

transfer in a subgroup of patients with impaired chances of pregnancy due to age and multiple IVF failures.

**DESIGN:** Case controlled retrospective study.

**MATERIALS AND METHODS:** Informed consent was obtained from all patients before MsFR. The study was comprised of 2 groups who failed at least 2 IVF treatments: A) 20 women aged 28–42 (36.1±3.9) yr who had 2.8±1.2 IVF treatments. MsFR was performed in 76 embryos that exhibited fragmentation ranging from 15–50%; B) 20 women aged 28–43 (35.1±4.4) yr who had 2.5±1.4 IVFs. 87 embryos from these patients were transferred without MsFR. The pairing of the groups was based on age, infertility factors, quality of embryos, and the interval of the procedures. The technique utilized for MsFR included an initial assisted hatching (AH) step using acid solutions, followed by the removal of some or all fragments after placing a new AH micropipette. Fragments were finally removed via controlled gentle mouth suction. Study endpoints included # of embryonic cells at ET, degree of fragmentation, improvement after MsFR, IR and PR. The Mann-Whitney and chi-square were used as appropriate. Levels <0.05 were considered significant.

**RESULTS:** All types of fragmentation were removed and there were no visible blastomere damage after MsFR. A statistically significant improvement on embryonic morphology was observed after MsFR ( $p < 0.001$ ) as noted by the improvement in the standard morphologic classification used (documented photographic embryonic improvement of 37%). There were no differences regarding PR (A=45% and B=20%,  $p = 0.177$ ); however, IR were significantly greater in group B (A = 26.5% and B = 49.6%,  $p = 0.011$ ). Conversely, the incidence of single gestation was 78% in group A and 25% in group B. Additional results are shown in the Table.

**CONCLUSION:** These findings support the applicability of MsFR to selected patients. The significant morphologic improvement in the embryonic appearance along with a tendency to an improved PR may imply that MsFR provided intracellular mechanisms to “fight” cell derangement and death. Although some indicators showed benefits to MsFR, the fact that the IR observed in the MsFR group is perplexing. The small sample size, lack of strict criteria for fragment removal, use of chemical/mechanical technique instead of hydraulic, and lack of biomarkers for this microsurgical approach provide some reasoning for such evidence.

*Supported by:* None.

	A	B	Sig
# oocytes	10.2±4.2	11.4±5.5	0.646
# 2 PN	7.1±2.8	8.0±3.6	0.803
# cells	6.4±1.6	6.7±1.5	0.158
# ET	4.5±1.7	4.4±1.4	0.728

#### P-542

**Time- and media-dependent improvements in motility of testicular spermatozoa retrieved from non-obstructive azoospermic men.** D. E. Davis, T. G. Schuster, M. R. Hiner, D. A. Ohl, G. D. Smith. University of Michigan, Ann Arbor, MI.

**OBJECTIVE:** Testicular-derived spermatozoa motility, reflecting viability, is key to intracytoplasmic sperm injection success. Experiments were conducted to compare number of motile sperm isolated from testicular aspirations incubated in different media over time.

**DESIGN:** Prospective side-by-side comparative laboratory study.

**MATERIALS AND METHODS:** Diagnostic and/or therapeutic fine needle testicular aspirations were performed on 14 men with non-obstructive azoospermia. Seminiferous tubules were manually dissected and seminiferous epithelial content dispersed into a single cell suspension. Number of non-motile and motile sperm/20 high-powered fields was assessed initially at the time of cell dispersion and following incubation at 37°C for 24 and 48 hours in either HEPES buffered human tubal fluid + protein (H-HTF) in atmospheric conditions or Ham’s F10 + protein (F10) in 5% CO<sub>2</sub> and air. Statistical analyses were performed with paired Student’s t-test and ANOVA with repeat measures.

