6)
Delivery≤37 weeks (%)	35 (35)‡	9 (11.8)
Birthweight≤2500 grams (%)	23 (23)‡	9 (11.8)
Birthweight≤1500 grams (%)	5 (5)	4 (5.3)

Parameter	Controls (n=528)	P-value
Age (y)	32.1±4.1	NS
Delivery week	38.3±3.3*	0.000
Birthweight (g)	3178±684†	0.005
Pregnancy induced hypertension (%)	13 (2.5)	NS
Preterm premature membrane rupture (%)	2 (0.4)‡	0.000
Preterm labor (%)	81 (15.3)	0.039
Karyotypic/structural anomaly (%)	3 (0.6)	NS
Fetal/neonatal exitus (%)	19 (3.6)	NS
Delivery≤32 weeks (%)	27 (5.1)	NS
Delivery≤37 weeks (%)	103 (19.5)	0.000
Birthweight≤2500 grams (%)	46 (8.7)	0.000
Birthweight≤1500 grams (%)	25 (4.7)	NS

Note. NS=not significant. *,† Significantly different from each other by Bonferroni test. ‡ Significantly different than the others by chi-square test.

Conclusion: Singleton pregnancies achieved by IVF and ICSI are not associated with severe perinatal complications.

P-065

Ultrasonographic Visualization of Uterine Contractions at the Moment of Embryo Transfer and Its Correlation With Assisted Reproduction Technique Outcome. G. Fiszbajn, A. Grabia, R. Lipowicz, M. Albamonte, J. Hamer, S. Papier, C. Chillik. Center of Studies in Gynecology and Reproduction. Buenos Aires—Argentina.

Objective: The aim of this study is to quantify the endometrial activity before and after the time of embryo transfer, with or without the use of a grasping forceps to stabilize the uterine cervix and to correlate it with the outcome of assisted reproduction techniques (FIV or ICSI).

Design: Observer blind prospective clinical study.

Material and Methods: Fifty-one patients undergoing assisted reproduction cycles between April and October 1998 fulfilling the following inclusion criteria: ≤38 years old, normal hormonal levels on the third day of the menstrual cycle and at least 2 good quality embryos to transfer, were recruited. Exclusion criteria included: recurrent miscarriage, hydrosalpinx, endometrioma, previous uterine interventions and difficult transfers. Patients were divided into 2 groups: 1 (n=31) in which we performed 3 pelvic vaginal sonographies, 5 minutes each; one before application of the speculum, the second after grasping of the cervix with a forceps and previous to transfer, and the third after transfer. Group 2 (n=20) transfer was performed without a grasping forceps, thus only two sonographies were required (before and after embryo transfer).

Results: There were no significant differences between the two groups regarding the number of contractions post-transfer (5,16±2,74 vs. 5,45±2,58), implantation rate (15,7% vs. 18,6%), pregnancy rate (35% vs. 45%) and miscarriage rate (18,1% vs. 22,2%). There was no significant difference in the number of contractions between patients who became pregnant and the nonpregnant ones (5,3 vs. 5,4 respectively).

Conclusions: The use of a grasping forceps does not alter the frequency of uterine contractions, and it does not have any impact on implantation and pregnancy rates.

P-066

Laser Assisted Hatching at the Extremes of the IVF Spectrum: First Cycle and After 6 Cycles. A Randomized Prospective Trial. [1]S. Antinori, [1]C. Versaci, [1]L. Dani, [1]E. Barbaro, [1]M. Antinori, [1]C. Cerusico, [2]A. Vidali. [1]R.A.P.R.U.I., Rome, Italy and [2]Columbia University, New York, N.Y.

Objectives: To assess the benefit of assisted hatching in a good prognosis and in a poor prognosis group of patients undergoing IVF-ET.

Design: Randomized prospective trial.

Patients and Methods: Patients in the two groups (First Cycle or >6 cycles) were randomly assigned to either hatching or no hatching. There were no statistically significant differences within the two groups as far as age, number of oocytes retrieved, number of embryos transferred between the treatment group and the controls. We used a compact laser microbeam system (PALM Wolfrathausen, Germany) consisting of a pulse nitrogen laser (LSI, Newton, MA, USA) at a wavelength of 337 nm operating through the epifluorescence path of an inverted microscope (Zeiss Axiovert 135, Germany).

Results: Results are summarized in the tables below.

>6 Cycles	Hatching	No Hatching
Patients	73	69
Age	mean 37.5	mean 36
Oocytes	581 (7.9)	552 (8)
Embryos	321	307
Embryos/Patients	4.3	4.4
Clinical Pregnancies	19 (26%)	11 (15.9%)
Miscarriage	3 (15.7%)	2 (18.1%)
Multiple	1	0

First Cycle	Hatching	No Hatching
Patients	96	103
Cycles	96	103
Age	mean 27.5	mean 27
Oocytes	932 (9.7)	1110 (10.7)
Embryos/patients	2.3	2.4
Clinical pregnancies	33 (30.2%)	30 (29.1%)
Miscarriage	3 (10.3%)	3 (10%)
Multiple	4	1

In the multiple IVF failure group the relative risk (RR) of pregnancy was 1.6 (95% CI 0.79-3.2) suggesting a possible advantage for assisted hatching. In the first-timers the RR was 1.04 (95% CI 0.89–1.2).

Conclusion: Laser assisted hatching may beneficial after repeat IVF failures. No benefit was found in the first time good-prognosis group. Assisted hatching should be reserved to poor prognosis IVF patients.

P-067

Prediction of Severe Ovarian Hyperstimulation Syndrome (OHSS) by Measurement Of Free But Not Total VEGF (Vascular Endothelial

Growth Factor) Serum Concentration–Results From a Prospective Study. [1]M. Ludwig, [2]W. Jelkmann, [1]O. Bauer, [1]K. Diedric. [1]Department of Obstetrics and Gynecology and [2]Institute of Physiology, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany.

Objective: VEGF (vascular endothelial growth factor) is proposed to be involved in the pathogenesis of ovarian hyperstimulation syndrome (OHSS) after controlled ovarian stimulation (COS). No data exist regarding the difference of free (fVEGF) and total VEGF serum concentration (tVEGF) in patients with subsequent OHSS. In two publications a significant increase of VEGF serum concentration from the day of hCG onwards in patients with subsequent OHSS, but not in those without OHSS was described. However, the data were measured in small (n <5) and heterogeneous groups (OHSS grade II and III) with different kits.

Design: fVEGF and tVEGF were measured in serum on days of hCG, oocyte pick up (OPU), and embryo transfer (ET). Serum was collected prospectively over a period of 19 months. 10 patients with severe OHSS were compared to 15 randomly selected patients without OHSS.

Material and Methods: All serum samples were stored at −20°C. Samples were measured within a one month period after completing collection. VEGF was measured by means of two different commercially available kits for assay of either VEGF (Quantikine human VEGF ELISA, R&D Systems Europe, Abingdon, Oxon, UK) or tVEGF (Accucyte VEGF Elisa, Cyt-Immune, College Park, Maryland, USA) in serum. Statistics were calculated using a one-sided heteroscedastic Student's t-test.

Results: The concentration of fVEGF was significantly higher on the days of hCG administration (309.4 ± 165.0 vs. 190.3 ± 127.8 pg/ml, $p<0.05$) and ET (315.0 ± 125.2 vs. 209.3 ± 137.2 pg/ml, $p<0.05$) in the OHSS compared to the control group. No such difference existed with respect to tVEGF. There was no significant rise in fVEGF or tVEGF concentration in the OHSS patients or the controls from the day of hCG administration up to the days of OPU or ET. A cut-off level of 200 pg/ml serum free VEGF concentration on the day of hCG treatment resulted in positive and negative predictive values of 75% and 92% for the development of an OHSS, respectively.

Conclusion: This is the first report on parallel measurement of fVEGF and tVEGF in OHSS patients. fVEGF seems to be a good parameter to predict the onset of OHSS. This is of special importance, when more recombinant FSH preparations were used in COS, since these preparations will lead to lower estradiol levels - the classical parameter to predict OHSS - on the day of hCG. The observation of unchanged tVEGF serum levels lead to the hypothesis, that the removal of albumin, a possible VEGF binding site, will lead to an increase of fVEGF, which trigger the onset of OHSS in these patients. This can explain, why the administration of albumin helps to avoid an OHSS only in those cases, when it is given at the day of oocyte pick up.

P-068

Psychological Profile During the Luteal Phase of Patients Undergoing Controlled Ovarian Stimulation–Results of a Prospective, Randomized Study. M. Ludwig, A. Finas, I. Kowacek, K. Diedrich. Department of Gynecology and Obstetrics, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany.

Objective: Luteal phase support (LPS) following controlled ovarian stimulation (COS) seems to be a necessary tool. There is an ongoing debate, whether hCG has to be used for LPS. Therefore, a prospective, randomized study was performed, to evaluate the outcome after different LPS protocols. A subgroup of patients (pts) was separately analysed for their psychosomatic profile during the luteal phase.

Design: 79 subsequent patients filled out a questionnaire on each day according to the Befindlichkeits-Skala (von Zerssen). The data of 71 of the questionnaires could be included in this study. On each day of the luteal phase one questionnaire was filled out, each daily questionnaire consisted of 24 items, two different questionnaires were used, which changed each other day. The test is proven to be useful for determination of psychosomatic complaints during a pharmacological treatment.

Materials and Methods: Pts at low risk for OHSS (<12 oocytes and/or estradiol on the day of embryo transfer (ET) <2.500 pg/ml) were prospectively randomized to one of three groups: first group (I) received three times hCG (5.000 IU on the day of ET, 5.000 IU three days later, and 2.500 IU again three days later; second group (II) received 5.000 IU hCG only on the day of ET, but additionally started with vaginal progesterone (Utrogest®) 600 mg daily on the evening before ET and up to the day of a positive hCG or onset of menstrual bleeding; third group received only vaginal progesterone (Utrogest®) 600 mg daily. Exclusion criteria were age >40 years, and/or estradiol on day of hCG >5.000 pg/ml and/or complaints of OHSS on the day of ET. Pts at high risk for OHSS (≥12 oocytes and/or estradiol on the day of ET ≥2.500 pg/ml) were randomized either to group IV (same protocol as group II) or group V (same protocol as group III).

Results: 50, 42, 42, 48 and 59 pts were randomized to group I to V, respectively. There was no significant difference regarding the pregnancy rates in the five groups. However, there were two severe OHSS observed, one in groups IV and V, each. Three moderate OHSS occurred in the low risk group, all in group I, and one in group V. 19 mild OHSS were recorded, two in each of the low risk groups, six in group IV and seven in group V. By chance, only four pts in group II received a questionnaire. Therefore, only groups I (n=21), III (n=15), IV (n=13), and V (n=22) could be analyzed for the psychological profiles. In groups III, IV, and V there was no change of subjective estimation of well feeling over the whole luteal phase. There was also no difference between pregnant and non pregnant pts from all groups. Only in group I, there was a tendency towards more complaints towards the end of the luteal phase. The most frequent complaints were very similar in all three groups, and were tiredness, abdominal pain and breast tenderness.

Conclusions: Since no positive effect of hCG administration on the pregnancy rate could be seen, but more cases of OHSS occurred in the hCG groups and the complaints during a luteal phase, substituted by several hCG administrations, increased over the time, there is no more reason to use hCG for LPS.

P-069

The Value of Pre-oocyte Retrieval Human Chorionic Gonadotropin (hCG) Determination as a Quality Improvement Tool in In Vitro Fertilization-Embryo Transfer (IVF-ET). V. T. Goudas, R. Bennett, D. Navot. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, New York Medical College at Valhalla, Valhalla NY, and Center for Human Reproduction-New Jersey, Westwood NJ.

Objectives: Assess the value of pre-retrieval serum hCG determination as a quality improvement tool during IVF-ET.

Design: Retrospective cohort study in a free-standing IVF Center.

Materials and Methods: The study comprised of 82 IVF-ET cycles, performed on 73 couples. The cycles were divided according to serum hCG level on the day after injection to a "Low hCG group" (Serum hCG <90 IU/L, group I) and a "Normal hCG group" (Serum hCG ≥90 mIU/mL, group II). Demographic, anthropometric and IVF-ET parameters were compared between the groups, using unpaired t-test and chi square as appropriate).

Results: Group I comprised of 12 IVF-ET cycles, performed on 12 patients. Mean age was 34.7 ± 3.21 years [Mean ± standard error (SE), NS] and mean height was 162.35 cm ± 1.96 cm (NS). The mean weight of group I was 81.44 Kg ± 6.52, compared to group II whose mean weight was 66.38 ± 4.76 ($P<0.05$). Mean body mass index (BMI) was significantly higher in group I than in group II [Group I: 30.8 ± 2.2; Group II: 25.1 ± 1.6; $P<0.03$]. The retrieval yield, defined as the number of oocytes retrieved over the number of follicles exceeding 10 mm in maximum diameter was 0.89 in group I and 1.23 in group II ($P<0.03$). The fertilization rate, was 44% in group I and 61% in group II, ($P<0.05$). The mean number of fertilized oocytes per 1000 pg/mL estradiol (E_2) was 3.88 and 5.83 for groups I and II respectively, a 33.5% decrease ($P<0.03$). The clinical pregnancy per transfer and implantation rates were 42% and 21.1% for group I and 41.4% and 20.8% for group II (NS).

Conclusions: Low serum hCG level after hCG injection, a possible result of a higher distribution volume due to higher BMI, lower absorption due to subcutaneous and not intramuscular administration, or injection error, results in lower retrieval and fertilization rates, yielding 33.5% fewer fertilized oocytes than what would be expected according to serum E_2. Nevertheless, implantation and clinical pregnancy rates are not affected, indicating that embryos carry the same pregnancy potential. Serum hCG assessment after hCG injection is an important quality improvement tool in IVF-ET practice. Low hCG levels prior to oocyte retrieval may indicate the

need for a 2nd dose of hCG and modification of oocyte retrieval by use of short-beveled low pressure retrieval system.

Monday, September 27, 1999

P-070

Ovulatory Dose of 5,000IU (5K) and 10,000IU (10K) of Human Chorionic Gonadotropin (hCG) Produces Similar Oocyte Maturation, Fertilization Rate and Embryo Quality. P. J. Beauchamp, C. Acevedo, M. Cortes, M. Crespo, R. Mendez, P. J. Beauchamp. IVF Program, Bayamon, PR.

Objective: To compare oocyte parameters of a midcycle ovulatory dose of 5K hCG and 10K hCG in patients undergoing IVF with ICSI procedure.

Design: Prospective randomized.

Materials and Methods: During 1–12/98, 78 patients aged 39 or less undergoing IVF with ICSI due to male factor infertility were randomized to receive a midcycle ovulatory dose of 5K hCG or 10K hCG. Oocyte pickup was scheduled at 34–35 hrs. post hCG and the oocyte was stripped from the cumulus-granulosa cell complex with diluted hyaluronidase at 38–39 hrs. post hCG. ICSI procedures were done at 39 hrs. post hCG. Oocyte fertilization was assessed 19 hrs. post ICSI and embryo quality was evaluated according to the Veecks classification 43 hrs. Post ICSI. Embryo transfers were done on second or third day after follicular aspiration. The luteal phase was supplemented with progesterone in oil 25mg IM if peak E2 was <1500 or 50mg IM if E2 >1500pg/ml.

Results: Patients receiving 5K hCG had 550/733 (75%) of oocytes in metaphase II compared to 100/128 (78%) of those receiving 10K hCG (NS). Fertilization rate was 64% and 59% (NS) for 5K and 10K respectively. Serum levels of hCG (MIU/ml) at time of follicular aspiration were 152 (36–788) for 5K group and 259 (81–574) for 10K group. Good quality embryos were obtained in 67% and 72% (NS) of the oocytes injected in the 5K and 10K group respectively. Pregnancy rates were 40.9% (25/61) for 5K and 29.4% (5/17) for the 10K group. However, the 5K group had more embryos transferred than 10K (4.7 vs 3.35) as a possible explanation for the difference in pregnancy rates.

Conclusion: A midcycle ovulatory dose of 5K hCG compared to 10K hCG produces similar oocyte maturation rates, fertilization rates and good quality embryos and may possibly increase the pregnancy rates.

P-071

Experience With Extended Embryo Culture in Consecutive IVF Cycles: Improvement of Implantation Rates Without Significant Reduction in Multiple Pregnancy Rates. E. Riley, G. Lopez, J. Eisermann, K. Thompson, M. Bustillo. South Florida Institute for Reproductive Medicine, Miami, FL.

From September 1998, all consecutive unselected couples undergoing IVF with or without ICSI were given the opportunity to have extended embryo culture (five instead of three days) prior to embryo transfer (ET). Extended culture in S1/S2 sequential media was carried out in couples with three or more embryos of at least 8 cells, grade 1 or 2 on the third day following oocyte retrieval; otherwise, ET was performed on day 3 adhering to the ASRM/SART guidelines for number of embryos to transfer. 27 out of 77 women (35%) met the criteria for extended culture and all but one (n=26, 96%) generated at least one morphologically normal blastocyst. The characteristics of the two ET groups and the results are listed below:

	n	Age	#Ova	#emb/ET	Preg/ET
D3 ET	50	33.2 ± 6.3	9.1 ± 3.8	3.5 ± 1.2	46%
D5 ET	26	32.5 ± 5.2	14.0 ± 5.9	2.44 ± 0.6	69.2%

	Implant/emb	Multiple/Pregn	Twin	Triplet
D3 ET	22.3%	52.2%	43.5%	8.7%
D5 ET	48.5%	72.2%	66.7%	5.5%

Conclusions: In comparing Day 5 ET versus day 3 ET, although the mean number of embryos transferred were significantly less ($p < 0.0001$), the implantation rate ($p < 0.001$) and the pregnancy rate ($p < 0.05$) were significantly higher. However, there was no significant decrease in multiple pregnancy rate. Larger cohorts of couples will need to be observed with the above treatment strategy to be able to detect whether there will be a significant decrease in high order (triplet or more) multiple pregnancy rates with extended embryo culture. It appears that to decrease the rate of twins, it will be necessary to resort to the transfer of a single blastocyst which could compromise the overall pregnancy rate per transfer.

P-072

Increased Early Pregnancy Loss in IVF Patients with Elevated Body Mass Index (BMI). M. C. Bastias, J. M. Vasquez, A. M. Carrico, M. B. Mink, H. Clardy. The Center for Reproductive Health, Nashville, TN.

Objective: Increased BMI (>25) is a common finding in the workup of patients seeking infertility treatment. The purpose of this study was to evaluate if elevated BMI affects the IVF outcome of patients ≤ 39 years old with normal day 3 FSH and LH levels.

Design: Prospective analysis of 85 patients undergoing 90 consecutive IVF treatment cycles.

Materials and Methods: BMI was calculated immediately prior to the initiation of the IVF treatment cycle. IVF cycles were classified according to the patient's BMI in group A: >25 (n=34) and group B: ≤25 (n=56). All patients underwent pituitary down regulation with luprolide acetate and ovarian stimulation with gonadotropins. Once adequate follicular development was reached, patients received human chorionic gonadotropin (hCG). Progesterone supplementation started the day of the oocyte retrieval. Statistical analyses were performed using ANOVA and Chi-square to compare ratios. P values <0.05 were considered significant.

Results: Thirty eight percent of the cycles were allocated in the high BMI group. The cancellation rates were 0% for group A and 9% (5 cycles) for group B. The IVF outcomes of both groups are summarized below:

Variable	Group A: BMI > 25	Group B: BMI ≤ 25	P value
# Oocyte Retrievals	34	51	
Age (mean±SD)	32.1 ± 3.1	30.6 ± 3.5	NS
BMI (mean±SD)	30.4 ± 3.8	22.1 ± 1.6	0.001
Ampules of Gonadotropin (mean±SD)	36.1 ± 9.1	31.8 ± 9.4	0.017
E_2 prior to hCG (mean±SD)	3627 ± 1529	4212 ± 2136	NS
Mature Oocytes (mean±SD)	14.9 ± 5.3	15.2 ± 5.1	NS
Fertilized Oocytes (mean±SD)	11.6 ± 4.6	12.6 ± 4.6	NS
Embryos transferred (mean±SD)	3.8 ± 0.6	3.5 ± 0.8	NS
# +β hCG per Transfer (%)	25/34 (74%)	45/51 (90%)	NS
# Deliveries/Ongoing Pregnancy per Transfer (%)	15/34 (44%)	41/51 (80%)	0.001

Conclusions: Our results indicate that patients with elevated BMI have significantly higher spontaneous abortion rates than patients with normal BMI. In addition a significantly higher dose of gonadotropins was required to reach adequate follicular development.

P-073

Routine Day 5 Transfer of Human Embryos Reduces the Rate of Multiple Pregnancies. H. Asakura, K. P. Katayama, E. F. Stehlik, J. C. Stehlik, A. M. Dessart. The Advanced Institute of Fertility, Milwaukee, WI.

Objectives: Human pronuclear embryos can be cultured to blastocyst stage *in vitro* using defined culture systems. Recent studies have indicated that cultured human blastocysts have higher implantation efficiency than 4 and 8 cells embryos transferred. This study was conducted to determine the obstetrical benefit of routine day 5 human blastocyst transfer.

Design: Retrospective analysis of fresh, non-donor IVF and embryo transfer (ET) on either day 2 to 3 or day 5.

Materials and Methods: Between January, 1997 and March, 1998, fresh embryos were cultured in Quinn's HTF medium (Advanced Reproductive Technologies, Sacramento, CA) with 15% synthetic serum substitute (Irvine Scientific, Irvine, CA) and were transferred either on day 2 or 3 (n=122). Between November, 1997 and January, 1999, embryos cultured sequentially in either Quinn's XI HTF and D3+ HTF media (ART, Sacramento, CA) supplemented with 15% synthetic serum substitute (Irvine Scientific) or S1-20 and S2-20 (IVF Science, Gothenburg, Sweden) were transferred on day 5 (n=93). Since March, 1998, all ET were performed using embryos treated with these extended sequential culture methods.

Results: Between day 2–3 and day 5 ET groups, mean age of patients (33.2 y.o. vs. 33.5 y.o.), mean number of pronuclear embryos per patient (7.5 vs. 8.3), percentage of ICSI cases (58.2% vs. 51.6%) were not statistically different. No cancellation of ET occurred in either group. Significantly fewer embryos were transferred on day 5 than day 2–3 (mean 2.8 vs. 3.6, $p<0.01$). However, clinical pregnancy rate between day 5 ET and day 2–3 ET were equivalent (44.1% vs. 38.5%, NS). Compared to day 2–3 ET, significantly higher singleton pregnancy rate (82.9% vs. 55.1%, $p<0.01$) and lower twin pregnancy rate (7.3% vs. 24.5%, $p<0.05$) were found after day 5 ET per intrauterine pregnancy. Day 5 ET had a trend to have fewer pregnancy losses and ectopic pregnancies. In day 5 ET group, 71% had at least one embryo reached blastocyst stage and were transferred. Clinical pregnancy rate of day 5 ET with blastocyst(s) was higher, although not significant, than day 5 ET with dividing non-blastocyst embryo(s) (48.5% with average 2.7 embryos vs. 33.3% with average 3.0 embryos). By limiting the number of blastocyst(s) to two or less *and* total embryo number to three or less for day 5 ET, higher singleton pregnancy rate and lower multiple pregnancy rate were achieved than ET with three or more blastocysts or total more than four embryos (86.2% vs. 70.4%, 6.9% vs. 28.6%, respectively) while not compromising the clinical pregnancy rate (44.6% vs. 42.9%). No triplets pregnancy occurred with the former criteria.

Conclusions: Blastocyst culture can be routinely attempted using commercially available chemically defined sequential culture media. Compared to the day 2 to 3 ET, equivalent clinical pregnancy rate can be achieved with significantly fewer embryos by day 5 ET. Risk of multiple pregnancy after IVF/ET, especially triplets or higher, can be significantly reduced by day 5 blastocyst(s) transfer. Limiting the number of total embryos to three or less and blastocysts to two or less may maximize these obstetrical benefits. Absence of blastocyst on day 5 of embryo culture does not warrant cancellation of ET.

P-074

16-Cell Versus Blastocyst for Embryo Transfer: Which Is Better? [1]S. C. Lee, [1]D. M. Min, [1]R. S. Ryu, [1]Y. C. Park, [2]C. K. Park, [1]J. H. Kim. [1]Institute of Human Infertility and Genetics, Saewha Women's Clinic, Pusan, Korea. [2]Department of Animal Science College of Animal Agriculture Kangwon University, Chunchon, Korea.

Objectives: Many reports exist about embryo transfer of early cleavage stage through to the blastocyst stage in in vitro culture in other to increase pregnancy rate. However, no clear information is available regarding the effects of stage in in vitro culture on pregnancy rate. Therefore, the purpose of this study was to evaluate whether embryo stage in in vitro culture affects pregnancy rate.

Design: Retrospective case study utilizing records of pregnancy rate. Analysis of 16-cell (day 4; 75~80 hours post-insemination) versus blastocyst (day 5; 120~125 hours post-insemination) embryo transferred.

Materials and Methods: The subjects consisted of 161 embryo transfer (ET) cycles under the age of forty from January 1996 to December 1997 who underwent ET in Saewha Women's Clinic IVF Center. Medium for in vitro culture was modified Tyrode's solution (mTLP) without glucose, phosphate and phenol red. This medium was essentially the same as that used by Kim et al., (1993). There were no other differences between the culture condition for these two years. Pregnancy were defined as clinical pregnancy when fetal heat beating were confirmed and ongoing pregnancy when pregnancy persist more than 16 weeks. Data analyzed by chi-square method.

Results:

	16-cell (day 4)	Blastocyst (day 5)
No. of ET cycles	89	72
No. of transferred embryos	384	233
Mean No. of transferred embryos	4.3 ± 0.2	3.2 ± 0.3
Clinical pregnancy rate	49.4% (44/89)†	37.5% (27/72)†
Ongoing pregnancy rate	39.3% (35/89)‡	22.2% (16/72)‡

† $P < 0.05$, ‡ $P < 0.01$, χ^2 test.

Conclusions: Much higher pregnancy rate after IVF-ET observed in 16-cell stage than blastocyst stage reveals that implantation of embryos transferred from in vitro culture take place much easily in 16-cell stage. It is known that embryo before blastocyst stage is found in oviduct and blastocyst stage is found only in uterus. Owing to above results embryo before blastocyst stage, 16-cell stage, is strongly recommend to transferred in uterus in other to increase pregnancy rate. Reference: Kim JH, Niwa K, Lim JM, Okuda K. (1993) Effects of phosphate, energy substrates, and amino acids on development of in vitro-matured, in vitro-fertilized bovine oocytes in a chemically defined, protein-free culture medium. Biol. Reprod. 48:1320–1325.

P-075

Short Term Use of Gonadotropin Releasing Hormone Agonists (GnRHa) in In-Vitro Fertilization (IVF) Patients Does Not Seem to Effect the Bone Turnover Rate. H. Yilmaz, K. Ozgur, C. Sonmez, G. Abban, M. Uner, O. Erman. IVF Unit, Department of Obstetrics and Gynecology, Akdeniz University Medical School, Antalya, Turkey.

Objectives: N-telopeptides (Ntx) are the most sensitive and specific biochemical indicator of all currently known markers to show subtle, short timed changes of bone resorption. Ntx is specific to bone and found in the urine as a stable end product of osteoclastic activity. The purpose of our study was to evaluate the effect of short-term use of GnRHa (long protocol) on bone turnover rate in IVF patients.

Design: Prospective cohort study.

Materials and Methods: Forty-six IVF patients were enrolled into the study between April 1998 and January 1999. The exclusion criteriae were as follows; woman age over 35 years, weight over 30% of their ideal body weight, taking of any medication or having disorders that might influence bone or mineral metabolism. Serum estradiol and urinary Ntx levels were measured on the following days: 1) the day of first GnRHa administration at the midluteal phase 2) the day of first gonadotropin administration when serum estradiol level was suppressed below 50 pg/ml 3) the day after the human chorionic gonadotropin (hCG) injection 4. twelve days after the transfer of embryos. The difference among urinary Ntx levels were tested by repeated measure ANOVA.

Results: Mean (±SD) serum estradiol levels on the first day of GnRHa and gonadotropin administration, on the day after the hCG injection and on 12 days after embryo transfer were 119 (±67) pg/ml, 11 (±12) pg/ml, 2865 (±1614) pg/ml and 169 (±403) pg/ml respectively. Mean (± SD) urinary Ntx levels were 71 (±34) nM BCE/mM creatinine, 81 (± 39) nM BCE/mM creatinine, 80 (±50) nM BCE/mM creatinine, 82 (±46) nM BCE/mM creatinine respectively. There were no statistically significant difference among these urinary Ntx levels ($p=0.22$).

Conclusion: Our results suggest that short-term use of GnRHa in long protocol has no effect on bone turnover rate in reproductive aged IVF patients. This work was supported by Akdeniz University Research Fund.

P-076

The Use of hMG Versus rec-FSH With the Single Dose GnRH Antagonist (Cetrorelix) Protocol in IVF-ET: A Prospective Randomized Study. [1]F. Olivennes, [2]J. Belaich-Allart, [3]S. Alvarez, [4]P. Bouchard, [1]R. Frydman. [1]Department of Obstetrics and Gynecology, A. Beclere Hospital, Clamart, France. [2]Department of Obstetrics and Gynecology, J. Rostand Hospital, Sèvres, France. [3]Department of Obstetrics and Gynecology,

Tenon Hospital, Paris, France. [4]Department of Endocrinology, St Antoine Hospital, Paris, France.

Introduction: We compared in IVF-ET, in a multicentric randomized prospective study, the use of hMG and rec-FSH in controlled ovarian hyperstimulation with the single dose GnRH antagonist (Cetrorelix) protocol.

Design: Prospective and randomized.

Material and Methods: We included in the study 62 infertile patients between 23 and 39 years old, with normal menstrual cycle and no more than 3 previous IVF attempts. HMG (N=31) or rec-FSH (N=31) were randomly attributed and started on cycle day 2 with 2 ampules per day. Monitoring of the cycles was done with daily E2 and LH and ultrasounds. The single dose of 3 mg Cetrorelix was injected in the late follicular phase (Day 7).

Results: The IVF-ET results (mean values) are presented in Table 1.

	OPU	stimulation Days	amp	E2 on hCG pg/m	oocytes total IVF	oocytes total ICSI
hMG	30	8.8	21	1241	8.3	10.3
rec-FSH	30	9.3	24	1541	8.8	11.7

	oocytes matures IVF	oocytes matures ICSI	fertilization rate % IVF	fertilization rate % ICSI	embryos total	embryos transferred
hMG	7.0	7.0	69	69	5.3	2.4
rec-FSH	7.1	7.8	57	56	5.3	2.7

No significant differences were observed in the IVF-ET results between the 2 groups.

Conclusion: The majority of the studies published so far with GnRH antagonists were made using hMG for ovarian stimulation. In this study, we did not find any significant difference in the IVF-ET results when we compared the use of hMG or rec-FSH associated with the single dose antagonist administration protocol.

P-077

Repeated Assessment of Day-3 FSH/E2 in IVF Patients with Poor Ovarian Response or Repeated Implantation Failures. F. Olivennes, C. Virelizier, N. Lédée, R. Fanchin, C. Righini, R. Frydman. Department of Obstetrics and Gynecology, A. Beclere Hospital, Clamart, France.

Introduction: Poor IVF outcome is associated with elevated FSH and E2 on cycle day 3 (Day-3). Inter-cycle variation of the Day-3 hormonal assessment have also been described and the higher values are predicting the outcome. Repeated Day-3 assements is not proposed routinely in patients undergoing IVF. This study was designed to propose 3 consecutive Day-3 FSH and E2 in patients with poor ovarian response or repeated implantation failures.

Design: Prospective evaluation of IVF patients.

Material and methods: We included in this study IVF patients with poor ovarian response (N=30) or repeated IVF failure (N=16). Only patients with normal Day-3 FSH and E2 assessed before the IVF attempts were included in this study. Poor response was defined as patients with less than 3 oocytes or with cycle cancellation. Repeated implantation failure was defined as patients with at least 4 fresh embryo transfers of a total of more than 10 embryos. FSH was measured by means of a monoclonal immunometric assay (BioMérieux, Marcy-l'Etoile, France). The range of FSH levels in the follicular phase was 1.7–6.5 IU/L (5th-95th percentiles). Elevated FSH was defined has levels >8 IU/l. Elevated E2 was defined as level >80 pg/ml.

Results: 60% (18/30) of the patients with poor response had at least one of the 3 assessments showing an elevated FSH or E2. In 12 cases, the 3 FSH levels were normal (mean ± SD: 5.3 ± 1.2 IU/L), 1 out 3 FSH levels was elevated in 12 cases (6.5 ± 2.6 IU/L), 2 FSH levels were elevated in 2 cases (9.2 ± 2.7 IU/L), the 3 levels were elevated in 3 cases (11.0 ± 3.3 IU/L) and E2 was elevated in 1 case. Elevated FSH or E2 was observed in 25% (4/16) of the patients with repeated implantation failures. No difference was observed between the groups of poor responders and implantation failures for age (37.0 ± 3.5 vs 35.1 ± 3.3 years) and pre-IVF basal Day-3 FSH (5.2 ± 1.7 vs 5.9 ± 1.2 IU/L) and E2 (28.1 ± 19.7 vs 22.8 ± 8.2 pg/ml).

Conclusion: The inter-cycle variation of Day-3 hormonal assessment is confirmed in this study. Sixty percent of the patients with poor ovarian response exhibit at least one elevated FSH or E2 despite the normal basal evaluation. This allowed us to identify patients who might not undergo further IVF attempts since they are likely destined for failure. The proportion of patients with elevated FSH/E2 was significantly lower (25%) in the group of repeated implantation failures directing our interest for potential uterine or male factors contribution involved in their poor implantation rate.

P-078

Perivitalline Space Granularity (PVSG) Does Not Develop During Maturation In Vitro (MIV). [1,3]Hassan Aly Hassan, [2,3]D. El Guiziry, [1,3]H. Azab, [1,3]A. Abel Rahman, [3]I. Bagdady. [1]Department of Obstetrics & Gynecology Faculty of medicine Alexandria University [2]Department of Clinical Pathology Faculty of medicine Alexandria University. [3]Alexandria IVF & ICSI center.

Objective: we recently reported the first description of the presence of dark coarse granules in PVS by light, inverted microscopy during ICSI, emphasizing that it is a maturational related process enhanced by high doses of HMG[1]. The true nature and origin of PVSG is unknown. They could be related to apoptosis[2], remnants of corona cell processes[3], or even protein and hyaluronic extra cellular matrix[4]. Earlier studies reported maturational related cortical cytoplasmic vesicles that extrude their glycoprotein into the PVS[5]. In the present study we evaluated the temporal relationship between expression of PVSG and germinal vesicle breakdown (GVBD) and first polar body extrusion (oocyte maturation in vitro MIV).

Design: Descriptive, prospective study.

Materials and Methods: The study included 610 oocytes obtained from 783 follicles punctured during 76 ICSI cycles (all follicles, large and small were aspirated).

Results: At retrieval 69.4% were M2, 19.5% were M1, 11.1% were GV. The incidence of PVSG at retrieval was 46.8%, 15.1% and 2.9% for M2, M1 and GV respectively ($p<0.01$). The rate of PVSG development during MIV was observed. Out of 66 GV oocytes without PVSG at OR, 59.1% matured to M2 in vitro. Only 5.1% of these M2 (MIV) developed PVSG (observation time 48 hours). As regards oocytes retrieved at M1 without PVSG (n=101), 92.1% matured to M2 in vitro, of these only 3.2% developed PVSG in vitro (observation time 24 hours). None of the agranular M2 at retrieval (n=225) developed de novo PVSG before injection (observation time 6 hours). Therefore the incidence of granularity after MIV (5.1%, 3.2%) was significantly lower when compared to the inherent granularity of M2 oocyte at retrieval (46.8%).

Conclusion: PVSG could be a contribution from the somatic cell component, hence they were missing during MIV because oocytes were denude prior to culture. Alternatively, maturation related PVSG expression in vivo may depend on exogenous and/or endogenous hormones that are missing from the in vitro system. References: 1) Hassan Aly et al Hum Reprod. 13(12) 3425–30, 1998. 2) Tilly GL Rev. Reprod 1, 162–17, 1996. 3) Sathananthan-AH- Hum cell 20,21–38, 1997. 4) Dan Dekar P. Hum Reprod 7, 391–98, 1992. 5) Stasna JZ. Mikrosk. Anat. Forsch 97, 675/87, 1983.

P-079

Early Prediction of Non Gonadotropic Ovarian Suppressive Action of GnRh Agonist (NGOSA). [1,3]Hassan Aly Hassan, [1]A. Abdel Aziz, [2,3]D. El Guiziry, [1,3]H. Azab, [1,3]A. Abdel Rahman, [3]I. Bagadady. [1]Department of Obstetrics & Gynecology Faculty of medicine Alexandria University [2]Department of Clinical Pathology Faculty of medicine Alexandria University. [3]Alexandria IVF&ICSI center.

Objective: A certain proportion of women with good ovarian reserve, are susceptible to inhibitory action of GnRha unrelated to gonadotropin suppression (NGOSA). These include direct ovarian suppression and inhibition of FSH mediated signal transduction,[1,2] growth hormone suppression and inhibition of growth hormone response to GHRH[3,4]. This study investigates the potential use of flare response to a dynamic test in order to predict non gonadotropin ovarian suppression by the agonist (NGOSA).

Design: Prospective randomized study.

Materials and Methods: The study included 190 ICSI women characterized by age <37, FSH <10 and antral follicle count more than 6. They were randomly allocated into 3 protocols, daily buserelin acetate (n=64), depot goserelin (n=62), and depot triptorelin (n=64). Initial E_2 response (first 3 days) to the agonist was evaluated and related to: final E_2 values, oocyte number, fertilization, cleavage and pregnancy rates.

Results: Cycles were canceled in 38 women (20%) due to absence of response. Only with buserelin was the initial E_2 response predictive of the poor outcome. In canceled cycles, E_2 value were (70 ± 36, 46 ± 41, 80 ± 29 pg/ml) in day 1, 2 and 3 respectively and were significantly lower ($p< 0.05$) as compared to respective values in good responders on the same buserelin protocol (120±40, 202±39, 241±45 pg/ml). On the other hand initial E_2 responses to the administration of the depot agonist (goserelin and triptorelin) were not predictive of the final ICSI outcome. The performance of initially canceled patients (n= 38) was evaluated in repeat attempts without GnRha. Good ovarian response (5.2±1.3 oocyte, 806±241 pg/ml E_2) was obtained in 81.6% using a non agonist protocol with a pregnancy rate of 22.6%. The outcome, in repeat attempts, was not dependent on the type of agonist in the initial cancellation.

Conclusion: 20% of potential good responders are candidate for NGOSA. Early prediction is possible only with buserelin but not with depot forms. It could therefore be recommended to use a busereline dynamic test to select good candidates for the depot forms of the GnRH agonist. References: 1) Kowalik A et al J Reprod Med. 43(5): 1998. 2) Furger et al Mol Hum Reprod 2 (4): 1996. 3) Tropiano et Fertil Steril 68 (6), 1997. 4) Kaltsas et al Hum Reprod 13 (1) 1998.

P-080

Empty Follicle Syndrome and ICSI: Sparing Class V and VI Follicle for Double Aspiration. [1,3]Hassan Aly Hassan, [2,3]D. El Guiziry, [1,3]H. Azab, [1,3]A. Abdel Rahman, [3]I. Bagdady. [1]Department of Obstetrics & Gynecology Faculty of Medicine Alexandria University. [2]Department of Clinical Pathology Faculty of Medicine Alexandria University, [3]Alexandria IVF and ICSI center.

Objective: Several etiologies were proposed for the empty follicle syndrome (EFS)[1,2,3]. The incidence of recurrence and subsequent fertility potential are not well defined. Saving the cycle by rescheduling a second retrieval[4,5] (double aspiration)[6] has been reported but not properly characterized. The present study compares 2 techniques that could be applied to (EFS): a) aspirating all follicles or b) sparing small follicles for a second retrieval.

Design: Prospective randomized.

Material and Methods: Patients were randomly allocated to 2 groups: group A n=7: all follicles were aspirated (class V to class VIII), group B n=8: only class VII and VIII were aspirated (V & VI were spared). Blood was withdrawn for estradiol, hCG and progesterone measurements (to assure hCG bioavailability). A booster hCG (10,000 units) was administered and a second retrieval 24 hours later was performed according to our previous publication[6].

Results:

	Oocytes	M2	M1	GV	MIV
Group A	33	70%	18%	12%	70%
Group B	57	51%	30%	19%	71%

	M2 fertilized	MIV fertilize	Pregnancy
Group A	61%	43%	14%
Group B	48%	40%	25%

Conclusion: In some women, with empty follicle syndrome, small follicles require longer duration of hCG bioavailability. Oocytes from those follicles are probably lost when considered for the first retrieval. If spared for a second retrieval, however, the immature oocytes are competent for both MIV and fertilization.

References: [1]Awonuga et al Hum Reprod, 13 (5)1998. [2]Korosi et al Orv Hetil 139 (42) 1998. [3]Meniru GI et al Hum Reprod 12 (11) 1997. [4]Quintans CJ et al Hum Reprod 13 (10) 1998. [5]Ubaldi F et al Hum Reprod 12(3) 1997. [6]Hassan HA et al Fertil Steril 69 (1), 1998.

P-081

Separation of HIV-1 from Motile Sperm Fraction: Comparison of Commercial Sperm Separation Media. J. A. Politch, C. Xu, L. Tucker, D. A. Anderson. Fearing Laboratory, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA.

Objectives: Until recently, Percoll, a polyvinylpyrrolidone (PVP)-coated silica particle colloidal suspension, was widely used as a sperm separation medium in ART clinics. It was also found to be highly effective in removing HIV-1 from semen, especially when used in conjunction with swim-up or double tube techniques (Lancet 340:1317, 1992; JRI 41:127–136, 1998). In 1996, Pharmacia issued a statement that Percoll is not intended for clinical use; subsequently, alternative sperm processing products, many using silane-coated silica particle suspensions, were introduced. Recent studies have demonstrated that these substitute sperm processing solutions compare favorably with Percoll for recovering motile sperm. The present study was designed to determine whether the new sperm separation media are as effective as Percoll in removing HIV-1 from semen.

Materials and Methods: Semen samples from HIV seronegative normal donors were spiked with high concentrations (range: 500–30,000 $TCID_{50}$) of HIV-1 (MN and JR-CSF HIV-1 strains) propagated in H9 cell cultures. Spiked samples were loaded on 15 ml tubes containing gradients (45%/90%) or single concentrations (90%) of separation media and spun for 20 minutes at 400 × g. Upper layers were carefully removed and pellets containing motile sperm were resuspended, placed in clean tubes and washed with 3 ml Ham's F-10/HSA. The motile sperm fractions were analyzed for HIV-1 DNA and RNA by PCR.

Results: In 3/3 separate experiments, neither HIV DNA nor RNA was detected in sperm fractions obtained by gradient or single concentration Percoll fractionation procedures. On the other hand, parallel experiments using Isolate (Irvine Scientific) or PureCeption (Pacific Andrology), two silane-coated silica particle separation media, resulted in detection of high levels (median=10,000 copies/ml) of HIV-1 RNA in motile sperm fractions. Statistical analysis by Fisher Exact Test indicated a statistically significant difference between the PVP and silane-coated products ($p<0.05$). HIV-1 DNA was not detected in the samples processed with silane-coated products. Preliminary tests of Sil-Select (another silane product; Ferti Pro) and IxaPrep (Media-Cult) also indicate considerable HIV RNA contamination of motile sperm fractions. Use of silane-coated silica separation media in conjunction with a swim-up step or double tube separation technique decreased but did not eliminate HIV-1 RNA levels. These data indicate that Percoll is the most effective sperm separation medium for excluding HIV from the motile sperm fraction. It is recommended that a secondary procedure (i.e., double tube or swim-up) be used to further safeguard against HIV contamination of motile sperm preparations.

P-082

Individual Variation in Biochemical Properties of Follicular Fluid (FF) of Lead Follicles from IVF. [1]A. Hossain, [1]B. Rizk, [2]W. Korzun, [1]I. Thorneycroft. Departments of [1]Obstetrics and Gynecology, and [2]Clinical Laboratory Sciences, University of South Alabama, Mobile, AL.

Objectives: Knowledge of the chemical composition of FF, the immediate micro environment of the oocyte, may be useful in understanding the factors that affect the quality of the oocyte and its postfertilization stages (zygote, embryo and blastocyst). Unlike in animal models, data on the chemical composition of human FF are scarce. In this study, 20 non-hormonal analytes of FF were measured to know their distributional ranges in the lead follicles of IVF, and to know how the values of these analytes differ between FF and serum.

Design: The biochemical profile of FF and serum were measured in patients undergoing controlled ovarian hyper stimulation (COH).

Materials and Methods: FF, retrieved from lead follicles that developed in patients undergoing COH for IVF, and free from blood contamination, was briefly centrifuged at 350× g, then stored at −20° C until analyzed. All samples (FF and serum) were analyzed with a Dimension Clinical Chemistry Analyzer (Dade-Behring, Inc.). The 20 analytes that were measured in 43 samples included glucose, blood urea nitrogen (BUN), creatine, calcium, phosphorus, magnesium, total protein (TP), albumin, cholesterol, triglyceride, alkaline phosphatase (ALP), alanine amino transferase (ALT), γ glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate amino transferase, creatine kinase, amylase, uric acid, and iron.

Results: FF pH was slightly acidic. FF osmolarity was similar to that of the plasma. In all FF samples, the concentrations of cholesterol, uric acid, GGT, and bilirubin were within the reference ranges for serum. In > 90% of FF samples, all 20 analytes were either within or below the references for serum. Eight analytes were above the serum range in < 10% FF samples. The summary statistics of the eight analytes, in which the values of ≥ 50% FF samples fell outside (below or above) the serum range, are shown:

Analyte	Mean	Median	Min.	Max.	25%	75%
Glucose (mg/dl)	72.4	64.5	38.0	134.0	52.0	92.0
Calcium (mg/dl)	6.8	7.1	1.7	11.7	6.0	8.6
Albumin (g/dl)	3.3	3.4	0.1	5.6	2.3	4.2
Triglyceride (mg/dl)	9.6	8.0	0.1	22.0	7.0	12.8
ALP (U/L)	26.3	24.0	0.0	74.0	17.8	36.0
ALT (U/L)	17.4	18.0	0.0	24.0	16.8	19.3
LDH (U/L)	95.3	98.5	2.0	193.0	77.0	118.0
TP (g/dl)	5.3	5.5	2.0	7.7	3.9	6.5

Conclusion: Our results showed a wide variation in the level of the analytes among the follicles. This variation could be a reflection of the biochemical kinetics of the follicles. Future study are needed to determine which analyte would predict the quality of oocyte and its postfertilization stages.

P-083

A Nitric Oxide Scavenger, Hemoglobin (Hb), Enhances Preimplantation Development of Mouse 1-Cell Embryos Cultured in Preimplantation (P)-1 Medium. J. M. Lim, S. E. Park, T. E. Shin, S. K. Cha, J. J. Ko, K. Y. Cha. Infertility Medical Center of CHA General Hospital and College of Medicine, Pochon CHA University.

Objective: It appears that amino acids (aa) and cysteine (cys) are embryotropic factors for preimplantation development of embryos in various species (Park et al., 1999). Further, the addition of Hb, a nitric oxide scavenger, to culture medium greatly stimulates bovine embryo development to the blastocyst stage (Lim and Hansel, 1998). It was therefore evaluated whether Hb could improve development of 1-cell mouse embryos cultured in medium containing either aa or aa + cys.

Design: Development of 1-cell embryos to the 4-cell (2-cell block overcome), 8-cell, blastocyst (BL) and hatched BL stages were examined at 48, 72, 96 and 120 h post-hCG injection, respectively.

Materials and Methods: ICR mouse 1-cell embryos were collected at 18 h after hCG injection following PMSG administration. The embryos were randomly divided into five groups and continuously cultured up to 120 h post-hCG in BSA (3 mg/ml)-containing P-1 medium supplemented with as follows; 1) no addition, 2) aa (19 aa included in 1% of MEM essential and nonessential aa solutions), 3) aa + Hb (1 μg/ml), 4) aa + cys (0.6 mM) and 5) aa + Hb + cys.

Results: More 1-cell embryos overcome the 2-cell block after addition of aa, aa+Hb or aa+Hb+cys (45 to 60%) than after no addition (19%). Development to the 8-cell (42 to 45%), BL (30 to 38%) and hatched BL (21 to 22%) stages was further enhanced after addition of Hb or aa + Hb + cys than after no addition or aa addition. More than a half of blastocysts developed to the hatched BL stage after addition of Hb to P1 with aa or aa + cys.

Table: Effects of the addition of Hb to P1 medium on development of ICR mouse 1-cell embryos (a-c: P<0.05).

P-1 medium supplemented with	No. embryos cultured	No (%) of block overcame embryos
No addition	57	11 (19)[a]
aa	64	31 (48)[b]
aa + Hb	67	40 (60)[b]
aa + cys	61	19 (31)[ac]
aa + Hb + cys	55	25 (45)[cd]

P-1 medium supplemented with	No (%) of 4-cell embryos developed to		
	8-cell	Blastocyst(BL)	Hatched BL
No addition	3 (5)[a]	0 (0)[a]	0 (0)[a]
aa	18 (28)[b]	9 (14)[b]	4 (6)[a]
aa + Hb	28 (42)[c]	20 (30)[c]	14 (21)[b]
aa + cys	2 (3)[a]	2 (3)[ab]	1 (2)[a]
aa + Hb + cys	25 (45)[c]	21 (38)[c]	12 (22)[b]

Conclusion: The results of this study demonstrated that Hb exerts as an embryotropic factor for preimplantation development of mouse embryos. Use of Hb in embryo culture system could contribute to establishing an effective culture medium.

P-084

What is the Best Stage for Freezing Human Blastocysts? H. J. Cho, S. H. Yoon, H. G. Yoon, H. J. Yoon, J. B. Lee, S. P. Park, K. A. Kim, W. D. Lee, J. H. Lim. Maria Infertility Clinic, Seoul, Korea.

Objective: This study was carried out to investigate the survival rates of human blastocysts frozen by either slow freezing or vitrification according to each developmental stage.

Design: The blastocysts used in this study were derived from 3PNs or surplus embryos post-ET in human IVF-ET program. On day 6, the blastocysts co-cultured with cumulus cells in YS medium containing 20% hFF were classified into early blastocysts (ErB: < 165 μm), expanding blastocysts (EgB: 165–180 μm), expanded blastocysts (EdB: >180 μm) and hatching blastocysts (HgB) according to the size and appearance of blastocysts.

Materials and Methods: All 3PN zygotes and surplus embryos removed from IVF-ET were routinely cocultured with cumulus cells in 20 μl YS medium containing 20% hFF until day 6 after OPU. If all 6-day blastocysts were classified into ErB, EgB, EdB and HgB depending on their diameter measured with a micrometer, and then cryopreserved by either slow freezing or vitrification. In slow freezing, the pre-freeze equilibration of blastocysts was performed in 5% glycerol and 9% plus 0.2 M sucrose. The slow freezing was carried out by cooling at −2°C/min to −7°C and −0.3°C/min to −38°C. At vitrification, the pre-freeze equilibration of the blastocysts was performed by transferring the blastocysts to 25% glycerol + 25% ethylene glycol (within 1 min). The vitrification was carried out by immediately plunging the straw into LN_2. The frozen-thawed blastocysts were washed twice in PBS with 20% hFF and YS with 20% hFF, respectively, cocultured with cumulus cells in a YS medium containing 20% hFF for 18 hours and then we morphologically observed the survived blastocysts.

Results: The result was as follows:

	Slow Freezing		
	No. of Frozen Bla.	No. of Survived Bla	% of Survived Bla.
ErB	96	46	47.9%
EgB	74	59	79.7%
EdB	57	53	93.0%
HgB	27	21	77.8%
Total	254	179	70.5%

	Vitrification		
	No. of Frozen Bla.	No. of Survived Bla	% of Survived Bla.
ErB	101	42	41.6%
EgB	77	60	77.9%
EdB	53	47	88.7%
HgB	12	9	75.0%
Total	243	158	65.0%

Conclusion: This study had shown that the survival rates of early blastocysts frozen-thawed by slow freezing or vitrification were significantly

lower as compared to the other 3 groups. However, there was no difference in the survival rate between slow-freezing and vitrification. There is the highest survival rate in the expanded blastocyst stage of both methods. Our results suggest that human blastocyst cryopreservation should be carried out in the higher developmental stage (>165 μm) than the early blastocyst stage.

P-085

Estimation of Permeability Coefficients of Human Oocytes in the Presence of the Cryoprotectant Propane-1,2-Diol. S. J. Paynter, L. O'Neil, B. J. Fuller, R. W. Shaw. Department of Obstetrics and Gynaecology, University of Wales College of Medicine, Cardiff, S. Wales, United Kingdom.

Objectives: Equilibration of oocytes with cryoprotectant (CPA) prior to subzero storage reduces damage from ice formation and improves survival. However, CPA exposure can, if performed inappropriately, induce damage via osmotic stress and chemical toxicity. This study aims to determine the cell membrane permeability characteristics of human oocytes in the presence of the current CPA of choice, propane-1,2-diol (PD). Such data would facilitate the design of cryopreservation protocols which minimise oocyte damage.

Design: Human oocytes, donated with informed consent by patients undergoing assisted fertility treatment at University Hospital of Wales, Cardiff, were perfused with PD and cell volume was determined from measurements of oocyte diameter during perfusion.

Materials and Methods: Cumulus cells were removed from donated oocytes using hyaluronidase and gentle pipetting. Each oocyte was placed in a 5μl droplet of phosphate buffered medium and held in position by suction generated using a fine glass pipette. The oocyte was then perfused with 1ml of 1.5M PD at 30°C, 24°C or 10°C. The volume of the oocyte before, during and after perfusion was recorded by videomicroscopy until osmotic equilibrium was reached. Oocyte volume excursions were calculated from diameter measurements, only oocytes which remained circular in cross section throughout perfusion were included. From these measurements the Kedem-Katchalsky (K-K) passive coupled transport coefficients namely L_p (water permeability), P_{PD} (CPA permeability) and σ (reflection coefficient) were derived.

Results: Mean±SEM K-K coefficients generated at 30°C, 24°C and 10°C, respectively, were L_p = 2.49±0.28, 0.53±0.04, 0.50±0.12 μm min^{-1} atm^{-1}, P_{PD} = 1.11±0.04, 0.28±0.02, 0.15±0.02 μm sec^{-1} and σ = 0.75±0.08, 0.94±0.02, 0.98±0.01.

Conclusions: At 24°C there was very little variability in the response of oocytes (n=9) to the presence of PD. At 30°C and 10°C the response was more variable even with the low numbers of oocytes studied (n=4 at each temperature). Variability could be due to differences in oocyte quality, although only oocytes which were morphologically normal were included in the study, or due to differences in oocyte maturity. Eight of the oocytes perfused at 24°C had extruded a polar body, at the other temperatures the oocytes were a mixture of those with and without a polar body present. Alternatively, variability in response to CPA may be an inherent feature of human oocytes, a factor which could have important consequences for the cryopreservation of human oocytes.

P-086

Comparison of ICM/Trophectoderm Development in Blastocysts Arising from Different In Vitro Culture Regimens: Effect of FGF-4, LIF and a Growth Factor Cocktail. N. Desai, J. Lawson, J. Goldfarb. Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH 44106.

Objective: The routine culture and transfer of all patient embryos at the blastocyst stage demands that culture systems maximally support the developing embryo. Supplementation with specific growth factors may be necessary to further refine culture formulations, and better simulate the in vivo environment. In this study, we contrasted the influence of growth factor supplementation on blastocyst development and differentiation.

Design: Computer assisted image analysis along with immunofluorescent staining and immunosurgery were utilized to assess total cell number and distribution of cells amongst the ICM and trophectoderm.

Materials/Methods: Zygotes were isolated from B6C3F1 mice and cultured overnight. Two cell embryos were pooled and randomly distributed between the following five treatment groups: (1) control (2) coculture on Vero cells (3) growth factor cocktail containing IGF II (1 ng/ml), EGF (4 ng/ml), TGF α(2 ng/ml) and PDGF (1 ng/ml) (4) LIF (1 ng/ml) and finally (5) FGF-4 (10 ng/ml). In a separate experiment, the specific effect of FGF-4 was challenged by simultaneous incubation with anti-FGF-4 antibody (200 μg/ml). Embryo development in each culture regimen was monitored for 72 hrs and the following morphologic parameters were assessed: embryo stage, blastocyst development and maturity (based on cavity size), and lastly the degree of embryonic hatching. At termination of the experiment, differential labelling of the ICM and the trophectoderm was accomplished using polynucleotide specific flourochromes and complement mediated lysis (Hardy et al, 1989).

Results: The effect of the culture regimen on ICM and TE development is depicted below. Only embryos with at least 60 cells were evaluated. All treatments significantly enhanced ICM development compared to the control (paired T-test, $p<0.001$). Anti-FGF-4 was effective in neutralizing the positive effect of FGF-4 on ICM formation.

Treatment	No. embryos	Total Cells (TC)	ICM	TE	Ratio ICM/TE	Ratio ICM/TC	Ratio TE/TC
Control	76	85	15	70	0.21	0.18	0.82
Vero	120	106	24	81	0.30	0.23	0.76
Cocktail	119	95	20	75	0.27	0.21	0.79
LIF	114	94	21	73	0.29	0.22	0.78
FGF-4	135	98	21	77	0.27	0.21	0.79
Anti-FGF-4	38	103	15	88	0.17	0.15	0.85
FGF-4	27	113	26	87	0.30	0.23	0.77

Conclusions: Blastocyst differentiation and inner cell mass development during in vitro culture can clearly be modulated by exogenous growth factor supplementation.

P-087

Ultrastructural Study of Immobilized Spermatozoa Used for ICSI. T. Takeuchi, M. C. Tsai, J. Hariprashad, Z. Rosenwaks, G. D. Palermo. The Center for Reproductive Medicine and Infertility, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY, USA.

Objective: To assess membrane and organelle characteristics of mechanically immobilized human spermatozoa immediately prior to the ICSI procedure.

Design: Membrane integrity and organelle characteristics of immobilized human spermatozoa were analyzed by transmission electron microscopy (TEM).

Material and Methods: Ejaculated semen samples obtained from consenting men (under the Internal Review Board No. 0696-389), were processed by density gradient centrifugation. One microliter of the final sperm suspension at a concentration of approximately 1×10^6/ml was placed in a 4 μl droplet of polyvinyl pyrrolidone (10%; w/v) in the center of a standard petri dish used for ICSI. Under inverted microscope at 400× magnification, spermatozoa were positioned perpendicularly to the ICSI pipette. The tool was gently lowered to compress and roll the sperm flagellum in a repetitive manner until the tail was clearly kinked, looped, or convoluted (Palermo *et al., Hum Reprod* 11:1023–1029, 1996). Since individual immobilization is labor intensive, the pellet yielded by the limited number of cells processed would be inadequate for TEM analysis. Thus, immobilized sperm cells were injected into the perivitelline space of mouse oocytes to serve as a tag to facilitate localization of spermatozoa in the resin. Intact, motile spermatozoa, subzonally injected into a single mouse oocyte were used as control (n = 80). Stuffed oocytes were fixed and processed for TEM. Membrane integrity and sperm organelles characteristics were observed and recorded.

Results: A total of 300 spermatozoa from samples of three different individuals were mechanically immobilized by micropipette and subzonally inserted into 5 mouse oocytes (approximately 60 spermatozoa per oocyte). The entire process, including sperm immobilization and oocyte stuffing and fixing, required about one hour per oocyte. TEM sections evidenced that immobilized spermatozoa underwent alterations in the plasma membrane

ranging from localized vesiculation to complete disruption and/or fusion with the outer acrosomal membrane allowing consequent leakage of acrosomal content. No changes in the chromatinic material or centriolar structures were observed.

Conclusion: It is believed that spermatozoa for use with ICSI do not require specific pretreatment except for mechanical immobilization prior to injection. These findings support the need for immobilization of spermatozoa prior to sperm injection, since it expedites crucial structural changes as observed in the spontaneous acrosome reaction.

P-088

The Presence of Antisperm Antibodies that Bind to Spermatozoa Does Not Impair ICSI Results. J. Hariprashad, M. C. Tsai, P. N. Schlegel, T. Takeuchi, S. S. Witkin, Z. Rosenwaks, G. D. Palermo. The Center for Reproductive Medicine and Infertility-New York Presbyterian-Weill Medical College of Cornell University, New York, NY, USA.

Objective: The presence of the antisperm antibodies has been correlated with impaired fertilization rates after standard in vitro insemination. In the present study we attempted to discover whether antisperm antibodies on spermatozoa affect the reproductive outcome after intracytoplasmic sperm injection (ICSI).

Design: The fertilization and pregnancy rates in ICSI couples with antisperm antibodies bound to the spermatozoa were compared to those in antibody-negative ICSI patients with congenital bilateral agenesis of the vas deferens (CBAVD).

Materials and Methods: The immunobead binding assay was used to detect the presence of IgG, IgA and IgM on the sperm surface. A "positive" patient was defined as one in which more than 20% of the spermatozoa bound the beads for one or more of the immunoglobulin isotypes tested. A total of 19 normozoospermic but "positive" patients were selected for this study based on the presence of the antibodies bound to spermatozoa. The control group consisted of 35 antibody-negative couples with CBAVD. Ejaculated and epididymal spermatozoa were used for ICSI in the positive and control groups respectively. The female patients were down-regulated using a long GnRH analog protocol then superovulated using gonadotropins. Following ICSI, embryo transfer was performed on day 3. Fertilization and pregnancy outcomes were assessed and compared for the two groups.

Results: The average female age in the positive group was 36.0 ± 3 years (M ± SD) and 36.1 ± 3 in the control group. In immunologically positive patients, the location of sperm surface binding was variable between spermatozoa and between patients. For example, twelve patients had IgG bound to the sperm head, 10 patients had bound IgA, and 4 patients had bound both IgG and IgA over the sperm head. In no patient did spermatozoa bind beads for IgM. As shown in the following table, there were no differences in fertilization or pregnancy rates between the immunologically positive and control groups.

Number of	Study group	Control group
Cycles	24	35
Fertilized/injected oocytes (%)	192/246 (78%)	279/376 (74.6%)
Clinical pregnancies (%)	15/24 (62.5%)	23/35 (65.7%)

Conclusion: The presence of the antisperm antibodies apparently has no deleterious effects on the fertilization rate or the pregnancy outcome in procedures involving ICSI. In addition, neither the type nor the location of the immunoglobulins on the sperm cells appeared to have predictive value regarding the reproductive outcome among the immunological couples in this study. Based on the results, ICSI should be considered as the treatment of choice when dealing with immunological factor infertility.

P-089

Higher Frequency of Mitochondrial DNA Deletion Noticed in Unfertilized Oocytes than Cleavage Stage Embryos. S. H. Lee, J. H. Han, S. W. Cho, I. P. Kwak, Y. S. Nam, K. Y. Cha. Department of Obstetrics and Gynecology, Human Genetics Laboratory of Infertility Medical Center of CHA General Hospital, College of Medicine, Pochon CHA University.

Objective: A mature human oocyte contains 10^5 mitochondrial genomes (mtDNAs). Accumulation of mitochondria is a central function of oocyte maturation for energetic demands of embryogenesis with the mode being maternal transmission. And also there are measurable levels (up to 0.1%) of the so-called common deletion, which removes 4977bp between the ATPase8 and ND5 genes in some oocytes. The stage of deletion is thought to be occurring before the division of the precursor cells and may be randomly segregated during early oogenesis, according to the bottleneck theory. Strikingly, a mtDNA molecule containing a 4977bp rearrangement is present in high amounts in many patients with Kearns-Sayre syndrome (KSS) and progressive external ophthalmoplegia (PEO). The objective of this study was to determine the frequency of common deletion in human unfertilized oocytes and cleavage stage embryos obtained from women undergoing IVF.

Design: The "common" 4977bp mtDNA deletion was assayed in unfertilized oocytes and cleavage stage embryos (8-cell stage) derived from the trinucleated oocytes (3PN).

Materials and Methods: After removing zona pellucida of unfertilized oocytes (n=88) and embryos (n=86) obtained from patients underwent IVF-ET program, lysis has been performed with lysis buffer to release DNA. The common deletion of mtDNA was assayed by a PCR strategy that depends on primers flanking the mtDNA deletion hot spot between np 8470 to 13447. Designed primers were MT1/MT4 (amplified the area around the deleted mtDNA), MT1/MT3 (nested PCR product), and MT2/MT4 (amplified undeleted mtDNA). One percent agarose gel was used for analysing PCR products. χ^2 analysis of the numbers of oocytes versus the numbers of embryos that harbour the common deletion was performed.

Results: The total amplification efficiency was 97.7%. The frequency of the common deletion in 88 oocytes and 86 embryos was found to be 33.7 and 18.6% respectively. There was a statistical difference between the presence of deletion in oocytes versus embryos ($p < 0.02$).

Conclusions: There was a higher frequency of common deletion in oocytes than cleavage stage embryos found in this study. The results suggests several points: 1) Defective mitochondrial function including common deletion would interfere with fertilization. 2) Although unfertilized oocytes showed a higher frequency of common deletion, cleavage stage embryo also harbour common deletion. It could interprete a high degree of heteroplasmy for common deletion in oocytes. However, further investigation on fertilization, and/or IVF pregnancy rate in the same individual followed by embryo development, should be performed to filter mutated mtDNA, to prevent transmission to the next generation.

P-090

Inhibin-B Levels During Ovarian Stimulation for IVF Correlate with Number of Oocytes Retrieved in Patients with Endometriosis. A. Dokras, A. Habana, J. L. Giraldo, E. E. Jones. Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.

Objectives: Inhibin-B secretion increases during the follicular phase of normal and gonadotropin stimulated cycles. One of the proposed mechanisms by which endometriosis causes infertility is impairment of follicular development, resulting in a suboptimal response to ovarian stimulation. The aims of this study were: (1) to determine the levels and pattern of Inhibin-B secretion during ovarian stimulation in patients with endometriosis undergoing IVF and (2) to evaluate the correlation between inhibin-B levels and number of oocytes retrieved.

Design: Retrospective cohort study.

Materials and Methods: Patients (n=18) with endometriosis as the only cause of infertility had 4 inhibin-B (I) and estradiol (E) levels measured during gonadotropin stimulation for IVF: day 1 (I_1, E_1), between days 8–10 (I_{8-10}, E_{8-10}), day of human chorionic gonadotropin (hCG) administration (I_{hCG}, E_{hCG}) and day of oocyte retrieval (I_{or}, E_{or}). All patients had surgical treatment for endometriosis prior to their IVF cycle. Patient characteristics and treatment parameters analyzed were age, days of gonadotropin stimulation and total ampules used, oocytes retrieved, number of grade 1&2 embryos, fertilization and pregnancy rate. Statistical analysis was performed by Anova, Kruskal-Wallis and Pearson's test.

Results: The secretion of inhibin-B in patients with endometriosis increased in the early follicular phase and in 8 of the 18 patients peaked prior to the day of hCG administration although E levels increased over this time period. In these 8 patients, I_{hCG} and I_{or} values were lower ($p<0.007$, $p<0.04$) compared to the 10 women in whom inhibin-B secretion continued to rise till the day of hCG. Inhibin-B area under the curve (AUC) and I_{hCG} correlated positively with number of oocytes retrieved ($r=0.66$, $p<0.005$;

r=0.61, p<0.01 respectively) whereas there was no correlation between EAUC and E_{hCG} with oocytes retrieved. The total gonadotropin ampules used correlated inversely with I_{hCG} levels (r=−0.58, p<0.02). Also, there was a positive correlation between E_1 and I_1 (r=0.7, p<0.002) and E_{hCG} and I_{hCG} (r=0.54, p<0.05).

Conclusion: This is the first report on the pattern of inhibin-B secretion in patients with endometriosis during an IVF cycle. It demonstrates that I_{hCG} and IAUC correlates with the number of oocytes retrieved, hence indicating that follicular development may not be impaired in these patients treated for endometriosis. Although estradiol levels corresponded with inhibin-B levels, there was no correlation between estradiol levels and number of oocytes retrieved in this study. These findings suggest the possibility of using inhibin-B as an alternative marker for follicular development.

P-091

In Vitro Development of Mouse Zygotes Following Reconstruction by Sequential Transfer of Germinal Vesicles and Female Pronuclei. H. Liu, L. C. Krey, J. A. Grifo, A. Blaszczyk, J. Zhang. Program for In Vitro Fertilization, Reproductive Surgery and Infertility, New York University School of Medicine, New York, NY 10016.

Objective: In the human and mouse oocyte morphologically normal meiosis can take place following transfer of its nucleus into a heterologous cytoplast of the same developmental stage. However, information about the developmental ability of such female nuclei (i.e., pronucleus formation and early embryonic development upon fertilization) is limited.

Design: Using a sequential nuclear transfer technique, we evaluated the behavior and maturation of a GV following electrofusion into an enucleated GV oocyte and subsequently into an in vivo fertilized zygote.

Materials and Methods: Two strains of mice were used: black (C57BL/6) mice were used to donate cytoplasts from immature GV oocytes and from zygotes whereas white mice (FVB/M) were nuclear donors (GV and sperm). The GV was removed from an oocyte of a white mouse and transferred by electrofusion into an enucleated GV oocyte from a black mouse. Each reconstructed oocyte was then matured in vitro to metaphase II and activated with an ionophore and anisomycin. After 4–6 h, each female pronucleus that formed was removed and transferred into a haploid zygote obtained by mating a white male with black female and then removing the female pronucleus; embryonic development of these reconstructed zygotes was then monitored in vitro for the next 72 h. Our micromanipulation and electrofusion methods for nuclear transfer have been described.

Results: A total of 45 oocytes were reconstructed by GV transfer. Fusion and maturation rates were, respectively, 89% and 80%. Second polar body extrusion and pronuclear formation occurred in 94% of the matured oocytes following treatment with an ionophore and anisomycin. The haploid female pronucleus that formed in each of the 30 activated oocytes was transferred to a haploid zygote. The fusion rate was 97% and each reconstructed zygote divided after 24 h in culture. Most of the resultant embryos (78%) developed to morula or hatching blastocyst stage, a maturational rate no different than that of non-manipulated zygotes fertilized in vivo and cultured in vitro.

Conclusion: The nucleus of a GV oocyte can develop normally following multiple transfer at the GV and zygote stages. This cellular model allows one to elucidate the developmental and functional competence of the female genome following transfer to oocytes at different maturational stages.

P-092

Development of a Sensitive Fluorescent Multiplex PCR for the Combined Detection of SMA Mutations and DNA Contamination in Single Cells. D. L. Blake, S. L. Tan, A. Ao. Department of Obstetrics and Gynecology, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada.

Objective: Spinal muscular atrophy (SMA) is the second most common autosomal recessive genetic disorder in Caucasian populations, affecting 1/10000 people. All three forms of SMA have been linked to mutations in the survival motor neuron (SMN) gene, where 95% of SMA patients lack the telomeric copy. Our primary objective is to develop preimplantation genetic diagnosis (PGD) for SMA using multiplex PCR. Co-amplification of informative polymorphic DNA markers will lower the rate of misdiagnosis caused by amplification of contaminating exogenous DNA.

Design: Development of multiplex fluorescence-PCR to detect the SMN telomeric deletion and chromosome 21 STRs, D21S226 and D21S11, in single cells.

Materials and Methods: Isolated normal blastomere, lymphocyte and SMA affected lymphocyte cells were subjected to PCR amplification to detect the SMN gene mutation and the polymorphic tetranucleotide repeats, D21S226 and D21S11. The centromeric and telomeric copies of SMN were distinguished by digesting exon 7 and 8 PCR products with either HinfI or DdeI restriction enzymes, respectively, prior to gel electrophoresis. All amplified PCR products were visualized on an ALFexpress fluorescence DNA sequence analyzer.

Results: From pooled data, the multiplex PCR amplification efficiencies were 93% for SMN's exon 7 and 75% for D21S11, in single cells. Accuracy rate of mutation detection was calculated to be 100%. In experiments where all four primer sets were used, the amplification efficiencies in normal cells were 81% for exon 7 and 60% for SMN's exon 8 and the two STRs.

Conclusion: Our results demonstrate that optimal amplification efficiencies are obtained when SMN exon 7 and D21S11 primers are used in combination in single cells, making this test sensitive enough to be used for future PGD of SMA. Furthermore, the data demonstrates that polymorphic STRs are ideal internal controls for detecting contamination in sensitive genetic tests.

P-093

Effects of Aging on Ovarian Fecundity in Terms of the Incidence of Apoptotic Granulosa Cells. H. Saito, S. H. Sadraie, T. Kaneko, T. Saito, K. Nakahara, M. Hiroi. Department of Obstetrics and Gynecology, Yamagata University, School of Medicine, Yamagata, Japan.

Objective: Apoptosis is the underlying mechanism of ovarian follicle atresia and the incidence of apoptotic granulosa cells is a very sensitive indicator to detect oocyte quality and consequently the fecundity, on per follicle and per patient bases. Aged women have been indicated to have lower fecundity in many studies which pointed out that the poor quality of oocytes is one of the major determinants. However, there is little information about the effects of age on ovarian fecundity assessed by apoptosis. The aim of the present study was to investigate the effects of aging on the incidence of apoptosis in granulosa cells of normo-ovulatory women in different age groups undergoing an IVF due to severe male factor.

Design: The incidence apoptosis in granulosa cells and the number of oocytes retrieved, the number of mature oocytes, endometrial thickness and the follicular fluid hormones were determined.

Materials and Methods: Twenty-eight normo-ovulatory women of different ages underwent ovulation induction for IVF due to severe male factors of their husbands. The women were divided into four groups according to their ages (A: ≤31, n= 7, B: 32–35, n= 6, C: 36–39, n= 8, D: ≥ 40 years, n= 7). The patients underwent a controlled ovarian hyperstimulation by using a GnRH analogue + hMG + hCG. The mural granulosa cell masses were picked out from the follicular fluids, and placed on a glass slide, and dispersed thoroughly by hyaluronidase, and fixed with neutral buffered formalin. After fixation, the slides were stained with Hoechst 33258 (fluorescent dye). The apoptotic cells were identified and counted among 1,000 granulosa cells at random at ×400 magnification. Each sample of FF was stored at −20 C until the assay of sex steroids hormones.

Results: The number of oocytes retrieved from the youngest groups (A), was significantly higher than those of groups B, C and D {9.8 ± 1.4 versus 6.0 ± 1.6 (p<0.05); 4.5 ± 0.8 (p<0.01) and 4.1 ± 0.9 (p<0.01) respectively, (mean ± SEM)}. The number of mature oocytes in group A was significantly higher than those of groups B, C and D {7.8 ± 1.1 versus 4.5 ± 1.3 (p<0.05); 3.7 ± 0.9 (p<0.01) and 3.1 ± 0.7 (p<0.01) respectively}. The incidence of apoptotic cells [per 1,000] in group A was significantly lower than those of groups C and D (3.5 ± 0.6 versus 6.1 ± 0.3 and 6.4 ± 0.5 respectively, P< 0.01). The incidence of apoptotic cells in group B was significantly lower than that of group D (4.7 ± 0.3 versus 6.4 ± 0.5, P< 0.05).

Conclusion: Age increases the process of follicular atresia through apoptotic changes in granulosa cells, and consequently decreases the ovarian fecundity.

P-094

The Experience of "Coasting in Calgary." J. O'Keane, J. Fleetham, A. Kenefick, D. Billay, S. Servis, S. Scott, C. Greene. Foothills Hospital Regional Fertility Programme, Calgary, Canada.

Objective: To evaluate the results from coasting (withdrawal of hMG treatment for 2 or more days prior to hCG) during IVF stimulation to prevent cancellation of the cycle because of the high risk of ovarian hyperstimulation syndrome (OHSS).

Design: Retrospective series from August, 1996 when coasting was initiated, to December, 1998. Estradiol (E_2) levels, days coasted, numbers of follicles, oocytes and pregnancy outcomes were analysed.

Materials and Methods: During this period, 93 patients who underwent routine late luteal phase GnRHa down-regulation and stimulation with hMG were coasted for 2 or more days due to E_2 levels $\geq$10,000 pmoles/L. HCG was given when E_2 levels fell to approximately 10,000 pmoles/L or less. All patients had embryo transfer.

Results: The mean patient age was 32.8. The mean E_2 levels at the start of coasting was 14,433 pmoles/L, the mean peak E_2 was 17,438, and the mean E_2 on the day of hCG was 9,412 pmoles/L. The average length of coasting was 2.5 days. A mean of 27.2 follicles were observed, yielding on average 23.8 oocytes. The fertilization rate was 74.5% and an average of 2.5 embryos were transferred. A mean of 10.5 embryos were frozen. The pregnancy rate as confirmed by ultrasound was 59/93 (63.4%), with 58/93 (62.4%) observed to have a fetal heart. There were 7/59 (11.9%) losses, resulting in 51/93 (54.8%) delivered or ongoing. The embryo implantation rate was 44.0% and the multiple pregnancy rate was 30/59 (50.8%) with 19/59 (32.2%) twins and 11/59 (18.6%) triplets or higher. Ten of the patients (10.8%) suffered OHSS severe enough to require a period of hospitalization. Nine of these patients were pregnant.

Conclusions: Coasting is a technique that allows further follicular growth and maturation of oocytes with a reduction of estradiol after discontinuation of gonadotropins. Despite excellent implantation and pregnancy rates, these patients are at risk for OHSS and multiple pregnancy. It is not known if this technique reduces the incidence of OHSS in this at risk population. It is now our policy to replace maximally two embryos in these stimulation cycles.

P-095

Evaluation of Meiotic Spindle Apparatus in Human Oocytes Following Cytoplasmic Donation. E. Jones, C. A. Boyd, D. Dowling-Lacey, J. Mayer, S. Muasher, S. E. Lanzendorf. The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA.

Objectives: To determine if the removal of cytoplasm from a metaphase II (MII) human oocyte for cytoplasmic transfer will have an impact on the meiotic spindle apparatus of the donating oocyte.

Design: MII donor oocytes were cryopreserved and thawed for use in the cytoplasmic transfer procedure. Following the removal of the cytoplasm, oocytes (test) were fixed and meiotic spindle and chromosomes evaluated. Oocytes which were thawed but not used for cytoplasmic transfer were also evaluated as controls.

Materials and Methods: Full IRB approval was obtained before the study was initiated. MII oocytes, obtained from egg donors, were cryopreserved in 1.5M propanediol added in a step-wise fashion. Prior to cytoplasmic transfer, oocytes were thawed and incubated for 3 to 4 hrs. Cytoplasm was aspirated the full length of the ICSI pipet that was visible with the 20X objective. The aspirated cytoplasm, along with a sperm, was then placed into the egg(s) of the recipient (patient). Following cytoplasmic removal, test oocytes as well as controls, were fixed using a microtubule-stabilizing buffer. To visualize the meiotic spindle, tubulin was localized using anti-tubulin monoclonal antibody. Chromosomes were identified by counterstaining with 4'6'-diaminodino-2-phenylindole. Oocytes were evaluated and photographed using a Nikon epifluorescent microscope.

Results: Forty-seven oocytes were thawed with 28 (59.6%) surviving. Following the cytoplasmic transfer procedure, seven of the donor oocytes were available for fixation and staining. Six (86%) contained a normal meiotic spindle, while one was found to have no alignment of the meiotic spindle and chromosomes. Four oocytes served as controls and, following staining, all 4 (100%) contained a normal spindle.

Conclusions: An abnormal spindle apparatus was observed in an oocyte which had been used for cytoplasmic donation. Because of the small sample size, it is yet too early to determine if the procedure may have an adverse effect on the oocytes donating cytoplasm. Should there be no effect, these oocytes could be used for insemination. Evaluation of these oocytes may also allow us to determine the risk of transferring chromosomal material to the recipient oocytes. Data will continue to be collected as more patients receive the cytoplasmic transfer procedure. This work was supported in part by a grant from Serono Laboratories.

P-096

Evaluation of the Effect of Hydrosalpingeal Fluids on Mouse Blastocyst Development in Relation to Cytokines Levels. [1]E. Puigdomenech, [1]R. Inza, [1]M. Tiveron, [1]A. Piazza, [1]E. Young, [1,2]R. I. Barañao. [1]IFER (Instituto de Ginecología y Fertilidad, Bs.As., Argentina), [2]IByME (Instituto de Biología y Medicina Experimental-CONICET, Bs.As., Argentina).

Objective: To evaluate if hydrosalpingeal fluid (HF) is embryotoxic or produces an adverse microenvironment for the embryo development. We assessed the effect of HF on mouse embryos and its relationship with Th1 and Th2 cytokine concentration.

Design: A prospective study in a cohort of infertile patients with tubal infertility.

Materials and Methods: HF was obtained simultaneously with oocyte retrieval. HF was used to evaluate its effect on mouse blastocyst development and to measure IL-2, IFNγ, IL-4 and IL-10 concentrations. HF was collected from 9 tubes in 7 patients (mean age 36.3 years, range 33–40). Individual HF aliquots were centrifuged and maintained at -196°C until their use. An average of 15 mouse embryos (Balb/c) at the stage of 2 cells were cultured in each assay in HTF medium supplemented with 0% (control), 30% and 100% of HF. Embryo cultures were observed daily until day 5 to verify blastocyst development. All the cytokines were assessed employing ELISA commercial kits (R&D). Ten samples of peritoneal fluid (PF) from patients with neither hydrosalpinx nor endometriosis were used as controls for cytokine determinations.

Results: At a 30% concentration 4/9 samples of HF produced an stimulatory effect, 4/9 produced inhibitory effect and 1/9 did not alter blastocyst development, in relation to the control media. However, all culture with pure concentration of HF produced a significant inhibition of embryo development. We did not find differences in Th1 cytokine levels between HF and PF. Values obtained were: IL-2: 28.2 $\pm$ 5.5 pg/ml in HF vs. 27.5 $\pm$ 4.2 pg/ml in PF (NS) and IFNγ: 42.0 $\pm$ 14.1 pg/ml in HF and 50.0 $\pm$ 19.7 pg/ml in PF (NS). With respect to Th2 cytokines levels we observed that none of PF had detectable levels of IL-4 neither IL-10 while we found IL-4 in 5/7 samples of HF (41.8 $\pm$ 9.0 pg/ml) and IL-10 in 3/7 samples of HF (8.1 $\pm$ 3.2 pg/ml).

Conclusions: We observed that at a concentration of 30%, some of HF did not affect embryo development while others produced an inhibitory effect. At 100% all HF produced inhibition of blastocyst development. This inhibitory effect is not attributable to the presence of IFNγ. In addition, no relationship with Th2 cytokines levels and blastocyst development was observed. We can not discard that the inhibitory effect of HF could be due to osmolarity, pH or another type of biochemical alterations.

P-097

Survival, Maturation and Chromosomal Normality of Human Oocytes Retrieved from Unstimulated Ovaries Following Vitrification at the Germinal Vesicle (GV) Stage. H. M. Chung, J. M. Lim, S. Y. Han, J. J. Ko, C. J. Chung, K. Y. Cha. Infertility Medical Center of CHA General Hospital and College of Medicine, Pochon CHA University.

Objective: Successful cryopreservation of human oocytes is essential for establishing an effective ovum bank program. Recent studies informed that chromosome and spindle abnormalities and developmental retardation were often found in cryopreserved oocytes. The purposes of this study was to evaluate whether immature human oocytes could maintain viability and normal chromosomal configuration after vitrified and thawed by our standard protocol (Hong et al., 1999).

Design: Survival (morphological normality), and maturation (extrusion of first polar body) and chromosome configuration of oocytes vitrified at the GV stage were evaluated at immediately after thawing and 48 hours after maturation culture, respectively.

Materials and Methods: Oocytes were retrieved from consented women with unstimulated cycle by direct follicular aspiration during c/s. Only GV stage oocytes with compacted cumulus cells were selected and divided into two groups. In Group 1 (n=9), oocytes were cultured in maturation medium (TCM-199 with PMSG, hCG and FBS) for 48 hour. In Group 2 (n=24), oocytes were vitrified in PBS containing 5.5 M ethylene glycol and 1.0 M sucrose and, after thawed by a 4-step method, the oocytes were cultured in maturation medium for 48 hours.

Results: Fifty-eight % (14/24) of GV stage oocytes were survived after vitrification. There was no significant difference in maturation rate between fresh (7/9=78%) and vitrified (9/14=64%) oocytes. Total 11 oocytes were karyotyped, but no chromosomal abnormality was found in vitrified oocytes.

Table: Survival and maturation rates of vitrified GV stage oocytes and their karyotypes.

Groups	No. oocytes examined	Rates (%) of Survival	Rates (%) of Maturation
Fresh	9	9 (100)	7 (78)
Vitrified	24	14 (58)	9 (64)

Groups	No. (%) oocytes Karyotyped	Haploidy (normal)	Aneuploidy	Polyploidy
Fresh	6	4 (67)	1 (17)	1 (17)
Vitrified	5	5 (100)	0 (0)	0 (0)

Conclusions: The results of this study indicated that vitrification of oocytes at the GV stage might not induce chromosomal abnormalities. Other reasons except for chromosome abnormality might be more responsible for post-thaw developmental retardation of vitrified oocytes.

P-098

Differential Gene Expression of Cumulus Cells and Their Encompassing Oocytes. H. C. Liu, Z. Y. He, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, New York, NY.

Objectives: During folliculogenesis, oocytes and somatic cells require complex intracellular communication to reach its full competence. Factors such as connexin 37 (critical gap junction protein between granulosa cells and oocytes), growth differentiation factor-9 (GDF-9) (a member of the TGF-β family), $P34^{CDC2}$ kinase and Cyclin B1 (dimer of maturation promoting factor), luteinizing hormone receptor (LHR), and heat shock protein-70 (HSP-70) (one of the first proteins produced during embryogenesis) are involved in controlling follicular maturation and communication between somatic cells and oocytes. In this study, we analyzed the differential expression of these studied genes in cumulus cells and their encompassing oocytes in order to understand their origin and communication patterns.

Design: Cumulus cells and oocytes separated from cumulus-oocyte-complexes (COCs) were used to detect the studied genes by semiquantitative RT-PCR.

Materials and Methods: Preantral follicles isolated from Day 14 B6D2-F1 mice were matured by culturing in microdroplets (20 μl) containing 5% fetal calf serum (FCS) and recombinant gonadotropins in vitro for 12 days. HCG (100 IU/ml) and EGF (100 μg/ml) were administered to induce final maturation and ovulation on day 12. Gene expression of cumulus cells and oocytes, separated from ovulated COCs, were evaluated by RT-PCR.

Results: The frequencies of gene expression (+/n) are shown in the following table:

	Connex. 37 (+/n)	GDF-9 (+/n)	$P34^{CDC2}$ (+/n)	Cyclin B1 (+/n)	LHR (+/n)	HSP-70 (+/n)
GVBD oocyte	8/8	0/8	8/8	7/8	0/8	3/8
Cumulus cells	5/5	3/5	5/5	6/6	5/5	4/4

Conclusion: There are distinct gene expression patterns in cumulus cells and their encompassing oocytes. Almost all of the studied genes were expressed in cumulus cells (100%) except GDF-9 (60%). GDF-9 and LHR were not detected in oocytes whereas cyclin B1 and HSP-70 were only detected in some (87.5% for cyclin B1 and 37.5% for HSP-70) of the oocytes. Whether the differential patterns are related to the maturation and quality of oocytes requires further study. Interestingly, the intensity of amplified amplicons of connexin 37 after ethidium bromide staining was significantly higher in oocytes than in cumulus cells. This may indicate that oocytes were the major origin of connexin 37 gap junction protein. On the other hand, the ratio of $P34^{CDC2}$/cyclin B in cumulus cells was significantly higher than in oocytes. The reduction of $P34^{CDC2}$ kinase mRNA in germinal vesicle breakdown (GVBD) oocytes may hint that $P34^{CDC2}$ was transported from cumulus cells.

P-099

Prediction of Embryo Implantation Potential After ICSI by a Simple Evaluation of Pronuclear Morphology. J. Tesarik, A. M. Junca, A. Hazout, F. X. Aubriot, C. Nathan, P. Cohen-Bacrie, M. Dumont-Hassan. Laboratoire d'Eylau, 55 rue Saint-Didier, 75116 Paris, France.

Objective: We previously defined criteria that can be used to predict the quality of the early preimplantation development of human embryos by a single static observation on pronuclear-stage zygotes (in press). Here we extend those observations by application of these criteria to the study of embryo implantation potential.

Design: The implantation potential of embryos developing from zygotes with different patterns of pronuclear morphology is evaluated by analyzing the pregnancy (PR) and implantation (IR) rates in 115 intracytoplasmic sperm injection (ICSI) treatment cycles in which embryos developing from zygotes with different pronuclear patterns were transferred.

Materials and Methods: Zygotes at the pronuclear stage were inspected once, 14–17 h after ICSI. The number and spatial distribution of nucleolar precursor bodies (NPBs) in each pronucleus were noted. However, these observations were not taken into account for the selection of embryos for transfer. Instead transferrable embryos were selected by using the conventional criteria of cleavage speed and morphology. PR and IR were calculated for cycles resulting in a clinical pregnancy.

Results: PR and IR in the whole study group were 31% and 16%, respectively. According to our previous observations, embryos showing the best preimplantation development result from zygotes with the following characteristics. (1) The difference in the number of NPBs between both pronuclei does not exceed 3. (2) The distribution of NPBs (random versus polarized) is similar in both pronuclei. (3) There are at least 3 NPBs in each pronucleus. Following embryo selection for transfer, based on the conventional criteria, only embryos developing from zygotes conforming to these characteristics were transferred in 32 treatment cycles (group A), whereas only embryos resulting from non-conforming zygotes were transferred in 18 cycles (group B). In group A, both PR (41%) and IR (21%) were higher than in group B (11% and 8%, respectively). This difference was significant ($P < 0.05$). Cleavage speed and morphology of the transferred embryos was similar in both groups.

Conclusion: Although some patterns of pronuclear morphology are more frequently associated with poor preimplantation development than others, any pattern can give rise to embryos that would be classified as "high-grade" according to the conventional evaluation of cleaving embryo quality and would thus be likely to be selected for transfer. Pronuclear morphology is thus an important auxiliary criterion for the selection of embryos for transfer and can also be used as the only criterion in programs in which this decision is taken as early as the pronuclear stage.

P-100

Role of Lipid Peroxidation and Total Antioxidant Capacity in Follicular Fluid and Pregnancy Outcome of Women Undergoing Assisted

Reproduction. [1,3]E. B. Pasqualotto, [4]N. J. Joshi, [1,2,3]R. K. Sharma, [4]B. I. Rose, [4]N. B. Maher, [1,2]A. Agarwal. [1]Center for Advanced Research in Human Reproduction and Infertility, Departments of [2]Urology and [3]Gynecology-Obstetrics, The Cleveland Clinic Foundation, Cleveland, OH; [4]Infertility Solutions, Allentown, PA.

Objectives: Assisted reproductive procedures involve ovarian hyperstimulation to retrieve larger number of oocytes. Healthy metabolically active oocytes may result in healthy embryos and successful pregnancies. Our objective was to evaluate the relationship between the lipid peroxidation (LPO) and total antioxidant capacity (TAC) in the follicular fluid (FF) of women undergoing assisted reproduction with fertilization rate, embryo quality, and pregnancy outcome.

Design: Prospective study in which follicular fluid LPO and TAC levels were compared between patients who became pregnant with those who did not.

Material and Methods: Following ovarian stimulation, macroscopically clear FF specimens were obtained at the time of oocyte retrieval from 36 patients undergoing assisted reproductive procedure. Eleven patients underwent intracytoplasmic sperm injection and the remaining 25 underwent conventional *in vitro* fertilization. Levels of lipid peroxidation in the FF were determined by malonaldehyde assay and reported as μmol MDA/mL. Total antioxidant capacity was measured by an enhanced chemiluminescence reaction and expressed as molar Trolox equivalents. The embryo quality index was determined using a ratio of cumulative embryo score and the total number of embryos in culture.

Results: Mean female age was significantly lower in patients who became pregnant (30.09 years ± 0.83) than in patients who did not (34.16 years ± 0.89) ($P = 0.01$). The mean number of oocytes recovered (13.91 ± 1.32 vs 11.96 ± 1.05) and percentage of oocytes fertilized (77.17 ± 8.82 vs 70.35 ± 4.78) were comparable in both groups of patients. After adjusting for age, non-pregnant patients demonstrated depressed LPO levels in the FF compared to patients who achieved pregnancy (0.80 μmol/mL ± 0.12 vs 1.48 μmol/mL ± 0.17, $P = 0.02$). There was no difference in the TAC levels between non-pregnant patients and those who achieved pregnancy (816.09 ± 105 vs 933.67 ± 101.49, $P = 0.67$). The embryo quality was significantly higher in pregnant than in non-pregnant patients (33.45 ± 1.67 27.51 ± 1.41, $P = 0.009$). LPO and TAC levels in FF showed no correlation with the embryo quality or fertilization rates.

Conclusion: High levels of LPO in the follicular fluid of pregnant women and a positive correlation of LPO with pregnancy may reflect more intense oxidative metabolism of healthy developing follicles which may be responsible for successful pregnancy. This finding may reflect a functional buffering system for oocyte metabolic activity. The lack of association between embryo quality and LPO and TAC levels in the FF found in our study could be due to the fact that the embryo is an interaction of sperm and oocyte therefore the LPO and TAC levels in sperm may play a critical role in the embryo quality.

P-101

Oxidative Stress in Normospermic Men Undergoing Infertility Evaluation. F. F. Pasqualotto, R. K. Sharma, H. Kobayashi, [1]D. R. Nelson, A. J. Thomas, Jr., A. Agarwal. Center for Advanced Research in Human Reproduction & Infertility, Departments of Urology and [1]Biostatistics & Epidemiology, The Cleveland Clinic Foundation, Cleveland, OH.

Objectives: Chronic exposure to elevated levels of reactive oxygen species (ROS) in semen can result in oxidative stress and male infertility. The role of oxidative stress in normospermic men with infertility is unclear. We measured the levels of ROS, total antioxidant capacity (TAC), and a composite ROS-TAC score in an attempt to examine the ability of these variables to detect oxidative stress in normospermic men attending our infertility clinic.

Design: Prospective study.

Material and Methods: Semen specimens from 299 subfertile men were examined between 1997 to 1998, according to the World Health Organization criteria. Of these, 34 patients were considered as normospermic. Volunteers with normal semen characteristics served as controls (n = 19). Patients were divided into three groups: group I, varicocele and no female factor (n = 12); group II, only female factor (n = 16); group III, idiopathic infertility (n = 6). ROS production in the semen specimens was measured by the chemiluminescence assay and the results were expressed as Log (ROS + 1) × 10^6 counted photons/minute/20 × 10^6 sperm. Total antioxidant capacity was measured in the seminal plasma by an enhanced chemiluminescence assay and results were expressed as molar Trolox equivalent. A composite ROS-TAC score was generated to examine the oxidative stress. An ROS-TAC score lower than 45 were considered abnormal.

Results: The higher levels of ROS were seen in group III (2.33 ± 0.3), followed by group II (1.62 ± 0.2), and group I (1.51 ± 0.2), compared to controls (1.3 ± 0.2) ($P = 0.03$). Compared to controls (1653.98 ± 105.03), lower TAC levels were seen in groups I (877.73 ± 144.35), followed by group II (951.87 ± 111.81), and group III (1033.06 ± 176.8) ($P < 0.001$). Similarly, compared to controls (50.0 ± 3.0), lower ROS-TAC score were seen in group III (29.1 ± 4.7), followed by group II (36.0 ± 3.3), and group I (37.7 ± 4.4) ($P = 0.002$).

Conclusions: The etiology of infertility in some couples with pure female factor (based on normospermic semen characteristics of the male partner), or idiopathic infertility may involve oxidative stress as it's underlying cause. Normospermic men who are evaluated for infertility should also be tested for the ROS and TAC levels in semen as oxidative stress may play an important role in their infertility. Antioxidant supplementation can be a potential treatment strategy in these men. This work was supported by a research grant from the Cleveland Clinic Foundation.

P-102

Cytogenetic Analysis of Epididymal and Testicular Spermatozoa in Azoospermic Patients Considered for ICSI. J. Hariprashad, P. N. Schlegel, M. C. Tsai, T. Takeuchi, Z. Rosenwaks, G. D. Palermo. The Center for Reproductive Medicine and Infertility-New York Presbyterian-Weill Medical College of Cornell University, New York, NY, USA.

Objective: Although ICSI provides a way to treat the vast majority of azoospermic men, this approach has raised concern about the potential risk for transmission of genetic abnormalities to the offspring. In this study we attempted to quantify the incidence of chromosomal abnormalities in epididymal and testicular spermatozoa retrieved from azoospermic patients undergoing the ICSI procedure.

Design: Epididymal and testicular spermatozoa, retrieved from obstructive and non-obstructive azoospermic patients respectively, were analyzed cytogenetically and the results were compared with ejaculated spermatozoa.

Materials and Methods: In patients with non-obstructive azoospermia (age: 43.3 ± 11 yrs), spermatozoa were mechanically microdissected from testicular biopsies. These spermatozoa were individually collected with an ICSI pipette and transferred onto a microslide. Epididymal spermatozoa retrieved from patients (age: 34.8 ± 3 yrs) undergoing microsurgical epididymal sperm aspiration were subject to single layer density gradient centrifugation. Ejaculated spermatozoa from proven fertile donors (age: 38.3 ± 9 yrs) were selected by multilayer density gradient centrifugation. After proper fixation and decondensation, spermatozoa were processed by fluorescent in situ hybridization (FISH) for chromosomes 18, 21, X and Y. The incidence of disomy, nullisomy and diploidy was recorded, with only unequivocal fluorocrome signals related to each chromosome being identified and scored for each experimental group.

Results: Approximately 200 testicular spermatozoa and 5,115 epididymal spermatozoa were evaluated. In the control (ejaculate) group 12,132 spermatozoa were scored. The low number of (testicular) spermatozoa analyzed in the non-obstructive group was due to the extremely limited number of cells recoverable from these patients. The chromosomal abnormality rate in the non-obstructive azoospermia group was 13.6% and 1.5% in the obstructive group. In the ejaculated group, 0.7% of the spermatozoa had a chromosomal abnormality. Thus, the incidence of chromosomal abnormalities in the obstructive and non-obstructive azoospermia groups was significantly higher ($P = 0.0001$) than in the control group. Overall, autosomal and gonosomal disomies were the most recurrent abnormality.

Conclusion: Although relatively fewer testicular cells were evaluated, these findings point to a higher incidence of sperm chromosomal abnormalities in spermatozoa of obstructive as well as non-obstructive men. This suggests that where sperm from obstructive and non-obstructive men are used, these patients should receive appropriate counseling as well as adequate screening of the resulting pregnancies.

P-103

Development of Human Oocytes After Nuclear Transplantation. T. Takeuchi, M. C. Tsai, J. Gong, L. L. Veeck, Z. Rosenwaks, G. D. Palermo. The Center for Reproductive Medicine and Infertility, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY.

Objective: To assess their fertilizability and potential for cleavage, human oocytes reconstituted after nuclear transplantation were inseminated by ICSI.

Design: Cell survival, fertilization patterns, and embryonic characteristics of oocytes inseminated by ICSI were measured.

Material and Methods: Human GV stage oocytes were obtained from consenting patients undergoing the ICSI procedure (IRB No. 0198-082). After exposure to cytochalasin B, the GV nucleus was removed with a micropipette and reinserted into the perivitelline space of a previously enucleated immature oocyte. Each grafted oocyte was then subjected to electrofusion. Such reconstituted oocytes were incubated for up to 48 hours to allow extrusion of the first polar body, those that reached metaphase II were inseminated by ICSI. After 16–18 hour incubation, the number and characteristics of pronuclei and polar bodies were recorded. Embryo cleavage and quality was evaluated up to day 3 by assessment of the number of blastomeres and quantifying the amount of anucleated fragments. Some embryos that were not fixed for cytogenetic analysis were allowed to grow in culture up to day 5.

Results: A GV nucleus was successfully removed in 93.8% of 64 oocytes. Isolated GVs were successfully transferred beneath the zona pellucida of 57 cytoplasts (95.0%), nucleo-cytoplasmic reconstitution was successful in 84.2% of the oocytes, and a total of 30 oocytes extruded the first polar body (62.5%). After a majority were inseminated by ICSI, their survival and fertilization characteristics were assessed (see table).

Number of (%)	
Oocytes injected	29
Oocytes survived	22 (75.9)
Oocytes with 2PN	14 (63.6)
1PN	1 (4.5)
≥3PN	1 (4.5)

The cleavage rate for the normally fertilized zygotes was 92.8% (13/14). The mean blastomere number and frequency of anucleate fragments on day 3 was 4.8 ± 2 (M ± SD) and 21.1 ± 12 (M% ± SD), respectively. Only two embryos were kept in culture up to day 5, while one arrested at 8 cells the other reached the morula stage.

Conclusion: These preliminary results indicate that human oocytes transplanted with GV nuclei will extrude the first polar body, and can be successfully inseminated and undergo early embryonic cleavage. Although their survival rate and embryo quality appeared poorer, their fertilization patterns were no different from those observed with *in vivo* matured oocytes.

P-104

Larger Trinucleotide Repeat Size in the Androgen Receptor Gene of Infertile Males with Severely Disturbed Spermatogenesis. [1]P. Patrizio, [2]A. O. Trounson, [3]K.-L. Chen, [4]S. Hernandez-Ayup, [3]D. G. B. Leonard. [1]Center for Reproductive Medicine & Surgery, University of Pennsylvania, Philadelphia, PA, [2]Institute Reproduction & Development, Monash University, Melbourne, Australia, [3]Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, [4]Instituto Estudio Concepcion Humana, Monterrey, Mexico.

Objective: Androgens are significant regulators of human spermatogenesis. Their action is mediated through the androgen receptor (AR) which binds to the AR element on DNA and regulates gene transcription. Males with spinobulbar muscular atrophy (Kennedy's disease) caused by a trinucleotide repeat expansion, ≥40 CAG repeat, in the AR gene located on X chromosome, are infertile. We speculated that the variable size of this trinucleotide repeat could interfere with AR function and result in impaired spermatogenesis.

Design: Prospective correlation between AR trinucleotide repeat size and semen analysis.

Material and Methods: A total of 45 predominantly Caucasian infertile males were studied. Clinical and laboratory analysis showed idiopathic, non obstructive azoospermia in 6, extremely severe oligozoospermia in 19 (few sperm up to 1 mill/mL) and severe oligozoospermia in 20 (>1 to <5 mill/mL). Fertile control males (n=45) were selected by documented paternity proven by linkage analysis. Leukocyte DNA was analyzed by PCR amplification across the androgen receptor repeat region. Accurate size determination of the PCR product using an ABI 373 DNA sequencer allowed precise calculation of CAG repeat sizes. The AR gene was not analyzed for other types of mutations.

Results: Patients with extremely severe oligozoospermia had significantly longer CAG repeat tracts (mean 24.6 ± 3.2, $P=0.01$, range 20–35) than controls (mean 22 ± 2.8, range 12–30). There was no statistical difference between patients with azoospermia (mean 23 ± 2.2, range 20–25) or severe oligozoospermia (mean 21.9 ± 2.3, range 18–26) and controls. None of the infertile males with azoospermia or with sperm counts <1 mill/mL had ≤19 CAG repeats compared to 6/45 controls (13%, $P=0.06$).

Conclusions: This study suggests that some men with severe impairment of spermatogenesis have longer trinucleotide repeats in the AR gene. Lower affinity between androgen and the AR protein or decrease in AR protein availability with longer repeats could be responsible for a diminished androgen effect on spermatogenesis. One of the patients in the extremely severe oligozoospermia group had 35 CAG repeats (normal range is 11 to 33). Although not yet considered a mutation, longer trinucleotide repeats are unstable and may expand between generations. Therefore, using ICSI, it is possible that in two generations, the son of an ICSI daughter could be affected not only by infertility but also by a neurodegenerative disease (Kennedy's disease).

P-105

Simultaneous Analysis of Individual Human Blastomere for Gene Transcription by RT-PCR and Chromosome Abnormalities by FISH. Y.-X. Tang, L. C. Krey, J. A. Grifo. Program for In Vitro Fertilization, Reproductive Surgery and Infertility, New York University School of Medicine, New York, NY 10016.

Objective: To develop a novel diagnostic method to analyze simultaneously both cytoplasmic gene transcripts and nuclear chromosome number in individual human blastomeres.

Design: Blastomeres from human embryos were individually separated into cytoplasmic lysate and intact nucleus fractions. Using the housekeeping gene B-actin as an internal control, transcripts of a tissue specific gene α-globin in the cytoplasmic lysate could be determined by single-cell sensitive reverse transcription-polymerase chain reaction (RT-PCR). Concurrently, the nucleus was fixed to detect aneuploidies of chromosomes X,Y,18 by triple color fluorescent in-situ hybridization (FISH).

Materials and Methods: Blastomeres were isolated from discarded day 4 human embryos (3–12) cells. After removing the zona pellucida with acidified Tyrode's solution, the blastomeres were dispersed and individually incubated in a cellular membrane lysis buffer drop for a few seconds. The nucleus was identified under phase contrast microscopy, transferred to a slide and then fixed with methanol:acetic acid (3:1). FISH was performed using X. Y and 18 CEP direct-labeled probes (Vysis) for 30 minutes in HYBrite. The residual buffer drop containing the cytoplasmic lysate was then transferred to a PCR tube. Single-cell-sensitive RT-PCR was performed to monitor expression of the α-globin and B-actin genes.

Results: A total of 80 blastomeres from 13 embryos were analyzed. Following RT-PCR, each blastomere (100%) gave a positive band for B-actin and 93% a band for α-globin. In the FISH study, 96% of the nuclei were successful fixed and 95% showed signals. Eight embryos were X,Y/18,18 or X,X/18,18. The other five were aneuploid (n=3) or mosaic (n=2) for these chromosomes.

Conclusion: A sensitive and efficient technique is described to analyze chromosome number and gene expression in the nucleus and cytoplasm of individual human blastomeres. This method may contribute significantly to preimplantation genetic diagnosis since a single blastomere can generate information about different chromosomes and genes. Combining RT-PCR and FISH also has the potential to identify relationships between chromosome abnormalities and gene expression.

IMAGING IN REPRODUCTIVE MEDICINE

Monday, September 27, 1999

P-106

Magnetic Resonance Image Attributes of Ovarian Follicles at Specific Phases of Development and Regression: I. The Follicle Antrum. J. L. Hilton, G. E. Sarty, G. P. Adams, R. A. Pierson. WHIRL, Obstetrics-Gynecology, University of Saskatchewan, Saskatoon, SK, Canada.

Objective: To determine whether MR image attributes of the follicular antrum would reflect the physiologic status of dominant and subordinate ovarian follicles.

Design: Mean numerical pixel values (NPV), relaxation rates, proton densities and apparent diffusion coefficients of the dominant and largest subordinate follicular antra at precise phases of development and regression were determined *in vitro* from MR images and maps.

Materials and Methods: The ovaries of sexually mature cows were removed on either day 3 of wave 1 (D3W1, n=10), day 6 of wave 1 (D6W1, n=9), day 1 of wave 2 (D1W2, n=9) or on at least day 17 (D≥17, n=8; pre-ovulatory) of the estrous cycle. Images were acquired using a 1.5 Tesla SP Magnetom MR imager with sequences weighted by proton density (PD) and T_1(TR/TE=480, 1000, 2000, 4000/15), 16 echo T_2, as well as weighted and unweighted diffusion sequences (b=0 and 17776 s/cm^2, in the z-direction). T_1, T_2, PD and apparent diffusion coefficient (ADC) maps were computed. The NPV and standard deviation (pixel heterogeneity, PH) of a spot comprising 90% of the follicular antrum were quantified in the weighted images (T_1W and T_2W). Mean nuclear relaxation rates, PD, ADC and their heterogeneities were determined from the maps. Statistical analyses were performed using ANOVA with protected LSD.

Results: Anovulatory and ovulatory dominant follicles of the same size were differentiated by T_1 relaxation rates calculated from the maps. Ovulatory dominant follicles (D≥17) exhibited higher T_1 relaxation rates than anovulatory dominant follicles (D6W1) (2739 ± 350 vs 2139 ± 195 ms; $P<0.05$). T_1 PH and T_2 NPV and PH of the anovulatory dominant follicle were higher when regression started (D1W2) than as healthy growing follicles at D3W1 and D6W1 ($P<0.002$). T_2 heterogeneity, determined from maps, of anovulatory dominant follicular antra was lower at D1W2 than at D3W1 (15.1 ± 1.2 vs 20.2 ± 1.2 ms; $P<0.01$). The regressing anovulatory dominant follicle antrum (D≥17) had a higher T_2 relaxation rate than growing and static phase anovulatory follicles (595 ± 138 vs 460 ± 14 and 425 ± 23 ms respectively; $P<0.07$). The T_1 relaxation rate, T_1 heterogeneity, PD, and the PD heterogeneity of the subordinate follicle in the presence of a preovulatory dominant follicle (D≥17) were higher than anovulatory wave subordinate follicles (D6W1) (2540 ± 220 ms, 160 ± 34 ms, 5250 ± 130, and 140 ± 22 vs 1880 ± 140 ms, 80 ± 13 ms, 4900 ± 69, and 80 ± 13 respectively; $P<0.04$). T_1W NPV of D≥17 subordinate follicles were lower than those of anovulatory wave subordinate follicles (D3W1; $P<0.03$). Subordinate follicles showed higher T_2W NPV at D3W1 and D6W1 than dominant follicles at those phases ($P<0.05$). D6W1 subordinate follicles had lower ADC than dominant follicles at D6W1 ($P<0.002$).

Conclusion: The physiological status (viability or atresia) of ovarian follicles was quantitatively reflected in MR image attributes. Supported by the Medical Research Council of Canada.

P-107

Intrauterine Cavity Sonographic Findings After First Trimester Abortion. I. Bar-Hava, I. S. Aschkenazi, R. Orvieto, J. Shalev, R. Bardin, D. Dicker, A. Dekel, I. Meizner, Z. Ben-Rafael. Department of Obstetrics and Gynecology, Rabin Medical Center, Golda Campus, Petah Tiqva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Introduction: Only little information is available regarding the sonographic characteristics of the normal uterine cavity following a first-trimester abortion. The purpose of this study was to define the acceptable range of sonographic patterns following a first-trimester abortion so that unnecessary invasive reinterventions (due to a presumed diagnosis of retained products of conception) may be avoided.

Design: Prospective clinical study.

Materials and Methods: Women who underwent uterine evacuation due to first-trimester abortion were referred for endovaginal sonographic examination within the week following the procedure. Special attention was directed to characterize the intrauterine cavity. Demographic and clinical parameters were collected. A repeated postmenstrual examination was carried out in selected cases.

Results: In all, 74 ultrasound examinations were performed, 57 after termination of pregnancy, 10 after incomplete abortion and 7 after missed abortion. Fifty-seven examinations (77%) demonstrated a considerable amount of intrauterine content with various echogenicities (anterior-posterior thickness range of 7 to 25 mm). No association could be documented between the pattern of appearance and gravity, parity, gestational age and type of abortion procedure. All postmenstrual reevaluations of the patients with excessive amounts of intrauterine material on the initial examination (n=7) demonstrated an empty intrauterine cavity.

Conclusion: Within the week following first-trimester abortion, the uterine cavity is seldom empty. This usually does not indicate clinically significant retained products of conception. By being familiar with this normal range of appearances, clinicians can avoid unnecessary invasive reevacuation procedures.

P-108

Women with PCOS Undergoing IVF-ET Demonstrate a Higher Incidence of Endometrial Echo Patterns That are Associated with Lower Implantation Rates. M. Abaé, H. Goulding, W. K. Firisin, M. H. Majercik. Center for Advanced Reproductive Endocrinology, Plantation, FL.

Objective: Comparative study of the uterine endometrial echo patterns on the day of hCG for IVF-ET in women with PCOS and those without.

Design: Retrospective analysis.

Patients: 37 women without PCOS (mean age 33.0 years, range 25–39) and 22 women with PCOS (mean age 31.4 years, range 25–37) undergoing fifty nine IVF-ET cycles.

Main Outcome Measure: Trilaminar endometrial echogenicity vs. non-trilaminar patterns and their associated implantation rates.

Materials and Methods: Patients underwent COH with a GnRH agonist and FSH and/or hMG. Endometrial pattern was assessed on the day of hCG administration. All transvaginal scans were performed and patterns evaluated by a single operator. Full thickness and sagital plane echogenic patterns were grouped into either (1) clear trilaminar echo, or (2) non-trilaminar echo patterns.

Results: On the day of hCG administration, the endometrial trilaminar pattern was observed in 63% of patients with PCOS and 86% of those without. The implantation rates for patients with this endometrial pattern were similar in both groups, 14% and 17%, respectively. The non-trilaminar endometrial echo pattern was present in 37% of the patients with PCOS and 14% in those without PCOS ($P=0.08$). The implantation rates here were similar as well, 8% and 6%, respectively, and were lower than when the trilaminar patterns were present.

Conclusions: PCOS was associated with a higher incidence of non-trilaminar endometrial patterns that lead to lower implantation rates.

P-109

Comparison Between Singleton and Multiple Gestations Using Three Dimensional Volumetrically Determined Nomograms. [1]T. Mukherjee, [2]A. Babinszki, [2]T. Nyari, MD, [1]S. Jordan, [1]A. Nasseri, [1]A. B. Copperman. [1]Division of Reproductive Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, Mount Sinai School of Medicine, New York, USA and [2]Department of Medical Informatics, Albert Szent-Gyorgyi Medical University, Szeged, Hungary.

Objectives: It has been current practice to apply normative data derived from singleton pregnancies to determine the normalcy of a multiple gestation. In fact, there have been few attempts made to prospectively evaluate a cohort of patients as such using first trimester transvaginal sonography. Three dimensional ultrasonography improves the ability of the sonographer to assess certain aspects of the early pregnancy. It is possible that through the establishment of normative data, early embryonic demise and other adverse outcomes can be predicted by analyzing gestational sac(GS) and yolk sac (YS) volumes during the first trimester.

Design: Prospective study.

Materials and Methods: Volume calculations of CRL, YS, and GS were carried out in 98 cases of normal singleton pregnancies and the volumes were plotted against GA to create nomograms. 22 examinations were performed in twins with normal outcome. 9 examinations were performed on triplets. Every volume measurement was repeated 3 times using 3 different planes, and the means of these values were calculated. GS and YS volumes of multiple gestations were fitted in the nomograms of singletons to evaluate whether those nomograms can be applied for twin pregnancies.

Results: Nomograms of gestational sac volume and yolks sac volume are not significantly different from those of twin gestations and can be used to reliably evaluate twin pregnancies (GS: $P>0.05$, YS: $P>0.05$. Examination of triplet pregnancies was carried out in 9 cases. There was a trend towards significance in triplet GS volumes ($P=0.07$) with GS volumes of greater than that of the singletons. This suggests that singleton GS cannot be used as the basis of evaluation in case of triplets. Nevertheless, the majority of YS volumes of the triplets were found inside the normal range of the singleton YS nomogram. GS volumes of abnormal twin pregnancies were found to be significantly smaller than that of normal ones ($P<0.05$), while no significant difference could be observed in case of YS volumes. No abnormal triplet pregnancies were found during the observation period.

Conclusions: The nomograms of normal singleton pregnancies can be used to evaluate twins with normal and abnormal outcome. The finding of increased GS in triplet pregnancies may be artifactual and due to the small number of observations. GS volumes predicted abnormal outcome in twin pregnancies, while no significant difference was found when YS volumes of twins with adverse outcome as compared to normal gestations. Using 3D ultrasonography, we have demonstrated that there is no difference in volumetric measurements between singleton and twin pregnancies, and suggest that already existing singleton nomograms are reliable in the evaluation of first trimester twin gestations.

MENOPAUSE

Monday, September 27, 1999

P-110

Raloxifene Effects on Quality of Life in Healthy Postmenopausal Women in Comparison to Unopposed Estrogen and Placebo, at 3-month and 12-month Periods. [1]D. W. Stovall, [2]B. W. Walsh, T. E. Melchione, [3]S. Weiss, [4]D. Merritt, [5]W. H. Scheele, [5]K. R. Srikanth, [5]S. L. Silfen. [1]Virginia Commonwealth University, Department of Obstetrics and Gynecology, Richmond, Virginia, [2]Brigham and Women's Hospital, Department of Obstetrics and Gynecology, Boston, Massachusetts, [3]San Diego Medical Endocrine Clinic, San Diego, California, [4]Barnes Jewish Hospital, St. Louis, Missouri, [5]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

Objectives: To evaluate the effects of raloxifene, conjugated equine estrogen and placebo using a self-assessed quality of life (QOL) questionnaire in healthy postmenopausal women who were not seeking relief from menopausal symptoms.

Design: This was a multicenter, double-blind, placebo-controlled, randomized, parallel study lasting 12 months. A total of 415 subjects were randomly assigned to one of four daily therapies: 101 to raloxifene HCl 60 mg/day (RLX60) (Evista®), 105 to raloxifene Hcl 150 mg/day (RLX150), 100 to conjugated equine estrogen (CEE) 0.625 mg/day (Premarin®), and 109 to placebo.

Materials and Methods: The Women's Health Questionnaire© (WHQ), a validated quality-of-life instrument for peri- and postmenopausal women, was adminstered at baseline and 3-month intervals. The WHQ consists of 36 questions measuring nine predetermined domains relating to QOL. Each domain was evaluated separately in these analyses. To consider both short and long-term effects of treatment, this report includes the 3- and 12-month evaluations. Therapy differences for change in domain scores (from baseline to endpoint) were analyzed using an ANOVA model with fixed effects for therapy and investigator, and changes over time within each therapy group were analyzed using paired *t*-tests.

Results: The domains with significant overall treatment effects were anxiety/fears ($P=.027$), menstrual symptoms (breast tenderness, bloating, bleeding and cramping) ($P=.006$) and vasomotor symptoms ($P<.001$) at 3 months, and menstrual symptoms ($P=.016$) and vasomotor symptoms ($P<.001$) at 12 months. Mean scores for memory/concentration, depressed mood, somatic symptoms, sexual behavior, sleep problems, and attractiveness domains were not different among treatment groups. Mean vasomotor symptoms scores improved significantly from baseline on CEE ($P<.001$) at both 3 and 12 months, but did not worsen on RLX60 at either time point. Mean menstrual symptoms scores worsened significantly from baseline on CEE at both 3 months ($P=.003$) and 12 months ($P<.004$), and on RLX150 at 12 months ($P=.044$). Mean scores in anxiety/fears improved significantly from baseline on RLX60 ($P<.001$), at both 3 and 12 months.

Conclusions: In healthy postmenopausal women who were not seeking treatment for symptoms, RLX60 was superior to CEE in self-assessed anxiety levels in both the short and long term. As expected, CEE was superior to raloxifene and placebo for relief of vasomotor symptoms, but worse for menstrual symptoms. Further research is needed to evaluate the clinical significance of unexpected anxiolytic benefit detected by the WHQ during RLX60 therapy.

P-111

The Effects of Testosterone (T), Tibolone (OD) and Hormone Replacement Therapy (HRT) on Diastolic Cardiac Functions and Lipid Peroxidation in Postmenopausal Women: A Randomized Placebo Controlled Study. [1]O. Taskin, [1]F. Burak, [1]R. Gokdeniz, [2]S. Sadik, [2]A. Onoglu, [3]H. Muderrisoglu. [1]Department of Obstetrics and Gynecology, [2]SSK Tepecik, [3]Cardiology, Inonu University, Malatya, Baskent University, Ankara, Turkey.

Objective: Most studies show a reduction in coronary heart disease incidence and mortality which the underlying mechanism are not fully understood. Although not directly linked, androgens are shown to exert effects on cardiac mass and function. This study is designed to investigate and compare the effects of T, OD, HRT on diastolic cardiac functions and lipid peroxidation in postmenopausal women.

Design: Prospective, randomized placebo controlled double blind study in a university clinic.

Materials and Methods: Fifty non-smoking, otherwise healthy postmenopausal women who did not receive any kind of HRT at least for three years within the onset of menopause were included in the study. The patients were randomly allocated to either 2.5 mg per day OD (n:12), daily combined 0.625 mg conjugated estrogens/2.5 mg medroxyprogesterone acetate pill (EP, n:13) and EP/2.5 mg methyl T (n:10) or a vitamin pill (n:15) in a double blinded fashion. Antioxidant activity and cardiac functions (with HP Sonos-1000 echocardiography using standard positions and windows) were assessed before and 6 months after the initiation of therapy.

Results: Mean age, weight, length of postmenopausal period were similar between the groups. The heart rate, systolic and diastolic pressures were similar during the pre- and post-treatment periods among the study groups, demonstrating lower blood pressure in OD, EP and T groups. At the end of 6 months, left ventricular end systolic and diastolic volumes were decreased significantly in T, OD and EP groups compared with placebo (56.5 ± 9.2 mL 62.2 ± 10.2 mL, 59.3 ± 21.5 mL vs 74.5 ± 12.4 mL, 84.1 ± 12.9 mL, 109.6 ± 18.8 mL, 118.1 ± 13.2 mL vs 140.4 ± 25.1, respectively). Left ventricular ejection fraction was increased in all steroid treated groups than placebo ($60.8\pm5.2\%$ $45.0\pm3.8\%$, $54.8\pm4.7\%$ vs $42.1\pm2.6\%$). Improvement in diastolic functions was significant in T, OD and EP groups compared to pretreatment period and placebo (E/A 1.31 ± 0.2,1.21 ± 0.4, 1.12 ± 0.3 vs 0.88 ± 0.4, deceleration time 198 ± 19.8,206 ± 20.6 msec, 208 ± 17.7 msec vs 242 ± 29.7 msec, respectively). Antioxidant activity was greater in the steroid treated group than placebo ($P<.05$). T did not impair the observed antioxidant activity observed with OD and EP. Although improved in all groups, addition of T to HRT revealed significantly higher changes in left ventricular ejection fraction and diastolic functions ($P<.05$).

Conclusion: Based on our preliminary results we may conclude that addition of low-dose T to HRT regimens may optimally improve cardiac performance and age related dysfunctions. The present results may further support that androgens are involved both OD and EP exert many direct effects on cardiovascular system other than metabolic changes regarding lipoproteins. The greater improvement in diastolic functions and ejection fraction in the T group and weak androgenic OD group is consistent with the in vitro data that androgens are potent relaxing agents on coronary arteries

and restores cardiac myosin isoenzyme and ATPase patterns which mandates further clinical studies.

P-112

The Effects of Estrogen/Progesterone (EP) Replacement Therapy With/Without Testosterone (T) and Tibolone (OD) on Endothelium-Dependent Relaxation in Isolated Aortic Rings From Ovariectomized Rats. [1,4]O. Taskin, [2]F. Burak, [2]R. Gokdeniz, [3]G. Saade, [3]J. M. Wheeler. Department of Obstetrics and Gynecology, [1]Akdeniz University, [2]Inonu University, Turkey, [3]UTMB, Galveston, [4]Texas Women's Hospital, Houston, Texas.

Objective: It appears that Estrogen restores coronary circulation and protects myocardium against ischemia in menopausal women. In order to elucidate sex hormones' circulatory effects, we studied the influence of sex hormones on endothelium-dependent relaxation of ovariectomized rat aortic rings.

Design: Prospective controlled study in the primate center of an university clinic.

Materials and Methods: Female Wistar rats at 70 days of age weighing 250–350 g were ovariectomized (OVX) or sham operated at 70 days of age. Twenty days following ovariectomy the rats (each group n:15) were randomized to receive either 7 μg/100 g body weight estradiol and 4 μg/body weight progesterone (EP), with (EPT) or without (EP) testosterone propionate (50 μg/100 g body weight), OD 14(0.04 mg/kg) or vehicle (V, sesame oil) intraperitoneally for another 20 days. Following replacement the rats were anesthetized and killed by exsanguination. Ring of aortas with endometrium and controls were mounted in organ baths for isometric tension recording. Indomethacin (10^{-5} M) and L-NAME (10^{-4} M) alone or in combination were used to block nitric oxide (NO) synthase (NOS) respectively. Mean data of contraction induced by KCL (60mM), relaxation by acetylcholine (10^{-6} M) in KCL contracted rings, tension induced by phenylephrine and the negative logarithm of the concentration of acetylcholine producing a 50% relaxation (IC_{50}) and the area under curve were calculated.

Results: All the treatment groups displayed significant relaxing responses in aorta than control group by decreasing the KCL induced tension and increased the acetylcholine induced in the rings with endothelium precontracted with KCL. The relaxing response of T was significantly improved compared to OD and EP groups. OD, EP and T groups counteracted the inhibition of endothelium-dependent relaxation by L-NAME in terms or IC_{50} and the area under curve. This counteraction was prominent in T added EP group than the others ($P<.05$).

Conclusion: Treatment with sex hormones particularly T increased production/release of endothelium-derived relaxing factor and increased the sensitivity of aorta to NO counteracting the NOS blocking activity. Above findings support sex hormones' direct effects on vascular tonus and endothelial vasodilatatory function. And further demonstrate androgen's additive role in stabilizing cardiovascular activity predominantly through NO, since T effect is independent of a classic receptor.

P-113

The Impact of Hormone Replacement Therapy (HRT) and Tibolone (OD) on Urodynamic Function in Postmenopausal Women: a Randomized Controlled Study. [1]R. Gokdeniz, [1,2]O. Taskin, [1]R. Atmaca, [1]F. Burak, [2]J. M. Wheeler. Department of Obstetrics and Gynecology, [1]Inonu University, Turkey and [2]Texas Women's Hospital, Texas.

Objective: Since vagina, urethra and trigone are all estrogen dependent, this study is designed to elucidate the effects of HRT on urodynamic function and subsequent genitourinary complaints in postmenopausal women.

Design: Prospective, randomized double blind study in a university clinic.

Materials and Methods: Forty-two non-smoking, otherwise healthy postmenopausal women without urinary incontience who did not receive any kind of HRT at least for three years within the onset of menopause were included in the study. The patients were randomly allocated to either 2.5 mg per day OD (n:20), daily combined 0.625 mg conjugated estrogens/2.5 mg medroxyprogesterone acetate pill (EP, n:22) in a double blinded fashion. All the women underwent a thorough evaluation including multichannel urodynamic testing and instrumented pressure-flow voiding before and 6 months after initiation of either therapy. All the measured parameters were compared with and within the study groups.

Results: All the patients were free of any genitourinary atrophy at the end of treatment period. Although higher in HRT group, both groups displayed significant increase in urethral pressure compared to pretreatment period. Both groups resulted in significant improvement in all the cystometric functions studied compared to pretreatment ($P<0.05$). Bladder capacity was found to be higher in HRT group than OD group compared to pretreatment (289 ± 92 vs 422 ± 89 mL and 254 ± 73 vs 402 ± 86, respectively, $P<0.05$). Bladder volume at first sensation of filling significantly increased with the treatment group compared to baseline (91 ± 34 vs 173 ± 73 mL and 93 ± 27 vs 154 ± 44 mL, respectively). Uroflowmetric measures were significantly improved by both HRT and OD treatments. Besides, the pressure transmission ratios were increased in the both treatment groups compared to pretreatment measurements.

Conclusion: Estrogens and synthetic steroid OD have resulted in improvement in the genitourinary anatomy and function in postmenopausal women. The above results are consistent with the fact that estrogen has a major role in the integrity of genitourinary tract. Furthermore, in menopausal urinary continence problems should be approached initially and with local and/or sytemic HRT and then should proceed with urodynamic or related studies, since most symptoms are atrophy related.

P-114

Dosimetric Absorption of Micronized 17 B-estradiol in Postmenopausal Women Using Varying Doses and Routes of Administration. M. M. May, C. A. Walters. Department of Obstetrics and Gynecology, Loma Linda University, Loma Linda, California.

Objectives: Estrogen replacement therapy (ERT) is known to prevent postmenopausal symptoms. For many women standard doses of estrogen fail to prevent the onset of these symptoms. This study was designed to determine serum estrogen levels following the administration of micronized 17B-estradiol and to observe the dosimetric absorption in postmenopausal women. Understanding the serum levels following estrogen administration may be helpful in determining why some women on standard doses of estrogen may have persistence of menopausal symptoms.

Design: The dosimetric curve of serum 17 B-estradiol levels was determined using varying doses and routes of administration over a 24 hour period in postmenopausal women.

Materials and Methods: Full IRB approval was obtained for eleven postmenopausal women (defined as having a serum estrogen level less than 50 pg/ml and a FSH level greater than 40 mIU/ml) to enter into the study. Participants were randomized to receive micronized 17 B-estradiol, 0.5 mg orally, 1.0 mg orally or 0.5 mg sublingually. A serum level was drawn at time zero, after which the randomized dose of micronized 17 B-estradiol was given. Blood was drawn every hour for the first eight hours and at 24 hours after administration. Each participant was randomized to the remaining doses and routes of administration in nonconsecutive days until all participants had entered all three arms of the study, thus serving as their own control. The serum from each draw was frozen for assay. Total serum 17 B-estradiol levels were measured using the Elisa technique. To avoid interassay variation, the serum from all three arms of the study for each participant was done on the same assay.

Results: For each dose of micronized 17 B-estradiol, 0.5 mg orally, 1.0 mg orally and 0.5 mg sublingually, mean peak serum levels for all participants were achieved approximately one hour after administration and were 50 pg/ml, 87 pg/ml and 325 pg/ml, respectively. Over the course of the next two to three hours all three arms of the study had rapid declines of serum estradiol levels to an average nadir of 40 pg/ml, which remained steady with no difference between the three study arms over the remaining time. Additionally, there was no difference in serum levels of 17 B-estradiol at the 24-hour time point in all three arms of the study with an average serum level of 26 pg/ml.

Conclusions: We conclude regardless of the dose and route of administration of estrogen that there is a peak and decline of serum 17 B-estradiol at the same times. Because sublingual dosing of estrogen appears to elevate significantly serum 17 B-estradiol, long and short-term complications related to ERT should be considered before using this method. With the average nadir at 40 pg/ml seen at a steady state in all three arms of the study, it may be beneficial to some patients with persistent menopausal symptoms to use twice daily dosing. Though serum levels of 17 B-estradiol may be

important in ERT management, it remains to be determined if this may have clinical applications.

MENTAL HEALTH

Monday, September 27, 1999

P-115

The Impact of Charges for Professional Services of ART Procedures and Resulting Stress on Pregnancy Rate in Infertile Women. [1,2]H. S. Kashaf, [2]M. Aghahosseini, [2]A. Aleyaseen, [2]M. Vahid Dastjerdi, [1,2]H. Saidi, [1]N. Ghalavand, [1]G. H. Amini, [1]D. Etemadi, [1]Sh. Mohajeri, [2]A. Khademi, [1]S. S. Kashaf. [1]Navid's Institute of Infertility and [2]Department of Infertility and Endocrinology Shariati Hospital Tehran University, Tehran, Iran.

