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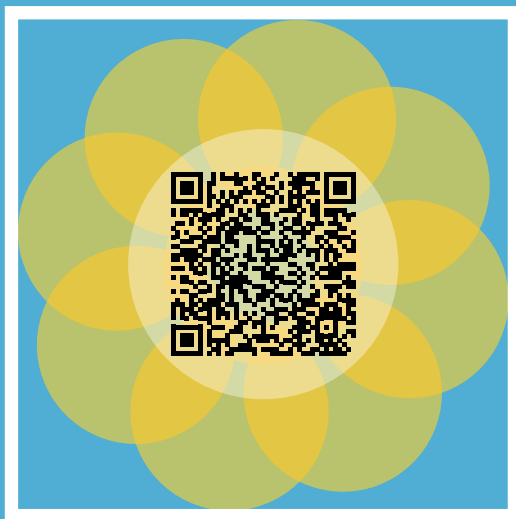
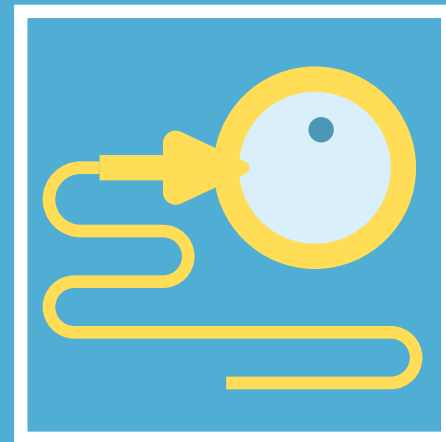
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## BACK TO THE FUTURE



# ALPHA BIENNIAL CONFERENCE 15.–17. SEPTEMBER 2006 IN LUZERN (ABSTRACTS\*)

## O1

### THE EFFECT OF IGF 2 ON HUMAN CRYO-PRESERVED OVARIAN TISSUE IN LONG-TERM CULTURES

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**Introduction:** Oocyte maturation of cryopreserved ovarian tissue followed by *in-vitro* fertilization would be an important option to treat infertility of young women undergoing premature ovarian failure due to anticancer treatment. The aim of this study was to evaluate the effect of insulin-growth factor 2 (IGF 2) on follicular and stromal cell preservation of human cryopreserved ovarian tissue cultured for 16 weeks.

**Material and Methods:** The ovarian cortex of five patients was collected and immediately cryopreserved, as previously described [Fabbri et al., 2003]. After thawing, a specimen of ovarian cortex from each patient was fixed for histological and ultrastructural analyses (control  $t_0$ ). The other pieces were cultured at 37 °C in an atmosphere of 6 % CO<sub>2</sub> for 16 weeks, in minimum essential medium supplemented with insulin-transferrin-selenium (ITS), follicle stimulating hormone (FSH), human serum (HS), N-acetyl-L-cysteine (NAC), antibiotics and IGF 2 (medium A) or without IGF 2 (medium B). The medium was changed every second day. After 16 weeks, pieces of ovarian tissue were fixed for histological and ultrastructural analyses ( $t_{16}$ ). The developmental stage of follicles was classified using light microscopy (LM); follicular and stromal cell integrity was evaluated using transmission electron microscopy (TEM).

**Results:** TEM showed that the percentage of healthy follicles was increased in medium A (100 %) compared to medium B (50 %) after a 16-week culture period.

\* Peer-reviewed and compiled by Mag. Marc Van den Bergh, IVF Laboratory Director, Kantonsspital Baden A.G., CH-5404 Baden, Switzerland (24 credits by SGGG, 15 EUMS credits).

Furthermore, more growing follicles (i.e., secondary and preantral) were observed in tissue cultured in medium A (LM observation). Conversely, TEM did not show any difference in stromal cell preservation between medium A and B, even if a slightly better structural stromal cell integrity was observed in medium A after 16 weeks of culture with respect to control  $t_0$ .

**Conclusions:** Our results suggest that IGF 2 may promote the functional integrity of the follicles and stimulate follicular growth in an *in-vitro* culture. The advantage of preserving the integrity of the oocyte-granulosa-stroma interaction would be an important task in improving the development of early follicles in culture.

## O2

### THE EFFECT OF FSH AND NAC ON HUMAN CRYOPRESERVED OVARIAN TISSUE CULTURED FOR 32 WEEKS

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**Introduction:** The fertility of women with premature ovarian failure due to anticancer treatment might be preserved by cryopreservation and *in-vitro* culture of ovarian tissue. The aim of this study was to evaluate the effect of follicle stimulating hormone (FSH) and N-acetyl-L-cysteine (NAC) on human frozen-thawed ovarian tissue cultured for 32 weeks.

**Material and Methods:** The ovarian cortex of a patient suffering from breast cancer was collected and immediately cryopreserved, as previously described [Fabbri et al., 2003]. After thawing, pieces of ovarian cortex were cultured at 37 °C in an atmosphere of 6 % CO<sub>2</sub> for 32 weeks, in minimum essential medium supplemented with insulin-transferrin-selenium (ITS), human serum (HS), antibiotics, FSH and NAC. The medium was changed every second day. The developmental stage of follicles was classified by using light microscopy (LM); follicular and stromal cell integrity was evaluated using transmission electron microscopy (TEM).

**Results:** LM examination of cortical pieces after 32 weeks of culture showed a healthy early preantral follicle; TEM showed that the follicle was characterized by a polarized multilayer of granulosa cells surrounding the oocyte and several erect microvilli extended from the oolemma into the zona pellucida; zonula adherens-like and gap junctions were observed between granulosa cells and oolemma; the oocyte showed a regularly-shaped, outlined euchromatic nucleus; mitochondria were mainly located close to the nuclear side; clusters of cortical granules were found in the ooplasm.

**Conclusions:** These data suggest that the synergy in action of NAC and FSH plays an important role in follicle growth of ovarian tissue cultures. This is the first report showing morphological evidence of a well-preserved preantral follicle recovered from human frozen-thawed ovarian tissue cultured for 32 weeks.

## O3

### OOCYTE NUMBER AT PICK-UP IS A RELIABLE PREDICTIVE FACTOR TOWARDS CLINICAL PREGNANCY RATES IN ART

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**Introduction:** Maternal age is thought to represent the most important factor in determining clinical pregnancy rates when using fresh eggs. Other factors, such as infertility factor, laboratory culture conditions and the number of transferred embryos have also been shown to influence clinical pregnancy rates in ART. The objective of this study was to evaluate if the number of oocytes retrieved per aspiration constitutes a clear determinant of clinical pregnancy rates independent of maternal age.

**Material and Methods:** Between July 2000 and December 2005, 337 patients underwent 414 consecutive IVF/ICSI cycles. Inclusion criterion was the obtention of at least one oocyte at pick-up. Patients were between 19 and



46 years of age, with a mean of 33.4 years. Cycles were divided in groups according to number of oocytes collected at pick-up as follows: 1–5 (n = 97), 6–11 (n = 148), 12–17 (n = 104), 18–23 (n = 37) and 24 or more (n = 24) oocytes. IVF/ICSI outcomes including fertilization rate, cleavage rate, frozen embryos and clinical pregnancy rates were compared between the groups. A multivariable logistic analysis was performed using SPSS v13.0 software.

**Results:** Patients with less than 6 oocytes at pick-up obtained a clinical pregnancy rate that was arbitrarily attributed the value 1. Patients with 6–11, 12–17 and 18–23 oocytes at pick-up reached significantly higher clinical pregnancy rates (2.4x, 3.1x and 2.4x, respectively;  $p < 0.05$ ) than the first group. Patients with 24 or more oocytes collected at pick-up did not show a significant increase in clinical pregnancy rate when compared with the first group. These results were found to be independent of women's age.

**Conclusions:** This study identifies the number of oocytes at pick-up as a reliable predictive factor towards clinical pregnancy rates in ART since it does not seem to be significantly influenced by maternal age. Furthermore, it identifies 6 as the minimum number of oocytes to collect at pick-up when aiming for the best clinical pregnancy outcome. Oocyte number at pick-up may reflect, in a particularly effective way, the biological age of the ovary, becoming thus more significant than maternal chronological age. Future studies should be aimed at confirming if oocyte numbers at pick-up still hold as a reliable predictive factor towards more comprehensive parameters of success in ART such as "take home healthy baby" rates.

#### O4

##### EVALUATION OF EMBRYO QUALITY ON DAY 2 VERSUS DAY 3

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**Introduction:** Delaying the transfer from day 2 to day 3 increases the morphol-

ogy data available for embryo selection and allows to relocate the embryos in the uterus at a more natural stage. However, some embryos do not develop properly from day 2 to day 3. The aim of this study was to compare the morphological quality of the embryos evaluated on day 2 and later on day 3; and to decide if the differences reflected variations in the implantation potential or else were due to an incorrect evaluation of the morphological parameters.

**Material and Methods:** The 231 IVF cycles with fresh embryo transfer included in this study were performed during 2005 and 2006, all of them had more than 3 embryos and the patients received less than 400 FSH units per oocyte retrieved. The embryo transfers were done either on day 2 or day 3 depending on the day of the week. The day-2 transfer group included 102 cycles performed on patients with an average age of 34.2 years and 6.7 embryos per cycle, while 119 day-3 transfers were done on patients with an average age of 34.6 years and 6.8 embryos per cycle.

**Results:** A total of 608 embryos were evaluated on day 2 and day 3 in cycles of day-3 transfer. For 342 (56.3 %) of those embryos, the grade attributed on day 3 was worse than on day 2, in 104 (17.1 %) embryos it was better, while in 162 (26.6 %) cases it was the same. Moreover, when we select among the 226 embryos showing good quality on day 2 (4 cells, < 26% fragments, no multinucleated nor unevenly sized blastomeres, no defects in cytoplasm or ZP), only 154 (68 %) reached the correct 7–8 cells on day 3 and 16 (7.1 %) became arrested in 4 cells. When we selected the 87 embryos of good quality on day 3 (8 cells, < 26 % fragments, no multinucleated nor unevenly sized blastomeres, no defects in cytoplasm or ZP), 82 of them (94.3 %) had 4 cells on day 2.

