In the USA and the UK more than half of all women are either overweight or obese. Many are in the reproductive age group and a significant number present with infertility. An increasing body of literature has highlighted issues of concern with respect to poor clinical outcomes, increased health risks and expense in relation to assisted reproduction treatment for this group of patients. In addition obstetric data point to significant health risks for mother and child in association with obesity. Public funding of fertility treatment demands that limited resources are used to maximum effectiveness with the safety of women and children a prime concern. Women who are overweight should thus be encouraged to lose weight in advance of initiating treatment.

INVITED SESSION
SESSION 24: THE PATERNAL GENOME - MHR SESSION
Monday 4 July 2011 17:00 - 18:00

O-091 Sperm chromatin packaging
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During spermiogenesis a remarkable series of changes occurs in most cellular elements of germ cells. Perhaps most striking is the complete reorganization of the nuclear material as germ cells progress from round spermatids to spermatozoon. Most of the histones are removed and replaced, first by transition proteins and then protamines, highly basic proteins rich in SH groups that will form cross-links as the spermatozoon mature during epididymal transit. The condensation of sperm chromatin during spermiogenesis to less than one sixth its size appears to be a highly regulated, orderly process. While components of each chromosome remain attached to a nuclear matrix, the rest of the chromatin is organized as loops that are connected to this matrix.

Although assessing the number, motility and morphology of spermatozoon in semen samples provides valuable information, such data provide no insight into the quality of the chromatin that the sperm will deliver to the oocyte upon fertilization. Extensive evidence has accumulated demonstrating that spermatozoon with damaged chromatin are not at a disadvantage in fertilizing oocytes. Therefore, robust tests that provide insight into different aspects of sperm chromatin packaging and integrity are essential. The development of a number of such tests for spermatozoon allows us to assess single and double stranded DNA breaks, the amount of protamine and of free SH groups, the rate of decondensation, template function, and response to treatment with acid or base. Other aspects of chromatin packaging, such as the retention of histones and the nature of histone modifications, the localization of these histones on chromatin, the cytosine methylation pattern, and the extent of RNA retention (both mRNA and non-coding RNAs) are under active investigation by several groups.

We have investigated the impact of age and of treatment of males with chemotherapeutic agents on sperm chromatin packaging. Using a rat model, we have found that sperm chromatin is less tightly packaged with advanced age. This decreased condensation is associated with altered expression of glutathione peroxidase 4, an increased incidence of pre-implantation loss and altered progeny outcome. In addition, rat models have allowed us to demonstrate that, depending on the chemotherapeutic regimen used, different aspects of chromatin packaging are affected. Treatment with cyclophosphamide affects sperm chromatin packaging most dramatically during mid-spermiogenesis, when condensation is occurring most rapidly and there is an active exchange of transition proteins for protamines. In contrast, exposing males to the drug combination proteins for protamines. In contrast, exposing males to the drug combination used to treat testicular cancer (bleomycin, etoposide and cis-platin, BEP) affects germ cells as early as spermatogonia.

In men who have been diagnosed with either testicular cancer or Hodgkin lymphoma, we have found that sperm chromatin quality is affected even prior to the initiation of chemotherapy. Surprisingly, we found that in those males in whom spermatogenesis returned within two years after the completion of chemotherapy, several aspects of sperm chromatin quality, including aneuploidy, remained well below that of a control population. This indicates that treatment with chemotherapeutic agents may affect the quality of germ stem cells. These studies were supported by grants from the Canadian Institutes for Health Research.

O-092 The Human Sperm Epigenome and Vertebrate Comparisons
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In mature human sperm, genes of importance for embryo development (i.e. transcription factors) lack DNA methylation and bear nucleosomes with distinctive histone modifications (Hammoud et al., Nature 2009), suggesting the specialized packaging of these developmental genes in the germline, and the possible poisoning of these genes for expression in the embryo. Here, we extend on these observations in humans, and examine conservation for these principles in vertebrates by examining zebrafish sperm.

In zebrafish, we find conceptual conservation in regard to the packaging of developmental genes, as well as new features for the bulk genome (Wu et al., in press). Biochemical and mass spectrometric approaches reveal the zebrafish sperm genome packaged in nucleosomes and histone variants (and not protamine), and we find linker histones high and H4K16ac absent - key factors which we think contribute to genome condensation. We then examined several activating (H3K4me2/3, H3K14ac, H2AFV) and repressing (H3K27me3, H3K36me3, H3K9me3, hyperacetylation) modifications/compositions genomewide, and find developmental genes (for the embryo) packaged in large blocks of distinctive chromatin with coincident activating and repressing marks and DNA hypomethylation, revealing complex ‘multivalent’ chromatin, similar in concept and composition to human sperm chromatin. Notably, genes that acquire DNA methylation in the soma (i.e. muscle), compared to sperm, are mainly transcription factors for alternative cell fates, paralleling our human sperm-soma comparisons.

