Conclusions: The size of the 2–5 mm follicle pool is an independent and important contributor to the FA of PCOS. This result could be explained by an exaggerated physiological inhibitory effect from this pool on the terminal follicle growth, presumably involving the Anti-Müllerian Hormone. The metabolic derangement of PCOS that also contributes to the FA would act through a different mechanism.

O-266 Oral Effects of ghrelin administration on growth hormone secretion and metabolic parameters in obese patients with polycystic ovary syndrome

M. Giuliani[1], D. Romualdi[1], B. Costantini[1], G. Campagna[1], F. Macrì[1], A. Lanzone[1], M. Guido[1]
[1]*Universita Cattolica del Sacro Cuore, Obstetrics and Gynaecology, Roma, Italy*

Introduction: The exogenous administration of ghrelin, a hormone with a paramount role in the regulation of energy balance, is able to markedly increase GH secretion, to enhance glycemic levels, to inhibit insulin secretion and to induce hunger and imagination of food. The polycystic ovary syndrome (PCOS) represents an interesting model for the study of ghrelin functions. In fact, obesity and hyperinsulinemia are common features of this disorder. Furthermore, these patients show a blunted GH response to a variety of stimuli, particularly when obesity is present. Few studies were published on ghrelin levels in PCOS, with discordant results. This study was performed in order to evaluate the presence of abnormal endogenous ghrelin levels and to assess the effects of the exogenous ghrelin administration in obese patients with PCOS.
Materials and methods: We enrolled 20 obese PCOS women and 15 obese controls. Basal hormonal assays, including ghrelin, were performed. Glucose, insulin and C-peptide were assessed in fasting condition and during the OGTT. All patients underwent an oral glucose tolerance test, (OGTT) with the measurement of glucose, insulin and C-peptide plasma levels, and a ghrelin test (1 ?g/ kg i.v. bolus), with the measurement of GH, insulin and glucose every 15 minutes for 90 minutes after ghrelin injection.
Results: Both groups resulted hyperinsulinemic. Significantly lower ghrelin levels were detected in PCOS patients compared with controls (108.96 ± 27.65 vs 162.47 ± 42.23 Fmol/ml, $P<0.01$). Ghrelin administration markedly enhanced GH levels in both the groups (1888.59 ± 1209.53 and 1639.95 ± 631.79 ng/ml*90 min as GH area under the curve respectively), with a peak occurring 30 minutes after injection. Ghrelin also induced a trend towards increase in plasma glucose levels and a significant decrease in insulin concentrations in both groups.
Conclusions: Ghrelin injection seems to be able to override GH secretion defect in PCOS obese women and to induce gluco-insulinemic changes in both controls and PCOS obese patients. In the complex picture of the endocrine-metabolic alterations of the polycystic ovary syndrome, ghrelin may represent the link between the gluco-insulinemic imbalance and the profound derangement of GH secretion.

O-267 Oral Adrenal androgen production capacity remains high until menopause in PCOS

J. Kesti[1], T. Piltonen[1], P. Jaakkola[1], L. Morin-Papunen[1], A. Ruokonen[2], J.S. Tapanainen[1]
[1]*Oulu University Hospital, Department of Obstetrics and Gynecology, Oulu, Finland,* [2]*Oulu University Hospital, Department of Clinical Chemistry, Oulu, Finland*