The implantation rate was 35.9 % (74/206) for day-2 and 33.9 % (87/257) for day-3 transfers ( $p > 0.05$ ). The clinical pregnancy rate (fetal heart beat) was 51 % for day-2 (52/102) vs. 52.1 % (62/119) for day-3 transfers ( $p > 0.05$ ). There were no abortions after day-2 and 6 (9.7 %) after day-3 transfers ( $p < 0.05$ ). The ongoing pregnancy rate was 51 % (52/102) for day-2 and 47.1 % (56/119) for day-3 transfers ( $p > 0.05$ ).

**Conclusions:** The overall morphological quality grade attributed to the embryos on day 3 was lower than on day 2. Significantly more embryos seemed to deteriorate than to improve ( $p < 0,01$ ). Good quality day-2 embryos which could have been chosen for transfer evolved to lower-grade embryos and even 7,1 % were arrested at the 4-cell stage. If we were mistaken by the evaluation of day-2 morphology, we would expect a better choice of embryos for transfer when the selection is done on day 3. However, the efficacy of day-2 transfer was similar to that of day-3 transfer. The ongoing pregnancy rate was slightly higher (not significantly) for day-2 transfers.

These results suggest that in good-prognosis patients, the morphological data obtained from day-2 embryos are sufficient for a correct evaluation of their implantation potential. Further culture until day 3 does not improve the accuracy of the embryo selection for transfer while some of the embryos may deteriorate during this extra *in-vitro* incubation period. Day-2 transfer seems to be preferable for these patients.

#### O5

##### PRACTICE OF MORPHOLOGICAL EVALUATION OF OOCYTES, PRONUCLEAR STAGES AND EMBRYOS IN GERMAN ART LABORATORIES

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**Introduction:** Identification of the embryo(s) with optimal developmental capacity is an internationally recognized method to increase pregnancy rates. At the same time, it lowers multiple pregnancy rates by applying SET or DET. Although this possibility does not exist in Germany (German Embryo Protection Law), standardized evaluation of oocytes, pronuclear stages and embryos from clinical and scientific views and in the context of quality management is necessary. However, little is known about the application of



different morphological parameters in individual ART laboratories.

**Material and Methods:** By means of questionnaires, members of the AGRBM – the German Society of Clinical Embryologists – were asked to anonymously specify their practice of evaluating oocytes, pronuclear stages and embryos. Different established scoring systems as well as individual morphological criteria were considered.

**Results:** Out of 141 members of the AGRBM 65 (46.1 %) participated in the survey. For the specific stages, scoring systems were used in different frequencies: oocytes –81.5 %, pronuclear stages –96.9 %, day-2 embryos –75.3 %, and day-3 embryos –80.8 % of participants.

For oocytes, evaluation of polar body (81.5 %) and cytoplasm (64.6 %) was most frequently performed while perivitelline space (49.2 %) and zona pellucida (49.2 %) characterization seemed to be considered less important. For pronuclear stages, position (73.8 %) and size (78.5 %) of pronuclei were preferentially evaluated. In addition, number (67.7 %), size (60.0 %) and distribution (60.0 %) of nucleoli and presence of the halo phenomenon (60.0 %) were frequently determined. On the other hand, appearance of cytoplasm (57.0 %), polar body (55.4 %) and perivitelline space (41.5 %) were less often considered.

In the case of embryo evaluation on days 2/3, degree of fragmentation (63.8 %) and number of blastomeres (63.0 %) were the preferred morphological parameters. Less commonly size (54.6 %) and multinucleation (29.9 %) of blastomeres were assessed.

**Conclusions:** Assessment of morphological parameters and their application as scoring systems during IVF/ICSI is possible for oocytes, pronuclear stages and embryos. In Germany, the pronuclear stage is the crucial phase for prognostic evaluation. Therefore, the most intensive morphological evaluation activity is reported for this level. Considerable differences exist in the frequency and priority of individual morphological parameters between German ART laboratories. A standardized procedure of morphological evaluation is therefore urgently required. The AGRBM sees its task in appropriate training of its

German reproductive biologist members and in the establishment of inter-laboratory tests for the characterization of gametes and embryos to ensure comparable standards.

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## O6

### REVIEW OF ESTIMATION OF FRAGMENTATION OF HUMAN EMBRYOS USING AN INTERNET-BASED QUALITY ASSURANCE SCHEME

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**Introduction:** The estimation of fragmentation is a component of all embryo grading assessments, yet there are no clear guidelines or training process to ensure all embryologists are uniform in assessments. Variation in assessments may result in differences between staff both in reporting and decision aspects, e. g. freezing or discarding.

**Method:** QAPonline is an international, online, image-based quality assurance programme that allows individual participation. One scheme from the 2005 series was selected for review for the assessment of embryo fragmentation. Fifteen images were available over the year and up to 63 professional embryologists from 25 laboratories participated each month. The images were either animated GIF files with multiple sections through the embryo or short videos and were between the 2 and 8 cells (early day 2 to late day 3).

**Results:** Participation ranged between 54 and 62 and the mean %-fragmentation ranged from 1 % to 44 %. The CV was above 15 % for all samples and where the mean value was in the critical range for allocation to A or B grade embryos, the CV was above 25 % in all cases. Estimates for mean values for images of 1–10 % fragmentation had replies ranging from 0–20 and CV > 68 %. Of more importance, for images with mean value between 11 % and 30 % the range drifted between 10 % and 80 % and CV of 28–48 %. The range in these embryos was such that the same embryo may have been variably allocated to either GOOD or POOR status depending on the embryologist. The same schemes in 2004 and 2006 exhibit similar variability. Preliminary

morphometric analysis from selected images indicates that the embryo volume within the perivitelline space is close to 60–70 % and that the actual volume of fragmentation as a % of total cell volume is closer to the lower estimates. Therefore, the criteria for fragmentation of % of cell volume or % of zona volume will consequently significantly influence each participant's estimation.

**Conclusion:** In this brief QA programme, the variability in the assessment of fragmentation raises concern over the training and certification of competency. All participants were experienced embryologists with many years' experience and this reflects that the description of embryos may have been assumed to be largely self-evident but this data suggest otherwise. Description of embryos always includes an estimate of fragmentation, and variations as described above may translate into potential disparities in deciding the fate of embryos both within and between clinics. Preliminary morphometric assessment suggests that fragmentation is overestimated. This review reports significant and concerning variation in the estimation of fragmentation and suggests that closer attention is required for its definition and assessment of fragmentation.

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## O7

### sHLA-G IN EARLY HUMAN EMBRYO CULTURE AFTER ART

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**Introduction:** HLA-G is a non-classical HLA class I molecule that can be expressed in membrane-bound or soluble form and is well known for its tolerogenic properties. Increasing interest is now being addressed to the soluble forms because they might have prognostic value for the implantation and pregnancy process. The aim of our study was to evaluate the correlation between embryo cleavage, morphology and sHLA-G levels, to estimate the impact of sHLA-G concentration on pregnancy outcome.

**Material and Methods:** The study was performed in a group of infertile patients



with various infertility indications. IVF or ICSI procedure was used for insemination. After 72 h, on the day of transfer, embryo morphology was evaluated, ET according to embryo morphology was performed and supernatants of embryos were collected and stored at  $-90^{\circ}\text{C}$  until screening. For the quantitative measurement of soluble forms of Human Leukocyte Antigen G in cell culture supernatants we used ELISA. The ELISA kit from EHBIO Praha measures total sHLA-G and the components are ready-to-use.

**Results:** A total of 46 couples participated in this study. In all, 258 embryo culture supernatants were tested for sHLA-G. The soluble form of Human Leukocyte Antigen G was detected in 49 (19 %) embryo culture samples (range 0,4–9,9 IU/ml). The production of Human Leukocyte Antigen did not depend on morphological criteria of the embryo. No significant differences were found between sHLA-G concentrations in patients from IVF and ICSI groups. Soluble HLA-G had an impact on embryo cleavage rate and pregnancy outcome. Soluble HLA-G levels did not differ significantly with respect to the age of women and infertility indication.

**Conclusions:** Day-3 embryos secrete sHLA-G into the surrounding medium; the concentration of which can be detected using ELISA. The concentration of Human Leukocyte Antigen G correlates with the number of embryo blastomeres but not with morphological criteria. sHLA-G might be a marker of embryo implantation potential after IVF/ICSI procedures, a factor which helps to achieve and maintain pregnancy.

## O8

### EMBRYO QUALITY ASSESSMENT BY RESPIRATION RATE MEASUREMENTS AND IMAGE ANALYSIS OF TIME-LAPSE IMAGES DURING EMBRYO DEVELOPMENT

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**Objectives:** Evaluate novel approaches to quantitative embryo quality assessment using unattended real-time image analysis of time-lapse images and non-invasive online measurement of metabolic activity.

**Material and Methods:** Bovine immature cumulus-oocyte complexes were aspirated from slaughterhouse-derived ovaries, matured for 24 h before fertilization for 22 h. Cumulus cells were then removed and presumptive zygotes were transferred and cultured in synthetic oviduct fluid medium. A novel high-resolution microsensor technology was used to automatically monitor the respiration rates of individual embryos throughout a 6-day culture period. Time-lapse images and respiration rates were recorded automatically by an EmbryoScope (Unisense FertiliTech, Aarhus) twice per hour during this 6-day incubation.

**Results:** 99 bovine embryos were evaluated by time-lapse microscopy (48 % blastocyst rate). The complex automated image analysis procedure generated a quantitative measure of blastomere activity for each image in the time-lapse series. Pronounced peaks in blastomere activity were found to be associated with cell divisions and the exact onset and duration of cell divisions could be quantified directly based on position, shape and size of the recorded peaks. (Correctly identified divisional events by the fully automated routine > 95 %; n = 350). The derived timing of the first cell divisions was shown to be related to the subsequent fate of the particular embryo: more than 90 % of the embryos that developed to expanding blastocysts could automatically be identified. The respiration of 88 embryos was investigated in a different experiment. The respiration rate was initially stable around 5 fmol/s and remained largely constant

until the 8-cell stage at about 75 hours after fertilization. Then the respiration rate increased gradually as the embryo compacted and developed into a blastocyst. A relationship was found between morphological events and respiration patterns.

