Trial registration number: Not available

P-218 Transfer of frozen/thawed embryos: pregnancy comparison between blastocysts and cleavage stage embryos

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Study question: To compare the pregnancy rate in patients submitted to FET (Frozen Embryos Transfer) of cleavage stage embryos (Group 1), or at the blastocyst stage (Group 2).

Summary answer: Our data showed that the transfer of frozen blastocysts has a better pregnancy rate than cleavage embryos.

What is known already: The first birth of a baby arising from a cryopreserved embryo occurred in 1983. Since then, improvements in embryo cryopreservation have occurred. However since early 90's a vitrification technique has also been developed to cryopreserve embryos in any stage of development.

Study design, size, duration: Retrospective cohort study during 2012, with a total of 132 cycles.

Participants/materials, setting, methods: In the group 1 the embryos had been frozen on the 2nd, 3rd or 4th day after fertilization, while in the group 2 the embryos were frozen on 5th or 6th day at blastocyst stage. All embryos were cryopreserved by vitrification followed Kuwayama et al, 1998 protocol. The pregnancy rates were compared through the chi-square test (p < 0.05).

Main results and the role of chance: The Group 1 consisted of 74 patients, which pregnancy and clinical pregnancy rate were 27.0% (20/74) and 21.6% (16/74), respectively. The Group 2 had 58 patients, and pregnancy and clinical pregnancy rate were 50.0% (29/58) and 43.1% (25/58) respectively. There was statistical difference between the groups (P = 0.0107). The mean age were 34.8 and years old for groups 1 and 2 respectively.

Limitations, reason for caution: The study was conducted during a short period of time.

Wider implications of the findings: This result suggests that the transfer of embryos at the blastocyst stage would be the best choice for IVF clinics.

Study funding/competing interest(s): There were no competing interests in this study.

Trial registration number: Not Available

Endometriosis, endometrium, implantation and fallopian tube

P-219 Down-regulation of dipeptidyl peptidase 4 under hypoxia could enhance endometrial stromal cell migration in endometriosis

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Study question: To determine whether hypoxic microenvironment might be a factor in influencing cell migration of endometrial stromal cells in endometriosis

Summary answer: Endometrial stromal cells (ESCs) derived from endometriotic patients could migrate and invade more aggressively than control ESCs under hypoxia and this phenomenon may act through a downregulation of dipeptidyl peptidase 4 (DPPIV/CD26)- involved pathway and enhanced expression of angiogenesis-related genes.

What is known already: Previous reports showed that hypoxia-inducible-factor-1(HIF-1) was significantly higher in endometriotic women. Besides, increased expression of focal adhesion kinase (FAK) in endometriotic tissue also alluded to altered cell motility in endometriosis, yet there is little known about the relationship between hypoxia and cellular migration in the pathogenesis of such lesions.

Study design, size, duration: ESCs were isolated from endometrial curettage samples obtained from women with endometriosis (Stage IV AFS, n = 3) or other benign gynaecological disease (Stage 0 AFS, n = 3) undergoing laparoscopic surgery.

Participants/materials, setting, methods: Cells were exposed to hypoxia (2% O2) and assayed for motility with Oris Pro Cell migration/ invasion assay. Total DNA was extracted for PCR Array Human Cell Motility (SA Biosciences). DPPIV/CD26 expression was determined by flow cytometry whereas conditioned medium was applied to Human Angiogenesis Antibody Array (R&D Systems).

Main results and the role of chance: Endometriotic ESCs could migrate and invade through collagen gel (p < 0.005) more under hypoxic condition as compared to ESCs derived from healthy patients. PCR array revealed down-regulation of migration inhibitors (Fibroblast activation protein (FAP), DPPIV/CD26) in patient ESCs under hypoxia and was confirmed via flow cytometry (normoxia: 30.62% vs hypoxia: 12.37%; p = 0.004) Protein array studies showed that angiogenesis-related genes such as TIMP-1, angiogenin and IGFBP-3 was aberrantly upregulated in endometriotic cells when being exposed to hypoxic environment. This could imply that ESCs from endometriosis patients may be enhanced their cell motility under hypoxic microenvironment, leading to up-regulation of migration/ angiogenesis-related genes while keeping the migratory inhibitors at low concentration.

Limitations, reason for caution: One limitation is the ability to test downstream gene expression of DPPIV/CD26 pathway. Since DPPIV/CD26 is proven to modulate Stomal Cell Derived Factor-1 (SDF-1 / CXCL12) and its receptor, CXCR4, inhibiting DPPIV/CD26 may be possible to test whether DPPIV/CD26 down-regulation of endometriosis is SDF-1 / CXCL12-CXCR4 axis dependent.

Wider implications of the findings: This is the first study to show a statistically significant difference in DPPIV/CD26 expression associated with this disease. DPPIV/CD26, a membrane-bound extracellular peptidase, is found to be able to cleave CXCL12 and thereby immobilize hematopoietic stem and progenitor cell (HSCs/HPCs) populations. Overexpressing DPPIV/CD26 to modulate SDF-1 / CXCL12 and their subsequent chemotactic activity may represent new targets for novel therapies in women with endometriosis.

Study funding/competing interest(s): The authors declare no competing financial interests.

Trial registration number: N.A.

P-220 Inhibition of annexin A2 by prostaglandin E2 results in reduced phagocytic ability of peritoneal macrophages and contributes to the development of endometriosis

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Study question: Is annexin A2 involved in the reduced phagocytic ability of macrophages in endometriosis?