Introduction: Hyperandrogenism is one of the main features in polycystic ovary syndrome (PCOS). It has been estimated that 25% of androstenedione (A) and testosterone (T) production is of ovarian origin, 25% is of adrenal origin and 50% is produced in peripheral tissues, whereas dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) are thought to be almost uniquely of adrenal origin. According to our previous studies the ovarian androgen production capacity is enhanced in women with PCOS and it remains high until late reproductive age. To study whether this also applies to adrenal androgen production, adenocorticotrophin (ACTH) test was performed in healthy women and in women with PCOS.
Materials: 53 Healthy women (aged 19–48 yr) and 49 women with previously diagnosed PCOS (aged 18–50 yr) participated in the study. PCOS diagnosis was based on Rotterdam's criteria. The women in control group were healthy and had regular menstruation and normal ovaries in ultrasonography. The women were devided in three age-groups <31 (18–30 years), <41 (31–40 years) and <51 (41–50 years) years. The mean ages were 24.38 ± 3.38 (SD), 34.92 ± 3.15 and 45.00 ± 2.77 in control women and 26.14 ± 2.97, 34.44 ± 2.74 and 44.42 ± 2.02 in women with PCOS and the BMÍs were 22.93 ± 3.46, 25.50 ± 5.65 and 25.39 ± 3.31 in control women and 26.04 ± 5.79, 24.75 ± 3.40 and 29.63 ± 4.27 in women with PCOS, respectively.
Methods: All subjects underwent ACTH stimulation 2–4 days after spontaneous or progestin-induced menstrual bleeding. All ACTH tests were carried out after overnight fasting between 0700–0900. Serum samples for 17-OHP, A, T, DHEA, DHEAS, and cortisol measurements were drawn before, and 30 and 60 minutes after a single intramuscular injection of 250 ug tetracosactide hexacetate (Synacten®, Ciba-Geigy, Basel, Swizerland).
Results: The basal A production was higher in women with PCOS aged<31 years ($p=0.009$) and <51 years ($p=0.046$) when compared to control women. Also basal serum T levels were elevated in PCOS women<31 ($p=0.006$) and <41 years ($p=0.043$) when compared to control women of same age and the difference remained until the age of 50, although the difference in the oldest age group did not reach statistical significance probably due to individual variation. Basal serum levels of DHEAS levels tended to be elevated in PCOS women <31 years when compared to control women and were significantly increased in PCOS women<41 years ($p=0.037$). The basal cortisol levels were comparable in PCOS and control women <31 and <41 years, but were elevated in PCOS women <51 years ($p=0.047$).

Serum levels of all steroids, except T, increased significantly in response to ACTH injection. The area under the curve (AUC) was calculated to reflect the steroid secretion capacity of adrenals. The AUC of A was higher in women with PCOS<31 years compared to control women ($p=0.012$) and the difference remained until the age of 50 ($p=0.048$). Similarly to basal levels, AUC of DHEAS tended to be elevated in PCOS women <31 years and were increased significantly in PCOS women <41 years ($p=0.028$). The AUC of cortisol level was higher in PCOS women <51 years compared to control women ($p=0.004$).
Conclusions: Similarly to ovarian endocrine function serum adrenal steroid levels and adrenal steroid production capacity remained enhanced until menopause in women with PCOS.

FREE COMMUNICATION

Session 69: Endometriosis 2

Wednesday, 4 July 2007 **14:00–15:15**

O-268 Oral Effect of a statin on angiogenesis in an experimental endometriosis model

D. Izquierdo[1], E. Novella-Maestre[2], A. Pellicer[1]
[1]*Dr. Peset Hospital, Ob/Gyn, Valencia, Spain,* [2]*IVI Valencia, Fivier, Valencia, Spain*

Introduction: Endometriosis is a common disorder characterized by ectopic presence of endometrial glands and stroma. it is a multifactorial condition associated with oxidative stress and chronic inflammation within the peritoneal cavity. statins, 3-hydroxy-3-methylglutaryl-coenzyme a (hmg-co a) reductasa inhibitors (enzime limiting step in cholesterol synthesis) have antinflammatory and antioxidative actions and been shown to be effective in inhibiting the mechanisms of cell proliferation and angiogenesis in vitro in endometriosis models. thus, in this study we tested the hypothesis that lovastatin (mevinolin) may ameliorate the endometriosis lesions diminishing angiogenesis in an endometriosis mouse model.
Material and methods: A total of 16 immature and ovariectomized 4-week old female nude mice received a sc implant of 18mg 17β-estradiol pellet. four days later, an entrance was made to the peritoneal cavity in the abdomen, 4 fresh human endometrium fragments per mouse were stuck in the peritoneum, obtained from oocyte donors at ovum pick-up. after establishment of lesions,

