according to our original protocol which seems promising and appropriate for routine clinical application. We now plan to evaluate the functional quality of frozen/thawed ovarian tissue (by culture in vitro and/or xenotransplantation) before finally considering application of this procedure for therapeutic cryopreservation.

O-037 The perspective of fertility preservation helps patients to cope with the burden of cancer treatments

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Introduction: All indicate that the potential of fertility represents an important issue on the human psychological welfare. Since this potential is often altered by gonadotoxic cancer treatments, thereby engendering additional anxiety over the cancer prognosis itself, we hypothesized that the perspective of fertility preservation, which offers the opportunity to patients to become a genetic mother after healing, could help women to cope with their future treatments. Previous studies performed in young male survivors suggest that patients feel less distressed and more able to cope with their cancer treatment just by knowing that they have gametes in storage (Schöver et al., 2002). The lack of data available in women encouraged us to analyze the possible psychological impact of fertility preservation on how patients cope with cancer treatment.

Material and Methods: To address this issue, a standardized questionnaire was filled out by 44 women aged 20 to 40 years suffering from malignant diseases and who postulated to fertility preservation at our Center. The questionnaire assessed demographic data, the type of cancer, and how women experienced both the information on the possible negative impact of cancer treatments on their fertility and the different approaches of fertility preservation proposed.

Results: Median age of patients at the diagnosis of cancer was 29 (20-40) years. 39 of 44 patients (89.0%) were childless and 17 of them (39.0%) had already started chemotherapy or/and radiotherapy. Overall, 23 (52.0%) mentioned that their oncologist did not inform them on the presumable detrimental effects of cancer treatments on fertility. Among the 42 patients (95.0%) having accepted to undergo fertility preservation at our Center, 38 (86.4%) reported that such a possibility could be instrumental to improve their coping with the burden of cancer treatment. This proportion did not change significantly (88.0% vs. 85.0%, respectively) between women having started or not chemotherapy or/and radiotherapy before the inquiry.

Conclusions: The present results indicate that the simple fact of undergoing fertility preservation improves the patient subjective experience of cancer treatments. This pilot data spurs us to comparing psychological and medical outcome of cancer treatments in patients having or not decided to undergo fertility preservation.

O-038 Vitrification of testicular tissue: to mince.

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Introduction: With childhood cancer being treated more successfully, the focus of treatment needs to shift towards the quality of life after treatment. One aspect commonly overlooked is the prevention of sterility in childhood cancer survivors. In adults and adolescents, semen banking or cryopreservation of testicular tissue before radiation/chemotherapy treatments are valuable preventive measures. However, no such prevention is possible before puberty since no active spermatogenesis is present. Up to 30% of children cured of cancer remain sterile for life. Standard cryopreservation methods ensure sperm survival and although often used to cryopreserve testicular tissue, inevitably neglect immature germ cell survival. The purpose of this study is to determine the optimal technique to cryopreserve spermatogonia stem cell (SSC’s) and other premeiotic stages by using a mouse model.

Materials and Methods: Testes were obtained from adult (12-18wks) B,D,F mice. The study design indicates the allocation of the testes to whether they were minced or kept as individual tubules (Figure).

Conclusions: The results indicate that our proposed vitrification method can cryopreserve spermatogonia stem cell (SSC) for autologous transplantation or in vitro maturation purposes.

Figure: Specimen allocation

Testicular tissue was minced following the same procedure the urologist uses to process human specimen or tubules were kept intact in a 3-5 mm segments. Vitrification was a 3-step procedure either utilizing H199 (HEPES based) or standard M199 with 20% SSS (1: 10% ethylene glycol (EG); 2: 15% EG; and 3: 30% EG, 5% PVP and 0.5 mol/L sucrose). Warming was performed by exposing specimens to decreasing concentrations of sucrose. Both minced and tubular sections were digested with a sequential collagenase/trypsin solution. Cell suspensions were counted and viability assessed. Thereafter, cells were maintained in basic spermatogonia stem cell culture medium consisting of stem cell medium supplemented with 0.2% BSA, 5 μg/ml insulin, 10 μg/ml iron-saturated transferrin, 7.6 μeq/L free fatty acids, 3 x 10^-4 M H_2SeO_3, 50 μM 2-mercaptoethanol, 10 mM HEPES, 60 μM Putrescine, 2 mM glutamine, and antibiotics. Growth factors used were LIF, GDNF, and BMP4.

