8 difficult, 3 very difficult). The overall pregnancy rate was 32.6% (14 of 43 patients). Of these conceptions, 12 (86%) occurred in patients in whom the embryo transfer was easy, and 2 occurred in patients in whom the embryo transfer was moderately difficult. None of the patients in whom the embryo transfer was classified as difficult or very difficult achieved a pregnancy.

The pregnancy rate in those in whom the cervical dilatation resulted in a subsequently easy embryo transfer was significantly greater than in the group in which the embryo transfer remained difficult despite cervical dilatation (46.2% v 11.8%) ($p=0.03$).

Conclusions: Cervical dilatation carried out at the beginning of an IVF or FET treatment cycle results in a significant improvement in outcome with regard to both ease of embryo transfer and pregnancy rate, in patients whose previous IVF failure was associated with a difficult or failed embryo transfer.

P-204

The Significance of Human Embryo Fragmentation on Pregnancy Rates in Assisted Reproductive Technologies (ART). S. Cuneo, A. Bermúdez, L. Diaz, J. J. Stern, J. R. Verez, A. J. Gutiérrez-Nájar. Grupo de Reproducción y Genética AGN y Asociados, Mexico City, D.F., Mexico.

Objectives: To analyze the effect of aging and ovulation induction protocols in developing embryo fragmentation and its influence in embryo cleavage, blastocyst development, pregnancy and implantation rates in ART procedures.

Design: Prospective, ongoing, comparative study.

Materials and Methods: From May 1st to December 31st, 75 cycles of ART: 28 IVF, 11 IVF-ICSI and 36 ICSI, with embryo transfer were studied, to evaluate the percentage of embryo surface affected with fragmentation, embryo cleavage rate and blastocyst development on the 2nd and 3rd day after fertilization. We compared the type of drugs used for controlled ovarian hyper stimulation (COH), age, pregnancy and implantation rates, between Group I (none fragmentation or <10% of embryo surface affected), Group II (mild, 10–30%), Group III (moderate, 40–50%) and Group IV (severe, >50%). Statistical analysis of the results was performed using χ^2 and ANOVA test, with significance when $p<0.05$.

Results: A total of 494 developed embryos were studied, and 93 of them were place on sequential medium and culture to blastocyst stage. We did not observe any statistical differences between I, II and III groups in: fertilization rate, embryo cleavage, blastocyst development, pregnancy and implantation rates. However, the presence of severe fragmentation (group IV) was correlated with a none pregnancies and none blastocyst development. The relevant results were as follows:

	Group I (n=48)	Group II (n=12)	Group III (n=7)	Group IV (n=8)	*p*
Age (years & DS)	35.0 ± 4.92	29.9 ± 6.11	32.7 ± 5.18	33.8 ± 5.95	NS
Embryo cleavage (%)	85.7	93.3	95.8	100.0	NS
N° blastocyst	19	12	20	0	0.001
N° embryos arrested	20	3	5	14	0.001
Pregnancies & rates (%)	12 (25.0%)	4 (25.0%)	5 (71.3%)	0 (0.0%)	0.001

Conclusions: In our study, the degree of embryo fragmentation showed no correlation with age, drugs and COH protocols. Severe human embryo fragmentation decreased significantly pregnancy rates and blastocyst development in ART procedures.

ART: CRYOPRESERVATION

Tuesday, October 24, 2000

P205

Fertilization and Pregnancy Rates After Intracytoplasmic Sperm Injection Using Cryo-Thawed Ejaculated Semen and Surgically Retrieved Spermatozoa. A. K. Taha, I. Fahmy, R. T. Mansour, M. A. Aboulghad, G. Serour. The Egyptian IVF-ET Center, Maadi, Cairo, Egypt.