animals were treated with vehicle, 5, 25 and 50 mg/kg/day oral lovastatin during 14 days. then, the number of implants was recorded and subsequently removed and processed for immunofluorescence and confocal microscopy employing antibodies raised against the von willebrand factor (vwf) present in endothelial cells and vascular smooth muscle cells (α-sma) to test the anti-angiogenic action of lovastatin. the anova one way and kruskal-walllis tests were applied for statistics (graphpad instat v3.0).
Results: increasing doses of lovastatin resulted in decreased presence of immature vessels as markers of neoangiogenesis in a dose-dependent manner. in the group treated with vehicle, the ratio mature/immature vessels was 24.1/75.9%. in the lovastatin-treated animals, this ratio was respectively 66.7/33.3%, 75/25% and 86.2/13.8% in the 5, 25 and 50 mgrs/kg, significantly ($p < 0.05$) different than the controls.
Conclusions: Lovastatin inhibits proliferation of new vessels in a dose-dependent manner on endometriotic lesions in an animal model. the present finding provides a rational for further studies evaluating statins as potential therapeutic agents in endometriosis.

O-269 Oral Evaluation of endometrial biomarkers for semi-invasive diagnosis of endometriosis

C.M. Kyama[1], A. Mihalyi[1], O. Gevaert[2], P. Simsa[1], E. Waelkens[3], R. Van de Plas[2], J.M. Mwenda[4], C. Meuleman[1], T.M. D'Hooghe[1]
[1]University Hospital Gasthuisberg, Leuven University Fertility Centre Department of Obstetrics & Gynaecology, Leuven, Belgium, [2]Katholieke University of Leuven, Department of Electrical Engineering ESAT-SCD Kasteelpark-Arenberg, Heverlee, Belgium, [3]University Hospital Gasthuisberg, Biochemistry section Department of Molecular Cell Biology, Leuven, Belgium, [4]Institute of Primate Research, Division of Reproductive Biology, Karen -Nairobi, Kenya

Introduction: The early detection of endometriosis is crucial for its ultimate control and prevention. Currently, the diagnosis of endometriosis is through a laparoscopy with subsequent histological confirmation of endometrial stroma and glands in the biopsy that is collected. A non- invasive diagnostic test in serum or endometrium would be beneficial to both physicians and patients. This study tested the hypothesis that specific protein/peptides are differentially expressed in eutopic endometrium of women with and without endometriosis and at specific stages of the disease (minimal, mild, moderate or severe) during the secretory phase.
Materials and methods: A total of 29 samples of secretory phase eutopic endometrium collected previously from women undergoing laparoscopies for infertility and pain and frozen at $-80\ ^0$C were used in the study. The tissues included secretory phase endometrium from women with (n=19) and without (n =10) endometriosis. All women with endometriosis had minimal to mild (I-II, n= 9) and moderate to severe (III-IV, n= 10) endometriosis, according to the classification system of the American Society of Reproductive Medicine (ASRM, 1997). All endometrial samples were dated between days 16 – 26 of a 28-day menstrual cycle according to the Noyes criteria. A blind approach for a wide screening experiment was applied on these samples using Surface Enhanced Laser Desorption Ionization Time-of-Flight Mass spectrometry (SELDI-TOF - MS) to search for potential biomarkers. Analysis was done using Ciphergen's ProteinChip Software v3.1.1. A differentially expressed mass peak with *P*-value <0.05 was considered to be statistically significant. Diagnostic models were developed and validated using a Leave-One-Out- Support Vector Machine (LOO-SVM) algorithm and logistic regression classification models with Leave-One-Out –Cross Validation (LOO – CV) towards ranking the significant mass peaks according to their classification power.
Results: A total of 103 qualified mass peaks were upregulated or downregulated in endometrium from women with endometriosis (stages I-IV, ASRM classification 1997) when compared to endometrium from controls. Women with endometriosis had 73 mass peaks detected with significant differences when compared to controls. Using LOO-SVM algorithm ranking and logistic regression classification models (LOO – CV), 5 downregulated mass peaks (8.650kDa, 8.659kDa, 13.91kDa, 5.183kDa and 1.949kDa) were selected as potential endometrial biomarkers for the diagnosis of endometriosis with a high sensitivity (89.5%) and specificity (90 %). Women with Stage I - II endometriosis, when compared to controls, generated 30 differentially expressed mass peaks. Using LOO-SVM algorithm ranking and logistic regression classification models (LOO – CV), 4 mass peaks (2 upregulated: 90.675kDa and 35.956 kDa) and 2 downregulated: 1.924kDa and 2.504kDa) were selected as potential biomarkers for the diagnosis of Stage I - II endometriosis with maximal sensitivity (100%) and specificity (100%).
Conclusions: SELDI-TOF –MS ProteinChip technology combined with bioinformatics analysis tools may help develop a diagnostic model test with a high sensitivity especially for minimal to mild endometriosis. Further studies are needed to optimise and validate this semi-invasive diagnostic model to rule out women without endometriosis and to rule in those with endometriosis, especially minimal to mild disease.