Objective: The relationship between stress and infertility is complex. Infertile women report elevated levels of depression and anxiety when compared to fertile women. One of the reasons for stress is the charges for professional services. The effect of no charge for professional services on increasing pregnancy rate has not been adequately addressed.

Design: A prospective comparative study.

Methods and Materials: From Jan. 1, 1998 to Dec. 31, 1998, women who failed in their first attempt for IVF, GIFT, ICSI-IVF, and ICSI-ZIFT and were planning for second attempt were randomized to either attend a no charge for professional services or full charges for professional services.

Result: A total of 100 women were recruited and randomized. There were fifty in charge and fifty in no charge group. Clinical pregnancy rate for professional services charge group was 52% (26/50) and for no charge group was 26% (13/50) on going pregnancy rate for professional services charge group was 42% (21/50) and for no charge group was 12% (6/50). There was a significant difference of clinical and ongoing pregnancy rate between the two groups ($P<.001$ VS. charge for professional group two independent proportion test). There was no difference in the incidence of Ectopic or multiple gestation between the two groups.

Conclusions: There appears to be a connection between charges for professional services and resulting stress and pregnancy rates in ART Procedures. This study supports the complexity of the relationship between stress and infertility.

P-116

Alternative Medicine and Infertility: What Infertility Patients Are Using and Why. J. P. Galst, RESOLVE NYC, New York, NY.

Objectives: Research suggests that significant numbers of people are using various forms of alternative medicine. The purpose of the current investigation is to determine if infertile individuals are using alternative therapies to treat their infertility and if so, what they are using and their reasons for such use.

Design: Survey methodology. A sample of infertile individuals completed a survey on conventional medical and alternative treatments for infertility which was distributed in the RESOLVE NYC newsletter.

Materials: A checklist was designed examining respondents' use of conventional medical and alternative treatments to treat infertility. In open-ended questions, respondents were asked their reasons for using alternative treatments and whether or not they felt conventional and/or alternative treatment had helped them.

Results: 43 women and 3 men returned their surveys (return rate of approximately 5%). Average: age of respondents 38.8 (women), 47 (men); years married 6.7; years trying to conceive 3.6; years of education 17.5; income $160,400. In this sample, 91% of the respondents used a combination of conventional and alternative treatments (2% used neither; 2% used alternative only; 4% used conventional only). Forty per cent of the sample reported that conventional medical treatment had helped them; 55% reported that alternative medical treatment had helped them. The most frequently used conventional treatments were inseminations with medications (19%) and IVF (19%). The most frequently used alternative treatments were vitamins (74%), counseling and/or support groups (70%), mind-body techniques (59%), exercise (48%), herbs (48%), and acupuncture (37%). Reasons for using alternative treatments included dissatisfaction with conventional medical treatment, to help get pregnant, to re-gain control, to feel healthier, to try everything, and a personal belief in the approach.

Conclusions: This survey represents what some infertile individuals are using to treat their infertility. Despite little or no scientific proof of the efficacy of various alternative therapies for infertility treatment, well-educated patients are nevertheless turning to alternative therapies. It is suggested that alternative treatment and practitioners are meeting needs that currently go unmet by conventional medical treatment for infertility (e.g., need for control; a kinder, gentler approach; treatment of the whole person, including their emotional needs; empowerment of the individual to self-heal). Great advances have been made in the scientific technology of reproductive medicine, helping increasing numbers of people create the babies they so desire. However, comprehensive infertility treatment needs to heal the psychological wounds created by the infertility experience, as well, and here the science of reproductive medicine falls short. These needs are currently being met by alternative therapies.

P-117

Personal Infertility Experience Among Nurses and Mental Health Professionals Working in Reproductive Medicine [1]S. N. Covington, [2]K. R. Marosek. [1]The Shady Grove Fertility Centers, Rockville, MD and [2]Columbia Hospital for Women IVF Program, Washington, DC.

Objectives: Infertility is experienced by 17% of the general population; however, it is unknown how many professionals working in reproductive medicine experience infertility. The purpose of this study was to determine: 1) the prevalence of infertility among nurses and mental health professionals working in the field; 2) if the infertility occurred before or after their entry into reproductive medicine; and 3) where they obtained fertility treatment. Further, we sought to identify how respondents perceived the advantages, disadvantages, and the presence of ethical concerns in receiving treatment at their place of employment.

Design: An anonymous, confidential survey of reproductive endocrinology nurses and members of the Mental Health Professional Group (MHPG) of the American Society of Reproductive Medicine (ASRM).

Materials and Methods: A survey was included in the Serono *Insights into Infertility* Newsletter and distributed at an annual Serono Symposia USA Nurses Conference attended by approximately 250 nurses. Six months later, the same survey was mailed to 170 members of the MHPG of the ASRM. The survey contained 25 questions assessing demographics; reproductive experiences, particularly perceptions of treatment at the workplace; patient versus employee rates; and 3 open-ended items about practice policies and ethical concerns. Descriptive statistics were used to summarize the data.

Results: A total of 100 surveys were received, 70 from nurses and 30 from the MHPG. 94% of the respondents were women, and 9 years the average time working in the field. 52% of respondents reported a personal infertility history, with 67% of infertile respondents reported having received infertility treatment at the clinics which employed them. 71% of infertile respondents began working in reproductive medicine *after* diagnosis. The three major advantages to obtaining medical treatment at the workplace were (1) easier access to physicians (72%); (2) financial discounts in cost of treatment (67%); (3) less time away from work (61%). The three major disadvantages were (1) compromised confidentiality (59%); (2) the perception that the infertile professional did not need emotional support and/or explanation of treatment (37%); and (3) less opportunity for a spouse to be involved the treatment process (28%). Respondents were equally divided on the question of ethical conflicts when an infertile professional receives treatment at their work; 45% expressed concerns, 44% stated "no", and 11% were unsure. However, 66% of the MHPG felt there were ethical conflicts while only 41% of nurses thought this was problematic. Ethical concerns of respondents included boundary issues; confidentiality; dual role as professional and patient; preferential treatment; potential conflict of interest; and financial discounts.

Conclusions: Personal infertility experience appears to be prevalent among nurses and mental health professionals working in reproductive endocrinology in this non-representative sample. The majority of these infertile professionals sought treatment where they worked and felt that the advantages outweighed the disadvantages. The key disadvantage was compromised confidentiality. Since the overwhelming majority of infertile professionals started working in the field after diagnosis, their personal experience with infertility may have been a factor in choosing to work in reproductive medicine. It is speculated that the training of mental health professionals regarding maintaining professional boundaries in a therapeutic

relationship was a factor in their ethical concerns about receiving treatment at the workplace.

P-118

Withdrawn

P-119

Analysis of Minnesota Multiphasic Personality Inventory-2 (MMPI-2) Scores of Anonymous Oocyte Donors Based on Donation Outcome. [1]S. C. Klock, [2]J. Elman Stout, [3]M. Davidson. [1]Department of Obstetrics and Gynecology, Northwestern University School of Medicine, Chicago, IL. [2]Private Practice, Chicago, IL. [3]IVF Illinois, Chicago, IL.

Objectives: The MMPI-2 is frequently used as part of the psychological screening of anonymous oocyte donors although how MMPI-2 scores coincide with clinical and donation outcome data is not known. The purpose of this study was to examine MMPI-2 scores among three donor outcome groups: 1) accepted and donated; 2) accepted but did not donate and; 3) rejected based on psychological concerns.

Design: Observational study of 150 consecutive anonymous oocyte donors.

Materials and Method: 150 women volunteering as oocyte donors were identified from 2 donor recruiters and one IVF program. All donors underwent preliminary screening for health status, family history and understanding of the donation process. Women wishing to donate oocytes were seen for a 90 min. psychological interview and completed the MMPI-2. The decision to accept or reject a donor from a psychological perspective was based on interview findings. Demographic (ethnicity, marital status, education, religion), reproductive (number of children, elective abortions) and psychosocial history (psychological counseling, psychoactive medication use, sexual assault, parental loss) data were collected. MMPI-2 subscale scores were obtained from test data. Donation outcome (Group 1=accepted/donated; Group 2=accepted/did not donate and Group 3=rejected) was followed over time. The data were analyzed using Chi-square and t-tests. The study was approved by the institutional Human Subject Review Board.

Results: Demographic characteristics for the sample were: 65% single, 60% no children, 83% Caucasian, 61% high school graduates and 37% Catholic. 43% had at least one previous elective abortion. Psychiatric history included 10% with psychoactive medication use and 34% with out-patient psychological counseling. 17% reported a history of sexual assault and 50% reported parental loss (death or divorce). In terms of outcome, 71 (47%) women were accepted and completed at least one donation cycle (Group 1). 47 (31%) donors were accepted but did not donate (Group 2). For Group 2, reasons for not donating were: 10 (21%) could not be matched to a recipient or relocated prior to matching; 19 (40%) were excluded based on a failed medical exam/genetic screening or poor stimulation cycle; 18 (38%) were non-compliant with the donation process. 32 (21%) women were rejected for psychological reasons (Group 3). Comparison of demographic, reproductive and psychological variables showed significant differences between groups with more women in Group 3 reporting history of psychoactive medication use, psychological counseling and sexual assault. There were significant differences between groups on validity scales F and K with Group 3 having higher scores on F and lower scores on K compared to Groups 1 and 2. On the clinical scales an aggregate 5–9 profile was found. Significant differences were found between groups on scales 1, 2, 4, 7, 8, and 0 with Group 2 higher on scale 1, and Group 3 higher on scale 2, 4, 7, 8 and 0. Despite these differences, all group subscale mean scores were in the average to low average range and differences between group means were between 3 to 7 points.

Conclusions: In a large sample of donors MMPI-2 scores generally were in the average range. Significant differences between outcome groups were found but the magnitude of the differences were small. These results underscore the need for trained mental health professional to screen potential oocyte donors and for psychologists to interpret MMPI-2 results in the context of other clinical information.

P-120

Withdrawn

P-121

Cytoplasmic Transfer—A Means to an End. D. Batzofin, J. Wilcox, J. Nelson, M. Feinman, D. Potter, C. Tran, T. Tan, J. Batzofin. Huntington Reproductive Center, Pasadena, CA.

Introduction: Attention has recently been focused on Cytoplasmic Transfer (CT) from donor eggs to the eggs of recipients who are either older or whose major cause of infertility is poor oocyte and/or embryo quality.

Objective: To investigate the benefit of enabling patients (with input from the IVF team) to control and make an informed decision regarding the ultimate destiny in terms of transfer of embryos that were achieved from both CT and from pure egg donation.

Design: Informal prospective observational study.

Materials and Methods: Nine patients agreed to enter a preliminary trial for a CT treatment cycle. The mean duration of infertility for the group was 8 years, mean age 42 years old and all patients had undergone 3–6 previous failed IVF cycles. Since this treatment is still experimental all patients underwent psychological screening, intensive counseling and signed specific informed consents. Standard stimulation protocols were employed, however, monitoring of these recipient/donor cycles was more intensive than a regular IVF or donor cycle to ensure that synchronicity and concurrent maturity of eggs in both recipient and donor was achieved. All patients had oocyte retrieval performed in the office under IV sedation and local anesthesia. Only morphologically mature oocytes (the presence of the first polar body) were involved in the actual CT. This procedure was achieved through microinjection of aspirated cytoplasm from the donor oocytes together with an immobilized sperm into the recipients' oocytes. One donor oocyte was used per two to three recipient oocytes. The remaining donor oocytes were fertilized with the husband's sperm and were cultured along with the CT embryos.

Results: A total of 41 embryos were achieved after the recipients oocytes had undergone CT. All nine patients obtained CT embryos for transfer and in addition all nine patients had pure donor embryos available as well. The patients were presented with two photographs, one of the CT embryos and the other of the donor embryos. The ultimate decision as to which and how many embryos to transfer was left up to the patients with input from the IVF team. Three patients who observed that their CT embryos were clearly superior in quality to both the donor embryos as well as other embryos that had been obtained from previous IVF cycles, chose to transfer only CT embryos and freeze the donor embryos. The remaining six patients when confronted with the reality that even after CT their embryos were obviously still of inferior quality, chose to transfer a mix of both the CT embryos and the donor embryos. Six out of nine patients presently have frozen donor embryos in storage. Five pregnancies were achieved: two miscarried, one is ongoing and there was a twin and a triplet livebirth. Patients have agreed to allow any babies born from this study to undergo genetic testing.

Conclusion: Even though controversial in nature, by allowing patients to control the decision as to which embryos to transfer, their ultimate choice proved to be congruent with and affirmed the clinical findings. In addition, patients expressed a high degree of satisfaction and comfort with both the treatment process and the ultimate outcome.

P-122

Motivations for Seeking Reproductive Assistance in HIV Discordant Couples. S. M. Kavic, R. C. Zimmermann, G. Brown, J. Rosenthal, S. R. Lindheim, M. V. Sauer. Departments of Obstetrics & Gynecology and Psychiatry, Columbia University College of Physicians & Surgeons, New York, NY.

Objectives: To characterize the demographics and understand the motivations of HIV discordant couples who seek assistance for reproduction and family planning.

Design: A prospectively designed descriptive study of information gathered from open-ended interviews by specialists in reproductive endocrinology, maternal fetal medicine, and psychiatry of patients wishing to undergo reproductive therapy.

Materials & Methods: From 1/98–1/99, 27 couples known to be HIV discordant (male (+)/female (−), n=20; male (−)/female (+), n=7) contacted the fertility center inquiring about the possibility of receiving assisted reproductive services. Of these, 23 couples elected to proceed with care, which included IVF/ICSI for couples with male (+)/female (−) status

(n=19); washed IUI for couples with male (−)/female (+) status (n=4). Suspected causes of HIV status included prior drug use, blood transfusion, and heterosexual contact. Information regarding the patients' background, medical and reproductive histories, and current health status were abstracted from a series of one-on-one interviews.

Results: Demographic characterization of the enrollees were as follows:

	Age males ± SEM	Age females ± SEM	Duration HIV ± SEM
Male (+)	43 ± 1.32	38 ± 1.35	5.6 ± 0.9
Female (+)	43.5 ± 4.5	34.5 ± .5	8 ± 4

	Years together	Hx AIDS
Male (+)	8.7 ± 1.38	4.3% (1/23)
Female (+)	2.25 ± .75	0

All couples had previously been denied care by a health care professional in the United States. All had seriously considered timed intercourse as a means for procreating. All couples were aware of potential risks of infection transmission and accepted these risks in order to have a child. One partner died of AIDS during the course of therapy (banked specimens were being used for IVF/ICSI). Each HIV (−) female expressed an interest in continued therapy even if their partners became ill or died.

Conclusions: 1) Many couples have a strong desire to start a family in the presence of diagnosed HIV, and are willing to accept risks of disease transmission to accomplish this goal; 2) Many HIV (+) patients have extended disease-free intervals and improved quality of life using anti-viral therapy, and wish to experience biologic pregnancy and parenthood; 3) Even in the face of a potentially fatal illness or death, surviving partners continue to express an interest in posthumous reproduction with banked specimens.

P-123

Withdrawn

P-124

Oocyte Donation (OD): From 'Sparing' to 'Sharing' Models. D. Seibel, E. L. A. M. Motta, J. R. Alegretti, P. Serafini. Center of Human Reproduction—Huntington, São Paulo, Brasil.

Objectives: OD has been a successful treatment; however, because of different laws, ethics, religious beliefs, practice profiles, and financial hardships several models of OD have been used. Due to the recent introduction of non paid designated OD through oocyte sharing; we wish to compare clinical outcomes considering relevant psychological aspects of practice.

Design: Clinical study.

Materials and Methods: The # of eggs retrieved, embryos transferred and clinical pregnancy rates (PR) were evaluated in 63 patients who received spared eggs and in 20 women who had OD with shared eggs. The results of the spared program were collected over a 3 year period, while the shared eggs were gathered from 12/97–12/98. Medical screening outlined by ASRM was provided to all patients. Psychological screening and extended counseling was rendered to OD and recipients of shared eggs. Recipients were provided with the OD's history, physical exam, photographs, and medical screening before selection of the OD. 2–5 embryos were transferred after signing IRB consent.

Results: Contrary to the 'hiring' of a paid OD, the sharing model used subfertile women aged ≤32 years old (26.0 ± 3.9) who were unable to pay for IVF. The average age of the women who spared eggs was 31.4 ± 3.7 years ($P<0.05$). The sparing and sharing egg recipients were 39.6 ± 4.2 and 38.6 ± 3.9 years old (NS), respectively. The # of eggs recovered, the # of eggs donated, the # of embryos transferred to the donors and the recipients are shown in the Table.

	# Eggs Recovered	# Eggs Recipient	# Embryos Donor
Shared	21.3 ± 9.3**	8.1 ± 3.4**	3.1 ± 1.0*
Spared	14.3 ± 4.9	4.0 ± 1.2	4.8 ± 1.6

	# Embryos Recipient	
Shared	3.0 ± 1.3	*$P<0.05$
Spared	3.6 ± 1.5	**$P<0.01$

In the sharing program, PR were 37.5% for the OD and 38.1% for the recipients; further, a 38.0% PR was achieved for sparing OD, and 27.3% for the recipients, respectively.

Discussion: The sharing OD model allowed for the concurrent treatment of the OD and recipient, while providing a greater # of eggs and frozen embryos. Moreover, a reduced # of embryos were transferred, providing similar PR between the shared partners. Although there was a tendency for lower PR among the of spared OD recipients; the costs with the shared oocytes were greater than in the sparing model, and the ODs were also subfertile. Extensive psychological testing and counseling were needed, but these requirements contributed to the model's success by allowing for healthier transference.

P-125

Withdrawn

P-126

Withdrawn

P-127

Coping with Infertility: What are Patients Initial Concerns? K. Bevilacqua, D. Barad, J. Youchah, B. Witt. Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY.

Objectives: The aim of this study was to identify psychological strengths and vulnerabilities related to coping with infertility and how these concerns relate to the course of treatment.

Design: Subjects completed a psychosocial questionnaire regarding their emotional experiences; in previous medical settings, with infertility, and about infertility within their relationship.

Materials and Methods: Between May 1995 and January 1999, a sample of 475 female infertility patients returned completed questionnaires at the time of their 1st appointment at a metropolitan reproductive endocrinology center. The structured questionnaire containing open-ended questions about their emotional experiences with infertility prior to this appointment augmented the standard demographic/medical intake form given to new patients.

Results: Patients considered their psychological concerns to be almost as important as their medical needs. Several major themes emerged from their narratives: The emotional connection to their medical provider could foster trust and hope. The most desired qualities in medical staff were being a good listener, exhibiting positive and supportive attitudes as they proceeded with treatment, and genuine warmth. Patients wanted straightforward information about their condition and chances for becoming pregnant explained at their educational level. A strong relationship with their partner could help a patient through the more stressful aspects of treatment. A partner's most helpful quality was to be a good listener when they talked about fears and frustrations. Nearly all women wanted their partners with them when they underwent medical procedures. The most salient fears expressed by patients were never having children, current age limiting the possibility of becoming pregnant, and physical discomfort during medical procedures.

Conclusions: The study questionnaire helped create a qualitative description of the patient and couple being treated. Patients were willing to write about both positive and negative events that impacted on their decision to come to the particular facility for treatment. By expanding standard medical and demographic questionnaires to ask about past experiences and current

feelings, patients are given an opportunity to share emotions they may not feel comfortable expressing verbally. Through the use of open-ended questions clinicians may identify issues that can be used to build a therapeutic alliance before active treatment begins. When patients write information about themselves in a brief narrative format, touch points for further discussion about sensitive issues and fears are created. By using the patient's own language medical personnel can get a sense of the patients literacy level which may impact on treatment compliance. This alternative type of questioning identifies what has been previously helpful to individual patients. By building on strengths and addressing fears in a couple, a therapeutic alliance may be established to help patients remain in treatment long enough to become pregnant.

P-128

The Cognitive Abilities of In-Vitro Fertilization (IVF) Patients are Reduced Prior to Oocyte Retrieval. [1]M. Z. Onal, [2]C. Sonmez, [1]S. Ozkaynak, [2]K. Özgür, [1]H. Aydin, [2]M. Uner, [2]O. Erman. [1]Department of Neurology and [1]IVF Unit in Department of Obstetrics and Gynecology, Akdeniz University Medical School, Antalya, Turkey.

Objectives: Chronic exposure to exogenous estrogen have been suggested to have a role in preventing, delaying as well as treating dementia. P300 is an endogeneous event related brain potential that has become a very useful tool to identify the underlying neuropsychiatric diseases associated with cognitive dysfunction. The P300 is thought to reflect neuroelectic activity related to cognitive processes such as state of arousal, attention allocation and activation of immediate memory as whole. P300 amplitude and P300 latency closely reflect cognitive functions such as task relevance and stimulus evaluation time respectively. We have evaluated the cognitive functions prior to oocyte retrieval in an IVF population by using P300.

Design: Prospective cohort study.

Materials and Methods: Eighteen IVF patients (mean age=31.39 ± 5.01) were enrolled into the study without any detectable cognitive disorder. P300 traces were recorded during the lowest (after ovarian suppression achieved with leuprolide acetate) and the highest blood level of estrodiol (on hCG day). Mean interval between two recordings was 8.61 ± 2.50 days. We measured N1OO, P200, N200, P300 latencies and P300 amplitudes from isoclectric line and peak to peak points of N200 and P300 waves at C_z and F_z recordings of subjects. We evaluated the P300 potentials obtained from target tones. Paired-t-test was used to determine the significant difference of variables.

Results: Peak to peak P300 amplitudes at C_z are 17.42 ± 5.66 μV and 14.93 ± 6.20 μV and at F_z are 14.94 ± 4.42 μV and 12.06 ± 4.97 μV during the lowest (28.25 ± 23.04 pg/mL) and the highest (1845.56 ± 1415 pg/mL) blood levels of estrodiol respectively. P300 amplitudes were significantly reduced during the highest levels of estrogen both at F_z ($P<0.01$) and C_z ($P<0.03$) recordings of subjects. The other subcomponents of P300 wave measurements were not significantly different from each other at C_z and F_z recordings.

Conclusion: Our results demonstrate that the cognitive abilities of the patients are reduced when the E_2 levels have peaked in an IVF patient group. We believe that this contradictory effect of high endogeneous estrogen may be due to some other factors such as stress prior to oocyte retrieval.

P-129

Some Psychological Consequences of Multiple Pregnancy. G. Basso. Asociacion Psicoanalitica Argentina, Buenos Aires, Argentina.

Objective: The aim of the present work is to show the consequences over the development of the bond mother-child in a multiple pregnancy.

Design: This study is based on the conceptual psychoanalytical model, as frame of reference, regarding the constitution of the psyche and its way of acting.

Materials and Methods: Over a sample of 15 mothers and 46 children born towards multiple pregnancy (triplets and more). Initial individual interviews with the parental couples, once a week during the whole process including the first year after birth.

Results: The bond may seem simultaneous but it takes place one baby at a time, and which baby comes first depends on multiple causes in the mother babies relationship. 10 out of 15 mothers accept better the healthiest baby. 1 out of 15 mothers accept better the smallest baby. 2 out of 15 mothers accept better the baby with whom the mother identifies best. 2 out of 15 mothers accept better the baby with whose sex (male or female) was the one they wish for. Attachment takes place in a slower way.

Conclusion: 1) Pregnancy: The psyche process became slower, while the physical one with the fast uterine growth gives as a result a disadjustment between both processes. 2) Prematurity: premature delivery has been recognized as a severe life-stressor. 3) Attachment between mother and babies: The complementary series and the unconscious fantasies of the mother have to be taken into account. The baby's behaviours and health are very important factors to the beginnings of relationship. Early multidisciplinary supervision favours the better adaptation of bond mother-child in a multiple pregnancy.

P-130

Withdrawn

P-131

Psychological State of Infertile Women in Japanese Population: A Cross-Sectional Study. [1]H. Matsubayashi, [2]T. Hosaka, [1]S.-I. Izumi, [1]T. Suzuki, [1]K. Yoshikata, [1]T. Makino. [1]Department of Obstetrics and Gynecology and [2]Department of Psychiatry, Tokai University School of Medicine, Kanagawa, Japan 259-1193.

Objectives: Recent clinical papers have suggested the psychological involvement in infertility women. Whether stress and infertility are linked as cause (Psychogenic Hypothesis) or consequence (Psychological Consequences Hypothesis) is still uncertain. In Japan, historically, women are easily affected by such questions as 'Are you married? Do you have a child?', because these are kind of greeting. Therefore, this study was done to clarify whether Japanese infertile women are stressful.

Design: Cross-sectional questionnaire study in infertile women was performed to assess their psychological state.

Materials and Methods: Eighty-nine infertile women received the questionnaire when they attended the infertility clinic in Tokai University Hospital. Depression and Anxiety (DA) score were calculated from the Hospital Anxiety and Depression Scale (HAD), and the scores of Depression (D), Aggression-Hostility (AH), Lack-of-Vigor (LV), Fatigue (F), Tension-Anxiety (TA), Confusion (C) and Total Mood Disturbances (TMD) were measured by the Profile of Mood States (POMS).

Results: In our previous study, we found that patients with more than 11 scores of DA showed psychiatric disorders (sensitivity; 97%, specificity; 74%). Of 89 infertile women 42 (47%) had positive DA score, whereas depression/anxiety scores were not always parallel. Similarly, scores of D, TA and TMD in POMS test were correlated to the score of DA in HAD test with following correlation coefficient; 0.84, 0.82 and 0.86, respectively ($P<0.0001$).

Conclusions: In Japanese population, about half of infertile women are emotionally disturbed, especially suffered from depression and anxiety. What kind of factors in their daily life are relevant is underway at Tokai University Hospital.

P-132

Coping Strategies, Psycho-Social Functioning, Well Being and Specific Optimism During In-Vitro Fertilization Treatment. [1]M. Bar-Hava, [2]K. Ginzburg, [1]E. Geva, [1]F. Azem, [1]I. Yovel, [1]D. Kovalski, [1]B. Neiman, [1]J. B. Lessing, [1]A. Amit. [1]IVF Unit, Lis Maternity Hospital and [2]Social Service, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Israel.

Objectives: To examine the correlation between coping strategies, psychosocial functioning, general well-being, and specific optimism of treatment outcome and the likelihood to conceive from in vitro fertilization (IVF).

Design: Prospective assessment study.

Materials and Methods: Self-reported questionnaires evaluating the study parameters were randomly distributed among 102 women enrolled in our IVF program.

Results: The study group consisted of 96 women whose mean age was 34 ± 5.2 years. Positive reinterpretation and growth, acceptance, willingness to seek instrumental and social support and resorting to humor were found to be adaptive coping strategies that contributed to psychosocial functioning and well being. In contrast, planning, mental and behavioral disengagement, focus on and venting of emotions, denial and restrain coping all had an adverse effect. A significant association was found between the level of specific optimism of treatment success and the treatment stage. Women who conceived in the current cycle tended to express higher level of specific optimism.

Conclusions: Specific optimism of successful outcome in the current cycle seems to be positively associated with the likelihood to conceive. Coping strategies could be grouped into those with an advantageous and a deleterious influence on the infertility treatment process. Knowing which ones being used by a woman can help in improving the planning of her counseling and support system throughout the IVF process.

REPRODUCTIVE BIOLOGY

Monday, September 27, 1999

P-133

Expression and Chromosomal Localization of the Human Gcm1 Gene. A. B. Copperman, B. Nait-Oumesmar, R. A. Lazzarini. Mount Sinai Medical Center, New York, NY.

Objective: The Drosophila gene glial cell missing (Gcm) plays a pivotal role in glial cell fate determination. Recently, genes encoding highly homologous proteins with conserved "Gcm1" DNA binding domains have been identified in mouse and man. Our objective is to assess the expression of the human gene in placental tissues, evaluate the potential role of the protein in this tissue and to map the gene's chromosomal position.

Materials and Methods: A fusion protein consisting of glutathione S transferase and the unique carboxy terminal portion of the mouse Gcm1 was prepared in *E. coli* with the aid of a plasmid expression vector. The purified fusion protein was used to raise Gcm1 specific antisera in rabbits. The polyclonal antisera recognized both mouse and human Gcm1 with about equal intensity. The antisera recognized a single band of protein of the expected size (51kD) in western blot analyses of placental extract, demonstrating the specificity of this reagent. The cell type specificity of Gcm1 expression was investigated by immunocytochemistry using 10 micron paraffin sections of first trimester and term placentae. Human chromosomal localization was performed by fluorescence in situ hybridization (FISH). Chromosomal localization of human Gcm1 (hGcm1) gene was achieved by superimposing FISH signals with DAPI banded chromosomes.

Results: Comparison of mGcm1 and hGcm1 protein sequences revealed a high level of homology in the Gcm DNA binding motif and in the transactivation domain. We prepared antisera against the carboxy terminal epitopes of mouse Gcm1 and verified the specificity of this reagent by western blot analysis of third trimester placenta protein extracts: the anti-mouse Gcm1 recognizes a single protein band of 51kD, the predicted molecular weight of hGcm1. Using immunocytochemistry, we found that Gcm1 was highly expressed in the nuclei of syncytiotrophoblast cells of the placenta during first trimester, while in the third trimester placenta, the Gcm1 immunoreactivity was less apparent. Using a human cDNA clone of Gcm1 we successfully mapped the gene to the p12 region of chromosome 6.

Conclusions: We report the chromosomal assignment of hGcm1 to 6p12, and temporal expression of Gcm1 that suggests down-regulation of Gcm1 expression in syncytiotrophoblasts by third trimester. Further studies must be performed to decipher the precise role that this well conserved transcription factor plays in the cell fate specification of human syncytiotrophoblasts, the differentiation of these cells, and its potential role in disease processes.

P-134

Angiotensin II Induces Calcium Influx and Human Sperm Acrosome Reaction. [3]C. Müller, [1,2]G. Wennemuth, [2]N. Bagus, [2]A. Meinhardt, [2]H. Renneberg, [3]W. B. Schill, [2]G. Aumüller, [4]F. M. Köhn. [1]Institute of Physiology and Biophysics, Seattle, WA, USA; [2]Department of Anatomy and Cell Biology, University, Marburg, Germany; [3]Center of Dermatology and Andrology, Justus Liebig University, Giessen, Germany; [4]Department of Dermatology, Technical University, Munich, Germany.

Objective: Angiotensin II (AII) has been shown to stimulate sperm motility and to induce the acrosome reaction (AR) in vitro. Since previous experiments demonstrated that calcium is required for the induction of AR by AII, the purpose of the present study was to examine the effects of AII on the calcium influx in human spermatozoa.

Design: In vitro study.

Material and Methods: Ejaculates were produced by healthy donors. Experiment 1: After glass wool filtration and two washing steps in HTFM (1% HSA), spermatozoa were treated with 100nM AII for 4 h at 37°C. In order to study whether the induction of acrosome reaction was mediated by calcium, the experiments were performed in medium with and without calcium. The acrosome reaction was determined by triple staining. Experiment 2: Motile spermatozoa were separated (percoll), washed in HTFM and resuspended in HHBSS medium. After incubation with 100 nM AII for 1h, the calcium influx in single human spermatozoa (n=70) was investigated by the Fura-AM method.

Results: Compared to the controls, AII increased the percentage of acrosome reacted spermatozoa significantly (9.5 ± 3.6% vs. 4.6 ± 2.6%; $P<0.01$). The inducibility of AR by AII was significantly decreased in the absence of calcium (6.2 ± 3.3% vs. 0.8 ± 1.0%; $P<0.001$). Angiotensin II provoked a calcium influx in 30% of investigated spermatozoa.

Conclusion: Angiotensin II stimulates the human sperm acrosome reaction by inducing an intracellular calcium influx. Since A II is present in secretions of the female genital tract, it may modulate functions of spermatozoa during their migration to the oocyte.

P-135

Withdrawn

P-136

Cigarette Smoking Inhibits Apoptosis (Programmed Cell Death) in Early Human Embryos. [1]M. T. Zenzes, [2]T. E. Reed. [1]Department of Obstetrics and Gynaecology, and [2]Departments of Zoology and Anthropology, University of Toronto, Toronto, Ontario, Canada.

Objectives: Benzo(a)pyrene (BP), is a carcinogen in cigarette smoke. It is known to inhibit apoptosis of preneoplasic lung cells, thus promoting tumorgenesis. Its reactive metabolite, a diol epoxide (DE), binds covalently to DNA, forming adducts (e.g., premutational changes). Recently, Zenzes et al. (1999) detected BPDE-DNA adducts in early embryos of IVF couples in association with parental smoking, transmitted mainly by sperm of smoking fathers. Here we analyze whether maternal smoking affects the quality of early embryos.

Design: Fertilization rate (FR) and embryo quality were analyzed in relation to cotinine, a marker for smoking.

Material and Methods: 271 women in our IVF program, between ages 24 to 45y, participated after signing a consent. Their 1682 embryos, at 2- to 8-cell stages, were classified as: a) good-quality (grades 1+2; with even size blastomeres, no fragments; 0 to 25% fragments), and b) poor-quality (grades 3 + 4; 25 to 45% fragments; >40% fragments). Cotinine in follicular fluids was assessed by radioimmunoassay.

Results: After correcting for maternal age (and for age x cotinine interaction), the FR (from 2886 oocytes) was increased with increasing cotinine levels ($P=.0005$) until age 36.3y, but this cotinine effect becomes negative thereafter. The proportion of good-quality embryos was also increased with increasing cotinine levels ($P=.0001$) without age limit.

Conclusions: 1) An inhibiting effect of cotinine on embryo apoptosis during early development, manifested in smokers by increased proportions of good-quality embryos, may prevent sure identification of good-quality embryos for transfer. This effect may explain the higher risk of early abortion known to occur in smokers. 2) A higher fertilization rate with increased cotinine confirms similar previous findings by us and others. This "positive" effect of cotinine on FR, elimination of damaged maturing oocytes, increases the proportion of mature, fertilizable oocytes. A negative effect of cotinine on FR is detectable only after age 36y. Supported by the Medical Research Council, Ottawa, Canada.

P-137

Regulation of the FSH Receptor Promoter Activities by Upstream Stimulation Factors in Ovarian Granulosa Cells. [1]W. Xing, [1]M. R. Sairam, [2]J. Chedrese. [1]Molecular Reproduction Research Laboratory, Clinical Research Institute of Montreal, Montreal, Quebec, Canada and [2]Department of Obstetrics and Gynecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Objectives: The follicle stimulating hormone receptor (FSH-R) is tissue specifically expressed in ovarian granulosa and testicular Sertoli cells. Receptor activation is required in the ovary for follicular development and ovulation. As the factors controlling the expression of this gene is not fully understood, we have begun the analysis of its promoter.

Design: Study activation of a reporter gene driven by FSH-R promoter in ovarian cells.

Materials and Methods: Selections were made from the cloned 8 Kb ovine FSH receptor promoter region for their ability to activate the luciferase reporter gene. Suitable constructs were tested in a spontaneously immortalized cell line from pig granulosa cells. Mutations in the promoter region were made as required for studying protein-DNA interactions and promoter activities.

Results: Deletion studies revealed a regulatory region (+10 to +70) downstream from the transcription start site is required for FSH-R promoter activity. Sequence analysis and database search found that this region contained a consensus E-box to which a number of the transcription factors could potentially bind. Gel mobility shift assay with a 26 bp probe (+32 to +57) and nuclear extracts from granulosa cell line (JC410) demonstrated a sequence specific DNA-protein complex. Gel supershift with upstream stimulatory factor-1 and -2 antibodies (USF-1 and -2) confirmed their participation in the complex.

Conclusions: 1) These novel data establish the utility of the porcine ovarian granulosa cell line for studies on receptor gene regulation. 2) The FSH-R gene is one of the USF-1 and USF-2 target genes in the ovary. (Supported by MRC of Canada).

P-138

Molecular Size of Embryotoxins in Humans. M. P. Marynick, L. Zhang, S. P. Marynick. Baylor Center for Reproductive Health, Baylor University Medical Center, Dallas, TX.

Objectives: It is known that some women who have infertility or repeated spontaneous miscarriage have substances in their serum that are toxic to embryos of other species. The embryotoxicity of some but not all of these subjects sera is eleminated with affinity chromatography separation of gamma globulin (IgG) from the sera. The size of the embryotoxic molecules in patients where embryotoxicity is not removed by IgG affinity chromatography is not known. To determine the size of embryotoxins in humans the following study was performed.

Design: Serum from five women with known embryotoxicity as determined by previous mouse embryotoxicity assay (ETA) was dialysed against embryo culture medium using cellulose tubing with a pore size of molecular weight (M.W.) of 12,000. After one week of dialysis the sera was tested with mouse ETA and compared to sera from the same patient collected simultaneously and placed in contact with dialysis tubing but not dialysed. Embryo growth and development was observed and recorded daily until blastocyst hatching occurred.

Materials and Method: IRB approval was obtained to use the sera from this group of patients for this study. Two cell mouse embryos (B6C3F1, Charles Rivers Laboratories, Wilmington, MA) were used for ETA. The embryos were cultured in Human Tubal Fluid (Irvine Scientific, Irvine, CA) with 10% dialysed or non-dialysed patient serum at 37°C in 5% CO_2 in air. Dialysis tubing was standard pore tubing (Union Carbide, Chicago, IL). The significance of the embryo development at different stages was determined by χ^2 test. A level of $P<0.05$ was considered statistically significant.

Results: Dialysed sera were significantly less embryotoxic than sera that were not dialysed at all stages of embryo development ($P<0.001$). Three of the dialysed sera allowed embryo development to hatching blastocyst (HBLST) and four of the five dialysed sera allowed development to the blastocyst (BLST) stage. All dialysed sera allowed embryo development to the morula stage. Conversly, non-dialysed sera did not allow embryo development past the 8-cell stage with the exception of one sample studied which allowed development to the morula stage.

Treatment Group	No. 2 cell	4 cell%	8 cell%	Morula %	BLST %	HBLST %
Negative control	11	100a	100a	100a	100a	100a
Dialysis	55	100a	100a	95a	58b	47b
Non-Dialysis	55	87b	47b	16b	0c	0c
Positive Control	11	18c	0c	0c	0c	0c
P-valve		<0.001	<0.001	<0.001	<0.001	<0.001

Conclusions: 1). Dialysis membrane with a pore size of M.W. 12,000 daltons removed or decreased embryotoxicity from the sera of some but not all patients with known embryotoxicity. 2). Embryotoxic substances in humans appear to be comprised of multiple compounds of varying molecular size.

P-139

Xanthine/Xanthine Oxidase Increase DNA Fragmentation of Human Sperm Nuclei? [1]T. Hamano, [1]H. Miyata, [1]S. Fukumoto, [1]K. Honma, [1]Y. Fujino, [2]O. Kato. [1]Towako Reproduction Center Osaka, Toyotu, Suita, Osaka, Japan, and [2]Kato Ladies Clinic, Nishishinjuku, Shinjuku-ku, Tokyo, Japan.

Objectives: The relationship between mechanisms of apoptosis of sperm and Thiol-Disulfide status (-S-S-) in sperm was investigated. The reactive oxygen species from Xanthine/Xanthine Oxidase (X/XO) increase DNA fragmentation of sperm nuclei. We examined the relationship between thiol-disulfide status and DNA fragmentation on human sperm.

Design: The reactive oxygen-sensitivity for thiol-disulfide status in the ejaculated sperm was verified by the method of terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin end-labeling (TUNEL) and evaluated the relationship between DNA fragmentation and thiol status by thiol-specific fluorescence staining.

Materials and Methods: After approximately 30 min of liquefaction at room temperature, semen was washed in PBS and centrifuged for 10 min at 1000 rpm three times. Sperm samples were treated with Dithiothreitol (DTT) for (-S-S-) active status, and treated with N-ethylmaleimide (NEM) for (-S-H) active status. X/XO-induced DNA fragmentation rate were examined in DTT-treated (1mM, 30 min) sperm or NEM-treated (10mM, 10 min) sperm. After treatment of sperm with DTT or NEM, these sperms were exposed to reactive oxygen species generated with X (200uM)/XO (50 mM), or pretreated with catalase before the X/XO treatment. The percentage of sperm with DNA fragmentation was examined TUNEL method. Thiol-disulfide status were stained with monobromobimane (mBBr), a fluorescent thiol labeling agent.

Results: The rate of DNA fragmentation for original sperm was 7% with TUNEL. The rate of DNA fragmentation for DTT-treated and NEM-treated sperms were 36.3% and 8.2%, respectively. The DTT-treated sperms were labeled with mBBr, but the NEM-treated one were not labeled with mBBr. The X/XO exposure for DTT-treated sperm decreased in the rate of DNA fragmentation. However, X/XO exposure for NEM-treated sperm no decreased in DNA fragmentation. At that time, X/XO exposure decreased in the percentage of DTT-treated sperm labeled with mBBr from 100% to 82%. In the presence of catalase, the percentage of DTT-treated sperm with DNA fragmentation and that of mBBr positive sperm were not affected by the X/XO-exposure.

Conclusions: X/XO exposure for DTT-treated sperm decreased nuclear DNA fragmentation until 30 min. It's decrease was related to the number of mBBr-positive sperm. NEM treated sperm was not influenced both DNA fragmentation or mBBr-stain. While the thiol in DTT-treated sperm exhibited the high untoxicity for the reactive oxygen generated with X/XO, the results were suggested with oxidation of the thiol to the disulfide in sperm nuclei. We considered that the thiol in the sperm nuclei maybe necessary in untoxicity to oxidation, but the disulfide in sperm nuclei could be subsequently interfered with the reactive oxygen. From the inactivation experiment of reactive oxygen with catalase, the thiol in the sperm nuclei could be interfered with X/XO-induced DNA fragmentation.

P-140

Transfer of Zona-Free Embryos Improves Outcome in Poor Prognosis Patients: A Prospective Randomized Controlled Study. R. G. Mansour, M. A. Aboulghar, G. I. Serour, A. Kamal, C. A. Rhodes, N. Tawab. The Egyptian IVF-ET Centre, Maadi, Cairo 11431, Egypt.

Objective: Assisted zona hatching (AZH) has been used in IVF for several years, most successfully in women with a poor prognosis. Pregnancies using zona-free blastocysts have been reported. Our objective was to evaluate outcomes after transfer of zona-free embryos.

Design: A prospective randomised controlled study was done on the effect of removal of the zona pellucida (ZP) on clinical pregnancy rates after the transfer of human embryos on day 3.

Materials and Methods: Group A consisted of 52 women under the age of 40 years undergoing their first ICSI attempt. Group B included a poor prognosis group of 71 women aged 40 years or more in their first ICSI cycle, or those of any age with two or more previous failed attempts. In Group A alternate cases were randomised to undergo embryo transfer with or without complete ZP removal on day 3 after oocyte retrieval. In Group B the randomisation was in a 3:4 ratio. The embryos were exposed to acidic Tyrode's solution for a few seconds to remove the ZP, followed by repeated rinsing in tissue culture medium to remove any traces of the Tyrode's solution. The mean ages in the zona-free and zona-intact groups were not significantly different within Groups A and B.

Results: Results are shown in Table I. In Group A the differences in fertilisation rate (FR) and pregnancy rate (PR) were not statistically significant. In group B, the FR and PR in the zona-free group were 63% and 23%, compared with 61% and 7% in the zona-intact group. The PR was significantly higher when zona-free embryos were transferred in the poor prognosis group ($p < 0.05$, Fisher's exact test).

Table I: Results	Group A: <40 yrs and first trial of ICSI,	
	Group A	
	ZP removed	ZP intact
Cycles (no.)	27	25
Mean age (years ± S.D.)	32.1 ± 2.5	33.2 ± 1.4
No. oocytes retrieved	285	294
No. MII oocytes	237	231
No. 2PN oocytes	150	148
Fertilisation rate (%)	63%	64%
Mean no. embryos per transfer	3.2	3
No. clinical pregnancies (%)	12 (44)	10 (40)

Table I: Results	Group B: >=40 yrs or repeated failures.	
	Group B	
	ZP removed	ZP intact
Cycles (no.)	30	41
Mean age (years ± S.D.)	37.3 ± 5.6	36.4 ± 5.2
No. oocytes retrieved	268	330
No. MII oocytes	233	270
No. 2PN oocytes	147	165
Fertilisation rate (%)	63%	61%
Mean no. embryos per transfer	3.9	3.8
No. clinical pregnancies (%)	7 (23)*	3 (7)*

* $p < 0.05$.

Conclusions: The complete removal of the zona pellucida before embryo transfer improved pregnancy rates in a group of poor prognosis women undergoing ICSI. In patients under the age of 40 years in their first cycle of ICSI, zonal removal did not significantly improve outcome, but complete ZP removal using acid Tyrode's solution had no deleterious effect on implantation.

P-141

Precise Expression Pattern of the Growth Differentiation Factor-9 (GDF-9) mRNA in the Mouse Ovarian Follicles at the Different Developmental Stages. [1,2]K.-A. Lee, [2]S. H. Lee, [2]S. J. Yoon, [3]D. Geum, [3]K. H. Choi, [3]K. Kim. [1]College of Medicine, Pochon CHA University, [2]Infertility Medical Center, CHA General Hospital, and [3]Department of Molecular Biology, Seoul National University, Seoul, Korea.

Introduction and Objective: GDF-9, as an oocyte-derived growth factor, has been known to play a crucial role in the folliculogenesis. The present study was accomplished to determine the precise expression pattern of the GDF-9 mRNA in the mouse follicles at the different developmental stages.

Materials and Methods: To determine the exact expression of GDF-9 mRNA at the different sizes, follicles were isolated mechanically from 4 weeks old mice ovaries in the Leibovitz medium and sorted into 4 groups by their diameters, such as 1) <50 μm, 2) 50–100 μm, 3) 100–150 μm, and 4) >150 μm. Comperitive RT-PCR for GDF-9 mRNA was prepared as follows. After RT-PCR (the upper primer, 5′-tccttgctgctgcctgtagatg-3′; the lower primer, 5′-gggtgcttgtgtcgttgagata-3′), the product was cloned into the pGem vector. Mutant competitor RNA was transcribed in vitro from a cDNA clone with 133 bp deletion. To measure the relative amounts of competition, RNA isolated from 2 primordial follicles (<50 μm) was reverse transcribed with serially diluted mutant cRNA (1fg, 0.1fg, 0.01fg, 0.001fg, 0.0001fg), and then 4 μL aliquot of RT product was used for PCR. The pre-PCR denaturation was at 95°C for 10 minutes, first PCR was done 53 cycles (94°C for 40 seconds, 60°C for 1 minute, 72°C for 1 minute), second PCR was done 5 cycles (85°C for 40 seconds, 60°C for 1 minute, 72°C for 1 minute), and post-PCR denaturation was at 72°C for 10 minutes. To quantify the GDF-9 mRNA in the mouse primodial follicles, serially diluted native GDF-9 cRNA (1fg, 0.3fg, 0.1fg, 0.03fg, 0.01fg, 0.003fg, 0.001fg) was subjected to RT-PCR in the presence of 0.05 fg mutant cRNA. Each PCR products were analyzed on 2% agarose gel followed by ethidium bromide staining. Total RNA from 8 follicles at each sizes were subjected to competitive RT-PCR with 0.05fg of mutant GDF-9 cRNA. The log value of ratio between competitor RNA and native RNA was quantified in each sample and the results were plotted against the log amount of native RNA. In situ localization of the GDF-9 mRNA expression is under investigation.

Results: By RT-PCR with serial dilution of mutant RNA, it was determined that the GDF-9 mRNA of the primordial follicles compete 1:1 with mutant cRNA between 0.01fg and 0.001fg concentration, so 0.05fg was used for following experiments. GDF-9 mRNA was observed from the smallest primordial follicles and the amount of GDF-9 mRNA was abruptly increased in group 3 (follicle sizes of 100–150 μm).

Conclusion: Results of the present study suggest that: 1) GDF-9 mRNA expressed at all sizes of the early developmental stage follicles, and 2) the GDF-9 may play an important role during the differentiation of the primary follicles to the secondary follicles (at the size of 100–150 μm) in the mouse ovaries.

P-142

ERα Activation by Brx, a Novel Dbl Oncoprotein Family Member, is Ras-Independent and Specifically Requires the Small GTPase, Cdc42Hs. D. M. Rubino, J. H. Segars, P. H. Driggers. Dept. OB/GYN, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814.

Background: A pathway of estrogen receptor activation by growth factor receptors has been defined. Epidermal growth factor (EGF) reproduces many of the effects of estrogen and the mechanisms by which these pathways converge is actively being studied. Activation of estrogen receptor alpha (ERα) by EGF was shown to involve phosphorylation of serine 118 (Ser 118) in the amino terminus of ERα through a Ras-Raf-mitogen activated protein kinase (MAPK) signaling pathway. Our published data demonstrated that Brx, a novel Dbl family member, bound to human ERα and augmented ligand-dependent gene activation by ERα. We showed that activation of ERα by Brx required Cdc42Hs, a Rho GTPase family member, revealing a novel signaling pathway for estrogen action. Additionally, Rho GTPase family members have been shown to be required for Ras transformation, an observation placing Rho GTPases downstream of Ras in some signaling pathways and suggesting the possibility that Ras could play a role in the activation of ERα by Brx.

Objective: To determine if the Ras-MAPK pathway is involved in modulating Brx action on ERα in Ishikawa human endometrial cells.

Method: Plasmids encoding ERα, Brx, and an estrogen-response element (ERE) containing luciferase reporter were transiently cotransfected using lipofectamine into Ishikawa human endometrial cells. For the experiments involving EGF the cells were serum starved overnight prior to transfection and subsequently treated with media containing no serum. A mutant form of ERα in which serine 118 was mutated to alanine (ERαSA) was generated by overlap PCR, subcloned, sequenced, and found to be functional in our transient transfection assay system. An expression plasmid encoding a dominant interferring mutant for Ras was cotransfected to determine if activation by Brx could be inhibited.

Results: 1) The growth factor EGF did *not* modify the activity of Brx on ERα ligand-dependent action in transient transfection assays; 2) co-transfection experiments with Brx, ERα, and ERαSA demonstrated that Serine 118 of ERα was *not* required for enhancement of ERα activity by Brx; and 3) the small GTPase Ras was *not* involved in the activation of ERα by Brx. Ectopic expression of the dominant interferring Ras mutant did not inhibit activation of ERα by Brx. In addition, we tested the effects of PD98059, a specific mitogen-activated protein kinase kinase (MAPKK) inhibitor, on Brx action and found that Brx action was not diminished, lending further support for the lack of a role for Ras.

Conclusion: Ligand-dependent activation of ERα by Brx specifically required the small GTPase Cdc42, and did not appear to involve the Ras-MAPK pathway.

REPRODUCTIVE IMMUNOLOGY

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P-143

Levels of Antiphospholipid Antibodies (APA) in Serum During Controlled Ovarian Hyperstimulation (COH); Relationship to Serum Estradiol (E2) Level, Day of Stimulation, and Pregnancy Outcome. [1,2]B. A. Stone, [3]J. M. Vargyas, [3]G. E. Ringler, [3]A. L. Stein, [2,3]R. P. Marrs. [1]Reproductive Technology Laboratories, [2]Institute for Fertility Research & [3]California Fertility Associates, Santa Monica, CA.

Objectives: Venous thrombotic events have been reported following COH of patients with SLE, and elevated baseline APA levels. With respect to the established thrombogenic action of E2, it has been postulated that E2-induced APA rise during COH may mediate the increased thrombotic risk in SLE. This study analyses APA against serum E2 during COH in patients undergoing COH for IVF.

Design: Analysis of APAs in 318 sera drawn from 32 patients (19 of whom conceived) during COH.

Methods: Sera were drawn through GnRHa down-regulation, during COH, and on days 7 and 14 after oocyte retrieval. Anticardiolipin (aCL) IgG/M, and antiphosphatidylserine (aPS) IgG/M were determined by microplate ELISA. E2 was measured by RIA. APA values were analysed against day of downregulation/stimulation and pregnancy outcome by ANOVA. Relationships between E2 and APAs were analysed by linear regression. Projection of outcomes from serum APA levels was analysed by ROCs.

Results: Serum levels of APAs did not differ between days ($P=0.42$), and levels of APAs and E2 were not correlated across the E2 range <10–5032 pg/mL. Mean (±SE) levels of aCL IgM were lower ($P<0.0001$) in patients who maintained pregnancy (2.24±0.12 MPL/mL) or miscarried (1.81± 0.24) compared with patients who did not conceive (4.10±0.27). Levels of aPS IgM also differed between these outcome groups ($P<0.0001$), with similar relative differences between groups (10.91±0.59, 9.36±2.15 and 17.56±1.09 MPS/mL respectively). Levels of neither aCL nor aPS IgG differed between the groups of different outcome. By ROC analysis, a serum aCL IgM cutoff of 3.0 MPL/mL (pregnant lower, non-pregnant higher) yielded a 89% sensitivity in projecting pregnancy outcome, with 69% specificity and 81% accuracy. A serum aPS IgM cutoff of 16 MPL/mL yielded respective ROC values of 76, 62 and 75%. These derived cutoffs for projecting a pregnancy outcome are substantially lower than established limits for the population-at-large (11 and 22 MPL/mL respectively).

Conclusions: High levels of aCL IgM (>3 MPL/mL) or aPS IgM (>16 MPL/mL) project a low likelihood of pregnancy following COH and IVF. IgG levels are not predictive. APA levels do not change significantly across a broad range of serum E2 values, through GnRHa down-regulation, during COH, or in early pregnancy. Levels of aCL IgM are highly predictive (81%) of the likelihood of pregnancy, and this testing can therefore be performed at any stage of the patient's workup.

P-144

White Blood Cell Subsets Among Women Undergoing IVF. [1]B. R. Witt, [1]H. K. Amin, [1]J. J. Stangel, [2]A. Rubenstein, [1]D. H. Barad. [1]Departments of Obstetrics & Gynecology and Women's Health and [2]Pediatrics, Albert Einstein College of Medicine, Bronx, NY.

Objective: Immunologic factors have been implicated in implantation failures with IVF-ET. The purpose of this study was to prospectively evaluate white blood cell (WBC) immunophenotypes among patients prior to IVF-ET and to correlate these results with clinical pregnancy establishment.

Materials and Methods: Sixty-six women had blood obtained for WBC immunophenotyping prior to undergoing an IVF-ET cycle. Analysis was performed using fluorescently labeled monoclonal antibodies and FACSsort (Becton & Dickinson, San Jose, CA). The cell surface markers included those for total lymphocytes, T-helper cells, T-suppressor cells, total NK cells, and cytotoxic NK cells. Leukocyte subsets were reported as % of total lymphocytes and in absolute numbers. Statistical analysis including descriptive statistics and Students t-test was performed using SPSS statistical software.

Results: Among the 66 participants, 60 began stimulation for an IVF cycle, 11 were cancelled prior to retrieval, and 2 patients had no transfer. Thus, 47 patients underwent ET and were divided into 2 groups. Group I ($N=16$) became pregnant and Group II ($N=31$) did not. There were no significant differences between the groups with regard to age, gravidity, or baseline FSH levels. There was a trend ($p=0.056$) toward higher mean (± SE) total WBC counts ($\times 10^3$/ul) in Group I (6.9 ± 0.4) vs. Group II (5.8 ± 0.3). Mean (± SE) total T-cell count was significantly higher ($p = 0.02$) in Group I (1618/ul ± 93) vs. Group II (1335/ul ± 69), and mean (± SE) T-helper cell count was also significantly higher ($p = 0.03$) in Group I (1030/ul ± 76) vs. Group II (816/ul ± 52). There were no significant differences with regard to T-suppressor cells, total NK cells, or cytotoxic NK cells, or between any of the subsets as a percentage of the total. Cytotoxic NK cell absolute counts and percentages were 183/ul ± 16 and 8.6% for Group I and 157/ul ± 18 and 8.8% for Group II.

Conclusions: Among 47 patients who underwent ET, patients with failed implantation (non-pregnant) had lower T-cell and T-helper cell counts than those who became pregnant. There were no differences with regard to natural killer cell populations in this preliminary study. The significance of these findings is unclear and warrants further investigation.

P-145

Ovarian Antibodies, FSH and Inhibin: Independent Markers Associated with Unexplained Infertility. [1]J. Luborsky, [1]B. Llanes, [2]R. Roussev, [2]C. Coulam. [1]Obstetrics & Gynecology, Rush Medical College, Chicago IL, and [2]Center for Human Reproduction, Chicago, IL.

Objective: Ovarian autoimmunity has been shown to be associated with a significant proportion of unexplained infertility (Luborsky, *J Clin Immunol Immunopath* 90(3), 1999). The expectation was that ovarian antibodies (evidence of autoimmunity) would be associated with elevated FSH. However, FSH levels are normal in a majority of patients with unexplained infertility. The objective was to determine if ovarian autoimmunity was associated with elevated FSH and decreased inhibin, markers of follicle function, in unexplained infertility. The hypothesis that ovarian antibodies (OVAB) and markers of follicle function are not correlated was tested.

Design and Methods: Serum was obtained on day 3 of a natural cycle with IRB approval. OVABs were detected by immunoassay (*JCEM*, 1990, 70: 69) and results considered positive at 3SD above the mean of control sera (normally cycling women) ($p<.01$). FSH was measured by a chemiluminescence assay (Chiron) and inhibin B by immunoassay (Serotec).

Results: Ovarian antibodies were detected in 19/52 women with unexplained infertility, 0/3 males, 0/4 premenopausal and 2/7 postmenopausal women. The women with unexplained infertility (mean age, 34.9±4.9, range 20–44), had normal day 3 FSH (<10 mIU/ml, mean 6.3±1.9) or

elevated day 3 FSH (>10 mIU/ml, mean 15.8±4.7). Of these 3/10 (30%) had both elevated day 3 FSH and OVAB, while 17/42 (40%) had both normal FSH and OVAB. Of the women with normal FSH, only 2/7 (29%) had low inhibin B levels (<30pg/ml) and OVAB, while 15/35 (43%) had normal inhibin B levels (>30pg/ml) and OVAB. Pregnancy outcome data is currently available for 18 of 52 women with unexplained infertility. Of these, no pregnancies resulted in women with OVAB (n=6), and 4 pregnancies occurred in women negative for OVAB (n=12) (p=.06).

Conclusions: Thus, (1) OVAB are associated with elevated FSH, *but* (2) OVAB are also significantly correlated with normal FSH and inhibin B levels (and not normal FSH and decreased inhibin B levels). The results suggest that OVABs are an independent marker of unexplained infertility and may provide an additional predictor of poor IVF outcome and potential ovarian failure.

P-146

Effects of Cytokines and Leptin on Trophoblastic Expression of Invasive Markers. [1]R. R. Gonzalez, [1]L. Devoto, [2]A. Campana, [2]P. Bischof. [1]Inst. Maternal & Child Res. (IDIMI), Univ. Chile, Santiago, Chile, P.O. Box 226-3. [2]Dept Obst Gyn, WHO Collab. Centre Hum. Reprod., Univ. Hosp. Geneva, Geneva, Switzerland.

Objectives: Integrin switching and proteinase (metalloproteinase, MMP) secretion are characteristic markers of invasive human cytotrophoblast (CTB). We used CTB cultures to investigate the effects of interleukin-1 alpha (IL-1α), interleukin-6 (IL-6), transforming growth factor beta (TGF-β) and the novel placental hormone leptin, on the expression of the α2, α5 and α6 integrin subunits and on the activity of MMP-2 (gelatinase A) and MMP-9 (gelatinase B).

Design: CTB cultures from first trimester legal abortions were used to study the effects of tested compounds on expression of integrins and MMPs.

Materials and Methods: Immunocytochemistry was used to study the integrin expression. MMP-2 and MMP-9 activities were determined in the culture supernatants (24 h) by zymography. Statistical analysis was made by ANOVA using the Statview program (Abascus).

Results: The α2 subunit was only marginally upregulated by IL-1α. All cytokines significantly upregulated α5 expression and leptin did so only at the high dose tested. The α6 integrin subunit was massively upregulated by the interleukins, TGF-β and leptin. None of the factors tested affected MMP-2 activity but the activity of MMP-9 was upregulated by IL-1α and leptin. TGF-β and IL-6 remained without effect on this parameter.

Conclusions: IL-1 and leptin actively induce some of the changes that CTB undergo to achieve a more invasive phenotype. TGF-β and IL-6 have similar upregulatory effects on α5 and α6 integrin subunit expression but remain without effects on MMP-9 activity. A novel role for leptin is proposed during early pregnancy: leptin might be an autocrine/paracrine regulator of CTB invasiveness during implantation and placentation.

P-147

Expression of the Interleukin-1 System (IL-1α, IL-1β and IL-1 Receptor Antagonist) in Mature and Immature Mouse Testicular Cells. [1,2,3]M. Huleihel, [1,3]E. Lunenfeld, [1,4]D. Zeyse, [1,3]I. Prinsloo, [1,3]G. Potashnik, [1,3]M. Mazor. [1]Dept. of Obstetrics and Gynecology, Soroka University Medical Center of Kupat Holim and [2]Department of Microbiology and Immunology, [3]Faculty of Health Sciences Ben-Gurion University of the Negev, Beer-Sheva, Israel. [4]Christian-Albrechts-University of Kiel Medical School, Kiel, Germany.

Objectives: To characterize the cellular source(s) and the levels of IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra) in the testis of mature and immature mice under normal and pathological conditions.

Design: The study included mature and immature Balb/c male mice.

Materials and Methods: Mature (8 weeks) and immature mice (2 weeks) were injected (i.p.) with saline or LPS (10μg/mouse). Mice were killed after 4, 16 and 24 hours, following which their testicular tissues were examined by immunohistochemical staining for cell source and level of expression of IL-1α, IL-1β and IL-1ra. Formaline- and/or B5-fixed paraffin-embedded testicular tissues were stained using polyclonal rabbit anti-mouse IL-1α, or anti-mouse IL-1β, or anti-mouse IL-1ra primary antibodies. As a negative control for IL-1α, IL-1β and IL-1ra tissues were stained with the secondary antibody only.

Results: Testicular tissues from mature and immature mice highly expressed IL-1α in both seminiferous tubule cells and Leydig cells. However, low expression of IL-1β (compared to the expression of IL-1α) was found in seminiferous tubule cells of mature and immature mice. IL-1β was also expressed in Leydig cells of mature mice but not in immature mice. IL-1ra was shown to be highly expressed in seminiferous tubule cells but less than in Leydig cells of mature and immature mice. In addition, prominent expression of IL-1ra was observed in the Golgi apparatus of tubular and Leydig cells. The levels of IL-1α, IL-1β and IL-1ra in testicular tissues from mature and immature mice were not affected by LPS injection.

Conclusions: Our results may indicate that IL-1, IL-1β and IL-1ra are expressed in germ cells and/or somatic cells in mature and immature mice. However, IL-1β is not expressed in Leydig cells of immature mice, but it is expressed in germ and/or Sertoli cells. IL-1α was expressed in germ cells and/or Sertoli cells of immature mice. IL-1α was more pronounced than IL-1β in testicular tissues. LPS injection to the mice did not affect the expression of the IL-1 system in the testicular cells. Thus, IL-1 may be involved in the physiological functions of testicular tissue and the process of spermatogenesis.

P-148

A New Anti Ovary Antibody Test Kit, Based on Enzyme Linked Immunosorbent Assay (ELISA). B. Rivnay, D. MacCorkle. Repromedix Fertility Lab, Woburn.

Objectives: Infertility has commonly been associated with endocrine dysfunction or anatomic defects in the reproductive system. Immunological causes for infertility have also been well established through studies showing that various auto-antibodies interfere with normal reproductive functions and that patient's own immune system may reject the developing embryo. High levels of auto-antibodies to the ovary interfere with normal ovarian function, affecting induced ovulation in assisted reproductive technology (ART) and, in extreme cases, leading to premature ovarian failure (POF). In light of these findings our objective was to develop an ELISA kit for anti ovary antibodies and thus enable a widespread standardized testing for the presence of these auto-antibodies in serum of patients with POF, or patients with failed *in vitro* fertilization (IVF) cycles.

Design: We selected the ELISA format using ovarian membrane fragments as antigen and the alkaline phosphatase as the conjugated enzyme.

Materials and Methods: Positive and negative patient sera, previously tested in a home-brewed laboratory assay, were used to standardize and determine performance of the various components of the kit. Enzymatic activity was tested with phenyl phosphate as substrate, and read at 492 nm. All the components were examined for long-term stability using an accelerated stability paradigm of 10 days at 37°C. Absorbance was converted to units of μg IgG/ml using purified human IgG as standard.

Results: The kit is based on detachable 8 well strips of a 96 well plate, coated with ovarian membranes. It includes a positive control, 4 calibrators with a predetermined level of antibodies (IgG), the enzyme (Alk.Phos.) conjugated to anti human IgG, substrate and buffers. The assay has a sensitivity of 1 μg IgG/ml, and the detection is linear up to 40 μg IgG/ml. The intrarun variability is 7%. The assay involves 3-hrs and 20 min incubation and 4 pipettings. Measurements done by this assay yield results that are predictive of a clinical disorder. Recent clinical data compiled in our lab show that 40–50% of patient serum samples submitted for testing due to POF or failed/canceled IVF cycles were positive in the AOA test, suggesting this to be a clinically important laboratory test. Immunosuppressive treatment for patients with positive AOA results has been shown to effectively open a fertility window in such patients.

Conclusions: We present here a new 96 well plate ELISA kit comprising the full complement of reagents (9 components) necessary for measuring anti ovary antibodies. This assay, together with existing endocrine and autoimmune tests, would be helpful in identifying the etiology of infertility, and expedite selection of treatments.

REPRODUCTIVE SURGERY

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P-149

Laparoscopic Oophoropexy and Ovarian Function in the Treatment of Hodgkin's Disease. [1]R. S. Williams, [1]R. D. Littell, [2]N. P. Mendenhall.

[1]Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville FL, and [2]Department of Radiation Oncology, University of Florida College of Medicine, Gainesville, FL.

Objective: High survival rates in the treatment of Hodgkin's Disease (HD) have permitted the consideration of salvaging of reproductive function in female patients who are premenopausal. Total nodal irradiation (TNI) of the pelvis invariably will result in premature ovarian failure (POF) unless the ovaries are shielded. Transposition of the ovaries away from the pelvic nodal fields at staging laparotomy has had mixed success, probably because of migration of the ovaries to their original position, as irradiation is often delivered months after the procedure. We have previously described a laparoscopic method of oophoropexy that may be performed immediately prior to pelvic irradiation. We now report follow-up of twelve women who underwent laparoscopic oophoropexy at the University of Florida College of Medicine.

Design: Rates of intact ovarian function, POF, and pregnancy were determined from clinical follow-up of all women who underwent laparoscopic oophoropexy at the University of Florida.

Materials and Methods: Twelve patients underwent laparoscopic oophoropexy from 1989 to 1995. A permanent suture was used to approximate the uterovarian ligaments posterior to the uterine fundus. Medical records were reviewed of all 12 patients. Two patients were excluded from analysis: one for a death soon after treatment, and one for a second malignancy where radiation was aborted. The endpoints of ovarian function and fertility were documented by menstrual history, gonadotropin levels, presence of menopausal symptoms, pregnancies achieved, and pregnancy attempted.

Results: At follow-up, 5 patients had evidence of ovarian function, and the 4 women who desired children achieved pregnancies. All had to 0 to 2 courses of chemotherapy. Two patients who subsequently had pregnancies had staging laparotomy with oophoropexy five and six months, respectively, before laparoscopy. In both cases the ovaries had migrated back to their original position by the time of laparoscopy. Five patients had ovarian failure at follow-up. Four of the 5 had received multiple courses of combination chemotherapy; the other had pelvic primary disease and received 3500 cGy to the pelvis with little central shielding.

Conclusions: 1) Laparoscopic oophoropexy performed immediately prior to pelvic irradiation is effective in preserving ovarian function and fertility in nearly all patients who are to undergo total nodal irradiation for Hodgkin's Disease, and who receive minimal or no chemotherapy. 2) Total nodal irradiation would have resulted in ovarian failure in the 2 women who had oophoropexy performed at staging laparotomy 5 to 6 months before receiving treatment, had the laparoscopic repeat procedure not been performed.

P-150

The Impact of Insulin Resistance on the Outcome of Laparoscopic Electrocautery for the Induction of Ovulation in Infertilite Women With the Polycystic Ovary Syndrome. P. O. Dale, T. Tanbo, T. Åbyholm. Department of Gynecology and Obstetrics, The National Hospital, University of Oslo, Oslo, Norway.

Objective: Insulin resistance is established as an important pathophysiological mechanism in the development of the polycystic ovary syndrome (PCOS). We have earlier shown that insulin resistance may correlate with poor pregnancy rates following ovulation induction with gonadotropins in PCOS infertility[1]. In this study we wanted to assess the impact of insulin sensitivity on the outcome of ovarian electrocautery.

Design: Ongoing prospective cohort study, so far 22 patients have completed the follow-up period.

Materials and Methods: PCOS was defined as the presence of polycystic ovaries on vaginal ultrasound combined with menstrual disorders and anovulation. All women had tried ovulation induction with clomiphene citrate for at least 3 cycles and all partners semen samples were considered normal. Patients were included in a consequetive fashion. Insulin sensitivity was assessed by means of the Continuous Infusion of Glucose with Model Assessment (CIGMA) test[2]. A test value >4 was considered indicative of insulin resistance. Laparoscopic electrocautery of the ovaries on at least 8 points per ovary was performed under general anesthesia. If the woman did not conceive spontaneously during the first 6 months, she got an additional 3 cycles of clompihene induction of ovulation for a total of 12 months follow-up period.

Results: Following the CIGMA test 10 women were classified as insulin resistant while 12 women were classified as non-insulin resistant. There were no complications following the laparoscopy. Both groups achieved a more regular menstrual pattern and lower levels of LH and androgens following the ovarian electrocautery. Frequency of ovulation as judged by progesteron levels was similar between groups while 2 of the 10 insulin resistant women and 3 of the 12 non-insulin resistant women achieved pregnancy.

Conclusions: Outcome of ovarian electrocautery seems similar in insulin resistant and non-insulin resistant infertile women with PCOS.

[1]Dale PO et al. The impact of insulin resistance on the outcome of ovulation induction with low-dose FSH in women with PCOS. Hum Reprod 1998;13:567–70.

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P-151

Low Malignant Potential Ovarian Tumor and the Desire for Future Fertility. [1]C. H. Nezhat, [1]A. A. Alperin, [1,2]F. R. Nezhat, [1]C. R. Nezhat. [1]Dept of GYN/OB, Stanford University School of Medicine, Stanford, CA, [2]Dept of OB/GYN Mount Sinai Medical Center, New York, NY.

Objective: Ovarian carcinomas of low malignant potential account for 8–20% of epithelial cancers[1] and are associated with an excellent prognosis regardless of stage. The majority of the tumors occur in women of childbearing age often resulting in dilemmas in regard to treatment options versus fertility. We report a case of stage III-C ovarian cancer of low malignant potential and subsequent pregnancy after in vitro fertilization.

Design: Case report.

Materials and Methods: A 28 year old nulligravida woman underwent diagnostic laparoscopy for evaluation of chronic pelvic pain and infertility. Findings included, adhesions involving the posterior cul-de-sac and both adnexa. The left side was more severe with hydrosalpinx and vegetation of the left tube. Both ovaries had cystic structures with simple characteristics. The left ovary had multiple cortical papillary lesions similar to the peritoneal lesions involving the posterior cul-de-sac and pelvic sidewall. Left oophorectomy as well as peritoneal washings and multiple biopsies were performed. The left ovarian lesion revealed low malignant potential ovarian tumor. Frozen biopsy of the right ovarian cyst revealed serous cystadenoma with focal atypia only. The procedure was terminated pending final pathology. The later revealed serous papillary cystadenoma of low malignant potential of the left ovary with serous papillary implants of the left tube, omentum and peritoneal biopsies. Right ovarian biopsy confirmed frozen section report and was negative for malignancy. The patient was fully counseled regarding options and risks. She elected to preserve her remaining reproductive organs and pursue in vitro fertilization (IVF). She underwent 4 cycles of IVF in 11 months.

Results: She conceived during her fourth cycle of IVF resulting in the delivery of twins by elective cesarean section at 41 weeks. Two adhesive bands found on the uterus and adnexa were excised. Pathological diagnosis was negative for malignancy. Subsequently she underwent laparoscopic hysterectomy and removal of her remaining ovary, which was negative for residual disease. Follow-up examinations up to 2 ½ years have been within normal limits.

Conclusions: Patients with tumors of low malignant potential and the desire for future fertility require extensive counseling on the nature of the disease and the potential morbidity associated with conservative management. Careful follow-up is necessary. Although, the potential carcinogenesis of controlled ovarian hyperstimulation remains a concern, it did not appear to have a stimulating effect on the low malignant tumor in this patient.

Reference: [1]The Ovarian Tumor Panel of the Royal College of Obstetrics and Gynecologists. Ovarian epithelial tumors of borderline malignancy: pathological features and advanced states. Br J OB/GYN 1983;90:743–750.

P-152

The Value of Laparoscopy in Unexplained Infertility. [3]A. T. Cheng, [1–3]S. L. Corson, [1–3]J. N. Gutmann. [1]Women's Institute for Fertility, Endo-

crinology and Menopause, [2]Thomas Jefferson University, [3]Pennsylvania Hospital, Philadelphia, PA.

Objective: Historically the work up for the "unexplained infertility" patient has included diagnostic laparoscopy. This study focused on the value of diagnostic laparoscopy in patients unlikely to have abnormal pelvic findings at surgery: no prior surgery, no previous history of pelvic infection, normal hysterosalpingogram, normal pelvic ultrasound, no symptoms or signs of endometriosis, and normal seminal parameters.

Design: Retrospective chart review of primary and secondary infertility patients from private infertility practice.

Materials and Methods: Medical records of the first one hundred consecutive patients seen from January 1, 1994 to December 31, 1997 who met inclusion criteria were retrospectively reviewed. Patients were followed to pregnancy or cut off date period. Findings at the time of laparoscopy with subsequent surgical therapy, IV/GIFT, intrauterine insemination, and ovulation induction cycles were analyzed and correlated with reproductive outcome. Statistical analyses include Kruksal-Wallis analysis, life table analysis, Cox regression, T-tests, and chi-square performed on SYSTAT software.

Results: The mean female age was 32.7 ± 4.5 years, range 23–42. The duration of infertility was 28.0 ± 19.3 months, and the treatment/observation interval was 17.1 ± 12.4 months. Infertility was primary for 79% of couples. Pelvic anatomy was normal in 32% of patients including 8 with PCOS-like ovaries and 12 with seedling subserosal myomas. Tubes were normal in 76%, mildly abnormal in 8%, moderately abnormal in 14%, and severely damaged in 2%. Endometriosis was staged I/II (graded by ASRM criteria) in 23%, stage III in 13%, and stage IV in 6% despite the negative history for pelvic pain. Surgical therapy was performed in 60/68 patients with abnormal findings. Pregnancy was achieved by 14/30 with GIFT, 13/26 with IVF, and in 40 with combination IUI ± ovulation induction with or without operative intervention. Patients with negative findings on laparoscopy had a pregnancy rate of 69.9% compared with 48.0% for those with pelvic pathology (excluding those who conceived with IVF or GIFT). Patients with operative treatment of pelvic pathology also took longer to conceive by lifetable analysis although the difference did not reach statistical significance.

Conclusion: The current trend towards health cost containment as well as rationed health care caused us to take a second look at the value of laparoscopy in an infertile population thought to be at low-risk for significant pathology. In some cases laparoscopic findings significantly accelerated the decision to move to ART; in others operative intervention may have been responsible for increased fecundity. In light of the high prevalence of pathology, omitting laparoscopy from the work-up seems ill-advised; on the other hand considering the number of interventions, office laparoscopy is not an attractive alternative screening/therapeutic technique.

P-153

Ovarian Remnant Syndrome: A Challenging Diagnosis for the Advanced Laparoscopic Surgeon. A. Vidali, H. Reich, N. Husami, J. Evanko, S. Ribeiro, J. Rosemberg. College of Physicians and Surgeons of Columbia University, New York, NY.

Objectives: To identify the risk factors in the laparoscopic treatment of ovarian remnant syndrome, a rare complication following total abdominal hysterectomy. The incidence of ovarian remnant syndrome is unknown and only small series of cases have been published in the literature. Most common symptoms are pelvic pain, pelvic pressure or rectal pressure. A pelvic mass is usually either palpable or visible by ultrasound and serum estrogen levels can be premenopausal.

Design: Analysis of a series of 29 cases ovarian remnant syndrome that were treated laparoscopically.

Patients: 29 patients underwent laparoscopic with a diagnosis of ovarian remnant. Mean patient age was 37.7 years. Additional Diagnoses included severe pelvic pain (100% of patients). Pelvic mass in 41% of cases (12/29). 75% of the patients (22/29) had a pathologically documented history of severe endometriosis. All of the patients had a prior Bilateral Salpingo Oophorectomy (BSO) either in conjunction with a hysterectomy or as a subsequent procedure. Average number of prior laparoscopies was 2.3 (range 0–8) Average number of prior laparotomies was 2.6 (RANGE 0–8).

Results: Average surgery time was 185 mins (range 70–640). Associated procedures were the following: resection of deep rectal endometriosis 44% (13/29), ureterolysis 58% (17/29), Appendectomy 10.3% (3/29), ureteral stent placement 3% (1/29). Associated bowel surgery was performed in 5 patients (15%); procedures included endoscopic repair of small bowel enterotomy 6% (2/29), minilaparotomy and small bowel resection 3% (1/29), laparoscopic anterior rectal resection 6% (2/29).

Ovarian tissue was confirmed at pathology in 75% of cases (22/29). All negative cases had a pathologically confirmed diagnosis of fibromuscular tissue and endometriosis.

Conclusions: Ovarian remnant syndrome is frequently associated with endometriosis in patients who have undergone multiple surgeries. Concomitant procedures at laparoscopy frequently include bowel surgery. Treatment of this condition is challenging and should be carried out only by skilled surgeons in a multidisciplinary setting.

P-154

Reduction in Post-Surgical Adhesion Formation by Neutralization of the $\alpha v\beta 3$ Integrin in the Rabbit Model. [1]D. N. Sacks, [1]M. J. Murray, [1]Y. Gui, [1]A. Mohamad, [2]M. J. Illera, [3]D. A. Cheresh, [1]B. A. Lessey. [1]University of North Carolina, Department of Obstetrics and Gynecology, Chapel Hill, NC, [2]Universidad Complutense, Madrid, Spain and [3]Scripps Research Institute, La Jolla, CA.

Objective: Adhesion formation occurs in 50–100% of patients after abdominal surgery, of which appendectomy and gynecologic procedures are the two most commonly cited. Clinically significant complications of adhesions include infertility, chronic pelvic pain and bowel obstruction. The $\alpha v\beta 3$ integrin is a membrane-bound glycoprotein important for cell-cell adhesion and migration. The objective of this study was to assess the potential for intraperitoneal (IP) administration of neutralizing antibodies to the $\alpha v\beta 3$ integrin as a method to reduce post-surgical adhesion formation.

Design: A randomized, masked study with 21 New Zealand white rabbits.

Materials and Methods: This study was conducted in accordance with UNC IACUC regulations. All rabbits underwent laparotomy and were randomly assigned to have either an "open and close" sham procedure (n=3) or a procedure in which pelvic abrasions were intentionally created in a standardized fashion (n=18). The rabbits in the pelvic abrasion group were further randomized to receive 2 mL of IP saline (n=6), 2 mg/2 mL of IP anti-$\beta 1$ integrin antibody (n=6) or 2 mg/2 mL of IP anti-$\alpha v\beta 3$ monoclonal antibody, LM609 (n=6). Animals were killed three weeks postoperatively and IP adhesions were scored by a single masked observer. Statistical analysis was performed using ANOVA with Scheffe's correction (α=5%).

Results: No statistical difference was seen in adhesion scores between the sham procedure group and those treated with the anti-$\alpha v\beta 3$ monoclonal antibody (p=0.582). A significant difference was found between the sham and saline groups (p=0.0262) and the sham and anti-$\beta 1$ integrin antibody groups (p=0.038) in terms of adhesion number and density.

Conclusion: These data suggest that intraoperative instillation of a solution containing neutralizing antibodies to the $\alpha v\beta 3$ integrin may reduce adhesion formation.

Supported in part by NIH grant HD 34824 (BAL) and Subprograma general de estancias en el extrajero grant from the Spanish Government, Ministry of Science and Education, Madrid, Spain (MJI).

P-155

Predictors of Operative Complications During Hysteroscopic Surgery. [1]A. M. Propst, [2]R. Liberman, [2]B. L. Harlow, [1]E. S. Ginsburg. [1]Department of Obstetrics, Gynecology and Reproductive Biology and the [2]Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objectives: Hysteroscopic surgery provides less invasive and expensive options for patients with gynecological and reproductive problems. With an increasing number of hysteroscopic procedures available, concerns have arisen over the incidence of complications with hysteroscopic surgery. Our objective was to determine the incidence and predictors of complications during hysteroscopic surgery.

Design: Retrospective chart review.

Methods: We obtained demographic information and medical history on 376 women who underwent hysteroscopic surgery at Brigham and Wom-

en's Hospital between 1995 and 1996. Advanced hysteroscopic surgery (hysteroscopic myomectomy, endometrial ablation or resection, resection of uterine septum or adhesions) was performed in 109 patients. Categorical analysis was used to compare differences in the rates of operative complications and the influence of specific hysteroscopic procedures on the risk of adverse complications.

Results: Indications for hysteroscopic surgery were abnormal uterine bleeding (48.7%), postmenopausal bleeding (34.2%), and reproductive difficulty (13.6%). Operative complications were defined as uterine perforation (n = 2), termination of the procedure due to perceived excessive fluid absorption (n = 7), a measured fluid deficit of greater than 1000cc (n = 5), an inability to dilate the cervix (n = 2), and post-operative infection (n = 1). Operative complications occurred in 4.5% of all hysteroscopic surgeries and 10.1% (OR 4.1; 95% CI 1.4, 11.5) of advanced hysteroscopic surgery. The crude risk for operative complications for patients undergoing a hysteroscopic myomectomy was 13.7% (OR 4.5; 95% CI 1.7, 12.1) and resection of uterine septum 20% (OR 6.0; 95% CI 1.2, 31.1). The fluid deficits were less than 200 cc in 75% of surgeries, but were significantly higher for advanced hysteroscopy (249cc vs. 45cc, $p < 0.0001$). None of the patients required a transfusion or an unplanned laparoscopy or laparotomy due to a complication.

Conclusions: The rate of serious complications from hysteroscopic surgery was low in this patient population. Advanced hysteroscopic procedures, particularly hysteroscopic myomectomy and resection of a uterine septum, have a significantly higher rate of complications and fluid deficits.

P-156

Adhesion Reformation and Pregnancy Rates Following Hysteroscopic Lysis of Intrauterine Synechiae. [1]H. Yarali, [1]O. Bükülmez, [1]A. Al, [1]T. Gürgan. Hacettepe University, School of Medicine, Dept of Obstetrics and Gynecology, Ankara, Turkey.

Objective: The objective of this study was to evaluate the adhesion reformation and pregnancy rates following hysteroscopic lysis of intrauterine synechiae.

Design: Retrospective case-series.

Materials and Methods: Hysteroscopic adhesiolysis was performed in 56 consecutive infertile patients. The mean age was 27.4 ± 4.5 (range 23–40). 42 patients had a history of vigorous curettage and 14 had biopsy-proven genital tuberculosis. Synechiae were classified according to the American Fertility Society classification. Glycine was used as the distending medium and adhesions were lysed with the resectescope in all patients. A 8F Foley catheter was used as an intrauterine splint in moderate and severe cases. All patients received post-operative conjugated equine estrogen (1.25 mg/day) for 20 days. Post-operative HSG was performed in all patients in the second or third post-operative month. A second attempt of adhesiolysis was undertaken if adhesion reformation was noted at post-operative HSG. Chi-square test was used for statistical analyses.

Results: The results are given in Table 1. 22 patients had a second attempt for hysteroscopic lysis of reformed adhesions. Uterine perforation occurred in 4 cases with severe adhesions.

Type of adhesion at initial hysteroscopy	Adhesions at post-operative HSG/hysteroscopy			
	None	Mild	Moderate	Severe
Mild (n=16)	16	0	0	0
Moderate (n=25)	17	6	2	0
Severe (n=15)	0	2	1	12
Total (n=56)	34	7	3	12

Table 1. Adhesion reformation following hysteroscopic lysis of intrauterine synechiae.

12 (75%) of 16 patients with initial mild adhesions, 16 (64%) of 25 patients with initial moderate adhesions and 2 (13%) of 15 patients with initial severe adhesions conceived ($p=0.002$ for mild-severe groups; $p=0.005$ for moderate-severe groups; no significant difference between mild-moderate groups). None of the 12 patients with severe adhesions due to genital tuberculosis conceived and severe adhesion reformation was noted in all these 12 patients.

Conclusions: Hysteroscopic lysis of intrauterine synechiae is a safe and effective procedure for restoration of the uterine cavity. The reproductive outcome correlates with the severity of the initial adhesions. Severe adhesions due to genital tuberculosis carry a very poor prognosis and uterine surrogacy should be considered in such patients.

P-157

Cost of Diagnostic Laparoscopy and Hysteroscopy and the Impact of Site of Service and Anesthetic Technique-Cost Minimization/Sensitivity Analysis. S. Palter, MD, D. Patterson, BS, G. Kieffer, MBA, E. Ricarte, MD, D. Olive, MD. Division of Reproductive Endocrinology, Dept of Obstetrics/Gynecology, Yale School of Medicine, New Haven, CT, Patterson Group Chicago, IL, Private Practice Ohio.

Objectives: Many studies of health care costs suffer from methodological deficiencies and are criticized for incompletely capturing costs or for substituting proxies such as charges for costs. To make informed decisions about the relative cost-effectiveness of various competing therapeutic options, true cost data must be compared. There is debate regarding the cost-differential of the site of service (hospital OR, surgicenter, or office-based surgery). Confusion also exists regarding the differential of general anesthesia vs. sedation.

Design: A prospective cost-minimization analysis of diagnostic laparoscopy and hysteroscopy designed to capture all personnel, overhead, equipment, and supply costs using the perspective of the surgical site. Costs were compared for a traditional hospital operating room, surgicenter, office-based procedure room associated with a University, and office procedure room in a private office.

Materials and Methods: Actual costs were determined for each procedure via cost-minimization techniques. Sensitivity analysis was performed to validate the findings and determine cost decision shift points. Costs were compared to actual global hospital, surgeon, and anesthesia reimbursements by insurance payer type. Models were created to assess the impact of volume of cases and on varying anesthesia technique and provider (general versus sedation; anesthesiologist versus nurse) on per procedure costs. Equipment, supply, and time data were obtained from prospectively use databases validated by real-time observations. Per procedure overhead was determined by analysis of total expenses and facility Medicaid Cost Reports corrected for volume. Office overhead was calculated from total expenses adjusted for footage and patient visits. Capital equipment was depreciated using AHA Depreciable Assets guidelines. 12 major cost categories were identified with over 150 cost elements for each procedure.

Results: There is a marked reduction in procedure costs under local vs. general anesthesia. Office surgery is associated with a 42–56% cost reduction vs. traditional sites of service. Use of the surgicenter was not associated with cost reduction vs. hospital OR. Office procedure volume has a minor impact on procedure costs. Often reimbursement does not meet global costs. Sensitivity analysis shows the model to be robust for variables studies. Each site's cost differential and potential targets for reductions were identified.

Conclusions: Office-based surgery and the use of sedation (vs. GA) are both associated with cost reductions. Current reimbursement schemes for office-based procedures do not recognize these savings and need to be revised. The use of alternative anesthetic methods in traditional operating settings is another method of dramatic cost reduction. Cost-analysis should be performed for all surgical procedures to identify global costs of service delivery and ensure that reimbursement is reasonable. It is also a useful technique for identifying areas to target for cost reduction. The methodology developed for surgical procedures can be used for further cost studies.

P-158

Falloposcopic Characterization of the "Normal Tube"—Validation of Transcervical Falloposcopy and Scoring Systems in Fertile Volunteers Using a New Falloposcope. S. Palter, MD, M. Bosynak, RNC. Division of Reproductive Endocrinology, Department of Obstetrics/Gynecology, Yale University School of Medicine, New Haven, CT, USA.

Objectives: Traditionally, the fallopian tube is assessed indirectly via hysterosalpingography, sonohysterography, or laparoscopy. These techniques are indirect measurement of patency only do not address the functional lumen of the tube. Falloposcopy is a new technique claimed to better assess tubal function. Despite extensive worldwide use in infertile patients

and a standardized scoring system this technique has not been validated in normal fertile controls. One aspect of the scoring system in particular (dilation) is highly subjective with normative values unknown. Others have suggested Chlamydial serology is an equally accurate method of determining the risk for tubal infertility.

Design: A prospective surgical study. Multiparous volunteers underwent transcervical falloposcopic evaluation of their fallopian tubes, laparoscopy and Chlamydial serology. The protocol was approved by the institutional review board and informed consent was obtained.

Materials and Methods: All procedures were performed in the ambulatory surgical center of a tertiary-care University affiliated hospital. 30 healthy multiparous patients undergoing laparoscopic tubal ligation underwent concomitant transcervical falloposcopy. Subjects also had laparoscopic scoring of pelvic adhesions and pathology and history taken for sexually transmitted diseases, pelvic pathology, or previous surgery. Transcervical falloposcopy was performed using a linear everting catheter and a modified prototype 0.5 mm fiberoptic falloposcope under direct visualization ("Proboscis" scope designed to improve ease of tubal catheterization and visualization). Intramural, isthmic, ampullary, and fimbrial segments of the tube were separately scored, video images captured, digitized, and measured. Tubes were scored in real-time during the procedures using the standardized tubal falloposcopy scoring system (based upon patency, epithelium, vascularity, adhesions, and dilation). Chlamydial serology was obtained in all subjects. Procedures were videotaped and high resolution still images captured using a Truevision Targa 2000pro video capture board for the PC. Internal diameters were measured on the digital stills using the falloposcope proboscis tip as an internal gauge and calipers with normal values calculated as mean +/± 2 SDs.

Results: Isolated "Abnormal findings" on falloposcopy including internal tubal adhesions and alterations in mucosal epithelium and vascularity were observed in fertile women however, all fertile women fell into the fertile range. Those with surgery or pelvic infections since their last birth had more abnormal scores. Falloposcopy was more predictive than Chlamydia trachomatis serology of normal tubes. Normal anatomic measurements of tubal diameters for the intramural, isthmic, and ampullary regions of the tube have been established.

Conclusions: The use of falloposcopy for assessing tubal anatomy and the range of "normal" appearances was validated in fertile women. The falloposcopic scoring system should be revised to classify the mild observed abnormalities as a variant or normal rather than of potential decreased fertility. Chlamydial serology is of less value than falloposcopy. Objective values for tubal diameter and segmental cut-offs for the diagnosis of dilation are provided.

P-159

Falloposcopy vs. Salpingoscopy. A. Putz. Dept of OB/GYN, A.O.P.H., Dortmund, Germany.

Objectives: After appropriate basic diagnostic and therapeutic steps have been taken in the workup of infertile woman an operation clarification should follow. Is there an important difference in the falloposcopic and salpingoscopic examination of the intratubal region?

Design: The results of falloposcopy are compared with the results of salpingoscopic examination.

Materials and Methods: Within the scope of a prospective study from July 1995 until February 1999 two groups of female patients were recorded. The first group included 1000 infertile women whereby the interior of fallopian tubes was visualised using a linear-everting catheter which allowed insertion of a thin flexible 0,5 mm endoscope. The other group included 100 infertile women whereby the fimbrial and ampullary part of the tubal lumen was visualised with an 1.8 mm salpingoscope after catheterizing the tube with a thin flexible 0.8 mm catheter. Laparoscopy and hysteroscopy were also performed. The findings were assessed according to 5 criteria and transcribed into a point system from which a classification into 4 stages resulted.

Results: In the first group (falloposcopy-group) 36% of the patients had normal tubal epithelium, 29% showed mild tubal damage. Moderate and extensive tubal damage were exhibited by 16% and 19% of the patients respectively. Treatment of tubal pathology by ballon tuboplasty was successful on patients with mild, moderate and severe disease in 25%, 11% and 4%. In the second group (salpingoscopy-group) 32% of the patients had normal tubal mucosa, 22% showed mild tubal damage, 20% showed moderate and 26% severe tubal damage. Treatment of proximal tubal occlusion was successful on patients with mild, moderate and severe tubal disease in 27%, 10% and 4%. The results of both groups shows no significant difference ($p>0,05$).

Conclusions: Falloposcopy and salpingoscopy provides more complete information concerning the tubal lumen and may be therapeutic in proximal tubal occlusion. Both methods don't differ in their results. Salpingoscopy with catheterizing the tubes is more economic and practicable than falloposcopy. Indeed the combination of laparoscopy, hysteroscopy and salpingoscopy should be considered a standard in the invasive infertility diagnosis.

P-160

Predictors of Treatment Failure for Ectopic Pregnancy Treated With Methotrexate. A. Agameya, A. Tawfiq, P. Claman. Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Ottawa Hospital, University of Ottawa, ON, Canada.

Objective: To determine variables that predict treatment failure after methotrexate (MTX) for ectopic pregnancy.

Design: Retrospective study of 60 patients diagnosed with early ectopic pregnancy and treated with MTX at the University of Ottawa, Ottawa Hospital.

Material and Method: A total of 60 patients diagnosed with ectopic pregnancy where given MTX 50 mg/M^2 IM. Serial serum total βhCG assay (3rd IS, WHO, Abbott Axym MEIA) and follow up was done for all patients.

Results: Tubal rupture was observed following MTX in 65% (13/20) of cases when βhCG level was >4000 IU/L on the day of MTX. Tubal rupture only occurred in 7.5% (3/40) of patients when serum βhCG was <4000 IU/L on the day of MTX ($p < 0.0001$, OR 22.9, 95%CI 4.4–140). Patients complaining of some pelvic pain (without tenderness) had tubal rupture 56% (9/19) of the time vs only 17% (7/42) in patients without pain ($p=0.025$, OR 4.5, 95% CI 1.15–18.21). Tubal rupture also occurred in 53% (9/17) of patients presenting with vaginal bleeding versus 16% (7/43) of those without vaginal bleeding ($p=0.006$, OR 5.8, 95% CI 1.4–24.7). The first post treatment week resolution pattern of βhCG (immediate decline versus initial rise followed by a decline in βhCG) did not predict treatment success or failure. The time to complete resolution of ectopic pregnancy following MTX ranged from 7 to 43 days. Side effects of MTX included: nausea and/or vomiting ($n = 11$); stomatitis/or oral pain ($n=0$); cystitis ($n=0$).

Conclusion: There is a very high risk of tubal rupture after MTX when treatment day serum βhCG is >4000 IU/L. Pelvic pain (even without tenderness) and/or vaginal bleeding also predict a higher risk for tubal rupture following MTX therapy of ectopic pregnancy. MTX is a safe and effective treatment of ectopic pregnancy when βhCG <4000 IU/L especially when the patient presents without pain or bleeding.

P-161

Laparoscopic Section of Uterosacral Ligaments for Pelvic Pain. S. Ouhilal, M. Sergerie, F. Bissonnette. Department of Obstetrics and Gynecology, Saint-Luc Campus, University of Montreal, Quebec, Canada.

Objectives: To evaluate the effect of laparoscopic section of uterosacral ligaments on pelvic pain.

Design: A prospective randomized double blind trial.

Materials and Methods: 36 patients undergoing laparoscopy for chronic pelvic pain refractory to medical treatment were recruited between January 1991 and 1999. Patients were randomized to either uterosacral ligament section or the control group. Uterosacral ligament section was done with electrocautery and scissors approximately 1.5cm from the posterior cervical junction. Any areas of endometriosis were cauterized, independent of randomization group. A questionnaire of symptoms was filled before, and 4 months after, surgery.

Results: Both groups were comparable in age, parity, past surgeries, endometriosis and preoperative symptoms. There was no statistically significant difference between the uterosacral section group and the control group. However, a statistically significant improvement of pelvic pain was noted post-operatively, independent of randomization group ($p<0.001$).

Conclusion: Uterosacral ligaments section is of no benefit in patients with pelvic pain. However, laparoscopic surgery and cauterization of endome-

triosis provides improvement of pelvic pain up to at least 4 months post-operatively.

P-162

Efficacy of Microlaparoscopy - Laparoscopic Surgery by a Small-Diameter Scope Insertion Reduces Post-Operative Pain and Promotes Quick Recovery. T. Kamijo, K. Yamada, Y. Ibuki. Department of Obstetrics and Gynecology, Gunma University School of Medicine, Maebashi, Gunma, Japan.

Objectives: Office laparoscopy becomes civilized in the clinical practice and is an easy way to evaluate the disorders around uterus. To compare the efficacy of laparoscopy by a small-diameter scope with that by a regular-sized scope in the operation, either of the scopes was randomly assigned, by blinded approach, to the patients with gynecologic disorders.

Design: Differences in surgical performance by each scopes were compared. Effects of the scope size, or the wound size in umbilicus, on post-surgical pain and recovery were also investigated.

Materials and Methods: Laparoscopic operations were performed to the patients with infertility, endometriosis, uterine myoma and ovarian tumors, using a microlaparoscope (1.75 mm in diameter) or a regular-sized scope (10 mm in diameter). Either of the scopes was randomly assigned to the patients. Disease, type of operation, rAFS score and total length of incision except for the size of scopes were matched in 12 patients (6 cases in each group). Completeness of the operations and effects of the scope size on post-operative pain were evaluated by operation time, post-operative rAFS score and opioid drug (pentazocine 15 mg per ampoule) injections. Recovery of physical activities and appetite after laparoscopy and hospitalization period were also examined to compare the damage of operations by wound size in umbilicus.

Results: One case of laparoscopically assisted vaginal hysterectomy (LAVH), 1 laparoscopically assisted myomectomy (LAM) and 4 cases of resection of ovarian endometrioma, including adhesiotomy, were performed by a microlaparoscope or a regular-sized scope. There is no significant differences in the operation time and blood loss. Operation time (min) was 153 ± 90 (mean ± SD) and 161 ± 72, respectively. The severity of endometriosis was scored (rAFS score: 6-121 and 9-131, respectively) and changed 0-8 and 1-9 in post-operative scores, respectively. Operations were performed sufficiently by a microlaparoscope without difficulties, as well as those by a regular scope. In addition, 2 of 6 patients needed pentazocine injections after microlaparoscopy, while 6 all patients had the injections after surgery by a regular scope. Although there was no significant difference in the hospitalization period between the 2 groups (1.5 and 2.0 post-operative days in average, respectively), recovery in physical activities and appetite were observed half to one day earlier in the patients with small-diameter scope insertion than those in the cases with regular scope insertion into umbilicus.

Conclusion: A microlaparoscope is an useful instrument and is sufficient to apply for many laparoscopic operations. Laparoscopic surgery with a small-diameter scope insertion into umbilicus reduces post-operative pain and promotes quick recovery from surgical damage.

P-163

Inhibition of Postoperative Adhesions by Adept™, a Non-viscous Carbohydrate Polymer Solution. [1]S. J. Verco, [2]K. E. Rodgers, [2]N. Roda, [1]E. M. Peers, [1]C. B. Brown, [2]G. S. diZerega. [1]ML Laboratories PLC, Leicestershire, UK and [2]Livingstone Research Laboratories, Los Angeles, CA, USA.

Objectives: A glucose polymer solution, 4% icodextrin (Adept), has been developed for use in abdominal surgery by both laparoscopy and laparotomy, to reduce adhesion formation. This non-viscous solution is easy to handle and is resorbed and metabolized by the body. The aim of this series of studies was to evaluate the effect of icodextrin on reduction of adhesion formation and reformation in a rabbit model.

Design: The studies were conducted to evaluate the effect of administration of 50ml of 4% icodextrin solution on adhesion formation or reformation after adhesiolysis in a rabbit model of adhesions between the sidewall, cecum and bowel.

Materials and Methods: Model: Following preparation for sterile surgery, a midline laparotomy was performed, cecum and bowel exteriorized and digital pressure exerted to create subserosal hemorrhages over all surfaces. Damaged intestine was lightly abraded with gauze until punctate bleeding was observed. Cecum and bowel were returned to position and an area of peritoneum and transversus abdominus muscle removed on the right lateral abdominal wall. Prior to abdomen closure, either 50ml icodextrin (n=13) or no fluid (control, n=20) was instilled. For the reformation study, all adhesions found at second surgery (one week later) in the control group were lyzed prior to closure of the abdomen with concurrent instillation of 50ml icodextrin (n=10) or no solution (control, n=10); necropsy took place one week later. Adhesions to the sidewall were measured in terms of percentage of area involved, and the tenacity scored on a 0-3 scale. A reduction in either parameter was considered beneficial in this model.

Results: Adhesion formation was significantly reduced in the icodextrin group with 77% treated animals adhesion free. There was also a significant reduction in adhesions in the reformation model in the icodextrin group, with 45% adhesion free. In the remaining treated animals a 70% reduction in adhesions was seen. All control animals in the both studies had adhesions, with no overall reduction in tenacity or in the % area involved in adhesions after adhesiolysis.

Conclusion: Application of a 4% icodextrin solution following surgery as evaluated in the rabbit sidewall model significantly reduced the incidence, extent and severity of adhesions. All animals exhibited normal healing with no adverse effects, and no excess fluid or inflammation was observed in the abdominal cavity at necropsy.

We have previously reported a significant reduction in adhesion formation using icodextrin in the rabbit double uterine horn model, and have demonstrated that icodextrin poses no increased risk of infection in the abdomen. Adept is currently being evaluated for safety and efficacy in clinical trials in the USA and Europe.

P-164

Fertility-Sparing Surgery, With Subsequent Pregnancy, in Persistent Gestational Trophoblastic Neoplasia. A. M. Case, E. M. Greenblatt. Reproductive Biology Unit, The Toronto Hospital, University of Toronto, Toronto, Ontario, Canada.

Objective: Gestational trophoblastic neoplasia (GTN) is usually cured by surgical evacuation of the uterus, with persistent disease being very sensitive to chemotherapy. Hysterectomy, recommended for persistent chemotherapy-resistant uterine disease, may be unacceptable to the woman who wishes to maintain her fertility. Uterine resection of localized disease, with uterine reconstruction, may be a viable alternative. We present a case of a woman with persistent uterine GTN, treated with localized uterine resection and reconstruction, followed by successful pregnancy and delivery.

Design: Case report.

Materials and Methods: A 30 year old G5P3SA1M1 presented with recurrent choriocarcinoma. Previous treatment included dilatation and curettage, and two courses of chemotherapy. Transvaginal ultrasonography and MRI revealed a persistent chemoresistant disease focus in her anterior uterine wall. Hysterectomy was discussed, however she chose to undergo uterine resection of the tumor, with uterine preservation, because of a desire for future fertility.

Results: Hysteroscopy was performed initially, and a lesion was clearly seen anteriorly, pushing into the endometrial cavity. At laparotomy, the lesion was visually unidentifiable, therefore intraoperative uterine ultrasound was used to delineate the lesion, and margins were marked out using electrocautery. To minimize blood loss, a tourniquet was tied around the lower uterine segment to occlude the uterine arteries, and rubber-shod vascular clamps were placed across each infundibulopelvic ligament. A full thickness, ellipse-shaped wedge was removed from the right anterior fundal region of the uterus, and the margins were evaluated by frozen section. The uterus was reconstructed in three layers. The patient conceived 14 months postoperatively, and was followed with biweekly ultrasound starting in the third trimester to assess anterior uterine wall thickness. At 28 weeks, ultrasound showed a 3.3 mm thick 8cm × 8cm patch in the anterior right upper uterine fundus. Because of the concern for uterine rupture, she was admitted to hospital for observation. Intravenous access was maintained, and blood was crossmatched. At 31.5 weeks gestation, the defect was 2.2 mm thick. An uneventful elective lower segment Cesarean section was performed, delivering a healthy 1700 gm male infant. At surgery, the right tube and round ligament appeared to come from the anterior wall of the

uterus, presumably due to the previous excision of anterior myometrium. Because of concern for bleeding, no attempt was made to oversew the uterine defect.

Conclusions: Fertility-sparing surgery for uterine GTN is an acceptable alternative in selected patients. Preoperative counseling regarding risks of bleeding, uterine rupture, need for Cesarean section, and hysterectomy in subsequent pregnancy is essential.

ASSISTED REPRODUCTIVE TECHNOLOGY

Tuesday, September 28, 1999

P-165

High-Dose Ovarian Stimulation for IVF in Young (Under 40-Years) Patients with High Basal FSH. K. Sharif, M. Afnan, M. Elgendy, I. Yassen. Assisted Conception Unit, Birmingham Women's Hospital, Birmingham, UK.

Objectives: Patients with abnormally high basal l(menstrual-day 3) FSH have poor outcome after IVF, manifesting in higher cancellation rate (CR), fewer oocytes collected and lower pregnancy rate (PR). High-dose stimulation is sometimes used to improve the outcome. This will increase the cost but, as yet, has not been shown to improve the results. Women over 40y often receive high-dose stimulation because of the age-related low response. The question whether women with high basal FSH would benefit from high-dose is more relevant to those under 40y. In this study we have evaluated the use of high dose gonadotropin in patients under 40-year-old with abnormally high basal FSH for IVF.

Design: Historical cohort-control study.

Materials and Methods: 90 patients under 40-year-old with high (> 10.8 IU/L; WHO 2nd IRF) basal FSH were included in the study. All were undergoing their first IVF cycle and were given long-protocol gonadotropin-releasing hormone agonists prior to ovarian stimulation. The first 42 (47%) patients were given our standard gonadotropin dose for stimulation; 225 IU/day for those under 35y and 300 IU/day for those 35–39y (normal dose group; A). The other 48 (53%) patients were given double that dose (high dose group; B). All the other aspects of their treatment were similar as were their distribution of age, cause and duration of infertility, and basal FSH.

Results: The total gonadotropin dose used in group A was 38.4 ±8.6 ampoules (mean ±SD) and in group B was 73.4±11.2 ($P<0.0001$). For group A, the CR for poor response was 38%, and for group B it was 18.7% ($P<0.04$). The number of oocytes collected per procedure in group A was 6.5±4.3 and in group B was 6±5.1 ($P>0.05$). The fertilization rate (59% in A and 62% in B) and number of embryos transferred (2±0.6 in A and 2±0.8 in B) were not statistically significantly different ($P>0.05$). For group A the PR was 23.8%, the clinical PR was 16.6% and the implantation rate was 18%. For group B, the PR was 20.8%, the clinical PR was 14.6% and the implantation rate was 14.5%. All corresponding values were not statistically significantly different ($P>0.05$).

Conclusion: The use of high-dose stimulation in patients under 40-year-old with high basal FSH undergoing IVF has significantly reduced the cancellation rate (from 38% to 18.7%), but this has not led to an increase in the pregnancy rate or clinical pregnancy rate per cycle started. This would suggest either a lower pregnancy potential of oocytes collected after high-dose stimulation or that the design and size of the study were not adequate to address this question. A randomised controlled trial is underway to address this issue more appropriately.

P-166

Audit of a Urinary Luteinizing Hormone Test for Synchronisation of Frozen Embryo Replacement? L. G. Mowat, M. E. Jamieson, W. McNally, R. W. S. Yates, R. Fleming. ACS Unit, Royal Infirmary, Glasgow, UK.

Objectives: Women with normal menstrual rhythm may use a urine testing kit for determining the pre-ovulatory luteinizing hormone (LH) surge, and this point may be used to synchronise the days at which frozen embryos should be thawed and transferred. In a pilot study, a commercial kit (ClearPlan one step, Unipath, UK), accurately predicted the LH surge in over 90% of cases, and was adopted for routine use. However, it appeared to yield inconsistent results when compared with menstrual period and/or follow-up serum progesterone (P) concentrations. The aim of the study was to determine the efficacy of the LH surge predictor kits in the routine FER clinical setting.

Design: Phase 1 was a prospective study in which serum P concentrations were used to assess the precision of the apparent LH surge as identified the kit. The results were related to establishment of pregnancy. Phase 2 utilised prospective monitoring of plasma P concentrations prior to FER.

Materials and Methods: In phase 1, blood samples were taken on the day of FER in 64 consecutive procedures in women with normal menstrual rhythm (K=27-33) in whom the timing was based upon the detection of a LH surge by the kit. The concentrations of P and LH were determined and compared with laboratory normal ranges for the day of the cycle determined by the kit. In phase 2, prospective monitoring of the P concentrations prior to embryo thawing was employed to time the procedure after the LH predictor kit has identified a LH surge.

Results: During phase 1, there were 64 thaws following detection of the LH surge by predictor kit, yielding 15 pregnancies. The P concentration on the day of the FER fell within the normal limits in 39 cases (61%), of which 14 (36%) conceived. The P concentration was outside the normal data in the remaining 25 cases (39%: 7 higher and 18 lower). This group showed a single pregnancy (4%), which is significantly lower (chi square, 2 tailed: $p<0.01$). Results from the phase 2 study of 39 consecutive cases revealed that the P value was outwith the normal ranges relative to the predicted LH surge in 11 of 39 cases (28%), resulting in cancellation of the thaw. In the 28 thaws which were carried out, 6 pregnancies (24%) were established. Three of these pregnancies had their predicted thaw date altered as a result of the P concentration.

Conclusion: We conclude that the use of LH predictor kits, alone, for the timing of FER's demonstrates a failure rate that is too high for routine use in women in the embryo freezing program: even if they normally demonstrate regular menstrual patterns. Rapid prospective P estimations offer a more reliable approach.

P-167

IVF Treatment in Women With 'Normal' Day 2 FSH Levels (<12miu/ml) Who Had Previously High Basal FSH Levels. A. Lass, A. Gerrard, P. Brinsden. Bourn Hall Clinic, Bourn, Cambridge CB3 7TR, UK.

Objectives: Numerous studies have confirmed that raised early follicular phase FSH levels are correlated with poor response to superovulation for IVF, high cycle cancellation rates and low pregnancy rates. Increased FSH levels indicate reduced ovarian reserve. Based on evidence from our historic data, women whose FSH level is >12miu/ml (upper limit of normal 10miu/ml) are excluded from IVF treatment. They are advised to repeat the FSH test in the following months with the intention of starting a treatment cycle if the FSH level falls below <12miu/ml. The response of these women, with evidence of reduced ovarian reserve is not clear. This study was therefore undertaken: 1) to evaluate whether follicular FSH levels fluctuate in different cycles and 2) to test the hypothesis that if FSH levels have been raised >12miu/ml, the response to follicular stimulation for IVF is reduced, in spite of a 'normal' FSH level at the start of the treatment.

Design: A retrospective analysis of all patients whose day-2 FSH levels were >12miu/ml.

Materials and Methods: In a period of 3 years from January 1996 to December 1998, 303 women aged 38 years and above and/or who had previously responded poorly to superovulation for IVF gave blood samples for FSH, LH and E2 on day 2 of their cycle before commencing treatment. In 116 (38.2%) of these women, FSH levels were >12miu/ml (range 12–114miu/ml). 65 of these women gave a further 126 blood samples for FSH measurement in the following months. Seventy-nine of these tests returned raised FSH >12 miu/ml (60.4%). Twenty nine women whose repeat FSH levels were <12miu/ml underwent 37 IVF cycles. Ovarian stimulation was induced using the "short" GnRH-a protocol with 300–450iu recombinant FSH daily (n=29) or the "long luteal phase" GnRH-a protocol with a similar daily dose of recombinant FSH (n=8).

Results: In 11 cycles (29.8%) the response to follicular stimulation was suboptimal and the treatment cycle was abandoned. Of the 26 oocyte retrieval cycles, four women (15.4%) had complete failure of fertilisation due to poor quality oocytes and in another cycle the single embryo failed to cleave. Twenty patients had embryo transfer and 7 achieved clinical pregnancies (18.9% per cycle, 35% per transfer). One cycle was abandoned in

the group of women <40 years (7.1%) compared to 10 cycles in women 40 years old and above (43.5%, p=0.02).

Conclusions: Women whose day 2 follicular phase FSH levels are raised >12miu/ml have an increased risk (>50%) that in subsequent cycles levels will remain raised. If the cycle day 2 FSH level returns to a 'normal' level of <12miu/ml, patients who achieve the stage of embryo transfer have a good chance (35%) of conceiving, but women aged 40 and above have high (>40%) cycle cancellation rates.

P-168

Embryo Cryopreservation At The Pronuclear Stage And Efficient Embryo Utilization Optimizes The Chance For A Liveborn From A Single Oocyte Retrieval. [1]M. A. Damario, [1,2]D. G. Hammitt, [1]D. R. Session, [1]D. A. Dumesic. [1]Department of OB/GYN, Mayo Clinic, Rochester, MN and [2]Department of OB/GYN, Mayo Clinic, Scottsdale, AZ.

Objective: The ability of embryo cryopreservation to enhance the total pregnancy potential following a single oocyte retrieval is underappreciated. Many infertility clinics primarily emphasize pregnancy outcomes after fresh embryo transfer(s). Our objective was to evaluate our unique IVF-ET approach which emphasizes efficient embryo cryopreservation at the pronuclear stage.

Design: To estimate the potential for a liveborn (LB) in our program achieved through either fresh or frozen embryo transfers utilizing oocytes from a single cohort.

Materials and Methods: All couples undergoing IVF-ET at our center are counseled regarding a specific embryo transfer number after oocyte retrieval based on demographic and historical factors. All normally fertilized (2PN) oocytes exceeding this specified embryo number are immediately cryopreserved at the pronuclear stage. With this approach, we have found that the majority of normally fertilized oocytes left out for fresh transfer cleave (>98%) and still maintain a high implantation potential (implantation rate >25%). For couples who fail to conceive after fresh embryo transfers, frozen embryo transfers are subsequently performed by usually thawing only the number of embryos intended for transfer. Exclusive use of cryopreserved pronuclear-stage embryos permits high embryo thaw survival (>90%) and implantation rates (>20%), despite this very conservative thaw policy. The conservation of remaining embryos often allows for additional frozen embryo transfer attempts.

Results: From 1/1/96 to 6/30/97, 327 consecutive IVF-ET cycles were initiated (n = 259). There were 66 cancellations (20.2%). Two hundred sixty-one retrievals were performed resulting in 245 fresh and 103 frozen transfers (until 6/30/98-excluding transfers following the first liveborn delivery). Sixteen retrievals resulted in cryopreservation of all embryos. Embryos were cryopreserved after 75.9% of retrievals. Nearly all 2PN oocytes (99.0% of total) were utilized for either fresh transfer or cryopreservation.

Age	# retrievals	# fresh transfers (LB/ET)
<35 years	157	144 (67/144 = 46.5%)
35–39 years	77	75 (39/75 = 52.0%)
>39 years	27	26 (3/26 = 11.5%)
Total	261	245 (109/245 = 44.5%)

Age	# frozen transfers (LB/ET)	LB/retrieval
<35 years	71 (29/71 = 40.8%)	61.1%
35–39 years	22 (7/22 = 31.8%)	59.7%
>39 years	10 (2/10 = 20.0%)	18.5%
Total	103 (38/103 = 36.9%)	56.3%

Conclusion: This approach is highly effective for women less than 39 years of age. In these women, the efficient use of embryo cryopreservation at the pronuclear stage optimizes potential frozen embryo transfer outcomes without jeopardizing fresh embryo transfer results. This approach vastly increases the chance for a liveborn in a cost-efficient manner and also permits a significant potential for additional pregnancies.

P-169

Anonymous Oocyte Donation Performed Exclusively With Embryos Cryopreserved At The Pronuclear Stage: An Extended Series. [1]M. A. Damario, [1,2]D. G. Hammitt, [1]S. A. Stevens, [1]D. R. Session, [1]D. A. Dumesic. [1]Department of OB/GYN, Mayo Clinic, Rochester, MN and [2]Department of OB/GYN, Mayo Clinic, Scottsdale, AZ.

Introduction: We have recently published the preliminary results of our anonymous oocyte donation (AOD) program in which we have exclusively utilized embryos cryopreserved at the pronuclear stage (*Fertil Steril*, in press). Because of the unique and novel nature of this program, we are presently reporting our continuing experience with this approach.

Design: Retrospective analysis.

Materials and Methods: In all cases of AOD performed at our center, all normally fertilized (2PN) oocytes are immediately frozen at the pronuclear stage. Recipients are then treated at a later date, undergoing standard hormonal replacement protocols followed by embryo thaw and transfer. In most instances, frozen embryo transfers are performed by thawing only the number of embryos intended for transfer. This conservative thaw policy permits the potential for multiple frozen embryo transfer attempts for most AOD recipients.

Results: From 10/10/95 to 12/31/98, 59 oocyte retrievals were performed for 57 oocyte recipients. Previously cryopreserved sperm was used for insemination in 98.3% of cycles. Mean numbers of oocytes retrieved and pronuclear stage embryos frozen were 17.3 and 11.0, respectively. To date, eighty-four embryo thaw-transfer cycles have been undertaken with an ongoing pregnancy (>12 weeks) rate of 45.2% per transfer. Mean numbers of embryos thawed and transferred were 3.5 and 3.1, respectively. Embryonic thaw survival and implantation rates were 93.1% and 26.9%, respectively. At least one ongoing pregnancy (>12 weeks) has been achieved following 61.0% of retrievals to date. Of the 23 recipients who have not yet achieved an ongoing pregnancy from AOD, 15 still have 3–16 cryopreserved embryos (mean = 6.2) remaining for future thaw-transfer cycles. Of the 36 recipients who have achieved an ongoing pregnancy from AOD, 31 still had 2–16 cryopreserved embryos (mean = 5.9) available for potential future thaw-transfer cycles at the time the ongoing pregnancy was established. Four recipients who have delivered have returned for treatment attempts utilizing their remaining cryopreserved embryos. Two additional ongoing pregnancies (>12 weeks) have been established in these latter patients (4 embryo-thaw transfer cycles).

Conclusion: AOD can be conducted efficiently with the exclusive use of embryos cryopreserved at the pronuclear stage. This approach facilitates synchronization of the donor-recipient pair as well as protection of patient confidentiality. Recipients have the option of delaying embryo transfer until repeat infectious disease testing on the donor is completed. This approach not only produces an acceptable ongoing pregnancy rate per oocyte donation, it also permits many successful patients an opportunity to return for additional pregnancies in the future.

P-170

Sperm Washing by Swim-up Promotes *In Vitro* Capacitation as Assessed by Hyperactivation in Fresh and Cryopreserved Human Spermatozoa. [1,2]S. C. Esteves, [2]R. K. Sharma, [2]A. J. Thomas Jr, [2]A. Agarwal. [1]ANDROFERT-Andrology & Human Reproduction Clinic, Campinas, SP, Brazil and [2]Center for Advanced Research in Human Reproduction and Infertility, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH.

Objectives: Sperm capacitation is a fundamental step for *in vivo* fertilization. This process comprises a series of biochemical and structural changes, and includes an increase in the frequency and amplitude of sperm head and tail movements. Such change in the motion pattern is termed hyperactivation (HA), which constitutes a marker of capacitation. We studied to what extent the swim-up technique is effective in promoting *in vitro* capacitation.

Design: HA was determined in fresh and cryo-thawed sperm specimens processed by swim-up technique, and then incubated *in vitro* under capacitating conditions.

Materials and Methods: IRB approval was obtained for this study. Donor semen samples (n=15) were divided into two equal aliquots: the first aliquot was treated by "swim-up" (fresh); the second one was cryopreserved using the liquid nitrogen vapor method, and then treated by "swim-up" after thawing (frozen). After processing, the fresh and frozen specimens were incubated in a BWW culture media containing 3% bovine serum albumin at

37°C under 5% CO_2 in air for 3 hours. The percentage of spermatozoa exhibiting HA according to the Robertson criteria (star-spin + transitional) was determined using a computer assisted semen analyzer immediately after processing and after 3 hours incubation period. The Wilcoxon rank sum test was used to detect differences in HA.

Results: The frequency of spermatozoa exhibiting HA in unprocessed, processed and incubated specimens is shown in the table.

	Fresh specimens		
	unprocessed[1]	swim-up[2]	*in vitro* incubation[3]
HA (%)	1.1 (0.0–1.8)	1.4 (0.0–2.1)	5.2 (1.7–8.1)
p	0.88[1,2]; <0.01[1,3]	<0.01[2,3]	—
	Frozen specimens		
	unprocessed[4]	swim-up[5]	*in vitro* incubation[6]
HA (%)	0.14 (0.0–0.41)	0.46 (0.0–1.3)	1.0 (0.0–1.9)
p	0.59[4,5]; 0.02[4,6]	0.07[2,5]; 0.09[5,6]	<0.01[3,6]

Values are median (25%–75% interquartile range).

Conclusions: 1) Sperm washing by swim-up does not promote sperm capacitation *per se*, as assessed by HA, in fresh or frozen specimens; 2) In vitro incubation of processed specimens under capacitating conditions for 3 hours significantly increases HA in fresh specimens, but not in frozen ones; 3) The frequency of spermatozoa exhibiting HA is significantly lower in frozen specimens than fresh ones either after processing or incubation.

This work was supported by a research grant from The Cleveland Clinic Foundation.

P-171

Elevated Serum Estradiol Concentrations in IVF-Patients: Fresh Embryo Transfer or Cryopreservation? [1]A. Piazza, [1]G. Speranza, [1]L. Kopcow, [1]M. Vilela, [1]R. Quintana, [1]E. Young. [1]Instituto de Ginecología y Fertilidad (IFER), Buenos Aires, Argentina.

Objective: There exists controversy whether to transfer fresh embryos or to cryopreserve all of them in patients with high levels of estradiol. Some believe that the effect of cryopreservation reduces the chances of pregnancy while others sustain that estradiol levels may harm embryo development and implantation. Besides, deferring the transfer would diminish the risk of severe ovarian hyperstimulation syndrome. The aim of the present study is to compare the outcome of fresh embryo transfer vs. cryopreserved thawed embryo replacement cycles in patients with serum estradiol >/=3000pg/ml on the day of HCG.

Design: Retrospective analysis of clinical and laboratory data from 649 consecutive embryo transfers.

Materials and Methods: We analyzed 104 embryo transfers of patients who presented serum E2 concentrations >/=3000pg/ml on the day of HCG, which took place between October 1 1996 and September 30 1998. They were divided into three groups:

A-51 fresh embryo transfers

B-25 cryopreserved thawed embryo transfers in hormone replacement therapy cycles in patients who had previously received fresh embryos (23 patients)

C-28 cryopreserved embryo transfers in hormone replacement therapy cycles in patients who had not received fresh embryos (they had cryopreserved all the embryos)

The three groups were compared in terms of number and quality of embryos per transfer, implantation rate, and pregnancy rate per cycle and per patient and estradiol serum concentration on the day of HCG.

Results: Average serum estradiol concentrations on the day of hCG were for group A: 6018pg/ml, B: 6126pg/ml, and C: 7229pg/ml. Embryo quality was better in group A (47.8% Type I embryos vs. 19.5% in group B, and 19% in group C). The average number of embryos per transfer was 3.73 in group A, 3.68 in group B and 3.78 in group C. There was a lower but not significantly different (NS) implantation rate in group A (A=8.4%, vs. B=9.7% and C=9.5%). There was a higher, but NS pregnancy rate per transfer with cryopreserved embryos (A=27.4%, B=32% and C=35.7%).

Conclusions: In this study cryopreservation does not seem to worsen the outcome of embryo transfer in patients with high levels of estradiol. It is useful in preventing ovarian hyperstimulation syndrome. There remains the need to evaluate in a prospective study its possible beneficial effect in high responder patients, as it would avoid the detrimental effect of high estradiol concentration on embryo implantation.

P-172

Inhibin B During Controlled Ovarian Stimulation in Oocyte Donors is Predictive of Follicular Recruitment. G. Lee, J. L. Giraldo, A. Habana. Yale University School of Medicine, New Haven, CT.

Objectives: Inhibin B has been shown to directly reflect ovarian reserve since it is a product of the granulosa cells of the pre-antral follicle. Its assessment may help prognosticate IVF outcome and assist patient counseling. Our objective was to determine the pattern of Inhibin B levels during controlled ovarian stimulation of oocyte donors with normal ovarian reserve.

Study Design: Stored sera of anonymous donors in our oocyte donation program on at least three points in the cycle of donation (on the day of maximal suppression, day 8 to 10 of stimulation and day of hCG administration) were assayed for Inhibin B.

Materials and Methods: Anonymous donors from the oocyte donation program during the 1996 year were analyzed. Stored sera of donors on the day of maximal suppression, day 8 to 10 of gonadotropin stimulation and day of hCG administration were assayed for Inhibin B. These levels were plotted and were correlated with estradiol level, number of mature oocytes, fertilization rate and pregnancy rate. Pearson correlation's, chi square and Fisher's exact test were used with a p value <0.05 to indicate significant difference.

Results: Twenty-six cycles using 18 different anonymous donors were analyzed. There were 18 donors with 26 cycles and 23 recipients. Ovarian stimulation resulted in 14.08± 6.12 oocytes per cycle of which 82.92± 16.36% were mature oocytes. There was a 76±22.06% fertilization rate overall. A median of four embryos was transferred per patient. Amongst these donor cycles, the overall pregnancy rate per cycle was 38%. We found a linear increase in the serum Inhibin B levels from the day of maximal suppression (41.04± 22.89 pg/ml) to values more than 1000 pg/ml on the day of hCG administration. Serum Inhibin B levels on the day of maximal suppression were divided into low (<40 pg/ml) and high (≥40 pg/ml) groups. Both inhibin groups were comparable in donor's and recipient's age, diagnosis of infertility, number of ampoules of gonadotropin used, peak estradiol, number of oocytes retrieved, embryos transferred and percentage of mature oocytes. There was no difference in fertilization or pregnancy rates. However, there was a trend for those with serum Inhibin B on the day of hCG that was greater than or equal to 1000 pg/ml to have more than 10 oocytes retrieved than those were with levels below 500 pg/ml.

Conclusions: Levels of serum Inhibin B showed a gradual increase during the controlled ovarian stimulation cycle reaching peak levels on the day of hCG administration. Serum Inhibin B levels on the day of maximal suppression with GnRH analogues did not predict pregnancy outcome in an oocyte donation cycle. However, increasing Inhibin B levels are indicative of follicular recruitment.

P-173

Prospective Randomized Analysis of the Impact of Two Different Transfer Catheters on Clinical Pregnancy Rates. J. F. Mayer, F. Nehchiri, E. L. Jones, V. M. Weedon, H. L. Kalin, S. E. Lanzendorf, S. C. Oehninger, J. P. Toner, S. J. Muasher. The Jones Institute for Reproductive Medicine, Dept Ob/Gyn, Eastern Virginia Medical School, Norfolk, Virginia.

Objective: The success rates of ART can be influenced by a variety of the procedures employed by the clinical team. The objective of this study was to compare two different transfer catheters for their impact on clinical pregnancy rates.

Design: Prospective randomized study of two transfer catheters. The

study includes all patients undergoing a fresh transfer of at least 3 and no more than 5 embryos during the period from April 1998 to February 1999.

Materials & Methods: All patients were treated by our standard stimulation and laboratory culture protocols. However at transfer, patients were randomly divided into two groups by alternating between one of two transfer catheters, the Edwards-Wallace catheter (Cooper Surgical, Connecticut, USA) or the Cook Ob/Gyn soft-pass catheter (Cook, Indiana, USA). Both catheters types were hydraulically loaded with a 1 cc tuberculin syringe. Embryos were drawn up into the tip of the catheter in approximately 30 μl of culture media. All transfer procedures were identical between the two groups.

Results: The results are summarized in the following table.

Table 1 Comparison of Edward-Wallace and Cook Catheters

	Cook	Wallace	Statistics
Number of transfers	107	106	
Average Age	33.5 ± 0.5	33.2 ± 0.5	NS
Average # Mature oocytes	10.2 ± 0.5	10.7 ± 0.6	NS
Average # Transferred	3.7 ± 0.1	3.6 ± 0.1	NS
Average Grade Transferred*	2.3 ± 0.1	2.2 ± 0.1	NS
Clinical Pregnancy	36% (39/107)	39% (42/107)	NS (p = 0.73)

* embryo morphology grade 1–5 (with 1 = best), ± = SEM, NS = not significant.

Conclusion: The results of this randomized study indicate there was no significant difference in the clinical pregnancy rates between the two catheters.

P-174

Prospective Randomized Study of the Influence of Two Culture Media on Pregnancy Rates with Cryopreserved Zygotes. J. F. Mayer, F. Nehchiri, H. L. Kalin, E. L. Jones, V. M. Weedon, S. E. Lanzendorf, S. C. Oehninger, J. P. Toner, S. J. Muasher. The Jones Institute for Reproductive Medicine, Dept Ob/Gyn, Eastern Virginia Medical School, Norfolk.

Objective: Culture media has always been considered of critical importance in a clinical ART laboratory. The objective of this study was to compare two different culture media for their impact on post-thaw development and clinical pregnancy in a freeze/thaw treatment cycle.

Design: Prospective randomized study of two culture media. The study includes all patients undergoing a transfer of cryopreserved zygotes from Oct 1998 to February 1999.

Materials & Methods: All patients were treated by our standard stimulation and laboratory culture protocols. A standard 1-cell freezing protocol with 1,2 propanediol was employed for all cryopreservation. All storage and thaw protocols were also identical. At thaw patients were randomly divided into two groups by alternating between one of two culture media, Ham's F10 + 15% Synthetic Serum Substitute (SSS - Irvine Scientific CA, USA) or S1 media (IVF Scandinavian, Sweden). Both groups were cultured overnight and transferred on Day 2. Transfer procedures were identical between the two groups.

Results: The results are summarized in the following table. Pregnancy rates were compared with Fisher's Exact test.

Table 1 Comparison of Ham's F10 + 15%SSS and S1 media

	Ham's F10 + 15% SSS	S1	Statistics
Number of transfers	11	12	
Average Age	31.8 ± 1.7	31.1 ± 1.3	NS
Average # Transferred	3.5 ± 0.3	3.5 ± 0.5	NS
Average # of Blastomeres	3.1 ± 0.2	3.2 ± 0.2	NS
Average Grade Transferred*	2.5 ± 0.2	2.3 ± 0.2	NS
Clinical Pregnancy Rate	18% (2/11)	67% (8/12)	S (p = 0.03)

* embryo morphology grade 1–5 (with 1 = best), ± = SEM, S = significant, NS = not significant.

Conclusion: The results of this prospective randomized study indicate there was a significant difference in the clinical pregnancy rates between the two media. The clinical pregnancy rates with Ham's F10 (18%) during this study are similar to our historic cryo. pregnancy rates. The change of culture media to S1 resulted in a significantly higher pregnancy rate.

P-175

Is There Any Value in Splitting Sibling Oocytes Between Intracytoplasmic Sperm Injection (ICSI) and Conventional In Vitro Fertilization? [1,2]V. Karande, [1]C. Chapman, [1,2]N. Gleicher. [1]The Center for Human Reproduction-Illinois and [2]The University of Illinois at Chicago, IL, USA.

Objectives: ICSI is treatment of choice and minimizes the risk of failure of fertilization in patients with severe male factor. There, however, remain some patients without severe male factor who also experience complete failure of fertilization. A retrospective analysis of our historical data revealed that males whose wives had failed to achieve pregnancy with up to 6 cycles of ovulation induction (OI) and those with so-called mild male factor were most at risk for failed fertilization. We, therefore, during 1997 prospectively offered this group of patients the opportunity to split sibling oocytes between ICSI and conventional IVF.