Objectives: Cryopreservation of spermatozoa is a routine practice in most centers dealing with assisted conception. The aim of this study is to compare the fertilization rates (FR) and pregnancy rates (PR) in intracytoplasmic sperm injection (ICSI) using cryo-thawed spermatozoa from ejaculates of normal and abnormal semen, epididymal sperm, and testicular sperm from patients with obstructive and non-obstructive azoospermia.

Design: Retrospective review of 196 ICSI cycles using cryo-thawed spermatozoa from 1995 to 1999 at our center was performed.

Materials and methods: Patients were divided into four groups according to the source of sperm; ejaculated semen (ES) cryo-thawed epididymal sperm (EIPD), cryo-thawed testicular sperm from obstructive azoospermia TOB, and from non-obstructive azoospermia (TNOB). FR and PR were compared in 4 groups and with results of 2143, ICSI cycles using fresh spermatozoa during same period of time.

Results: The FR and PR are shown in the table. There was no statistical significant difference in the FR between fresh and cryo-thawed semen in all groups. The PR was lower in all the cryo-thawed groups as compared to fresh except in TNOB grouping which the PR was higher in the cryo group but it does not reach a significant difference.

	ES		EIPD		TOB		TNOB	
Parameter	F	C	F	C	F	C	F	C
Number	1486	112	150	20	269	40	238	24
FR %	54.03	54.8	56.5	57.5	53.9	54.5	46.0	50.7
PR %	29.54	22.3	37.3	35.0	35.3	20.0	22.3	33.3

Conclusion: The fertilizing ability of cryo-thawed spermatozoa is comparable to that of fresh samples irrespective to the source or quality of semen. There was even a trend for a better fertilization in the cryo-thawed group. However, the pregnancy rate is lower in all groups except for non-obstructive azoospermia.

P-206

Effect of Spermatozoa Aneuploidy in Recurrent Intrauterine Insemination Failures. W. W. Lin[1], W. Chuang[1], D. J. Lamb[1,2], J. DeLara[1], F. J. Orejuela[1], L. I. Lipshultz[1]. Department of Urology[1], Department of Molecular and Cellular Biology[2], Department Obstetrics and Gynecology[3], Baylor College of Medicine, Houston, TX.

Objectives: Intrauterine insemination (IUI) for oligoasthenospermia has a pregnancy rate of only 33–18%[1], depending on whether or not ovarian stimulation is employed. The pathophysiology of this poor success rate is unclear. The objective of the current study is to determine whether there is a positive correlation between the rate of aneuploidy in ejaculated sperm of men whose female partners failed more than 3 cycles of inseminations.

Materials and Methods: Semen smears were obtained from the male partners of couples with male factor infertility who failed more than three cycles of IUI's. Semen smears from donors of proven fertility were obtained as controls. Three-color fluorescence in-situ hybridization (FISH) procedure was performed with probes to chromosome 18, X, and Y. The incidence of germ-cell nondisjunction was determined by the fluorescence pattern. 2000 cells per smear were scored.

Results: Semen smears from 10 couples with male factor infertility whose female partners failed more than 3 cycles of insemination were subjected to FISH analysis. 6 donor smears were analyzed as controls. The germ-cell nondisjunction rate was 2.3% for the IUI failure group and 0.65% for the control group respectively.

Conclusions: Germ-cell nondisjunction may be a significant contributing factor to recurrent IUI failures. FISH analysis may be an important screening tool before recommending IUI for couples with male factor infertility. If found to have a high rate of spermatozoa nondisjunction, couples should be counseled accordingly regarding the efficacy of IUI.

1. D. S. Guzick et al., Efficacy of Superovulation and Intrauterine Insemination in the Treatment of Infertility. N Engl J Med 1999;340:177–83.

P-207

Improving Oocyte Cryopreservation: Osmotic Stress and Aquaporins in Human Oocytes. [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 Medical Institute, Department of Reproductive Medicine, Buenos Aires, Argentina.

