**Results:** Histological sections of vitrified-thawed cortical strips before culture showed that 85% of follicles were at the primordial or transitory stage, with the remainder being at the primary stage of development. The proportion of atretic follicles observed ranged from 3–12% between individuals. After 6 days in culture, follicles had been initiated to grow within strips (55% primordial and transitory present) and secondary follicles with 2–4 layers of granulosa cells (15%) could be observed in histological sections. The proportion of atretic follicles in strips cultured for 6 days ranged from 5–15% and this was comparable with previously observed freshly cultured tissue.

48 intact secondary follicles were isolated from the cultured vitrified-thawed tissue for further development *in vitro*. A significantly higher proportion of isolated follicles showed an increase in diameter during the first two days of culture in the presence of activin-A (65%) compared to control (40%) ($P > 0.05$). Activin-A also significantly improved the viability of isolated follicles after 4 days *in vitro* (45% compared with 10% in control ($P > 0.005$)) and the incidence of early antral formation.

**Conclusions:** The results reported here demonstrate that 1) the developmental potential of follicles within vitrified ovarian tissue is maintained post thawing 2) it is possible to achieve accelerated oocyte/follicle development from human primordial/primary follicles derived from vitrified ovarian tissue 3) activin promotes the development/survival of isolated preantral follicles. This culture system provides a powerful technique for assessing the potential of frozen tissue and represents significant progress towards achieving full *in vitro* development of human oocytes.

## O-240 Oral Altered gene expression profile in cumulus oophorus cells of mature oocytes from ART patients with polycystic ovary syndrome

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**Introduction:** Patients suffering from Polycystic Ovary Syndrome (PCOS) present hormonal dysfunctions with arrested follicle development and anovulation. These patients benefit from assisted reproduction techniques (ART) with the support of multiple follicle maturation under controlled ovarian stimulation. Although sufficient oocytes numbers are usually retrieved for *in vitro* fertilization (IVF), we do not know if their maturation process is optimal for the ensuing embryo development and implantation. The aim of this study is to analyze the gene expression profile of cumulus cells associated to mature metaphase II oocytes in PCOS patients.

**Materials and methods:** Samples were collected with the informed consent of patients undergoing controlled ovarian stimulation. The control ($n = 4$) and PCOS ($n = 6$) patients were respectively $32.3 \pm 1.4$ and $32.7 \pm 4.7$ years old and their respective FSH/LH ratio at J3 was $2.1 \pm 0.6$ and $0.7 \pm 0.2$. On the pick up day, the control and experimental cumulus cells were dissected from MII oocytes selected for ICSI, and frozen individually prior to total RNA extraction. Their messenger RNA (mRNA) content was analyzed on Affymetrix™ HG-U133 plus 2.0 GeneChip oligonucleotide microarrays, which combine 54675 probe sets representing roughly 30 000 genes. The Affymetrix™ GeneChip Operating Software 1.2 (GCOS) was used to evaluate signal intensities. The Significance Analysis of Microarrays (SAM) software measured the differential expression between sample groups. A gene was considered decreased or increased when it presented an absolute fold change $\geq 2$ with a False Discovery Rate (FDR) corrected $P$-value $\leq 0.05$.

**Results:** Unsupervised principal component analysis (PCA) of 10217 probes (selected after removing undetected probes and probes showing unvarying expression across all samples) segregates control and experimental samples into distinct groups. The GCOS analysis revealed higher gene expression in the control versus PCO groups with an average detection of expressed genes at $44.1 \pm 2.9\%$ and $30.7 \pm 5.8\%$ ($P < 0.002$) respectively. Differential expression analysis was performed on a selection of 2645 genes filtered for signal intensity. In accordance with the detection results, we mostly identified genes with decreased expression in PCO samples. Only 16 genes were found with a higher expression in PCO samples with fold changes (FC) ranging from x3 to x14. The top gene group, ribosomal proteins *RPS11* and *RPL23* (FC x11) and translation factor *EIF5A* (x3.8), is involved in translation control. Others, such as *ZNF548* (x14) are mostly involved in transcriptional control. In contrast, 177 genes were found with decreased expression (FC from x-2 to x-14.8) in PCOS samples. The top genes are another zinc finger transcription factor, *ZNF718* (x-14.8) and an inhibitor of the IL1R pathways, *IL1RN* (x-10.8). Others, are the glucose transporter *SLC2A6* (x-6.4) and many genes participating in the extracellular matrix (*ADLICAN/MXRA5* x-10.8, *TIMP3* x-3.9, *COL6A2* and *CTHRC1* x-4.0, *COL1A1* and *-6A1* x-2.5) and the WNT signaling (*SFRP1* and *SFRP4* x-3).