Design: Prospective study in medical school-affiliated infertility center.

Materials and Methods: The study population consisted of consecutive patients who failed OI in the absence of any male factor (Group I, n=20) and those with mild male factor (defined as one or more of the following: count of 10–19 million/ml, motility of 30–49%, morphology of 5–29% normal forms) (Group II, n=45). All patients had normal day 3 FSH levels.

Results: Oocyte and embryo data for study patients (N=65). The fertilization rate was higher with ICSI ($p<0.05$).

Patients	No. of mature oocytes	No. of fertilized oocytes	Fertilization rate (%)
Study Group (n=65)			
Conventional IVF	504	274	54.4
ICSI	570	372	65.3
Failed OI (Group I, n=20)			
Conventional IVF	148	83	56.08
ICSI	174	120	69.0
Male Factor (Group II, n=45)			
Conventional IVF	356	191	53.7
ICSI	396	252	63.6

Patients	No. of viable embryos	No. of cryopreserved embryos
Study Group (n=65)		
Conventional IVF	248	105
ICSI	357	148
Failed OI (Group I, n=20)		
Conventional IVF	73	16
ICSI	109	31
Male Factor (Group II, n=45)		
Conventional IVF	175	89
ICSI	248	117

Amongst conventional fertilizations, six patients (9.2%) failed to fertilize and an additional six (9.2%) fertilized only 1 oocyte. None of the patients failed fertilization with ICSI. Two patients who failed conventional fertilization conceived due to ICSI-generated embryos. Overall for the entire study group, so far there have been a total of 91 ETs with 29 pregnancies (PR = 31.9%, implantation rate = 16.3%). The multiple pregnancy rate was 44.8% (13/29).

Conclusions: We conclude that the splitting of oocytes between ICSI and conventional IVF makes it possible to evaluate the fertilizing ability of sperm and at the same time increases the chance of embryos being available for transfer. The clearly improved fertilization rate with ICSI, however, also raises the strategic question of whether the routine fertilization with ICSI

should be evaluated in prospective fashion for cost-effectiveness. Such a strategy may turn out to be especially cost-effective in couples with mild male factor infertility and after repeated failures of OI.

P-176

A Prospective Evaluation of the Value of Blastocyst Transfer in Low Responders Undergoing IVF-ET. [1,2]V. Karande, [1]C. Chapman, [1,2]N. Gleicher. [1]The Center for Human Reproduction-Illinois and [2]The University of Illinois at Chicago, IL, USA.

Objective: Low responders to ovarian stimulation are known to have poor pregnancy rates with IVF-ET. Following various criteria, many such patients are often not allowed to enter IVF cycles and/or are canceled during stimulation. This study was designed to evaluate whether embryo culture to blastocyst stage in such patients would either improve pregnancy rates or reveal new diagnostic information which potentially could be useful to patient counseling about future treatments.

Design: Prospective study of 30 patients at medical school-affiliated infertility center.

Materials and Methods: Women had to be ≥ 42 years old, have a peak E_2 level of ≤ 500 pg/ml or ≤ 3 dominant follicles on day of human chorionic gonadotropin (hCG) administration in order to qualify for this IRB-approved study. Patients also had to demonstrate normal day 3 FSH levels (≤ 15 mIU/ml) and at least one (1) dominant follicle prior to retrieval. Cycle stimulation involved low-dose stop Lupron downregulation with gonadotropin usage of 6 ampules (75 IU) daily. Embryos were cultured in sequential media (PI media, Irvine Scientific, Santa Ana, CA, followed by S2 media, Zander IVF, Vero Beach, FL) to blastocyst stage and ET took place on day 6 if embryos survived.

Results: 30 patients underwent oocyte retrieval (eight with female age ≥ 42 years, 24 with ≤ 3 dominant follicles, and eight with a peak E_2 ≤ 500 pg/ml). The number of oocytes retrieved was 5.9 ± 4.7 (range 1–24), number of oocytes fertilized was 2.77 ± 3.4 (range 0–19). The fertilization rate was 46.9% (83/177). Two patients had complete failure of fertilization. 28 patients had thus 76 embryos that were further cultured, with 20 forming blastocysts (26.3%). 18 patients did not have any viable blastocysts for transfer. 10 patients (33.3%) underwent transfer of 1–3 blastocysts resulting in one pregnancy. The pregnancy rate per transfer was thus 10%, the implantation rate was 12.5% (2/16). The only pregnancy (twin) occurred in a 43 year old who otherwise had a normal response (peak E_2 was 2,028 pg/ml, 24 oocytes retrieved, 18 fertilized, and 4 cryopreserved). During that same study period, the regular IVF program had a pregnancy rate of 55% with blastocyst transfer in patients who responded normally.

Conclusions: 1) Women with low peak E_2 levels and ≤ 3 preovulatory follicles at time of hCG administration have a dismal pregnancy rate even if their embryos reach blastocyst stage; 2) Whether older women (≥ age 42) who otherwise have normal appearing ovarian function may benefit from blastocyst culture remains to be determined; 3) A high degree of unsuccessful blastocyst culture in low responders suggests an embryo quality problem in these patients and may, therefore, serve as an additional diagnostic test leading towards oocyte donation.

P-177

How Does Female Age Affect Medication Utilization in IVF? [1,2]N. Gleicher, [1]J. Rinehart, [1,2]D. Pratt, [1,2]R. Morris, [1,2]R. Rao, [1,2]M. Balin, [1,2]V. Karande. [1]The Center for Human Reproduction-Illinois and [2]The University of Illinois at Chicago, Chicago, IL, USA.

Objectives: It is well known that medication usage increases in IVF cycles with advancing female age. What the age-specific medication usages are, however, has not been conclusively established. This was the purpose of our study.

Design: Medication usage in (75 I.U.) ampules of gonadotropins was investigated in 657 consecutive IVF cycles during 1998. Patients were stimulated with various gonadotropin products, through only a small minority of products were recombinant in nature.

Materials and Methods: At CHR-Illinois IVF cycle data are placed real-time into a computerized database which serves the institutional continuous quality process on an ongoing basis. From this database gonadotropin ampule usage was extracted and assessed for four age groups: less than 35 years; 35–37 years; 38–40 years; and 41–42 years.

Results: The table below summarizes the results.

	<35	35–37	38–40	41–42
Number of IVF Cycles	329	173	124	31
Number of Ampules	14,366	8,422	6,549	1,874
Ampules per Patient	43.7	48.7	52.8	60.5
Pregnancies per cycle start (%)	29.3	27.3	18.0	16.4
Pregnancies per embryo transfer (%)	41.5	42.8	31.2	25.7

Conclusions: These data suggest a gradual 38.4% increase in medication utilization between ages less than 35 and 41–42 years. Concomitantly, pregnancy rates per embryo transfer declined by 38.1% between these age groups. These almost identical data suggest that increasing medication demand and decreasing pregnancy rates with advancing female age denote a similar ovarian factor which can be seen as an adverse prognostic factor of IVF success.

P-178

Application of Cumulus Cells as Factors to Predict the Outcomes of In Vitro Fertilization and Embryo Transfer. K. S. Lee, Y. J. Na, W. W. Kim. Department of Obstetrics & Gynecology, Pusan National University, College of Medicine, Pusan, Korea.

Objectives: It has been well known that apoptosis, which is programmed cell death for homeostasis, is closely involved in most reproductive processes including follicular atresia, and many studies for apoptosis in ovarian cells have emerged. On the other hand, co-culture has been applied to improve the culture conditions and implantation ability of the embryo.

Design: This study was performed to establish the evaluation method of oocytes on the basis of apoptosis incidence in cumulus cells and to understand the relationship between the status of cumulus cells and the outcome of co-culture.

Methods: This study was analyzed in 34 cycles undergoing IVF-ET between Jan. 1998 to July 1998 at Pusan Nat'l Univ. Hospital. COH & IVF-ET were carried by conventional methods. After maturation of oocytes was graded, some cumulus cells were removed from oocyte-cumulus complex and provided for apoptosis detection using apoptotic detection kit (Oncor Inc.) and co-culture.

Results:

1. The incidence of apoptosis in cumulus cells markedly increased in patients aged 40 or over, while the fertilization rate was greatly decreased in those age group.
2. Apoptosis in cumulus cells was found in both the fertilized oocytes and unfertilized oocytes, but the incidence of apoptosis was higher in unfertilized oocytes.
3. There is no clear correlation between apoptosis in cumulus cells and the number of oocytes retrieved. However, the incidence of apoptosis was increased when the number of oocytes retrived was 5 and fewer in comparison with 6–10.
4. Embryo grade was significantly affected by the incidence of apoptosis in cumulus cells.
5. Pregnancy rate of IVF-ET per cycle was 29.4% and the pregnant group had the higher fertilization rate and a significantly lower incidence of apoptosis in cumulus cells compared with the nonpregnant group.
6. When cumulus cells were used as helper cells in the co-culture of the embryo, in vitro activity of cumulus cells based on morphological changes and proliferations did not influence the quality of embryo, but was closely associated with the implantation rate and pregnancy rate, which was enhanced when morphological changes and proliferations of cumulus cells were more active.
7. This difference in the outcome of IVF-ET according to in vitro activity of cumulus cells used for co-culture was not associated with the incidence of apoptosis in cumulus cells, but rather had likely relations with the different secretion pattern of protein, which may be an embryotropic factor by cumulus cells.

Conclusions: These results suggest that the incidence of apoptosis in cumulus cells can be used in predicting oocyte qualities and the outcomes

of IVF-ET. And the effect of co-culture largely depends on the in vitro activity of cumulus cells as well.

P-179

Retrospective Review of Pregnancy Rates Following On-Site Versus Off-Site Cryopreservation: A Nine-Year Experience. J. Wilcox, D. Potter, J. Batzofin, M. Feinman, T. Tan, C. Tran, J. Nelson. Huntington Reproductive Center, Pasadena, CA.

Objective: Increasingly, IVF programs are required to explore options to reduce costs of maintaining and operating their centers. Recent studies have been published with encouraging success rates for IVF-ET following transport of oocytes. Transport of oocytes and embryos may allow for reduced costs for maintaining multiple clinical sites by reducing redundancy of services. We performed a retrospective evaluation of our IVF live birth per ET, embryo survival rates and embryo transfer rates for off-site cryopreserved zygotes compared to on-site data over a 9-year interval.

Materials & Methods: From 1990–1998, cryopreservation of zygotes has been performed using a standard protocol with 1,2 propanediol with sucrose methodology. On-site cryopreservation was performed at our center between 1990–1994 and off-site from 1995–1998. Zygotes were transported in a vehicle to an off-site location via a Labotect-thermo-cell transporter maintained at 37.1°C for a 30-minute interval.

Results:

	On-Site			Off-Site		
	<35	35–39	>40	<35	35–39	>40
# of Cycles	172	116	119	96	58	57
# of ET	168	111	115	93	53	49
Live births/ET (%)	35.1	22.5	17.4	37.6	20.7	20.4
Ave # ET	3.3	3.6	2.8	3.5	3.7	3.1

There were no differences in the average number of ET, live birth rates, embryo survival rates or embryo transfer rates between on-site and off-site cryopreservation.

Conclusion: Zygote transport during a 30-minute interval appears to be effective for centers wishing to reduce costs by minimizing redundancy of services for multiple site centers. Embryo survival rates, transfer rates, live birth per ET rates following off-site cryopreservation of zygotes compares favorably to on-site cryopreservation.

P-180

Provider-Dependent Success at Embryo Transfer During In Vitro Fertilization. R. M. Hearns, B. T. Miller, P. K. Chakraborty, J. H. Segars. Combined Federal Program in Reproductive Endocrinology at WRAMC, NNMC, USUHS, and NIH, Bethesda, MD.

Objectives: Success at IVF is influenced by a number of critical factors, and correct placement of embryos into the uterine cavity is essential for establishment of pregnancy. One variable not well characterized is the influence of an individual provider upon establishment of pregnancy at IVF. The objective of this study was to address the variable of the individual provider at embryo transfer.

Design: Analysis data from 576 embryo transfers at a university-based tertiary care center from January 1996 to January 1999.

Materials and Methods: Clinical outcomes of embryo transfers from eight different providers were prospectively collected and analyzed. Only embryo transfers of either grade one and/or grade two embryos were included in the analysis. All embryos were graded according to the criteria of Veech (Ann. NY Acad. Sci. 541:259, 1989). The number of embryos transferred adhered to recommendations of the *American Society for Reproductive Medicine*. The principal clinical outcome variable was the establishment of a clinical pregnancy at embryo transfer, as determined by ultrasound at 6–8 weeks gestation. Minor outcome variables included: number of gestational sacs per established gestation (high order multifetal gestation), spontaneous abortion per embryo transfer, ongoing pregnancy rate, and ectopic pregnancy rate. All embryo transfers were performed using a Wallace catheter with ultrasound guidance. All patients underwent at least one mock embryo transfer with an empty catheter prior to entering the IVF cycle to establish uterine depth, and confirm that no cervical obstruction existed. Each provider received instruction from experienced clinicians prior to performing solo embryo transfers. The average age of patients receiving embryo transfers did not differ significantly between providers. Chi-square analysis was used to test for differences between providers. An alpha error of 0.05 was considered significant.

Results: 301 clinical pregnancies resulted from 576 embryo transfers, for an overall clinical pregnancy rate of 52.2% per embryo transfer. 262 pregnancies were ongoing (45.5%). For most providers, an average of 2.8 grade one and/or grade two embryos were required to produce a gestational sac. Substantial differences were observed between providers with respect to the number of high-grade embryos required to produce a gestational sac (2.8 versus 6.2). Of note, the clinical pregnancy rate per transfer varied greatly between providers and could not be explained by the number of transfers performed; for example, 23.4% (47 transfers) versus 54.3% (57 transfers); $p<0.05$; X^2. The differences in outcome between providers could not be explained by grade or number of embryos transferred, since the provider with the lowest pregnancy rate per transfer had the highest rate of high-order multifetal gestations per pregnancy established.

Conclusions: We observed significant differences in the ability of different providers to establish a pregnancy at embryo transfer, suggesting that embryo transfer technique can greatly influence pregnancy outcome at IVF.

P-181

Impact of Implementation of an Embryo Storage Fee on Embryo Disposal Activity. R. G. Brzyski, P. A. Binkley, J. D. Pierce, C. A. Eddy. Department of OB/GYN, The University of Texas Health Science Center–San Antonio, San Antonio, TX.

Objectives: Embryo cryopreservation has increased the opportunities for conception for patients requiring assisted reproductive technology (ART). However, cryopreserved embryos have increased the complexity of administration of ART programs and present a number of potential ethical and legal problems. It has been the policy the ART program at the University of Texas Health Science Center-San Antonio (UTHSCSA) to require patients who desire disposal of cryopreserved embryos to sign a specific disposal request at the time they wish to discontinue embryo storage. We examined the pattern of requests for disposal before and after implementing an embryo storage fee. We speculated that the fee would generate an increase in disposal activity.

Design: The number of embryo disposal requests before and after implementation of an embryo storage fee was compared.

Materials and Methods: In January 1997, ART patients began paying a fee for the process of embryo cryopreservation, separate from other embryology laboratory fees. In addition, beginning in July 1997, patients with cryopreserved embryos were assessed a semi-annual storage fee of $100 to cover administrative and laboratory costs. The records of the embryology laboratory of the ART program at UTHSCSA from the time embryo cryopreservation was implemented in 1986 until December, 1998 were examined. The occurrence and date of a request for embryo disposal was noted for each case.

Results: Seven requests for embryo disposal were received by the ART program between 1986 and January 1997: two in 1992, three in 1995, and two in January 1997. In contrast, fourteen disposal requests were received between July 1997 and December 1998, for an annualized rate of 9.3 requests per year. The number of cases of cryopreservation and frozen embryo transfer during that time period was essentially stable at approximately 50 cases each. After the implementation of semi-annual billing, the ART program began receiving change of address information from the postal service for patients with undeliverable bills. For the last billing cycle (December 1998) this amounted to 13.8% (8/58) of billed patients.

Conclusions: Despite the positive medical benefits of embryo cryopreservation, the technology poses significant problems for ART program administration. This study, examining the effect of a unique implementation of a new billing policy, suggests the magnitude of effect that economic considerations may have on patients' decisions regarding maintenance of cryopreserved embryos. Regular contact with patients provided a mechanism to update patient address records, and may represent an alternative explanation for patient behavior independent of economic considerations.

P-182

Day 5 Estradiol (E2) is Predictive of Pregnancy Outcome in IVF Cycles Using Donor Oocytes. S. M. Kavic, M. A. Cohen, S. R. Lindheim, M. V. Sauer. Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York, NY.

Objectives: Various parameters have been shown to be predictive of cycle outcome, including day 3 FSH/E2, peak estradiol (E2), and number of oocytes retrieved in IVF-ET cycles. We sought to assess an early predictor of cycle outcome in donor IVF cycles by evaluating the predictive value of serum E2 levels on day five of gonadotropin stimulation.

Design: Retrospective chart review of all donor IVF cycles in a university based practice from January 1998 to December 1998.

Materials and Methods: Donor oocyte cycles (n=140) were evaluated for day 5 E2 levels, peak E2 levels, number of oocytes obtained at aspiration, and pregnancy outcome. Receiver operator curve analysis was used to evaluate various threshold levels of day 5 E2 to assess the greatest diagnostic predictive value for pregnancy outcome. Statistical analysis was done with the SPSS statistical package.

Results: Day five serum estradiol levels were positively correlated with peak E2 levels ($r=0.646$, $p<0.05$) and number of oocytes obtained at aspiration ($r=0.554$, $p<0.05$). Pregnancy rates were significantly greater when day 5 E2 was $\geq$ 90pg/mL (58.1% [50/86] vs. 34.2% [13/38]) ($p>0.05$). Incremental threshold values of 10pg/mL, from 50–120pg/mL, failed to demonstrate a diagnostic parameter with significantly greater negative predictive value. At reevaluation on day 7, patients with a day 5 E2 $\leq$ 90pg/mL showed no differences in estradiol levels between pregnant (mean: 165.83±37.81 [±SEM]; CI: 90–242) and nonpregnant (mean: 162.57+24.61; CI: 113–212) cycles. No pregnancies occurred when the day 7 E2 < 67pg/mL or day 8 E2 < 125pg/mL.

Conclusion: Day 5 serum E2 levels $\geq$90 pg/mL in donor IVF cycles are associated with enhanced success rates. The positive predictive value at this threshold is significant, however, a significant negative predictive value was not found at any incremental value. Therefore, cycle cancellation is not justified based on a low day 5 E2 level, unless the value remains low on cycle day 7 or day 8.

P-183

Transmission of Y Deletion to Male Offspring by ICSI. [1]S. J. Silber, [2]L. G. Brown, [2]D. C. Page. [1]Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, MO, USA, [2]Howard Hughes Medical Institute, White Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

Objectives: 13% of azoospermic and 7% of severely oligospermic infertile men have been found to have Y chromosomal deletions that are not found in their fathers and brothers. These deletions are found most commonly in the DAZ region of the Y (interval 6D-F), but often involve other areas of the Y as well.

Design: Our purpose was to report on the children derived from ICSI or from TESE-ICSI of these sterile Y-deleted men.

Materials and Methods: A prospective review of seven pregnancies, and eight children arising from ICSI with sperm from azoospermic or severely oligospermic (<500,000 sperm) Y-deleted men was performed. Five Y-deleted azoospermic men with sperm recoverable from the testes, and five Y-deleted men who were severely oligospermic, underwent TESE-ICSI or ICSI procedures. A total of seventeen ICSI cycles were performed for these ten couples, resulting in seven pregnancies. These pregnancies were followed to see if there would be any unusual outcome in the children of Y-deleted infertile men.

Results: Seven of the ten Y-deleted couples eventually became pregnant. One miscarried. Of the remaining six pregnancies, two were twins. Four of the eight babies were girls and four were boys. Of the eight delivered babies, the four girls had a normal XX karyotype, were healthy, and had no congenital abnormality. The four boys had exactly the same Y deletion as their father, but otherwise all had a normal XY karyotype. Three of the four boys appeared completely normal (aside from the Y deletion). One of the four boys had severe tricuspid and pulmonary atresia, and died in the first week after an attempt at surgical correction. All boys had normal-appearing genitalia.

Conclusions: 1) De novo Y-chromosomal deletions in azoospermic or severely oligospermic men do not prevent the delivery of normal offspring from ICSI using their sperm. 2) We have demonstrated that the Y deletion is transmitted to the male offspring of these azoospermic or oligospermic men via ICSI. We presume, therefore, that these offspring will also be infertile or sterile. 3) The heart malformation in one of these eight children is probably not related to the Y deletion, but does point to the need for careful counseling and caution.

P-184

Testicular Histology of Men With Non-Obstructive Azoospermia or Severe Oligospermia Caused by Y Chromosomal Deletions. [1]S. J. Silber, [2]R. Alagappan, [2]L. G. Brown, [2]D. C. Page. [1]Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, MO, USA, [2]Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

Objective: It has been suggested that deletions in specific regions of the Y chromosome cause specific histologic testicular defects. We wished to determine whether the type of Y deletion had any impact on the specific histologic defect in azoospermic or severely oligospermic men.

Materials and Methods: Twenty-one fertile men with Y chromosomal deletions were studied with testicular sperm extraction (TESE)-ICSI procedures and/or diagnostic testicular biopsy for quantitative analysis of spermatogenic defect. A total of sixteen azoospermic and five severely oligospermic patients were found to have Y chromosome deletions that were not present in their fertile fathers, brothers, or paternal uncles. The common deletion in all twenty-one men was AZFc. In two cases, AZFa was also deleted, and in three cases, AZFb was also involved. In sixteen cases, only AZFc was deleted. In four of the AZFc deleted cases (severe oligospermia), no histology was available. In seventeen cases histology was available.

Results: Of the twelve AZFc only deleted patients, five had Sertoli cell only, five had maturation arrest, and two had a mixed combination of Sertoli cell only and maturation arrest. In the three cases where AZFb was also deleted, two were Sertoli cell only and one was maturation arrest (in contrast to what has been proposed). In the two cases where AZFa was also deleted, both were Sertoli cell only. Sperm were found (often in minute quantities) in the sixteen cases where only AZFc was deleted. In the five cases involving AZFa and AZFb, no sperm were found.

Conclusions: We could not demonstrate for AZFb or AZFc a specific histologic defect in the testis other than a dramatic overall reduction in mature sperm. Deletions involving AZFa were unusual, and caused Sertoli cell only. The size and location of the Y deletion predicted the presence or absence of sperm but not the histology.

P-185

Y Chromosome Deletions and the Feasibility of ICSI. [1]S. J. Silber, [2]R. Alagappan, [2]L. G. Brown, [2]D. C. Page. [1]Infertility Center of St. Louis, St. Luke's Hospital, St. Louis, MO, USA, [2]Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA.

Objectives: Y chromosome deletions which are not found in normal, fertile men are found in 12% of azoospermic men and 7% of severely oligospermic men. Does this affect the feasibility of ICSI?

Design: In this updated study, we wished to determine the severity of testicular defects in azoospermic men with different Y chromosomal defects, and what impact different types of Y-deletions might have on the results with ICSI.

Materials and Methods: 205 infertile men with azoospermia or severe oligospermia underwent Y chromosomal mapping. 135 were azoospermic, with a histologic diagnosis of either maturation arrest or Sertoli cell only. 51 of these azoospermic men (10 with Y deletions) chose to have testicular sperm extraction with ICSI performed. 70 men with severe oligospermia ($<2\times10^6$/cc) also underwent Y DNA testing, and 31 (5 with Y deletions) of them underwent ICSI with ejaculated sperm.

Results: 16 of the 135 azoospermic men (12%) and 5 of the 70 severely oligospermic men (7%) were found to have deletions of the Y chromosome. None of the 100 controls had Y deletions, and none of the parents or male

relatives of these men had these Y deletions. 10 of the 16 azoospermic men who were Y-deleted underwent TESE-ICSI. Of those 10, 5 (50%) had sperm retrievable from the testis, and 2 of those 5 became pregnant in nine cycles and delivered. There was an additional pregnancy that miscarried. 4 of the 5 oligospermic couples that were Y-deleted eventually became pregnant and delivered in eight cycles. Thus there were 7 pregnancies, and 6 deliveries (two were twins) and 8 offspring in 17 cycles of 10 Y-deleted couples with sperm undergoing ICSI. Of the total of 21 infertile men who were Y-deleted, 6 of whom did not undergo ICSI, 16 had deletions limited to the AZFc region of interval 6. 14 of those 16 (89%) had some small but easily discernable degree of spermatogenesis. The other two had only one or two sperm noted in the testes. 5 of the Y-deleted infertile men had deletions that extended proximal to the AZFc region, and none of these 5 had any spermatogenesis at all, despite an extensive testicular search.

Conclusion: 1) Our studies continue to suggest that there are several genes on the non-recombining portion of the Y chromosome (NRY) in addition to DAZ that impinge on spermatogenesis. 2) In those azoospermic men who were Y-deleted, deletions limited to the (AZFc) region were associated with the presence of very small numbers of testicular sperm that were sufficient for ICSI. Larger Y deletions were associated with a total absence of testicular sperm. 3) This is consistent with the view that the Y chromosome has collected, during evolution of higher primates, many previous autosomal spermatogenesis genes, with results amplification and subsequent degeneration. This can help to explain why the human male has relatively poor spermatogenesis compared to other animals. 4) This molecular data may eventually be used to prognosticate the success or failure of TESE in azoospermic men who are not obstructed.

P-186

Nafarelin Acetate Vs Leuprolide Acetate in Women With Ovarian Function Undergoing Oocyte Donation. [1,2]A. Gutierrez, [1]F. Hernandez, [1,2]S. Mendoza, [2]E. Monroy, [2]E. Perez-Peña, [1,2]E. Gallardo. [1]Instituto de Medicina Reproductiva del Bajio (IMER), Hospital Aranda de la Parra, Leon Guanajuato, Mexico. [2]Instituto de Medicina Reproductiva de Occidente. Guadalajara Jalisco, México.

Objectives: Women with ovarian function undergoing oocyte donation (OD) without ovarian desensitization, have lower pregnancy rates than those without ovarian function, suggesting the importance to perform ovarian desensitization to have a better cycle control. Some gonadotrophin-releasing hormone analogues (GnRH-a) have been used for this purpose as leuprolide acetate and busereline. Nafarelin has been successfully used to down regulate women in *in vitro* fertilization programs, but to our knowledge, nobody has report the use in recipients of the (OD) program. The aim of this study was to determine the effectiveness of nafarelin acetate in the oocyte donation program when compared with leuprolide acetate, a well-known and probed GnRH-a in OD.

Design: Prospective randomized clinical study.

Materials and Methods: We analyzed the results of 97 consecutive embryo transfer cycles in our OD programme at the Instituto de Medicina Reproductiva del Bajio. Patients with ovarian function (n=68) were randomly allocated in one of two groups in the study. In both cases, the GnRH-a started in the mid luteal phase of the previous cycle. For group I (n=35), 100 μcg twice a day daily of nafareline acetate and for group II (n=33) 1 mg/day s.c of leuprolide acetate. Patients without ovarian function (n=29) included in the study as a control group conformed group III. All patients received exogenous administration of oestradiol valerate (EV) as hormonal replacement therapy, beginning with 2 mg. from day 1 to day 8 of natural or induced menses, 4 mg. from day 9 to 11 and 6 mg. from day 12 onwards. The day of oocyte donation, 400 mg. of intravaginal micronized progesterone were added. Embryo transfer was performed on day +2. The regimen of EV and progesterone were maintained for 15 days, then a β-hCG analysis was performed. In case of positive result, EV was increased to 8 mg/day and progesterone maintained at the same dose until day 100 of pregnancy. Data were expressed as mean ± SE. For statistical comparison among groups, analysis of variance (ANOVA) and chi squared test were applied.

Results: There was no difference among the groups regard age of recipients, number of oocytes and transferred embryos (3.7 ± 0.89, 3.8 ± 0.7 and 3.5 ± 0.6, respectively). Between group I and II, there was also no difference in the reason entering the OD program. Pregnancy rate per transfer was 54.3, 48.5 and 44.8%, and no statistically differences were found among groups, neither with implantation (16.9, 16.8 and 16.6%), nor with abortion rates (15.7, 18.7 and 15.3%).

Conclusions: Nafereline acetate can be used safely and with good clinical results in recipients of OD programe. The easy administration of nafareline and its lower price when compared with leuprolide makes nafereline an interesting option for use in hormonal replacement therapies in women with ovarian function undergoing OD.

P-187

Recombinant FSH (rec-FSH) Alone vs rec-FSH and Menotropins for Multiple Follicular Recruitment for *In Vitro* Fertilization (IVF). [1,2]A. Gutierrez, [1]F. Hernandez, [1,2]S. Mendoza, [1]C. Gonzalez, [2]E. Perez-Peña, [1,2]E. Gallardo. [1]Instituto de Medicina Reproductiva del Bajio (IMER), Hospital Aranda de la Parra, Leon Guanajuato, Mexico. [2]Instituto de Medicina Reproductiva de Occidente. Guadalajara Jalisco, México.

Objectives: There is evidence concerning an increased bioactivity of rec-FSH, when compared with urinary FSH, resulting in more oocytes, higher quality embryos and higher pregnancy rates in women undergoing in vitro fertilization. The aim of this study was to compare the results, in terms of days of stimulation, number of ampoules used, retrieved oocytes, embryo quality, pregnancy, implantation and abortion rates, of two ovarian hiperestimulation protocols for IVF, rec-FSH alone vs. rec-FSH plus menotropins.

Design: Retrospective and comparative study.

Materials and Methods: We analyzed the results of 322 IVF cycles at the Instituto de Medicina Reproductiva del Bajio. All patients received long gonadotropin releasing hormone analog protocol, started in the mid luteal phase of the previous cycle. Patients with rec-FSH alone (Group I) (n=156) started with 250 unit/day for three days, followed with 200 units/day for another three days. Group II was composed of patients with rec-FSH and menotropins (n=166). Ovarian stimulation was made with 225 units/day of menotropins and 100 units/day of rec-FSH for 3 days. From day 4 onwards 150 units/day of menotropins were administered. The daily dosage was adjusted in response to follicular growth. In both protocols, gonadotropins were administered until at least two follicles were >18 mm in diameter, then 10,000 IU of hCG was given IM. Oocyte retrieval was scheduled 37 hr. after hCG administration. Embryo transfer was performed on day +2. All patients received 400 mg. of oral micronized progesterone for 15 days, and then a β-hCG analysis was performed.

Data were expressed as mean ± SD. For statistical comparison among groups, analysis of Student's *t* and chi-squared test were applied.

Results: There was no difference among groups regard age of patients, time and causes of infertility. We found no difference in the average of stimulation duration (7.9 ± 1.9 and 8.2 ± 1.7) and total number of gonadotropin units used to achieve follicular growth. Similar number of retrieved oocytes (11.9 ± 6.6 and 12.7 ± 7.4), mature oocytes (8.2 ± 4.4 and 9.32 ± 6.1) and good quality embryos (4.9 ± 2.7 and 5.4 ± 3.8) were obtained from both groups. There was a higher pregnancy (35.3%) and implantation (11.3%) rate in group I when compared with group II (24.7% and 8.1% respectively) with similar number of good quality embryos transferred in both groups (3.5 ± 0.9 and 3.6 ± 1.0). These differences were statistically significant ($p<0.0001$). There was no difference in abortion rate within the two groups (16.3 and 12.2%).

Conclusions: Follicular response was similar in both groups with the same number of gonadotropin units employed. Higher pregnancy and implantation rates were achieved with rec-FSH alone, even when the same number of good quality embryos were transferred. These results suggest that large amounts of LH existing in menotropins may have a detrimental effect on endometrial-embryo interaction.

P-188

Numerical Abnormalities for Chromosomes 1, 13, 16, 18, 21, 22, X and Y From 59 Preimplantation Embryos Using Multi-Color Fluorescence *In Situ* Hybridization (FISH). [1,2,3]W. G. Kearns, [1]M. Franzitta, [2]S. Gitlin, [1]M. W. Stacey, [2]W. E. Gibbons. [1]Center for Pediatric Research, [2]Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, Virginia, [3]The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Objective: This study determined aneuploidy in blastomeres from 59 cleaving preimplantation embryos in which two pronuclei were observed 1 day after insemination. With IRB approval, embryos were donated by patients after informed consent was obtained.

Study Design: Multi-probe, multi-color FISH was performed on blastomeres to diagnose aneuploidy for chromosomes 1, 13, 16, 18, 21, 22, X and Y.

Materials and Methods: Blastomeres from 6–8 cell preimplantation embryos were isolated. Individual blastomeres were dropped onto microscope slides and fixed. For all embryos, all available blastomeres were studied. First, simultaneous five-probe, four-color FISH was performed for chromosomes 13, 18, 21, X and Y using direct labelled chromosome specific alpha satellite or contig DNA using a 2 hour hybridization protocol. All hybridization's and stringency washes were between 37 and 42°C. Microscopy was performed using an epi-fluorescent microscope. Following microscope analyses, all slides were destained and dehydrated. Second, three-probe, three-color FISH was performed for chromosomes 1, 16, and 22 using direct labelled alpha satellite DNA. For all hybridization's, multiple fluorescent signal detection was accomplished using a Ludl wheel with six excitation filters. Images were captured using a cooled CCD camera controlled by a MacIntosh computer using Image and FISH analysis software.

Results: Twenty-six of 59 (44%) embryos were diploid. Four of 59 (6.8%) embryos were mosaic. Two trisomy 18's, one trisomy 13 or 21, and one XXY mosaic were observed. Three of 59 (5.1%) embryos were classified as chaotic. Twenty-four of 59 (40.7%) embryos, diagnosed by the analysis of just one blastomere, were either aneuploid or mosaic. Five trisomy 13 or 21's, three trisomy 18's, three Turner syndrome, one Klinefelter syndrome, two monosomy 13 or 21's, five trisomy 16's, one trisomy 1, and four trisomy 22's were observed. One embryo (1.7%) was triploid and one mis-diagnosis for a gonosome occurred (1.7%).

Conclusions: Of the 59 embryos evaluated for preimplantation diagnosis, 54% were aneuploid, mosaic, chaotic or triploid. FISH analysis for chromosomes 1, 16, and 22 identified an additional 12.5% aneuploid embryos not detected when analyzed for chromosomes 13, 18, 21, X and Y.

P-189

Transfer Catheter Effect on In-Vitro Fertilization Pregnancy Rates, After Forty. M. F. Kamis, D. Harris, A. S. Penzias, M. M. Alper, M. J. Berger, S. P. Oskowitz, J. R. Stelling, R. H. Reindollar. BIDMC, Harvard Medical School, Boston IVF, Boston, MA, USA.

Objective: Significant improvement in IVF pregnancy rates has continually been reported for women under 40 years of age. For women over 40, pregnancy rates have remained low and have been attributed to poor egg quality and poor implantation. Identification of the specific age(s) over 40 at which success rates are clinically acceptable and continue to improve may well delineate those ages for which continued investigation of new treatments is valid and demarcate a natural cut-off for IVF utilization. We have examined the changes in success for women over 40 during introduction of the Wallace catheter.

Design: In women aged ≥40, clinical pregnancy and implantation rates were compared between the Wallace and other catheter.

Materials and Methods: The Boston IVF database was searched for all women aged ≥40, undergoing IVF from January 1, 1995 to December 31, 1998. The groups were separated with respect to the type of embryo-transfer catheter used, Wallace or other. Calculations of pregnancy and implantation rates per initiated cycle were adjusted for year of age (40–46.9) and summarized for all women ages forty or greater. Statistical analysis was performed with chi square tests and ANOVA with Bonferroni correction.

Results: Clinical Pregnancy Rates and Implantation Rates: *1995–1998*

Age	Wallace® # cycles	pregnancy	Other # cycles	pregnancy
40–40.9	142	26.8%	203	16.2%
41–41.9	172	19.2%	173	15.0%
42–42.9	99	16.2%	93	8.6%
43–43.9	48	4.2%	71	1.4%
Total	483	18.4%	579	12.3%

Age	p	Wallace® implantation	Other implantation	p
40–40.9	.018	12.6%	7.4%	.004
41–41.9	.305	9.1%	6.4%	.103
42–42.9	.113	5.6%	4.4%	532
43–43.9	.346	1.7%	0.5%	.264
Total	.005	8.4%	5.4%	<.0005

A statistically significant decrease in clinical pregnancy rate is seen at age 43–43.9, with both catheters (Wallace, p=.001; other, p=.015). Implantation rates decreased at age 42–42.9, for the Wallace catheter (p=.006) and age 43–43.9 for the other catheter (p=.003).

Conclusions: In women aged forty and over, pregnancy and implantation rates increased following the introduction of the Wallace embryo-transfer catheter. The difference in both rates is statistically significant for women aged 40–40.9. The trend for improved clinical pregnancy rates continues through age 42.9 and for implantation rates, through age 43.9. It is now evident that women over forty may benefit from improved technology and therefore should not be excluded from treatment. The clinical pregnancy rate of 16.2% for women aged 42–42.9 should encourage medical insurance companies to cover IVF treatment at least up to age 43.

P-190

Aspiration and Ethanol Sclerosis of Endometriomas in Women Undergoing In Vitro Fertilization. J. C. Petrozza, M. Guarnaccia, M. Summers, P. Huang, R. I. Hardy, V. Cardone. Fertility Center of New England, Reading, MA and Division of Reproductive Endocrinology, New England Medical Center, Boston, MA.

Objectives: Ovarian cysts are known to be detrimental to folliculogenesis. Aspiration of these cysts, especially before starting gonadotropin therapy for in vitro fertilization, is beneficial. However, endometriomas in particular recur or persist 50% of the time. Options to alleviate this issue include a repeat aspiration if the patient fails to conceive or an ovarian cystectomy. Ethanol sclerosis has been used successfully to treat renal and intraperitoneal cysts. The purpose of this study was to determine if ethanol sclerosis would prevent the recurrence or persistence of endometriomas detected at the onset of ovarian stimulation for in vitro fertilization.

Design: Prospective study.

Materials and Methods: All women undergoing IVF between January, 1998 and January, 1999, with the presence of an ovarian cyst greater than 10 mm in diameter at their baseline evaluation and having ultrasonographic characteristics of an endometrioma were recruited. Cysts were unilocular with a "ground-glass" appearance. Patients were given intravenous sedation. A 16-gauge oocyte aspiration needle was used to drain each cyst. The cyst was flushed until the returning fluid was clear. Ethanol 95% was then instilled up to a volume that represented 50% of the initially aspirated cyst volume. The ethanol was allowed to remain in the cyst for 5 minutes, at which time the ethanol was aspirated. Recurrence or persistence of an endometrioma was documented as well as ovarian response on the aspirated side.

Results: Twelve women had aspiration of their endometriomas. Nine underwent ethanol sclerosis and 3 had simple aspiration and flushing of their cyst. Mean size of the endometrioma was 21.5 mm with a mean volume of 27 cc aspirated fluid. All instilled ethanol was removed after the allotted 5 minutes of sclerosis. There were no complications and patients in both groups had similar post-aspiration discomfort. The endometrioma recurred in only two of the nine (22%) women who had ethanol sclerosis as compared to all three women (100%) who underwent the simple flush procedure. Ovarian response in the aspirated side was similar to the contralateral ovary in all 12 women with a mean of 4.5 oocytes retrieved from the aspirated ovary and 5.0 oocytes aspirated from the contralateral side (P>0.05).

Conclusion: Cyst aspiration with ethanol sclerosis provides an ideal option for women with ultrasound evidence of an endometrioma, with a decreased recurrence rate when compared to simple aspiration techniques. There appears to be no detrimental effects to the ovary based on response to gonadotropin stimulation.

P-191

Day 5 Transfer of Donated Cryopreserved Embryos: An Attractive Alternative Reproductive Option. H. Asakura, K. P. Katayama, K. Edwards, S. Milosavljevic, E. F. Stehlik, J. C. Stehlik, A. M. Dessart. The Advanced Institute of Fertility, Milwaukee, WI.

Objectives: Conventional reproductive options for women with predicted poor IVF outcome using own oocytes have been IVF/ET with donated oocytes or simply infant adoption. Recent studies have indicated that cultured human blastocysts have higher implantation efficiency. We determine the efficacy of day 5 embryo transfer using donated cryopreserved embryos.

Design: Retrospective analysis of day 5 transfer with donated cryopreserved embryos and comparison with day 5 transfer of fresh embryos created with donor oocytes.

Materials and Methods: The candidates of donated embryos or oocytes recipient were extensively counseled and underwent psychological evaluations. Cryopreserved embryos were released from couples who had IVF at our program and signed consent as anonymous donors. Oocytes donors received extensive infectious disease screenings, karyotyping and psychological analysis. Donated cryopreserved early stage embryos thawed and cultured sequentially in either Quinn's XI HTF and D3+ HTF media (ART, Sacramento, CA) supplemented with 15% synthetic serum substitute (Irvine Scientific) or S1-20 and S2-20 (IVF Science, Gothenburg, Sweden) were transferred on day 5 (n=7). Pronuclear stage embryos in donor oocytes IVF were cultured in the same sequential culture system until day 5 and were transferred (n=8). The recipient cycles were either spontaneous or controlled with GnRH-analog and estrogen/progesterone replacement regimens.

Results: Maternal indications of donated embryos transfer were: premature ovarian failure (3), diminished ovarian reserve (2), balanced translocation of maternal karyotype (1), habitual abortion (1). Average 6.6 donor embryos were thawed and 5.3 survived (80.3% cryosurvival rate). Between embryos and oocytes recipients, mean age of recipients (39.6 y.o. vs. 38.4 y.o.), donors (31.9 y.o. vs. 26.6 y.o.), number of total embryos and blastocysts transferred (3.3 vs. 3.1, 1.0 vs. 1.3, respectively) were not statistically different. No cancellation of ET occurred in either group because of embryo quality. 42.9% (3/7) donated embryo cases and 62.5% (5/8) of donated oocyte cases had at least one blastocyst available for transfer. Clinical pregnancy rates of donated embryos and oocytes recipients were 71.4% (5/7), 50% (4/8), respectively. 60% (3/5) of donated embryos pregnancies were singleton gestation and 40% (2/5) were twins. 50% (2/4) of donated oocytes pregnancies were singleton and 50% (2/4) were multiple gestations (1 twins, 1 triplets).

Conclusions: Blastocyst culture of cryopreserved dividing embryos can be routinely attempted using commercially available chemically defined sequential culture media. High clinical pregnancy rate, equivalent or greater than that of donated oocytes IVF, can be achieved with resulting embryos transferred on day 5. In view of this highly efficient and significantly less expensive "embryo adoption" would be an attractive alternative method of reproduction to conventional adoption or IVF/ET with donated oocytes.

P-192

Effect of Age on Frozen Embryo Transfer (FET) Pregnancy Rates: Day 3 Multi-cell Embryo Transfer vs. Day 5 Blastocyst Embryo Transfer. D. Marek, M. Langley, N. Confer, L. Cram, L. Underwood, K. M. Doody, K. J. Doody. Center for Assisted Reproduction, Bedford, TX.

Objective: Previous experience has suggested that pregnancy rates (PR) following FET are influenced by the stage of embryo development at transfer. This study was undertaken to evaluate the impact of maternal age on outcome of FET of Day 3 multi-cell embryos and Day 5 blastocyst embryos.

Design: A retrospective analysis of frozen embryo transfer results from January 1, 1998 through December 31, 1998.

Materials and Methods: Embryos thawed for transfer at the multi-cell stage on Day 3 or blastocyst stage on Day 5, were placed into a four-well multi-dish (Nunc) containing S-2 Media (IVF Science) and cultured approximately four hours prior to transfer. Blastocyst and multi-cell embryos were cultured in 5% CO_2 and air at 37°C and evaluated prior to Day 5 or Day 3 transfer. Multi-cell embryos were frozen and thawed using propanediol protocols previously reported (Testart et al. 1986, Tucker et al. 1997). Embryos frozen and thawed at the blastocyst stage utilized glycerol protocols as previously described (Menezo et al. 1992, Menezo et al 1996). Estrogen and progesterone supplementation was administered to all patients in preparation for embryo transfer. An abdominal ultrasound (5 MHz) was utilized for all transfers to assist intrauterine placement of the embryo transfer catheter (Wallace).

Results:

Transfer Day:	Age ≤39 Day 3	Age ≥40 Day 3	Total Day 3
Mean Patient Age:	34.0	44.3	35.8
Thaws/Transfers:	39/39	9/9	48/48
Pregnancies (+ hCG)/Ongoing:	13/9	3/1	16/10
Biochemical/Miscarriages:	2/2	1/1	3/3
PR (+ hCG) per Thaw/Transfer (%):	33.3/33.3	33.3/33.3	33.3/33.3
Ongoing Pregnancy Rate per Thaw:	23.1%	11.1%	20.8%
Embryos Thawed/Transferred:	162/105	36/24	198/129
Average Embryos Transferred:	2.7	2.7	2.7
No. Clinical Sacs (1/2/3):	10/1/0	1/0/1	11/1/1
Implantation Rate:	11.4%	16.6%	12.4%*

Transfer Day:	Age ≤39 Day 5	Age ≥40 Day 5	Total Day 5
Mean Patient Age:	33.4	43.3	35.5
Thaws/Transfers:	32/32	12/12	44/44
Pregnancies (+ hCG)/Ongoing:	17/10	8/4	25/14
Biochemical/Miscarriages:	5/2	1/3	6/5
PR (+ hCG) per Thaw/Transfer (%):	53.1/53.1	66.6/66.6	56.8/56.8
Ongoing Pregnancy Rate per Thaw:	31.3%	33.3%	31.8%
Embryos Thawed/Transferred:	107/73	54/32	161/105
Average Embryos Transferred:	2.3	2.7	2.4
No. Clinical Sacs (1/2/3):	8/3/0	4/3/0	12/6/0
Implantation Rate:	19.2%	31.3%	22.9%*

Significant difference using Fisher's Exact test for implantation rate for Day 3 compared to Day 5: * $p<.05$.

Conclusion: This data suggests that an increase in ongoing pregnancy rates may be obtained from frozen embryo transfer of Day 5 blastocyst embryos compared with transfer of Day 3 embryos irrespective of maternal age. A significant increase in implantation rate was noted for total Day 3 and Day 5 transfers.

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The Scheduled Ovarian Hyperstimulation for *In Vitro* Fertilization and Embryo Transfer was Useful to Avoid Oocyte Retrieval in the Weekend. S. Goto, K. Takakura, K. Takebayashi, K. Nakanishi, Y. Masuda, T. Kimura, M. Hirose, M. Akiyama, Y. Noda. Department of Obstetrics and Gynecology, Shiga University of Medical Science, Otsu, Japan.

Objective: The scheduled method of ovarian hyperstimulation for IVF-ET in which the day of oocyte retrieval was determined in advance to avoid oocyte retrieval during the weekend was evaluated and compared to the conventional method.

Design: A retrospective study.

Materials and Methods: Twenty cycles in patients undergoing ovarian hyperstimulation for IVF-ET were stimulated according to the scheduled method (scheduled group). Fifty-three cycles in patients with similar indications of IVF-ET and similar age-group were stimulated by the conventional method of ovarian hyperstimulation (conventional group). In the scheduled method, injection of FSH and hMG under GnRHa long protocol was started 12 days before the day of oocyte retrieval. The day of oocyte retrieval was scheduled on Monday, Tuesday or Wednesday. In the conventional method, FSH and hMG under GnRHa short protocol were administered daily until the second dominant follicle reached 18mm in diameter. Other procedures were similar in the two group. ET was conducted 48 hours after oocyte retrieval. The cancellation rates (CR), the clinical pregnancy rates (PR), the day of oocyte retrieval and the grade of embryos transferred were evaluated in the two groups.

Result: The PR in the scheduled group and the conventional group were 40.0% and 13.9%, respectively. In 86.4% of cycles in the scheduled group, oocyte retrievals were performed on the scheduled day. The CR and the grade of embryos were comparable between the two groups.

Conclusion: The scheduled method of ovarian hyperstimulation for IVF-ET brought preferable clinical outcome and was the useful method for avoiding oocyte retrieval on the weekend.

P-194

Assessment of the Accuracy of Rapid Fluorescence In-Situ Hybridization for the Detection of X and Y Chromosomes in Uncultured Amniocytes. X. Z. Zheng, J. Liu, Y. L. Tsai, [1]J. L. Hwang, [1]T. A. Baramki, R. A. Yazigi, G. Compton, E. Katz. The Greater Baltimore Medical Center Fertility Center, the Greater Baltimore Medical Center, Baltimore, MD. [1]Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, Republic of China.

Objective: To assess the accuracy of a rapid fluorescence in-situ hybridization (FISH) method for detection of chromosomes X and Y in uncultured amniocytes for prevention of X-linked disorders. The FISH results were compared to the karyotype in each patient.

Design: A rapid FISH procedure with five minute hybridization time for detection of chromosomes X and Y in uncultured amniocytes was carried out. Accuracy of the rapid FISH procedure was determined by checking the karyotype in each sample.

Materials and Methods: Fresh amniotic fluid was obtained after amniocentesis, which was carried out at 16–18 weeks of pregnancy. One mL of fresh amniotic fluid was transferred into an Eppendorf tube; the tube was centrifuged for 10 min at 300g. After removal of most of the supernatant, the pellet was resuspended in about 100 μL of the fluid. Fifty μL of the cell suspension was used to make a smear on a glass slide. The slide was left at room temperature for 30 min. The slide with dried uncultured amniocytes was put into a jar containing 0.01% pepsin and 0.01N HCl. The jar was kept in a 37°C waterbath for 10 min; the slide was then rinsed in water. The FISH procedure was the same as described by Liu et al (Fertil Steril 1998;70: 927–32) except that 5-minute hybridization time was used. Direct label fluorescence Vysis CEP probes for chromosomes X and Y were used. After FISH, approximately 50 nuclei of amniocytes were examined in each slide. Chromosomes were analyzed in the cytogenetics laboratory at the Greater Baltimore Medical Center using a standard G-banding method.

Results: Fresh uncultured amniocytes from one hundred pregnant women were analyzed. The results of the rapid FISH for detection of chromosomes X and Y corresponded to the karyotype. The whole FISH procedure can be completed in 40 min.

Methods	No. of samples with chromosomes XX	XY
Rapid FISH	52	48
Karyotype	52	48

Conclusion: Chromosomes X and Y of uncultured amniocytes can be detected by using rapid FISH with 100% accuracy. This FISH procedure is quicker than conventional karyotype analysis for detection of sex chromosomes and it is useful for prenatal diagnosis for prevention of X-linked disease.

This study was supported by a grant from the Greater Baltimore Medical Center.

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Improved Clinical Pregnancy Rates Using a Sequential Media Embryo Culture System (G1.2/G2.2). J. M. Jones, S. S. Shapiro, O. Khorram. Department Obstetrics/Gynecology, University of Wisconsin Hospital and Clinics, Madison, WI.

Objective: To report our preliminary experience over a one year period using a sequential media embryo culture system (G1.2/G2.2) for the culture of human embryos in a clinical IVF setting.

Design: Ninety-two consecutive embryo transfer cycles from January to December of 1998.

Materials and Methods: The culture media used in this study were prepared at the University of Wisconsin Hospital and Clinics using formula developed by David Gardner and Michelle Lane. This sequential embryo culture system consisted of five different media: Hepes-buffered Flush Medium (FM), Insemination Medium (IM), G1.2 Medium (G1), G2.2 Medium (G2) and Transfer Medium (TM) which were supplemented with Synthetic Serum Substitute (SSS) at concentrations of 5%, 5%, 5%, 2% and 8% respectively. After standard ovarian stimulation, oocytes were retrieved in FM, washed with IM, placed in 1.0ml of IM under oil and inseminated with sperm prepared by a standard swim-up procedure using IM. After 12 to 16hrs co-incubation, the resulting zygotes were washed in G1, placed in 10ul drops of G1 under oil and cultured for an additional 48hrs under 7% CO_2, 5% O_2, 88% N_2. For patients receiving a Day 3 (6-8 cell) embryo transfer, up to four embryos were placed in TM for immediate transfer. Any remaining embryos were washed in G2, placed in 10ul drops of G2 under oil and cultured for an additional 48hrs with 7% CO_2, 5% O_2 88% N_2 and any resulting blastocysts were then frozen. For patients receiving a Day 5 blastocyst transfer, all cleaving embryos of were washed in G2, placed in 10ul drops of G2 under oil and cultured for an additional 48hrs under 7% CO_2, 5% O_2, 88% N_2. No more than two blastocysts were transferred using TM and any remaining blastocysts were frozen.

Results: Prior to the development of sequential media culture systems, the ART Laboratory at the University of Wisconsin Hospital and Clinics utilized a single step culture medium for the growth of human embryos. From 1992 until 1995 our laboratory used Ham's F-10 supplemented with fetal cord serum and obtained an average clinical pregnancy rate of 23.9%. In 1996 and 1997 an increased pregnancy rate was observed (Mean = 37.3%) after switching to Human Tubal Fluid (HTF) medium supplemented with SSS. Then as described above, in January of 1998 our laboratory began using a sequential media culture system (G1.2/G2.2) and again observed an increase in the overall pregnancy rate to 50.0% (46/92). The pregnancy rate for Day 3 embryo transfer cycles was 44.3% (21/70) and the pregnancy rate for Day 5 embryo transfer cycles was 68.2% (15/22). No (0) Day 5 embryo transfers were canceled and we observed an implantation rate of 55.8% (24 sacs from 43 blastocysts transferred).

Conclusions: Our preliminary experience suggests that the G1.2/G2.2 sequential embryo culture system may provide improved clinical pregnancy rates for Day 3 embryo transfer cycles. Additionally the relatively high clinical pregnancy and implantation rates observed for blastocyst stage transfer cycles in this study suggests a mechanism to reduce the high number of multiple gestation pregnancies associated with Day 3 embryo transfer cycles.

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Does Blastocyst Development in Remaining Embryos Predict the Pregnancy Rate? M. A. Henry, W. L. Gentry, E. S. Critser. Clarian Health Partners, Indianapolis, IN.

Objectives: Although there seem to be advantages to transferring blastocyst stage embryos on day 5, there is concern that many patients do not have embryos that develop to this stage. The natural assumption would be that these patients would not likely become pregnant, even if the embryos were transferred at the cleavage stage. To address this issue, we compared the pregnancy rates between patients whose remaining, or "extra", embryos developed to the blastocyst stage with those whose remaining embryos did not develop to blastocysts.

Design: Retrospective comparison of pregnancy rates between patients whose remaining embryos developed to blastocysts with those patients whose embryos did not develop to blastocysts.

Materials and Methods: Records were reviewed from all patients who underwent an IVF procedure with a day 3 transcervical embryo transfer in 1998. Only patients who had all their remaining embryos maintained in culture until day 5 or day 6 were included. Embryos that progressed to blastocyst stage were frozen. Those embryos that did not develop to blastocysts by day 6 were discarded. Ongoing pregnancy rates between these two groups were compared via chi-squared analysis.

Results: There were 133 patients who met the inclusion criteria. The ongoing pregnancy rate (defined as fetal heart activity) was 40% (33/83) in those patients whose embryos developed to blastocysts. When blastocyst

stage embryos were not attained, the ongoing pregnancy rage was 42% (21/50). These rates were not different ($p>0.05$).

Conclusion: The development of the remaining embryos to the blastocyst stage (or lack of development) does not predict whether or not a patient will become pregnant following transfer of day 3 embryos within that cycle. Even though the embryos selected for transfer on day 3 consisted of those that appeared most likely to produce a viable pregnancy, these data suggest that some patients conceive following a day 3 transfer who may otherwise not even achieve a day 5 blastocyst transfer.

P-197

Does Blastocyst Development of Non-Transferred Embryos Predict Pregnancy During IVF Cycle? [1]D. G. Diaz, [2]F. A. Morales, [1]B. D. Smith, [1]M. C. Karl. [1]West Coast Fertility Centers, Fountain Valley, CA, USA and [2]Division of Reproductive Biology and Endocrinology, Department of Obstetrics and Gynecology, Universidad Autónoma de Nuevo León, Monterrey, México.

Objectives: In human in- vitro fertilization (IVF) embryos area routinely transferred to the uterus on day 2 or day 3 of development. Resultant implantation and pregnancy rates are 10-15% per embryo transferred. Transfer of early cleavage stage embryos to the uterus is performed in preference to blastocyst stage transfer primarily because of concern that prolonged culture of embryos in laboratory conditions could compromise their viability. The purpose of this study was to determine the predictive value of the non-transferred embryos to reach blastocyst stage, on the pregnancy rate for the embryos transferred on day 2 or 3.

Design: Retrospective clinical study in a private IVF unit.

Materials and Methods: 75 patients were studied between December 1996 and December 1997 resulting in 83 cycles. The inclusion criteria were controlled ovarian hyperestimulation (COH), oocyte retrieval, transfer of fertilized embryos on day 2 or 3, and culture of remaining embryos to blastocyst stage. The patients were included in the study regardless of age or infertility causes. Oocyte retrieval was performed 34 hr after the administration of hCG 10 000 IU. Oocyte culture, insemination and early embryo culture was performed in QB XI medium suplemented with 5% H.S.A. Oocytes were inseminated 4-7 hrs after the retrieval, depending on their maturity; fertilization scoring was done 15-18 hrs after the insemination. The best embryos were choosen for transfer. The number of embryos transferred depended on age and infertility history. All other embryos were transferred to HAM'S F-10 medium suplemented with 5% H.S.A. and cultured for an extra 2-3 days to obtain blastocysts. Blastocysts were cryopreserved on day 5 or 6. We correlated the blastocyst development with positive pregnancy outcome in each cycle.

Results: 55 out of 83 cycles (66.3%) achieved at least one expanded blastocyst. 28 cycles did not develop blastocysts (33.7%). There was no difference in the pregnancy outcome between both groups. In the blastocyst group, 20 pregnancies occurred (36.3% pregnancy rate, n=55), while in the group without blastocyst developed 10 pregnancies occurred (35.7% pregnancy rate, n=28).

Conclusions: The development of blastocyst stage from embryos not used at embryo transfer, does not have positive predictive value on the pregnancy rate for the embryos transferred on day 2 or 3.

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Advantages of Intracytoplasmic Testicular Sperm Injection in the Inflammatory Obstructive Azoospermia. K. Mahmoud, M. H. Ben Aribia, F. Zhioua, F. Meherzi, J. Nemsia, H. Zayani, M. Elouakdi. Centre de Medecine de la Reproduction et de Diagnostic Prenatal, Clinique El-Manar, Tunis, Tunisia.

Objectives: The aim of our study is to determine the influence of the chronic inflammation on the epididymial sperm performance within a group of infertile patients with obstructive azoospermia, treated with ICSI, and for whom the interest to use of testicular spermatozoa recovered by percutaneous aspiration.

Design: Prospective study during 1998.

Materials and Methods: From January 1998 to December 1998, the results of ICSI procedure were compared between three groups of patients: group 1: Congenital bilateral absence of the vas deferens treated with epididymial spermatozoa, group 2: chronic inflammatory obstructive azoospermia treated with epididymial spermatozoa, group 3: chronic inflammatory obstructive azoospermia treated with testicular spermatozoa. We have used GnRHa & gonadotropins (long protocol) for ovarian stimulation for the 63 women. The following table shows a summary of the clinical characteristics of couples:

	Group 1	Group 2	Group 3	Total
No of cycles	16	22	25	63
Age of women (y)	22.9 ± 2.1	25.9 ± 4.4	26.8 ± 5.2	25.5 ± 4.6
Age of men (y)	33.9 ± 7.8	37.7 ± 8.1	39.2 ± 8.7	37.3 ± 8.4
Duration of infertility	3.8 ± 1.4	6.2 ± 3.9	6.04 ± 3.0	5.5 ± 3.2

Results: The results are shown through the below table:

	No of cycles	# Ova Inject.	No of 2PN	% Fertil.	No of Emb.	% Cleav.
Group 1	16	153	89	58.2	85	95.5
Group 2	22	216	97	44.9	77	79.4
Group 3	25	237	161	67.9	147	91.3

	#Good Emb.	#Emb. Transf	No of IUsacs	% Impl.	#Clin Pregn	% Pregn
Group 1	65	44	7	15.9	5	31.2
Group 2	36	56	6	10.7	5	22.7
Group 3	124	61	10	16.4	8	32

Conclusions: Testicular spermatozoa yields a better fertilization, cleavage, pregnancy and implantation rate than epididymal spermatozoa in chronic obstructive genital tracts. It seems that chronic inflammatory reduce the epididymal sperm performance. Our results and the easy retrieval testicular spermatozoa (TESA) suggest that they should be preferred in ICSI procedure for the treatment of men with chronic inflammatory obstructive azoospermia.

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The Difference in Fertilization, Cleavage, Implantation and Pregnancy Rates Between Standard ICSI Procedure and Day 1 'Rescue' ICSI Following Total Fertilization Failure. N. Dean, S. J. Phillips, M. M. Biljan, S. L. Tan. McGill Reproductive Center, Royal Victoria Hospital, McGill University, Montreal, Canada.

Objectives: Since the introduction of ICSI the proportion of cycles with total failed fertilization has considerably decreased. However, unexpected failed fertilization still occurs up to 10% of cases following standard IVF treatment. It has been suggested that in these patients, ICSI performed on the day following oocyte collection (day 1) could result in fertilization and pregnancy ('rescue ICSI'). This study compares in, a prospective manner, the fertilization, cleavage, implantation and pregnancy rates between ICSI performed on the day of oocyte collection ('standard ICSI') and rescue ICSI.

Design: Prospective, observational study. We have previously observed a pregnancy rate of 35% for standard ICSI in our centre. 196 controls and 18 study patients were required to have 80% power ($\alpha=0.05$) to detect a decrease of pregnancy rate to 5% which was previously reported following rescue ICSI.

Materials and Methods: Between October 1, 1997 and December 1, 1998 22 patients undergoing IVF had total fertilization failure on day 1 (6.9%). Of these 18 consented to have all mature oocytes injected between 19 and 26 hours after oocyte collection. The outcome of these cycles was compared to all 230 standard ICSI cycles performed during the same period of time.

Results: Initial diagnosis was not predictive of likelihood of total fertilization failure. Fertilization (49.6% vs 66.8% Odds Ratio (OR)=2.1 95% Confidence Interval (CI)=1.4–3.0), cleavage (86.0% vs 95.6% OR=3.7 95%CI=1.5–8.3), and pregnancy rates (5.6% vs 33.5% OR=5.9 95%CI=1.2–175.7) were significantly lower in the patients who had rescue ICSI. Additionally, in the study group a tendency towards lower implantation rates (5.4% vs 17.9% OR=3.1 95%CI=0.9–23.3) was observed. Fol-

lowing an unsuccessful cycle with rescue ICSI, seven patients proceeded with standard ICSI in subsequent cycles and four achieved a pregnancy (57.1%).

Conclusions: Contrary to previously published data, patients with unexplained infertility did not have an increased chance of total fertilization failure. When compared with standard ICSI, rescue ICSI results in significantly lower fertilization, cleavage, and pregnancy rates. Therefore, rescue ICSI should be performed only in patients fully aware of its limitations. Moreover, in subsequent cycles patients who had total fertilization failure yield good pregnancy rates following standard ICSI procedure.

P-200

The Inefficacy of Cervical Mucus Aspiration Prior to Embryo Transfer. A Prospective Randomized Trial. [1]C. Ruhlmann, [1]C. Bisioli, [1]G. Terrado, [1]E. D. Rolla, [1]R. E. Nicholson, [1]D. Gnocchi, [1]P. Mentasti. [1]Unidad de Fertilidad San Isidro, San Isidro, Buenos Aires, Argentina.

Objective: To assess the efficacy of cervical mucus aspiration previous to fresh embryo transfer.

Design: Prospective randomized trial.

Materials and Methods: Ninety seven patients, scheduled for IVF or ICSI procedures, were prospectively randomized at the time of oocyte retrieval. In group A (N=48), cervical mucus aspiration was performed with an insulin syringe immediately before embryo transfer. In this group one patient had no embryos available for transfer. In group B, cervical mucus aspiration was obviated (N=49). Both groups were comparable regarding: female age (34.7 ± 4.3 vs. 34.6 ± 4.9 years), peak estradiol levels (736.7 ± 411.9 vs. 752.9 ± 419.8 pg/ml), number of metaphase II oocytes retrieved (7.0 ± 4.9 vs. 6.1 ± 4.1), number of transferred embryos (3.5 ± 1.1 vs. 3.3 ± 1.0), respectively for groups A and B. For statistical comparison Student's "t" test and Chi square were used as appropriate. Implantation and clinical pregnancy rates were the main outcome measures.

Results: There were no significant differences between group A and B in the implantation rate (13.3% vs. 12.3%, respectively), neither in the clinical pregnancy rate (34.0% vs. 32.6%, respectively).

Conclusion: Cervical mucus aspiration seems an unnecessary procedure at the time of embryo transfer.

P-201

Microdose GnRH-a Flare Protocol For Poor Responders (Flare Protocol). D. Billay, J. Fleetham, A. Kenefick, S. Servis, C. Greene, S. Scott, J. O'Keane. Foothills Hospital Regional Fertility Programme, Calgary, AB, Canada.

Objective: To determine the pregnancy rate (PR) and implantation rate (IR) for patients ≤ 38 years of age and ≥ 39 years of age who underwent microdose GnRHa Flare protocol for IVF stimulation during 1997 and 1998 at the Regional Fertility Programme (RFP). The Flare protocol used at our clinic consisted of 21 days of oral contraceptives (Cyclen 21) followed by microdose GnRHa (s.c. Lupron 40 ug bid) starting on Day 3 and gonadotrophins beginning on Day 6 at a dosage of 400 to 450 IU daily until oocyte retrieval. Embryos were transferred on Day 3 and all underwent assisted hatching.

Design: Retrospective chart review.

Materials and Methods: All IVF cycles at the RFP between 1997 and 1998. Patients eligible for the Flare protocol were those that had a cancelled IVF cycle with the standard protocol (≤ 6 follicles on Day 7) and those who consistently demonstrated reduced ovarian reserve by Day 3 FSH and Estradiol values. The mean number of oocytes retrieved, embryos transferred, the clinical PR confirmed with a fetal heart and IR were calculated.

Results:

	No. of patients	Mean age	Mean no. oocytes	Mean no. ET	Clinical Pregnancies	PR(%)/ ET	IR(%)/ Embryo
≤38 years	78	34.9	8.2	3.0	32	41.0	21.8
≥39 years	62	40.7	7.7	3.7	18	29.0	13.0

Conclusion: Microdose GnRH Flare protocol for poor responders achieves acceptable ongoing pregnancy rates in a traditionally very difficult group of patients to treat with a poor prognosis for IVF success. Flare protocol increased the number of oocytes retrieved allowing for a better choice of embryos for ET and the possibility to cryopreserve supernumerary embryos.

P-202

Comparison of Fertilization, Cleavage and Pregnancy Rates of ICSI with Mature Spermatozoa Obtained from Different Origins. [1]O. Karabulut, [1]M. Karacan, [1]H. Erkan, [1]I. Daskaya, [1]M. Benhabib, [2]E. Çoskuner. [1]Department of Obstetrics and Gynecology, [2]Department of Urology, International Hospital-Istanbul, Yesilkoy, Istanbul, Turkey.

Objectives: Since the introduction of ICSI and with the advent of sperm retrieval techniques (either from the testis or the epididymis) azoospermic (obstructive or non-obstructive) patients can be treated successfully. Since sperm retrieval from the epididym is an unpredictable process, we prefer to retrieve spermatozoa from the testis, either by aspiration or extraction. The aim of this retrospective study is to compare the fertilization, cleavage and pregnancy rates of ICSI with ejeculated and testicular motile and mature spermatozoa.

Design: Retrospective analysis of 141 consecutive cycles in which fresh ejeculated sperm (118 cycles) in Group I and testicular sperm (23 cycles) in Group II were used for injection.

Materials and Methods: Patients were divided according to the origin of sperm injected. Group I was treated with ejeculated sperm and Group II with testicular sperm obtained from obstructive and non-obstructive patients. There was no significant difference in age or duration of infertility between groups. Testicular sperm recovery procedure were scheduled on the day of oocyte retrieval. In Group II if the testis volume and consistency were within normal limits, testicular aspiration was performed. In cases where no sperm retrieved by aspiration, testicular extraction was carried out. Only mature and motile spermatozoa were used for injection. Cases with immature and immotile spermatozoa were excluded from the study.

Results: Fertilization rates were 64% (843/1307) and 56% (117/206) and cleavage rates were 62% (813/1307) and 53% (111/206) in Group I and Group II, respectively. No significant differences in either fertilization or cleavage rates were observed after ICSI with spermatozoa from ejeculate or testis. There was also no difference in pregnancy rates between groups, 38% (46/118) vs 30% (7/23).

Conclusion: ICSI with motile and mature spermatozoa irrespective of its origin either from ejaculate or testis yields comparable results.

P-203

The GnRH Antagonist (Cetrorelix) Single Dose Protocol in 78 Cycles of Ovarian Stimulation for IVF-ET. [1]F. Olivennes, [1]R. Fanchin, [1]C. Righini, [1]N. Lédée, [2]S. Hamamah, [2]N. Frydman, [2]C. Conord, [3]P. Bouchard, [1]R. Frydman. [1]Department of Obstetrics and Gynecology, A. Beclere Hospital, Clamart, France. [2]IVF Center, A. Beclere Hospital, Clamart, France. [3]St Antoine Hospital, Paris, France.

Introduction: New GnRH antagonists will be available shortly in IVF-ET. We have designed a GnRH antagonist single dose protocol in which a single injection of Cetrorelix is administered in the late follicular phase. In previous studies, we have chosen the dose of 3 mg which is efficient in preventing LH surge for at least 4 days. We are presenting the results of 78 IVF-ET cycles using this protocol.

Design: Retrospective analysis.

Material and methods: Patients between 20 and 38 years old, with normal ovulation and no more than 3 previous IVF attempts were included in the study. The stimulation was started on day 2 of the menstrual cycle with 2 ampules of hMG or rec-FSH. A single dose of 3 mg Cetrorelix (ASTA Medica, Frankfurt, Germany) was injected systematically on stimulation day 7 except if the ovarian response was too slow. Monitoring of the cycle was done with ultrasounds and plasma levels of LH, E2, FSH, P. Triggering of ovulation was obtained with hCG administered when 2 follicles were ≥ 18 mm. Luteal support was systematically administered with vaginal natural progesterone (600 mg/day).

Results: No LH surge was observed after the administration of the 3 mg

Cetrorelix. The IVF-ET results are presented in the Table (mean ± SD).

Stim length	N Amp.	E2 dayhCG pg/ml	Oocytes (total)	Mature Oocytes (%)	Fertilization rate (%)
8.9 ± 1.5	22.8 ± 6.4	1547 ± 744	6.8 ± 4.0	83.4	74.3

Stim length	Embryos (total)	Embryos transferred	Ongoing PR./OPU (%)	OHSS
8.9 ± 1.5	4.4 ± 3.2	2.5 ± 1.0	23.7	0

Conclusion: The GnRH antagonist (Cetrorelix) protocol allows us to prevent premature LH surge and ovulation in all the patients treated. The IVF-ET results were good. These results, on a large number of patients, confirm the interest of our single dose Cetrorelix protocol which allies efficiency and simplicity.

P-204

Proportions of Morphologically Normal Sperm as a Predictor for Fertilization and Pregnancy. [1,2]G. S. Hamilton, [1]C. J. Faraci, [1,3]F. F. W. Lee, [1,3]W. G. McTavish, [1,3]J. V. Kredentser. [1]Heartland Fertility & Gynecology Clinic, Winnipeg, MB and Departments [2]Physiology and [3]Obstetrics & Gynecology, University of Manitoba, Winnipeg, MB.

Objectives: Like many fertility clinics, our laboratory routinely determines the proportion of morphologically normal sperm (%MNS) as part of our semen analysis. Since a number of previous studies have indicated that this information may help to predict the success of various fertility treatments, patients with low %MNS may consider intracytoplasmic sperm injection (ICSI) despite having adequate total motile sperm counts (TMC) for invitro fertilization (IVF) or intrauterine insemination (IUI). We have examined the results of treatment cycles involving male partners with %MNS assessments previously done in our laboratory. The study was intended to identify ways to use sperm morphology and other semen analysis results to best counsel patients on appropriate fertility treatment.

Design: A retrospective study was done to correlate the %MNS with fertilization rates for IVF and ICSI patients and with pregnancy rates for IVF (n=73), ICSI (n=57) and IUI (n=32) cycles. Sperm morphology and TMC data were collected from semen analysis performed during preliminary fertility work-ups. Fertilization and pregnancy data were derived from subsequent treatment cycles.

Materials and Methods: Semen was collected by masturbation. Concentration, percentage motility and progression were determined in the raw sample and following processing by swim-up. Stained smears of raw semen were observed (by 2 trained observers) at high magnification (~1000x) and the number of normal sperm (according to strict criteria of Kruger; Fertil Steril 49:112, 1988) was recorded per 100 total sperm counted. When patients advanced to subsequent treatment cycles, IVF and ICSI sperm samples were processed by swim-up and IUI samples were processed by wash, swim-up, or density gradient centrifugation. Pregnancy was determined by measurable serum hCG 14 to 16 days after insemination. Fertilization rates (IVF and ICSI patients) were calculated as the ratio of bipronucleate eggs (16–20 hours post-insemination) to the number of eggs retrieved.

Results: Regression analysis failed to show a significant correlation between the %MNS and pregnancy rates for subsequent IUI, IVF and ICSI treatment cycles ($p>0.05$). Fertilization and pregnancies occurred in all programs, even at the lowest range of %MNS. The %MNS correlated significantly with IVF fertilization rate ($p<0.05$) but not with ICSI fertilization rate ($p>0.05$).

Conclusions: While we have found it useful to determine proportions of morphologically normal sperm (%MNS) during semen analysis, this information, in itself, has only limited ability to predict the success of various forms of fertility treatment. Our results indicate %MNS may predict low versus high fertilization rates in our IVF program and that ICSI effectively neutralizes the effect of low %MNS on fertilization rates. The high numbers of eggs commonly retrieved for IVF likely prevented the reduced fertilization rate from significantly affecting pregnancy rates, although numbers of embryos frozen would be expected to be reduced. We believe that high TMC following semen processing may compensate for low %MNS in IUI programs thus preserving pregnancy rates.

P-205

Withdrawn

P-206

Comparison of Follicle Stimulating Hormone Preparations: Results of Women Undergoing GnRH-a Down Regulated IVF Cycles. J. L. Morris, J. L. Fratterelli, S. D. Ernst, T. Smith, P. A. Bergh, R. T. Scott Jr. Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, Livingston, NJ.

Objectives: To determine the impact of different gonadotropin preparations on ovarian responsiveness and pregnancy rates in women undergoing stimulation for IVF following GnRH-a down regulation.

Design: Retrospective review of stimulation and pregnancy data.

Materials and Methods: Patients received gonadotropin therapy after GnRH-a down regulation for ovarian stimulation during IVF cycles. Patients were divided into three categories based on the type of gonadotropin they received: 1.) Urinary gonadotropins delivered IM; 2.) Urinary gonadotropins delivered SC; and 3.) Recombinant gonadotropins given SC. Data compared included age, basal FSH level, days of gonadotropin therapy, total number of ampules received, peak E_2 level, number of oocytes retrieved, fertilization rate, pregnancy rate, and sustained implantation rate. Data were evaluated by ANOVA, MANCOVA, and contingency tables.

Results: The study populations had equivalent ages and basal FSH levels. Many stimulation parameters were equivalent with no differences in duration of stimulation, or number of oocytes retrieved. Women receiving recombinant FSH had lower peak E_2 levels ($P<0.01$) and received fewer ampules of medication ($P<0.02$). Most importantly, there were no differences in fertilization rate, initial or ongoing pregnancy rate, or sustained implantation rate (ongoing sacs per ET). Covariate analysis accounting for age did not change any of the statistical conclusions.

	Age (yrs)	Day 3 FSH	Peak E2 (pg/mL)	# of Amps	Oocytes (n)	Pos hCG (%)	Sustained Implant (%)
Urinary - IM	35.4	4.5	2625	44.2	16.5	66.5	30.2
Urinary - SC	35	4.8	2595	41.6	17.2	69.4	30.9
Recombinant - SC	34.9	6.3	2348	39.7	16.4	66.3	32.1

Conclusions: These data indicate that the type of gonadotropin preparation (urinary vs recombinant), and the route of administration (SC vs IM) did not impact clinical outcomes for the population as a whole. These data do not preclude the possibility that various clinical subsets of patients may have differing responses to these medications. Specific gonadotropin stimulation protocols may be needed to optimize clinical responses and outcomes in specific settings.

P-207

Day 3 Estradiol Values are Prognostic of In Vitro Fertilization (IVF) Outcome. B. Miller, J. Seagars, R. Alvero, J. Fratterelli, J. MacKeeby, J. Broussard, L. Scott. RSC of the Combined Federal Program of WRAMC, NNMC, USUHS, Washington, DC 20307.

Objectives: It is well known that elevated serum follicle stimulating hormone (FSH) levels on day 3 of the cycle are counter indicated for IVF with resulting low oocyte retrieval rates and low implantation and pregnancy rates. There is no reliable data on the prognostic value of elevated estradiol on day 3 and if it is pronostic what the cut off point should be. The purpose of this study was to elucidate the value of day 3 E2's as a prognostic tool, taking into account E2 levels, age and day 3 FSH level.

Design: Data from 198 IVF cycles was retrospectively analysed with respect to day 3 E2 and FSH, age, and outcome.

Materials and Methods: Day 3 blood work was obtained the month prior to entering the program and the patient was then placed on continuous low dose oral contraceptive pills (OCP's) without the use of the placebos. A base-line (BL) appointment is set up and if the E2 is not elevated OCP's are discontinued and a microdose GnRH flare followed by gonadotropins commences. Oocyte retrieval is performed 12–15 days later, 36 h post-hCG. Embryo transfers (ET) were performed on day 3 or day 5 according to age and number of embryos obtained. Only clinical pregnancies confirmed by ultrasound 6 weeks after OR were counted in the data. The data was analysed with regression analysis and chi square analysis for significance between groups.

Results: On regression analysis there was a correlation between age, E2 level and pregnancy. The intersecting point for age and E2 levels was 35 years and an E2 level of 75. E2 values had no significant impact on any fertilization rates or FSH levels. In women <35 Day 3 E2 levels >75 were prognostic for pregnancy ($P<0.01$ cf <75). In women over 35 there was an overall reduction in pregnancy rate that was age related. There was a further reduction when E2 values were elevated ($P<0.001$). The results are presented below:

Age	<35		>35	
Day 3 E2	<75	>75	<75	>75
#	101	19	66	12
pregnant (%)	44 (44)	14 (74) $P<0.01$	26 (39)	1 (8) $P<0.001$

Conclusions: Day 3 E2 values >75 are prognostic of pregnancy in younger women but not older women, where a lower E2 level is more desirable.

P-208

Use of Crinone* for Luteal Support in *In vitro* Fertilization Cycles. S. J. Chantilis, K. M. Zeitoun, S. I. Patel, D. A. Johns, V. A. Madziar, D. D. McIntire. The University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

Objectives: Luteal phase support with progesterone is a commonly used treatment for prevention of luteal phase deficiency following *in vitro* fertilization and embryo transfer (IVF-ET). In this study, the results of Crinone 8%, a sustained release vaginal progesterone gel formulation, administered for luteal phase support were compared to those with intramuscular (IM) progesterone following IVF-ET.

Design: Pregnancy outcome and midluteal progesterone levels among Crinone users, obtained in a prospective nonrandomized manner, were compared to a historical control group.

Materials and Methods: The Crinone group consisted of 100 patients undergoing IVF-ET who agreed to participate in an IRB-approved study using Crinone (90 mg once daily), a vaginal gel, for luteal phase support beginning the evening of oocyte retrieval. The historical control group ($N=106$) consisted of all IVF-ET patients using homologous, fresh oocytes who completed treatment in 1996. All patients in the control group received a standard regimen of daily IM progesterone treatment, 50 mg, beginning the evening of oocyte retrieval. Luteal phase serum progesterone levels were obtained 6–7 days after oocyte aspiration. Serum β-hCG was obtained 14 days after oocyte retrieval. Clinical pregnancy was determined by sonography conducted at 4–6 weeks conceptual age. The two groups were compared with respect to age, number of embryos transferred, luteal phase progesterone levels, pregnancy rates and miscarriage rates.

Result(s): The age (34.2 versus 33.5 years old) and number of embryos transferred (3.7 versus 3.5) were similar between the Crinone and control groups, respectively. Positive β-hCG rates (53.2% versus 49.2% for patients <35 years old; 48.9% versus 44.4% for patients 35–39 years old), clinical pregnancy rates/transfer (40.4% versus 44.4% for patients <35 years old; 33.3% versus 44.4% for patients 35–39 years old), and ongoing pregnancy rates (36.2% versus 36.5% for patients <35 years old; 33.3% versus 36.1% for patients 35–39 years old) were similar for the Crinone and IM progesterone groups, respectively. Women using Crinone had higher rates of biochemical pregnancy loss (24.0% versus 4.8% for patients <35 years old; 15.6% versus 0.0% for patients 35–39 years old), but lower clinical pregnancy loss versus spontaneous abortion (10.5% versus 22.7% for patients <35 years old; 0.0% versus 18.8% for patients 35–39 years old) than those women using FM progesterone. Midluteal serum progesterone concentrations were significantly higher ($p=0.0015$) in the IM progesterone group (94.3 ± 8.8 versus 57.7 ± 7.4 ng/mL). Several women using Crinone experienced vaginal bleeding 11 to 13 days after oocyte retrieval.

Conclusion(s): For all ages categories, positive β-hCG, clinical and ongoing pregnancy rates were similar when Crinone or IM progesterone were given for luteal phase support in IVF-ET, despite lower serum progesterone concentrations seen with Crinone. Women treated with Crinone suffered more biochemical pregnancy loss than women treated with IM progesterone, however, clinical pregnancy loss occurred less, resulting in similar ongoing pregnancy rates. While bleeding was not a measured outcome, several women treated with Crinone experienced vaginal bleeding prior to cycle day 28, the pregnancy test day. While the results of this study support the use of Crinone as an acceptable alternative for luteal support following IVF-ET, differences in bleeding patterns and biochemical pregnancy loss demonstrate the need for a prospective randomized study. *Study supported by Columbia Research Laboratories, Inc.

P-209

Double Embryo Transfer (48–72 hours + blastocysts) vs. Single Embryo Transfer (48–72 hours) in ART. I. Ben-Shlomo, J. Golan, V. Eyali, Z. Wiener-Megnagi, J. Geslevich, E. Shalev. Division for Reproductive Endocrinology and ART, Department of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula and the Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Objectives: Recent reports indicate that growing embryos in vitro to the blastocyst stage may improve implantation and conception rates, alongside reduction in multiple gestation rate. We have succeeded in growing embryos to the blastocyst stage in good proportion of the allocated embryos. This series was analyzed to evaluate whether double embryo transfer (ET) of both 48–72 hour embryos and blastocysts can improve pregnancy rate (PR) when compared to single transfer of 48–72 hours' embryos in patients undergoing ART.

Design: Patient-elected design of ET out of three available options.

Materials and Methods: From August 1997 through August 1998, 360 fresh and 110 frozen/thawed ET cycles were performed. Patients with more than 8 cleaving embryos after 48 hours were offered three options: 1) Conventional ET after 48–72 hours. 2) 48–72 hours' transfer of 2-3 embryos, culture of 3-5 embryos for transfer of 1-2 blastocysts and cryopreservation of supernumerary embryos. 3) Culture of 3-5 embryos for transfer of 2 blastocysts and cryopreservation of the remaining embryos.

Results: Of 360 fresh ET cycles, 83 (23%) had more than 8 cleaving embryos. Patients chosen either double ET (25) or single ET (58). None elected to receive only blastocysts. Mean ages of patients with 8 or more embryos (double ET: 28 ± 3.6 years, single ET: 30.3 ± 5 years) were lower than those of patients with 7 embryos or less (33.7 ± 5.5 years). Gravidity, parity, number of previous cycles and number of days of stimulation did not differ between the three groups. Mean number of ampules of hMG used was higher in the group with 7 or less embryos (43.9 ± 16) than in the groups with 8 or more embryos (double ET: 35.1 ± 8.7, single ET: 35.1 ± 10.5). Mean numbers of oocytes retrieved (8.9 ± 5.9), M2 oocytes in the cohort (5.9 ± 4.2), and cleaving embryos (3.6 ± 2.6), were lower in patients with 7 or less embryos, compared those with 8 or more embryos (double ET: 20.8 ± 6.9, 16.2 ± 5.7, 12.0 ± 3.3; single ET: 20.4 ± 7.2, 14.9 ± 4.9, 11.7 ± 4.3, respectively). Mean number of blastocysts transferred in the double ET group was 1.56 ± 0.65. Pregnancy rates (PR) per ET were 52.0% for double ET, 34.5% for single ET and 27% for the group with 7 or less embryos ($p = 0.01$). During the same period, out of 110 cryopreservation cycles, PR was 32% and projected cumulative PR for fresh and thawed ET's was above 50%.

Conclusions: Double transfer of 48–72h embryos and later blastocysts

was not detrimental to the treatment cycles. Double transfer did not result in PR, higher than the projected summation of fresh ET with later frozen/thawed ET.

P-210

Early vs. Late Injection of Sperm to Oocytes After Removal of the Cumulus Granulosa Cells: A Split Study. I. Ben-Shlomo, J. Golan, J. Geslevich, V. Eyali, E. Shalev. Division for Reproductive Endocrinology and ART, Department of Obstetrics and Gynecology, Ha'Emek Medical centre, Afula, and Rappaport Faculty of Medicine, Technion-Israel Institute of technology, Haifa, Israel.

Objectives: There have been inconclusive reports as to the appropriate time at which oocytes should be injected after their retrieval in assisted reproduction (ART). Due to concerns regarding nuclear-cytoplasmic concordant maturity, it is unclear what time is preferable for cumulus cells removal and later injection of sperm cells to the oocytes. To address the issue of the most appropriate time for injection of the oocytes after their denudation, we conducted a study in which we allocated the oocytes of each patient for either late or early injection.

Design: A prospective, comparative split-study.

Materials and methods: Twenty-four patients with an expected large cohort, who underwent oocyte retrieval for assisted fertilization were included. Oocytes were denuded of the surrounding cumulus cells within the first hour after retrieval and those in second meiosis (M2) were split to two groups. Oocytes in one group were injected immediately and those in the other were injected four hours later. Embryo transfer was performed 72 hours after oocyte retrieval. In vitro results were compared between the two groups.

Results: The mean number of oocytes in the whole group was 18.25 (±7.67), with 15 (±6.5) in the M2 stage, 1.8 (±1.4) in M1 and 1.0 (±1.0) in the germinal vesicle stage. A mean number of 7.2 (±3.0) vs. 7.3 (±3.2) oocytes were allocated for early injection and late injection, respectively. Of these 5.4 (±2.8) vs. 5.5 (±2.7) fertilized and 4.9 (±3.1) vs. 5.0 (±2.6) cleaved, respectively. The mean cell numbers in the resultant embryos were 5.7 (±2.1) vs. 5.5 (±1.7), respectively. All these were not statistically different. There was also no difference between the groups of oocytes in embryo quality. In a few cases there was a sharp difference between the two halves of the cohort, but this was distributed equally between early and late injection times.

Conclusions: When oocytes are denuded immediately after retrieval, there does not seem to be an advantage to a specific interval to injection within five hours. There may be women, in whom either late or early injection could improve results. If the latter is characteristic to the woman or the specific cycle remains to be determined.

P-211

Subsequent Embryo Transfers on Day 2 and Day 5: Is it Safe and Effective? T. H. Lee, K. S. Park, S. S. Chun. Department of Obstetrics and Gynecology, Kyungpook National University Hospital, Taegu, Korea.

Objectives: In vitro fertilization (IVF) and a prolonging the time of culture may be helpful in establishing a viable pregnancy through a selection effect. Some embryos do not develop beyond the 4-cell stage and some may not develop to the blastocyst stage. We have evaluated the safety of subsequent embryo transfers (SET) on day 2 and day 5 and the outcomes of pregnancy.

Design: A prospective randomized study.

Materials and Methods: Sperms were treated with Ham's F-10 supplemented with 10% human follicular fluid (hFF). Oocytes were cultured and fertilized in Dulbecco's Modified Eagle Medium (DMEM) with 10% hFF. Pregnancy rate was determined after the subsequent transfer of human embryos at the two to four cell stage on day 2 and at the blastocyst stage on day 5. For statistical analysis, student's *t*-test was used. Results were considered statistically significant when *P* value was less than 0.05.

Results: Results are presented in the following table.

	Day 2 ET	SET	*P* value
No. of cycles	19	18	
No. of used oocytes (/cycle)	121 (6.4)	220 (12.2)	
Fertilization rate (2PN, %)	99 (81.8)	180 (81.8)	NS
No. of cultured embryos	98	178	
Cleavage rate	94 (95.9)	174 (97.8)	NS
No. of ET cycles	19	18	
No. of transferred embryos (/cycle)	78 (4.1)	108 (6.0)	
Pregnancy rate	5 (26.3)	12 (66.7)	0.014

Conclusion: The results of this study showed that SET is safe and effective and significantly increases the pregnancy rate.

P-212

Developmental Capacity of Oocytes Cultured on Vero Cell Monolayers from Day 0 on *In Vitro* Fertilization-Embryo Transfer (IVF-ET) Cycles. T. H. Lee, K. S. Park, S. S. Chun. Obstetrics and Gynecology, Kyungpook National University Hospital, Taegu, Korea.

Objectives: To evaluate whether co-culturing of oocytes on vero cell monolayers from day 0 after egg retrieval resulted in an increase in cleavage rate and embryo grade compared to co-culturing from day 1.

Design: A prospective randomized study.

Materials and Methods: Oocytes were co-cultured from day 0 (group I, n=10) and 2PN oocytes were co-cultured from day 1 (group II, n=23) on vero cell monolayers after egg retrieval. The grading of embryos has been described by Veek (1991). For statistical analysis, student's *t*-test was used. Results were considered statistically significant when *P* value was less than 0.05.

Results: Results are presented in the following table.

		Group I	Group II	*P*-value
Used oocytes		64	208	
Fertilization rate (2 PN, %)		56 (87.5)	175 (84.1)	NS
Cultured Embryos		56	171	
Cleavage rate (%)		56 (100)	157 (91.8)	0.027
Grade of embryos	1	5 (8.9)	27 (15.8)	NS
	2	0	11 (6.4)	0.042
2~3 cell	3	11 (19.6)	18 (16.5)	NS
	4	0	1 (0.6)	NS
	5	0	0	
	Total	16 (19.6)	57 (33.3)	NS
	1	24 (42.9)	77 (45.0)	NS
	2	1 (1.8)	4 (2.3)	NS
4~6 cell	3	9 (16.1)	13 (7.6)	NS
	4	2 (3.6)	5 (2.9)	NS
	5	1 (1.8)	0	NS
	Total	37 (66.1)	99 (57.9)	NS
	1	1 (1.8)	1 (0.6)	NS
	2	0	0	
8 cell	3	0	0	
	4	2 (3.6)	0	0.017
	5	0	0	
	Total	3 (5.4)	1 (0.6)	0.025

Conclusions: This study shows that co-culturing of oocytes from day 0 on vero cell monolayers increases the developmental capacity.

P-213

A Comparison Between the Implantation Rates of Day Two Embryos Resulting from Intracytoplasmic Sperm Injection (ICSI) and Embryos Obtained After Short Exposure of Oocytes to Sperm In Vitro. [1]Y. Menezo, [2]Y. Barak. [1]Laboratoire Marcel Merieux, Bron, France and [2]IVF Unit, Herzliya Medical Center, Herzliya-on-Sea, Israel.

Objectives: Short incubation time allows in vitro fertilization (IVF) embryos to avoid the deleterious effect of degenerating cumulus cells and

excess spermatozoa. The aim of the current study was to compare the developmental potential of embryos resulting from ICSI and embryos obtained in IVF after short exposure to sperm and incubated with a minimal mass of cumulus cells.

Design: Prospective study.

Materials and Methods: A short incubation protocol of 3 hours of exposure of oocytes to spermatozoa in vitro, was performed in 204 patients in our IVF program (IVF group). Oocytes were rinsed from sperm cells after 3 hours into a fresh 0.5ml incubation medium covered with oil. Embryo transfer (ET) was performed on day 2. Supernumerary embryos were with sequential medium for freezing at the blastocyt stage. Fertilization, polyploidy and cleavage rates were assessed. Pregnancy rates per transfer and implantation rates per embryo were determined and compared to the outcome in 163 patients who entered our ICSI program (ICSI group) and had the ET on day 2. Results were compared between the two protocols according to three groups of maternal age: <30 years, 30–35 years and >35 years of age.

Results: An increased rate of polyploidy was age related in the IVF-group, but not in the ICSI patients. According to the assigned age group a decrease in the number of oocytes retrieved in relation with maternal age was observed - 12.5, 10.2 and 6.6 oocytes per patient respectively ($p<0.05$). Such a difference was not observed regarding the number of MII oocytes in the ICSI group. Embryos showed a higher cleavage rate in the IVF group, when compared to the ICSI embryos (66.9% vs 55.3%; $p<0.01$). An age related decrease in implantation rate was observed in the IVF-embryos but not in the ICSI embryos (32.5, 17.6 and 18.9% vs. 20, 17.6 and 18.6% respectively). However, implantation rates were similar for ICSI and IVF embryos in the overall population (18.2 vs. 18.9%, respectively). A decrease in blastocyst formation, from supernumerary embryos, was noticed in the ICSI embryos in comparison to IVF [130/380 (34.2%) vs. 543/1241 (43.8%) respectively; $p<0.05$).

Conclusions: Short incubation does not decrease fertilization and cleavage rates. Since blastocyte formation rate is higher in those embryos and as it is also accepted that short exposure to sperm might reduce zona hardening and avoid exposure to free radicals, we suggest the short exposure as a routine method for IVF patients.

P-214

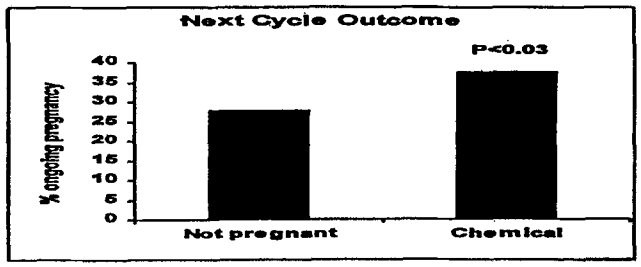
Biochemical Pregnancy and Subsequent IVF Outcome. G. W. Bates, E. S. Ginsburg. Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objectives: Biochemical pregnancies are a frequent occurrence following in vitro fertilization (IVF). Our objective was to determine the significance of a biochemical pregnancy on outcomes of future cycles of IVF.

Design: Retrospective study.

Materials and Methods: The outcomes of 2651 IVF cycles between 1996 and 1998 were analyzed. A serum pregnancy test was obtained 18 days after oocyte retrieval. IVF cycles following unsuccessful attempts and biochemical pregnancies were evaluated. A biochemical pregnancy was defined as a positive serum βhCG (>5 mIU/ml) which resolved spontaneously with no ultrasound evidence of an intrauterine or an ectopic pregnancy. Next cycle outcomes were divided into unsuccessful attempts and ongoing pregnancies. Cycle parameters including age, total no. IVF attempts, peak serum E_2, no. follicles, no. embryos transferred, and no. cells per embryo transferred were compared using Mann Whitney U test. The Fisher's Exact Test were employed to evaluate the outcome of the next cycle initiated.

Results: Of the 2651 cycles analyzed, 836 did not conceive and 72 cycles had a biochemical pregnancy. Analysis of the next cycle showed a significantly greater ongoing pregnancy rate in the cycle following a biochemical pregnancy (37.5 vs 27.8, $P < 0.04$). No significant difference was noted in cycle parameters between groups except for the total number of IVF attempts in women with a prior biochemical pregnancy.



Parameters	Chemical (N = 72)	Not Pregnant (N = 836)	P-value
Age	35.1 ± 4.3	35.9 ± 4.2	NS
Attempts	2.42 ± 1.43	1.94 ± 1.26	p<0.001
Peak E2	1866 ± 885	1784 ± 972	NS
# Follicles	12.5 ± 7.1	12.5 ± 6.4	NS
# Transferred	2.9 ± 1.7	2.8 ± 1.1	NS
# Cells	5.5 ± 1.7	5.5 ± 1.8	NS

Conclusion: A biochemical pregnancy is a positive marker for a successful outcome in the next IVF attempt.

P-215

Comparison of Flare Protocol and Luteal Downregulation In IVF Poor Responders. M. Yao, R. F. Liberman, D. W. Cramer, B. L. Harlow, E. S. Ginsburg. Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objectives: Poor response to controlled ovarian hyperstimulation in IVF occurs in 10–26% of IVF cycles. It is clinically significant because it is associated with high cancellation rates and is predictive of poor clinical pregnancy rate in the subsequent cycle. Our objective was to determine whether the flare protocol (FP) improved IVF outcomes in poor responders.

Design: Retrospective cohort study.

Materials and Methods: We identified women from 2571 IVF cycles in 1996–1998 who had a poor response on the luteal downregulation protocol (LP) in their first IVF cycle (C1) with no ongoing pregnancy. These women were then divided into 2 groups: Group 1 had LP in Cycle 2 (C2) and Group 2 had FP in C2. Our definition of a poor response was either a serum estradiol level on the day of hCG (E2) < 750 pg/mL or ≤ 5 oocytes retrieved. In C2, the dosage of gonadotropins was routinely increased for poor responders on LP. Age, ampules of gonadotropins used, the number of embryos transferred, E2 level, the number of oocytes retrieved, and the fertilization rate were compared between C1 and C2 for each group using paired or unpaired Student's T-test. Ongoing clinical pregnancy rates (PR) were compared using Chi-Square Test and Fisher's Exact Test. Logistic regression was used to determine the effect of FP on PR.

Results: There were 1257 first cycles and 200 of those (15.9%) had a poor response. Of the poor responders, 30 had LP in C1 and C2 (Group 1) and 23 had LP in C1 and FP in C2 (Group 2). In a logistic regression model with C2 outcome as the dependent variable, FP had a trend of negative effect on PR (OR 0.25, 95% CI 0.03–1.83). Increasing age (OR 0.71, 95% CI 0.55–0.92) and estradiol level in C1 (OR 0.28, 95% CI 0.09–0.90) were significant predictors of cycle failure while ampules of gonadotropins, oocytes, and the number of embryos transferred were not significant predictors.

Variables/Outcomes	Group 1/C1	Group 1/C2	P-value
Age	36.8 ± 3.6	37.3 ± 3.4	NS
No. Ampules	49.2 ± 15.8	64.4 ± 19.0	<.001
No. Embryos Trans.	2.6 ± 1.3	3.3 ± 1.3	<.05
E2 on Day of HCG	842 ± 286	1323 ± 811	<.01
No. Oocytes	6.2 ± 3.6	9.9 ± 7.1	<.001
Fertilization Rate	60 ± 30%	60 ± 20%	NS
PR per Cycle Start	N/A	23.3%	N/A

Variables/Outcomes	Group 2/C1	Group 2/C2	P-value
Age	38.5 ± 3.5	38.9 ± 3.5	NS
No. Ampules	69.8 ± 22.3	74.2 ± 20.3	NS
No. Embryos Trans.	2.2 ± 0.9	3.0 ± 1.4	NS
E2 on Day of HCG	576 ± 342	1050 ± 562	<.001
No. Oocytes	3.8 ± 1.4	6.1 ± 4.0	NS
Fertilization Rate	50 ± 30%	70 ± 30%	NS
PR per Cycle Start	N/A	17.4%	N/A

Variables/Outcomes	Grps 1 vs. 2/C1	Grps 1 vs. 2/C2
Age	NS	NS
No. Ampules	<.001	NS
No. Embryos Trans.	NS	NS
E2 on Day of HCG	<.01	NS
No. Oocytes	<.05	<.05
Fertilization Rate	NS	NS
PR per Cycle Start	N/A	NS

Conclusions: Women who had poor response to the luteal regimen in their first IVF cycle have no apparent increase in success when placed on a flare regimen in the second cycle. The flare protocol showed a trend towards lower clinical pregnancy rates when compared to the luteal protocol.

P-216

Endometrioma at the Inception of an IVF-ET Cycle: A Negative Predictor of Outcome. S. D. Spandorfer, O. K. Davis, J. Navarro, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility. Department of Obstetrics/Gynecology. The New York Presbyterian Hospital-Cornell Medical Center, New York, NY, USA.

Objective: Endometriosis is a chronic and progressive disease. Patients with severe (Stage III or IV) endometriosis, despite obtaining fewer oocytes at retrieval, do not appear to have a worse prognosis than patients with milder disease do. However, moderate and severe endometriosis is represents a heterogenous group of patients. Some have ovarian involvement (endometriomas) while others are clear of ovarian disease. The purpose of this study was to evaluate the effect of the presence of an endometrioma at the time of stimulation in patients with severe endometriosis (Stage III or IV) and its association with IVF-ET outcome.

Design: Retrospective study.

Materials and Methods: 286 consecutive cycles of patients with severe endometriosis (Stage III or IV) undergoing IVF-ET at The New York Hospital-Cornell Medical Center from 1989–1997 were reviewed. All patients underwent a sonogram before initiation of gonadotropins for the determination of the presence of endometrioma(s). Patients were treated with standard ovulation induction protocols and underwent IVF-ET according to previously published guidelines. Analysis of variance, Chi square, and non-parametric *t-test* were utilized when appropriate. $P < 0.05$ was considered significant.

Results: 31.1% (89/286) of the cycles were notable for endometriomas on the baseline sonogram. Table 1 demonstrates the characteristics and results of the cycles based on the presence or absence of an endometrioma.

Variable	Endometrioma (n=89)	No Endometrioma (n=197)	P Value
Age (years)	35.8 ± 3.5	35.4 ± 4.1	0.38
Cancellation rate	11.2%	13.2%	0.70
Peak Estradiol (pg/mL)	1214.9 ± 612	1240.4 ± 837	0.85
Mature Oocytes at harvest	8.9 ± 6.1	9.7 ± 5.3	0.32
# ET	3.1 ± 1.1	3.1 ± 1.2	0.90
Positive pregnancy[1]	38.0%	55.5%	0.01
Clinical pregnancy[1]	34.2%	46.2%	0.07

[1] Per retrieval.

Conclusion: We have demonstrated, for the first time, that in patients with severe endometriosis, the presence of an endometrioma at the inception of an IVF-ET cycle has no adverse affect on stimulation, but is significantly associated with a decreased pregnancy rate.

P-217

Endometriosis: Analysis of 1417 Consecutive Cycles of IVF. S. D. Spandorfer, I. Kligman, H.-C. Liu, A. Neuer, S. S. Witkin, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility. The New York Hospital-Cornell Medical Center, NY, NY.

Objective: Endometriosis is a very common cause of infertility and often an indication for IVF-ET. The purpose of this study was to evaluate the effect of the presence of endometriosis and the stage of disease on IVF-ET outcome at a single institution.

Design: Retrospective study.

Materials and Methods: 1417 consecutive cycles in 872 patients undergoing IVF-ET at The New York Hospital-Cornell Medical Center from 1989–1997 were analyzed. Patients were identified by the presence of endometriosis as a diagnosis (primary, secondary or the presence of endometriosis) in a computer database. All charts were reviewed for the stage of endometriosis and classified according to ASRM (Fertil & Steril 1997; 67:817). Patients were treated with standard ovulation induction protocols and underwent IVF-Et according to previously published guidelines. Analysis of variance, Chi square, and student's *t-test* were utilized when appropriate. $P < 0.05$ was considered significant.

Results: 1196 (84.4%) of the 1417 initiated cycles went to retrieval. 1105/1196 (92.3%) underwent an embryo transfer. An overall clinical pregnancy rate/transfer of 44.7% (495/1105) and an overall ongoing pregnancy rate/transfer of 37.1% were noted. In analyzing all cycles that went to transfer, the stage of disease had no significant effect on peak estradiol levels, number of mature oocytes or embryos transferred, or pregnancy outcome. As expected, age was found to have a significant effect on IVF-ET outcome. Patients under 40 years old had a significantly higher clinical pregnancy rate/transfer than women 40 and over (48.4% vs. 31.8%; $p < 0.001$). To remove the effect of individual patients with multiple failed cycles, we next analyzed the first cycle of each patient with endometriosis. Once again, the stage of disease had no significant effect on peak estradiol levels, number of mature oocytes or embryos transferred, or pregnancy outcome. In analyzing the population by stimulation protocol, patients with lupreolide acetate down-regulated cycles were found to be younger, stimulate better, and had significantly higher clinical pregnancy rates (48.9% vs. 27.2%; $p < 0.001$). Given that patients with lupreolide acetate down-regulated cycles were found to have better outcomes, we than analyzed these patients to detect if stage of disease had any effect on IVF-ET outcome. In these patients, lower stages of endometriosis were associated with better stimulations, more oocytes retrieved, and more oocytes normally fertilized. Despite this, no differences in the ultimate number of embryos transferred or, more importantly, pregnancy outcomes were detected.

Conclusion: We have demonstrated in this large study that the stage of endometriosis may have a slight effect on stimulation (lower stage disease in good prognosis patients had better responses), but, more importantly, no differences in pregnancy outcomes were detected.

P-218

Autologous Endometrial Coculture (AECC) in Patients with IVF Failure: Correlations of Outcome with Leukemia Inhibiting Factor (LIF) and Calcitonin. S. D. Spandorfer, D. Levy, I. Bagchi, H.-C. Liu, L. Veeck, O. K. Davis, S. S. Witkin, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility, The New York Hospital/Cornell Medical Center, New York, NY.

Objectives: CC of embryos with monolayers of AECC has been shown to improve outcome for patients with multiple implantation failures after IVF-ET. Presumably, this effect is mediated through the production of trophic factors expressed by these helper cell lines. LIF expression by coculture cells, in a murine model, was associated with embryonic development (JARG 1995). Calcitonin is an early marker of endometrial receptivity. The specific aim of this study was to determine if Calcitonin and LIF levels as measured in the supernatants of conditioned media (CM) of embryos cocultured in AECC is predictive of IVF outcome.

Design: CM from the ECC cells exposed or non-exposed to human

embryos was collected in 42 patients and assayed for the presence of LIF and calcitonin and correlated with outcome.

Materials and Methods: During a luteal phase biopsy (5–10 days after LH surge) made prior to the treatment cycle, glandular (G) and stromal (S) endometrial cells were isolated by enzymatic digestion and separated based on differential sedimentation rates. These cells were cryopreserved, then plated as a 50%/50% combination of G and S cells prior to embryo exposure. The conditioned medium (CM) was changed every 2 days. Embryos were randomly grown on ECC or conventional media if more than 6 oocytes normally fertilized. Otherwise, all embryos were grown on AECC. LIF and calcitonin levels were measured utilizing an immunoenzymetric assay. Background levels of LIF and calcitonin were also determined from media alone (Hams F-10 supplemented with 15% patient's serum). Statistics included Wilcoxon signed rank test, Mann-Whitney U test and Chi-square.

Results: Exposure or non-exposure to an embryo did not result in differing levels of Calcitonin or LIF in the CM. LIF levels were significantly greater in the CM than in the serum controls (LIF was not found in the serum controls). There was no difference in the calcitonin levels in the CM as compared to the serum control. Embryos grown on ECC demonstrated a significant improvement in number of blastomeres and fragmentation (frag) when compared to embryos grown in conventional media without ECC (6.7 ± 1.3 vs. 5.6 ± 1.2 blastomeres and $17.6\% \pm 9.3$ vs. $26.4\% \pm 9.8$ frag; $P < 0.05$). When LIF levels were detectable in the CM, the embryos grown in ECC were of improved quality as compared to the embryos grown only in conventional media and a non-significant increase in pregnancy rates was found (60 vs. 48%, p=0.50). Conversely, Calcitonin levels in the CM were not associated with embryo quality or pregnancy outcome.

Conclusions: We have demonstrated a significant improvement in blastomere number and fragmentation with ECC. LIF is expressed by the cells in the AECC. The presence of LIF in the CM was associated with embryonic development and clinical pregnancy. The presence of Calcitonin in the CM was not associated with embryonic development or outcome.

P-219

Cervical IgA Antibodies to *Chlamydia Trachomatis*, the Chlamydial 10 KDA Heat Shock Protein (HSP 10) and the Human 60 KDA Heat Shock Protein (HSP60) in Women Undergoing IVF-ET: Correlations with Poor Outcome. S. Spandorfer, S. S. Witkin, A. Neuer, P. Giraldo, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility. Department of Obstetrics/Gynecology. The New York Presbyterian Hospital-Cornell Medical Center, New York, NY, USA.

Objective: To evaluate the relation between local (cervical) immunity to C. Trachomatis, hsp10 and hsp60 and IVF outcome.

Design: Prospective study.

Materials and Methods: At the time of oocyte harvest, endocervical swabs were obtained from 187 women, shaken into a saline solution and supernatant and pellet fractions were obtained. The supernatants were tested by a commercial ELISA (SeroCt, Savyon Diagnostics) for IgA antibodies specific for *trachomatis* antigens. Antibodies to recombinant chlamydial hsp10 (provided by G. Byrne) and human hsp60 (StressGen) were also detected by ELISA. The pellets were tested for *C. Trachomatis* by PCR (Amplicor, Roche Diagnostics). To minimize the effect of age on outcome, only women $\leq$ 38 years were included.

Results: Embryo transfers occurred in 179 of the subjects (95.7%). 18 (10.1%) of these women were positive for cervical IgA to *C. trachomatis*, 10 (5.6%) were positive for antibodies to the chlamydial hsp10 and 8 (4.5%) were anti-human hsp60 IgA positive. None of the women were cervical PCR positive. The clinical pregnancy rate among the 149 women negative for the above antibodies was 57.1%. This was significantly greater than the 12.5% pregnancy rate in women positive for antibodies to hsp60 ($p = 0.02$), the 20% pregnancy rate in women with anti-hsp10 (p=0.01), and the 33.3% pregnancy rate in women with *C. Trachomatis* antibodies (p=0.04). The presence of IgA antibodies to human hsp60 and chlamydial hsp10 were highly correlated (p=0.003). Hsp60 antibodies were detected in 40% of women with hsp10 antibodies as opposed to only 2.4% of women negative for anti-hsp10 IgA. Similarly, hsp10 antibodies were present in 22.2% of women with antibodies to *C. Trachomatis* and in only 3.7% of women who were chlamydial antibody negative (p=0.01). There was no relation between hsp60 antibodies and *C. Trachomatis* antibodies.

Conclusion: Cervical antibodies to *Chlamydia Trachomatis*, the Chlamydial hsp10 and the human hsp60 all correlate with poor IVF outcome after transfer. The data suggest that active immunity to Chlamydia and/or the human hsp60 is taking place, perhaps in the endometrium.

P-220

Microdose GnRH Analog Flare Protocol Does Not Improve Outcome of IVF in Patients with Diminished Ovarian Reserve. G. Bremner, L. Siano, D. Maier, J. C. Nulsen, C. A. Benadiva. University of Connecticut Health Center, Farmington, CT.

Objectives: Previous studies have shown that the microdose GnRH analog flare protocol has been successful in improving response to ovarian stimulation in poor responder patients who have failed prior luteal phase leuprolide acetate (Lupron) protocols. Because poor responders are a heterogeneous group, it is unclear if all patients in this group will benefit from the microdose protocol. The purpose of this study was to identify which group of patients benefitted from changing to the microdose protocol.

Design: Retrospective study of patients stimulated using microdose GnRH flare after failing standard luteal phase lupron (LPL) protocols.

Materials and Methods: Twenty-seven patients (Group A) with a mean age of 35 years +/−4 underwent 51 cycles of standard LPL stimulation before changing to the microdose flare protocol (Group B; n=28 cycles). Oral contraceptive pills (OCP) were started on day 5 of the menstrual cycle and continued for 21 days. Lupron 40 micrograms twice daily was started three days after the last OCP, followed by six ampules of gonadotropins per day. Lupron was continued until the end of stimulation. The paired student's t-test and chi-square were used for statistical comparison of clinical parameters between the two groups.

Results: Cancellation rate (35% vs. 25%; p=.05), mean number of eggs retrieved (9.5 vs. 10.4; p>.10), and estradiol level on day of HCG administration (1366 vs. 1727; p>.10) did not differ significantly between group A and group B respectively. However, the fertilization rate (49% vs. 67%; p<.01), implantation rate (6.38% vs. 17.1%; p<.05) and ongoing pregnancy rate (0% vs. 33%) were significantly greater in group B. In those patients who conceived, the miscarriage rate (100% vs. 12.5%; p<.007) in group A was significantly higher than in group B. When patients on the microdose protocol were divided by baseline day 3 FSH, no patients with a baseline FSH $\geq$ 10 mIU/ml (n=3) achieved a successful pregnancy.

Conclusions: Microdose GnRH flare protocol improves fertilization, implantation and pregnancy rates in poor responders. Nevertheless, it does not improve outcome of IVF in patients with an FSH $\geq$ 10 and they should be directed to oocyte donation.

P-221

Blastocyst Formation Subsequent to Day 3 Embryo Transfer Is Not Predictive of Pregnancy. R. Margalit, D. Smith, R. P. Buyalos. Martin Luther Hospital, Anaheim, CA and the University Of California School of Medicine, Los Angeles, CA.

Objective: High order multiple gestation is the principal contributor to both maternal and perinatel morbidity in assisted reproductive technology (ART). The ability to transfer only one or two preimplantation embryos while maintaining high pregnancy rates remains a goal of ART. We speculated that blastocyst development after 5 days in culture might be indicative of superior embryo quality for a particular ART cycle. The objective of this analysis was to determine whether patients whose untransferred (extra) embryos were capable of blastocyst development had higher clinical pregnancy rates following day 3 transfer compared with those whose extra embryos did not produce blastocysts.

Design: Retrospective.

Materials and Methods: Eighty-four consecutive patients undergoing in-vitro fertilization and transfer of 6-8 cell embryos after 72 hours in culture were studied. After embryo transfer (mean 4.2) all remaining embryos were cultured in chemically defined blastocyst media for an additional 48 hours to assess their ability to reach the blastocyst stage.

Results: Forty of 84 (48%) patients showed development of one or more blastocyst stage embryos which were cryopreserved for future cycles. Forty-four of eighty-four (52%) patients had no embryos which survived to the blastocyst stage. There was no difference in pregnancy rates (44% vs. 40%) between patients with and without subsequent blastocyst formation (P >

0.6). In patients having blastocyst development, there was no difference in the percentage of embryos surviving to blastocyst between the pregnant (53% + 23%) vs. nonpregnant patients (55% + 23%, P > 0.5). Maternal age, number of oocytes retrieved, fertilization rates, peak estradiol levels, edtradiol levels per oocyte both before and after hCG injection, and the number of embryos transferred did not differ between patient groups or between pregnant and nonpregnant patients (P > 0.3).

Conclusions: Pregnancy rate is not correlated with development of blastocysts 48 hours following day 3 transfer. Prospective randomized clinical trials comparing one or two embryo transfers on day 3 compared to day 5 are needed.

P-222

Estrogen Supplementation During the Luteal Phase Improves Pregnancy Rate in IVF-ET. J. Farhi, Z. Steinfeld, M. Shorer, H. Nahum, A. Weissman, M. Glezerman, D. Levran. IVF Unit, Department of Obstetrics and Gynecology, Wolfson Medical Center, Holon and Sackler Faculty of Medicine, Tel Aviv University, Israel.

Objectives: The role of progesterone supplementation in the luteal phase of IVF-ET cycles has been clearly established. It was introduced following the wide use of gonadotropin releasing hormone agonist (GnRH-a) in controlled ovarian hyperstimulation protocols, which resulted in corpus luteum dysfunction. Little is known on the significance of estradiol levels and/or estradiol supplementation during the luteal phase following pituitary down-regulation. Our objective was to study the effect of the addition of estrogen to routine luteal progestin supplementation on pregnancy rate in IVF-ET treatment cycles.

Design: Prospective randomized study.

Materials and Methods: All IVF patients who underwent controlled ovarian stimulation using GnRH-a (short or long protocol) between August 1997 and December 1998, and in whom E2 levels at the time of hCG administration were >2500 pg/dl were included in the study. Patients were prospectively randomized into two luteal support protocols: Protocol A - progesterone-only supplementation, consisting of 150 mg daily divided into 50 mg IM injection and 50 mg vaginal tablets BID starting on the day following OPU. Protocol B - Same regimen of P supplementation as in protocol A, with the addition of estradiol, 2mg BID, P.O. daily starting from day 7 following ET. In both groups, E2 and progesterone levels were measured on days 7, 10, 12 following embryo transfer (ET) and compared. Pregnancy rate was used as the main outcome measure. Data were analyzed for the entire study population and further stratified according to the GnRH-a protocol used (short and long).

Results: Both groups were found comparable in terms of patient characteristics, response to stimulation, and the mean number of transferred embryos. Estradiol supplementation resulted in significantly higher serum E2 levels on day 10 following ET. A significant difference in pregnancy rates was found only in cycles in which a long GnRH-a protocol was used, 39.6% (40/101) vs. 25.6% (29/113), for groups B and A, respectively ($p<0.05$).

Conclusions: The addition of estradiol to progestin as luteal support improves pregnancy rates in patients treated with the long regimen of GnRH-a, and should thus be offered to this subgroup of patients.

P-223

Testicular Sperm Extraction from Previously Cryopreserved Tissue: Clinical Results and Follow-up from Couples With and Without Transport of Oocytes and Testicular Tissue. [1]M. C. W. Scholtes, [1]D. G. van Hoogstraten, [1]A. Schmoutziguer, [2]G. H. Zeilmaker. [1]Center for Reproductive Medicine, Genetics and Clinical Chemistry, Düsseldorf, NRW, Germany and [2]Department of Endocrinology and Reproduction, Erasmus University Rotterdam, The Netherlands.

Objective: Evaluation of results from IVF and ICSI with testicular sperm extraction from frozen-thawed testicular tissue.

Design: Retrospective follow-up study.

Materials and Methods: Thirty-five couples with transport of testicular tissue from a transport clinic and 125 regional couples. Testicular sperm extraction by maceration and enzymatic digestion from frozen thawed testicular tissue prior to ICSI. Clinical pregnancy rate and implantation rate in couples with obstructive/non-obstructive azoospermia, completely immotile sperm and elevated male serum-FSH values. Statistics were done by X-square analysis.

Results: The clinical pregnancy rate per ET and implantation rate per embryo in couples with transport of testicular tissue in obstructive azoospermia was 40% and 18% respectively and in non-obstructive azoospermia 37% and 26%. In the regional couples these rates were 42% and 19% for obstructive azoospermia and 18% and 10% for non-obstructive azoospermia. The implantation rate for ICSI with motile/immotile sperm was 26% versus 11% in the transport group and 16% versus 8% in the regional group. Male serum-FSH did not have a clear correlation with implantation.

Conclusions: Clinical pregnancy/implantation rate is not affected by transport of testicular tissue but significantly by non-obstructive azoospermia and by immotile sperm. No major increase in chromosomal aberration or congenital malformation was noted in the offspring of this limited group.

P-224

Does Embryo Quality Correlate with Sperm Quality in Male Factor IVF/ICSI Cases? [1]D. M. Nudell, [1]D. M. Lee, [2]E. D. Schriock, [2]C. A. Givens, [1]P. J. Turek, [2]J. Conaghan. Departments of [1]Urology and [2]Obstetrics-Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA.

Objective: The role of sperm quality or source in defining embryo quality after IVF-ICSI is controversial. To address this issue, we compared patterns of embryo quality in 2 groups of male factor IVF-ICSI couples: in one group, "good" epididymal sperm was obtained from previously fertile, vasectomized men, and in the second, "poor" sperm was obtained from men with nonobstructive azoospermia (NOA).

Design: Retrospective study of male factor IVF-ICSI cases at a single institution.

Materials and Methods: We identified 28 men who underwent epididymal sperm aspiration after prior vasectomy (Group 1) and 24 men with NOA who had testis sperm extraction (Group 2). Following IVF-ICSI, scoring of embryo quality was assessed with a standard grading system (1 = best quality to 5 = worst quality) by a single embryologist. Differences were assessed with unpaired t tests.

Results: There was no statistical difference between the 2 groups with respect to the number of oocytes/patient, fertilization rate or overall embryo quality (see Table). In Groups 1 and 2, 59.5% and 60.6% of embryos, respectively, were scored as Grade 1 or 2 (high quality). Since female partners in Group 1 were older than in Group 2 ($p=0.02$), a subset analysis was performed with female age-matched cohorts; again, no differences in embryo quality were detected.

	Group 1-Good Sperm	Group 2-Poor Sperm
Median female age	38.5	33.0
Median # eggs/patient	14	16.5
Median # embryos/patient	7.5	8.0
# Grade 1 embryos (%)	41 (15.3)	39 (19.6)
# Grade 2 embryos (%)	111 (44.2)	82 (41.0)
# Grade 3 embryos (%)	65 (23.7)	40 (19.1)
# Grade 4 embryos (%)	41 (15.3)	35 (16.2)
# Grade 5 embryos (%)	4 (1.6)	9 (4.5)

Conclusion: Embryo quality does not appear to be compromised by the source of aspirated sperm in male factor IVF-ICSI cases. This is evidence against aspirated sperm source as a determinant of embryo quality in these cases.

P-225

Predictive Value for Pregnancy of the Blastocyst Stage Supernumerary Embryos in In Vitro Fertilization Patients Transferred on Day 2 or 3. M. Ruy Sánchez, G. Villafaña, M. Tucker, P. Galache, S. Hernández, R. Santos, V. Batiza, D. Montoya, A. Hernández, J. F. Vélez. Instituto para el Estudio de la Concepción Humana (IECH). Monterrey, Nuevo León, México.

Objective: Human preimplantation embryos conceived through In Vitro Fertilization (IVF) are routinely transferred to the uterus on day 2 or 3,

around the 4- to 8-cell stage of development. In vivo, these embryos enter the uterus postcompaction. At the present time, there are some culture media which allow to transfer embryos in the blastocyst stage.

To assess the predictive value for pregnancy of the supernumerary embryos allowed to reach the blastocyst stage in IVF patients transferred on day 2 or 3.

Design: Retrospective study. The pregnancy rate was determined in the developed blastocyst stage and in the non-developed blastocyst stage supernummerary embryo groups.

Materials and Methods: 36 patients underwent conventional IVF, being transferred on day 2 or 3. The best embryos were selected for transfer and freezing. The mean number of embryo transferred to patients was 3.4. There were 18 positive serum pregnancy tests. There were 16 clinical pregnancies with 22 gestational sacs.

157 supernummerary embryos were cultured to day 5, 50 (31.8%) reached the blastocyst stage. They were frozen.

Twenty four (66.6%) patients developed blastocysts. There were 12 pregnancies in this group (50%).

12 of the 18 (66.6%) pregnant patients developed blastocysts while 11 of the 17 (64.7%) non pregnant patients developed blastocysts.

Conclusions: 1) The development of supernummerary embryos to blastocyst stage does not have a predictive value for pregnancy. 2) Embryo quality on day 3 did not reflect the ability to form blastocysts, neither their potentiality for a clinical pregnancy.

P-226

The Effect of Mechanical Assisted Hatching on Progression of Cleavage Stage Embryos to the Blastocyst Stage. A. Isiklar, B. Balaban, S. Aksoy, C. Alatas, R. Mercan, A. Nuhoglu, B. Urman. Assisted Reproduction and Fertility Unit, American Hospital of Istanbul, Turkey.

Objectives: Assisted hatching (AH) has been proposed as a means to increase the implantation rate of cleavage stage embryos. The rational behind its application is to circumvent the zona hardening effect of prolonged in vitro culture. Assisted hatching may also be beneficial in terms of nutrient transfer from and metabolic waste disposal to the culture medium. This may influence the survival of embryos during culture with the aim to transfer at the blastocyst stage. We performed this prospective randomized study to observe the effect of mechanical AH on progression of cleavage stage embryos to the blastocyst stage.

Design: Prospective randomized study.

Materials and Methods: A total of 44 patients planned to undergo blastocyst transfer were randomized to mechanical AH versus no intervention on the third day of insemination. Assisted hatching was performed as described by Cohen. Blastocyst culture was performed in sequential media (S1 + S2; Scandinavian Science). Three to four blastocysts were transferred according to their morphology.

Results:

	Assisted hatching (+)	Assisted Hatching (−)	P value
Number of cycles	22	22	
b	32.7	31.9	
Number of oocytes	358	405	
Fertilized oocytes	224	246	
Cleaved embryos on D3	211	233	
Blastocyst on day 5–6	102 (48.3%)	106 (45.4%)	NS
Blastocysts transferred	3.7	3.5	NS
Clinical PR/ET	16/22 (72.7%)	10/22 (45.5%)	NS
Implantation/embryo	32/83 (38.6%)	13/78 (16.7%)	0.001
Singleton Pregnancy	6/16 (37.5%)	8/10 (80%)	NS
Twins	6/16 (37.5%)	0	<0.05
>Twins	4/16 (25%)	2/10 (20%)	NS

Conclusions: Mechanical AH appears to increase the implantation potential of blastocysts. The increase in clinical pregnancy rates may have reached statistical significance had more number of subjects were included in the study. The beneficial effect of AH may be mediated through providing a better nutrient exchange between the embryo and the culture medium.

P-227

Clinical use of Immature Oocytes from Normal and PCO Women In Non-Stimulated IVF-ET Program. B. R. Do, H. R. Paik, D. R. Lee, S. J. Yoon, J. S. Jeon, J. H. Cho, S. I. Roh, H. S. Yoon. Infertility Research Center, Jeil Women's Hospital, Seoul, Korea.

Objectives: Although the controlled ovarian hyperstimulation (COH) is generally used to retrieve the mature oocytes in human IVF-ET program, some disadvantages have been reported. In recent ART program, *in vitro* maturation (IVM), fertilization, and implantation using the immature oocytes is proposed as one methods of the infertility treatment. However, this protocol is not firmly established. Therefore, the present study was conducted using the immature oocytes obtained from normal and PCO women to evaluate (1) the clinical feasibility of IVM and IVF, (2) the increase of pregnancy rates in the non-simulated cycle, and (3) the effect of hCG and/or estradiol (E) on the pregnancy outcome.

Design: The number of the retrieved, fertilized, and cleaved oocytes, and the pregnancy outcome rate were observed in the prospective randomized studies after the administration of hCG and/or E in the non-stimulated immature oocytes program.

Materials and Methods: Seventy immature oocytes were retrieved from forty-six patients (age: 31.5 ± 4.3). In mid-follicular phase, the transvaginal ultrasound guided aspiration was performed with a 17-gauge short beveled needle and a 20-gauge transvaginal ultrasound injection needle. The oocytes were cultured in the maturation media supplemented with gonadotropins (10 iu/ml hMG and 10 iu/ml hCG or 10 iu/ml FSH and 20 iu/ml hCG), 10 ng/ml estradiol, and 20% human follicular fluid. Intracytoplasmic sperm injection was performed on maturated oocytes after 24 h to 48 h *in vitro* culture. For the endometrium preparations, estradiol valerate (6 mg/day) was treated before or after OPU date. Embryos were transferred into uterus at 2- to 8-cell stage or into fallopian tube at pronucleus stage. After ET, progesterone (50 mg/day) was concomitantly treated with or without hCG (5000 iu).

Results: The number of the immature oocytes aspirated from the PCO patients was more than that of the regular cyclic patients (11.3 ± 6.0 vs. 4.0 ± 2.0; mean ± SD, $p < 0.001$), and healthy oocytes number was decreased by the treatment of E before ovum pickup (2.9 ± 1.7 vs. 5.8 ± 4.8, mean ± SD, $p < 0.05$). The rates of maturation, fertilization, and cleavage of immature oocytes were 67.7% (373/551), 78.6% (293/373), and 85.4% (211/247), respectively. Eleven pregnancies (11/50, 22.0%) were obtained using the immature oocytes retrieved from the patients who did not received E before OPU, but received hCG after OPU. The endometrial thickness was not different between pregnant and non-pregnant groups. Pregnancy rates were higher after ZIFT (37.5%) than IVF-ET (12.9%).

Conclusion: From the above results, it can be concluded that the immature oocytes have a potency to mature, fertilize, and implant with the reproductive consistencies in the non-stimulated ART program. And E administrated before OPU has a detrimental effect on oocytes. However, hCG administration is necessary for the proper preparation of endometrium in the immature oocyte program. Establishment of good culture condition and understanding of regulation of endometrium receptivity remain to be assessed to improve further pregnancy success.

P-228

Scheduled Weekly Physician-Patient Time Blocks in an IVF Program Does Not Impact on Pregnancy Outcomes. C. L. Librach, S. Eltayab, C. Palomares, S. B. Benson, J. Silverman, L. Anderson. Department of Obstetrics and Gynecology, Women's College Hospital, University of Toronto and the S.T.A.R.T. Clinic, Toronto, Canada.

Objectives: IVF programs require a number of physicians to allow for time off and weekend coverage. Therefore individual couples may have contact with physicians during their cycle that may not be familiar with their history and whom they had not previously met. This could potentially result in errors and decreased patient satisfaction and comfort level. The objective of this study was to evaluate a system of scheduled weekly physician-patient blocks on IVF outcome.

Design: Patients were scheduled into a week that their attending physician was also scheduled for IVF duty by modifying their length of time on lupron suppression prior to initiating HMG therapy to fit into this week.

Materials and Methods: 139 consecutive long lupron suppression IVF cycles under a single physician's care during a one year period were

analyzed. The starting day for lupron was the first day that was one week prior to an anticipated period that allowed at least 7 days on lupron before HMG could be initiated on one of the designated HMG start days. Statistical comparisons were performed using χ^2 analysis.

Results: The average age was 34.9 (range 24–44). Lupron suppression time prior to HMG therapy ranged from 7 to 39 days with a mean of 18.1 days. The overall pregnancy rate per transfer for all patients was 38.1%. The pregnancy rate per transfer for women <38 was 45.8% (n = 96) and for women 38 and older was 23.3% (n = 43). The overall pregnancy rate per transfer for patients on lupron for <20 days was 39.3% (n = 84) and for patients on lupron for ≥20 days was 38.2% (n = 55) (ns). No significant differences were seen between women on shorter or longer lupron periods for those <38 (47.4% vs 44.8%, ns) or ≥38 (23.1% vs 23.5%, ns).

Conclusions: There was no difference in outcome for women on different lupron suppression periods prior to initiating HMG therapy. Therefore a system of scheduled weekly physician-patient blocks does not appear to adversely affect IVF outcomes.

P-229

ICSI Outcome In Patients with Previous Low or Failed Fertilization On In-Vitro Fertilization. S. Papier, R. Lipowicz, W. Rawe, S. DeVincentiis, S. Brugo Olmedo, F. Nodar, G. Fiszbajn. Center of Studies in Gynecology and Reproduction (CEGyR), Buenos Aires—Argentina.

