granulose cells by ovarian histology. Nonfunctional grafts were evidenced by dominant parabasal cells of vaginal cytology and necrosis of ovarian histology.
Results: Of the 15 rabbits, 13 (86.7%) restored ovarian function as short as a week after transplantation. Among the 13 functional rabbits, 7 revealed in the lutenized stage and 6 in the non-lutenized follicular stage by H&E stain, both corresponding to intermediate and superficial cells of vaginal cytology, respectively. Despite vascular anastomosis, two (13.3%) failed to restore function due to fragile mesenteric fat during thawing, in contrast to functional rabbits with intact mesenteric fat. These failed rabbits demonstrated all parabasal cells of vaginal cytology and necrosis of ovarian histology.
Conclusions: Cryopreservation of whole rabbit ovary followed by vascular anastomosis of transplantation may overcome revascularized ischemia and last at least 7-month longevity in the majority of transplanted ovaries. Fragile mesenteric fat after freezing process seems to affect intact architecture of graft complex or to predict the freezing outcome. Before establishing an optimal protocol, more indicators are needed to define the freezing efficiency. This study supports the promising role of whole ovarian cryopreservation and transplantation.

POSTER SESSION

ART, laboratory: embryo selection

P-413 A proteomic analysis of embryonic development

M.G. Katz-Jaffe, D.W. Linck, J.R. Blakesley, W.B. Schoolcraft, D.K. Gardner
Colorado Center for Reproductive Med., Research, Englewood, CO, USA

Introduction: Genetic studies on the mammalian preimplantation embryo are providing a wealth of information regarding gene expression and development. However, only an in depth understanding of the protein composition can lead to a true indication of embryonic cellular function and metabolism. The need to distinguish between viable and non-viable preimplantation embryos is a pre-requisite for moving to single embryo transfers. Knowledge of the regulation, formation and development of mammalian preimplantation embryos will be essential to developing viability assays. The ability to monitor the production of key developmental proteins in a non-invasive fashion should provide a better predictor of embryonic viability than the current use of morphology alone. From a clinical perspective, quantification of viability potential will result in an increase in IVF pregnancy rates and live births, while reducing the number of embryos transferred.
Aim: To investigate the protein constitution of mammalian embryos and culture media throughout the preimplantation period, and to determine the effect of oxygen concentration on developmental processes.
Materials and methods: Novel mass spectrometry was used to determine the protein profiles of in-vivo and in-vitro preimplantation F1 mice embryos. Samples of media (n=20 replicates) were also analysed and statistically evaluated. Zygotes (n=16 replicates) were cultured in vitro in G1/G2 sequential media with recombinant albumin (2.5 mg/ml) in 6% carbon dioxide and oxygen concentrations of either 5 or 20%. In vivo developed embryos (n=16 replicates) were flushed from the reproductive tract.
Results: Using a combination of invasive and non-invasive analysis, numerous panels of statistically significant proteins/biomarkers were identified. For each oxygen concentration and developmental stage, significant differences were observed both within embryos and in the proteins secreted into the media ($p<0.05$). There were proteins observed only at specific developmental time points, as well as the down and up regulation of proteins (n>30) from zygote to blastocyst ($p<0.05$). However, embryos cultured under high oxygen showed consistent down-regulation of 10 proteins between 4000 to 20,000 Dalton ($p<0.05$), while the protein profiles of embryos cultured under low oxygen conditions were more comparable to the in vivo state.
Conclusions: These data show for the first time the analysis of proteins/biomarkers both within mammalian preimplantation embryos and those detected in the surrounding media. Down-regulation of proteins under high oxygen conditions further confirms the pathological effects of oxygen during embryonic development. The advent of proteomics and the identification of critical proteins and biochemical pathways during mammalian embryonic development may provide the potential for developing non-invasive viability assays that can assist in achieving routine single embryo transfer, the elimination of multiple pregnancies and increase the live birth rate of human IVF.

P-414 Spermatozoa and correlation with early embryo development

A. Iaconelli Jr, L.M. Rossi, T.C.S. Bonetti, C.V. Locambo-Freitas, P. Guilherme, E. Borges Jr
Fertility Assisted Reproduction Center, Sao Paulo, Brazil

Introduction: A number of non-invasive methods have been proposed to evaluate embryo viability in human in vitro fertilization (IVF)/intracytoplasmatic sperm injection (ICSI) programs. Recently, assessment of the time of cleavage to the 2-cell-stage has proven to be a reliable parameter for the selection of embryos with the highest capability of implantation and successful pregnancy after transfer. This prospective study was planned to investigate the effect of the quality and origin of sperm on embryo development after ICSI, taking into account the early cleavage (EC) evaluation. Fertilization and pregnancy rates were also compared.
Methods: One hundred ninety-two ICSI cycles, performed in 175 patients were analyzed. The study groups were divided in: (A) normal semen (control, n=680 embryos/96 cycles); (B) semen with oligozoospermia (n=337 embryos/36 cycles); (C) semen with asthenozoospermia (n=171 embryos, 31 cycles); (D) epididymal sperm from obstructive azoospermia (n=58 embryos, 11 cycles) and (E) testicular sperm from non-obstructive azoospermia (n=122 embryos/15 cycles). In all cases the early cleavage embryos (embryos that have shown 2-cell stage, 26 h after ICSI) were transferred when available.
Results: A total 1368 normal fertilization embryos were evaluated and early cleavage was noted in 21.3%. In the evaluation of total EC-embryos we could note that 76.7% were selected to be transferred providing a total pregnancy rate around 47.7%. Pregnancy rate doubled when, at least one, EC-embryo was transferred (60% versus 33.3%; with or without EC-embryos transferred, respectively, p=0.025). Results are shown in the table below.