Objectives: It has been recently reported that rat oocytes lost a mercury sensitive water channel during their arrival to maturity (aquaporine 9). The presence of aquaporins in human oocytes has not been described. During cryopreservation oocyte are under osmotic stress resulting in serious structural changes. The aim of this study was to evaluate the presence of water channels in human oocytes and its potential benefit in improving cryopreservation.

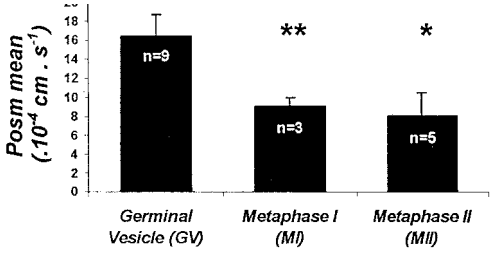
Design: Prospective study of human oocyte membrane properties.

Materials and Methods: 17 oocytes at different maturity stages, were donated by patients who underwent ART procedures. The controlled ovarian hyperstimulation protocol was luteal phase analogue in association with gonadotropin therapy. Patients signed an informed consent. All the oocytes were analyzed individually by the same observer. The assays were per formed creating an osmotic gradient, and volume changes in relation to time were assessed by an original video microscopy technique. Osmotic water permeability (Posm) was calculated as follows:

$$\frac{JV_0}{V_w \cdot S(Osm_a - Osm_i)}$$

JV_0: volume flow through the membrane ($cm^3 \cdot s^{-1}$). V_W: partial molar volume of water (18 cm^3/mol). Osm_e and Osm_i: external and internal osmolarities. S: oocyte surface.

Results:



**p<0.02; *p<0.05.

Conclusions: Osmotic water permeability was significantly higher in GV than in mature stages. These results suggest that an aquaporin can be lost during the transition from GV to MII. This basic biophysical research would be useful in better freezing of oocytes and freezing of ovarian tissue.

P-208

In Vitro Culture of the Human Adult Ovarian Tissues After Vitrification: Comparison Among Detection Methods of the Culture Effect*. [1]S. H. Lee, [1]C. S. Shin, [1,2]J. J. Ko, [1,2]H. C. Lee, [1,2]C. Park, [1,2]K.-A. Lee. [1]Infertility Medical Center, CHA General Hospital, [2]College of Medicine, Pochon CHA University, Seoul, Korea.

Objective: It is necessary to establish the stable in vitro culture method for ovarian tissue banking, however, it is in its infancy. One of the major limits is to observe the follicles during culture of the ovarian tissue. The present study was conducted to evaluate their usefulness among three different methods: lactate dehydrogenase (LDH) assay, vital staining of isolated follicles after culture, and histological analysis.

Materials and Methods: The human ovarian cortex tissues were sliced into 1×1 mm sizes, and cultured on Millicell-CM insert in 24-well culture dishes. Ovarian tissues were pretreated with 5.5 M ethylene glycol containing 1 M sucrose and 10% FBS (EG5.5) for 10 minutes, followed by transferred onto the stainless steel mesh and plunged into the liquid nitrogen immediately. Vitrified tissues were thawed through serial dilution of sucrose solutions, and cultured in vitro for 14 days. Culture medium was α-MEM supplemented with ITS, 5% FBS, and 100 mIU/ml rhFSH. After 14-day culture, tissues were divided into three groups for analyzes. LDH levels were compared with fresh and frozen-thawed cultured tissues, and follicles were isolated and viability was checked with 0.4% trypan blue staining. Finally, histological configuration was analyzed after H&E staining.

Results: Similar levels of LDH values were resulted in fresh (29 mIU/mg) and frozen-thawed (25 mIU/mg) tissues after culture, but the higher values in frozen-thawed control tissues (42.5 mIU/mg). By vital staining, it has observed that the isolated follicles from both of fresh and frozen-thawed tissues were healthy and alive after 14-day culture. By histological observations, conditions for follicles and stroma were nearly the same between fresh and frozen-thawed tissues after culture, and it was not different with the fresh controls, too.