Objective: Main indications for intracytoplasmatic sperm injection (ICSI) are severe male factor and fertilization failure in previous in vitro fertilization (IVF) treatments. The fertilization and pregnancy rates after ICSI are seldom reported separately for these two different indications. To compare the treatment outcome and pregnancy rate after ICSI in-patients with previous failed fertilization or low fertilization rate (below 50% of the inseminated oocytes) and patients with primary severe male factor.

Design: Retrospective clinical study.

Materials and Methods: 368 couples undergoing ICSI in our clinic between September 1997 and September 1998 were reviewed and the results related to the indications. The indications were absent or low fertilization in previous IVF attempts (group 1: n = 21), severe impaired semen quality (group 2: n = 294) and patients with azoospermia, requiring surgical intervention to obtain sperm (group 3: n = 53).

Results: Results can be summarized as follows:

	Group 1 (n = 21)	Group 2 (n = 294)
No. of oocytes collected (mean ± SD)	11,58 ± 5,59	11,3 ± 8,04
No. of oocytes injected (mean ± SD)	6,47 ± 3,55	6,02 ± 3,87
No. of oocytes fertilized (mean ± SD)	4,00 ± 2,55	3,98 ± 3,01
Fertilization rate (%)	55	63
No. of good quality embryos (mean ± SD)	1,95 ± 1,38	2,12 ± 1,39
No. of embryos transferred (mean ± SD)	2,58 ± 1,43	2,61 ± 1,38
Pregnancy rate per cycle (%)	9,52	29,93
Implantation rate (%)	6,12	15,23

	Group 3 (n = 53)	p
No. of oocytes collected (mean ± SD)	13,51 ± 8,64	NS
No. of oocytes injected (mean ± SD)	6,45 ± 4,46	NS
No. of oocytes fertilized (mean ± SD)	3,58 ± 2,56	NS
Fertilization rate (%)	52	NS
No. of good quality embryos (mean ± SD)	2,21 ± 1,49	NS
No. of embryos transferred (mean ± SD)	2,57 ± 1,49	NS
Pregnancy rate per cycle (%)	41,51	NS
Implantation rate (%)	21,32	NS

Conclusions: Patients with previous failed fertilization or low fertilization rate in a standard IVF without male factor have a smaller but not statistically significant chance of becoming pregnant after subsequent ICSI than patients with a primary male factor.

P-230

Coaxial Embryo Transfer Catheter: A New Catheter Set for Replacing Embryos Through Difficult-to-Pass Cervix. E. Confino, R. R. Kazer, M. F. Milad, E. E. Puscheck, X. J. Zhang. Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL.

Objective: Due to anatomical variations the cervical canal in some women are difficult to pass by plastic catheters. This can increase anxiety in both the patient and clinician and may compromise the outcome following in vitro fertilization-embryo transfer. A new combination of existing embryo transfer catheters was developed in this trial in an attempt to reduce the time between loading the embryos to a catheter and expelling them into the uterine cavity in patients with "difficult" cervix.

Design: The new combination of embryo transfer catheters, named "coaxial catheter set", is consisted of a TEFCAT catheter and MARRS laparoscopic catheter for intrafallopian gamete transfer (GIFT). Both catheters were purchased from COOK OB/GYN, Spencer, IN). In this coaxial combination, the TEFCAT catheter is used as the outer catheter and passed through the cervical canal, with the tip of the catheter 1 cm away from the uterine fundus. The GIFT catheter is used as the inner catheter and contains the embryos for transfer. A stopper is positioned on the GIFT catheter so that 2 mm of its tip is protruded into the uterine cavity when it is inserted through the TEFCAT. For embryo transfer, the outer catheter is passed through the cervix and held in place; the embryos are loaded into the inner catheter which is then threaded into the lumen of outer catheter into the uterine cavity; after the embryos are expelled by a syringe attached to the inner catheter, both catheters are withdrawn at the same time and examined for possible retaining embryos.

Materials and Methods: This was not a randomized study. The decision to use the coaxial combination was made during the mock transfer just before the embryos were replaced. The first choice was Wallace catheter followed by TEFCAT catheter. If it took more than 5 minutes to insert the TEFCAT catheter during the mock transfer, the "mock" catheter would be held in place and the embryos would be loaded into the GIFT catheter for the coaxial combination.

Results: Nine embryo transfers utilized the coaxial catheter set between August 1998 and January 1999 in eight patients at ages ranging from 32 to 42 years old (average = 36), resulting in five clinical pregnancies (56%). During the same time period, twenty-nine pregnancies were produced from 62 embryo transfers (47%) using either Wallace or TEFCAT catheters, in 58 patients at ages from 29 to 43 years old (average = 36).

Conclusion: The new coaxial catheter set can produce a pregnancy rate similar to that obtained by conventional catheters.

P-231

Decreased 17-Beta Estradiol; A Marker for Decreased Ovarian Reserve? J. J. Shelnutt, [2]G. W. DeVane, [2]R. A. Loy, [2]S. B. Jaffe, [1]S. J. Carlan. Orlando Regional Medical Center, Orlando FL and [2]The Center for Infertility and Reproductive Medicine (CIRM), Orlando, FL.

Objective: Baseline serum 17-beta estradiol (E_2) levels have been used to predict ovarian reserve in women undergoing in-vitro fertilization (IVF). It has been assumed that markedly elevated levels of estradiol indicate poor ovarian reserve, and thus identify women likely to have a poor response to hyperstimulation. Recently it has been suggested that low baseline serum estradiol, in the presence of normal follicle stimulating hormone (FSH) levels, also predicts which women are likely to have decreased ovarian reserve. To study this proposition we examined the baseline serum estradiol levels and outcome of all IVF cycles at the Center for Infertility and Reproductive Medicine over a four-year period.

Design: Baseline estradiol levels were compared to various measures of IVF outcome in this retrospective chart review study.

Materials and Methods: Every IVF cycle between January 1, 1994 and June 1, 1998 was reviewed for patient demographics, baseline serum estradiol and FSH, peak serum estradiol concentration, number of days until hCG administration, number of oocytes retrieved, number of grade 1 or 2 embryos produced, and pregnancy outcome. A total of 571 cycles were reviewed.

Results: Two groups of baseline estradiol levels were compared; $E_2 < 20$

(N = 30) and E_2 > 20 and <50 (N = 247). No statistically significant difference could be found in either group with regard to patient demographics, number of oocytes retrieved, number of grade 1 or 2 embryos produced, number of days until hCG administration, chemical or clinical pregnancy rates.

Conclusion: Low baseline serum 17-beta estradiol levels, in the presence of normal FSH levels, do not correlate with decreased ovarian reserve in the outcome variables we studied. We conclude that low baseline estradiol levels should not be used to exclude patients from undergoing ovarian hyperstimulation. The Center for Infertility and Reproductive Medicine supported this work.

P-232

Comparison of Pregnancy Complications After First-Trimester, and Late, Selective Multifetal Pregnancy Reductions. [1]E. Geva, [1]I. Yovel, [1]A. Amit, [2]L. Lerner-Geva, [3]Y. Hartoov, [3]J. Jaffa, [3]I. Wolman, [1]F. Azem, [4]H. Yavetz, [1]J. B. Lessing. [1]Sara Racine IVF and [3]Ultrasound Units, [4]Institute for the Study of Fertility, Lis Maternity Hospital, Tel Aviv Sourasky Medical Center, and [2]Department of Clinical Epidemiology, The Chaim Sheba Medical Center, and Sackler Faculty of Medicine, Tel Aviv University, Israel.

Objective: The aim of our study was to evaluate the pregnancy outcome of late, selective multifetal pregnancy reduction (MFPR) compared to first-trimester MFPR.

Design: Cohort analysis.

Materials and Methods: The study groups comprised 38 and 70 patients who underwent late, selective MFPR and first-trimester MFPR, at mean gestational ages of 19.7 ± 3.3 weeks, and 11.7 ± 0.7 weeks, respectively. The two groups were compared with regard to pregnancy loss and obstetric complications.

Results: In the first-trimester MFPR group, 40% of the patients delivered prior to 36 weeks of gestation, compared to only 21% of the patients in the late, selective MFPR group (P = 0.046), although the mean gestational age was not statistically different (35.4 ± 3.4 and 35.9 ± 3.1, respectively). No difference was found in birth weight between the two groups (2138 ± 529.4 and 2318.9 ± 565.7, respectively). The incidence of obstetric complications in the early MFPR group was higher than that of the late, selective MFPR group (although not statistically significant), i.e: pregnancy loss (spontaneous abortion and intra-uterine fetal death; 15.7% and 5.2%, respectively), pregnancy-induced hypertension (10% and 0%, respectively), discordancy (18.4% and 12%, respectively), intra-uterine growth restriction (40% and 0%, respectively) and gestational diabetes (6% and 0%, respectively).

Conclusions: First-trimester MFPR may be associated with an increased rate of pregnancy loss and obstetric complications, when compared to late, selective MFPR. Moreover, late MFPR may facilitate detection of structural and chromosomal anomalies prior to the procedure and selective MFPR of the affected fetus.

P-233

Density Gradient Centrifugation and Cryopreservation of Spermatozoa Obtained by Microepididymal Sperm Aspiration (MESA). S. E. Smith, J. R. Richard, W. W. Brockman, J. D. Wininger, M. A. Witt. Reproductive Biology Associates, Atlanta, GA.

Objectives: Male factor infertility can be overcome with a combination of MESA and intracytoplasmic sperm injection (ICSI). Currently, for IVF/ICSI cycles, the criteria for sperm selection is based on motility and morphology. In order to increase fertilization, pregnancy and implantation rates we investigated the use of a double selection criteria for epididymal sperm. We hypothesized that a double selection of sperm which included density gradient centrifugation and cryopreservation would possibly make higher quality sperm available for injection.

Design: A study comparing fertilization rates, pregnancy rates and implantation rates using frozen epididymal sperm from 1997 and 1998. During 1997 (group A), surplus sperm from MESA cases were cryopreserved for subsequent IVF cycles. During 1998 (group B), epididymal sperm were processed using density gradient centrifugation and cryopreserved using controlled rate freezing.

Materials and Methods: Twenty men with obstructive azoospermia including congenital absence of the vas deferens, failed reversal or obstruction were included in the study. Sperm processed in group A (n = 12) was washed 3× in HTF sperm washing media at 300 × g for 5 minutes. Sperm obtained from patients in group B (n = 8) was layered over a 55%, 80%, and 90% Puresperm gradient and spun for 25 minutes at 100 × g. The pellet was removed and washed 3 times at 300 × g for 5 minutes. In both groups egg TEST Yolk Buffer with glycerol was added to the final preparation. Samples were cryopreserved using controlled rate freezing. On the day of egg retrieval, sperm was thawed, washed and prepared for ICSI. Embryo transfer occurred on Day 3 followed by a pregnancy test 12 days post transfer. At 5–6 weeks of gestation, fetal cardiac activity was assessed.

Results: In groups A and B all thawed sperm were easily processed and all samples displayed motility from 1% to 10%. However, more debris was found in group A. The fertilization rate was significantly lower in group A when compared to group B, 47% vs. 62%, respectively. No significant differences were found in positive βhCG pregnancy rates between groups A & B, 47% vs. 54%, respectively. The implantation rates in group A (10%) and B (50%) were significantly different. Thus far there have been 4 live singleton births in group A. In group B, two sets of triplets, and two sets of twin pregnancies have been reported. In one patient (group B) a fetal demise occurred at 8 weeks.

Conclusions: In centers where sperm retrievals must be performed on a separate day prior to egg retrieval, cryopreservation of epididymal sperm is essential. We have demonstrated that density gradient centrifugation of epididymal sperm prior to cryopreservation may yield higher quality sperm as demonstrated by higher fertilization and implantation rates.

P-234

A Comparison of Fresh Versus Cryopreserved Epididymal Spermatozoa Obtained by Microepididymal Sperm Aspiration (MESA). S. E. Smith, J. R. Richard, W. W. Brockman, J. D. Wininger, M. A. Witt. Reproductive Biology Associates, Atlanta, GA.

Objectives: MESA in conjunction with ICSI is now a commonly used procedure for the treatment of male factor infertility. In the past, we performed epididymal sperm aspirations on the day of egg retrieval. Our clinic now also cryopreserves epididymal sperm prior to egg retrieval. In this study we examined how fresh epididymal sperm compared to cryopreserved epididymal sperm over a one year period.

Design: A retrospective study comparing fertilization, pregnancy and implantation rates using fresh and frozen epididymal sperm.

Materials and Methods: Thirty two men with obstructive azoospermia including congenital absence of the vas deferens, failed vasectomy reversal or obstruction were divided into two groups. Twelve patients underwent MESA on the day of egg retrieval and 20 patients underwent MESA with cryopreservation one week before egg retrieval. Sperm processed for fresh IVF cycles were washed 3 times in HTF sperm washing media at 300 × g for 5 minutes. The final pellet was resuspended and prepared for ICSI. Sperm obtained from patients undergoing a frozen cycle were washed as described above. TEST Yolk Buffer with glycerol was added to the final sperm preparation and cryopreserved using controlled rate freezing. On the day of egg retrieval, sperm were thawed, washed and prepared for ICSI. Embryo transfer occurred on Day 3 followed by a pregnancy test 12 days post transfer. At 5–6 weeks of gestation, fetal cardiac activity (FCA) was assessed.

Results: The mean number of sperm retrieved was 14 × 10^6 with 25% motility and a 1.5 progression. The fertilization rate for fresh and frozen epididymal sperm was not significantly different, 45% vs. 40%, respectively. No significant differences were found in positive βhCG pregnancy rate (33% vs. 55%) or implantation rate (24% vs. 30%) between fresh and frozen epididymal sperm, respectively. Of the 12 patients in the fresh category, 11 underwent an embryo transfer and 8 FCA have been detected. Embryo transfers occurred in 18 of 20 patients undergoing a frozen IVF/ICSI cycle with 19 FCA detected.

Conclusions: We have demonstrated that fertilization, pregnancy and implantation rates are similar between fresh and frozen epididymal IVF/ICSI cycles. Therefore, if a fresh IVF/ICSI cycle is not an option, epididymal sperm can be cryopreserved and used at a later date with similar IVF outcomes.

P-235

Comparison of Fertilization, Subsequent Development, and FISH Efficiency in First Polar Body Biopsy Before and After ICSI. [1]M. H. Han, [1]C. K. Lim, [1]J. H. Jun, [1]H. K. Byun, [1]H. W. Youm, [2,3]M. K. Koong, [2,3]E. C. Paik, [2,3]I. S. Kang. [1]Infertility Research Laboratory, [2]Department of Obstetrics and Gynecology, Samsung Cheil Hospital & Women's Healthcare Center, [3]College of Medicine, Sungkyunkwan University, Seoul, Korea.

Objective: FISH analysis of the first polar body identifies chromosomally normal oocytes. A polar body biopsy is carried out along with ICSI, which should be conducted carefully to minimize damage on oocytes by injection on the right point where should be decided according to the position of the polar body of the oocytes. The objective of this study is to determine the influence of first polar body biopsy before and after ICSI on fertilization rate, subsequent development, and efficiency of FISH analysis.

Design: The first polar body biopsy for FISH analysis was conducted on 3 patients who had chromosomal translocation and required PGD.

Materials and Methods: A group of first polar bodies of 37 mature oocytes, collected form 2 patients (30 and 7 oocytes), were biopsied before ICSI, and another group of first polar bodies of 9 mature oocytes, collected from the other patient, were biopsied within an hour after ICSI. We used a drilling/biopsy micropipette to drill a hole in the zona pellucida by expulsion of acidified Tyrode's solution and to aspirate the first polar body by gentle suction with the same micropipette. Biopsied first polar bodies were analysed by FISH using probes simultaneously for chromosomes 2, 21 or 13, 21 or 1, 14 in each patient.

Results: For the case of first polar body biopsy followed by ICSI, 37 mature oocytes were polar bodies biopsied and injected with sperm. The fertilization rates were 86.7% (26/30) and 57.1% (4/7) of oocytes in each patient and all of these fertilized oocytes developed normally. The FISH results were obtained in 93.3% (28/30) and 100% (7/7) of polar bodies and indicated that six and three of the cleaving embryos to have arisen for normal oocytes which were transferred to each patient. In the other case of ICSI followed by first polar body biopsy, 9 mature oocytes were biopsied and injected with sperm. In 66.7% (6/9) of oocytes had two pronuclei and developed normally. FISH results were obtained in 88.9% (8/9) of polar bodies and indicated that three of the cleaving embryos to have arisen from normal oocytes which were transferred to the patient. No pregnancy was established. First polar body chromosomes were fuzzy and short, but FISH results showed that it was possible to detect whole chromosome, centromeres and unique sequences.

Conclusions: To summerize, there was no significant difference in both cases in terms of fertilization rate, development rate, and FISH efficiency. This study shows that the timing of first polar body biopsy in relation with ICSI does not affect fertilization, subsequent development and FISH efficiency.

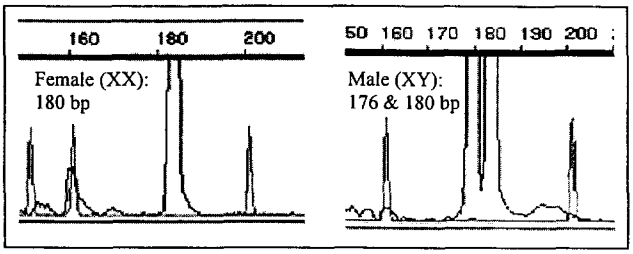
P-236

Single Cell Sexing by Fluorescent Polymerase Chain Reaction. K. P. Xu, Z. M. Shi, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, New York, NY.

Objectives: Preimplantation embryo sexing was first succeeded by single cell DNA amplification with polymerase chain reaction (PCR). Sexing by PCR has recently been replaced by fluorescent in situ hybridization (FISH) because it is less risky with DNA contamination. However, for certain purposes, such as individual sperm sex determination, PCR is still a method of choice.

Design: To take advantage of high sensitivity of fluorescent PCR and automated genetic analyzer, a new PCR system was designed and tested to determine the feasibility of the approach.

Materials and Methods: The original PCR procedure was previously published (Hum. Reprod, 1994, 9:716), in which X- and Y-chromosome-specific regions of steroid sulphatase gene (on X chromosome) and steroid sulphatase pseudogene (on Y chromosome) were amplified by a nested-PCR (two rounds). A female cell will produce one band of 180 bp (X-specific), whereas a male cell will produce a 176 bp band (Y-specific) and a 180 bp band (X-specific). For fluorescent PCR, one of the primers was labeled with a fluorescent dye. A single PCR with 50 cycles were carried out. PCR products were then analyzed with the Genetic Analyzer (ABI PRISM™ 310, CA).



Results: Examples of the fluorescent PCR products from the female and male cells detected by the Genetic Analyzer is shown below.

Positive signals were obtained from all 16 single lymphocytes and 16 DNA samples (~6 pg/PCR reaction) with only a single round of PCR, and both sexes were correctly diagnosed by this new approach.

Conclusion: Single cell sexing with fluorescent PCR is a fast, reliable and accurate procedure. Therefore, the technique can be used for clinical preimplantation genetic diagnosis and other applications, where FISH may not be suitable.

P-237

Cigarette Smoking and Male Sexual Behavior: Effects on Quantitative and Qualitative Measurements of Seminal Parameters. [1,2,3]P. M. Zavos, [1,2]C. N. Zarmakoupis, [1,2,3]P. N. Zarmakoupis-Zavos. [1]Andrology Institute of America, Lexington, Kentucky; [2]Greek-American Institute of Andrology, Athens, Greece and [3]The Kentucky Center for Reproductive Medicine and IVF, Lexington, KY, USA.

Objectives: It has been shown that smoking can impair male reproduction and spermatogenesis, as well as the quantity and quality of sperm produced during spermatogenesis. No substantial epidemiological data exists on adverse effects of smoking on various components of sexual behavior. The current study was designed to assess the possible effects of smoking on male sexual behavior and seminal parameters.

Materials and Methods: Two hundred and ninety couples undergoing infertility evaluation at our facilities (Andrology Institute of America) participated in the current study. During the first visit, each couple was asked to fill out a questionnaire pertaining to their clinical profile and sexual history. The requested information included: age of the husband and wife, number of years of marriage and years trying to conceive, sexual frequency per month and sexual satisfaction/rating (0–10; 0 = poor and 10 = extremely satisfactory). They were also asked to reveal the male's smoking habits, specifically the number of years that they have been smoking (duration) and number of cigarettes smoked per day. In all couples that participated in the study the females were non-smokers. Also, from the smoking males, only those that smoked 30 cigarettes per day or more were considered in the study. Data from the two patient groups were compiled and compared.

Results: The results obtained in this study are shown in the table below:

Patient Groups	Men's Age (yrs)	Women's Age (yrs)	Years Married	Trying to Conceive (yrs)
Smokers (n = 158)	31.2 ± 2.3	29.3 ± 3.1	7.6 ± 1.3	3.1 ± 0.5
N-smokers (n = 132)	33.4 ± 2.7	29.5 ± 3.4	6.2 ± 0.8	2.6 ± 0.4

Patient Groups	Sexual Frequency (per month)	Smoking Duration (yrs)	Cigarettes smoked per day	Sexual Satisfaction (0–10)
Smokers (n = 158)	5.7 ± 1.8	11.6 ± 3.7	37.6 ± 4.1	5.2 ± 1.1
N-smokers (n = 132)	11.6 ± 2.1*	0.0 ± 0.0	0.0 ± 0.0	8.7 ± 1.2*

The results of the seminal parameters assessed between the two groups showed significant differences ($P < 0.05$) in all sperm quantitative and qualitative measurements performed except for seminal volume. The clinical data show that the two patient groups studied were similar ($P > 0.05$).

However, it is shown that nonsmoker males were found to experience higher sexual frequency and higher sexual satisfaction* ($P < 0.05$). Also, of biological significance, is the fact that smoker couples were trying to conceive for a longer time when compared to non-smokers.

Conclusions: The biological trends established in this study are of great significance and should be used as a warning for anyone wishing to reproduce and particularly for those having difficulty in conceiving or experiencing infertility problems. At the present time, we do not completely understand the mechanism of action of cigarette smoking on the male reproductive tract, the spermatogenetic pathways and the sexual habits and sexual performance of these men that smoke. However, it is possible to postulate that smoking could be acting at different levels in the body and the male reproductive tract, diminishing the smoker's seminal parameters, sexual frequency and satisfaction. Additional studies are currently underway at our Centers to further delineate and understand the effects of smoking on these factors and mechanisms.

P-238

Artificial Generation of Single Pronuclear Haploid Oocytes: A Noval Approach to Preservation of Femal Fertility Potential. J. L. Hall, Y. L. Feng. Center for Reproductive Research and Testing, Inc., Rockville, MD.

Objective: Unfertilized human oocytes have been successfully frozen and thawed, but serious concerns regarding the safety and efficacy of human oocyte freezing exist. The mature oocyte is in the metaphase II (MII) stage of meiosis, when chromosomes are arranged on the temperature-sensitive microtubular spindle. Disruption of this structure during freezing may impair subsequent mitosis and cause chromosomal loss. Since freezing currently is most successful with pronuclear stage one-cell embryos, we sought to generate pronuclear haploid oocytes without involvement of fertilization with the male gamete. These single-pronuclear oocytes should then successfully withstand cryopreservation similar to those with the conventional two pronuclei.

Design: Parthenotes which contain a single female pronucleus can be obtained by exposing freshly collected oocytes to a variety of agents, including isotonic mannitol, 7% alcohol, hyaluronidase, the Ca++ ionophore A23187, Ca++/Mg++-free medium and electric shock. We now report a rapid, and very efficient method for the production of single pronuclear haploid oocytes.

Materials and Methods: Oocytes were collected from superovulated mature golden hamsters. The cumulus cells were removed with 0.1% hyaluronidase and washed with Tyrode's medium. The mature MII oocytes were placed into freshly prepared 7% ethanol in isotonic PBS for 5 minutes. After rinsing, the oocytes were transferred into 200 microliter drops of Tyrode's medium and incubated in a 37°C humidified chamber with 5% CO_2 in air. After 2–2.5 hours, the formation of a single female pronucleus along with a second polar body was assessed.

Results: Parthenogenetic activation can result in four classes of oocytes: (A) A single pronuclear haploid oocyte with extruded second polar body (uniform haploid); (B) a two-pronuclear presumptive diploid oocyte (heterozygous diploid); (C) an immediate cleaving embryo with two approximately equal-sized blastomeres (mosaic haploid); (D) a single pronuclear diploid oocyte (heterozygous diploid). In our protocol where we removed the cumulus cells and subsequently stimulated the hamster oocyte with ethanol, we achieved all Class A parthenogenetic oocytes. Single pronuclear haploid oocytes with an extruded second polar body were observed in 110 of 120 treated oocytes (92%). These were normal-appearing female pronuclear oocytes devoid of the male pronucleus. Concurrent treatment of 90 control oocytes whereby only ethanol was omitted, yielded no (0%) artificially activated oocytes.

Conclusion: These data demonstrate with a high degree of success the artificial generation of single pronuclear haploid oocytes potentially making cryopreservation and storage more efficient than methods currently available and possibly routine if similar results can be achieved with human oocytes. This technique may require pronuclear transfer to or from donor ooplasm in order to reconstitute a diploid oocyte, importantly, containing the genome from each biological parent. If proven safe and effective in the future, however, the ability to successfully preserve unfertilized oocytes would be an especially needed advancement for women leaving normal child bearing age and for women undergoing radiation treatment or chemotherapy. We report for the first time the potential application of this method to human IVF.

P-239

A Subpopulation of One-Pronuclear Zygotes Can Be Rescued by Cysteine and Glutathione Supplement. G. M. Grunert, H. Su, M. Gil, R. C. Dunn, C. T. Valdes, R. Mangal, L. Shenk, C. C. Wun, W. S. A. Wun. Obstetrical and Gynecological Associates, Houston, Texas.

Objectives: Zygotes with a single pronucleus are felt to be the result of abnormal fertilization. The mechanism could be genetic or cytoplasmic. We postulate that one of the cytoplasmic deficiencies could be due to a relative deficiency of glutathione, necessary for reduction of the sperm head. In this study we examined the effect supplementation of the culture medium with cysteine and glutathione on growth of one-pronuclear zygotes to the blastocyst stage.

Design: IVF cases from 03/01/98 to 01/31/99 were included in the study. One-pronuclear zygotes were randomly allocated to the control or experimental group. The blastocyst formation rate of the two groups was compared.

Materials and Methods: In the control group, zygotes were cultured in Minimum Essential Medium alpha (MEM-a). The experimental group used MEM-a, supplemented with 2 μl of cysteine (100 mM) and 2 μl of glutathione (100 mM) in 750 μl. The embryos were cultured until blastocyst formation or day 7 following aspiration. Statistical analysis was done by the Chi Square method.

Results:

	IVF	ICSI	Total
Case #	183	165	348
Occurring %	93/2538 (3.6%)	62/1783 (3.5%)	155/4321 (3.6%)
Control	5/51 (9.8%)	3/43 (6.9%)	8/94 (8.5%)
Experiment	13/43 (30.2%)*	2/20 (10%)	15/63 (23.8%)***

(Control vs. Experiment *: $P < 0.05$; ***: $P < 0.005$)

Conclusion: The results suggest that supplementation of MEM-a with cysteine and glutathione can significantly improve the blastocyst formation rate in one-pronuclear zygotes. These zygotes may be deficient in cysteine and/or glutathione, which are responsible for reduction of the sperm head. The lack of significance in the ICSI group may be due to the small population size.

P-240

In Vitro Maturation of Human Oocyte Retrieved from Unstimulated Ovaries: Fertilization and Embryo Development. R. Fabbri, E. Porcu, P. M. Ciotti, T. Marsella, O. Magrini, S. Venturoli, C. Flamigni. Infertility and IVF Center, Human Reproductive Medicine Unit, Institute of Obstetrics and Gynaecology, University of Bologna, Bologna, Italy.

Objectives: The establishment of a clinical feasibility to retrieve immature oocytes from unstimulated ovaries could be an important technique in assisted reproduction due to its potential for reducing the use of fertility drugs.

Design: The purpose of this study was to assess the ability to retrieve transvaginally unstimulated immature oocytes and to determine their competence to mature, fertilize, cleave and be transferred as viable embryos in IVF programs.

Materials and Methods: Seven patients with different diagnoses of infertility were enrolled in this study. Baseline ultrasound was performed on day 5 or 6 along with E2 levels. All patients received increased doses of 17-β oestradiol between cycle days 7 and 12, followed by progesterone administration initiated the day after the oocyte retrieval. Transvaginal ultrasound aspiration was performed between the ninth and the eleventh day of the cycle with a 30 cm 17 gauge needle. The collected oocytes were graded based on the appearance as NUDE (with a few corona cells surrounding the oocyte), CO (with only the corona cells surrounding the oocyte) and CEO (with cumulus and corona cells strictly adherent to oocyte). All immature oocytes were allowed to mature in Chang medium added with 0.075 IU FSH and 0.5 IU HCG in 5% CO_2 at 37°C. Insemination was performed by ICSI. All the developed embryos were cultured for three days before transfer, and scored daily for cleavage, EDR rate and for morphological quality.

Results: Seven patients (mean age of 36.6 ± 1.7), had 17 (mean 2.4 ±

1.4/patient) oocytes retrieved. Within a mean time of 43.2 ± 17.6 hours after collection, all the oocytes had the polar body extruded with an overall rate of meiotic maturation of 100%. The oocytes classified as NUDE showed the extrusion of the polar body after a mean of 47.5 ± 20.5 hours of culture, while those classified as CO and CEO showed the extrusion of the polar body after a mean of 55.5 ± 12.1 and 36.5 ± 11.8 hours respectively. The fertilization and the cleavage rates were of 50% and 100% respectively. The mean of EDR rate, evaluated 40–42 hours after insemination, was of 105.5 ± 16.0 while 65 hours after ICSI was of 81.5 ± 11.5. Five embryos out of six showed a good morphology with their blastomeres regularly shaped and a fragmentation rate less than 20%. All the embryos were transferred in five patients, whose endometrial thickness ranged from 7 mm to 12 mm. None of the patients became pregnant.

Conclusions: The ability to retrieve immature oocytes transvaginally with ultrasound guidance was accomplished in each patient with different diagnoses of infertility. In this preliminary study the immature oocytes were able to mature, fertilize and cleave with reproducible consistency. Further investigations are required in order to better synchronize endometrial receptivity for embryo cleavage stage, and in order to increase the implantation rate.

P-241

Prediction of Assisted Reproduction Treatment (ART) Outcome Using Artifician Neural Networks. [1]S. Jacob, [2]P. Cunningham, [1]R. F. Harrison. [1]Human Assisted Reproduction Ireland and Royal College of Surgeons in Ireland, Rotunda Hospital, Dublin 1. [2]Department of Computer Sciences, Trinity College Dublin.

Objective: Major technological advances have been made in ART since the birth of the first IVF baby Ms. Louise Brown in 1978. But these have not resulted in statistically significant improvement in pregnancy rates worldwide, which is about 33% per cycle started and take home baby rate of 20%. Unfortunately we don't know the critical factor(s) which determine successful pregnancy, though vast amount of clinical data are generated during the course of the treatment. Artificial Neural Networks (ANN) are highly successful in pattern recognition in databases where conventional statistical methods fail and are successfully applied in medical image analysis, automated ECG analysis, critical care and also in industries like banking, air traffic control etc. The aim of this study was to predict ART outcome using ANN and thus help in decision making and patient counselling.

Design: Prospective data analysis.

Materials and Methods: The backpropagation ANN was developed using C++ programming language. Randomly selected 471 IVF/ICSI cycles were used to learn the ANN. A total of 51 attributes (including female age, cause of infertility, day 3 hormone profile, stimulation protocol, no. of oocytes, number and quality of embryos etc) from each treatment cycle was used in the learning algorithm. The ANN was optimised by identifying the ideal number of iterations, learning rate, momentum and error rate. The trained optimised machine was then challenged with the data set of 979 completed treatment cycles performed in the unit. The treatment outcome predicted by the ANN was compared with the actual outcome. The sensitivity, specificity, positive and negative predictive values and the total performance of the ANN machine was evaluated.

Results: Total of 1450 IVF/ICSI cycles were completed in the unit since January 1997 with a pregnancy rate of 21%. The backpropagation neural net was optimised at 250 iterations, learning rate of 0.4, momentum of 0.2 and error rate of 0.0001. 206 of the 979 cycles tested on the ANN achieved pregnancy. The machine was able to positively identify 144 true successful cycles and 695 true failed cycles giving a sensitivity of 70.1% and specificity of 89.4%. The positive predictive value was 87.5% and negative predictive value was 75%. The over all prediction accuracy of the ANN was 70% (CI: 65%–78%).

Conclusions: 1. The backpropagation ANN can be used in the prediction of ART outcome with an accuracy of 70%. 2. The specificity of prediction was higher than sensitivity. 3. There is potential for the application of ANN in clinical practice. 4. The determination of individual weights of the attributes in ANN are unknown and they could be identified by further research using lazy learning machines like *k*–Nearest Neighbour.

P-242

Effects of Low Levels of Ascorbic Acid (AA) On Survival of Human Sperm, and on Outcomes of the Zona-Free Hamster Penetration Assay (SPA). [1,2]B. A. Stone, [2]E. Ong, [2]R. Faridi, [1,2]R. P. Marrs. [1]Institute for Fertility Research and [2]Reproductive Technology Laboratories, Santa Monica, CA.

Objectives: Separation of sperm from seminal plasma during processing predisposes to DNA fragmentation through oxidative attack, verified by single-cell gel electrophoresis. Recent studies have shown that AA can preserve sperm nuclear DNA during X-irradiation challenge, the level of protection being dose-dependent at ≤600 μM (Hum Reprod *13*, 1240; 1998). Before supplementing culture media with AA, we have examined its dose-responsive effects on motile sperm survival, and function.

Design: Motile sperm survival and SPA outcomes following culture in graded concentrations of AA.

Materials and Methods: Semen samples from each of ten patients were processed through Percoll and resuspended to a final concentration of ca. 40 million motile sperm/mL in bicarbonate HTF containing 5% (v/v) SSS. Each suspension was then aliquoted and diluted ×2 to provide final concentrations of L-ascorbic acid (sodium salt, Sigma culture grade) of 0 (control), 75, 150, 300 and 600 μM. At time 0, and after each of 3 consecutive 24-hr culture intervals at 37°C under 5% CO_2 in air, concentrations of motile sperm were determined (CASA). The percentage survival of motile sperm was then analysed by full factorial ANOVA (culture day × ascorbic acid concentration).

Results: In the absence of AA, 79 (±5, SE)% of motile sperm survived 24 hr culture. By ANOVA, day of culture and AA concentration influenced motile sperm survival ($P < 0.0001$), without interaction ($P = 0.4$). Motile sperm survival fell to 67 (±3)% as AA levels increased to 300 μM (NS), and to 44 (±11)% at 600 μM AA ($P < 0.05$). The survival of motile sperm was also lower with increasing AA after 48 and 72 hrs culture, but was significantly lower at 600 μM only (9 ± 4 c.f. 68 ± 8% for control at 48 hrs; 5 ± 4 c.f. 36 ± 7% for control at 72 hrs). While, therefore, 600 μM reportedly provides optimal DNA preservation, effects of this AA level on sperm survival are unacceptable. 300 μM provides 10% less DNA protection (Hum Reprod, *13*:1240;1998) without significant impact on survival. The SPA was performed with further sperm suspensions cultured overnight in HTF supplemented with 300 μM AA, yielding an average (±SE) penetration rate of 91 (±5)%, c.f. 32 (±4)% for controls ($P < 0.0001$).

Conclusion: Supplementation of sperm culture media with a level of AA which reportedly maximizes DNA-protection (600 μM) is associated with low motile sperm survival. 300 μM AA does not significantly impact motile survival, promotes high hamster ovum penetration outcomes, and remains effective in diminishing DNA fragmentation of human sperm during X-irradiation challenge.

P-243

Withdrawn

P-244

Multinuclear Blastomeres (MNB): Incidence, Distribution Pattern and Influence on Embryo Development and Quality. L. Chi, A. Chin, A. Adler, M. Clarke, A. Goldstein, P. Labella, C. McCaffery, L. C. Krey. Program for In Vitro Fertilization, Reproductive Surgery and Infertility. New York University School of Medicine, NY, NY 10016.

Objectives: To assess the incidence and the distribution patterns of MNB, and its influence on embryo development, embryo quality and pregnancy rate.

Design: The incidence and type of multinuclear blastomere (MNB) in the human embryos at day 2 stage were recorded. MNB embryo development rate and quality were assessed. The pregnant rate of patients with MNB embryos was evaluated and compared with the control group.

Materials and Methods: All IVF patients from April–June 1998 were included. Eggs and embryos were cultured in HTF medium supplemented with maternal serum or Plasmanate. MNB, embryo quality and cleavage rate were monitored under inverted microscopy at 40–46 h and 61–67 h after insemination. Only normally fertilized embryos containing MNB at 40–46 h are described; bi-nuclear blastomeres (2N) and multi-nuclear (≥3N)

blastomeres were recorded separately. The influence of MNB on pregnant rate was also determined.

Results: Embryos with MNB (n = 82) were found in 56 of 311 patients (18%). MNB were found in 10% of the patients undergoing ICSI (11/117) and in 23% patients in which the eggs were inseminated normally (45/194). The pregnancy rate for patients with MNB in their embryo cohort was 63% (35/56); this was not different from the rate for patients without MNB (56%; 140/256). Of the MNB observed, 68%, 13%, 11% and 7% appeared in 2-cell, 3-cell, 4-cell and 5-cell stage embryos, respectively. A single MNB was noted in 76% of the embryos and 24% of embryos had two. Embryos with 2N or ≥3N MNB at the 2-cell stage displayed a slow cleavage rate; 52% and 67%, respectively, were <5 cells the following day. No effect on embryo quality was noted. Embryos often cleaved within 6 h after MNB detection; 40% did not display MNB by this time.

Conclusions: MNB incidence was higher following normal insemination than ICSI, suggesting that fertilization mechanisms may be important for MNB generation. Embryos with a single 2N blastomere were most commonly observed. Significantly, these embryos often cleaved with the MNB disappearing within 6 h of detection and displayed little influenced on cleavage rate or quality. However, the presence of a ≥3N blastomere did impact on subsequent embryo development. Pregnancy outcome was not compromised when embryos containing MNB were observed.

P-245

Sex Chromosomes in Sperm and Spare Embryos of a Male with Klinefelter's Syndrome. M. M. Bielanska, S. L. Tan, A. Ao. McGill University, Royal Victoria Hospital, Department of Obstetrics and Gynecology, Montreal, Quebec, Canada.

Objective: Klinefelter's syndrome is usually associated with azoospermia or oligospermia. Most patients are infertile. Recently it has been suggested that 47, XXY germ cells are able to progress through meiosis to produce aneuploid spermatozoa. In order to determine whether a 46, XY/47,XXY Klinefelter patient had an increased risk of a chromosomally abnormal conceptus, we used fluorescence in situ hybridization (FISH) to examine the sex chromosomes in his sperm and in spare embryos fertilized by intracytoplasmic sperm injection (ICSI).

Study Design: Sperm cells and spare preimplantation embryos produced by intracytoplasmic injection of spermatozoa from a 46 XY/47 XXY male were analyzed by 3-color fluorescence in situ hybridization using probes specific to chromosomes X, Y, and 18.

Materials and Methods: Blastomeres from 10, day-4 spare preimplantation embryos were spread on glass slides using HCl/Tween 20. Sperm was washed in Tris/NaCl, smeared on glass slides, and sperm nuclei decondensed by treatment with Tris/DTT and Tris/LIS/DTT solutions. Blastomere and sperm nuclei were treated with pepsin and dehydrated. Nuclei were hybridized with directly labeled fluorescence probes specific to chromosomes X, Y and 18.

Results: The proportions of X18 and Y18-bearing sperm was 48% and 45%, respectively. Abnormalities for sex chromosomes were detected in a total of 6.25% of cells analyzed, and included XY18 (2.84%), XX18 (2.27%), YY18 (0.57%) XXY18 (0.57%) chromosome complements. Of 10, 5-cell to 11-cell spare embryos analyzed, 3 were normal for chromosomes tested, 1 with a XX1818, and 2 with a XY1818 pattern. Two embryos were diploid mosaic, with majority of blastomeres normal for the three chromosomes tested. Five embryos consisted of mostly abnormal blastomeres, with chaotic chromosome XY18 patterns. None of the 67 blastomeres analyzed showed a XXY1818 complement.

Conclusions: Our results confirm that 47 XXY germ cells are able to complete meiosis and produce sperm carrying an XY complement. The sperm of the mosaic Klinefelter patient had a higher frequency of sex chromosome disomy than sperm of normal fertile males, however none of the spare embryo analyzed by FISH showed a XXY chromosome complement.

P-246

Aquisition of Metaphase Chromosome in Single Blastomere of Human Preimplantation Embryos: a Suitable Method for Preimplantation Genetic Diagnosis. [1]M. H. Han, [1]J. H. Jun, [1]J. W. Kim, [1]H. K. Byun, [1]H. W. Youm, [2,3]I. P. Son, and [2,3]J. Y. Jun. [1]Infertility Research Laboratory, [2]Department of Obstetrics and Gynecology, Samsung Cheil hospital & Women's Healthcare Center, [3]College of Medicine, Sungkyunkwan Univ., Seoul, Korea.

Objectives: Individuals, heterozygous for reciprocal translocations, are at an increased risk for reproductive failure and chromosomally abnormal progeny. However, current FISH technique is not suitable for detection of chromosome structural abnormalities of nuclei in blastomere for these patients. The objectives of this study were to determine the reliability of arrest agent, colcemid, for halting blastomere division at metaphase in human embryos; and to evaluate nucleus morphology at the beginning of colcemid treatment and determine its relationship with metaphase arrest after treatment.

Design: The surplus 2PN and 3PN embryos, obtained from patients undergoing IVF-ET programme, were treated with various concentrations of colcemid for 16–18 hrs and observed metaphase chromosome at the end of colcemid treatment.

Materials and Methods: The zona pellucida of human surplus 2PN and 3PN embryos was removed by acidified Tyrode's solution. Each blastomere was detached by mouth pipetting and treated with various concentrations of colcemid (0.2, 0.4, 1.0, 2.0, 4.0, and 5.0 μg/ml) for 16–18 hrs. At the beginning of colcemid treatment, blastomeres were microscopically evaluated for the presence of intact nuclear membrane and the multinucleation. At the end of the colcemid treatment, the blastomeres were fixed in 10% formalin and stained with hoechst 33342. The nuclear morphology and cleavage of the blastomeres were examined with an inverted microscope equipped with epifluorescence. Metaphase arrest rates were then compared with nuclear parameter.

Results: It was observed that no blastomere was metaphase arrested by colcemid concentration at 0.2 μg/ml and 0.4 μg/ml, and 16.1% (9/56) of blastomeres were arrested at 1.0 μg/ml, 26.8% (11/41) at 2.0 μg/ml. The highest arrest rate, 65.1% (54/83) was obtained at 4.0 μg/ml, and 61.5% (16/26) was obtained at 5.0 μg/ml. In surplus 2PN embryos, 62.9% (17/27) of blastomeres were arrested at 4 μg/ml. Metaphase arrest rate of blastomeres with a visible intact nucleus (65.1% vs 2.08%) was significantly higher ($p<0.001$) than that with either no visible nucleus or multinucleation.

Conclusions: This experiment shows that the colcemid concentration at 4 μg/ml is optimal condition for arresting cleavage at metaphase in human blastomeres. Since blastomeres containing intact nucleus were observed to be suitable for arresting cell cycle, nucleus morphology of the blastomeres should be examined before colcemid treatment. This cell cycle arrest method would be used effectively for FISH analysis for structural and numerical chromosome abnormalities.

P-247

Production of Mature Oocyte from Frozen-Thawed Primordial Follicular Oocyte by Reconstruction with Cytoplasm of Full Grown Germinal Vesicle(GV)-Stage Oocyte. [1]D. R. Lee, [1]B. R. Do, [1]J. E. Lee, [1]S. J. Yoon, [1]S. I. Roh, [2]M. K. Kim, and [1]H. S. Yoon. [1]Infertility Research Center, Jeil Women's Hospital, [2]Department of Biology, Hanyang University, Seoul, Korea.

Objectives: Primordial follicle was suggested as a future source of oocytes for infertility treatment in premature ovarian failure (POF) and perimenopause patients. Although the only live birth reported after the culture of primordial follicles in the mouse, it was required a lot of time for new technology of assisted reproduction in human because a defect of culture system. This study was performed to develop a new method for treatment of infertility patients required oocyte donation as mouse model system.

Designs: Reconstruction of frozen-thawed primordial follicular oocyte and enucleated ooplasm of germinal vesicle(GV)-stage oocyte from antral follicle, and analysis of maturation rate and chromosomal status of reconstructed oocytes in mouse.

Materials and Methods: Primordial follicular oocytes (30–40 μm) were obtained from frozen-thawed ovarian tissues of 21-days female mice (ICR strain) by enzymatic method, and used for nuclear donor after remove of zona pellucida by pronase treatment. For the cytoplasm recipient, full grown GV-stage oocytes were acquired from ovarian puncture at 46 hours after PMSG injection and were cultured for 3 hours in CZB medium containing dbcAMP(250 μM). After incubation for 10 minutes in medium containing cytochalasin B (5 μg/ml), GV was removed from oocytes. And then primordial follicular oocytes were inserted into perivitelline space of enu-

cleated oocyte and reconstructed by electrical stimuli. Fused oocytes were transferred into maturation medium (TCM 199 + 20% FF, 10 iu/ml FSH, 20 iu/ml hCG, 10 ng/ml E_2) and cultured for 24 hours. Chromosomal statuses of mature oocytes were analyzed by air-drying and Giemsa staining method.

Results: In preliminary study, in vitro maturation of primordial follicular oocytes from frozen-thawed tissues was not initiated when they simply cultured in maturation medium. After electrofusion and in vitro maturation, 66.7% (10/15) of the reconstructed oocytes underwent the breakdown of GV and 53.3% (8/15) of these produced first polar body, and decreased when compare with sham-operated GV-stage oocyte obtained from ovarian puncture (83.3% (25/30); 80.0% (24/30). And, all of mature oocytes showed normal karyotype.

Conclusions: From these results, reconstruction of primordial follicular oocyte and enucleated ooplasm of GV stage oocyte from antral follicle can produce mature oocyte with normal karyotype. Therefore, application of primordial follicular oocytes by transfer technique may be suggest a useful tool for oocyte production in infertility patients, who have poor ovarian reservoir.

P-248

Presence of Water Channels at Different Maturity Stages in Human Oocytes. [1]P. Ford, [1]M. S. Parisi, [2]J. Notrica, [1]M. N. Parisi, [2]E. Polak de Fried. [1]University of Buenos Aires, School of Medicine, Department of Physiology and [2]CER Instituto Medico, Department of Reproductive Medicine, Buenos Aires, Argentina.

Objectives: Important structural and functional modifications occur in mammalian oocytes during their arrival to maturity. During this process the cell switches from a high activity level, implying an important metabolic rate and a coordinated movement of water and solutes, to a lower functional state characterized by significant changes in its membrane structure and function. Full activity will start again after fecundation. It has been recently proposed that a broad selectivity "water channel" (aquaporins 9, AQP 9) is responsible for solute and water transfer in highly active cells. We have previously demonstrated that rat oocytes express a water channel that disappears during their arrival to maturity. Because of its potential significance for fertility and cryopreservation, similar studies were performed in human oocytes.

Design: The osmotic water permeability in human oocytes was studied in different stages of maturation.

Materials and Method: Oocytes were isolated at different maturity stages: germinal vesicle (GV, n=9), metaphase I (MI, n=3); metaphase II (MII, n=2), metaphase II after ICSI failure (MII-ICSI, n=14) and metaphase II after IVF failure (MII-IVF, n=6). In all cases an informed consent from donors was obtained. Volume changes, induced by an osmotic gradient, were followed by video microscopy and osmotic water permeability was calculated and expressed as Posm, cm $\times 10^{-4}.s^{-1}$.

Results: Osmotic permeability was significantly higher in GV (16.46 $\pm$ 2.24) than in MII-ICSI (6.75 $\pm$ 0.88), mean difference 9.71 $\pm$ 2.40, $p<0.001$ and than in MII-IVF (10.04 $\pm$ 1.55, mean difference (6.42 $\pm$ 2.72, $p<0.05$). It was also observed that Posm was higher in GV than in MI (9.10 $\pm$ 0.90, mean difference 7.32 $\pm$ 2.41, $p<0.02$).

Conclusions: A water channel seems to have been lost during transition from germinal vesicle to metaphase I human oocytes. Furthermore, after unsuccessful ICSI or IVF attempts, water permeability was strongly reduced as compared with the germinal vesicle stage. The molecular characterization of the putative water channels (aquaporins) expressed in mammalian oocytes is the subject of an on-going assay. All of the above contributes to the better understanding of the inner physiological mechanism involved in oocyte cryopreservation and will eventually allow for the improvement of human oocyte cryopreservation at different stages.

P-249

Rapid, Repeated FISH (Fluorescence in Situ Hybridization) in Human Amniocytes and Fibroblasts for Preimplantation Genetic Diagnosis. L. J. Evenson, K. C. Drury, L. Kovalinskaia, R. S. Williams. Department of Obstetrics and Gynecology Research Laboratory, University of Florida College of Medicine, Gainesville, Florida.

Objective: To determine the potential of repeated FISH applications on the same human amniocyte or fibroblast.

Design: Two consecutive FISH applications were performed on human amniocytes and fibroblasts to enumerate specific chromosomes.

Methods and Materials: Prepared slides of mosaic male fibroblasts and normal male amniocytes were provided by Vysis, Inc., IL., USA. The FISH procedure (as described by Vysis, Inc.) was performed utilizing the Aneu-Vysion Multicolor DNA probe kit. LSI probes for chromosomes 13 and 21 were initially used. After examination of the results of the first FISH, the nuclei were washed in buffered formamide and Tween 20 solutions. Slides were then sequentially dehydrated in 70%, 85% and 100% ethanol. A second FISH procedure was then performed on the same nuclei with the CEP alpha satellite probes for chromosomes X, Y, and 18. This second FISH application was carried out by an ultra rapid FISH technique using microwave technology as described by Drury et al (Abstracts of the American Society of Reproductive Medicine, 1997). Analysis was performed with the aid of Vysis Quips image analysis software. The two FISH procedures took approximately five hours.

Results: One hundred nuclei were examined after application of the 13, 21 probes. The signal detection rate was 97% after the first FISH application and 93% after the second FISH application. There was no statistically significant difference between the rate of nuclear loss, present signals or absent signals with the subsequent FISH treatment.

Conclusions: Repeated FISH applications may performed quickly and with high efficiency on the same nuclei. This may have a significant role in preimplantation genetic diagnosis.

P-250

Comparison of Cell Lysis Conditions for Single Cell PCR Using Molecular Beacons to Monitor Reactions in Real Time. K. E. Pierce, J. E. Rice, J. A. Sanchez, L. J. Wangh. Department of Biology, Brandeis University, Waltham, MA.

Objectives: Preimplantation genetic diagnosis using PCR requires cell lysis to free the DNA from other components of the cell. Poor lysis conditions can result in a high frequency of amplification failure, allele dropout, and possible misdiagnosis. We have compared different lysis protocols with the aid of new PCR technologies to establish which method maximizes target gene amplification.

Design: Real-time PCR with fluorescent probes provides a quantitative measure of target availability in terms of the initial time of signal detection (threshold cycle, C_t). The highly-conserved TSPY genes were chosen for amplification, since each male cell contains approximately 30 copies as possible targets for PCR. Thus, comparison of C_t value indicates the relative availability of TSPY genes following different lysis protocols.

Materials and Methods: Single male lymphocytes were lysed using a variety of protocols and the TSPY genes were amplified using a specific pair of primers. Molecular beacons, a new type of fluorescent oligonucleotide probe, were used to monitor product accumulation during PCR (i.e., in "real time"). Fluorescent measurements were taken at each annealing step with an ABI Prism 7700 Sequence Detector. The mean and standard deviation of C_t values were calculated for each group of samples prepared with a particular lysis protocol.

Results: Lysis buffers containing proteinase K (PK) and SDS yielded the lowest C_t values, indicating the highest number of targets available for amplification. Decreasing the lysis incubation temperature from 50° to 37° increased the mean C_t by 0.6 cycles. The standard deviation of each mean was 0.4 cycles. The presence of Mg^{2+} in the lysis buffer increased the mean C_t by 3.5 cycles and standard deviation to 1.6 cycles, possibly due to inhibition of PK. Alkaline lysis (KOH/DTT) increased the mean C_t by 2.5 cycles compared to the optimal PK/SDS lysis protocol.

Conclusions: Molecular-beacon-monitored PCR with the multi-copy TSPY genes provided direct comparison of several protocols for single cell lysis. Best results were obtained with a PK/SDS lysis at 50°C. Other protocols, including alkaline lysis, yielded higher C_t values, indicating that fewer copies of the gene were available as targets for PCR. It is likely that the optimized protocol will provide lower rates of allele drop-out when used with single-copy genes.

This work was supported by a grant from Hamilton Thorne Research, Inc.

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Cryopreservation of Human Germinal Vesicle (GV) Stage and In Vitro Matured M II Oocytes: Influence of Cryopreservation Media on the Survival, Fertilization and Early Cleavage Divisions. A. P. Goud, P. T. Goud, C. Qian, J. Van der Elst, M. Dhont. Infertility Center, Department Obstetrics and Gynecology, University Hospital, Ghent, Belgium.

Objective: Cryopreservation of oocytes has a significant potential for clinical application. However, the current level of success of immature as well as M II stage oocyte freezing is low. Recently, lowering of sodium (Na) concentration in the cryopreservation media has been suggested as an alternative for better results for in vivo matured M II stage oocytes (Stachecki *et al.*, Biol Reprod 59, 395–400, 1998). The current study evaluates the applicability of low Na freezing medium in comparison to the Na based conventional freezing medium (CFM) for immature and in vitro matured oocyte cryopreservation.

Design: GV stage oocytes were subjected to in vitro maturation (IVM) or cryopreservation using Na based or low Na cryopreservation media. In another experiment, in vitro matured M II stage oocytes were cryopreserved using the low-Na protocol. The oocytes were thawed and subjected to IVM followed by intracytoplasmic sperm injection (ICSI) and the rates of cryosurvival, maturation, fertilization and cleavage were compared.

Materials and methods: IRB approval was obtained for this experimental study. Cumulus-corona cell enclosed sibling GV stage oocytes obtained from ICSI patients were assigned for IVM (n=112) or cryopreservation using the slow-freeze and rapid thaw procedure using either one of the following protocols: 1.5 M PROH in PBS-20% FBS/0.1 M sucrose (group A, n=63) or 1.5 M PROH in low-Na choline based medium-10% FBS/0.1 M sucrose (Group B, n=47). In a third group, 35 GV stage oocytes were subjected to maturation and the resultant M II stage oocytes (n=25) were frozen using the same protocol as group B (group C). The medium for IVM was M-199 containing HSA, pyruvate, FSH, hCG, 17β-estradiol and epidermal growth factor (Goud *et al.*, *Hum Reprod* **13,** 1638–1644). The mature oocytes from all the groups were subjected to ICSI with donor spermatozoa and cultured for 42–45 hours in Human tubal fluid medium (HTF, Irvine Scientific). The incidence of maturation, fertilization and cleavage were compared using the Fisher's exact test.

Results: The survival of the GV stage oocytes frozen with either protocol were similar in groups A and B (57.1 and 42.5% respectively). The rates of GVB and M II stage oocytes were significantly lower in group A compared to unfrozen controls (GVB: 72.2 vs 90.2%, $p<0.02$; M II: 33.3 vs 74.1%, $p<0.001$). However, these rates were similar in the surviving oocytes from group B in comparison to the unfrozen controls(GVB: 90.0% and M II: 50.0%). The rates of fertilization and cleavage among the surviving thawed oocytes were similar in all the groups. However, the percentages of cleavage per GV oocyte were significantly lower in groups A and B (4.8 and 8.5% respectively) but not group C (28.6%) compared to the unfrozen controls (44.6%, $p<0.001$).

Conclusions: 1. Reduction of Na from the cryopreservation medium significantly improves the rate of maturation of the frozen-thawed GV stage oocytes. 2. Overall success of cryopreservation of in vitro matured M II oocytes is higher than that for the immature oocytes. (Support: Grant awarded by the University of Ghent).

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Sperm Decondensation and Oocyte Activation After Intracytoplasmic Injection of Epididymal and Ejaculated Human Sperm. [1]W. S. O, Y. Ying, [1]M. P. L. Cheung, [2]W. S. B. Yeung. [1]Department of Anatomy and [2]Department of Obstetrics & Gynaecology, The University of Hong Kong, Hong Kong, China.

Objectives: The fusion of a fertilizing sperm with an oocyte is thought to give the physiological trigger of oocyte activation. The process of intracytoplamic sperm injection can by-pass the fusion of the gametes and yet gives a reasonable success rate of fertilization and pregnancy. As hamster oocytes are readily available, we try to compare human ejaculated and cauda epididymal sperms in nuclear decondensation and oocyte activation after ICSI in hamster oocytes.

Design: Oocytes after intracytoplasmic injection of ejaculated and epididymal sperms were incubated for 8 h and then stained with propidium iodide to determine the stage of sperm decondensation and oocyte activation.

Materials and Methods: Sperms were obtained from patients undergoing a routine semen analysis at IVF Centre in Queen Mary Hospital, The University of Hong Kong. Samples showing normal values of sperm concentration, motility and morphology were used for ICSI. Ejaculated sperms and epididymal sperms from the epididymal biopsy were processed by Percoll gradient centrifugation and resuspended in EBSS medium. Natural ovulated golden hamster oocytes with cumulus removed were washed and stored in TALP medium. ICSI was performed using a micromanipulator mounted Nikon Diaphot inverted phase contrast microscope with a heated plate. Just before injection, sperms were pelleted and resuspended in TALP medium with 5% PVP and injection was done under mineral oil in TALP medium. After injection oocytes were incubated for 8 h and were air-dried on polylysine coated slide, fixed in 70% ethanol, permeated with 0.1% Tween-20 and Triton X-100 in PBS for 20 min, stained with 10 μg/ml propidium iodide and observed with a Zeiss epifluorescent microscope (510–560 nm excitation; 590 nm emission filter).

Results: A total of 135 oocytes were injected with ejaculated sperms from 15 patients and 54 oocytes injected with epididymal sperms from 6 patients. At 8 h after injection, 26.1 and 22.2% of the ejaculated and epididymal sperm developed into male pronuclei respectively. A significantly lower % of the oocytes injected with epididymal sperm (75.9%) formed female pronuclei (FPN) compared with those injected with ejaculated sperm (92.8%; $p<0.01$ χ^2 test). A higher % of oocytes in the former group were in MIII (with polar body II but FPN not formed; 16.7%) compared with the latter group (3.6%; $p<0.01$ χ^2 test).

Conclusion: At 8 h after intracytoplasmic injection into hamster oocytes, both ejaculated and epididymal human sperm decondensed and formed male pronuclei. However, ejaculated and epididymal sperm showed different patterns of activation of oocytes to form female pronuclei.

This study was supported by a grant from the Faculty of Medicine, The University of Hong Kong.

CLINICAL FEMALE INFERTILITY AND GYNECOLOGY

Tuesday, September 28, 1999

P-253

Smoking Risks to Reproductive Health: Assessment of Women's Knowledge. L. Roth, H. S. Taylor. Yale University School of Medicine, New Haven, CT, USA.

Objective: Cigarette smoking is the largest single avoidable cause of death in our society. The public is generally aware of the risks of smoking related disorders, such as cancer, heart disease, and chronic respiratory ailments, however public health measures have not addressed the health risks specific to women. Smoking has deleterious effects on the reproductive health of women, knowledge of which may impact on their smoking behavior. The goal of this study is to assess the knowledge and beliefs of women regarding those health risks of smoking which are specific to women.

Design: Cross-sectional survey.

Materials and Methods: A self reporting anonymous questionnaire was mailed to all 388 female employees of a small community hospital and a 75% response rate was achieved. The questionnaire assessed women's knowledge of the links between smoking and several health risks specific to women. 20% of the women were smokers (equal to the State average). Multiple linear regression analysis was used to associate personal background with knowledge of smoking risks.

Results: Nearly all of the respondents identified the risk of smoking on lung cancer, respiratory disease, and heart disease. Most respondents realized that smoking in pregnancy was associated with risks. Nearly half of the respondents mis-identified all cancers as linked to smoking, including breast cancer. However, only 27% correctly identified a link between smoking and infertility. Only 39% identified smoking as a risk factor for spontaneous abortion, and a mere 17% correctly stated that smoking can lead to early menopause. Only 30% related smoking to osteoporosis in women or to ectopic pregnancy. Those identifying themselves as "health care professionals" were no more likely to identify these risks, nor was education level a predictor of correct response.

Conclusion: The effects of smoking on heart and lung disease is well known and has received a great deal of public attention and funding. However, with the exception of pregnancy, only 21% of the women in this study realized that there are health risks associated with smoking that are specific to women. These risks are significant enough to warrant individual attention. Health care professional and all women need to be informed of these risks. By increasing the knowledge of risks that may be of more concern to women, we may have greater impact on their smoking behavior.

P-254

Laparoscopy With Hysteroscopy or With Ultrasound in the Evaluation of Patients With Chronic Pelvic Pain. G. Ventolini, S. L. Fremont. University of Cincinnati, (OH) USA.

Objectives: Diagnostic hysteroscopy and/or pelvic ultrasonography are often used in the evaluation of patients with chronic pelvic pain. It is plausible that one is more accurate than the other and few studies have addressed this question in the primary care setting.

Design: This prospective cohort study included 39 patients from a large family medicine practice (>50,000 visits per year) between October 1995 and October 1998.

Materials and Methods: The 39 patients were divided in two groups: 20 patients were evaluated with laparoscopy and ultrasound (Group A). 19 patients were evaluated with laparoscopy and hysteroscopy (Group B). All women of child bearing age (24 to 39 mean 30.7) had failed medical therapy with non-steroidal anti-inflamatories for more than three months and oral contraceptives for more than three months. The patients underwent complete history and physical exam and had normal laboratory values for complete blood count with differential, sedimentation rate, urine analysis and culture, cultures for Gonorrhea and Chlamydia renal profile, PT and PTT.

Results: The two groups were comparable with respect to age, race, parity, smoking and socio-economic status. Results from the diagnostic laparoscopy were also non-statistically significant regarding: endometriosis, pelvic congestion, polycystic ovaries, tubal occlusion, multiple diagnosis and the ones with normal findings. Group A had more pelvic adhesions 6/20 (30%) than Group A 4/19 (21%) $P<0.01$. Regarding endometrial findings: The two groups were comparable with respect to submucous fibroids and adhesions. Hysteroscopy identified polyps in 7/19 (36.8%) versus ultrasound in 2/20 (10%) $P<0.01$, cervical stenosis in 5/19 (26.3%) versus ultrasound in 1/20 (5%) $P<0.01$ and septated uterus 3/19 (15.8%) versus none with ultrasound $P<0.01$.

Conclusions: Hysteroscopy seems to be more accurate than ultrasound in the diagnosis of endometrial polyps, cervical stenosis and intrauterine anomalies in our study. The added value of hysteroscopy or pelvic ultrasound to the diagnostic laparoscopy in the diagnosis of chronic pelvic pain requires larger studies.

P-255

Metformin Increases the Ovulatory Response to Clomiphene Citrate (CC) in Patients Resistant to CC Alone. [1]D. T. Vandermolen, [2]V. S. Ratts, [3]W. S. Evans, [4]J. E. Nestler. [1,4]Departments of Obstetrics/Gynecology and [4]Internal Medicine, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA, [2]Department of Obstetrics/Gynecology, Washington University, St. Louis, MO, and [3]Department of Internal Medicine, University of Virginia, Charlottesville, VA.

Objectives: CC is the first line of therapy for anovulatory infertility. Anovulatory women resistant to CC often have obesity, hyperandrogenism, and/or hyperinsulinemia, factors associated with polycystic ovarian syndrome (PCOS). Correction of hyperinsulinemia in women with PCOS can correct anovulation. We tested the hypothesis that in women resistant to CC alone treatment with metformin to decrease hyperinsulinemia will increase the ovulatory response to CC and lead to pregnancy.

Design: Randomized, double blinded, placebo controlled, prospective study in progress.

Materials and Methods: Eligible for study were euestrogenic women aged 18 to 35 who desired pregnancy and had failed to ovulate despite treatment with CC 150 mg daily for five days. Study participants received placebo or metformin, 500 mg three times daily, for seven weeks. Baseline and post treatment reproductive steroids, gonadotropins, and oral glucose tolerance testing were obtained. Participants were continued on metformin or placebo and treated with CC 50 mg daily for five days. Serum progesterone (P4) was assayed on days 21 and 28 of each CC treatment cycle. A P4 level greater than 4 ng/ml defined ovulation. Treatment was advanced through treatment cycles at 100 and 150 mg CC doses when ovulation failed. With ovulation, the same CC dose was continued. A total of 6 ovulatory cycles, pregnancy, or failed ovulation on the CC 150 mg dose completed a participant's study.

Results: In the metformin and placebo groups, 7 of 9 participants (77%) and 1 of 11 participants (9%) ovulated, respectively ($P < 0.005$, Fisher's exact test). In the 9 participants ovulating in response to metformin and CC, 20 ovulatory cycles occurred (2.2 ovulations per participant, range 1–5). Of these 20 ovulatory cycles, 7, 7, and 6 occurred at the 50, 100, and 150 mg CC doses, respectively. Pregnancy (gestational sac on ultrasound) per ovulatory cycle was 3 of 20 (15%). Two of these 3 pregnancies are ongoing. In response to placebo only one ovulatory cycle occurred, this on the CC 150 mg dose, and it resulted in pregnancy. Means of the ages, weights, body mass indexes, and waist hip ratios were not significantly different between the metformin and placebo groups ($P > 0.05$, Student's t-test). Excluded from this analysis is the one participant who ovulated on metformin alone.

Conclusions: In euestrogenic anovulatory women resistant to CC alone, metformin use significantly increases the ovulatory response to CC and can result in pregnancy. This ongoing study will assess for hormonal factors that may predict an ovulatory response to combined metformin and CC. This work was supported in part by NIH grants GCRCM01 RR000: 65[1,4] and 36[2], and NIHHD96008[4].

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Single Dose Miconazole Nitrate Vaginal Ovule in the Treatment of Vulvovaginal Candidiasis: Two Single-Blind, Controlled Studies Versus Miconazole Nitrate 100 mg Cream for Seven Days. D. H. Upmalis. Advanced Care Products, North Brunswick, NJ.

Objective: To determine the efficacy and safety of a miconazole nitrate (1200 mg) vaginal ovule with miconazole nitrate 2% topical cream administered for a single night compared with the efficacy and safety of MONISTAT7 (miconazole nitrate 2%) Vaginal Cream administered for seven consecutive nights in the treatment of women with vulvovaginal candidiasis (VVC).

Design: Two equally randomized, single-blind, multicenter, controlled, parallel group, comparative Phase III studies.

Materials and Methods: 558 patients received either a single dose miconazole nitrate (1200 mg) ovule or seven consecutive doses of MONISTAT7 (miconazole nitrate 2%) vaginal cream. Patients in the ovule arm also received miconazole nitrate 2% cream to apply up to twice daily for symptom relief, as needed. Assessments were upon admission and at two subsequent visits. The primary endpoint was the therapeutic cure of VVC, determined by clinical symptoms, physical examination, and microbiology. Time to complete relief of symptoms and safety were evaluated, and patients were asked to complete a questionnaire indicating regimen preference.

Results: Miconazole nitrate (1200 mg) ovule had therapeutic cure rates of 71.7% (71/99 patients) and 61.5% (64/104 patients). MONISTAT7 had cure rates of 70.1% (68/97 patients) and 61.1% (55/90 patients). Difference between treatments was not significant ($p = 0.96$ and $p = 0.775$). The proportion of patients experiencing complete symptom relief by Day 3 was significantly greater in the ovule arm ($p=0.008$ and $p=0.025$). Time to complete relief of symptoms was significantly faster with the miconazole nitrate ovule (4 versus 5 days, and 3 versus 4 days). Overall safety results were consistent between groups in both studies. Less than 10% of adverse events classified as serious or severe were considered highly or probably attributable to the medication.

Conclusions: Miconazole nitrate vaginal ovule is as safe and efficacious in curing VVC as MONISTAT7 Vaginal Cream, while providing complete symptom relief significantly faster. Compared with prior therapy, patients preferred the single dose ovule. These studies were supported by Advanced Care Products, North Brunswick, NJ.

P-257

Etiology In Patients With Recurrent Reproductive Failure. F. P. G. Leone, A. Bulfoni, E. Garzia, V. Savasi, S. Giuntelli, P. Antonazzo, S. Di Grazia, A. E. Semprini. Department of Obstetrics and Gynaecology, San Paolo Biomedical Institute, University of Milan, Medical School.

Objective: Evaluate the role of etiology in patients with two or three pregnancy loss.

Design: Retrospective study of 601 patients affected by recurrent pregnancy loss (RPL) visited in out-patient's department.

Materials and Methods: We screened six hundred unpregnant women with a history of almost two consecutive early pregnancy loss for antinuclear antibodies (ANA), antiphospholipid antibodies (APLA), mainly anticardiolipin antibodies (ACA) and lupus anticoagulant (LAC), antithyroid antibodies. Furthermore, they underwent serum test to evaluate hormone profile of ovarian and thyroid, hysteroscopy and endometrial biopsy, vaginal and cervical swab, chromosomal map of the couple. Two hundred eighty-eight patients had two previous abortions, while three hundred thirteen women suffered three or more pregnancy loss.

Results: One hundred forty seven (51%) patients of the first group suffered autoimmune disorders, one hundred thirty eight (44%) in the second group. Infective problems are very common in each group.

Etiology	Two pregnancy loss	Three or more pregnancy loss
Abnormal kariotype	6 (2%)	9 (3%)
Uterine abnormalities	11 (4%)	19 (6%)
Genital tract infections	78 (27%)	109 (35%)
Iperprolactin	23 (8%)	19 (6%)
Autoimmune disorders	147 (51%)	138 (44%)
Unexplained	23 (8%)	19 (6%)
Total	288	313

Conclusion: It is important to evaluate autoimmune disorders in the women with two or more pregnancy loss because it represent the first ground. Furthermore our date suggest that the genital tract infections have an important role: one third of our population suffer of this problem. So we think that at the first approach it is necessary to seclude the present of patogeny in genital tract, especially Chlamydia, Mycoplasma. This tests are simple and cheap and can resolve a long story of recurrent reproductive failure.

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Hydrosalpinx Fluid Fails to Negatively Affect Sperm Motility or Survival. [1]K. D. Traynor, [1]S. A. Beyler. [1]Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC.

Objectives: To evaluate whether infertility associated with hydrosalpinges may be mediated, in part, by a deleterious effect of hydrosalpinx fluid on sperm motility and survival.

Design: Blinded, in vitro videomicroscopic analysis of the effect of hydrosalpinx fluid on sperm motility, progression, and longevity.

Materials and Methods: Donor sperm was exposed to hydrosalpinx fluids, which had previously been shown to have a deleterious effect on murine embryo survival. Identical aliquots of sperm were combined with hydrosalpinx fluids in concentrations of 0% (control), 25%, 50% and 75%. Semen analysis of each sample was then performed at time points 0, 2, 4, 6, 12, and 24 hours to assess for sperm viability, percent motility, and progression. Sperm longevity was determined by calculation of a survival index for each dilution and time point. Motility was graded on a 1–4 scale (1 = Minimal or no movement, 4 = Rapid forward progression).

Results: Our data failed to show a statistically significant impairment of sperm motility or longevity, even with minimal dilution of hydrosalpinx fluids.

Conclusions: The association between hydrosalpinx and infertility is well documented. The etiologies for this association may include; mechanical obstruction, endometrial effects, embryotoxicity, or other mechanisms not yet defined. Our study attempted to determine if a negative effect on sperm cell motility and survival might also be an etiology. Despite the well-documented association between the presence of hydrosalpinx and decreased fecundity we were unable to demonstrate an effect of hydrosalpinx fluid on sperm cell motility or longevity. However, this does not rule out other possible functional impairment (i.e. sperm/egg binding, etc.). This work was supported in part by the UNC Medical Alumni Endowment Fund.

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Non Responsive Defective Endometrium May be Corrected by Improving Uterine Blood Flow With the Use of Pentoxifylline. A. Husulak, D. Vasquez. Department of Reproduction, Centro Médico S.A. Corrientes, Argentina.

Introduction: Endometrial receptivity has been correlated with peripheral estradiol (E_2) values, endometrial thickness (ET) and blood flow patterns in the uterine and ovarian arteries. ET in turn, correlates, among other factors, with E_2 levels and uterine perfusion. Pentoxifylline, a methylxanthine derivative, improves blood flow in patients with chronic peripheral arterial disease by decreasing blood viscosity and consequently increasing microcirculation; it may potentiate the effects of endogenous prostacyclin which have an antiaggregatory fibrinolytic and vasodilatory properties through an increase in cAMP levels. Preembryo implantation in Assisted Reproductive Technologies (ART), depends in part, on endometrial thickness. In high complexity as well as in low complexity ART, patients have been identified with very thin endometria, that do not correlate with E_2 levels and can not be corrected by changing stimulation protocols. Pentoxifylline, by improving endometrial blood flow, may correct this problem.

Objectives: To analyze the changes in ET by improving uterine blood flow with pentoxifylline and its response in the conception rate.

Material and Methods: Patients: From January 1996 through December 1997 (2 years) 465 patients with history of infertility (3 to 12 years) were routinely investigated on day 11–13 of the cycle using peripheral E_2 levels, transvaginal ultrasound (US) for endometrial and ovarian status and color Doppler to determine uterine and ovarian arteries pulsatility index (PI) **(basal determinations)**. Two hundred and sixteen of them had normal PI (<3); 249 were abnormal. Most of them (225) had a history of pelvic problems (surgery, uterine synechiae, endometriosis, vascular factors) or were above the age limit of 38 years selected for this study. Only 24 had no identifiable factor for an abnormal PI pattern (>3) and thin endometrium (<6mm); one patient had to be eliminated because of E_2 values at ovarian stimulation far above the mean; therefore 23 patients form the basis of this study. Investigations: E_2 levels were measured in duplicate using enzyme chemiluminescent immunoassay system. Conventional and Doppler US was done using Toshiba, SSH 140 with a transvaginal probe of 6 MHz (Toshiba, Japan). The observer was unaware of patient's histories. ET was measured in a longitudinal section using maximal diameter from one myometrial - endometrial interphase to the opposite one. Mean PI of both uterine arteries was used to measure uterine blood flow. Interventions: The whole group was stimulated first using Frydman's protocol (Clomiphene-hMG-hCG) during which same investigations were repeated **(Pre-treatment group)**. No patient got pregnant in this cycle using timed intercourse. After 1 month rest, the group was treated again with the same stimulation protocol additioned with oral pentoxifylline (1200 mgs/day in 3 divided doses from the third through the 17th day of the cycle). **(Post-treatment group)**. Same end points were reinvestigated; if the patient did not get pregnant the same treatment was repeated 4 more times for a total of 5 cycles. Eight patients got pregnant in this group; 6 went to term (pregnant group). Statistical evaluation: Pre-treatment and post-treatment results were evaluated using matched-pairs "t" test. Values in pregnant and non-pregnant groups were compared using two-way repeat measure design.

Results: When pre and post-treatment groups were compared E_2 levels were significantly higher in the latter (202 ± 27pg/ml vs. 332 ± 226pg/ml respectively [mean ± SD] $p<0.05$). ET was also significantly higher in post-treatment group (8.26 ± 1.8mm vs. 5.17 ± 0.8mm $p<0.001$). Mean PI was significantly lower in post-treatment group (2.6 ± 0.38 vs. 3.7 ± 0.8 $p<0.001$) indicating an improved blood flow. Same variables were then compared in pre and post-treatment cycles in pregnant group. Again E_2 values were significantly higher in the latter (326 ± 215 pg/ml vs. 202 ± 16pg/ml); endometrium was significantly thicker (9.4 ± 2.1mm vs. 5.2 ± 0.8mm) and PI was lower (2.4 ± 0.4 vs. 3.8 ± 1.1). Results in non-pregnant group were similar (E_2: 335 ± 230pg/ml vs. 200 ± 32pg/ml; ET: 7.6 ± 1.4mm vs. 5.2 ± 0.8mm; PI: 2.4 ± 0.4 vs. 3.8 ± 0.8). When same variables were compared in pregnant and non-pregnant groups during treatment

cycle, no significant differences were found in E_2 values (326 ± 216pg/ml vs. 335 ± 230pg/ml); ET was higher (9.4 ± 2.1mm vs. 7.6 ± 1.5mm) and the PI were similar (2.4 ± 0.4 vs. 2.7 ± 0.3).

Conclusions: Results of this study seem to indicate that pentoxifylline, by improving blood flow, induce positive changes at the endometrial level directly or indirectly through an increase in E_2 levels favoring implantation.

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Uterine Artery Embolization as the Treatment for Cervical Ectopic Pregnancy. K. M. Berkowitz, S. M. Pfeifer. Center for Reproductive Medicine & Surgery, University of Pennsylvania Medical Center Health System, Philadelphia, PA.

Objective: To describe a case report of uterine artery embolization as the primary treatment for cervical ectopic pregnancy, and to review the literature.

Design: A case report.

Materials and Methods: Review of the Medline literature from 1966–1999.

Results: A 40 year old gravida 3, para 0 woman was transferred to our institution at 7 weeks gestation with intermittent vaginal bleeding and suspected cervical ectopic pregnancy. Transvaginal ultrasound confirmed an intracervical ectopic pregnancy with fetal cardiac activity and crown rump length consistent with 6 5/7 weeks gestational age. Mild elevation of liver transaminases precluded use of methotrexate, and so bilateral uterine artery embolization was performed with a slurry of Gelfoam. Transvaginal ultrasound was repeated 48 hrs later, and revealed perigestational hemorrhage and decreased sac vascularity with demise of the pregnancy. Serum B-hCG level decreased from 33,745 mIU/ml on admission to 1,329 mIU/ml four days post treatment. The patient experienced some cramping and vaginal bleeding post-embolization, but she remained hemodynamically stable with a stable hemoglobin and hematocrit throughout her hospital course, and through resolution of the cervical ectopic. Quantitative B-hCG levels were followed to a value of < 5 mIU/ml, noted at 4 weeks post-embolization. Post-treatment the patient failed to resume her normal menses, and she did not bleed in response to progestin or estrogen and progestin therapy. She was found to have cervical stenosis with a deformed cervix and an eccentrically placed os. Intra-operative dilatation of the internal os was successful, and she has resumed cyclic menstruation. Review of the literature reveals several small series and case reports where uterine artery embolization was utilized in conjunction with methotrexate, intra-amniotic sac KCl injection, and dilatation and evacuation. There have been no reports of bilateral uterine artery embolization as the primary and only treatment of cervical ectopic pregnancy.

Conclusion: Single therapy with bilateral uterine artery embolization as a treatment for cervical ectopic pregnancy may be an effective means of treatment. However, further study is needed to investigate longterm effects on cervical integrity, and subsequent pregnancy outcome.

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Application of Antral Follicle Count Assessment in the Prediction of One-Year Conception Rate of Women With Infertility. M.-Y. Chang, C.-S. Hsiao, C.-H. Chiang, H.-C. Hou, T.-T. Hsieh, Y.-K. Soong. Department of Obstetrics and Gynecology, Reproductive Center, Chang Gung Memorial Hospital, Taipei, Taiwan, Republic of China.

Objectives: We have reported two investigations that determination of the prestimulation antral follicle count (AFC) can predict the response of controlled ovarian hyperstimulation as well as the pregnancy rates of the treatment. The prestimulation AFC may represent the state of ovarian reserve. The current methods to estimate patient's ovarian reserve is still insatisfactory.

Design: To evaluate the characteristics of ovarian antral follicle counts in women with different infertility groups and its effect to their reproductive outcome.

Materials and Methods: Women aged less than 40 years old visited our infertility clinic during January 1st and December 31st, 1996 were assessed of ovarian antral follicle count (AFC). The evaluation of their infertility causes were performed in the next two cycles with complete hormonal assay, preovulatory follicular ultrasonography, hysterosalpingography, semenalysis and laparoscopy if indicated. The history of any intraabdominal surgeries were recorded as detail as possible including the stage of endometriosis, type of surgeries and ovarian involvement. Individualized and proper treatment of infertility were performed to each patients and one-year pregnancy rate were recorded for all patients with regularly follow-up. Twenty-nine fertile women without history of infertility and any surgeries involving ovaries were enrolled as control group.

Results: The antral follicle count (AFC) was significantly higher (19.8 ± 10.7) in the group of oligo-anovulatory patients than the AFC in the control group (14.2 ± 7.0), while AFC were lower in groups of advanced endometriosis (9.5 ± 5.2) and previous partial ovarian cystectomy or oophorectomy (7.6 ± 4.7). The pregnancy rate in the group of oligo-anovulation is significantly higher than those with utero-tubal factors and endometriosis. After subdivided the infertile group of patients into 3 groups according to their previous ovarian surgery history: no surgery, minor ovarian surgeries, and major ovarian surgeries, such as ovarian cystectomy/oophorectomy and found that the more advanced surgeries were performed previously, the lower the number of AFC were detected, also the one-year pregnancy rate was lower than the other two groups. Multivariate analysis of the confounding factors associated with one-year pregnancy rate found that antral follicle count and major ovarian surgeries obtained significant difference.

Conclusion: Our investigation proves that ovarian surgeries decrease the number of ovarian antral follicles in patients with infertility. While the lower number of AFC, esp. under 4, and major ovarian surgeries predicts significantly lower one-year conception rates of infertile women.

ENDOMETRIOSIS

Tuesday, September 28, 1999

P-262

Endometriosis Continues to Burden Healthcare Systems—Updated Findings. J. M. Wong, L. M. Arguelles. G. D. Searle & Co., Skokie, IL.

Objective: To determine trends over time in the prevalence and cost of endometriosis by comparing results from an earlier study using 1991 and 1992 data with more recent data from 1994 and 1995.

Design: A cross-sectional database analysis.

Materials and Methods: Data for years 1994 and 1995 were obtained from the Nationwide Inpatient Sample (NIS) from the Health Care Cost and Utilization Project (HCUP-3), a 20% sample of all U.S. hospital discharges. Women, 15 to 54 years, with a primary diagnosis of endometriosis (ICD-9 codes 236.0, 617.0 to 617.9) were included in the analysis. Costs, as represented by hospital charges, were adjusted to reflect 1995 dollars.

Results: In 1994 and 1995, a total of 13,606 (8.3/1000) and 15,012 (8.8/1000) hospital admissions in this database were for a primary diagnosis of endometriosis. Most endometriosis admissions occurred in women between 30 and 44 years of age. Nearly all (95%) of the endometriosis hospitalizations were routine admissions. The most common diagnosis was endometriosis of the uterus (>50%); the most common procedures were total abdominal hysterectomy (52%) and vaginal hysterectomy (23%). The length of stay (LOS) and mean total charge increased with older age. African-American patients had significantly longer LOS compared with women of other races ($p<0.05$) while Asian/Pacific Islanders had significantly higher total charges ($p<0.001$). The average LOS and total hospital charges were 3.1 days and $8,401 for 1994, and 2.9 days and $8,149 for 1995. The estimated total hospitalization costs for women with endometriosis as the primary diagnosis in the U.S. were $624 million for 1994 and $622 million for 1995. In comparison, in 1991 and 1992, the rate of hospital admissions for endometriosis as a primary diagnosis was 9.4/1000 and 9.6/1000; the average LOS was 3.8 days and 3.5 days; total hospital charges were $8,491 and $8,796; and the estimated total hospitalization costs on a national level were $719 million and $725 million, respectively.

Conclusion: Hospital admissions for endometriosis remain prevalent among women of reproductive age. The diagnosis rate, length of hospitalization stay, and total hospitalization costs for endometriosis have decreased over time; however, endometriosis and its treatment continues to be a burden on U.S. health care systems.

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Familial Risk of Endometriosis: An Evaluation of the Family History of 98 Cases of Endometriosis from Two Tertiary Hospitals in Montreal Recruited Through Fertility Clinics. [1]P. N. Tonin, [1,2]C. Serruya, [1]S. Jananji, [1]K. Morgan, [2]T. Tulandi. [1]Departments of Medicine & Human Genetics, McGill University & Montreal General Hospital Research Institute, & [2]Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada.

Objectives: Recent studies strongly suggest a familial tendency for endometriosis and support the hypothesis that endometriosis has a genetic basis. Clinical manifestations of the disease include pain, menstrual disorders, infertility and pelvic masses. The objective of this study is to establish the occurrence of endometriosis in mothers and sisters of women with documented endometriosis identified from the fertility clinics of two tertiary hospitals in Montreal.

Design: Participants were recruited through the fertility clinics affiliated with two McGill teaching hospitals. They were selected on the basis of a diagnosis of endometriosis by laparoscopy that occurred between 1993 and 1996. Disease severity was assessed according to the revised American Fertility Society classification and divided into Stage A (Stage I–II) and Stage B (Stage III–IV). The participants were interviewed for a family history of endometriosis.

Participants: A total of 195 women with laparoscopy confirmed endometriosis were contacted: 52 were not interested in participating in the study, 9 were removed from the study due to ineligibility, and 37 have not responded. A total of 98 participants were eligible for the study.

Results: The mean age for participants (cases) at diagnosis by laparoscopy was 36 years (median age = 36 years). There were 57 cases with Stage A disease and 31 cases with Stage B disease: in 10 cases staging is pending confirmation. There was 105 female siblings included in the study. A family history of endometriosis was reported in 16 cases: 7 reported a sister with either Stage A (2 cases), Stage B (3 cases) or unknown stage (2 cases); 4 reported a mother with endometriosis with Stage A (1 case), Stage B (1 case) or unknown stage (2 cases); 1 reported both a mother and sister with endometriosis (Stage A); and 4 reported cousins or aunts with endometriosis with Stage A (3 cases) or unknown stage (1 case).

Conclusions: There is a familial tendency of endometriosis in first-degree relatives of women with proven endometriosis recruited through infertility clinics of Montreal. Taking 1% as an estimate of the population prevalence (0.5–2.5%) then the estimate of relative risk ratios are 5.1 and 7.6 for mothers and siblings, respectively. This is consistent with published studies reporting an increased prevalence of endometriosis in mothers and sisters of patients. We are currently investigating additional cases and controls (women with no endometriosis by laparoscopy recruited through the same clinics) to more precisely estimate the incidence and relative risk ratios, and for genetic studies. *This study was funded in part by a research grant from Abbott Laboratories.*

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Modulation of Fas Ligand Expression by Integrin Dependent Cell Adhesion in Endometrial Stromal Cells: A Mechanism for the Survival of Endometrial Implants in Endometriosis. Belgin Selam, J. A. Garcia-Velasco, Naciye Mulayim, Aydin Arici. Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.

Introduction: Integrins, a class of cell adhesion molecules, are membrane bound proteins that function in cell to cell and cell to matrix adhesion with their extracellular matrix (ECM) ligands. Integrins establish connections between retrogradely menstruated endometrial cells and ECM in endometriosis. The presence of Fas ligand (FasL) expression on the surface of ectopic endometrial cells may help these cells to escape local immune rejection by inducing apoptosis of cytotoxic T cells. We hypothesize that integrin-dependent attachment of endometrial cells will up-regulate FasL and thus allow the survival of the implants.

Design: Regulation of FasL protein production by integrin-dependent adhesion to extracellular components was analyzed in human endometrial stromal cells in culture.

Material and Methods: Endometrial tissue samples were obtained from human uteri after hysterectomy for benign disease. Endometrial stromal cells were prepared by standard enzyme digestion and filtration and were replicated to confluence in Ham's F12:DME medium. Cells were incubated in serum-free, phenol red-free medium for 24 hours prior to each experiment. Stromal cells were trypsinized and plated on 100 mm Petri dishes previously coated with fibronectin (1 $\mu g/cm^2$), laminin (2 $\mu g/cm^2$), collagen IV (6 $\mu g/cm^2$) and poly-L-lysine (0.1 mg/ml). Uncoated plates were used as controls. Forty-eight hours after incubation protein from cells were extracted and the level of FasL protein was analyzed by Western blot following SDS-polyacrylamide gel electrophoresis.

Results: FasL protein expression was detected at low levels in endometrial stromal cells after 48 hours. Adherence of stromal cells to integrin-dependent matrix; laminin, fibronectin and collagen IV up-regulated the FasL expression in endometrial cells. No effect on FasL expression was observed in cells plated on poly-L-lysine, a non-integrin dependent adhesion matrix.

Conclusion: Adhesion of endometrial stromal cells to extracellular matrix by way of integrins increases the expression of FasL, a mediator of apoptosis. This demonstrates the potential role of integrin dependent attachment of endometrial cells in the survival of endometrial implants.

P-265

Discrepant Immunologic Findings in Peripheral Blood and Peritoneal Fluid in Patients with Endometriosis. [1]E. Jeremias, [2]J. P. Andrus, [2]S. C. DeRosa, [2]I. M. Tjioe, [3]J. S. Kruessel, [1]K. S. Smith, [1]C. R. Nezhat, [2]L. A. Herzenberg, [2]L. A. Herzenberg, [1]M. L. Polan. [1]Department of Gynecology and Obstetrics and [2]Department of Genetics, Stanford University School of Medicine, Stanford, California 94305 and [3]Department of Obstetrics and Gynecology, Heinrich-Heine-University Medical Center, Duesseldorf, Germany.

Objectives: Evidence has been accumulated suggesting immunological mechanisms in the pathology of endometriosis. This ongoing study is designed to evaluate alterations in the lymphocytic population in peripheral blood and peritoneal fluid in patients with different stages of endometriosis.

Design: A prospective clinical study.

Material and Methods: Blood and peritoneal fluid samples were obtained from patients with clinical symptoms of endometriosis and control patients undergoing laparoscopy for diagnosis/treatment. Leukocytes were separated on Ficoll-paque density gradients, and lymphocyte subpopulations were defined as following: B cells (CD3, CD20 and CD5), NK cells (CD3, CD56 and CD16) and T cells (CD3, CD4, CD8, CD62L and CD45RA) with activation markers (CD11a, CD25, CD57, CD69, HLA-DR and CD121a). All samples were analyzed by 10-color flow cytometry in 5 patients with endometriosis and 4 controls.

Results: As a percentage of lymphocytes, CD3+ T cells in the peritoneal fluid and peripheral blood of endometriosis patients were similarly distributed. However, in this group, the CD4/CD8 ratio in peritoneal fluid (0.56 ± 0.06) was inverted as compared to the peripheral blood (2.23 ± 1.61). B lymphocytes (CD3-CD20+) as a percentage of lymphocytes were elevated in peripheral blood in patients with endometriosis compared to the control group, but their matched blood and peritoneal fluid samples showed a considerable decrease in the fluid. Both findings indicate a difference between the local and systemic immune response. Additional observations suggest disparities in other lymphocytic subpopulations, which will be further examined.

Conclusions: These pilot data suggest substantial alterations in the B cell population and a reversed CD4/CD8 ratio in patients with endometriosis exhibiting a marked discrepancy in the immunologic response regarding the peripheral blood and the matched peritoneal fluid.

P-266

Does Superovulation Change the Stage of Endometriosis? B. Amir, G. Graves. Reproductive Endocrine Centre, IWK/Grace Health Centre, Halifax, NS.

Objective: Superovulation is a recognized treatment for infertility. Endometriosis, a female factor in infertility is treated through pharmacologic suppression of estrogen or surgical management. Although, it has been uncertain whether superovulation can exacerbate endometriosis, no study

has yet addressed this question. The aim of this study was to determine whether superovulation changes the stage of endometriosis.

Design: Retrospective chart analysis for women who underwent superovulation in the Reproductive Endocrine Clinic from 1988 to 1997.

Materials and Methods: 296 charts of women involving 437 superovulation cycles were reviewed. 97 (33%) women with documentation of endometriosis were selected. Operative reports were used to stage endometriosis by the American Society for Reproductive Medicine (ASRM) revised classification of endometriosis. A significant change in endometriosis was considered as one ASRM stage. The superovulation protocols included the following medications; clomiphene, gonadotropins and hCG. Our statistical analysis involved using the SPSS statistical package to give confidence intervals for the prevalence of exacerbation.

Results: Of the 97 women with documented endometriosis prior to superovulation the meanage was 32.87. 79 (81%) women were not surgically restaged following their superovulation cycles since they have not presented with a clinical change in symptoms or pelvic mass up until the most recent clinical visit. 18 (19%) women required another laparoscopy after superovulation due to pain or pelvic mass. (Meanage 31.1 yrs). There were 10 women [55.6% with 95% C.I. (30.76%, 78.47%)] who experienced a change in the stage of endometriosis over an average of 47 months.

No disease to minimal	3	Mild to severe	1
Minimal to mild	3	Moderate to severe	2
Minimal to moderate	1		

There were 8 women (44.4%) who experienced no change in stage (5 minimal, 3 mild) over 66 months in spite of symptoms.

Conclusion: There are wide confidence intervals around the percentage of women with an aggravation of endometriosis. This is due to the small numbers of women investigated with a second laparoscopy. For the majority of women the superovulation did not appear to worsen the symptoms of their endometriosis.

MALE REPRODUCTION AND UROLOGY

Tuesday, September 28, 1999

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Correlation Between Sperm Quality of Patient Suffering from Cancer and the Aetiology of Their Malignancy. A. Lass, F. Akagbosu, N. Abusheikha, A. Burnley, M. Robbins, P. Brinsden. Bourn Hall Clinic, Bourn, Cambridge CB3 7TR, United Kingdom.

Objectives: In many cancer patients, sperm quality is already reduced at the time of diagnosis of their illness. There are conflicting reports on sperm quality in different types of cancer, mainly testicular carcinoma and lymphoma. The purpose of this study is to investigate, in a large series of patients suffering from different cancers, whether the origin of the malignancy has any effect on the sperm quality.

Design: Retrospective study in a single tertiary referral centre.

Materials and Methods: From August 1989 to December 1998, 281 men diagnosed with malignant disease were referred to our unit for semen cryopreservation (mean age 28.4; range 15–56 years). Thirty eight men who had chemotherapy treatment before obtaining the sample and eleven men who could not produce sperm and one patient who had undergone vasectomy were excluded from the study. Of the 231 eligible patients, 87 men had testicular tumours (seminoma = 21 and non-seminoma = 66). 113 men suffered from haematological malignancy (leukaemia = 16, Hodgkin's disease = 64 and Non-Hodgkin's lymphoma = 33). 31 had cancer of different causes (gastrointestinal = 8, neuroendocrine = 7, sarcoma = 10 and 6 from other systems or origin unknown). 60 male partners of infertile women having IVF treatment served as a control group. The first pre-freeze semen sample of each patient was analysed according to WHO guidelines (1992) and cryopreservation was performed on any sample containing motile spermatozoa. Sample contain $< 5\times10^6$ and/or $<2\times10^6$ motile sperm/ml was defined as poor quality sperm.

Results: Seventy two patients (31.2%) had poor quality sperm. Eighteen patients were azoospermic, 44 had severe oligospermia ($<5\times10^6$/ml) and another 10 patients had less then 2×10^6 motile sperm/ml. Men suffering from cancer had lower sperm counts (median = 88.5×10^6 vs 22.0×10^6, $p<0.001$) and fewer motile sperm/ml than the healthy control group (36.7×10^6 vs 10.0×10^6 motile sperm/ml, $p<0.001$). Sperm density was significantly reduced in non-seminoma compared to seminoma testicular cancers (9.8×10^6 vs 23.0×10^6, $p=0.04$) and in Hodgkin's disease versus non-Hodgkin's lymphoma and leukaemia (23.0×10^6 vs 57.0×10^6 and 48.5×10^6, respectively, $p<0.05$). In the non-testicular, non-haematological malignancy, there was no difference in sperm quality between the variant types of cancer.

Conclusions: Men with malignant disease generally have a reduced sperm quality at the time of diagnosis of their illness. Non-seminoma testicular cancer and Hodgkin disease affect sperm quality more than other types of cancer.

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Fertilization and Pregnancy Outcome of Cryopreserved Spermatozoa From Patients With Cancer. A. Agarwal, F. F. Pasqualotto, H. Kobayashi, A. J. Thomas, Jr., J. Hallak. Center for Advanced Research in Human Reproduction & Infertility, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH.

Objectives: Sperm cryopreservation before treatment has been recommended for men with newly diagnosed malignancies who desire to have children or may not have completed families. However, the value of sperm banking in these patients population remains controversial. The purpose of this study was to assess fertilization and pregnancy outcome of cryopreserved specimens from cancer patients following the use of assisted reproductive techniques.

Design: Retrospective study involving 19 patients with cancer who cryopreserved their sperm at our sperm bank and used outside *in vitro* fertilization (IVF) programs for assisted reproduction.

Material and Methods: Patients (n = 19) were divided into 3 groups according to the type of cancers: group I, testicular cancer (n = 5); group II, Hodgkin's disease (n = 9); and group III, included patients with prostate cancer (n = 2), leukemia (n = 1), metastatic neuroendocrine cancer (n = 1), and thyroid cancer (n = 1). The mean length of sperm storage in patients group was: group I, 46.8 ± 15.2; group II, 77.33 ± 15.34; and group III, 20.4 ± 11.63 months. Intrauterine insemination (IUI) was used in 5 patients, *in vitro* fertilization (IVF) in 4 and intracytoplasmic sperm injection (ICSI) in 10 patients.

Results:

Patients	Fertilization	Pregnancy	Live Birth
Testicular cancer (Group I, n = 5)	4/5 (80%)	3/5 (60%)	1/5 (20%)
Hodgkin's disease (Group II, n = 9)	5/9 (55.5%)	2/9 (22.2%)	2/9 (22.2%)
Other tumors (Group III, n = 5)	3/5 (60%)	1/5 (20%)	1/5 (20%)
All neoplasms (n = 19)	12/19 (63.1%)	6/19 (31.6%)	4/19 (21.05%)
p*	0.83	0.44	1.00

* $p < 0.05$ was significant when compared between the groups.

There were no differences in the mean pre-freeze: sperm motility, total motile sperm count, and the mean post-thaw: sperm motility, and total motile sperm count between the three groups. The fertilization rate, pregnancy rate, and the live birth rate showed no differences between the three cancer groups.

Conclusions: These results indicate that cryopreserved spermatozoa from cancer patients, irrespective of the type of tumor, are able to fertilize and initiate pregnancy with assisted reproductive techniques. Our data should encourage the oncologists to advise cryopreservation of semen to their patients before cancer treatment.

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Impact of Testosterone Levels on Erectile Response to Trimix Intracavernosal Infections. A. Spitz, E. D. Kim, M. N. Witte, R. E. Brannigan, F. Orejuela, S. G. Moreira, Jr., L. I. Lipshultz. Scott Department of Urology, Baylor College of Medicine, Houston, TX.

Introduction: We have observed, in a case-specific pattern, that patients with decreased serum testosterone (T) respond less favorably to identical

doses of intracavernosal trimix (papaverine, phenylephrine, prostaglandin E1), than do patients with normal serum T. Animal studies have demonstrated that the androgen effect on erections is, in part, mediated by nitric oxide. The purpose of this study was to determine whether a relationship exists between serum testosterone levels and erectile response measured with duplex ultrasonography.

Methods: Over a 23 month period, 139 men presenting with erectile dysfunction had an evaluation consisting of a history and physical exam, a hormonal profile (T, prolactin), and a penile duplex ultrasound using 0.25 ml of Trimix. Peak systolic flow velocity (PSFV) in both cavernosal arteries was measured at 0, 5 and 15 minutes after injection.

Results: Patients with testosterone levels >225 ng/dl (normal 225–1000) had a significantly higher median PSFV at 5 and 15 minutes ($p<0.05$; Mann-Whitney rank sum test) than those with lower testosterone levels. No significant differences were present in patient co-morbidities related to erectile dysfunction, although lower T was noted in the older age group.

Testosterone Level (ng/dl)	# of Patients (n)	Age	PSFV (cm/s) 0′	PSFV 5′	PSFV 15′
<225	25	59.2 ± 12.5	8.0	19.0	21.0
>225	114	52.9 ± 13.3	7.0	22.8	28.3
		p=.033	p=.668	p=.038	p=.046

Conclusion: Patients with lower serum testosterone concentrations were found to have poorer responses to identical trimix injection as demonstrated by color flow Doppler ultrasonography measuring PSFV. This relationship between declining circulating androgen levels and decreased penile vascular responsiveness may be related to impaired nitric oxide release or end organ responsiveness. These findings could help explain the decline in erectile function associated with aging and the need for higher doses of trimix in the older patient experiencing erectile dysfunction.

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Results of Microsurgical Anastomosis in Men With Seminal Tract Obstruction Due to Inguinal Herniorrhaphy. J. A. Daitch, F. F. Pasqualotto, A. Agarwal, A. J. Thomas, Jr. Center for Advanced Research in Human Reproduction & Infertility, Department of Urology, The Cleveland Clinic Foundation, Cleveland, OH.

Objectives: Herniorrhaphy is the most common cause of iatrogenic vasal obstruction. The incidence of injury to the vas deferens during hernia repair ranges from 0.3% to 2.0%. The purpose of our study was to assess the patency rates and long-term fertility outcome after microsurgical repair of vasal obstruction due to inguinal herniorrhaphy.

Design: Retrospective study.

Material and Methods: Twenty procedures were performed on 13 men diagnosed with infertility and vasal injury due to inguinal hernia repair. Eight patients had bilateral and 5 unilateral inguinal herniorrhaphy. Six underwent a second microsurgical procedure due to primary vasovasostomy failure, and one patient underwent two procedures simultaneously. Of the 20 procedures, 12 were inguinal vasovasostomies (IVV), 3 crossover vasovasostomies (COVV), 2 inguinal vasoepididymostomies (IVE), and 3 crossover vasoepididymostomies (COVE). The indications for the reanastomosis were infertility (n = 12) and testicular pain (n = 1). Prior to the initial procedure, eight patients were azoospermic, three were oligospermic, and two asthenospermic. Four patients had a unilateral atrophic testis, 3 had unilateral congenital absence of the vas deferens, 1 had an orchiectomy due to undescended testis, and 1 had an undescended testis. The mean patient age was 32 years (range 26–36), and the mean obstruction interval was 25.4 years (range 3–40). Follow-up was obtained via chart review and telephone interview. Patency was defined as the presence of long-tailed sperm in the postoperative semen analysis. Patency data was obtained on all 13 patients, and pregnancy data was available for 10 couples (77%) at a mean follow-up of 69.5 months (range 13–181).

Results: For the 20 procedures, the overall patency rate was 65% (13/20). In the vasovasostomy group, the patency rate was 60% (9/15) and in the vasoepididymostomy group it was 80% (4/5). Among eight azoospermic patients, thirteen procedures were performed (7 VV, 4 VE, and 1 simultaneous VE and contralateral VV). For these azoospermic patients, patency rate was 42.9% for the VV procedure (3/7), and 100% for the VE procedure (4/4). The patient who underwent simultaneous VV and VE had a patent anastomosis on initial follow-up, but subsequently became azoospermic. The overall pregnancy rate was 40% (4/10). Of interest, none of the six patients who had undergone only vasovasostomy fathered a pregnancy. In contrast, four of the five (80%) patients who underwent VE did father a pregnancy. The singular failure was in the patient who had undergone simultaneous left VE and right VV.

Conclusions: Microsurgical VV after inguinal vas injury results in high patency rates, but poor pregnancy rates. This may be due to concurrent partial epididymal obstruction or recurrent partial or complete vasal obstruction. VE may be needed as a secondary procedure and COVE appears to be a better surgical alternative than inguinal VV when appropriate. We are currently pursuing new techniques which allow more extensive mobilization of the abdominal vas with its blood supply. This may improve the results of VV after injury to the inguinal vas deferens.

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Successful Use of Cryopreserved Sperm Obtained by Electroejaculation for IVF/ISCI. R. Dolgina, G. S. Prins, L. S. Ross, C. S. Niederberger. Department of Urology, University of Illinois at Chicago, Chicago, IL.

Objectives: Electroejaculation (EEJ) has allowed for sperm retrieval from anejaculate men, however the pregnancy results using intrauterine insemination (IUI) have been disappointing. To maximize chances of fertilization, fresh EEJ sperm is currently used in patients undergoing assisted reproductive techniques including traditional IVF and IVF/ICSI. Our recent experience with non-EEJ patients demonstrated that cryopreserved sperm can be used for IVF/ICSI without a decline in outcomes. Therefore the objective of the present study was to evaluate the efficacy of cryopreservation of EEJ sperm for patients undergoing IVF/ICSI.

Design: Sperm concentration and motility in fresh EEJ specimens were determined and sperm were cryopreserved. The pregnancy rate after IVF/ICSI using cryopreserved EEJ sperm was assessed.

Materials and Methods: EEJ sperm were obtained in the operation room under general anesthesia. Antegrade and retrograde portions were collected in warm Ham's F-10 medium in separate containers. Specimens were transferred to the laboratory for evaluation and processing. Specimens were washed in fresh medium, concentrated and cryopreserved in TEST-Yolk buffer. On a later date, frozen specimens were transferred to IVF facilities for IVF/ICSI procedures.

Results: Eleven patients underwent a total of 15 EEJ procedures. Ten specimens contained detectable sperm in both ante- and retrograde portions, four cases contained sperm in only the retrograde fraction and one specimen presented with sperm in the antegrade portion only. The total amount of sperm ranged from 0.8 to 840 $\times 10^6$ per patient with an average of $191.3 \pm 159.1 \times 10^6$. Sperm motility was low, ranging from 0 to 48% (average $14.43 \pm 10.7\%$). After processing, 1 to 7 vials per patient (average 3.9 ± 1.9) were cryopreserved for future use. Nine patients underwent nine cycles of IVF/ICSI with frozen EEJ sperm. A total of five pregnancies (55.5%) were achieved with one twin and four singletons. One pregnancy resulted in a first trimester miscarriage, one pregnancy is ongoing, and three healthy babies were delivered. Two patients who failed to conceive after IUI with EEJ sperm became pregnant after one cycle each of IVF/ICSI using frozen EEJ sperm.

Conclusions: 1) All 15 EEJ procedures resulted in successful sperm retrieval. 2) Sperm specimens obtained by EEJ can survive cryopreservation and fertilize oocytes. 3) IVF/ICSI using cryopreserved EEJ sperm resulted in 5 pregnancies out of 9 cycles for a 55.5% pregnancy rate. 4) The advantage of cryopreserved sperm makes this a valuable option in patients who require EEJ.

Results: The full length coding region of the normal LH receptor mRNA that contained all 11 exons was initially identified. Isoform 1 had a deletion of exon III, and isoform 2 had exon IX omitted. Isoform 3 had both exons III and IX deleted. Isoform 4 had exons III through VI and part of exon XI missing, and isoform 5 had exons III through VI, IX, and part of exon XI deleted. An aberrant migration pattern of the different LH receptor mRNA isoforms was observed for the granulosa cell tumors. Bands representing the PCR products showed a different migration pattern for at least four of the tumor samples, and the band that is believed to represent isoform 1 migrated differently for at least five

tumor samples. The band that represents isoform 4 was not observed for four tumor samples. No bands were observed for three tumor samples when the PCR reaction included sequences in exons I through X but were observed in the PCR reaction product that included sequences in exons X through XI. This suggests that a portion of exon I is missing in these three granulosa cell tumors. The bands that represent the LH receptor mRNA isoforms with exon IX deleted (isoforms 2, 3, and 5) had similar migration patterns in normal ovaries and granulosa cell tumors.

Conclusions: 1) Alternately spliced forms of LH receptor mRNA exist for normal human ovary. 2) Alternately spliced forms of LH receptor mRNA exist for granulosa cell tumors. 3) Aberrant migration patterns of LH receptor mRNA isoforms were observed for the granulosa cell tumors. Further DNA sequencing for the granulosa cell tumors is underway. This work was supported by HD7108, HD9140, and the Mayo Foundation.

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Impaired Spermatogenesis in Men With Congenital Absence of the Vas Deferens. M. V. Meng, P. J. Turek. Department of Urology, University of California San Francisco, San Francisco, CA.

Objectives: It is generally assumed that patients with congenital absence of the vas deferens bilaterally (CAVDB) have azoospermia secondary to obstruction and that sperm production is normal. This study examines spermatogenesis in CAVDB men to determine whether it is indeed uniformly normal.

Design: Retrospective study of infertile, azoospermic men with CAVDB.

Materials and Methods: The study cohort included all men with CAVDB who had undergone either a diagnostic or therapeutic fertility procedure. Procedures included fine needle aspiration (FNA) mapping of the testes, diagnostic testis biopsy, microscopic epididymal sperm aspiration (MESA) and testis sperm extraction (TESE). We evaluated all findings from these procedures, including testis histology, cytology, or extracted sperm quality, to assess the status of spermatogenesis.

Results: Of 26 CAVDB men, 19 had MESA, and 7 had diagnostic FNA mapping. On evaluation of these procedures, normal spermatogenesis was present in 24 men (92%). Two men (8%) exhibited impaired spermatogenesis. One patient underwent both MESA and TESE due to extremely low sperm production and the second patient had FNA testis cytology consistent with late incomplete maturation arrest. Within this cohort, both had mild testicular atrophy, but normal serum FSH and testosterone levels. Potential etiologies for impaired spermatogenesis included varicocele and an underlying cytogenetic abnormality. No Y chromosome microdeletions were observed.

Conclusions: Although patients with CAVDB are assumed to have normal spermatogenesis and infertility simply from obstruction, cases with impaired sperm production can be encountered. In the presence of testis atrophy, diagnostic evaluation of spermatogenesis may be warranted and TESE may be the only option for successful sperm retrieval.

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Male Partner Screening Before IVF: The Sperm Penetration Assay (SPA) Predicts Fertilization in IVF With High Diagnostic Accuracy That is as Predictive as Previous IVF Fertilization for Couples with Multiple Cycles. M. R. Freeman, A. A. McAlister, K. J. Morgan, K. G. Howard, M. S. Hinds, G. A. Hill. Nashville Fertility Center, Nashville TN.

Objectives: The screening of the male partner prior to the initiation of an IVF cycle usually involves the assessment of semen parameters (sperm concentration, motility and morphology). However, unless two or more of these parameters are abnormal, the semen analysis has little value for predicting successful fertilization in IVF. Although the most accurate *in vitro* analysis of the fertility of a couple is the fertilization occurring with their own eggs and sperm, the SPA answers some important questions left unanswered by the standard semen analysis. The SPA evaluates the ability of human sperm to undergo capacitation, acrosome reaction, fusion with and penetration through the oolemma, and decondensation within the cytoplasm of an oocyte. The purpose of this study was to determine the diagnostic accuracy of the SPA and standard semen parameters for subsequent fertilization in IVF. In addition, for couples with multiple cycles, to determine the diagnostic accuracy of fertilization in the first IVF cycle for fertilization in subsequent IVF cycles.

Design: Prospective study to determine the diagnostic accuracy of the standard semen analysis and the SPA in predicting subsequent fertilization in IVF compared to the diagnostic accuracy of the first cycle of IVF in predicting fertilization in subsequent IVF cycles.

Materials and Methods: The ability of the standard semen analysis and the TEST-yolk buffer enhanced SPA to predict subsequent normal IVF fertilization was evaluated in 216 couples who completed 265 IVF cycles during the study period (1994–1998). In addition, the ability of IVF fertilization to predict subsequent normal IVF fertilization was determined in 76 repeat cycles of 63 couples. Couples showing previous evidence of fertility (miscarriage, ectopic, etc.) within two years of their initial IVF cycle were not tested with the SPA and were excluded from the SPA comparison. Couples using donor oocytes or semen, or with sperm antibodies in the male or female partner were excluded from the study. Normal SPA percent penetration was considered to be 20% or greater, while normal IVF fertil ization was considered to be 50% and above. χ^2 test was performed for proportion differences and multiple regression analysis was made to select the most contributory variables to fertilization in IVF.

Results: The SPA predicted IVF fertilization with high negative (100%) and positive (73%) predictive rates, and correct prediction in 80% of couples. This compares well to the diagnostic accuracy of previous IVF fertilization, which also had high negative (78%) and positive (80%) predictive rates and correct prediction in 79% of couples. Sperm count and motility had good negative predictive (77%) but poor positive (55%) predictive rates, while sperm morphology had poor negative (59%) and positive (57%) predictive rates.

Conclusions: Abnormal sperm concentration and/or motility were good predictors of poor IVF fertilization, however, normal semen parameters were not predictive of successful IVF fertilization. The Sperm Penetration Assay is a useful screening tool that predicts IVF fertilization with high diagnostic accuracy. We have shown that the SPA performed prior to the first IVF cycle predicts IVF fertilization as well as previous IVF fertilization in couples with repeat IVF cycles. The SPA may be useful in couples with normal semen parameters to discriminate between those with a high probability of normal fertilization in IVF and those with a low probability of normal fertilization that may benefit from assisted fertilization by ICSI.

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Semen pH in Patients with both Normal and Abnormal Sperm Parameters are Higher than the Normal Range Specified by World Health Organization. C. L. Harraway, N. B. Berger, N. H. Dubin. Union Memorial Hospital, Baltimore, MD.

Objectives: World Health Organization (WHO) and various textbooks state that the normal pH of semen ranges from 7.2–8.0. Our experience over the last 5 years has been that our patient population was consistently higher than this. For this reason we reviewed over 1000 of our patients' pH values and determined how these values compared to the WHO guidelines.

Design: Assay validation, and retrospective cohort study.

Methods: Initially, accuracy and precision of various brands of pH paper were compared to pH measurement by a meter for a range of standards. All patient records on a computer assisted semen analyzer data base from January 1994 to December 1998, which had pH measurements, sperm concentration, and motility measurements, were included in this study. All pH measurements were from raw semen ejaculates at the time of semen analyses, screens, or sperm preparations for intrauterine inseminations (IUI) and ART procedures. We determined the distribution of pH values for our patient population and compared those with normal (concentration $>$ 20 million/ml, motility $>$ 40%) and abnormal sperm parameters. We also determined the distribution of pH in a subgroup of patients who underwent sperm preparations for IUIs which resulted in documented pregnancies. Correlations of pH with other parameters were determined as noted. Chi squared tests and regression were used to analyze data.

Results: Precision was 1.43% and 1.57% (coefficient of variation) for the pH paper used (pHydrion, Brooklyn, NY and ColorPhast, Gibbstown, NJ, respectively) compared to 0.24% for the pH meter. Accuracy for the meter was within .004 units of the standards and was −0.01 and 0.08 for the pH papers used. For all patients (n=1199), pH was 8.2 ± 0.3 (mean ± SD). The range was 7.3 to 9.5 with 32% of the samples less than 8.0. The mean pH was 8.2 for both those patients with normal (n=602) and abnormal (n=597)

sperm parameters. In a small group of patients (n=19), whose sperm preps were documented to result in a clinical pregnancy following IUI, the semen pH was 8.3 ± 0.3 with a range of 7.9 to 8.7. For all semen samples studied, there was a significant ($p<.001$), but relatively weak correlation between pH and time of abstinence ($r = -.22$) and volume ($r = -.15$). There was no relationship between pH and patient age, interval from collection to measurement, nor was there a change in average pH over the time period studied, ruling out changes in quality of pH paper.

Conclusions: The distribution of semen pH in our patient population was higher than that described as normal by WHO. Even semen with normal sperm parameters was in this high range. Patients, who were proven fertile following IUI procedures, had pH readings in this elevated range as well. This study questions the reliability of the normal pH range described by WHO, as it clearly does not reflect the values we observe in our population, including those with proven fertility.

P-275

The Outcome of the Extraction of Testicular Sperm (TESE) and the Possibilities of its Prediction. [1]K. Kočí, [2]M. Trnková, [2]I. Juliš, [1]M. Mrázek, [1]Z. Mayer, [1]M. Lachman, [1]K. Jarolímková, [1]O. Teplá, [1]J. Míka, [1]L. Hybnerová, [1]J. Jirmanová. [1]ISCARE IVF and [2]BioLab, Prague, Czech Republic.

Objective: A prediction of a probability of successful TESE can prevent an unnecessary ovarian stimulation. The aim of this study was to evaluate possible predictors of the outcome of TESE.

Design: Histological findings, serum FSH concentrations and testicular volume were retrospectively correlated with the outcome of TESE.

Materials and Methods: A retrospective study of the group of 124 men with dg. azoospermia. 37 patients had the obstructive azoospermia, remaining 87 men suffered from testicular azoospermia. In this second group of patients we analyzed following parameters: testicular volume, serum FSH concentration and histological finding. Samples of testicular tissue were sent in histological laboratory where the classification and quantitative evaluation using the image analysis was made. On average ten small tissue samples from different locations were taken from every testicle for TESE. In IVF lab they were washed, mechanically dissected and checked for spermatozoa up to 7 hours.

Results: Serum FSH concentrations did not correlate neither with histological finding nor with the outcome of TESE. At normal FSH concentrations were in several patients no spermatozoa found while at the concentration 41.4 IU/L we found 6 spermatozoa/HPF and achieved the delivery of healthy twins. The testicular volume did not correlate with the outcome of TESE as well. The elongated spermatids were found in 48.6% of the histological samples. Spermatozoa were obtained per TESE in 51.7% men. In no patient with the true histological diagnosis of Sertoli cell only syndrome any spermatozoa was found per TESE.

Conclusions: Neither the elevated serum FSH concentration nor small testicular volume exclude the presence of spermatozoa in testicular azoospermia. The most accurate predictor seems to be the testicular biopsy but its results validity depends on the number of seminiferous tubules explorated. For the reliable evaluation is necessary to examine at least 100 tubules. This number is already sufficient to detect even small focus of the spermatogenesis by our experience. The recognition of the elongated spermatids in biopsy sample indicate the positive finding in TESE in at least 84% patients, while in patients without finding of germinal cells in representative number of tubules we can practically exclude obtaining any spermatozoa usable for ICSI in TESE. We demonstrated the same oocyte fertilization rate at using fresh and frozen spermatozoa before now. Regarding these findings our strategy was changed—the material taking for biopsy and for cryopreservation for subsequent TESE we perform simultaneously before the launch of the ovarian stimulation which we do not initiate until we have the positive peroperative finding or the conclusion of the histological examination.

P-276

Regular Semen Donors: A Unique Population of Men That Allowed to Assess the Effects of *Ureaplasma urealyticum* on Pre-freeze and Post-thaw Semen Parameters. A. P. Del Valle, A. Bhowmik, M. H. Javed, M. A. Shaikh, C. Ruberto. ReproMed Ltd./AVR Andrology Inc., Toronto, ON, Canada.

Objectives: *U. urealyticum* is isolated with considerable frequency from the male urogenital tract. Conflicting reports of the effects of *U. urealyticum* on human reproduction and semen parameters have been documented. The lack of conclusive evidence is due to the absence of meaningful comparative studies investigating *U. urealyticum* positive and negative semen specimens in the same individual. The objective of this study was to determine the effects of *U. urealyticum* on pre-freeze and post-thaw semen characteristics in regular semen donors.

Design: A comparative retrospective study of semen characteristics in *U. urealyticum* positive and negative semen specimens within the same donor and amongst regular donors in a large clinical sperm bank.

Materials and Methods: A regular semen donor, an individual who after completing the required screening as per official standards of Health Canada, Canadian Fertility and Andrology Society, American Society for Reproductive Medicine and American Association of Tissue Banks was accepted into the semen donor program for scheduled donations after 3 days of abstinence of ejaculation. Thirty-eight regular semen donors were found to be positive for *U. urealyticum* from 1992 to 1998. *U. urealyticum* positive (Arginine culture broth, London Health Sci. Ctr, Univ. Campus London, ON, Canada) semen specimens were compared for their semen characteristics with the mean ± SD of six preceding *U. urealyticum* negative semen specimens of the same donor. Values of all positive and negative donors were also compared. Comparisons were made for volume, sperm concentration, pre-freeze motility, post-thaw motility and motile concentration post-thaw (WHO Manual 1992). T-test was used as a statistical tool to compare the observed mean values for each parameter analyzed.

Results: The comparison between positive and negative *U. urealyticum* semen specimens did not reveal any statistical difference ($P<0.05$) for all the parameters observed. Mean ± SD values for percentage pre-freeze motility in *U. urealyticum* positive and negative specimens were 54.68 ± 5.54 and 55.0 ± 4.58 and for sperm concentration (M/ml) 62.33 ± 19.82 and 65.25 ±14.45 respectively. Mean ± SD values for percentage post-thaw motility in *U. urealyticum* positive and negative specimens were 30.5 ± 0.5 and 31.33 ± 3.83 and for post-thaw motile concentration (M/ml) 30.21± 10.65 and 31.5 ± 6.34 respectively. The volume for *U. urealyticum* positive and negative specimens was 3.59 ± 1.51 and 3.67 ± 1.38 ml respectively.

Conclusions: The semen parameters evaluated did not appear to be affected by the presence of *U. urealyticum*. The detrimental effects of *U. urealyticum* on semen parameters observed in other studies may have been minimized in the present study by giving the opportunity to compare the ureaplasma positive specimens with the mean ± SD of the six preceding negative specimens of the same donor. Regular semen donors are an ideal population to investigate the effects of *U. urealyticum* on semen parameters.

P-277

IGF-II Gene Expression in Human Testes with Spermatogenic Failure. [1]W. Küpker, [1]P. Kressin, [2]R. Johannisson, [1]S. Al-Hasani, [1]K. Diedrich. [1]Department of Obstetrics and Gynecology, Medical University Lübeck, [2]Institute of Pathology, Medical University, Lübeck Ratzeburger Allee 160, 23538 Lübeck, Germany.

Objective: IGF (Insulin like growth factor) II is a monoallelic expressed gene product of paternal origin. Uniparental gene expression in adult tissues is based on the principle of genomic imprinting. Testicular sperm extraction provides not only mature spermatozoa but also immature round and elongated spermatids for injection into the oocyte. Until now the status of imprinted genes has not clearly been characterized for the development of germ cells nor its relevance for embryonic development. Objective of this study was to evaluate the expression of IGF-II in spermatogenesis by means of immunhistochemistry.

Design: Development of gene expression of imprinted genes in spermatogenesis.

Materials and Methods: 15 azoospermic patients with different patterns of spermatogenic failure were examined (Sertoli cell only pattern, n=5, Spermatogenic arrest, n=5, Hypospermatogenesis, n=5). Semi thin sections were taken from cryopreserved testis biopsies and incubated with specific IGF II antibodies. IGF II expression was detected by means of the EPOS system (Enhanced Polymer One Step Staining).

Results: IGF II gene expression could be detected in all cases. IGF II could be visualized in germ cells whereas Sertoli and Leydig cells displayed no signs of immunostaining. High activity of intranuclear IGF II expression

could be demonstrated in meiotic primary spermatocytes whereas in spermatogonia IGF II was not detectable.

Conclusion: IGF II is detectable in germ cells. IGF II expression begins during meiosis. The early onset of IGF II gene expression in testicular tissue reflects the imprinting status in early spermatogenesis. The establishment of gene specific imprinting is most relevant under therapeutic aspects of early spermatid injection and to achieve normal embryonic development.

P-278

Comparative Cryorecoveries of Three Procedures After Cryopreservation of Human Spermatozoa. M. H. Javed, M. A. Shaikh, C. Ruberto, A. P. Del Valle. ReproMed Ltd./AVR Andrology Inc., Toronto, ON, Canada.

Objective: Human semen cryopreservation is carried out either by standard protocol (STD, seminal plasma not removed) or Pre-wash (PW, seminal plasma removed by centrifugation). For intrauterine inseminations, semen is usually processed through a cell isolation medium (sperm passed through discontinuous colloidal suspension and washed again in fresh medium) to remove dead spermatozoa and seminal debris. Information on freezing characteristics of specimens passed through cell isolation media is scanty. The objective of this study were (1) to compare pre-freeze values, post-thaw recoveries and kinematics of spermatozoa cryopreserved by STD, PW using Tyrode's Salt Solution (Sigma Chem. CO., USA) and Enhance-S Plus (EP) protocol and (2) to compare time spent and cost on these procedures.

Design: Normal fresh semen ejaculates from patients who had undergone fertility assessment (n=15) were split into three aliquots and cryopreserved as follows: (1) STD, (2) PW, and (3) EP method. Raw, extended and post-thaw motility, cryosurvival factor, motile sperm concentration and kinematics were compared. Semen analyses were performed manually (WHO manual, 1992) and by CASA (Cell Trak/S).

Materials and Methods: Semen samples of ≥ 3ml volume were split into three 1ml aliquots. Volume frozen, cryoprotectant and thawing protocol were identical for the 3 groups. The specimens were processed as follows: (1) STD, addition of cryoprotectant only; (2) PW, 1:1 dilution with Tyrode's, centrifuged 300g for 10 min, pellet re-suspended in Tyrode's then cryoprotectant added and (3) EP, layered on two phase Enhance-S Plus silica gradient, centrifuged 300g for 20 min, pellet aspirated, re-suspended in modified human tubal fluid (HTF), centrifuged 300g for 10 min, pellet re-suspended in HTF and cryoprotectant added. Duplicate observations were made on raw, extended and at 0, 1 and 2 hr post-thaw specimens. The data were analyzed by ANOVA and t-test.

Results: Pre-freeze total sperm recovery (M/ml) was significantly low ($P<0.01$) in EP, 28.20 ± 3.73 as compared to PW, 44.61± 4.88 and STD, 45.80 ± 5.15. Pre-freeze motile Conc. (M/ml) in EP, 18.69, PW 20.0 and STD 21.93 was not different statistically. Post-thaw motile Conc. (M/ml) in EP, 9.74, PW, 10.59 and STD, 12.45 were not different ($P<0.05$). Pre-freeze and post-thaw motility in EP, 66.29 ± 4.49 and 34.54 ± 3.12 % was significantly higher ($P<0.05$) than in PW, 44.83 ± 3.24 and 23.75 ± 2.23 % and in STD, 47.89 ± 3.30 and 27.19 ± 2.37% respectively. Cryosurvival factor, sperm survival over 2 hour period and kinematic parameters evaluated were also not different ($P<0.05$) in 3 methods.

Conclusions: (1) Pre-freeze and post-thaw motile concentration, cryosurvival factor, post-thaw sperm survival and kinematics in EP, PW and STD were similar. (2) EP procedure required significantly more time and cost as compared to STD or PW. (3) These results indicate no advantage of processing semen before freezing by EP procedure over STD or PW.

P-279

Expression of the Cyclin Dependent Kinase 5 Protein in Testicular Cells. [1]D. R. Session, [2]M. Fautsch, [1]R. Avula, [3]W. Jones, [3]A. Nehra, [2]E. Wieben. Departments of [1]Obstetrics and Gynecology, [2]Biochemistry and Molecular Biology and [3]Urology, Mayo Clinic, Rochester, MN.

Objectives: Cyclin dependent kinase 5 (Cdk5) is expressed in multiple sites including testis, ovary, muscle and neural tissue. While the physiologic role of Cdk5 is unknown, studies have shown that Cdk5 phosphorylates microtubules in neural tissue. The cells of the seminiferous tubule also contain microtubule structures, which include microtubule networks, mitotic and meiotic spindles and flagella: this study evaluated the expression pattern of Cdk5 in the seminiferous tubules.

Design: The expression of the Cdk5 protein was determined using immunohistochemical and immunoblot analysis.

Materials and Methods: Immunohistochemistry was performed on adult mouse and human testis sections with a rabbit polyclonal anti-Cdk5 antibody. Detection was performed with anti-rabbit IgG peroxidase labeled antibody, which was stained with diaminobenzidine. Controls included pre-blocking with the immunizing peptide and affinity purified rabbit IgG. Double immunohistochemistry was performed with anti-Cdk5 (stained brown with diaminobenzidine) and α-tubulin (stained purple with Vector VIP) antibodies. Lysates from multiple mouse tissues were examined for Cdk5 expression by immunoblotting. Specificity for Cdk5 was confirmed by pre-blocking with the immunizing peptide.

Results: Cdk5 was localized specifically to microtubules within the cytoplasm of Sertoli cells and meiotic metaphase germ cells. Double immunohistochemistry demonstrated co-localization of Cdk5 and α-tubulin within the Sertoli cells, strengthening the association between Cdk5 and microtubules. Expression of Cdk5 in Sertoli cells also was identified in human testis. Western blot analysis of mouse tissues demonstrated a high level of expression of Cdk5 in the testicular lysate.

Conclusions: Cdk5 localized to the cytoplasm of Sertoli cells and meiotic spindle of germ cells. The cyclin dependent kinases are known regulators of the cell cycle; however, Cdk5 expression previously has been described in terminally differentiated cells of the brain and now testis. The present evidence of an association between Cdk5 and microfilaments of Sertoli cells and meiotic metaphase germ cells suggests a role of Cdk5 in seminiferous tubule function and meiosis.

This work was supported by a CR20 award from the Mayo Foundation.

P-280

Azoospermia With Seminal Vesicle Enlargement in the Voltage Dependent Anion Channel-3 (VDAC3) Knockout Mouse. [1]E. C. Schatte, [2]W. K. Decker, [1]L. I. Lipshultz, [1]E. D. Kim, [1]D. J. Lamb, [2]W. J. Craigen. [1]Scott Department of Urology and [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX (Presented by Dr. Schatte).

Objective: Our objective was to develop a mouse model for distal seminal obstruction.

Design: The murine voltage dependent ion channel (VDAC) gene family consists of small voltage gated channels on the mitochondrial membrane (Manella, et al., J. Bioenerg. Biomembr., **24:**7, 1992). One family member, VDAC3, has its highest level of expression in the testes. In order to understand physiologic differences among the VDAC isoforms, a null mutation was introduced in the VDAC3 gene in ES cell clones and VDAC3-deficient mice were generated as described by Sampson et al., (submitted). The mice are viable and healthy, however, on electron microscopic exam, the mitochondria in sperm exhibit abnormal morphology and axonemal defects. In over 100 matings, the male mice exhibited infertility secondary to isolated asthenozoospermia.

Materials and Methods: A VDAC3 knockout (KO) mouse was created (Sampson et al., submitted) and the epididymal sperm of 7 VDAC3 KO mice older than 10 months were analyzed by standard semen criteria. Standard necropsy was performed after sacrifice. Fifteen wild type mice were sacrificed and examined in the same manner.

Results: Three mice had normal sperm counts compared to same strain of wild type animals, but four of the seven VDAC3 KO mice were azoospermic. The 3 KO mice with normal counts also had significantly decreased motility compared to wild type. On necropsy, these animals displayed massively enlarged seminal vesicles with cloudy retained seminal fluid. On aspiration, there was sperm present in the seminal vesicle fluid. No wild type animals displayed enlarged seminal vesicles or had sperm in their seminal fluid.

Conclusions: The VDAC3 knockout mouse provides a unique model of male infertility secondary to poor motility in normal reproductive age mice. The finding of azoospermia and post-seminal vesicle obstruction in the older VDAC3 mice suggest a variable penetrance mouse model for ejaculatory duct obstruction. On necropsy, the obstruction does not appear to be anatomic. Current studies are underway to determine whether the enlarged seminal vesicles are due to a functional defect or from inspissated sperm.

