formation rates among three groups. Below table summarized the results in each group.

	Group I (w/o PTXF)	Group II (with PTXF)	Group III (Combined) w/o PTXF	with PTXF
No. of cycles	34	28	29	
Mean age	33.9 ± 5.6	33.6 ± 4.8	32.9 ± 3.5	
Fertilization rate (%)	69.9 ± 30.5	74.9 ± 28.3	57.4 ± 21.4[a]	68.0 ± 22.5[b]
Pregnancy rate (%)	12/32 (37.5)[c]	17/28 (60.7)[d]	13/27 (48.1)	

a vs b: $P<0.05$, c vs d: $P<0.01$.

Conclusions: The PTXF treatment is very simple and certainly enhances the motility of TE sperm from poor quality samples. This treatment can reduce the time for selection of viable TE sperm in ICSI, and increase the pregnancy rate in our results. We suggest that the PTXT treatment should be useful in ICSI with the sperm from severe male factor infertility.

P-238

Pattern analysis of aneuploidy in 8 pairs of chromosomes of day 3 human embryos. Kelly Pagidas, Ying Ying, James R. Trimarchi, Rick J. Hachett, Ben N. Cao, David L. Keefe. Women & Infants Hosp of RI/Brown Univ, Providence, RI.

Objective: Chromosome abnormalities may cause abnormal embryonic development, failure of implantation and spontaneous abortions. Aneuploidy screening with multi-color FISH enables detection of chromosomally abnormal embryos. This study evaluates the pattern of aneuploidy in 8 pairs of chromosomes and their relationships in day 3 human pre-embryos.

Design: Retrospective analysis.

Materials and Methods: Thirty-one patients with advanced age and/or repeated implantation failures were included in this study. The average maternal age was 37.5 ± 5.3 (range from 24 to 46). 46.9% of patients were > 40 years of age. 70% of patients had undergone >3 cycles. One cell was biopsied from each day 3 embryo and a total of 315 embryos from 37 cycles were analyzed. Fixed cells were analyzed by multicolor FISH for chromosomes 13, 15, 16, 17, 18, 21, 22, X and Y.

Results: Two hundred six of the 315 embryos analyzed with FISH for the 8 chromosome pairs were detected to have chromosomal abnormalities. Analysis of the given type of abnormalities did not demonstrate any significant difference in the percentage of pre-embryos with a given abnormal chromosomal compliment among the 8 chromosome pairs analyzed with one exception. Chromosome 21 and Chromosome 22 has the highest rate of trisomy (14.0%, 11.4%, respectively), which was significantly higher than that of Chromosome 17 (6.3%). 113 pre-embryos had an abnormal gonosomy compliment. No difference was noted in the frequency or distribution of sex chromosome abnormality among the X or Y chromosomes. Analysis of a relationship between these 8 pairs of chromosomes demonstrated that aneuploidy in chromosome 22 had the highest power to predict other chromosome abnormalities, about 3 times higher than aneuploidy in chromosomes 15, 16 and 21.

Conclusion: There was no statistical difference in the occurrence of homologous pairs of individual chromosomes. However, the type of aneuploidy can differ for individual chromosomes. Trisomies of Chromosomes 21 and 22 were the most common aneuploidy identified, suggesting that segregation errors could occur at different rates for each chromosome. Moreover, aneuploidy in Chromosome 22 had more power to predict the pattern of other chromosome abnormalities. Analysis of chromosome 22 in a 5 panel FISH for aneuploidy may enable a greater detection rate of abnormal pre-embryos prior to embryo transfer without having to resort to an 8 panel FISH to improve the reproductive outcome.

Supported by: No support.

P-239

Blastocyst formation is not an indication of genetically normal embryos. George M. Grunert, Wan-Song A. Wun, Sau Wai Cheung, Randall C. Dunn, Cecilia T. Valdes, Leah Schenk. Obstetrical & Gynecological Assoc, Houston, TX; Baylor Coll of Medicine, Houston, TX.