**RESULTS:** Initially following seminiferous tubule micro-dissection, total sperm found was 93 ± 22 (mean ± SE) and mean number of motile sperm/20 high-powered fields was 3 ± 2. In H-HTF sperm motility increased in 8 of 14 samples after 24 hours of culture (18 ± 9) and 11 of 14 samples after 48 hours of culture (19 ± 9), although these increases were not significant. Mean number of motile sperm/20 high-powered fields significantly increased in all 14 samples following incubation in F10 for 24

hours (30 ± 11, paired t-test,  $P = 0.03$ ) and 48 hours of culture (33 ± 13,  $P = 0.04$ ). Testicular sperm motility increased significantly after incubation in F10 compared to HTF at both 24 and 48 hours of culture (repeated measures ANOVA,  $P = 0.05$ ).

**CONCLUSION:** Incubation of spermatozoa from fine needle testicular aspirations facilitates motile spermatozoa isolation. More motile spermatozoa were isolated after incubation in F10 than in H-HTF. There was no significant increase in motile sperm isolated between 24 and 48 hours. Following seminiferous tubule micro-dissection, culture of testicular spermatozoa for 24 hours in Ham’s F10 is an efficient means of collecting motile spermatozoa for ICSI.

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#### P-543

**Marker chromosome identification in infertility.** A. Abderazek, H. Kayed, R. Mansour, O. Kamal, M. Aboughar. Egyptian IVF-ET Center, Cairo, Egypt.

**OBJECTIVE:** A marker chromosome is a structurally abnormal chromosome in which no part can be identified. So far the identification of marker chromosomes has been of interest for cancer cytogenetics only. Molecular cytogenetics (fluorescence in situ hybridization:FISH) has been considered a very powerful research tool, in the understanding of many aspects of human biology. The aim of this study was to identify the origin of chromosome markers detected by G-banding in patients with infertility entering ICSI program. This is of great importance for genetic counseling and offering these patients chance to enter Perimplantation Genetic diagnosis (PGD) program.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** A total of 3540 patients, between August 2000 – January 2004, 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. Chromosomal analysis of peripheral blood lymphocytes culture was performed by standard cytogenetic technique, and banding using Giemsa trypsin banding (GTG) was done. In all cases, 12 metaphases were karyotyped. When any apparent chromosomal aberrations were detected, 20 metaphases were analyzed. Computerized Karyotyper (Cytovision 2.7) was used for observation and detection of chromosomal abnormalities. FISH with alpha satellite DNA (centromeric) and whole chromosome painting probes for chromosomes 13,14,15, 21 and 22 were used because the markers appeared to be a part of acrocentric chromosomes.

**RESULTS:** Cytogenetic results were successfully obtained from all patients. Overall, 95.02 % of patients (3250 patients) had a normal karyotype, and 4.98% (170 patients) had abnormal karyotypes. In 6 cases (0.001 %), 4 males and 2 females a marker chromosome was identified. All of these marker chromosomes by G-banding appeared to be part of acrocentric chromosomes, so by using probes for these chromosomes we could identify these markers to be parts of chromosomes 13,15, and 22. Two males and one female had partial trisomy 13, a male and a female had partial trisomy 15, and one male had partial trisomy 22.

**CONCLUSION:** The combined use of classical banding and FISH techniques is a useful tool in the identification of the origin of the unknown chromosome markers. It could be of significant importance for diagnosis and offering PGD for these patients to prevent the transmission of this anomaly to their offspring.

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#### P-544

**A microfluidic chemotaxis system to select motile and mature sperm.** L. Karakoc Sokmensuer, S. Palaniappan, M. Toner, T. L. Toth, D. L. Wright. Massachusetts General Hospital, Boston, MA.

**OBJECTIVE:** The evolution of IVF technology has been weighted heavily in the area of embryo culture with limited attention to sperm processing. Many of the current sperm processing techniques may be less than desirable due to damaging effects inflicted on the sample. This study describes the development of a microfluidic chemotaxis device for the purpose of selecting a highly motile, mature sperm fraction for potential use in IVF/ICSI.