pathology werefound in 2 (6.6%) patients (1 polyp and 1 myoma). Only one patient (3.3%) reported moderate pain but did not need any medication. There were no long-term complications and post-procedure pain.

CONCLUSIONS: Our results confirm that the PSM preparation offers a simplified more user-friendly way of having HySo-like images, while retaining the simplicity of regular ultrasounds with no instruments present. Our finding of 6.6% incidence of intrauterine pathologies in asymptomatic women undergoing IVF is consonant with data from other groups and confirms the need to explore the uterine cavity prior to IVF.

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P-151

HUMAN NATURAL CYCLE: NEW BIOMARKERS OF THE HUMAN ENDOMETRIUM RECEPTIVITY. K. Mahmoud, D. Haouzi, M. Fourar, K. Bendhaou, B. Hedon, S. Hamamah. Centre de Fecondation In Vitro, Tunis, Tunisia; Insitut de Recherche en Biothérapie—INSERM, Montpellier, France; Institut de Biologie et Chimie des Protéine, Montpellier, France; Centre de Fecondation In Vitro, Montpellier, France; Hôpital Arnaud de Villeneuve, CHU Montpellie, Montpellier, France.

OBJECTIVE: In order to identify news markers of the endometrium receptivity, we performed the gene expression profiles between pre-secretory (LH+2) and secretory (LH+7) stages using endometrial biopsies (n=62) from the same normo-responders patient during a natural menstrual cycle before to be referred for COS.

DESIGN: The messenger RNA (mRNA) content of each biopsy was analyzed by DNA chip microarray to evaluate the mRNA expression profile at the LH+2 and LH+7 stages. The differential gene expression between stages and a gene list exclusive to our analyses were then performed with bioinformatics methods.

MATERIALS AND METHODS: The mRNAs extracted from LH+2 and LH+7 biopsies were analyzed on Affymetrix HG-U133 plus 2.0 GeneChip oligonucleotide microarrays. The Affymetrix GeneChip Operating Software 1.2 (GCOS) was used to evaluate signal intensities and to perform pair wise gene expression comparison between samples. Gene expression profiles associated to the implantation window were computed and were compared to four others studies using the same approach to identify new biomarkers of the endometrium receptivity.

RESULTS: Five specific genes of the implantation window were selected and validated by quantitative PCR. These genes are LAMB3, MFAP5, ANGPTL1, EG-VEGF and NLF2, which are up-regulated in LH+7 sample group and play a role in the extracellular matrix components remodeling of the endothelial cell microenvironnement, angiogenesis and the formation of endothelial fenestration. This group of predictive genes shows the potential to be useful prognostic indicators of the endometrium receptivity.

CONCLUSIONS: Five selected genes have been validated and are suggested as new biomarkers for endometrium receptivity. As this biopsy procedure is available during natural cycle and has been shown to improve the implantation rates and the clinical pregnancies, this approach may offer a novel treatment protocol that will precede the treatment of IVF in poor implanted patients, and thereby limiting both the number of IVF attempts and the risk of hyperstimulation.

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P-152

THE AGE OF RECIPIENTS DOES NOT AFFECT THE PREGNANCY DERIVED FROM DONOR OOCYTES. T. Tao, W. Zhang, A. Del Valle. The Toronto Institute for Reproductive Medicine, Toronto, ON, Canada.

OBJECTIVE: The use of donated oocytes in assisted reproductive technology has increased in the past years. One of the most common reasons for using donor oocytes as a treatment for infertility is advanced reproductive age of the female partner. However, most of the advanced age women considering using donor oocytes concern about whether their age will affect the pregnancy. The objective of this study was to investigate the impact of recipient age on the pregnancy derived from donor oocytes.

DESIGN: Retrospective study of donor oocyte cycles from 2003-2007.

MATERIALS AND METHODS: The oocyte donors ranged in age from 21 to 35 years old, with an average age of 28.6 ± 4.5 years. All of the oocytes were inseminated by using intracytoplasmic sperm injection regardless of sperm quality. The recipient ages were within the range of 22 to 48 years. All of the patients used the same protocol for endometrium preparation. Embryo transfers were performed on Day 3 post aspiration. Recipients were retrospectively divided into four groups according to their age: <35; 35-39; 40-44 and >44. Statistical analysis was done by using chi-square test.

