chromosome condensation (PCC) in the penetrating sperm nucleus. It may be because of fertilization failure in IVF and the formation of PCC may be associated with the immaturity of oocyte. The objective of this study was to determine if ICSI could alter the incidence of PCC.

Design: The success ICSI in terms of fertilization rate and resulting pregnancies using two methods of oocyte activation and sperm immobilization was evaluated. The incidence of PCC occurring in unfertilized oocytes from two groups was compared.

Materials and Methods: ICSI cycles (n=53) was performed for couples suffering from severe male infertility and dysfunction of fertilization. According to the states of sperm used and treatment for oocyte activation, ICSI cycles were retrospectively analyzed in two groups. In group A (38), sperm was immobilized by touching the tail either at its "tip" or in the "midpiece". The ooplasm was slightly aspirated into the pipette. In group B, sperm was immobilized by a forceful immobilization of the sperm so that a bending of the tail was observed ("hard" touching). The ooplasm was aspirated gently until an outflow of cytoplasm was visualized in the injection pipette. Outcome of ICSI in terms of fertilization and pregnancy rates in each group were analyzed with χ^2. Unfertilized oocytes (n=38, 10) from two groups were studied with cytogenetic method.

Results: A total of 396 metaphase II oocytes out of 481 were used for ICSI. Results were divided into two groups (n=38, 15). Oocyte damage dropped from 14.2% in group A to 8.4% in group B. Normal fertilization for each group was 55%, 90%, respectively ($p<0.05$). Pregnancy rate per egg retrieval was 13.2% in group A and 46.7% in group B ($p<0.05$). There were 19.4% of PCC occurring in group A and none in group B.

Conclusion: This study indicates that ICSI could not only yield high fertilization rates (90%), but also minimize the incidence of PCC. It may directly relate to two crucial steps (immobilization of sperm and oocyte aspiration) used in ICSI procedure. This study also suggests that it is possible to overcome one cause of IVF fertilization failure resulting from the formation of PCC by using improved ICSI technique in the future.

O-098

The Linear Increase in Pregnancy Loss After Clinical Pregnancy in IVF Is Related to Chromosomal Abnormalities. S. D. Spandorfer, O. K. Davis, I. Kligman, L. I. Barmat, H. C. Liu, A. Kowalik, Z. Rosenwaks. The Center for Reproductive Medicine and Infertility. The New York Hospital-Cornell University Medical Center, New York, NY.

Objective: The fetal loss rates following a documented fetal heart (FH) during a first trimester trimester U/S in spontaneous, non-stimulated conceptions have been shown to be approximately 3% (JAMA 258:2555). To date, no large studies evaluating the outcome of IVF pregnancies after demonstrating a FH have been described. The purpose of this study is determine the fetal loss rate after a documented FH and to evaluate the chromosomal makeup of these losses.

Design: Retrospective chart review

Materials and Methods: 2346 consecutive IVF clinical pregnancies (positive fetal heart) were reviewed. Results of pregnancy outcomes were analyzed by age group. Chromosomal studies when obtained were reviewed. Statistical analysis was accomplished by utilizing the Chi-square test for trend and Student's T test. P values <0.05 were considered significant.

Results: 39 pregnancies were not included in the analysis because these losses were related to amniocentesis complications, elective termination after findings of chromosomal or congenital anomalies, or losses due to incompetent cervix. The overall pregnancy loss rate after demonstrating a FH during a 7 week U/S was 11.31% (261/2307). A highly significant trend demonstrated an increase in fetal loss when comparing the four age groups ($\leq$30 yrs=4.95% vs. 31–34 yrs=9.46% vs. 35–39 yrs=11.57% vs. $\geq$40 yrs=21.28%; $P < 0.0001$). Of the 261 losses in the study period, cytogenetic analysis was obtained on 71 (27.2%). Three specimens were non-diagnostic due to trophoblastic nonproliferation. Only 15 (22.03%) were normal (46XX or 46XY). Of the 53 chromosomally abnormal specimens, 45 (80.4%) were trisomies, 4 (7.14%) had 48 chromosomes, 2 were mosaics (3.57%), 2 were Turner's Syndrome (3.57%), 1 was a translocation (1.79%) and 1 was a triploidy (1.79%). The most common trisomies were 21 (7), 16 (5), 15 (5), 22 (5) and 18 (3). No differences were noted in the average age of the group with normal chromosomal losses as compared to the group with an abnormal chromosomal makeup (37.6 years old vs. 39.4 years old, $P = 0.40$). 91.3% of the losses in women over the age of 40 were chromosomally abnormal as compared to 71.1% of the losses in women under the age of 40 years.

Conclusion: We have demonstrated a highly significant increase in pregnancy loss after demonstrating a FH during a 7 week U/S with increasing maternal age. The overwhelming explanation for these losses appears to be chromosomal in nature with almost 80% having an abnormal chromosomal composition.

O-099

Low Dose Aspirin Treatment Improves Implantation and Pregnancy Rates in IVF Patients: A Prospective, Randomized, Double Blind Study. M. Rubinstein, A. Marazzi and E. Polak de Fried. CER Instituto Medico, Buenos Aires, Argentina.

Objective: Previous studies have reliably demonstrated that low dose aspirin is an effective inhibitor of platelet aggregation and an immunomodulator by the irreversible inhibition of the enzyme cyclooxigenase. Low dose aspirin is widely used in the treatment and prevention of cardiovascular disease, in pregnant patients with preeclampsia and fetal growth retardation and its efficacy has also been proven in women with antiphospholipidic syndrome and recurrent miscarriages. However, it has not been evaluated in the area of assisted reproduction. The aim of this study was to evaluate whether low dose aspirin treatment improves the outcome of IVF cycles.