This work was supported by NIH grant RO1 GM055713-03.

P-281

Differential Capacitation Monitored by the Chlortetracycline (CTC) Fluorescence Assay in Ejaculates from Fertile and Infertile Males. [¶]J. M. Vasquez, [†]J. J. Parrish, [¶]M. B. Mink, [¶]M. C. Bastias. [¶]The Center for Reproductive Health, Nashville, TN and [†]The University of Wisconsin-Madison.

Objectives: Previously we confirmed the use of CTC fluorescence to monitor human sperm capacitation and acrosomal loss. We reported that clear perimeter head fluorescence (CP) and acrosome reaction (AR) CTC patterns are correlated positively with the capacity of human sperm to bind and penetrate zona-free hamster ova (SPA). In contrast, midpiece early bright fluorescence (EF) was correlated negatively with SPA. Thus, differences among individuals in these parameters could be related to fertilizing capacity. The purpose of the present study was to evaluate whether CTC binding patterns differed between fertile and infertile males.

Design: Prospective controlled study.

Materials & Methods: To minimize intersubject variation 24 semen samples were collected from one control with proven fertility and compared with single ejaculates from 47 males with infertility. The samples were allowed to liquify (fresh) and subjected to Cellsoft computer analysis. Sperm swim-ups (SU) were collected after 2 h. SU samples were also subjected to Cellsoft computer analysis. Aliquots were taken from fresh ejaculates, SU's, and at 3 and 9 h after incubation in capacitating medium. A 5 μl aliquot of various sperm preparations was placed on a microscope slide, followed by 5 μl of CTC stock solution (500 μM CTC-HCl, 20 mM Tris, 130 mM NaCl, 5 mM cysteine) and 0.05 μl 12.5% paraformaldehyde in 1 M Tris. CTC fluorescence was examined within 24 h at 100X by random selection of 100 sperm cells. Fluorescence patterns already described (Fertil Steril 48: 649, 1987) were designated as EF, dark postacrosomal band (DP), CP, and AR.

Results: total cell number (tot_{cn}), % motility, mean velocity, and morphology were significantly increased in control samples as compared with infertile males ($p<0.01$). In fresh ejaculates, EF pattern prevailed in infertile males and DP pattern prevailed in control samples. Pattern CP prevailed in infertile males and pattern AR prevailed in control samples at 3 & 9 h. A significant increase in pattern AR was observed in control samples at 3 & 9 h. In contrast, AR was not affected by incubation time in infertile males.

Conclusion: These data indicate that spermatozoa from infertile males are not able to undergo the acrosome reaction with the same frequency as do spermatozoa from a donor with proven fertility. The CTC assay may be a good predictor of fertility in the human. However, further research is warranted to establish the utility of this assay, particularly in a clinical context.

	Infertile Males * different from control ($p<0.001$).			
	EF	DP	CP	AR
Fresh	93.2 ± 1.9*	2.1 ± 0.6*	4.2 ± 1.8	0.7 ± 0.3*
SU	69.2 ± 6.8*	3.5 ± 2*	23.1 ± 5.6	4.7 ± 1.9*
3 h	5.4 ± 1.5	0.9 ± 0.4*	88.4 ± 2.1*	4.8 ± 1.5*
9 h	3.6 ± 1.4	1.0 ± 0.4*	91.0 ± 1.8*	4.3 ± 1.2*
	Control			
	EF	DP	CP	AR
Fresh	0 ± 0	80.7 ± 11.5	0.7 ± 0.5	17.2 ± 10.8
SU	1.3 ± 1	71.7 ± 12.4	2.3 ± 1.9	23.7 ± 10.7
3 h	2.3 ± 1.5	13.6 ± 7.4	3.6 ± 3.1	81.5 ± 8
9 h	1.3 ± 0.9	4.7 ± 2.6	1.9 ± 1.4	87.7 ± 4.9

P-282

Swim Up Separation of the More Motile Fraction Does Not Separate Aneuploid From Haploid Sperm. [1]M. W. Stacey, [2]J. Pfeffer, [3]M. G. Pang, [4]S. F. Hoegerman, [5]S. Oehninger, [1]L. Lunsford, [5]G. Doncel, [5]A. A. Acosta, [1,5,6]W. G. Kearns. [1]Center for Pediatric Research, Eastern VA Med Sch, Norfolk, VA, [2]DHUYS IVF Center, Zerah-Taar-Pfeffer Laboratory, Bagnolet, France, [3]Biomedical Research Center, Korea Advanced Institute of Science and Technology, Taejon, Korea, [4]College of William & Mary, Williamsburg, VA, [5]Jones Institute for Reproductive Med, Eastern VA Med Sch, Norfolk, VA, [6]Inst of Genetic Med, Johns Hopkins Univ Sch of Med, Baltimore, MD.

Objective: We summarize data on aneuploidy in sperm from twenty-three infertile males with oligoasthenoteratozoospermia (OAT) undergoing intracytoplasmic sperm injection (ICSI).

Design: Prospective study to determine whether isolation of the motile sperm fraction successfully separates aneuploid from haploid sperm.

Materials and Methods: Cytogenetic analyses by fluorescence *in situ* hybridization (FISH) to determine aneuploidy frequencies for chromosomes 1, 13, 18, 21, X and Y in sperm from swim-up and pellet fractions.

Results: In all cases, chromosome aneuploidy levels in patients were significantly ($p < 0.05$) greater than in controls. Per chromosome disomy for patients ranged between 0.0 and 3.8% in swim up fractions and 0.0 to 2.1% in pellets; for controls, the frequencies were 0.0 to 0.3% in swim up versus 0.0 to 0.7% in pellets. Per chromosome nullisomy for patients ranged from 0.0 to 6.3% in swim up and 0.1 to 3.1% in pellets; in contrast, for controls the frequencies were 0.0 to 0.3% in swim up and 0.0 to 0.7% in pellets. The frequencies of diploid sperm in patients were 0.0 to 1.7% in swim up versus 0.0 to 1.4% in pellets; for controls the rates were 0.0 to 0.3% in swim up versus 0.3 to 1.1% in pellets. Per chromosome aneuploidy for patients ranged from 0.0 to 10.0% in swim up versus 0.3 to 3.9% in pellets; in controls the rates were 0.0 to 0.6% in swim up versus 0.0 to 0.8% in pellets. Total aneuploidy in sperm from patients' whole semen ranged between 32 and 69%. In contrast, total aneuploidy for controls ranged between 4.1 and 7.7%. Patient-to-patient heterogeneity was shown.

Conclusions: The data show significantly higher rates of diploidy, autosomal disomy and nullisomy, sex chromosome disomy and nullisomy and total aneuploidy in sperm from all separated fractions from all OAT patients versus controls. The type and percent of aneuploid sperm for all OAT patients studied found in both swim-up and pellet fractions was not different, with the exception of diploid sperm, which remained in the pellet fraction. Isolation of the more motile sperm fraction, using the swim-up technique, does not separate aneuploid from haploid sperm in these patients studied. This population of infertile males may be at an increased risk of transmitting genetic abnormalities to their offspring.

P-283

Detection and Quantification of Early and Late Stages of Apoptosis in Human Semen: Analysis of Sperm Fractions With High and Low Motility and Relationship With the Production of Reactive Oxygen Species (ROS). G. Barroso, M. Morshedi, S. Oehninger. The Jones Institute for Reproductive Medicine, Dept. Ob/Gyn, Eastern Virginia Medical School, Norfolk, VA, 23507.

Objective: (1) To detect and quantify early and late stages of apoptosis in spermatozoa obtained from separated fractions of high and low motility; and (2) to analyze the relationship between apoptosis and the generation of ROS.

Design: Prospective studies.

Materials and Methods: Semen samples from subfertile men (n=5) were subjected to processing by gradient centrifugation (Percoll™). The highly motile (95% layer, HMF) and low motility (40% layer, LMF) sperm fractions were separated and evaluated. Sperm concentration and motion parameters (% progressive motility and % hyperactivated motility, HA) were assessed with a computer-assisted semen analyzer. The translocation of plasma membrane phosphatidylserine (a sign of early apoptosis) was detected using annexin V (AnV) with the simultaneous application of propidium iodide to identify necrosis. The selective binding of the monoclonal antibody (Mab) F7-26 to single stranded DNA was used to identify decreased stability of DNA to thermal denaturation in early apoptotic nuclei. Late nuclear apoptosis (internucleosomal DNA fragmentation) was detected with TUNEL (Tdt-mediated dUTP nick end labeling of DNA strand breaks). Apoptosis was detected and quantified (as %) in single cells using indirect immunofluorescence. ROS production ($\times 10^3$ counts per minute) was determined using a chemiluminiscence technique after luminol addition.

Results:

	Motility	HA	ROS	Necrosis	AnV	Mab	TUNEL
HMF(95%)	87±8	7±3	5.6±0.8	7±2	23±6	3±1	1±.05
LMF (40%)	4±2	2±2	7.7±.5	75±8	10±3	9±2	13±4
p-value	0.004	0.04	0.03	0.002	>0.5	0.07	0.02

The generation of ROS was significantly correlated with apoptosis (TUNEL) (r= 0.91, p= 0.02). There was a good correlation between techniques measuring nuclear apoptosis (TUNEL and MAb, r= 0.93, p= 0.01).

Conclusions: These preliminary data demonstrate that: (1) early (membrane and nuclear) and late (nuclear) stages of apoptosis can be detected in ejaculated spermatozoa from subfertile men; (2) highly motile sperm fractions have significantly lower number of spermatozoa with nuclear signs of apoptosis and necrosis as well as lower generation of ROS when compared to low motility sperm fractions; and (3) the strong association of ROS and apoptosis, if confirmed, may suggest a causal relationship.

P-284

Inducible Nitric Oxide Synthase (iNOS) Activity is Upregulated in the Epididymis of Rats With Long Term Varicoceles. L. S. Cho, M. A. Wheeler, S. C. Honig, R. M. Weiss. Section of Urology, Yale University School of Medicine, New Haven, CT, U.S.A.

Objectives: The pathophysiological changes in the testes and epididymis as a result of a clinical varicocele have not been well elucidated. The objective of this study is to determine whether changes in reproductive physiology, in a rat model of long term varicocele, are associated with upregulation of nitric oxide synthase.

Design: iNOS and neuronal (nNOS) nitric oxide synthase activities were measured, and caudal epididymal sperm analysis was evaluated in an in vivo rat model of long term varicocele.

Materials and Methods: Varicoceles were induced by partial ligation of the left renal vein in sexually mature, male Sprague-Dawley rats. After 96–117 days, Ca^{++} independent iNOS and Ca^{++} dependent nNOS activities were measured in the soluble cytosolic tissue fractions of the ipsilateral kidney and epididymis. NOS activity was quantified by [^{14}C]-L-arginine conversion to [^{14}C]-L-citrulline. Sperm samples were obtained by diffusion into physiologic medium after puncture of the cauda epididymis, and were observed for motility and viability by propidium iodide (PI) and SYBR-14 dye exclusion under the fluorescent microscope.

Results: A significantly greater percentage of nNOS and iNOS activity was found in the soluble fractions of the kidney and epididymis compared to the particulate fraction. In the soluble fraction, nNOS activity in the kidney (0.29 ± 0.03 pmole/min./mg) and epididymis (0.73 ± 0.07 pmole/min./mg) of rats with a varicocele (n=3) did not significantly change when compared to the kidney (0.30 ± 0.06 pmole/min./mg) and epididymis (0.58 ± 0.15 pmole/min./mg) of sham operated rats (n=3). In the soluble fraction, the iNOS activity in the kidney (0.75 ± 0.02 pmole/min./mg) of rats with a varicocele did not significantly change when compared to the kidney (0.41 ± 0.24 pmole/min./mg) of sham operated rats. However, in the soluble fraction, epididymal iNOS activity of rats with a varicocele (0.15 ± 0.002 pmole/min./mg) was significantly higher ($p<0.05$) than in sham operated rats (0.005 ± 0.003 pmole/min./mg). The mean forward progression (2.9 ± 0.1) of rats with varicocele was significantly less ($p < 0.05$) than the forward progression (3.3 ± 0.1) of sham operated rats by light microscopy. The mean percent sperm motility of rats with a varicocele (66% ± 3) was less, but not significantly lower than the mean percent motility of sham operated rats (72% ± 6). Similarly, the mean percent moribund sperm (14% ± 6) of rats with a varicocele was greater, but not significantly greater than the mean percent moribund sperm (2% ±1) of sham operated rats by PI and SYBR-14 dye exclusion fluorescence. The mean percent dead sperm (22% ± 8) of rats with a varicocele was similar than the mean percent dead sperm (24 ± 3) of sham operated rats by PI and SYBR-14.

Conclusions: A statistically significant change in sperm physiology associated with diminished forward progression correlates with an increased iNOS activity of the ipsilateral epididymis in the long term rat varicocele model.

Source of Funding: R01-DK38311, R01-DK47548

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Relationship Between Sperm Maturity and Frequency of Chromosomal Aneuploidies in Oligospermic and Normospermic Men. [1]E. Kovanci, [1]E. Moretti, [2]T. Ozcan, [2]P. Ward, [1]J. Stronk, [1]L. Vigue, [1]G. Huszar. [1]Sperm Phys. Lab. and [2]Dept. of Genetics, Yale School of Medicine, New Haven, CT.

Objectives: Diminished sperm maturity is characterized by increased cytoplasmic retention, low CK-M ratio and larger and rounder sperm heads. Immature sperm also lack the zona-binding site, and thus, they are deficient in fertilization based on sperm-zona pellucida interaction. However ICSI, overrides the sperm-zona interaction process. To evaluate the impact of fertilization with immature sperm, we studied the relationship between the incidence of chromosomal aneuploidies and sperm maturity in normospermic (NS) and oligospermic (OS) men who differ in the proportion of mature and immature sperm in their ejaculates.

Design and Methods: Mature and diminished maturity sperm were prepared by 80% and 28% Percoll gradients (the 28% pellet contains immature sperm with cytoplasmic retention that are excluded by 80% Percoll). The 8 pairs of fractions (5 normospermic men: all >20million sperm/ml, 3 oligospermic men: 14.2±2.2million sperm/ml, all data are mean±SEM) were evaluated by CK-immunocytochemistry (300 sperm each) which highlights the retention of cytoplasm. In the fluorescent in situ hybridization (FISH) experiments, we used centromeric probes for chromosomes X, Y and 17. For the detection of disomies, we examined 4000 sperm and 2000 sperm in each NS and OS men, respectively (the higher incidence of aneuploidies in the diminished maturity 28% Percoll fractions compensates for statistical power). Sperm were scored according to Martin and Rademaker (MRD, 1995). Analysis of frequency was carried out by the Pearson's X^2 analysis.

Results: The incidence of sperm with cytoplasmic retention was 9% and 29% in the immature 28% Percoll fractions of NS and OS men, respectively. Reflecting diminished sperm maturity, the incidence of aneuploidies were significantly higher in the 28% vs. 80% fractions (disomy X: 0.3% vs. 0.17%, $p=0.005$; disomy Y: 0.2% vs. 0.08%, $p<0.001$; disomy XY: 0.31% vs. 0.15%, $p<0.001$; disomy 17: 0.29% vs. 0.15%, $p<0.001$, N=8 men). In OS vs. NS men, who had a higher and lower incidence of immature sperm, respectively, there were additional aneuploidy differences between two 80% and two 28% fractions (Table 1).

	28% Percoll		
	Oligospermic men		Normospermic men
Disomy X (%)	0.80	p<0.001	0.15
Disomy Y (%)	0.42	p<0.001	0.13
Disomy XY (%)	0.46	p=0.003	0.22
Dismoy 17 (%)	0.56	p<0.001	0.21

	80% Percoll		
	Oligospermic men		Normospermic men
Disomy X (%)	0.42	p<0.001	0.01
Disomy Y (%)	0.15	NS	0.06
Disomy XY (%)	0.27	p=0.006	0.11
Dismoy 17 (%)	0.39	p<0.001	0.08

Conclusions: We detected overall differences in chromosomal aneuploidies in sperm originating in diminished maturity and mature fractions. In addition, we also found an increased incidence of aneuploidies in the respective 28% vs. 28% and 80% vs. 80% fractions of OS vs. NS men. This is important from the practical point of view, because the sperm of severely OS men is primarily of diminished maturity. Thus, arbitrary ICSI sperm selection in OS and severely OS men further increases, in proportion with the decrease in sperm concentration, the likelihood that a sperm with chromosomal aneuploidy will be selected. (Supported by HD-32902).

P-286

Antisperm Antibodies Affect Sperm Zona Binding and Not Sperm Oolema Fusion. [1]S. Friedler, [2]Y. Soffer, [1]A. Raziel, [2]A. Umanski, [2]S. Kaufman, [1]D. Strassburger, [3]L. Yogev, [1]R. Ron-El. [1]IVF Unit and [2]Male Infertility Unit, Assaf Harofeh Medical Center, Zerifin and Institute for the Study of Fertility, Liss Maternity Hospital, Tel Aviv, Israel.

Objectives: Sperm-bound antibodies may interfere with in vivo and in vitro sperm fertility potential at two levels of interaction, the sperm-mucus and the sperm-egg interaction and thus, require ART appropriate treatments. As many sperm variables may concomitantly influence these interactions, a multifactorial approach is necessary. The objective of this study was to distinctly analyze the in vitro effects of IgA and IgG sperm-bound antibodies on sperm-egg interactions, sperm zona binding in human hemizona assay (HZA) and sperm oolema fusion in zona-free hamster egg penetration test (SPA).

Materials and Methods: A group of 127 infertile men underwent a full male work-up including semen analysis with morphology Kruger strict criteria (NF%), antisperm antibodies screening using SpermMar™ IgA and IgG. Semen samples with or without sperm-bound antibodies were tested in HZA and SPA. To eliminate confounding factors, semen samples with and without IgA and/or IgG antibodies were statistically compared using uni and multivariate analysis.

Results: Sperm-bound IgG was significantly found in 13.8% of semen samples, IgA in 23.1% and both in 7.4%. In the whole group, mean HZA% was 56.0±37.6. It was 59.2% if antibodies were absent and significantly dropped to 22.6 if both IgA and IgG were detected ($p<0.04$). In logistic regression, NF, IgG, motility and IgA were, in decreasing order, the significant factors influencing HZA%. On the other hand, mean SPA% was 59.4 ± 41.9. SPA% was not significantly different with IgA or IgG sperm-bound antibodies, 57.2%, or without, 60.3%. In logistic regression, sperm motility and sperm count were the only factors that significantly influenced SPA%.

Conclusions: HZA and SPA integrate different sperm variables and both are complementary. Antisperm antibodies may interfere in sperm egg interaction through sperm zona binding mainly, as tested by HZA and not sperm-oolema fusion, as tested by SPA. If antisperm antibodies are detected, sperm functional assays are required to choose proper ART (IUI, IVF or ICSI) treatment.

MENOPAUSE

Tuesday, September 28, 1999

P-287

Correlation Between Plasma Estradiol (E_2) and Estrone Sulfate (E_1-S) Levels Following Long-Term Oral and Transdermal Administration of Estradiol in Healthy Postmenopausal Women (PMW). C. Coulam, B. D. Acacio, H. N. Hodis, R. J. Paulson, F. Z. Stanczyk. Depts. of Ob/Gyn and Medicine, University of Southern California School of Medicine, Los Angeles, CA.

E_1S is quantitatively the most important circulating estrogen in women. E_1S can be readily transformed to E_1 and E_2 by sulfatases in different body tissues. Recent reports have suggested the importance of E_1S and E_1 sulfatase in regulating the supply of estrogens to estrogen-dependent breast cancers. Estrogen sulfatase inhibitors have been found to open new possibilities in breast cancer treatment. The objective of this study was to measure the linear relationship between E_1S and E_2 levels in PMW during long-term treatment with oral and transdermal E_2. Group 1 (n=10) received 1 mg of micronized E_2 daily for 16 months, and their blood was drawn at 0, 7, and 15 months. Group 2 consisted of 23 PMW randomized into 3 subgroups. Two of the subgroups (n = 8, and n=7) received E_2 delivered at a rate of 0.05 mg/day and 0.1 mg, respectively, by transdermal patch (changed twice weekly), and the third subgroup received a placebo patch for 9 continuous months. Blood samples were obtained at 0, 6, and 9 months. E_1S and E_2 were quantified by direct RIA. Statistical analysis was done by the Pearson correlation coefficient. Results are shown below.

Oral E_2	0 Months	7 Months	15 Months
E_1S (ng/ml)	0.76 ± 0.12	24 ± 4	39 ± 11
E_2 (pg/ml)	11.2 ± 1.5	72.3 ± 22.4	48.7 ± 21.1
r	0.539	0.602	0.508
p	NS	0.05	NS

	0 Months		
T-dermal E_2	Placebo	0.05 mg	0.1 mg
E_1S (ng/ml)	0.81±0.24	0.69±0.1	0.81±0.07
E_2 (pg/ml)	11±5.6	6±5.2	11.6±7.4
r	0.718	0.464	0.347
p	NS	NS	NS

	6 Months		
T-dermal E_2	Placebo	0.05 mg	0.1 mg
E_1S (ng/ml)	1.0±0.4	1.6±0.3	2.3±0.6
E_2 (pg/ml)	12±6.7	70±22	155±80
r	0.982	0.755	0.829
p	0.01	0.04	NS

	9 Months		
T-dermal E_2	Placebo	0.05 mg	0.1 mg
E_1S (ng/ml)	1.2±0.46	1.8±4.2	3.2±0.52
E_2 (pg/ml)	15±11	74±22	146±83
r	0.798	0.277	0.667
p	0.05	NS	NS

We conclude that: (1) a large accumulation of E_1S develops after long-term oral estrogen treatment; (2) there is only a small E_1S accumulation after transdermal E_2 therapy; (3) plasma E_1S levels and E_2 levels do not appear to be significantly correlated in PMW on oral and transdermal E_2 replacement. Monitoring serum E_1S levels may be important in patients receiving oral estrogen replacement.

P-288

Raloxifene Does Not Stimulate the Uterus in Postmenopausal Women as Compared to Continuous Combined Hormone Replacement Therapy Following 24 Months of Treatment. [1]P. Fugère, [2]W. H. Scheele, [2]K. R. Srikanth, [3]G. Anglin, [3]T. R. Strack, [2]A. B. Nauden, [3]L. J. Jonnavithula, [3]S. Kaptein, [4]P. Kenemans, [5]E. E. Jolly. [1]Hôpital Saint-Luc, Montreal, Dept. of Obstetrics and Gynecology, PQ, Canada. [2]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA. [3]Lilly Research Laboratories, Eli Lilly Canada, Inc., Toronto, ON, Canada. [4]Vrije Universiteit, Dept. of Obstetrics and Gynecology, Amsterdam, Netherlands. [5]Ottawa General Hospital, Dept. of Reprod. Endocrin., Ottawa, ON, Canada.

Objectives: To determine the effect of raloxifene HCl (RLX) as compared to continuous combined hormone replacement therapy (ccHRT) on endometrial histology, endometrial thickness, and uterine volume in subjects with no baseline endometrial abnormalities.

Design: A 24-month, multicenter, double-blind study of 136 healthy postmenopausal women who were randomized to either ccHRT (Premarin 0.625 mg/day with Provera 2.5 mg/day) or RLX (150 mg/day), a selective estrogen receptor modulator (SERM).

Materials and Methods: Endometrial biopsy samples were obtained by Pipelle™ biopsy at baseline, 12 and 24 months. Endometrial thickness and uterine volume were measured by transvaginal ultrasonography at the same time intervals.

Results: Sixty nine subjects were randomized to ccHRT and 67 subjects were randomized to RLX. All subjects were between 1 and 15 years postmenopause and 45 through 65 years of age, inclusive. Biopsy samples were evaluated by an external lab and diagnoses were grouped into four categories: (1) normal, benign, (2) benign, stimulatory, (3) benign, abnor-

mal, or (4) premalignant/malignant. Throughout the study, no biopsy samples were classified as premalignant/malignant. At baseline, 94.1% of subjects were classified as category 1, 5.0% as category 2, and 1% as category 3, with no differences between therapy groups. At endpoint, 78.7% of biopsies in the ccHRT group and 94.4% in the RLX group were classified as category 1; 19.1% in the ccHRT group and 5.6% in the RLX groups were classified as category 2; and 2.1% in the cc-HRT and 0% in the RLX group were classified as category 3 ($p = 0.056$). From baseline to endpoint, mean endometrial thickness decreased by 0.1 mm in the RLX group ($p=0.597$) and increased by 0.6 mm in the ccHRT group ($p=0.077$), and there were no significant differences between therapy groups ($p=0.067$). Mean uterine volume decreased by 1.9 cm^3 in the RLX group ($p=0.153$) and increased by 14.7 cm^3 in the ccHRT group ($p<0.001$), and there was a significant difference between the therapy groups ($p<0.001$). A significantly higher incidence of vaginal bleeding was reported in the ccHRT group (68.1%) relative to the RLX group (9.0%) ($p<0.001$). There was also a higher incidence of breast pain in the ccHRT group (43.5%) than in the RLX group (9.0%) ($p<0.001$).

Conclusion: RLX treatment at 150 mg/day for 24 months resulted in no endometrial stimulation and no increase in endometrial thickness or uterine volume.

P-289

Influence of Estrogen With or Without Methyl-Testosterone Therapy on Cognitive Functions in Post-Menopausal Women. [1]T. T. Nguyen, [2]A. B. Wisniewski, [1]A. S. Dobs. [2]Department of Medicine, Johns Hopkins University, Baltimore, MD and [1]Department of Psychology, Johns Hopkins University, Baltimore, MD.

Objectives: The nature of the relationship, if any, between cognitive abilities in humans and circulating sex hormones still remains unclear. Our group has previously reported a significant influence of testosterone treatment on patterns of cerebral lateralization exhibited by hypogonadal men (Wisniewski, Nguyen & Dobs, 1998). Lateralization has been use as an indicator of cognitive abilities. The goal of the present double-blinded randomized study was to determine if esterified estrogen alone, Estratab, (1.25 mg) [E-group] or testosterone treatment in conjunction with estrogen, Estratest, [MT-group] (2.5 mg methyl-testosterone and 1.25 mg estrogen) exerted any effects on cognitive functions.

Design: Forty women (age 56.48 ± 8.10 years) on a stable 0.625 mg estrogen dose for 3 months were randomized to either the E-group or the MT-group. The tests for cognitive abilities, which included the area of spatial ability, perceptual speed, and memory, were given to these post-menopausal women before they started the study medications and then repeated after four months on the medications.

Materials and Methods: Full IRB approval was obtained before the start of the study and each patient signed a consent form before any test began. Morning serum (between 8:00 and 11:00 am) was obtained from each women before each visit. Hormone profiles were sent away to be analyzed by Quest Diagnostics. Spatial abilities were assessed using the Map Test (M) and Cube Comparisons Test (CC), perceptual speed with Identical Pictures Tests (IP), and memory by a Shape Memory Test (SM). All standardized tests were developed by Educational Testing Services.

Results: Both groups started with compatible hormone profiles (E and MT-group respectively, estradiol 93.42 ± 60.24 vs. 115.06 ± 77.55 pg/ml; Total Testosterone [TT] 21.06 ± 8.98 ng/dl vs. 21.56 ± 10.73 ng/dl; Biovailable testosterone [BT] 1.28 ± 1.25 ng/dl vs. 1.25 ± 2.23 ng/dl). After four months, both groups had an elevated estradiol (E-group 191.82 ± 86.13 pg/ml, $p < 0.0002$ and MT-group 229.71 ± 140.24 pg/ml, $p < 0.01$). The E-group had no changes in TT (23.44 ± 8.85 ng/dl) and BT (1.422 ± 0.97 ng/dl) while the MT had significant increase in TT (137.82 ± 116.76 ng/dl, $p < 0.0004$) and BT (25.56 ± 25.26 ng/dl, $p < 0.001$). No changes were observed for the CC and the IP tests in both groups. However, in the M test, there was a decrease of 27% ($p = 0.01$) in the E-group, but no change in MT-group. The E-group showed a 53% increase ($p = 0.05$) while the MT-group had a 44% increase ($p = 0.005$) in the SM test.

Conclusions: We found that women randomized to high dose estrogen exhibited improved memory, decreased in spatial ability, without a change in perceptual speed. In comparison to estrogen alone, the addition of testosterone had an additive effect on memory, prevented the decrement in spatial ability, without affecting perceptual speed. In conclusion, the administration of sex hormones, both estrogen and estrogen with testosterone to post-menopausal women for four months elicited changes in cognitive functions.

P-290

The FORKO Mouse as a Genetic Model for Hormone Replacement Therapy. M. R. Sairam, N. Danilovich. Molecular Reproduction Research Laboratory, Clinical Research Institute of Montreal, Montreal, Quebec, Canada.

Objectives: The need to compensate the severe loss of estrogen during menopause in women to derive its beneficial actions on the skeletal, cardiovascular, neural, and other systems has led to an intense drive to develop the so-called selective estrogen receptor modulators. The ovariectomized rodent has been extensively used in such developmental studies. We recently observed that genetic disruption of the FSH receptor in the mouse (the FORKO mouse) induced severe estrogen deficiency in females beginning at birth and persisting into adulthood. Our objective in this investigation was to evaluate whether these mutants respond to estrogen therapy.

Design: Estrogen therapy in the form agonist or antagonist injection was initiated at different times following birth.

Materials and Methods: All three genotypes (+/+, +/− and −/−) were investigated at comparable ages. Mice were given subcutaneous injection of test compounds. Reproductive tissue response, adipose tissue mass and histological changes were examined.

Results: Within 36–48 hrs of agonist administration water imbibition, uterine, and vaginal growth became evident and this was confirmed by histological examination, which included evaluation of estrogen responsive genes such as lactoferrin. Estrogen action was clearly evident by reopening of the vagina and presence of cornified cells in the smear. Even older animals responded to estrogen during a ten-day test period. The beneficial effect of such a short treatment in these obese mutants was apparent by a decrease (60%) in adipose tissue in all areas. Tamoxifen, which acts as an antagonist (weak agonist), had only marginal effects on the uterus and body fat in conformity with previous observations.

Conclusions: 1. In the FORKO mice the principal source of estrogen (ovary) has been eliminated by a gene disruption. 2. The repertoire of estrogen receptors (α and β) remains functional in responsive tissues. 3. Our results are sufficiently encouraging to propose that the FORKO mouse is a suitable model to evaluate the molecular basis of hormone replacement therapy and aid the design of site selective agents for osteoporosis, obesity, cardioprotection, and restoring cognitive function in menopause. 4. These animals could also be used to evaluate Xenobiotics. (Supported by MRC of Canada).

P-291

Role of Nitric Oxide (NO) Metabolism in Estrogen Related Effects on Cardiovascular Function in Ovariectomized and Estrogen Treated Rats. [1]M. Birincioglu, [2]I. Yilmaz, [1]E. Olmez, [1]A. Acet, [2,3]O. Taskin, [3]J. M. Wheeler. Dept. Ob&Gyn/Pharm, [1]Inonu University, Turkey and Texas Women's Hospital, TX, USA.

Objective: To elucidate the role of NO in estrogen related effects on cardiovascular function.

Design: Prospective controlled follow-up study in the primate center of an university based clinic.

Materials and Methods: Female Wistar rats at 70 days of age weighing 250–350 g were ovariectomized (OVX) or sham operated at 70 days of age. Twenty days following ovariectomy the rats (4 groups, n: 15) were randomized to receive either 7 μg/ 100 g body weight estradiol(E), L-ARG 5 g/L, E +L-NAME 500mg/L(EL), E or vehicle (V, sesame oil) intraperitoneally for another 20 days. Then the animals were sacrificed and the uterus, aorta and heart tissues were homogenized. The levels of scavenger (FRS) enzymes(SOD, CAT, GSH-Px) were determined and compared within the groups.

Results: Estrogen treated and Sham operated groups had significantly higher levels of SOD, CAT and GSH-Px compared to the OVX group in cardiovascular tissues and uterus(1.8±0.1 ng, 8.4±0.7 umol, 0.14±

0.02umol vs 0.7±0.05ng, 5.3±0.6umol, 0.08±0.01 umol, respectively, P<0.05). A similar increase in FRS levels was observed with L-Arginin which is known to have positive effect on NO system. Moreover, when NOS blocking agent L-NAME was administered to estrogen treated OVX rats, the increase in FRS levels was completely blocked resulting in tissue levels similar to OVX group lacking estrogen (P<0.05).

Conclusion: Based on the above results we may conclude that estrogen modulates the cell protective system which is reflected by increase in cardiovascular tissues- FRS levels. Furthermore, this modulatory effect of estrogen was blocked by NOS blocking agents and attenuated by NOS activity(L-Arg). The above positive and negative effects despite estrogen have further showed that NO is one of the major pathway that estrogen exerts its cardiovascular protective effects.

REPRODUCTIVE ENDOCRINOLOGY

Tuesday, September 28, 1999

P-292

Elevated Interleukin-10 and Sex Steroid Levels in Peritoneal Fluid of Patients With Ovarian Hyperstimulation Syndrome. K. Manolopoulos, U. Lang, H. Gips, G. A. Braems. Department of Obstetrics and Gynecology and Institute of Reproductive Medicine, Justus-Liebig University Giessen, Germany.

Introduction: The ovarian hyperstimulation syndrome (**OHSS**) after ovulation induction is characterized by enlargement of the ovaries with an acute third space fluid sequestration. Recent studies ascribed cytokines that mediate the inflammatory response (IL-1, IL-2, IL-6, IL-8, TNF), a crucial role in the prediction and pathogenesis of OHSS. Other cytokines, such as the anti-inflammatory interleukin-10 (**IL-10**), are potent modulators of T-lymphocyte function and could play a role in OHSS, although this has not been examined. 17-estradiol and progesterone are also involved in OHSS, but an relationship with IL-10 has not been investigated. Furthermore, early pregnancy is a known source of IL-10 and increases serum IL-10 levels. The effect of early pregnancy on peritoneal fluid IL-10 is unknown and might serve as a positive control.

Aim: Aim of this study was to determine the role of IL-10 in OHSS and its correlation with 17-estradiol and progesterone in patients with OHSS after ovulation induction in an IVF programm.

Patients and Methods: Peritoneal fluid (**PF**) and serum (**S**) samples for IL-10 were collected from 9 patients with severe OHSS after ovulation induction by administration of GnRH-analogues followed by hMG (n=5) or recombinant FSH (n=4). Abdominal puncture was performed for relief from abundant ascites. Patients in early pregnancy (n=14) were between 7–16 weeks of gestation. Patients (n=19) without pathological findings during laparoscopy served as control. The medical reasons of laparoscopy were tubal ligation, interruptions or chromopertubation. The samples were centrifuged (2600 rpm-1 × 10 min) at 4°C and stored at −80°C until analysis. The ELISA-kit for IL-10 was commercially available (PerSpective Biosystems), 17-estradiol and progesterone were measured by RIA (Biermann). Statistical analysis was performed by Mann Whitney-U test and results are presented as the median and range.

Results: OHSS patients had significantly higher peritoneal fluid IL-10, 17-estradiol and progesterone levels than patients during early pregnancy and the control group. However, no correlations were found between peritoneal fluid or serum IL-10 and 17-estradiol or progesterone in the different groups. Serum 17-estradiol and progesterone, but not serum IL-10 levels were elevated in OHSS and early pregnancy.

Conclusions: High concentrations of IL-10 in peritoneal fluid suggest a role of this anti-inflammatory cytokine during OHSS. 17-estradiol and progesterone were elevated in peritoneal fluid and serum during OHSS but no correlation with IL-10 concentrations was found. Therefore, we assume that IL-10 has an role in OHSS, but indicates as a local mediator of inflammation stands for different aspects of the OHSS than 17-estradiol and progesterone.

		OHSS (n=9)	early pregnancy (n=14)	control (n=19)
IL-10 (pg/ml)	PF	104,6[c,*] [46,5-336,2]	12,0[b] [6,3-220,8]	6,3 [4,2-18,7]
	S	1,5 [1,2-26,0]	6,3 [1,9-220,5]	5,5 [1,2-16,8]
17-estradiol (pg/ml)	PF	4400[c,***] [2510,0-5400,0]	416,5[b] [47,0-1819,0]	67,5 [41,0-3930,0]
	S	2185,0[b,*] [541,0-4220,0]	452,0[a] [20,0-1560,0]	55,0 [27,0-299,0]
progesterone (ng/ml)	PF	381,0[c,**] [15,0-460,0]	25,0[b] [1,3-46]	1,4 [0,7-328,0]
	S	293,0[c,**] [32,0-710,0]	22,4[b] [5,7-33,8]	5,7 [3,7-7,7]

[a] p<0,05; [b] p<0,01; [c] p<0,001 vs. control; * p<0,05; ** p<0,01; *** p<0,001 vs. early pregnancy.

P-293

Long-term Follow-up of Children Born After Inadvertent Administration of a Gonadotropin-Releasing Hormone Agonist in Early Pregnancy. [1]R. Ron-El, [2]E. Lahat, [1]A. Raziel, [1]D. Strassburger, [1]M. Schachter, [1]S. Friedler. [1]IVF and [2]Pediatric Neurology Units, Assaf Harofeh Medical Center, Tel Aviv Uni., Israel.

Objectives: Administration of gonadotropin-releasing hormone agonist (GnRHa) in mid luteal phase for down regulation can be during the establishment of a very early spontaneous conception. Our aim was to evaluate long-term outcome of children born after such a pregnancy.

Design: Data concerning the course of pregnancy, delivery and post-natal course of all inadvertent pregnancies were collected. The children's medical history was taken. Physical, neurological and psycological evaluations were performed on the children of at least 4 years of age by the same pediatric neurologist and psychologist.

Materials and Methods: Serum βhCG was measured in any patient whose progesterone (P) was >1ng/nl 14 days after mid-luteal administration of GnRHa (Decapeptyl, Depo or daily preparates). The GnRHa was discontinued and P if needed, was supplemented when pregnancy was documented. The WISC-R test was used for all children except for the youngest one (4 y) in this case Wipsi was performed.

Results: Six children from 6 pregnancies were included. Mothers' age was 35.8±5.1 y (29 to 41) with a median age of 33y. Infertility period was 5.5±1.4 y. Exposure to GnRHa was estimated to have lasted from 4 to 14 days, calculated retrospectively according to the assumed day of ovulation. Five children were vaginally delivered and one abdominally at the gestational age of 34 to 39 wks. Weights ranged from 2130 to 3780 grams. One was small for dates. Apgar scores were normal and postnatal course was uneventful except for premature infant who developed transient tachypnea which resolved after 24 h. The mean age at examination was 7.8±2.0 y; median of 8.4y. The only major congenital malformation was cleft soft palate which was successfully corrected at the age of 14 months. Another child had gastro-esophageal reflux which was not surgically corrected due to parents' refusal. Three children were evaluated by occupational therapists at the age of 3 to 4 y because of significant impairment in motor skill performance. Two of them and another child were diagnosed as having Attention Deficit Hyperactivity Disorder (ADHD).

Conclusions: The observation justifies the necessity for long-term follow-up of more or all children previously exposed to GnRHa in order to correctly estimate the risk of the exposure to the drug at very early pregnancy.

P-294

Endocrine and Sonographic Response to Immediate Gonadotropin Releasing Hormone Agonist (GnRHa) After Oral Contraceptives (OCs) in Women of Advanced Reproductive Age for Maximal Follicular Recruitment. [1]H. J. Yeon, [1]W. I. Park, [2]H. R. Chung. [1]Dept of Ob Gyn, [2]Dept of Clinical Pathology, Eulji Medical College, Seoul, Korea.

Objectives: We hypothesized that earlier administration of GnRHa after OCs may be beneficial in synchronization of follicular cohort. As a pilot

study, the endocrine and sonographic response in advanced reproductive aged women was investigated to see if the immediate administration of low dose GnRHa after pretreatment with short and long term OCs could increase or sustain the beneficial effect of OCs in COH, such as better homogeneity of follicular cohort and avoidance of corpus luteum rescue.

Design: Prospective clinical trial.

Materials and Methods: Fifteen selected women aged 38–45 (mean 41.7) with regular cycles were assigned to 3 groups according to their menstrual phases at the day of entry. OCs containing ethinyl estradiol 0.020mg and desogestrel 0.150mg were given BID starting in follicular phase for 7 days (FS;follicular short, n=5), for 21–22 days (FL;follicular long, n=5) or in midluteal phase for 8–10 days (ML;midluteal, n=5) followed by GnRHa (Decapeptyl) 50ug, SC qd for 2 days from the next day of the last pill. Serum FSH, LH, E2, P, T were measured on the Initial Day of entry (ID), Final pill Day (FD), 1 day after FD (FD 1) and 2 days after FD (FD 2) before GnRHa injection and on 3 days after FD (FD 3). As a control, 2 women among group FL was not given GnRHa to see the recovery pattern after OCs administration (no GnRHa). And 2 women among group ML was given GnRHa on FD 2 (FD 2 GnRHa). Transvaginal sonography (TVS) was performed on ID and on FD 3 to measure the largest follicular diameter.

Results: OCs pretreatment successfully suppressed the levels of gonadotropins, E2 (<10pg/ml), P (<1ng/ml) and T (<0.4ng/ml) in all three groups regardless of the day of entry and the duration. Spontaneous recovery of FSH began from FD 2 in group FL and in group ML. Flares of gonadotropins but not of E2, P, T were noted on FD 2 after GnRHa injection in group FS (LH 246.4%, FSH 197.3% of baseline MCD#3) and ML (LH 357.9%, FSH 283.2% of baseline). But in group FL, gonadotropins were recovered only 50% & 64% of baseline in LH and FSH, each. TVS on FD 3 showed follicular diameters less than 4mm in all three groups. Withdrawal bleeding was appeared after FD 3 (4–6) in group FS, ML. And in group FL, menstrual bleeding was also delayed 3 to 6 days than expected menses.

Conclusions: Immediate use of low dose GnRHa after OCs suggested earlier rise in gonadotropins and no adverse endocrine response in follicular growth such as P or T rise in advanced reproductive aged women. TVS didn't show discrimination in follicular size in all groups on FD 3. And it was suggestive that this method may cause follicular-uterine dys-synchrony by delaying withdrawal bleeding. The actual increase in number and homogeneity in follicular cohort should be further investigated in IVF. And also, it should be answered the possible dys-synchrony. But this method may be applied in case of oocyte donation program or low responders with uterine factor in which separation of follicular and endometrial growth is possible.

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Recurrent Early Pregnancy Loss: High Basal LH or Low Midluteal Progesterone? H. N. Sallam, A. N. Sallam, P. Ezzeldin. Department of Obstetrics and Gynaecology, the University of Alexandria in Egypt, and the Alexandria Fertility Center, Alexandria, Egypt.

Objectives: Recurrent early pregnancy loss is associated with high basal serum LH levels. However, the exact mechanism by which high LH leads to pregnancy loss is not exactly understood. Many explanations have been suggested including inhibition of the ovulation maturation inhibiting (OMI) factor, abnormal prostaglandin synthesis, altered androgen production or the production of abnormal glycoforms of LH.

Design: A group of patients with recurrent early pregnancy loss was studied by measuring both the basal serum LH and midluteal plasma progesterone levels before and after treatment with human menopausal gonadotrophins (hMG) to clarify whether the elevated LH or the diminished progesterone is the factor responsible for the pregnancy loss.

Materials and Methods: Forty eight patients with recurrent early pregnancy loss and a basal serum LH level of 10 IU/L or more were studied. The mean age (+/−SD) was 33.25 years (+/−7.48) and the mean number (+/−SD) of early pregnancy losses was 3.25 (+/−1.16). The basal level of serum LH was determined on day 3 of the cycles and the plasma progesterone concentration on day 21 in the pre-treatment cycle and 7 days after HCG administration in the treatment cycles. The patients were treated with hMG for 3 months and monitored with ultrasound.

Results: Thirty one patients became pregnant giving a 3-months cumulative pregnancy rate of 64.6%. Of these, 6 pregnancies ended in first trimester pregnancy loss (20.1%) while 25 pregnancies continued beyond 28 weeks. In these 25 patients, the mean basal LH (+/−SD) was 18.57 IU/L (+/−6.72) during the pre-treatment cycle and was still high during the pregnancy cycle at 17.11 (+/−7.36). This difference is not statistically significant ($P=0.657074$). The mean (+/−SD) midluteal plasma progesterone concentration in the pre-treatment cycle was 6.97 (+/−4.84) ng/mL but increased significantly to 21.14 (+/−11.62) ng/mL during the pregnancy cycle ($P<0.05$).

Conclusions: The results show that repeated early pregnancy loss in these patients with high basal LH levels is a manifestation of the low plasma progesterone levels rather than the elevated basal serum LH levels. Repeated early pregnancy loss in patients with elevated basal LH levels and low midluteal plasma progesterone concentration can therefore be seen as a manifestation of LPI and can be treated successfully with hMG.

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Elevated Serum Progesterone (Day hCG) During Gonadotropin (hMG) Stimulation for IUI or IVF is Not Associated With Diminished Ovarian Reserve. G. E. Hofmann, C. Mitchner. Bethesda Hospital, Cincinnati, OH, USA.

Objective: To determine if an elevated serum P on the day of hCG administration (serum P ≥ 1.1 ng/mL) during gonadotropin stimulation for intrauterine insemination (IUI) or IVF is associated with diminished ovarian reserve (DOR).

Design: Retrospective chart review.

Materials and Methods: A previous study suggested that a serum P ≥ 1.1 ng/mL was associate with DOR (Younis et al. Fertil Steril 1998;69:461–5). To evaluate this possibility further 286 women who underwent ovarian reserve screening with a clomiphene citrate challenge test (CCCT), were later stimulated with hMG for IUI (n=98) or IVF after luteal pituitary suppression with a GnRHa (n=188). Women in each group were evaluated on the basis of their CCCT (abnormal test, FSH ≥ 25 mIU/mL on cycle day 3 or 10) and the value of their serum P on the day of hCG administration. Comparisons were made between women with normal and abnormal CCCTs based on the serum P on the day of hCG administration, their ages, day 3 FSH, day 10 FSH and serum P, peak E_2, and ampules of hMG used. Comparisons were made with a student t-test, Chi-Square and linear regression as appropriate. Statistical significance was defined as $P<0.05$.

Results: For the IUI group, 74 women had a normal CCCT, while 24 had an abnormal CCCT. For women undergoing hMG stimulation for IVF, 171 had a normal CCCT and 17 had an abnormal CCCT. Women in both groups with a normal CCCT were younger, had lower day 3 FSH and day 10 FSH and P, required more ampules of hMG to achieve lower peak E_2, but had similar serum P levels on the day of hCG administration (IUI: normal CCCT P=0.7±.3, abnormal CCCT P=.9±.7: $P=0.17$, CI −0.48 to 1.08) (IVF: normal CCCT P=0.9±.3, abnormal CCCT P=1.0±1.1, $P=0.4$ CI −.4 to 1.16). For women undergoing IUI, 19/74 (21.6%) (normal CCCT) had a serum P≥1.1 ng/mL on the day of hCG, while 5/24 (20.8%) (abnormal CCCT) had a serum P≥1.1 ng/ml, ($P=0.5$ CI 0.4 to 6.9). Similarly, for women doing IVF 72/171 (42.1%) (normal CCCT) had serum P≥1.1 ng/mL, and 7/17 (41.2%) (abnormal CCCT) had a serum P≥1.1 ng/mL ($P=.85$, CI 0.3 to 2.9). For both groups, there was no association between serum P on the day of hCG administration and day 3 FSH or day 10 FSH or P during the CCCT. However, for women undergoing IVF, higher serum P levels on the day of hCG administration was associated with higher peak E_2 levels.

Conclusions: As anticipated from the literature on CCCT, women with abnormal tests respond to hMG less favorably than women with a normal CCCT. For women stimulated with hMG for either IUI or IVF, serum P on the day of hCG administration, is not associated with diminished ovarian reserve.

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Laparoscopic Treatment of Polycystic Ovaries with Insulated Needle Cautery. A. Felemban, T. Tulandi. Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada, H3A 1A1.

Objectives: To evaluate the results of laparoscopic treatment of polycystic ovarian syndrome in clomiphene-resistant infertile women.

Setting: University teaching hospital.

Materials and Methods: 214 clomiphene-resistant anovulatory women with polycystic ovarian syndrome. Laparoscopic treatment was done using an insulated needle cautery, 8 mm depth, 40 Watts for 2 seconds.

Results: The mean age of the women was 30.2±years, body mass index: 27.8±0.6, serum LH: 12.7±0.8mIU/mL, FSH 5.9±0.3 mIU/mL and total testosterone: 2.8±0.2. The cumulative probability of conceptions at 12, 18 and 24 months after surgery were 54%, 68% and 72% respectively (median: 10.2 months). Using Cox's proportional hazards model, the effects of age, body mass index and duration of infertility were evaluated. These factors were not associated with the pregnancy rate (P: not significant). Of 15 women who underwent a second-look laparoscopy, 11 women were found to be free of adhesions. In 4 women with periadnexal adhesions, filmy and minimal amount of adhesions were found on the ovarian surface only.

Conclusions: Laparoscopic treatment of polycystic ovarian syndrome in clomiphene-resistant infertile women is associated with a pregnancy rate of 54% and 72% at 12 and 24 months follow-up respectively. The use of an insulated needle cautery is associated with minimal amount of adhesion formation.

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The Relationship of Body Mass Index (BMI) and Serum Estrogen Levels to Biochemical Bone Markers in Untreated Postmenopausal Women. [1]G. R. Kraemer, [2]B. A. Tawwater, [3]R. R. Kraemer, [1]E. R. Brooks, [1]K. Moreau, [2]T. L. Gimpel. [1]Women's Health Research Institute, Baton Rouge, LA, [2]Texas Tech University Health Sciences Center, Amarillo, TX, [3]Southeastern LA University, Hammond, LA.

Objectives: Estrogen replacement therapy (ERT) is documented to decrease the risk of osteoporosis in postmenopausal (PM) women and to reduce biochemical bone degradation markers in these subjects.

Design: In the present study we have examined biochemical bone markers (serum osteocalcin and urinary deoxypyridinoline (DPD) for their relationship to BMI and serum estrogens in untreated PM women).

Materials and Methods: PM women, either surgical (n=10) or natural (n=32), were enrolled in the study. Ages range from 33 to 79 years of age. Fasted morning serum and first morning voided urine samples were obtained in addition to height and weight. Serum samples were analyzed for E2 using an ultrasensitive assay (Third Generation Estradiol), E1 and E1S using a specific immunoassay (Diagnostic Systems Lab, Webster, TX). Serum osteocalcin was measured with a sensitive IRMA (Diagnostic Systems Lab., Webster, TX) and urinary DPD was measured by chemiluminescent assay (Immulite, Diagnostic Products Corp., Los Angeles, CA).

Results: There was a positive correlation between urinary DPD levels and BMI (r=0.51, P=.0006) although most (35/42) values were elevated above reproductive age control levels of 7 pg/ml. Only E1 was significantly related to DPD. Serum osteocalcin showed a significant but weak inverse relationship with BMI (r=.34). There were significant relationships between osteocalcin and E1, E1S, and E2 (r=−0.35, −0.39, −0.37 respectively). The weak correlation between DPD levels and BMI was probably related to low estrogen levels in these women. In other studies we have observed that ERT will decrease bone degradation markers. Osteocalcin revealed a negative correlation between BMI and serum estrogen levels as was expected.

Conclusions: Estrogen levels in untreated PM women may be too low to affect bone degradation markers, although serum osteocalcin may represent a more sensitive indicator of these low serum estrogen levels. Levels of these bone markers do not always correlate with serum estrogen levels and other osteoporosis risk factors may play a significant role in regulation of these substances.

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Serum Leptin Changes and Relationship to Pregnancy Weight Gain. M. A. Franken, V. Vas, T. Gimpel. Department of Ob/Gyn, Texas Tech University Health Sciences Center, Amarillo, TX.

Objectives: In an earlier study, we have observed that serum leptin levels increased in pregnancies with normal weight gain. The obstetrical risk of excessive weight gain has been well documented. In the present study we wish to examine serum leptin changes throughout pregnancy with different degrees of weight gain.

Design: Normal singleton pregnant women were followed through pregnancy (n = 31). Body weight was recorded at each prenatal visit and blood samples obtained roughly every 4 weeks for serum leptin determination. Baseline body weight and calculated BMI (body mass index) were based on pre-pregnancy weight and height (or earliest pregnancy weight available). Subjects were divided by weight gain (< 20 lbs, n=10; 20–30 lbs, n=12; > 30 lbs, n=9).

Materials and Methods: Serum was assayed for leptin using a highly specific and sensitive (0.25 ng/ml) IRMA method (Diagnostic Systems Lab., Webster, TX).

Results: In those subjects with the lowest weight gain, who tended to be obese, there was generally a decline in serum leptin levels throughout pregnancy. In the group with the intermediate weight gain during pregnancy, there was an increase in serum leptin throughout the course of pregnancy. In the group with the highest weight gain, there was an increase in serum leptin which declined slightly over the last 10 weeks of gestation.

Conclusions: Changes in serum leptin are related to levels of weight gain in pregnant women. Further studies are needed to determine any relationship of leptin to increased obstetrical risk.

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Intercycle Variability of Day 3 Serum FSH Levels in Normal Eumenorrheic Young and Older Women. T. Jain, D. M. Lee, N. A. Klein, M. R. Soules. Division of REI, Department of Ob/Gyn, University of Washington, Seattle, WA.

Objectives: Early follicular phase FSH (and estradiol) levels are frequently used to approximate ovarian follicular reserve in the clinical setting. The intercycle variability of day 3 FSH levels has been incompletely characterized. This variability is significant since values obtained from a single cycle are frequently used to counsel patients regarding their reproductive potential and probable response to ovulation inducing drugs (e.g. ART).

Design: Prospectively assess the consecutive cycle to cycle variability of day 3 FSH levels between normal women aged 20–25 (control group, n=25) and women aged 40–45 (study group, n=31).

Materials and Methods: All women were required to be eumenorrheic, ovulatory, in good health, not on medications, and without endocrine problems or infertility. Blood samples from these subjects were obtained by venipuncture on day 3 of two consecutive menstrual cycles (cycles 1 & 2) between 0700 and 1000 h. Serum was separated and frozen in aliquots at −20°C for subsequent analysis. FSH levels were determined in a 2-site monoclonal ELISA assay (Delfia, Wallac Inc.). For inclusion purposes, all subjects had a simultaneous day 3 estradiol level < 80 pg/ml. Coefficient of variation (CV) was used to assess the variability between cycle 1 and cycle 2 levels of day 3 FSH for each subject. A two-tailed t-test was used to compare the mean CV and FSH levels between the young and older subject groups.

Results:

	Age 20–25 mean +/− SD (n=25)	Age 40–45 mean +/− SD (n=31)	P value (two-tail)
FSH (Cycle 1)	5.8 +/− 1.3	10.4 +/− 4.6	0.000006
FSH (Cycle 2)	5.6 +/− 1.6	10.5 +/− 5.3	0.00002
CV	0.09 +/− 0.06	0.22 +/− 0.19	0.002

The upper limit of normal day 3 FSH (2 SD above the mean) based on the control group is 8.8 mIU/ml. In subjects aged 20–25, all day 3 FSH levels were < 8.8 mIU/ml. In subjects aged 40–45: 1) 48% of cycle 1 & 52% of cycle 2 day 3 FSH levels were > 8.8 mIU/ml, 2) 39% of the subjects had a day 3 FSH > 8.8 mIU/ml in both cycles, and 3) 23% of subjects had a day 3 FSH level ≤ 8.8 mIU/ml in one cycle followed by a day 3 FSH level > 8.8 mIU/ml in the next cycle (or vice versa).

Conclusions: 1) The upper limit of normal day 3 FSH is 8.8 mIU/ml in young eumenorrheic women. 2) Day 3 FSH varies by 22% between consecutive menstrual cycles of normal eumenorrheic women aged 40–45. This variability is significantly different from the 9% variability of the control group of women aged 20–25. If the initial day 3 FSH level in an older woman is normal, then a second level should be obtained in a subsequent cycle.

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Relationship of Chlamydia Antibody Titers to Tubal Disease in Unexplained Infertility. [1–3]B. Gocial, [3]A. Cheng, [1–3]J. N. Gutmann, [3]L. W.

Thomson, [1–3]S. L. Corson. [1]Women's Institute for Fertility, Endocrinology and Menopause, [2]Thomas Jefferson University, and [3]Pennsylvania Hospital, Philadelphia, PA.

Objectives: This study was designed to determine if testing for exposure to chlamydia might add useful information to an infertility work up in a population at low risk for tubal disease. Low risk was defined as those patients with a normal pelvic ultrasound, normal hysterosalpingogram, and a negative sexual history of having been infected with chlamydia or other sexually transmitted diseases.

Design: Retrospective chart review of patients with primary or secondary infertility from a private infertility practice.

Materials and Methods: Medical records from January 1, 1994 to December 31, 1997 were reviewed and those patients who met selection criteria were included. All patients had serologic assays for chlamydia antibody determined as a routine study during their infertility evaluation using a Wampole chlamydia antibody assay kit on a Biowhitaker microplate reader. Chlamydia antibody titers were reported as negative, low, mid, or high positive. The extent of pelvic and specifically tubal disease was determined by laparoscopic findings and compared to the level of chlamydia antibody. Statistical analysis was performed using chi-square analysis as determined by Systat software on an IBM compatible PC.

Results: 100 consecutive patients qualified for inclusion to the study. Comparing chlamydia antibody titers to laparoscopic findings for intrinsic fallopian tube and peritubular disease, a high chlamydia antibody titer proved to be 100% predictive of disease (16/16 patients) compared with negative titers (46/66), low titers (2/5), or mid-positive titers (5/13), $p = 0.002$. Furthermore, intrinsic tubal disease occurred in 68.8% of those with high antibody titers compared with 19.7% with negative titers and zero percent with low or mid-level titers, $p = 0.001$. Differences in the comparison between peritubular adhesions and chlamydia antibody titers did not reach statistical significance $p = 0.64$.

Conclusion: Determinations of chlamydia antibody titers in a low risk population of patients with unexplained infertility aids in the diagnostic work up when high titers are found. Patients with tubal disease can be identified even when there is no historic or other objective evidence suggestive of tubal factor infertility. The hysterosalpingography and/or pelvic ultrasound is not adequate alone or in combination to ensure an absence of tubal disease. Assessment of prior chlamydia exposure by serologic assay should be a routine part of the initial infertility workup.

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Intramural Leiomyomata Not Associated With Lower Pregnancy or Implantation Rate Following In Vitro Fertilization (IVF). J. H. Check, C. Dietterich, J. K. Choe, D. Lurie. UMDNJ, Robert Wood Johnson Med. School at Camden, Cooper Hosp./Univ. Med. Cntr., Dept. OB/GYN, Div. Repro. Endo. & Infertility, Camden, NJ.

Objectives: To confirm or refute recent conclusions that intramural fibroids reduce pregnancy and implantation rates following IVF-embryo transfer (IVF-ET).

Materials and Methods: Prospective matched-control study (age and number of embryos transferred in next patient without any fibroids) comparing clinical and ongoing pregnancy and implantation rates.

Results:

	Intramural Uterine Fibroids (n=35)	Control Group (n=35)
Total number of cycles	43	48
Average age	36.5	36.5
Pregnancy rate (clinical)	41.9% (18/43)	41.7% (20/48)
Implantation rates	18.05% (24/133)	18.30% (28/153)
Outcomes		
Chemical/ectopic	3	2
Ongoing/delivered	10 (23.3%)	15 (31.25%)
Spontaneous abortion	5	3

Conclusions: These data do not suggest that intramural leiomyomata adversely affect pregnancy or implantation rates following IVF-ET. A more extensive study is needed to see if the trend for a highest spontaneous abortion rate in those with intramural fibroids can be substantiated.

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A Prospective Study of Intrauterine Insemination: Immediate Discharge Versus Delayed Discharge. A. Saleh, S. L. Tan, M. Biljan, T. Tulandi. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada.

Objective: To compare the fecundity rate after intrauterine insemination (IUI) and immediate discharge and IUI and 10 minutes lying supine.

Design: Fifty six participants with unexplained infertility were prospectively randomized into two groups: Group I, Ovarian stimulation with clomiphene citrate (CC), IUI and immediate discharge. Group II, Ovarian stimulation with CC, IUI and 10 minutes lying supine.

Materials and Methods: A total of 56 couples attending the McGill Reproductive Center for husband's sperm IUI as the treatment of unexplained infertility. After informed consent, couples were randomized into two groups using a computer generated random table numbers with sealed envelops at the time of insemination. Group I (n = 28): IUI and immediate discharge. Group II (n = 28): IUI and lying supine for 10 minutes before discharge. Each participant had a maximum of three treatment cycles.

Result(s): Despite normal semen analysis, three patients were excluded due to post-washed sperm concentration of <5million/ml. One couple went for invitro fertilization and four couples had spontaneous pregnancies. They were not included in the analysis. Group I: consisted of 23 couples (43 cycles) and group II: consisted of 25 couples (51 cycles). One pregnancy was found in group I (1/23): pregnancy rate (PR) of 4.3% per couples and 2.3% per cycle. 5 pregnancies occurred in group II (5/25): PR 20% per couples and 9.8% per cycle. There was no significant difference in PRs between the two groups.

Conclusion(s): Our preliminary results showed a trend of lower PRs in immediate discharged patients following IUI.

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Preliminary Experience With Metformin Treatment of Polycystic Ovary Syndrome: Reproductive Outcomes Among 20 Consecutive Patients. [1]E. S. Sills, [2]D. P. Levy, [3]K. M. Wittkowski, [1]M. Perloe. [1]Atlanta Reproductive Health Centre, Atlanta, Georgia USA; [2]Center For Reproductive Medicine & Infertility, Dept. of Obstetrics & Gynecology, New York Presbyterian Hospital-Cornell Medical Center, New York, New York USA; and [3]General Clinical Research Center, The Rockefeller University, New York, New York USA.

Objective: To describe reproductive outcomes following treatment of polycystic ovary syndrome (PCOs) using Glucophage/metformin.

Design: Observational clinical report.

Materials & Methods: Twenty anovulatory or oligoovulatory women presenting for infertility evaluation were found to have PCOs as defined by clinical examination, ultrasonographic assessment of ovaries, and/or laboratory evidence of hyperandrogenism. Fasting serum insulin and glucose levels were determined before therapy and patients were stratified by body mass index (BMI). No patient was diabetic, and PCOs was established as the only infertility factor in all patients prior to treatment. After obtaining informed consent, oral therapy with metformin (850mg/d bid) was initiated according to a supervised incremental dosing schedule. Except for one patient with artificial insemination, treatment was followed by timed intercourse in all cycles. In 2 patients with evidence of high androgens, supplemental decadron therapy was administered; treatment of 3 patients included "ovarian drilling."

Results: Pre-treatment patient characteristics and outcomes are summarized below. Following metformin administration, all 20 patients achieved clinical pregnancy (including 8 uncomplicated deliveries). Median [IQR 25;75] time to conception after metformin therapy was 3.0 [1;6] months. In the >32 BMI group, prolonged metformin therapy was necessary to establish pregnancies and reproductive losses were higher. Only one patient experienced untoward GI symptoms while on metformin, but severity was not sufficient to cause discontinuation of the drug.

median [IQR 25;75]	age (yrs)	infertility (yrs)	fasting insulin (μu)
BMI[2] ≤32 (n=10)	30.9 [29;33]	2.8 [2.0;3.0]	13.0 [9;16]
BMI >32 (n=10)	32.1 [27;33]	3.0 [2.0;4.8]	30.0 [15;40]
p (WMW/U-test)[3]			0.009

median [IQR 25;75]	androstenedione (ng/dL)	FBS[1] (mg/dL)	Metformin (months)	pregnancy losses
BMI[2] ≤32 (n=10)	234 [157;281]	86.0 [79;95]	1.5 [1;3]	10%
BMI >32 (n=10)	185 [137;240]	91.5 [84;99]	5.5 [2;7]	40%
p (WMW/U-test)[3]	0.165	0.042	0.042 (exact)	0.366

IQR=interquartile range [1] fasting serum glucose [2] body mass index (kg/m^2) [3] Wilcoxon-Mann-Whitney U-test

Conclusion: Pregnancy was established for all patients in this group receiving metformin therapy for PCOs; conceptions were achieved within six months of treatment for most patients. Pregnancies occurred irrespective of BMI or age, but lower BMI was associated with earlier conceptions and better reproductive outcomes. Initial treatment experience with metformin treatment of PCOs appears encouraging, although larger controlled clinical trials are needed to confirm these initial results.

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Increased Risk of NIDDM, Arterial Hypertension and Coronary Artery Disease in Perimenopausal Women With a History of Polycystic Ovary Syndrome: A Survey of 28 Women Age-Matched With 752 Controls. [1]D. Cibula, [2]R. Cifková, [1]M. Fanta, [1]J. Bartošová, [1]J. Kobilková, [4]R. Poledne, [1]J. Živný, [3]J. Skibová. [1]Department of Obstetrics and Gynecology, General Teaching Hospital, Charles University, Prague, [2]Department of Preventive Cardiology, Institute for Clinical and Experimental Medicine, Prague, [3]Department of Statistics, Institute for Clinical and Experimental Medicine, Prague, [4]Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, Prague, CZ.

Objective: To determine the prevalence of non-insulin dependent diabetes mellitus, arterial hypertension, coronary artery disease, and the risk factors for these diseases in perimenopausal women with a history of PCOS treatment.

Setting: Department of Obstetrics and Gynecology, General Teaching Hospital, Charles University, Prague, Czech Republic.

Design: A group of 28 women were selected according to strict inclusion criteria from a large group of patients who had undergone wedge ovarian resection in our department. 752 controls were selected by age (45–59 years) from a female population random sample.

Methods: Physician-completed questionnaire, clinical examination, fasting venous sampling.

Results: Although the control group was selected exclusively on the basis of age range, there was no difference between the two groups in BMI, waist circumference, WHR, proportions of BMI > 28.9, or proportions of women with WHR > 0,85. Both groups were found to have identical family histories of NIDDM, hypertension, and coronary artery disease and identical smoking habits. We did not find a difference between the mean levels of lipids and fasting glucose levels. The two groups did not differ in the proportions of women with elevated lipid levels. The prevalence of NIDDM and coronary artery disease was significantly higher in women with PCOS. The difference in the prevalence of hypertension did not reach statistical significance.

Conclusion: Women in the general population have the same level of risk factors in perimenopausal age as women with PCOS. Patients with clearly pronounced clinical symptoms of PCOS create a subgroup in general population at high risk for developing non-insulin dependent diabetes mellitus, coronary artery disease and arterial hypertension.

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The Expression of Apoptosis-Related Genes and the Presence of Apoptosis in Human Granulosa-Luteal Cells Under the Regulation by Gonadotropin-Releasing Hormone (GnRH) Analogue. H. F. Chen, H. N. Ho, J. C. Shu, Y. S. Yang. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei 100, Taiwan.

Objectives: The growth and atresia of ovarian follicles have been confirmed to be regulated by apoptosis in animals. However the role of apoptosis process has not been as well studied in human ovary. Since gonadotropins and GnRH analogue (GnRH-a) are frequently used in ovulation induction and that these hormones may play paracrine and autocrine roles in human follicles, it is therefore clinically important to identify the effect of these hormones on the apoptosis of human ovary. To address this issue, the expression of apoptosis-related genes and the apoptosis process in human granulosa-luteal cells were examined, especially when under the treatment with gonadotropins and GnRH-a.

Design: The expression of pro- and anti-apoptosis genes and the presence of apoptosis were examined in fresh and cultured human granulosa-luteal cells (GLC) co-treatment with gonadotropins and GnRH-a.

Materials and Methods: Human GLC were obtained during oocyte retrieval in *in vitro* fertilization programs. The cells were examined when freshly collected or after in vitro culture for 2–5 days in the presence of various doses of FSH, LH and GnRH-a. The transcripts and protein production of apoptosis-related genes (Bcl-2, BAD, Fas, Fas L, Bcl-x, P53) were examined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting. Presence of apoptosis was examined by TdT-mediated 3′-end labeling of fragmented DNA (TUNEL method) and subsequently checked either with flowcytometry or fluorescence microscope.

Results: Fresh human GLC expressed predominantly Fas and Fas L both at the transcript and protein levels, but apoptosis was not apparent at this point (5%). In vitro treatment with GnRH-a significantly increased the apoptosis after 5 days (35% compared to 23% in controls) in a dose-dependent manner, but did not significantly alter the expression of apoptosis-related genes. In contrast, LH and FSH both decreased apoptosis (12% and 16%, respectively), but this effect was counteracted by concomitant GnRH-a treatment. Again LH and FSH did not alter apoptosis gene expression.

Conclusions: 1) Apoptosis process is present in human GLC and these cells express pro-apoptosis genes Fas and Fas L, suggesting the major role of Fas system in regulating apoptosis in human follicles. 2) GnRH-a increases but gonadotropins decreases the apoptosis in human GLC. This indicates that apoptosis is likely a hormonally-controlled process functioning in human follicles to regulate the development of granulosa cells, at least at the postovulatory stage. Further study is ongoing in this lab to examine the granulosa cells at the pre-antral and antral follicles. This work was supported in part by grants from the National Science Council of R.O.C. and National Taiwan University Hospital.

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Identification of Alternately Spliced Luteinizing Hormone Receptor Messenger RNA in Granulosa Cell Tumors of the Human Ovary. M. M. Reinholz, M. A. Zschunke, P. C. Roche. Department of Experimental Pathology. Mayo Clinic and Foundation, Rochester, MN 55905.

Objectives: Luteinizing hormone (LH) is a glycoprotein and is involved in hormone-dependent signal transduction via its membrane-associated receptor and the G-protein/adenylyl cyclase system. Messenger RNA (mRNA) for the LH receptor undergoes alternate splicing and multiple isoforms have been reported in a number of species except for the human. This study characterizes the alternately spliced forms of LH receptor mRNA for the normal human ovary as well as for human granulosa cell tumors.

Design: LH receptor mRNA isoforms in normal human ovary and granulosa cell tumors were characterized by RT/PCR and direct DNA sequencing.

Materials and Methods: Normal human ovarian tissue and 12 granulosa cell tumors, considered to be surgical waste, were obtained with approval of the Institutional Review Board at the Mayo Foundation. Total RNA was then isolated from the tissue. Messenger RNA was reverse transcribed to cDNA and amplified by PCR with specific primers to the human LH receptor. Two PCR reactions were performed that included sequences in exons I through X and in exons X through XI. The PCR products were separated using agarose gel electrophoresis. The DNA was then extracted and sequenced.

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The Role of the Endothelium in the Ovarian Hyperstimulation Syndrome (OHSS). C. Albert, N. Garrido, A. Mercader, J. Remohí, C. Simón, A. Pellicer. Instituto Valenciano de Infertilidad and Department of Obstetrics/Gynecology, Valencia University School of Medicine, Valencia, Spain.

Objective: OHSS is an iatrogenic and potentially life-threatening complication of treatment with fertility drugs. Certain vasoactive substances produced in response to hCG during induction of ovulation initiate the cascade of events resulting in the syndrome. In previous studies we observed that supraphysiological E_2 and hCG levels stimulate IL-6 and VEGF secretion by endothelial cells. Our aim was to analyze the kinetic of endothelial IL-6 and VEGF production in response to hormonal stimulation.

Design: Time-course experiments with 10^{-4} M E_2 and 1000 IU hCG, in human endothelial cells in culture. VEGF and IL-6 levels were determined by ELISA.

Materials and methods: Human lung microvascular endothelial cells (HLMEC) purchased from Clonetics^R, were grown in endothelial cell growth medium (Promocel) until they reached 70–90% of confluence. Then we added 10^{-4} M E_2 and 1000 IU hCG and cells were cultured for 48 hours. The conditioned media and controls without hormonal stimulation were collected at 0, 3, 6, 12, 24 and 48 hours.

Results: Endothelial VEGF secretion was immediately stimulated (2–3 min) after E2 and hCG administration. Then VEGF production decreased significantly in a time-course manner ($p<0.05$). Unlike VEGF, endothelial IL-6 increases at 3 to 6 h after hormonal stimulation, peaking at 48 hours ($P<0.05$).

Conclusions: Our results showed that high E_2 and hCG levels induce immediate secretion of VEGF by endothelial cells, suggesting that these hormones mediates VEGF secretion by endothelial cells. VEGF levels were decreased while IL-6 secretion in human endothelial cell culture was increased in a time-course manner and may be VEGF stimulate IL-6 secretion. Therefore, we consider the possibility that VEGF initiate the cascade of events resulting from hCG administration in OHSS. Supported by Ares-Serono Foundation.

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Expression of Aryl Hydrocarbon Receptor (AHR) and Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) in the Female Reproductive Tract. O. Khorram, M. Garthwaite, T. Golos. Department of Obstetrics and Gynecology, University of Wisconsin, Madison, WI, USA.

Objectives: Recent emerging evidence suggests that environmental contaminants such as dioxin can act as endocrine disrupters through inappropriate modulation of target responses to hormones. These compounds produce their biological and toxicological effects by binding to the AHR. Binding of AHR by agonists like dioxin initiates a series of events resulting in dissociation of hsp90, movement into the nuclear compartment and hetrodimerization with another protein termed ARNT. The resulting AHR-ARNT complex binds to cis-acting DNA sequences called dioxin-response elements to modulate transcription of a number of genes such as drug metabolizing enzymes, estrogen receptor, and growth factors like IL-1β, TGF-α and TGF-β. The goal of this study was to characterize the expression of AHR/ARNT in the female reproductive tract and elucidate their potential function in human reproduction.

Design: Reproductive tissues were obtained from women undergoing total hysterectomy and bilateral salpingoophorectomy for benign gynecologic disease.

Materials and Methods: Following homogenization and extraction of RNA, ribonuclease protection assay (RPA) was used to quantify the AHR and ARNT mRNA levels. Immunohistochemistry using polyclonal antibodies to AHR and ARNT were used to determine the tissue localization of these proteins.

Results: AHR and ARNT mRNA were readily detectable in the endometrium, myometrium, ovary, fallopian tube and placenta. The highest expression of mRNA for AHR/ARNT was found in the fallopian tube followed by the placenta, ovary and uterine tissues. IHC revealed both AHR and ARNT were present predominantly in the endometrial glands in the basiglandular areas and luminal surface of the epithelium. In the myometrium a diffuse distribution in the myocytes, and in tunica media of spiral arterioles was found. A statistically significant variation in endometrial AHR and ARNT mRNA was not found during the menstrual cycle, although there was a trend for higher AHR expression in the proliferative phase and in specimens with adenomyosis ($P=0.1$). Greater expression of AHR was found in the endometrium of postmenopausal women treated with continous hormone replacement therapy as compared to women on no hormones ($P=0.01$).

Conclusions: The differential tissue specific expression of AHR/ARNT in the reproductive tract suggests a physiologic role for these proteins in reproductive processes, and in pathologic processes such as adenomyosis. Exogenous sex steroids upregulate AHR but not ARNT in the endometrium.

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Calcium Imaging of Transiently Transfected Human Oxytocin Receptors in HEK-293 Cells. D. P. Cohen, Z. Li, E. Stein, L. C. Layman. University of Chicago, Division of Reproductive Endocrinology and Infertility, Chicago, IL.

Objective: The human oxytocin receptor (hOTR) is a guanine nucleotide binding protein-coupled receptor with seven transmembrane domains. It couples to G_q and provokes inositol phosphate intracellular second messengers that lead to the release of intracellular calcium after oxytocin binds. The purpose of this study is to assess the ability of the hOTR to couple to calcium release in HEK-293 cells, to provide a means to study hOTR signal transduction.

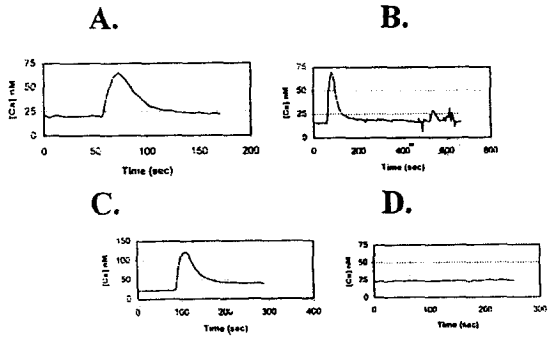
Design: A transient transfection system. hOTRs are identified in vitro after incubation with Fura-2, a calcium binding molecule that absorbs light at different wavelengths.

Materials and Methods: HEK-293 cells were transfected using the Ca-phosophate method. Cells were seeded onto 10 cm tissue culture dishes (1.5E5 cells/dish) and transfected overnight with HBSS/Ca-phosphate containing the oxytocin or bradykinin receptor cDNA (+ control). The next morning the dishes were rinsed with HBSS and 10% FBS-containing DMEM was added. For imaging, the cells were seeded onto coverslips 6 hours later and used the next day. Light emission at 590 nM was recorded every 2 seconds following ligand application and the ratio of bound to free Fura-2 assessed.

Results: Confirmation of oxytocin receptor-specific calcium release was documented. The data are presented in figure 1.

FIGURE 1

Intracellular calcium curves generated in response to 1 μM oxytocin (A and B), 100 nM bradykinin (C) and 1 μM oxytocin in mock transfected cells (D). All transfections used a calcium phosphate protocol and HEK-293 cells. Intracellular calcium measurements were obtained following incubation with Fura-2 and image capture of fluorescent emission.



Conclusion: hOTR can be identified at the cell surface following transient transfection into HEK-293 cells, providing a technique to elucidate the molecular mechanisms of hOTR signal transduction.

P-311

Expression of Receptor Genes for Gonadotropins and Estrogens, and Biosynthesis of Estrogen in Mid-Gestational Stage of Human Ovarian Tissues. [1]B. R. Do, [1]B. G. Yang, [2]C. J. Lee, [2]Y. H. Lee, [1]S. J. Yoon, [1]S. I. Roh, [2]Y. D. Yoon, [1]H. S. Yoon. [1]Infertility Research Center, Jeil Women's Hospital, [2]Department of Biology, Hanyang University, Seoul, Korea.

Objectives: At present, it is not fully demonstrated the effects of gonadotropins and steroids on the primordial follicle formation and ovarian morphogensis. In human ovary, the peak number of germ cells was reached at about 20 week of gestation. At this stage, most of oogonia developed into primordial follicles, and interestingly, the levels of pituitary gonadotropins were the highest among the entire fetal life. It has been reported that from the eight week of gestation, human fetal ovary has aromatization capacity and synthesized estrone and estradiol. However, before follicular differentiation, action mode of gonadotropin and the regulation factor of steroidogenesis are not clear in human fetal ovary. This study performed to investigate the action mode of gonadotropin on fetal ovarian folliculogenesis and the relationship between primordial follicular differentiation and estrogen synthesis at mid-gestational stage in human fetal ovary.

Design: The activities of steroidogenesis were determined. And gene expression of FSH receptor, LH receptor, Estrogen receptor and aromatase were analyzed by RT-PCR, and also the localities of mRNA of these genes were observed in ovarian tissue by PCR *in situ* hybridization method.

Materials and Methods: Human fetal ovarian tissues were used on this experiment at gestational stage 18, 19, 21, 21.5, 22 and 31 weeks. Ovarian tissues were sliced and cryopreserved by modified PROH two step method until use. After thawing, the ovarian tissue were cultured in TCM-199 supplemented with ITS (5pg/ml insulin, 5pg/ml transferrin and 5ng/ml selenium), 0.23 mM pyruvate, 0.6% BSA and with or without 10 mIU/ml recombinant human FSH. After 1–3 weeks cultivation, the concentration of estradiol (E) synthesized were determined by radioimmunoassay. Gene expression of FSH receptor (FSH-R), LH receptor (LH-R), estrogen receptor α (ERα), ER β and aromatase (AR) were analyzed by RT-PCR. Also localization of these gene expression were observed using PCR *in situ* hybridization.

Results: Concentration of estrogen was increased by supplementation of FSH. Genes of FSH-R, LH-R, ERα, ERβ, and AR were expressed from all of the fresh 18, 19, 22 week human fetal ovarian tissues. Localization of FSH-R, LH-R, E-Rα, E-Rβ, and AR genes detected by PCR *in situ* hybridization were in fibroblast-like formed interstitial cells (outer portion of sheath of ovigerous cord), but no gene expression was detected in germ cells or other portion of tissues in 21–22 week human fetal ovarian tissues. Especially, FSH-R was strongly detected in all 18–22 week human fetal ovarian fibroblast-like interstitial cells. Primordial follicles was abundantly observed in 21.5 and 22 week fetal ovarian tissues. And at this stage, genes for FSH-R, LH-R, E-Rβ, and aromatase were expressed in the differentiated primordial follicular granulosa cells (GC) near the fibroblast-like interstitial cells, but no gene expressions were observed in germ cell cord and primordial follicular oocytes.

Conclusion: FSH affects the estradiol biosynthesis in fetal ovarian tissues of mid-gestational stages in vitro. Gene expression of FSH-R, LH-R, E-Rα, E-Rβ, and AR were detected in undifferentiated fetal ovarian tissues and location of gene expression site was not a portion of germ cell or germ cell nest, but interstitial cells. Therefore it can be supposed that mid-gestational stage of human fetal ovarian interstitial cells may be affect to regulate the ovarian morphogenesis and primordial follicle formation by modulation of FSH-FSH-R and estrogen synthesis.

P-312

Pregnancy Associated Plasma Protein A (PAPP-A) and Free Beta hCG Throughout Pregnancy: A Cross-Sectional and Longitudinal Study of New Methodologies. V. D. Castracane, B. A. Tawwater, T. L. Gimpel. Department of Obstetrics/Gynecology, Texas Tech University Health Sciences Center, Amarillo, TX, USA.

Objectives: Recent interests in both the levels of PAPP-A and free β hCG have resulted in new diagnostic testing available for these substances.

Design: In the current study we have utilized new commercially available sensitive ELISA kits for the assay of PAPP-A and free β hCG in serum (Diagnostic Systems Lab., Webster, TX). Cross-sectional study for both PAPP-A (n = 107) and free β hCG (n = 122) using serum samples from normal singleton pregnancies was carried out to establish the range of normal values in this large population. Samples were uniformly distributed from 5 to 40 weeks of gestation. In addition, longitudinal samples obtained at frequent intervals through 3 normal singleton pregnancies were analyzed to study the pattern of both PAPP-A and free β hCG. Samples were obtained from 5 weeks of gestation through term and sometimes postpartum. Luteal phase samples from normally cycling women were assayed to establish nonpregnant values.

Results: Serum PAPP-A levels in cross-sectional samples are undetectable in the nonpregnant luteal phase for the first few weeks of gestation. Levels rise continuously through pregnancy starting at 8–9 weeks and reaching levels as high as 50 mIU/ml in some subjects in the weeks before delivery. The same pattern is confirmed in a series of 3 longitudinally sampled pregnant subjects. Serum free β hCG is also undetectable in nonpregnant samples and is detectable as early in pregnancy as 4–5 weeks, to reach peak levels of about 100 mIU/ml at about 8–12 weeks of gestation and generally declines to low levels in late pregnancy by 15 weeks through term, although an occasional sample may still have moderately elevated levels in late pregnancy. This pattern is also confirmed in longitudinally sampled pregnant subjects where the peak level is more clearly defined being between 8 to 10 weeks. The pattern for free β hCG parallels that for intact hCG in these longitudinally sampled pregnant individuals.

Conclusions: These new sensitive specific assays are rapid and convenient to measure serum levels of PAPP-A and free β hCG throughout pregnancy and will be invaluable in understanding their role and diagnostic utlity during gestation.

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There is No Effect of Age or Estrogen on the Gender Differences in Morning Growth Hormone (GH) Levels. V. D. Castracane, B. A. Tawwater, T. L. Gimpel. Department of Obstetrics/Gynecology, Texas Tech University Health Sciences Center, Amarillo, TX, USA.

Objectives: It has been reported that morning serum values of GH are higher in young females than in young males (Engstrom, et al 1998, Clin Chem 44:6, 1289). There is extensive literature on the stimulatory effect of estrogens on serum GH levels and this might account for the higher levels in female subjects.

Design: In order to study this effect further, we have examined morning (0800–1000 hours) GH levels in young ($\leq$40 years) males (n = 45), untreated females (n = 32) and females on E/P OC's (n = 38). We have also studied postmenopausal (>50 years) females without ERT (n = 35) or with ERT (n = 48) and older males (>50 years) (N = 45).

Materials and Methods: Serum was measured with a sensitive IRMA chemiluminescent assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA) with a sensitivity of 0.05 ng/ml.

Results: In young males, the median value of serum GH levels was always less than the sensitivity of the assay (0.05 ng/ml) whereas in young females, either with or without oral contraceptives, levels were almost always elevated above that value with medians of 0.235 and 0.0575 ng/ml respectively. In older males, the median value for serum GH was 0.06 ng/ml. In older females without ERT or with ERT, the median values were 0.63 and 0.68 ng/ml respectively.

Conclusions: These studies demonstrate that in males at any age group, a morning blood sample is almost always below the sensitivity of this very sensitive assay. The median values in females are always higher with very few females below the sensitivity of the assay. These results indicate that estrogen would not seem to be the causative factor for these levels of GH that are several fold higher than levels in males. Similarly, the morning levels of GH do not change with age in either males or females. These studies demonstrate that females have higher basal levels of GH than males and that this is unrelated to age or to estrogen administration in these groups. The nature of other gender related factors that might be responsible for changes for gender differences in GH levels warrants further investigation.

P-314

Intravenous Immunoglobulin (IVIG) Therapy for Patients with Phospholipid Antibodies Undergoing IVF: Does it Improve Pregnancy Rates? V. Sahakian. Pacific Fertility Medical Center, Los Angeles, CA.

Objective: Data has shown that patients with the Antiphospholipid Antibody (APA) syndrome might suffer from lower fecundity rates and higher spontaneous pregnancy losses. Similarly, it has been suggested that Intravenous Immunoglobulin (IVIG) therapy in patients harboring some of these antibodies increases IVF success rates. Our objective was to evaluate the effect of IVIG therapy on IVF success rates in patients with serum phospholipid antibodies (phosphoethanolamine and/or phosphoserine).

Design: A retrospective analysis in a private practice setting.

Materials and Methods: Between January and June 1998, patients undergoing IVF whose serum contained antibodies (IgG and/or IgM) against phosphoethanolamine and/or phosphoserine phospholipids ("APA positive") were treated with Aspirin 81 mg/day, Heparin 5,000 U SQ bid, and IVIG 20gms administered one week after the start of Menotropins (Group 1). Between July and December 1998, patients undergoing IVF who tested "APA positive", received Heparin and Aspirin only in similar doses, without IVIG (Group 2). The inclusion criteria were: (1) Age $\leq$ 36 years, (2) No previous IVF cycles (3) Less than 2 previous spontaneous abortions (SAB), (4) Absence of any thyroid antibodies and (5) Cycle day 3 FSH of less than 10. All patients were treated with a luteal phase GnRH agonist (long) protocol and embryo transfer was done 3 days after egg retrieval. Patients who were past 12 weeks of gestation were considered pregnant. Student-t and Chi-square was used for data analysis.

Results: In Group 1, 36 patients met the inclusion criteria versus 27 patients in Group 2. Outcome data is summarized in the table below. The pregnancy rate in Group 1 was 36% versus 44% in Group 2 (p=0.503).

	N	Age	Day 3 FSH	SAB	Pregnancy Rate
Group 1 (IVIG)	36	32.3 ± 3.0	6.6 ± 1.4	0.18 ± 0.39	36%
Group 2 (No IVIG)	27	31.2 ± 2.7	6.5 ± 1.8	0.16 ± 0.32	44%

Conclusion: The addition of IVIG therapy did not increase IVF pregnancy success rates in this population of infertile patients.

P-315

Evaluation of Cytokines (IFN-γ, TNF-α, IL-6 and IL-10) in Exocoelomic Fluid from Normal and Complicated First Trimester Pregnancy. [1]J. A. Lee, [2]B. C. Choi, [2]K. J. Yoo, [2]E. C. Paik, [2]J. H. Song, [1]J. W. Kim, [3]Y. Y. Cho, [2]I. S. Kang. Recurrent Miscarriage Clinic, [1]Infertility Research Laboratory, [2]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Samsung Cheil Hospital and Women's Healthcare Center, College of Medicine, Sungkyunkwan University, [3]Dr. Cho's Women's Clinic, Seoul, Korea.

Objectives: The first trimester human gestational sac contains two physiologic fluid cavities (i.e., the amniotic cavity surrounding the fetus and the extraembryonic coelom between the amniotic cavity and the placental chorionic plate). The semi-allogeneic fetus is in indirect contact with the uterine and blood-borne cells of the mother. Recent reports suggest that a local imbalance of cytokines, in materno-fetal interface, are proposed for immunologic reproductive failure. The goal of our study was to evaluate cytokines in exocelomic fluid, between normal and complicated first trimester human pregnancies.

Design: Cytokine assessment (IFN-γ, TNF-α, IL-6 and IL-10) in exocoelomic fluid.

Materials and Methods: The exocoelomic fluid was obtained by transvaginal puncture, under ultrasonographic guidance, from 12 healthy women with apparently normal pregnancies, who were undergoing termination for psychologic reasons between 7 and 10 weeks of gestation and from 26 women presently with missed abortion. Samples of exocoelomic fluid were frozen at −20°C until assayed. The cytokines were assayed by a two step sandwich enzyme immunoassay technique (Biosource, CA; lower limit of sensitivity, 4 pg/mL for IFN-γ, 1 pg/mL for TNF-α, 2 pg/mL for IL-6, 5 pg/mL for IL-10). The data are presented as mean ± SEM. Statistical analysis was performed by Mann-Whitney U test and *t*-test.

Results: Characterization of cytokines (IFN-γ, TNF-α, IL-6 and IL-10) in exocelomic fluid

	Missed Abortion (n=26)	Surgical Termination (n=12)	*p* value
IFN-γ (pg/mL)	3.86 ± 1.75 (n=5/26)	2.43 ± 1.54 (n=2/12)	NS
TNF-α (pg/mL)	35.70 ± 13.20 (n=26/26)	21.22 ± 7.77 (n=12/12)	NS
IL-10 (pg/mL)	4.53 ± 2.12 (n=8/26)	6.95 ± 5.01 (n=5/12)	NS
IL-6 (pg/mL)	1.85 ± 0.72 (n=6/26)	0.17 ± 0.15 (n=1/12)	0.004

NS; Not significant.

In our study, the exocoelomic fluid from the majority of normal pregnancies was related to Th2 cytokine (IFN-γ and TNF-α) secretion, whereas Th1 cytokine (IL-6 and IL-10) was related with missed abortion. Furthermore, inflammatory mediated cytokine (IL-6) was significantly increased in missed abortion (P=0.004) and its level depends on the presence of embryo demise in uterus (embryonic pregnancy vs. anembryonic pregnancy; 2.36pg/mL±0.91pg/mL vs. 0.19pg/mL±0.19pg/mL, p=0.02).

Conclusions: These data show that exocoelomic fluid may have a unique immune previlege surrounding developing embryo in early pregnancy. Further studies are needed to determine the growth factors in coelomic fluid from normal pregnancies and missed abortions, and to evaluate the influence on the development of early pregnancy complications.

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Cytokine Profiles in Autologous Peritoneal Fluid and Peripheral Blood of Women With Deep and Superficial Endometriosis. [1,2]T. M. D'Hooghe, [1]L. Xiao, [2]P. R. Koninckx, [1]J. A. Hill. [1]Fearing Research Laboratory, Division of Reproductive Immunology, and Division of Reproductive Medicine, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, USA; [2]Department of Obstetrics, and Gynecology and Leuven University Fertility Center, University Hospital Gasthuisberg, B-3000 Leuven, Belgium.

Objective: Preliminary study to test the hypothesis that cytokine profiles in peripheral blood were higher in women with deep infiltrating endometriosis and cytokine profiles in peritoneal fluid were higher in women with superficial endometriosis.

Study Design: Case-control study.

Materials and Methods: Thirteen women of reproductive age having laparoscopy for infertility (n=9), pain (n=3) or combined pain and infertility (n=1). Peripheral blood and peritoneal fluid were obtained and analyzed by ELISA kits for Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Interleukin-10 (IL-10), Transforming Growth Factor-beta1 (TGFβ1), and Interferon-gamma (IFN-γ).

Results: No significant cytokine differences were observed in either peritoneal fluid or peripheral blood between IL-6, TGFβ1, IFN-γ, TNF-α and IL-10 of women with superficial endometriosis and women with deeply infiltrating endometriosis.

Conclusion: The results of this preliminary study do not show significant differences in peripheral blood and peritoneal fluid cytokine levels between women with deep infiltrating endometriosis compared to women with superficial disease. Future studies with increased sample size are required to either confirm or refute these preliminary findings. This work was supported by the Collen Research Foundation/Faculty of Medicine, University of Leuven, Belgium; grants HD00915, HD023547 and AI38515 from the National Institutes of Health, Bethesda, MD, USA; Fearing Research Laboratory Endowment, Department of Obstetrics/Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, USA.

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Assessment of the Human Endometrial Production of Leukemia Inhibitory Factor by a Simple Method of Uterine Flushing and Correlation With Endometrial Production of Explant in Culture. [1]N. Lédée, [2]G.

Laprée-Delage, [3]J. L. Taupin, [2]S. Dubanchet, [3]J. P. Moreau, [1]S. Hamamah, [1]R. Frydman, [2]G. Chaouat. [1]Department of Obstetrics-Gynecology and IVF Center, Antoine Béclère Hospital, Clamart, France, [2]Inserm U131, Clamart, France, [3]CNRS, UMR 5540, Bordeaux, France.

Objectives: Leukemia inhibitory factor (LIF) is a pleiotropic cytokine with a remarkable range of biological actions in various tissue system. In the murine model, LIF has been shown to be one of the essential cytokine for implantation. In human endometrium, LIF is expressed in a menstrual cycle-dependent manner with a maximal expression coinciding with the time of implantation. LIF production index measured in supernatants of explant culture were significantly lower in infertile women than in fertile women. Uterine flushing and endometrial biopsy were performed in an infertile population to address if a correlation could be established between those two data.

Design: Uterine flushing and endometrial biopsy were performed during laparoscopy for infertility, during surgery for other purpose in fertile women independently of the cycle and under oestroprogestative substitution in the late luteal phase for women undergoing a preimplantation cycle in a program of oocyte donation.

Material and Methods: Uterine flushing was performed using a catheter for embryo transfer and endometrial biopsy with the classical Cormier pipelle. Production of LIF was assessed on the flushing and on the culture of endometrial explant with an without fetal calf serum (FCS) using an highly sensitive and specific ELISA assay. The Man Whitney U test was used for statistical analysis.

Results: 1) This technic of flushing, easy to perform and unpainfull, allows to detect with our reagents and methods large amount of LIF (0-7469 pg/ml). 2) No correlation could be established between the production of LIF in uterine flushing and in supernatants of explant culture. 3) Significant difference could be observed on the production of LIF detected culture media with FCS between infertile women with and without hormonal substitution ($p=0,0423$).

Conclusion: The absence of correlation between the LIF detected in flushing and in explant cultures suggests another origin than the endometrium for the LIF detected in flushings. Studies are ongoing to refine this working hypothesis. This data also question the use of flushing for assessment of the production of LIF, and raise questions about methodology to be used in clinics.

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The Level of Leukemia Inhibitory Factor in Human Follicular Fluid is Decreased Among Polycystic Ovary Like Responders. [1]N. Lédée, [2]G. Laprée-Delage, [3]J. L. Taupin, [2]S. Dubanchet, [3]J. P. Moreau, [1]N. Frydman, [1]R. Frydman, [2]G. Chaouat. [1]Department of Obstetrics-Gynecology and IVF Center, Antoine Béclère Hospital, Clamart, France, [2]Inserm U131, Clamart, France, [3]CNRS, UMR 5540, Bordeaux, France.

Objectives: Follicular growth and maturation is a complex process that is regulated by autocrine and paracrine/autocrine factors. Numerous studies have revealed that a variety of cytokines are capable of affecting ovarian function and are implicated as regulator of steroid secretion. Leukemia inhibitory factor (LIF) is a pleiotropic cytokine with a remarkable range of biological actions in various tissues systems. Addition of LIF into embryo culture media enhances the blastocyst development and his presence in human follicular fluid with expression of LIF mRNA by human granulosa cells from preovulatory follicles has been reported. Polycystic ovaries like (PCO-like) responders represent a specific ovarian profile for the clinician if compared with normal or poor responder. This ovarian profile seems to be partially related to functionnal abnormalities of granulosa cells. To address if particular expression pattern of LIF could be detected, dosage of LIF in these PCO-like and non PCO-like population were performed.

Design: Concentration of LIF was assessed in pooled follicular fluid collected during oocyte pick-up by a highly sensitive and specific ELISA assay in non PCO-like and PCO-like patients undergoing in vitro fertilization (IVF).

Material and Methods: PCO-like responders have at least 3 of the four criteria: irregular cycle or amenorrhea, LHSH>1, previous hyperstimulation, ultrasound pattern of OPK. Follicular fluid collected during the oocyte pick-up was pooled and concentration of LIF was assessed using an ELISA assay directed on both free LIF and LIF linked to his soluble receptor. 9 PCO-like responders were compared to 39 non PCO like responders. The Man Whitney U test was used for statistical analysis.

Results: In our hands, concentration of LIF in the follicular fluid with our assay reagents and methods is dramatically higher than previously described by others with a median of 653 pg/ml (78-2894). Among 9 PCO-like responders, median of concentration of LIF found is 443 (78-594) pg/ml. This concentration is significantly lower if compared with the median concentration of 718 (240-2894) pg/ml found among the 39 non-OPK-like responders ($p=0,002$).

Conclusion: Significance of those great amount of LIF in the preovulatory follicles still need to be assessed. The difference of LIF concentration between PCO-Like and non PCO-Like responders suggest a specific role of LIF *in vivo* on the aromatase activity of granulosa cells. Studies are ongoing to refine this working hypothesis.

REPRODUCTIVE LABORATORY

Tuesday, September 28, 1999

P-319

FDA Reclassification of Medical Devices Used for In Vitro Fertilization—Implications for the Reproductive Laboratory. R. C. Courtney. Irvine Scientific, Santa Ana, CA, USA.

Objective: Effective October 13, 1998, The United States Food and Drug Administration has reclassified medical devices used for in vitro fertilization (IVF) and related assisted reproduction technology (ART) procedures. Under the Final Rule, the generic type of device, instrumentation intended for use in IVF and related ART procedures is reclassified into class II (special controls), and assisted reproduction microscopes and microscope accessories are classified as class I (general controls). The reclassification directly effects manufacturers of products marketed and promoted in the US for use in IVF/ART procedures. Indirectly, the reclassification also has implications for the Reproductive Laboratory. The implications will be discussed in this presentation.

Design: The Final Rule will be summarized. Special controls developed for affected IVF/ART instrumentation will be summarized for each device category. Emphasis will be given to the information most relevant to decision makers in the Reproductive Laboratory.

Materials and Methods: The principal source material will include: 1. The Final Rule, 21 CFR Part 884, Obstetric and Gynecologic Devices; Reclassification and Classification of Medical Devices Used for In Vitro Fertilization and Related Assisted Reproduction Procedures. 2. FDA Draft Guidance—Devices Used for In Vitro Fertilization and Related Assisted Reproduction Procedures.

Results: Labeling requirements provide the Reproductive Laboratory an understanding of information to expect of the manufacturer. Details of the special controls offer assistance in determining standard of care.

Conclusions: The reclassification has implications to the Reproductive Laboratory both as a consumer and a care provider.

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Embryo Cleavage Rate Improves After Intracytoplasmic Sperm Injection (ICSI) When the Polyvinylpyrrolidone (PVP) Concentration is Reduced From 10% to 7.5%. [1]P. P. Risch, [1,2]F. Akerman, [1,2]A. J. Carrillo, [1,2]C. L. Cook, [1,2]A. C. Eblin, [1,2]S. T. Nakajima, [1,2]M. L. Swanson. [1]Fertility Center at University Obstetrics/Gynecology Associates, P.S.C., [2]Department of Obstetrics/Gynecology, University of Louisville, Louisville, KY.

Objective: To examine the effect of reducing the concentration of PVP on ICSI fertilization, embryo cleavage and pregnancy rates.

Design: Retrospective study of ICSI laboratory and clinical outcomes.

Materials & Methods: Oocytes from 31 patients undergoing IVF-ICSI treatment were divided into 2 groups based on the following concentration of PVP used for ICSI:10% and 7.5%. Patient management and IVF lab procedures were described previously (Fertil & Steril, 70:676, 1998). Ejaculated semen was used in 28 patients and testicular tissue was used in 3 patients. Two of the testicular sperm samples were in the 7.5% PVP group and the other one was in the 10% PVP group. After processing through PureSperm and/or washing, ejaculated sperm pellets were resuspended in P1 medium with 3 mg/ml of HSA. Testicular sample pellets were resus-

pended in Ham's F-10 medium with 3 mg/ml of HSA. Sperm and testicular samples were placed in an incubator with 5% CO_2 in room air until the time of ICSI. Twelve–18 hours after ICSI oocytes were examined for the presence of pronuclei. When there were large numbers of zygotes, some were frozen on that day. Embryo cleavage was determined on the morning of day 2 and 3. Embryos were returned to patients on the morning of day 3. Pregnancy was confirmed 5 weeks after oocyte retrieval by the prescence of an embryo sac and a heart beat.

Results: Reducing the concentration of PVP did not result in a noticable change in our ability to pick up and inject sperm, nor did it result in a significant change in fertilization rate or damage rate. There was a significant increase in embryo cleavage rate on day 2 in the 7.5% PVP when compared to 10%. Most of the zygotes produced with 10% PVP that had not cleaved by day 2 began cleaving on day 3 such that there was no difference in cleavage rate between the two groups on day 3. However, embryos in the 10% PVP group with delayed cleavage had fewer blastomeres on day 3 than embryos that began cleaving on day 2. Reducing the concentration of PVP also resulted in a non significant increase in pregnancy rate.

Group	No. of oocytes	No. of 2PN	No. of >2PN	Fert. rate
10% PVP	189	130	10	74%
7.5% PVP	121	83	4	72%

Group	Damage rate	Cleavage Rate on Day 2	Cleavage Rate on Day 3	Pregnancy rate
10% PVP	10%	84% (91/108)	96% (104/108)	22% (4/18)
7.5% PVP	12%	96% (73/75)*	100% (75/75)	31% (4/13)

*, $p<0.01$ when compared with 10% PVP.

Conclusion: Decreasing the concentration of PVP used for sperm pick up during ICSI from 10% to 7.5% does not interfere with the sperm handling and injection, but does improve embryo development and possibly pregnancy rates. Because of these data we are now using 7.5% PVP for all ICSI procedures.

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A System of Quality Control and Assurance for the Non-Automated Andrology Lab. E. Taylor. Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, The University of Vermont College of Medicine, Burlington, VT, USA.

Objectives: Although a standardized model for quality control (QC) and quality assurance (QA) has not been established, the Joint Commission on Accreditation of Health Care Organization (JCAHO) mandates adequate documentation of systematic controls by labs undergoing inspection. In this abstract we present our system of QC and QA for routine semen analysis.

Design: Inter- and intra-laboratory variation was calculated for count, motility, and morphology using clinical specimens.

Methods and Materials: In our lab one andrologist performs all the semen analyses, roughly 300 per year. Counts and motility are obtained with a Makler chamber. Normal values have been specified by the World Health Organization, 1992. Controls for low (<10, 10–20 million/ml), medium (20–60), and high (>80) were collected from clinical samples with corresponding counts and volumes >3 ml. Aliquots were kept in a −20°C freezer. Control counts are recorded twice a week. Morphology controls were obtained from clinical slides representing low (<30%) and acceptable (>30%) levels of normal forms. Control slides are read prior to reading clinical morphology, usually twice a week. Quarterly the andrologist takes serial counts of density and motility on normal and subnormal clinical specimens to evaluate repeatability. We test inter-observer proficiency and consistency by comparing assessments of count, motility, and morphology on the same samples by both the andrologist and IVF embryologist. For quality assurance, we check the following documentation on all in-house semen analyses: 1) receipt form signed by andrologist, 2) presence of result form in patient's chart, 3) result signed by andrologist and patient's physician.

Results: We constructed a Levy-Jennings chart for each level of counts and normal and abnormal morphology. Values are entered daily onto the charts, and deviations greater than 2 SD noted. Bar graphs are used to display intra- and inter-observer consistency and proficiency. We calculate % signed and charted forms, and put missing forms in the charts.

Conclusions: By using clinical samples as controls, we can document QC for count, motility, and morphology, the most important components of semen analysis. We can also confirm custody of specimens from arrival at the clinic until completion of report. Semen analysis results accurately predict quality of intrauterine insemination for patients who proceed with treatment. These methods provide internal confirmation of the high caliber of our andrology lab, as well as excellent records which JCAHO can use in the recertification process.

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Enhancement of Fertilization Rates After Sperm Immobilization by Laser System Technology (Fertilase) Used During Intracytoplasmic Sperm Injection (ICSI) Technique. S Melo-Abreu, J. Abreu Roriz, C. Ceccatelli. IN-VITRO, Andrology and Assisted Fertilization Clinic of Goiania, State of Goias, Brazil.

Objectives: The Fetilase - laser system technology is primarily aimed to treated gametes from couples who have encountered repeated failured during in vitro fertilization procedures. As a consequence, significant attention has been focused to investigate the efficacy and the time less consume to immobilize the sperm during the micromanipulation intended for use in an intracytoplasmic sperm injection technique (ICSI).

Design: In the present study we evaluated whether laser system (fertilase) technique could minimize the time of the sperm immobilization during ICSI procedure.

Materials and Methods: Samples from male factor patients (n=20) were evaluated for motility, progression and sperm count. Semen samples were divided into two aliquots and washed with modified Human Tubal Fluid Medium (Irvine Scientific), containing 0.5% bovine serum albumin using microcentrifuge (Denver Instrument, Italy) and incubated for one hour at 37°C. Sperm from one aliquot was treated with laser system (Fertilase) and the immobilization of the most motile and normal spermatozoa was obtained instantly by pressing a button which releases a few millisecond laser irradiation in the last portion of the sperm tail. Sperm from the other aliquots was treated using the conventional mechanical sperm immobilization technique. Fertilization rates was compared in all oocytes treated with that two different techniques.

Results: Oocytes treated with sperm that was immobilized by laser system technique preserved a higher percentage of fertilization rates (90%) when compared with oocytes treated with sperm immobilized by conventional mechanical immobilization technique (65%). Also, the most important results was the time of the treatment that was reduced in more than 90% (only < 1 second) compare with the conventional sperm immobilization technique. (1 to 3 minutes; $p<0.001$).

Conclusion: These results show that laser system (Fertilase) technology may be useful to reduce significantly the time of sperm micromanipulation in the laboratory and consequently new hopes to improve higher fertilization rates with better results in most of the patients treated with ICSI technique.

P-323

Routine Use of Normal Saline as Flushing Media Has No Impact on Fertilization, Embryo Development and Pregnancy Rates in Assisted Reproductive Technologies. E.-A. M. Khalifa, K. F. S. H. Buraidah, Al-Quassim. Infertility/IVF Center, Kingdom of Saudi Arabia.

Objective: The current trend to harvest good number of oocytes after moderate controlled ovarian hyperstimulation necessitates the flushing of the ovarian follicles during oocytes' retrieval. This study evaluates the impact of non-heparinized normal saline when used as flushing media in IVF in a 2-phase study.

Design: Phase I, a prospective randomized and comparative study between non-heparinized EBSS and non-heparinized normal saline as flushing media. Phase II, a prospective clinical study to evaluate the impact on pregnancy and implantation rates after the use of normal saline as flushing media.

Materials & Methods: Phase I; 40 IVF cycles for infertile couples. Cases

with >10 follicles were included and randomized to have the 1st half of follicles (≥14mm) flushed with non-heparinized EBSS (Cat No. 14055-040, "without phenol", Gibco-UK) and the 2nd. half with non-heparinized normal saline and vice versa. Both fluids are colorless to avoid any bias from the operator performing the oocyte retrieval. The 2 groups of oocytes collected were separated and rinsed in culture media (EBSS, Cat No. 14050-033) supplemented with 10% human serum albumin (Biotest, Pharma GmbH, Germany) 5 times before putting them in separate culture dishes (4-well, Nunc, Denmark). The comparison includes, oocyte retrieval, fertilization and cleavage rates and embryo grading. Phase II; includes 38 IVF-ET, 50 ICSI and 13 ICSI/PESA-TESE cycles, evaluating the pregnancy and implantation rates.

Results: In Phase I, a total of 372 oocytes were retrieved. One hundred and eighty five oocytes out of 276 follicles (67%) were retrieved when EBSS flushing media was used (Group I) and 187 oocytes were retrieved out of 284 follicles (65.8%) when normal saline was used (Group II). Normal fertilization and cleavage rates were (150/185, 81% vs. 153/187, 82%; NS, and 136/150, 90.6% vs. 141/153, 92%, NS) in Group I and II respectively. Grade I embryos showed no significance difference at 48 hours and 72 hours (74% vs. 76%) and (68% vs. 67%) in Group I and II, respectively. In Phase II, 38 IVF cycles reached the ET stage with 44.7% pregnancy rate (4 deliveries, 9 ongoing pregnancies, 3 abortions and 1 ectopic) and 16.7% (23/138) implantation rate. While 49 ICSI cycles reached ET stage with 36.7% preg. rate (7 deliveries, 9 ongoing pregnancies and 2 abortions) and 14.9% implantation rate (24/161). Twelve cycles of ICSI/TESE-PESA reached the ET stage and resulted in 3 ongoing pregnancies (1 twin and 2 singleton) and 2 first trimester abortions (41.7% pregnancy rate and 13%, 6/46, implantation rates).

Conclusions: The brief exposure of oocytes to normal saline when used as flushing media has no untoward effect on the fertilization, cleavage rates or the early pre-embryo development or the pregnancy rate.

P-324

Implantation Rate of Day Five Versus Day Six Blastocysts. D. A. Occhino, C. J. Zaloudek, C. W. Chapman. The Center for Human Reproduction (CHR)-Illinois, Chicago, IL, USA.

Objective: To determine if day 5 blastocysts have a greater implantation rate than day 6 blastocysts.

Design: A retrospective analysis of blastocyst implantation rates of patients that had undergone a fresh IVF cycle.

Materials and Methods: Data was obtained from patients that received either a day 5 or day 6 transfer of one to three blastocysts. Day 5 and day 6 blastocysts were defined as those embryos which formed a blastocoel cavity in the presence of an inner cell mass and trophoblastic cells after 120 and 144 hours post insemination, respectively. Implantation rates were calculated by dividing the sum of the blastocysts transferred by the sum of IUP detected approximately 23 days post transfer. Demographics were analyzed to determine if age, the number of 2PN's or E_2 levels prior to oocyte retrievals differed significantly between the two patient populations. In addition, the clinical pregnancy rate per transfer and the mean number of embryos per transfer were calculated for the two patient populations.

Results:

	Implantation Rate	Age	2PN
Day 5	42% (18/43)[a]	33.1 ± 5.5[b]	9.2 ± 4.8[c]
Day 6	24% (30/123)	34.2 ± 4.0	7.7 ± 5.8

	E_2 (pg/ml)	Clinical Preg. Rate/ET	Mean # Embryos Trans.
Day 5	1906 ± 802[d]	63.2% (12/19)[e]	2.3 ± 0.6[f]
Day 6	1996 ± 1090	29.7% (19/64)	1.9 ± 0.8

[a,e] $p < 0.05$; [b,c,d,f] $p > 0.05$.

Conclusions: The implantation rate of day 5 blastocysts (42%) is significantly higher than that of day 6 blastocysts (24%). This difference could not be found to be due to a difference in age, the number of 2PN's, or E_2 levels between patients receiving day 5 embryos versus those receiving day 6 embryos.

P-325

The Onset of Cytoplasmic Fragmentation Can Occur Within 24 Hr of Insemination or ICSI and Appears to be an Important Indicator of Subsequent Preembryo Quality. N. Zaninovic, R. N. Clarke, L. L. Veeck. The Center for Reproductive Medicine and Infertility, Cornell University Medical College, New York, NY.

Objectives: Morphology and cleavage rates are commonly used as indicators of preembryo (PE) quality. In IVF Programs transferring preembryos (PEs) on Day 3, these parameters are usually assessed on Day 2 and again immediately before intrauterine transfer. Little attention has been given to morphologic and developmental characteristics of earlier stages. The objective of the present study was to determine if the onset of early fragmentation and/or developmental delay at 24 hr post-insemination or ICSI were related to PE quality later in development.

Design: A prospective analysis of 422 PE from 71 patients was performed during a 3 month period. The stage of development and degree of cytoplasmic fragmentation was observed in singly-cultured PEs at exactly 24 hr and again before transfer on Day 3.

Materials and Methods: PEs were cultured in 75-100 ul droplets of HTF + 15% maternal serum under oil. At 24 hr post-insemination or ICSI, they were evaluated for both morphology and onset of the first cleavage. PEs were classified as either being at the pronuclear (2PN) stage, in syngamy, or having undergone the first mitotic division. The appearance of cytoplasmic fragments was recorded. Before transfer on Day 3 (approximately 72 hr in culture), PEs were again evaluated for blastomere number and degree of fragmentation. Fragmentation, if observed, was estimated and classified as either mild (1-10%), moderate (11-19%), or excessive (≥20%). PEs on Day 3 were considered to be of morphologically good quality if they exhibited only mild fragmentation and of poor quality if excessive fragmentation was found.

Results: A significantly ($P<0.05$) lower percentage of good quality PEs on Day 3 resulted from PEs that exhibited any degree of fragmentation at 24 hr (36.6%) as compared to PEs with no early fragmentation (49.8%), regardless of the developmental stage attained. When these data were reanalyzed according to the stage of development at 24 hr, it was found that fragmenting PEs at the pronuclear stage resulted in a significantly ($P<0.05$) lower percentage of good quality embryos (15.8%) and a higher percentage of poor quality PEs (63.2%) as compared to their unfragmented 2PN counterparts on Day 3 (42.3% and 32%, respectively). This trend was also observed with fragmenting PEs that cleaved by the 24 hr observation, but the results were not significant. Data were additionally analyzed according to the stage of development attained at 24 hr, regardless of the presence or absence of fragments. PEs failing to enter syngamy or failing to cleave within 24 hr resulted in significantly fewer good quality PEs and more poor quality PEs on Day 3 of culture as compared to more rapidly developing PEs.

Conclusions: 1) The onset of cytoplasmic fragmentation can occur within 24 hr of ICSI/insemination. 2) The appearance of cytoplasmic fragmentation before the first cleavage or a delay in the first cleavage often leads to poor PE quality in subsequent days; PEs exhibiting both characteristics are most likely to exhibit poor morphology on Day 3 of culture. 3) Microscopic evaluation at 24 hr may serve as a useful tool for predicting subsequent PE quality.

P-326

Relationship Between Sperm Processing Technique and Recovery Rate of Nuclear Mature Spermatozoa and Its Predictive Value in an ICSI Program. M. E. Hammadeh, A. Kühnen, P. Rosenbaum, W. Schmidt. Department of Obstetrics & Gynecology. University of Saarland. Germany.

Objective: To compare the improvement of spermatozoa with respect to recovery rate of mature spermatozoa (Chromatin condensed) and to determine the relationship between sperm selection methods and ICSI-outcome in this regard.

Design: Comparison of sperm selection methods and the effect of these selection methods on the enhancement of the percentage of chromatin condensed spermatozoa and their relationship to ICSI-outcome.

Material and Methods: Semen samples were obtained from patients husband undergoing ICSI-therapy. The semen samples were subjected either to glass wool Filtration (G.1=33) or to percoll gradient centrifugation (G.2=29). Many smears were made before and after sperm selection. The slides were stained with Chromomycin (CMA-3) which differentiate between normal (unstained) and abnormal (Stained) chromatin condensed spermatozoa (Protamin deficiency). The result were expressed as a percentage after a counting 200 spermatozoa per slide.

Result: The mean percentage of chromatin condensed spermatozoa enhancement after sperm selection with (G.1) was (11.9±2.2%) from 49.4±17.3% in the native semen sample to 61.3±19.5% after sperm selection (p=0.0001). After sperm selection with percoll gradient (G.2) the mean chromatin condensed spermatozoa enhancement was 6.5±2.1%. There was an increase from 50.3±18.1% in the neat semen sample to 56.8±20.2% after sperm selection. No significant difference between the two groups with this regard was shown (p=0.356). On the other hand, the fertilization, and pregnancy rates the G.1 were 56.7±20.5% and 24.2±43.0% and the corresponding results in the second group G.2, were 58.0±19.9% and 29.6±46.5%. No significant difference was found between the two groups with respect to fertilization and pregnancy rate. (p=0.152 and p=0.642 respectively).

Conclusions: 1) The proportion of chromatin condensed spermatozoa was significantly higher after sperm selection either with G.1 or with G.2 in comparison to native semen samples. However, this proportion was higher after sperm selection with glass wool Filtration in comparison to value obtained by percoll gradient centrifugation. 2) Both sperm selection methods are effective for selecting chromatin condensed spermatozoa and the fertilization and pregnancy rate did not differ significantly between the two sperm processing method and fertilization and pregnancy rates after ICSI do not seem to be influenced by the percentage of chromatin condensed spermatozoa in the ejaculate before processing or after sperm selection.

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Study on Prediction of Ovarian Hyperstimulation Syndrome During hMG-hCG Treatment Using a Damped Oscillation Rheometer. K. Aisaka, T. Watanabe, F. Tamiaki, S. G. Liang, M. Kaibara, H. Mori. Department of Obstetrics & Gynecology, Teikyo University, Ichihara Hospital, School of Medicine, Tokyo, Japan.

Objectives: It is well known that ovarian hyperstimulation syndrome (OHSS) is a common but sometimes severe side effect during hMG-hCG treatment. Present study was performed to elucidate whether OHSS was predictable during hMG administration by precise measurement of the early stage of the blood coagulation using a damped oscillation rheometer (DOR).

Design: Blood samplings were performed during hMG administration and the time of the onset of blood coagulation (Ti) was measured with the DOR.

Materials and Methods: Ten cases of severe hypothalamic anovulations were subjected in this study under an enough informed consent. Clomiphene citrate was not effective in all of the subjects. Then, 150iu of hMG (Pergonal™) was administered from the third day of the menstrual cycle under the monitoring of the transvaginal ultrasonography. The dosage of hMG was increased up to 450iu/day until the diameters of main follicles grew at least 20mm, and 5000 to 10000iu of hCG was administered for the ovulation induction. The subjected patients were divided into two groups by their clinical courses after hCG administration; OHSS(+): 4 cases, group A, and OHSS(−): 6 cases, group B. Blood samples were corrected at the beginning, 5th day after hMG administration, and before hCG administration. And the onset of coagulation (Ti) was measured with a high sensitivity DOR using whole blood. Prothrombin time (PT), hematocrit (Ht) values and serum estradiol levels were also examined at the same time.

Results: There was almost no change in PT during hMG administration. Ht values tended to increase, but no significant change was observed between group A and B (5th day; 45.6 ± 4.9 vs. 45.3 ± 4.5%, before hCG administration; 48.0 ± 5.1 vs. 47.5 ± 5.6%). Serum estradiol levels increased during hMG treatment (5th day; 356.2 ± 78.4 vs. 362.5 ± 81.2pg/ml), and there was a significant change before hCG administration (1887.1 ± 510.0 vs. 1563.8 ± 477.9pg/ml, p<0.05). Ti values were more sensitive, and a significant change was observed even in the 5th day after hMG administration (19.2 ± 5.4 vs. 23.9 ± 5.6min., p<0.02).

Conclusions: It is suggested that the occurrence of the OHSS may be predictable from the early stage of the hMG administration by the measurement of Ti values using the high sensitive DOR.

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The Benefit of Performing Precautionary Intracytoplasmic Sperm Injection (ICSI) to Ensure Fertilization. G. K. Adaniya, P. A. Schnarr, A. R. Hall, J. C. Ketner, K. A. Buss. Midwest Reproductive Medicine, Indianapolis, IN.

Objectives: ICSI has become the fertilization method of choice for couples with male factor infertility or for couples with a history of poor fertilization in previous assisted reproductive technology (ART) cycles. However, our ART facility is located in a state without mandated infertility insurance coverage, and many of our patients can only afford to do a single ART cycle. A cycle with complete fertilization failure for these couples is devastating. Therefore, we have examined the benefit of injecting three of the mature oocytes retrieved and inseminating the remaining oocytes in the conventional manner. This "To Be Sure" (TBS) ICSI (performed at no additional cost to the patient) was offered to couples with normal semen parameters who were undergoing their first ART cycle.

Design: Retrospective analysis of 101 cycles of couples undergoing TBS ICSI.

Materials and Methods: After obtaining informed consent to perform the ICSI procedure, retrieved oocytes were cleaned with a brief exposure to 80 IU/ml hyaluronidase until three mature oocytes were obtained. Approximately three hours post retrieval, the three mature oocytes were injected using standard ICSI procedures, and the remaining oocytes were inseminated with 1×10^5 motile spermatozoa. Oocytes were checked for fertilization approximately 14–19 hours post insemination.

Results: In 5.9% (6/101) of the cases in which TBS ICSI was performed, no fertilization was obtained by conventional insemination. In all six cycles fertilization was observed with the ICSI oocytes. Fertilization rates were significantly different (p<0.05) between ICSI (11/14, 78.6%) versus regular insemination (0/32). In addition to the six cycles with no fertilization using conventional insemination, there were seven other cycles in which only one oocyte fertilized. In these seven cycles, ICSI fertilization was 80.0% (16/20) while conventional insemination was only 12.7% (7/55). In the 101 TBS ICSI cycles, fertilization was 75.8% (216/285) with ICSI and 60.0% (569/948) with insemination.

Conclusions: Although there are risks in doing TBS ICSI, almost 6% of our couples would have had no chance of becoming pregnant if TBS ICSI had not been done. In a state where most couples must pay for all ART expenses out of pocket and multiple attempts may not be possible, TBS ICSI offers all couples a chance of achieving pregnancy.

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Successful Twin Pregnancy in a Dual-Transplant Couple Resulting From In Vitro Fertilization and Intracytoplasmic Sperm Injection. A. M. Case, A. Weissman, M. Sermer, E. M. Greenblatt. Reproductive Biology Unit, The Toronto Hospital, University of Toronto, Toronto, Ontario, Canada.

Objective: There are numerous case reports of pregnancy following liver transplantation. Little information is available regarding the incidence and management of infertility in transplant recipients, in particular the use of advanced reproductive technologies. We present what is believed to be the first case of a successful twin pregnancy resulting from in vitro fertilization with intracytoplasmic sperm injection (IVF/ICSI) in a liver transplant recipient, whose partner was a renal transplant recipient with severe oligospermia.

Design: Case report.

Materials and Methods: A 22 year old nulliparous woman who underwent a liver transplant for Budd-Chiari syndrome at age 18 presented for management of primary infertility. Her 26 year old partner who had previously undergone an unsuccessful renal transplant for polycystic kidney disease, and was now on dialysis, was diagnosed with retrograde ejaculation and profound oligospermia (<5000 motile sperm/ml). In

consultation with andrology, it was felt that pregnancy could best be achieved in this couple by ovulation induction, and intracytoplasmic sperm injection of retrieved oocytes, using sperm obtained from epididymal aspiration. Prior to initiating a treatment cycle, consults were obtained from perinatology, genetics and hematology, in particular to assess her fitness for pregnancy, and management of her anticoagulation. She was started on a long GnRH agonist protocol, followed by gonadotropins. Prior to initiation of treatment, epididymal aspiration was performed, and sperm were cryopreserved.

Results: Seven oocytes were retrieved, 5 successfully fertilized with ICSI, and 3 embryos were transferred. An intrauterine twin pregnancy was confirmed 6 weeks post transfer. At 28 weeks gestation, she was hospitalized for mild preeclampsia and discordant fetal growth. Preterm premature rupture of membranes and preterm labour occurred at 34 weeks, and healthy twin boys weighing 1460 grams and 2120 grams were delivered by Cesarean section. Postpartum course was complicated by delayed postpartum hemorrhage from her uterine incision, requiring reoperation and blood transfusion.

Conclusions: The desire for pregnancy is likely to become more common among liver transplant recipients of reproductive age. With careful evaluation and monitoring, and the involvement of appropriate consultants, the use of advanced reproductive technologies can be safely considered in couples experiencing infertility.

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Ovarian Stimulation with Recombinant FSH in the Intrauterine Insemination with Husbands Sperm: A Randomized Study in Comparison with Highly- Purified Urinary FSH. R. Matorras, V. Recio, B. Corcóstegui, F. J. Rodríguez-Escudero. Hospital de Cruces, País Vasco University, Vizcaya, Spain.

Objective: To compare the results of an intrauterine insemination with husbands sperm (IUIH) program when the ovarian stimulation is performed with highly-purified urinary FSH (UF) or with recombinant FSH (RF).

Design: Prospective randomized trial.

Material and Methods: 93 women receiving IUIH (285 cycles): 46 with ovarian stimulation with UF (155 cycles), and 42 with RF (130 cycles). The main inclusion criteria were: woman < 40 years, at least 1 patent tube, and the recovery after sperm preparation of at least 5,000,000 motile sperm/cc. The starting dose was in both cases 150 IU of FSH on day 2. Ovarian stimulation was monitorized by vaginal ultrasound and plasma estradiol. One insemination per cycle was performed, 36 h after ovulation triggering with 5,000 IU of HCG. Sperm preparation was performed with Puresperm.

Results:

	Recombinant FSH	HP Urinary FSH
Days of ovarian stimulation	10.5	10.5
FSH ampoules/cycle	19.4	23.7
Pregnancy rate/cycle (%)	19.2 (25/130)	15.5 (24/155)
Pregnancy rate/woman (%)	59.5 (25/42)	52.2 (24/46)
Estradiol on day of HCG (pg/ml)	1,007.2	921.3
Estradiol/FSH ampoules ratio	51.91	38.87

Conclusion: In IUIH cycles recombinant FSH showed a 33% increased potency in regard to urinary FSH, expressed by the ratio estradiol/FSH ampoules. A trend to higher per cycle pregnancy rates was observed.

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Baseline Serum Estradiol Values and the Presence of Follicular Cysts: Is a Baseline Sonogram Necessary? J. M. Groll, T. N. Hickman, R. D. Robinson, G. S. Neal, J. Y. Phelps. Department of Obstetrics & Gynecology, Wilford Hall Medical Center, San Antonio, Texas.

Objectives: To evaluate the utility of baseline serum estradiol (E_2) values obtained on the third day of the menstrual cycle in predicting the likelihood of follicular cysts.

Design: A retrospective analysis of menstrual day three E_2 values and follicular cysts.

Materials and Methods: Women underwent serum E_2 determinations and transvaginal ultrasonography on day three of their menstrual cycle.

Results: Estradiol values obtained on day three of the menstrual cycle correlate positively with the presence of follicular cysts > 10 mm in diameter ($\chi^2 = 15.0$, $P < 0.001$). Women with baseline serum E_2 values ≥ 100 pg/mL had a significantly higher rate of follicular cysts > 10 mm in diameter compared to women with baseline serum E_2 values < 100 pg/mL, 66% (21/33) versus 28% (41/146) respectively. Although elevated baseline serum E_2 values ≥ 100 pg/mL were correlated with the presence of follicular cysts > 10 mm in diameter, this cut-off value was not useful in excluding the presence of follicular cysts. This was also true when lower E_2 values were used; 27% (29/109) of patients with baseline serum E_2 values < 75 pg/mL had follicular cysts while 29% (12/42) of patients with baseline serum E_2 values < 50 pg/mL had follicular cysts.

Conclusion: Estradiol values obtained on the third day of the menstrual cycle are correlated with the presence of follicular cysts. However, the predictive value of this relationship is questionable since a significant percentage of patients had follicular cysts > 10 mm in diameter despite normal baseline E_2 values. For this reason, we believe a transvaginal ultrasound is necessary prior to the initiation of ovulation induction.

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A Randomized Study Comparing the Efficacy of Recombinant FSH in Different IVF Protocols. A. Ravhon, H. Lawrie, A. Ellenbogen, G. Trew, R. Margara, R. M. Winston. Wolfson Family Clinic & Flick Laboratories, Department of Reproductive Medicine & Science, Imperial College School of Medicine, Hammersmith Hospital, London, UK.

Objective: The use of HMG for controlled ovarian hyperstimulation in IVF has shown that the long protocol is more successful than the short protocol. The complete absence of LH in recombinant FSH (rFSH) may alter the optimal protocol in IVF. The aim was to compare the efficacy of rFSH in the following three IVF protocols: long protocol started either on mid-luteal phase or early follicular phase and short protocol.

Design: A single center, randomized, prospective clinical trial.

Materials and Methods: 150 patients were included: 44 patients were treated by short protocol (SP), 61 patients by long protocol started on day 2 of the cycle (D2 LP) and 45 patients by long protocol started on day 21 (D21 LP). All patients received buserelin acetate (Suprecur, Hoechst, UK) and rFSH (Puregon, Organon, UK or Gonal F, Serono, UK). Cycles were monitored using ultrasound and plasma estradiol levels. The dose of rFSH was adjusted according to ovarian response.

Results: The results (±SD) are summarized in the table below.

	SP	D2 LP	D21 LP
Age (y)	34.7 (±4.0)	35.0 (±3.9)	34.3 (±3.9)
Stimulation days (d)	10.6 (±1.7)	12.9 (±2.1)	13.1 (±2.4)
Total dose (iu)	2043 (±892)	2996 (±1241)	2925 (±1437)
E2 (day of hCG) (pmol/l)	7705 (±3256)	6030 (±2512)	6318 (±2179)
LH (day of hCG) (iu/l)	4.3 (±3.0)	1.1 (±1.2)	0.9 (±0.3)
No. of oocytes	8.4 (±3.95)	9.5 (±3.96)	7.8 (±2.9)
No. of embryos	4.9 (±2.8)	5.6 (±2.6)	4.3 (±2.4)
Embryos transferred	2.03 (±0.45)	2.31 (±0.6)	2.05 (±0.6)
Implantation Rate (%)	4.1	11.8	13.3
Clinical Preg. (per ET) (%)	8.3	19.6	18.6

Conclusions: Using rFSH for controlled ovarian hyperstimulation leads to similar implantation and clinical pregnancy rates in the two long protocols. The implantation rate is significantly higher in the long protocols compared with the short protocol with a tendency for a higher pregnancy rate as well.

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Serum Screening for Down Syndrome Among Pregnancies Following Assisted Reproduction Technology. R. Wainer[1], L. Malagrida[2], P. Rozenberg[1], J. Nizard[1], F. Merlet[3], Z. Lahna[1], Y. Giudicelli[2], Y. Ville[1]. [1]Depart-

ment of Obstetrics-Gynecology [2]Department of biochemistry, [3]Department of Assisted Reproduction technology. Centre Hospitalier de Poissy/Saint-Germain 78300 France.