**Conclusions:** Abnormal folliculogenesis in PCOS patients could alter the cumulus cells expression of genes important for oocyte maturation, even under therapeutic COS. Many genes decreased in PCOS are part of the ECM or short range signaling (WNT pathway) or involved in the proliferative control of cumulus cells. We will show next whether this dysregulation affects primarily the cumulus compartment or if it also has an impact on oocyte maturation and developmental potential during the course of the IVF procedure.

Supported by Ferring Pharmaceuticals and Organon France.

## O-241 Oral Feasibility of xenogeneic porcine small intestinal submucosa for ovarian defect repair, maintaning ovarian reserve and renewal of primordial follicles

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**Introduction:** Porcine small intestinal submucosa (SIS) is an exciting material, which has been shown to provide temporary support for regenerative process of native tissues. The purpose of this study is to investigate the feasibility of porcine SIS as a scaffold for ovarian defect repair and possibility of follicle renewal in an in situ xenograft model.

**Materials and methods:** Fourteen female rabbits were put into two subgroups: Group 1 rabbits whose unilateral ovarian defect was repaired with SIS graft, and Group 2 rabbits whose unilateral ovarian defect was repaired with 7–0 vicryl as the controls. Contralateral ovary of each animal was assigned as a local control to its pair. The animals were killed at 4, 8, 12, 16, 20, 24 and 28 weeks following the repair. During laparotomy, the extent and severity of adhesions in the operation site and contralateral nongrafted site were evaluated using Mazuji's scoring system. Afterwards, bilateral ovaries were removed and their volumes were measured using ellipsoid formulae. The graft site, along with the adjacent ovarian tissue was disected and processed for microscopic examination. The sections were stained with hematoxylin and eosin, Masson's trichrome and also proliferating cell nuclear antigen (PCNA) and inhibin-α subunit immunohistochemistry. Microscopic images of these sections were further processed for de-noising and identification of tissue using image processing techniques.

**Results:** The ovarian volumes where SIS graft was applied were found of similar size compared to the volumes of contralateral ones ($140.25 \pm 8.78$ mm$^3$ versus $151.21 \pm 9.96$ mm$^3$, $P > 0.05$). In contrast, in the control group, the volume of the operated ovary was small compared to the volume of contralateral one ($109.14 \pm 8.15$ mm$^3$ versus $238.30 \pm 8.97$ mm$^3$, $P < 0.05$). The total volumes of the SIS grafted ovary was found as larger than the volume of damaged ovaries of control group ($P < 0.05$), and the adhesion was lower in SIS grafted rabbits ($1.14 \pm 0.14$ vs $3.85 \pm 0.26$, $P < 0.001$). Until 4 weeks, the response of host tissue to the SIS graft involved hemorrhage and polymorhonuclear leukocytes infiltration, but this is rapidly diminished to a negligible level by the next time point of evaluation (8 weeks). From 12 to 16 weeks the SIS graft and ovarian tissue were well organized and the primordial follicles were accumulated about the boundary of the SIS-ovary. Most of the primordial follicles appeared to be dragging out from the ovarian site toward adjacent SIS graft. However, there also were some isolated primordial follicles showing no apparent connection to the adjacent normal ovarian tissue. These primordial follicles were stained by PCNA and interestingly both primordial follicles and some cells that could be of stromal cells in the SIS graft were stained by inhibin-α. Being staining with inhibin-α

and PCNA raises the possibility of these cells being of pre-granulosa cells which are rendering signs of follicle formation. At 24–28 weeks the primordial follicles collaborated in the majority of the SIS graft and organized alike ovarian structure so that the SIS material could not be identified under light microscopy. Epithelization in SIS graft was partial till 16 weeks, around 75% at 20 weeks and completed by 28 weeks. Interestingly at 28 weeks, control animals showed incomplete epithelization. Granulation tissue had resolved in SIS group by 16 weeks but it was still present in control animals at 28 weeks. Signs of graft rejection were not found in tissue samples of the SIS grafted ovary.

**Conclusions:** This study provided favorable results which indicate that porcine SIS can be used as a reliable scaffold for repairing of ovarian defect. SIS graft remodeled into the native ovarian tissue and demonstrated primordial follicles which are probably newly formed.

**INVITED SESSION**

## Session 61: Contraception

**09 July 2008** **12:00–13:00**

### O-242 Oral Potential new targets for contraception

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Despite the availability of a range of contraceptive methods, unintended pregnancy is common and abortion rates are increasing in many countries. New methods of contraception that have become available in the last decade have all been variations on the theme of exisiting hormonal methods. It is often argued that contraceptive development should concentrate on finding methods which act specifically and selectively on reproductive processes thus reducing the likelihood of unwanted side effects leading to health risks and discontinuation of the method. This is hard to achieve and commonly targets which are thought to be specific prove not to be so.