Summary answer: Data from women with endometriosis and a murine model of the disease show that expression of annexin A2 in peritoneal macrophages is inhibited by prostaglandin E2 (PGE2) and this impairs the phagocytic ability of macrophages.

What is known already: Endometriosis is a chronic inflammatory disease that recruits many immune cells, especially macrophages, to the peritoneal cavity. The phagocytic ability of peritoneal macrophages isolated from women with endometriosis is reduced.
Study design, size, duration: A laboratory study. Thirty-five patients (20 with and 15 without endometriosis) of reproductive age with normal menstrual cycles were recruited.

Participants/materials, setting, methods: Peritoneal macrophages isolated from women with or without endometriosis were cultured and treated with vehicle, PGE\textsubscript{2} and different EP receptor agonists, and the expression of annexin A2 was quantified by RT–PCR and western blotting. Annexin A2 was knocked down (by small interfering RNA) in normal macrophages or overexpressed (by treatment with recombinant protein) in endometriotic macrophages and their phagocytic ability was measured by flow cytometry. Peritoneal macrophages were isolated from a mouse model of endometriosis and treated with PGE\textsubscript{2} or cyclo-oxygenase (COX) inhibitors, and annexin A2 mRNA was quantified.

Main results and the role of chance: Levels of annexin A2 were markedly reduced in peritoneal macrophages from women with endometriosis versus controls (mRNA: \( P < 0.01 \)). The level of annexin A2 mRNA in the macrophages was reduced by PGE\textsubscript{2} (\( P > 0.01 \) or \( < 0.05 \) in women without endometriosis versus control) via the EP2/EP4 receptor-dependent signaling pathway. Treatment with PGE\textsubscript{2} or knockdown of annexin A2 inhibited the phagocytic ability of macrophages (\( P < 0.05 \) versus control), while treatment with annexin A2 recombinant protein enhanced phagocytosis. Autologous transplantation animal studies further confirmed that levels of annexin A2 in peritoneal macrophages were markedly reduced in mice treated with PGE\textsubscript{2} (\( P < 0.01 \) versus control). In contrast, treatment with COX inhibitors to inhibit PGE\textsubscript{2} production enhanced annexin A2 expression in peritoneal macrophages (\( P < 0.05 \) versus control).

Limitations, reason for caution: We have provided no direct demonstration that phagocytic activity is indeed decreased in peritoneal cells from patients with endometriosis or that their endometriotic fluid contains increased amounts of PGE\textsubscript{2} when compared with control subjects.

Wider implications of the findings: Inhibiting PGE\textsubscript{2} signaling, in order to restore or enhance the phagocytic capability of macrophages, may represent a new direction of thinking in developing novel strategies against endometriosis.

Study funding/competing interest(s): This work was supported by grants from the Chang Gung Memorial Hospital, Taiwan, Republic of China (CMRPG8A0531) to P.-C.C., and grants from National Science Council of Taiwan, Republic of China (NSC97-2314-B-006-020-MY3) to M.-H.W. and (NSC98-2320-B-006-026-MY3) to S.-J.T. None of the authors have any conflicts of interest.

Trial registration number: undefined

P-222 Functional analyses of biomarkers of human endometrial receptivity under natural cycle

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Study question: To date, numerous genes or proteins have been identified as biomarkers of human endometrial receptivity to improve the clinical outcome under \textit{in vitro} fertilization (IVF) conditions. However, the determination of the function(s) of these biomarkers remains indispensable to the understanding of the regulation cascade controlling human endometrial receptivity.

Summary answer: Using transcriptomic and proteomic technologies, we identified pertinent biomarkers of endometrial receptivity. The over-expression of one candidate, during the implantation window, has been validated in independent fertile patients. This biomarker is mainly expressed in epithelial cells and plays probably a central role in the acquisition of the receptive endometrial phenotype.

What is known already: Numerous biomarkers of human endometrial receptivity have been previously reported. However, few studies have been performed to identify their role(s)/function(s) during the implantation window.

Study design, size, duration: We performed the gene and protein expression profiles between pre-secretory and secretory stages from the same patients (n = 9) during natural cycles. New biomarkers have been selected for functional analyses by loss of function using primary endometrial cell culture isolated from endometrial biopsies performed during the implantation window of fertile patients.

Participants/materials, setting, methods: DNA and proteins content were simultaneously extracted from each endometrial biopsy. Gene and protein expression profiles of LH + 2 and LH + 7 were analyzed by DNA microarray chips and SELDI TOF respectively. One biomarker over-expressed during the implantation window (at mRNA and protein level) was selected for functional analyses by shRNAs.

Main results and the role of chance: Western blot analyses of the selected biomarker, belonging to the S100 protein family, in independent samples (pre-
receptive and receptive samples from fertile patients) confirmed the over-expression of the candidate during the implantation window. Immunofluorescence staining of sections from paraffin-embedded endometrium, as well as western blot analyses of purified epithelial and stromal cells, revealed a localization mainly epithelial of the candidate. An approach by loss of function via the use of shRNA (3 shRNAs/candidate, vector pLKO.1-puro-CMV-tGFP) was used in the target cellular type. The obtained phenotypes were analyzed in regard of the morphology, proliferation, survival/death, migration and the decidualization.

Limitations, reason for caution: Validation of the over-expression of the selected candidate must be performed in a large cohort of fertile patients.

Wider implications of the findings: This study should open new perspectives in the understanding of molecular mechanisms regulating human endometrial receptivity.