Objective: To compare the median levels of free β-hCG and α-fetoprotein (AFP) assayed during serum screening for Down Syndrome (DS) among a population of pregnancies obtained by assisted reproduction and a control population of spontaneous pregnancies.

Study Design: Between July 1996 and December 1998, in 70 singleton pregnancies obtained by assisted reproduction (37 ICSI and 33 IVF), maternal serum was screened for DS by assays of Free β-hCG and AFP between 14 and 17 weeks' gestation. Each sample was matched with 10 serum samples from spontaneous pregnancies of the same gestational age, collected immediately after the samples from the IVF-ICSI patients. The multiples of medians' means (MoM's means) were compared by Student's t-test.

Results:

	FIV + ICSI n = 70	
	FIV n=33	ICSI n=37
Patient's age (years)	32.0 ± 2.9	
	33.4±2.7	32.5±3.1
Free β-hCG (mean of MoM ± SD)	1.31 ± 1.30	
	1.31±1.40	1.25±1.14
AFP (mean of MoM ± SD)	1.03 ± 0.49	
	1.02±0.35	1.04±0.61

	Témoins n=700	t-test
Patient's age (years)	29.7 ± 4.2	NS
Free β-hCG (mean of MoM ± SD)	1.13 ± 0.76	NS
AFP (mean of MoM ± SD)	1.08 ± 0.40	NS

The screen positive rate was 11.4% (8/70) in IVF/ICSI population versus 5.6% (39/700) in control population.

Discussion: Although the mean of the free β-hCG median in the IVF-ICSI group was higher than that of the control population, the difference was not significant. This lack of significance contrasts with the results published for total hCG in four studies of the standard triple-marker screening test (total hCG, AFP, E3) among IVF-pregnancy populations of similar size (41–70 patients). Nonetheless, in view of the relatively small size of our IVF-ICSI population, a lack of power cannot be ruled out.

Conclusion: If the better specificity of free β-hCG (compared with total hCG) is confirmed among a larger population of IVF-ICSI patients, the use of free β-hCG (instead of total hCG) should help reduce the abnormally high false-positive rate observed in these populations and thus the risks inherent to amniocentesis among this especially vulnerable population.

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Low Dose Aspirin Plus Low Molecular Weight Heparin Treatment in Women Undergoing Repeated Failed Assisted Conception Treatment Cycles. R. Kazem, A. Bunkheila, M. Aloum, G. Ndukwe. School of Human Development, Nottingham University Research and Treatment Unit, "NURTURE", B Floor, East Block, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

Objectives: To determine whether treatment with low dose aspirin (LDA) and low molecular weight heparin (LMWH) leads to an improved outcome in women undergoing repeated failed In Vitro Fertilisation (IVF) and Intracytoplasmic Sperm Injection (ICSI) cycles.

Design: Clinical pregnancy rates were compared in women who received treatment with LDA & LMWH in their 4th or subsequent cycle of IVF treatment to those who did not.

Materials and Methods: Women undergoing their 4th or subsequent cycle of IVF or ICSI were offered empirical treatment with LDA & LMWH regardless of their antiphospholipid and anticardiolipin antibody status. LDA (75 mg daily) was commenced on the first day of gonadotrophin releasing hormone analogue treatment and LMWH (Clexane 20 mg sc. daily) was commenced after embryo transfer in 30 women. Routine ovarian stimulation protocols were followed in all cases. A retrospective analysis was performed to compare the clinical pregnancy rates in the study group (n=30) with the clinical pregnancy rates in all other women undergoing their 4th or subsequent cycle of IVF or ICSI treatment during the same time period (control group, n=119).

Results: The clinical pregnancy rate per cycle and per embryo transfer in women treated with LDA & LMWH was 33.33% (10 pregnancies in 30 cycles, all cycles had embryos transferred) compared to 21.84% and 23% respectively (26 pregnancies in 119 cycles and 113 embryo transfers) in women who did not receive LDA & LMWH.

Conclusion: Treatment with LDA & LMWH may improve clinical pregnancy rates in women with repeated failed IVF or ICSI cycles. Although the improvement in the pregnancy rate in our study did not reach statistical significance further larger scale trials are warranted.

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The Quality of Sperm Transfer: Another Variable to Consider in Intrauterine Insemination Cycles? A. Manzur, A. Magendzo, P. Labra, C. Almendra, G. Durruty, M. Bianchi, R. M. Zeydan. Unidad de Reproducción Humana, Departamento de Obstetricia y Ginecología, Pontificia Universidad Católica de Chile.

Objectives: To evaluate if the quality of sperm transfer has an impact by itself in the success rate of homologous intrauterine insemination cycles.

Design: A prospective study of infertile patients undergoing induced ovulatory cycles for intrauterine inseminations (IUI) with husband's sperm. Women's age, sperm concentration and quality of sperm transfers were taken as independent variables when analyzing pregnancy rates with this technique.

Materials and Methods: From September 1997 to January 1999, every patient undergoing homologous IUI was classified as *A*, *B* or *C* according to the quality of sperm transfer. The classification considered *A* as a smooth and easy transfer, with no reflux nor pain referred by the patient; *B* as a transfer in which some resistance or difficulty was experienced by the physician, with/without a variable degree of sample reflux or discomfort referred by the patient, but no endometrial bleeding; *C* was a very difficult transfer with evident bleeding from the endometrial cavity, with/without pain experienced by the patient. All transfers were performed by the same two physicians during the whole period, using a Makler long-cannula catheter (Sefi-Medical Instruments, Haifa, Israel). Women older than 38 years, or undergoing the procedure in spontaneous cycles or with severe tuboperitoneal factor were excluded from the study, together with semen samples with less than 1 million motile sperms recovered by swim-up or percoll gradient separation technique. Timing of IUI was determined by vaginal ultrasound follow-up (Aloka 630, 5 Mhz) and urinary LH surge detection or hCG 5000 I.U. administration. Only one insemination, 24 hrs after LH surge or 36 hrs after hCG, was programmed per cycle. For statistical analysis Chi square was selected, defining significance as $p < 0.05$.

Results: From 167 IUI cycles that met the selection criteria, 100 were classified as *A*, 60 as *B*, and 7 as *C*. Overall 27 clinical pregnancies were obtained (16.2% pregnancy rate) distributed as follows: *A* 17/100 (17%), *B* 10/60 (16.6%) and *C* 0/7 (0%). No significance differences were found between groups. The mean age of the female patients was 31 ± 3.7 years, with a range of 23 to 38 years. Women < 30 y.o. exhibited the highest pregnancy rate, with 14/59 clinical pregnancies (27.2%), compared to women aged 30–34 with 10/70 pregnancies (13.7%) and women 35–38 y.o. with 3/38 pregnancies (7.8%). Statistical difference was reached between women <30 and 35–38 y.o. ($p < 0.05$). The sperm count used to inseminate did not show statistical differences in pregnancy rates when comparing samples with 1–5 million motile sperms transferred (13/90 pregnancies, 14.4%) and those with >5 million motile sperms (14/77, 18.2%).

Conclusions: The quality of sperm transfer technique does not influence the results of IUI, however, C transfers were too few to allow final conclusions. When >1 million motile sperm are recovered, an acceptable pregnancy rate should be expected, that will not increase with higher sperm counts. The age of the female partner remains as the most important variable conditioning the results of IUI.

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The Effect of Human Follicular Fluid on Embryo Development and Pregnancy Rate in A.R.T. (Assisted Reproductive Technology) Programs. A. Aflatoonian, M.D., M. A. Karimzadeh Meibodi, M.D., M. H. Amirarjmand, M.S., M. Soleimani, M.S. Madar Hospital, Yazd, Iran.

Objective: To increase the pregnancy rate in ART (Assisted Reproductive Technology) different methods such as transfer of blastocyst on the fifth day after the puncture (egg recovery) or many co-cultures have been proposed. Recently use of follicular fluid instead of umbelical cord serum or albumin has been suggested. In this study we wish to compare pregnancy rate with the use of follicular fluid instead of protein supplements.

Design: Two groups of ART cycles are compared. In one group follicular fluid from the patients' follicles is added to the media and in the other group conventional method without follicular fluid the same procedure was performed. The embryo quality and the pregnancy rate were compared in the two groups.

Materials and Methods: One handred couples who referred to our center for ART were divided in two groups with same cause of infertility and similar age groups. In group one (50 cycles) follicular fluid of the patients at the time of follicular aspiration after filtration were added to the media (Han, F10) and in group two the procedure was performed without adding follicular fluid and only with fetal cord serum. The embryo quality and pregnancy rate in the two groups were compared.

Results: In group one (with FF) significant increase in cell stage development of embryos also increase in pregnancy rate in comparison with group two was observed (15 out of 50 or 30% in group one and 10 out of 50 or 20% in group two).

Conclusion: The use of human follicular fluid is effection in increasing the pregnancy rate in ART programs.

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Clinical and Treatment Variables Affecting Growth of Human Embryos to the Blastocyst Stage in IVF Patients with Recurrent Implantation Failure. H. Pinkas, O. Rufas, O. M. Avrech, A. Stein, R. Orvieto, S. Amit, A. Berkovich, Z. Ben-Rafael, B. Fisch. Infertility and IVF Unit, Department of Obstetrics and Gynecology, Rabin Medical Center-Beilinson Campus, Petach-Tikva, Sackler Faculty of Medicine, Tel Aviv University, Israel.

Introduction: Recently, several in vitro fertilization (IVF) and embryo transfer (ET) programs reported encouraging results relating to their experience with the transfer of embryos at the blastocyst stage. Replacement of embryos to the uterus at this stage is more physiological, thus permitting better embryo-uterine synchronization. Furthermore, it provides an additional method for evaluating embryo quality and viability before transfer.

Objective: To determine clinical and treatment variables of IVF and ICSI cycles that might affect blastocyst development and implantation rates in a group of patients with recurrent implantation failure.

Materials and Methods: Sixty-four patients (mean age 34.4±4.95 years, range 25–44 years) with previous repeated failed ET (at least 4), who underwent 80 treatment cycles (46 IVF cycles and 34 ICSI cycles) in which all embryos achieved were designated for blastocyst growth and transfer, were selected for the study.

Results: Blastocyst development was observed in 73 cycles (91.2%). Forty percents of all fertilized oocytes developed to blastocysts. No significant difference in the rate of blastocyst formation was observed between IVF and ICSI cycles. Significant negative correlation between the number of blastocysts developed was observed with patient age and number of previous IVF attempts ($p=0.0470$ $r=-0.22563$ and $p=0.0039$ $r=-0.32297$, respectively). Positive correlation was observed between the number of blastocysts formed and implantation rate ($p=0.0012$ $r=0.37386$). Long GnRH-agonist protocols achieved better implantation rate and pregnancy outcome than short GnRH-a protocol ($p=0.0042$, $r=0.333348$ and $p=0.0324$, $r=0.49187$, respectively). Pregnancy rate was significantly influenced by blastocyst development rate ($p=0.0009$) and embryo morphology ($p=0.0049$). Overall implantation rate was 16.4% per embryo and clinical pregnancy rate per ET was 26.5%.

Conclusions: In a group of patients with repeated implantation failure the method of fertilization (IVF or ICSI) did not affect the blastulation rate. Patient age and number of previous treatment attempts were in negative correlation with blastocyst development. The number and quality of blastocysts formed significantly affected clinical pregnancy rate. Treatment protocol (long GnRH-a protocol) was found to be superior to short protocol regarding implantation rate and pregnancy outcome.

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Impairment of IVF Outcome Due to Clomiphene Citrate Concentration in Follicular Fluid. E. Strehler[1], M. Abt[2], M. Walter[1], K. Sterzik[1]. [1]Institute for Reproductive Medicine Ulm, [2]Institute for Mathematics, Augsburg, Germany.

Objective: Clomiphene citrate (CC) is widely used for ovarian stimulation in IUI as well as in IVF cycles. In comparison to gonadotropins, with or without GNRH analogs, clomiphene citrate is cheaper and easier to use but often is discredited to have a detrimental impairment on oocyte quality due to the antiestrogenic effect. In this study, we analyzed the relationship between the clomiphene citrate (CC) concentration in the follicular fluid and the IVF outcome.

Design: Prospective study, examining the relationship between the clomiphene citrate (CC) concentration in the follicular fluid measured at the day of the ovum pick up, and the occurrence of pregnancy rate after IVF treatment.

Materials and Methods: Seventy-one patients (mean age 31.7 years) undergoing an IVF procedure with embryo transfer, were recruited into this study. The reason for infertility was a tubal factor. For ovarian stimulation patients were treated with hMG and CC (Dyneric 100 mg cycle days 5–9). HCG was administered when at least one follicle reached 20 mm in diameter. Oocyte retrivial was carried out 36–38 h later. In all 71 patients clomiphene citrate concentration was measured in the follicular fluid. The embryo transfer was carried out two days after ovum pick up. Clinical pregnancy was defined by the presence of a fetal sac at ultrasound examination. Statistical analysis was done by the Wilcoxon test.

Results: In the study group, the mean concentration of CC in the follicular fluid was 99.4 ng/mL. After IVF therapy 21 patients (29%) became pregnant. Comparison of pregnant and non-pregnant patients showed significant differences of CC concentration in follicular fluid ($P=0.00$). The mean values were 46.6 ng/mL in the group with, and 121.6 ng/mL in the group without pregnancy, respectively. Age of patients and mean number of recovered oocytes per cycle were similar in both groups. Only one cycle per patient was evaluated.

Conclusion: The results suggest that there is a clinically detectable impairment of IVF outcome due to clomiphene citrate concentration in follicular fluid. Clomiphene citrate might be detrimental to oocyte quality.

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Fluorescent PCR is More Effective Than Classic PCR and FISH in Pre-Implantation Diagnosis (PGD) of Sex Chromosomes. [1]R. Poverini, [1]M. Sbracia, [2]G. Di Cola, [3]M. Baldi, [1]F. Scarpellini, [4]G. Micara, [5]F. Morgia, [5]M. Iacobelli, [4,5]C. Aragona. [1]Centro di Endocrinologia e Medicina della Riproduzione, [2]Bio-Tech Lab., Parma, [3]Consultorio di Genetica, [4]Department Obstetrics and Gynecology University "La Sapienza", [5]European Hospital Centro della Medicina della Riproduzione, Rome, Italy.

Objective: Preimplantation genetics diagnosis has been suggested as an alternative to chorionic villus sampling or amniocentesis in patients carrier of sex-linked disorders to investigate the chromosomal status of offsprings. The preimplantation diagnosis is performed on one blastomere extracted generally from 8 cells embryo, using FISH or PCR techniques. Recently, a new technology has been proposed as an alternative methodology in this cases, the fluorescent PCR. In order to investigate which of these three different techniques is more affordable in sex diagnosis of preembryo we studied in a series of no viable embryos, using three blastomeres of each embryos, the performances of PCR, FISH and Fluorescent PCR.

Materials and Methods: 25 human pre-embryos, no viable for uterine replacement, originated from patients treated with either intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF) techniques were used as samples in our experiments. From the same pre-embryo, at 6–8 cell stage, three cells were removed by micromanipulation to be tested for sex with the three different techniques, PCR, fluorescent PCR and FISH (florescent in situ hybridization). For FISH the blastomeres were fixed on slides and

hybridized with X and Y chromosomes specific fluorescent probes (Vysis). For PCR and Fluorescent PCR the blastomeres were collected in 0.5 microfuge tube containing 5μl of hypotonic solution and stored in liquid nitrogen until to used. For PCR specific published primers for X and Y chromosomes were used. The quantitative fluorescent PCR was performed using "small tandem repeat" specific for X and Y chromosomes. The products of amplification have been analyzed with an automatic DNA analyser (ABI-310 Perkin Elmer).

Results: With FISH we obtained an valuable signal in 20 cases (80%), whereas with PCR in 21 cases (84%) and with fluorescent PCR in 23 cases (92%). With fluorescent PCR we obtained a correct sex diagnosis in 23 (92%) cases whereas with the other techniques we obtained a correct sex diagnosis in 17 cases (68%) ($P<0.05$).

Conclusions: Our findings showed that fluorescent PCR is an affordable technique for preimplantation diagnosis. It was more accurate and efficient than FISH and classical PCR in the assessment of sex in pre-embryo.

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Effect of Partial Laser Assisted Hatching on Mouse Embryos. [1]D. H. Kim, [1]H. G. Kang, [1]S. W. Han, [2]W. I. Park, [2]H. J. Yeon, [1]H. J. Lee. [1]Medical Science Institute and [2]Obstetrics/Gynecology of Eulji General Hospital, Seoul, Korea.

Objective: The present study was performed to investigate the efficiency of partial laser assisted hatching (p-LAH; lased 1/2 ZP width from ZP edge) on hatching on mouse blastocysts.

Design: To compare the effect of coventional laser assisted hatching (c-LAH) and partial laser assisted hatching (p-LAH), assisted hatching was performed mouse embryos at 8-cell stage. Hatching rate, hatching time and blastocyst diameter and zona pellucida thickness at hatching time were examined.

Materials and Methods: We used an non-contact 1.48 μm diode laser (MTM, Switzland) to create a precise hole on zona pellucida. 2-cell embryos collected from the mouse (ICR) oviduct at 48 hours after hCG administration. Collected 2-cell embryos were cultured in the P-1 medium supplemented with 0.4% BSA. For experiments, embryos at 8-cell stage were used after 20–22 hours in culture. After conventional or partial laser assisted hatching, the embryos were further cultured in P-1 medium supplemented with 0.4% BSA for 3 days. To compare efficiency of complete and partial laser assisted hatching, hatching rate, hatching time and blastocyst diameter and zona pellucida thickness at hatching time were investigated. Embryos were examined every 12 hours. Blastocyst diameter and zona pellucida thickness at hatching time were measured with an ocular micrometer.

Results: Hatching rates of p-LAH group (84.2%) was significantly higher than that of control group (39.3%), but there was no difference between the p-LAH (84.2%) and c-LAH (91.2%). p-LAH group was hatched 12 hours earlier than control group, but hatched 12 hours later than c-LAH group. The diameter of blastocyst at hatching time of p-LAH group (113.1±6.4 μm) was smaller than that of control group (122.2±5.0 μm). Zona pellucida thickness at hatching time of p-LAH group (6.4±0.9 μm) was thicker than that of control group (4.5±1.5 μm), but thinner than that of c-LAH group (10.0±0.8 μm).

Conclusion: These results suggest that p-LAH may maintains the cell arrangement of early embryos to ensure successful development and prevent precocious hatching of blastocyst when compare to c-LAH and conventional (acidic tyrode) AH. Thus, p-LAH may provide a valuable and effective AH technique for human ART program.

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Human Amniotic Fluid (HAF) Induces Spontaneous Hardening of the Zona Pellucida (ZP) of Mouse Immature Oocytes During Maturation *In Vitro*. [1]K. S. Park, [1]S. S. Chun, [1]T. H. Lee, [2]K. Y. Cha, [3]H. B. Song. [1]Department of Obstetrics and Gynecology, Kyungpook National University Hospital, Taegu, [2]Institute of Infertility Medical Center, CHA General Hospital, Seoul and [3]Department of Animal Science, Taegu University, Kyungbuk, Korea.

Objectives: ZP has been thought to be the barrier of egg to sperm penetration before and after fertilization. The phenomenon of ZP hardening has been considered as a post-fertilization event until now, and it is generally accepted that it is caused by the secretory products of cortical granules released during the cortical reaction. De Fellici and Siracusa (Gamete Res 1982;6:107–113) reported that irrespective of fertilization or activation, hardening of ZP could occur "spontaneously" in mammalian oocytes in standard culture conditions, and that it is probably not a consequence of cortical reaction. The purpose of our study was to investigate the effect of HAF on nuclear maturation (NM) and fertilization ability of mouse oocytes.

Design: NM and fertilization ability were determined after *in vitro* maturation of mouse immature oocytes in the medium supplemented with HAF.

Materials and Methods: HAF was obtained from patients undergoing amniocentesis at 16–20 weeks of gestation. HAF from five to ten patients was centrifuged and the supernatants were pooled. Cumulus-enclosed mouse immature oocytes were incubated in the medium containing HAF, and examined to confirm NM and fertilization. Female ICR mice (about 3 weeks old) were stimulated with 7.5 IU pregnant mare's serum gonadotropin (PMSG). Immature oocytes were isolated at 48–52 hrs post PMSG injection and cultured in Tissue Culture Medium-199 supplemented with 20% HAF for 18 hrs. Fetal bovine serum (FBS) was used as a control for the examination. Matured oocytes (M II) were fertilized with sperms collected from the epididymis of male mice (over 10 weeks old). Fertilization was conducted in T6 medium containing 15 mg/ml bovine serum albumin (BSA), and confirmed at 6 hrs post-insemination. Evaluation of NM and fertilization was carried out by rapid staining method as described by Byun *et al.* (Kor. J. Anim. Sci. 1991;33:25–31). ZP hardening was assessed by incubating cumulus cell-free mature oocytes in 0.001% chymotrypsin at 37°C as described by George *et al.* (Hum. Reprod. 1992;7:408–412). For statistical analysis, student's *t*-test was used. Results were considered statistically significant when P value was less than 0.05.

Results: There was no significant difference between the effects of HAF (85.6%) and FBS (87.6%) supplements on NM of immature oocytes. When maturation medium was supplemented with HAF, total fertilization rates (7%) were significantly lower ($P<0.01$) than that of FBS (85.1%). In HAF group, fertilization rate was increased ($P<0.01$) in zona-free oocytes (7% versus 100%; denuded by trypsin). The resistance of mouse oocyte ZP to digestion by chymotrypsin after maturation *in vitro* was significantly higher ($P<0.01$) in HAF group (86.7%) than in FBS (6.7%). To culture oocytes in FBS were very effective in preventing ZP hardening. However HAF cultured oocytes showed high rate of ZP hardening ($P<0.01$).

Conclusions: These results suggest that HAF can be used as a supplement for the NM of mouse immature oocytes *in vitro*. However, HAF induces spontaneous hardening of ZP of mouse immature oocytes during maturation *in vitro*.

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Transfer of Polar Body DNA to Enucleated Oocytes in Bovine and Non-Human Primates. R. R. Yeoman, N. Ouhibi, S. Mitalopov, J. M. Larson, D. P. Wolf. Department Obstetrics/Gynecology, Oregon Health Sciences University, Portland, and Oregon Regional Primate Research Center, Beaverton, OR.

Objective: To evaluate the ability of polar body (PB) DNA to support embryo development when transferred into enucleated oocytes. When used with sperm activation, this procedure could expand the number and quality of embryos and may have clinical application. Though used successfully to produce fertile offspring in mice (Wakayama et al., BOR 59:100, 1998), this approach has yet to be evaluated in higher mammals.

Design: Retention of transferred polar bodies, cytoplasmic intactness and ability to cleave after chemical activation were evaluated in bovine and rhesus monkey enucleated oocytes.

Materials and Methods: Bovine oocytes were commercially obtained (BOMED, Madison, WI). Monkey oocytes were collected by follicular aspiration after ovulation induction with recombinant human FSH and LH in the presence of an LHRH antagonist followed by hCG. Preparation of donor cytoplasts by micromanipulation was performed in TALP-Hepes media with 5 μg/ml cytoclasin B. Complete enucleation and PB removal using a beveled, spiked 22 μM pipette was confirmed by brief UV illumination showing an absence of Hoechst stained DNA material. Additional oocytes were utilized for collection of intact PB without exposure to UV. Rupture of PB prior to transfer occurred during aspiration from a collection drop into an unpolished, blunt 8 μM i.d. micro pipette previously coated

with silicone. Successful transfer of PB into cytoplasts was facilitated by a piezoelectric microdrive. The reconstructed oocytes were chemically activated by 5 μM ionomycin (4 min) and 1.9 mM 6-dimethylaminopurine (4 hr). Activated bovine oocytes were cultured in CR1aa medium with 0.3% BSA and activated monkey oocytes were co-cultured on buffalo rat liver cells in CMRL + 10% FBS.

Results: Present use of smaller pipettes with piezo microdrive accomplishes intracytoplasmic PB retention compared to negative results in earlier bovine attempts with beveled 18 μM pipettes using an hydraulic microdrive (n=27) or with subzonal insertion followed by electrofusion (n=18). Chemical activations of PB constructs have produced bovine embryos (11/21) cleaving to the 8 cell stage though presently with inconsistent blastomere DNA distribution. Activated cytoplasts without PB transfer have not cleaved (0/6). Monkey cytoplasts chemically activated after PB transfer have also cleaved.

Conclusions: These results suggest that the bovine and monkey first PB may support early embryonic development. Clinical application could expand the number and improve cytoplasmic quality of embryos. Supported by Ares Serono and Humagen.

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Influences of Different Concentrations of EGTA on Vitrification of *In Vitro* Produced Bovine Blastocysts. [1,2]M. H. Javed, [1]B. G. Brackett, [2]A. P. Del Valle. [1]Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA, USA, [2]ReproMed Ltd./AVR Andrology Inc., Toronto, ON, Canada.

Objective: Many cryoprotective agents result in depolymerization of cytoskeletal components prior to cooling. Pre-freeze treatment of rhesus monkey and bovine oocytes with EGTA (Ethylene glycol-bis-beta-amino ethyl ether-N,N,N′,N′-tetra acetic acid) was useful in enhancement of cryopreservation. EGTA may stabilize oocyte/embryo cytoskeletal elements and make the plasma membrane less rigid and more elastic to avoid injury during the osmotic stress of vitrification. The objectives of this study were (1) to investigate the effect of pre and post-thaw addition of different concentrations of EGTA on post-thaw development of in vitro produced bovine blastocysts and (2) to determine the effect of post-thaw sucrose dilutions on development of in vitro produced bovine blastocysts.

Design: Development of in vitro produced bovine blastocysts after vitrification and thawing in solutions containing 0, 1, 5 or 10 mM EGTA (Exp. 1), no post-thaw EGTA (Exp. 2) and no passage through serial sucrose dilutions (Exp. 3) was investigated. Post-thaw blastocyst re-expansion was compared at 0, 24 and 48 hours (h) after in vitro culture.

Materials and Methods: Bovine blastocysts were produced *in vitro* in chemically defined conditions. Vitrification and thawing solutions were prepared similar to Vajta et al. (Theriogenology 45:683–689, 1995) either in TCM alone or TCM with 0, 1, 5 or 10 mM EGTA. Blastocysts were placed in 200 μl TCM-Hepes for 5 min, shifted to 200 μl vitrification solution consisting of 12.5% ethylene glycol, 12.5% dimethyl sulfoxide at 22°C for 60 sec and then at 4°C in vitrification solution consisting of 25% ethylene glycol, 25% dimethyl sulfoxide for 60 sec and loaded into 225 μl straws. Straws were placed horizontally in liquid nitrogen vapors for 2 min and immersed in liquid nitrogen. They were warmed in a water bath at 30°C for 10 sec, contents poured into 200 μl drop containing (Exp. 1) 0, 1, 5, or 10 mM EGTA; (Exp. 2) no EGTA; (Exp. 3) no serial sucrose dilutions. Data were compared by Chi-square.

Results: Percent blastocyst re-expansion after 24 h in culture for Exp. 1 (N=107) for 0 (control), 1, 5, and 10 mM EGTA was 48, 66, 68 and 63; Exp. 2 (N=89), 46, 56, 60 and 58; and Exp. 3 (N=96), 45, 58, 57 and 55, respectively. Significantly more embryos re-expanded when vitrification and thawing solutions were supplemented with EGTA ($P<0.05$) as compared to the control. Although differences among 1, 5 or 10 mM EGTA were not statistically significant, blastocyst morphology appeared more normal in the 5 mM group. Blastocyst re-expansion observed at 24 or 48 h in culture was not different.

Conclusions: 1) Bovine blastocysts grown in chemically defined protein free medium can be vitrified successfully. 2) EGTA supplementation of vitrification and thawing solutions improves bovine blastocyst re-expansion. 3) Passage of blastocysts through serial sucrose dilutions is not required when thawing solutions contain EGTA. (Genex/CRI, Ithaca, NY, Shapiro Packing CO, Augusta, GA, and IDB, Jeddah, Saudi Arabia are gratefully acknowledged).

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Vitrification of Mouse Ovarian Tissue in Ethylene Glycol Based Solution, Thawing, In Vitro Maturation of Follicles and Fertilization of Oocytes. [1]N. Nematollahi Mahani, [2]H. Saito, [2]M. Hiroi. [1]Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran, and [2]Department of Obstetrics/Gynecology School of Medicine, Yamagata University, Yamagata, Japan.

Objectives: Preantral and antral follicles from different species have successfully been matured in vitro during recent years. Combination of follicle in vitro maturation (IVM) techniques with freezing may help patients who suffer from cancer of ovary and other ovarian disease to retain fertility at desired time. Vitrification is a low cost and fast method of freezing that has successfully been applied for embryo and oocyte cryopreservation. Present study was designed to investigate the potential of vitrified ovarian follicles to undergo IVM and fertilization in vitro.

Design: Mean diameter of vitrified in vitro matured follicles, the rate of M11 oocytes and the rate of fertilization was determined and compared with non-vitrified follicles.

Materials and Methods: Ovaries were obtained from 8-days old mice in HTF with 3 mg/ml BSA. Each ovary was bisected into fragments of 300–500 μm. Tissues were equilibrated in 6M ethylene glycol, 0.3 M trehalose and 18% w/v ficoll at RT. for 2 minutes, loaded into french straws and immersed in liquid nitrogen. After thawing and stepwise dilution, tissues transferred into bottles of 5 ml α-MEM with 100 mIU FSH. Bottles were attached to a rolling incubator with 5% CO^2 in air at 37°C and 12–15 RPM. Induction of ovulation was performed by addition of 1.5 IU hCG/ml to culture medium. The rate of MII oocytes was detected by presence of polar body. MII oocytes were fertilized with conventional method of IVF. Development to 2-cell embryos was criterion used to confirm fertilization. Non-vitrified mechanically isolated follicles were cultured singly in drops of α-MEM as control.

Results: Sixty-six percent of follicles survived after thawing. Mean diameter of oocytes inside the zona was 43.1±4.38 μm at the time of recovery. After 12-days culture the mean diameter of oocytes increased to 65.5±5.11 μm that is comparable with control (66.7±4.31). Seventy-five percent of vitrified follicles underwent germinal vesicle break down (GVB). From these, 23% developed to MII oocytes. In control 64% and 44% underwent GVB and MII respectively. After insemination 11% of Vitrified IVM Oocytes and 22% of control developed to 2-cell embryos.

Conclusions: Mouse ovarian tissues can successfully be vitrified in ethylene glycol based solutions. Vitrified follicles can tolerate freeze-thawing procedure. Lower rate of cleavage in vitrified oocytes shows that vitrification has some long-term deleterious effects on ovarian tissues. Rolling system is a good tool to prevent attachment of ovarian tissues to the culture dish. Besides, nutrition will be well maintained in a rolling system. However, presented method requires more refinement to achieve higher results and Further experiment is required to apply this method for human ovary banking.

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In Vitro Maturation of Ovarian Follicles in Presence of Hyaluronic Acid. [1]N. Nematollahi Mahani, [2]H. Saito, [2]M. Hiroi. [1]Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran, and [2]Department of Obstetrics/Gynecology School of Medicine, Yamagata University, Yamagata, Japan.

Objectives: Hyaluronic acid (HA), an extracellular glycosaminoglycan, has been shown to contribute to the maintenance of cell membrane integrity, movement and morphogenesis through attachment to CD-44, an integral membrane glycoprotein. HA has been detected in follicular fluid giving rise to evidence that HA may regulate some events during folliculogenesis. To assess this, HA was added to the culture medium from the initial steps of culture period to survey the effect of HA on in vitro maturation (IVM) of preantral follicles.

Design: Events during IVM of preantral follicles in presence of HA were recorded for 10–12 days and fertilization rate of IVM oocytes was compared with control.

Materials and Methods: Follicles were mechanically released from ovaries of 12-days old mice into HTF medium. Diameter of intact follicles and germinal stage oocytes inside the follicles were carefully measured under microscope equipped with a calibrated ocular micrometer. Follicles were

randomly allocated into drops of α-MEM, 100 mIU/ml FSH, with and without (control) 5 μg/ml HA. Follicles were observed daily for 10–12 days. The rate of monolayer formation (MF) and cavitation (CAV) was assessed. After hCG administration the rate of mucified (MUC) follicles and polar body extrusion (MII oocytes) were determined. MII oocytes were inseminated with conventional method of IVF and the rate of oocytes that developed to 2-cell embryos were assessed.

Results: HA addition had no positive effect on cytoplasmic maturation comparing to control. In HA group 100%, 96% and 58% of follicles underwent MF, CAV and MUC respectively that is nearly identical to control, 100%, 96% and 69% respectively. Mean diameter of follicles following culture increased from 54.12±7.25 to 68.53±2.71 for HA group and from 55.84±3.51 to 68.78±2.86. The rate of nuclear maturation to MII oocytes was identical in HA (73%) and control (68%). However, fertilization rate was higher in HA (33%) than control (24.6%).

Conclusions: HA may exert some role in nuclear maturation during IVM of preantral oocytes leading to higher fertilization rate. However, HA at concentration used in present study had little positive effects on follicular maturation. More extensive study is required to illuminate the definite role of HA in follicular maturation.

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Transvaginal Hydrolaparoscopy: Feasibility, Safety and Accuracy in the Exploration of the Subfertile Patient. S. Gordts, I. Brosens, R. Camp. Leuven Institute for Fertility and Embryology: Tiensevest 168,3000 Leuven, Belgium.

Objectives: For the exploration of the subfertile patients without obvious pelvic pathology, standard laparoscopy is a rather invasive and not innocious procedure. Findings at laparoscopy are normal in 30–50% of the patients. Although considered as the golden standard, the umbilical approach is not the ideal angle for a good visualisation of the tubo-ovarian structures and the high intra-abdominal pressure caused by the CO2 pneumoperitoneum masks the presence of filmy adhesions. For the exploration of the female pelvis we evaluated the possibilities of a vaginal approach, performing the procedure as a simple needle puncture technique and using warm saline as distention medium. The technique is called transvaginal hydrolaparoscopy (THL).

Design: Performed as an outpatient procedure the feasibility, safety and accuracy of the THL is evaluated.

Materials and Methods: As part of their subfertility exploration patients were referred for a THL under local anesthesia. The procedure was performed only in patients with negative findings at vaginal examination and vaginal ultrasound. Once installed in a horizontal decubitus position, a local anesthesia is done at the posterior lip of the cervix and at the center of the posterior fornix. After the posterior lip of the cervix has been lifted up with a forceps, a specially designed trocar system (Circon, U.S.A.) is placed in the midline 15 mm below the insertion of the vaginal wall to the cervix. The system consist of three parts: a Veress needle, a dilating device and the outer cannula. Rather briskly the Veress needle is inserted in the pouch of Douglas, followed by the insertion of the outer cannula which is facilitated by the dilating device. After removal of the Veress needle and dilator, a 2.7 mm rigid endoscope with a 30° optical angle and a 105° wide angled view (Circon, USA) is introduced through the remaining sheet. After confirmation of the correct intra-abdominal position a continuous infusion with warm saline at 37° is started. Using the posterior wall of the uterus as landmark, the tubo-ovarian structures are identified by rotating and lateralizing the endoscope.

Results: As an office procedure THL was performed in a consecutive series of 147 patients. Entrance to the pouch of Douglas failed in 6 (4%) patients and impossibility of visualization in 1 patient due to the presence of blood in the pouch of Douglas. Findings were normal in 62.3% (n=86) of the patients; endometriosis was diagnosed in 16% (n=22) and adhesions in 18% (n=25). In 3.7% of the patients other pathology was identified. Two complications occurred: one hematoma formation of the round ligament, conservatively treated, and one perforation of the rectum, treated conservatively, because extraperitonally localised, with antibiotics for 7 days. The pain score was recorded on a linear pain scale from 0 to 100 and compared favourably with the pain at ambulatory hysteroscopy. The procedure can easily be completed by an hysteroscopy, dye test and salpingoscopy, if indicated. Only in 26.4% (n=37) of the patients an operative laparoscopy was indicated because of the severity of the lesions diagnosed at THL.

Conclusions: THL offers a comprehensive outpatient infertility investigation that can be performed in the early stages of fertility work-up avoiding delay in diagnosis and accurate treatment. The vaginal access allows the inspection of the tubo-ovarian structures in their natural position and the aqueous distention medium improves the detection of filmy adhesions. Performed under local anesthesia the procedure is easy repeatable.

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Comparison of Utilization Patterns in Consecutive Years: Is There Value in a Formal Utilization Review Program for Infertility Services? [1,2]N. Gleicher, [1]C. Coulam, [1,2]D. Pratt, [1]J. Rinehart, [1,2]R. Morris, [1,2]R. Rao, [1,2]M. Balin, [1,2]V. Karande. [1]The Center for Human Reproduction-Illinois and [2]The University of Illinois at Chicago, Chicago, IL, USA.

Objectives: Infertility services are increasingly offered under contract conditions, which represent risk contracts to provider organizations or provider networks. Utilization of resources thus becomes an important cost consideration in calculating contract fees and contract risks. Whether such review within infertility care can affect utilization and consequently cost patterns is an important question within such a contracting environment.

Design: Average charges per patient under treatment (Avg. Chg./Pt.) and percentage of patients under treatment who receive a particular service (% Pt. rec. Tx) were compared between service years 1997 and 1998 for 11 different types of services. Of those, three were persistently under a utilization review process and eight were not.

Design: Average charges per patient under treatment (Avg. Chg./Pt.) and percentage under treatment who receive a particular service (% Pt. rec. Tx) were compared between service years 1997 and 1998 for 11 different types of services. Of those, three were persistently under a utilization review process and eight were not.

Materials and Methods: All CHR-Illinois utilization data are entered in real-time into an electronic database. Monthly reports are generated as part of CHR's continuous quality improvement program. Data for this study were derived from a dedicated patient population of between 491,135 and 596,802 lives monthly during 1997 and between 610,657 and 707,737 lives during 1998. This patient population was contracted to CHR-Illinois under a full-risk capitation contract (including risk for medical, lab and imaging services, for surgery inclusive facility charges and for injectable medication costs. 11 services were assessed. Three services (laparoscopy, hysteroscopy, medications) were managed under utilization review processes. The other eight services were not.

Service	Avg. Chg./Pt. '98 vs. '97 (%)	% Pt. rec. Tx '98 vs. '97
Laparoscopy*	−24.4	−54.1
Hysteroscopy*	+4.9	−9.1
Medication*	−5.4	+1.5
D&C	−10.8	+23.3
Sperm Wash/IUI	+8.2	+7.2
X-ray	−12.0	+12.5
IVF	+32.3	+4.6
Ultrasound	+5.0	+9.0
Phlebotomy	−5.1	+6.6
Doppler	+10.4	+64.3
Consults	+13.5	+8.6

* Services under utilization review.

Results: The table summarizes changes in utilization between 1997 and 1998. Services that were under utilization review demonstrated either utilization declines or basically flat utilization patterns. In contrast, services outside of utilization review demonstrated almost uniformly utilization increases, some of major proportions.

Conclusions: 1) Even in an at-risk fertility program, there is a strong trend towards increasing utilization of services over time; 2) This trend appears better controllable if utilization review processes are put into place; 3) Since

clinical outcome data for CHR-Illinois significantly improved between 1997 and 1998 (data not shown), decreasing utilization patterns do not appear to have adversely affected program outcomes. Whether increasing utilization patterns contributed to the improved outcomes is under investigation but seems unlikely. 4) The introduction of utilization review processes may help to control the cost of infertility care.

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Impaired Implantation and Pregnancy Women Exposed to DES In-Utero Undergoing IVF-ET. [1]U. Ulug, [1]O. Karabacak, [1]R. A. Yazigi, and [1]E. Katz. [1]The GBMC Fertility Center, Department of Gynecology, The Greater Baltimore Medical Center, Baltimore, MD.

Objectives: The exposure in-utero to DES may result in anatomical abnormalities of the female genital tract. The impact of the exposure to this drug on future reproduction is not entirely clear. This study was designed to describe the reproductive performance of in-utero DES exposed women undergoing IVF-ET.

Design: Retrospective study of IVF cycles initiated between 1986 and 1997 among in-utero DES exposed women and women with unexplained infertility.

Materials and Methods: DES exposed patients were compared to women with unexplained infertility. Male infertility and ICSI cases were excluded. Among 44 DES-exposed women with available information about their uterine configuration, the reproductive performance was reported according to whether the configuration was normal or affected. Statistical analysis was carried out with Student's-test and chi-square. Significance was set at $P < 0.05$.

Results:

	DES-Exposed			Unexposed
	Total	Normal uterus	Abnormal Uterus	
No. patients	53	18	26	174
No. cycles	126	47	65	290
Fertilization rate	80	81	85	77
Implantation rate	5.2*	7.1	6.6	11.5*
Pregnancy rate	17.7*	21.5	27.6	30.6*
First trimester abortion rate	42.9*			21.5*

* $P < 0.05$.

Conclusions: In-utero DES-exposed women undergoing IVF-ET present impaired implantation and pregnancy regardless of the anatomical configuration of the uterus. In addition, they suffer a higher rate of first trimester pregnancy loss.

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Evidence-Based Definition of Infertility. [1]A. Hershlag, [2]E. H. Kaplan. [1]Department of Obstetrics/Gynecology, North Shore University Hospital—New York University School of Medicine, Manhasset, NY and [2]Yale University School of Organization and Management, New Haven, CT.

Objective: To develop a new definition of infertility based on a sequence of specific biologic events. This approach is aimed at replacing the existing arbitrary denotation of twelve months of unprotected intercourse.

Design: A simple probability model using efficiency rates of embryonic development *in vitro*.

Materials and Methods: In order to compute the time required to progress from gametes to a baby, we assumed that for each step, the efficiency *in vitro* equals that *in vivo*. Our model, therefore, was based on the following assumptions: 1) The female partner has regular ovulatory cycles, and 12 times a year she releases one mature oocyte; 2) The male partner produces sperm of normal fertilizing potential; 3) Well-timed intercourse occurs 12 times a year, and there is always sufficient sperm in contact with the oocyte in the ampullary part of the fallopian tube; 4) 80% of all mature oocytes fertilize; 5) The cleavage rate of pronuclear zygotes is 90%; 6) 50% of cleaved embryos grow to blastocyst stage; 7) 70% of blastocysts achieve implantation; 8) Of all implantations (+hCG), 70% will develop into a baby.

Results: The probability that an oocyte in a given month will become a baby can be calculated by multiplying all the above-stated probabilities: $0.8 \times 0.9 \times 0.5 \times 0.7 \times 0.7 = 0.1764$ probability per month. Consequently, the expected time required to conceive a viable baby is $(1 - 0.1764)/(0/1764)^2 = 26.47$, and thus the standard deviation of the number of months required to become pregnant equals SQRT(26.47) = 5.14 months. The probability of becoming pregnant within t months is simply given by $p(t) = 1 - (1 - .1764)^t = 1 - .8236^1$. So the probability to conceive, for each month, is the following:

T	p(t)	T	p(t)	T	p(t)	T	p(t)
1	.1764	4	.5399	7	.7430	10	.8564
2	.3217	5	.6211	8	.7883	11	.8817
3	.4413	6	.6879	9	.8256	12	.9026

Conclusions: Under optimal biologic conditions, the average time to conceive a viable baby is just under 6 months. A viable baby should be conceived by 90% of couples attempting pregnancy for 12 consecutive months. This probability model is based on the following assumptions: a) *In vivo* efficiency of embryonic milestones is identical to respective *in vitro* events; and b) Normal sperm and oocyte interact monthly. Since it is highly unlikely that the latter condition would exist in most patients, it is expected that the achievement of a viable pregnancy in 90% of the normal population would take significantly longer.

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Success Rates and Cost-Effectiveness of Methotrexate for Ectopic Pregnancy: A Direct Comparison with Laparoscopic Salpingotomy. D. I. Eisenstein, R. Morlock, J. Elston-Lafata. Henry Ford Health System, Detroit, MI.

Objective: To evaluate the cost-effectiveness of intra-muscular methotrexate as a first line treatment for unruptured ectopic pregnancies compared to laparoscopic salpingostomy.

Design: A decision tree model was developed to estimate the health outcomes and economic costs expected for each of the two treatment approaches. The model was developed in TreeAge; multi- and one-way sensitivity analyses were performed in Crystal Ball to evaluate the model parameters.

Materials and Methods: IRB approval was granted for chart and administrative data review. Model parameters including resolution, complications, and side effects were established from the scientific literature and when necessary supplemented with data from a large multi-specialty group practice. The model evaluates parameters both individually and jointly estimating their economic impact to a provider group.

Results: Using methotrexate as a first line therapy reduces the average cost incurred by over $2,900 per resolved ectopic pregnancy. Estimates of cost savings ranged from $1,913 to $4,382. Model findings of cost savings were insensitive to extreme parameter estimates including best and worst case scenarios. Monte Carlo simulations found the magnitude of cost savings to be most sensitive to estimates of methotrexate resolution and complication rates. Model results illustrate the potential for immediate cost savings to provider groups in the treatment of unruptured ectopic pregnancy.

Conclusions: Combined with its known clinical efficacy, its favorable economic impact recommends methotrexate as first-line therapy for small-unruptured ectopic pregnancy. While cost savings persist across the entire range of modeling assumptions, the magnitude of cost savings—and clearly the impact on patients—are most effected by initial methotrexate resolutions rates. Key to ensuring optimal clinical and economic outcomes is the use stringent criteria for patient selection.

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The Use of Early Pregnancy Monitoring Algorithms Improves Care for Disorders of the First Trimester. J. M. Storment, E. Taylor, J. R. Brumsted, J. H. McBean. Department of Obstetrics and Gynecology, University of Vermont, Burlington, VT, USA.

Objectives: To determine the efficacy of the standard application of diagnostic algorithms in the first trimester in early identification of normal (IUP) and abnormal (early abortions, AB; ectopic pregnancy, EP) pregnancy outcomes.

Design: Prospective study applying a screening algorithm to all patients who presented for antepartum care to a university practice between July 1997 and December 1998.

Methods: The diagnostic algorithm was applied as follows: If no EP risk factors (RF) were present (vaginal bleeding, pain, prior ectopic, tubal surgery, pelvic inflammatory disease, recent IUD use or greater than one year history of infertility) and the gestational age (GA) was known, an antepartum visit was scheduled. If the GA age was unknown or at least one risk factor for EP was present, subjects were monitored with serial HCG and ultrasound (US) measurements until location and viability of the pregnancy was determined. Subjects were grouped according to presence of RF and pregnancy outcome and the number of USs and HCGs per person compared using Kruskal-Wallis analysis. Sensitivity, specificity and predictive values were calculated.

Results: 455 subjects were enrolled and divided into 6 groups: ***Group 1*** (n=275) No RF/IUP; ***Group 2*** (n=42) No RF/AB; ***Group 3*** (n=4) No RF/EP; ***Group 4*** (n=70) RF/IUP; ***Group 5*** (n=25) RF/AB; ***Group 6*** (n=34) RF/EP. The algorithm resulted in early identification of 95% (34/38) of EP, 76% of whom were treated with methotrexate. The average number of US and HCGs per person in Group I (0.29, 0.06, respectively) was significantly less than in all other groups. The average number of USs and HCGs in all other groups was 1.4 and 2.2. The use of risk factor assessment to predict abnormal outcome (AB, EP) had an overall sensitivity and specificity of 56% and 80% respectively, but was more sensitive in identifying EP (89%). The overall positive (PPV) and negative predictive (NPV) values were 45% and 86% respectively. With respect to EP alone, risk factor assessment has a negative predictive value of 98%.

Conclusions: This study shows that the use of risk factor-based diagnostic algorithms with the selective use of HCG levels and US successfully results in the early identification of abnormal pregnancies. As a result, medical management of EP was utilized in 76% of cases. With a high NPV, this algorithm results in the identification of normal pregnancies with minimal additional testing and therefore can be broadly applied in the first trimester to improve the management of early pregnancy disorders.

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The Changing Demographics of Women Diagnosed with Endometrial Polyps: 1990 to 1996. E. O. Garner, W. Khan, D. Rodriguez-Thompson, N. Sharif, F. Syed, E. A. Stewart. Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Objective: Endometrial polyps are often associated with abnormal uterine bleeding, particularly in postmenopausal women. We hypothesize that the demographics of endometrial polyps are changing primarily due to new modalities of diagnosis. To test this hypothesis we identified the bleeding patterns present before diagnosis and the diagnostic modality leading to the pathologic identification of endometrial polyps between the years 1990 and 1996.

Design: Retrospective chart review of hospital medical and pathologic records.

Materials and Methods: The pathologic reports of all women diagnosed with endometrial polyps in 1990 (220 patients) and in 1996 (386 patients) were obtained and reviewed. The medical records of each of these women were reviewed for age, bleeding pattern and method of diagnosis. Comparisons of binomial data were carried out using Chi Square analysis utilizing Stata statistical software (CRC, College Station, TX).

Results: The percentage of patients reporting no abnormal bleeding increased from 12.3% in 1990 to 23.6% in 1996 ($P<0.001$) and the percentage of patients having postmenopausal bleeding decreased from 36.4% to 25.1% ($P<0.03$). Mean age was not significantly different between the two groups. Diagnostic modalities that identified the polyps were also significantly different. Diagnosis by ultrasound increased 5-fold (3.6 vs. 16.8%, $P<0.001$), hysteroscopy performed in an operating room increased 3-fold (6.4 vs. 19.7%, $P<0.0001$) and office hysteroscopy was introduced to diagnose these lesions (0.0 vs. 4.2%, $P<0.002$) from 1990 to 1996. Incidental finding of previously undiagnosed polyps at the time of the index procedure decreased over the same interval (79.6 vs. 46.63%, $P<0.0001$).

Conclusions: Endometrial polyps are increasingly identified in women with no abnormal uterine bleeding using ultrasound and hysteroscopy. Further study is necessary to determine if the evaluation of processes such as infertility contribute to these demographic changes.

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The Effect of Acetyl Salicylic Acid and Prednisolone Before and During Pregnancy in Reducing Unexplained Recurrent Abortions. M. Vahid Dastjerdi, A. Moini, A. Aleyasin, H. Kashaf, V. Marsoosi, A. Aghahosscini. Arash Women's Hospital, Tehran, Iran.

Objectives: Approximately 15% of over 1000 recurrent pregnancy loss patients have recognized autoimmune factors. The aim of this study was to compare the use of acetyl salicylic acid (ASA) plus prednisolone before and during pregnancy in the treatment of recurrent abortions.

Design: A randomized prospective clinical trial comparing three groups of recurrent pregnancy loss patients.

Materials and Methods: 75 patients with a history of 3 or more unexplained recurrent abortions were randomly divided into three groups. The first group (n: 28) were given ASA 100mg/day and prednisolone 10mg/day three months before pregnancy. After positive pregnancy test, this group received ASA 100mg/day up to 36 weeks of pregnancy and prednisolone 30mg/day up to 16 weeks of pregnancy. The second group (n: 22) received ASA and prednisolone (like above) during pregnancy. The third group (n: 25) received ASA alone during pregnancy.

Results: Abortion rate in the first, second and third group were 15%, 36.3% and 52%. Ratio of term and preterm labor in the first group were 67%, 18%, for the second group: 41%, 22.7% and for the third group: 28%, 20%.

Conclusions: This study suggests that treatment with ASA and prednisolone before pregnancy in patients with recurrent abortion improves the results by decreasing the abortion rate and in creasing term delivery but the difference between the second and third group was not significant. This maybe explained by improvement of antiphospholipid antibody syndrome before pregnancy by affecting the immune and coagulation systems.

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Proven Fertile Control Patients Have a Low Incidence of Histologic or MAG Mucin Expression Abnormalities. [1]H. J. Kliman, [2]L. Elberger, [2]A. A. Acosta, [3]G. F. Doncel, [1]H. S. Taylor, [1,4]E. A. Nannenberg, [1]K. M. Mitchell. [1]Department of Obstetrics/Gynecology, Yale University, New Haven, CT, [2]Centro de Estudios en Ginecologia y Reproduccion, Buenos Aires, Argentina, [3]Department Obstetrics/Gynecology, Eastern Virginia Medical School, Norfolk, VA, [4]University of Groningen, Netherlands.

Objectives: We have previously shown that MAG mucin expression abnormalities in endometrial biopsies are correlated with infertility. The majority of biopsies examined have been from infertile patients, making it difficult to assess the prevalence of these abnormalities in the general population. Therefore, we studied biopsies from proven fertile patients to estimate the prevalence of histologic and MAG expression abnormalities in this population.

Design: MAG mucin immunohistochemistry was performed on endometrial biopsies collected from healthy parous volunteers and women undergoing tubal ligation. The patterns of glandular MAG staining were compared to standard histologic dating criteria for each cycle day (CD).

Materials and Methods: At least two endometrial biopsies from 13 normal volunteers were collected within 1–2 days and ~10 days after the LH surge, or one biopsy randomly in the follicular phase from 11 tubal ligation patients. All patients were 25–35y and had a parity of at least one. Biopsies were fixed in formalin, paraffin embedded and immunohistochemically stained for MAG (AJP, 146:166–181, 1995), ABO blood group antigens, MUC1 (positive control), and NMA (neg. control). Less than 10% glandular MAG reactivity was considered negative. H&E sections were used for endometrial dating of the stroma and glands according to the criteria of Hendrickson and Kempson.

Results: All the biopsies except 1 (which was 3 days within the CD) from the volunteers were dated within 2 days of actual CD (27/28; 96%). Two biopsies showed significant (>50%) luteal phase glandular-stromal dyssynchrony (14%). Six of the 13 volunteers (46%) and 4 of the 11

tubals (36%) were blood group A, and could therefore be evaluated for MAG reactivity. Two of these tubal patients were excluded (one was on OCP, one was immediately postpartum). Of the 8 remaining patients, all except 1 expressed MAG appropriately: high expression up to day 18, no expression after day 19. The one abnormal patient expressed 10% glandular MAG reactivity (the lowest level regarded as positive) with a stromal dating of d24-25. Of note, 10% of the glands exhibited d18 vacuolization.

Conclusions: Using histologic criteria alone, only 1 biopsy (4%) was greater than 2 days out of phase while 2 (14%) showed significant glandular-stromal dyssynchrony. MAG, a marker of endometrial receptivity, was only minimally abnormal in one patient in one biopsy (17%). Additional samples will be required to calculate the true prevalence of histologic and receptivity marker abnormalities in fertile women, however, this initial study suggests that these abnormalities will be infrequent in this population. Supported in part by the Donaghue Foundation (HJK).

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Incidence of Local Reactions with Subcutaneous Administration of Conventional Vs Highly Purified Urinary Gonadotropins. M. P. Steinkampf, C. W. Childers, K. R. Hammond, R. E. Blackwell. Department of Obstetrics/Gynecology, University of Alabama at Birmingham, Birmingham, AL.

Objectives: Although highly purified urinary and recombinant gonadotropins are routinely administered subcutaneously, conventional first-generation urinary gonadotropin preparations have been limited to intramuscular use because of concerns about injection reactions. The purpose of this study is to compare the incidence of adverse effects with subcutaneous administration of conventional (Repronex) vs highly purified (Fertinex) urinary gonadotropin preparations.

Design: Prospective trial with each subject serving as her own control.

Materials and Methods: Twenty-eight women undergoing ovarian stimulation for assisted reproduction (27 cycles) or intrauterine insemination (one cycle) injected their daily gonadotropin doses subcutaneously in the paraumbilical region, with equal doses (150-300 IU each) of Fertinex on one side of the umbilicus and Repronex on the other side. Patients were offered the option of switching to intramuscular injections if either drug produced unacceptable reactions when given subcutaneously. The incidence of injection reactions and treatment discontinuation were monitored, and patients scored the pain on subcutaneous injection using an ordinal pain score (0=no pain, 10=unbearable pain).

Results: Local reactions on subcutaneous administration occurred in 27 of 28 cases with Repronex, compared with 0 of 28 with Fertinex ($P<0.001$); erythema at the injection site was the most common reaction (80% of patients). Pain on subcutaneous injection was significantly greater with Repronex than with Fertinex (Pain score means: Repronex 4.59, Fertinex 1.23; $P=0.0001$). No life-threatening reactions occurred, and despite the increased discomfort only four patients (14.2%) converted subcutaneous Repronex injections to intramuscular administration, all without further reactions. Of 23 treatment cycles completed at the time of this submission, 13 pregnancies had been established (56% per cycle).

Conclusions: Highly satisfactory pregnancy rates can be achieved with urinary gonadotropins, but the subcutaneous administration of conventional gonadotropins yields a high incidence of minor side effects.

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Prevalence of Chronic Endometritis in Women Undergoing Infertility Therapy. J. C. Petrozza, M. Guarnaccia, P. Huang, M. Summers, R. I. Hardy, V. Cardone. Fertility Center of New England, Reading, MA and Division of Reproductive Endocrinology, New England Medical Center, Boston, MA.

Objectives: The evaluation of endometrial integrity is often employed at some point in the evaluation of an infertile couple. Benign endometrial pathologies such as chronic endometritis can have a negative effect on endometrial receptivity, yet the prevalence of these pathologies in infertile women has not been clearly evaluated, except in a few small studies in women proceeding on to in vitro fertilization. In these women, the incidence of chronic endometritis is reported to be only 1%. However, it is likely that this percentage is even higher if women were screened earlier in their evaluation. Over a two year period in our center, all women undergoing a diagnostic hysteroscopy as part of their infertility evaluation also had endometrial biopsies done as part of their procedure. The prevalence of chronic endometritis was then calculated as well as its association with other pelvic pathology.

Design: Retrospective analysis of all patients undergoing a hysteroscopic procedure as part of their infertility evaluation.

Materials and Methods: Patient charts from January 1995 to December 1996 were evaluated. It has been the protocol at our center that endometrial biopsies are taken at all hysteroscopies. The biopsies were reviewed by one pathologist and classified into three categories: 1.) Chronic endometritis with definite plasma cell infiltration; 2.) Plasmacytoid lymphocyte infiltration suggestive of chronic endometritis, and; 3.) Can't exclude chronic endometritis due to masking inflammatory cell infiltration. Associated pelvic pathology such as hydrosalpinges, endometrial polyps, uterine myomas, and intrauterine adhesions were gathered from operative reports or prior hysterosalpingograms.

Results: Seven hundred and twenty-two (722) patients underwent a hysteroscopy. 85% were done prior to gonadotropin/intrauterine insemination therapy. Plasma cell endometritis was seen in 58 (8%) of specimens, plasmacytoid lymphocyte infiltration in 30 specimens (4.2%), and 38 (5.3%) with a diagnosis of "can't exclude chronic endometritis". Of those women with plasma cell endometritis, 19% had endometrial polyps, 8.6% had hydrosalpinges of one or both tubes, 13.8% had intrauterine adhesions, 15.6% had evidence of at least one occluded fallopian tube, and 12.1% had uterine fibroids. At the time of the hysteroscopy, chronic endometritis was suspected in only 17.2% of those patients with histologic evidence of plasma cell endometritis. This percentage increased to 30.5% if all inflammatory specimens are included.

Conclusion: Chronic endometritis is more prevalent in infertile women than previously reported. Endometrial biopsies should be done at the time of a hysteroscopy in these patients and evaluated for the presence of plasma cells. In women with a prior hysteroscopy where no endometrial biopsy was done, an office endometrial biopsy should be considered. A prospective trial of treatment for chronic endometritis and pregnancy outcomes is underway at our center.

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Location of Anterior Abdominal Vessels at Risk in Laparoscopy of the Obese Patient. [1]L. Dundoo, [1]A. Auyeung, [1]I. Brondz, [1]R. R. Odem, [2]H. F. Bennett. [1]Department of Reproductive Endocrinology, [2]Department of Radiology. Washington University School of Medicine, St. Louis, MO.

Objective: Vessels of the anterior abdominal wall are commonly at risk of injury in laparoscopy; therefore, knowing the location and course of these vessels would help to minimize injury. Prior studies that mapped these vessels suffer from small numbers and included few obese patients. As laparoscopic surgery becomes more routine, obese patients excluded from laparoscopy in the past are frequently operated on. This is especially true of the infertility patient for whom laparotomy is not a reasonable alternative to diagnostic laparoscopy. The purpose of this study is to determine the location and course of anterior abdominal wall blood vessels in patients' whose BMI is ≥ 30 kg/m^2, and to locate the aortic bifurcation relative to the anterior abdominal wall.

Design: This is a prospective observational study of patients on whom abdominal and pelvic computed tomography (CT) scans were performed for various medical conditions.

Materials and Methods: Patients presenting for abdominal and pelvic CT scans were measured for height and weight and included for study if their BMI's were ≥ 30 kg/m^2. Exclusive criteria were: distortion of abdominal wall by tumor or ascites, gross anatomic deformities from prior surgery and scarring, and inability to identify vessels of interest and the ipsilateral landmarks on at least one side of the CT film. The distance from the midline of the following vessels were measured: the inferior epigastric artery, superficial epigastric artery and the superficial circumflex iliac artery. Vessels from both right and left sides were measured at the symphysis pubis, at 3 and 5 cm cephalad from the symphysis, and at the umbilicus. The lateral borders of the rectus muscles from the midline were similarly mapped. The location of the aortic bifurcation relative to the umbilicus and its distance from the anterior abdominal wall were also measured. The position of vessels and muscles at the specified distances from the symphysis are

reported as the mean distance from the midline ± the standard deviation. Correlation between BMI, lateral border of the rectus muscle and depth of aortic bifurcation were examined using linear regression analysis. Student's t-test was used to compare measurements from the right and left sides, and those between males and females.

Results: Forty women and 24 men were included in the study. Their BMI was 37.0 ± 5.2 kg/m^2. There were no differences between the measurements between males and females, so the data were pooled. Similarly, no differences were found between right and left sides ($p>0.05$). Vessels were found within the range of previously published distances, but there was no correlation between BMI and the lateral rectus border ($R=0.23$) at the level of the umbilicus, and the distance between aortic bifurcation and anterior abdominal wall (12.9 ± 2.3 CM) did not correlate with BMI ($R=0.28$). The bifurcation was 0.63 ± 2.9 cm caudal to the umbilicus in 50 patients measured. Fifteen patients (30%) had bifurcations cephalad to the umbilicus, 24 (48%) were caudal, and 9 (18%) were level with the umbilicus.

Conclusion: In the obese patient, the locations of anterior abdominal wall vessels from the midline do not differ substantially from those of non-obese patients. Contrary to published data, the distance of the lateral border of the rectus muscle does not increase with BMI, and the aortic bifurcation is much closer to the level of the umbilicus than previously published. The location and technique for trocar placement in the obese patient should be adjusted according to these new data.

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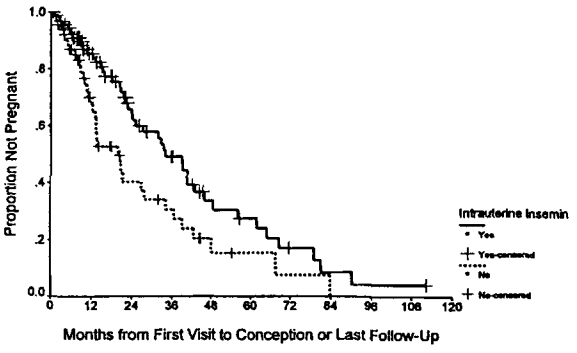
Assisted Reproduction in Infertile Patients of Different Etiologies. [1]E. Confino, [2]S. Watson. Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, Illinois.

Objective: Patients with severe endometriosis (E), tubal factor infertility (TF) and male factor infertility (MF) are less likely to have a spontaneous conception when compared to mild to minimal E, unexplained infertility (U) and ovulatory dysfunction (OD). The purpose of this study was to compare the outcomes of all five infertility groups.

Design: Retrospective case analysis.

Materials and Methods: Demographics, diagnoses, treatments, pregnancies and time to conception were analysed for 169 patients with E, TF, MF, OD and U infertility. All patients were treated in escalating infertility treatment regimens. The data was subjected to analysis of variance. Time to conception was subjected to Kaplan Meyer survival analysis.

Results: All five groups did not differ in their demographic characteristics, and pregnancy rates. Time to conception was not statistically different among all five groups subjected to clomiphene, gonadotropins, or IVF. Only intrauterine inseminations resulted in a longer time to conception (graph 1). At 1, 2, 3 years of treatments 26%, 47% and 58% of the patients who continued treatments conceived respectively.



Conclusion: Escalating ART regimens resulted in comparable PR in these series and comparable time to conception, except for IUI. A consistent substitution of IUI with IVF/ICSI for MF infertility may eliminate this observation.

P-359

Human Embryonic Heart Rates Measured by Ultrasound Progressively Rise Three-fold and Rapidly in Earliest Gestation; Normative Data. [1]S. L. Feigenbaum, [2]M. Fluker. [1]Kaiser Permanente, Northern California Region and University of California San Francisco, San Francisco, CA and [2]University of British Columbia and Genesis Fertility Center, Vancouver, BC, Canada.

Objectives: Growth tables for accurate mensuration of early embryonic features including fetal pole and other structural features have existed for many years. However, developmental progression of the normal fetal heart rate has not been described. With the implementation of M-mode and more advanced doppler techniques, accurate detection of fetal heart tones (FHTs) and their measurement are now possible. Recent studies have described slow heart rates to be predictive of adverse pregnancy outcome in some small series of normal and infertility populations although normative FHT data for the early gestational ages, between 5.5 and 8.5 weeks (by last menstrual period (LMP) are lacking. Two datasets comprised of patients having ultrasound documentation of normal early gestations were used to describe the developmental progression of early FHTs.

Design: Descriptive study.

Materials and Methods: Two datasets were used. Set 1 included all patients who underwent IVF/ET or controlled ovarian hyperstimulation with hCG ovulation induction. Patients who conceived on this regimen had early ultrasound with real-time doppler determination of FHTs performed between 5.5 and 8.5 weeks gestation by adjusted LMP. Set 2 consisted of all patients presenting for elective termination of normal pregnancy during the first trimester. These patients had doppler or M-mode determination of FHTs prior to termination. Pregnancy dating was done using conventional obstetric and gynecologic history as well as by sonographic measurement of fetal pole and average gestational sac diameter. For both sets of patients, only those patients who had a normally progressing singleton pregnancy were included. Excluded were patients with multiple gestations, bleeding and/or cramping suggestive of an impending spontaneous miscarriage, or other features, e.g. extremely small sac for dates, suggestive of an abnormal gestation. Means, ranges, standard deviations and 95% confidence intervals were determined. A test for homogeneity of variance was performed.

Results: Data for both data sets were compared and found to have similar means and variances. The data were then pooled. Heart rates were as follows. Weeks 5.5–6.5: Mean 68, S.D.=26, Weeks 6.6–7.5: Mean 132, S.D.=54, Weeks 7.5–8.5: Mean 178, S.D.=42. Examination of the data revealed a truncated distribution in the first and last weeks of measurement, i.e., no FHTs less than 48 were seen in the first week and no FHTs exceeding 196 were seen in the third week. The most rapid progression of FHTs occurred during the period from 6.6 to 7.5 weeks gestation when FHTs nearly tripled. A regression curve with confidence intervals describing FHTs of normally progressing singleton gestations can be plotted.

Conclusions: Developmental progression of FHTs in normal early gestation occurs very rapidly. Because these observations were limited by the periods examined, it was not possible to determine when in gestation the rapid heart rates seen in the eighth week of gestation slow somewhat to the rates observed later in gestation. Prospective studies comparing outcomes of all early pregnancies can be compared to determine the utility of these measurements for prognostic studies in both fertility and other populations.

P-360

Prevention of Acute Pelvic Inflammatory Disease After Hysterosalpingography: A Follow-up Report on the Effectiveness of Doxycycline Prophylaxis. D. E. Pittaway, J. O'Neill, D. Rondinone. Brookview Women's Center and Department of Obstetrics/Gynecology, Wake Forest University School of Medicine, Winston-Salem, N.C.

Objectives: An earlier study (Am J Obstet Gynecol 1983;147:623–626) found that 11% of women with dilated tubes developed an acute pelvic infection (PID) after hysterosalpingography (HSG), and that doxycycline administration prevented a clinical infection in 56 women at risk for PID. To examine further the effectiveness of oral doxycycline prophylaxis for patients at risk for PID following HSG, all women with dilated tubes were treated with doxycycline.

Design: A prospective 14-year study of all women (1300) undergoing 1379 HSG's performed by the first author.

Setting: University-based gynecologic-infertility practice.

Interventions: For prevention of pelvic inflammatory disease following HSG, patients with dilated fallopian tubes with or without blockage received oral doxycycline 100 mg. twice daily for 5 days.

Main Outcome Measures: Clinical pelvic infection (PID) following HSG.

Results: In the 1186 HSG's without dilated tubes, no cases of PID were observed. One case of PID developed after the 193 HSG's (0.5%) performed on the 176 women who were at risk for post-HSG infection and had received doxycycline.

Conclusion: The risk of infection after hysterosalpingography was very low in this population when nondilated tubes (0/890), peritubal disease (0/124) or proximal occlusion (0/172) are present. In the highest risk group (dilated tubes), doxycycline administration is effective, and is a cost-effective intervention for preventing infection after hysterosalpingography.

P-361

Serum Human Chorionic Gonadotropin (hCG) Dynamices in Surgically Proven Ectopic Pregnancies. D. E. Pittaway, D. Ginsberg, D. Rondinone. Brookview Women's Center, Wake Forest University School of Medicine, Winston-Salem, NC.

Objectives: Since hCG concentrations rise exponentially at predictable rates in normal pregnancy, serial determinations have been useful in establishing the normalcy of pregnancies. However, some ectopic pregnancies (EP) have been observed to have normal rates of hCG rise. Consequently, we studies the types and frequency of hCG patterns in asymptomatic women with EP.

Design: Serial hCG determinations were performed prospectively in 782 consecutive pregnancies at risk for EP and were evaluated after surgical confirmation.

Materials and Methods: Serum hCG concentrations were measured by an automated microparticle enzyme-immunoassay technique using the Third International Standard. EP were surgically proven in 89 women of whom 54 fulfilled the entry criteria which includes: absence of symptoms, initial hCG<1000 mIU/ml and having at least two hCG determinations. A doubling time (DT) of 2.4 days was considered normal (95% upper limit), and hCG dynamics were classified as normal, abnormal, or falling.

Setting: University-based gynecologic-infertility practice.

Results: With initial hCG concentrations ≤500mIU/ml, 20 of 48 (47%) women with an EP had a normal DT, 19 (44%) had an abnormal DT and 4 (9%) had falling levels. Two of 11 (18%) women with initial hCG levels between 501 and 1000 mIU/ml had a normal DT, 5 (45%) an abnormal and 4 (36%) a falling hCG. Eighteen women with a normal initial DT had a second DT of which two (11%) was normal, eleven (61%) were abnormal and five (28%) had falling hCG concentrations. Thirteen of 17 (76%) women with initial normal DT had abnormally low initial hCG concentrations.

Conclusions: These observations suggest that: (1) normal hCG dynamics, frequently occur very early in the gestation of EP; (2) most EP with an initially normal DT will be abnormal in the second DT; (3) a common pattern of hCG dynamics in EP is an initially normal DT with lower than anticipated hCG for the gestational age; (4) continued monitoring of hCG beyond an initial normal DT is necessary in women at risk for EP.

P-362

Glucose Screening in an Infertile Population. K. L. Durinzi and R. P. Buyalos. Kaiser Los Angeles, Section of Reproductive Endocrinology and Infertility, Los Angeles, CA.

Objective: Patients who are diabetic at the time of conception are at an increased risk for fetal malformations unless they are in good metabolic control during organogenesis of the fetus. Because most major malformations occur in the first 9 weeks of pregnancy, we prospectively screened the fasting glucose levels of all patients prior to initiating an infertility evaluation from 2/1/97–8/31/98. All patients with fasting plasma glucose levels ≥95 mg/dl were referred to our Perinatology Department for further evaluation, diet counseling and insulin therapy when indicated.

Design: Prospective.

Materials and Methods: 531 infertility patients were screened during the period of study. Of these patients, 42 had plasma glucose levels ≥95 mg/dl (Carpenter and Coustan criteria for gestational diabetes), 15 had plasma glucose levels ≥105 mg/dl (National Diabetes Data Group criteria for gestational diabetes), 7 had plasma glucose levels between 109–125 mg/dl (impaired fasting glucose) and 6 women had plasma glucose levels ≥126 mg/dl (diabetes). The data collected from these patients included chronological age, Body Mass Index (BMI), race/ethnic origin, and menstrual cyclicity.

Results:

	Mean Age	Mean BMI	Race/Ethnic Origin %			
			Cauc.	Asian	Black	Hisp.
Controls	32.8	24.9	37	10	24	29
Glucose ≥95	33.9	27.1*	23	29	19	29
Glucose ≥105	32.7	28.7*	27	33	13	27
Glucose 109–125	33.7	26.9	28	44	14	14
Glucose ≥126	30.2	30.2*	33	17	0	50

	Menstrual Cyclicity %	
	Regular	Irregular (≥35 days)
Controls	68	32
Glucose ≥95	69	31
Glucose ≥105	60	40
Glucose 109–125	57	43
Glucose ≥126	67	33

* P<0.05 compared to Controls

Conclusion: Of the infertility patients screened 8% (42/531) had glucose levels requiring further evaluation. Of the variables analyzed, BMI was the most predictive of elevated fasting glucose levels. Prospective glucose screening in an asymptomatic infertility population may be of benefit in reducing the diabetic complications of pregnancy particularly in women with BMI >25.

P-363

Trilaminar Pattern, Follicle Stimulating Hormone Concentration, and Maximum Estradiol Levels are not Good Predictors of Pregnancy Outcome in Intrauterine Insemination. [1]A. N. Wakim, [1]A. J. Karam, [1]A. Sandu, [2]J. Schlosser. [1]Department of Obstetrics/Gynecology and [2]Department of Research Computing, Allegheny University of Health Sciences, Allegheny General Hospital, Pittsburgh, PA.

Objective: It is believed that the presence or absence of a trilaminar pattern (TP) is a good predictor of pregnancy outcome in in-vitro fertilization. In this study, we tested this hypothesis in cases of superovulation with human menopausal gonadotropins and intrauterine insemination (HMG-IUI).

Design: This is a retrospective analysis of 230 cycles from 130 women who received HMG-IUI at our center in 1995.

Materials and Methods: For each cycle, the variables assessed included: the presence or absence of TP, amount of HMG, and maximal Estradiol (E2) levels. Cycle data were grouped by presence or absence of male factor as well as by TP, HMG concentration, and E2 maximum level. Analysis of this data was done using T-test, Anova, and logistic regression modalities to assess the correlation between the above factors and pregnancy.

Results: The appearance of a trilaminar pattern in all cycles analyzed was 57.4% in the positive pregnancy outcome group versus 55.7% in the negative pregnancy outcome group, a difference that was not statistically significant $X2=0.968$. The mean total HMG used and the E2 maximum level achieved in the two groups was also not statistically significant ($P=0.444$ and $P=0.074$, respectively).

In those cases with documented male factor infertility (130 cycles), the group that achieved pregnancy received a higher total HMG and had a higher E2 maximum level than the group with negative pregnancy outcome. Again, this was not statistically significant $P=0.143$, and $P=0.075$ but highly suggestive of a possible correlation (HMG mean = 2214.18 ±

383.37 vs 1713.61 ± 132.15 and E2 mean 1233.73 ± 100.94 vs 1044.95 ± 42.84).

In those cases with no male factor infertility (100 cycles), 26.9% of cases with a trilaminar pattern achieved pregnancy versus 18.2% of cases that did not. Although not statistically significant (P=0.578), this is obviously clinically significant.

Conclusion: 1. Our analysis shows that a trilaminar pattern is not a good predictor of pregnancy outcome except in cases of no male factor infertility. 2. Both HMG concentration and E2 maximum levels seemed to indicate that the higher the concentrations, the better the chance of achieving pregnancy in cases of male factor infertility. If indeed that is the case, perhaps certain levels should be targeted before performing intrauterine insemination. More cases are needed to strengthen the power of this study.

P-364

High Doses of Tamoxifen Versus HMG Therapy in Clomiphene Citrate Resistant Patients. H. N. Sallam, A. N. Sallam, F. Ezzeldin, A. Khanfour. Department of Obstetrics/Gynecology, Alexandria University, Alexandria, Egypt.

Objectives: Anovulatory patients resistant to 200 mg of clomiphene citrate are usually required to receive the more expensive HMG therapy. The aim of this study was to explore the possibility of inducing ovulation with high doses of tamoxifen as compared to conventional HMG therapy.

Design: Anovulatory patients not responding to clomiphene citrate were randomly allocated to two groups. Group A was treated with increasing doses of tamoxifen and group B with HMG.

Materials and Methods: Fifty two patients belonging to WHO group II of functional infertility and who failed to ovulate on clomiphene citrate (200 mg/day for 5 days) were studied. They were randomly allocated to 2 therapy groups. The first group (group A, n=26) received ascending doses of tamoxifen (Nolvadex) for 3 months as follows 40 mg/day for 7 days, 60 mg/day for 7 days and 60 mg/day for 10 days during the first, second and third cycles respectively. The patients were monitored with ultrasound scanning of their ovarian follicles and with mid-lutea plasma progesterone concentration, and the dose was only increased if anovulation persisted. The second group (group B, n = 26) received standard HMG therapy monitored with ultrasound scanning of the follicles as described by ourselves elsewhere (Sallam et al, 1982) for 3 cycles.

Results: Seven pregnancies resulted in group A (26.9%) and 9 pregnancies in group B (34.6%). Six patients reported temporary headaches and/or blurring of vision in group A and six patients developed mild and moderate hyperstimulation in group B.

Conclusion: It is concluded that high doses of tamoxifen offer a cheaper alternative to HMG therapy in selected cases.

Sallam HN, Marinho AO, Rodeck CH, Collins WP and Campbell S. Br J Obstet Gynaecol 89:1515, 1982.

P-365

Serum Progesterone as a Marker of Non-Viable Pregnancy in Emergency Department Patients and after Assisted Reproductive Technology. [1]P. Claman, [2]S. L. Perkins, [3]M. Al-Ramahi. [1]Division of Reproductive Medicine, Department of Obstetrics and Gynecology and [2]Department of Pathology and Laboratory Medicine, The Ottawa Hospital and University of Ottawa, ON., Canada. [3]Department of Obstetrics and Gynecology, Jordan University, Aman Jordan.

Objective: To determine the sensitivity and specificity of serum progesterone (P) in predicting non-viable pregnancy in patients pregnant after ART vs spontaneously pregnant women.

Design: Prospective study of pregnant patients.

Materials and Methods: Group A included ART patients who underwent I) superovulation, ii) ovulation induction or iii) spontaneous ovulation. Group B included women who had conceived spontaneously presenting to the Emergency Department with bleeding &/or pain. Serum was collected from women in both groups and analyzed for BhCG and P (Abbott AxSYM MEIA). Linearity of P assay is 0.6–127 nmol/L. Pregnancy outcomes were determined by chart review and telephone interview and were classified as: Miscarriage (Sab), Ectopic (Ect.) or Viable intrauterine pregnancy (VIUP). Sensitivity and Specificity were calculated for two different cutoffs of Progesterone with a positive result defined as Ect. Or Sab when P < 45 or 70 nmol/L.

Results: Table: Prediction of Non-viable Pregnancy (P=[Progesterone] in nmol/L)

	Viable Intrauterine Pregnancy		Miscarriage	
Group:	N	Median P (range)	N	Median P (range)
A-i (n=55)	38	>127 (42.6–>127)	16	23.4 (1.3–>127)
A-ii (n=23)	15	91.5 (34.2–>127)	6	16.8 (1.6–112)
A-iii (n=45)	24	48 (17.8–>127)	12	23.9 (0.5–68.6)
B (n=137)	40	60.5 (3.3–127)	72	16.8 (1.2–>127)

	Ectopic Pregnancy		For P <45 predicting non-viable pregnancy	
Group:	N	Median P (range)	Sensitivity	Specificity
A-i (n=55)	1	N/A	58.8%	100%
A-ii (n=23)	2	N/A	62.5%	80%
A-iii (n=45)	9	14.6 (0.6–82.0)	71.4%	58.3%
B (n=137)	25	24.7 (2.2–>127)	83.7%	87.5%

Conclusions: A serum progesterone <45 nmol/L is a sensitive and specific indicator of non-viable pregnancy after superovulation ART, non superovulation ART and spontaneous pregnancies. However, in non-superovulation pregnancies there is considerable overlap of serum P between non-viable and viable pregnancies. Serum Progesterone levels should be interpreted with caution in patients who have not undergone superovulation therapy.

P-366

Can the Beta-3 Integrin Assay be Used for Clinical Management Prior to Oocyte Donation? [1]S. J. Chantilis, [2]W. F. Howard, [1]Meintjes, [1]D. Doherty, [1]D. Ward. [1]ARTS Program, Presbyterian Hospital of Dallas, [2]Trinity ART Program, Carrollton, TX.

Objective: The beta-3 (β3) integrin subunit is an endometrial protein which is present in glandular epithelium during the menstrual cycle, and has been purported to serve as a marker of the window of implantation with expression occurring during days 20 through 24. Indeed, a variety of entities causing infertility including luteal phase dysfunction, presence of hydrosalpinx, and endometriosis have been associated with diminished or absent β3 integrin expression. While an abundance of data exist regarding this assay obtained during natural spontaneous cycles, relatively little is known about β3 integrin assay obtained during exogenous hormone replacement cycles. Using the oocyte donation model, we tested the reliability of a β3 integrin assay in a woman who repeatedly failed to demonstrate the presence of β3 endometrial integrin during mock cycles by treating her with oocyte donation and embryo transfer.

Design: Implantation and pregnancy was determined in a woman who underwent an oocyte donation IVF cycle.

Materials and Methods: A 35-year-old $G_2P_1A_1$ woman with secondary infertility due to endometriosis and poor ovarian response during three IVF attempts underwent three mock hormone replacement cycles in preparation of a donor oocyte IVF cycle. All mock cycles were completed following midluteal phase Lupron down regulation. Hormone replacement for the first and third cycles consisted of estradiol valerate, 5 mg IM q week × 7 days, then q 4 days until biopsy. After sonogram confirmed an adequate endometrium, progesterone in oil, 50 mg (IM) q AM, and progesterone vaginal suppositories, 100 mg q PM, was initiated. Endometrial biopsy (EMB) was conducted after 8 days of progesterone, *viz.*, cycle day 22. After six months ovarian suppression, hormone replacement for the second mock cycle consisted of 17β-estradiol, 2 mg po × 6 d, then 4 mg q d × 3 d, then 6 mg × 4 d, then 4 mg until the day of endometrial biopsy. Progesterone vaginal gel, 90 mg bid, was initiated for 8 days before EMB. The first specimen was

snap frozen, while the last two specimens were fixed in a 10% formalin solution and delivered to Adeza Biomedical (Sunnyvale, CA) and analyzed by the E-tegrity Test.® This test is an immunohistochemical stain using the SSA6 antibody to the β3 integrin. During the treatment cycle, the patient received 17β-estradiol po, 2 mg q d × 5 d, 4 mg q d × 4 d, 6 mg q d × 5 d, then 4 mg q d. Progesterone vaginal suppositories, 200 mg bid and oral progesterone, 100 mg tid was administered starting the day after the donor received HCG.

Results: EMB specimens obtained during all 3 mock cycles failed to identify β3 integrin. Histologic dating of the endometrium was interpreted as CD 19–20 (out of phase), CD 17–18 (out of phase), and CD 22–23 (in phase), respectively, for the first, second and third mock cycles. During the treatment cycle, 2 blastocysts were transferred after 6 days (doses) of progesterone. At 5 weeks conceptual age, a singleton intrauterine pregnancy with FHM's (136 bpm) was confirmed.

Conclusion: Relatively little information is known concerning the β3 integrin assay with mock cycles using exogenous hormone replacement. Pregnancy with oocyte donation was established after three repeated attempts during mock cycles failed to demonstrate β3 integrin. Although this discrepancy between the mock (pretreatment) and treatment cycles could be explained by a variety of circumstances, clinicians should interpret the results of the β3 endometrial integrin assay obtained during mock cycles with caution.

P-367

Long-term Effects of Laparoscopic Ovarian Cauterization (LOC) on Endocrine Changes in Women With PCOD. [1]S. Sadik, [1]A. Onoglu, [1]N, Karayilanoglu, [1]E. Turan, [1]O. Taskin, [1]J. M. Wheeler. Department Obstetrics/Gynecology, [1]SSK Tepecik, Izmir, Turkey, [2]Texas Women's Hospital, Houston, USA.

Objective: To study long-term LOC effects on endocrine changes in women with clomiphene (CC)-resistant PCOD.

Design: Prospective controlled study in a tertiary patient care center.

Materials and Methods: Thirty women with CC-resistant PCOD were included. Serum concentrations of LH, FSH, androstenedione (A), T, and sex hormone-binding globulin (SHBG) were determined before and, 7 days, 3 months and 6 months after surgery.

Results: All the patients (aged 24–34 yrs) had increased serum LH-to FSH ratio, elevated A-T ratio and/or clinical evidence of androgen excess. All were oligomenorheic or amenorheic with Feriman Gallwey scoring above 9. Serum concentrations of LH, A, T, and SHBG began to decrease in the first week after LOC. All the measured hormone levels decreased significantly compared with pretreatment levels and maintained for 6 months after surgery ($P<0.05$). Menstruation occurred in 3 to 4 weeks in more than 93% (n:28) of patients and was regular in 80% (n:25) of patients at 6 months. Ovulation was observed in 27 (90%) patients in the first 11 weeks after LOC and 24 (89%) of them were still ovulatory at the postoperative 6th month.

Conclusion: Although the mechanism of ovulation is unclear in LOC, normalized endocrine milieu is the probable factor. Our results showed improved clinical/hormonal parameters which was maintained more than 6 months following surgery which may be cost-effective compared to ovarian hyperstimulation with gonadotropins revealing similar ovulation/pregnancy rates.

P-368

A Randomized Trial of Clomiphene Citrate plus Intrauterine Insemination Versus Recombinant Follicle Stimulating Hormone Plus Intrauterine Insemination for the Treatment of Unexplained Infertility. A. K. Nakajima, L. L. Smith, B. Wong, J. Z. Scott. D. C. Cumming, I. V. Tataryn, M. A. Sagle, D. McAra, and L. Nordstrom. Department of Obstetrics and Gynecology, University of Alberta, and Regional Fertility and Women's Endocrine Clinic, Edmonton, Alberta, Canada.

Objectives: Controlled ovarian hyperstimulation with clomiphene citrate (CC) for the treatment of unexplained infertility is a well-established regimen in both the literature and clinical practice. More recently, there has been a growing trend towards the use of Follicle Stimulating Hormone (FSH) in treating this group of patients, supported by literature which suggests superior pregnancy rates associated with FSH. There is clearly a need for a randomized trial comparing CC and FSH, as there are wide ranges of efficacy published for both drugs and there is a distinct lack of studies comparing the two in a randomized population.

Design: A single center open randomized trial of CC plus intrauterine insemination (IUI) versus recombinant FSH (rFSH) plus IUI for the treatment of unexplained infertility was performed, and the pregnancy rates were compared.

Materials and Methods: After obtaining informed consent, 22 consecutive patients with unexplained infertility presenting to the Regional Fertility and Women's Endocrine Clinic, from October 1997 to January 1999, were randomized into one of 2 treatment arms, either CC plus IUI or rFSH plus IUI, for 4 cycles in alternate months. These couples had undergone complete investigation, and had a minimum of 18 months' duration of infertility. Ovulation monitoring was performed using transvaginal ultrasound and serum estradiol levels. IUI was timed using a urinary ovulation prediction kit in the CC group, and was performed 28–36 hours after the administration of an ovulatory dose of human chorionic gonadotropin in the rFSH group.

Results: The CC plus IUI group completed 27 cycles with 4 pregnancies, including 2 singletons, 1 twin pregnancy delivering at 33 weeks gestation, and a spontaneous abortion. Two additional singleton pregnancies occurred in the "off-cycle" period. Twenty-eight rFSH cycles were performed, resulting in 4 singleton pregnancies. An additional singleton pregnancy occurred in a non-treatment cycle. Minor side effects were common, and encountered with equal frequency in both groups; however, no serious adverse condition occurred. Two patients, one from each treatment arm, withdrew from the study.

Conclusions: Equal numbers of pregnancies were achieved in both the CC and the rFSH groups. Pregnancies in the non-treatment cycle occurred in both groups. Both CC and rFSH demonstrated very acceptable side effect profiles. The average cost of a rFSH cycle was 5-fold greater than that of CC. As our patient numbers are small, no definite conclusions can be drawn and larger multi-centered randomized trials are warranted to clarify the optimum first-line treatment of unexplained infertility. We wish to thank Berry Technologies of Calgary, Canada, for their kind donation of clomiphene citrate, Serono for their assistance with the recombinant FSH, and Novartis for their assistance with the urinary ovulation prediction kits.

P-369

Uterine Contractility, Irritable Bowel Disease and Dysmenorrhea. C. Bulletti, D. De Ziegler*, E. Del Ferro, V. Polli, M. Stefanetti, G. Grazia, L. Diotallevi, P. Farnelli, C. Flamigni. Dept of Obst, Gynecol and Physiopathology of Reproduction, Rimini's Hospital and Univ of Bologna, Italy. *Nyon Medical Center, Nyon, Switzerland. **Columbia Laboratories, Paris, France.

Objectives: Dysmorrhea occurs in close relationship with Irritable Bowel Disease. The present study was undertaken to evaluate the relationships between abnormal uterine contractility and both irritable bowel disease and dysmenorrhea.

Design: Forty-two nulliparous women were recruited for the study: 21 patients having both dysmenorrhea and Irritable Bowel Disease were admitted to the uterine contractility measurements as well as 21 controls.

Materials and Methods: Intra Uterine Pressure (IUP) for 10 minutes and UltraSound (US) for 3 minutes were used as standard methods to measure intrauterine basal pressure tone, frequency of contractions and type of contractions depending on their front of migration (antegrade, retrograde and localized). The exams were done in early and late follicular and luteal phases of the menstrual cycle and in periovulatory phase. One IUP per patient and 5 US per patients were performed. Analysis was performed by using the T-Student test and the linear correlation test.

Results: The frequency of IUP and US were significantly higher in patients versus controls all cycle long. The basal tone was significantly higher in patients versus controls all cycle long, while the dominant type of patients' contraction was the "localized" versus the antegrade of the controls.

Conclusions: Human uterus in vitro exhibits spontaneous electrical and mechanical activity in relationship with the production of progesterone and estrogen. The abnormal uterine contractility of smooth muscle based organs,

such as uterus and bowel, may have similar origin and may account for several other gynecological pathologies, such as endometriosis and spontaneous abortion.

P-370

Early Diagnosis of Genital Tuberculosis to Prevent Infertility. [1]B. Vural, [1]F. Vural, [2]C. Erçin, [3]I. Katircioğlu, [1]G. Yücesoy, [1]I. Yücesoy, [1]A. Erk. Kocaeli University School of Medicine, [1]Department of Obstetrics & Gynecology, [2]Department of Pathology and [3]Department of Microbiology, Kocaeli, Turkey.

Objective: Women are at increased risk of progression to disease during their reproductive years, but in many cases clinical presentation is obscure and diagnosis is delayed. In spite of advances in antituberculosis treatment, pregnancy rates is low and when it occurs it is likely to be ectopic or spontaneous abortion. This study was planned to determine genital tract involvement in patients with active pulmonary tuberculosis, before extensive damage of female genital tract.

Design: The bacteriological and histological examination of endometrial samplings and hysterosalphingographic evaluation were performed in newly diagnosed untreated active pulmonary tuberculosis cases.

Materials and Methods: Twenty-eight cases of bacteriologically positive, newly diagnosed, uncomplicated and untreated pulmonary tuberculosis were included in this study. Patients were submitted to a detailed gynecological history, physical examination, hysterosalphingography (HSG) and endometrial curettage for bacteriological and histological examination.

Results: The findings of pelvic examination, HSG and endometrial samplings did not determine any genital tuberculosis at the very early stage of pulmonary tuberculosis.

Conclusions: Genital tuberculosis is rarely seen at the early stage of pulmonary tuberculosis, unless the bacilli is resistant and patients come to clinics at later stages of the disease. It is suggested that, the early diagnosis and treatment of pulmonary tuberculosis cases may be associated with more favorable results in female fertility, before extensive genital organ damage occurs.

P-371

***Chlamydia* (C.) Antibody Testing (CAT) in Screening for Tubal Factor Subfertility: Added Value of Determining C. Pneumoniae Antibodies?** [1]A. P. Gijsen, [1]J. A. Land, [2]C. A. Bruggeman, [2]V. J. Goossens, [1]J. L. Evers. Departments of [1]Obstet and Gynaecol and of [2]Med Microbiol, Academisch ziekenhuis Maastricht, Maastricht, The Netherlands.

Objectives: CAT by micro-immunofluorescence (MIF) has been introduced into the fertility workup as a screening test for tubal factor subfertility due to previous *C. trachomatis* infections. A correlation between MIF and tubal pathology at laparoscopy has been established, using 64 as a cut-off titre (Land et al. 1998). The aim of the present study was to investigate the presence of *C. pneumoniae* in different patient groups, using a recently introduced *C. pneumoniae* IgG assay. In these groups the results were related to the presence of tubal pathology.

Design: In 151 subfertility patients serum was sampled and a laparoscopy was done as part of their fertility work-up.

Materials and Methods: CAT was performed by MIF (*Chlamydia trachomatis*-Spot IF; Biomerieux) and specific *C. pneumoniae* IgG antibodies were determined by ELISA (Bioclone EIA Elegance). An index of ≥ 1.1 is considered positive. An IgG pneumoniae index of 3 was arbitrarily taken to differentiate between high and low positive results. The percentage of patients with tubal pathology (defined as extensive periadnexal adhaesions and or distal occlusion of one or both tubes) was determined, as was the tubal pathology score (0= no abnormalities, 6= severe pathology).

Results: Subdivision of patients according to their *C. trachomatis* IgG (MIF) and *C. pneumoniae* IgG (ELISA) titres gave the following results (within columns, different superscripts reflect significant differences ($p \leq 0.05$)):

MIF titre	C. pneumoniae IgG index	% with tubal pathology	tubal pathology score
<64	<3	7%[a]	0.6 ± 1.5[a]
<64	≥3	4%[a]	0.5 ± 1.2[a]
≥64	<3	36%[b]	2.2 ± 2.8[b]
≥64	≥3	72%[c]	4.3 ± 2.6[c]

Conclusion: *C. trachomatis* IgG antibodies (MIF) correlate with tubal pathology. Within a group of 122 patients with low MIF titre (<64), there is no correlation between IgG *pneumoniae* antibodies and tubal pathology. In a group of 29 patients with high MIF titres (≥64) tubal pathology was more frequent in patients with a high positive index using Bioclone EIA Elegance kit. Additional studies, with specific *C. trachomatis* and *C. pneumoniae* serology, are needed, to investigate the contribution of testing for different *Chlamydia* species in screening for tubal pathology.

References: Land et al. (1998) *Hum. Reprod.*, **13,** 1094–1098

CONTRACEPTION

Wednesday, September 29, 1999

P-372

Contraceptive Effects of Quinacrine (Q) and Erythromycin Lactobionate (EL) in Adult Female Sprague-Dawley Rats. [1]P. A. Fail, [2]P. M. Martin, [2]D. C. Sokal. [1]Reproductive and Endocrine Toxicology, Research Triangle Institute and [2]Clinical Trials Group, Family Health International (FHI), Research Triangle Park, NC 27709.

Objective: Surgical sterilization, a commonly used method of family planning worldwide, is not accessible to all women because of cost, safety, or lack of access to medical care. One nonsurgical method extensively studied is Q sterilization. Q is a mutagen, however, and has a higher failure rate than surgical sterilization. Clearly a nonsurgical sterilizing agent which is less toxic and more effective than Q is needed. We compared the efficacy of EL with that of Q in producing sterilization.

Design: In Experiment 1 (Exp1), females mated at 21 days after sterilization treatment, were observed for toxicity and fetal status. In Experiment 2 (Exp2), the histopathology of the Q and EL sterilization was evaluated.

Methods and Materials: Five groups of female Sprague-Dawley rats (20/group) were given 70 or 280 mg/kg EL, 350 mg/kg Quinacrine dihydrochloride dihydrate, or the appropriate vehicle, administered transcervically (1/2 into each uterine horn). They were mated 21 days later, then sacrificed 14 days after mating. The number of fetuses, corpora lutea, implants, pregnancy rates and fertility measures were evaluated (Exp1). Five more groups (4/group) were given identical treatments, but were sacrificed 21 days later without mating. Hematoxylin/eosin stained uterine sections were examined for fibrosis, lumen closure, and other abnormalities (Exp2). Using the Statistical Analysis Systems package, an Analysis of Variance (parametric) or a Kruskal-Wallis Test (non-parametric) was performed in Exp 1. Next, pairwise comparisons to the appropriate controls were made using Dunnett's, Mann-Whitney U, Chi-Square or t-tests as appropriate.

Results: Neither drug altered the number of corpora lutea in pregnant animals ($P>0.05$). EL decreased pregnancy and fertility rates ($P<0.05$) in a dose-related manner, and both doses were more effective than Q treatment. EL also caused a dose-dependent decrease in number of implantations ($P<0.05$), but did not increase resorptions. In contrast, number of resorptions was increased after Q ($p<0.05$). The number of dead fetuses was minimal overall, and did not differ due to treatment. In Exp. 2, uterine pathology included lumen dilatation, fibrosis and chronic inflammation, which was more extensive and frequent after high dose EL than after Q. The extent and severity of the lesions increased over time from 21 to 33+ days. Several rats at 280 mg/kg EL had bloody vaginal discharge, rough coats, and inactivity during the first week after EL, indicating that this dose caused some toxicity.

Conclusions: Thus, EL was more effective in preventing pregnancy than Q, without causing endocrine toxicity (as indicated by corpora lutea and vaginal cytology), or fetal toxicity, (as indicated by resorption frequency).

Supported in part by FHI.

P-373

Experience With Self-Administered Emergency Contraception in a Low-Income, Inner City, Family Planning Program. L. K. Endres, M. Beshara, G. Squires, K. Gendron, S. Sondheimer. University of Pennsylvania Medical Center, Philadelphia, PA.

Objective: The Yuzpe method of emergency contraception was stated to be safe and effective in preventing pregnancy after unprotected intercourse by the FDA in February 1997. Historically, access to the pills has been limited. Since July 1997, the University of Pennsylvania Family Planning Clinic (FPC) has dispensed free packets of emergency contraceptive pills (ECP) to interested, low-income, inner city women. This study evaluates patients' use, knowledge, and attitudes on self-administered ECP.

Design: Patients completed a standardized phone interview six to eight months after receiving an ECP packet from the FPC.

Materials and Methods: Full IRB approval was obtained to perform a phone survey. The University of Pennsylvania FPC is a Title 10, publicly funded clinic serving urban, low-income women. All women attending the clinic were offered ECP packets. Exclusion criteria for ECP were current pregnancy or newly diagnosed hypertension. Oral and written instructions were reviewed with a trained counselor and consent forms signed. Women then received 21 Nordette birth control pills without the inert pills packaged with specific instructions to use the ECP in the standard Yuzpe method. Patients who received the pills between 4/21/98 and 5/29/98 were contacted between 12/1/98 and 2/18/99. 192 patients received the packets. 144 patients had moved, had disconnected phones, or were unreachable after multiple attempts. 48 patients were reached and completed the survey.

Results: Average age of patients was 23.5 years, average parity was 1.4. Eleven of 48, or 22.9%, had used the ECP. One of 11, or 9.1%, had become pregnant despite using the pills. Thirty-seven of 48, or 77.1%, had not used the pills. Four of 37, or 10.8%, had an unplanned pregnancy. Of the four women who experienced unplanned pregnancies, two forgot they had the ECP packets, one was using oral contraceptive pills incorrectly, and one had recent Norplant removal and did not know she could get pregnant. Two of 11, or 18.2%, used the pills correctly. The other nine women took a range of pills from all 21 at one time to only four pills without a repeat dose. Thirty-three of 48, or 68.8%, are sexually active and 30 of 48, or 62.5%, are using some form of birth control. Women on average were willing to pay $15 for the pills. Thirty-three of 48, or 68.8%, reported a positive attitude toward having the pills available at home and stated they felt "safer."

Conclusions: Emergency contraception utilization was far lower than anticipated, suggesting that ready access is not the only issue. Most of the women did state that they were glad to have the pills, but of women who had not used them, only 25 of 37, or 67.6%, could locate them and only 9 of 37, or 24%, could recall how to use them correctly. Compared to Glasier and Baird's paper on self-administered emergency contraception in Scotland in which 69% of women used the ECP and 98% used them correctly, our study revealed dramatically different results in a low-income, inner city, family planning clinic population. Patients will require new and creative approaches to encourage their use of emergency contraception. Recent media attention and new packaging may increase usage and correct dosing. Additional studies are needed.

P-374

Histological Features of Endometrial Biopsies in Patients Using Parenteral Progestin Contraceptives. [1]I. P. Ryan, [2]J. L. Shifren, [1]D. I. Lebovic, [1]A. P. Korn, [3]C. J. Zaloudek, [1]P. D. Darney, [1]R. N. Taylor. [1]Dept. Obstetrics, Gynecology & Reproductive Sciences and [3]Dept. Pathology, University of California, San Francisco, CA; [2]Dept. Obstetrics & Gynecology, Massachusetts General Hospital, Boston, MA.

Objectives: Parenteral progestin contraceptives are one of the most effective methods of hormonal contraception. Unfortunately, breakthrough bleeding occurs in up to 70% of women and is a major reason for discontinuation of this contraceptive method. We have conducted studies to understand the biology underlying this side effect. Here, we describe the histological features of endometrial biopsies in women using parenteral progestin contraceptives, and suggest correlative findings which may account for the breakthrough bleeding.

Design: A prospective case controlled study was used to compare endometrial histology in patients using parenteral levonorgestrel-releasing contraceptives (Norplant) (n=47), and control patients using non-hormonal contraceptive methods (n=14). University IRB approval was obtained and informed consents was given by all study subjects.

Materials and Methods: Pipelle biopsies were performed and fundal endometrial tissues were fixed in Histochoice, stained with H&E, and histological features assessed using a detailed semi-quantitative scoring system. One pathologist scored all biopsies, and was blinded to the clinical history of the subjects. Categorical data were compared by X^2 and continuous data by Mann-Whitney tests.

Results: The majority of biopsies from patients using Norplant featured a mixed or indeterminate histological pattern, while the control biopsies showed the spectrum of menstrual stage appropriate features. The endometrial index, which provides an indication of proliferative activity (Darney et al., 1996), did not differ between the two groups (Norplant=0.54 vs Control=0.47, P=0.44). When stratifying for specific histological features, biopsies from Norplant patients showed a significant difference is characteristics of proliferative phase endometria such as tubular gland configuration (P=0.005), compact stroma (P=0.01), and mitotic figures of the glands (P=0.006). As well, these biopsies showed some features of progestin exposure such as glandular nuclear localization (P=0.005), and presence of glandular cell cilia (P=0.002). There was an absence of secretory activity, pre-decidualization, degenerative changes and apoptosis (all P>0.05). We failed to observe specific vascular changes or differences in tissue hemorrhage.

Conclusions: Instead of a histological pattern predominated by secretory changes, we found a preponderance of mixed, indeterminate or inactive patterns in Norplant users. These histological features appear to represent an unstable or fragile endometrium, which predisposes to increased breakthrough bleeding commonly seen with these hormonal contraceptive techniques. This work is supported by NIH grant R01-HD33238.

ENDOMETRIOSIS

Wednesday, September 29, 1999

P-375

Prolonged Suppression of Circulating Estrogen Levels Without an Initial Hormonal Flare Using Abarelix-Depot, a Pure GnRH Antagonist in Women With Endometriosis. P. M. Martha, M. E. Gray, M. Campion, B. Kuca, M. B. Garnick for the FASTER Study Group. PRAECIS Pharmaceuticals, Inc., Cambridge, MA.

Objective: GnRH *superagonists* have proven to be useful for managing several clinical conditions in women. However, an inherent drawback to all GnRH superagonists is the requirement that the patient tolerate the unwanted, but unavoidable, initial hormonal stimulation phase before the desired suppression phase takes effect. Abarelix-Depot is a sustained-release formulation of the potent, pure GnRH *antagonist*, abarelix. Abarelix is totally devoid of the initial stimulatory activity typical of all the GnRH superagonists. Therefore, we are conducting a clinical trial of Abarelix-Depot in women to identify doses that are safe and provide both rapid and prolonged suppression of circulating estrogen levels following once monthly s.c. injection.

Design: Randomized, parallel arm trial comparing the dose-response relationships between Abarelix-Depot (A-D) or leuprolide (Lupron Depot 3.75 mg; L-D) and circulating levels of reproductive axis hormones.

Materials and Methods: Forty women with endometriosis-associated pain were randomized to receive either Abarelix-Depot, 30, 60, 90 or 120 mg s.c. or Lupron® Depot 3.75 mg i.m. once monthly × 6 doses. Blood samples are collected frequently during the first study month then at 4-week intervals thereafter for determination of serum hormone levels.

Results: Estradiol - By 24 hrs after injection (study Day 2), serum estradiol levels were <50 pg/mL in 29/29 (100%) of women receiving A-D but in none (0%) receiving L-D. All patients receiving L-D had E_2 levels at 24 hrs post-dose that were 70%–400% higher than the respective pre-dose values. In contrast, no A-D pt. experienced an increase in E_2 on Day 2. Further, in the A-D group, the E_2 levels were <30 pg/mL in 27/29 (93%) at 24 hrs and in all 29 (100%) by 72 hrs. The duration of E_2 suppression through 4 weeks was dose-dependent with the percentage maintaining suppression being 47%, 67%, 86% and 100% for the 30, 60, 90 and 120 mg doses respectively. At this time, E_2 values are available for 21 pts. from study week 8 and from 10 pts. at study week 12. Of these, 20/21 (95%) and

10/10 (100%) are <30 pg mL. A-D has been well tolerated at all doses studied.

Conclusions: Abarelix-Depot is capable of inducing a rapid suppression in circulating estradiol with total avoidance of the initial hormonal flare effect of GnRH superagonists and this suppression appears to be able to be maintained long-term with once monthly s.c. administration. Due to its rapidity of action, lack of hormonal flare, subcutaneous route of administration and attractive initial safety profile, Abarelix-Depot should offer therapeutic advantages over use of conventional GnRH superagonists where prolonged suppression of serum estrogen levels is clinically indicated.

P-376

Effectiveness of Photodynamic Ablation for Destruction of Experimental Endometriosis in the Rat. [1]A. A. Krzemien, [1,2]D. A. Van Vugt, [1]R. L. Reid. [1]Department of Obstetrics and Gynaecology, [2]Department of Physiology, Queen's University, Kingston, ON, Canada.

Objective: Photodynamic therapy (PDT) is a treatment that may allow for selective and complete destruction of certain tissues. It involves application of a photosensitizer followed by exposure of the tissue to photoactivating light. In this study we sought to determine if systemic 5-aminolevulinic acid (ALA) followed by exposure to photoactivating light would result in ablation of endometriotic explants in the rat, and to assess permanency of this destruction.

Design: The extent of ablation of endometrial explants in Sprague-Dawley female rats was assessed 3–4 days or 3 weeks following systemic administration of a photosensitizer and exposure to photoactivating light.

Materials and Methods: Endometriosis was surgically created in rats by transplanting uterine sections on both sides of peritoneal cavity. In all rats estradiol capsules were implanted subcutaneously one to three weeks prior to the photodynamic treatment. 12–14 weeks following induction of endometriosis rats were injected intravenously with 400 mg/kg ALA or saline. Three hours later the abdomen was opened and implants were exposed to photoactivating light for 0, 5, 10, or 15 min. Endometrial explants were harvested and their histology was assessed 3–4 days or 3 weeks following ALA and light exposure.

Results: Systemic ALA followed by exposure to photoactivating light for 10 or 15 min resulted in ablation of all endometrial explants harvested 3–4 days after the treatment. No damage was observed in ALA exposed implants exposed to 5 min of photoactivating light. Permanency of ablation was confirmed by absence of endometrial tissues on histological examination of ALA and light exposed implants harvested 3 weeks following light treatment. In contrast implants exposed to ALA only or light treatment only did not exhibit any histological damage.

Conclusions: We conclude that systemic ALA followed by exposure to photoactivating light at relatively low power densities for periods as brief as 10 minutes resulted in ablation of endometriotic implants in the rat. This observation supports the potential use of this approach for treating endometriosis clinically.

This work was supported by the Medical Research Council of Canada

P-377

Long-Term Treatment of Symptomatic Endometriosis With Norethindrone Acetate. O. Muneyyirci-Delale, S. Jalou. Department of Obstetrics and Gynecology, SUNY/Health Science Center at Brooklyn and Kings County Medical Center, Brooklyn, NY.

Objectives: To evaluate long-term effectiveness of norethindrone acetate (NA) for treatment of symptomatic endometriosis and frequency of side effects.

Design: Retrospective study.

Materials and Methods: 40 women who had surgically diagnosed endometriosis were treated with NA for at least 2 years. All patients were symptomatic with complaints of dysmenorrhea, dyspareunia, with or without noncyclic pelvic pain. The incidence of symptoms prior to and side effects during treatment; such as breakthrough bleeding, weight changes, hyperandrogenic side effects and others were recorded at each visit. Weight changes were compared with endometriosis patients who were treated with other medications (non-NA).

Results: The mean ages of the patient were 29.35 ± 5.26 years. The mean duration of follow-up was 5.48 ± 3.02 years. Dysmenorrhea and noncyclic pain were relieved in 39/40 (97.5%) of patients. Dyspareunia and pelvic tenderness on examination were relieved in all patients (100%). When the weight changes of NA patients were compared with those of non-NA patients, the weight prior to treatment was 65.26 ± 15.39 kg vs. 67.86 ± 14.45 kg, respectively. Weight during treatment (NA vs. non-NA) was 74.38 ± 16.87 kg vs. 74.47 ± 14.75 kg, respectively. The difference in weight in both groups was not significant ($p=0.2$). Breakthrough bleeding was experienced by 30/40 (75%) of patients, only 2/40 (5%) had severe bleeding. However, 39/40 (97.5%) of patients developed amenorrhea during treatment.

Conclusions: NA seems to be a cost-effective alternative in the long-term treatment of symptomatic endometriosis with relatively mild side effects.

P-378

Expression of Telomerase Activity in Ectopic Endometrium of Women with Endometriosis and Its Significance in Endometriosis Pathogenesis. [1]S. D. Chang, [1,2]Y. M. Lai, [1]H. I. Liu, [1]C. L. Lee, [1]Y. K. Soong. [1]Dept. of Obstetrics and Gynecology Chang Medical Center and Medical College of Chang Gung University, and [2]Formosa Fertility Center, Taipei, Taiwan.

Objectives: The association of endometriosis and infertility has been observed for quite a long time and numerous hypotheses have been proposed to explain the pathogenesis of endometriosis. Although pathologically, endometriosis is a benign disease, clinically, its invasive behavior mimics a malignant tumor. Telomerase activity is known to be present in malignant tumor and absent in benign tumor. The telomerase activity has been detected in human eutopic endometriumm especially during proliferative phase. We have previously reported that telomerase activity is detected in the individual blastomeres of human IVF embryos, because of their proliferation potential. In the case of endometriosis, the wandering endometrial cells can implant and proliferate in ectopic endometrial lesions, which is believed to be moderated by telomerase. In order to investigate the relationship between the telomerase activity and the proliferation potential of wandering endometrial cells, the telomerase activity in ectopic endometrial tissue was assayed.

Design: The expression of telomerase activity in ectopic endometrium of women with endometriosis was studied by a polymerase chain reaction-based telomeric repeat amplification protocol (TRAP) assay.

Materials and Methods: Twenty-two patients that received enucleation of ovarian endometrioma and twelve patients that received hysterectomy for adenomyosis were included in the study. Each case had histological confirmation and the menstrual cycle days were recorded. The tissue specimens of ectopic endometrium obtained from the capsules of endometrioma and the myometrial lesions of adenomyosis were studied with TRAP assay. Human leiomyoma tissues and cervical cancer cell line SiHa were used as negative and positive control of TRAP assay, respectively.

Results: The telomerase activity was found in 36% (8/22) of ovarian endometrioma cases and 25% (3/12) of adenomyosis cases, respectively. Both in the endometrioma group and the adenomyosis group, the incidences of active telomerase activity were higher in the proliferative phase: 50% (6/12) and 43% (3/7), respectively than in the secretory phase: 20% (2/10) and 0% (0/5), respectively.

Conclusions: 1) The data appear to indicate that telomerase activity was indeed expressed in ectopic endometrium of endometrioma and adenomyosis. 2) The positive rate of telomerase activity was higher in the proliferative phase than in the secretory phase because of the active mitosis in proliferative phase. 3) These results suggested that the endometrial cells, after leaving their original site in uterine endometrium, implant and proliferate in other pelvic tissues is in part related to telomerase activation.

P-379

Endometrial Macrophages May Be Involved in the Regulation of Endometrial Apoptosis. [1,2]J. Shen, [1]J. Ding, [1,2]D. P. Braun, [1,2]N. Rana, [3]A. Dombrowski, [1,2]W. P. Dmowski. [1]Institute for the Study and Treatment of Endometriosis, Chicago, IL, [2]Rush Medical College, Chicago, IL, [3]Pathology Laboratory, Grant Hospital, Chicago, IL.

Objectives: We have demonstrated previously that endometrium in women with endometriosis resists spontaneous apoptosis (Gebel et al,

1998), and macrophage-mediated cytolysis, and is stimulated to proliferate by autologous monocytes/macrophages (Braun et al, 1994, 1998). Considering that TNFα, a major secretory product of the monocytes/macrophages initiates apoptosis in a variety of cell systems, the objective of this study was to investigate correlation between the number of apoptotic cells and macrophages in the endometrium of women with and without endometriosis.

Design: Uterine endometrial tissues from women with and without endometriosis were evaluated for the apoptosis and macrophages. Paired data were analyzed for correlation between the number of apoptotic cells and number of macrophages.

Materials and Methods: Paraffin blocks from 33 endometriosis and 9 control women were retrieved from the pathology laboratory. Tissue sections were made and apoptotic cells were determined by TUNEL (TdT-mediated DUTP-biotin nick end-labeling) staining and macrophages were identified by detection of specific CD68 antigen with a CD68 antibody. Apoptotic cells and CD68+ cells were counted with a cytometer in an area of 10 to 50 mm^2 depending on the size of tissue samples. The data were presented as the number of cells per 10 mm^2 unit area. T-test or correlation analysis were applied to analyze the data.

Results: The (mean ± SD) number of apoptotic cells/10 mm^2 in the uterine endometrium of controls was significantly higher than in women with endometriosis (92.3 ± 70.6 vs 58.2 ± 33.8, P = 0.042). The mean number of macrophages/10 mm^2 in the uterine endometrium of controls was also higher than in women with endometriosis (751.8 ± 244.8, n = 9 vs 657.3 ± 304.1, n = 33) but the difference was not statistically significant. There was also no significant difference in the concentration of macrophages in different stages of the disease. There was a significant correlation ($r = 0.739$, $P < 0.0001$) between the number of macrophages and apoptotic cells when the data from both patients and controls were pooled together. The correlation remained significant when the same data were analyzed separately for glandular and stromal cells ($r = 0.62$, $P < 0.0001$, and $r = 0.592$, $P < 0.0001$, respectively).

Conclusions: This study demonstrates a significant correlation between the number of apoptotic cells and the number of macrophages in the uterine endometrium of women with and without endometriosis, suggesting that the macrophage-secreted TNFα may be a mediator of spontaneous apoptosis in both glandular and stromal cells. A decreased macrophage colonization of the endometrium through the decreased TNFα secretion may cause reduction in spontaneous apoptosis. When spontaneous apoptosis is reduced during the menstrual phase of the cycle the shed endometrial cells may have increased ability of implantation in the ectopic sites resulting in the development of endometriosis.

P-380

In Vivo Expression and Localization of Haptoglobin in Endometrium and Endometriotic Lesions. K. L. Sharpe-Timms, E. A. Ricke, G. M. Horowitz. Department of Obstetrics and Gynecology, University of Missouri, Columbia, MO.

Objectives: We have previously described *in vitro* synthesis and secretion of endometriosis protein-I (ENDO-I) by peritoneal endometriotic tissue explants and demonstrated ENDO-I amino acid and nucleotide sequences are almost identical to hepatic haptoglobin. This study evaluated *in vivo* expression and localization of haptoglobin in endometrial and endometriotic tissues.

Design: Immunohistochemistry and *in situ* hybridization were used to identify site specific localization and expression of haptoglobin mRNA, respectively, in endometrial and endometriotic tissues throughout the menstrual cycle.

Materials and Methods: Matched endometrial and peritoneal endometriotic lesions from women with endometriosis (n = 12) were acquired via endometrial biopsy and intraoperatively at laparoscopy. Additional endometrial tissues were obtained from women without endometriosis (n = 12) at laparoscopic sterilization. Informed consent, as approved by our Institutional Review Board, was obtained from all patients prior to collecting tissues. Tissues were formalin fixed, paraffin embedded, sectioned at 5 μm and immunohistochemically stained with anti-haptoglobin using the avidin-biotin peroxidase procedure. Haptoglobin mRNA was localized in serial tissue sections by non-isotopic *in situ* hybridization using digoxin-labeled riboprobes generated from cDNA. Expression and localization differences were evaluated and scored by blinded observers and statistical differences determined with McNemar's, Fisher's and Wilcoxon's tests.

Results: Haptoglobin immunohistochemically localized in a gradient fashion in endometrial stromal cells of the functionalis, but not the basalis. Haptoglobin immunohistochemically localized in the stroma of endometriotic lesions, with preferential localization noted towards peritoneal surfaces. *In situ* hybridization of haptoglobin mRNA mirrored protein localization patterns in the endometrial and endometriotic stroma. Haptoglobin protein localization and message expression did not vary throughout the menstrual cycle (p = 1.00). There was a strong trend for greater haptoglobin protein localization (p = 0.130) and message expression (p = 0.051) in endometrium from women with endometriosis as compared to endometrium from women without endometriosis. Haptoglobin protein localization (p = 0.025) and message expression (p = 0.037) was greater in endometriotic lesions than eutopic endometrium.

Conclusions: These *in vivo* results support our earlier *in vitro* studies showing that ENDO-I (haptoglobin) is produced by endometriotic lesions throughout the menstrual cycle. Hepatic haptoglobin, induced by acute phase stimuli, modulates immune and angiogenic activity. If ENDO-I haptoglobin possesses similar activities, it may be involved with endometriosis-associated anomalies of the immune system or the disease process of endometriosis.

This work was supported in part by NICHD 29026 and TAP Holdings, Inc.

P-381

Expression of the α2β1 and α3β1 Integrins at the Surface of Mesothelial Cells: a Potential Attachment Site of Endometrial Stromal Cells. [1]C. A. Witz, [2]A. Takahashi, [1]I. A. Montoya-Rodriguez, [1]J. Fellows, [1]R. S. Schenken. Departments of [1]Obstetrics and Gynecology and [2]Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX.

Objective: We recently described a model of endometriosis using explants of human peritoneum and endometrium. Preliminary observations demonstrated that endometrial stromal cells attach to intact peritoneal mesothelium (F and S 1999;71:56). We also described the expression of α2 and α3 integrin subunits in peritoneal mesothelium in vivo and in monolayer culture using immunohistochemistry and immunoelectron microscopy (J Soc Gynecol Invest 1998;5:93). However, study of integrin subunits does not allow for specific localization on the cell surface as expression in the plasma membrane is dependent on heterodimerization. The purpose of this study was to localize intact α2β1 and α3β1 integrins in the cell membrane of peritoneal mesothelium in vivo and in vitro.

Design: Immunohistochemical study of α2β1 and α3β1 integrins in peritoneal mesothelium in vivo and in monolayer culture.

Materials and Methods: Biopsies of anterior abdominal wall peritoneum were obtained from reproductive-age women without endometriosis undergoing laparotomy for benign conditions. Samples of peritoneum were immediately frozen in the vapors of liquid nitrogen. Mesothelial monolayer cultures were also established following enzymatic digestion of peritoneum biopsies. Sections of peritoneum and cultured mesothelial cells were incubated with mouse monoclonal antibodies to the intact α2β1 and α3β1 integrins (Chemicon, Temecula, CA) followed by fluorescence labeled anti-mouse antibodies. Non-immune mouse serum substituted for primary antibody served as a negative control. Specimens were examined for expression of α2β1 and α3β1 integrins in mesothelial cells using a two-photon laser scanning microscope (TPLSM) with a Ti:sapphire laser.

Results: The α2β1 and α3β1 integrins were identified with the greatest intensity at the base of the mesothelial cells (i.e. toward the basement membrane). In addition, there was expression of the α2β1 and α3β1 integrins between mesothelial cells and at the cell surface (i.e. toward the peritoneal cavity). There were similar patterns of expression in vivo and in monolayer culture.

Conclusions: The resolution of the TPLSM allowed for localization of intact integrins in mesothelial cells. The presence of α2β1 (collagen/laminin receptor) and α3β1 integrins (collagen/laminin/fibronectin receptor) at the base of mesothelial cells suggests a role for these molecules at the cell surface suggests a potential locus for cell adhesion in such processes as endometriosis and cancer metastasis.

P-382

Iron, Ferritin, Total Iron Binding Capacity and Electrolyte Concentrations in Peritoneal Fluid and Serum in Patients with Endometriosis. T. C. Sheen, L. C. Pung, J. Y. Wen, S. J. Chang, K. Y. Wu, C. R. Tzeng. Department of Obstetrics & Gynecology, Taipei Medical College Hospital, Taipei, Taiwan, R.O.C.

Objectives: In order to investigate the pathophysiology of endometriosis, we try to evaluate the iron related molecules and electrolyte concentrations from serum and PF in patients with endometriosis and to explore the possible role of iron in PF of endometriosis induced infertility.

Design: Prospective case-control clinical study.

Materials and Methods: From Apr. 1998 to Nov. 1998, PF and serum were obtained from 47 infertile patients during laparoscopic examination. Based on the classification of rAFS, cases were categorized as no endometriosis (group 1, n=10), stage I + II endometriosis (group 2, n=27), stage III + IV endometriosis (group 3, n=10). The concentrations of iron, ferritin, TIBC, Na+, K+, Cl^- and Ca+2, both in PF and serum, were measured in each group. Student t test was used for statistic analysis.

Results: The concentrations of iron, ferritin and TIBC in PF were on the upward trends along with the severity of endometriosis (Table). The concentrations of Na+, K+, Cl^- and Ca++, however, was not different in serum and PF in each group.

	Iron (μg/dl)		Ferritin (ng/ml)		TIBC (μg/dl)	
	PF	serum	PF	serum	PF	serum
Control	55 ± 7*	104 ± 11	338 ± 86	56 ± 9	146 ± 19*	270 ± 12
Endometriosis stage I + II	68 ± 4	92 ± 5	437 ± 52	75 ± 12	179 ± 8*	272 ± 12
Endometriosis stage III + IV	71 ± 5*	108 ± 10	542 ± 88	62 ± 11	184 ± 14	275 ± 10

* $p<0.05$.

Conclusions: Endometriosis is thought to be a consequence of retrograde menstruation. Hemorrhagic PF is commonly observed during laparoscopic examination caused by cyclic bleeding of ectopic endometrial foci. The PF of patients with endometriosis has been demonstrated to be embryo-gamete toxic, but the mechanism is still under investigation.

Iron is highly susceptible to oxidative-reductive reaction. While released from the breakdown of hemoglobin, it facilitates not only the formation of superoxide radical but also the hydroxyl radical. These free radicals have been shown to induce lipid peroxidation and damage embryo and gametes. The levels of iron in PF appear to be related to the severity of endometriosis and is detrimental to the fecundity.

P-383

Progesterone Receptor Immunostaining Is Inversely Related to Matrix Metalloproteinase 9 Expression In Human Ovarian Endometriosis. [1]L. O'Neil, [2]B. Jasani, [2]S. Ismail, [1]S. J. Paynter, [1]B. J. Fuller, [3]A. C. Newby, [1]R. W. Shaw. [1]Department of Obstetrics and Gynaecology, [2]Department of Pathology, University of Wales College of Medicine, Heath Park, Cardiff, Wales; [3]Bristol Heart Institute, University of Bristol, Bristol, U.K.

Objectives: Over-expression of matrix metalloproteinases (MMPs), specifically the gelatinases, has been associated with the malignant phenotype and more recently, endometriosis. The aim of this study was, therefore, to analyse expression of constitutive and inducible forms of gelatinases (MMP 2 and 9) in ovarian endometriosis and to examine the possibility that progesterone plays a role in the regulation of their expression.

Design: A retrospective analysis of 9 cases of ovarian endometriosis using immunohistochemical techniques.

Materials and Methods: Endometriotic tissue was obtained from patients with histologically confirmed ovarian endometriosis. The archived tissue was stored in paraffin wax blocks and these were sectioned and stained with haematoxolin and eosin and with antibodies to reveal expression of progesterone receptors (PR) and MMP 2 and 9. The staining intensity was graded for each factor on a scale of 0–3, 0 = no stain, 1 = faint, 2 = moderate and 3 = strong. The number of cells positively stained was graded on a scale of 0–3, 0=0%, 1=1–30%, 2=31–70%, 3=71–100%. An overall intensity distribution score was obtained by multiplying the two grades. Stromal and epithelial cells were scored separately and the resultant grades were totaled to give a final score. Results were analysed using Spearman's rank correlation coefficient.

Results: In cases where the expression of PR was high (n=7), the expression of MMP 9 was low, indicating an inverse correlation between the two (PR = 10, MMP 9 = 1; PR = 8, MMP 9 = 1; PR = 18, MMP 9 = 1; PR = 11, MMP 9 = 2; PR = 10, MMP 9 = 7; PR = 13, MMP 9 = 1; PR = 12, MMP 9 = 5). Concomitantly, when MMP 9 levels were high (n = 2), few PR were present (PR = 4, MMP 9 = 10; PR = 1, MMP 9 = 7; ($r = -0.676$). MMP 2 was expressed to varying degrees in all cases and was not correlated to PR expression ($r = -0.174$).

Conclusions: The expression of PR did not correlate with MMP 2 expression in ovarian endometriosis. However, PR expression was inversely related to MMP 9 expression in human ovarian endometriosis. We believe that this is the first *in situ* evidence of this relationship.

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Aromatase Inhibitors in the Treatment of Low Responders Women With Severe Endometriosis. F. Scarpellini, M. Sbracia. Centro di Endocrinologia e Medicina della Riproduzione, Rome Italy.

Objective: Endometriosis is one of the most important causes of pelvic pain of the fertile female population and plays an important role in several cases of apparent unexplained infertility. Till now, the treatment of this disease is based on pharmacological approach with progestogens or GnRH analogues, surgical intervention with laparotomic or laparoscopic ablation of endometriomas. Recently, we have shown as aromatase inhibitors, a new class of drugs, is effective in the treatment of severe endometriosis. In our study we have shown as endometriosis patients with ectopic endometriotic tissue lacking expression of EGF-R and cERB2 have a worse prognosis than other women which express these antigens. In this study we have treated women affected by endometriosis in which immunohistochemical analysis of endometriotic lesions showed a lack of expression of EGF-R and cERB2 with aromatase inibitor plus GnRH analogues compared to the GnRH treatment alone in order to evaluate the role of this substances in the therapy of endometriosis in low responder patients.

Material and Methods: We studied 45 patients affected by the class IV endometriosis, diagnosed during operative laparoscopy, referred to us for infertility. After immunohistochemistry study for IGF-1, EGF-R and cERB2 we divided the patients in two groups: the first group of patients of 14 women was positive to all the antigens tested (group A), and the second one of 31 women tested positive for IGF-1 and negative to EGF-R and cERB2. This last group of 31 patients was randomly divided in two further groups of 15 (group B) and 16 patients (Group C). Group B was treated for six months with GnRH analogue, Goserelin 3.6 mg s.c. every 28 days and an aromatase inhibitor, Anastrozole 1 mg per day for 6 months. Groups A and C were treated only with GnRH analogue at the same dose for the same time. Number of relapse, resolution of infertility and frequency of treatment side effects were evaluated. Patients were re-evaluated after one years from the termination of therapies.

Results: After one year from the termination of therapies, patients of group B showed relapse of disease only in 2 cases (13.3%), whereas in group C relapse of disease was observed in 12 cases (75%) ($P<0.01$). Patients of group A showed relapse of disease in 3 cases (21.4%) (A versus B $P=0.16$; A versus C $P<0.05$).

Conclusions: These findings suggest that aromatase inhibitor supplementation in low responder patients with endometriosis is more effective than GnRH analogue treatment. Furthermore, aromatase inhibitor therapy reduces relapses in low responder patients during the follow up as well as the high responder patient's rate.

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Endometriosis Patients with Ectopic Endometrium Positive to C-ERB2 or EGF-R Have a Better Prognosis Than the Negative Ones. [1]E. Zupi, [2]M. Sbracia, [2]F. Scarpellini, [2]R. Poverini, [3]P. Alò, [1]D. Marconi, [1]B. De Vivo, [1]C. Romanini. [1]Department of Obstetrics/Gynecology Università "Tor Vergata", [2]Centro di Endocrinologia e Medicina della Riproduzione, [5]Department Experimental Medicine University "La Sapienza", Rome, Italy.

Objective: We have previously shown that epithelial cells of eutopic endometrial and ectopic endometrium of endometriotic lesions have a different pattern of expression of several growth factors such as IGF-I, IGF-II, EGF, Growth Hormone (GH), and their receptors as IGF-1R, EGFR, cERB2. Especially IGF-1 seems to be the antigen always expressed in the ectopic endometrium whereas the other factors are not expressed on the ectopic endometrium in most of the cases, even though they are normally expressed in the eutopic endometrium of the same women. In order to investigate the relationship between the pattern of expression for these growth factors and the prognosis of the patients, the two years follow up of the women with endometriosis was studied.

Materials and Methods: We evaluated tissue specimens of eutopic and ectopic endometrium obtained from 20 patients with endometriosis class IV who underwent operative laparoscopy in different phases of the menstrual cycle. The indirect avidin-biotin complex immunoperoxidase assay was performed on dewaxed and dehydrated sections of 10% formalin fixed, paraffin embedded tissue. Anti IGF-I, anti IGF-I-R, anti-cERB-2, anti-EGF-R monoclonal primary antibodies with a dilution 1:50 phosphate-buffer solution were used. After the surgery the patients were treated with GnRH-analogue for three months and followed up for two years in order to know if they develop relapses of the disease or they solved the infertility problem.

Results: After 2 years follow up we observed in the group of patients studied patients with relapses were 13, with presence of endometriotic ovarian cyst, pelvic pain, infertility. When we compared the group of patients with relapses with the patients without for the expression of growth factors in the tissue of ectopic lesions we observed a significant differences in the lack of expression for some of the antigens tested, EGF-R and c ERB2. Twelve out of 13 (92.5%) patients with endometriosis relapse were negative for EGF-R or cERB2, whereas all patients without relapse were positive for these antigens ($P<0.01$).