Results: In our TESE samples, the proportions of germ cells/round cells that survived cryopreservation are negligible at < 0.1%. In previous mouse experiments, we have cryopreserved testicular cells with standard ESC cryopreservation with DMSO as a cryoprotectant and was capable of yielding after thawing an average of 68.6% viability. In culture, germ cells began to grow and continued to proliferate for an average of 30 days. Following vitrification, the survival rate was granted to be about 80%. Moreover, we did not see any difference between the HEPES-buffered medium compared to the standard. Minced tissue had a survival rate of 75.1% (H199) and 84.7% (M199) but the growth after thawing was minimal. A limited number of cells were seen growing in culture. The best survival rate came from intact tubules of 85.6% (H199) and 87.3% (M199) along with significantly more cells proliferating.

Conclusions: Current methods of cryopreservation of testicular tissues are not aimed at sparing immature germ cells. Tweakng vitrification methods would allow to devise a way to preserve spermatogonia stem cell integrity for autologous transplantation or in vitro maturation purposes.
nutrition from the endometrial glands during this period. Furthermore, a servomechanism has been identified in the sheep and other species by which signals emanating from the conceptus upregulate the expression of uterine milk proteins in the glands during early pregnancy. This study sought evidence of whether equivalent nutritional pathways are active during human early development.

**Materials and Methods:** An archival collection of human placenta-in-situ histological material, the Boyd Collection, was examined to investigate the relationship of the endometrial glands to the developing placenta during the first trimester. Immunohistochemistry (IHC) was performed on unoverslipped slides from the collection using primary antibodies against MUC-1 and glycodelin to follow the passage of these secreted maternal glandular proteins.

Secretory phase endometrium and first trimester decidua were collected with written consent and local ethical approval. Sections were stained with a panel of antibodies against growth factors, and lectins to assess changes in glycossylation with pregnancy.

An immortalized glandular epithelial cell line, EM-E6/E7-hTERT-3, was exposed to increasing doses of prolactin, and synthesis of MUC-1 was assessed by immunofluorescence and western blotting.

**Results:** The endometrial glands in the decidua basalis of the earliest specimen available, a 28 somite embryo of approximately 28 days post-conception, appeared highly active. They could be seen discharging their secretions into the intervillus space of the developing placenta through the maternal-fetal interface. The secretions were rich in carbohydrates and lipid droplets, and IHC confirmed their uptake by the syncytiotrophoblast where they entered the lysosomal digestive pathway. Communications between the glands and the intervillus space could be traced throughout the first trimester, although the thickness of the endometrium, and the activity of the glands, declined over this period.

IHC revealed that the glandular epithelial cells express a wide range of growth factors that may play important roles in modulating placental cell proliferation and differentiation. The pattern of sialylation of the secretions changed between the secretory phase and early pregnancy in that the terminal sialic acid cap was lost during pregnancy. This change will facilitate uptake by the trophoblast, but will also ensure that any growth factors that enter the maternal circulation via the placental venous openings and the uterine veins will be rapidly cleared in the liver.

Prolactin was found to stimulate MUC-1 in the immobilised glandular cell line in a dose dependent fashion.

**Conclusions:** These findings confirm that the endometrial glands remain highly active during human early pregnancy, and that their secretions enter the placenta through the developing basal plate. The secretions are phagocytosed by the trophoblast, and may provide a source of amino acids and other nutrients in a manner analogous to that of the rodent yolk sac during the period of organogenesis. The secretions are also a rich source of growth factors that potentially stimulate development of the placenta. The change in sialylation of the secretions provides the first evidence that activity of the glands is regulated by pregnancy, and may enable a proliferative microenvironment to be created within the placenta without placing the mother at risk of undue stimulation. Pilot data suggest that lactogenic hormones, prolactin from the decidual cells, or human placental lactogen from the trophoblast, may stimulate activity of the glandular epithelium. This raises the possibility of the human conceptus upregulating the supply of nutrients via a servomechanism similar to that in domestic species.