O-270 Oral Expression of Toll-like Receptor 3 (TLR3) and induction of apoptosis in patients with endometriosis and healthy controls

S. Dogan[1], F. Koester[1], A. Agic[1], S. Djalali[1], K. Diedrich[1], R. Pries[2], B. Wollenberg[2], D. Hornung[1]
[1]University of Schleswig-Holstein Campus Luebeck, Gynecology and Obstetrics, Luebeck, Germany, [2]University of Schleswig-Holstein Campus Luebeck, Otorhinolaryngology, Luebeck, Germany

Introduction: Endometriosis is a chronic disease associated with local inflammation und reduced apoptosis. Toll-like receptors are the basic signalling receptors of the innate immune system. Toll-like receptor 3 (TLR3) responds to dsRNA and heterologous RNA and initiates the production of proinflammatory and antiviral cytokines to induce pro- and antiapoptotic signalling pathways. Functional TLR3 is expressed in endometrial epithelial tissue, dendritic cells, macrophages, fibroblasts, keratinocytes, endothelial and epithelial cells. However it is unknown so far, whether TLR3 is expressed in endometriotic tissue and if TLR3 could influence apoptosis in endometriosis. We investigated the expression of TLR3 and the regulation of cell death in tissue and cells of women with and without endometriosis.
Materials and Methods: Endometrium, endometriomas and benign ovarian tissue were obtained from patients undergoing laparoscopy after providing written informed consent under a study protocol approved by the local Ethics committee. Tissues from 24 patients with endometriosis and 21 healthy controls were examined. Westernblotting for TLR3 was performed with mouse monoclonal antibodies raised against human TLR3, whereas beta-actin served as housekeeping gene. Total RNA from tissue was extracted using the RNA purification kit and reverse-transcribed with the SuperScript first-strand synthesis system. Quantitative real-time RT-PCR was performed with specific oligonucleotide primers, which were designed to amplify sequences from human TLR3 mRNA. HPRT served as housekeeping gene. Endometrial cell cultures were stimulated with Poly I: Poly C (polyinosinic-polycytidylic acid). Apoptosis was detected using westernblot with mouse anti-PARP [Poly (ADP-ribose) polymerase] monoclonal antibody.
Results: Westernblot analysis showed similar expression of TLR3 in endometriotic tissue, normal ovaries and in endometrium of patients with and without endometriosis. Quantitative real-time RT-PCR revealed a reduced TLR3/HPRT mRNA ratio in endometrioma from patients with endometriosis compared to benign ovaries from women without endometriosis. Cultured endometrial stromal cells from patients with and without endometriosis were examined for apoptosis after stimulation with Poly I:C for 0, 24 and 48 hours. Westernblot showed no apoptosis in endometrial stromal cells from women with endometriosis, whereas apoptosis was significant after stimulation in endometrial stromal cells from healthy controls.
Conclusions: In this study, we demonstrated for the first time TLR3-expression in endometriosis tissue and in endometrial tissue of women with and without endometriosis. A reduced TLR3/HPRT mRNA ratio might be one of the important pathophysiological reason for decreased apoptosis in endometrial stromal cells from women with endometriosis compared to healthy controls. TLR3 might also have the potential to alter the cytokine milieu in this disease. Future studies should show, if TLR3-agonists are able to enhance the apoptosis rate in endometriosis as a new treatment modality similar to ovarian cancer therapy models.

