mined by the failure to amplify and/or detect a Y PCR band from male cells or those blastomeres which failed to demonstrate a Y band when others from the same embryo were diagnosed as male by PCR or FISH.

Materials and Methods: PCR was carried out with an initial denaturation by KOH. A full nested primer design was used to amplify the Amelogenin gene from both X and Y chromosomes. Specific details of the reactions will be provided. However the first 7 cycles of the initial reaction was carried out using a denaturation temperature of 97C which was then lowered to 95C for the remaining 17 cycles. This was to enhance Y gene amplification. The nested reaction was carried for 35 cycles. PCR products were analyzed by 2% agarose gel electrophoresis and detected with 0.5 μg/ml ethidium bromide (EB) or 1:10000 SYBR Green I fluorescent stain (Molecular Probes).

Results: ADO was reduced in male single cells when detected by fluorescent DNA staining methods. In 62 cells using EB 47/62(75%) gave amplified signals; 3/38(7.9%) = Y ADO and 5/38(13.2%) = X ADO. In contrast using SYBR staining 115/135(85.2%) gave amplified signals; 1/60 (1.7%)=Y ADO and 11/60(18.3%)= X ADO. When blastomeres were analyzed by EB, 33/42(78.6%) amplified; 3/42(7.1%) = Y ADO and 8/42(19%) = X ADO. This was contrasted to SYBR staining where 54/68(79.4%) amplified; 1/68(1.5%) = Y ADO and 8/68(11.8%) = X ADO.

Conclusion: Fluorescent DNA staining of PCR products may be a useful tool in increasing the sensitivity of signal detection especially in single cell amplification reactions. The incidence of Y allele ADO decreased when the fluorescent DNA stain was used to detect PCR products from both known male cells and donated human embryos. In no case was a misdiagnosis of a donated embryo made when two blastomeres were analyzed separately from a single embryo. The use of fluorescent DNA stain enhances detection roughly 25 fold over that of EB at a cost well within most laboratory budgets. Fluorescent staining clearly enhances Y detection as seen when the same sample is detected by both methods. However, complications may still arise due to mosaicism within an embryo which may be confused with ADO.

P-126

Effect of Head or Tail First Sperm Injection into Oocytes in Intracytoplasmic Sperm Injection.

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Objectives: Immobilization of spermatozoa prior to intracytoplasmic sperm injection (ICSI) sometimes results in crooked tail and this makes it difficult to aspirate sperm into an injection pipette tail first. Head first sperm aspiration into an injection pipette avoid this problem due to the bigger size of the sperm head. The effect of head or tail first sperm injection into an oocyte in ICSI program has been studied.

Design: Fertilization, cleavage, percentage of grade 1 embryos and development to blastocyst stage were examined from oocytes sperm injected by head first or tail first.

Materials and Methods: The patients in ICSI program received gonadotrophin releasing hormone agonist (GnRHa) + human menopausal gonadotrophin (HMG) regimens. A dose of 10000 IU human chorionic gonadotrophin (HCG) was administered when two dominant follicles reached 18 mm diameter. Thirty six hours after HCG, the oocytes were recovered transvaginally using ultrasound guidance. Aspirated oocytes were matured for 3 hours in mHTF supplemented with 10% synthetic serum substitute (SSS) at 37°C. Cumulus cells surrounding oocyte were removed with 0.1% hyaluronidase and repeated pipetting. Liquified semen from oligoasthenozoospermic patients was put on a two-layer percoll (90% and 40%) and was centrifuged for 30 minutes at 300 ×g. The 90% fraction was collected, washed with mHTF Hepes at 300×g for 10 minutes, and the pellet was used for injection. A single living immobilized spermatozoa was injected into an oocyte head first or tail first according to the treatment. Eighteen hours after microinjection, oocytes were inspected for survival and fertilization. Fertilized oocytes with two pronuclei were cultured in 30 μl drop of mHTF supplemented with 10% heat-inactivated follicular fluid (FF) at 37°C. On day 2, embryo transfer was performed with cleaved embryos. The remaining 2–8 cell stage embryos were cultured with BRL cells in mHTF + 10% FF for 72 hours and developmental stage was observed. The data were analyzed by Analysis of Variance.