Objective: Blastocyst formation is an important marker of embryo development. While embryo transfer at blastocyst stage improves implantation rate, development to the blastocyst stage may not indicate that the embryo is genetically normal. Preimplantation genetic diagnosis (PGD) can be used to screen for numerical chromosomal abnormalities and for single gene defects embryos at the 6-8 cell stage. This study examines the relationship between blastocyst formation and PGD results.

Design: Retrospective study.

Materials and Methods: All PGD cases, either screening for aneuploidy by fluorescent in situ hybridization (FISH) or single gene defect by polymerase chain reaction (PCR), were included. There was a total of 71 PGD cases from March 1, 1994 to April 10, 2003. The indications were advanced maternal age, translocation carrier, single gene defect, and previous child with birth defects. Patient ages ranged from 25-46 (mean 36.1). FISH analysis using probes for chromosomes 13, 16, 18, 21, 22, X and Y were performed in 46 cases. PCR based for detection of single gene defect were performed in 25 cases using specific primer for Duchenne muscular dystrophy, Huntington's chorea, cystic fibrosis, alpha thalassemia, SCID, fragile X syndrome, and hemophilia. Pearson Chi-square test with Logistic Regression was used for statistical analysis. The significance level was $p<0.05$.

Results: There is no significant difference in the percentage of blastocyst and non-blastocyst in genetic constitution.

PGD and blastocyst formation results

	Number of embryos	Genetic normal	Developed to blastocyst	Genetic abnormal	Abnormal to blastocyst	Inconclusive	Inconclusive to blastocyst	Carrier	Carrier to blastocyst
FISH	400	108	43	185	63	107	23	n.a.	n.a.
PCR	203	67	17	59	18	38	5	23	9
Total	603	175	60	244	81	145	28	23	9

Comparison of genetic constitution between blasstocyst and non-blastocyst group

	Number of embryos	Normal	Abnormal	Carrier
Blastocyst	159	60 (38%)	81 (51%)	18 (11%)
Non-blastocyst	299	115 (38%)	163 (55%)	21 (7%)
Odds ratio		0.97 (95% CI 0.65-1.44)	0.87 (95% CI 0.59-1.27)	

Conclusion: The results showed 29% (175/603) of embryos had normal PGD results. 60 of these embryos (34.2%) of these reaches blastocyst compared with 81/244 (33.2%) with abnormal PGD results. Only 38% (60/159) of blastocyst stage embryos were normal. Culture of embryos to the blastocyst stage does not select out a population of normal embryos.

P-240

Chromosomal abnormalities of 1,022 couples entering ICSI program. Hesham F. Kayed, Ragaa T. Mansour, Alaa E. Amer, Ashraf Abdelrassek, Gamal I. Serour, Mohamed A. Aboulghar. The Egyptian IVF-ET Ctr, Cairo, Egypt.

Objective: Chromosomal abnormalities are believed to be a major contributor to the genetic risks of infertility treatment associated with In-vitro Fertilization (IVF). Apart from abnormalities arising de novo, abnormal karyotypes in pregnancies conceived through assisted reproductive technology may be directly derived from predisposing parental aberrations. Our objective was to prospectively assess the frequency of chromosomal aberrations in couples that were candidates entering our IVF/ICSI program.

Design: Prospective study.

Intervention(s): Heparinized blood samples (5 ml) were collected 3-4 weeks before a scheduled ICSI cycle.

Setting: The Egyptian IVF-ET Center.

Participants & Methods: A total of 1022 infertile couples (2044 patients), between August 2000 and April 2003, underwent chromosome analysis and genetic counseling before entering our IVF/ICSI program. Chromosomal analysis was indicated for couples with severe male factor infertility, azoospermia and/or other factors. Heparinized blood samples were cultured and harvested according to standard methods. Chromosomes were GTG banded (G banding by Trypsin Giemsa Stain) in 1022 couples (2044 patients). In all cases, 10 metaphases were karyotyped. When any apparent

chromosomal aberrations were detected, 20 metaphases were analyzed. When mosaicism was detected, an additional 50-100 metaphases were analysed to detect the percentage of mosaicism. Computerized Karyotyper (Cytovision 2.7,...) was used for observation and detection of chromosomal abnormalities.

