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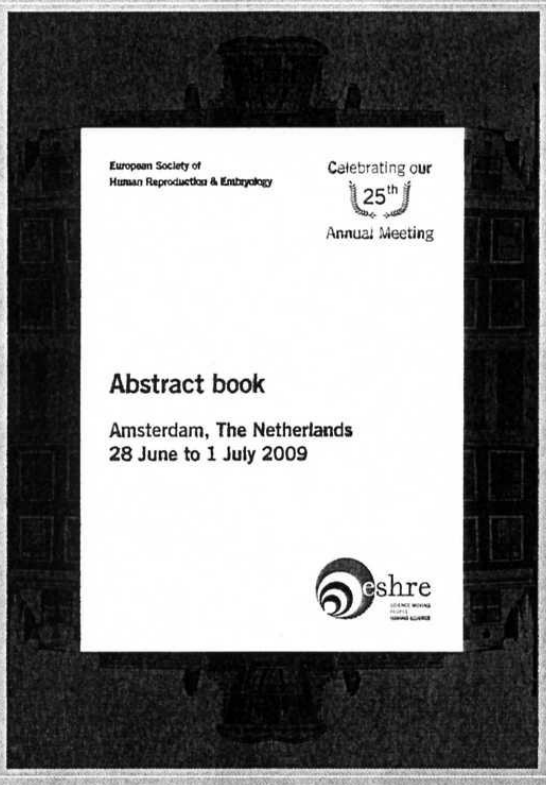

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P-410 Poster Outcome of intracytoplasmic sperm injection using zona pellucida-bound sperm and conventional selected sperm

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Introduction: The interaction between oocyte and sperm implies a series of physiological events involving species recognition, adhesion and fusion between gametes. The first interaction between the spermatozoon and the oocyte is at the zona pellucida (ZP), however in the process of intracytoplasmic sperm injection (ICSI) a single spermatozoon is selected and delivered into the oocyte. The injected fertilizing spermatozoon therefore bypasses several physiological barriers compared to *in vivo* or conventional *in vitro* fertilization. It has been described that defective sperm-ZP interaction is the major cause for low fertilization rates, and that this is usually due to defects in the spermatozoa rather than defective oocytes. It has indeed been found that oligozoospermic men have a higher frequency of defective sperm-zona interactions. It was also observed that sperm morphology was significantly related to the percentage of sperm capable of binding to the ZP. Moreover it was demonstrated that sperm with single stranded or denatured DNA generally do not bind to the ZP. The present study aimed to investigate whether the sperm-ZP binding test was able to select spermatozoa with higher fertilization potential and a higher rate of successful embryo development.
Material & Methods: This randomized prospective study was performed in 388 metaphase-II (MII) oocytes retrieved from 40 couples undergoing ICSI. For each patient, half of the MII oocytes were injected with our routine ICSI method (control group, n = 194)) and the other half were injected with previously ZP-bound sperm (ZP-binding group, n = 194). For the ZP binding testa 5 μL sperm sample (concentration: 1×10^6 motile sperm per mL) was incubated for two hours with one immature oocyte, retrieved from the same patient. The sperm bound to the oocyte ZP were removed with a microinjection needle, under an inverted microscope at 400x magnification, and used for ICSI. Successful fertilization and embryo quality were compared between the groups. The cycles were additionally split into two groups based on the group origin of transferred embryos: a ZP-binding transfer group (n = 15), in which only embryos derived from the ZP-binding group were transferred, and a combined-transfer group (n = 25), in which embryos derived from both the ZP-binding and control groups were transferred. Implantation, pregnancy and miscarriage rates were compared among the embryo transfer groups.
Results: No significant difference was observed in the fertilization rate regardless of whether oocytes were injected with ZP-bound sperm. An increased percentage of high quality embryos was observed, however, when ZP-bound sperm were injected (70.0% *vs* 83.5%, P = 0.003). In addition, when embryo selection was performed while ignoring the experimental group origin, it was observed that embryos from the ZP-binding group were more commonly selected for transfer when compared to the control group (43.5% *vs* 54.6%, P = 0.004). When the treatment success was evaluated according to the embryo transfer group, a trend for higher implantation rate in the ZP-binding transfer group was noted (20.8% *vs* 42.2% *P* = 0.075). These most likely did not reach statistical significance because of the small number of cycles evaluated in this trial. However, no difference in the pregnancy (32.0% *vs* 40.0%, *P* = 0.673) and miscarriage rates (37.5% *vs* 33.3%, *P* = 0.633), for ZP-binding transfer and combined transfer groups, respectively, was observed.
Conclusion: We have shown here that the sperm-ZP interaction is extremely important in the selection of sperm with the higher embryo developmental capacity, and that the sperm-ZP binding test may be an efficient method to identify the most competent sperm for ICSI.

