study (2:1). A group of 22 patients with ECF during stimulation (Group A) was compared with 44 women without fluid accumulation (Group B). Cases with ECF and hydrosalpinges or polyps were excluded from the study. In all cases no abnormalities were detected in the uterine cavity before ovarian stimulation. In our center when ECF was diagnosed, careful ultrasound assessment of the endometrium was performed in every examination and when fluid accumulation was persistent, we performed fluid aspiration before oocyte retrieval.

Results: The ECF and controlled groups were matched for age, basal FSH, ethnicity, BMI and stimulation protocol. The mean number of oocytes retrieved was 13 in group A and 12 in group B (P=NS), the fertilization rate was 46% in Group A and 50% in Group B (P<0.05). No statistically significant differences were found between the two groups according to endometrial thickness, embryo quality and mean number of embryos transferred. The pregnancy rate per oocyte retrieval was 52% in group A and 57% in group B, with the difference not being statistically significant. Four patients out of five with transient fluid accumulation were pregnant (75%).

Conclusions: Although fluid accumulation within the endometrial cavity is not a common finding, we believe that if found, careful ultrasound monitoring is required to confirm if the fluid accumulation is transient. If ECF is persistent, we believe that aspiration of the fluid before oocyte collection might be a successful treatment technique.

FREE COMMUNICATION
Session 59 – ART/Laboratory/ICSI – MESA – TESE
Wednesday 30 June 2004 14:00-15:15

O-249 The relationship between the mode of oolemma breakage and the clinical outcome in ICSI
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Introduction: Previous studies have reported a relationship between the mode of oolemma breakage on one hand and the fertilization rate and embryo quality on the other hand in patients treated with intracytoplasmic sperm injection (ICSI). The aim of this work was to study the relationship between the mode of oolemma breakage during ICSI and the clinical outcome in those patients.

Materials and methods: A total of 295 treatment cycles were studied in patients treated with intracytoplasmic sperm injection due to male factor infertility. A total number of 1717 oocytes were retrieved. Of these, 1572 oocytes were in metaphase II (91.6%) and were injected with spermatozoa. The mode of oolemma breakage of each oocyte during the ICSI procedure was recorded and was assigned to one of 3 grades. Grade A refers to oocyte exhibiting oolemma penetration without need of cytoplasmic aspiration (no elasticity). Grade B refers to oocytes exhibiting oolemma penetration requiring mild or moderate cytoplasmic aspiration (average elasticity), while grade C refers to oocytes exhibiting oolemma penetration requiring strong cytoplasmic aspiration (excessive elasticity).

Results: The fertilization rate was 73.5% in grade A oocytes, 78.3% in grade B oocytes and 82.4% in grade C oocytes and these differences are not statistically significant. However, the pregnancy rate was 8.3% in patients with embryo transfers arising from grade A oocytes compared to 44.1% in patients with embryo transfers arising from grade B oocytes (P< 0.05). The pregnancy rate was 22.2% for patients with embryo transfers arising from grade C oocytes and 33.3% for patients with embryo transfers arising from mixed-grade oocytes (P> 0.05 and P< 0.05 compared to grade B). Similarly, the implantation rate was 5.6% in patients with embryo transfers arising from grade A oocytes, 15.7% in patients with embryo transfers arising from grade B oocytes, 10.3% in patients with embryo transfers arising from grade C oocytes and 8.4% in patients with embryo transfers arising from mixed-grade oocytes. These differences are not statistically significant.

Conclusions: It is concluded that the mode of oolemma breakage affects the clinical outcome of ICSI and that an optimum elasticity of the oolemma is required for optimum clinical results.

O-250 The contribution of spindle birefringent imaging by PolScope to the outcome of ICSI
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Introduction: Studies show higher fertilization and embryo development rates in oocytes where a birefringent spindle was visualized. Our aim was to examine whether the ICSI outcome of oocytes in which spindle imaging was applied differed from the ICSI outcome of sibling oocytes in which no search for spindle was performed.

Materials and methods: 219 oocytes from 16 consecutive patients (each having ≥10 MII oocytes) were included in the study. Patients’ mean age was 28 ± 4.36 and mean interval from HCG injection to ICSI procedure was 40.5h ± 28.12. Half of the oocytes of each patient, a total of 114 (study group) were tested with the PolScope (LC-PoScope, CRL, MA, USA). Once spindle was viewed (SP+), the spermatozoon was injected perpendicularly to the spindle site. In oocytes where spindle was not visualized (SP−), injection was performed at 3 o’clock when polar body was at 6 o’clock. In the remaining 105 oocytes (control group) ICSI was performed at 3 o’clock when polar body was at 6 o’clock. Fertilizations were assessed 18-20h post insemination, and embryo transfer was done on day 3. embryo quality was evaluated according to cleavage and amount of fragmentation (1-best, 4-worst). Supernumerary embryos were either frozen or cultured to theblastocyst stage and then frozen. Contingency analysis, t-test, and Anova with Tukey as a post test analyzed the data.