Design: Prospective, randomized, double blind placebo controlled study.

Materials and Methods: 74 infertile patients underwent their first IVF attempt. They were randomly divided into two groups: the study group received 100 mg/d Aspirin orally and the control group received placebo. Both groups started aspirin or placebo cotreatment on the initial day of luteal phase analogue GnRh treatment in association with gonadotrophin therapy. The study was double blinded to patients and clinical staff. Pregnant patients continued the medication through 12 weeks gestation. Ovarian responsiveness and IVF outcome variables were analysed. The monitorization of cycles was performed as usual by adding transvaginal color and pulsed Doppler ultrasound to evaluate ovarian and follicle blood flow. Data were analysed using 2-tailed student t, Mann Whitney rank sum and Fisher's exact test. $p < 0.05$ was considered statistically significant.

Results:

	Study Group (Aspirin)	Control Group (Placebo)	*P*
Patients	35	39	
Age ($\bar{x} \pm$ SD)	35.2 ± 5.2	36.0 ± 6.2	NS
Cancelation Rate (%)	5.7	10	NS
Follicles ($\bar{x} \pm$ SD)	17.8 ± 10.5	12.0 ± 7.3	0.01
Oocytes retrieved ($\bar{x} \pm$ SD)	15.2 ± 9.6	10.6 ± 7.6	0.4
E_2 on HCG day ($\bar{x} \pm$ SD)	2792.8 ± 1083.4	1917.3 ± 861.7	0.003
$\bar{x}$ Embryos transferred	3.6	3.6	NS
Implantation rate (%)	17	8.2	0.04
Pregnancy rate (%)	45	20	0.04

Conclusion: Our results show that a low dose of aspiring significantly improves the number of follicles, oocytes retrieved, serum estradiol levels, and diminishes the cancelation rate. This allowed us to obtain statistically significant higher implantation and pregnancy rates. Aspirin significantly increases follicular blood flow, which improves folliclegenesis. Aspirin would appear to be a useful, effective and safe treatment in patients who undergo assisted reproductive technology procedures.

O-100

Novel 5 Minute Hybridization Procedure for FISH Preimplantation Diagnosis. K. C. Drury, L. Kovalinskaia, P. Clark, D. Zhaoheng, R. S. Williams. Dept OB/GYN, University of Florida College of Medicine, Gainesville, FL.

Objective: The objective of this study was to develop a rapid and simple Fluorescent In-Situ Hybridization (FISH) procedure that allows simultaneous detection of human chromosomes (in the present case X, Y and 18) of various cell types including preimplantation human embryos.

Design: Male and female lymphocytes, amniocytes, fibroblasts, sperm and embryo blastomeres were used to assess conditions for rapid FISH. Embryos were donated for research by patients undergoing infertility therapy (IVF) under IRB informed consent.

Materials and Methods: Direct fluorescent labeled DNA probes for X, Y and 18 chromosomes were obtained from Vysis Inc. Cell nuclei were fixed to slides with 3:1 methanol-acetic acid and used immediately or stored at −20°C. Prior to denaturation, slides were passed through a 1 minute each dehydration step of 70–85–95% ethanol and allowed to dry. A mixture of probes was prepared by adding 1 ul of each to 7 ul hybridization buffer. Ten microliter of probe mixture was overlaid on sample nuclei and sealed with an 18×18 plastic coverslip and co-denatured at 75–80C for 8–10 minutes (depending on cell type) using a covered slide warmer or computer controlled Hybrite (Vysis). Samples were then hybridized for 5 minutes using microwave technology and washed twice. The first wash was performed at 42°C for 10 minutes in 50% formamide, 2× SSC. The second wash was performed at room temperature for 1 minute in 2× SSC + 0.1% NP-40. Slides were dried for 5 minutes in an air flow drying box and counter stained with 10ul DAPI containing antifade. Samples were sealed with a glass coverslip for viewing with a Nikon epifluorescent microscope with Quips analysis software (Vysis).

Results: When viewed with a 60× non-oil objective, strongly fluorescent signals could be observed easily using single, double or triple bandpass filter systems. For male diploid cells, 2 green signals (chromosome 18), 1 red signal (X chromosome) and 1 aqua signal (Y chromosome) were observed in all cell types. For female diploid cells, 2 green and 2 red signals were observed. X bearing sperm cells showed 1 green and 1 red (18 + X) and Y bearing sperm showed 1 green and 1 aqua (18 + Y).

Conclusions: This novel rapid (5 minute) hybridization procedure allows various types of cells, including those from human early embryos, to be diagnosed for sex and potentially a variety of chromosomal abnormalities. The entire FISH procedure takes approximately 30 minutes to perform. Further use of this procedure will be made to analyze additional chromosomal and specific loci probes as they become available.

O-101

Preimplantation Diagnosis of a β1 Integrin Targeted Mutation in Mouse Embryos. [1]R. A. Pedersen, [1]H. S. Kim, [2]I. V. Klimanskaya, [1]R. V. Lebo and [2]C. K. Damsky. [1]Reproductive Genetics Unit, Dept of Obstetrics, Gynecology and Reproductive Sciences, and [2]Dept of Stomatology, University of California, San Francisco, CA.

Objectives: Preimplantation diagnosis can be used to characterize specific gene mutations in blastomeres of human embryos by polymerase chain reaction (PCR). However, the limited numbers of available human embryos has made it difficult to assess the efficiency of these methods. We estimated the error rates in genotyping wild-type (AA), heterozygous (AB) and mutant (BB) embryos by pre-