

zation rate during this period of 65%.) Pregnancy and implantation rates were the same for the TESE/ICSI and oligoteratozoospermic groups (52% and 22% respectively).

**Conclusion:** Previous studies have shown that normal fertilization rates with ICSI using testicular sperm are generally lower than with ejaculated sperm. In contrast to these results, our data indicates that higher fertilization rates may be achieved with testicular sperm. A possible cause of the high fertilization rate achieved with testicular sperm is that the majority of the TESE patients had obstructive azoospermia, in which spermatogenesis is normal. Sperm extracted from these patients may have a higher fertilization potential than ejaculated sperm from severe male factor patients. In addition, improved protocols used during the processing and injection of testicular sperm (e.g. separation on a density gradient, incubation of processed sperm prior to injection and minimal exposure to PVP) may increase its fertilizing capability.

#### P-214

**Effect of Hyaluronic Acid (HA) as a Substitute for Polyvinyl-Pyrrolidone (PVP) on ICSI Outcome.** J. H. Moon, Y. S. Heo, S. H. Yoon, J. H. Jeong, S. P. Park, J. H. Lim. Maria Infertility Clinic, Seoul, Korea.

**Objectives:** Synthetic macromolecule polyvinylpyrrolidone (PVP) is routinely used in conventional ICSI procedure. However, it has been reported that PVP induces detrimental effect on embryo development including chromosomal abnormality. It suggests that the avoidance of PVP in ICSI procedure should be a reasonable choice. HA, a kind of Glycosaminoglycans (GAGs), is found at high concentration in the fluid of the female reproductive tract of several mammalian species. The aim of this investigation was to examine whether HA could be used as a substitute for PVP in ICSI procedure.

**Design:** ICSI was performed in PBS supplemented with either PVP or HA. After ICSI, fertilization and development were evaluated in two treatments.

**Materials and methods:** After oocyte retrieval, oocytes were enzymatically denuded. MII oocytes derived from one patient were randomly divided into group A (n=28) and group B (n=22) and ICSI were performed with husband spermatozoa which immobilized in either 5% PVP or 2.5 mg/ml HA, respectively. At 18 hours after microinjection, 2PNs were co-cultured with cumulus cells in YS medium containing 10% hFF until day 3 or day 5. Quality of embryos was evaluated on day 3 into four grades (good, medium+, medium and poor) based on their fragmentation and morphology. The blastocyst rates were also evaluated in two groups.

**Result:** Viscosity of HA allowed for reduction of sperm motility and control of sperm movement inside injection pipette like PVP. Fertilization rates were not different between group A (78.6%) and group B (81.8%). On day 3, however, the rate of good quality embryos of group B (61.1%) was higher than those of group A (45.0%). The blastocyst rate of group B (77.8%) was also higher than that of group A (59.0%).

**Conclusion:** This result suggests that HA should be a good candidate for reducing the potentially harmful effects derived from PVP in ICSI.

#### P-215

**Three Successful Pregnancies Resulting from Intracytoplasmic Injection of Elongated Spermatids.** R. T. Mansour, I. Fahmy, A. Kamal, M. A. Aboulghar, G. I. Serour. The Egyptian IVF-ET Center, Cairo 11431, Egypt.

**Objectives:** In approximately 35% of patients with non-obstructive azoospermia undergoing testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI), failure of spermatozoa retrieval leads to cycle cancellation. In these cases, every effort should be made to find spermatids for injection. The aim of this study was to report our experience in using elongated spermatids for ICSI.

**Design:** Retrospective chart review.

**Materials and Methods:** Four hundred and eighty-eight cycles of TESE and ICSI were performed in patients with non-obstructive azoospermia. In 179 cycles (26.7%), no spermatozoa suitable for injection were found after an extensive search and spermatids were sought. Spermatids were identified as described by de Kretser and Kerr (1994). Elongated spermatids were identified by their darker condensed nucleus, which resembles the head of a spermatozoon. The cytoplasm is shed from around the nucleus at one side, giving the elongated spermatid its characteristic 'ice-cream cone' shape. In

22 of these 179 cycles (12%), elongated spermatids were found and used for micro-injection.