Conclusions: Our study showed that in patients with endometriosis the expression of some receptor for growth factors, cERB2 and EGF-R, in ectopic endometrium is associated with a better prognosis, whereas patients where the ectopic endometrium of endometriotic lesions expresses only the IGF-1 and there is a lack of expression for the other growth factors, show a higher rate of relapses.

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Adhesion of Endometrium to Peritoneal Membranes *In Vitro*: a Preliminary Study. S. Debrock, J. A. Hill, C. Meuleman, T. M. D'Hooghe. Leuven University Fertility Center, UZ Gasthuisberg, Leuven, Belgium.

Objectives: The purpose of this study was to develop an *in vitro* culture model for the pathogenesis of endometriosis; the hypothesis was tested that endometrium can adhere to autologous endometrium *in vitro*.

Design: Experimental study using tissue culture and light microscopy.

Materials and Methods: Seven patients with infertility underwent diagnostic hysteroscopy and laparoscopy during the luteal phase of the cycle. During laparoscopy, a peritoneal biopsy of 2×2 cm was surgically excised by CO_2 laser. An endometrial biopsy was taken during hysteroscopy. After collection, the biopsies were washed in PBS to remove debris and excess blood cells. The endometrial biopsy was mechanically fragmented and placed on top of the peritoneal biopsy and cultured in DMEM/F12 supplemented with 10% fetal calf serum for 48 h at 37°C in 5% CO_2. Culture medium was changed every 24 hours. After incubation, the peritoneum with the endometrial fragments was rinsed in PBS. Tissue samples were fixed in 4% formaldehyde and embedded in paraffin or snap frozen in isopentane embedded in dry ice. The morphology and the adhesion of the endometrial fragments to the peritoneal biopsy was studied using hematoxylin-eosin stained sections. Adhesion was defined to be present when uninterrupted contact between the peritoneal biopsy and endometrial fragments was noted at light microscope.

Results: In 4 of the 7 patients, adhesion was observed between the endometrial fragments and the peritoneum. The mesothelial layer was uninterrupted up to the area of adhesion, and at least partially intact mesothelium was observed beneath the attached fragments in 2 out of 4 cultures. The area of endometrium adherent to peritoneum consisted mainly of cells morphologically identified as stromal cells.

Conclusions: Endometrial fragments can adhere to autologous peritoneum *in vitro*.

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The Oxford Endometriosis Gene (OXEGENE) Study. [1]S. Kennedy, [1]R. Hadfield, [1,2]S. Nakago, [1]H. Mardon, [3,4]D. E. Weeks, [1]D. Barlow & The OXEGENE Collaborative Group. [1]Nuffield Department of Obstetrics and Gynaecology, University of Oxford, UK; [2]Department Obstetrics & Gynecology, Kobe University, Japan; [3]University of Pittsburgh, USA; [4]Wellcome Trust Centre for Human Genetics, Oxford, UK.

Background: There is mounting evidence that genetic predisposition plays a role in the aetiology of endometriosis. The disease is probably a complex genetic trait, like diabetes or asthma, e.g. a condition without a clear Mendolian pattern of inheritance. In such complex traits, a number of predisposition genes interact with each other and environmental factors to produce the disease phenotype. The evidence for a genetic basis is that: i) there is familial clustering in humans and rhesus monkeys; ii) there are some case reports of concordance in monozygotic twins; iii) the age at onset of pain symptoms is similar in non-twin sisters concordant for the disease; iv) the prevalence of endometriosis and/or adenomyosis is 6–9 times greater in the 1st-degree relatives of affected women; v) the risk of developing endometriosis may be up to 15 times greater in the relatives of women with rAFS moderate-severe disease, when MRI is used as the diagnostic test, and vi) certain genetic polymorphisms are more prevalent in affected women than controls. The polymorphisms studied to date include the N314D mutation in galactose I-phosphate uridyl transferase (GALT), an enzyme involved in galactose metabolism, and a null mutation in glutathione *S*-transferase M1 (GSTM1), an enzyme involved in detoxification.

Methods: The OXEGENE Study is an international, collaborative project which aims, using affected sib-pair and TDT analysis, to identify susceptibility loci involved in the development of endometriosis. Families containing at least two sisters with surgically confirmed rAFS moderate-severe disease are being recruited: i) through advertisements placed in the newsletters of national endometriosis self-help groups, in Australia, Ireland, New Zealand, Scandinavia, UK and USA; ii) through an MRI screening programme in Oxford; iii) with the help of leading research centres throughout the world, and iv) via the OXEGENE web-site (http://www.medicine.ox-.ac.uk/ndog/oxegene/oxegene.htm). To date, 986 families (1,465 women) have joined the study. The web-page alone has been hit 21,600 times since March 1997. DNA has been extracted from blood obtained from over 200 sister-pairs and their parents. A genome-wide screen using 400 microsatellite markers is currently being conducted, and candidate gene studies involving the GALT N314D mutation and the null mutation in GSTM1. Genotyping and clinical data from these studies will be presented.

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Development of an Endometriosis Specific Quality of Life Instrument. [1,2]G. Jones, [2]C. Jenkinson, [1]S. Kennedy. [1]Nuffield Department of Obstetrics and Gynaecology, and [2]Health Services Research Unit, Department of Public Health, University of Oxford, UK.

Objectives: Endometriosis undoubtedly affects quality of life in terms of the pain and infertility it causes, but the disease also results in emotional distress, relationship problems, time off work and reduced productivity. However, there is no way at present of quantifying the effect of endometriosis on quality of life using a scale that has been generated by sufferers themselves. The aim of this study is to develop and validate such a quality of life instrument in the form of a questionnaire for use in health-care settings to (i) assess patient-centred evaluation of care, and (ii) evaluate the effect of treatment.

Methods: The questionnaire will be developed in 3 stages using established methods (Jenkinson C, Fitzpatrick R, Peto V. Manual for the Parkinson's Disease Questionnaire. Health Services Research Unit, Oxford, 1998). *Stage I - Item generation*: Women with endometriosis are having exploratory in-depth interviews to generate a large number of candidate questionnaire items. A long form measure will then be developed based on these items. The interviews will be undertaken until no new themes or concerns are uncovered. *Stage II - Item reduction and scale generation (n=200)*: A postal survey will be conducted using the questionnaire generated at Stage I to determine the measure's acceptability. This phase will allow (i) development of a more practical instrument with fewer items, and (ii) identification of instrument sub-scales which address different dimensions of the endometriosis experience. The instrument sub-domains will be

developed using factor analytic techniques. Internal reliability will be assessed using the Cronbach's α-statistic. *Stage III - Assessing construct validity and test re-test reliability (n=200)*: Women will complete the SF-36, a generic health status measure. It is thought that scales addressing similar issues on the two questionnaires will be significantly correlated, although such associations will not be perfect given the specific nature of the endometriosis questionnaire. Women will be asked to complete the questionnaire twice in a week. Responses from those who complete the measure at time two and indicate no change from baseline will be used to assess test re-test reliability (the measure's ability to provide the same results at different times for those who have not changed). A sample size of 200 assumes that 100 women will report no significant changes between two administrations of the same instrument, and that the intra-class correlation will be $\geq$0.9. All subjects have surgically confirmed endometriosis. The methodology will be presented in detail, as well as the results of Stage I, and the preliminary results of Stage II (This work is being supported by an educational grant from Searle).

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Influence of Peritoneal Fluid of Endometriosis Patients on Zona Pellucida Binding Capacity (ZPBC). [1]J. P. van der Merwe, [1]D. R. Franken, [2]S. C. Oehninger, [1]T. F. Kruger. [1]Reproductive Biology Unit, Tygerberg Hospital, Tygerberg 7505, South Africa and [2]Jones Institute for Reproductive Medicine, Norfolk, VA 23507.

Objective: The aims of this ongoing study are: (1) to determine zona pellucida binding capacity after sperm exposure to peritoneal fluid of patients with endometriosis; (2) to assess the effect of the degree of endometriosis; and (3) to evaluate the effect of surgical-medical therapy on gamete interaction.

Design: Prospective, controlled cohort study.

Patients and Methods: Study group consisted of 23 patients with endometriosis. This group was subdivided into two groups: group 1, patients with stage I and II disease (ASRM), and group 2, patients with stage III and IV disease. The control group (group 3, n=7) consisted of patients without endometriosis. Sperm were exposed to peritoneal fluid of patients and hemizona index (HZI) was recorded in a blind fashion. The HZI was repeated on sperm exposed to peritoneal fluid of patients who had fulgeration of endometriotic lesions and three months treatment with gonadotrophin releasing hormone agonist. Sperm binding was recorded using standard procedures for the hemizona assay. Peritoneal fluid samples were collected laparoscopically and stored at $-20°C$ after centrifugation to remove red blood cells. Samples were thawed and diluted 1:5 using 5×10^6 spermatozoa/mL suspended in synthetic human tubal fluid medium with 3% BSA.

Results:

		Group 2		
	Group 1	Before treatment	After treatment	Control group
N	3	12	8	7
HZI	83.0 (±15.5)	86.3 (±9.8)	93.4 (±10.3)	92.9 (±10.52)

No significant difference between the groups.
HZI: hemizona index

Conclusion: Preliminary results of this ongoing, prospective study demonstrate that exposure of sperm to peritoneal fluid from patients with different stages of endometriosis does not appear to influence sperm-zona pellucida binding. The possibility of a beneficial effect of therapy on gamete interaction remains to be clarified.

P-390

Differential Gene Expression in Endometrioma and Normal Endometrial Cells. S. Z. A. Badawy, L. Kaufman, V. Cuenca. Department of Obstetrics and Gynecology, State University of New York, Health Science Center, Syracuse, New York.

Objective: Endometriosis in general and endometrioma in particular have shown invasive characteristics similar to tumor-like processes. Some studies have shown certain cytologic atypia present in the glandular epithelium that lines the ovarian endometrioma. DNA aneuploidy was observed in some endometrioma cysts with severe atypia. The present study attempts to show there are differences in gene expression that underlie these morphological and physiological changes in endometrioma cells as compared to normal endometrial tissue.

Design: Using the method of fluorescence-based differential display, m-RNA isolated from patient cells was reverse-transcribed to c-DNA and amplified. Differences in RNA display were observed after automated sequencing electrophoresis, by use of computer-generated electropharograms.

Materials and Methods: Specimens of endometriomas and endometrial tissue were either placed in culture or used directly for RNA isolation. RNA isolated from both cultured cells and tissue was treated with DNAse, Phenol/chloroform-extracted, ethanol-precipitated and quantified by UV spectrophotometry at OD_{260}. RNA was reverse-transcribed to c-DNA and amplified by polymerase chain reaction (RT-PCR). Each RNA specimen was amplified with three anchored (fluorescent-labeled) oligo-dT primers and eight arbitrary 13mer oligo-dT primers, for a total of twenty-four amplifications per RNA sample. Electrophoresis of amplified c-DNA was performed by automated sequencing on a Model 377 PRISM DNA sequencer and results were observed as peaks, which reflected base-pair size and relative intensity of the fluorescent signal on electropharograms. This study was IRB-approved.

Results: Distinct differences were observed in the pattern and number of RNA sequences displayed between endometrioma cells and those of their normal counterparts. We further observed an increase in the number of RNA sequences in endometrioma cells not seen in the normal endometrium.

Conclusion: We speculate that the differences in RNA sequences seen between endometrioma and normal endometrial cells are reflective of variations in gene expression in the tissues. This may explain the morphological and biochemical changes in endometriomas.

MALE REPRODUCTION AND UROLOGY

Wednesday, September 29, 1999

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Motility of Testicular Sperm Obtained From Men With Obstructive Azoospermia Improves After Incubation In Vitro for 1–2 Days. [1,2]A. J. Carrillo, [1]P. P. Risch, [1,2]C. L. Cook, [1,2]A. C. Eblen, [1,2]S. T. Nakajima, [1,2]M. L. Swanson, [4]S. Yoffe, [3,4]A. M. Belker. [1]Fertility Center at University OB/GYN Associates, P.S.C., [2]Dept. of OB/GYN and [3]Division of Urology, University of Louisville School of Medicine, [4]Jewish Hospital, Louisville, KY.

Objective: To examine the effects of maintaining testicular tissue in vitro for 1 to 3 days on sperm motility.

Design: Retrospective study of testicular sperm motility in vitro.

Materials and Methods: Nine samples were obtained by testicular exploration and sperm aspiration (TESA) from 8 men with obstructive azoospermia to retrieve motile sperm for IVF-ICSI. Samples were washed $\times2$ in Modified Human Tubal Fluid with 3 mg/ml HSA (MHTF3). Pellets were resuspended in 1 ml of erythrocyte-lysing buffer for 6 minutes (Hum Reprod 10:2956, 1995). Three ml of MHTF3 then were added to stop cell lysis and the sample was washed $\times2$ in MHTF3. Pellets were resuspended in 50–100 μl of Ham's F-10 with 3 mg/ml of HSA and placed in an incubator with 5% CO_2 in room air. Part of these samples were used to retrieve sperm for ICSI that same day. Two-20 μl aliquots of each sample were placed in an ICSI dish, overlaid with mineral oil and returned to the CO_2 incubator. Sperm motility was determined that day (day 0). The samples were placed on the stage of an inverted microscope, examined under 200$\times$ magnification and non-motile and motile sperm were counted. Samples then were returned to the CO_2 incubator. Motility determinations were repeated in the same manner on days 1, 2, and 3.

Results: The total number of sperm per sample was variable (43 to 625). Overtly motile sperm were found in 6/9 samples in day 0. Between days 0 and 1, 7/9 samples had an improvement in motility, including the development of overt motility on days 1–3 in samples with no motility on day 0; 1/9 had no change and 1/9 had a decrease in motility. Between days 1 and 2, 6/7 samples had a further increase in motility. Regardless of these changes in motility, motile sperm could be readily identified in 9/9 samples on day 1, in 8/9 samples on day 2 and in 3/3 samples examined on day 3. Furthermore,

sperm motility on days 1 & 2 was much more evident than the subtle motility observed on day 0.

	Day 0	Day 1	Day 2	Day 3
Samples with motility	6/9	9/9	8/9	3/3
% motility (avg)	9	25	29	23
% motility (range)	0–37	3–68	0–79	5–35

Conclusions: TESA samples obtained from men with obstructive azoospermia develop marked improvement in motility after 1 to 2 days in vitro. Scheduling of the TESA 1 or 2 days prior to the oocyte retrieval reduces the time required to find and retrieve sperm because motility is more vigorous and thus more apparent on days 1 and 2 than on day 0. We now are examining the effects of using testicular sperm incubated for 1 to 2 days on fertilization and pregnancy rates.

P-392

Sperm Longevity and Semen Cultures. E. E. Gottenger, H. M. Nagler, N. E. Medley, N. Virji. Beth Israel Medical Center, New York, NY.

Objectives: The impact of infection on infertility as well as the potential mechanism of this reported effect remains controversial. It has been shown that infection can adversely affect sperm motility. We observed decreased survival after swim-up (SU) separation and overnight (O/N) incubation of sperm in men with infection. We retrospectively studied a population of men referred for infertility who had poor O/N survival after SU separation of sperm.

Design: Results from semen analyses, SU separation with O/N incubation of sperm and semen cultures from infertile patients were retrospectively evaluated.

Materials and Methods: Sperm from fifty men who had all of the above tests performed were selected. Semen was separated by multi-tube swim-up after routine semen analysis. Motility was determined post-SU and after O/N incubation at 37°C in modified Ham's F-10 medium supplemented with antibiotics. Microbiological evaluation of semen was performed for routine organisms, chlamydia trachomatis, ureaplasma urealyticum and mycoplasma hominis on a fresh ejaculate by a reference laboratory within 2–8 weeks of SU and poor O/N survival.

Results: The mean percent motility of the initial, post-SU and O/N incubated sperm was 38.6±17.8, 82.2±15.8 and 23.9±22.9 respectively. The overall mean difference between post-SU and post-O/N motility was 69.2%. Of the 50 patients, 31 (62%) had positive cultures for pathogenic bacteria, 6 (12%) grew nonpathogenic bacteria and 13 (26%) had negative cultures. The mean difference in sperm motility between post-SU and post-O/N samples was 76.2% for patients with positive cultures, 76.9% with nonpathogenic bacteria, and 49.1% with negative cultures. The difference in motility between the positive and negative cultures was significant ($p < 0.05$).

Conclusions: O/N incubation of SU separated sperm is an integral part of our algorithm prior to therapeutic use of semen. This retrospective analysis showed that a correlation exists between poor overnight survival of sperm and infection with either pathogenic or nonpathogenic bacteria. Patients with a mean motility difference of 69.2% between the post-SU and O/N incubated sperm had a 74% incidence of positive cultures. In these patients semen culture and subsequent treatment with antibiotics when required could be of benefit prior to clinical use of the sperm in assisted reproductive techniques. Semen cultures have been recommended when pyospermia is detected on semen analysis. We have expanded this indication to include patients with poor survival after O/N incubation of SU sperm.

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Side Effect Profile of Sildenafil Citrate (VIAGRA™) in the Treatment of Erectile Dysfunction in a Large Community Practice. R. E. Brannigan, A. Spitz, F. Orejuela, E. D. Kim, E. C. Schatte, L. I. Lipshultz. Scott Department of Urology, Baylor College of Medicine, Houston, TX.

Objectives: Sildenafil citrate (Viagra™) has been shown to be an effective treatment for erectile dysfunction. Initial studies reported a high tolerability and low incidence of certain characteristic adverse reactions. We sought to re-evaluate the incidence of side effects of sildenafil citrate utilizing a heterogeneous cohort of patients from a community-based practice. The patients were explicitly questioned about the occurrence of headache, flushing, dyspepsia, nasal congestion and visual changes.

Design: A prospective, open label, flexible dose study of 267 patients treated with sildenafil citrate for erectile dysfunction at a single institution.

Materials and Methods: A total of 256 patients with erectile dysfunction of various etiologies received open label sildenafil citrate. Patients were specifically asked if they had experienced the more common, previously determined known side effects of sildenafil including headache, flushing, dyspepsia, nasal congestion and visual changes. The incidence of adverse events was calculated in our patient cohort and compared with the published incidences of these events from data obtained in studies of controlled patients prior to community based care.

Results: The most commonly reported adverse events were facial flushing, headache, nasal congestion, heartburn, and visual disturbances. All side effects occurred more frequently than initially reported (Morales et al., Int. J. Impot. Res., 10:69,1998).

	Flushing	Headache	Congestion
Baylor (267 pts)	30% (80/267)	26% (69/267)	19% (51/267)
Morales et al (734 pts)	10% (73/734)	16% (117/734)	4% (29/734)
P value	p< 0.001	p< 0.001	p< 0.001

	Heartburn	Visural Changes
Baylor (267 pts)	11% (30/267)	6% (18/267)
Morales et al (734 pts)	7% (51/734)	3% (22/734)
P value	p=0.039	p=0.013

At Baylor, 56.5% of patients experienced one or more adverse events. However, no one withdrew from the study due to the severity of adverse events. Also, there was no significant change in the incidence of adverse events in relation to dosage of sildenafil citrate.

Conclusions: The incidence of adverse events due to sildenafil citrate may be higher than initially reported. An explanation for this may be the way in which the data is obtained. Whereas initial studies typically ask open-ended questions about any adverse events experienced, our study elicited information about specific adverse events. The adverse events do not appear to be dose related, and sildenafil citrate remains a safe and well tolerated treatment for erectile dysfunction.

P-394

Relationship Between Pellet Sperm Analysis (Virtual Azoospermia) and Serum Folicule-Stimulating Hormone (FSH) Levels in Patients With Non-Obstructive Azoospermia (NOA). F. Orejuela, A. Spitz, R. E. Brannigan, S. G. Moreira Jr, E. D. Kim, L. I. Lipshultz. Scott Department of Urology, Baylor College of Medicine, Houston, TX.

Objective: Our aim in the present study is to determine if serum FSH levels are predictive of the presence of sperm in the sperm pellet analysis of the patient with NOA.

Design: A prospective consecutive study of 62 patients with NOA was conducted between March 1996 and February 1999.

Materials and Methods: Semen centrifugation for sperm pellet analysis was performed in 62 consecutive men with NOA. NOA was defined as the absence of sperm on routine semen analysis in men who demonstrated moderate to severe testicular atrophy with markedly elevated FSH. This finding of extremely low sperm density has been termed "virtual azoospermia".

Results: Sperm was identified in the centrifuged pellets of 15 of 62 patients (24.1%) with NOA. Motile sperm was seen in all 15 samples. FSH levels in patients with absent sperm in the pellet were neither clinically nor statistically significantly different from those with sperm present in the pellet.

Sperm in pellet	# of Patients	Serum FSH Levels		
		Mean	Median	SD
Absent	47	21	25.3	18.5
Present	15	21	24.5	14.0

p:0.88

Conclusions: We have previously demonstrated the presence of sperm in the pellet of patients traditionally believed to be completely azoospermic. In the present study we did not find any correlation between FSH levels and the presence of sperm in the pellet of men with non obstructive azoospermia. Consequently, semen centrifugation (sperm pelleting) should be performed in all men considered to be azoospermic by routine semen analysis, regardless of the serum FSH levels.

P-395

The Role of Diagnostic Testis Biopsy in the Modern Treatment of the Azoospermic Patient. E. Onel, C. S. Niederberger, L. S. Ross. The University of Illinois at Chicago, Chicago, IL.

Objectives: The placement of azoospermic patients into obstructed and non-obstructed categories can be accomplished with a high degree of accuracy by a complete history, physical examination and hormonal screening. Absolute resolution of the cause of azoospermia in the non-obstructed patient has traditionally required testis biopsy. Since intra-cytoplasmic sperm injection (ICSI) may allow patients with severe forms of non-obstructive azoospermia (including Sertoli Cell Only) to achieve a biologic pregnancy owing to patchy areas of spermatogenesis, we have questioned whether purely diagnostic testis biopsy as a separate procedure has any value in the modern treatment of the azoospermic patient.

Design: Sperm retrieval rates, along with clinical pregnancy rates and live birth rates with IVF/ICSI, were evaluated in patients with various forms of non-obstructive azoospermia. The cost of diagnostic testis biopsy alone was compared with that of diagnostic biopsy combined with sperm retrieval and cryopreservation.

Materials and Methods: A meta-analysis of prior reports of sperm retrieval rates, clinical pregnancy rates, and live birth rates was performed.

Results: In non-obstructed azoospermic patients, testis biopsy yielded sperm in 34% to 57% of patients; retrieval rates approached 100% in obstructed patients. Clinical pregnancy rates in the non-obstructed patient approached 60%, with live birth rates nearing 50%; these were not significantly different than in the obstructed patient. Assuming fixed costs of $2000 for a testis biopsy, $300 for tissue processing and $150 for tissue storage for one year, performing a purely diagnostic testis biopsy costs the health care system an additional $50,000 per 100 patients when the sperm retrieval rate is only 34%. When the retrieval rate increases to 57%, the added cost of purely diagnostic testis biopsy increases to $92,550 per 100 patients. This simple cost analysis does not take into account the additional days of lost work or recovery time in a patient required to have two procedures.

Conclusions: It is not cost effective to do separate diagnostic testis biopsy in the non-obstructed azoospermic patient. In addition, performing a single procedure on patients decreases emotional strain, scheduling difficulties and testicular risk. The traditional algorithm can thus be modified, saving patients from unnecessary procedures while preserving success in the measured outcomes of the male infertility patient.

P-396

A Prospective Randomized Study Comparing ISolate™-Processed Versus Washed Semen in Intrauterine Insemination (IUI) Therapy Using Husband's Sperm: Efficiency and Pregnancy Outcome. M. Morshedi, S. Taylor, K. Duru, C. Montgomery, G. Barroso, S. Oehninger. The Jones Institute for Reproductive Medicine, Dept. Ob/Gyn, Eastern Virginia Medical School, Norfolk, VA.

Objective: To compare sperm parameters and pregnancy outcome in IUI using two different methods of semen processing.

Design: Prospective, randomized, ongoing study performed at a tertiary care institution.

Materials and Methods: All couples participating in IUI therapy using husband's sperm during September, 98 through February, 99 were enrolled. Exclusion criteria were: original sperm concentration $<1 \times 10^6$/ml, progressive motility <10%, $>1 \times 10^6$ leukocytes and positive anti-sperm antibodies as detected by the direct immunobead test. Subjects were randomized according to the day of the week (in an alternating fashion) to semen processing with ISolate (gradient centrifugation) or washing (in human tubal fluid supplemented with 0.2% human serum albumin). Sperm motion parameters were assessed with a computer analyzer; morphology was evaluated with strict criteria. A final volume of 0.25–0.4 ml was used for the IUI. Sperm and female variables as well as pregnancy rates were analyzed by the Anderson-Gill extension of the Cox proportional hazard regression method. Comparison of sperm parameters in original and processed samples according to the method used was carried out by the generalized estimating equation approach to longitudinal data.

Results: A total of 131 cycles (63 ISolate and 68 wash cycles) performed in 86 different couples have been analyzed so far. There were no significant differences in the various sperm parameters of original samples. Sperm parameters of the processed samples over 3 consecutive IUI cycles were significantly higher in the ISolate group in regard to motility, velocity and linearity ($p< 0.0001$ for all). However, the total number of motile spermatozoa recovered and the recovery rate were significantly higher in the wash group ($p< 0.0001$ for both). There were no significant changes for the sperm parameters analyzed over 3 cycles. Among all covariates analyzed for impact on pregnancy rate (male factors: sperm parameters of the original ejaculate or of the processed samples; female factors: age, duration and type of infertility; semen processing: ISolate or wash) only female's age demonstrated a significant effect (hazard ratio 0.87, $p= 0.01$). The estimated cumulative pregnancy rate (over 3 cycles) ranged from 39% in 30 year-old women to 15% in women 39 years of age.

Conclusions: Analysis of the data obtained so far revealed that ISolate processing allows for the recovery of sperm of superior quality in motion parameters but at the expense of a lower recovery rate when compared to a simple wash. The use of these methods does not appear to have an impact on pregnancy outcome. Female's age is corroborated as a significant covariate for conception.

P-397

Does Testis Injury From Diagnostic Fine Needle Aspiration Mapping Affect Later Sperm Retrieval Procedures? [1]P. J. Turek, [1]M. Meng, [2]I. Cha, [3]J. Conaghan. Departments of [1]Urology, [2]Pathology, [3]0b-Gyn and Reproductive Sciences, University of California San Francisco, San Francisco, CA.

Objectives: Systematic testis fine needle aspiration (FNA) mapping is an alternative to biopsy to determine sperm presence within infertile testes. To assess whether this diagnostic technique impairs subsequent sperm retrieval from the epididymis or testis, we analyzed the success of post-FNA sperm extraction procedures in obstructed (OBSTR) and nonobstructed (NOA) azoospermic men.

Design: Retrospective study of infertile, azoospermic men.

Materials and Methods: Testis FNA was performed diagnostically in 110 men suspected of having either OBSTR (n=23) or NOA (n=87) to either confirm or localize spermatogenesis within the testis. All OBSTR men and 40/87 (46%) of NOA men had sperm detected by FNA. The success, quality and timing of all subsequent TESA, TESE and MESA procedures were analyzed in these 63 men.

Results: The mean number of diagnostic FNA sites was 7.8 and 16 per patient in OBSTR and NOA men, respectively. Subsequent sperm retrieval procedures were undertaken in 17/23 OBSTR and 25/40 NOA men. The mean time to sperm retrieval was 4.1 months (range 1 week–12 mos.) in OBSTR men and 5.1 months (1 week–14 mos.) in NOA patients. Among OBSTR men, 14/17 (82%) underwent MESA, 2/17 (12%) had TESA, and 1/17 had electroejaculation. Sperm was retrieved in all OBSTR cases. With MESA, the mean total motile sperm count retrieved was 20 million sperm, and mean motility 30%. In NOA men, a total of 28 procedures were performed: 16/28 (57%) unilateral TESE, 6/28 (21%) bilateral TESE and

6/28 (21%) TESA only. Mean total and total motile counts retrieved in NOA patients were 147,000 and 8,000 sperm respectively. In two procedures (7%), no sperm were recovered by TESE.

Conclusions: Excellent success at sperm retrieval is possible with prior confirmation of spermatogenesis by FNA in obstructive and nonobstructive azoospermia. Contrary to reports with diagnostic testis biopsy, there appears to be no need to wait for testis recovery after diagnostic FNA before initiation of subsequent sperm retrieval procedures.

P-398

Transurethral Resection of the Ejaculatory Ducts (TURED): Success Rates, Options and Complications of REPEAT Resection, Seminal Vesicle Aspiration (SVA) and Sperm Retrieval After Unsuccessful Repair. [3]Luke Cho, [3]Ithar Derweesh, [2]Michael Witt, [1,3,4]Stanton C. Honig. [1]Urology Center, New Haven CT, [2]Reproductive Biology Associates, Atlanta GA, [3]Yale New Haven Hospital Urology, New Haven CT, [4]Division of Urology, University of Connecticut, Farmington CT.

Objectives: To determine the success rates and complications of repeat TURED in cases of either unsuccessful resection or restenosis of ejaculatory ducts. We present an algoithm for management of these patients after resection.

Design: Retrospective study at a university based infertility center and private practice tertiary referral program.

Materials and Methods: Patients were included in this study if they meet the initial criteria of obstructive azoospermia secondary to ejaculatory duct obstruction. All patients initially presented with low volume azoospermia (one with severe asthenospermia), normal size testes and NO evidence of epididymal induration to suggest a secondary epididymal obstruction. Mean age was 36 yrs. All patients had testis biopsies which revealed normal spermatogenesis and FSH values within normal limits. Diagnosis of ejaculatory duct obstruction was made by either transrectal ultrasonographic evidence of ejaculatory duct obstruction (ejaculatory duct cyst, seminal vesicle dilatation) or intraoperative vasography showing obstruction at the distal ejaculatory duct. All patients underwent a TURED (3) or balloon dilatation of the ejaculatory duct (1). Postoperatively, all patients had either no sperm in the ejaculate or return of sperm followed by evidence of restenosis (decrease in ejaculate volume, decrease in sperm concentration). All patients underwent subsequent repeat TURED or balloon dilatation with or without SVA or vasography.

Results: Four patients met the inclusion criteria. Two patients had evidence of azoospermia. Two patients had evidence of sperm in the ejaculate (range 1–65 million) that subsequently diminished significantly. After REPEAT TURED, one patient had significant improvement in semen quality and achieved a pregnancy through natural intercourse. Two patients had improvement in ejaculate volume, one with improved semen quality and one without improvement. One of these subsequently went on to microscopic vasal sperm retrieval/ICSI and is pregnant with triplets. One patient developed a post operative complication of a seminal vesicle abcess (in a dilated seminal vesicle cyst) requiring hospitalization, IV antibiotics and percutaneous drainage.

Conclusions: REPEAT TURED may be a viable option for treatment of persistent or recurrent ejaculatory duct obstruction. An algorithm for treatment of the unsuccessful relief of obstruction is necessary to determine the appropriate next step in treatment. This should include transrectal ultrasound, and possibly SVA or microscopic vasography prior to repeat resection. Good results are possible however significant risks do exist and patients must be counseled regarding all options, success rates and complications prior to therapy.

P-399

Clomiphene Citrate Improves Sperm Quality and Fertility in Hypogonadal, Oligospermic Males. K. M. Silverberg, R. A. Ormand, L. J. Hansard, T. C. Vaughn. Texas Fertility Center, Austin, TX.

Objectives: Male factor infertility has been implicated as a contributing factor in up to 50% of cases of infertility. Although any abnormality in the semen analysis may lead to a diagnosis of "male factor," the most common abnormality is oligospermia. The endocrinologic evaluation for oligospermia includes serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. Although clomiphene citrate has been used empirically in males with oligospermia, to our knowledge, its use has never been prospectively evaluated using both endocrinologic and semen analysis parameters in hypogonadal, oligospermic men. The purpose of this study was to evaluate the effect of daily clomiphene citrate on serum FSH, LH and testosterone levels, as well as sperm concentration, motility, and morphology in hypogonadal, oligospermic males.

Design: Prospective trial of clomiphene citrate 25 mg/day for a minimum of 90 days.

Materials and Methods: Twenty-four men with hypogonadal oligospermia received clomiphene citrate 25 mg/day for a minimum of 90 days. FSH, LH, and testosterone levels as well as semen analysis were then repeated in order to determine the effectiveness of the clomiphene treatment. Clomiphene was continued until either the couple conceived or infertility therapy was discontinued.

Results: Using the paired t-test, mean FSH levels increased significantly during clomiphene treatment (2.9 vs. 4.9 mIU/mL, $p = 0.01$), as did LH levels (3.6 vs. 6 mIU/mL, $p < 0.001$), and testosterone levels (259.8 vs. 556.2 ng/dL, $p < 0.001$). In addition, the mean sperm concentration rose significantly (29.6 M/mL vs. 65 M/mL, $p < 0.05$), as did sperm motility (33.3% vs. 47.7%, $p < 0.01$). There was no significant increase in normal sperm morphology. Fourteen couples conceived while on treatment.

Conclusion: Clomiphene citrate represents a viable alternative for the treatment of hypogonadal oligospermia.

P-400

Correlation Between Improvement in Strict Sperm Morphology and Unassisted Pregnancy After Subinguinal Microsurgical Varicocele Repair For Male Factor Infertility. S. C. Esteves, L. T. Nakazato. ANDROFERT-Andrology and Human Reproduction Clinic, Campinas, SP, Brazil.

Objectives: Strict sperm morphology results are well correlated to IVF outcome. However, the predictive value of this seminal parameter *in vivo* is not established. We studied whether the improvement in strict sperm morphology after microsurgical varicocele repair for male factor infertility is associated with better unassisted pregnancy rates.

Design: Strict morphology results and pregnancy outcome were prospectively obtained after subinguinal microsurgical varicocele repair over a 12-month period.

Materials and Methods: Twenty-six teratozoospermic men submitted to microsurgical correction of clinical varicoceles were studied over a 12-month period. Preoperative evaluation included at least 2 seminal analyses including sperm morphology according to the Tygerberg criteria. Infertility in the female partner was excluded. Postoperative evaluation included serial semen analyses at 3 month-intervals and pregnancy reports. The subjects were divided according to the preoperative morphology results in two groups: A) <4% normal forms ($n = 7$), and B) between 4% and 14% normal forms ($n = 19$). Chi-square or Fisher exact test were used for statistical analyses.

Results: Thirteen men (50%) demonstrated significant postoperative improvement in strict sperm morphology ($P = 0.004$). All men from group A were reclassified as group B ($n = 6$) or reached normal morphology values ($n = 1$). Six of 19 (32%) men from group B had normal sperm morphology postoperatively. The mean values obtained 3 months after surgery remained unchanged in the follow-up period ($P = 0.59$). Unassisted pregnancy rate (PR) for the partners of men who had improvement in morphology post-varicocelectomy was 42% (5/12), as compared to 31% (4/13) of those whose morphology remained unchanged ($P = 0.68$).

Conclusions: 1) Our results suggests that microsurgical varicocele repair enhances strict sperm morphology mainly in men with profound morphology abnormalities. 2) Although this cohort is too small to obtain statistically significant results, unassisted PRs seems to be higher in the partners of men who had improvement in strict morphology after varicocelectomy.

P-401

Does *U. Urealyticum* Adversely Affect Semen Characteristics in Random Potential Semen Donor Candidates? A. Bhowmik, M. H. Javed, M. A. Shaikh, C. Ruberto, A. P. Del Valle. ReproMed Ltd./AVR Andrology Inc., Toronto, Ontario, Canada.

Objectives: Conflicting observations relating *U. urealyticum* infection to human infertility have been reported. The objectives of this study were (1) to evaluate effects of *U. urealyticum* infection on pre-freeze and post-thaw semen characteristics of semen donor candidates and (2) to determine annual incidence and prevalence of *U. urealyticum* infection in semen donor candidates.

Design: The effects of *U. urealyticum* on semen parameters of a population of semen donor candidates were evaluated retrospectively in a large clinical semen cryobank.

Materials and Methods: The data of 44 potential candidates from 1990 to 1998 who had both positive and negative *U. urealyticum* cultures were analyzed. Candidates were divided into two categories, accepted (n = 17) and rejected (n = 27). Semen specimens were collected by masturbation after a period of 3–5 days of abstinence of ejaculation. Each specimen was sent for *U. urealyticum* isolation (Arginine culture broth, London Health Sci. Ctr, Univ. Campus London, ON, Canada). Specimens were frozen by standard vapor freezing using SMMG (Irvine Scientific, CA, USA) cryoprotectant. Comparisons were made for volume, sperm Conc., pre-freeze motility, motility grade, post-thaw motility and motile Conc. (WHO Manual, 1992) within and amongst candidates. Motility was recorded as percent and Conc. M/ml. Means were compared by t-test. Annual incidence and prevalence of *U. urealyticum* positive candidates were also calculated.

Results: Comparison of positive and negative specimens of the same accepted or rejected candidate and amongst candidates did not reveal any significant difference ($P < 0.05$) in any of the parameters observed. In accepted candidates, mean ± SD values for raw motility in *U. urealyticum* positive and negative specimens were 56.87 ± 7.29 and 59.75 ± 9.22; for total sperm concentration 68.25 ± 29.54 and 72.0 ± 31.51; for post-thaw motility 26.33 ± 6.47 and 31.33 ± 3.2 and for post-thaw motile concentration 21.63 ± 9.16 and 21.83 ± 8.3 respectively. In rejected candidates, mean ± SD values for raw motility in *U. urealyticum* positive and negative specimens were 63.15 ± 9.80 and 61.69 ± 6.50; for total sperm concentration 72.93 ± 47.91 and 71.20 ± 32.50; for post-thaw motility 32.0 ± 7.79 and 29.55 ± 11.10 and for post-thaw motile concentration 22.78 ± 16.09 and 22.0 ± 20.20 respectively. Annual incidence (%) of *U. urealyticum* in candidates was 16.6 (9/54) and 8.97 (7/78) and 7.35 (5/68) and annual prevalence (%) was 24.07, 16.66 and 14.70 for the year 1996, 1997 and 1998 respectively.

Conclusions: (1) The presence of a positive *U. urealyticum* culture in semen donor candidates does not appear to have a detrimental effect on semen volume, raw, pre-freeze and post-thaw motility. (2) A decreasing trend for the incidence and prevalence was observed during 1996 to 1998. (3) Further research is required to address the effect of *U. urealyticum* in human reproduction to evaluate the need for screening of semen donor candidates.

P-402

Applying Fluorescence In Situ Hybridization to Detect Spermatids in Testis Biopsy [1]H. S. Wei, [1]W. M. Lin, [1]J. Y. Wen, [2]H. S. Chiang, [1]C. R. Tzeng. [1]Department of Obstetric and Gynecology and [2]Department of Urology, Taipei Medical College Hospital, Taiwan.

Objective: Fluorescence in situ hybridization was applied to detect the spermatids in the testis biopsy. The result can serve as criteria to evaluate the successful rate for a non-obstructive azoospermia patient going through IVF+ ICSI program.

Design: Testis biopsy is scheduled for non-obstructive azoospermic patients. FISH was done on the section by applying X and Y dual labeled probe or chromosome 18 alpha satellite probe to detect spermatids.

Materials and Methods: There are 25 non-obstructive azoospermic patients going through testis biopsy. Before going through this procedure, every patients have blood chromosome examination and Y chromosome microdeletion detection. After dividing the specimen into three, we fixed two sample in formaldehyde and bowine solution respectively. FISH was applied to detect spermatid on the section fixed by formaldehyde. HE stained was used on the sections which were fixed by bouin's solution. The results from FISH and bouin's stain were compared. We flushed out the inner cell mass from the tubules in the rest one specimen. We tried to find the sperms in these tubules.

Results: There are 5 chromosome anomaly patients were included in this study. Three are Klinefelter's syndrome. One is 46,X,t(6;Y). One is 46,Xidic (Y)(p11.2). Also there are two patients were detected to have Y chromosome microdeletion but their chromosome studies were normal. Sperms were discovered in only two patients after needle dissection of the tubules. However, from the pathology study no spermatozoa were found. There are two patients shown to have 1–2 sperms in each tubules after FISH detection. But from the pathology study, there are only spermatids seen. One of these two patients also had microdeletion at SY153-277. There are no spermatids found after FISH detection in 16 patients. However, from pathology study, 3 patients had spermatids, 2 patients had germ cells but no spermatids discovered. The pathology report of the rest 11 patients is sertoli cell only. The other one patient who has Y chromosome microdeletion showed no spermatids on both FISH and pathology study.

Conclusion: 1.-FISH is a technique that can detect haploid germ cells on testis sections. The results can serve as a good indicator for NOA patients to go through IVF with microinjection of sperms or spermatids if sperms are not discovered during TESE. 2. Patients who have no spermatids or sperms found on the pathology section still have the chance to go through assisted reproduction technology.

P-403

Platelet-Activating Factor-Receptor mRNA Quantification Between Normal and Abnormal Spermatozoa. [1]M. D. Wild, [1]X. Cui, [2]M. Gibson, [2]C. Simmons, [2]T. Thompson, [1]W. E. Roudebush. [1]Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, and [2]Southeastern Fertility Center, Mt. Pleasant, SC.

Introduction: Platelet-activating factor (PAF) plays a significant role in spermatozoa motility, fertilization and subsequent preimplantation embryo development. PAF's mechanism of action is a receptor-mediated event. We have recently reported on the presence and distribution of the PAF-receptor along the human spermatozoa and that PAF-receptor distribution is significantly altered (depressed) in abnormal (poor motility) human spermatozoa.

Objective: To quantify PAF-receptor mRNA in spermatozoa between normal and abnormal human spermatozoa.

Design: Measure PAF-receptor mRNA between normal and abnormal human spermatozoa by competitive RT-PCR.

Methods: Total RNA was purified by acid-phenol extraction and ethanol precipitation from normal (n = 12) and abnormal (n = 6) human spermatozoa as defined by World Health Organization criteria. Complementary DNA were synthesized by reverse transcriptase and RNA primed with oligo-dT at 42°C, 60 min; 95°C, 5 min. The RT products with PAF-receptor MIMIC [an internal standard that is a nonhomologous DNA fragment of the PAF-receptor; has the same primer template as the target PAF-receptor cDNA but generates a PCR product of a different size (310 bp) than the target cDNA (610 bp); Clontech Labs, Palo Alto, CA] were amplified with *Taq* polymerase and PAF-receptor specific primer pair at 94°C, 30 sec; 60°C, 1 min; 72°C, 2 min for 30 cycles followed by 72°C, 10 min. The RT-PCR products were analyzed by agarose gel-electrophoresis. Data were analyzed by Student's t-test.

Results: The amount of PAF-receptor mRNA in abnormal spermatozoa (7.48×10^{-3} attomoles/10^6 spermatozoa) was significantly ($P < 0.01$) higher than that found in normal spermatozoa (2.53×10^{-3} attomoles/10^6 spermatozoa).

Conclusion: Abnormal human spermatozoa have more PAF-receptor mRNA than normal human spermatozoa. The alteration of PAF-receptor distribution in abnormal spermatozoa may be the result of poor or inefficient mRNA translation and or post-translational modifications. Additional studies are warranted to determine if and how posttranscriptional defect(s) in PAF-receptor mRNA translation affect spermatozoa function.

P-404

The Role of LDHC-4 in Sperm Energy Metabolism and Motility. S. C. Sikka, J. S. Armstrong, W. J. G. Hellstrom. Department of Urology, Tulane University School of Medicine, New Orleans, LA, USA.

Objectives: Sperm motility and movement is a prerequisite for normal fertilization. In this study, we have identified important sites of sperm intermediary metabolism in the mitochondria, in order to better understand the regulatory mechanisms involved in the maintenance of sperm motility.

Design: Human sperm from fertile donors were Percoll washed and

suspended in HAM's F-10 nutrient media and then selectively exposed to various pharmacological compounds to identify metabolic sites involved in sperm movement.

Materials and Methods: Human sperm were treated with various uncouplers of energy metabolism, e.g., 3 bromopyruvate (3BP), potassium cyanide (KCN), m-chlorocarbonyl cyanide phenylhydrazone (CCCP), or iodoacetate (IOA). Sperm motility (using μ cell) and ATP (by luciferase chemiluminescence assay) were evaluated in the absence and presence of additional pyruvate added to HAM's media. Sperm mitochondrial membrane potential was assessed by using flow cytometry.

Results: The uncoupler CCP which blocks ATP formation by oxidative phosphorylation did not inhibit either sperm motility or ATP levels but significantly compromised sperm mitochondrial membrane potential, indicating its redundancy in energy production for sperm motility maintenance. Exogenous pyruvate [20 μM] preserved motility in both IOA- and KCN-treated sperm, indicating two important points: (1) the requirement of citric acid cycle substrate level phosphorylation for sperm motility, and (2) the requirement of a high-ratio of NAD^+/NADH for maintenance of sperm motility.

Conclusions: In conclusion, energy generation in sperm mitochondria by the citric acid cycle, independent of oxidative phosphorylation, is sufficient to maintain sperm motility even when glycolysis is inhibited. Pyruvate maintains a high ratio of NAD^+/NADH, provided by mitochondrial lactate dehydrogenase isozyme C-4 (LDHC-4) activity via a lactate-pyruvate shuttle which stimulates the citric acid cycle and generates energy.

P-405

Effects of Sildenafil on Motility and Viability of Human Spermatozoa. [1]C. Teloken, [1]M. Badalotti, [2]T. J. Bivalacqua, [2]S. C. Sikka, [2]W. J. G. Hellstrom. [1]Fund. Fac. Federal Ciencias Medicas, Porto Alegre, Brazil, and [2]Department of Urology, Tulane University School of Medicine, New Orleans, LA, USA.

Objectives: Since its launch, sildenafil (a type 5 cGMP-specific phosphodiesterase inhibitor) has been widely prescribed for the treatment of erectile dysfunction. Many couples of childbearing age are using this medication for sexual enhancement with untoward exposure to the male gametes. The aim of this study was to evaluate effects of sildenafil on the motility and viability of human sperm, *in vitro*.

Design: Human sperm from fertile donors were incubated with HTF medium supplemented with Hepes buffered salt solution (HBSS) and then selectively exposed to various doses of sildenafil in order to test the viability and motility.

Materials and Methods: Ten healthy volunteers provided semen samples. After allowing the samples to liquefy, these were divided into two groups: A and B. Samples from *Group A* were subdivided into 0.5 ml aliquots and incubated respectively in modified HTF medium (control), sildenafil 250 ng/ml and sildenafil 750 ng/ml. The motility (Makler chamber) and viability (with 5% eosin) assessments were carried out in 1 and 3 hours. Sildenafil was previously diluted with HTF containing 10% SSS (synthetic substitute serum). *Group B* samples were washed and re-suspended in HTF with 10% SSS and subsequently underwent the same routine described above. Statistical studies were done by student t test.

Results: Sperm motility after 1 h and 3 h in Group A incubated with HTF was 57.5% and 53.8%, respectively. In the presence of sildenafil, sperm motility was measured at 68.8% (1 h) and 60% (3 h) for 250 ng/ml and 63.8% (1 h) and 50% (3 h) for 750 ng/ml of sildenafil. There was no statistically significant ($p > 0.05$) correlation for these groups. However, Group B (washed sperm) after 1 h and 3 h showed a statistically significant decrease ($p < 0.01$) in sperm motility in the presence of sildenafil (250 and 750 ng/ml). Sperm motility after 1 h and 3 h in Group B incubated with HTF was 52.1% and 41.4%, respectively. In the presence of sildenafil in a dose of 250 ng/ml, sperm motility decreased after 1 h to a value of 57.1% and after 3 h to 51.4%. Sildenafil in a dose of 750 ng/ml also decreased sperm motility after 1 h and 3 h to 53.6% and 51.4%, respectively. Sildenafil in all doses tested had no significant effect ($p > 0.05$) on sperm viability in both groups.

Conclusions: The results demonstrate a statistically significant decrease in sperm motility but no statistically significant impact of sildenafil on the viability of human spermatozoa, *in vitro*. However, further studies are warranted to test the effect of sildenafil on sperm membrane integrity.

P-406

Catalase- and Superoxide Dismutase-Like Activities in Human Semen: Clinical Considerations. V. Mak, D. Phang, A. Zini. Division of Urology, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Objectives: Human spermatozoa are highly susceptible to oxidative injury but they are naturally protected from such injury by seminal plasma. It is known that human seminal plasma is rich in antioxidants, however, little is known about the source of this antioxidant activity. We measured catalase- and superoxide dismutase (SOD)-like activities in the semen of fertile and vasectomized men in order to gain insight into the potential source(s) and function(s) of these antioxidants in semen.

Design, Materials and Methods: Semen samples were obtained from fertile men (n = 17) and men postvasectomy (n = 16). Seminal plasma was recovered by centrifugation of semen (10,000 × g, 10 minutes) and stored at −20°C for subsequent evaluation of antioxidant activity. Catalase-like activity was measured by the decrease in hydrogen peroxide concentration after incubation with seminal plasma. SOD-like activity was measured as the inhibition of nitroblue tetrazolium reduction due to superoxide anion generation by xanthine plus xanthine oxidase.

Results: Mean seminal catalase-like activity (±1 SD) in the fertile group was not significantly different from that of the post-vasectomy group (365 ± 136 and 315 ± 116 U/mL, respectively). Similarly, mean seminal SOD-like activity (±1 SD) in the fertile group was not significantly different from that of the post-vasectomy group (35.0 ± 9.5 and 37.6 ± 7.8 U/ml, respectively).

Conclusions: Our data suggest that the testis and epididymis are not an important source of catalase- and SOD-like activities in ejaculated semen but rather that semen antioxidants are mainly derived from the seminal vesicle and/or prostate. These findings support the notion that semen antioxidants are primarily designed to protect spermatozoa in the female reproductive tract. In addition, our data do not support the argument that the possible increase in prostate cancer in men who have had a vasectomy is due to defective antioxidant defense in semen.

P-407

Determining Aneuploidy for Chromosomes 12, 13, 17, 18, X and Y and Deletions on the Y Chromosome in Sperm from Twenty Oligoasthenoteratozoospermic (OAT) Patients Undergoing Intracytoplasmic Sperm Injection (ICSI). [1,2]S. F. Hoegerman, [3]M. G. Pang, [1]M. K. Rudd, [1]N. K. Dahiya, [1]M. Stacey, [1]L. Lunsford, [4]G. Doncel, [4]A. A. Acosta, [5]L. Brown, [5]D. C. Page, [1,4,6]W. G. Kearns. [1]Center for Pediatric Research, Eastern Virginia Medical School, Norfolk, VA, [2]The College of William and Mary, Williamsburg, VA, [3]Biomedical Research Center, Korea Advanced Institute of Science and Technology, Taejon, Korea, [4]Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, [5]Whitehead Institute for Biomedical Research, Cambridge, MASS, and [6]Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Objective: To determine aneuploidy frequencies and Y chromosome deletions from twenty OAT patients and six proven fertile donors.

Study Design: Multi-probe, multi-color fluorescence *in situ* hybridization (FISH) and STS-PCR was performed on sperm to determine aneuploidy and Y chromosome deletions.

Materials and Methods: Aneuploidy frequencies were determined in sperm from twenty OAT patients and six fertile control donors. Three-probe, three-color FISH was performed using direct labeled DNA specific for chromosomes 12, 13 and 17 (probe set I) and chromosomes 18, X and Y (probe set II). Over 104,000 sperm were scored in this study. STS-PCR was performed to determine genomic deletions within the euchromatic region of the Y chromosome. Where appropriate, Chi-square analysis and Fischer's Exact Test were performed.

Results: In OAT patients, the per chromosome disomy for chromosome 12 ranged from 0.3 to 4.3%, for chromosome 13 from 0.2 to 3.5%, and per chromosome disomy for chromosome 17 ranged between 0.09 and 2.4%. In controls, the mean per chromosome disomy frequency was 0.8% for chromosome 12, 0.2% for chromosome 13, and 0.2% for chromosome 17. The per chromosome disomy for the sex chromosomes ranged between 1.8 and 5.3% and the per chromosome disomy for chromosome 18 ranged between 0 and 1.5%. In controls, the mean disomy frequency for the sex chromo-

somes was 0.4% and the mean disomy frequency for chromosome 18 was 0.2%. Diploidy ranged between 0.1 and 2.6% in OAT patients, with a control mean of 0.3%. Total aneuploidy ranged from 31 to 70% for OAT patients. Total aneuploidy in controls ranged between 4.1 and 7%. Patient to patient heterogeneity for aneuploidy was noted. STS-PCR studies from 20 OAT patients identified one Y chromosome deletion, from both sperm and somatic cells of one patient. From this same patient, a significant increase ($p < 0.05$) in autosomal and sex chromosome aneuploidy was found in his gametes.

Conclusions: These findings showed significant increases of genetic abnormalities in sperm of all OAT patients studied.

P-408

Specific Expression of A-myb Protein in Mouse and Human Testis. [1,4]W. Y. Son, [1]J. H. Lee, [4]J. T. Han, [1,2]S. Kim, [1,3]Y. C. Kim. [1]Center for Reproduction and Genetics, [2]Department of Obstetrics and Gynecology, [3]Department of Urology, Pundang Je-Saeng General Hospital, Kyungki-do, Korea. [4]Department of Life Science, Sogang University, Seoul, Korea.

Objective: Spermatogenesis is the process by which immature male germ cells develop into mature spermatozoa, through a series of mitosis, meiosis, and cellular differentiation. The myb gene family consists of three members, A-, B- and C-myb. The proteins encoded by these genes bind DNA in a sequence-specific manner and regulate transcription of target genes. Recently, Toscany et al. (1997) demonstrated that the knockout of A-myb gene in the mice leads to maturational arrest of the pachytene spermatocytes at the stage of meiosis I. This observation indicate that A-myb protein might have important function in spermatogenesis. This study was conducted to examine the testis-specific expression of A-myb protein in mouse and human.

Design: The expression level of A-myb protein in mouse and human tissues were determined by western blot analysis. A-myb cellular specificity in mouse testis was examined by immunohistochemistry.

Materials and Methods: Mouse tissues were collected from adult ICR male and female mice. Human tissues were stored in liquid nitrogen. Western blot analysis was performed using antiserum (generous gift from M. Introna, Italy) raised against human A-myb protein. To study cell-specific expression pattern of A-myb protein in the testis, immunohistochemistry was performed using the same antibody. Immunohistochemical staining in mouse testis was performed using the kit, using an avidin-biotin immunoperoxidase technique.

Results: Western blot analysis of adult mouse tissue revealed a predominant A-myb expression in the testis, with very low expressions in the ovaries, spleen, liver, muscle, kidney, lung, stomach, uterus, and brain. In human, significant A-myb protein expression was also observed in testis, whereas a small amount of A-myb was detected in breast, stomach, prostate, colon, liver, ovary, and epididymis. Immunohistochemical analysis of adult mouse testis shows that this gene is expressed at high levels in spermatogonia, and preleptotene and pachytene spermatocytes, with concomitant down-regulation during terminal differentiation of these cells into mature spermatozoa.

Conclusion: These results demonstrate that A-myb might play a specific role during the early process of spermatogenesis, i.e. proliferation and/or differentiation, in mouse and human. Further studies to determine the functions of A-myb in the testis should improve understanding of the molecular events associated with spermatogenesis.

P-409

Seminal Plasma Inhibits Progesterone-Promoted but Enhances Follicular Fluid-Promoted Calcium Influx in Human Spermatozoa. [1]R. Minaguchi, [1]Y. Shimizu, [1]T. Aso, [2]R. A. Bronson. [1]Department of Obstetrics and Gynecology, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Obstetrics & Gynecology, State University of New York, Stony Brook, New York, USA.

Objectives: The increase of cytosolic free calcium concentration ($[Ca2^+]i$) induced by follicular fluid, progesterone and prostaglandin E_1 (PGE_1) promotes an acrosome reaction in human spermatozoa. We previously reported refractoriness in the response to a second addition of PGE_1 but not following prior exposure to progesterone. This suggests that different mechanisms exist between progesterone and PGE_1 in mediating $[Ca2^+]i$ of human sperm (Shimizu *et al.* Mol Hum. Reprod. 1998; 4:555–561). High concentrations of PGE_1 exist in human seminal plasma. In this study, the response of $[Ca2^+]i$ induced by progesterone, PGE_1, follicular fluid and seminal plasma was measured using fura-2 in order to investigate the interaction of several agonists, which increase $[Ca2^+]i$ of human sperm.

Design: The increase of $[Ca2^+]i$ evoked by progesterone, PGE_1, follicular fluid and seminal plasma was measured using fura-2, a calcium-sensitive dye.

Materials and Methods: Sperm and seminal plasma were obtained from ejaculates submitted for semen analysis to Tokyo Medical and Dental University Hospital and from proven fertile donors. After swim-up for 60 minutes and incubation for 8–10 hours, spermatozoa were loaded with 5 μM fura-2 for 45 minutes. $[Ca2^+]i$ changes induced by progesterone, PGE_1, follicular fluid and seminal plasma were measured on a Hitachi spectrofluorometer F-2000 (Tokyo, Japan) at an excitation wavelength 340/380 nm and an emission wavelength 510 nm. Human follicular fluids were obtained at the time of oocyte retrieval, as part of the IVF-ET program in our hospital, with patient's permission.

Results: $[Ca2+]i$ increased rapidly after the administration of 1 ug/ml progesterone, 60 ug/ml PGE_1, 1% follicular fluid or 1% seminal plasma. The response reached a peak within 10–20 seconds and then slowly declined to the plateau phase. Refractoriness of the increment of $[Ca2+]i$ arose when 1% seminal plasma was administered 100 seconds after the prior addition of 60 ug/ml PGE_1. Refractoriness also occurred when 1% follicular fluid was administered 100 seconds after the addition of 1 ug/ml progesterone. The increment of $[Ca2+]i$ induced by 1 ug/ml progesterone was reduced when administered 100 seconds after the addition of 1% seminal plasma. Refractoriness did not occur when follicular fluid was administered 100 seconds after the addition of seminal plasma, and the peak of $[Ca2^+]i$ induced by follicular fluid administered 100 seconds after the addition of seminal plasma was higher than that of follicular fluid alone.

Conclusions: This study suggests that seminal plasma contains substances which inhibit the progesterone-mediated calcium influx yet enhance follicular fluid-mediated calcium influx. Seminal PGE prostaglandins may play a major role in these phenomenon.

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Germ Cells Assessment in the Ejaculate of Oligospermic Men: Quantitative Analysis by Flow Cytometry. [1]S. Hamamah, [1]L. Corcia, [1]M. Benkhalifa, [2]Y. Levergne, [2]D. Kerboeuf. [1]Centre de Fécondation in vitro, hôpital A. Béclère, 157 rue de la Porte de Trivaux 92141 Clamart, France, and [2]INRA, service de Cytométrie en flux, 37380 Nouzilly, France.

Objectives: It has been reported since 4 years, that the injection of spermatids recovered from the ejaculate into human oocyte can lead to fertilization, embryonic development and births. The identification of round spermatids in human ejaculate is still unclear today. The quantification by flow cytometry of germ cells at different stages of maturation in the human ejaculate can be used as a diagnostic method before intracytoplasmic sperm injection (ICSI) with round spermatids recovered in the ejaculate. The aim of this study is to evaluate by flow cytometry analysis the germ cells population present in the ejaculate of moderate and sever oligospermia patients.

Design: Germ cells population as well as somatic cells present in the ejaculate of moderate and sever oligospermic patients.

Materials and Methods: Germ cells populations present in the ejaculate were obtained from 13 moderate, and 10 sever oligospermia. 4 normospermia used as control. The round cells of each sample has been performed according Tesarik et al (1995). Specimens preparation for DNA flow cytometry analysed on a flow cytometer using FACS Star plus (Becton Dickinson). The cell pellet was resuspended in citrate buffer solution and adjusted to a cell count of 1×10^6 cells/ml. The DNA flow cytometry analysis has been performed according to Kostakopoulos et al (1997). Every DNA histogram obtained with FACS analysis had 4 regions including: R1 haploid cells (spermatozoa), R2 sub-haploid cells (spermatids), R3 diploid cells (primary, secondary spermatocytes and Sertoli's cells), and R4 tetraploid cells (spermatogonia G2 phase and Somatic cells in G2 phase).

Results: Analysis by FACS of germ cells populations present in the ejaculate are presented in the table:

	haploid cells %	sub-haploid cells %	diploid cells %	tetraploid cells %
normospermia	88.6 ± 2.9*	8.8 ± 1.9	1.1 ± 0.4**	0.3 ± 0.02**
moderate oligospermia	66.1 ± 3.1	21.1 ± 1.7	7.9 ± 0.8	4.9 ± 1.2
severe oligospermia	34.2 ± 5.9	19.1 ± 4.4	18.2 ± 7.0	28.6 ± 9.7

values are mean ± SEM, *p=0.002, **p=0.03. A significant correlation was noted of haploid cells % according sperm concentration (p=0.01).

Conclusions: DNA flow cytometry of the germ cells populations presents in human ejaculate seems to be an objective and quantitative method that can be used to investigate round spermatids in severe oligospermia and azoospermia in infertile patients before to refer to ICSI. Quantitative histometric parameters may have a role in the selection of haploid cells either spermatozoa or spermatids of patients for treatment by ICSI. The DNA analysis of different cells population will be assessed by multicolour FISH for aneuploidy.

REPRODUCTIVE BIOLOGY

Wednesday, September 29, 1999

P-411

Relaxin Regulation of Vascular Endothelial Growth Factor (VEGF) Expression in the Endometrium. [1]E. N. Unemori, [1]M. Lewis, [1]B. H. Grove, [1]M. E. Erikson, [2]A. Einspanier, [1]U. Deshpande. [1]Connetics Corp., Palo Alto, CA and [2]German Primate Center, Gottingen, Germany.

Objectives: Relaxin is produced by the corpus luteum and endometrium during the menstrual cycle and pregnancy. Its role in human reproduction, however, is unclear. When previously tested in clinical trials for efficacy as a topically applied cervical ripening agent, relaxin failed to show any effect on modified Bishop score, length of labor, or oxytocin requirement. However, relaxin administered by continuous sub-cutaneous infusion has recently been correlated ($p<0.005$) with menometrorrhagia in female systemic sclerosis patients in a clinical trial. We have hypothesized that one potential mechanism for this finding may lie in the induction of a potent angiogenic agent, VEGF, in the endometrium. To further investigate this possibility, expression of VEGF in normal human endometrial (NHE) cells was studied.

Design: Firefly luciferase reporter vectors containing two regions of the VEGF promoter, from −42 to −2336 (pGL3B.hV-2336), and from −42 to −564 (pGL3B.hV-564), were constructed. NHE cells were transfected with these reporter constructs. Reporter expression was measured in the presence and absence of recombinant human relaxin (rhRlx).

Materials and Methods: NHE cells were transfected with 1 μg of pGL3B.hV-2336 or pGL3B.hV-564 using Effectene reagent (QIAGEN). One μg of Renilla reniformis luciferase reporter vector (Promega) was cotransfected with each VEGF reporter construct into NHE cultures as a control for transfection efficiency. Cells were treated with 10ng/mL rhRlx for 36–40h. Total cellular extracts were assayed for Firefly and Renilla luciferase content. Conditioned media were collected and assayed for VEGF protein, using an ELISA (R&D Systems).

Results: rhRlx caused a two-fold elevation over baseline expression of endogenous VEGF protein following 36–40h of treatment, as assayed by ELISA. PCR analysis of VEGF transcripts demonstrated rapid induction of endogenous VEGF mRNA (30 min-2h). Following transfection, cell lysates, when normalized for Renilla luciferase expression, demonstrated a roughly two-fold increase in Firefly luciferase expression through the promoter elements in pGL3B.hV-2336. In contrast, rhRlx did not drive transcription of the Firefly luciferase reporter gene through the promoter sequence in pGL3B.hV-564, demonstrating the critical nature of sequences between −564 to −2336.

Conclusions: rhRlx caused transcriptional activation of the VEGF promoter following transfection of reporter constructs into NHE cells. These studies suggest that rapid induction of the VEGF gene may represent a fundamental regulatory pathway for relaxin. Consistent with this finding is the induction of menometrorrhagia in women following relaxin treatment. Furthermore, preliminary evidence from studies in the marmoset monkey demonstrates rhRlx-induced endometrial thickening. (This work was supported by Connetics Corporation.)

P-412

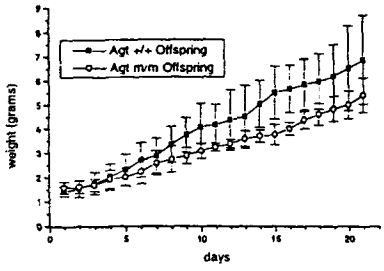
Reduced Fertility and Perinatal Survival Due to Angiotensinogen Deficiency. [1]C. B. Tempfer, [1]R. Moreno, [2]W. E. O'Brien, [1,2]A. R. Gregg. [1]Department of Obstetrics and Gynecology and [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

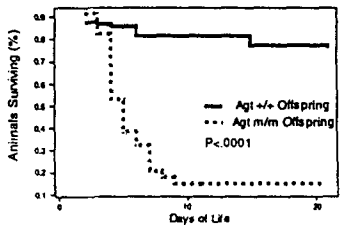
Objectives: To evaluate the impact of Angiotensinogen (*Agt*) deficiency on maternal reproduction and neonatal outcomes.

Design: Observational study on reproductive outcomes in a mouse model with targeted mutagenesis of the *Agt* gene.

Materials and Methods: Mice, genetically engineered to have 0 ($Agt^{m/m}$), 1 ($Agt^{m/+}$), and 2 ($Agt^{+/+}$) copies of the *Agt* gene on a C57BL6 background were studied. Three breeding schemes were evaluated: 1) $Agt^{m/m} \times Agt^{m/m}$, 2) $Agt^{m/+} \times Agt^{m/+}$, and 3) $Agt^{+/+} \times Agt^{+/+}$. Each scheme consisted of sexually mature mice. There were 4 breeding pairs per group. Cages were surveyed for 4 months. Breeding pairs were time-mated. Female mice and litters were weighed daily. Weaning and genotyping were performed on day of life 21. Maternal and paternal reproductive organs were harvested and histologically examined.

Results: Time to first litter, pregnancy duration, and maternal weight gain were not different for $Agt^{+/+}$ and $Agt^{m/m}$ matings. The median number of litters was lower in $Agt^{m/m}$ compared to $Agt^{+/+}$ matings (2 and 4, respectively, $P=0.002$). The ratios of postcoital plugging to subsequent litter were 4.0 and 1.2 for $Agt^{m/m}$ and $Agt^{+/+}$ matings, respectively ($P=.03$). There was a reduced number of pups born per litter for $Agt^{m/m}$ compared to $Agt^{+/+}$ matings (4 and 7, respectively, $P=.003$). Of interest, among $Agt^{m/+} \times Agt^{m/+}$ matings, the proportions of $Agt^{+/+}$, $Agt^{m/+}$, and $Agt^{m/m}$ offspring differed significantly from the expected 1:2:1 mendelian inheritance pattern ($P=0.002$). Pup weights throughout days of life 1–21 (Figure 1) and overall survival (Figure 2) were reduced for $Agt^{m/m}$ vs. $Agt^{+/+}$ offspring. Histology of testicles in males and uteri, fallopian tubes, and ovaries in females was not different for either group.





Conclusions: Maternal *Agt* deficiency results in decreased fertility and reduced fecundity. Our data demonstrate an *in utero* lethal effect due to *Agt* deficiency. Furthermore, neonatal outcome measures suggest absence of *Agt* results in developmental delay and increased mortality.

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***NOS3* Deficiency Negatively Impacts Responsiveness to Ovarian Hyperstimulation, But Not Reproductive Outcome.** [1]C. B. Tempfer, [2]W. E.

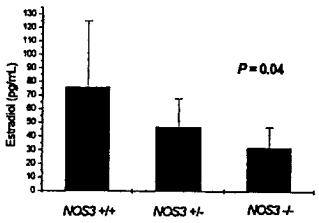
O'Brien, [1]R. Moreno, [1]Z. Liu, [1,2]A. R. Gregg. [1]Department of Obstetrics and Gynecology and [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

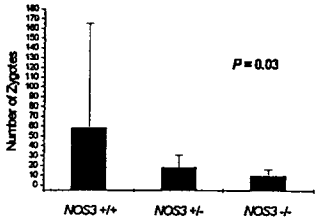
Objectives: To elucidate the influence of Endothelial Nitric Oxide Synthase Gene (*NOS3*) deficiency on gonadotropin induced ovulatory hyperstimulation and reproductive outcome.

Design: Hyperstimulation protocol and observation of breeding outcomes in a mouse model with targeted mutagenesis of *NOS3*.

Materials and Methods: F1 generation *NOS3* heterozygote mice were backcrossed over 8 generations to obtain a pure C57BL6 genetic background. Prepubescent females (days 21–28 of life), wild type ($NOS3^{+/+}$, n=11), heterozygous ($NOS3^{m/+}$, n=17), and homozygous deficient ($NOS3^{m/m}$, n=4) for *NOS3* were studied. Mice underwent a superovulation protocol, employing intraperitoneal injection of pregnant mare serum (PMS, 7.5 Units) and human chorionic gonadotropin (HCG, 7.5 Units). After mating with a C57BL6 male, mice were sacrificed, egg cells were recovered from the fallopian tubes and counted, blinded to genotypes. Serum samples, obtained at the time of sacrifice, were evaluated for estradiol (E2). For evaluating reproductive outcomes, 2 breeding schemes were established: $NOS3^{+/+} \times NOS3^{+/+}$ and $NOS3^{m/+} \times NOS3^{m/+}$. Each scheme consisted of 5 cages containing one male and two females. Cages were surveyed daily for 7 months and twice weekly for an additional 11 months.

Results: E2 serum levels after hyperstimulation were significantly higher in $NOS3^{+/+}$ compared to $NOS3^{m/+}$ and $NOS3^{m/m}$ mice, respectively (Figure 1). The median number of eggs harvested was significantly higher for $NOS3^{+/+}$ compared to $NOS3^{m/+}$ and $NOS3^{m/m}$ mice (Figure 2). $NOS3^{+/+} \times NOS3^{+/+}$ and $NOS3^{m/+} \times NOS3^{m/+}$ breedings showed no significant differences with respect to age at first litter (84 vs. 75 days), number of litters per week in the cage (0.2 vs. 0.15), interpregnancy intervals (17.4 vs. 18.0 days), and age at reproductive senescence (327 vs. 388 days), respectively.





Conclusions: Our data suggest *NOS3* deficiency is associated with reduced responsiveness to ovarian hyperstimulation. Our observation of breeding outcomes suggests that *NOS3* deficiency has no effect on the hypothalamo-pituitary-ovarian axis.

P-414

Haploidization of Somatic and Germ Cell Nuclei. T. Takeuchi, M. C. Tsai, K. P. Xu, Z. Rosenwaks, G. D. Palermo. The Center for Reproductive Medicine and Infertility, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY.

Objectives: In order to assess and compare the haploidization ability, enucleated immature oocytes were transplanted with nuclei from somatic and germ cells.

Design: We used enucleated immature mouse oocytes transplanted with germinal vesicles (GV) or granulosa cell nuclei, to evaluate cell survival, grafting, nucleo-cytoplasmic reconstitution, and the ability to undergo haploidization. To assess chromatin distribution between ooplasm and polar bodies, oocytes were exposed to a DNA stain.

Materials and Methods: B6D2F1 mice were superovulated with PMSG and hCG, and cumulus cells were isolated from the oocyte-cumulus complexes. Cumulus cells were plated in three sequential passages and cultured for up to 30 days in a long term culture medium supplemented with 10% fetal bovine serum. Mouse GV oocytes were retrieved by puncturing follicles of unstimulated ovaries of the same mouse strain. GVs were removed by a single enucleation procedure to produce GV karyoplasts and ooplasts simultaneously. Intact cumulus cells or GV karyoplasts were then inserted subzonally into the previously enucleated oocytes. After electrofusion, grafted oocytes were incubated for up to 14–16 hours to allow reconstitution and extrusion of the first polar body, then were anchored between microslide and coverslip and fixed with a solution of methanol and acetic acid (3:1; v/v), followed by aceto-orcein staining.

Results: All of 73 intact GV oocytes were successfully enucleated and transplanted with either GV karyoplasts or cumulus cells. The different steps of nuclear transplantation are shown in the following table:

No. of oocytes	GV karyoplasts (%)	Cumulus cells (%)
Enucleated	38	35
Grafted	38	35
Reconstituted	32 (84.2)	26 (74.3)
With First polar body extruded	31 (96.9)*	15 (57.7)*

* χ^2, 2 × 2, 1 *df*, Effect of nuclear origin on maturation rate, $P<0.001$

Conclusions: These findings show that immature cytoplasts can support haploidization of somatic nuclei. Transplanted cumulus cells reconstitute at the same rate as transplanted GV nuclei but had a lower rate of haploidization. Although perhaps less efficient, somatic cell haploidization thus may represent an alternative source of female gametes.

P-415

Effect of Ultraviolet (UV) Light on Human Spermatozoa and Development of Human Conceptuses Derived from These Spermatozoa after ICSI. N. Zaninovic, D. Liotta, C. A. Cook, L. L. Veeck, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility, Cornell University Medical College, New York, NY.

Objectives: The role of chromosomal defects in human spermatozoa and their developmental capabilities are unknown. UV light primarily affects cell DNA by forming specific dimers, and their effect primarily depends on the UV wavelength (UV-A, UV-B, UV-C) and exposure time. The effect of UV light on human sperm and their DNA is not well understood. We studied the effect of UV light on human spermatozoa and their capability to undergo decondensation, fertilize (through ICSI), and promote development of human oocytes.

Design: Experiment I: Motile sperm samples with normal WHO characteristics were subjected to short (UV-C) and/or long (UV-A and UV-B) waves. The sperm samples were assessed immediately and 24 h after treatment. Experiment II: ICSI using UV treated (short and/or long), and control spermatozoa were performed on *in vitro*-matured oocytes cultured for at least 24 h after retrieval. Fertilization results and preembryo (PE) development were recorded and DNA studies were performed on developing PE.

Materials and Methods: Sperm samples (n=6) were treated with short UV (9.3 $\mu J/m^2/min$) and/or long UV (7.1$\mu J/m^2/min$) using a UV lamp for 1, 5, 10, and 15 min and 1 hour at room temperature. The percentages of sperm motility, rapid progressive motility, and plasma membrane integrity (eosin Y) were assessed. ICSI was performed using spermatozoa that remained motile after treatment for 5 minutes with either short or long UV light. DNA studies were performed using a DNA counterstain (DAPI).

Results: *Experiment I:* Sperm exposed to short UV light showed a significant drop in motility after 5 min of treatment and were completely immotile after 10 minutes. They also demonstrated a higher percentage of damaged membranes as compared to long UV exposure. On the other hand, long UV treatment required a lengthier exposure (>10 min - one hour) to significantly affect motility. In brief, the effect of short UV light was more detrimental on sperm motility and membrane integrity than that of long UV light. The effect of treatment varied between sperm samples.

Experiment II: A significantly lower 2PN rate was observed using short UV-treated sperm versus control sperm (61.5% vs. 81.5%,$p<0.05$). Also, significant impairment in cleavage was noted after injection of oocytes with short UV-treated sperm; 75% of conceptuses did not cleave and proceeded to fragment in culture. Developing PEs showed dispersed DNA in the cell cytoplasm without visible nuclear membranes. In long UV ICSI experiments, no differences were observed in fertilization or PE development. Interestingly, we obtained two healthy-looking blastcysts from long UV-treated sperm.

Conclusions: 1) Short UV light had more detrimental effects on sperm motility and membrane integrity than long UV light. 2) UV-treated sperm are capable of decondensing and activating human oocytes through ICSI. 3) PE development is highly impaired using short UV-treated sperm, while long UV-treated sperm possessed the capability to promote PE development.

P-416

Protein Kinase C (PKC) Inhibition Arrests Leiomyoma Cell Growth: A Role for Vitamin E Therapy? S. L. Young, P. Goluszko, J. A. Copland. Department of Obstetrics and Gynecology, The University of Texas Medical Branch at Galveston, Galveston, TX.

Objective: Uterine leiomyomata are an abnormal proliferation of smooth muscle responsible for more than 25% of hysterectomies performed in the United States. Despite this enormous impact on women's health, the factors causing formation and growth of these benign tumors remain largely enigmatic and pharmacologic treatment remains limited. Two lines of evidence led us to investigate the role of PKC in leiomyoma growth: (1) PKC signaling may be activated by paracrine factors thought to be involved in leiomyoma growth (e.g. basic fibroblast growth factor and insulin-like growth factor I). (2) PKC activation has been shown to cause abnormal proliferation of vascular smooth muscle. We therefore tested the effects of PKC inhibitors on leiomyoma cell growth in vitro.

Materials and Methods: Leiomyoma tissue and adjacent myometrium were obtained from surgical specimens under a protocol approved by the hospital institutional review board. Cells were isolated from tissue by collagenase and DNAse I digestion, grown in 10% fetal calf serum, and characterized by immunofluoresence microscopy using antibodies for α-smooth muscle actin, connexin-43, and oxytocin receptor. During each experiment, the cells were grown in 12-well plates in 5% fetal calf serum in the presence or absence of candidate growth inhibitors with a starting concentration of approximately 25,000 cells/well. Multiple wells were treated in parallel and each analyzed separately. Cell growth was estimated by measuring DNA content in each well using the fluorescent reagent, Hoechst 33258.

Results: The PKC inhibitors, α-tocopherol succinate (vitamin E) and the GF109203X both caused a dose-dependent inhibition of growth of leiomyoma cells. Treatment with succinate was no different than serum alone over the range of concentrations studied. Complete growth inhibition occurred at about 40–50 μM (vitamin E) and 8–10 μM (GF109203X). At increased concentrations, both agents caused cell death. Since vitamin E also has potent antioxidant activity, the effects of another antioxidant, vitamin C, was investigated. Vitamin C had little effects at concentrations where pH was not affected ($\leq 50\mu$M). Another vitamin E analogue, troglitazone showed similar effects at similar concentrations.

Conclusion: (1) In vitro treatment with the protein kinase C inhibitors, vitamin E or GF109203X, results in a dose-dependent inhibition of leiomyoma cell growth. (2) Effective levels of vitamin E are clinically achievable with low toxicity, thereby suggesting a potential new therapy for leiomyomata.

P-417

Calcitonin Promotes Invasiveness of Human Trophoblast Cells Through Intracellular Calcium Signaling. R. Dinsay, J. Wang, B. A. Kilburn, [1]R. Romero, D. R. Armant. C.S. Mott Center for Human Growth and Development, Departments of Obstetrics & Gynecology and Anatomy & Cell Biology, Wayne State University School of Medicine, Detroit, MI. and [1]Perinatology Research Branch, NICHD, NIH, Bethesda, MD.

Objectives: Blastocyst implantation in the receptive uterus depends on trophoblast differentiation to an invasive phenotype. Calcitonin is secreted in the uterus during the peri-implantation period under control of steroid hormones. Calcitonin accelerates mouse preimplantation development through its ability to elevate intracellular calcium levels. Therefore, we hypothesized that calcitonin may also induce calcium signaling and promote invasive activity of human trophoblast cells.

Design: To examine the interaction of calcitonin with human trophoblast cells, we have used a cell line with an extended life span, HTR-8/Svneo (HTR), derived from first trimester human cytotrophoblast cells.

Materials and Methods: Intracellular calcium levels were estimated in HTR cells using the fluorescent probe, fluo-3-AM, and an image analysis system. Invasion was assayed using membrane inserts by culturing HTR cells on Matrigel in DMEM/F12 medium containing 0 to 10 nM calcitonin for 72 h. The invasive activity was determined by counting the number of HTR cells that penetrated through the Matrigel and into the lower chamber.

Results: Intracellular calcium levels were elevated in most HTR cells within seconds by exposure to 10 nM calcitonin, rising from approximately 160 nM to more than 800 nM. Intracellular calcium increased variably with 2 and 5 nM calcitonin. Exposure of HTR cells to calcitonin significantly increased ($p<0.05$) the number of cells that penetrated the Matrigel from 1.3 ± 1.6 (control) to 3.2 ± 2.4 (2 nM), 5.5 ± 2.9 (5 nM) or 6.4 ± 2.7 (10 nM) cells/field.

Conclusion: HTR cells respond appropriately to calcitonin, as evidenced by its ability to activate calcium signaling pathways. Calcitonin significantly stimulates the invasive activity of HTR cells, suggesting that HTR cells may express calcitonin receptors and that calcitonin may play a central role in the human implantation process.

Supported by the National Institute of Health grant HD 36764.

P-418

The Effect of Hypoxia and TGF-β1 on the Expression of Tissue Inhibitors of Metalloproteinases (TIMP-1) in Human Peritoneal Mesothelial Cells. G. Saed, W. Zhang, P. Falk*, L. Holmdahl*, M. P. Diamond. Departments of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, and *Department of Surgery, University of Gothenburg, Gothenburg, Sweden.

Objective: Studies of tissue inhibitors of metalloproteinases (TIMPs) suggest that one of their main functions is to inhibit metalloproteinases (MMP) activity and thus help preserving extracellular matrix (ECM) integrity. Since chronic low-grade hypoxia has been implicated in the pathogenesis of fibrosis and postoperative adhesion formation, we hypothesized that hypoxia may stimulate ECM accumulation in the development of postoperative adhesions. A potential mediator for these effects is transforming growth factor-beta 1 (TGF-β1), a major pro-fibrogenic factor produced by peritoneal tissues.

Design: To test this hypothesis, we utilized the multiplex RT/PCR technique to determine the effect of hypoxia and/or TGF-β1 treatments on the mRNA levels of TIMP-1 in human peritoneal mesothelial cells in culture (HMC).

Materials and Methods: Primary cultures of HMC were treated with hypoxia by incubating the cultured HMC in a 2% oxygen chamber for 24 hours. TGF-β1 treatment was performed on cultured HMC by the addition of 1ng/ml of TGF-β1 for 24 hours. A combination of hypoxia and TGF-β1 treatment was performed by incubating the cultured HMC in a 2% oxygen chamber in the presence of 1ng/ml TGF-β1 for 24 hours. Total RNA was extracted from HMC and converted to cDNA by reverse transcriptase. Multiplex RT/PCR simultaneously amplifying β-actin with TIMP-1 mRNAs in the same tube was developed in our laboratory to quantitate TIMP-1 mRNA level in response to these treatments. PCR products were analyzed by agarose gel electrophoresis and density of each ethidium bromide stained band was measured by a scanning densitometer.

Results: Multiplex RT/PCR showed that hypoxia treatment resulted in a

2-fold increase in TIMP-1 mRNA level, while TGF-β1 treatment resulted in a 7-fold decrease in TIMP-1 mRNA level. A combination treatment of hypoxia and TGF-β1 resulted in a 1.75-fold decrease in TIMP-1 mRNA level.

Conclusion: Exogenous TGF-β1 (1 ng/ml) did not mimic hypoxia, as it decrease the mRNA level for TIMP-1. Our results suggest that hypoxia may be an important pro-fibrogenic stimulus independent of TGF-β1. However, this does not exclude the fact that stimulation of endogenous TGF-β1 production by hypoxia may be the mechanism of action of hypoxia. (Supported in part by Genzyme Corporation).

P-419

Expression of Activin-A and Matrix Metalloproteinases (MMP) in Human Cytotrophoblasts. [1,2]F. D. Yelian, [1]W. W. Zhang, [3]L. Z. Zhuang, [1]K. A. Wei, [1]G. M. Saed, [4]D. B. Seufer, [1]M. P. Diamond. [1]Dept of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, [2]Dept of Obstetrics and Gynecology, Michigan State University College of Human Medicine, Saginaw, MI, [3]Reproductive Biology, Chinese Academy of Science, Beijing, China, and [4]Dept of Obstetrics and Gynecology, UMDNJ, New Brunkswick, NJ.

Objectives: The invasion of extravillous trophoblast cells into the maternal endometrium is one of the key events in human placentation. In contrast to tumor cell invasion, cytotrophoblast invasion is highly regulated both spatially and temporally. Successful trophoblast invasion depends on its ability to synthesize and secrete various MMPs, especially MMP-2, and MMP-9. A recent study has shown that the first trimester cytotrophoblasts invasion is induced by activin-A. To determine the relationship between activin-A and MMPs expression in trophoblast differentiation, we analyzed the activin-A levels in various stages of CTB and studied their relationship with MMP activities.

Design: Cytotrophoblast cells were isolated from various stages of human placenta. These cells were cultured in a serum-free medium, and protein levels of activin-A, MMP2 and MMP9 were analyzed.

Materials and Methods: Full IRB approval was obtained for the collection and culture of human placental tissues. Various stages (6, 7, 8, 9, 13 weeks) of human cytotrophoblast cells were cultured in a serum-free medium for 48 h. The culture supernatants were collected for measuring activin A, MMP-2, and MMP-9. Activin-A ELISA kit was purchased from Serotec Inc., and activin-A levels were measured according to the manufacturer's recommendations. An established zymogram was used to measure the MMP levels. Briefly, samples were applied on 10% polyacrylamide gels containing 1 mg/ml gelatin. After SDS-PAGE, gels were washed in Triton X-100 and incubated in substrate buffer. The gels were stained, and the clear bands of proteolytic zones were then analyzed by the densitometry.

Results: Our study demonstrated the presence of activin-A in all culture supernatants. The levels of activin-A showed a gestational age-related change. The concentrations of activin-A were 0.10, 1.23, 0.64, 4.96, and 5.14 ng/ml for gestation 6, 7, 8, 9, 13 weeks, respectively. In addition, both MMP-2 and MMP-9 activities were also detected from these samples. The activities of MMP-9 were increased with gestational age, while the activities of MMP-2 were decreased as pregnancy progresses. There was an approximately two- to three-fold change in each gestational stage. However, the combined MMP-2 and MMP-9 activities for each gestational age remained relatively constant. These results demonstrated a positive correlation between activin-A level and MMP-9 activity.

Conclusion: 1) We have demonstrated that first trimester human cytotrophoblast cells secrete activin-A and the levels increase with gestational age. 2) There is an inverse relationship between MMP-2 and MMP-9 activities in trophoblast culture. 3) We have also found a positive correlation between activin-A level and MMP-9 activity in early cytotrophoblast cells. These results suggest that activin-A may play a regulatory role in MMP expression during embryo implantation.

P-420

The Efficacy of Blastocyst Culture and Transfer in a High Volume IVF Center. J. D. Wininger, A. E. Jones, G. Wright, S. Smith, W. W. Brockman, J. Johnson, J. B. Massey. Reproductive Biology Associates, Atlanta, GA.

Objective: To assess the efficiency of blastocyst culture and transfer using commercially available defined medium.

Design: Retrospective analysis of all embryos cultured until Day 6, and of non-donor patients receiving embryo transfer. The main outcome measures were predictive value of day 3 embryo morphology, blastocyst development rate, pregnancy and implantation rate.

Materials and Methods: Oocytes and embryos were cultured in P1 (Irvine Scientific) until seventy-two hours post egg retrieval. Embryos designated for blastocyst culture were transferred into Blast Medium (Irvine Sci.) and cultured another 48 to 72 hours depending development. On the morning of day 3 all embryos were graded based on cell stage and fragmentation and grouped according to cell number. Patients with three or more eight-cell embryos on the morning of Day 3 were given the opportunity to have a blastocyst transfer. Those patients who did not meet the criteria or who declined blastocyst transfer received a day three transfer. Three patient populations were evaluated: 1. Patients meeting blastocyst transfer criteria and having a day 5 or 6 transfer. 2. Patients meeting blastocyst transfer criteria and having a day 3 transfer. 3. Patients not meeting the blastocyst transfer criteria and having a day 3 transfer.

Results:

	Pregnancy Rate (+βhcg)	Imp. Rate
Group 1	63% (38/60)	34% (15/45)
Group 2	62% (28/45)	28% (38/132)
Group 3	33% (42/127)	13% (53/412)

Conclusions: A total of 37 patients requested and met the criteria for a day 5 or 6 transfer, and only 2 couples failed to develop blastocysts. This data demonstrates that production of at least three 8 cell embryos on day 3 is a good predictor for both continued embryo development to the blastocyst stage and pregnancy. Patients who met the day 3 blastocyst criteria had similar pregnancy and implantation rates regardless of whether they had a day 3 or day 5 transfer. However, patients who did not meet the day 3 criteria and received a day 3 transfer had significantly lower blastocyst formation and pregnancy rates. This study also demonstrates that blastocyst culture and transfer can be easily implemented in a high volume IVF center and is a useful method in preventing high-order multiples.

P-421

HLA-G Gene Expression in Chorionic Villi Tissues from Early Normal Pregnancy and Missed Abortion. [1]H. S. Lee, [2,3]B. C. Choi, [2,3]I. O. Song, [2,3]S. J. Hong, [2,3]J. H. Yang, [1]J. H. Jun, [2,3]J. Y. Jun, [4]J. P. Lee, [2,3]I. S. Kang. Recurrent Miscarriage Clinic, [1]Infertility Research Laboratory, [2]Dept of OB/GYN, Samsung Cheil Hospital and Women's Healthcare Center, [3]College of Medicine, Sungkyunkwan University, [4]Ilsan Cheil Women's Clinic, Seoul, Korea.

Objectives: HLA-G is a nonclassical class I major histocompatibility complex molecule with a restricted pattern of expression, that includes the placental extravillous cytotrophoblast cells in direct contact with maternal tissues. Preliminary evidence suggest that expression of HLA-G protects cells against natural killer cell lysis and that it does not stimulate an allogeneic response by peripheral blood T cells. These features suggest that expression of HLA-G could be a crucial factor for fetal survival in the face of a potentially hostile maternal immune system. The goal of our study is to investigate whether HLA-G gene expression is associated with early pregnancy outcome.

Design: The expression level of HLA-G gene in placental extravillous cytotrophoblasts were measured by reverse transcription-polymerase chain reaction analysis.

Materials and Methods: Placental tissue was obtained by currettage, after informed consent from women undergoing surgical termination of pregnancy (n=8) and from women with a history of recurrent pregnancy loss undergoing a further miscarriage at first trimester (n=40). RNA was extracted from tissues with the TRIsol Reagent (GIBCO BRL). Extraction was performed as described by the manufacture. Total RNA extracted was used for reverse transcription. The first-strand cDNA reaction was performed using reverse transcriptase, M-MuLV (BM). The HLA-G-specific primer pair, which amplify a part of exon 2 and the first half of exon 3, was used for PCR amplification. All amplified DNA fragments were analysed by electrophoresis on 2% agarose gels and stained with ethidium bromide.

Results: RT-PCR analysis of RNA from human placental tissues, with HLA-G-specific primer pair, resulted in the expected 299-bp HLA-G product. Transcripts of GAPDH gene as a positive control was detected in all tissues tested. HLA-G gene was detected in 21/40 (52%) chorionic villi samples from missed abortion and 4/8 (50%) in surgical abortion ($p > 0.05$, Fishers exact test).

Conclusions: In our study, HLA-G mRNA expressed in about 50% of chorionic cytotrophoblast, about the same percentage from complicated pregnancy and normal first trimester pregnancy. As a result the expression of HLA-G mRNA in human placental tissues is not associated with early pregnancy outcome. For further study, comparison of NK cell expression in decidua and HLA-G mRNA in human placental tissues are might be helpful to investigate the influence of HLA-G mRNA on the development of early pregnancy complication.

P-422

Cytoskeletal Organization and Chromatin Configuration in Abnormally Fertilized Human Oocytes. V. Y. Rawe, S. De Vincenttis, S. P. Brugo-Olmedo, F. N. Nodar, A. D. Vitullo, A. A. Acosta. Centro de Estudios en Ginecología y Reproducción-CEGyR-Buenos Aires, Argentina.

Objectives: Despite the continuous improvement of assisted reproduction techniques (ART), abnormal fertilization is a common phenomenon in humans. Extrusion of the second polar body (2PB) and the simultaneous formation of a single pronucleus (1PN) occurs in 2–5% of human oocytes after conventional IVF and is increased after ICSI, ranging between 5.3 to 26.8%. We analyzed the distribution of α and β tubulin, acetylated tubulin and chromatin configuration in oocytes showing abnormal fertilization after IVF and ICSI in order to understand the reasons for this in each particular case.

Design: Prospective study.

Materials and Methods: A total of 86 abnormally fertilized oocytes (1, 3 or ≥4PN) were examined 20–40 hr after IVF (n=52) or ICSI (n=34). Oocytes were immunostained using a modified protocol already described [1]. After fixing and permeabilization, anti α-acetylated tubulin antibody was used to identify the sperm tail. Oocytes were then incubated with fluorescein-conjugated goat antimouse IgG. Meiotic spindle was analyzed with anti β-tubulin-Cy3 monoclonal antibodies. Chromatin was identified by counterstaining with Hoescht 33258. Processed material was examined using an epifluorescent microscope.

Results: The distribution of the different types of abnormal zygotes were as follows:

Nuclear content	IVF	ICSI
• 1PN (large), 1PB	4 (spz −)	3 (spz −)
• 3PN (two large/one small or one large/two small), 1PB	12 (spz +)	8 (spz +)
• 4PN, 1PB (Digynic/ Diandric)	2 (spz −)	–
• ≥ 4PN, 1PB (Karyomeres)	2 (spz ++)	–
• 1PN, 2PB	7 (6 spz +, 1 spz −)	17 (4 spz +, 13 spz −)
• 3PN, 2PB	25 (18 spz ++, 7 spz +)	6* (spz ++)

Spz −: no sperm found, Spz +: one sperm found, Spz ++: two sperms found. * Three pronucleated embryos from ICSI 24 hr after IVF.

Conclusions: The etiology of zygotes' abnormal fertilization is completely dependent on the technique used (IVF or ICSI). According to the distribution of the frequencies observed, the most common cause of abnormal fertilization was dispermic fertilization (IVF) and parthenogenetic activation (ICSI). The high incidence of retention of the 2PB seems to indicate that partial or insufficient oocyte activation are the basis of abnormal fertilization.

[1] Messinger S. M. and Albertini D. I. *J. Cell. Sci.*, 100:289, 1991.

P-423

No Impact of Cryopreservation After Laser Assisted Polar Body Biopsy on the Hatched Blastocyst Rate in Mice. M. Ludwig, B. Schöpper, S. Al-Hasani, K. Diedrich. Department of Obstetrics and Gynecology, Medical University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany.

Objective: According to the German Embryo Protection Law (Embryonenschutzgesetz, ESchG), embryo biopsy is not allowed. Furthermore, it is not allowed to culture more than three fertilized oocytes. Therefore, we tried to choose another way to perform preimplantation genetic diagnosis or preconception genetic diagnosis (PGD). The idea was to biopsy all oocytes at the pronuclear stage (PN), remove the polar bodies (PB) for genetic diagnosis, cryopreserve the PN oocytes, and thaw electively those which are suitable for transfer after confirmation of the genetic diagnosis. To prove, that cryopreservation has no impact on the further developmental potential, these studies were first done in mice.

Design: Hatched blastocyst rate (HBR) was calculated in mice PN after biopsy combined with consecutive cryopreservation at the PN stage and compared to different control groups.

Materials and Methods: 377 pronuclear stages of 13 mouse F1 hybrids (C57Bl/j X CBA) were harvested and divided into several experimental groups after ovarian hyperstimulation using PMSG (pregnant mare serum gonadotropins) and ovulation induction using hCG (human chorionig gonadotropin). All oocytes were fertilized in vivo. Biopsy was done after opening of the zona pellucida using a laser system (Fertilase®). HBR in six different groups were compared: (I) control; (II) sham biopsy, i.e. opening of the zona without removing of polar bodies; (III) cryo, i.e. cryopreservation of PN without any manipulation procedures; (IV) sham-cryo, i.e. sham biopsy and subsequent cryopreservation; (V) biopsy-cryo, i.e. cryopreservation after biopsy of PB; (VI) biopsy, i.e. biopsy of PB without cryopreservation. Cryopreservation was done using the open freezing system. Statistics were calculated using unpaired, two-tailed t-test.

Results: The HBR in the groups without cryopreservation were 63.8% (control), 68.3% (sham biopsy), and 69.0% (biopsy). In the groups with cryopreservation the HBR was 54.5% (cryo), 49.1% (sham-cryo), and 58.9% (biopsy-cryo) after a rapid thawing procedure by plunging the frozen straws into a water bath at 37°C. No statistical significant difference was seen between the groups. However, the results after cryopreservation were somewhat lower compared to those groups without cryopreservation.

Conclusion: PB biopsy can be combined with subsequent cryopreservation without any impact on the HBR after thawing in the mouse. This provides a possible approach for PGD in Germany which is in accordance with the embryo protection law.

P-424

Biological Role of Coelomic Fluid in the Embryonic Development During the Human Early Pregnancy. [1]H. K. Byun, [2]C. S. Hwang, [1]J. A. Lee, [1]J. W. Kim, [3]H. J. Lee, [4]B. C. Choi. [1]Infertility Research Lab., [2]Endocrinology Research Lab., [4]Recurrent Miscarriage Clinic, Samsung Cheil Hospital & Women's Healthcare Center, [3]Department of Physiology, Eulji Medical College, Seoul, Korea.

Objective: The 1st trimester human gestation sac consists of amniotic cavity surrounding the fetus and extraembryonic coelom. Coelomic fluid encloses to contact amniotic cavity but the amniotic membrane divides into the two cavities. Biochemical analysis of coelomic fluid has been studied to identify the physiological condition at the early pregnancy; its biological role is still unknown. Coelomic fluid is thought to be a reservoir of nutrients and metals essential for the embryonic development. The present study was carried out to investigate the biological role of coelomic fluid in the embryonic development. In addition, coelomic fluid obtained from patients with anembryonic and missed abortion was compared with that of selective abortion to determine the causes of anembryonic and missed abortion.

Materials and Methods: Coelomic fluids were aspirated from the patients undergoing anembryonic (n=4), missed (n=5), and selective (n=3) abortion at 7–10 weeks of gestation in Recurrent Miscarriage Clinic, Samsung Cheil Hospital. Fluids were centrifuged and stored at −20°C before use. Mouse 1-cell embryos (CBA/C57BL F1 hybrid) were cultured in the presence of each fluid (10, 50, and 100%) in order to test the cytotoxicity or nutrophic effect of the fluids on the embryonic development. Proliferation assay of Jeg3 cell with coelomic fluid (0, 25,

50, 75, 90, and 100%) was also performed using colorimetric immunoassay based on BrdU incorporation into DNA in order to evaluate the capacity of the fluid whether it can regulate the proliferation of trophoblast. Chi-square test and Fisher's exact test was used for statistical analysis.

Results: Of the result of bioassay of mouse embryos with coelomic fluid, there was no significant difference in mean developmental rate between anembryonic, missed, and selective abortion (81.0, 84.9 and 86.5%, respectively). In the case of coelomic fluids from anembryonic and missed abortion, developmental rate was decreased in a concentration-dependent manner ($p<0.005$). However, developmental rate in the fluid from selective abortion was not related to the concentration of the fluid, suggesting that the coelomic fluid from selective abortion (normal pregnancy) has no detrimental effect on the development of mouse preimplantation embryo and it plays a role of reservoir of nutrients. In addition, the proliferation of Jeg3 cell line was significantly suppressed in the coelomic fluid from selective abortion, compared with anembryonic and missed abortion ($p<0.005$), which means that the coelomic fluid from normal pregnancy can regulate the proliferation of trophoblast in contrast with those of anembryonic and missed abortion. The suppression of proliferation in the three groups also showed concentration-dependency.

Conclusions: We conclude that coelomic fluid obtained from the 1st trimester human gestation plays a role of reservoir of nutrients for the embryonic development. On the contrary to normal pregnancy, the coelomic fluid from anembryonic and missed abortion decreases the developmental rate of mouse embryo, which is caused by the imbalance of cytokine levels and embryonic miscarriage in the uterus. Furthermore, coelomic fluid from normal pregnancy regulates the proliferation of trophoblast, compared with anembryonic and missed abortion, which is required to maintain a normal fetal development. Therefore, coelomic fluid has an important biological role in the embryonic development in human early pregnancy.

P-425

Development of Positive Morphological Predictors for Human Blastocyst Implantation. L. Scott, R. Alvero, J. Broussard, J. McKeeby, B. Miller. RSC of the Combined Federal Program of WRAMC, NNMC, USUHS, Washington, DC, 20307.

Objectives: Implantation and pregnancy rates in human in vitro fertilization utilizing day 5 blastocysts is higher than obtained with day 3 embryo transfers (ET). However, there is little information on what constitutes a "good" day 5 blastocyst. The objective of this study was to establish a grading system for blastocysts which could be correlated with implantation.

Design: Archived photographs of D5 blastocysts used for ET where outcome was known were used to establish a positive correlation between morphology and implantation. This morphological grading system was then applied prospectively in an attempt to increase implantation and predict outcome.

Materials and Methods: Oocyte retrieval (OR) was performed at 36 hours post-hCG, inseminations 4 hours later and all subsequent scoring of embryos at 24 hour intervals after OR. Photographs of Day 5 embryos from 39 cycles at 120 h post OR (156 h post-hCG; 116 h post-insemination) and prior to transfer, 1–5 h later were analyzed. A scoring system was developed that had a 90% correlation with implantation: Baby-Grade embryos (BGE): intact cells; no granular cells; continuous well defined outer perimeter of cells with good cell-cell contact and no long thin cells. Cells in the next layers had clear membranes, good cell-cell contact, clear cytoplasm and a blastocoel was beginning to appear. Over the following 2 hours embryos expanded by at least 1/3 of their size, had a well defined innercell mass without giant cells or cells with spider like projections spreading out from the innercell mass, had at least 80 cells which could be counted by scanning through the embryo on the inverted microscope. By 5 h later many were well expanded blastocysts. Non-Baby-Grade (NBG) embryos presented with one or many of: too few cells; granular darkened areas; too few cells in the outer layer resulting a long thin cells expanding over a large portion of the blastocyst; inner cell mass disorganized or with cells of very uneven sizes; spidery cellular projections through the blastocoel cavity; many small fragments localizing to the outside of the embryo. Some embryos were only compacted morulae at ET and implantation for these was correlated with good cell-cell contact, rounding up of the outer cells, no granular dark areas and a central well forming. This scoring system was then applied prospectively to a further 57 cycles.

Results: The results for both the retrospective and prospective analyses are shown below:

	Retrospective	Prospective		
	<35	<35	>35	Total
Age	30.7 (2.8)	31.1 (3.1)	37.8 (1.4)	35.1 (3.2)
# of patients	39	35	22	57
Ongoing/delivered pregnancies (%)	29 (74)	21 (60)	14 (64)	35 (61)
Total Embryos ET (mean)	85 (2.1)	68 (1.9)	47 (2.6)	115 (2.0)
Total Implantation (%)	51 (60)	36 (53)	15 (32)	51 (44)
ET with BGE/# embryos ET	33/50	35/39	6/19	41/58
Pregnancy rate/implantation rate (%)	91/96[a]	80/90[a]	100/79[a]	83/86[a]

a= $P<0.01$ vs. total

Conclusions: It is possible to increase and predict the implantation of Day 5 blastocysts to the 95% confidence level using the new scoring system which could lead to the ability to perform single-blastocyst transfers in patients desiring singleton pregnancies.

P-426

Nitric Oxide as a Regulator in Pre-implantation Embryonic Development. H. W. Chen, W. S. Jiang, C. R. Tzeng*. Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Taipei Medical College, Taipei, Taiwan.

Objectives: Previous study demonstrated that nitric oxide synthase (NOS) expression in the endometrium was upregulated in the endometriosis and adenomyosis, this effect was suggested to correlate with the early embryo loss. In this study, the regulation of NO in the mouse preimplantation embryo development and apoptosis was examined.

Design: The development of two- and four-cell mouse embryos in medium containing N^G-nitro-L-arginine (L-NAME, NO synthase inhibitor) or sodium nitroprusside (SNP, NO donor) was observed by the rates of blastocyst formation and embryo hatching.

Materials and Methods: The degeneration of mouse embryo was examined by the TUNEL technique and annexin-V/propidium iodide staining. The expression of NOS protein products in mouse embryo was examined by immunofluorescence staining with specific antibody.

Results: The preliminary result indicated that iNOS and eNOS were expressed in the blastocyst. Table summarized the percentage of embryos that attained at blastocyst and hatching stages by day 3 and day 4 of incubation, in the presence of different concentrations of L-NAME or SNP. The data indicated that L-NAME inhibited the development of mouse embryos was concentration-dependent (10^{-7}-10^{-5} M). SNP also showed the inhibitory effect in the embryonic development and induced embryo fragmentation at the higher concentration (10^{-6}-10^{-5} M), but has no effect at lower concentration (10^{-7} M). The further study showed that following 10^{-5} M L-NAME pre-treatment for 24 h, the blastocyst formation rates were restored by washout or supplementation of SNP (10^{-7} M). The TUNEL assay illustrated that 10^{-5}-10^{-4} M SNP induced embryo apoptosis and further fragmentation.

Addition	Conc. (M)	Blastocyst (%) on day 3
Control	–	77 (73/95)
L-NAME	10^{-7}	41 (36/85)*
	10^{-6}	29 (23/82)*
	10^{-5}	4 (2/59)*
SNP	10^{-7}	69 (54/80)
	10^{-6}	30 (20/54)*
	10^{-5}	2 (1/35)*
L-NAME (10^{-5} M) + washout (at day 2)		71 (41/58)**
L-NAME (10^{-5} M) + SNP (10^{-7} M)		62 (59/98)**

Addition	Hatching (%) on day 4	Fragmentation (%) on day 4
Control	48 (45/95)	8 (6/95)
L-NAME	18 (16/85)*	12 (10/85)
	10 (8/82)*	35 (27/82)*
	0 (0/59)*	48 (29/59)*
SNP	51 (40/80)	0 (0/80)
	35 (19/54)*	56 (30/54)*
	2 (1/35)*	79 (32/35)*
L-NAME (10^{-5} M) + washout (at day 2)	57 (33/58)**	9 (5/58)**
L-NAME (10^{-5} M) + SNP (10^{-7} M)	27 (24/98)**	31 (27/98)**

Each data represented the mean ± s.e. mean. * $P<0.01$ when compared to the control group. ** $P<0.01$ compared to the 10^{-5} M L-NAME-treated group.

Conclusions: In this study, the results demonstrated that NO regulated mouse embryo development *in vitro*. These studies suggest that proper NO production is necessary for the embryonic growth, but excessive NO generation mediated by inflammatory factors in reproductive tissues may inhibit embryonic development and thus lead to infertility.

P-427

Intracellular pH Regulation in Human Preimplantation Embryos. [1,3]Karen P. Phillips, [4]Marie-Claude Léveillé, [2,4]Paul Claman, [1,2,3,4]Jay M. Baltz. [1]Loeb Research Institute, and [2]Departments of Obstetrics and Gynecology, (Division of Reproductive Medicine), and [3]Cellular and Molecular Medicine, University of Ottawa, and [4]Human IVF Laboratory, Ottawa Hospital, Ottawa, Ontario, Canada.

Objectives: To examine intracellular pH (pH_i) regulation in human immature eggs and preimplantation (PI) embryos in order to determine how pH_i is regulated and identify the transporters involved.

Design: Human immature eggs and PI embryos, excess to requirements for in vitro fertilization (IVF) protocols at Human IVF Clinic, Ottawa Hospital were obtained on Day 0-1 (eggs) or Day 2-4 post-egg retrieval (embryos) and assessed for the ability to recover from imposed intracellular alkalosis or acidosis. HCO_3^-/Cl^- exchanger, Na^+/H^+ antiporter and $Na^+,HCO_3^-/Cl^-$ exchanger activity were examined.

Materials and Methods: pH_i was measured by fluorescence imaging using an intracellularly loaded pH_i-sensitive fluorophore SNARF-1. pH_i measurements were recorded for each individual egg/embryo. pH_i transporters were examined using appropriate inhibitors (DIDS inhibits HCO_3^-/Cl^- exchange and $Na^+,HCO_3^-/Cl^-$ exchanger; amiloride/EIPA inhibits Na^+/H^+ antiporter) and manipulations of external ion composition to reveal which of these transporters mediate pH_i regulation in human eggs and embryos. HCO_3^-/Cl^- exchange was detected by measuring the rate of intracellular alkalinization (HCO_3^- influx) upon external Cl^- removal, as well as the rate of recovery of pH_i following alkaline load induced with NH_4Cl. Na^+/H^+ antiporter activity was assessed in cleaved embryos by measuring the rate of recovery of pH_i following induced acid load (NH_4Cl pulse method) in the presence and absence of external Na^+.

Results: Steady-state pH_i was slightly different between eggs and embryos ranging from pH_i 7.05 in eggs to pH_i 7.2 in 6–8 cell embryos GV, MI eggs and cleaved embryos (2 cell-8 cell) demonstrated DIDS-sensitive alkalinization upon external Cl^- removal and exhibited robust recovery from induced intracellular alkalosis which was appropriately inhibited by the absence of external Cl^-. In addition, cleaved embryos were able to recover from induced acidosis. Recovery depended upon external Na^+ and appeared insensitive to amiloride and EIPA.

Conclusions: pH_i in human PI embryos is regulated in both the alkaline and acid range. This dual regulation maintains steady-state pH_i throughout embryo development. pH_i in human eggs and embryos is regulated by HCO_3^-/Cl^- exchange for the alleviation of alkalosis. Cleaved embryos use a Na^+-dependent mechanism for the alleviation of acidosis although the precise regulatory mechanism is unclear. This work was supported by the Division of Reproductive Medicine, Department of Obstetrics and Gynecology, University of Ottawa. KP Phillips is supported by the Bombardier Foundation and Ontario Graduate Science and Technology Studentship (OGSST).

P-428

Immunolocalisation of Cadherins and Catenins in the Human Placenta. S. Getsios, G. T. C. Chen, C. D. MacCalman. British Columbia Research Institute for Children's and Women's Health, University of British Columbia, Vancouver, BC, Canada.

Objectives: The cadherins are a gene superfamily of calcium-dependent cell adhesion molecules (CAMs). The regulated expression of these CAMs are believed to govern the developmental fate of cells and the subsequent formation of tissues during embryonic development. Recent studies indicate that two cadherin subtypes, E-cadherin (E-cad) and cadherin-11 (cad-11), are differentially expressed in the human placenta. In particular, E-cad has been localised to the mononucleate villous cytotrophoblasts whereas cad-11 expression is restricted to the syncytial trophoblast. These observations have led to the hypothesis that E-cad and cad-11 play a central role in the formation and organisation of this dynamic tissue. Although cadherin function is regulated by the family of cytoskeletal-associated proteins, known as the catenins, the identity and expression patterns of the catenins present in the human placenta have not been determined. To address these outstanding issues, we have examined the expression of the catenin subtypes, known as α-, β-, γ-catenin and $p120^{catenin(ctn)}$ in human chorionic villi.

Design: The expression of these catenin subtypes was correlated with the cadherins present in the chorionic villi of the first trimester placenta.

;Materials and Methods: First trimester placentae (8–12 weeks gestation) were obtained from women (n=6) undergoing elective termination of pregnancy in accordance with a protocol approved by the Committee for Ethical Review of Research involving Human Subjects, UBC. Frozen sections (8 μm) were prepared from chorionic villi isolated from the placental tissue and processed for immunoperoxidase histochemistry. Immunohistochemistry was performed using monoclonal antibodies directed against either human E-cad, cad-11, α-, β-, γ-catenin or $p120^{ctn}$.

Results: Intense immunostaining for α-, β-, γ-catenin and $p120^{ctn}$ was detected in the villous cytotrophoblasts but not the syncytial trophoblast of the first trimester placenta. Similarly, E-cad was localised to the mononucleate villous cytotrophoblasts. In contrast, cad-11 immunostaining was only detected in the multinucleated syncytial trophoblast.

Conclusions: The expression of the catenin subtypes examined in these studies is restricted to the mononucleate villous cytotrophoblasts. As E-cad was also localised to this cell layer, it would seem likely that these cytoplasmic protein regulate the function of this placental CAM. In contrast, these catenin subtypes do not appear to mediate cad-11 function in the syncytial trophoblast suggesting that other unidentified catenins and/or cytoplasmic proteins associate with this CAM in chorionic villi. Future studies will characterise the cytoplasmic proteins capable of regulating cad-11 function in the human placenta.

This work was supported by a grant from the Medical Research Council (MRC) of Canada. CDM is also the recipient of an MRC scholarship. SG is the recipient of a Doctoral Research Award from the MRC.

P-429

Chromosomal Studies in Infertile Men. P. Kalantari, [1]H. Sepehri, [2]F. Behjati, Z. Ousati Ashtiani, [2]M. T. Akbari. [1]Department of Biology, University of Tehran, Tehran, Iran and [2]Department of Genetics, Tehran University of Medical Sciences, Tehran, Iran.

Objectives: Among the various etiologic factors of male infertility, chromosomal anomalies play an important role. Chromosomal abnormalities contribute to male infertility, asserting their effect though gametogenic impairment. The elucidation of such correlations helps us to achieve a more through understanding of male reproductive dysfunction.

Design: We report the occurrence of chromosomal aberrations and chromosomal variants, by high-resolution banding method in 70 infertile azoo- and oligozoospermic (<20 million/ml) men. We compare the clinical and cytogenetic findings and report the association between minor chromosomal anomalies and sperm counts.

Materials and Methods: Chromosomal analyses were done from cultures of peripheral blood lymphocytes by High-Resolution banding (Thymidine method). From each patient, 10 well-spread metaphases were analyzed by G-banding (and when required C-banding, NORs and Q-banding). We compared mean sperm count of infertile men with

chromosomal variants with a control group of infertile men with normal somatic chromosomes that had no history of treatment or ingestion of drug and cigarette smoking.

Results: 11.42% of patients had chromosomal abnormalities. Azoospermia was found in 6 patients with klinefelter syndrome, 1 with mosaic 47,XXY/46,XY and 1 with mosaic 45,X/46,XY. A sperm count of <20 million per ml was found in a patient with Y chromosome deletion (46,XY, del (Y) (q11.2q11.2)). All patients with numerical chromosomal anomalies had azoospermia. The most frequent anomaly was 47,XXY, (8.57%). The highest frequency of abnormal karyotypes (10%) was found among patients with azoospermia. Chromosomal variants (21.38% of patients) comprised: (46,XY, fra(2)(q31)), (46,XY, 22pss), large heterochromatin regions on either chromosome 1(46,XY, 1qh+), 9(46,XY, 9qh+), 16(46,XY, 16qh+), and Y chromosome (46,XY, Yqh+ and 46,XY, Yqh−). Anomalies of the Y chromosome was the most frequent variant. Mean sperm count of infertile men with chromosomal variants in comparison with mean sperm count of infertile men with normal chromosome is not significantly different ($P<0.05$).

Conclusions: The rate of chromosomal aberrations observed among infertile men was 11.42%. The highest frequency of abnormal koryotype (10%) was found among patients with azoospermia. We conclude that all patients with azoospermia should be referred for cytogenetic investigation. The frequency of chromosomal abnormalities of azoospermic and oligospermic men in the world, range from 7.3% to 14%. This figure compares rather well to that found in this study. Comparison of the mean sperm count of infertile men with chromosomal variants with infertile men with normal chromosome constitution indicates that "Minor chromosomal aberrations" may not alter the carrier's sperm count. In this study, comparison between the mean sperm counts of infertile men with variants of the Y chromosome (46,XY, Yqh+ and 46,XY, Yqh−) and infertile men with normal chromosome constitution, indicates that "variants of the Y chromosome" has no influence on the sperm count of men.

P-430

Expression of mRNA for Colony Stimulating Factor-1 (CSF-1) and Its Receptor (CSF-1R in Human Granulosa Cells and the Stimulation of Progesterone Production by Recombinant CSF-1. [1,2]F. R. Tekpetey, [2]A. K. Papay, [2]A. J. Watson. [1]Reproductive Endocrinology & Infertility Program, London Health Sciences Centre and [2]Department of Obstetrics and Gynaecology, The University of Western Ontario, London, Ontario, Canada.

Objectives: High concentrations of CSF-1 have been reported to be present in human follicular fluid, suggesting a role for CSF-1 in the regulation of follicular function. In the mouse and pig, expression of mRNA for CSF-1 in follicular cells have been observed. To assess the potential role of CSF-1 in human follicular function, the present study investigated the expression of mRNA for CSF-1 and CSF-1R in human granulosa cells undergoing luteinization, as well as progesterone production by these cells in response to rCSF-1.

Materials and Methods: Granulosa cells were obtained, from the follicular aspirations of women undergoing in vitro fertilization. For mRNA analysis, the reverse transcription polymerase chain reaction (PCR) was applied to total RNA extracted from the granulosa cells. Published CSF-1 and CSF-1R oligodeoxynucleotide primer sets, derived from human cDNA sequences, were used for PCR amplification. To assess steroidogenesis cells were seeded overnight, washed and cultured with rCSF-1 ± LH. Medium was changed every 24h for 3 days.

Results: PCR products for CSF-1 and CSF-1R were detected as 407 and 519 base pair (bp) amplicons respectively. To confirm the PCR product identity, CSF-1 amplicon digestion with Pvu II produced two fragments of 295 bp and 112 bp in length as expected. Likewise, Hind III digestion of the CSF-1R amplicon produced two fragments of 348 bp and 149 bp in length. In the cell culture experiments, basal progesterone accumulation per 24h period was enhanced dose-dependently on each day of culture, by rCSF-1 treatment (0, 0.5, 5, 50 ng/mL) alone or in the presence of LH (50 ng/mL). LH alone had a marginal stimulatory effect of progesterone production, but the combined effects of LH and rCSF-1 appeared to be synergistic on days 2 and 3 of culture at the highest dose of rCSF-1 tested.

Conclusion: These data suggest that CSF-1 may have an autocrine or paracrine role in corpus luteum formation and function.

P-431

Increased DNA Synthesis in Oocyte-Cumulus Complexes (OCCs) of Rats Treated In Vivo with an Insulin-Like Growth Factor-I (IGF-I) Analogue. [1]S. Roberge, [1]Y. Yavas, [1]L. Nicot-Montalvo, [1]K. Gopalasundaram, [1]M. Endman, [1,2]F. Khamsi. [1]Toronto Fertility Sterility Institute; [2]Department of Medicine, University of Toronto; [2]Division of Endocrinology, The Toronto Hospital, Toronto, Ontario, Canada.

Objective: To evaluate the in vivo actions of an IGF-I analogue on in vitro DNA synthesis response of OCCs to FSH and IGF-I in vitro.

Design: ^{3}H-thymidine incorporation as an indicator of DNA synthesis was measured in OCCs cultured with FSH, IGF-I, or none.

Materials and Methods: Day-24 immature rats received subcutaneous mini-osmotic pumps releasing either 1 mg/kg per day of IGF-I analogue (LR-^{3}IGF-I) or saline. At day 26, rats were injected with either equine chorionic gonadotropin (eCG) or saline, and were sacrificed 48 h later for isolation of OCCs. Groups of 10–15 OCCs from each in vivo treatment were cultured for 20 h in BTCM-199 media with either IGF-I (10 ng/ml), FSH (5 ng/ml) or no hormone. At that time, 1 μCi ^{3}H-thymidine was added into each well and cultured for another 8 h. OOC cells were measured for ^{3}H-thymidine incorporation.

Results: The results are shown in the table below. In vitro treatment of FSH increased DNA synthesis in OCC over all in vivo treatment ($P<0.05$). Treatment with LR-^{3}IGF-I tended to decrease the in vitro stimulatory effect of FSH ($P<0.10$).

In Vitro Treatment	In Vivo Treatment			
	Control	eCG	LR^3-IGF-I	eCG + LR^3-IGF-I
Control	450 ± 50	340 ± 40	500 ± 50	275 ± 50
FSH	825 ± 75*	1,250 ± 100*	800 ± 50*	1,200 ± 100*
IGF-I	550 ± 50	460 ± 40	575 ± 50	500 ± 50*

Values are dpm/OCC (mean±SEM; n=12 per in vivo treatment). *Different from control within columns ($P<.05$).

Conclusion: Treatment in vivo with eCG enhanced DNA synthesis in OCCs cultured in the presence of FSH. Treatment with LR-^{3}IGF-I alone tended to counteract the in vitro stimulatory effect of FSH. The combined treatment of eCG+LR-^{3}IGF-I gave the highest increase in DNA synthesis after an in vitro stimulation with FSH and IGF-I, suggesting that cumulus cells are regulated differently by both hormones.

P-432

Increased DNA Synthesis of Isolated Rat Follicle Cells by Insulin-like Growth Factor-I (IGF-I). [1]S. Roberge, [1]I. Lacanna, [1]D. N. Afzal, [1,2]J. Wong, [1]P. Shirazi, [1]A. Agrawal. [1]Toronto Fertility Sterility Institute; [2]Department of Obstetrics and Gynecology, University of Toronto; [2]Sunnybrook Health Science Centre, Toronto, Ontario, Canada.

Objective: To study the role of IGF-I on DNA synthesis of rat granulosa cells and oocyte-cumulus complexes (OCC).

Design: Measurements of ^{3}H-thymidine incorporation in granulosa cells and OCC after in vitro stimulation with IGF-I.

Materials and Methods: Day-26 immature rats were injected with equine chorionic gonadotropin (eCG; n=20) or saline (n=20), and sacrificed 48 h later for isolation of granulosa cells and OCC. Groups of 10–15 OCCs and granulosa cells were cultured separately in BTCM-199 media. IGF-I was added into each culture well at one of the following concentrations: 0, 0.78, 1.56, 3.12, 6.25, 12.5, 25 and 50 ng/ml, and incubated for 20 h at 37°C. Then 1 μCi ^{3}H-thymidine was added into each well and cultured for another 8 h. Cells (OCC and granulosa) were then measured for ^{3}H-thymidine incorporation as an indicator of DNA synthesis.

Results:

	IGF-I Concentrations (ng/ml)			
	0	0.78	1.56	3.12
OCC (control)	550±50	600±50	680±45*	750±51*
OCC (eCG)	400±35	480±42	400±40	550±40*
Granulosa (control)	40±3	52±5*	48±5*	55±8*
Granulosa (hCG)	57±2	60±3	65±3*	75±5*

	IGF-I Concentrations (ng/ml)			
	6.25	12.5	25	50
OCC (control)	650±40	800±50	700±50	680±50
OCC (eCG)	725±50*	550±55	450±50	435±50
Granulosa (control)	57±5*	74±5*	80±5	92±2
Granulosa (hCG)	83±3*	85±3*	88±3*	92±2

Value units are dpm/OCC and dpm/10^3 granulosa (means±SEM). *Different from control (0 ng/ml) within rows ($P<0.05$).

Conclusion: Two types of isolated rat follicles cells responded differently to in vitro stimulation with IGF-I. Granulosa cells were more responsive to IGF-I with a higher increase in DNA synthesis than were the cumulus cells (OCC), which exhibited higher cell proliferative activity. Differences in responsiveness to IGF-I between the 2 cell populations could be related to an influence of the oocyte in the OCC.

P-433

The Molecular Basis of the Role of Tissue Plasminogen Activator in Oogenesis. F. Khamsi. Toronto Fertility Sterility Institute; Department of Medicine, University of Toronto; Division of Endocrinology, The Toronto Hospital, Toronto, Ontario, Canada.

Objective: Tissue plasminogen activator (tPA) plays an important role in oogenesis. The objective was to evaluate the role of transcription and translation in tPA production during oogenesis.

Design: Oocytes collected from hyperstimulated rat ovaries were subjected to in vitro maturation (IVM); transcription and translation were prevented by α-amanitin and Cycloheximide, respectively, and tPA activity was measured.

Materials and Methods: The ovaries of immature rats of 26–27 days old were stimulated with equine chorionic gonadotrophin. Subsequently the oocytes were retrieved and subjected to IVM. To culture medium, α-amanitin was added to prevent transcription, and Cycloheximide to prevent translation. tPA was also measured in pregerminal vesicle phase oocytes when it is known that the tPA values are low. In each group, comparison was made between oocytes with cumulus intact and oocytes with no cumulus. In one group, the tPA activity was measured with a chromogenic assay, and in all four experiments it was assessed with signalling on the basis of a gel chromatographic technique.

Results: The pregerminal vesicle phase oocytes showed minimal tPA production with an average signal of 1/4. The control group with IVM showed average signal of 2.9/4, the α-amanitin group a signal ratio of 3.1/4, and the Cycloheximide group a signal ratio of 2.1/4. When the data for all the groups were pooled with presence of cumulus oophorum, the signal was 2.9/4 and in the absence of cumulus the signal was 2.5/4.

Conclusion: We found that at the final stages of oocyte maturation blockage of transcription using α-amanitin did not change the production of tPA. This indicates that the messenger RNA for tPA production is already in the cell. Therefore, we confirmed in the rat the observations made previously by Huarte and colleagues in mice. These authors also found that blockage of translation completely halts production of tPA in mice. Our results in the rat were different and blockage of translation appeared to reduce tPA production, but did not completely abolish it. This indicates that in the rat oocytes some precursors of tPA may be present which can lead to production of tPA without necessarily having to rely on translation from mRNA.

P-434

Co-culturing Oocyte-Cumulus Complexes (OCCs) with Varying Amount of Autologous Granulosa Cells Does Not Improve IVF Rate and Embryo Development. [1]A. Agrawal, [1]P. Shirazi, [1]S. Krakofsky, [1]I. Lacanna, [1]V. Mauricio, [1]M. Endman. [1]Toronto Fertility Sterility Institute, Toronto, Ontario, Canada.

Objective: We had previously demonstrated that co-culturing of OCCs with a fixed large amount of autologous granulosa cells did not change development to 2–4 cell stage embryos. The objective was to determine if varying the amount of granulosa cells added may influence oocyte fertilization and/or development to 2–4 cell stage from fertilized oocytes.

Design: OCCs were cultured either alone, or with small (6×10^6) or large (18×10^6) amount of autologous granulosa cells. Fertilization rate and embryo quality at 48 h post-retrieval were evaluated.

Materials and Methods: In nine women, OCCs were cultured in 100 μl HTF-type media drops covered by oil. Within women, OCCs were either left intact (control), or co-cultured with small or large amount of autologous granulosa cells. These did not include any cumulus type of granulosa cells and were all mural granulosa cells. Oocytes were inseminated with sperm by standard procedure. At 24 h post-retrieval, fertilized oocytes displaying two pronuclei were transferred into fresh drops containing no autologous granulosa cells, or small or large amount of autologous granulosa cells, respectively. At 48 h post-retrieval, ratio of good-quality embryos (equal-size blastomeres with no or minor fragmentation) and the mean blastomere number were determined.

Results: Fertilization rate did not differ among OCCs cultured alone, or with small or large amount of autologous granulosa cells (89±12, 81±12 and 63±12%, respectively; mean±SEM; $P>0.13$). Ratio of good-quality embryos did not differ among the three groups (83±13, 63±14 and 100±17%, respectively; $P>0.10$). The mean blastomere number also did not differ among the three groups (3.9±0.4, 3.6±0.6 and 4.8±0.6, respectively; $P>0.14$).

Conclusion: Co-culturing OCCs with varying amounts of autologous granulosa cells did not affect IVF rate and embryo development.

P-435

Increasing Number of Oocyte-cumulus Complexes (OCCs) Co-Cultured in a Small Volume Does Not Change IVF Rate and Embryo Development. [1]I. Lacanna, [1]A. Agrawal, [1]K. Gopalasundaram, [1]M. Endman, [1]L. The, [1]L. Nicot-Montalvo. [1]Toronto Fertility Sterility Institute, Toronto, Ontario, Canada.

Objective: We had previously demonstrated that co-culturing oocytes in 100 μl volume did not influence development to 2–4 cell stage embryo. The objective was to determine if reducing the volume to 25 μl and increasing number of OCCs co-cultured improved embryo development in this context.

Design: OCCs were cultured either individually or in groups of four in 25 μl culture volume. Fertilization rate at 24 h post-retrieval and embryo quality at 48 h post-retrieval were evaluated.

Materials and Methods: Within eight women, OCCs were cultured individually in 25 μl HTF-type media drops, and in groups of four in 25 μl culture volume, covered by oil. Oocytes were inseminated with sperm by standard procedure. At 24 h post-retrieval, fertilized oocytes displaying two pronuclei were transferred into fresh drops of the same volume, with number of fertilized oocytes adjusted to four. At 48 h post-retrieval, ratio of good-quality embryos (equal-size blastomeres with no or minor fragmentation) and the mean blastomere number were determined.

Results: Fertilization rate did not differ between OCCs cultured individually or in groups of four (91±9 vs. 74±9%; mean±SEM; $P=0.18$). Ratio of good-quality embryos did not differ between the two groups (87±7 vs. 88±7%; $P=0.91$). The mean blastomere number also did not differ between the two groups (3.9±0.2 vs. 3.9±0.2; $P=0.88$).

Conclusion: Increasing number of OCCs and reducing culture volume did not affect IVF rate and development of embryos to 2–4 cell stage.

P-436

Effects of Hypo-Osmotic Swelling Test on the Outcome of ICSI for Patients with Only Non-Motile Spermatozoa Available for Injection. A. M. El-Nour, H. A. Al-Mayman, A. M. Atared, K. A. Jaroudi, J. M. G. Hollanders, S. Coskun. King Faisal Specialist Hospital and Research Center, Departments of Pathology and Laboratory Medicine, and Obstetrics and Gynecology, Riyadh, Saudi Arabia.

Objective: Hypo-Osmotic Swelling Test (HOST) has been shown to be an effective method for selection of live sperm. On-going pregnancies were obtained by using HOST-selected sperm. The aim of this study was to evaluate the effect of using HOST-selected live sperm versus non-selected sperm on the outcome of ICSI cycles when only non-motile sperm are available for injection.

Design: Prospective randomized study.

Materials & Methods: Twenty-four patients were included into this study. For the HOST group a single spermatozoon was collected by an ICSI needle and incubated in a hypoosmotic saline of 150 mOsm for 1 minute. Live spermatozoa detected by hypoosmotic reaction of the tail were injected into oocytes after washing in medium. For the NO-HOST group the sperm were injected directly into the oocytes. The fertilization, cleavage, embryo quality, pregnancy and implantation rates were assessed for the two groups.

Results: Among 24 cycles, 10 patients fall into the HOST group and 14 patients into the NO-HOST group. The following table summarizes the results:

Study	*Cases*	*Oocytes*	*Injected*	*Fertilized*	*Cleaved*
HOST	10	102	82	42 (51)	41 (98)
NO-HOST	14	142	97	45 (46)	43 (96)

Study	*Good Embryo*	*Transferred*	*Pregnancy*	*Fetus*
HOST	19 (45)	28	5 (50)	7 (21)
NO-HOST	20 (44)	27	2 (14)	2 (7.4)

Numbers in parenthesis are percentages.

Fertilization, cleavage rates and the number of good quality embryos were similar between two groups. Pregnancy and implantation rates tend to be higher in HOST group compared to NO-HOST group. However, differences were not statistically significant ($p=0.08$ and 0.1 for pregnancy and implantation rates, respectively).

Conclusions: HOST-selected live spermatozoa can be safely used for ICSI to establish pregnancies. There is a tendency for higher pregnancy and implantation rates although not significant. More cases are needed to conclude the effectiveness of HOST over random injection of nonmotile spermatozoa.