RESULTS: Results were shown in table 1. High clinical pregnancy rate (50%) and implantation rate (29.5%) were obtained in the group of recipients with >44 years old. There were no statistical differences among four groups in the rates of chemical pregnancy, clinical pregnancy and implantation ($p>0.05$).

TABLE 1. Outcome by age groups

Recipient age	< 35	35-39	40-44	>44
Number of patients	21	20	47	20
Number of chemical pregnancy (%)	12 (57.1)	13 (65.0)	25 (53.2)	10 (50.0)
Number of clinical pregnancy (%)	10 (47.6)	13 (65.0)	23 (48.9)	10 (50.0)
Number of embryos transferred	42	39	100	44
Number of embryos per transfer	2.0	2.0	2.1	2.2
Number of fetus	17	20	37	13
Implantation rates	40.5%	51.3%	37.0%	29.5%

CONCLUSIONS: Our results show that the age of recipient does not affect the rates of chemical pregnancy, clinical pregnancy and implantation. It is the ability of the aging oocyte that is the most important factor in the decreasing fertility of older women. The endometrial factor in terms of aging was not of importance on the outcome of oocyte donated cycles.

Supported by: None.

P-153

MORPHOLOGICAL AND GENOMIC ENDOMETRIAL DEVELOPMENT WITH LONG-ACTING DEGARELIX STARTING IN THE MID-LUTEAL OR EARLY FOLLICULAR PHASE COMPARED TO DAILY GANIRELIX STARTING IN THE MID-FOLLICULAR PHASE. J. A. Martínez-Conejero, C. Bourgain, A. Pellicer, P. Devroey, J.-C. Arce, C. Simón. Research Department, IVI Foundation, Valencia, Spain; Department of Pathology, UZ Brussels, Brussels, Belgium; Reproductive Endocrinology, IVI Valencia, Valencia, Spain; Centre for Reproductive Medicine, UZ Brussels, Brussels, Belgium; Clinical Research & Development (OB&GYN), Ferring Pharmaceuticals, Copenhagen, Denmark.

OBJECTIVE: To compare the histological and genomic endometrial effect of three GnRH antagonist regimens starting in the mid-luteal, early follicular and mid-follicular phase.

DESIGN: Randomized, controlled, assessor-blind, multicenter, multinational trial.

MATERIALS AND METHODS: Eighty-five oocyte donors were randomly allocated to one of three groups: 1) degarelix mid-luteal regimen: degarelix (Ferring Pharmaceuticals) 2.5 mg SC on day LH +7 and day 6 of gonadotropin stimulation; 2) degarelix early follicular regimen: degarelix 2.5 mg SC on days 1 and 6 of stimulation; 3) ganirelix 0.25 mg SC starting on day 6 of stimulation onwards. Highly purified menotropin (MENOPUR) 225 IU SC was used throughout ovarian stimulation, starting on cycle day 2. As soon as ≥ 3 follicles of ≥ 17 mm were observed, rhCG 250 μg SC was administered. Vaginal progesterone 200 mg twice daily was provided from the day after oocyte retrieval. Endometrial biopsies from natural cycles (LH +7) (n=65) and stimulated cycles (hCG +7) (n=63) were evaluated histologically according to Noyes' criteria and for gene expression using microarrays (15 and 15 samples, respectively) centrally by independent assessors blinded to treatment allocation. For the labeling and hybridization of each sample 1 μg of total RNA (RIN >7) was used. Data were analyzed for gene expression patterns and gene ontology terms. The results were validated by qPCR.

RESULTS: In-phase endometrial maturation was observed in the natural cycles and similar in-phase endometrial dating was seen in the stimulated cycles for all three regimens. The degarelix mid-luteal regimen showed a trend towards the most exact matching with the expected chronological cycle day. Dissociated maturation with stromal advancement was present and similar for all regimens. Endometrium from natural and stimulated cycles in all regimens followed similar genomic patterns in the receptive phase. The only group with minor differences was the degarelix mid-luteal regimen, showing 37 genes statistically up-regulated and 32 genes down-regulated at the stimulated cycle versus natural cycle (FC>2; $p<0.05$). However, functionally none of the gene ontology terms were statistically represented and therefore no endometrial genomic differences can be concluded.

CONCLUSIONS: Endometrial histology and gene expression profile of degarelix starting in the mid-luteal or early follicular phase do not differ from those observed with the standard fixed GnRH antagonist regimen starting in the mid-follicular phase.

Supported by: Ferring Pharmaceuticals.