	A	B	C	D	E
Cycles/patients	96/89	36/32	31/30	11/9	18/15
Mean maternal age±SD (years)	35.5±4.9	34.5±5.4	36.3±4.6	36.3±4.9	35.3±3.2
Normal fertilization rate (%)	77.9	75.7	78.3	64.3*	63.5*
Non-fertilization rate (%)	12.1	14.9	13.2	17.1	24.5**
EC embryos (%)	24.7	24.0	22.7	24.4	24.7
Pregnancy rate per patient (%)	38.2	53.1	31.3	55.5	53.3
Miscarriage rate (%)	26.5	17.6	31.3	20.0	37.5

*p<0.03 when group D was compared to groups A, B and C; **P<0.03 when group E was compared to groups A, B and C (ANOVA, Tukey comparison)

Conclusions: The quality and origin of sperm seems to influence the fertilization rates. Despite the non-ejaculated spermatozoa providing lower normal fertilization rates as well as higher fertilization failure, it has not influenced the EC-embryos. Since embryos present good morphology they result in satisfactory pregnancy rates irrespective of the source of spermatozoa.

P-415 Exclusion of embryos with multinucleated blastomeres at day 2 improves the pregnancy

S. Takeishi[1], Y. Akamatsu[1], H. Oku[1], S. Hashimoto[1], A. Ohgaki[2], T. Nishihara[1], M. Okazaki[1], F. Nagata[2], Y. Murata[1], Y. Nakaoka[2], A. Fukuda[2], Y. Morimoto[1]
[1]IVF Namba Clinic, Osaka, Japan; [2]IVF Osaka Clinic, Osaka, Japan

Introduction: It has been suggested that the development of embryos containing multinucleated blastomeres (MNBs) is low compared with that of embryos without MNBs. To assess the developmental competence of embryos with MNBs, we examined the cell number, the morphology and the pregnancy rates of embryos with or without MNBs.
Materials and methods: Embryos were divided into 2 groups – with or without MNBs on day 2 after in vitro fertilization. The morphology of embryos

was categorized into five groups on day 2 or 3 after IVF according to Akamatrsu et al. (2004). Furthermore, the number of blastomeres was compared on day 2 or 3 after IVF. To estimate the pregnancy rates, we divided the IVF-ET cycles into three groups. The control group consisted of cycles in which only embryos without any MNBs were produced and transferred. Group A consisted of cycles in which multinucleated embryos were present but only the sibling embryos without MNBs were transferred. Group B consisted of cycles in which the majority of transfer embryos were multinucleated. We assessed the pregnancy rates in these three groups. Moreover, the relationship between the incidence of embryos with MNBs and the age of the patient was examined by assessment of Pearson's correlation. The morphology and the number of embryos were compared by t-test. The pregnancy rates were compared by χ^2 test.
Results: There was a significant decrease in the average cell numbers of embryos containing MNBs as compared to embryos without MNBs (day 2: 3.04 versus 4.07, $p<0.05$; day 3: 6.00 versus 7.38, $p<0.05$). However, there was no difference in the morphology between embryos with and without MNBs. The pregnancy rate in group B was significantly lower than that in group A (11.1% versus 53.3%), and the pregnancy rate in group A was the same as in the control group (53.3% versus 45.9%). Moreover, the rates of embryos with MNBs increased as the age of the patient increased in the cycles with embryos with MNBs ($P<0.05$). Consequently, the frequency of the transfer of embryos with MNBs increased in the cycles of patients of advanced age.
Conclusions: The results of this study suggest that excluding embryos with MNB from transfer improves the fertility rate.

P-416 Does first polar body morphology influence subsequent embryo development?

A. Nishigaki, A. Egashira, M. Sugioka, E. Nagafuchi, K. Ohtsu, H.N. Hori, N. Yoshioka, T. Kuramoto
Kuramoto Women's Clinic, Fukuoka, Japan