**Conclusions:** The novel methods and instrumentation give unprecedented quantitative information about the first cell divisions. Based on our preliminary findings with bovine embryos, it is likely that the techniques can improve embryo selection in human IVF treatments.

## O9

### CORRELATION OF SPERM DNA FRAGMENTATION AS ASSESSED BY THE HALO SPERM ASSAY WITH IVF OUTCOME PARAMETERS

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**Introduction:** The introduction of intracytoplasmic sperm injection (ICSI) proved to be highly efficacious for severe forms of male infertility. However, a male factor not resolved by the application of ICSI may be contributing to infertility in some patients. Subtle male factor may be identified by low normal morphology despite otherwise normal semen characteristics; low recovery of sperm after gradient separation, arrested embryo development, and lack of pregnancy. The concept of sperm DNA fragmentation was introduced with the SCSA by Evensen et al. [Science 1980; 210: 1131–3]. Since then, numerous assays have been developed in order to accurately and easily assess the degree of DNA fragmentation in human sperm. The halo assay is commercially available to analyze sperm DNA integrity in-house and may offer insights into the sperm contribution to IVF/ICSI failure in mild male-factor couples.

**Methods:** Halo Sperm Kits were obtained from Indas Laboratories (Madrid, Spain) and used to evaluate DNA fragmentation in pre-washed sperm samples from control and subject men. Assays were performed according to the instructions. DNA damage was measured by chromatin dispersion or



halo. A large or medium halo (similar to or larger than the smallest core diameter) represented little or no fragmentation; a small or no halo (smaller than 1/3 of the smallest diameter of the core) showed large amounts of DNA damage. Control samples were collected from the male partners of IVF couples who were < 37 years old and presenting for treatment of female tubule pathology. Test samples were collected from the males of couples experiencing unexplained IVF failure (n = 22) in the presence of low sperm recovery and/or poor embryo development in combination with borderline oligospermia and/or teratospermia (10–20 mil/ml and less than 10 % normal forms) in at least 2 previously failed IVF cycles.

**Results:** Control samples (n = 6) had an average DNA fragmentation rate of 24.8 %; mean sperm recovery rate of 66 %; fertilization rate of 79 %; mean age of 33.2 and female age 31.3; 57 % good day-1 score; 54 % good day-2 score (even cell size and lack of multinucleation); low day-3 developmental arrest (5.7 %) and 50 % clinical pregnancy rate. Twenty-two test samples were analysed and showed an average rate of DNA fragmentation of 45.9 % (p < 0.01); mean sperm recovery of 39 %; 72 % fertilization rate; 56 % good day-1 score, 44 % good day-2 score, and 39 % developmental arrest rate on day 3 (p < 0.01). The mean male age was 36.0 and the mean female age 34.1. Patients with low recovery (< 20 %) had a mean fragmentation rate of 55 % and a 43 % developmental arrest rate. Patients with poor day-3 embryo development (> 20 % arrested) had an average fragmentation rate of 44 % and a mean recovery rate of 32 %. In all cases, patients were repeat IVF failure despite the replacement of morphometrically normal embryos. Five test patients' partners had a positive pregnancy test. Four resulted in early biochemical losses, one is ongoing. The ongoing pregnancy rate for all under 38-year-old patients in this practice is 42 %.

**Conclusions:** These data indicate that even in the presence of normal or only sub-optimal sperm samples, the degree of DNA fragmentation in the sperm could be a cause of pregnancy failure. In cases of poor embryo development and low sperm recovery, investigation

of the sperm DNA fragmentation rates may be a useful clinical test for unexplained IVF failure patients. More data are needed to validate this test and to develop methods of treatment once diagnosis is established.

## O10

### PRELIMINARY RESULTS OF A PROSPECTIVE STUDY ABOUT SEMEN QUALITY IN VARIOUS GEOGRAPHIC REGIONS OF SWITZERLAND

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**Background:** Over the last decades, exposure to estrogens or related compounds in the environment has been suspected to induce detrimental effect on male fertility and to be responsible for a decline in sperm concentration and quality. In the absence of reference values for Swiss men, we started a prospective trial to better understand the parameters affecting male fertility. This study will extend over a period of 3 years among voluntary young men, originating from all regions of Switzerland, attending recruitment for military service (conscripts), with the aim to include 3000 subjects.

**Methods:** A careful anamnestic evaluation of each subject was performed using a validated questionnaire (Danish model), referring among others to the origin of the parents, geographic location of the mother during pregnancy, medical history (cryptorchidism or other congenital malformations), exposure to toxic agents, lifestyle and fertility history of the subject. A complete urological status including clinical examination and testicular ultrasound was performed. Sperm, urine and blood samples were obtained from each volunteer. A Computer Assisted Sperm Analyzer (CASA) (SCA, Microptic SL, Spain) was used for sperm analysis. A video sequence of each sperm sample was recorded and stored for later possible re-examination. The sperm morphological analysis was performed by a trained technician (Kruger's criteria). A 10-year

follow-up is scheduled regarding fertility and urogenital health history of each subject.

**Results:** Between September 2005 and April 2006, 253 volunteers (5 % of the conscripts) accepted to participate in the trial. Most volunteers originate from the French-speaking part of Switzerland. Demographic parameters of the cohort are (mean ± SD): age = 19.5 ± 1 years, weight = 72.4 ± 12 kg, height = 179 ± 7 cm and BMI = 22.6 ± 3. Two azoospermic (0.8 %) and 3 cryptozoospermic (1.2 %) samples were detected. Sperm values (except two azoospermic cases; n = 251) were (mean ± SD/median/range): abstinence length: 4.1 ± 4.6/3.1/0.5–31.1 days, volume: 3.05 ± 1.37/3.00/0.29–6.88 ml, sperm concentration: 71 ± 69/53/0.2–570 × 10<sup>6</sup>/ml, total sperm count: 226 ± 333/151/8–3862 × 10<sup>6</sup>, motile forms: 58 ± 20/59/7–96 %, rapidly progressive forms: 39 ± 15/39/2.6–77 % and morphologically normal forms: 7.8 ± 5.0/7.5/0–25 %. Regarding the subjects participating in the clinical examination (n = 141), 2.9 % had a total testicular volume < 30 ml, 25.5 % had a volume between 30–40 ml and 71.6 % were normal (≥ 40 ml); the mean ± SD sperm concentration in these categories were 10.6 ± 13.9 × 10<sup>6</sup>/ml, 59.9 ± 56.6 × 10<sup>6</sup>/ml and 76.6 ± 82.8 × 10<sup>6</sup>/ml. Sperm values below WHO references for volume, concentration and motility were found in 56 (22 %) volunteers. In 31 of them, the low sperm concentration was correlated to a clinical finding: 6 cryptorchidisms (unilateral or bilateral), 7 varicoceles, 7 other uro-genital diseases and 11 cases with a testicular volume ≤ 30 ml. The 25 remaining cases need further investigation to determine causes of low sperm values.

**Conclusions:** The preliminary results of this national prospective study show that 22 % of the first 253 subjects have sperm parameters for volume, concentration or motility below the WHO reference values. Cryptorchidism, urogenital diseases or decreased testicular volumes were frequently associated with the low sperm values. These preliminary results confirm the importance of widening this study to the other geographic regions of Switzerland and of pursuing the follow-up of the subjects in terms of fertility and urogenital diseases.



## O11

### IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED IN HUMAN GRANULOSA CELLS FROM COMPETENT OOCYTES

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**Introduction:** To increase pregnancy rates in human IVF, usually two or more embryos are transferred in each patient. Unfortunately, multiple pregnancies can occur with potentially harmful consequences for the mother and the babies. The main objective of the project is to find a way to predict embryo quality in order to reduce the number to be transferred. We made the hypothesis that the embryo quality depends on the final maturation of the follicle leading to a competent or incompetent oocyte. Therefore, we believe that there are markers in granulosa cells from the follicles bearing good oocytes.

**Material and Methods:** Granulosa cells from consented patients were recovered by individual follicle puncture with a special double lumen needle. Populations of follicles (n = 5) leading to pregnancy were subtracted from the negative ones (n = 5) using SSH (Suppressive Subtractive Hybridization) with the PCR-Select cDNA Subtraction Kit. ESTs subtracted products from SSH were sequenced and compared against the GenBank database. Purified PCR products from granulosa cells library, cumulus cells library were spotted on glass slides using a VersArray Chip WriterPro robot. Microarray analyses were performed in order to identify true granulosa cell specific genes. Forward PCR products were used as probes for the first hybridization. For the second hybridization, populations of follicles (n = 15) leading to pregnancy and follicles (n = 15) from the negative ones were used. To improve the genome coverage, improve the number of candidate genes and confirm candidate genes already found in our libraries, hybridizations with the human HG-U133 Plus 2 Affymetrix GeneChip® with the 2 pools of patients were done (Plateforme des Biopuces, CREMO, Centre de recherche du CHUL, CHUQ).

**Results:** In the granulosa and cumulus cells library, 89 % and 68 % of the sequences were from genes with known function respectively, leading to a total of 465 and 645 unique sequences in each library. Some genes found in the two libraries as Cox-2, StAR, Interleukin and 3bHSD known in the literature to be potential markers of granulosa cells from competent follicle procedure, were found in our subtracted library. A total of 305 (46 % from human cells libraries) and 229 (78 % from human cells libraries) sequences had a strong hybridization (ratio more than 2 between competent and non-competent follicle) in the first and second hybridization. For the human Affymetrix GeneChip®, a total of 325 and 505 genes had a strong ratio (more than 2) for the two hybridizations, respectively. With the four hybridizations of the granulosa chip and the human Affymetrix GeneChip®, a comparison between common genes has been done and 96 candidate genes have been identified. The differential expression of these genes is now being validated with real-time PCR to identify markers with high sensitivity and low false positive rate. The selected markers will then be used within patients to assess their predictive value for single embryo transfer.

**Conclusions:** The microarray technology is a very useful tool to discover new genes and to provide information in the context of oocyte competence. This technology will help to define the transcriptome of granulosa cells present in a competent oocyte and improve the selection of healthy oocytes and the selection of a single embryo with high pregnancy rates. (Research supported by CIHR/IRSC and FRSQ).

