in DCS (7%) versus SCS (19%; p < 0.002); the likelihood of formation of PQE on day 3 in SCS was 3.27 times higher than in the DCS (95% CI = 1.51-7.06; p = 0.001).

Conclusions: Microfluidic dynamic culture of human embryos for two days significantly improved grade of embryos, reduced fragmentation, and enhanced collective embryo quality. Interestingly, these studies allowed insight into potential benefits of dynamic culture not seen in mouse embryos, which have very little spontaneous fragmentation. Whether use of this dynamic culture system for extended human embryo culture (>48hrs) will improve cell count, blastocyst formation, and pregnancy rates remains to be determined.

O-096 Effects of a new fluidic culture system on in-vitro folliculogenesis of ovarian follicles as part of fertility protection

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Introduction: Oncological treatments still bear the risk of gonadal arrest with loss of fertility as consequence. Thus cryopreservation of ovarian tissue for later retransplantation provides a potential option for fertility preservation in young women by now. However, concerning malignant tumors, retransplantation of cryopreserved ovarian tissue is contraindicated. Therefore in-vitro culture of ovarian cortex to get meiotic competent oocytes is in focus of current research. This trial investigated the initial follicle recruitment by gene expression assays and the growth of in-vitro cultured follicles by hormone assays in two different culture systems.

Material and Methods: Ovarian cortical tissue from 10 postpuberal women, aged 26.1 ± 1.3 years, were freshly prepared, divided in three equally sized tissue pieces and were cultured for 6 days in a conventional (CC) and in a new fluidic culture system (FC). One piece served as control. Underlying stromal tissue was removed. Culture medium was not exchanged and supplements like growth factors or gonadotropins were not added. Tissue and follicle viability was evaluated by calcein staining. Concentrations of Estradiol and Progesterone were analyzed in the supernatant using a chemiluminescent-microparticle-assay. Early follicular growth was also determined with the aid of gene expression profiles created by use of real-time PCR. RNA was extracted from histological sections of ovarian tissue embedded in paraffin wax. For the relative quantification of the particular mRNA-products we used the housekeeping gene ribosomal protein L 19 and the follicle specific of every embedded ovarian tissue probe.

Results: Calcein stainings of controls and cultured cortical pieces (FC and CC probes) showed healthy follicles in different growth stages. However the number of follicles decreased over the culture period compared with the controls. The least number of follicles was found in samples from the CC system. Early follicles which increased in size during the culture period produced high levels of Estradiol (E2) and minimal basal levels of Progesterone (P4). In the supernatant of both groups the rise of E2 was significant (P < 0.05). Furthermore in the CC probes the increase of P4 was also significant (P < 0.05). The ratios of E2 and P4 as an indicator of luteinization induced by premature stress were significantly different in both groups (P < 0.05). FC pieces showed a normal result (level of the basal P4 level) and the CC group showed a notably drop by high P4 levels. Gene expression profiles were investigated for GDF9 and BMP15, members of the early oocyte specific TGF family, as well as Kit-Ligand and Inhibin B. Kit Ligand, a potential marker for the initial recruitment of primordial follicles, exhibited in average the highest expression level in samples of the FC system. The GDF9 expression profile showed a significant increase over the culture period in both groups (P < 0.05) compared to controls with a higher expression level of GDF9 in FC samples versus CC samples. The results for BMP15 gene expression were inversely correlated to GDF9 due to the negative feedback loop between both gene products. Inhibin B, a marker for preantral follicles, showed a significant increase in the expression level of FC probes (P < 0.05) compared to controls but not in CC probes.

Conclusions: We could demonstrate that the new established fluidic culture system showed a better performance in all parameters compared to the conventional static culture system. Different stages of folliculogenesis (from primordial to late preantral stage) were found in the tissue cultures alive and growing. The parameters investigated enabled an estimation of the developmental potential of follicles in ovarian cortical tissue in-vitro. These findings are important in view of the possible use of such culture systems for later in-vitro follicle growth.