Study funding/competing interest(s): This work was partially supported by a grant from the Ferring Pharmaceutical Company. The authors of the study have no competing interests to report.

Trial registration number: Not applicable

P-223 Pale cells might be involved in the development of adenomyosis

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Study question: Are there any ultrastructural differences of the basal endometrial glands and stroma in uteri with and without adenomyosis?

Summary answer: The basal layer of the endometrium of adenomyosis patients did not show micro-rupture. However the morphology of the endometrial cells seems to be affected. Abnormal ultrastructure of the basal endometrial glandular cells could reflect abnormal behaviour. Pale cells seem to invade into the myometrium and develop into adenomyotic lesions.

What is known already: Adenomyosis develops after microscopic tissue injury in the endometrium secondary to iatrogenic injury or uterine hyperperistalsis. Such hyperperistalsis may induce a cascade of multiple enzymatic reactions, leading to an increased estradiol synthesis, which induces in turn more hyperperistalsis, promoting dislocation of the basal endometrium into the underlying myometrium. Cellular transformation follows and adenomyotic foci develop.

Study design, size, duration: In a prospective study 20 uteri of women with and without adenomyosis were obtained during laparoscopically-assisted vaginal hysterectomy, done at Charité Universitätsmedizin in the period from 2012 to 2013. Specimens obtained were histopathologically examined.

Participants/materials, setting, methods: The anterior, posterior uterine wall and fundus at the midline, at the level of the fallopian tubes were obtained from 10 adenomyosis and 10 non-adenomyosis uteri from premenopausal patients and were examined using light and transmission electron microscopy and immuno stained for E-cadherin and Transforming Growth Factor beta Receptor 3(TGFBR3).

Main results and the role of chance: The basal endometrial epithelial cells in adenomyosis uteri exhibited infolding of the nuclear membranes, in contrast to smooth one in non-adenomyosis uteri.

The electron microscopical analysis reveals the presence of morphological abnormal cells, eccentrically positioned in the basal endometrial glands. Some pale cells were enriched in mitochondria and ribosomes, suggesting their role as endometrial stem cells. Other were full of variable vacuoles, which may represent apoptotic epithelial cells.

Both groups showed DESosomes between the epithelial cells, this finding was confirmed by equal E-cadherin expression. The “Pale cells” may lack these DESosomes.

The straight interface between the basal endometrium and inner myometrium in adenomyosis uteri is lost.

No significant difference in TGFBR3 expression either in the glands or stroma in both groups was found.

Limitations, reason for caution: Marginal acquisition of uteri with histological proven adenomyosis. Accurate orientation and immediate fixation of the specimens (less than 10 min).

The effect of different phases of the menstrual cycles and the hormonal contraception on the endometrial basal glands.

Wider implications of the findings: Pale cells have been described before in peritoneal endometriotic lesions. We suggest that these pale cells may be forced by uterine hyperperistalsis either through the Fallopian tubes to the peritoneal cavity and initiate endometriosis, or forced into the myometrium and initiate adenomyosis. Furthermore, the presence of desmosomes suggests the lack of microdisruption in the junctional layer. In addition, nuclear membrane infolding, like intestinal cell, may reflect abnormal metabolism.

Study funding/competing interest(s): The study is funded by Bayer pharmaceuticals and a scholarship from Ernst Schering foundation.

Trial registration number: Basic Science

P-224 The frozen-thawed blastocyst transfer at 4 days after ovulation present an effective method in the natural cycles


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Study question: Would the frozen-thawed blastocyst transfer (FT-BT) in natural cycles on 4 days after ovulation affect the IVF outcome in assisted reproductive technology?

Summary answer: The result showed FT-BT in natural cycles of 4 days after ovulation might be the equivalent to that of 5 days after ovulation for the pregnancy rate.

What is known already: Because synchronization between embryo and endometrial receptivity is a major consideration in preparation for FT-BT, FT-BT usually has been performed at 5 days after ovulation. Although there is conjecture concerning the implantation window that follows between six to eight days after luteinizing hormone surges, there have been few reports or little investigation about the timing of BT-FT, especially FT-BT at 4 days after ovulation.

Study design, size, duration: This is a retrospective study of IVF outcomes between April 2003 and November 2012 at the Advanced Fertility Center of Fuchu Nozomi, based in Izumi Fuchu, Osaka. 888 women who were up to the age of 43 at the time of oocyte retrieval underwent WT-BT with natural cycle.

Participants/materials, setting, methods: FT-BT was performed from 4 to 5 days after ovulation. Participants were divided into two groups according to the timing of FT-BT: Day4-BT cycles; FT-BT at 4 days after ovulation and Day5-BT cycles; FT-BT at 5 days after ovulation. Statistical analyses were carried out by t-test or fisher’s exact test.

Main results and the role of chance: The two groups were comparable with respect to their characteristics and reproductive history. The two groups, respectively the Day4-BT cycles and the Day5-BT cycles, were homogeneous for the age of the patients at the time of cryopreservation (mean age 34.6 ± 0.4 vs 34.9 ± 0.1), based FSH levels (6.9 ± 0.2 vs 6.7 ± 0.2), the number of previous oocyte retrieval attempt (2.05 ± 0.15 vs 1.98 ± 0.04), numbers of embryos transferred (1.01 ± 0.01 vs 1.04 ± 0.01) and endometrial thickness (9.1 ± 1.2 vs 9.91 ± 0.05mm). Pregnancy and miscarriage rates were 47.8% (43/90), 7.0% (3/43) in the Day4-BT cycles and 39.0% (557/1428), 21.7% (121/557) in the Day5-BT cycles, respectively. A significant difference was observed only in the miscarriage rate (p < 0.05).