Results: A birefringent spindle was viewed in 68% (78/114) of the oocytes. The distribution of the oocytes according to the angle of spindle deviation from first polar body was: 0°-9°, 6°-45°, and 46°-90°-21% of the oocytes. There were more post-mature oocytes (maturation time from HCG injection >40h) among the ones in which spindle was not viewed 21/36 (58%) comparing to 40/78 (51%) and 54/105 (51%) in the SP+ and control groups (P=0.05). Fertilization rate was similar both in the study and control groups: 76/114 (67%), 70/105 (67%). Fertilization rate in the SP+ group was 78% (61/78), significantly higher than in the SP- group, 42% (15/36) (p<0.001). SP- oocytes had also significantly less fertilizations in comparison with the controls (p<0.05). The cleavage rate was similar among the groups. Morphology of day 3 embryos that derived from SP- oocytes was significantly poorer than the control (p=0.01). Injection according to the spindle at 6 o’clock took place in 14/78(18%) of the oocytes, of them 9/14 (64%) were normally fertilized.

Conclusions: Overall fertilization and cleavage rates were comparable in the PolScope examined and the control groups. However, the SP- group showed significantly poorer embryo morphology on day 3. Birefringence imaging by PolScope may be useful in the oocytes in which the angle of deviation of the spindle from the first polar body is large and their injection is thus according to spindle site.

O-251 Increased fertilization rates after in-vitro culture of frozen-thawed testicular sperm
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Introduction: The efficacy of using cryopreserved spermatozoa extracted from the testes is slightly questionable with regard to fertilizing capacity. The aim of this study was to optimise the use of freeze/thaw testicular spermatozoa from obstructive azoospermia patients and to analyse the outcome of intracytoplasmic sperm injection (ICSI) of such spermatozoa.

Material and methods: Testicular specimens were retrieved and cryopreserved from twenty-two patients with obstructive azoospermia and underwent one cycle with thawed spermatozoa (Group I) that led to pregnancy in eight cases. Fourteen patients of group I subsequently underwent treatment with the same batch of thawed spermatozoa (Group II). Washed spermatozoa were cultured in microdroplets under paraffin oil using bicarbonate-buffered bicarbonate-buffered medium. The ECF and controlled groups were matched for age, basal FSH, ethnicity, BMI and stimulation protocol. The mean number of oocytes retrieved was 13 in group A and 12 in group B (P=NS), the fertilization rate was 46% in Group A and 50% in Group B (P<0.05). No statistically significant differences were found between the two groups according to endometrial thickness, embryo quality and mean number of embryos transferred. The pregnancy rate per oocyte retrieval was 52% in group A and 57% in group B, with the difference not being statistically significant. Four patients out of five with transient fluid accumulation were pregnant (75%).

Conclusions: Although fluid accumulation within the endometrial cavity is not a common finding, we believe that if found, careful ultrasound monitoring is required to confirm if the fluid accumulation is transient. If ECF is persistent, we believe that aspiration of the fluid before oocyte collection might be a successful treatment technique.
medium containing 10% human serum in a 5% CO2 incubator at 37°C. For the first ICSI attempt (Group I), injection was performed when motile spermatozoa were found. In most cases, viable sperm began to twitch after 2-4 hours in culture, and these cells were selected for ICSI. In all the fourteen couples of group II, one portion of the specimen was thawed the day before ICSI, and changes in post-thaw motility after 24 h of culture at 37°C were recorded. In group II, injection was performed when maximum motility was reached.

The data for each cycle with regard to oocytes collected, injected and fertilized and pregnancy outcomes were analysed by paired r-test to assess differences between each of the two ICSI cycles.

Results: In the Group II, the proportion of frozen/thawed spermatozoa which achieved motility was 10-30% and this was estimated to be 20-25 spermatozoa per droplet. The motility improved markedly on the sixth hour of culture and it peaked around eight hours. In three cases testicular spermatozoa became motile at 18 hours.

There was no significant difference in number of oocytes collected or injected, number of grade 1 embryos per cycle or number of embryos transferred per cycle between any of the two groups. However, cryopreserved testicular spermatozoa in Group I showed a significant decrease in fertilization rates when compared with cryopreserved testicular spermatozoa in group II (68.5% vs. 82%, p<0.05, respectively). No difference was noted between the clinical pregnancy rates (8/22 (36.3%) vs. 5/14 (35.7%),

Conclusion: These data, although from a small group, suggest fecundation rate can be significantly improved after in-vitro culture and sperm selection of frozen-thawed testicular spermatozoa in patients with obstructive azoospermia.

O-252 ICSI results in men with cystic fibrosis

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Introduction: Life expectancy and health of men with cystic fibrosis have been largely improved recently so they are more likely to marry and wanting to become father. Unfortunately men with cystic fibrosis are sterile because an abnormal development of vas deferens. We report results of ICSI performed in men with cystic fibrosis with surgically retrieved sperm.