P-437

X-linked Inhibitor of Apoptosis Protein Prevents Tumor Necrosis Factor α-induced Apoptosis in Rat Granulosa Cells. C. W. Xiao, B. K. Tsang. Reproductive Biology Unit, Department of Obstetrics & Gynecology and Cellular & Molecular Medicine, University of Ottawa, Loeb Health Research Institute, Ottawa, Ontario, Canada.

Objective: The fate of the developing ovarian follicles (growth/ovulation vs atresia) is the consequence of the fate of granulosa cells within them (proliferation vs apoptosis). Whereas tumor necrosis factor α (TNFα) produced by granulosa cells and oocytes induces apoptosis in ovarian follicles, the mechanisms involved are not well understood. X-linked inhibitor of apoptosis protein (Xiap) is an important suppressor of apoptosis in various cell types. Although its gene is present in ovary, its physiological roles in follicular development and atresia remain unclear. The purpose of the present studies were to examine the role of Xiap in the modulation of TNFα-induced apoptosis in rat granulosa cells.

Design: Granulosa cells isolated from 24 to 25-day old immature rats 24 hr after PMSG treatment by follicular puncture were plated for 24 hr in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), and subsequently cultured in serum-free RPMI in the absence or presence of TNFα (100 ng/ml), cycloheximide (CHX; 1–10 μM) and/or adenovirus containing the Xiap antisense full length cDNA.

Materials and Methods: Apoptosis was assessed by Hoechst nuclear staining and in situ TdT-mediated dUTP nick 3′ end-labeling (TUNEL, DNA fragmentation analysis) and Xiap protein contents were measured by Western blot.

Results: Although TNFα alone failed to induce granulosa cell death, it remarkably increased the apoptotic cell number in the presence of the protein synthesis inhibitor CHX, suggesting that TNFα may be involved in the induction of an intracellular apoptosis-preventing factor. To determine if the Xiap is a possible candidate, granulosa cells were infected with adenovirus containing the Xiap antisense full length cDNA to downregulate Xiap expression and subsequently by challenged with TNFα. Xiap protein content was increased markedly by TNFα alone and reduced significantly by adenoviral Xiap antisense infection. Downregulation of Xiap expression induced granulosa cell apoptosis which was further enhanced by the presence of TNFα.

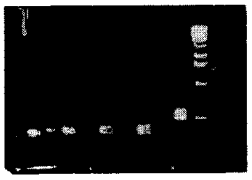
Conclusion: 1) TNFα alone increased the Xiap expression and failed to induce granulosa cell apoptosis; 2) Downregulation of Xiap expression not only induced granulosa cell apoptosis and also facilitated the induction of apoptosis by TNFα. Collectively, these finding suggest that TNFα is both a death and survival signals and an important cytokine for the control of both ovarian follicular development and atresia. The role of TNFα as a determinant of granulosa cell fate may be dependent on the expression of Xiap. The physiological modulator (s) of granulosa cell Xiap expression remains to be determined.

This work was supported by Medical Research Council of Canada.

P-438

Jagged1, a Ligand for the Endothelial Specific Notch4 Receptor, is Expressed in the Ovary and Uterus in Mice. [1,2]R. C. Zimmermann, [1]E. Gugliominetti, [1]M. V. Sauer, [1]J. Kitajewski. [1]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and [2]Department of Psychiatry, Columbia University, New York, New York.

Objective: The female reproductive organs i.e. ovary and uterus are some of the few adult tissues that exhibit periodic vascular growth and regression. The identification and characterization of endothelial cell-specific receptor tyrosine kinases (RTK) (Flk-1, Flt-1; Tie1, Tie2) and their ligands (VEGF; Ang1 and Ang2) has allowed us to begin to understand the vascular biology of reproductive organs. For example treatment with truncated soluble Flt-1 receptors, which inhibit vascular endothelial growth factor bioactivity, results in virtually complete suppression of corpus luteum angiogenesis. Based on in-situ hybridization and immunohistochemistry results we have identified a new endothelial specific receptor, Notch4, a member of the cell fate decision family Notch. Notch4 is expressed in reproductive organs as demonstrated by Northern blot (data not shown). To start to characterize the role of this new gene family in reproduction, it is pertinent to first establish that the Notch 4 ligand Jagged1 is expressed in reproductive organs.



Design: Animal study.

Materials and Methods: The ovaries and uteri from 2 hormonally treated immature mice were frozen in liquid nitrogen, immediately after having been sacrificed. Tissue was stored −80°C. Total RNA was prepared by using TRIZOL extraction, and subjected to denaturing agarose gel electrophoresis and the integrity of the RNA by the 28S and 18S ribosomal RNA bands was visually verified. The RT reaction was performed using the

SUPERSCRIPTPreamplification System for first strand cDNA synthesis. Random nonamer primers were used together with 1 μg of RNA in a total volume of 20 μl. The following published primers were used: sense 5′-TGCAGCTGTCAATCACTTCG-3′ derived from rat (bp 1761–1781), antisense 5′-CAGAATGACGCTTCCTGTCG-3′ (bp 2102–2122), product size 361 bp; RT-PCR was performed using Taq DNA polymerase under the following conditions for jagged 1: 37 cycles, 94°/20s, 60°C/30s, 72°C/90s. The amplified DNA was fractionated by electrophoresis and stained with ethidium bromide.

Results: Jagged1 is expressed consistently in the ovaries and uteri of the 2 animals studied. Figure: Lane 1 ovary 1 +, lane 2 ovary 1 −; lane 3 uterus 1 +, lane 4 uterus 1 −; lane 5 ovary 2 +, lane 6 ovary 2 −; lane 7 uterus 2 +, lane 8 uterus 2 −; lane 9 positive control; lane 10 size ladder; + with RT, − without RT.

Conclusion: We have shown by RT-PCR that the Notch4 ligand Jagged1 is consistently expressed in uteri and ovaries of hormonally treated mice. Based on our preliminary results and the examples provided by the discovery of endothelial specific RTKs, we have every reason to believe that the Notch/Jagged receptor/ligand pair plays an important role in the vascular biology of reproductive organs. A distinct role for Notch/Jagged is also suggested by the fact, that it does not signal through tyrosine kinase as the other endothelial specific receptors, but through a new signaling pathway, in which the intracellular domain of notch seems to have a direct nuclear function. We are in the process of studying the expression pattern of Notch4 and Jagged1 in the ovary and uterus of mice by in-situ hybridisation and immunohistochemistry.

P-439

Estrogen and Progesterone and Androgen Receptors in the Reproductive Tract of the Human Female Fetus. D. E. Goldschlag, L. M. Kump, J. Jacobson, K. P. Xu, O. K. Davis, Z. Rosenwaks. Center for Reproductive Medicine and Infertility, Cornell Medical Center—The New York Hospital, New York, NY.

Introduction: Factors involved in the prenatal development of the female fetal reproductive tract are poorly understood. Sex steroid hormones may play critical roles in reproductive organ development. Identifying estrogen, progesterone and androgen receptor expression in the various cell types contained within these organs should further elucidate the developmental process.

Design: Immunolocalization of estrogen, progesterone, and androgen receptors in the 21–22 week female fetal reproductive tract.

Materials and Methods: Under IRB approval, ovaries, uteri, and fallopian tubes were collected from mid-trimester TOP's. Specimens were dissected; fixed in 10% formalin; and imbedded in paraffin blocks. Immunohistochemical staining was performed using primary antibodies specific for the estrogen receptor (ER) progesterone receptor (PR) and androgen receptor (AR) on 3 μm sections. Sections of adult premenopausal ovarian tissue and controls for the ER, PR and AR antibody were stained simultaneously. Specificity was confirmed by the absence of staining in sections incubated with primary antibody preabsorbed with antigen.

Results:

	ER	PR	AR
Fetal Ovary (n=8)			
Surface epithelium	++	++	−
Oocyte	+°	−	++
Oogonia	+°	−	++
Follicular cells	−	−	−
Sex cord stromal cells	−	++	++°
Fetal Uterus (n=4)			
Endometrium	+	+	+
Stroma/Myometrium	++*	+°	++**
Fetal Fallopian Tube (n=4)			
Luminal epithelium	++	−	−
Stroma	++	++	++
(+) control (n=1)	++	++	++
(−) control (n=1)	−	−	−
Adult Ovary (n=1)			
Follicular cells	++	++	+
Stromal cells	−	−	−

(+) Indicates mild staining
(++) Indicates strong staining
(−) Indicates negative staining
(°) Occasional positive staining
(*) The uterine stoma/myometrium stain very strongly centrally with ER
(**) The uterine stoma/myometrium stain very strongly peripherally with PR

Conclusions: Within different tissue types each receptors is expressed in uniquely reproducible patterns. In this study, we have identified the estrogen, progesterone, and androgen receptor status of the 21–23 week female fetal reproductive tract. Estrogens, progesterones, and androgens may play critical roles in the development of the human reproductive tract.

P-440

Inhibin and Activin Expression in the Reproductive Tract of the Human Female Fetus. D. E. Goldschlag, J. Jacobson, L. M. Kump, S. D. Spandorfer, O. K. Davis, Z. Rosenwaks. Center for Reproductive Medicine and Infertility, Cornell Medical Center—The New York Hospital.

Introduction: A role of inhibin and activin in prenatal development of the female fetal reproductive tract has not been previously described. Many factors play critical roles in reproductive organ development. Identifying the presence of specific growth factors in various cell types contained within these organs should further elucidate the developmental process.

Design: Immunolocalization of inhibin and activin in the human 21–22 week ovary.

Methods: Under IRB approval, ovaries, uteri, and fallopian tubes were collected from mid-trimester TOP's. Specimens were immediately fixed in 10% formalin and imbedded in paraffin blocks. Immunohistochemical staining was performed using primary antibodies specific for inhibin α, inhibin βA, and inhibin βB on 3 μm sections. Sections of paraffin embedded adult premenopausal ovarian tissue and positive controls for the inhibin antibodies were stained simultaneously. Specificity was confirmed by the absence of staining in sections incubated with primary antibody preabsorbed with antigen.

Results:

	Inhibin α	Inhibin βA (Activin A)	Inhibin βB (Activin B)
Fetal Ovary (n=8)			
Primary Oocyte	−	++	−
Oogonia	−	++	−
Follicular epithelial cells	++	+/−	++
Sex cord stromal cells	++°	−	−
Fetal Uterus (n=4)			
Endometrium	−	++	++
Stroma/Myometrium	−	++	++
Fetal Fallopian Tube (n=4)			
Luminal epithelium	−	++	++
Stroma	−	++	++
	α	**βA**	**βB**
(+) control	++	++	++
(−) control	−	−	−
Adult Ovary			
Oocyte	−	−	−
Follicular cells	++	++	++
Stromal cells	−	−	−

(+) Indicates mild staining
(++) Indicates strong staining
(−) Indicates negative staining
(°) Indicates occasional staining

Conclusions: In this study, we have identified the presence of inhibin B in female fetal follicular cells; the presence of activin A within the oogonia and oocytes; activin B and/or inhibin B in follicular cells; and the free inhibin α in occasional sex-cord stromal cells. This finding may implicate activin A in early oogenesis and both inhibin b and activin A in early folliculogenesis. Throughout the uterus and fallopian tube, both activin A, B, and/or AB was expressed. Inhibin expression was not detected in the uterus and tube, limiting its role to oogenesis, early oocyte development, and early follicle formation.

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The Regulation of Oogenesis and Early Follicle Formation in the Humans: A Look at Genes Controlling the Cell Cycle and Apoptosis. D. E. Goldschlag, L. M. Kump, J. Jacobson, K. P. Xu, O. K. Davis, Z. Rosenwaks. Center for Reproductive Medicine and Infertility, Cornell Medical Center—The New York Hospital New York, NY.

Objectives: The regulation of oogenesis and early folliculogenesis in the human is poorly defined. Cell cycle regulatory genes are activated and deactivated in specific sequences to allow for the transition of primordial germ cell to primordial follicle. Oogonia can continuously cycle through mitosis (G_1, S, G_2, and M phases) without entering G_0, a stable quiescent state, or becoming senescent. Additionally, follicles and their oocytes may enter prolonged dormant stages (arresting in meiosis I) while others become atretic (a process utilizing apoptosis pathways). Though it is well documented that oogonia increase in number throughout the mid-trimester, the regulation of this process is not well understood. Cyclin D1 a key facilitator of transition from G_1 to S phase; p16 and p21 both inhibitors of cyclin D1; p53 an apoptosis inducer; bcl-2 an anti-apoptosis promoter, and Ki67 a marker of G_1, S, G_2, and M phases were all evaluated to ascertain the identity of cell cycle regulators important in oogenesis and early folliculogenesis. Understanding these processes should further elucidate the developmental process and perhaps illuminate pathological processes that lead to premature depletion of ovarian follicles.

Design: Immunohistochemical identification of cyclin D1, p16, p21, p53, bcl-2, and Ki67 in the 21–22 week fetal ovary.

Methods: Under IRB approval, ovaries were collected from four elective 2nd trimester TOP's; transported to the lab in Hanks balanced salt solution at 4°C; immediately fixed in 10% formalin; and imbedded in paraffin blocks. Immunohistochemical staining was performed on 2 sections from each of four different ovarian specimens on 3μm sections using primary antibodies specific for p53, p16, p21, bcl-2, and cyclin D1. Sections of paraffin embedded adult premenopausal ovarian tissue and positive controls for each respective antibody were stained simultaneously. Specificity was confirmed by the absence of staining in sections incubated with primary antibody preabsorbed with antigen.

Results:

	p53	bcl-2	cyclin D1	p16	p21	Ki67
Primary Oocyte	−	−	++	++	−	−
Oogonia	++°	−	++	++	−	++
Follicular cells	−	+	+/−	+	−	−
Stromal cells	−	++°	−	−	−	++°

(−) negative staining
(+) mild staining
(++) strong staining
(°) occasional staining

Conclusions: The high presence of cyclin D1 in oogonia and primary oocytes is indicative of active mitosis. The increase in p16 expression, a cyclin D1 kinase inhibitor, highlights the dynamic state of this regulated process. Similarly, cyclin D1 expression in the primary oocyte is consistent with progression through S phase and then to meiosis I arrest. This cyclin D1 and p16 interaction is likely to be critical in modulating the mitotic rate in oogenesis. Ki67, an antigen normally expressed in G_1, S, G_2, and M phases (not G_0) is detected in more than 90% of oogonia, further confirming their rapid mitotic rate in the second trimester. Interestingly, bcl-2, a common anti-apoptotic gene, was not detected at all. Finally, the occasional presence of p53 in some oogonia may herald the initiation of apoptosis and the end of oogenesis.

REPRODUCTIVE ENDOCRINOLOGY

Wednesday, September 29, 1999

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Resumption of Fertility with Diet in PCOS Patients. [1]P. G. Crosignani, [1]S. Piloni, [2]A. Gessati, [1]M. Colombo, [1]W. Vegetti, [2]D. Comi, [1]G. Ragni. [1]First Department of Obstetrics and Gynecology, University of Milan and [2]Clinical Nutrition Department, University Hospital "L. Sacco," Milan, Italy.

Objective: The aim of this study was to evaluate the effect of weight reduction on menstrual cycles, spontaneous ovulatory cycles and spontaneous pregnancies in anovulatory overweight patients with polycystic ovary syndrome (PCOS). The effects of weight loss on anthropometric characteristics and body fat distribution were also recorded.

Design: Prospective study.

Materials and Methods: Twenty-six anovulatory overweight patients with PCOS were enrolled in the study. Patients had a mean age of 30.3 ± 4 years (range 22–37), a mean infertility duration of 40 ± 30.9 months (range 12–120), a mean body weight of 80.6 ± 8.8 kg (63.5–100) and a mean body mass index (BMI) of 31.7 ± 4.3 (25.1–43.7). All patients had patent fallopian tubes and were infertile due to chronic anovulation: 5 patients were amenorrheic, 13 were oligoamenorrheic and 8 were eumenorrheic. The partners were normospermic. Patients were treated with a 1,200 Kcal/day diet (20% protein, 25% lipids, 55% carbohydrates and 30 g of fibers/week) and physical exercise was recommended. Body fat distribution by bioimpedance analysis (BIA) and anthropometric indexes were assessed at baseline and after a weight loss of 5% and 10%. Resumption of regular menstrual cycles in oligo- or amenorrheic patients and spontaneous ovulation were recorded using menstrual diary cards and by means of serum midluteal progesterone assessment.

Results: So far a weight loss of 5% compared to basal values has been reached by 18 out of 26 patients in 45.8 ± 12.8 days (range 21–56). Ten out of these 18 patients achieved a weight loss of 10% in 148.8 ± 72.4 days (range 70–273). Out of the 18 patients with oligo-amenorrhea, 12 had a resumption of regular cycles and 10 experienced spontaneous ovulation. Five spontaneous pregnancies were initiated, 4 after a 5% weight loss and 1 after a 10% weight loss. Patients who did not lose weight neither improved their menstrual irregularities nor resumed ovulation.

Among anthropometric indexes and body fat distribution parameters, body weight, BMI, Waist hip ratio and fat mass were significantly reduced after a weight loss of 5% and 10% ($p<0.001$, t-test for paired data); on the contrary free fat mass and total body water remained unchanged.

Conclusions: Weight loss through hypocaloric and controlled diet restores ovulatory cycles, and improves anthropometric characteristics in obese PCOS patients.

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CA-125 Tumor Marker Behavior During Induction of Ovulation, Oocyte Retrieval, Early Pregnancy and Ovarian Hyperstimulation Syndrome. I. Sherizly, I. Bar-Hava, R. Orvieto, J. Ashkenazi, A. Ferber, R. Bardin, Y. Yairi, O. Davidi, Z. Ben-Rafael. Department of Obstetrics and Gynecology, Rabin Medical Center, Petah Tikva, and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Introduction: Despite the evolution of sophisticated controlled ovarian hyperstimulation regimens, the complication of severe ovarian hyperstimulation syndrome (OHSS) has not been eliminated. Elevated CA-125 levels have been described in OHSS patients. The purpose of this preliminary study was to investigate the source of CA-125, to evaluate whether it can serve as a marker to predict the severity of OHSS, and what levels of this antigen can be found in affected patients.

Design: Prospective clinical study.

Material and Methods: Blood CA-125 levels were determined. Patients enrolled in our IVF program were divided into five groups as follows: A: day of human chorionic gonadotropin (hCG) administration; B: day of oocytes retrieval; C: 12 days after embryo transfer in those who did not

conceived. D: 12 days after embryo transfer in those who conceived and did not demonstrate clinical hyperstimulation findings; E: presence of severe OHSS (based on clinical, sonographic and laboratory criteria). In addition, follicular CA-125 levels were determined during the oocyte retrieval procedure.

Results: CA-125 levels were within normal range in groups A–C. However, a significant ($P<0.001$) elevation in mean CA-125 levels was detected in groups B and C in comparison with group A. Elevated (50–100 U/ML) CA-125 levels were detected in the follicular fluid. Significantly elevated CA-125 levels were detected during early stage of pregnancy (group D). Patients with severe OHSS (group E) were found to have elevated CA-125 levels (250–1900 U/ML) up until gestational week 20 (twins) that resolved as pregnancy progressed.

Conclusions: There is a significant elevation in CA-125 levels following hCG administration. It seems that its source is in the follicular fluid and probably triggered by hCG. High levels of CA-125 can be found in patients with severe OHSS. Based on our preliminary findings (the study is ongoing), we suggest that CA-125 levels correlate with the severity of the syndrome (as reflected by the amount of ascites fluid) and the gestational week. Physicians should be aware of these findings since the clinical picture can resemble ovarian carcinoma.

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Primary Aldosteronism and Pregnancy: A Case Report. F. P. G. Leone, *M. Leone. Department of Obstetrics and Gynecology, San Paolo Biomedical Institute, University of Milan Medical School and *Department of Obstetrics and Gynecology, S. Sebastiano Martire Hospital, Frascati (Rome), Italy.

Objective: Report the management of a rare case of pregnancy complicated by primary aldosteronism diagnosed before gestation.

Design: Case report.

Material and Methods: A 38-year-old white woman gravida 1 initially presented with hypertension and hypokalemia at age 21. The combination of suppressed plasma renin activity and high levels of serum aldosterone and of a 24-hour urine assay for aldosterone led to the diagnosis of primary aldosteronism. A thin-slice computed tomography scan evidenced a left adrenal lesion, consistent with the clinical suspicion of adrenal adenoma. She was successfully treated with spironolactone (300 mg/day) until 25 years old. Then, to avoid a chronic medical therapy, the patient underwent a bilateral emiadrenalectomy, without complications. Pathologic analysis of adrenal specimen revealed a bilateral cortical hyperplasia. She did not need further oral medication for hypertensive and electrolytic disorders. Twelve years later she became spontaneously pregnant. Throughout the pregnancy, the patient was asymptomatic, she did not assume antihypertensive medication and her blood pressure was stable. Repeated obstetric ultrasound exams at 8, 20 and 32 weeks showed an appropriate fetal growth with no anomalies. Early second trimester amniocentesis revealed an euploid female fetus. High-resolution ultrasonography of the maternal abdomen evidenced normal adrenal glands. Biochemical findings of Potassium (K), Plasma Renin Activity supine and upright (PRAs and PRAu), Serum Aldosterone supine and upright (SALDs and SALDu), Urine Aldosterone (UALD) and Blood Pressure (BP) values immediately before pregnancy, during gestation and in the post-partum period are evidenced on the table.

Parameters	Normal value	Before pregnancy	Gestational age (weeks) 12	16
K (mEq/L)	3.5–5.3	4.6	5	4.3
PRAs (ng/mL/h)	0.3–2.8	1.9	5.2	6.6
PRAu (ng/mL/h)	1.5–5.7	4.2	8	14
SALDs (ng/dL)	3–16	16	26	52
SALDu (ng/dL)	4–31	24	90	130
UALD, 24h (μg/day)	2.5–30	–	21	4.8
BP (mmHg)		100/70	105/75	100/75

Parameters	Gestational age (weeks) 22	27	32	36	Postpartum
K (mEq/L)	4.3	4.4	4.6	4.3	4.6
PRAs (ng/mL/h)	5.6	8.9	4.8	10.6	–
PRAu (ng/mL/h)	12.7	16.9	10.8	18.4	–
SALDs (ng/dL)	30	88	100	>150	15
SALDu (ng/dL)	150	>150	>150	>150	25
UALD, 24h (μg/day)	6.5	10.5	151	172	–
BP (mmHg)	100/70	115/70	115/80	130/85	120/80

At 37 gestational weeks she was admitted asymptomatic with hypertension. On admission, her blood pressure was 140/100 and her pulse rate was 80 beats per minute. Fetal well-being was verified with cardiotocography, ultrasonographic exam and Color-Doppler assessment. Blood pressure responded to bed rest. Two days later it rose slightly and an elective cesarean section was decided. A healthy female infant was delivered, weight 2800 g and Apgar score 10/10 at 1 and 5 minutes. No signs of virilization were present. Placental weight was 600 g. The postpartum and neonatal courses were uneventful and both were discharged on the sixth postoperative day.

Discussion: Hyperaldosteronism is an uncommon and potentially life-threatening disorder and there are few reports of its occurrence and management in pregnancy. In normal pregnancy, since the second trimester, adrenal glands augment aldosterone production to balance the natriuretic effects of the rising plasma progesterone levels. Aldosterone levels increased upright, as already described in patients with adrenal hyperplasia. As well, normal gestation is characterized by relatively high renin levels, generally attributed to placental production of this hormone. However, normal values in pregnancy have not been established. In our case, we had analogue biochemical findings. As previously referred, we did not observe hypokalemia (see table). Previous reports of emergency preterm delivery and cases of fetal and neonatal mortality in the setting of hyperaldosteronism in gestation confirm the significant risks associated with this condition. Our behaviour was guided by this background and an elective cesarean delivery was decided when blood pressure started rising, to avoid maternal and fetal complications. In fact, the electrolyte disturbances and hypertension may be first apparent in the peripartum period, coinciding with the removal of the protective antialdosterone effect of progesterone. We conclude that pregnant women previously surgically treated for aldosteronism need close observation throughout the pregnancy and good clinical outcome for both mother and infant can be anticipated.

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Randomized Study of Induction of Ovulation by Two Different Molecules with Antiestrogenic Effects, in Patients with Chronic Anovulation Disorders. W. Vegetti, A. Riccaboni, M. Colombo, E. Baroni, D. Diaferia, G. Ragni, P. G. Crosignani. First Department of Obstetrics and Gynecology, University of Milan, Italy.

Objectives: Antiestrogens are well-known as the first step in ovulation induction of normogonadotropic anovulatory women. Clomiphene Citrate (CC) is the most widely used and studied. Tamoxifen (Tx) has never reached the same popularity. Our aim was to compare these two molecules in inducing ovulation in infertile women suffering from chronic anovulation.

Design: Prospective randomized study in a tertiary infertility center.

Materials and Methods: To be recruited for our trial each patient had to fall within the following criteria: at least one year of duration of infertility; tubal patency proved either by a recent hysterosalpingography or by a laparoscopy; normogonadotropic anovulation; a normal semen analysis according to the World Health Organization criteria.

Both the molecules were administered for a period of five days, starting from the third day of each cycle. CC was given at the dose of 100 mg/day; Tx at the dose of 20 mg/day. These doses were duplicated in those patients who had two following anovulatory cycles. Follicular development was monitored by transvaginal ultrasound. Patients were asked to use a urinary immunosticks for the identification of the luteinizing hormone (LH) spontaneous surge. Serum progesterone was assessed 8 days after the positivization of the above sticks. We considered

ovulatory those cycles with progesterone levels ≥7 ng/ml. Only clinical pregnancies (gestational sac detected by ultrasound scan) were considered for this study.

Results: We have randomized 95 women. Fifty of them were administered CC; 45 Tx. A total of 262 cycles were performed: 129 with CC and 133 with Tx. CC was used at the dose of 100 mg/day in 107 cycles and at the dose of 200 mg/day in the remaining 22. Tx was given at the dose of 20 mg/day in 103 cycles and at the dose of 40 mg/day in 30 cycles. We obtained ovulation in 108 (83.7%) cycles induced by CC and in 92 (69.2%) induced by Tx ($p<0.001$). To duplicate the dose, in those patients who didn't respond in term of ovulation to the above standard doses, was useful in inducing ovulation in 80% of CC treated cycles and in 63.3% of cycles treated by Tx. Pregnancies in CC group were 12: nine with 100 mg and 3 with 200 mg. With Tx 8 women got pregnant: 5 with 20 mg and 3 with 40 mg. These data are not significantly different. No relevant side effects were recorded.

Conclusions: Despite Clomiphene induces a higher percentage of ovulatory cycles than Tamoxifen, these two drugs can be considered interchangeable according to the pregnancy rate. Moreover our study demonstrates the utility of duplicating drug doses in all those patients who do not respond to a daily dose of 100 mg of Clomiphene and 20 mg of Tamoxifen.

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Case-Control Study on Risk Factors for Premature Ovarian Failure. [1]W. Vegetti, [2]G. Testa, [3]F. Chiaffarino, [1]A. Nicolosi, [1]F. Alagna, [4]M. Taborelli, [1,3]F. Parazzini, [1]P. G. Crosignani. [1]I Department of Obstetrics and Gynecology, University of Milan, [2]Obstetrics and Gynecology Unit Department of Biology and Clinical Sciences, University of Insubria, Varese, [3]Istituto di Ricerche Farmacologiche Mario Negri, Milan, [4]Department of Biology and Clinical Science, University of Insubria, Ospedale di Circolo, Varese.

Objectives: The median age at menopause is currently around fifty in Western industrialized society. Recently, Torgerson et al., (1997) reported that the mean age at menopause is over 45 in 88% of women, under 45 in 9.7% (early menopause = EM) and under 40 years in only 1.9% (premature ovarian failure = POF). In most cases the etiology of premature onset of menopause is still unknown. The aim of this case-control study was to investigate potential risk factors for POF.

Design: Preliminary report of a case control study.

Patients and Methods: Patients were 73 women with idiopathic POF (23 of them showed a familial condition) who were part of a larger POF study recruited by Reproductive Endocrinology Services, Department of Obstetrics and Gynecology in Milan and Varese. Both Centers followed a preestablished protocol for patient selection. Included as control group were 146 women with acute, non-neoplastic, non hormone-related diseases matched to case patients by age. Informations about sociodemographic factors, personal characteristic and habits, age at menopause, gynecological and obstetric data, smoking habits, alcohol consumption and eating behavior were collected.

Results: A statistically significant association between high education level and POF was found ($p=0.006$) but this can be due to a selection bias. No association was observed with smoking habits, alcohol consumption and diet. Among the relationship between reproductive factors and POF, parity showed to reduce the risk of POF. This reduction is directly correlated with the number of livebirths ($p=0.008$) but low parity can be a consequence and not a determinant factor of POF. No association emerged between POF and age at menarche and menstrual pattern.

Conclusions: Our study confirms that there are no clear risk factors for POF. Nevertheless, this study strongly suggests that POF condition is an indicator of low fertility: POF patients show a reduced number of livebirths compared to age-matched control subjects.

Reference: Torgerson DJ, Thomas RE and Reid DM. Mothers and daughters menopausal ages: is there a link? Eur J Obst Gyn Reprod Biol 1997; 74:63–6.

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Withdrawn

P-448

Pregnancy Outcome After First Trimester Bleeding in Patients Treated for Infertility. K. Pezeshki, D. E. Stein, S. M. Lobel, R. V. Grazi. Department of Obstetrics and Gynecology, Maimonides Medical Center, Brooklyn, NY.

Objectives: Bleeding during early pregnancy may be of great concern to a patient and her physician, especially when it follows infertility and its treatment. Whether or not bleeding is more likely to complicate treatment-induced pregnancies than naturally occurring pregnancies is unknown. Although it is well known that bleeding does not always herald a poor outcome, it is unclear how often bleeding during early pregnancy does in fact lead to an adverse outcome. Certainly, many pregnancies resulting from medical intervention result in poor outcomes even in the absence of bleeding. The objectives of this study were, therefore, twofold: (A) to establish the frequency of bleeding during early pregnancy after various types of fertility therapy, and (B) to assess the value of bleeding in predicting pregnancy outcome.

Design: Retrospective analysis of pregnancy outcome in patients attending a private infertility clinic.

Material and Methods: All patient records were stored electronically, including laboratory values, obstetrical sonograms, patient complaints at the time of presentation and any phone calls that occurred in between clinical assessments, which were performed periodically through the twelfth week of pregnancy. Records of all patients who conceived were reviewed, as defined by serum hCG levels >10 mIU/ml, for a period of 28 months. Of 292 pregnancies, 57 (19.5%) occurred spontaneously (Group I). The remaining 235 patients conceived following treatment and were separated into three additional groups according to the method of treatment: Group II: ovulation induction (OI) (n=124; 42.5%); Group III: in vitro fertilization (IVF) (n=65; 22.3%) and Group IV: pregnancies resulting from surgery, intrauterine insemination and/or medical therapy other than OI (n=46; 15.7%). All patient reports regarding bleeding were recorded. Pregnancy outcome was recorded as either favorable (ongoing pregnancy beyond the first trimester) or adverse (spontaneous abortion or ectopic pregnancy).

Results: The overall occurrence of bleeding and adverse outcome during early pregnancy were not significantly different in those patients with treatment-induced pregnancies (Groups II–IV) as compared to those with spontaneous pregnancies (Group I) (32.8% vs. 24.6%, and 30.2% vs. 29.0%, respectively). The incidence of bleeding among groups was not statistically different (Group I - 24.6%, Group II - 36.9%, Group III - 32.3%, Group IV - 28.0%). Adverse outcome more often followed bleeding than no bleeding only in Group II (42.0% vs. 20.3%, $p < 0.05$).

Conclusion: In patients presenting with infertility, bleeding during early pregnancy is as likely to occur when the pregnancy is the result of treatment than when it occurs spontaneously. Adverse outcome is not different among groups and occurs independently of a history of bleeding. Bleeding during early pregnancy is associated with adverse outcome only when the pregnancy has resulted from ovulation induction without IVF. With other common pregnancy-inducing treatments, including IVF, the occurrence of bleeding is not predictive of adverse outcome.

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Evaluation of Ovarian and Uterine Blood Flow by Transvaginal Color Doppler Ultrasonography in Patients with Polycystic Ovaries. M. Vasiljevic, M. Djukic, N. Antic, S. Rakic, E. Garalejic, A. Markovic, S. Dikic, B. Arsic, B. Vasic. Clinic of Gynecology and Obstetrics "Narodni Front" Belgrade, Yugoslavia.

Objective: The aim of this research was to analyze the haemodynamic changes of the ovarian and uterine blood vessels in polycystic ovaries.

Materials and Methods: We have examined 26 patients with polycystic ovaries. The diagnosis was set on the basis of hormone analysis, ultrasound examination and laparoscopic findings. The mean age of the patients was 26,4 years. Twenty patients had a menstrual cycle disorder out of which 14 (70%) patients had oligomenorhea and 6 (30%) patients had amenorhea. Signs of mild hirsutism were noticed in 8 (30,77%) patients. The ultrasound examination for all patients was done by the Aloka Color Doppler SSD 2000, with transvaginal probe 5,5MHz.

Results: In 8 (30,7%) patients normal ovary volume was found with average values of 4,2 ccm and range of 3,6 to 5,9 ccm. 18 (69,23%) patients

had enlarged ovary volume with average values of 12,6 ccm and a range of 9–16 ccm. The color Doppler analysis of the blood flow at the level of uterine artery showed high values of the Pulsatility index (x>3,1) and Resistance index (x>0,82). At the stromal ovary blood vessels low values of resistance index (RI =0,52) and high values of pulsatility index (PI =0,98) were found. At the level of a. arcuate, a. radialis and a. spiralis the average mean values of RI were somewhat lower: 0,68; 0,56; 0,54 respectively and values of PI: 1,8; 1,5; 1,28 for each respectively. The values of the PI in the stromal ovary blood vessels were in negative correlation with the values of estradiol and progesterone in serum, and in positive correlation with the lh/FSH ratio.

Conclusion: The presence of the stromal ovarian vascularisation with a low resistance index and high pulsatility index has a significant diagnostic meaning in the diagnosis of polycystic ovary.

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Fertility Care and Severe Polycystic Ovarian Syndrome (PCOS): New Lessons From Our Patients. [1,2]R. F. Feinberg, [1]J. Biancosino, [1]B. A. McGuirk. [1]Reproductive Associates of Delaware, Wilmington, DE, [2]Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT.

Objectives: In women with severe clomiphene-resistant PCOS, we proposed that the insulin-lowering agent troglitazone (Rezulin) could have a positive impact on the ovarian microenvironment by supporting normal follicular growth, ovulation, and successful pregnancy.

Design: Observational and prospective, in an academic-affiliated subspecialty private practice.

Methods: Clomiphene-resistant women (n=22) were referred by their gynecologists for subspecialty fertility care, with a desire to avoid gonadotropin therapy. All women were evaluated in detail for objective evidence of anovulation, polycystic ovaries, insulin resistance, androgen excess, and dyslipidemias, and written informed consent was obtained prior to prescribing troglitazone.

Results: Of 22 patients, 21 (95%) had documented ovulatory cycles, and 19 continued in active treatment beyond one cycle. Of 16/19 women continuing with troglitazone ± clomiphene, 13/16 (81%) achieved a pregnancy within 6 months; 10/16 (62%) had healthy ongoing gestations (9 singletons, 1 twin); 4/16 (25%) had spontaneous losses, but one subsequently achieved a normal pregnancy. Of the 3/19 patients who ovulated with troglitazone ± clomiphene but didn't conceive, 2 pursued IVF (both singletons) and 1 patient had gonadotropins in conjunction with troglitazone (singleton).

Conclusions: A high percentage of women with clomiphene-resistant PCOS manifest treatable insulin resistance and hyperinsulinemia. For many of these women, the insulin-lowering agent troglitazone is effective in supporting ovulatory function, leading to ongoing pregnancies. Beneficially, the risks and costs associated with gonadotropin use in these women can potentially be avoided.

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Lack of Correlation Between Antiphospholipid Antibodies and Beta 2-Glycoprotein 1 Antibodies in Patients with Recurrent Pregnancy Loss. [1]N. Hollier, [1]R. D. Franklin, [1]W. H. Kutteh, [2]D. J. Thompson. [1]Department of and Gynecology, University of Tennessee, Memphis, TN, and [2]Center for Reproductive Medicine of New Mexico, Albuquerque, New Mexico.

Objectives: Antiphospholipid antibodies (APAs) are important etiologic factors that are detected in 15–20% of women with recurrent pregnancy loss (RPL). Several studies suggest that antibodies in Antiphospholipid Syndrome (APS) actually bind to a cofactor known as β2-glycoprotein 1 (β2-GP1) rather than to the actual phospholipid, thus making it a better indicator of APS. We tested the APA ELISA in conjunction with the β2-GP1 ELISA in a group of women with RPL and with and without APAs.

Design: Through a chart review 45 women with RPL and positive APA titers and 45 women with RPL but no APAs were identified. All women were of similar age, had a history of RPL (≥2 losses), and a complete examination for RPL. Excluded were women with SLE or prior thrombosis.

Materials and Methods: Serum samples were obtained from the reproductive immunology lab. A standard APA ELISA was employed to detect the presence of IgG, M, and A antibodies in serum against the phospholipids cardiolipin, glycerol, inositol, serine, and ethanolamine. Samples were also assayed with a commercial β2-glycoprotein 1 ELISA for IgG antibodies (Quanta Lite β2-GP1, INOVA Diagnostics, San Diego).

Results: Only 10 out of 45 women (22.2%) women with APA and RPL had positive IgG antibodies for β2-glycoprotein 1. Similarly, 1 out of 45 women (2.2%) were positive among the control group of women with RPL but no APAs (Table 1.). There was no correlation among the β2-glycoprotein 1 positive patients for a specific phospholipid.

APA status	Age at entry	prior pregnancies/patient	prior live births/patient	prior losses/patient
+ APA	33.3 ± 4.7	3.9 ± 2.2	0.4 ± 0.5	3.4 ± 2.0
− APA	32.5 ± 4.2	3.6 ± 1.4	0.6 ± 0.8	2.9 ± 0.9

APA status	prior losses <12 wks (%)	prior losses 13–19 wks (%)	prior losses >20 wks (%)	+ β2-GP1 (%)
+ APA	97.4	1.3	1.3	22.2
− APA	86.8	7.8	5.4	2.2

Conclusions: These data suggest that β2-glycoprotein 1 is less sensitive than APAs for the diagnosis of APS. APAs are a more sensitive laboratory method for diagnosing APS.

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High-Order Multiples, A Preventable or Unpreventable Complication of Controlled Ovarian Stimulation with Gonadotropins? [1,2]N. Gleicher, [1]G. Kaberlein, [1]J. Rinehart, [1,2]R. Morris, [1,2]D. Pratt, [1,2]R. Rao, [1,2]M. Balin, [1,2]V. Karande. [1]The Center for Human Reproduction-Illinois and [2]The University of Illinois at Chicago, IL USA.

Objective: Because of the associated risk of premature delivery, multiple births and especially high-order multiples (≥3) represent one of the most feared complications of controlled ovarian stimulation with exogenous gonadotropins. While various stimulation criteria have been widely published in attempts to minimize the occurrence of multiples, and especially grand multiples, the question whether grand multiples are at all preventable has not been answered. This question is especially relevant since our ability to control multiple births through the transfer of limited numbers of embryos during IVF may warrant a rethinking of current treatment algorithms if controlled ovarian stimulation was unable to achieve the same goal. This study was designed to answer this question.

Design: Analysis of 4,035 consecutively attempted gonadotropin cycles between 1/1/97 and 11/30/98 at a medical school-affiliated infertility program.

Materials and Methods: All controlled ovarian cycle stimulations are entered real-time into a fully computerized electronic database. At CHR-Illinois these data are maintained and analyzed monthly as part of an ongoing continuous quality improvement (QI) process under the control of a full-time QI coordinator (G.K.). This study included all consecutively performed gonadotropin cycles during the study period. During cycle stimulation, patients were monitored with follicular ultrasounds and estradiol (E_2) levels, as determined by their physician. Ovulation was triggered through the administration of hCG when at least 1 follicle was judged to be preovulatory (≥16 mm and E_2 ≥250 pg/ml). CHR treatment guidelines recommended that cycles should not be triggered with hCG if E_2 levels ≥2,000 pg/ml and must not be triggered ≥2,500 pg/ml. Guidelines also mandated cycle cancellation if ≥6 follicles were at ≥16 mm and recommended cancellation if ≥4 follicles were at ≥16 mm.

Results: Amongst 4,035 attempted cycles, 682 were canceled before stimulation start and 210 were canceled during stimulation. Out of 3,143 completed cycles, 447 clinical pregnancies were established (14.2% per cycle). 320 pregnancies were singletons (71.6%), 88 were twins (19.7%), 22 were triplets (4.9%), 10 were quadruplets (2.2%), 5 were quintuplets (1.1%) and 2 were sextuplets (0.4%). The incidence of high-order multiples (≥3) was thus 1.2% of all cycles. Estradiol (E_2) levels at time of hCG administration ranged (95% C.I.) for singletons from 927–1,075 pg/ml,

for twins 1,152–1,504 pg/ml, for triplets 1,372–1,693 pg/ml, for quadruplets 881–2,131 pg/ml, for quintuplets 601–1,735 pg/ml and for sextuplets from 0–7,431 pg/ml (NS by Student-Newman-Keuls Multiple Comparison Test and Dunnett Multiple Comparisons Test). The number of follicles ≥16 mm at hCG administration ranged between 1 and 6 and the mean was not statistically different between singleton and various multiple pregnancies. Only 1 pregnancy exceeded an E_2 level of 2,500 pg/ml at time of hCG (quadruplets) and was thus judged as potentially preventable.

Conclusion: Even if conservative standard stimulation criteria are upheld, high-order multiple pregnancies are unpreventable with controlled ovarian stimulation by gonadotropins. Considering the associated cost due to prematurity from up to 1.2% of such cycles, a reconsideration of current treatment algorithms in favor of earlier IVF cycles may be indicated at least in younger patients with significant risk for multiple births.

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Vascular Endothelial Growth Factor Receptor (VEGF-R1) Is a Key Regulator in Endometrial Angiogenesis. [1]D. R. Grow, [1]M. S. Ahmed, [1]L. A. Adams, [2]G. D. Hodgen, [1]M. T. Reece. [1]Baystate Medical Center, Tufts University School of Medicine, Springfield MA and [2]Jones Institute for Reproductive Medicine, Norfolk, VA.

Objective: VEGF is critically involved with the construction and remodeling of blood vessels throughout the body via binding to its transmembrane tyrosine kinase receptor, VEGF-R1. We describe changes in VEGF and its receptors in the primate endometrium under different hormonal conditions in an attempt to better understand endometrial vascular growth and dysfunction.

Materials and Methods: Adult female cynomolgus monkeys with a history of menstrual regularity underwent endometrial biopsy; during the natural menstrual cycle (N=11), after 3 months of GnRH-agonist for prolonged hypoestrogenism (N=9), or after 3 months of weekly mifepristone injections (N=12). Immunohistochemical analysis of the endometrium was performed for VEGF (anti-human goat polyclonal Ab293-Na, R and D Systens), and for VEGF-R1 (anti-human goat polyclonal Ab C-17, Santa Cruz Biotechnology, Inc.), Immunoreactivity was scored by three evaluators blinded to the treatment groups with Score = Intensity × Proportion of cells reacting (where Intensity = 0,1,2,3).

Results: Immunoreactivity for VEGF showed abundant expression in both proliferative and secretory endometrium. Similarly, VEGF immunoreactivity was strong in both estrogen deprived (GnRH-a treated) and atrophic appearing mifepristone treated endometrium. VEGF-R1 expression was variable. VEGF-R1 though abundant in the endometrium of all cycling monkeys, showed slightly more glandular expression in the secretory phase, ($P>0.1$). Severe sex steroid deprivation during 3 months of GnRH-a resulted in nearly complete suppression of VEGF-R1. (Kruskall-Wallis, $P<0.0004$). Suppression of VEGF-R1 with mifepristone was also profound, ($P<0.05$).

Mean ± SEM	VEGF	VEGF	VEGF-R1	VEGF-R1
	Glands	Stroma	Glands	Stroma
Proliferative	246 ± 25	224 ± 13	108 ± 21	74 ± 23
Secretory	253 ± 22	204 ± 22	166 ± 23	71 ± 11
All Cycling	250 ± 15	213 ± 15	139 ± 18	72 ± 11
GnRH-a	183 ± 71	233 ± 17	12 ± 6	20 ± 5
Mifepristone	168 ± 16	208 ± 8	49 ± 10	33 ± 8

Conclusions: Subtle variation in VEGF itself is unlikely to affect control over the monthly sloughing and regeneration of endometrial vasculature. VEGF control of endometrial angiogenesis is exercised primarily via regulation of its transmembrane receptor VEGF-R1 by the ovarian steroids. Severe hypoestrogenism and the progesterone/estrogen antagonism of mifepristone both profoundly suppress receptor expression.

P-454

Brain Regions Associated with the Predisposition To and Occurrence of Severe Premenstrual Syndrome. E. M. Reiman, J. H. Mattox, L. Don, J. T. Frost; K. Chen, D. J. Bandy, K. S. Matt, R. A. Reiman, E. J. Perla. Department of Psychiatry, University of Arizona; Obstetrics and Gynecology Department and PET Center, Good Samaritan Regional Medical Center, Phoenix, Arizona.

Positron emission tomography (PET) was used to investigate brain regions that are associated with the predisposition to and occurrence of premenstrual syndrome (PMS) in 8 women with premenstrual dysphoric disorder (PMDD). Eight women with PMDD had PET measurements of the cerebral metabolic rate for glucose (CMRgl) in the mid-follicular phase (5–9 days after the onset of menstruation) when they had minimal PMS symptoms and in the late-luteal phase (9–13 days after ovulation) when they had relatively severe PMS symptoms. Automated brain mapping algorithms were used to compare mid-follicular PET images in the women with PMDD and ten previously studied female controls and to compare late-luteal and mid-follicular PET images in the women with PMDD. Although the women with PMDD were not distinguished from the previously studied female controls in PMDD ratings, depression ratings, or plasma reproductive hormone concentrations at the time of the mid-follicular PET session, they had abnormally increased CMRgl bilaterally in the hippocampal formation, visual cortex, midbrain, and cerebullum, a trend for increased CMRgl in the vicinity of the hypothalamus, and abnormally decreased CMRgl bilaterally in medial and lateral prefrontal, anterior and posterior cingulate, and parietal cortex. In the women with PMDD, the premenstrual PET session was distinguished from the mid-follicular PET session by increased PMDD ratings and serum progesterone levels, further CMRgl increases bilaterally in posterior cingulate and parietal cortex, a trend for further increased CMRgl in the vicinity of the hypothalamus, increased CMRgl in the cerebellar vermis, further CMRgl decreases in medial, left lateral and left orbital frontal cortex, and decreased CMRgl in the vicinity of left anterior temporal cortex. These findings raise the possibility that women with PMDD have functional brain abnormalities independent of their menstrual cycle phase or symptomatology and that these abnormalities conspire with intrinsically normal hormonal changes in the late-luteal phase to produce severe PMS.

P-455

Relationship of BMI and the Effect of Abdominal vs. Peripheral Fat Distribution on Serum Leptin Levels in Untreated Postmenopausal Women. [1]V. D. Castracane, [2]G. Kraemer, [2]E. Brooks, [3]R. R. Kraemer, [1]T. L. Gimpel. [1]Department of Obstetrics/Gynecology, Texas Technology University Health Sciences Center, Amarillo, TX, [2]Women's Health Research Institute, Baton Rouge, LA and [3]Department of and Physical Education, Southeastern Louisiana University, Hammond, LA.

Objectives: Numerous studies have demonstrated the relationship between serum leptin and adiposity and that serum leptin levels increase with increasing BMI or % body fat.

Design: We have compared the distribution of serum leptin levels with BMI in untreated postmenopausal (PM) women. A comparison between serum estrogens and serum leptin levels was also investigated. The question of whether abdominal or peripheral fat is more potent in its production of leptin was also investigated by subdividing the population based on waist-hip ratio (WHR) into those with predominantly abdominal fat (≥0.85) and those with subcutaneous fat (<0.85).

Materials and Methods: PM women, surgical (n=12) or natural (n=30), without ERT were enrolled in this study. Serum samples were taken, height, weight and WHR were recorded. Patients were divided into abdominal obesity (n=17) or peripheral obesity (n=26) for further analysis. Serum leptin was analyzed with a sensitive (0.5 mg/ml) and highly specific IRMA assay (Diagnostic Systems Laboratories, Webster, TX).

Results: Serum leptin was compared with BMI and showed a significant positive correlation (r=0.88). Leptin was also significantly correlated to WHR, although the relationship was weak (r=0.38). To further examine the relationship between fat distribution and leptin, women with greater abdominal obesity (WRH≥0.85, n=17) and women representing more lower body fat distribution (WHR<0.85, n=26) were compared. Using an ANCOVA and adjusting the leptin concentrations for BMI, the difference in means approached significance (P=0.0588). Serum levels of individual estrogens were compared with leptin. There was a positive significant correlation between all 3 estrogens and leptin with the strongest correlation seen for E1

(r=0.77, 0.47, 0.56 for E1, E1S and E2, respectively. This would be expected since serum E1 levels predominate over E2 in the PM.

Conclusions: Serum leptin increases with increasing BMI and the suggestion of a greater increase in the abdominal adiposity group is seen. Patient enrollment continues in this study.

P-456

Circadian Leptin Rhythm in the Postmenopausal Subject and the Effect of Estrogen Replacement. [1]V. D. Castracane, [2]N. Santoro, [1]B. A. Tawwater, [2]G. Adel, [1]T. L. Gimpel. [1]Department of Obstetrics/Gynecology, Texas Tech University Health Sciences Center, Amarillo, TX, and [2]Department of Obstetrics/Gynecology, UMDNJ-New Jersey Medical School, Newark, NJ.

Objectives: Leptin secretion in reproductive age women has a distinct circadian rhythm which is lost in hypothalamic amenorrhea subjects.

Design: In the present study, we have examined postmenopausal women and established this diurnal variation in leptin. Naturally menopausal women (n = 7), with an age range of 51 to 70 years were enrolled in this study.

Materials and Methods: After placement of an intravenous catheter, q 10 minute samples were obtained for two 24 hour cycles. These were either untreated or with a transdermal estrogen patch to achieve concentrations of 100 pg/ml. Subjects were randomized to these treatment groups. Serum leptin, insulin and cortisol were measured at 60 or 30 minute intervals using commercially available reagents (Linco, DSL and Immulite respectively).

Results: Both in individual subjects and the mean results from all subjects, there was no difference in the leptin circadian rhythm in the untreated vs the ERT cycle. Maximum levels were obtained at about 0100 hours, declining through the night to reach nadir levels in the early morning about 0800 hrs., which were almost half of peak levels, and then arise again in the late afternoon through the evening back to peak levels. Postprandial insulin levels were increased following breakfast, lunch and dinner and resulted in increased insulin concentrations in the daylight hours and the nocturnal rhythm of cortisol was associated with a late afternoon, evening increase in all subjects.

Conclusions: These results are similar to an earlier study in POF subjects in which leptin secretion was essentially unchanged in younger women and that the same estrogen replacement was without effect in those subjects. These studies demonstrate that postmenopausal women either with or without estrogen replacement show a distinct circadian rhythm similar to that seen in POF patients as well as in normally cycling patients, but distinctly different from hypothalamic amenorrhea subjects. Neither age nor estrogen administration seems to dampen the circadian rhythm. The circadian patterns of insulin and cortisol may represent significant regulatory agents in the establishment of this circadian rhythm.

P-457

Effect of Estrogen on Presynaptic Cardiac Nerve Endings. [1,2]B. A. Eskin, [2]J. Roberts, [2]D. Snyder. [1]Department of Obstetrics/Gynecology and [2]Center for Aging Research, Medical College of PA and Hahnemann School of Medicine, Philadelphia, PA.

Objective: Increased sympathetic activity in the presynaptic cardiac nerve endings may result in clinical hypertension, cardiac failure and arrhythmias. Sympathetic neural discharges increase with age and in hypoestrinism. Estrogen (E) replacement, particularly long-term, provides protection from heart failure and coronary disease in the menopause. Norepinephrine (NE) release induced by K+ is measured in this study and the effect of estrogen on this release is studied.

Design: Using sealed nerve endings or synaptosomes from rat hearts, norepinephrine (NE) release is measured. Estrogen presence is evaluated by serum LH and estrogen testing, vaginal smears and uterine weights.

Material and Methods: Female rats (F344) were sham operated, ovariectomized or made severely hypoestrin with depot leuprolide (GnRH agonist) injection. The ovariectomized and sham operated groups were given equivalent estrogen replacements and were treated for short-term (90 days) and long-term (270 days). Rats in which low estrogen was induced chemically were only studied over the short term. Our laboratory technique for NE release from synaptosomes has been previously described (J Gerontology 50:358–67, 1995).

Results: In both the long-term and short-term E replacement groups, NE release was significantly reduced below the vehicle controls. Both sham animal groups were similar to the E treated groups. In the chemically hypoestrin groups when E decreased to its lowest levels, NE release was highest. Vaginal smears obtained in all hypoestrin animals were in diestrus.

Conclusions: When estrogen is experimentally low in female rats, levels of NE released from synaptosomes induced by K+ is increased. Replacement E therapy reduces NE release. The decrease in NE release is greatest after prolonged treatment. This activity could be due to either an E effect on release and/or reuptake mechanisms in the nerve terminals. Clinical studies have shown that an increase in NE may cause serious disturbances in the heart. Further studies into the effect of estrogen on the adrenergic neuroeffector junction is warranted.

P-458

Effects of Type I Collagen Matrix on Progesterone Production and Cytochrome P450 Cholesterol Side-Chain Cleavage Enzyme Gene Expression in Primary Cultured Porcine Granulosa Cells [1]X. Wang, [1]H. Saito, [2]K. Otsu, [2]K. Ishikawa, [1]H. Hiroi. [1]Department of and Gynecology, and [2]Department of Biochemistry, Yamagata University School of Medicine, Japan.

Objectives: Type I collagen (TIC) is a major protein of ovarian extracellular matrix. It has been reported that its abundance in granulosa cell layers increases during follicular growth, and that TIC might be involved in the regulation of steroidogenesis of granulosa cells. The cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc) that converts cholesterol to pregnenolone is the rate-limiting enzyme in progesterone biosynthesis. We examined the effects of TIC on progesterone production and P450scc gene expression in primary cultured porcine granulosa cells.

Design: Progesterone production and P450scc gene expression were determined in the cells overlaid with or without TIC gel.

Materials and Methods: The granulosa cells of the prepuberal porcine ovaries were aspirated from middle size follicles (diameter: 3–5 mm) using needle aspiration method. The granulosa cells were cultured on 0.03% TIC coated 35 mm dishes with 5% FBS in MEM. After 24 h, the dishes were divided into two groups. One group was overlaid with 0.5 ml of 0.1% TIC gel and another group was not (control). After the gel was coagulated by 1 h incubation, all of the dishes were cultured in serum-free medium containing 10 mg/ml low density lipoprotein for 3 additional days. Progesterone production was determined by measuring concomitant progesterone accumulation in the culture medium every 24 h. Total RNA was extracted from granulosa cells by the acid guanidium thiocyanate phenol chloroform method. P450scc mRNA abundance was determined by Northern blot hybridization using a ^{32}P-labeled 1.2 kilobase porcine cDNA as a probe and measured by Fuji Film FLA2000.

Results: The progesterone production in the cells overlaid with TIC gel decreased on day 3 (0.34-fold) and day 4 (0.16-fold), compared with that in the control ($p<0.01$). The P450scc gene expression in the cells with TIC gel was suppressed on day 3 (0.62-fold) and day 4 (0.36-fold) compared with that in the control cells. When pregnenolone (10^{-5}M), the precursor of progesterone, was added to the culture media, progesterone production increased in the cells of both groups, and no difference in progesterone production was observed between both groups. Treatment with hFSH enhanced progesterone production in a dose-dependent manner in the cells with and without TIC gel. At the concentrations of hFSH from 0.1 mIU/ml to 1 mIU/ml, the level of progesterone production was lower in the cells with TIC gel than that in the cells without TIC gel ($p<0.01$). At the concentration more than 10 mIU/ml, there was no significant difference between two groups.

Conclusions: These results indicate that TIC gel reduces the progesterone production in granulosa cells by suppressing P450scc expression. It is probable that TIC gel prevents the luteinization of granulosa cells in vitro culture. The fact that hFSH overcomes the inhibitory effect of TIC gel on progesterone production may explain the mechanisms of subtle P rise in the late follicle phase of IVF program.

P-459

Titering Individualized GnRH Antagonist Doses to Amenorrhea/Oligomenorrhea: Maintaining Basal Tonic Ovarian Estrogen Secretion for

Extended Therapeutic Regimens. [1,2]J. T. Queenan Jr, [3]A. Phillips, [2]K. Gordon, [2]R. Williams, [2]G. D. Hodgen. [1]Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC, [2]The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, and [3]Robert Wood Johnson Pharmaceutical Research Institute, Raritan, NJ.

Objective: To demonstrate how a GnRH antagonist can be utilized to achieve therapeutic benefit, yet conserve tonic endogenous ovarian secretion at levels likely to minimize the severe hypoestrogenism known to accompany extended GnRH agonist regimens.

Design: Cohort study of 34 adult female, normally cycling cynomologous monkeys (Macaca Fascicularis). Twenty six were treated with the GnRH antagonist Azaline B (Ortho Pharmaceuticals, Raritan, NJ) and 8 monkeys served as controls.

Materials and Methods: On menstrual day six subjects were treated with either 0.03, 0.015 or 0.01 mg/kg/day of Azaline B. After 4 days, treatment was continued when the serum E_2 was within the desired range of 25–50 pg/ml. If E_2 was >50 pg/ml, the dose was increased. If serum E_2 levels were <20 pg/ml, the dose was reduced. To test the functional status of the endometrium, a progesterone-containing silastic implant was inserted for 10 days. Daily observation for withdrawal bleeding was performed. Blood samples were continued following treatment to assess the recovery of ovulatory function.

Main Outcome Measure: Serum E_2 levels within the desired therapeutic window of 25–50 pg/ml.

Results: E_2 levels in all six monkeys treated with 0.03 mg/kg/d Azaline B were <20 pg/ml. One of six monkeys who received 0.015 mg/kg/d ovulated and five were suppressed to below 25 pg/ml. Four out of five monkeys initiated with 0.01 mg/kg/d demonstrated partial E_2 suppression within 24 hours. This cohort was expanded to 14 monkeys and treated for up to 70 days. Following Progesterone treatment monkeys with mean E_2 levels >50 pg/ml were more likely to exhibit a withdrawal bleed. Return of ovulation occurred 14.1 days following GnRH antagonist treatment.

Conclusions: In a primate model, the use of a GnRH antagonist can achieve and sustain partial ovarian suppression after individual titration of dose. The subjects demonstrated amenorrhea or oligomenorrhea while retaining tonic basal estradiol levels near 30 pg/ml. A progesterone challenge test provides a clinical indicator of the efficacy of therapy.

P-460

Müllerian Inhibiting Substance in Serum: A Comparison of Patients with Untreated Polycystic Ovary Syndrome and Normal Women. [1]A. G. Brenner, [1]C. L. Cook, [2]Y. Siow, [2]M. E. Fallat. Departments of [1]Obstetrics and Gynecology and [2]Surgery, University of Louisville Health Sciences Center, Louisville, KY, USA.

Objectives: To determine Müllerian inhibiting substance (MIS) serum levels in untreated women with polycystic ovary syndrome (PCOS). These levels will be compared with untreated normal women.

Design: Prospective study.

Materials and Methods: Patients with a clinical diagnosis of PCOS were seen in a fertility center by one of the authors. Random serum samples were obtained from 36 patients for measurements of MIS and luteinizing hormone (LH) levels by enzyme linked immunosorbent assay (ELISA). In the control group (n = 20), serum was sampled on menstrual cycle day 2 or 3. Statistical analysis was by the Student's t-test. A P value of < 0.05 was considered significant.

Results: Levels of MIS in sera of PCOS patients were significantly higher than those of controls (4.45 ± 3.31 versus 1.36 ± 1.00 ng/ml [mean ± SD] respectively. [$P < 0.0002$]). PCOS patients had higher serum LH levels than those of controls (13.51 ± 10.98 versus 6.65 ± 10.79 ng/ml [mean ± SD] respectively. [$P < 0.05$]).

Conclusion: Women with PCOS have significantly higher serum MIS levels. In an animal model, MIS has been shown to inhibit oocyte meiosis. In PCOS patients undergoing IVF procedures, higher serum and follicular fluid levels of MIS were associated with a greater number of immature oocytes at retrieval (Fallat et al, Fertil Steril 1997;67:692–5). These findings suggest that MIS may have a role in the regulation of follicular development and oocyte maturation. The disordered folliculogenesis characteristic of PCOS may be related to chronic elevation of MIS.

This work was supported by a grant from the Alliant Health Community Trust Fund, Louisville, KY.

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Dynamics of Leptin Secretion by Human Blastocyst and Endometrial Epithelial Cell. [1]R. R. Gonzalez, [2]P. Caballero-Campo, [2]M. Jasper, [2]A. Mercader, [1]L. Devoto, [2]C. Simón. [1]Instituto de Investigación Materno Infantil. Hospital San Borja Arriarán. Fac. de Medicina. U. de Chile. Chile. [2]Fundación Instituto Valenciano de Infertilidad. Depto de Pediatría, Obstetricia y Ginecología. Fac. de Medicina. U. de Valencia, Spain.

Objective: Leptin, the product of the ob gene, is a small peptidic molecule synthesized by white adipocytes with an important role in the regulation of body fat and food intake. Leptin has been linked to reproductive function and also detected in ovary, oocyte, pre-implantatory embryo and trophoblast. In the present study, we evaluated leptin secretion in conditioned media from human blastocyst and endometrial epithelial cell (EEC) cultures.

Materials and Methods: Human embryos (n = 30), obtained after ovarian superovulation and insemination employing routine IVF procedures, were cultured alone or co-cultured with EEC until blastocyst stage and transferred back to the mother. Leptin concentrations in lyophilised conditioned media from these cellular cultures at day of blastocyst transfer were measured by ELISA.

Results: The mean leptin concentrations from EEC cultures (without embryo) was 254.3 ± 16.3 pg/mL (mean ± SEM). Leptin concentrations from competent blastocyst cultured alone were significantly higher (453.3 ± 43.3 pg/mL; $p = 0.001$) than from arrested blastocyst cultures (238.6 ± 13.1 pg/mL). However, when blastocyst and EEC were co-cultured, leptin concentrations from arrested blastocyst cultures were significantly higher (360 ± 30 pg/mL; $p = 0.012$) than from competent blastocyst (247 ± 14.5 pg/mL).

Conclusions: We demonstrated that pre-implantation competent or arrested embryos and EEC differentially secrete leptin *in vitro*. Leptin concentrations where inversely related to IL-1 concentrations previously reported in these conditioned media. The IL-1 system has an important role in human implantation and induces leptin secretion in cytotrophoblast cells and may also regulate inflammatory cytokine secretion. These data might suggest an autocrine/paracrine regulatory mechanism involving the IL-1 system and leptin in the early phases of human implantation.

Supported by Programa de Cooperación Iberoamericana 1997–98. Instituto de Cooperación Iberoamericana. Ministerio de Asuntos Exteriores. Spanish Government.

P-462

Hormonal Regulation "In Vivo" of MUC1 In Human Endometrium. [1,2]M. Meseguer, [1,2]P. Caballero-Campo, [1]J. C. Martín, [1]A. Galán, [1,2]J. Remohí, [1,2]A. Pellicer, [1,2]C. Simón. [1]Instituto Valenciano de Infertilidad, [2]Department of Pediatrics, Obstetrics and Gynecology Valencia University School of Medicine.

Objective: During implantation, the interaction between the apical surface of luminal uterine epithelium and the surface of trophoectoderm is an event regulated by unknown molecular players. MUC1, a very large highly glycosilated transmembrane mucin, is a possible candidate involved in this process. The objective of this study was to analyze the hormonal regulation of MUC1 during the process of the acquisition of endometrial receptivity.

Design: MUC-1 protein and mRNA levels were analyzed in mock cycles from patients undergoing hormonal replacement therapy (HRT) as oocytes recipients.

Materials and Methods: Endometrial samples were obtained in the mock cycle from 10 patients undergoing HRT for ovum donation. Patients with ovarian function were desensitized with Gn-RH analogs. The hormonal regimen consists on E_2-valerate (2 mg per day) on days 1–8; 4 mg per day on days 9–11; and 6 mg per day on days 12–22. Natural micronized P (100 mg/day) was administered intramuscularly from day 16 to 22. Endometrial biopsies were performed on days 13 (with only E_2), 18 E_2 + P, nonrecep-

tive) and 21 (E_2 + P, receptive). Immunohistochemical analysis for MUC1 was performed on paraffin embedded sections using MAbs HMFG-1 (1:10 dilution) (kindly provided by J. Burchell), BC-2 (1:54 dilution) (Serotek) (against core protein extracellular domain) and CT-1 (1:50 dilution) (provided by J. Burchell) (against intracellular domain of MUC1). Also samples were pretreated with sialidase for increasing the binding of HMFG-1 to MUC1. mRNA expression was studied by Northern blot analysis in total RNA from biopsies with a specific MUC1 cDNA probe labeled with αP^{32} dCTP. Serum was collected on the day of the biopsies described above and E_2 and P were analyzed by MEIA.

Results: The summary of immunohistochemistry, Northern blot and MEIA results are presented in this table. MUC1 is up regulated in the uterine epithelium at the immunoreactive protein and mRNA levels during the window of receptivity. Intracellular staining was not observed with BC-2 antibody. L.E. = luminal epithelium. G.E. = glandular epithelium.

	HMFG-1		HMFG1+Sialidase		BC-2	
	L.E.	G.E	L.E.	G.E.	L.E.	G.E.
Day-13	0	0	+	0/+	+	+
Day-18	+	+	+	++/+++	0/+	+/++
Day-21	+	++	++	+++	0	++/+++

	MUC1 mRNA expression	Hormonal levels	
		E_2 pg/m	P ng/ml
Day-13	0.80 ± 0.12	333,9 ± 92.9	–
Day-18	1.08 ± 0.18	331.6 ± 59.1	9.5 ± 3.8
Day-21	2.40 ± 0.70	362.6 ± 78.5	10.5 ± 2.1

Conclusions: There is an intrinsic heterogeneity in MUC1 glycosylation which affects its detection in all the days studied. Nevertheless, we observe an up-regulation of MUC1 protein after P administration, that increases during the window of receptivity and is corroborated by mRNA levels. Subsequently, this high presence at the expected time of implantation is contradictory with an anti-adhesion function and opens the possibility that MUC1 with its heterogeneity could define the receptive endometrium which would be recognized by the embryo.

Supported by FPI98-VS-23-256 Generalitat Valenciana grant and FIS 98/1263 Spanish Government grant.

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Cyclic Guanosine 3′,5′-Monophosphate (cGMP) Prevents Apoptosis in Luteinized Human Granulosa Cells (GC): An Interleukin-1β (IL-1β)-Triggered Phenomenon? S. Kulshrestha, A. Makrigiannakis, J. Bucci, S. Bunso, C. Coutifaris. Division of Human Reproduction, Department of Obstetrics and Gynecology and the Center for Research on Reproduction and Women's Health, University of Pennsylvania Medical Center, Philadelphia, PA.

Objectives: Current experimental evidence suggests that apoptotic cell death is the molecular mechanism underlying the degeneration of ovarian follicles during atresia and of luteal cells during luteolysis. Since our work and that of others' has indicated a role for cyclic nucleotides in these processes, we hypothesized that cGMP also participates in the regulation of human GC apoptosis.

Design: Laboratory investigation approved by the Institutional Review Board.

Materials and Methods: GCs were isolated from follicular fluid after oocyte retrieval and cultured in serum free conditions for 24 h in the absence or presence of varying concentrations of cGMP (0.5–10 mM) or IL-1β (5–100 ng/ml). Apoptosis was assessed by flow cytometric cell cycle analysis and by the TUNEL assay.

Results: As we have shown previously, the rate of apoptosis was 2–3 fold lower among aggregated compared to single GC's ($p < 0.05$). Cell culture, under serum free conditions, increased cell apoptosis. Exposure of the cells to cGMP inhibited apoptosis in a dose-dependent manner. The greatest inhibition of apoptosis was observed at a concentration of 5 mM and was 53% of the control value ($p < 0.05$). Similarly, exposure of the cells to IL-1β prevented apoptosis.

Conclusions: We conclude that in human GCs, cGMP inhibits apoptosis. Given the pro-apoptotic function of cAMP, we propose that cGMP is critical for normal human follicular development and/or the maintenance of a functional corpus luteum possibly though activation of, as yet unknown, intracellular processes antagonizing the cAMP action within the cells. The data suggest that one possible intraovarian modulator of this process is IL-1β, which is known to utilize cGMP as a second messenger. [Supported by NIH grant HD-31903 (CC) and the Alexander Onassis Foundation (AM)]

REPRODUCTIVE LABORATORY

Wednesday, September 29, 1999

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Accuracy of Embryo Preselection at Pronuclear (PN) Stage. [1,2]D. G. Hammitt, [1]A. P. Singh, [1]D. L. Walker, [1]C. A. Sattler, [2]T. M. Galanits, [2]K. M. Barud, [2]M. A. Wentworth, [2]M. A. Damario, [2]D. A. Dumesic. Department of Obstetrics and Gynecology, Mayo Clinic, [1]Scottsdale, AZ and [2]Rochester, MN, USA.

Objective: To maximize pregnancy rates per oocyte retrieval, our IVF program traditionally has cryopreserved all embryos not required for the fresh embryo transfer (ET) at the PN stage rather than leaving excess embryos in culture for preselection (P). In attempt to optimize pregnancy rates in the fresh cycle, a PN grading system was established to help select the best quality embryos for ET. Using this PN grading system and freezing all extra embryos at the PN stage, pregnancy rates per transfer over a 5-year period (1993–1998) were 50% (444/896) and 40% (155/384) for fresh and frozen cycles, respectively.[2] Embryo quality at the cleavage stage has been shown to correlate well with implantation and pregnancy rates in our programs. The purpose of this study was to investigate the accuracy of our PN grading system for selecting the best quality embryos for ET as determined by embryo quality at the cleavage stage. This analysis began in 1997 when we[1] began leaving 2–4 extra embryos in culture for P on some patients.

Design: Patients <35 years of age were scheduled for a 3- or 4-ET and patients ≥35 years of age for a 4-ET. The best 3 or 4 PN grade embryos were placed in the transfer-select drop (TS) and the next best 2–4 embryos were placed in preselect drop (PS). All remaining embryos were frozen at the PN stage. Embryos were evaluated before ET for cleavage grade quality and cell number.

Materials and Methods: PN grades (0 best–3 poorest) were assigned at 16–18 hours following insemination: 0 - smooth vitelline membrane (VM), even granularity, small perivitelline space (PVS), even PN size; 1 - slight ruffling of VM/enlargement of PVS/uneven PN size; 2 - folded or very ruffled VM, large PVS, drastically uneven PN size, large sized oocyte; 3 - any fragmentation (F). Cleavage grades (0 best–3 poorest) were assigned immediately before ET: 0 - no F, cells even size and even granularity; 1 - F <25%, slightly uneven cell size; 2 - F 25–50% or dominant cell; 3 - F >50%, dark cytoplasm or multinucleated cells.

Results: A total of 288 zygotes from 37 patients were included in this study. The average age of the patients was 34.7 years. Based on the PN grades, 127 zygotes were selected as the best quality for TS and 101 additional zygotes for PS. Embryos assigned to TS had an average quality grade of 0.58 at the PN stage and 1.04 at the cleavage stage. Embryos assigned to PS had an average quality grade of 1.05 at the PN stage and 1.19 at the cleavage stage. The average cell numbers for embryos assigned to TS and PS were 4.0 and 3.8, respectively. Of the zygotes originally assigned to TS and PS, 69.3% and 44.5%, respectively, were found to be in the best quality group at the cleavage stage and were transferred.

Conclusion: It appears that the PN grading system described does not always accurately select the best embryos for ET based on cleavage grade embryo quality. Therefore, when freezing all extra embryos at the PN stage, some of the best quality embryos may be frozen rather than transferred in the fresh cycle. Data will be presented and compared for a new PN grading system that was just initiated in our program to improve the accuracy of PN embryo selection. This new grading system evaluates for uniformity in PN size, cytoplasmic clearing around the zygote periphery and nucleoli alignment at the PN interface.

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The Rate of Refreezing of Thawed Semen Significantly Affects Preservation of Motility and Viability. [1]B. R. Gilbert, T. A. Brown. [1]North Shore University Hospital, Manhasset, NY.

Objective: Cryopreservation has provided men with severely impaired semen quality or those about to undergo treatment that will adversely affect spermatogenesis, the means to preserve their gametes. However, often the total amount stored is also limited. Therefore, refreezing thawed specimens would provide additional opportunities for conception. The purpose of this study was to evaluate the effect that the rate of refreezing has on maintenance of sperm motility and viability after repetitive freeze/thaw cycles in specimens of varying semen quality.

Design: A prospective analysis of motility and viability of semen specimens after repetitive freeze/thaw cycles by either a slow (controlled rate freezer) or fast (vapor) refreeze method.

Methods: 22 semen specimens (4 paired and 7 unpaired specimens) were diluted with 12% glycerol, 20% egg yolk in a 1:1 ratio at room temperature. The specimens were initially cryopreserved in 1cc plastic cryovials, using a controlled rate freezer with a standard "slow" freeze cycle (−1°C/min until −30°C then 5°C/min until −80°C). All specimens were thawed using a standard thaw protocol (30 minutes at room temperature then 10 minutes at 37°C). The specimen then underwent repetitive cycles of either slow (n = 10; as described above) or fast (n = 12; −60°C/min) refreezes with the standard thaw protocol. The thaw/refreeze cycles were repeated until no motile and no viable sperm (eosin Y-nigrosin stain) remained. t-test was used for statistical evaluation.

Results: The mean sperm concentration and motility was 41.3 million/ml (range 8.3 to 98.5 million/ml) and 45% (range 28% to 60%). Motility and viability was present in all specimens through 2 thaw/refreeze cycles with a range from 2 to 7 thaw/refreeze cycles. Mean values for motility and viability, for both paired and unpaired specimens, were significantly greater for specimens undergoing repetitive fast refreezing ($p \leq 0.01$), with a linear decrease of motility and viability of 8.7%/cycle and 8.2%/cycle respectively for the slow refreeze cycles and 6.7%/cycle and 7.7%/cycle respectively for the fast refreeze cycles ($R^2 \geq 0.93$). A fast refreeze preserved motility for an average of 2.75 cycles longer and viability for an average of 2.0 cycles longer than a slow refreeze.

Conclusions: Multiple thaw/refreeze cycles are possible with even markedly impaired semen specimens. A fast rate of refreezing is significantly better than a slow rate in preserving motility and viability of cryopreserved semen specimens.

P-466

Clinical Results in ICSI Method and Characterization of Acrosomal Integrity of Applicated Spermatozoa. [1]O. Teplá, [2]J. Pěknicová, [1]M. Mrázek, [1]K. Koči, [1]J. Mika, [1]L. Hybnerová, [1]J. Jirmanová, [2]D. Chládek, [1]Z. Mayer. [1]Iscare IVF a.s., Prague, Czech Republic and [2]Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Objective: The intracytoplasmic sperm injection (ICSI) can be altered in combination with either microepididymal sperm aspiration (MESA) or testicular extraction (TESE), for successful in achieving good rates of ongoing pregnancies. Cell surface and intraacrosomal proteins of sperm were tested with monoclonal antibodies (MoAbs).

Design: Clinical results (transfer rate, pregnancy rate per transfer and delivery rate per transfer using ejaculated, epididymal, testicular fresh and frozen/thawed spermatozoa) were compared to physiological stage of spermatozoa.

Materials and Methods: Over a period three years a total of 1359 cases were included in this study. The epididymal sperm was obtained in 26 cycles by aspiration procedure (No. 1), these sperm after freezing/thawing procedures in 5 cycles were tested as experimental group (No. 2). The following group (No. 3) were sperm extracted from testes by extraction procedures in 29 cycles and last group (No. 4) were extracted spermatozoa after freezing/thawing procedures in 11 cycles. Fresh ejaculated spermatozoa were used as a control in 1304 cycles.

Results: The clinical results and immunocytochemical analysis are summarized in follow table:

Spermatozoa (Origin/Group No):	Aspirated		Extracted		Ejaculated
Studies:	1	2	3	4	5
Transfer rate (%)	89[a]	100	79[a]	63	90[a]
Pregnancy rate per transfer (%)	61[b]	25	30[b]	60	35[b]
Delivery rate per transfer (%)	35[c]		27[c]		26[c]
IF: Acrosome labelled cells (%):					
MoAb: H 8	38	36	25	48*	53
MoAb: H 14	34	34	19	14	54
MoAb: H 36	56	55	32	55*	58

* less than 100 cells were evaluated.

No statistically differences were found in transfer rate ($P^a = 0,14$) and delivey rate ($P^c = 0,68$). Pregnancy rate was different ($P^b = 0,03$). The best results achieved group No. 1 ($P = 0,05$)

Conclusion: Results indicated the application facility of all sperm categories in ICSI method. Clinical results are supported by our immunocytochemical findings.

This work was supported in part by the grant 3911/3 from Ministry of Health of the Czech Republic.

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Pronuclear Pre-Embryo Morphology Can Predict Pregnancy Outcome. A. S. Potts, R. Pyrzak, R. P. Dickey, S. N. Taylor, P. H. Rye, P. Y. Lu, S. Sartor. Fertility Institute of New Orleans, New Orleans, LA, USA.

Objectives: The purpose is to evaluate the morphological dynamics of a pronuclear (PN) stage embryo and on to transfer and cryopreservation. We wanted to find out if these morphological characteristics correlated with further embryonic development.

Design: Prospective randomized trial performed in a private infertility clinic.

Materials and Methods: 39 patients were chosen randomly and their subsequent 324 pre-embryos displaying two pronuclei were examined at 18 hours (± 2 hours) and rated according to the pattern of (1) cytoplasmic granularity, (2) pronuclear contact, and (3) nucleolar alignment and given a cumulative score. Embryos with central cytoplasmic granularity and a clear peripheral ring were scored 5,3 or 1 for pronounced, partial or no delineation, respectively. Pronuclei association ranging from compressed contact, minimal contact or no contact were scored 5, or 1. Nucleoli arrangement within pronuclei received a score of 5 if both sets were aligned, grade 3 if one set are in a line or clustering near the adjacent pronucleus and score 1 if scattered. These numbers were computed as a percent of a maximum possible score. Average age was 32.9 years (24.0–43.2). Superovulation was achieved by down regulation with GnRHa (Nafarelin nasal spray, Synarel) followed by ovarian stimulation with hMG or FSH. Oocyte retrieval followed 36–37 hours after hCG administration (10,000 IU).

Results: Of the 39 patients receiving embryos, 16 became pregnant. The pregnant patients received a total of 62 embyros (average 3.8 embryos/transfer). 56 (90%) embryos had a PN score of 51% or higher. Six embryos (10%) scored 0–50%. Of the 23 non-pregnant patients 65/86 embryos (76%) had a PN score of 51% or higher. The remaining 21 embryos (24%) scored 0–50%. An additional 147 embryos were scored but did not culminate in transfer or blastocyst development. No transfers took place in which all PN were scored <51%. The average age for pregnant and non-pregnant patients was 31.0 and 35.6 years, respectively. The average pronuclear score for ages <35 and ≥35 years was 70.7% and 70.8% for patients who became pregnant, and for patients not pregnant was 63.5% and 61.0%, respectively. Of 23 transfers in which all of the embryos in the transferred cohort had a PN score of 51% or higher, 12 pregnancies ensued (43%). Of the 16 transfers in which at least one of the embryos in each transferred cohort was scored below 51% four pregnancies resulted (25%).

Conclusions: Chance of pregnancy increases with better quality PN pre-embryos as defined by this scoring system. Morphological observation of PN pre-embryos can indicate the quality of the embryo in the next days. In addition this may help select embryos that can give higher chance of implantation and pregnancy resulting with live birth. Further study may be developed to predict blastocyst development for cryopreservation or for blastocyst transfer.

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Intracytoplasmic Sperm Injection (ICSI) in Cases of Non-Male Factor Infertility. V. M. Sopelak, R. S. Hines, J. D. Isaacs Jr, B. D. Cowan. Obstetrics and Gynecology Department, University of Mississippi Medical Center, Jackson, MS.

Objectives: The use of ICSI for male factor infertility is effective, while its benefit for additional patient populations is less clear. This retrospective study was done to evaluate whether fertilization (fert.) failure could be prevented by injecting half of the oocytes from a physician selected population of patients that excluded male factor infertility.

Design: Fertilization rates, embryo transfers, pregnancy rates and outcome were evaluated in these combination (COMBO) cycles (N = 34) where a portion of oocytes were inseminated (Insem) and a portion were injected (ICSI). Data were contrasted with concurrently done regular IVF cycles (N = 138) where oocyte insemination was standard.

Materials and Methods: Retrospective analysis was done on cycles performed at our institution between 1997 and January 1999. Any cycles with severe male factor ($<20 \times 10^6$ sperm/ml), with no oocytes recovered, or with all oocytes injected were excluded. For included cycles, the females age was 26–46 and the sperm count/ml ranged from 21×10^6 to 262.5×10^6. Criteria for selection into the COMBO group was based on a clinical suspicion of fert. failure after a documented long history of infertility, lack of a well defined etiology and an absence of prior conception with the current partner. Similar ($P < 0.01$) numbers (Mean ± SEM) and quality of oocytes were injected and inseminated in these COMBO cycles (injected = 7.2 ± 0.6; insem. = 6.7 ± 0.6). These numbers are also similar to those inseminated with our standard IVF (7.8 ± 0.4). This cycle was the first IVF attempt in 82% of cycles in the COMBO group.

Results: In the COMBO cycles:

	Inseminated	ICSI
Fertilization rate	26.1 ± 5.4%	62.4 ± 3.8%
Cycles (Yes Fertilization)	20	34
Cycles (No Fertilization)	14	0

No fert. of insem. oocytes occurred in 14/34 = 41.2% of cycles resulting in a lower fert. rate ($P < 0.05$), while successful fert. was achieved with ICSI in all 34 cycles. The use of ICSI in these 14 cycles, where standard insemination failed, made embryos available for transfer, resulting in 4 positive pregnancy tests and 3 deliveries. Overall, there were 10 pregnancies from 34 transfers in the COMBO group for a pregnancy rate/transfer = 29.4%. In contrast, the fert. rate in our regular IVF cycles was 56.8 ± 2.7% (due to complete fert. Failure in 17/138 = 12.3% cycles) with 121 embryo transfers, 46 pregnancies and a pregnancy rate/transfer of 38%.

Conclusions: Performing ICSI on a portion of oocytes can decrease the incidence of IVF cycles with complete fert. failure. While the use of ICSI may not increase the overall pregnancy rate, it can increase the number of cycles with embryos available for transfer. This approach is useful in cases of unexplained infertility with no prior history of conception.

P-469

In Vitro Blastocyst Formation In Two Embryo Culture Systems. M. Gvakharia, J. Hubbard, M. Jablonsky, A. H. Kim, H. P. Nelson, G. D. Adamson. Fertility and Reproductive Health Institute of Northern California, San Jose.

Objective: To evaluate the effect of Preimplantation stage one (P1)-Blastocyst medium embryo culture system versus Human Tubal Fluid (HTF)-M3 media on human blastocyst development by comparing blastocyst development rates between embryos derived from these two systems.

Design: Initial blastocyst culture protocol was based on the use of HTF and M3 media, which was subsequently replaced with P1-Blastocyst medium culture system.

The study is a retrospective analysis of 168 IVF patients, whose excess embryos were cultured for 2–3 days post-embryo transfer. The resulting blastocysts were cryopreserved for the future use.

Materials and Methods: Patients were divided into two groups - "HTF-M3" and "P1-Blastocyst medium" group (59 and 109 patients respectively). Depending on the laboratory protocol used, all normally fertilized oocytes were cultured in HTF or P1 medium until embryo transfer on day 3. Following embryo transfer, remaining embryos were transferred to M3 or Blastocyst medium respectively for subsequent development. In vitro blastocyst formation was assessed on day 5 or 6 of the embryo culture.

Results: There was no significant difference in mean maternal age (34.6 vs. 34.0), number of retrieved oocytes (16.0 vs. 17.5), fertilization (66.8% vs. 65.7%) and embryo cleavage rates (98.5% vs. 98.8%), mean number of blastomeres (5.2 vs. 5.1) and grades of the remaining embryos between the two groups.

Overall, 1082 supernumerary embryos were cultured for possible blastocyst development, resulting in 342 blastocysts (31.6% overall blastocyst development rate). A blastocyst development rate of 34.5% was observed in P1-Blastocyst medium group, versus 23.5% in HTF-M3 group ($p < 0.01$).

Conclusion: The blastocyst development rate of embryos cultured in P1-Blastocyst medium system is statistically higher than blastocyst development rate of embryos cultured in HTF-M3.

P-470

The Rate of Human Embryo Development to the Expanded Blastocyst Stage Influences Clinical Pregnancy Rate. E. Johnson, W. A. Caswell, R. I. Hardy, M. A. Lee, M. M. Guarnaccia, J. C. Petrozza, V. R. Cardone. Fertility Center of New England, Reading, MA.

Objective: This analysis was designed to assess the rate of embryo development to the expanded blastocyst stage on Day 5, Day 6 or Day 7 after oocyte retrieval and its relation to the overall clinical pregnancy rate per transfer.

Design: A retrospective study over a four month period of 352 IVF cycles from September 1998–January 1999. Inclusion criteria for this study were: 1) blastocyst transfer, 2) <39 years of age, 3) first attempted IVF cycle, and 4) no micromanipulation performed. These criteria produced 60 patients for analysis.

Materials and Methods: Ova were harvested via transvaginal follicular aspiration with ultrasound guidance. Ova were collected and inseminated with a concentration of 120,000 sperm/ml and cultured under oil in 500 μL of S-1 media. At 18–20 hours, ova were assessed for pronuclear development. Fertilized zygotes were cultured in fresh S-1 for days 1–3 and S-2 media for days 3–7 (Scandinavia IVF). All embryos were cultured at 37°C in a humidified environment of 5% CO_2, 95% air. Embryos were examined daily. Transfers were performed at the blastocyst stage. Blastocyst transfers occurred when at least two blastocyst were fully expanded on Day 5 (120 hrs), Day 6 (144 hrs) or Day 7 (168 hrs) post retrieval. Groups were statistically analyzed using analysis of variance (ANOVA).

Results: Clinical Pregnancy was determined by the presence of an intrauterine gestational sac. Blastocyst transfers occurred on Day 5 in 48% (29/60), on Day 6 in 38% (23/60) patients and on Day 7 in 13% (8/60). Clinical Pregnancy Rates per transfer were as follows: Day 5 = 66% (19/29), Day 6 = 34% (8/23) and Day 7 = 0% (0/8).

Conclusion: Based on our results, the rate of development of embryos to the expanded blastocyst stage correlates with Clinical Pregnancy Rates. According to our data, if embryos do not reach the expanded blastocyst by Day 6 of culture, an embryo transfer would not be warranted.

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The Effect on Clinical Pregnancy Rates of the Transfer of Human Blastocysts Following Intracytoplasmic Sperm Injection (ICSI). E. Johnson, W. A. Caswell, M. A. Lee, R. I. Hardy, M. M. Guarnaccia, J. C. Petrozza, V. R. Cardone. Fertility Center of New England, Reading, MA.

Objective: The aim of this study was to investigate the influence of ICSI on human blastocyst development and clinical pregnancy rates.

Design: A retrospective clinical study of 431 IVF cycles over a five month period. Inclusion criteria for this study were: 1) blastocyst transfer, 2) <39 years of age, 3) first attempted IVF cycle and 4) ICSI versus non-ICSI. These criteria produced 87 patients for this analysis.

Materials and Methods: A total of 61 patients from routine IVF

(non-ICSI) and 26 patients undergoing ICSI were studied. Ova were harvested via transvaginal follicular aspiration with ultrasound guidance. Ova were collected in IVF-50 (Scandinavian IVF). Ova for routine IVF were inseminated in S-1. Ova for ICSI were injected in modified HTF-Hepes buffered +5% bovine serum albumin (Irvine Scientific) and cultured in S-1 (Scandinavian IVF). At 18–20 hours, ova were assessed for pronuclear development. Embryos were cultured from Day 1–3 in S-1, and Days 3–6 in S-2. Only patients with blastocyst development by Day 5 or 6 with Day 0 being defined as day of retrieval were considered for this study. Blastocyst development was defined as the presence of a blastocele cavity. Groups were statistically analyzed using analysis of variance (ANOVA).

Results: A clinical pregnancy was determined by the presence of an intrauterine gestational sac. The clinical pregnancy rate (CPR) per transfer for non-ICSI patients was 50% (30/60) with 98% (60/61) having at least 1 embryo develop to the blastocyst stage; eighty-eight percent (53/60) has at least two fully expanded blastocysts available for transfer. The CPR per transfer for ICSI patients was significantly less with a CPR of 36% (8/22). In the ICSI group, 84% (22/26) had at least one embryo develop to the blastocyst stage and only 68% (15/22) had two fully expanded blastocysts available for transfer. Within the ICSI group, when at least two fully expanded blastocysts were available for transfer, the pregnancy rates were not significantly different between the ICSI and non-ICSI groups.

Conclusion: Clinical pregnancy rates in ICSI blastocyst transfers were significantly less that that of non-ICSI patients. Sperm quality may influence embryo development as measured by the overall progression to the blastocyst stage and negatively impact clinical pregnancy rates.

P-472

The Differences in Implantation and Pregnancy Rates of Different Forms of Male Factor Infertility Can Be Negated With Day 5 Blastocyst Transfers. L. Scott, R. Alvero, J. Broussard, J. Presto, J. McKeeby, B. Miller. RSC of the Combined Federal Program of WRAMC, NNMC, USUHS, Washington, DC 20307.

Objectives: It has been suggested that embryos arising from ICSI are of lower quality which may affect implantation rates. Further, that day 5 blastocyst transfers (ET) can increase implantation rates and reduce the numbers of embryos needed to establish a pregnancy. To address this issue, the data from ICSI and conventional in vitro fertilization (IVF) using day 3 or day 5 ET's was analyzed to establish whether difference did exist and if a day 5 ET could negate this.

Design: The fertilization rate, percent of high grade embryos and blastocysts, number of embryos transferred, implantation rate and ongoing pregnancy rate were determined for 3 different male infertility types: low count/motility (LCM), vasectomy reversal (VR) or MESA/TESA (MT) patients having day 3 or 5 ET's and compared with a similar group of IVF patients in the program over the same time period. All patients 40 y and less were included.

Materials and Methods: All sperm preparations were performed using a density gradient separation technique in HTF medium with 5% HSA. IVF and embryo culture (day 1–3) were in P1 medium (Irvine Scientific) or S1 (ZIVF) and extended culture (day 3–5) for blastocyst formation in S2 medium. ICSI was performed in hepes buffered HTF 3–5 h after retrieval (OR), 36 h post-hCG. ET's were performed on the morning of D3 (72 h post OR) or the morning of D5 (120 h post OR). A clinical pregnancy was documented after an ultrasound at 6 weeks gestation.

Results: 104 ICSI procedures were performed which included 69 LCM, 21 VR, 14 MT. The control group included 191 OR. There was no difference in the ages between groups or in the fertilization rates between the ICSI groups but the IVF group had a higher fertilation rate (65 vs. 76, $P < 0.05$). There was no difference in the number of high grade embryos between any group (46% to 51%) or the rate of blastocysts formation (39% to 42%). The mean number of embryos transferred on D3 was higher than on D5 for all groups ($P < 0.01$) with no difference between them. The implantation and ongoing/delivery pregnancy rates for the different groups were as follows:

	ICSI	Low count/ Motility	Vas Reversal	MESA TESA	IVF
Mean # embryos ET					
Total/D3/D5	3.0/3.2/2.1	3.2/3.3/2.3	2.8/3.1/2.0	2.7/3.0/2.0	3.0/3.4/2.1
Ongoing Rate (%)					
Total/D3/D5	55/47/51[a]	41/40/57	38/21/71	64/30/80	55/47/71
Implantation (%)					
Total/D3/D5	17[b]/14/39	16[b]/14/38[b]	17[b]/9[c]/43	32/30/38	25/19/46

a = NS vs IVF; b = $P < 0.01$ vs IVF; c = $P < 0.001$ vs IVF.

Conclusions: There was a significant difference in the implantation and pregnancy rates of the 3 ICSI categories. The use of extended culture and day 5 blastocyst transfers overcame this, resulting in rates equivalent to those obtained with IVF. The use of D5 ET's can substantially increase the positive prognosis for all ICSI patients.

P-473

The Effect of Assisted Hatching on the Outcome of Assisted Reproductive Technology Cycles in Women Under 39 Years of Age. I. S. Laffoon, J. E. Sokoloski, E. A. Volk, L. Hughes, D. M. Krivinko, J. S. Sanfilippo, A. N. Wakim. Department of Obstetrics and Gynecology, Allegheny University of the Health Sciences, Allegheny General Hospital, Pittsburgh, PA.

Objectives: It has been well established that performing Assisted Hatching (AH) on developing 8 cell embryos prior to transfer for women who are older than 39 years of age may improve the pregnancy and implantation rates. This study addresses the efficacy of AH in a patient population of women 39 years of age or younger.

Design: A randomized, prospective, IRB approved study design in which in vitro fertilization and embryo transfer (IVF-ET) patients were assigned to a Hatching protocol (Group A) or a Non-Hatching protocol (Group B) at the time of human Chorionic Gonadotropin (hCG) administration.

Materials and Methods: IVF-ET patients who met the age criteria and had given prior consent to the study, were randomly assigned, at the time of hCG administration, to either Group A in which the embryos had AH and a day 3 transfer or Group B in which the embryos were transferred on day 2 following insemination. The pregnancy rate for each group was the main outcome compared. AH was performed on 8 cell embryos by traditional partial zona dissection, making the slit slightly larger than the average blastomere diameter. Other cycle parameters analyzed included: female age, total cycle number, estradiol at time of hCG, total amps of medication used, days of stimulation, number of follicles aspirated, eggs retrieved, percent fertilization, number of embryos transferred and the semen parameters at the time of insemination. Student -t or chi-square analysis as appropriate, determined statistical significance.

Results: In this study, 56 patient couples completed IVF cycles. Each group was comprised of 28 patients. The pregnancy rates for the study groups were not statistically different. (Group A (9/28) 32.1%, Group B (10/28) 35.7%, p = ns). All of the other cycle parameters monitored also revealed no significant differences.

Conclusion: Assisted Hatching, although indicated by many published studies for women older than 39 years of age and/or observed zona criteria, offered no significant advantage for younger women undergoing initial cycles of IVF-ET. This work was supported in part by a grant from the Allegheny-Singer Research Institute.

P-474

Late Third Pronucleus (PN) Formation for Zygotes That Displayed Two Pronuclei During First Fertilization Assessment. G. K. Adaniya, P. A. Schnarr, A. R. Hall, J. C. Ketner, K. A. Buss. Midwest Reproductive Medicine, Indianapolis, IN.

Objectives: A recent study recommended observing normally fertilized embryos in the afternoon of the day of fertilization check in order to determine which embryos were the fastest developers. Zygotes in which the two PN had disappeared or those that had already cleaved to the two-cell stage were separated from the main cohort of zygotes, the thought being to transfer these embryos preferentially. While doing this at our assisted

reproductive technology (ART) facility, we noticed a secondary phenomenon, the late formation of a third PN. Since these zygotes are considered abnormally fertilized and are discarded, we have begun routinely checking for their presence. We report here the incidence of late third PN formation.

Design: Retrospective analysis of all cycles in which a late pronucleus was observed.

Materials and Methods: Oocyte retrieval was performed 36 hours after human chorionic gonadotropin injection, and the insemination done three hours later. Fertilization was assessed 14–19 hours post insemination. Zygotes exhibiting two pronuclei were separated from the other oocytes. Approximately 4–6 hours after the initial fertilization check, the zygotes were again examined and any zygotes with three pronuclei were discarded.

Results: Late third pronucleus formation was seen in 2.7% (13/482) of the cycles analyzed. In these 13 cycles, the polyploidy rate was 18.2% (26/143), while the polyploidy rate in the remaining 469 cycles was significantly lower at 8.0%. Average age for the late three PN patients was 35.3 years, not significantly different than the rest of the population at 34.6 years.

Conclusions: Although two PN zygotes are placed into fresh media drops without spermatozoa present, late third PN formation was observed in 2.7% of all cycles. Previous cytogenetic studies have shown that 82% of tripronucleated zygotes are chromosomally abnormal. Since these triploid zygotes undergo cleavage, it is virtually important to identify these zygotes prior to the first cleavage in order to distinguish them from normally fertilized zygotes. A second check for pronuclei helps to find these late developing polypoid zygotes.

P-475

Pregnancy Failure Is More Accurately Predicted by Split Ejaculate Semen Evaluation. [1]P. E. Lethin, [1,2]L. J. Burkman, [2]K. Crickard, [2]F. Gonzalez, [2]M. W. Sullivan. [1]Department of Urology and [2]Department of Obstetrics and Gynecology, SUNY at Buffalo, NY.

Objectives: Efficient and accurate assessment of sperm function is critical to appropriate assignment of infertile patient couples to either IUI, IVF or ICSI. We evaluated semen parameters from split ejaculates (2 portions) versus whole ejaculates. These data were assessed to determine whether split ejaculate analysis can *better* predict pregnancy failure.

Design: We examined the split and whole semen analysis data as well as the pregnancy outcome from 144 couples who had visited our andrology lab and infertility clinic. These included 59 fertile couples (PREGNANT) and 85 other couples (NOTPREG) who had not achieved a pregnancy by 1 year after their semen analyses.

Methods: Ejaculates were collected in holding medium. CASA sperm evaluation included count, log (count), motility (mot), velocity, amplitude (alh), hyperactivation (ha) and strict morphology (mor). Individual parameters were compared statistically within and between 4 groupings (PREG, NOTPREG, split ejaculate, whole ejaculate) using "t"-test, frequency distributions, and calculation of the lower 25th percentiles of the confidence intervals (cut-offs).

Results: As expected, the sperm were highly concentrated in Split 1 (PREG: 165 ± 32 M/ml in Split 1 versus 84 ± 9 in the whole ejaculate). The (ha) analysis confirmed our previous findings, that (ha) of <5% predicts failed pregnancy. For *whole* ejaculates, the PREG data were significantly higher than NOTPREG for log (count), mot and mor ($p = .004$, $p = .04$, $p = .03$, respectively). The Split 1 data for PREG versus NOTPREG were more informative. The NOTPREG group had significantly lower values for 5 parameters: count, log (count), mot, mor and alh. We then utilized the PREG data to define sperm criteria which could predict pregnancy failure. Therefore, identification of the lower 25th percentile for the 5 parameters yielded cut-off values. Separate cut-off criteria were derived from whole data versus Split 1 data and were then applied to the NOTPREG data file. The set of cut-offs derived from split ejaculate data (logcount ≤2.0, mot ≤58%, mor ≤3.6%, alh ≤4.7) was *more discriminating* than cut-offs taken from PREG whole ejaculate data. *Most importantly, 88% of all the NOTPREG cases were correctly identified by the Split 1 cut-offs*, compared to only 69% when using the whole ejaculate cut-offs.

Conclusions: Correct identification of semen with fertility problems is more likely using *split ejaculate* analysis. Within our data, 88% of the non-fertile cases were identified. Prospective use of these cut-offs may facilitate appropriate assignment of infertile couples to the most reasonable ART therapy (supported by departmental funding).

P-476

Does the Production of Blastocysts from Supernumerary Embryos Predict Pregnancy Outcome After Day 3 ET? [1]C. Chapman, [1,2]V. Karande, [1]J. S. Rinehart, [1,2]N. Gleicher. [1]The Center for Human Reproduction-Illinois and [2]The University of Illinois at Chicago, Illinois, USA.

Objective: To determine whether the ability to produce blastocyst stage embryos from a cohort of embryos was predictive of pregnancy potential for randomly selected embryos from the same cohort that were transferred on day 3.

Design: Retroactive analysis of 118 consecutive IVF cycles performed at a medical school affiliated infertility center.

Materials and Methods: Patients underwent standard down regulation and stimulation protocols followed by oocyte retrieval. Laboratory cultures were performed in a sequential medium regimen of P1 supplemented with synthetic serum substitute (Irvine Scientific, Santa Ana, CA) followed by S2 (ZIVF, Vero Beach, FL). Culture conditions were 5% CO_2 in air in a humidified atmosphere at 37°C. On day 3, ≥6 cell embryos of good quality were pooled. Embryos from the pool were randomly selected for transfer and the remaining embryos underwent additional culture for 72 hours in S2. Those embryos which formed good quality blastocysts with a distinct inner cell mass and healthy trophectoderm as observed under a 40× Nikon inverted scope were cryopreserved using a modified Menezo method. Data were analyzed by chi-square.

Results: Out of 118 consecutive IVF cycles resulting in supernumerary embryos after day 3 ET, 77 (65%) allowed for at least 1 embryo to reach the blastocyst stage, while in 41 cycles (35%) no embryo reached the blastocyst stage. Of the 77 cycles that reached the blastocyst stage, day 3 transfer resulted in 46 pregnancies (60%), while of the 41 cycles that failed to reach the blastocyst stage 18 (44%) achieved pregnancy. The difference in pregnancy rate was not statistically significant ($p = 0.4561$).

Conclusion: Whether supernumerary embryos after day 3 ET reach the blastocyst stage is not predictive of the pregnancy potential of embryos transferred on day 3. The culture of supernumerary embryos to the blastocyst stage would, therefore, appear economical only if it can be demonstrated that embryos frozen at the blastocyst stage generate better pregnancy rated than embryos frozen on day 3.

P-477

Embryo Cryopreservation on Day 1, 2 or 3: Transfer and Pregnancy Rates. W. D. Hazlett, P. A. Mangan, C. W. Chapman. ART Program, The Center for Human Reproduction, Chicago, IL.

Objective: To evaluate post-thaw survival of embryos cryopreserved on day 1, 2 or 3 and subsequent clinical pregnancy rates following day 3 transfer.

Design: Retrospective data analysis of 208 FET cycles.

Materials and Methods: Day 1, 2 or 3 conventional IVF- and ICSI-derived embryos were cryopreserved using a 2-step protocol of 1.5 M PrOH (15 minutes) followed by 1.5 M PrOH with 0.1 M sucrose (10 minutes). Embryos were loaded in 0.25 mL straws, cryopreserved in a Planer Kryo 10 programmable freezer using a standard slow freeze protocol. Upon thawing, the cryoprotectant was removed stepwise in 5 minute intervals in decreasing concentrations of PrOH (1.5, 0.5, and 0 M) and 2.0 M sucrose. All embryos were cultured in P1 supplemented with 10% Synthetic Serum Substitute until the time of transfer on day 3. Embryos ≥4 cells with ≤20% fragmentation were transferred to recipient patients. Embryo transfer and clinical pregnancy rates (positive hCG) was analyzed using Chi-square analysis.

Results:

Parameter	Day 1 (Pronuclear Stage)	Day 2 (2–4 Cell Stage)	Day 3 (4–8 Cell Stage)
# Thaw Cycles	111	74	23
# Embryos Thawed	629	359	95
# Embryos Transferred (%)	419 (66.6)	245 (68.2)	61 (64.2)
Clinical Pregnancy/Transfer (%)	45/105 (42.9)	24/67 (35.8)	5/19 (26.3)

Conclusion: Results suggests embryo cryopreservation on day 1, 2 or 3 does not effect transfer rate, however, there was a trend ($P = 0.176$) of

reduced pregnancy rates following transfer of embryos frozen on day 3 compared with day 1. While extending embryo culture beyond the pronuclear stage may provide further selection of good quality embryos, it may compromise embryo viability with regard to cryopreservation.

P-478

Initial Clinical Evaluation of the Fertilase (MTM) Laser For Assisted Hatching of Embryos of Male Factor ICSI Patients. J. Meriano, R. F. Casper, S. Visram, J. Alexis. Toronto Centre for Advanced Reproductive Technology, Toronto, Ontario.

Objective: To determine the efficacy of the Fertilase laser for assisted hatching of embryos in male factor patients undergoing ICSI.

Design: A retrospective sequential clinical evaluation comparing the outcomes for patients undergoing ICSI with the use of the Fertilase for assisted hatching as compared to those male factor patients who had no assisted hatching performed.

Patients: All women were stimulated using the long protocol of GnRH agonist downregulation with a luteal phase start. All male patients had at least one semen parameter below the reference ranges for normal specimens as described by the WHO (1992). Group 1: Patients who consented to have their embryos, for transfer, treated with assisted hatching with the Fertilase laser (MTM technologies), (n = 14). Group 2: Patients who did not have their embryos treated with assisted hatching using the fertilase (n = 26).

Methods: Oocyte retrieval, cumulus cell stripping, sperm preparation, ICSI, determination of oocyte and embryo morphology, and embryo transfer were all performed using standard techniques as we have previously described. In group 1, the assisted hatching was preformed at assessment for transfer (approx. 69 hrs post ICSI) on those embryos chosen for transfer. The hole made by the laser in the *zona pellucida* was 18 μm for all patients. *Zona pellucida* thickness was not taken into account in this comparison.

Results: There was no significant difference in patient age (36.2 ± 4.4 vs 32.2 ± 5.15) or estradiol concentration on the day of hCG. In group 1, 84/107 (79%) of the oocytes were MII and were injected with sperm. In group 2, 196/215 (92%) of the oocytes were MII and injected (p = 0.002). Fertilization (58.2% vs 62%) and cumulative embryos score (30.25 vs. 29.7) of those embryos transferred were not different in the two groups. At 70–72 hours after injection, there were 2.23 ± 0.7 vs 2.03 ± 0.6 (ns) embryos transferred in group 1 and 2 respectively. The clinical pregnancy rates were 62% for group 1 and 24% for group 2 (p = 0.044).

Conclusion: The number of patients was small for both groups. However the data did show a trend toward pregnancy difference for the fertilase group which is encouraging for further in depth study of this method of hatching.

P-479

Use of Atomic Force Microscopy (AFM) for Human Sperm Morphology Analysis. [1,3]A. Mai, [1]W. Weerachatyanukul, [4]M. Tomietto, [4]D. Wayner, [2]A. Leader, [1,2,3]N. Tanphaichitr. [1]Human IVF Program, Reproductive Biology Unit, Loeb Health Research Institute, [2]Fertility Center, Ottawa Hospital Civic Campus, Division of Reproductive Medicine, Department of Obstetric and Gynecology, [3]Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, [4]National Research Council, Ottawa, Ontario, Canada.

Objectives: Light microscopy of fixed and stained sperm is the technique used for conventional sperm morphology analysis to predict male fertilizing potential. However, the limitations of light microscopy are two-fold. Firstly, the fixing and staining could distort sperm morphology. Secondly, the resolution of light microscopy is only 0.1 μm, thus leading to variation of the analysis among interobservers or intraobservers. In contrast, AFM, having the atomic resolution (1 Å), does not require sample fixing and staining. Therefore, we, for the first time, employed AFM to analyze sperm morphology. The objectives of our studies were to detect acrosome-intact (AI) and reacted (AR) sperm, and to obtain three dimensional parameters of the sperm head using AFM functions.

Design: In order to decrease variability of sperm head parameters due to the heterogeneity of the ejaculated sperm population, Percoll-gradient centrifugation (PGC) was used to select motile sperm with oval-headed shape. Since the acrosomal cap is lost during the acrosome reaction, we expected the sperm head anterior height of AR sperm to be differed from that of AI sperm. Therefore, the height profile curves along the sperm major axis were used to differentiate between the AI and AR sperm. Although sperm size is variable from sperm to sperm, the maximum height at the equatorial segment (ES), and the distance (D) between the ES and the connecting piece do not change upon the acrosome reaction. To account for the sperm size variability, the change of the sperm anterior height as a result of the acrosomal cap loss was expressed as the ratio of the maximum height to the height in the sperm head anterior, located by 1D distance from the ES.

Materials and Methods: Semen collected from healthy, fertile donors was subjected to PGC. PGC sperm, treated with A23187 to induce an acrosome reaction, were plated onto a cover slip to assess the acrosomal status by FITC-Con A staining, which indicated 20–50% of sperm were acrosome-reacted. The same FITC-Con A stained (AR) sperm and unstained (AI) sperm were imaged by AFM using contact mode to obtain the height profile curves and three dimensional parameters of the sperm head. Localization of the same sperm was aided by their position in the numbered square of an electron microscopy grid, attached underneath the cover slip.

Results: Sperm showing negative staining of FITC-Con A (AI) possessed a height profile curve, having a gradual decrease from the ES to the head anterior, whereas FITC-Con A positively stained sperm (AR) displayed a height profile curve with a sudden decrease in the same region, as anticipated as a result of acrosomal cap loss. Accordingly, the height ratio of the AR sperm head anterior (4.4) was significantly greater than that of the AI sperm head anterior (3.2). As expected, the D and the maximum height were the same for AI and AR sperm, i.e., 2.1 μm and 1.0 μm, respectively. The width at the half maximum height of both AI and AR sperm was also the same, i.e., 2.7 μm, whereas, the length at the half maximum height of AR sperm was decreased significantly to 2.7 μm, as compared to 3.5 μm of AI sperm, suggesting that the human acrosomal cap is thin but long.

Conclusions: AFM was used to differentiate between AI and AR sperm, and to obtain three dimensional parameters of their sperm head.

P-480

In Vitro Culture Medium Without Human Albumin for an In Vitro Fertilization Program. [1]A. Ruiz, [1]A. C. Cobo, [1]I. Pérez-Cano, [1]R. Herrer, [1]S. Ochoa, [1]D. Moreno, [1,2]A. Pellicer, [1,2]J. Remohí. [1]Instituto Valenciano de Infertilidad and [2]Department of Pediatrics, Obstetrics and Gynecology, Valencia University, School of Medicine, Valencia, Spain.

Objective: To evaluate the possibility to use a new medium without human albumin, in order to avoid contamination in human IVF procedures.

Design: This is a prospective study of 85 patients included in our in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) program. In all cycles we used each medium in half twin oocytes. The parameters analized were fertilization and cleavage rates as well as mean number of blastomeres and fragmentation degree. We classified fragmentation in five grades, being grade 1 absence of fragmentation and grade 5 totally fragmented embryos.

Materials and Methods: In 85 IVF cycles with at least 8 oocytes, a total of 1147 oocytes were obtained. Twin oocytes were cultured with both mediums at random; half of them with each medium: A) universal IVF-medium, with human albumin. B) Synvitro medium, with synthetic albumin. Both of them from Medicult, Copenhagen, Denmark.

Results: Results and statistical differences were showed in this table.

				Embryo quality	
	No. of oocytes	2 PN rate	Cleavage rate	No. of blastomeres/ embryo	Degree of fragmentation/ embryo
Medium A	626	66.5	85.1	3.2 ± 0.06*	1.5 ± 0.03
Medium B	521	63.8	78.3	2.7 ± 0.06*	1.5 ± 0.04

* $p < 0.001$.

NS: No significant differences.

Conclusions: These results show that there is no significant differences in fertilization and cleavage rates between both culture mediums. However,

the study of embryo quality revealed a significantly higher number of blastomeres in those cultured with IVF-medium (with human albumin) while fragmentation degree was similar in both groups.

P-481

Improved Recovery of Motile Spermatozoa from Fresh and Frozen Poor Semen Samples and Testicular Spermatozoa Samples Using Micro Processing and Micro Cryopreservation Approaches. C. Lawrence, M.-C. Léveillé. Fertility Center, Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Ottawa Hospital, University of Ottawa, Ottawa, Ontario, Canada.

Objectives: The expanded use of intracytoplasmic sperm injection for male factor infertility has led to the investigation of new approaches for the processing and cryopreservation of poor spermatozoa specimens. In this study, we developed new micro processing and micro cryopreservation techniques and evaluated their efficacy in comparison with more standard approaches.

Design: Prospective experimental study.

Materials and Methods: Four semen samples with <1.5 million motile spermatozoa in the entire ejaculate were divided into five parts and used to compare five methods of sperm processing. (1) 0.5-mL layers or (2) 50-μL layers 45%/90% PureSperm density gradient, or (3) 0.5-mL layer or (4) 100-μL layer 80% PureSperm, or (5) washing/concentration with washing medium. Two testicular biopsy samples were divided into three parts and used to compare methods 2, 3 and 4. Additional poor semen samples (n = 2) and testicular biopsy samples (n = 2) were cryopreserved in 50–100-μL aliquots after proper dilution with a cryoprotective solution and loading into small plastic pockets made from 0.5-mL straws. These samples were used to evaluate the efficacy of the above first four processing methods on frozen-thawed samples and to compare them to a micro washing method by stepwise addition of medium to 5-μL aliquots of thawed sample.

Results: For fresh specimens, the micro processing methods on 100-μL 80% PureSperm layers or on 50-μL 45/90 gradients resulted in the highest number of motile spermatozoa recovered; up to six times the recovery obtained with the more standard methods (i.e. methods 1 and 3). In these cases, washing/concentration-only method also resulted in high recovery of motile spermatozoa but these latter preparations were heavily contaminated with other cell types and debris, in contrast to the preparations recovered after processing using any of the PureSperm methods. Similar results were obtained for the two frozen-thawed semen samples. However, for the frozen-thawed testicular specimens, the micro washing method was more effective and resulted in the recovery of >10 times more spermatozoa. Using the micro washing method, 68 motile spermatozoa washed free of cryoprotective solution were recovered from a 100-μL thawed aliquot of a testicular sample containing 100 motile spermatozoa at freezing. Only 21 motile spermatozoa were recovered after processing on a 100-μL 80% PureSperm layer.

Conclusions: Our micro processing methods are highly effective in improving the recovery of spermatozoa from fresh and cryopreserved poor semen samples and testicular biopsy samples. These methods allow the cryopreservation of specimens with very few spermatozoa and the subsequent recovery of sufficient numbers of spermatozoa after thawing to perform an ICSI procedure. We are currently extending these observations to more specimens and data from this second group will also be presented.

P-482

The Use of the Human Sperm Motility Assay to Assess the Toxicity of Latex and Non-Latex Gloves Used in an IVF Program. C. Lawrence, M.-C. Léveillé. Fertility Center, Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Ottawa Hospital, University of Ottawa, Ottawa, Ontario, Canada.

Objectives: With the introduction of universal precautions for handling human biological specimens and the need for sterility in procedures in an IVF program, gloves are routinely used. This study was performed to assess the potential toxicity to gametes of various types of gloves, latex or non-latex and powdered or powder-free.

Design: The human sperm motility assay was used to assess the toxicity of IVF media that had been exposed, by direct contact, with a variety of commercially available gloves.

Materials and Methods: 10-mL aliquots of Human Tubal Fluid Medium supplemented with 0.5% BSA (HTF-BSA) were each conditioned by 15-second direct contact exposure to one of the eleven brands of gloves under evaluation (4 types of powdered gloves and 7 types of powder free gloves). Powdered gloves were exposed to an aliquot of medium with and without prior rinsing with Milli-Q water. The conditioned media and a control aliquot of HTF-BSA were then allowed to re-equilibrate at 37°C under 5% CO_2/5% O_2/90% N_2 for 3 hours. Semen specimens were processed on 45%/90% discontinuous PureSperm gradients, the resulting sperm pellets were resuspended in control HTF-BSA and divided into the appropriate number of aliquots. Each aliquot was washed twice and resuspended with one of the conditioned media or the control HTF-BSA, to a concentration of 10 million total spermatozoa per mL. One-mL aliquots of these sperm suspensions were incubated at 37°C under 5% CO_2/5% O_2/90% N_2 for up to 48 hours. Sperm parameters, concentration and motility, were assessed at 0 hr, 24 hr and 48 hr. Viability assessment by Eosin Y staining was performed at 48 hr.

Results: At 24 hours, a drop in percent motility of more than 25% was observed for three conditioned media (N-Dex nitrile power free gloves, Curity latex powdered gloves and Gammex latex powdered gloves pre-rinsed with water). At 48 hours, the drop in sperm motility was >50% for these three media. However, the drop in motility in conditioned medium with Curity latex gloves pre-rinsed in water was only 7% at 24 hours. The drop in motility in control HTF-BSA was 0% at 24 hr and 23% at 48 hr. No significant drop in sperm motility was observed for 3 types of powdered gloves pre-rinsed with water and 6 types of powder free gloves, at 24 hours. No significant drop in sperm viability was observed at 48 hours after incubation in any of the conditioned media.

Conclusions: Using the Human Sperm Motility Assay, three out of eleven brands of gloves were shown to be toxic to spermatozoa. This has implications for an IVF program because the gloves come into contact with the dishes, tubes, catheters, media and all other related supplies that are used for handling and culturing gametes. Even if in one case, rinsing of the powdered gloves with water reduced their sperm toxicity, the availability of many non-toxic powder free gloves favors the use of these latter for routine use in an IVF program.

P-483

The Relationship Between Fertility Potential Measurements on Cryobanked Semen and Fecundity of Sperm Donors. A. Johnson, T. Navarrete, B. Mixon, [1,2]D. P. Wolf. Department of Obstetric and Gynecology, Andrology/Embryology Laboratory, [1]Department of Physiology/Pharmacology, Oregon Health Sciences University, Portland, OR, [2]Oregon Regional Primate Research Center, Beaverton, OR.

Objectives: As a response to the AIDS pandemic, quarantined donor semen is now used for therapeutic insemination (TI), however, recipients must attempt more TI cycles because of the exclusive reliance on frozen semen. A substantial burden is also placed on sperm banks in recruiting and screening donors. Here, we correlate sperm penetration assay (SPA) scores, obtained from cryobanked semen, with corresponding fecundity in a group of established sperm donors, thereby evaluating the efficacy of the SPA in screening donors for sperm banking. The optimized SPA measures sperm penetrating ability from either fresh or frozen semen to distinguish the infertile from fertile population. This assay was altered to distinguish performance among fertile donors.

Design: Frozen semen (3 different ejaculates) from 11 pregnancy-proven donors whose samples were used in at least 35 cycles of TI were analyzed for relationships between SPA scores and donor fecundity. The sperm concentration employed in the SPA was also varied in an effort to establish the most sensitive test condition. TI recipients were screened to identify detectable pathology.

Materials and Methods: All donors met minimum WHO semen criteria. Semen was frozen in cryotubes using a glycerol modified test yolk buffer and was stored for 1.1 to 5.1 years. Samples for SPA testing were thawed and held for 18 hours at 4°C before the rapid addition of 6 ml 37°C BWW. Swim-up sperm were tested at 5.00 (routine) or 1.00, 0.50, or 0.25 $\times$ 10^6 motile sperm/ml. Samples for TI were thawed at room temp. for 5 min, held at 37C° for 10 min, washed, and resuspended in 0.5 ml medium before use.

SPA scores were expressed as the mean number of penetrations/ovum (p/o). Aliquots of frozen semen were run in parallel in all SPA runs as controls. Students t-test and linear regressions were performed where indicated using SAS Institute software.

Results: Of 905 TI cycles, 275 recipients achieved 95 pregnancies. Eleven donors participated in from 35 to 134 TI cycles. There were no statistically significant relationships between fecundity and donor semen, washed sperm parameters, sperm recoveries, or recipient age (mean range 33.3 to 36.6). In contrast, a significant relationship ($p < 0.03$) was revealed between routine mean SPA scores (range 8.7 to 66.6 p/o) and donor fecundity (no. pregnancies/no. cycles, range 0.04 to 0.16). While 1.00 and 0.50 SPA concentrations showed the same trend, a highly significant relationship (linear regression, $p < 0.002$) was observed at 0.25×10^6 motile sperm/ml. Tested at this concentration the 4 donors with the lowest SPA scores achieved the 4 lowest fecundities.

Conclusions: A modified SPA can be used on frozen donor semen to estimate donor fertility potential. If applied routinely in donor semen banking, poor quality donors could be culled thereby increasing pregnancy rates while decreasing donor screening costs. A blind prospective trial comparing donor fresh/frozen semen SPA results to fecundity from therapeutic insemination is underway.

P-484

The Increased Risk of Monozygotic Twinning Caused by Mechanical Assisted Hatching is Probably Related to the Size of the Slit in the Zona: a Mouse Model. [1]I. Van der Auwera, [2]A. Wetzels, [3]R. Pijnenborg. [1]Division of Infertility, University Hospital Gasthuisberg, Catholic University Leuven, Leuven, Belgium, [2]IVF Laboratory, Department of Obstetrics and Gynecology, University Hospital Nijmegen, HB Nijmegen, The Netherlands, [3]Experimental laboratorium of Obstetrics and Gynecology, Department of Obstetrics and Gynecology, University Hospital, Gasthuisberg, Leuven, Belgium.

Objectives: After mechanical assisted hatching (AH) an increased risk of monozygotic twinning has been observed. This phenomenon can be related to the hatching blastocyst which stuck within the zona. Therefore, a mouse model was used to evaluate the hatching process after creating different sizes of holes (as extensive as possible) into the zona pellucida at specific stages.

Design: A prospective randomised study with C57BlxCBA mice (the female mice with c.p. were randomised over days 0, 1, and 2).

Materials and Methods: Pronucleate ova (2PN), 2c and 4–8c embryos of C57blxCBA F1 hybrid females were treated with mechanical assisted hatching at the time of collection and compared to untreated controls and to in vivo developing blastocysts. The slits created in the zona of the 2PN extended at least between 10a.m.–2p.m. length while for the 2c and 4–8c stages the slits were smaller (11a.m.–1p.m. length) due to a smaller perivitelline space. The embryos were co-cultured in HTF medium supplemented with 0.5% BSA on a monolayer of human skin fibroblasts. Hatching was evaluated at days 4 and 5 after copulation plug. Statistics were evaluated using Chi-square.

Results:

		in vitro controls			in vitro + AH		
	in vivo controls	2PN	2c	4–8c	2PN	2c	4–8c
N embryos	78	59	85	50	70	85	77
% hatching day 4	61$	31	58	80	94	90	96
% stuck within zona day 5	0	49	37	45	22*	60	55
% totally hatched day 5 (N)	86 (58)	20	38	43	75*	39	44

$ hatched
* $P < 10^{-6}$

After in vitro culture of embryos, the hatching process was delayed compared to in vivo controls ($P < 10^{-6}$). Moreover, hatching progressed totally differently in vivo versus in vitro. The typical 8-form has never been observed in vivo because the zona became thinner until it disappeared. After AH, the hatching process started earlier ($P < 10^{-6}$), but most of the embryos were stuck into the slit of the zona pellucida. Only blastocysts arising from manipulated 2PN were able to escape completely from the zona.

Conclusions: Stucked blastocysts within the zona pellucida due to the size of the slits after mechanical assisted hatching could be an explanation for a higher monozygotic twin rate in human assisted hatching programmes.

P-485

Embryo Quality and Implantation Rate After the Transfer of Cocultured Blastocysts. C. J. Quintans, M. J. Donaldson, M. G. Rocha, R. S. Pasqualini. Halitus Instituto Médico, Buenos Aires, Argentina.

Objective: To investigate the relationship between blastocysts (blcs.) quality assessed by morphological criteria and their ability to originate viable pregnancies after transfer.

Design: Retrospective study.

Materials and Methods: Seventy patients (mean age 32 ± 4 yr.) were stimulated with GNRHa, FSH, hMG and hCG. In 26 cases conventional IVF was used, in 36 ICSI was performed, both techniques were used in 8 cycles. HTF with 10% serum substitute (SSS) was used for fertilization. At pronuclear stage, embryos were cocultured on a Vero cell monolayer in α-MEM with 10% SSS. The obtained blcs. were graded in 3 groups: Group I (GI) (optimum morphology) expanded or fully expanded blastocele, no loose cells nor cellular fragments, continuous trophectoderm layer with well-defined intercellular limits as well as distinct inner cell mass (ICM). Group II (GII) (slightly suboptimal blcs.) show some of these characteristics one at a time: lack of precise cellular limits and/or presence of granulation in trophectoderm layer, less developed blastocele, not well defined ICM. Group III (GIII) (frankly suboptimal blcs.) exhibit two or three of the detrimental characteristics defined for Group II and in some cases added to the presence of isolated cells and other fragments in the blastocele. On day 5 or 6, two to three blcs. were transferred and supernumerary blcs. were cryopreserved.

Results: From all the fertilized oocytes (2 PN) 54.7% reached the blcs. stage after 5 to 6 days in culture, 20 clinical pregnancies (28.6% pregnancy rate (p.r.)) were obtained from a total of 70 patients and other 3 pregnancies were achieved after the transfer of cryopreserved blcs. In 46 patients embryos from a single morphological group were transferred; whereas in the remaining 24, blcs. from more than one group were replaced. In 11 patients, only G.I blcs. (28) were transferred, 16 implanted (59% implantation rate (i.r.)) and 6 pregnancies were obtained (54.4% p.r.). A total of 45 G.II blcs. were replaced to 19 women, 6 implanted and 4 pregnancies were achieved (13% i.r. and 21% p.r. respectively). Other 17 patients were transferred a total of 46 G.III blcs., of which 2 implanted (4.3% i.r.) and 2 pregnancies were obtained (12% p.r.). Three patients who received G. I. embryos did not achieve a pregnancy, and when later on they were transferred with cryopreserved blcs. of the same quality they did get pregnant. In all cases pregnancies belonged to day 5 transfers, we did not obtain any pregnancies after day 6 transfers. Day 6 blcs. always belonged to G. II and G. III.

Conclusions: Reaching the blastocyst stage reveals morphological features more indicative of embryo viability than when only attaining earlier stages. Overall p.r. obtained after blcs. transfer was not different from that obtained in our IVF program after transfer at earlier stages. Nevertheless, when morphology of the transferred blcs. is considered, important differences are observed between results obtained after the transfer of optimum blcs. and those showing different degree of imperfection. The speed of development also seems to be an indicator of viability since no pregnancies were obtained from the transfers performed on day 6.

P-486

Comparison of Three Methods for the Detection of Seminal White Blood Cells: Benzidine-Cyanosine Staining, Myeloperoxidase Staining and LeukoMARQ PLUS™ Test. G. M. Centola, E. Andolina, R. Herko. Andrology Laboratory, Department of Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, New York.

Objectives: WBC infiltration in semen is frequently associated with urogenital infection, antisperm antibodies as well as male factor infertility. This study was designed to evaluate three methods for determining WBC in semen: the standard histochemical assay employing benzidine-cyanosine

(benz-cyan), the myeloperoxidase method using tetramethylbenzidine solution (TMB), and the LeukoMARQ PLUS kit with the AutoMARQER analyzer (Embryotech Laboratories, Wilmington, MA).

Design: Human semen specimens were assessed for WBC concentration using the three methods by two separate technicians.

Materials and Methods: Semen specimens (n = 20) with various WBC concentrations were assayed at 30–45 minutes after ejaculation. For the benz-cyan method, an equal amount of semen and working solution (1 ml benzidine-cyanosine with 50 μl H_2O_2) were mixed, and incubated at 37°C for 30 min. The number of peroxidase positive WBC were counted on a wet mount slide. For the TMB method, 150 μl of tetramethylbenzidine solution was added to 50 μl of semen and incubated at RT for 2 min. Blue stained WBC were counted on a wet mount slide. For the LeukoMARQ assay, 50 μl of semen was loaded into the appropriate well of a test cassette, as was the positive control and indicator solution. The reagent added to the WBCs reacts with myeloperoxidase-containing granules in the WBCs to produce a blue color which is quantified colorimetrically by the AutoMARQER. The data was analyzed by analysis of variance (ANOVA) for between method and between technician variability. Graphs of the means and pooled SD were generated with individual 95% confidence intervals indicated. Statistical significance was set at $p < 0.05$.

Results: There was no significant difference between the LeukoMARQ, the benz-cyan or TMB assays or between the two technicians in performance of either assay. The p values (Tech # 1, Tech # 2) were as follows: LeukoMARQ vs TMB, 0.33, 0.29; LeukoMARQ vs benz-cyan, 0.91, 0.78; TMB vs benz-cyan, 0.30, 0.37. The between technician ANOVA p values were as follows: LeukoMARQ, 0.73; TMB, 0.44; benz-cyan, 0.54.

Conclusion: The LeukoMARQ assay, TMB assay and the standard benzidine-cyanosine assay were comparable for measurement of seminal WBCs. The LeukoMARQ Plus kit provides a rapid (<2 min) method for determining seminal WBCs that does not involve preparation of hazardous chemicals as in the benzidine-cyanosine assay. This assay is easy to perform, reproducible and cost-effective for busy andrology laboratories.

P-487

Effect of Vapors from Marking Pens on In Vitro Mouse Embryo Development. Y. Gong, N. H. Dubin. Union Memorial Hospital, Baltimore, MD 21218.

Objectives: We previously demonstrated that pen marks from some brands of marking pens made directly in incubation wells were toxic to mouse embryo development and some were not. As a more practical matter, we now investigate whether vapors from these pens could be toxic to the embryos.

Design: An experimental design examining effects of marking pen vapors on *in vitro* mouse blastocyst development.

Methods: Hybrid B6C3F1 mice were superovulated with PMSG and HCG and mated with B6D2F1 males. Embryos were harvested at the 2-cell stage and incubated in 0.75 ml Human Tubal Fluid (Q-HTF, In Vitro Care, CA) at 37C, 5% CO2/air in a 4 well dish without added protein or oil overlay unless indicated. In most cases there were 40 embryos per dish. A full circle (~1.5 cm in diameter), 1/2, 1/4, or 1/8 circle were drawn on the lid of the dish with a black marking pen such that it would be directly over a well containing embryos. The mark was made at the time of media equilibration unless otherwise noted. Both Sharpie (Sandford Corporation, Beltwood, IL) and Labcraft (Precision Dynamics Corporation, San Fernando, CA) pens were tested against control wells with no mark. Embryos were observed on day 4 and the stage of development and hatching were noted. Most treatment groups contained 40 embryos. Group differences were determined by chi-squared test.

Results: Control (HTF) embryos all developed from the 2 cell to the blastocyst stage. When a complete circle is drawn with a Sharpie marking pen on the lid above the well containing the embryos, none of the embryos reach the blastocyst stage. For 1/2 circle, 1/4 circle and 1/8 circle, the number reaching blastocysts stage were 0, 12.5, and 50% respectively. When a whole circle is drawn with a Labcraft pen, 96.7% reach the blastocyst stage. A Sharpie mark on the lid also prevented development of embryos in an adjacent well, although these embryos advanced to later developmental stages than those directly under the mark. Of the embryos reaching the blastocyst stage, 62.5% of HTF controls hatched by day 5. Of those blastocysts which developed in the Sharpie treated groups, none hatched. Although most embryos with the Labcraft mark on the lid did reach blastocyst stage only 37.9% hatched which is significantly less than the control ($P < 0.05$).

Conclusion: Vapors from Sharpie markers can affect mouse blastocyst development. Vapors from Labcraft pens do not affect development to the blastocyst stage, but hatching is impaired.