Limitations, reason for caution: The major drawback of our study was the retrospective design. Miscarriage rates in Day4-BT cycles were significantly lower than in Day5-BT cycles. However the number of Day4-BT cycles were not enough to conclude that miscarriages might be prevented by treating FT-BT at 4 days after ovulation. More research is needed.

Wider implications of the findings: Our results suggest that FT-BT dates can be adjusted from 4 to 5 days after ovulation. And it is useful in a clinical manner because the day of FT-BT in natural cycles could be chosen on 4 or 5 dates after ovulation according to the patients personal wishes or situations while in consultation within the clinic.
Study funding/competing interest(s): Nothing.
Trial registration number: Nothing.

P-225 Luteal phase support has a beneficial effect on natural frozen-thawed embryo transfer cycles
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Study question: In natural frozen-thawed embryo transfer (FET) cycles, does luteal phase support (LPS) with progesterone improve cycle outcomes?

Summary answer: Vaginal progesterone gel supplementation significantly improves pregnancy rate in natural frozen-thawed embryo transfer (FET) cycles.

What is known already: Luteal phase support (LPS) with progesterone improves outcomes in fresh controlled ovarian hyperstimulation (COH) in vitro fertilization (IVF) cycles and high dose progesterone supplementation in hormonally controlled FET cycle has resulted in higher clinical pregnancy rate. However, there is no consensus about the possible benefit of LPS in natural FET cycles.

Study design, size, duration: This retrospective cohort study included 432 women undergoing FET in natural cycles without hCG between Jan 2010 and Dec 2012.

Participants/materials, setting, methods: By ultrasound examination and monitoring of serum hormone levels, exact ovulation day was assessed. The cryopreserved embryos were transferred 3 days after ovulation. One hundred patients (Group A) received daily vaginal progesterone gel starting from the day of embryo transfer and 332 patients (Group B) did not receive it.

Main results and the role of chance: In each group, age, AMH level, number of transferred embryos and percentage of top quality embryos reflected no differences. Pregnancy rate was significantly higher in the group that received progesterone than in the other group [41.0% (41/100) vs. 29.2% (97/332), P = 0.013]. There was no significant difference between two groups in the spontaneous abortion rate. Ongoing pregnancy rate was also significantly higher in LPS group [40.0% (40/100) vs. 28.3% (94/332), P = 0.009].

Limitations, reason for caution: This is a retrospective study. A prospective randomized study would have minimized potential limitations.

Wider implications of the findings: In general, endogenous production of progesterone is sufficient to support implantation in a natural cycle of fertile women. In this case additional progesterone does not change IVF outcomes. In our study, pregnancy rate was improved by LPS in natural FET cycle, it is probably because the women who undergo IVF cycles are often subfertile, and they may have suboptimal progesterone level. Therefore, even though in natural cycle clinicians should consider LPS to women undergoing FET.

Study funding/competing interest(s): None
Trial registration number: None

P-226 The interaction between decidualized stromal cells and trophoblast stimulates expression of metalloproteinases to regulate trophoblast invasion in a novel three dimensional (3D) human implantation model

Study question: We investigated the expression and functions of molecules critically involved in the invasion of trophoblast cells to the endometrial stroma using an in vitro human model.

Summary answer: Results showed that decidualization conditions modulated the gene expression of matrix metalloproteinase protein 9 (MMP-9) by trophoblast cells, and its inhibitor TIMP-1 by the stromal cells, and facilitated trophoblast stromal-matrix invasion.

What is already known: Successful implantation requires highly complex and orchestrated interactions between the maternal endometrium and the embryo. A dynamic crosstalk between the trophoblast cells and the endometrial decidualis is crucial for the initiation of implantation. However, the functional role of previously identified key molecules involved in human implantation is not clear.

Study design, size, duration: This was an in vitro controlled study using human established cell lines: Jar cells to mimic trophoblast in co-cultured monolayers and immortalized human endometrial stromal cells in monolayers and also embedded in an agarose-fibrin matrix to construct a novel 3D human endometrial culture system.

Participants/materials, setting, methods: Stromal cell decidualization was promoted with E2 (10⁻⁸ M) and MPA (10⁻⁶ M) for 7 days. Gene expression of MMP-9, TIMP-1, and prolactin was examined by real time PCR. The invasion depth of Jar spheroids into the 3D stromal matrix was analyzed with Z-images captured by confocal laser scanning microscopy.

Main results and the role of chance: Stromal cell decidualization was confirmed by significantly enhanced prolactin gene expression on day 7 of E₂ plus MPA treatment (P < 0.05). Decidualized stromal-trophoblast interaction also enhanced the expression of prolactin (by stromal cells), suggesting that their interaction may synergize the implantation signals. Decidualization significantly increased the expression of MMP-9 in Jar cells (P < 0.05) and of TIMP-1 in stromal cells (P < 0.05). Analyses of Z-stack confocal demonstrated that Jar spheroids invaded into the 3D matrix in response to decidualization signals.

Limitations, reason for caution: It remains to be established whether these in vitro results may reflect the in vivo scenario at the time of implantation, and if they can be recapitulated in a 3D model with primary human endometrial cells.