P-154

SUCCESSFUL RECONSTRUCTION OF ENDOMETRIAL TISSUES IN-VIVO BY ADULT STEM CELLS ISOLATED FROM ENDOMETRIUM. H.-C. Liu, Z. He, Y. Tang, W. Wang, Z. Rosenwaks. Weill Medical College of Cornell University, New York, NY.

OBJECTIVE: Endometrium undergoes hormone-driven dynamic changes including self-renewal, proliferation, differentiation and shedding off. These characteristics suggest possible presence of adult stem/progenitor cells in endometrium. Here, we used mouse model to test this hypostasis.

DESIGN: The adult stem/progenitor cells were cloned from mouse endometrium and were tested their stem-cell like properties such as plastic, adherence, clonogenicity, the presence of stem cell markers and capability to reconstruct endometrial tissue.

MATERIALS AND METHODS: Endometrial cells isolated from CD1 female mice were used to test their clonogenic efficiency (CE) by seeding cells at concentration of 50 and 100 cells/cm^2. Colongenic cells were examined their origin by immunostaining and were detected the expression of embryonic stem cell markers by real-time RT-PCR. Clonogenic cells transfected by plasmid DNA containing EGFP and luciferase (pIRES2-EGFP-luc) were sorted by FACS. Cells with $PI^-/EGFP^+$ were autotransplanted into mouse kidney capsules implanted with E_2 pellets. Real-time growth of the reconstructed tissue was scanned using IVIS imaging system.

RESULTS: After 1 day culture, 36% of mouse individual endometrial cells were attached to the plates. The CEs of mouse endometrial cells were $0.2\pm0.03\%$ and $0.12\pm0.04\%$ at the seeding concentrations of 50 and 100 cell/cm^2 respectively. We had isolated 12 colonies. Of them, all were vimentin+ and cytokeratin-, indicating their stromal origin. All were Oct4+, 6 were Nanog+, 8 were Sox2+, and 3 revealed bioluminescence signals around the recipient's kidneys. The active growth of reconstructed tissues was demonstrated by progressive increase of signal intensity throughout in-vivo transplantation. Lesions with multicystic structure and fine surface vasculature were well integrated onto the recipient's kidneys. Immunohistological analysis revealed that these lesions were consisted of endometrial-like tissues.

CONCLUSIONS: We had isolated adult stem/progenitor cells from mouse endometrum. They were stromal origin, expressed embryonic stem cell markers and had potential of plastic, adherence, clonogenicity and capability to reconstruct endometrial tissues in mouse autograft model. Here, we also developed a novel noninvasive real-time assessment for the growth of the reconstructed tissues in living animals. Clinically, these adult stem cells can be used for cell/tissue therapy avoiding rejection, and be used to study pathological mechanism and treatment of endometrial diseases.

Supported by: None.

P-155

COX-2 AS A POSSIBLE PREDICTIVE MARKER FOR CLINICAL PREGNANCY IN IVF. I. Van Vaerenbergh, P. In't Veld, F. Schuit, H. Fatemi, P. Devroey, C. Bourgain. EXPA, Pathology Department, Vrije Universiteit Brussel, Brussels, Belgium; Pathology Department, Vrije Universiteit Brussel, Brussels, Belgium; Gene Expression Unit, Department of Molecular Cell Biology, KU Leuven, Leuven, Belgium; Centre for Reproductive Medicine, UZ Brussel, Brussels, Belgium; Pathology Department, UZ Brussel, Brussels, Belgium.

OBJECTIVE: Study the gene expression of human endometrium of patients undergoing an IVF treatment with GnRH antagonists/rec FSH.

DESIGN: Prospective study.

MATERIALS AND METHODS: Ovarian stimulation was performed with a fixed dose of 200 IU recombinant-FSH. To inhibit premature LH surge, daily GnRH-antagonist ganirelix was used from d6 onwards. Final oocyte maturation was achieved by administration of 10.000 IU of hCG when $\geq$ 3 follicles $\geq$ 17 mm were present. Oocyte retrieval was carried out 36 h after hCG administration. Biopsies were taken with a Pipelle® on the day of OPU in 47 patients with 1 or 2 embryos replaced on day 3 in the same cycle. Patients $>$ 37 years, with endometriosis $\geq$ III (AFS), PCOS or any other endometrial pathology were excluded from the study. Pregnancy was observed in 11/47 patients, with 8/47 ongoing pregnancies. The biopsy was divided into two parts for microarray and histological analysis. Gene expression from 4 patients with ongoing pregnancy and from 4 matched controls without ongoing pregnancy were compared with the Affymetrix Human Genome U133 Plus 2.0 Array. Affymetrix GeneChip Operating Software v1.2 was used to analyze the microarray data. qPCR was conducted using the TaqMan Gene Expression Assay Hs00153133_m1 (Applied Biosystems). Immunohistochemistry was performed on all samples and intensity was scored semiquantitatively from 0 to 3, blinded for clinical outcome. T-tests were used where appropriate. Significance level was set at $p \leq 0.05$.