P-411 Poster Effects of four different culture media on the quality of human zygotes and embryos

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Introduction: Since the establishment of in vitro fertilization (IVF) technologies worldwide, a large number of different media formulations and culture systems have been employed for the development of the human embryo. ' effects of the composition of culture media upon embryogenesis have b the subject of extensive studies. The aim of this study was to compare effects of four different culture media on the quality of zygotes and the mation of good quality embryos, defined as embryos TOP.
Material & methods: Retrospective study, in the Center for Human Reprod tion of Campinas-Brazil, where 2289 embryos were assessed for the per from September 2006 to September 2008. Long protocol was used ovarian stimulation in all cases. All embryonic culture plates were compo of microdrops of two different culture media, where HTF (Irvine) was se the default for all cycles and IVF Medium (Medicult), GGG 20 (Global) IVF 30 (Vitrolife) defined as secondary media. After ICSI, sibling oocy were divided in the two culture media. The confirmation of fertilization classification of the pronuclei were evaluated 18 hours after ICSI, using the teria described by Gianaroli et al, 2003. On the second day (day 2) of devel ment, the embryos were evaluated according to the number of cells, percent of fragmentation and number of nuclei. On day 2, the embryos that had cells with less than 20% of fragmentation and were mononucleated were cla fied as TOP.
Results: Regarding the formation of centralized pronuclei, classified as A was significantly higher in Vitrolife medium when compared to Irv medium zygotes [odds ratio (OR) (95% confidence interval (CI)-1.57 (1. 1.94)]. On the other hand, Global and Medicult medium showed differen (OR (95% CI): 0.79 (0.58, 1.07)) and (OR (95% CI): 1.07 (0.80, 1.42)), resp ively. Regarding the arrangement of nucleoli, Global medium was associa with a higher formation of pronuclei with scattered nucleoli (2) (OR (9 CI): 1.71 (1.18, 2.47)), and a lower number of pronuclei with juxtapo nucleoli (1) (OR (95% CI): 0.69 (0.51, 0.94)) when compared to Irvine. Vit life medium also produced higher number of centralized pronuclei with ju posed or scattered nucleoli (A1+A2) in relation to Irvine (OR (95% CI): 1 (1.05, 1.54)). The Global (OR (95% CI): 0.84 (0.62, 1.13)) and Medic medium (OR (95% CI): 0.82 (0.63, 1.08)) showed no significant differe when compared with Irvine medium for the same category (A1+A2). T number of TOP embryos on day 2 of development with the use of Glo and Vitrolife medium was higher (OR (95% CI): 2.94 (2.16, 4.00); OR (9 CI 1.32 (1.09, 1.61)), respectively, while no difference was observed with M icult medium (OR (95% CI): 0.61 (0.44, 0.82)).
Conclusions: The use of Vitrolife medium resulted in a higher number zygotes with centralized pronuclei with juxtaposed or scattered nucle when compared to Irvine. In addition, Global medium produced a gre number of morphologically best embryos (TOP) on the second day of devel ment when compared to Irvine.

P-412 Poster Oocytes with visualized meiotic spindle give higher f tilization rate and embryo scores

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Introduction: The introduction of polarization light microscopy was used assess oocyte quality and related IVF-ICSI results. High birefringence zona pellucida has been positively associated with embrionic better impl tation potentials. No conclusive results were reached for the meiotic spin visualization in relation with fertilization rate. This is the first study to correl birefringence pattern of zona pellucida and meiotic spindle metaphase oocytes with fertilization rate (FR), pronuclear stage Z1 oocytes (PNZ grade I embryos (EI) and clinical pregnancy rate (CPR).
Material & methods: 177 metaphase II oocytes were inseminated with c ventional ICSI procedure in 48 cycles. For this prospective study, no m factor cases of infertility were included. A maximum of 3 oocytes per cy were avalaible for ICSI according with the limitation of the Italian law ART. Mean maternal age was 37.2 ± 3.4. Zona and spindle imaging achieved using an inverted microscope equipped with a polarization filt The birefingence analysis was performed by a polarization imaging softw module (OCTAX ICSI Guard TM, OCTAX Microscience GmbH, German Oocytes were classified according with the high (HZB) or low birefringe (LZB) of the zona pellucida and with the presence (SP) or absence (aSP) meiotic spindle after usual decumulation. Ovum pick-up was performed at