O-097 Improved embryo development in a time-lapse incubator system evaluated by randomized comparison of surplus embryo development to the blastocyst stage

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1Erasmus MC University Medical Center, Obstetrics and Gynaecology, Rotterdam, The Netherlands

Introduction: Selecting embryos for transfer with an optimal implantation potential is still largely based on morphological criteria, such as the number and size of blastomeres and the percentage of fragmentation in the 4- to 8-cell stage of the embryo at fixed time points. Time-lapse observation of embryo development might improve criteria for embryo selection. The EmbryoScope™ Embryo Monitoring System (Unisens Fertilitech, Denmark) is an incubator designed for time-lapse embryo assessment, facilitated by a specialized built-in microscope. The EmbryoScope™ provides a controlled culture environment, while acquiring images of up to 72 embryos in different focal planes every 20 minutes.

Before introducing this instrument into our routine practice, we validated the performance of this new incubator by comparing surplus embryo development in the EmbryoScope™ to development in our regular CO2 incubator.

Material and Methods: Surplus embryos were collected from patients undergoing routine IVF/ICSI treatment at the Erasmus MC, University Medical Center between October and December 2010. Embryo(s) for transfer are routinely selected three days after ovum pick-up, followed by assessment for cryopreservation on Day 4. During the study period, 60% of fertilized oocytes were either transferred or frozen. Based on previous experience, we estimated that 5% of remaining poor quality surplus embryos will be able to reach the expanded blastocyst stage in our normal incubator, with an expected increase to 15% in the EmbryoScope™. In order to detect this 10% difference with a power of 80% at a 5% significance level, 280 surplus embryos are needed.

On Day 4, surplus embryos were pooled and randomly divided into two groups at a low magnification to avoid a selection bias based on morphology. The control group was cultured in our regular CO2 incubator (CO2-waterjacketed incubator, model NU-4950E, NuAire, Plymouth, MN) at 37°C and 5.8% CO2. The experimental group was cultured in the EmbryoScope™ at 37°C and 5% CO2. All embryos were cultured individually in G-2™ v5 PLUS medium (Vitrolife, Sweden) in EmbryoSlides™. These slides have a well-on-wall design to fit the EmbryoScope™. The primary outcome measure was the capacity of the embryos to develop to the expanded blastocyst stage after 168 hours post insemination. An estimated survival rate of the embryos, defined as a continued increase in cell number, combined with limited (~30%) fragmentation and degeneration, was used as a secondary outcome parameter. Morphology of embryos cultured in the regular CO2 incubator was assessed by daily microscopy at fixed time points. In the EmbryoScope™, embryo development was judged by evaluating the acquired images at the same time points.

Results: In total 315 embryos were randomized, 164 embryos to the EmbryoScope™ and 151 to the regular CO2 incubator. The expanded blastocyst rate was 23.8% in the control group, compared to 35.4% in the EmbryoScope™ (χ2; p = 0.026). The estimated survival rate of embryos in the control group was 67.5% at 120hr, 51.6% at 144 hr, and 36.0% at 168hr, compared to 83.5%, 64.9% and 44.5% in the EmbryoScope™.

Conclusions: The EmbryoScope™ allowed a significantly higher proportion of surplus embryos to develop to the expanded blastocyst stage in our setting. The estimated survival rate of the embryos during the culture period supports this conclusion. The EmbryoScope™ is a small volume incubator, with short recovery times for CO2-cycling. This system showed a better performance in all parameters compared to the conventional CO2 incubator. The expanded blastocyst rate in the control group was higher than expected. This is possibly due to the microwell culture system of the EmbryoSlides™. Microwell culture has previously been described to improve mammalian embryo development.
O-098 Dynamic analysis of early initiation of compaction in human embryos using two- and three-dimensional time-lapse cinematography
K. Yumoto1, K. Iwata1, A. Kawai1, M. Ueda1, Y. Tsuchie1, A. Imajo1, Y. Miura1, T. Mochida1, Y. Iba1, Y. Mio1
1Mito Fertility Clinic, Reproductive Centre, Yonago, Japan

Introduction: We have reported several novel aspects of human embryonic development in vitro using time-lapse cinematography (TLC)1. Compaction is the change in cellular adherence that indicates the first morphological event in embryonic differentiation. In a recent study, we demonstrated that compaction is initiated at the 8- to 16-cell stage in the vast majority of human embryos. However, we also found that several embryos initiate compaction earlier than the 8-cell stage and that these embryos showed poor development morphologically. This study therefore analyzed the etiology as well as the fate of those embryos showing early initiation of compaction in human embryos obtained from two- (2D) and three-dimensional (3D; a new system) TLC.