O-271 Oral c-Fos and matrix metalloproteinase-9 expression increase in eutopic and ectopic endometrium of patients with endometriosis

H. Pan[1], Q. Luo[1], J. Cai[1], R. Zhu[1], L. Tang[1], H.F. Huang[1]
[1]Women's Hospital School of Medicine Zhejiang University, Department of Reproductive Endocrinology, Hangzhou, China

Introduction: Endometriosis, albeit considered to be a "benign" one, shares the same characteristic of invasion behavior and biochemical mediators with malignant tumor. C-Fos, a transcription factor, regulates multifold invasion related genes expression in tumor tissue, including MMP-9, and subsequently facilitates degradation of extracellular matrix (ECM) and invasion of malignant tumor. We hypothesize that c-Fos plays an important role in invasion behavior of endometrial explants by controlling MMP-9 expression. In the present study we detected the expression of c-Fos and MMP-9 in paired eutopic and ectopic endometrium of patients with endometriosis and in endometrium from healthy controls to confirm our hypothesis.
Material and methods: Paired samples of eutopic and ectopic endometrium were obtained from 20 premenopausal women who suffered from ovarian endometriosis. Control endometrium was collected from 20 premenopausal women by endometrial curretage during gynecologic operations unrelated to endometriosis or endometrial disease. These three groups of endometrium were all acquired during the proliferative phase (n=10) and secretory phase (n=10). All patients were treated in the Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China. Endometrial tissue was homogenized in lysis buffer and protein was extracted. Western blot was employed to detect c-Fos and MMP-9 exression.
Results: C-Fos expression in both eutopic and ectopic endometrium from patients with endometriosis was significantly higher than that in control endometrium (eutopic vs control, $P<0.01$; ectopic vs control, $P<0.01$), MMP-9 expression in both eutopic and ectopic endometrium from patients with endometriosis was also significantly higher than that in control endometrium (eutopic vs control, $P<0.01$; ectopic vs control, $P<0.01$), and there was no significant difference in c-Fos and MMP-9 expression between the eutopic and ectopic endometrium from patients with endometriosis. We also found that there was a significant positive correlation between c-Fos expression and MMP-9 expression in all the endometrium. ($r=0.505$, $P<0.01$).
Conclusions: In the present study we found that c-Fos and MMP-9 expression in eutopic and ectopic endometrium from patients with endometriosis is higher than that in control endometrium, and there is a significant positive correlation between c-Fos expression and MMP-9 expression in all the endometrium. Our data suggest that c-Fos and MMP-9 might be involved in the pathogenesis of endometriosis. As a transcription factor, c-Fos might play an important role in development of endometriosis by promoting MMP-9 expression and subsequently the invasive potential of endometrial explants.

O-272 Oral Glandular lesions in endometriosis show ultrastructural and biochemical abnormalities

L.G. Nardo[1], C.J.P. Jones[2], P. Litta[3], A.T. Fazleabas[4]
[1]University of Manchester, Division of Human Development Reproductive Medicine St Mary's Hospital, Manchester, United Kingdom, [2]University of Manchester, Division of Human Development, Manchester, United Kingdom, [3]University of Padua, Obstetrics and Gynaecology, Padua, Italy, [4]University of Illinois, Obstetrics and Gynaecology, Chicago, U.S.A.