## O12

### EFFECT OF THE OCCURRENCE OF MULTINUCLEATED BLASTOMERES IN HUMAN IVF TREATMENT

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**Introduction:** Multinucleation occurs in a high number of embryos in human

IVF treatments. However, data are limited on the effect of multinucleated blastomeres on embryo development and outcome of IVF treatment. Our retrospective study analyses the effect of multinucleation on embryo quality and implantation in a human IVF programme.

**Material and Methods:** A total of 4041 embryos from 835 IVF cycles performed between December 2001 and July 2005 were analysed retrospectively. Embryo morphology and multinucleation were assessed 2 and 3 days after fertilization. Embryo transfer was performed on day 2 or 3, depending on the number of embryos available for transfer. Cycles were classified into 4 groups according to the frequency of embryos with multinucleation (group I: 0 %; group II: 0– < 25 %; group III: 25– < 50 %; and group IV: > 50 %). Correlation was analysed between frequency of multinucleation and embryo morphology (embryo-specific data), implantation and pregnancy rates (cycle-specific data). Pearson chi-square-test and Student-t-test were used for statistical analysis.

**Results:** Embryos containing multinucleated blastomeres had a significantly lower cell number ( $3.5 \pm 1.3$  vs.  $4.1 \pm 1.3$ ;  $p < 0,0001$ ) and a significantly higher amount of fragmentation ( $21.2 \pm 13.9$  vs.  $16.4 \pm 12.4$ ;  $p < 0,0001$ ) than embryos without multinucleation. There was no correlation between patients' age and frequency of multinucleated embryos. Pregnancy rate was the lowest in group IV (25.5 %) which was significantly lower ( $p < 0.02$ ) than in group I (44.9 %), group II (50 %) and group III (47.6 %). Implantation rate was also lower in group IV (10 %) than in group I (19.3 %), group II (20.6 %) and group III (19.6 %), however, the differences between the groups were not significant.

**Conclusions:** Embryo quality is strongly influenced by the occurrence of multinucleation. However, multinucleation seems to be independent of maternal age. Pregnancy rate was affected only if more than 50 % of the embryos contained multinucleated blastomeres, which can be because of the lower number of embryos with normal nucleation in those cycles.





### O13

#### SELF-CORRECTION OF CHROMOSOMALLY ABNORMAL EMBRYOS AND ITS IMPLICATION FOR PGS

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**Introduction:** Preimplantation genetic screening (PGS) has been proposed as a method for improving success rates in patients with repeated IVF failures. This approach is based on the hypothesis that such failures are the result of aneuploid embryos. It has been suggested that FISH analysis of blastomeres removed from pre-implantation embryos represents the chromosomal constitution of the entire embryo. Whether this also represents the chromosomal constitution of the implanted fetus is as yet not clear.

**Aim:** To ascertain PGS results of aneuploid day-3 embryos with FISH reanalysis at day 5 of development and evaluate whether self-correction occurs, and to what extent, during further cleavage.

**Material and Methods:** Fifteen repeatedly failed IVF patients with good-quality embryos underwent PGS. At day 3 of development, 1–2 blastomeres were aspirated and FISH analysis was performed, using probes for chromosomes 13, 15, 16, 17, 18, 21, 22, X, Y in two rounds of hybridization. Chromosomally normal embryos were transferred to the uterus on day 4/5. Chromosomally abnormal embryos were re-analysed on day 5, using the same probe panels.

**Results:** A total of 52 embryos diagnosed as aneuploid by PGS on day 3 were re-analysed on day 5. On day 3, since the average cell number of the biopsied embryos was  $7.4 \pm 1.3$ , the analysis could be performed on 2 biopsied blastomeres in 31 embryos (60%). Altogether, 46 (88.5%) of the aneuploid embryos underwent compaction or blastulation by day 5. FISH reanalysis on day 5 was based on an average of  $7.6 \pm 4.8$  blastomeres/embryo, demonstrating that 13 (28%) were partly or

entirely normally disomic (5 embryos had 10–25% disomic cells, 2 had 70% disomic cells and 6 were 100% normal).

**Conclusions:** PGS re-analysis on day 5 of embryos designated as “aneuploid” demonstrated that some of these embryos are, in fact, mosaic normally disomic and some even completely disomic for the chromosomes tested. This may be explained by 1) FISH errors that lead to a 7–15% mis-diagnosis, 2) a high degree of chromosomal mosaicism in day-3 embryos, which cannot always be detected by PGS of 1 or 2 blastomeres, and 3) self-correction, a mechanism of natural selection which operates in favor of normal cells, probably more significant during cleavage and propagation towards the blastocyst stage. These findings also suggest that PGS results must be interpreted with caution.

### O14

#### FF-MAS PROTECTS AGED OOCYTES FROM PREDIVISION AND MODULATES GENE EXPRESSION

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**Introduction:** Culture of denuded mouse oocytes in  $\alpha$ -MEM predisposes them to precocious chromatid separation (predivision) [1]. Follicular meiosis-activating sterol (FF-MAS) significantly reduces predivision of chromatids in young oocytes [1]. Since loss of chromosome cohesion [2], predivision [3], mitochondrial dysfunction [4], and permissive checkpoint control [5] are discussed in age-related aneuploidy influences of FF-MAS on chromosomal constitution, mitochondria and gene expression were analysed in young and aged mouse oocytes, particularly with respect to presence of chromatids and age-related aneuploidy at metaphase II.

**Material and Methods:** Oocytes from young (1–5 months) or aged ( $\geq 9$  months) CBA/Ca mice were matured in  $\alpha$ -MEM with and without 10  $\mu$ M FF-MAS and assessed for chromosomal constitution by spreading and C-banding [1]. Mitochondrial

distribution was analysed by staining with Mitotracker TM at meiosis I, at 6.5 h of culture with and without FF-MAS. Relative concentration of mRNA coding for the meiotic cohesion protein SMC1 $\beta$  and the checkpoint protein MAD2 was compared between metaphase II oocytes matured without and with FF-MAS by real time fluorescent PCR with  $\beta$ -actin as standard.

**Results:** Aged oocytes were especially susceptible to induction of predivision by maturation in  $\alpha$ -MEM, supporting the concept of transient loss of cohesion due to ageing. FF-MAS reduced predivision significantly in aged and young oocytes and restored clustering of mitochondria at the spindle. However, no reduction in age-related non-disjunction by FF-MAS was observed, while FF-MAS did not increase hyperploidy rates, either. FF-MAS increased relative SMC1 $\beta$ -mRNA-concentration in young and especially in aged oocytes, and it slightly increased MAD2 mRNA in the aged group.

**Conclusions:** Some of the beneficial effects of FF-MAS and its analogues on oocyte quality to improve developmental potential of mammalian embryos (e. g. [6, 7]) may rely on protection of oocytes from precocious chromatid segregation and second meiotic errors leading to aneuploidy in the embryo. FF-MAS appears to improve oocyte quality by supporting mitochondrial activity (for instance, by enhancing local supply of high energy substrates or calcium signalling during maturation). The protection from predivision may also relate to enhanced expression of meiotic cohesins that mediate tight attachment of sister chromatids, thus counteracting partially the effect of ageing, and, possibly, improving checkpoint control [8]. However, the postulated loss of cohesion between chromosomes during the long meiotic arrest of aged oocytes in primordial follicles prior to resumption of maturation [2] may be irreversible by the activity of FF-MAS during resumption of maturation, and therefore probably cannot prevent susceptibility to first meiotic non-disjunction. In practical terms, the observations suggest that aged oocytes are especially susceptible to aberrations due to sub-optimal media during *in-vitro* maturation, and that FF-MAS may be of clinical relevance to improve



oocyte maturation and quality by protection from predivision.

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## O15

### GENETIC MOSAICISM IN DONATED CRYOPRESERVED 8-CELL EMBRYOS OF GOOD QUALITY

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**Introduction:** The occurrence of genetic mosaicism is well documented among human embryos. However, only few studies analysed mosaicism on whole embryos with normal development on day 3. Most studies are confined to

embryos unsuitable for transfer or cryopreservation on day 3 or to blastocysts or to genetically abnormal embryos. The aim of this study was to determine the degree of mosaicism in donated good quality embryos on day 3 by analysing as many blastomeres as possible per embryo.

**Material and Methods:** Embryos included in this study were cryopreserved in 1.5 M DMSO and 0.1 M sucrose on day 3 after IVF or ICSI for later replacement in another IVF cycle, they had at least 6 blastomeres and less than 20 % fragmentation. Embryos were either donated for research before thawing or were unsuitable for transfer or overnight culture after thawing. In total, 34 embryos were thawed by stepwise dilution of the cryoprotectants and fixed as whole embryos in 0.1 % Tween, 0.01N HCl. FISH was carried out for chromosomes 13, 18, 21, X and Y.

**Results:** 185 blastomeres of 28 embryos (mean of 6.6 blastomeres per embryo) gave interpretable results for at least 4 chromosomes in at least 4 blastomeres.

Only 8 embryos (28.6 %) were uniformly diploid (n = 53 blastomeres) and 9 (32.1 %) were limited mosaics ( $\geq 75$  % 2N-cells; n = 53). In these 17 genetically "normal" embryos, 11 blastomeres out of 106 were found to be abnormal (10.4 % false positive results). 6 embryos (21.4 %) were extensive mosaics ( $< 75$  % and  $> 40$  % 2N-cells; n = 39) with a mean of 56.4 % diploid cells. 5 embryos (17.9 %) were abnormal ( $< 40$  % 2N-cells; n = 40). Of these only one was uniformly abnormal. In the remaining 4 abnormal mosaics, 5 out of 36 blastomeres were normal diploid (13.9 % false negative results). Of these embryos, 3 were chaotic embryos without implantation potential but 1 was a trisomy 13 with 1 diploid cell out of 6.