37–38 h hCG administration. For controlled ovarian hyperstimulation (COH), a short protocol with rFSH was used. Embryo grading at day 2 was performed according to Steer-Mills and pronuclear stage scoring according to Scott criteria. For statistical analysis Chi-square test was used.

Results: 104 oocytes showed HZB (58.7%) and 73 LZB (41.3%). FR was 80.7% with HZB oocytes and 79.5% with LZB oocytes (not significant).

54 PNZ1 pronuclear stage oocytes derived from HZB (64.3%) and 33 (56.9%) from LZB (not significant). 54 E1 derived from HZB oocytes (70%) and 29 (65.9%) from LZB (not significant).

For the presence of meiotic spindle, we observed that in 133 oocytes (75.4%) it was easily visible (SP), whereas in 32 (18%) it was not visible (aSP). FR was 87.9% with SP oocytes and 53.1% with aSP oocytes ($p < 0.0001$). SP oocytes giving ZP1 were 74 (63.2%) but with aSP oocytes ZP1 were 7 (41%) (not significant). Grade I embryos from SP oocytes were 78 (72.9%), whereas from aSP only 5 grade I embryos were obtained (37.5%) ($p < 0.01$).

Conclusions: Meiotic spindle pattern, as assessed by polarization microscopy, is correlated with main reproductive indexes of outcome in ICSI procedure FR and grade I embryos. However, zona birefringence does not influence FR nor embryo quality. Meiotic spindle birefringence is good selection criteria usefull for local situation where it is advisable and possibly for oocyte freezing procedures.

P-413 Poster A comparison of sperm yield following changes in HIV sperm washing laboratory practice

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Introduction: The safety and effectiveness of sperm washing as a risk reduction method in HIV discordant couples wishing to conceive has been recognized in several studies. Sperm washing using density gradient centrifugation followed by PCR for viral detection of sperm is a well established technique. We have routinely included an additional wash and 'swim-up' step in our standard operating procedure (SOP) for sperm washing as a means of further eliminating the presence of potentially infected non-sperm cells that could inhibit or contaminate the PCR technique and generate a positive result (Kim et al., 1999). This retrospective study aims to determine whether changes to our lab practice for sperm washing affects clinical outcome.

Materials & Methods: A total of 98 HIV semen samples were included in this study, 49 of which were prepared according to the "new" SOP by density gradient centrifugation followed by two washes. This compares to 49 samples that were matched for total motile count that had been prepared using the "old" SOP by density gradient centrifugation, three washes followed by a 'swim-up' step. An aliquot of prep sperm sample was tested for detectable HIV RNA. Nucleic acid extraction and HIV-1 RNA quantification was performed using a real-time PCR assay (ROCHE) with a detection limit of 50 RNA copies/ml. The remaining washed sperm was incubated at 37 C and used for treatment (IUI, IVF or ICSI) if the PCR result for HIV was negative.

Using the "new" and "old" SOP for sperm washing, pre- and post-prep semen parameters were compared in accordance to WHO criteria. The main outcome measures included volume (ml), concentration (x106/ml), total count (x106), motility (%), morphology (%), total motile count (x106), proportion of initial total motile count recovered for insemination (%), and PCR assay results. Statistical analysis was performed by Fisher's Exact Test and Mann Whitney Test using Analyse-It for Microsoft Excel.

Results: Pre-prep semen analyses were comparable between the "new" vs "old" SOP: volume: 2.19 ml vs. 2.59 ml, concentration: 55.3 vs. 47.9×106/ml, total count: 115.5 vs. 110.1×106, motility: 54.3 vs. 52.8%, abnormals: 82 vs. 77.1% and total motile count: 61.4 vs. 61.2×106. When post-prep parameters were examined, the data showed a significantly higher post-prep volume (0.63 ml vs. 0.47 ml; $p = < 0.001$), total count (9.4 vs. 4.2×106; $p = 0.03$), total motile count (8.6 vs. 4.0×106; $p = 0.04$) and proportion of initial total motile count recovered for treatment (21.6% vs. 14.6%; $p = 0.01$) using the "new" compared to the "old" SOP respectively. There was no difference in the incidence of positive PCR assay results with changes in SOP (4%: 2 of 49 in each of the two groups).