RESULTS: Histology showed secretory changes in all biopsies, dating ranging from luteal phase d2 to d4. In microarray experiments for the full human genome, 23 genes showed a significantly increased expression in non-pregnant patients. One of these genes was COX-2 (cyclooxygenase-2) or PTGS-2 (prostaglandin-endoperoxide synthase 2) with a fold-change of 1.65 ($p=0.024$). Quantitative real-time PCR resulted in a fold-change, comparable with the microarray results: 1.64 ($p=0.016$). Immunohistochemistry for anti-COX-2 showed 2+ to 3+ secretory cytoplasmic localization in the glandular and luminal epithelium and heterogeneous positivity in the stroma in both pregnant and non-pregnant patients.

CONCLUSIONS: COX-2 has been extensively studied as a crucial fertility element in both knock-out mice and human. It appears that increased gene expression of COX-2 on the day of oocyte retrieval in GnRH ant/rec FSH stimulated cycles coincides with a lower probability of achieving a clinical pregnancy in this cycle.

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P-156

IN VIVO AND IN VITRO EVIDENCE FOR ERβ REGULATION OF G PROTEIN-COUPLED ESTROGEN RECEPTOR (GPER) EXPRESSION IN NORMAL WOMEN AND THOSE WITH ENDOMETRIOSIS. B. J. Plante, L. Yuan, B. Lessey, M. A. Fritz, S. L. Young. Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, NC; Obstetrics and Gynecology, Greenville Hospital System, Greenville, SC.

OBJECTIVE: GPER, also known as GPR30, is a membrane-bound estrogen receptor that stimulates multiple signaling pathways. Previous work in our laboratory has demonstrated expression of GPER in normal human endometrial epithelium, with highest expression in the proliferative phase, suggesting regulation by estradiol in vivo. We also observed estradiol induction of GPER in vitro. The aims of this study were to evaluate the receptors conferring estrogen regulation as well as study patterns of expression in women with endometriosis.

DESIGN: Laboratory study.

MATERIALS AND METHODS: Urinary LH-surge timed endometrial specimens were obtained from healthy volunteers. Matched samples of eutopic and ectopic endometrium from women with endometriosis were collected and menstrual cycle stage was assigned using Noyes criteria. Real time quantitative RT-PCR analysis of GPER mRNA expression was performed using Taqman® probes for GPER and a constitutive control (PPIA). Estrogen receptor (ER) selective agonists were utilized to determine the role of each ER on GPER mRNA expression. Immunohistochemistry utilized a GPER C-terminal rabbit polyclonal antibody (Eric Prossnitz, U. New Mexico) and a mouse monoclonal ERβ antibody (NovoCastra). ECC-1 endometrial epithelial cell line was used for in vitro studies.

RESULTS: In ECC-1 cells, GPER mRNA expression was stimulated by treatment with estradiol and an ERβ-selective ligand, but not by agonists selective for ERα or GPER. In normal endometrium from the proliferative phase, strong immunostaining for GPER was seen in the epithelium and stroma. In the early secretory phase, the staining intensity in the epithelium was decreased, but the stromal staining remained strong. In the mid-secretory phase, stromal staining persisted, but staining was largely absent in the glandular epithelium. In the late secretory phase, minimal epithelial staining and moderate stromal staining was seen. In eutopic and ectopic endometrium from patients with endometriosis, immunostaining for GPER and ERβ closely correlated. Unlike normal endometrium, proliferative eutopic and ectopic endometriosis samples exhibited largely absent epithelial staining for both GPER and ERβ, but endothelium remained positive for both GPER and ERβ.

CONCLUSIONS: 1. Estradiol induces GPER mRNA expression in ECC-1 cells and this effect is mediated through ERβ. 2. ERβ staining is strongly correlated with GPER staining. 3. GPER expression appears to be aberrant in patients with endometriosis.

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