**Conclusion:** Mosaicism can be frequent among day-3 embryos of good quality.

## O16

### OBJECTIVE CHARACTERISATION OF PRONUCLEAR ZYGOTES USING AN IMAGE ANALYSIS SOFTWARE

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**Introduction:** Identifying embryos with high implantation potential is a prerequisite for decreasing the number of embryos transferred without compromising pregnancy outcome. In Switzerland, analysis of 2PN-zygotes constitutes, through legal constraints, the unique way to identify viable embryos. This situation offers a unique opportunity to study the value of early zygote scoring for prognostic evaluation of embryo implantation potential. In order to reduce both the time needed and the subjectivity of the 2PN-zygote scoring, a computer programme allowing a fast and semi-automatic quantitative measurement of a high number of features from digital images of 2PN-zygotes was developed. The purpose of this study was to determine to what extent this tool might be useful in a clinical setting.

**Material and Methods:** A plug-in for the image processing programme ImageJ (<http://rsb.info.nih.gov/ij/>) was developed to analyse 2PN-zygote digital images. Zygotes were photographed 17–20 h after insemination under Hoffman contrast (Eyeware camera, Octax, Germany). The programme detects automatically the oolemma and pronuclei outlines, measures distances and angles, and calculates surfaces within the zygote. All measurements are saved for subsequent statistical analysis. The main studied features were: PN sizes, PN position, cytoplasmic Halo area, distribution of nucleolar precursor bodies (NPB). Each PN and its respective NPB were analysed separately, PN1 being the PN with the highest number of NPB and possibly the male pronucleus. A sensitivity (% of true positives) and specificity (% of true negatives) analysis for different cut-off values of the measured parameters was done first



on 118 transferred zygotes with known individual implantation outcome (set A). To limit the number of false positives (zygotes not implanted despite a good score), threshold values associated with high specificity (80 %) were selected for each parameter and their respective positive predictive values calculated on basis of a mean implantation rate of 15 %. To determine the importance of the above parameters on implantation of zygotes randomly allocated for transfer, images of 188 zygotes transferred on day 2–3, in consecutive non-selected IVF cycles were prospectively recorded and analysed (set B).

**Results:** Specificity/sensitivity analysis showed that, for all studied parameters, high specificity of 80 % was associated with limited sensitivity ranging from 10 % to 31 % and positive predictive values ranging from 14 % to 27 %. In set B, the only parameter which appears to influence implantation was the dispersion of NPB in PN2. Although not significant ( $p = 0.062$ ), IR was higher (14/65, 21.5 % vs. 14/123, 11.4 %) when at least one zygote transferred presented NPB global dispersion  $< 11 \mu\text{m}$  in PN2.

**Conclusions:** Computer-assisted analysis of zygotes allows fast and precise measurements of a number of morphological parameters on digital images. When evaluated individually, the parameters considered in this study did not display a high predictive value of implantation. In a future study, we intend to use another statistical approach to evaluate the effect of combining several of the measured parameters for prediction of implantation.

## O17

THE OCCURRENCE OF HIGH PRONUCLEUS SCORES IS NOT AGE DEPENDENT AND IS NOT RELATED TO THE TOTAL NUMBER OF RETRIEVED OOCYTES

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**Introduction:** Pronucleus Z1-score as described by Scott et al. is associated

with a higher pregnancy rate. Little is known about factors that might influence the frequency of occurrence of the best Z-scores. We explored retrospectively if the patient's age and the total number of recovered oocytes influence the appearance of the highest Z-scores.

**Material and Methods:** For 215 consecutive ICSI treatment cycles with fresh ejaculated sperm, a total number of 1145 zygotes were scored and classified as Z1, Z2, Z3 and Z4; Z1 being the best score. The 215 cycles were divided in 3 age groups:  $< 34$  years,  $\geq 34$  and  $< 38$  years and  $\geq 38$  years old and the distribution of the different Z-scores was analysed. The same 215 cycles were divided in 3 groups according to the total number of retrieved oocytes:  $< 5$ ,  $\geq 5$  and  $< 10$  and  $\geq 10$  oocytes and the distribution of the Z-scores was analysed.

**Results:** The distribution in the three age groups was for Z1: 17.8 % (91/511), 16.6 % (61/368) and 19.2 % (51/266); for Z2: 16.6 % (85/511), 18.2 % (67/368) and 16.2 % (43/266); for Z3: 58.9 % (301/511), 53 % (197/368) and 56.7 % (151/266); for Z4: 6.6 % (34/511), 1.6 % (43/368) and 7.8 % (21/266). No significant differences were observed. The implantation rate in the 3 age groups was 27.4 % (43/157), 22.8 % (29/127) and 9.6 % (11/115). The frequency of Z1-score for less than 5 recovered was 9 % (29/150), for  $\geq 5$  and  $< 10$  oocytes also 19 % (88/476) and for more than 10 recovered oocytes 16 % (86/522); for Z2 the distribution was 15 % (23/150), 15 % (85/473) and 17 % (87/522); for Z3 56 % (84/150), 52 % (247/473) and 61 % (318/522); a Z4-score occurred in 9 % (14/150), 11 % (53/473) and in 6 % (31/522). The implantation rates for these groups were 14.7 % (16/109), 20.4 % (36/176) and 27.2 % (31/114). The overall clinical pregnancy rate was 32 % (69/215) including 9 twins and 1 triplet. The mean clinical pregnancy rate after ICSI in 2004 was 25.9 % as reported by FIVNAT-CH ([www.sgrm.org](http://www.sgrm.org)), the Swiss National Register.

**Conclusions:** The frequency of the best pronucleus score (Z1) appears to be independent of the patient's age and of the number of retrieved oocytes.

## O18

Z-SCORE IS NOT PREDICTIVE FOR THE POST-THAW SURVIVAL BUT FOR THE PREGNANCY RATE

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**Introduction:** The assessment of the pronuclear morphology according to a scale proposed by Scott et al. is a commonly used method to evaluate the competence of the obtained zygotes. This scoring system is especially valuable in countries like Switzerland, where a maximum of 3 zygotes can be kept in culture and embryo freezing is prohibited. We evaluated the impact of the pronuclear score on the post-thaw survival.

**Material and Methods:** For 438 zygotes frozen with the classical propanediol-sucrose method (Lasalle & Testart) we analysed the post-thaw recovery, the cleavage rate, the cleavage state, the embryo quality and the pregnancy rate in relation to the pronucleus Z-score observed 18 hours after ICSI just before cryopreservation.

**Results:** Of 44 zygotes with Z1-score 30 (68 %) were intact after thawing, of 47 with Z2-score 35 (74 %), of 306 with Z3-score 220 (72 %) and of 41 with a Z4-score 25 (61 %). There was no statistical difference in post-thaw survival. The cleavage rate was similar in all groups: 90 % (27/30) for Z1-score, 77 % (27/35) for Z2-score, 79 % (174/220) for Z3-score and 84 % (21/25) for Z4. No statistical differences were observed. The mean embryo score – defined by the number of blastomeres, their regularity and the presence of cytoplasm fragments – was  $2.1 \pm 0.86$  for Z1,  $2.5 \pm 1.28$  for Z2,  $2.4 \pm 1.0$  for Z3 and  $2.9 \pm 1.28$  for Z4. The difference in embryo scores between Z4 and Z3 or Z2 was significant ( $p = 0.02$ ;  $p = 0.01$ ). When at least 1 embryo was transferred after thawing from a zygote with Z1-score, 38 % (8/21) positive pregnancy tests per transfer were obtained, with a clinical pregnancy rate per transfer of



29 % (6/21). When only embryos issued from thawed zygotes with an initial score of Z3 were transferred, we obtained 17 % (18/106) positive pregnancy tests with a clinical pregnancy rate of 14 % (15/106). When no Z1s but at least 1 Z2-zygote contributed to the transfer we observed a clinical pregnancy of 8 % (2/24). The observed differences in pregnancy rates were significant. The overall clinical pregnancy rate per transfer obtained with those 438 thawed zygotes was 18 % (30/168) and corresponds to the mean value of 18.3 % reported by the national register FIVNAT in 2004.

**Conclusion:** Embryos obtained after thawing of zygotes with the best pronucleus score result in significantly higher pregnancy rates. The post-thaw survival and cleavage is not related to the pronuclear score.

## O19

### PREGNANCY RATES OF BIOPSIED CATTLE EMBRYOS

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**Introduction:** DNA analysis of an embryo biopsy is becoming an important method to diagnose embryos for the presence of particular genetic diseases in humans but also in cattle. However, it is important that the pregnancy rate of the embryo is not too much diminished by the biopsy procedure. With human embryos it is often difficult to obtain information about the effect of needle biopsy on pregnancy rates following embryo transfer. For cattle embryos, however, this information (after blade biopsy) is available for high numbers of embryos and these data will be presented here.

**Material and Methods:** Cattle embryos were obtained after superovulation (SO) or by in-vitro production (IVP). For SO, female donor animals were treated with FSH, inseminated with semen and subsequently embryos were retrieved from the uterus by flushing at day 7 of the estrus cycle. For IVP, immature oocytes were recovered by Ovum Pick Up, matured and fertilized *in-vitro* and subsequently cultured for 6 days.

Both SO and IVP embryos (both blastocysts and morulae) were biopsied by blade biopsy. During this procedure the embryo was immobilized on the bottom of a petri-dish and a small part (about 10 cells) was removed with a knife. The SO embryos (both biopsied and non-biopsied) were frozen using ethylene glycol as a cryoprotectant and stored in liquid nitrogen until transfer. The IVP embryos were transferred fresh.

The embryos were transferred non-surgically into recipient animals, which were at day 6, 7 or 8 of their estrus cycle and pregnancies were scored 5 months after transfer by rectal palpation. The (raw) data were analysed by chi-square-test.

**Results:** The pregnancy rate for the non-biopsied SO embryos was 60.4 % (n = 956) compared to 46.1 % (n = 421) for biopsied embryos (p < 0.001), indicating a 14.3 % lower pregnancy rate with biopsied embryos. Despite a numeric difference, the pregnancy rate of IVP embryos was not significantly affected (52.2 % [n = 697] and 44.4 % [n = 41] for non-biopsied and biopsied embryos, respectively).