Introduction: Intracytoplasmic sperm injection (ICSI) has been widely used to treat couples with infertility because of severely impaired sperm characteristic and for whom in vitro fertilization (IVF) had failed. The removal of the cumulus cells from the oocyte prior to ICSI allows investigation of many factors about oocyte morphology. Recent reports have suggested that the evaluation of the morphology of human oocytes can predict the outcome of ICSI. In the present study, the relationship between first polar body (PB) morphological evaluation and embryo development was investigated prospectively.
Materials and methods: The study included 81 patients who underwent 88 consecutive ICSI treatments. The identical embryologist performed ICSI and observed first PB morphology. MII oocytes were divided into three groups according to the first PB morphology (grade 1: round or ovoid, smooth or rough surface; grade 2: fragmented; grade 3: big). The fertilization rates and embryo quality (cleavage rates, blastomere number, percentage of fragmentation, appearance of multinucleated embryo on day 2 and day 3) were evaluated for each group of oocytes.
Results: A total of 443 MII oocytes were assessed in this study. The first PB morphology essentially observed at ICSI, were grade 1 (67.9%), grade 2 (30.0%), and grade three (2.0%). No significant differences were found between the three groups regarding fertilization rates (respectively, 78.2, 78.0, and 87.5%). Although, grade 1 embryos had no significant differences in cleavage rate (98.4% versus 96.5%), blastomere number (day 2: 3.6 versus 3.5, day 3: 7.1 versus 6.7) and the percentage of fragmentation (day 2: 14.0% versus 13.1%, day 3: 16.5% versus 19.2%) on day 2 and day 3 as compared with grade 2 embryos, yet the rate of good quality embryo was increased on day 3 (53.9% versus 36.1%; $p<0.05$). Surprisingly, embryos in the fragmented first PB group were significantly higher in the appearance of multinucleated embryo compared to the intact first PB group (4.3% versus 12.9%; $p<0.05$). Cleavage rate was 50% and subsequent embryo development was poor in grade 3 embryo (data not shown). The percentage of MII oocytes with first PB in grade 2 and 3 was observed to be lower in the patients who were younger than 35 years as compared with the ones who were older than 41 years (age $\geq$30 years: 26.7%, 31–35 years: 28.2%, 36–40 years: 32.9%, $\leq$41years: 42.0%, $p<0.05$)
Conclusions: The results suggested that human oocyte grading which is based on first PB morphology is significantly related to the quality of embryo and the appearance of multinucleated embryo after ICSI. First PB morphology at the time of ICSI seems to be a suitable indicator for the developing potential of zygotes. However we need to clarify the chromosomal situation and the implantation potential of embryos derived from oocyte with varying first PB. In conclusion, this is the first investigation on the relationship between morphology of the first PB and the appearance of multinucleated embryo.

P-417 Pronuclear scoring (Z-score) predicts the subsequent development to blastocyst for the zygotes derived from ICSI, not from conventional IVF

F. Nagata, A. Ohtani, T. Murata, Y. Nakaoka, A. Fukuda, Y. Morimoto
IVF Osaka Clinic, Osaka, Higashi-Osaka, Japan

Introduction: Pronuclear scoring (Z-score) developed by Scott et al. is a morphological evaluation system of the zygotes by assessing the number, size and distribution patterns of nucleolar precursor bodies in the pronuclei. Z-score has been reported to predict subsequent embryonic development. The first objective of the present study was to identify whether Z-score was useful to select cryopreserved zygotes to thaw for transfer at blastocyst stage by predicting subsequent development to blastocyst. The second objective was to determine if there were any differences in predictive value of Z-score between the zygotes derived from conventional IVF (cIVF) and those from ICSI.
Materials and methods: The zygotes from 64 patients with estradiol level above 5000 pg/ml in controlled ovarian stimulation cycles at HCG administration for oocyte retrievals were frozen after Z-score evaluation to avoid severe ovarian hyperstimulation syndrome from July 2002 through Oct 2003. Subsequent development to blastocyst from the zygotes evaluated by Z-score from 28 cycles (26 patients) of cIVF and 45 cycles (38 patients) of ICSI were analyzed. Z-score evaluation classified as Z-1 (excellent), Z-2 (good), Z-3 (fair) or Z-4 (poor) was performed 16–18 h after either cIVF or ICSI before freezing all the zygotes. Cryopreserved zygotes were selected for transfer by order from Z1 to Z4. Thawed zygotes were cultured for 4 days and transferred at blastocyst stage. Development to blastocyst, the rates of pregnancy, implantation and miscarriage were compared between cIVF and ICSI.
Results: There were no differences in the rates of blastocyst development of the zygotes derived from cIVF among any Z-score (Z1: 58.3%, Z2: 40.0%, Z3: 51.4% and Z4: 57.8%) However, significantly higher percentage of the zygotes from ICSI classified as Z1 (52.4%) or Z2 (50.0%) developed to blastocyst after thawing compared to either Z3 (38.6%) or Z4 (32.1%). There were no differences in the rates of pregnancy, implantation and miscarriage among Z-score classifications in either cIFV or ICSI.
Conclusions: Z-score before cryopreservation predicts subsequent development to blastocyst after thawing only in the zygotes derived from ICSI. On the other hand, Z-score has nothing to do with pregnancy, implantation and miscarriage rates in both cIVF and ICSI. The present study suggests that Z-sore is a useful tool to evaluate zygotes derived from ICSI, because the zygotes are evaluated with precise time interval from sperm entry in ICSI cases.