**O-040 Enhanced imaging of embryonic growth and development**

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Although three-dimensional ultrasound (3D) has substantial advantages compared to two-dimensional ultrasound (2D), the 3D images are currently still viewed and assessed using 2D media like ordinary computer screens. These 2D media do not offer depth perception, which is required for optimal interpretation of 3D and four-dimensional ultrasound images. The Barco I-Space virtual reality (VR) system at the Erasmus MC offers depth perception and three dimensional interaction, resulting in a truly 3D experience. This allows for more precise visualization, for example in some cases of congenital anomalies. A special volume rendering application, called V-Scope, is used to create the so called “hologram”. The length and volume measuring tools implemented in V-Scope proved to be accurate and reliable. Standard biometric measurements like the crown-rump length, biparietal diameter, occipito-frontal diameter and abdominal diameter can be performed by placing two calipers. This is also true for non-standard biometric measurements like the width of the hip, knee, elbow, shoulder and the length of the ear and foot. The length of the arm, umbilical cord and vitelline duct can be measured by placing multiple calipers. Volume measurements using VR can be conducted of the embryonic body, embryonic cerebral cavities, yolk sac and amniotic cavity. Associations were demonstrated between maternal age, the use of folic acid and smoking with embryonic growth using embryonic volume measurements. VR also enables scoring of embryonic development according to the external criteria of the Carnegie stages. In conclusion, VR enables a new way of studying embryonic and early fetal growth and development in vivo. This may contribute to a shift of prenatal diagnosis of congenital abnormalities and suboptimal intrauterine growth from the second to the first trimester of pregnancy, enabling embryonic medicine in the future.

INVITED SESSION

SESSION 09: DATA FROM THE PGD CONSORTIUM

Monday 4 July 2011 11:45 - 12:45

**O-041 Data from the ESHRE PGD Consortium**

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**ESHRE, on behalf of the ESHRE PGD Consortium, Grimbergen, Belgium**

**Introduction:** The ESHRE PGD Consortium was set up in 1997 and since then has been actively collecting data on PGD and PGS. The PGD Consortium currently has five Working Groups to look at important aspects of PGD: data collection and database, accreditation, misdiagnosis monitoring and audit, and sharing of molecular methods and the newly formed working group on array-based PGD. In 2010 the guidelines group (now dissolved) authored and published several PGD Guidelines Documents (Organization of a PGD Center, Amplification-based PGD, FISH-based PGD and Aspects of embryology and biopsy for PGD). In addition this year sees the introduction of a new Working Group on Array-Based PGD and PGS

**Methods:** There have been eleven collections of data on PGD/PGS cycles, mostly using a filemaker Pro database. Currently there are 115 registered centers worldwide, including from Europe, Argentina, Australia, Brazil, Egypt, India, Israel, Japan, Korea, Russia, Singapore, South Africa, Thailand, Taiwan, United Arab Emirates, Pakistan and the USA.

**Results:** The Consortium has analyzed eleven sets of data on 3372 cycles. The indications analyzed are inherited chromosomal abnormalities (5299 cycles), monogenic disorders (5961 cycles), sexing for X-linked diseases (1400 cycles) or for social reasons (676 cycles), and aneuploidy screening for infertility (PGS) (2027 cycles). Detailed analysis of 7521 clinical pregnancies and 4855 babies born has also been conducted. Over the eleven years there has been a change for embryonic biopsy for PGD). In addition this year sees the introduction of a new Working Group on Array-Based PGD and PGS

**Conclusion:** There have been eleven collections of data on PGD/PGS cycles, mostly using a filemaker Pro database. Currently there are 115 registered centers worldwide, including from Europe, Argentina, Australia, Brazil, Egypt, India, Israel, Japan, Korea, Russia, Singapore, South Africa, Thailand, Taiwan, United Arab Emirates, Pakistan and the USA.

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