**Conclusion:** The use of the blade biopsy technique on cattle embryos has a negative effect on the pregnancy rate. This effect is larger when embryos are frozen after biopsy as is done for the SO embryos. Perhaps the combination of taking a biopsy and subsequent freezing of the embryos is too harmful, resulting in a decrease in pregnancy rates. It is also conceivable that a needle biopsy, as is routinely done with human embryos, is less harmful and results in smaller effects on pregnancy rates. However, needle biopsies can not be done easily in a cost-effective manner on large numbers of embryos, while this is possible with blade biopsy.

Despite the effect on pregnancy rate, blade biopsy followed by different DNA tests (for disease and sex) is a routine procedure now in our company. Momentary work is in progress to minimize the effect of the biopsy method and to perform pre-amplification on the embryo biopsy in order to be able to do more (100–1000) different DNA tests.

## O20

### REPLACEMENT OF CRYOVIALS BY HIGH-SECURITY STRAWS IN A HUMAN ZYGOTE CRYOPRESERVATION PROGRAMME AFFECTS THE SURVIVAL RATE BUT NOT THE CLINICAL PREGNANCY RATE

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**Introduction:** Cryovials with or without ring, used for freezing of gametes and embryos, are not leak-proof. Up to 45 % of the vials leak after 3 hours of submersion in liquid nitrogen, generating a risk of microbial contamination and explosion. Cryovials were replaced by heat-sealed high-security straws and an analysis of the outcome in terms of survival and pregnancy rate was made.

**Material and Methods:** Three different protocols for zygote-freezing were used. Group 1: cryovials in combination with freezing with 1.5 Propanediol (PROH) in Phosphate Buffered Saline (PBS), group 2: high security straws in combination with 1.5 PROH in PBS and group 3: high security straws in combination with 1.5 M PROH in Hepes Buffered Human Tubal Fluid Medium (MHTFM) supplemented with 10 % Serum Substitute Supplement (SSS) and 0.1 M Sucrose. Freezing was performed according to the classical Lasalle-Testart protocol, with manual seeding at -7 °C and warming up in a 37 °C water bath. Dilution of the cryoprotectant was performed in 3 steps for the PROH-PBS groups and in 2 steps for the PROH-MHTFM group.

**Results:** In group 1: 765 zygotes were thawed, 553 (72 %) were intact and 455 (59.5) did cleave by the next day, corresponding to a cleavage rate of 82.2 % of the intact zygotes. A total of 73 clinical pregnancies were obtained after 254 transfers corresponding to a pregnancy rate (PR) per transfer of 28.7 % or 24.8 % per thawing cycle, taking in consideration that 41 patients did not have a transfer. The implantation rate (IR) per replaced embryo was calculated from the observed foetal heart activities as 19.7 % (90/455) or 11.8 %



(90/765) per thawed zygote. Eleven twins and 3 triplets were observed. In group 2: 297 zygotes were thawed, 137 (46.1 %) were intact and 130 (95.9 %) cleaved. The clinical pregnancy rate per transfer was 29.7 % (22/74) or 23.9 % (22/92) per thawing procedure. The I.R. per replaced embryo was 20.8 % (27/130) or per thawed zygote 9.1 % (27/297). Four twin pregnancies but no triplets were observed. In group 3: 159 zygotes were thawed, 117 (73.5 %) were intact and 105 (66 %) cleaved, corresponding to a 89.7 % cleavage rate. A 28 % (16/57) clinical pregnancy rate per transfer and a 26.6 % clinical pregnancy rate were obtained. The I.R. per replaced embryo was 16 % (16/105) and 10.2 % per thawed zygote. No multiple pregnancies were observed.

**Conclusion:** Replacement of cryovials by high-security straws for human zygote cryopreservation resulted in a significant ( $p < 0,01$ ) drop in the survival rate. This drop was compensated by changing to a Hepes-buffered medium containing 0.1 M sucrose and 10 % SSS and a long-term effect on the cumulative pregnancy rate has been reduced to a minimum.

## Poster Presentation

### P1

#### PGD IN COUPLES WITH 47,XXY AND 47,XYY KARYOTYPES AND/OR ABNORMAL SPERMATOZOA

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**Introduction:** Fluorescence *in-situ* hybridization (FISH) studies on sperm from patients with 47,XXY and 47,XYY karyotypes show a high incidence of sex chromosomal abnormalities and/or diploidy, similar to those observed in infertile patients with severe male factor and normal karyotypes. The objective of this study was to assess if the increased incidence of sperm chromosomal abnormalities observed in these

two groups of patients could be translated into a higher rate of embryonic chromosomal abnormalities on day-3 embryos.

**Material and Methods:** Preimplantation genetic diagnosis (PGD) for aneuploidy screening was performed and three groups of patients were included. Group 1 consisted of 8 PGD cycles in 5 patients with 47,XXY or 47,XYY karyotypes. Sperm FISH analysis for chromosomes 13, 18, 21, X and Y was also performed in 3 of these patients. Group 2 was formed of 48 PGD cycles in 36 couples with normal karyotypes and classified as severe male infertility because previous FISH analysis on sperm showed significant increases in sex chromosome disomy and/or diploidy compared to normozoospermic fertile donors. A control group of 35 PGD cycles in fertile young patients who underwent PGD for sex-linked diseases was also included. Female age in all PGD groups was  $\leq 37$ . Embryo biopsy was performed on day 3 and chromosomes 13, 15, 16, 18, 21, 22, X and Y were analysed by FISH. All probes were available from Vysis Inc., Downers Grove, Ill., USA. Mosaicism was estimated as discordant results when two blastomeres from the same embryo were analysed. Statistical analysis was done using chi-square-test, student-t-test and Welch-t-test when appropriate.

**Results:** The incidence of chromosomal abnormalities in sperm samples from 2 patients with 47,XXY and 47,XYY karyotypes was significantly ( $p < 0.0001$ ) increased compared to fertile donors, mostly due to higher sex chromosome disomy (1.5 % vs. 0.4 %) and diploidy (0.9 % vs. 0.2 %). These results were similar to our previous observations in severe male-factor patients with normal karyotype and abnormal FISH results (0.88 % sex chromosome disomy and 0.95 % diploid sperm).

In group 1, 52.1 % of embryos were chromosomally abnormal and even a higher percentage of abnormal embryos (85.7 %) was observed in the subgroup of patients with an increased incidence of sex chromosome disomy in sperm ( $p < 0.05$  compared to control group). Sex chromosome aneuploidy was significantly increased compared to

the control group (22.9% vs. 8.3%;  $p < 0.05$ ) and the incidence of mosaicism was also higher (25 % vs. 10.3 %). In group 2, there was an increased incidence of abnormal embryos compared to controls (59.7 % vs. 33.5 %;  $p < 0.05$ ). And again, there was a significant increase in embryos with sex chromosome disomy (15.8 %;  $p < 0.05$ ) and mosaicism (36.6 %;  $p < 0.05$ ). No differences in PGD outcome were observed among the three groups, in terms of clinical pregnancies (37.5 %, 55.0 % and 43.8 %, respectively), implantation (41.7 %, 45.1 % and 35.3 %, respectively), and miscarriage rates (0 %, 9.1 % and 14.3 %, respectively).

**Conclusions:** Couples with an abnormal 47,XXY or 47,XYY karyotype and with an additional abnormal FISH result on sperm showed the highest risk of abnormal embryos in a PGD programme. Intermediate risk was observed in patients with normal karyotype and increased incidence of sex disomies and diploidies in sperm. The high incidence of sex chromosome disomies and diploidies in sperm was positively correlated with an increased percentage of embryos with sex chromosomal abnormalities and mosaicism.

### P2

#### PGD OUTCOME AND EMBRYO DEVELOPMENT IN PATIENTS WITH STRUCTURAL CHROMOSOME ABNORMALITIES

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**Introduction:** The objective of this work is to evaluate the efficacy and clinical outcome of preimplantation genetic diagnosis (PGD) using fluorescence *in-situ* hybridization (FISH) in couples with structural chromosome abnormalities.

**Material and Methods:** A total of 38 PGD cycles was performed in 27 couples with Robertsonian translocations, and 46 PGD cycles in 41 couples with other chromosomal rearrangements, including reciprocal translocations, inversions and deletions (January 1997 to Decem-



ber 2005). Embryo biopsy was performed on day 3 and one or two cells were removed from each embryo. Interphase nuclei were analysed by FISH with different combinations of centromeric, locus specific and subtelomeric probes for each of the cases evaluated (Vysis Inc., Downers Grove, IL, USA). The embryos were cultured during the analysis and normal/balanced embryos were transferred on day 5.

**Results:** A total of 226 embryos were analysed from carriers of Robertsonian translocations, and 239 embryos from couples with other chromosomal rearrangements. A high percentage of unbalanced embryos were observed in both groups but it was significantly higher in patients with non-Robertsonian abnormalities than in patients with Robertsonian translocations (83.7 % vs. 68.6 % respectively;  $p = 0.0002$ ).

Regarding the effect of the chromosomal imbalance in embryo development, blastocyst rates were significantly reduced in unbalanced embryos from carriers of Robertsonian translocations compared to normal/balanced embryos (21.3 % vs. 49.1 %;  $p = 0.0006$ ). Embryos with monosomies showed the lowest blastocyst rate compared to normal/balanced embryos (17.5 % vs. 49.1 %;  $p < 0.0006$ ), whereas embryos with trisomies did not show differences with normal/balanced embryos (29.4 % and 49.1 %, respectively). Surprisingly, embryo development for other non-Robertsonian structural abnormalities did not follow this pattern, with similar blastocyst rates for both, normal/balanced and unbalanced embryos (61.3 % and 50.3 %, respectively).

Significant differences were observed in the percentage of cycles with at least one normal/balanced embryo for transfer (81.6 % in Robertsonian translocations vs. 47.8 % in non-Robertsonian structural abnormalities;  $p = 0.003$ ). Despite this, similar pregnancy (35.5 % and 54.5 %) and implantation (29.4 % and 35.3 %) rates were achieved in Robertsonian and non-Robertsonian carriers. Likewise, the percentage of miscarriages was similar in both groups, and the cytogenetic study after hysteroembryoscopy in one miscarriage revealed a trisomic foetus for chromosome 7 (chromosome not involved in the rearrangement). All the amniocentesis per-

formed showed normal or balanced karyotypes as well as the karyotype in two liveborns.