We have recently determined the complete CpG methylation of human sperm at base pair resolution, and at ~50-fold coverage, using ‘shotgun bisulphite high-throughput sequencing’. Our analyses are currently in process, and includes comparisons to ES cells and somatic cells. Many new features will be discussed – such as relationships between CpG island methylation and histone modifications, relationships between piRNA clusters and both DNA methylation and histone modifications, DNA methylation features at imprinted loci, and the extent and location of non-CpG methylation.

SELECTED ORAL COMMUNICATION SESSION
SESSION 25: TRANSLATIONAL RESEARCH
Monday 4 July 2011 17:00 - 18:00

O-093 Novel treatment for ovarian hyperstimulation syndrome (OHSS) using pigment epithelium derived factor (PEDF)
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Introduction: Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening complication of assisted reproduction, with a yet unknown pathophysiology. This syndrome appears to be induced by an ovarian release of vasoactive, angiogenic substances, mainly vascular-endothelial-growth-factor (VEGF). Several OHSS-managing treatments that affect the VEGF system were suggested; unfortunately, these treatments do not entirely eliminate the risk for OHSS and are associated with undesired side effects.

Aim: To develop a novel, physiologic approach for preventing OHSS, hypothesizing the existence of an ovarian negative angiogenesis mediator that antagonizes VEGF.

Methodology: OHSS was induced in 5 weeks old ICR female mice by 3 consecutive daily injections of 20IU PMSG, followed 24hrs later by 7IU hCG. Control mice were administrated with a single dose of 5IU PMSG, followed 48hrs later by 7IU hCG (standard superovulation protocol). We tracked, by qPCR analysis, the changes in the levels of pigment epithelium-derived-factor (PEDF) and VEGF in total ovarian lysates and isolated granulosa cells. In
order to test the therapeutic properties of PEDF we injected it at the same time of the first and last administrations of excess PMSG, and recorded the changes in body weight and in peritoneal vascular leakage that was quantified by the modified Miles vascular permeability assay.

**Results:** We have characterized the ovarian anti-angiogenic factor that negates VEGF as PEDF, a known potent anti-angiogenic factor. In murine-OHSS model we found that the delicate counterbalance between VEGF and PEDF was violated. We have shown that high doses of PMSG significantly reduced PEDF expression, compared to standard superovulation dose, and that VEGF levels increased dramatically following hCG stimulation. Administration of recombinant PEDF to OHSS-induced mice (16 mice per treatment) significantly reduced the measured OHSS symptoms, including weight gain (p < 0.05) and vascular hyperpermeability (p < 0.05). We have further shown that administration of PEDF to OHSS-induced mice reduced the level of VEGF mRNA and the overdeveloped ovarian angiogenesis.

**Conclusions:** These observations provide, for the first time, a comprehensive explanation for the pathophysiology of OHSS; namely, an imbalance between the high expression levels of the pro-angiogenic factor, VEGF, and the nearly undetectable levels of the anti-angiogenic factor, PEDF. A replacement therapy with recombinant PEDF is suggested as an innovative physiological treatment for OHSS. Furthermore, control of the PEDF-VEGF inverse relationship opens new therapeutic avenues for other angiogenic-related fertility pathologies.

**Abbreviations:** ovarian hyperstimulation syndrome (OHSS), vascular-endothelial-growth-factor (VEGF), pigment epithelium-derived-factor (PEDF).

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**O-094 Spermatozoa as carrier of large unilamellar vesicles into the oocyte**

G. Huszar1, N. Geerts2, K. Vanderlick1, J. McGrath3

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**Introduction:** In recent years, the interest in micron and sub-micron systems (i.e. nano-systems) in pharmacology has surged, specifically in the area of drug distribution and targeted delivery. Large unilamellar vesicles (LUV’s) are a class of most commonly studied among nano-sized carriers. While most research focuses on injectable LUVs, in a new approach we are focusing upon a new delivery paradigm by attempting to potentially transport spermatozoa-associated LUV’s into the oocyte.

**Material and Methods:** LUVs (~200 nm) are prepared by the self-assembly of amphiphilic lipids in solution with the use of an extrusion method. A lipid mixture (1 mM) in chloroform was placed into a glass vial and evaporated under vacuum (> 4 h) to remove all the solvent and leave a lipid film at the bottom of the vial. Two ml of media (composed of 125 mM NaCl and 10mM Hepes buffer, pH 7.2) was added, and the samples were vortexed, frozen, and then thawed five times before extruding through No. 2 Whatman Nucleopores (pore size 200 nm). LUVs of five different compositions were prepared: all neutral lipids and neutral lipids with positive, negative, cerebroside or ganglioside lipids (in a variable 4:1 to 1:1 ratio). For visualization of the LUVs, a low proportion of fluorescent lipid was also incorporated.

Human semen from left-over semen analysis samples was obtained within 90 minutes of ejaculation. Sperm concentration and motility were determined with computer assisted semen analysis (Hamilton-Thorn, Beverly, MA), and were within normal limits. The sperm were mixed with high levels of LUVs at room temperature. Sperm-LUV binding was confirmed with confocal microscopy.