Results: A total of 164 oocytes from 36 patients were assigned to each treatment and ICSI was performed (88 head first, 76 tail first). The rates of normal fertilization and cleaved embryos were 81.8% and 72.7%, 76.3% and 71.1% for head first and tail first, respectively. Of the cleaved embryos, the percentage of grade 1 embryo was 68.8% and 74.1% for head first and tail first, respectively. Of the 2–8 cell embryos cultured, 44.4% (16/36) and 50.0% (10/20) for head first and tail first, respectively developed to blastocyst stage. There were no differences in fertilization, cleavage, rates of grade 1 embryos, and development to blastocyst stage ($p > 0.05$).

Conclusion: Head first or tail first sperm injection into an oocyte in ICSI program does not affect fertilization and subsequent embryo development of blastocyst stage in vitro.

P-127

Testicular Sperm From Nonobstructed Azoospermic Males Does Not Appear To Alter Preembryo Morphology Or IVF Pregnancy Outcome.

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Objective: To determine if microsurgical fertilization using sperm obtained from testicular biopsy affects in vitro fertilization/embryo transfer (IVF/ET) preembryo quality and pregnancy outcome.

Design: Retrospective analysis.

Materials & Methods: Embryology and pregnancy records from 23 patients who underwent testicular sperm extraction (TESE) and subsequent IVF/ET between April 1995 and October 1996 were reviewed. A control group consisting of 62 patients using ejaculated sperm for IVF were matched for age, ovarian stimulation protocol, number of oocytes retrieved and fertilized, as well as number of preembryos transferred. For both groups, preembryo quality was assessed by standard morphologic grading as described by Veeck[1] (1 = best, 5 = worst) and compared. Rates of implantation, spontaneous abortion, and ongoing pregnancy were also compared.

Results: The table below summarizes data derived from TESE and control patients:

	TESE	Control	P
Age	34.9 ± 4.9[a]	34.9 ± 4.6[a]	0.97[c]
#Oocytes retrieved	14.7 ± 9.6[a]	12.4 ± 5.9[a]	0.19[c]
Inseminated or ICSI	10.7 ± 4.7[a]	9.7 ± 5.2[a]	0.44[c]
#Fertilized-2pn	6.0 ± 3.4[a]	7.1 ± 4.3[a]	0.25[c]
Grade	2.5 ± 0.90[b]	2.0 ± 0.69[b]	0.11[d]
#Embryo Transfer	3.3 ± 1.1[a]	3.4 ± 0.91[a]	0.78[d]
Implantation Rate	19/75 (25%)	60/212 (28%)	0.77[d]
Abortion Rate/ Clinical Pregnancy	2/12 (16%)	4/30 (13%)	0.56[e]
Ongoing Pregnancy Rate/Transfer	12/23 (52%)	30/62 (49%)	0.97[d]

Conclusion: There appears to be no significant difference in preembryo morphology, implantation rates, miscarriage rates or ongoing pregnancy rates with the use of sperm retrieved from the testicle compared to ejaculated sperm.

[1] L. L. Veeck: Atlas of the Human Oocyte and Early Conceptus. Williams and Wilkins, Baltimore, MD 1991.

P-128

Detection of Chromosomes 13 and 21 by FISH With Two Different Sets of Probes. K. P. Xu, T. Z. Liu, T. H. Huang, L. L. Veeck. The Center for Reproductive Medicine and Infertility. The New York Hospital-Cornell Medical Center, New York, NY.

Objectives: Incorrect segregation of chromosomes 13 and 21 occurs frequently in human preembryos. Detection of these aneuploidies before preembryos are transferred to women of advanced maternal age may have great significance in human assisted reproductive medicine.