**Conclusions:** We can conclude that there is a high incidence of unbalanced embryos in carriers of both Robertsonian and non-Robertsonian rearrangements.

Additionally, unbalanced embryos can reach the blastocyst stage in both groups of rearrangements. Although the blastocyst rates in normal/balanced and unbalanced embryos in non-Robertsonian rearrangements were similar, a significantly lower number of unbalanced embryos in Robertsonian translocation reached the blastocyst state, probably as a reflection of the comparatively higher chromosome imbalance in these embryos. Therefore, PGD is an effective and reliable approach in these couples to decrease the risk of miscarriage and unbalanced offspring.

### P3

#### A UK EXPERIENCE OF OOCYTE CRYOPRESERVATION – 5 YEARS ON

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**Introduction:** Oocyte cryopreservation is an attractive proposition for many patients, especially women whose fertility is at risk. Although the post-thaw survival rates in the past have been disappointing, recent developments, such as the increase in sucrose concentration and the use of ICSI, have shown a promising increase in oocyte survival and pregnancy rates, approaching those of embryo freezing.

**Material and Methods:** A slow-freeze protocol was adopted for the cryopre-

servation of Metaphase II oocytes using 1.5 mol/l PROH and 0.3 mol/l sucrose freezing solution. A total of 732 oocytes were stored for 66 patients for purposes such as fertility preservation (47 %) and religious objections to embryo cryopreservation (15.2 %). 11 patients underwent thaw cycles using the rapid-thaw technique and the surviving oocytes were inseminated using ICSI.

**Results:** Here we present the results of our first thaw cycles. 88 oocytes were thawed and 59 survived (67 %). 47 oocytes were injected, of which 30 fertilised normally (63.8 %). 28 embryos cleaved, giving a cleavage rate of 93.3 % and an average of 1.64 embryos were transferred in 14 embryo transfers for 11 patients. 3 clinical pregnancies resulted plus an additional biochemical pregnancy. To date, we report the birth of three girls (1 twin) and one boy. This gives a live birth rate of 21.4 % per embryo transfer.

**Conclusions:** Although still small numbers, our experience shows the survival rates and live birth rates for oocyte cryopreservation are encouraging to women whose fertility may become compromised.

### P4

#### IMPORTANCE OF AN AIR PURIFICATION SYSTEM IN THE IVF LABORATORY

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**Introduction:** The concentration of indoor pollutants can reach levels up to 8 to 10 times the outdoor environment. These pollutants consist of chemical air

Table 1: Janssen et al.

	Without Air purification filter Period 1		With Air purification filter Period 2		
Oocyte collections with embryo transfer	282		310		
Mean number of embryos per transfer	1.3		1.3		
Pregnancy rate per transfer	73/282	25.9 %	90/310	29 %	$p = 0.39$
Ongoing pregnancy rate	38/282	13.5 %	69/310	22.2 %	$p = 0.005$



contaminants (CACs), volatile organic compounds (VOCs) and heavy metals. The most common pollutants detected in an IVF laboratory are benzene, toluene, freon, isopropanol, acetone and carbon. This retrospective study evaluates the outcome of IVF/ICSI treatments during 1 year and this before and after the installation of air purification filters on gas bottles and in all incubators in the IVF laboratory.

**Material and Methods:** The outcome of 282 subsequent IVF/ICSI cycles (period 1) is compared with 310 subsequent IVF/ICSI cycles from the same year. The latter 310 cycles were performed in the laboratory where the air purification system (Coda filter) had been installed (period 2).

**Results:** Table 1.

**Conclusions:** The implantation rate of IVF/ICSI embryos significantly improved after the installation of an air purification system in incubators in the IVF laboratory. The presence of VOCs and CACs has a clear influence on the embryo nidation and development of the early pregnancy and has to be removed from the culture environment.

## P5

### SPERM PARAMETERS AND DNA FRAGMENTATION

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**Introduction:** Sperm DNA fragmentation (DF) can contribute to the abnormal development of embryos and to their implantation failure. The Sperm Chromatin Structure Assay (SCSA<sup>TM</sup>) can be used to determine the degree of DNA fragmentation (DFI) in human spermatozoa.

The aim of this study is to investigate if a correlation exists between sperm concentration, motility and morphology of

a sperm sample and the DNA fragmentation as measured by SCSA.

**Material and Methods:** 212 patients entering the IVF/ICSI programme were asked to produce a semen sample (after 2 days of abstinence) within 5–7 days of the actual IVF/ICSI attempt. Sperm samples were evaluated for count, motility and morphology (WHO criteria and Kruger strict criteria). They were frozen within 30 minutes and shipped to RMC (USA) for SCSA<sup>TM</sup> analysis. Statistical analysis included non-parametric linear regression.

**Results:** Table 2.

**Conclusions:** The data presented here demonstrate a direct inverse correlation between DFI values and sperm concentration, motility and morphology.

Table 2: Nijs et al.

Confidence interval	2.5 %	97.5 %
Concentration	0.00003841	0.0369586
Motility A+B	0.00012841	0.0006710
Morphology	0.00069184	0.0040031

## P6

### INTEGRATION OF MULTIMORPHOLOGICAL EMBRYO PARAMETERS FOR THE SELECTION OF TOP-QUALITY EMBRYOS WITH THE BEST IMPLANTATION POTENTIAL

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**Introduction:** Implantation rate (IR) per embryo in IVF is low (10–15 %) while the multiple pregnancy rate with its well-known complications, is high (20–30 %). Improved selection criteria for top-quality transferred embryos with the best implantation potential are needed. Various morphological structures in the embryo have been shown to correlate with implantation potential, and new parameters significantly improved selection criteria of top quality embryos.

**Aim:** To evaluate several morphological parameters from fertilization through day 3 of development in order to design an integrative embryo scoring system for improving selection criteria for top quality embryos with the best implantation potential.

**Methods:** All patients  $\leq 40$  years old with  $\leq 3$  previously failed IVF cycles, undergoing IVF/ICSI between October 2004–October 2005 were included. A subgroup of all cycles with 100 % implantation rate (64 embryos) was also analysed. Embryos were assessed at 4 time points during development and their pronuclear morphology, timing of first mitotic cleavage, cleavage rate and pattern, degree of fragmentation, the appearance of multi-nucleated blastomeres and compaction were recorded. All parameters, individually and integratively, were then correlated to clinical pregnancy rate (PR) and implantation.

**Results:** There were 183 study patients and 248 cycles. Significantly higher pregnancy rates were obtained with embryos displaying  $< 10$  % fragmentation and good cleavage patterns on days 2 and 3 ( $p < 0.05$ ). Embryos with  $> 7$  blastomeres on day 3 were more likely to implant than less advanced ones ( $p < 0.028$ ). Compacted embryos implanted 3-fold more than non-compacted embryos (OR: 3.116). Analysis of the subgroup of all cycles with a 100 % implantation rate revealed a better prognosis for patients with male and/or mechanical factor and for patients who had already experienced pregnancy. Among the implanted embryos, 71 % had early first cleavage,  $> 80$  % had normal cleavage rate (4 and 8 cells on days 2 and 3, respectively) and 95 % had  $< 10$  % fragmentation. Good cleavage patterns (85 % of implanted embryos) and a single visible nucleus (88 % of implanted embryos) were also good factors for predicting implantation.

**Conclusions:** Comprehensive assessment/documentation of embryo morphology at various stages of development increases our ability to choose top-quality embryos with the best implantation potential. Integrated analysis of these data will enable a more reliable embryo scoring system for better IR.



## P7

### REDUCED PERFORMANCE OF EMBRYOS FROM FROZEN OOCYTES

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**Introduction:** In the last few years, numerous pregnancies from cryopreserved oocytes have been achieved. However, detailed information on the possible impact of freezing-thawing on oocyte viability is still limited and in some cases controversial. In this study, we compared the fertilization potential and early development of sibling fresh and frozen-thawed oocytes stored with two alternative slow-cooling protocols.

**Material and Methods:** Oocytes included in this study were used for the treatment of infertile couples. Controlled ovarian hyperstimulation was induced with standard long protocol. After retrieval, oocytes were cultured 1–2 hours and assessed with respect to their meiotic status after removal of surrounding cumulus cells. Only oocytes showing an extruded polar body I were either used fresh or frozen after culture for a total period of time of about 4 hours following retrieval. Cryopreservation was conducted with a slow-cooling protocol based on a freezing solution containing 1.5 mol/l PrOH as intracellular cryoprotectant (CPA) and either 0.1 (A) or 0.3 (B) mol/l sucrose as extracellular CPA. Sperm microinjection was performed in fresh oocytes about 5 hours following retrieval, and in frozen oocytes about 1 hour after completion of the thawing procedure. Frequencies of fertilization, cleavage, as well as the proportion of good quality day-2 embryos generated from fresh and frozen-thawed oocytes were compared statistically with the chi-square-test.

**Results:** Concerning cryopreservation protocol A, 375 oocytes were thawed, with a survival rate of 21.9 %. Following sperm microinjection, fertilization was 69.8 % and 44.2 % in fresh and frozen-thawed oocytes, respectively ( $p < 0.05$  %). In the fresh group, 126 (94.8 %) embryos were obtained, while in the frozen co-

hort 29 (85.7 %) fertilized oocytes underwent cleavage. The proportion of good-quality embryos in the two classes was 36 % and 23.8 % ( $p < 0.05$ ), respectively. Eighty-four percent of embryos from fresh oocytes and 76 % of embryos in the cryopreserved group progressed beyond the 2-cell stage ( $p < 0.05$ ). With regard to cryopreservation protocol B (494 oocytes thawed), the survival rate was 59.1 %. The frequencies of fertilization and cleavage in fresh and cryopreserved oocytes were 73.1 % and 76.7 % ( $p > 0.05$ ), and 93.7 % and 89.5 % ( $p > 0.05$ ), respectively. In the fresh and frozen groups, respectively 28.6 % and 27.0 % embryos displayed good morphology, while percentages of embryos with more than two blastomeres were 91.8 % and 77.9 % ( $p < 0.05$ ), respectively.