**Results:** Sperm-LUV binding was variable depending on the LUV composition. No binding was exhibited by the neutral lipid LUV’s, all other LUVs tested bound to spermatozoa and sperm binding was increased in proportion of the non-neutral functional groups (either positive, negative, cerebroside or ganglioside). Maximum binding occurred when 50% of the LUV lipids were of the non-neutral type. With the use of positive LUVs there were large aggregates of LUV’s bound that enveloped the spermatozoa, thus we did not use the positive LUV’s. Of the remaining LUV types tested, the cerebroside LUVs bound with the highest affinity (within 10 min), and these also exhibited the brightest fluorescence. With respect to the pattern of binding, initially there was a random binding to all sperm regions, however after 30 minutes specific binding to the head and the midpiece (neck of sperm) regions were detectable.

Regarding sperm function, the spermatozoa loaded with LUVs, showed only minimal alterations in swimming characteristics (% motility, velocity, cross-beat frequency, and linearity), indicating that LUVs do not affect sperm kinematics. Ganglioside and cerebroside LUV loading did not affect sperm capacitation or acrosomal status, as followed by the fluorescence lectin-binding method. With the time-related “sequential swim-up” of sperm across an LUV-liquid phase, it was shown that especially cerebroside LUV loading goes quickly and efficiently. The highest binding was obtained at pH 7.2. Dissociating the sperm-LUV complex and reloading may be accomplished by altering the medium pH by ± 1.0 unit. After reversing the pH to 7.2, the LUV’s rapidly rebound. In recent experiments, we have also explored LUV binding to rodent sperm with an experience similar to that of human sperm.

**Conclusions:** Although LUVs are extensively studied as drug-carryers, until now no transport strategy with sperm-associated LUV was utilized. We are now exploring the utility of this new novel approach.

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**O-095 Development of human embryos in a dynamic microfluidic culture system: results from a prospective randomized study**

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**Introduction:** Embryo culture media have evolved greatly over time; yet the static physical relationship between embryo and culture media has remained constant. In the female reproductive tract preimplantation embryos are exposed to dynamic chemical and mechanical environments. A dynamic culture system (DCS) based on microfluidic technologies has been developed that improves mouse and bovine embryo development and mouse pregnancy rates. The aim of this study was to compare embryo development in a static culture system (SCS) and DCS using sibling human zygotes.

**Material and Methods:** This study was approved by the Institutional Review Board of Universidade Federal de São Paulo, Brazil and performed at Huntington Medicina Reprodutiva, São Paulo, Brazil. Women between 21 and 35 years old, with less than 2 previous failed in vitro fertilization (IVF) cycles, and a minimum of eight zygotes were invited to participate in the study. Normally fertilized zygotes were randomly divided and placed into the SCS or DCS. Culture media and incubator conditions were identical between groups; namely, embryos were kept in G-1 Plus (Vitrolife) at 37°C and the CO2 concentration was tailored to attain a pH of ~7.35. Outcome measures on Day 3 were: i) cell number; ii) fragmentation (fragment) assigned as grades (1 = 0% frag; 2 > 10% frag; 3 > 10% ≤ 30% frag; 4 ≥ 30% ≤ 50% frag; 5 > 50% frag); iii) and collective embryo quality (top quality embryo; TQE = 8/9 cell grade 1); (good quality embryo; GQE = 6-9 cell grade 2 or 1); (poor quality embryo; PQE ≤ 4 cell and/or ≥ grade 3). Cell number and grade were assessed statistically with Mann-Whitney Test and proportions of collective embryo quality by z-test. Differences were considered significant at p < 0.05.

**Results:** Twenty-five women (33 ± 2 years old) were treated with IVF due to ovarian factor (28%), tubal factor (24%), male factor (32%), endometriosis (8%) and multiple factors of infertility (8%). Patients had normal fertilization rates [85.2 ± 2.4% (mean ± se); Day 1] that yielded 315 zygotes for randomization. Outcome measures on Day 3 were: i) cell number (137 ± 21,000); ii) fragmentation (frag); 3 >10% frag); iii) and collective embryo quality (TQE = 8/9 cell grade 1); (good quality embryo; GQE = 6-9 cell grade 2 or 1); (poor quality embryo; PQE ≤ 4 cell and/or ≥ grade 3). Cell number and grade were assessed statistically with Mann-Whitney Test and proportions of collective embryo quality by z-test. Differences were considered significant at p < 0.05.

**Conclusions:** Twenty-five women (33 ± 2 years old) were treated with IVF due to ovarian factor (28%), tubal factor (24%), male factor (32%), endometriosis (8%) and multiple factors of infertility (8%). Patients had normal fertilization rates [85.2 ± 2.4% (mean ± se); Day 1] that yielded 315 zygotes for randomization.

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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 postpuberual 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-asay. 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 L19 and the follicle count 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 surge (result 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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Introduction: Selecting embryos for transfer with an optimized 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 (Unisense Fertitech, 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 novel 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 24-™x5 PLUS medium (Vitrolife, Sweden) in EmbryoSlides™. These slides have a well-on-platform 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 and temperature when opened, whereas our regular CO2 incubator contains a large volume with slow recovery of CO2 levels. Furthermore, the regular incubator was opened several times a day to allow inspection of morphology, whereas this is not needed for the EmbryoScope™. The 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.