Results: Blood samples were collected from 2044 patients and the results were successfully obtained from all. Overall, 97.2% of patients (1987 patients) had a normal karyotype. Of these 15 patients (0.75%) had a normal karyotype, but with normal variants. In 57 cases (2.8%) an abnormal karyotype was detected. Male patients constituted the majority of abnormalities; thirty-nine males (68%), eighteen females (32%). These chromosomal aberrations included 20 cases (1%) of sex chromosome abnormalities (18 males and 2 females); comprising hyperploid chromosomes X & Y, mosaicism, and Y deletion. Twenty-eight cases (1.41%) had autosomal aberrations, fifteen of them were male and thirteen were females. This included four cases of reciprocal translocations, four cases of robertsonian translocations, three cases showed pericentric inversion of chromosome 9, one case with pericentric inversion of chromosome 1. In addition, duplication was found in seven cases with duplication 1q and one case with duplication of chromosome 9. Low level of mosaicism was detected in two cases (0.1%), two cases (0.1%) showed single cell abnormality and two cases with an additional marker chromosome were also encountered.

Conclusion: Our data showed that chromosomal abnormalities affect a considerable number of infertile couples, and occur in both sexes. However, more frequently in the male due to the fact that our selection of cases was more directed towards male factor infertility. It is also noted that sex chromosome aberrations were more frequent in male while autosomal aberrations were almost equally distributed between both sexes. It is highly recommended that a chromosomal analysis should be preformed before the beginning of IVF/ICSI program. Most of these patients can be offered PGD/ICSI to prevent the transmission of their chromosomal abnormalities to their offspring.

P-241

A prospective randomized study comparing laser and tyrode's mediated methods of assisted hatching. Jacob Levron, Betty Ferber-Meiri, David Bider, Adrian Shulman, Tali Levin, Einav Shporn. IVF unit, Haim Sheba Medical Center, Tel-Hashomer, Ramat-Gan, Israel.

Introduction: During the last years new laser mediated micromanipulation stations have been introduced for clinical application in the IVF laboratory. The use of such devices significantly simplifies the procedure of zona manipulations in compare to the traditional drilling method using acidified solution. Nevertheless, there is still little information in the literature about the comparative efficacy of both methods. We have designed this study in order to adopt either one of the methods as our method of choice for zona manipulations.

Materials & methods: A total of 238 IVF cycles that were designated for AHA during the year 2002, were randomly divided into two study groups. The procedures were performed only on day 3 embryos. The embryos designated for AHA were treated by local administration of acidified Tyrod's solution (ZD-10, vitrolife™, Sweden) using micropipette (Cook, Australia) and Nikon-Narishige micromanipulation system. The embryos designated for laser AHA were treated by ZILOS laser module (Hamilton, Thorne) installed on Nikon-Narishige micromanipulation station. After performing AHA, embryos of both study groups were cultured for at least 30 minutes before ET. Statistical analysis was performed by using the χ^2 test on Medculc© software.

Results: Both study groups were identical for No. of embryos transferred. The pregnancy rates were 23.2% in the laser AHA group versus 22.2% in the Tyrode's solution group (NS). The ongoing pregnancy rates were 13.4% and 14.3% respectively (NS).

Conclusions: Our present study demonstrate that assisted hatching using laser is as efficient and safe as the traditional method of zona drilling by acidified Tyrode's solution.

P-242

Correlation between patient age, day 3 FSH levels and zona pellucida thickness. Danielle E. Lane, Shehua Shen, Victor Y. Fujimoto, Daniel H. Moore, Marcelle I. Cedars. Univ of CA San Francisco, San Francisco, CA.