Wider implications of the findings: Taken together, the data demonstrate that interactions of stromal cells and the trophoblast can modulate the balance between MMPs and TIMPs to set up new conditions to favor invasion. The newly developed 3D human implantation model provides a unique model for studying interactions between trophoblast and stromal cells, via soluble-paracrine signals as well as through cell to cell interactions, and is a useful tool to study molecular mechanisms of early embryo implantation.

Study funding/competing interest(s): Financial support for this study was provided by a Serono endowment of the Jones Institute Foundation.
Trial registration number: Not applicable

P-227 Comparison of GnRH agonist and antagonist protocols in stage III-IV endometriosis patients who underwent endometrioma resection surgery
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Study question: Do protocols, namely gonadotropin-stimulating hormone (GnRH) agonist and antagonist, that are utilized to achieve controlled ovarian hyperstimulation (COH) in in vitro fertilization IVF treatment affect the IVF outcomes in stage III-IV endometriosis patients who underwent endometrioma (>4cm in size) resection surgery?

Summary answer: GnRH agonist and antagonist protocols both present similar IVF outcomes in patients with severe endometriosis who underwent endometrioma resection surgery. GnRH agonist protocol may lead to higher number of MII oocytes and embryos, which can be cryopreserved.

What is already known: Both endometrioma and endometrioma resection surgery can have detrimental effects on the outcome of IVF as far as ovarian response and pregnancy rates are concerned. In literature, this was the first study to compare GnRH analog and antagonist protocols in infertility patients with specifically large endometriomas (>4cm).

Study design, size, duration: A total of 88 intra-cytoplasmic sperm injection (ICSI) cycles in 88 patients who underwent laparoscopic resection surgery for endometrioma in an university hospital setting between January 2000 and January 2010 were included in this retrospective study. 88 patients were selected from 610 patients, which matched our inclusion criteria.

Participants/materials, setting, methods: Endometrioma was detected by vaginal ultrasound and confirmed by the pathologic examination of cyst wall extracted during laparoscopy. Stage of endometriosis was confirmed during laparoscopy. The patients were divided into two groups according to the analogue that they received: 44 patients received GnRH agonist protocol; 44 patients received antagonist protocol.

Trial registration number: Not applicable
Main results and the role of chance: The number of follicles on hCG day (12.68 ± 7.09 versus 8.44 ± 6.09; p < 0.001), duration of hyperstimulation 11.00 ± 2.13 versus 9.76 ± 1.98; p < 0.001), number of follicles (12.68 ± 7.09 versus 8.44 ± 6.09; p < 0.001), number of retrieved MII oocytes (8.93 ± 5.43 versus 5.25 ± 5.1; p < 0.001), total number of grade 1 embryos (5.82 ± 3.00 versus 2.65 ± 2.14; p < 0.001) were higher in the GnRH agonist protocol. There were no significant differences in positive β-hCG pregnancy rates (25% versus 20.4%; p = 0.269) and ongoing pregnancy rates per patient (20.4% versus 18.1%; p = 0.302) between the two protocols.

Limitations, reason for caution: The selection of our study population was based on a surgical diagnosis. Women with an asymptomatic form of endometriosis are therefore not included in our study.

Wider implications of the findings: In the present study, GnRH agonist protocol revealed increased numbers of oocytes and embryos, so the patients who received this protocol could have a chance to cryopreserve and transfer embryos later. Additionally, cryopreserved embryo transfer might have an advantage for women with endometriosis, since ovarian hyperstimulation, which may potentially activate the endometriosis, is not required.

Study funding/competing interest(s): None.

Trial registration number: N/A.

P-228 A previous cesarean section does not impair IVF or intratubal insemination results

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Study question: To assess the influence of a previous cesarean section on pregnancy rate (PR) in IVF and intratubal insemination (IUI).

Summary answer: A previous cesarean section does not impair IVF or IUI results.

What is known already: Although there is universal agreement on the importance of uterine/endometrial factor in assisted reproduction, the importance of uterine scars (mainly cesarean section and myomectomy) have received little attention. A number of reports have studied the influence of myomas/myomectomy, but the impact of cesarean scar has been not analyzed in IVF/IUI.

Study design, size, duration: Retrospective study. All the ART cycles were performed at our center from 2007 to September 2012 where there was a history of previous newborn delivery. 3451 cases were revised.

Participants/materials, setting, methods: Pregnancy rates were compared between patients with a previous vaginal delivery (n = 2247) and patients with a previous cesarean section (N = 1204). The following subgroups were performed:

Group A: IVF (N = 563 and 1058), Group B: oocyte donation (N = 356 and 689), frozen embryo transfer (N = 75 and 104), Group C: IUI (N = 210 and 395) respectively.

Main results and the role of chance: Clinical PR were very similar in the previous cesarean section group and in the previous vaginal delivery group: 46.34% and 48.45% in IVF (Group A), 59.32% and 58.63% in oocyte donation (Group B), 18.1% and 16.4% in artificial insemination (Group C). When corrected for maternal age, somewhat higher in previous cesarean section group (34.9 ± 7.2 vs 33.7 ± 7.8), no significant differences were observed.

Limitations, reason for caution: Our study was not randomized and clinical characteristics of patients with a previous cesarean section or a previous vaginal delivery could not be the same. However it seems that characteristics of the cesarean group would be not better that those in previous vaginal delivery.

Wider implications of the findings: Although cesarean section is associated with a number of short, mean and long term consequences, embryo implantation is not negatively affected by a previous cesarean section scar.

Study funding/competing interest(s): None.