Conclusion: The data in this study demonstrates that omitting one wash and "swim-up" step from the sperm washing SOP produces a significantly higher yield of total sperm count that could alter the patients' treatment option without increasing the risk of a positive PCR result.

References: Kim et al., AIDS, 1999, 13: 645–651.

P-414 Poster Low frequence of spindle apparatus in metaphase II oocytes retrieved from women with a poor ovarian response

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Introduction: In women with a low ovarian response to gonadotropin treatment, there has been reported diminished embryo implantation and pregnancy rates after in vitro fertilization (IVF) treatment compared to average population in treatment. Low ovarian response is strongly correlated to female age and it is suggested that this condition interferes with the oocyte quality in the growing follicles. It has been shown that the secondary meiotic spindle often is disrupted in this category of oocytes. In this study, we investigated the frequence of appearance of spindle by birefringence analysis of oocytes retrieved from women with a low ovarian response and compared the developmental capacity of these oocytes to a average treatment group with similar diagnostic profile.

Materials & Methods: This prospective study included 46 women (N) in vitro fertilization treatment at Fertility Clinic, Holbaek Hospital, Denmark. The controlled ovarian hyperstimulation protocol consisted of an agonist down regulation and follicular stimulation by recombinant follicular stimulating hormone (rFSH) followed by human recombinant chorionic gonadotropin (rhCG). The group of a poor ovarian response defined by ≤ 5 oocytes per retrieval and ≥ 500 IU FSH per retrieved oocyte ($N = 22$) and referral average group ($N = 24$) excluding the former group.

Oocyte retrival was performed 36 hours after 5.000 IU rhCG injection. Oocytes were washed in Gamte Buffer (Cook) and cultured in Fertilization Medium (Cook). Two hours later, cumulus cells were removed by hyaluronidase (MediCult) treatment and the oocytes were placed individually in 5 µL droplets of Cleavage Medium covered by mineral oil (Cook) in glass bottom dish (WillCo).

Spindle imaging was performed at x20 and x40 optics in a inverted microscope (Leica DMI 6000), equipped with a heated glass plate (37.0 ± 0.5 C) and polarization filter, combined with polarization module polarAIDE® including software (MTG, Germany). Oocytes were rotated to favour spindle microtubules birefringence. Following image analysis, the metaphase II oocytes were microinseminated and cultured in Cleavage Medium (Cook) invidually in 500 µL vials (Nunclon) for two days. Oocytes were observed and digitally recorded 18, 24 and 40 hours post insemination. StatView for Mac® was used for statistical analysis: $p < 0.05$ was considered significant.

Results: There was a significant difference ($p < 0.05$) in rate of poor ovarian response according to female age (32% of women below 35 years ($N = 31$) and 68% women over 34 years ($N = 15$)).

In contrary, there was no significant difference between the two age groups concerning presence of spindle in retrieved MII oocytes (mean ± SEM, 58.6 ± 5.6% MII oocytes with visible spindle in women ≤ 34 years ($N = 31$) and 57.8 ± 8.8% in women ≥ 35 years($N = 15$)).

The oocytes retrieved from women with low ovarian response displayed a significantly ($p < 0.05$) lower rate of visible second meiotic spindle (mean ± SEM: 50.2 ± 8.5% in low ovarian responders ($N = 22$) and 73.2 ± 4.9% in average responders ($N = 24$)).

The metaphase II oocytes of low ovarian response group were not fertililized in a significantly lower rate than average (mean ± SEM, 55.8 ± 7.8% in low ovarian responders ($N = 22$); 57.6 ± 5.7% in average responders ($N = 24$))

The fertilized MII oocytes in same group were neither significantly retarded in respect to rate of four cell preembryos at 40 hours post insemination (mean ± SEM: 34.8 ± 7.7% rate of four cell preembryos in low ovarian responders ($N = 22$), and 35.0 ± 5.1% four cell preembryos in average responders ($N = 24$)).

Conclusions: Present study suggests that oocytes retrieved from women with a low ovarian response have a low rate of visible spindle apparatus but not a compromised fertilization rate by microinsemination and not a following retarded cleavage rate.