Objective: Published literature remains inconclusive as to the significance, and predictors of zona pellucida thickness. Current treatment strategies involve the use of assisted hatching (AH) for women of older reproductive age and/or with elevated FSH levels. This strategy assumes that these women are at increased risk for thickened zona pellucida and would benefit from AH. To test the hypothesis that zona pellucida thickness correlates with maternal age, day 3 follicle-stimulating hormone (FSH), and/or average peak estradiol per follicle, we performed a retrospective analysis of all embryos transferred between November 2001 and May 2002 at the University of California, San Francisco Center for Reproductive Medicine.

Design: A retrospective analysis. All patients, going through their first IVF cycle, were eligible. Patients who used donor eggs, and patients who did not undergo embryo transfer in their index cycle were excluded. A total of 91 patients were included in the study. Each patient had between one and six embryos transferred on Day 3 after oocyte retrieval. With a sample size of 91 patients, the power of this study was 90% to detect correlations of 0.3 or greater.

Materials and Methods: Each transferred embryo included in the data set was photographed using a Nikon Diaphot inverted microscope and a Sony VP 895MD printer at either 10x or 20x magnification. Digital photographs were measured using Mitutoyo Digit Dial Calipers. The average zona thickness was calculated by making four equidistant measurements from the inside to the outside of the zona pellucida of each embryo at 12, 3, 6, and 9 o'clock positions. A zona thickness score was calculated from the average of transferred individual embryo zona thicknesses.

All data were analyzed using Stata version 8.0 for Macintosh. Using Pearson correlations and locally weighted least squares (lowest) fits to the data, average zona thickness scores were compared with maternal age, day 3 FSH, and the peak estradiol divided by the number of follicles greater than 13 mm diameter (peak E2/follicle) on the day of hCG trigger. Implantation rates (IR) were also evaluated. An exact correlation between zona thickness and IR is not possible due to the inability to identify the specific embryo responsible for implantation.

Results: Zona pellucida thickness scores were not found to correlate with maternal age, day 3 FSH, or peak E2/follicle. Additionally, no correlation was found between the individual patient variation in zona pellucida thickness and maternal age, day 3 FSH or peak E2/follicle. In evaluating all eligible patients, zona thickness score does not predict implantation rates in individual patients. These findings were also true in patients less than 38 years of age, who do not routinely undergo assisted hatching in our program.

Conclusions: These findings suggest that neither maternal age, day 3 FSH nor peak E2/follicle is an accurate surrogate for zona pellucida thickness. Additionally, it unlikely that average zona pellucida thickness can be used to predict outcome when multiple embryos are transferred. These data raise questions about the identification of which patient populations may be most aided by assisted hatching techniques.

P-243

Biopsy technique may hold the key to cryosurvival of supernumerary blastocysts following preimplantation genetic diagnosis. Edward Stehlik, Joni Stehlik, K. Paul Katayama, Hiroyuki Asakura, Yutaka Sasabe. Advanced Institute of Fertility, Milwaukee, WI; Kitano Hosp, Osaka, Japan; Toho Univ, 1st Dept of Obstetrics and Gynecology, Tokyo, Japan.

Several reports have indicated poor survival and pregnancy rates are to be expected from blastocysts cryopreserved after biopsy for Preimplantation Genetic Diagnosis (PGD). Our clinic's retrospective study demonstrates that blastomere biopsy and cryopreservation of human blastocyst stage embryos can be accomplished with survival rates and pregnancy rates comparable to those acheived of the non-biopsied blastocysts in our laboratory.

In our study, day three embryos were biopsied by mechanically creating a small slice above the blastomere to be removed. A 30 micron biopsy pipette was used to exert external pressure against the zona pellucida opposite the desired blastomere. When the blastomere was 80-90 percent expelled, a 135 micron Stripper Tip was used to remove the adhering blastomere from the zona pellucida. The embryos were then cultured to the blastocyst stage while the fluorescent in-situ hybridization technique was utilized for PGD. Following a day five transfer, supernumerary blastocysts were cryopreserved using the conventional slow-freezing method.