Trial registration number: NCT0150863.

P-230 Prevalence and pelvic distribution of deeply infiltrative endometriosis (DIE) in 1141 consecutive infertile Brazilian women evaluated with transvaginal ultrasound after bowel preparation

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Study question: Does OCP have any impact on endometrial receptivity when is used for cycle programming?

Summary answer: OCP could be used for IVF cycles scheduling without any significant effect on the expression of endometrial gene related to implantation.

What is known already: Cycle scheduling is common in IVF units, in order to avoid weekend retrievals and/or to evenly distribute workload with a better efficiency and better results.

A recent meta-analysis showed that OCP pretreatment might be detrimental for live birth rate. We performed a RCT to assess the effect of OCP for cycle scheduling on pregnancy rate on women undergoing IVF-ICSI with the antagonist cycle, and comparing it with the long-agonist protocol no significant differences were found.

Study design, size, duration: For this prospective cohort study, customized microarray ERA™ data were obtained from endometrial biopsies of 20 egg donors undergoing ovarian stimulation that were randomized to four different protocols of oocyte maturation and luteal phase support. Comparisons were performed by using parametric test. Fold change ± 2 was considered significant (p < 0.05).

Participants/materials, setting, methods: In groups 1 and 2, final oocyte maturation was induced with GnRH agonist (GnRHa) 0.2 µg and received estradiol 6 mg/day p.o + progesterone 400 mg/12h vaginally for luteal phase support. Groups 3 and 4, received recombinant hCG 6500 IU for triggering and only used progesterone 200 mg/12h vaginally. In groups 1 and 3, OCP pretreatment was used, while stimulation in groups 2 and 4 was started on cycle day 3. Endometrial biopsies were collected 7 days after triggering.

Main results and the role of chance: Protocol 1 showed an up-regulation of 9 gene (TMEPA1, CRABP2, DHR35, IMPA2, SLC15A1, IGF2, MAFP2, ANG and KIAA0802) and down-regulation of 6 (BIRC3, STEAP4, OLFM4, GDF15, KIF20A, ADAMTS8) compared to its control without scheduling, group 2. In group 3, an increased expression of 11 (TH, ALPL, PAQR4, CTNNA2, OLFM4, NDRG2, GALNT12, SERPINA5, CRISP3, KCNJ2, AMIGO2) and decreased gene expression of 4 gene (GZMA, C14orf161, TAGLN, RARRE3) were observed compared to its control without OCP programming, group 4.

No one of these gene showed a fold change > ± 2 and adjusted p-value < 0.01, therefore differences in gene expression associated to endometrial receptivity were not significant.

Limitations, reason for caution: Inter-individual variations could be found but sample size was adequate for molecular analysis.

Wider implications of the findings: Cycle scheduling has some benefits associated, helping to avoid weekend retrievals and equally distribute the workload throughout the week. Controversy has arisen regarding a hypothetical negative impact of OCP pretreatment for scheduling on IVF cycles. By studying gene expression on endometrial samples, we confirmed that OCP used for cycle programming does not have any effect on endometrial receptivity gene expression, regardless if a GnRHa or hCG was used for final oocyte maturation.

Study funding/competing interest(s): Economical support for this study was provided by an unrestricted grant from MSD.

Trial registration number: NCT0150863.

P-229 Impact of oral contraceptive pill (OCP) for cycle scheduling on gene expression related to endometrial receptivity

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Study question: Does OCP have any impact on endometrial receptivity when is used for cycle programming?

Summary answer: OCP could be used for IVF cycles scheduling without any significant effect on the expression of endometrial gene related to implantation.
Study question: To determine the prevalence and the extension of DIE in a large population of infertile women who were unaware of having endometriosis. Further, to demonstrate themost frequent affected pelvic sites.

Summary answer: In this large series of infertile women, DIE was present in 72.7% of the patients. The posterior compartment was the most common area affected by pelvic endometriosis. The addition of the DIE lesions located in the posterior compartment to the intestinal disease make up for 75.4% of all diagnosed DIE.

What is known already: Clinical presentation of DIE includes dysmenorrhea, dyspareunia, dyschezia, and infertility. DIE typically appears as a nodular mass or an irregular thick, fibrotic reaction which arecharacterized by a difficult and delayed diagnosis. TVSBP allows for mapping of the pelvic sites affected like bladder, vesicouterine pouch, round ligaments, retrocervical space, vagina, ureters and rectosigmoid colon. TVSBP has been used by several radiologists and gynecologists as the first-line imaging for the evaluation of women with suspected endometriosis.

Study design, size, duration: Prospective observational study including 1141 consecutive infertile women. All patients were routinely submitted to TVSBP by the same radiologist regardless of the medical history and pelvic examination findings from 08/2010 to 12/2012. All women signed approved informed consent.

Participants/materials, setting, methods: Women aged 19-44 years old with infertility length varying 1-5 years. Bowel preparation was accomplished with the use of a low-residue diet, oral laxative and rectal enema 1 hour before the examination. Transvaginal US was performed with a Voluson E-8 and 5–9-MHz transducer at a Radiological Clinic.

Main results and the role of chance: Absence of DIE in 311 women (27.3%). Presence of DIE in 72.7% of women. Distribution according to the number of lesions: 1(347-30.5%); 2(181-15.8%); 3(140-12.3%); 4(97-8.5%); 5(48-4.4%); ≥6(5-3.3%). DIE presence distributed as compartments: anterior (237-20.8%); posterior(594-52.6%); endometriomas (179-15.7%); and intestinal (131-20.2%).