P-418 Early cleavage profile is valuable in choosing the embryo for transfer

V. Standaert, L. Vanhoutte, J. Van der Elst, P. De Sutter, M. Dhont
University Hospital Ghent, Infertility Center, Ghent, Belgium

Introduction: Prevention of multiple pregnancies by limiting the number of embryos for transfer is an important issue in IVF. For single embryo transfer (SET) to be acceptable, selection of the embryo with the highest implantation potential is crucial. Conventional selection criteria are the number of blastomeres, fragmentation and presence of multi-nucleation. We investigated whether the early cleavage profile might provide an additional prognostic factor to refine embryo selection criteria.
Materials and methods: We analysed the early cleavage profile in 155 cycles at 24–27 hours after IVF (35 cycles) or ICSI (120 cycles). Three groups of early cleavage profile were identified: pronuclear presence (2PN), pronuclear disappearance (0PN) or early cleavage (EC). Transfers were done on day 2 (28%), day 3 (69%) or day 5 (3%). Embryo selection for transfer was based only on the aforementioned conventional morphology criteria. Embryos were given a score from 5 (excellent) to 1 (poor). We analysed the outcome of embryo transfer according to the early cleavage profile. Only single embryo transfers (n=75) or transfers of embryos with the same early cleavage profile (n=49) were considered (total=124). Clinical pregnancy rate per transfer (CPR) was

assessed by the presence of an intra-uterine gestational sac with foetal heartbeat at 6 wks. Statistical analysis was done using Chi square test, Fisher's exact test or one-way ANOVA (Newman-Keuls post-test).
Results: Of the total of 124 transfers, 66 (53%) belonged to the 2PN group, 37 (30%) to the 0PN group and 21(17%) to the EC group. The mean maternal age (overall 34.5±4.9 yrs) and the distribution of the day of transfer were not different between the three groups. There was a significant difference, however, in the mean number of transferred embryos and the mean embryo score per transfer: (2 PN group) 1.7 embryos with mean score 5.9; (0PN group) 1.2 embryos with mean score 4.1; (EC group) 1.3 embryos with mean score 4.5. Clinical pregnancy rates per transfer were significantly different: 11%; 27 and 33%, respectively. Extracting from these data the SET gave the following results: 31 transfers with 6% CPR for the 2PN group, 30 transfers with 23% CPR in the 0PN group, 15 transfers with 27% CPR in the EC group. These values were not significantly different.
Conclusion: The early cleavage profile appears to have a strong predictive value for the embryo implantation potential. In view of SET it will be worthwhile to investigate the independent predictive value in a prospective study.

P-419 Importance of multinucleation on day 2 in embryos from ICSI

P. Guilherme, L.M. Rossi, T.C.S. Bonetti, C.V. Locambo-Freitas, W.C.P. Busato, M. Bibancos, L.G.L. Maldonado, A. Iaconelli Jr, E. Borges Jr
Fertility Assisted Reproduction Center, Sao Paulo, Brazil

Introduction: The selection of pre-embryos for uterine transfer is still based predominantly on morphological appearance according to various scoring systems. These scoring systems have in common that the main parameters judged are pronuclear evaluation, time of first division (early cleavage assessment), percentage of fragmentation, uniformity of blastomere size and presence of multinuclear cells. The aim of this study was to analyze whether the presence of multinucleation on day +2 after ICSI exhibit some relationship to other morphological characteristics.
Materials and methods: This study included 386 ICSI cycles performing in 321 patients. Pituitary blockage with GnRH agonists and ovary stimulation with recombinant FSH (Gonal-F®, Serono) were employed. After oocyte pick-up, all mature oocytes (MII) collected were fertilized by ICSI. Seventeen hours after ICSI, pronuclear (PN) arrangement was evaluated. Normal embryos (S0) showed aligned/aligning PN, with no discrepant number of nucleoli compared between male and female PN. Embryos with different conditions were classified as an abnormal (S1). Early cleavage assessment was done 26 h after ICSI and three situations were found: embryos with visible PN (EC-1), embryos non-cleaved without visible PN (EC-2) and cleaved embryos (EC-3). On day +2, multinucleation was observed and normal embryos were considered as having one nucleus in each blastomere (NB) and abnormal when two or more nuclei were noted (multinucleation – BMN). Subsequent classifications were done according to cell regularity (even and uneven) and fragmentation (FR-1 until 20%; FR-2 from 21% to 35% and FR-3 with more than 35%). Chi-square analysis was used to determine correlations between these characteristics and P-value <0.05 was considered statistically different.
Results: The mean maternal age in this study was 35.4±5.5 years. A total 5999 follicles and 3414 oocytes were retrieved. From this 3414 MII (56.9%) were injected and 2310 (67.7%) showed normal fertilization and were analyzed. There was no statistical correlation between multinucleation and PN arrangement (34.2% versus 38.8%, respectively; BMN+S0 and NB+S0, P=0.741). A strong correlation was established between multinucleation and early cleavage assessment (10.8% versus 24.3%, BMN+EC-3 and NB+EC-3, respectively, P=0.001). Moreover, more than 77.8% of BMN embryos had not shown two cells 26 h after ICSI compared with 37.3% of NB embryos (p<0.001). Thirty percent of NB embryos showed less than 20% of fragmentation on day +3 compared with 16.5% of BMN embryos (P=0.013). Multinucleation seems to provide more embryos with blastomere irregularity (81.8% versus 63.7%, BMN+uneven blastomere and NB+uneven blastomere, respectively, P=0.022).
Conclusions: Our study suggests that there is a significant relationship between multinucleation analyzed on day +2 and other morphological characteristics of early cleaving embryos such as EC assessment, fragmentation and regularity of blastomeres. Multinucleation is an observed phenomenon that seems to impair cleavage and increase fragmentation and because of this shall be part of embryo assessment mainly on day +2.

