After the establishment of a Centre of Excellence the number of patients suffering from severe (complex) endometriosis/month will increase dramatically. Therefore, you have to face several structural problems but also some problems of man power (human resources) and other resources (MRI, transrectal sonography, decrease of surgical support). In addition, the OR will be blocked longer, which might be not a primary interest of your hospital. Many Hospital or Departments – at least in Germany - are missing a clinical and/or basic research infrastructure, which is difficult to establish.

Conclusions: On the background of intense European discussions, the recent implementation of Endometriosis Centres of Excellence (ECoE) in Germany might be a useful attempt to improve the management of endometriosis. Given limited budgets, focusing on the existing national commitment may be the first step. Later on, this should lead to the unification of national endometriosis groups under European patronage.

INVITED SESSION

Session 35: Stem cells

Tuesday 30 June 2009 11:45–13:00

O-135 Human pluripotent stem cells: the state of the art

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Two decades of advances in human reproductive biology and in vitro fertilization enabled the first derivation of human embryonic stem cells in 1998. Since that time there has been significant progress in understanding the molecular mechanisms that underlying maintenance of the pluripotent state, and in developing cell culture systems to direct embryonic stem cells to differentiate into a wide variety of cell types of scientific and medical interest. It is likely that the first clinical trials of embryonic stem cell-derived products will begin soon. The recent stunning discovery of reprogramming of adult cells to the pluripotent state promises a solution to the problem of tissue rejection in stem cell therapy, and to provide powerful laboratory models for the study of human disease. Despite this, there is still much to learn about the cellular and molecular biology of pluripotent stem cells, and many hurdles to cross before they realize their potential in medicine. We will summarize the current status of pluripotent stem cell biology and highlight some fascinating questions and sharp challenges that still face the field.

O-136 Oral Spermtogonial stem cells

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Spermtogonial stem cells (SSC) are required for sperm production throughout adult life of each male individual. Studies in animal model systems have shown that it is possible to propagate SSCs in vitro and transplant them to recipient testes where they can re-establish complete spermatogenesis. SSC cryopreservation and autotransplantation is considered to be an elegant tool to preserve fertility for prepubertal boys diagnosed with cancer. As it is a prerequisite to have large numbers of SSCs available for successful autotransplantation, we have studied the possibility to culture and propagate human SSCs in vitro. We cultured testicular cells from normal adult testes, donated for research by prostate cancer patients. We found that human SSCs can efficiently proliferate in our culture system without losing their ability to migrate to the basal mem-

brane of seminiferous tubules after transplantation into recipient mouse testis. This is an important step forward to the clinical application of human SSC transplantation for preserving fertility of young boys diagnosed with cancer.

Besides being able to proliferate and self renew in vitro and produce mature sperm after transplantation, it has recently been discovered that mouse SSCs can also give rise to multipotent stem cells in vitro. We and others have now shown that human SSCs can form multipotent stem cells as well. These multipotent cells show molecular characteristics of human embryonic stem (hES) cells and have the ability to differentiate in vitro into cells of all three germ layers, including neuronal, osteogenic and pancreatic cells, the latter being able to secrete insulin and react on glucose shock. Their easy accessibility, ethical acceptability, and autogenic nature, make these cells an attractive alternative to hES cells or induced pluripotent stem cells (iPS) for future stem cell therapies. The trigger for SSCs to become multipotent is currently unknown and requires further research.

In conclusion, we have established a human SSC culture system in which SSCs can proliferate without losing their stem cell characteristics as well as form multipotent stem cells. These results show the great potential of human SSCs for future applications in reproductive and regenerative medicine.

O-137 Oral Morphological observations and kinetics of human embryonic stem cell derivation and culture

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Introduction: Our understanding of human embryonic stem cell (hESC) derivation, morphogenesis and development is incomplete, as it has been previously based almost solely on static observations of live or fixed material. The critical number of pluripotent inner cell mass (ICM) cells needed for successful ESC derivation and the mechanisms controlling the ICM to ESC transformation are unclear. However, differences between ICM and ESC cells include morphologic appearance, organization, proliferative capacity and gene expression. The dynamics of hESC development require continuous observations under optimal conditions. Therefore, we utilized digital imaging and time-lapse microscopy to observe hESC derivation and growth under non-invasive conditions.