**Results:** A total of 184 MII oocytes were injected using elongated spermatids and the 2PN stage was reached in 61 oocytes, giving a fertilization rate of 33.2%. Embryo transfer was performed in nineteen cycles (86.4%). In six cycles, a positive  $\beta$ -hCG test was observed after two weeks. Three clinical singleton pregnancies were established and resulted in the delivery of three healthy boys with a normal karyotype. Interestingly, in 16 of the 22 cycles of spermatid injection, a small number of abnormal and non-viable spermatozoa were found but not used for injection. In the remaining six patients, only spermatids were detected, in spite of a history of spermatozoa present in previous semen or TESE samples.

**Conclusions:** When no normal living spermatozoa can be found after TESE, elongated spermatids can be used to inject oocytes, resulting in the delivery of healthy babies.

#### P-216

**Aneuploidy in Sperm from Seventy-Four Oligoasthenoteratozoospermic (OAT) Patients Undergoing Intracytoplasmic Sperm Injection (ICSI).** M. G. Pang<sup>1</sup>, S. F. Hoegerman<sup>2,4</sup>, J. Pfeffer<sup>3</sup>, H. Kim<sup>4</sup>, S. Oehninger<sup>5</sup>, W. G. Kearns<sup>4,5,6</sup>. <sup>1</sup>Biomedical Research Center, Korea Advanced Institute of Science and Technology, Taejon, Korea, <sup>2</sup>College of William and Mary, Williamsburg, VA, <sup>3</sup>DHUYIS IVF Center, Zerah-Taar-Pfeffer Laboratory, Bagnolet, France, <sup>4</sup>Center for Pediatric Research, Norfolk, VA, <sup>5</sup>Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, <sup>6</sup>Johns Hopkins University School of Medicine, Baltimore, MD.

**Objective:** We summarize data on aneuploidy in sperm from 74 OAT patients undergoing ICSI.

**Design:** This study determined aneuploidy in sperm from OAT patients and tabulated fertilization and pregnancy rates following ICSI.

**Materials and Methods:** Aneuploidy frequencies were determined in sperm from 74 OAT patients and 28 proven fertile donors using multiprobe, multi-color fluorescence in situ hybridization (FISH). Direct labelled DNA specific for chromosomes 1, 4, 6, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y were used and over 300,000 sperm were scored. Where appropriate, Chi-square analysis or Fisher's Exact Test were performed.

**Results:** Per chromosome disomy frequencies for the gonosomes and autosomes ranged between 0 and 5.8% for patients and 0 to 0.3% for controls. The frequency of diploid sperm ranged from 0 to 9.6% in patients and 0 to 1.2% for controls. The OAT patient group was heterogeneous for the type and frequency of numerical chromosome abnormalities observed. Total aneuploidy in sperm from the 74 OAT patients was estimated, using formulae developed by Hoegerman et al, to range between 27 and 74%. In contrast, total aneuploidy in sperm from the 28 proven fertile donors was between 3.9 and 7.7%. Fifty-six ICSI cycles were performed. Sixty-nine percent (366/525) of oocytes fertilized. Following fresh embryo transfer, 6 preclinical abortion's, 1 first trimester loss and 3 healthy term deliveries resulted.

**Conclusions:** There were significant increases in the frequencies of diploidy, autosomal disomy, sex chromosome aneuploidy and total cytogenetic abnormalities in sperm from 74 OAT patients versus controls. The data suggest that meiotic errors occur at highly elevated frequencies in the germ cells of severely affected OAT patients. Males with severe OAT donating sperm for ICSI are at risk of transmitting genetic abnormalities to their offspring.

#### P-217

**Relevance of Fertilization Outcome with Conventional or ICSI Insemination.** <sup>1</sup>R. Porter, <sup>1</sup>T. Han, <sup>2</sup>M. Tucker, <sup>1</sup>L. Meincke, <sup>1</sup>S. Greenhouse, <sup>1</sup>G. Mottla. <sup>1</sup>Shady Grove Reproductive Science Center, Rockville, Maryland. <sup>2</sup>Georgia Reproductive Specialists, Atlanta, GA.

**Objectives:** The principal aim of this study was to monitor the tri-pronucleate formation rate following either conventional or ICSI insemination, to estimate the rate of 2<sup>nd</sup>, polar body retention giving rise to 3PN formation post-fertilization. Data were stratified according to maternal age to discern if 3PN formation increased with increasing age of the woman from whom the oocytes were collected.