P-420 Implantation potential of unselected embryos with multinucleated blastomeres

M.J. Pelinck, A. Van Leeuwen, N.E.A. Vogel, J. van Echten, E.G.J.M. Arts, A. Hoek, A.H.M. Simons, M.J. Heineman
University Medical Center, Reproductive Medicine, Groningen, The Netherlands

Introduction: In COH-IVF, embryos with multinucleated blastomeres (MNB) are usually discarded for transfer, unless no others are available. Since 2001, part of our IVF cycles are carried out in a modified natural cycle, where the oocyte from the one follicle that spontaneously develops to dominance is used for IVF. Because of the presence of one oocyte this protocol offers the unique opportunity to study the direct relationship between embryo quality and implantation after natural selection of the dominant follicle, without selection of embryos before transfer. Embryos with MNB are often transferred in the modified natural cycle protocol, since usually only one embryo is available. In this study, we compared implantation potential of embryos with and without MNB.
Materials and methods: All patients were younger than 38 years. When the dominant follicle reached a size of 14 mm, the GnRH-antagonist cetrorelix (0.25 mg daily) and rec-FSH (150 IU daily) were administered until the follicle had a diameter of 18 mm, followed by hCG (10 000 IU) for ovulation triggering. Conventional IVF was performed according to standard procedures. All normally fertilized oocytes were scored at days 2 and 3 after oocyte retrieval for number of blastomeres, percentage of anucleated fragments and the presence of multinucleated blastomeres (MNB). All embryos were transferred on day 3 without further selection except those that showed 3PN, 50% fragmentation or no cleavage. Only cycles in which one embryo was available for transfer were evaluated. Embryos were divided in four groups: group A, MNB visible on day 2 and not on day 3; group B, MNB visible on day 3 and not on day 2, group C, MNB visible both on day 2 and on day 3; group D, no MNB visible at any time.
Results: Between February 2001 and July 2004, in a total of 415 cycles of modified natural cycle IVF, a single embryo was available and transferred, resulting in 107 pregnancies (25.8%/ET) of which 88 are ongoing (21.2%/ET). In groups A, B, C and D, 8, 10, 39 and 358 embryo transfers led to 2, 0, 6 and 99 pregnancies (25.0, 0.0, 15.4 and 27.7%, respectively), of which 2, 0, 6 and 80 are ongoing (25.0, 0.0, 15.4 and 22.3%). In total, 57 embryos showing multinucleation at any time (groups A, B and C taken together) led to 8 pregnancies (14.0%), whereas 358 transfers of embryos without multinucleation (group D) led to 99 pregnancies (27.7%); Chi-square: P<0.05.
Conclusions: In modified natural cycle IVF, embryos with multinucleated blastomeres have impaired implantation potential, but still lead to pregnancy in 14.0% of transfers. It seems from our results that implantation is more severely impaired when MNB are visible on day 3 or on both day 2 and 3, as compared to MNB visible on day 2 only. However, groups were too small to draw conclusions.

P-421 First polar body morphology before ICSI is related to embryo quality and fragmentation

M. Maeda[1], N. Sugawara[1], R. Komaba[1], N. Fujii[1], Y. Araki[2]
[1]Iwaki Women's Clinic, Iwaki Fukushima, Japan; [2]The Institute for ARMT, Fujimi Seta Gunma, Japan

Introduction: Many factors are involved in assessing denuded oocytes prior to ICSI. One of these is first polar body (1st PB) morphology, but this optimal morphology for ICSI remains controversial. The present study analyzed relationships between 1st PB morphology, embryo quality and fragmentation.
Materials and methods: A total of 348 oocytes from 56 patients (85 ICSI cycles) were included in the present study.
At 2 h after retrieval, oocytes were denuded of cumulus by aspiration in medium that contained 70 IU/ml hyarulonidase, rinsed in culture medium, then used for ICSI. At that time, 1st PB morphology was observed for the first time, and embryo morphology was observed a second time at 67 h after ICSI.
Classification of 1st PB morphology was performed using inverted microscopy. Morphological classification was made according to two groups: group A, round or ovoid with smooth surface (intact polar body); or group B, fragmentation or separation into two parts (fragmented polar body). The cleavage blastomere was classified into four stages (G1–G2, G3, G4 and G5) according to modified Veeck criteria.

Semen treatment, ICSI and ovary stimulation procedures were performed as previously reported.
Results: Mean age of patients was 37.1 years. Mean number of retrieved oocytes was 3.5. In group A, frequency of good quality embryos (G1–G2) was 50.2% (103/205), compared to 20.5% for G3 (42/205), 10.2% for G4 (21/205), 5.9% for G5 (12/205) and 13.2% for other (degeneration or unknown fertilization; 27/205). In group B, frequency of good quality embryos (G1-G2) was 38.5% (55/143), compared to 14.7% (21/143) for G3, 26.6% for G4 (38/143), 7.7% for G5 (11/143) and 12.6% for other (degeneration or unknown fertilization; 18/143).
Frequencies of both G1-G2 and G4 differed significantly between groups A and B ($P<0.01$). Pregnancy rate of A group was higher than B group. Miscarriage rate was the same between A and B group.
Conclusion: In cases with normal 1st polar body morphology, incidence of good quality embryos is high after fertilization. Conversely, poor polar bodies are associated with more fragmented embryos than good polar bodies. Pregnancy rate of good quality embryo with normal 1st polar body morphology was higher than good quality embryo with abnormal 1st polar body morphology.