Design: To compare the results of aneuploidy for detection of chromosome 13 and 21 by different probes and procedures.

Materials and Methods: A total of 334 blastomeres from arrested preembryos (n = 92, averaging 3.6 cells/preembryo) were obtained from consenting IVF patients. In the first FISH procedure, numerical analysis of chromosomes 13 and 21 was performed in a 4-color FISH (no. of blastomeres = 172) with a combined 13/21 FISH probe (alpha satellite DNA probe, Oncor), together with 3 other specific probes (X, Y, and 18, Vysis). In the second protocol, chromosomes 13 and 21 were determined separately (no. of blastomeres = 189) by 13 and 21 specific probes (locus specific probes of 13 and 21 mixture, Vysis).

Results: With the first protocol, FISH signals were obtained in 124 of 172 cells (72.1%), in which 47.1% (81/172) of the blastomeres were diagnosed as normal (all 4 signals). The distribution of abnormal 13/21 chromosomal signals was 5.2% without signal (n = 9), 3.5% one signal only (n = 6), 8,1% two signals only (n = 14), 4.1% three signals only (n = 7) and 4.1% more than four signals (n = 7). In the second procedure, 127 of 162 cells (8.4%) gave rise to FISH signals, of which 50.6% (82/162) of the cells had a correct number of signals (two signals each for chromosomes 13 and 21). In addition, 4.9% (n = 8) showed monosomy 13 and trisomy 21, and 1.2% (n = 2) were trisomic for 13 and monosomic for 21. These monosomic and trisomic cells would have been diagnosed as normal ones if the first protocol had been used. The remaining 37 abnormal nuclei (22.8%) were diagnosed as follows: 1 × 13 & 0 × 21 (n = 1); 1 × 13 & 1 × 21 (n = 3); 1 × 13 and 2 × 21 (n = 2); 2 × 13 and 0 × 21 (n = 3); 2 × 13 & 1 × 21 (n = 9); 2 × 13 & 3 × 21 (n = 4); 3 × 13 & 2 × 21 (n = 2); 3 × 13 & 3 × 21 (n = 2); 4 × 13 & 2 × 21 (n = 3); 4 × 13 & 3 × 21 (n = 2); and 4 × 13 & 4 × 21 (n = 6).

Conclusions: Our results indicate that using a dual-color FISH to estimate individual chromosome composition of 13 and 21 has a great advantage over a single colored 13/21 alpha satellite probe, in that not only a more precise diagnosis was made in terms of each chromosome, but also fewer missing signal were observed, thus increasing the sensitivity and accuracy of aneuploidy detection in preimplantation preembryos.

P-129

First Pregnancy Resulting from Chromosome Analysis of Preembryos of a Patient with Previous Recurrent Miscarriages Due to Trisomies. T. Z. Liu, K. P. Xu. The Center for Reproductive Medicine and Infertility. The New York Hospital-Cornell Medical Center, New York, NY.

Objective: To achieve a normal pregnancy after aneuploidy analysis by fluorescent in situ hybridization (FISH) in preimplantation preembryos.

Design: Single cells were biopsied from preembryos obtained by IVF and FISH was applied to analyze chromosomes 16 and 22 to assess the normality of the preembryos.

Materials & Methods: A 41 year old patient who had four previous miscarriages, in which two chromosomal disorders were diagnosed (trisomy 16 and trisomy 22) was admitted for this trial. After a standard luteal phase leuprolide acetate downregulation and gonadotropin stimulation, 13 oocytes were retrieved and inseminated. Eleven oocytes were normally fertilized. Biopsy was performed on day 3 after oocyte retrieval. FISH analysis using chromosome specific probes for 16 (Oncor) and 22 (Vysis) was performed. Only chromosomally normal preembryos were replaced on day 4 post retrieval.

Results: Biopsy was successful on all 10 preembryos all of which had 4 or more blastomeres. One blastomere was