**Conclusions:** In addition to ensuring a higher survival rate, the use of increased sucrose concentration in the freezing solution (protocol B) coincides with a fertilization rate that appears unchanged compared to the unfrozen control. On the contrary, fertilization ability of oocytes stored with protocol A appears compromised to a certain extent. Cleavage rate results were moderately reduced with both protocols, although differences did not attain statistical significance. Furthermore, irrespective of the protocol used, stored oocytes develop into embryos with decreased morphological quality (protocol A) and ability to progress beyond the second cell-cycle division (protocols A and B). This may explain why in the large majority of the experiences conducted so far neither protocol has proven itself able to generate implantation rates comparable to those of fresh oocytes.

## P8

### QUALITY CONTROL OF IUI/IVF DISPOSABLES AND PRODUCTS: A TWO-YEAR SURVEY

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**Introduction:** Consumables, media and other products can be a source of toxic-

ity to the culture system of oocytes, spermatozoa and embryos.

It is crucial to identify those consumables/products that contain toxic substances and that will have a possible toxic effect during oocyte collection, sperm preparation, artificial insemination, embryo culture and transfer.

**Material and Methods:** All new consumables and new batches were submitted to a quality control (QC) programme before being accepted for use in the IVF culture during two years, from 2003 till 2005. Seventy-four products including plastics, consumables, tubings and surgical gloves were submitted to the human sperm survival test (SpST). The SpST monitors the sperm survival, i.e. motility, of a normal semen sample (WHO criteria) over 24 and 96 hours and this after being exposed to a consumable or product, and compares this outcome to the same sample that has not been exposed (control). When the SpST index dropped below 0.85, the products/consumables were considered to be toxic.

**Results:** Five types of products used during the oocyte collection procedure were found to be toxic: an SpST index of 0 was noted (zero sperm viability) 24 hours post exposure. The products were 8 brands of non-powdered surgical gloves, 2 types of hysteroscopes and one type of tubing used for the oocyte collection needle. These products and materials all contained silicone, latex or nitrile components. The uncoated polystyrene cover of a petridish, used up to that point for the ICSI procedure, also showed a toxic effect. The cover of one type of semen receptacle, coated with silicone, could also be identified as toxic by the SpST.

**Discussion:** The SpST clearly identified 5 types of products with absolute toxicity to human spermatozoa. As a consequence, these products/consumables were rejected for use in the IVF system and an alternative had to be sourced.

**Conclusion:** The SpST is an inexpensive and easy method to identify potentially toxic products and consumables used for all IVF procedures. The inclusion of the SpST in our continuous monitoring programme of IVF results (fertilization rate, embryo development, multinuclea-





tion, implantation rate and ongoing pregnancy rate) has been proven to be a valid strategy.

## P9

### ASSISTED HATCHING BY LASER FOR FROZEN-THAWED HUMAN EMBRYOS

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**Introduction:** Cryopreservation of human embryos could leave the embryos with a hardened zona pellucida (ZP), resulting in a lower hatching and subsequently lower implantation potential. Assisted hatching by laser prior to transfer could facilitate the hatching procedure of the frozen-thawed embryos. This prospective randomised study evaluates the possible beneficial effect of lasering of the ZP as an assisted hatching.

**Material and Methods:** Day-3 human embryos (grades A and B) were frozen and thawed using the slow Propanediol-Sucrose protocol. The embryos were placed in culture until day 4. Embryos were allocated to the control (zona intact) group or to the laser group (zona lasering) according to their thawing number. Prior to transfer, an opening was created in the ZP with two 300 mW laser pulses using the Hamilton Thorn Zilos-system.

#### Results: Table 3

**Conclusions:** Assisted hatching did not improve the implantation rate of frozen-thawed day-3 embryos. A trend towards

lower implantation rates could be observed when transferring one or two embryos with a lasered ZP. The negative impact of the laser pulse on further embryo development has to be investigated in more detail since this method is also used routinely for lasering the ZP prior to biopsy in a PGD procedure.

## P10

### PROSPECTIVE EVALUATION OF ICSI GUARD SPINDLE VISUALIZING HARDWARE FOR USE DURING ICSI

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**Hypothesis:** Visualization of oocyte spindle used in combination with ICSI may improve ART outcome.

**Objective:** We wished to determine whether most spindles were adjacent to polar body as first thought and whether the use of a spindle visualizing apparatus would help in the patient cycle.

**Introduction:** ICSI guard is a birefringence filter and software system allowing visualization of oocyte spindle. Theoretically, if the spindle is not adjacent to the polar body, the embryologist may make proper adjustments before ICSI.

**Design:** Prospective sequential analysis of 44 ICSI cycles in which the ICSI guard was used to visualize (group 1) the oocyte spindle before ICSI, and 44 ICSI cycles in which the ICSI guard was not used (group 2) (as a concurrent control). All ICSI patients (except PESA

and TESA)  $\leq 42$ yr enrolled were observed for spindle appearance before ICSI.

**Material and Methods:** The study was performed in a private health care facility. Glass bottom ICSI dishes were used. Oocyte retrieval, sperm preparation and ICSI were performed as previously described.

**Results:** Average age was 36.4 yrs in group 1 and 35.98 yrs in group 2. No difference in age, maturation, and fertilization or blastocyst rates was seen between the two groups. 476 oocytes were retrieved from 42 patients in group 1 of the study. Of these, 345 were mature (MII) and 257 (74.5 %) fertilized normally with 2 pronuclei. ICSI guard was used in group 1 before ICSI. Control group (group 2) was observed sequentially.

20.5 % of the oocytes examined did not show a spindle. A spindle was seen in 274 oocytes. We found that only 31 % (107) of the oocytes in which we did visualize a spindle had the spindle adjacent to the polar body (in the "12 o'clock" position relative to polar body). 48 % of spindles seen were at 1 and 2 "o'clock" position, 12 % were on or below the equator of the oocyte, which is a potentially dangerous position when the polar body is at 12 o'clock, possibly leading to chromosomal scatter and aneuploidy.

**Discussion:** The ICSI guard was useful in visualizing the spindle in 80 % of all eggs observed. It was unexpected to find that only 31 % of spindles were at 12 o'clock position and 12 % were on or below oocyte equator. ICSI position was adjusted for those oocytes. Extrapolating over a year's time where we would inject a projected 4500 oocytes, the number potentially "rescued" theoretically is approximately 540 eggs. More data is being added to this study.

Table 3: Nijs et al.

	Control Zona Intact		Laser group Assisted Hatching	
80 Single Embryo transfer	45		35	
Pregnancy rate per transfer	13/45	28.9 %	4/35	11.4 %
Implantation rate	10/45	22.2 %	4/35	11.4 %
60 Double Embryo transfer	31		29	
Pregnancy rate per transfer	9/31	29 %	6/29	20.7 %
Implantation rate	11/62	17.7 %*	5/58	8.6 %

\*2 twins



## P11

### IVF-LABORATORY PRACTICES IN FINLAND

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**Introduction:** Application of the EU tissue culture directive will start shortly in Finland. The quality of laboratories will be monitored by government authorities. How to define quality? How do Finnish IVF laboratories actually perform their activities? Are they all the same?

**Method:** Telephone interview was used to gather information of IVF laboratories in Finland. Seventeen out of 20 laboratories agreed to take part in this study. The responsible IVF biologist answered 45 questions in a telephone interview.

**Results:** The technical equipment varied a little among laboratories. Sperm count and study of follicular fluid is often done without heated microscope stage. Average number of oocytes per ovum pick-up is 7–13. Over 20 oocytes per patient are nowadays rare in most clinics. Pipetting using mouth piece suction is still in use in some clinics, as it is very ergonomic. Cells are cultured in microdroplets under oil, open 1 ml dish or 4-well dish with or without oil. Oil is always used to cover an ICSI dish, and

in one clinic to cover denuding solution in a 4-well dish.

Embryos are mostly evaluated through ICSI microscope. In some clinics, they are studied from a computer display in larger size. In some labs, the images of all embryos are stored, and in some the ones that are transferred. The classification of embryos has two major approaches: one concentrating on the level of fragmentation (valid day-2 embryos should not have more than 20 percent fragmentation), and the other on the quality and number of blastomeres (correct number of blastomeres plus normality and elasticity of them is vital, the amount of fragmentation is irrelevant).

Embryos with malformed, granular, vacuolised or unevenly sized blastomeres are not utilized in some labs. Some labs accept lower quality in fresh transfers, and some freeze them. The policy for embryos with multinucleated blastomeres differs: some disqualify embryos, some use them when the patients want so, and some use them as the last option.

Thawing of frozen embryos is done by dipping the ampule or by strawing in a 37, 30 or 20 °C water bath, or by thawing the straw in air. Also, self-made freezing and thawing solutions are used with superior results (90 % of embryos intact). Finishing the thawing protocol is

done either by moving the embryo from table-warm media straight to 37 °C media, or by allowing the last thawing media to gradually warm up. Not all laboratories utilize outside quality control for sperm analysis. Staff meetings are commonly held once a week, in some clinics daily, once a month or once a year. Half of the clinics utilize psychological counselling to discuss demanding cases or communication in work environment. The work load per person was very different between clinics.

The quality in IVF-laboratory work can be determined by fertilization and cleavage rates, amount of first-class embryos, biochemical and clinical pregnancies, and the “take home baby” rate. Some laboratories were not aware what their own rates are.

**Conclusions:** It will be difficult for government authorities to define quality in a Finnish IVF laboratory, as the labs are very different from each other. Pregnancy is a combination of many factors, and the laboratory is just one of them. The participants of the enquiry emphasized the importance of practice and team-work. IVF is modern handicraft, with ICSI being the master class. In practice, the laboratory can rely on its own work quality, if the fertility rate is higher than 65–70 %, cleavage rate of fertilised oocytes is 90 % and first-class embryos are regularly produced.

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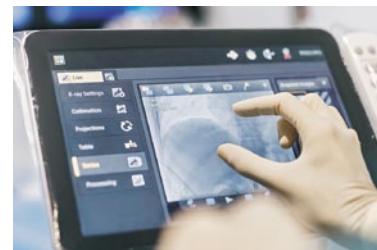
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