P-422 Early cleavage is associated with high ART pregnancy rates

K.E. Tucker, P.M. Rijnders, A.E. Bracelly, C.A.M. Jansen
Renier de Graaf Group, Department of IVF Diaconessenhuis, Voorburg, The Netherlands

Introduction: Non-invasive embryo evaluation is pivotal in identifying the embryo(s) that will implant and result in a healthy, singleton pregnancy. In an attempt to refine this procedure, we have assessed the incidence of cellular cleavage in embryos as early as one day after insemination or ICSI. Our study was designed to determine if the transfer of embryos displaying early cleavage (EC) or those simply from a cohort of embryos displaying EC would result in improved pregnancy rates for ART patients.
Materials and methods: A total of 605 IVF/ICSI patients undergoing IVF or ICSI in our centre, from August 2003 and throughout 2004, were included in this study. All embryos from each patient were evaluated on day 1 following follicular aspiration (FA) for the incidence of fertilization (presence of 2 pronuclei) and then later in the afternoon for the initiation of cell division (early cleavage – EC). The best quality embryos (with or without EC), based on universally published criteria, were transferred on day 2 or 3. Pregnancy was defined as a positive urinary test 15 days after FA. Differences between groups were analysed and values with $p<0.05$ were considered significant.
Results: Two groups of patients were analysed: Those with one or more EC embryos in the cohort (Group 1, n=341) and those with no embryos demonstrating EC at the time of evaluation (Group 2, n=264). There were no differences in the type of procedures performed (IVF: n=170 versus n=155; ICSI: n=164 versus n=108), mean (±STD) age (33.7±3.9 versus 34.6±4.8 years), the time to EC evaluation (26.4±1.1 h versus 25.8±1.8 h post-insemination/ICSI) and the number (1.9±0.42 versus 1.86±0.53) or quality (grade 2.0±0.62 versus 2.0±0.66) of the embryos transferred between Groups 1 and 2, respectively. Pregnancy rates, however, were significantly different between the groups (Group 1: 50.7% versus Group 2: 32.6%; $p<0.0001$).
Conclusions: The assessment of the day 1 embryo is a quick, relatively simple procedure, that can be done without harm to the embryo. The presence of EC embryos on day 1 appear to be highly prognostic of a successful IVF or ICSI cycle, whether or not these develop into the embryo(s) chosen for transfer.

P-423 Multinucleation is associated with high oocyte yield but comparable clinical pregnancy and implantation rates in ICSI cycles

L. Karakoc Sokmensuer, A. Sever, I. Esinler, G. Bozdag, S. Gunalp, T. Aksu, H. Yarali, M. Alagoz
Hacettepe University, Obstetrics and Gynecology, Ankara, Turkey

Introduction: The aim of this study was to evaluate the impact of the presence of, although not transferred, multinucleated embryo(s) in the cohort on ICSI outcome.
Materials and methods: Seventy-one consecutive ICSI cycles with one or more multinucleated embryos were included; in none of the cycles multinucleated embryos were transferred. Three hundred and fifty-six consecutive ICSI cycles without any multinucleated embryos served as the control group. All patients in the two groups had severe male factor necessitating ICSI. Patients with azospermia, polycystic ovary syndrome/polycystic ovaries or poor ovarian response were excluded. The two groups were matched for female age, body-mass index and number of previous ICSI attempts. Standard luteal-long leuprolide acetate with rFSH using the step-down protocol was employed. Standard culture conditions were used and day 3 ET was employed in all patients. The embryo grading was grade 1: evenly sized blastomeres and no fragmentation; grade 2: unevenly sized blastomeres and/or 0–20% fragmentation; grade 3: 21–50 % fragmentation; grade 4: >50% fragmentation. Grades 1–3 were considered as transferable. Values were expressed as mean±SD. Student t-test, Mann–Whitney U, chi-square and Fisher's exact tests were used. Type 1 error was set at 0.05.
Results: Duration of stimulation, total dose of FSH used, E2 level and endometrial thickness on the day of hCG administration were comparable between the two groups. The number of oocytes (14.7±6.9 versus 10.8±5.6) and the number of the M2-oocytes (13.1±6.2 versus 9.3±5.2) were significantly higher in the multinucleated group ($p<0.01$). Fertilization rates were comparable (74% and 73% in the multinucleated and control groups, respectively). The number of 2-pronucleated oocytes (9.7±5.6 versus 6.6±4.1; $p<0.01$) and grade 1 embryos available on day 3 (1.1±0.2 versus 0.6±0.1) were significantly higher in the multinucleated group. However, the mean number of total and Grade 1 embryos transferred were comparable among the two groups. The clinical pregnancy rates per ET of the multinucleated and control groups were 57% and 46%, respectively ($p>0.05$). The respective figures for implantation rates were 26% and 23%, respectively. Multiple pregnancy and miscarriage rates were comparable. The rate of cycles in which embryo cryopreservation was performed was significantly higher in the multinucleated group (41% versus 28%, $p<0.05$).
Conclusion: Multinucleation is associated with high oocyte and M-2 oocyte yield, but comparable with FSH consumption and E2 level on the day of hCG. When multinucleated embryos are present, but not transferred, the developmental capacity of the non-multinucleated sibling embryos are not impaired.