Patients and methods: Twenty three couples whose the male partner had a clinical cystic fibrosis requested medically assisted procreation. Nine men (39%) had two ∆F508 mutation of the CFTR gene, 11 (48%) had one ∆F508 mutation associated with another CFTR mutation, three had other CFTR mutations. No CFTR mutations have been detected in their partner and genetic counselling has been provided to all couples. All men were infertile.

In the fourteen couples of group II, one portion of the specimen was thawed the day before ICSI, and changes in post-thaw motility after 24 h of culture at 37°C were recorded. In group II, injection was performed when maximum motility was reached.

The data for each cycle with regard to oocytes collected, injected and fertilized and pregnancy outcomes were analysed by paired r-test to assess differences between each of the two ICSI cycles.

Results: In the Group II, the proportion of frozen/thawed spermatozoa which achieved motility was 10-30% and this was estimated to be 20-25 spermatozoa per droplet. The motility improved markedly on the sixth hour of culture and it peaked around eight hours. In three cases testicular spermatozoa became motile at 18 hours.

There was no significant difference in number of oocytes collected or injected, number of grade 1 embryos per cycle or number of embryos transferred per cycle between any of the two groups. However, cryopreserved testicular spermatozoa in Group I showed a significant decrease in fertilization rates when compared with cryopreserved testicular spermatozoa in group II (68.5% vs. 82%, p<0.05, respectively). No difference was noted between the clinical pregnancy rates (8/22 (36.3%) vs. 5/14 (35.7%).

Conclusion: These data, although from a small group, suggest fecundation rate can be significantly improved after in-vitro culture and sperm selection of frozen-thawed testicular spermatozoa in patients with obstructive azoospermia.

O-253 Over-night incubation after “sperm washing” does not affect IVF outcome in HIV-1 serodiscordant couples

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Introduction: In HIV-1 serodiscordant couples for male positivity the sperm washing technique followed by detection of HIV-1 RNA in swim up fraction allows safe procreation.

We keep the processed semen samples at 4°C for about 24 hours, time required for HIV-1 RNA detection, before insemination in IVP procedures. In this study we evaluated the fertilization rate, the percentage of abnormally fertilized oocytes and the pregnancy rate when over-night incubated spermatozoa are used for IVP insemination.

Patients and methods: We performed 26 IVF cycles in 23 serodiscordant couples. In 12/26 cycles, random selected, insemination was performed adding 150,000–400,000/ml spermatozoa (group A). In remaining 14/26 cycles (group B) oocytes were inseminated adding 30,000-100,000/ml spermatozoa. 94% oocytes inseminated were classed as type 1 in group A and 77% in group B. In group A basal semen analysis showed a motility (a+b) ranging from 2% to 62% and a morphology ranging from 2% to 26%. In group B semen samples showed a motility (a+b) ranging 69% to 11% and a morphology ranging from 5% to 17%.

Semen was processed by “sperm-washing” technique. The final aliquot (swim-up) was incubated about 24 hours at 4°C waiting for HIV-1 RNA assay result.

Results: Fertilization rate in group A was 89% whereas in group B was 77%. In group A 12/69 (18%) zygotes presented abnormal fertilization (six showing 3 pronuclei, two 4 pronuclei, four 1 pronucleus) and in these cases semen motility (a+b) was >45%. In group B one zygote out of 64 (2%) showed 3 pronuclei and semen motility (a+b) was >40%. Pregnancy rate was 25% (3/12) in group A and 27% (3/11) in group B.

Conclusions: When an excess of spermatozoa is added for insemination in IVF (group A) we observed a higher but not significative fertilization rate. On the contrary a higher even if not significative percentage of abnormal fertilization was present in group A when associated to a semen motility (a+b) > 45%. Pregnancy rate was similar in both groups. A high number of inseminated spermatozoa does not enhance fertilization and pregnancy rates but increases the risk of abnormal fertilizations. Our data suggest that spermatozoa might maintain the fertilization efficiency in spite of over-night incubation and that incubation does not affect the IVF outcome.

FREE COMMUNICATION

Session 60 – ART/Cryopreservation of Embryos

Wednesday 30 June 2004 14:00–15:15

O-254 Cryosystem assessment by glucose uptake of blastocysts

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Introduction: Glucose uptake is a measure of metabolic activity and implantation potential of both murine and human embryos. We evaluated vitrification and slow freezing in a prospective randomized trial using murine blastocysts as a model for human blastocysts. Quantitating glucose consumption could validate survival and implantation potential in a murine model, allowing implementation of more effective cryopreservation of human blastocysts.

Materials and methods: Frozen 2-cell murine embryos (n = 132) thawed and cultured for 48 hours were randomly divided into 4 groups: Control - not refrozen; slow freezing using a programmed rate (PR); vitrification using...