Introduction: Endometriosis is a common cause of infertility and pelvic pain affecting about 10% of women during their reproductive years. We recently studied a baboon model of endometriosis and, using electron microscopy and lectin histochemistry, reported progressive changes in the endometrial gland architecture and biochemistry (Jones et al., 2006 Hum. Rep. 21: 3068–80) which may contribute to the reduced fertility associated with the disease. We are now extending this work to human patients in order to establish whether a similar phenomenon can be found here.
Materials and Methods: Paired ectopic and eutopic endometrial biopsies have been taken from 10 women with endometriosis and 10 healthy controls and processed into epoxy resin for ultrastructural and lectin histochemical examination. The latter technique focussed particularly on the binding of the agglutinin from Dolichos biflorus (DBA) to fucosylated N-acetylgalactosamine which is normally a marker for the secretory phase of the menstrual cycle.
Results: It was found that, in the early/mid secretory stage of the cycle, binding of DBA did not occur in the glands of the endometriotic lesions, unlike those in eutopic endometrium and in the normal healthy control tissue. At the ultrastructural level, several morphological features were seen in the lesions that were not found in eutopic endometrium, such as a flattened surface epithelium, decrease in the expected amount of glycogen for the time in the cycle, increased numbers of secretory droplets and also indications of abnormal glandular morphogenesis as, in some cases, a population of undifferentiated cells could be seen streaming into the stroma from the surface epithelium, forming glandular structures. The epithelial nature of these structures was confirmed by immunocytochemistry; it was found that they stained positively for cytokeratin 7, an epithelial cell marker, but not with CD34 which stained the endothelial cells of blood vessels.
Conclusions: These findings indicate that the glands in the lesion show evidence of a biochemical and morphological delay in maturation. This might be indicative of progesterone resistance, as the glands do not appear to be responding to the increase in the levels of progesterone that normally occur in the second part of the cycle, after ovulation. In some cases there was also evidence of glandular neogenesis and the appearance of an undifferentiated population of cells that had invasive properties and showed signs of a possible epithelial-mesenchymal transformation.
Funded by: NIH grant HD 40093 (to ATF)

FREE COMMUNICATION

Session 70: Laboratory procedures / IVM

Wednesday, 4 July 2007 **14:00–15:15**

O-273 Oral Morphological integrity of human sperm nuclei and blastocyst formation after intracytoplasmic morphologically selected sperm injection (IMSI)

M. Bach[1], A. Neyer[1], A. Stecher[1], P. Uher[2], P. Vanderzwalmen[1], M. ZIntz[1], N. Zech[1]
[1]Institut für Reproduktionsmedizin und Endokrinologie, IVF, Bregenz, Austria, [2]Institut für Reproduktionsmedizin und Endokrinologie, IVF, Pilsen, Czech Republic

Introduction: Recent publications have demonstrated that selection of spermatozoa at high magnification is positively associated with pregnancy rates after day 3 embryo transfers for couples with previous failures of implantation. The aim of our work is to confirm (a) the usefulness of the IMSI procedure over the ICSI in terms of embryo development to day 5 and (b) to analyse if the existence of vacuoles in the nuclei of spermatozoa was associated with the ability of the embryos to develop to the blastocyst stage.
Material and Methods: The study included couples with at least 2 previous failures of implantation (2 to 5) after ICSI. For IMSI, spermatozoa were selected at magnifications ranging from 6000 to 12500 X under a Normarski interferential inverted microscope (Leica AM6000 Germany) equipped with a variable zoom lenz (HC VarioC-mount Leica). After sperm injection the oocytes were cultured to day 5 in Global medium (Lifeglobal). In order to classify and correlate the morphological status of the nuclei of spermatozoa with the development of the embryo, a picture of the selected spermatozoa was recorded at high magnification. The spermatozoa were classified in 4 groups. Gr I consists of spermatozoa free of any abnormalities. When no grade I spermatozoa were available for injection, spermatozoa were classified as Gr II with a maximum of two small vacuoles ($<$ 4% head surface) and normal shape, Gr III was defined with the presence of at least one large vacuole ($>$ 4%) and normal shape and Gr IV was characterized by the presence of large vacuoles and abnormal head shapes. For ICSI, spermatozoa were selected at magnification 400 X.
Results: From the first study (a), a total of 282 MII oocytes (19 oocyte retrievals) were selected and injected with ICSI (n= 151) or with IMSI (n=131) procedures. No difference in the fertilization (ICSI 72.2% IMSI 70.2%) and number of embryos that cleaved to day 3 (ICSI 79.5% IMSI 81.7%) were noticed. On day 5, as compared to ICSI, IMSI provided a significant higher proportion of blastocysts (ICSI 27.5% vs IMSI 52.0% $P< 0.001$) and good quality blastocysts, showing expanded blastocoele and well defined inner cell mass and trophectoderm (ICSI 12% IMSI 26% $P< 0.01$)

In the second study (b), a total number of 307 spermatozoa were selected after observation at 12500 X. Only 5.0% of them were free of any abnormalities,