Limitations, reason for caution: This is a descriptive study with only short term follow up of the primary human endometrial cells in vitro.

Wider implications of the findings: The surface marker SSEA1 enriches for an epithelial endometrial cell subpopulation from the basalis. Since the functional endometrium originates from these cells, it is now possible to study basalis epithelium for stem/progenitor cell activity to extend our current understanding of endometrial biology in health and diseases.

Study funding/competing interest(s): The work included in this manuscript was funded by Wellbeing of Women project grant RGI073 (DK1 & CG). The authors declare that they have no competing interests.

Trial registration number: Not applicable (REC references; 09/H1005/55 and 11/H1005/4)

P-232 Soluble fibronectin enhances trophoblast cell migration by epithelial-mesenchymal transition in a trophoblast stem cell model: possible applications in improving implantation rate in assisted reproductive technology

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Study question: Since successful implantation requires adequate migration of trophoblast cells for invasion into the endometrium and extracellular matrices (ECM) can stimulate cell migration, we aimed to investigate trophoblast cell migration under various soluble ECM proteins to find the ECM protein that maximally enhances trophoblast cell migration and study the underlying mechanisms.

Summary answer: Using trophoblast stem (TS) cells derived from mouse blastocysts, we found that, among various soluble ECM proteins including collagen I, collagen IV, osteopontin, hyaluronic acid and fibronectin, soluble fibronectin maximally enhanced TS cell migration by induction of epithelial-mesenchymal transition (EMT) through activation of integrin-linked kinase (ILK) and Akt.

What is known already: Previous studies have shown that cell migration is enhanced on ECM-coated surfaces through EMT and ECM receptors. ECM proteins may be added in the blastocyst transfer medium to enhance trophoblast cell migration; however, the relative enhancing effects of various ECM proteins on trophoblast cell migration have not been compared and the underlying mechanisms remain to be unraveled.

Study design, size, duration: We first determined the soluble ECM protein that maximally enhanced TS cell migration for subsequent experiments. Then we studied the involvement of EMT in the enhanced trophoblast cell migration by the soluble ECM protein found in the previous step. Finally, the molecular mechanisms mediating soluble ECM protein-induced EMT were explored.

Participants/materials, setting, methods: For cell migration analysis, TS cells derived from mouse blastocysts were used to form spheroids, which were then

P-231 Markers of human endometrial basal glandular epithelial cells: phenotypic and functional characterisation and implications in the pathogenesis of endometriosis

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Study question: Can the epithelial compartments of the human endometrium be defined by specific markers?

Summary answer: Human endometrial epithelial cells from the basalis express nuclear SOX9, cell surface marker SSEA-1 with some cells expressing nuclear β-catenin. Primary endometrial epithelial cells enriched for SSEA-1+ show some features expected of the basalis epithelium in vitro.

What is known already: The endometrial glands of the functionalis regenerate from the basalis gland stumps following menstruation. Endometriosis is thought to originate from abnormal dislocation of the basalis endometrium. In the highly regenerative intestinal epithelium, SOX9 and nuclear β-catenin are more highly expressed in the intestinal crypt, the stem/progenitor cell region. The embryonic stem cell surface marker SSEA-1, can be used to isolate early-differentiating human embryonic stem cells.

Study design, size, duration: A large prospective observational study analysing full thickness human endometrial hysterectomy samples from 315 premenopausal women, 15 post menopausal women, and ectopic endometriotic lesions from 20 women with endometriosis.
cultured in various soluble ECM proteins, followed by measurement of trophoblast outgrowth areas at 144 h. Western blot and appropriate inhibitors were employed to study the involvement of EMT and underlying mechanisms.

**Main results and the role of chance:** Among various soluble ECM proteins including collagen I, collagen IV, osteopontin, hyaluronic acid and fibronectin, soluble fibronectin maximally enhanced TS cell migration. Treatment of TS cells with soluble fibronectin induced morphological changes to mesenchymal types, down-regulation of E-cadherin expression and up-regulation of vimentin expression, indicating EMT. Pre-treatment of TS cells with ILK inhibitor or Akt inhibitor could block fibronectin-induced EMT and spheroid expansion. Moreover, ILK inhibitor could block fibronectin-induced Akt phosphorylation in TS cells. Our results demonstrated that soluble fibronectin maximally enhanced trophoblast cell migration by induction of EMT through ILK-stimulated Akt activation, findings that may be applied to increase implantation rate in ART.

**Limitations, reason for caution:** These findings were based on *in vitro* study using mouse TS cells. Further studies using human TS cells may be performed after ethical issues are settled. Mouse model of recurrent miscarriage (CBA/J female X DBA/2 male) may be utilized to examine the *in vivo* effects of soluble fibronectin on trophoblast invasion.

**Wider implications of the findings:** Since soluble fibronectin significantly enhanced trophoblast cell migration, it may be added in the embryo transfer medium, especially in combination with assisted hatching of the blastocysts, to enhance implantation rate in ART. The role of EMT in enhancing trophoblast cell migration may be applied to search other EMT-inducing molecules that may promote trophoblast migration and invasion.

**Study funding/competing interest(s):** This work has been supported by the National Science Council (NSC 100-2314-B-010-011-) and Taipei Veterans General Hospital (V101C-140). The authors indicate no potential conflicts of interest.