In reality most people are rather inconsistent when it comes to using any mediaction - including contraception - and the high failure rates are usually due to poor compliance. Existing hormonal contraceptives have non-contraceptive benefits which should in theory add to their acceptability. There is limited evidence that when users gain advantage from the non-contraceptive side effects of contraception, continuation rates are much better then when the method is used simply for preventing pregnancy. It seems likely that, rather than aiming for increasingly specific targets in the reproductive tract, researchers interested in contraceptive development should concentrate on producing methods which have beneficial side effects, particularly if they are immediate and clearly apparant.

### O-243 Oral Demographic consequences of contraception use

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**Introduction:** The impact of contraception can be measured at two levels: the individual level, and the population level. A high rate of failure at the individual level is yet compatible with a substantial reduction in population's fertility. In other words, even rather poorly effective contraceptive methods (e.g. withdrawal) can induce a significant decline in fertility in a country, as shown by historical examples. The new medical methods (developed in the 1960s) are more effective than the previous ones, but their key feature is that they are under the sole control of women and no longer linked to intercourse.

Fertility started to decline in many developed countries almost simultaneously to the spreading of the pill in the mid-1960s: the total fertility rate (TFR, sum of the age-specific fertility rates) of the 15 countries of the European Union decreased from an average of 2.72 children per woman in 1965 to 1.96 in 1975. Some countries showed a striking coincidence: in France, fertility peaked in 1964 at the end of the baby boom, and then rapidly declined just as hormonal contraception was increasingly being adopted by French women. Therefore, we can ask whether the diffusion of oral contraception caused the recent decline in fertility observed in Europe and other developed countries?

The word 'fertility' is used here in its demographic meaning, i.e. the number of births per woman, and not its biological sense (the ability to conceive).

**Materials and methods:** We first analyse the relationship between the spread of oral contraception use and the change in fertility in 21 developed countries over the last forty years. Data on fertility (the TFR) come from national vital statistics and are available for each year. Data on hormonal contraceptives'use come most often from demographic surveys. We selected countries where at least two surveys were available after having explored several bibliographic databases. The situation of specific countries is examined more in depth, on the basis of available literature. Finally we review the various theories attempting to explain these trends and see how the family planning variables are treated in these approaches.

**Results:** At the country level, the conclusion is unambiguous: within individual countries, there is no systematic negative correlation between fertility and contraceptive pill use. The development of hormonal contraception cannot be considered as responsible for either starting or the size of the fertility decline. A more subtle chain of causality must be considered, but there is no agreement on a general theory of fertility changes. Most authors however agree that the diffusion of modern contraception has certainly contributed to the reduction in the number of unwanted pregnancies, and has also facilitated and favoured the adoption of new (more restrictive) norms for the ideal family size.

**Conclusions:** 1- The diffusion of modern contraception has certainly contributed to the reduction in the number of unwanted pregnancies and, with abortion, to the reduction in the number of unwanted births. 2- These new methods have been adopted because of the major changes of attitude towards sexuality, the nature of marriage and other forms of union, the place of women in societies and, more specifically, the position of women in the work place. 3- These contraceptive techniques have also facilitated and favoured the adoption of new (more restrictive) norms for the ideal family size. 4- However, motivation always comes first: when couples are not worried about how many children they have, a baby boom may occur, with many not-really-wanted births. When couples want to avoid births for any reason, they can largely succeed even without elaborate contraceptive technology (but some unwanted births will still occur).

**INVITED SESSION**

## Session 62: ASRM session - Bleeding problems at the extremes of life-evidence based information

**09 July 2008** **12:00–13:00**

### O-244 Oral Abnormal uterine bleeding in the adolescent

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The onset of puberty is predicated upon increase in hypothalamic pulse generator activity complemented by genetic factors, circulating leptin change, growth factor and tumor related genes also play a role in timing of onset. There is a 10% increase in hypothalamic gene expression in addition kisspeptin, a potent regulator of GnRH release, is involved in the pubertal cascade. Inhibin B rises in correlation with an increase in LH and FSH. There is a steep increase in wake-time LH Level, ie a 60 fold increase (compared to pubertal levels) as well as development of multifollicular appearing ovaries; they are distinguished from PCOS by the presence of "scanty stroma". With the onset of puberty, the FSH/LH ratio is 10–20/1. Because of lability of the HPO axis, it is common to have menstrual disturbances in the adolescent. Abnormal uterine bleeding must involve determination of pregnancy, endocrinological causes, acquired defects such as stress related hypothalamic dysfunction, eating disorders as well as pathology involving the ovarian adrenal and/or pituitary gland. Other possible etiologies of abnormal uterine bleeding in the