Material and Methods: We previously developed a system of time-lapse cinematography (2D-TLC, as described elsewhere). We have now developed a 3D-TLC system equipped with an autofocus system using 10-μm intervals in vertical depth. Temperature was maintained at 37 ± 0.2°C and pH at 7.37 ± 0.05 by controlling CO2 flow. Digital images of the cultured embryos were acquired for 5 days with an exposure time of 1/20 seconds. In 3D-TLC, images captured in each depth were analyzed individually. Of 119 donated embryos frozen at an early developmental stage (pronuclear stage to 4-cell stage), 109 successfully developed to the blastocyst stage and so far, 11 embryos have been analyzed by 3D-TLC.

Results: From our TLC analysis, compaction occurred in 109 embryos. Of those embryos, 96 (88.1%) embryos initiated compaction after the 8-cell stage and 52 (54.2%) developed into good quality blastocysts. On the other hand, 13 (11.9%) embryos initiated compaction before the 8-cell stage. Of those, 12 (92.3%) embryos presented multinucleated blastomeres, and 10 of that 12 (83.3%) developed into poor-quality embryos.

Seven of the embryos that initiated compaction before the 8-cell stage (53.8%) showed interruption of blastomere cleavage after the cleavage furrow was formed, followed by multinucleation in those blastomeres.

Analysis of the 11 embryos using 3D-TLC indicated that compaction initiated at a focal site within the blastomeres and spread over the whole blastomeres in 8 of those embryos.

Conclusions: Our study suggested that early initiation of compaction is strongly associated with poor quality in human embryonic development. Because most of the multinucleated blastomeres observed here failed to complete normal cytokinesis, early initiation of compaction might be caused by the aberrant cytokinesis followed by karyokinesis. Our 3D-TLC study showed the first reported compaction initiating from a focal site in human embryo followed by gradual fusion from that point. Our 3D-TLC technique may therefore enable more detailed analysis of such developmental mechanisms, and further such investigations are clearly needed to elucidate this intriguing phenomenon of embryonic development.

Reference:

O-099 Single touch immobilization versus triple touch immobilization in ICSI, does this improve success rates? A prospective randomised controlled trial
A. Velaers1, G. Paternot1, S. Debrock1, T.M. D’Hooghe1, C. Spiessens1
1U.Z. Leuven, Fertility Centre, Leuven, Belgium

Introduction: Although different techniques for sperm immobilization before ICSI have been described, their value has not been assessed in an adequately powered randomised study. The aim of this study was to compare two types of sperm immobilization methods prior to intracytoplasmic sperm injection (ICSI) and to test the hypothesis that triple touch immobilization (TTI) would lead to a higher fertilization rate (FR) than single touch immobilization (STI).

Materials and Methods: All patients treated by ICSI were eligible for our prospective randomised controlled study, except patients treated with ICSI combined with pre-implantation genetic diagnosis (PGD), or with ICSI using either immotile sperm from fresh ejaculate, or frozen-thawed sperm from testicular biopsies. Mean age (± SD) was 31.7 (± 4.5) and 34.6 (± 6.0) for female and male partner respectively. Patients received treatment with ICSI mostly for severe male factor infertility (88%). A total of 3056 metaphase II (MII) oocytes, from 290 patients, were randomly assigned to the STI group (n = 1528 oocytes; 145 cycles) or to the TTI group (n = 1528 oocytes (TTI group, 138 cycles). Due to no d1 or d2 scoring, 50 oocytes (STI group) and 52 oocytes (TTI group) were excluded from the analysis. A total of 1478 oocytes (STI group) and 1476 oocytes (TTI) were used in the statistical analysis.