Conclusions: Results of the present study showed that 1) culture system of the present study may be appropriate to culture of the human adult ovarian tissues at least 14 days, 2) tissues after vitrification had the similar capacity in vitro compared to the fresh tissues, and 3) LDH levels of cultured tissues can be used as the indication of the tissue survival.

** Supported by a grant (HMP-98-M-5-0054) of the Good Health R&D Project, Ministry of Health & Welfare, Republic of Korea.*

P-209

High Survival Rate After Cryopreservation of Human Prophase I Oocytes (Germinal Vesicle). [1]E. Sereni, M. A. Bonu, A. Borini, R. Sciajno, [1]M. R. Trevisi, [2]C. Flamigni. [1]TECNOBIOS, Center for Reproductive Health, Bologna, Italy and [2]Department of Obstetrics and Gynecology, University of Bologna, Bologna, Italy.

Objective: The aim of the study was to investigate oocytes survival rate after cryopreservation at germinal vesicle stage (GV).

Design: Sovrannumerary GV oocytes were donated by consenting patients who underwent IVF-ET treatment.

Materials and Methods: GV were collected during oocytes pick-up 36h after HCG administration in patients undergoing ovarian stimulation regimens for IVF-ET cycles. The total cumulus and corona mass was removed with hyaluronidase (80 IU/ml) and oocytes were washed several times in Hepes buffered Human Tubal Fluid medium (HTF) supplemented with 0.5% human serum albumin (HSA). GV were cryopreserved using 1.5M propanediol (PROH) plus 0.1M sucrose in phosphate-buffered saline solution (PBS). Thawing was performed by holding straws at room temperature for 30s and then plunging them in a water bath at 30°C for 40s. The cryoprotectant was then removed by stepwise dilutions (1.0, 0.5, 0.0M PROH) at room temperature and in the presence of 0.2M sucrose in PBS. After thawing, survived oocytes (showing an intact plasma membrane and an intact germinal vesicle) were cultured in Tissue Culture Medium (M199) supplemented with 0.5% HSA, 0.05 mg/ml penicillin, 0.075 mg/ml streptomycin, 0.037 g/l sodium piruvate, 0.1 g/l L-Glutamine. After 35–40h culture period, maturation was assessed by observing the extrusion of the first polar body. Metaphase II (MII) stage oocytes were injected. Fertilization was assessed at 16h postinsemination.

Results: A total of 82 GV were cryopreserved. Sixty six were intact after thawing for a survival rate of 80.5%. Oocytes at MII stage after culture were 44 (66.6%). Seventeen GV arrested at MI stage (25.8%) and only five failed to mature (7.6%). After ICSI, 23 oocytes showed 2PN (52.3%), 11 showed 1PN (25%) and 2 showed 3PN (4.5%).

Conclusion: Our results show that human prophase I oocytes obtained from stimulated IVF cycles show good survival rates and maintain the capability of resuming meiosis after thawing.

P-210

Effect of Cryopreservation Technique on Acrosome Structure of Human Spermatozoa. M. E. Hammadeh, D. Szarvasy, T. George, P. Rosenbaum, W. Schmidt. Department of Obstetrics and Gynecology, University of Saarland, 66421. Homburg/Saar, Germany.

Objective: The freezing and thawing of human spermatozoa are associated with a decrease in sperm quality. Moreover, there is still controversy about the rapid method using uncirculated vapour in liquid nitrogen (fast freezing method) and controlled programmable biological freezer in which the semen is gradually cooled. Therefore this study was undertaken to compare the efficiency of slow computer-controlled freezing and fast vapour freezing, on the acrosome integrity and morphology of human spermatozoa.

Design: Prospective controlled study.

Materials and Methods: 75 semen samples were obtained from patients attending our IVF and Andrology laboratory for semen analysis. Each