Material and Methods: We used a continuous digital imaging system to capture daily images for hESC derivation and a computerized digital time-lapse microscopy system for hESC culture observations. hESC cells were cultured under mouse embryonic fibroblast feeder (MEF) and/or feeder-free (Matrigel) conditions in serum-free culture medium.

Results: ESC derivation: There were clear morphological differences between pluripotent inner cells (embryoblast) and the surrounding primitive endoderm cells of the ICM. Notably, there were differences in nuclear:cytoplasmic (Nu/Cytopl) ratios between ICM cells and ESC cells. In particular, ESC cells were characterized by a high Nu/Cytopl ratio, rapid proliferation (cell cycle < 24 hr), and tight compacted colony growth with shiny membrane appearance under phase contrast. During the derivation process, the earliest onset of cells with ESC morphology was observed after five to six days in culture. In addition, attachment to the surface/feeder matrix was essential for the ESC derivation with cells showing polarized morphology similar to polarized epithelium. Successful ESC derivation from intact trophoblasts required extraction and replating of the ICM outgrowth from the surrounding trophoblast cells and exposing ICM cells to feeders or matrix. Sometimes, ICM outgrowth often resembled tri-dimensional “epiblast-like” morphologic structure and therefore multiple passaging was necessary for successful ESC derivation. ESC culture growth: hESC demonstrated a similar growth pace under both feeder or feeder-free culture conditions. After initial attachment, cells divided rapidly, forming round-shaped colonies in both systems. In feeder cultures, the MEF were remarkably active, showing dynamic movements and migrations within the dish. After initial attachment on MEF, hESC attached and grew on the bottom of the dish by actively displacing MEF; resulting in hESC colonies surrounded by MEF. Similarly, hESC colony formation occurred in the feeder-free system although these cells appeared to exhibit a flatter cell morphology. The process included initial attachment followed by rapid cell division and round-shaped colony formation. In the feeder-free system, cells on the edge of the colony actively migrated by amoeboid-like movement out of the colony but required cell contact to survive. We also observed cell-cell communications within the colony along with dynamic apoptotic changes characterized by cell fragmentation and expulsion of dead cells to the surface.

Conclusions: Derivation and maintenance of hESC depends on the provision of an in vitro niche where conditions uncouple proliferating ICM cells from further differentiation and transforms them into self-renewing cells. There is a transition period when ICM pluripotent cells transform into stem cells (5–6 days). This transition is accompanied by morphologic as well as kinetic
cell changes. Time-lapse technology provides a valuable tool in understanding the active processes of hESC derivation, growth and differentiation under both feeder and feeder-free conditions.

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**SELECTED ORAL COMMUNICATION SESSION**

**Session 36: Male fertility 2**

**Tuesday 30 June 2009**

11:45–12:45

**O-138 Oral**

**Empirical oral antibiotic and anti-inflammatory treatment of recurrent subclinical prostate and seminal vesicles with high sperm DNA fragmentation**

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**Introduction:** Elevated sperm DNA fragmentation has been implicated with an increased interval to conception and poor assisted reproduction outcomes. Abortive apoptosis, failure of sperm DNA protamination and oxidative stress are causes of sperm DNA fragmentation. Oxidative stress can be caused by the intrinsic sperm production of reactive oxygen species (ROS) or by the oxidative burst of leukocytes involved in inflammatory processes. The empirical administration of oral anti-oxidant has become a common place in clinical practice; however, anti-oxidant therapies are yet to be proved beneficial for the reduction of sperm DNA fragmentation. Recent studies evaluating men with seminal leukocyte counts under 1.106 leukocytes/mL by PCR analysis demonstrated amplification of DNA from several pathogenic bacteria. Thus, subclinical infections of the prostate and seminal vesicles (annexes glands) are probably involved in the extrinsic pathway of ROS production. Reduction of annexes glands’ inflammatory processes and bacterial population might decrease sperm DNA fragmentation. Therefore, we aimed to prospectively evaluate the empiric effect of ciprofloxacin associated to etoricoxib in infertile men whom exhibited recurrent elevated sperm fragmentation rates without evidence of clinical infectious process.