The primary outcome variable was FR. To improve the FR with 5% (65% up to 70%) and to reach a statistical power of 0.8, at least 1416 injected oocytes were required in each group. Secondary outcome variables included: number of good quality embryos (GQE) on day 2 (4- cell stage embryos with less than 25% fragmentation and equally or slightly unequally sized blastomeres) and day 3 (7-, 8-, or 9- cell stage embryo with less than 25% fragmentation and equally or slightly unequally sized blastomeres), implantation rate (IR) and implantation with foetal heart beat rate (FHB). Statistical analysis was done using the Fisher Exact Chi-square test with a significance level of 0.05.

Results: The results of the study are listed in the following table.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>TTI group</th>
<th>P-value</th>
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<tbody>
<tr>
<td>FR (n normal fertilized embryos/total n of injected MII) 67.1% (992/1478)</td>
<td>66.7% (984/1476)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>GQE d2 (n GQE d2/total n of 2PN) 33.0% (126/387)</td>
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Conclusion: In this RCT, the hypothesis that the post-ICSI FR would be higher after TTI than after STI was not confirmed and the number of good quality embryos on day 3 was significantly lower in the TTI group than in the STI group. These data suggest that the more ‘aggressive’ TTI technique has no advantages compared to the STI technique before ICSI.

O-100 Clinical outcome after culturing human preimplantation embryos in incubators with individual chambers compared to standard incubators; randomised trial
R. Janssens1, R. Souffreau1, P. Haentjens1, H. Van de Velde1, G. Verheyen1
1Universitair Ziekenhuis Brussel, Centre for Reproductive Medicine, Brussels, Belgium
2Universitair Ziekenhuis Brussel Vrije Universiteit Brussel, Centre for Outcome Research and Laboratory for Experimental Surgery, Brussels, Belgium

Introduction: In IVF, the use of standard incubators with a single large incubation chamber is generalised since the 80s. These type of incubators has been criticised since each door opening results in fluctuations in temperature and gas composition. In the 90s, smaller desktop incubators with individual culture chambers flushed with premixed gas were developed. We aimed to validate a new desktop incubator with 10 individual incubation chambers (G-185, K-Sys) compared to the STI technique before ICSI. These data suggest that the more ‘aggressive’ TTI technique has no advantages compared to the STI technique before ICSI.

Material and Methods: From June to December 2010, oocytes of 312 patients were randomised for culture either in standard incubators (SI, HeraCell 240 or Thermodrorna) or in the G-185. Cycles with at least 6 cumulus-oocyte complexes and ejaculated (fresh and frozen) semen were included. PGD cycles were excluded. Culture conditions (37.0 ± 0.2°C and 6.0% CO2 – 5% O2) were identical in both groups. In this RCT, the hypothesis that the post-ICSI FR would be higher after TTI than after STI was not confirmed and the number of good quality embryos on day 3 was significantly lower in the TTI group than in the STI group. These data suggest that the more ‘aggressive’ TTI technique has no advantages compared to the STI technique before ICSI.

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Conclusion: In this RCT, the hypothesis that the post-ICSI FR would be higher after TTI than after STI was not confirmed and the number of good quality embryos on day 3 was significantly lower in the TTI group than in the STI group. These data suggest that the more ‘aggressive’ TTI technique has no advantages compared to the STI technique before ICSI.

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increment was not statistically significant. Pregnancy rates per started cycle were 50.4% (69/137) and 39.0% (53/136) for G-185 and SI with a between-group difference + 11.4% (95% CI, -0.3% to +23.1%; p = 0.0583) in favour of G185. Likewise, the pregnancy rates per embryo transfer were 51.1% (69/135) and 39.6% (53/134) for G185 and SI with a between-group difference + 11.6% (95% CI, -0.3% to +23.4%; p = 0.0569) in favour of G-185.

The implantation rate (sacs with foetal heart beat) per embryo transferred was significantly higher in the G185 group (27.1% vs 18.5%), with a between-group difference of + 8.5% (95% CI, +0.4% to +16.6%; P = 0.036) in favour of G185. However, a positive effect of a higher proportion of day 5 transfers cannot be excluded.