P-424 The contribution of spindle birefringent imaging by PolScope in characterizing oocyte morphology

D. Komarovsky, O. Bern, D. Strassburger, E. Kasterstein, A. Raziel, M. Schachter, S. Friedler, R. Ron-El
Assaf Harofeh Medical Centre, IVF and Infertility, Department of Obstetrics/Gynecology, Zerifin, Israel

Introduction: Studies show higher fertilization rate and better embryo development in oocytes where birefringent spindle is visualized. We examined whether spindle imaging can correlate with morphological appearance of oocyte and analyzed ICSI outcome in terms of fertilization, embryo cleavage and morphology.
Materials and methods: Two hundred seventy oocytes from 31 patients were included in the study. Inclusion criteria was previous IVF cycle with mean embryo morphology score of ≥ 2 (classification was according to the presence and quantity of fragments – one without fragments and four >50% fragments). The control group consisted of patients with embryo morphology <2. The study group included 156 oocytes and the control group 114 oocytes. Patients' mean age was 29.6±4.80 and 27.1±5.41 years in the study and the control groups, respectively. Mean interval from hCG injection to the beginning of ICSI procedure was 40.8±1.18 and 40.3±0.90 h. Oocytes were evaluated morphologically and photographed before spindle visualizing by PolScope (LC-PolScope, CRI, MA, USA) and ICSI was carried out successively. Fertilization (2PN) was evaluated 18–20 h later; embryos were assessed for cleavage rate and morphological score on day 2 and 3. Each step was documented and photographed. 'Good quality oocytes' (GQO) were MII oocytes with clear, moderately granular cytoplasm, small perivitelline space (PVS) and a clear colourless zona pellucida. 'Affected oocytes' (AO) had at least one of the following parameters. Intracytoplasmic parameters included dark granular cytoplasm, central granulation, transparent cortical cytoplasm, dark spots in the cytoplasm, SER – smooth endoplasmic reticulum, clear and granulated vacuoles, refractile body and irregular oolemma. Extracytoplasmic parameters were: large or granulated PVS, fragmented polar body and dark or irregular zona pellucida.

Results: The fraction of AO was significantly higher in the study group as compared with the controls (63% versus 41%, p<0.001). Birefringent spindle was viewed (SP+) significantly more in the GQO than in the AO ones both in the study and control groups (72% versus 47%, p<0.001 and 75% versus 55%, p<0.05, respectively). However, spindle was viewed in a similar percentage in the whole cohort of oocytes of both groups 56.4% (88/156) and 66.6% (76/114). The SP+ oocytes showed higher (but not significantly different) fertilization rates in the study and control groups (77% and 71%) as compared with the SP− oocytes (53% and 63%). The SP+ oocytes of the study group showed a mean cell number of 3.3±1.4 and 5.9±2.2 on day 2 and 3, respectively. Whereas the SP+ oocytes of the control group had a mean cell number of 4.0±0.9 and 7.5±1.7 on day 2 and 3 (p<0.01 and p<0.001). Embryo morphology was significantly better in SP+ oocytes of the control group on day 3 embryos, with a mean score of 2.0±0.6 versus 2.4±0.59 (P<0.001).
Conclusions: Although birefringent spindle imaging is more frequent in oocytes with better morphology and their fertilization rate is higher, spindle visualization by PolScope, does not predict embryo developmental potential in terms of cleavage and morphology. The use of the PolScope does not enable to differentiate among oocytes according to their morphology.

P-425 Are some human embryos unable to survive in vitro culture conditions?

J. Mandelbaum[1], O. Anastasiou[1], V. De Larouziere[1], C. Ravel[1], I. Berthaut[1], E. Mathieu[2], I. de Buretel[2], J.M. Antoine[2]
[1]Hôpital Tenon, Laboratoire de FIV et de Biologie de la Reproduction, Paris, France; [2]Hôpital Tenon, Service de Gynecologie/Obstetrique/Medecine de la Reproduction, Paris, France