**Materials and methods:** All consecutive infertile men undergoing infertility investigation at Huntington Medicina Reprodutiva, São Paulo, Brazil from February 2007 to December 2008 were invited to participate in this prospective observational study. Sperm DNA fragmentation was assessed by Sperm Chromatin Dispersion (SCD) Test and all men had initial sperm DNA fragmentation higher than 25% (normal range < 18%) and negative bacterial culture of semen. All treatment consisted in twice daily doses of ciprofloxacin (500 mg) and etoricoxib (120 mg) per oz for 15 days. Sperm DNA fragmentation test (SDFT) and sperm analysis (SA) were performed before and after treatment. Data are represented as mean ± standard error of the mean and compared with paired t-test. Additionally, Pearson’s correlation coefficient was calculated between sperm DNA fragmentation, sperm concentration, percent of motile sperm and normal morphology before and after treatment were compared with paired t-test. Furthermore, Pearson’s correlation coefficient was calculated between sperm DNA fragmentation, sperm concentration, percent of motile sperm and normal morphology. Significance was attained at p < 0.05.

**Results:** Thirty-three men (37 ± 5 years old) accepted to participate in the study and signed informed consent. Treatment resulted in a statistically significant reduction in the SDFT from 30 ± 11% to 20 ± 7%. Pre-and post-treatment sperm concentration (25.0± 7.106 and 17.0 ± 3.106 spermatozoid/mL); sperm motility (50.0± 22.0% and 55.0 ± 14.0%) and Kruger strict morphology (3.5 ± 0.8% and 2.8 ± 0.5%) were not significantly different. Sperm DNA fragmentation values did not correlate to sperm concentration, motility and strict morphology parameters.

**Conclusions:** The empirical use of an association between an oral antibiotic and anti-inflammatory agent significantly reduced sperm DNA fragmentation in a group of infertile men with subclinical recurrent infection and elevated fragmentation tests. This data also implies that a course of antibiotic and anti-inflammatory therapy of infertile men exhibiting an unrecognized cause could benefit for such empirical approach.

**O-139 Oral**

**Keep the river flowing. An exploratory study to assess the effect of daily ejaculation for 7 days on semen parameters and sperm DNA damage**

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**Introduction:** The optimal ejaculatory frequency for human fertility is yet to be determined. This study assessed semen quality after daily ejaculation for 7 days as compared to a standard day 3 abstention period. We hypothesised that frequent ejaculation (FE) may be a physiological mechanism to improve sperm DNA damage while enabling standard, WHO-defined, semen parameters to stay within the normal, and presumed fertile, range.

**Materials and methods:** 118 men with a history of infertility, recurrent miscarriage or repeated IVF failure were enrolled based on evidence of elevated sperm DNA damage as determined by the sperm chromatin structural assay (SCSA). This assesses sperm DNA integrity based on the percentage of sperm with a high susceptibility to low pH-induced DNA denaturation and is expressed as the DNA Fragmentation Index (DFI%). The entry criterion was a DFI > 15%. After 3 days of abstinence: semen parameters were assessed by strict WHO criteria.

Men were then instructed to ejaculate daily for 7 days with re-assessment on day 7. No other treatments or lifestyle change interventions were offered. All 118 men completed the 7 days of ejaculation.

**Results:** 96 men (81.4%) exhibited a decrease in DFI (mean decrease 12.1%), whereas 22 men (18.6%) had an increase in DFI (mean increase 9.6%). Frequent ejaculation significantly decreased semen volume and sperm density, without compromising sperm motility, which rose slightly but significantly. While there was no change in morphology on very strict WHO criteria, the changes in DFI were substantial in degree and statistically highly significant.

**Abstracts of the 25th Annual Meeting of ESHRE, Amsterdam, the Netherlands, 28 June – 1 July, 2009**