**Conclusions:** Among patients treated for IVF/ICSI, culture in the G185 incubator with small individual chambers resulted in a trend towards higher pregnancy rates. Since all culture variables were identical, we speculate that better temperature and gas stability may explain these findings.

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### INVITED SESSION

**SESSION 27: AVOIDABLE LOSS OF FERTILITY**

**Tuesday 5 July 2011 08:30 - 09:30**

**O-101 AVOIDABLE LOSS OF FERTILITY**

**W.L. Ledger**

*University of New South Wales, School of Women’s & Children’s Health, Sydney, Australia*

One of the most significant demographic changes seen in Europe in recent decades has been the right shift in age at first birth. In UK, for example, in 2009, 47% of women and 64% of men became parents for the first time beyond age 30. Given the failure of evolution of the female reproductive lifespan to keep up with this trend towards deferral of pregnancy, it is more important than ever to advise men and women of the risks of deferral and on strategies to defer loss of fertility where possible.

Most of the methods available to delay loss of fertility are well rehearsed: avoidance of smoking, recreational drugs or excess alcohol, possibly also maintenance of a normal body weight and regular exercise. However it is difficult to quantify the extent of the benefits that can be gained from such lifestyle changes and many younger people seem to over-estimate the impact of a ‘healthy’ lifestyle on their future fertility. In vitro fertilisation is frequently seen as a means of ‘rescuing’ a woman’s fertility if she is too old to conceive naturally although the chances of successful pregnancy after IVF for those over 40 are low and have not improved significantly over the last 20 years. It seems that the biological clock can be accelerated, most strikingly by chemotherapy and less so by smoking and exposure to environmental toxins, but it cannot currently be slowed.

Recent developments in methods for assessment of ovarian reserve combined with improved survival of oocytes following vitrification now permit an individualized measurement of ovarian reserve to be followed in some cases by oocyte freezing. The advantages of this approach over the current widespread utilization of donor oocytes by older women who wish to become parents are obvious, and this may become a commonplace strategy in the future.

Further longitudinal studies are required in order to validate use of measurements of anti-Mullerian hormone (AMH) and antral follicle count (AFC), possibly in combination with genetic testing, to identify the optimal age at which to recommend oocyte freezing to an individual. It seems likely that this age will vary considerably from one woman to the next. For example, those who have a strong family history of early menopause, low AMH and AFC will be advised to consider oocyte cryopreservation at an earlier age than those who have polycystic ovary syndrome and high AMH and AFC.

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**O-102 SURGICAL REDUCTION OF OVARIAN RESERVE IN WOMEN WITH ENDOMETRIOSIS**

**P. Vercellini**\(^1\), **E. Somigliana**\(^2\), **P. Vigano**\(^3\), **S. De Matteis**\(^4\), **L. Buggio**\(^5\), **L. Fedele**\(^6\)

\(^1\)University of Milan, Dept. of Obstetrics and Gynaecology, Milano, Italy

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**O-103 CHANGES IN GENETIC MANAGEMENT OF MTDNA DISEASES: IMPLICATIONS OF SCIENTIFIC ADVANCES**

**J. Poulton**\(^1\), **D. Wells**\(^2\)

\(^1\)John Radcliffe Women’s Centre, Nuffield Department of Obstetrics, Oxford, United Kingdom

One in 400 people carries pathogenic mitochondrial DNA (mtDNA) mutations. These may cause epilepsy, liver failure, cardiomyopathy or sudden death; or, more commonly, milder disorders such as age-related deafness and/or diabetes, and loss of vision. Yet management of mtDNA diseases has lagged far behind the genetics revolution.

Recent reports of strong selection of mitochondrial DNA (mtDNA) during transmission in animal models of mtDNA disease, and of nuclear transfer in both animal models and humans, have important scientific implications. These are relevant to the genetic management of mtDNA disease.

The risk of recurrence is difficult to estimate due to heteroplasmy, the existence of normal and mutant mtDNA in the same individual, tissue or cell. In addition the mtDNA bottleneck during oogenesis frequently results in dramatic and unpredictable inter-generational fluctuations in the proportions of mutant and normal mtDNA. I will outline the implications of recent advances for genetic management of these potentially devastating disorders.