Introduction: Preimplantation embryo development is dependent on stored maternal factors. The oocyte to embryo transition relies on maternal transcripts that accumulate during oogenesis. Animal studies have allowed the identification of specific maternal proteins(*hsf1*, *mater*) essential to ensure a normal early embryonic development (Christians et al., 2000; Zhi Bin, 2002; Xuemei et al., 2003). In animal knock out models fertilization is not impaired but embryos are arrested and/or fragmented before the first or second cleavage.
In IVF (in vitro fertilization) programs some patients display repeatedly an abnormal embryonic development with high early fragmentation rates and consequent recurrent IVF failures.
We have made the hypothesis that for various maternal reasons some embryos were unable to withstand the in vitro environment and an early transfer (day 1) has been proposed to these patients.
Materials and methods: We present here the data of the first 27 patients that had followed an IVF procedure at Tenon Hospital, Paris in 2003–2004 and had been included in this program. Their average age was 35.26+/− 4.5 years. All patients presented at least two IVF or ICSI failures, characterized by a normal response to ovarian stimulation, adequate oocyte retrieval and normal ferilization rates. The embryonic development however was drastically impaired: cleavage arrest and/or extended fragmentation (>30%). No pregnancy had been obtained for any of them in these previous attempts. Parental karyotypes and sperm DNA fragmentation rates were normal in all cases. The third or fourth cycle was performed with transfer and cryopreservation at the pronuclear stage (day 1 post insemination or microinjection).
Results: The mean number of oocytes retrieved was 11.96±4.66. The average fertilization rate was 65% and the mean number of zygotes per transfer was 2.46. Eight clinical pregnancies have been obtained, representing a pregnancy rate of 29% and an implantation rate of 14.5%. All pregnancies were evolutive. No pregnancy has yet been obtained from a transfer of cryopreserved 2PN embryos in these patients.
Conclusion: The occurrence of heavy embryo fragmentation in vitro is a common feature and is associated with a deleterious outcome. Unfortunately recurrent attempts with low quality embryos concern a significant number of IVF cycles. These preliminary data on fresh 2PN embryo transfer are encouraging and may provide a valid alternative solution for these patients.

POSTER SESSION

ART laboratory: ICSI, MESA, TESE

P-426 To evaluate the effect of PVP to replace by hyaluronate on intracytoplasmic sperm injection (ICSI) procedure

J.-T. Su, M.-H. Lin, Y.-M. Hwu, R.K.-K. Lee
Mackay Memorial Hospital, Obstetrics/Gynecology, IVF Laboratory, Taipei

Introduction: During the procedure of ICSI, PVP is routinely used in order to slow down the motile sperm and to facilitate the injection of the sperm into the cytoplasm of oocyte. However, the long term effect of PVP for injecting sperm into human oocyte has been concerned; the PVP will remain in the oocyte for a prolonged period after injection with sperm which may deliver some PVP into the oocyte and is not digestible by lysosomal enzymes. Therefore, it is reasonable to seek an alternative material to replace PVP to facilitate capturing of the sperm for ICSI. Hyaluronate, the major component of the extracellular matrix which appears in the cumulus cell of matured oocytes, is the natural material found in the mammalian reproductive tract. This study, therefore, was designed to evaluate hyaluronate-containing medium to replace PVP as a potential substitute that can be applied for ICSI procedure.
Materials and methods: In 22 ICSI cycles enrolled in this study, collected oocytes were denuded from the cumulus cells in Hepes-buffered medium containing the concentration of 80 IU/ml of hyaluronidase. In each patient, MII stage oocytes were randomly divided into two groups: Group A: oocytes were injected with sperm exposed to the hyaluronate-containing medium (SpermCatch^{tm}). Group B: oocytes were injected with sperm which was immobilized and was aspirated in 7% PVP solution. The fertilization rate and the quality of day 3 embryos were compared. Embryos were graded according to the amount of fragmentation and number of blastomeres: embryos with no fragmentation or <20% fragmentation were classified as good embryos; while embryos with >20% fragmentation or the number of blastomeres was less than five classified as poor embryos.
Results: In group A, 2PN were observed in 104 out of 137 injected MII stage oocytes (fertilization rate: 75.9%) and the rate of good embryos was 58.7% (in 61 out of 104 zygotes); In group B, 2PN were observed in 94 out of 126 injected MII stage oocytes (fertilization rate: 74.6%) and the rate of good embryos was 59.6% (in 56 out of 94 zygotes). No significant difference was found in the fertilization rate and the quality of embryo between the two groups.
Conclusion: Hyaluronate was found to be as efficient as PVP for the ICSI procedure and it can be a physiological alternative to replace PVP to slow down sperm motility prior to aspirating a single sperm into the injection pipettes in the ICSI procedure.

P-427 Age as a limiting factor for successful sperm retrieval in nonmosaic Klinefelter syndrome

H. Okada[1], O. Maruyama[1], S. Muto[1], M. Koshida[2], K. Tatsumi[3], Y. Odawara[4], S. Horie[1]
[1]Teikyo University School of Medicine, Urology, Tokyo, Japan; [2]Koshida Clinic, Osaka, Japan; [3]Umegaoka Women's Clinic, Tokyo, Japan; [4]Odawara Women's Clinic, Tokyo, Japan

Introduction: Intracytoplasmic sperm injection (ICSI) now permits fertilization of eggs using very small numbers of spermatozoa. Improvements in testicular sperm extraction (TESE) techniques have expanded indications from oligozoospermia to nonobstructive azoospermia. Klinefelter syndrome, which accounts for 5% of the latter cases, is characterized by sterility caused by lack of spermatogenesis. Isolated foci of spermatogenesis have been found in involved testes, explaining the rare cases of sperm production with appearance in the ejaculate. Several institutions perform TESE using either ordinary testicular biopsy techniques or meticulous microdissection to retrieve spermatozoa for ICSI. The reported success rate for TESE in patients with Klinefelter syndrome is 56%, but no predictive factors have been identified given the paucity of cases in each report. We analyzed results of TESE to elucidate success determinants for TESE in patients with Klinefelter syndrome.