**Trial registration number:** Not applied.

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P-234  **Analysis of the anti-STX5 autoantibody identified as a new serum marker for endometriosis**


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**Study question:** The sensitivity and specificity of CA125, as a sole serum marker of endometriosis, are not high enough for routine clinical assessment. To explore new markers for the diagnosis of endometriosis, serum autoantibodies in endometriotic patients were investigated employing a fibroblast cell line, two-dimensional gel electrophoresis and Western blotting.

**Summary answer:** We conclude that serum anti-STX5 autoantibody, which was discovered by a proteomic approach, is a potential new serum marker for the diagnosis of endometriosis.

**What is known already:** Previous studies have suggested that some autoantibody-reactions are provoked in patients with endometriosis. We have investigated autoantibodies as serum biomarkers for the diagnosis of endometriosis, in the course of this work, we have found anti-α-enolase and anti-PDK1IL autantibodies to be useful.

**Study design, size, duration:** Study design: Retrospective study. Patients: Sixty-nine patients with endometriosis, 38 disease control patients without endometriosis, and 44 healthy volunteers. Setting: Departments of Molecular Pathology and Obstetrics and Gynecology in Ehime University and University Hospital.

**Participants/materials, setting, methods:** Serum autoantibodies in patients were investigated employing a fibroblast cell line, two-dimensional gel electrophoresis and Western blotting. Proteins reacting with Western blotting were identified using MASCOT analysis. ELISAs were prepared using recombinant proteins and titers of serum autoantibodies were determined in the endometriotic patients, disease controls, and healthy subjects.

**Main results and the role of chance:** Western blot analysis revealed a spot that was produced by specific reaction in sera of endometriotic patients; however, no such spot was produced from the sera of healthy controls in preliminary Western blotting experiments. By MASCOT analysis, this spot was syntaxin 5 (STX5). Employing this recombinant, ELISA for estimating serum autoantibodies was established. By the screening ELISA analyses using a panel of sera from patients with endometriosis, disease controls, and healthy subjects. Anti-STX5 autoantibody levels were significantly elevated in endometriotic patients. Sensitivity (53.6%) and accuracy (72.2%) of the serum anti-STX5 autoantibody assay were better than those of serum CA125 levels (36.2% and 62.9%, respectively) for diagnosis. The sensitivity of anti-STX5 autoantibody was markedly high in Stage II (80.0%) compared with that of CA125 (40.0%).

**Limitations, reason for caution:** It is curious that some control subjects possess relatively high anti-STX5 autoantibody levels could be diagnosed with this disorder in the future. A cohort study in healthy subjects should be carried out to address this. Further clinical and basic analyses are needed.

**Wider implications of the findings:** The advantage of the anti-STX5 autoantibody assay for the diagnosis of endometriosis will be its high sensitivity in the
early stages. The sensitivity of anti-STX5 autoantibody appears to be higher than that of CA125. No correlation was seen in the serum levels of anti-STX5 autoantibody and serum CA125. The sensitivity of combined assays improved to 69.6% while keeping the specificity above 70%. Serum anti-STX5 autoantibody can be a novel clinical diagnostic marker for endometriosis.

Study funding/competing interest(s): The authors have no financial conflicts of interest to disclose concerning the presentation.

Trial registration number: This study is not clinical trial.

P-235 Progesterone receptor isoform (PR-B) is strongly expressed in rectosigmoid endometriosis and associated to HOXA-10 expression in the lesion

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Study question: To evaluate the expression of progesterone receptor isoform B (PR-B) and homeobox gene A10 (HOXA-10) proteins in rectosigmoid endometriotic lesion (rsg lesion) and its adjacent muscular tissue (rsg muscular), during both proliferative and secretory phases of the menstrual cycle.

Summary answer: PR-B is strongly expressed in the gland and the stroma of rsg lesion in both phases, while absent in rsg muscular. HOXA-10 is weakly to moderately expressed in the stroma of rsg lesion and rsg muscular in both phases, while absent in gland. PR-B and HOXA-10 expressions correlate positively.

What is known already: PR-B and HOXA-10 expressions were previously evaluated in peritoneal and ovarian endometriosis. In these tissues, PR-B was found to be absent or weakly expressed, while HOXA-10 was weakly expressed. It is suggested that lower levels of PR-B in endometriosis could explain its progesterone resistance. Also, HOXA-10 expression outside its domain would be necessary to impart endometriosis developmental identity in endometriosis.

Study design, size, duration: Retrospective analysis of pathological specimens from 18 patients (9 in proliferative and 9 in secretory phases) who underwent laparoscopic resection of endometriosis, including rectosigmoidectomy, from March 2003 to October 2007.

Participants/materials, setting, methods: Surgeries were performed in Sao Paulo, Brazil. Samples were included in tissue microarray (TMA) construction and immunostaining for the protein. Morphometric analysis was performed by specific computer software. Mean and SD values were estimated. Bonferroni’s comparisons were used to evaluate differences in expressions between sites and menstrual cycle phases.

Main results and the role of chance: Microscopic PR-B expression results were: glandular rsg lesion (8.1 ± 5.1) and stroma rsg lesion (10.2 ± 2.9) in proliferative; glandular rsg lesion (10.3 ± 3.8) and stromal rsg lesion (11.4 ± 1.1) secretory phase. Microscopically, there was no expression of PR-B in rsg muscular. Morphometric results were: rsg lesion (1.5 ± 1.6) and rsg muscular (3.3 ± 5.0) in proliferative; rsg lesion (2.2 ± 2.6) and rsg muscular (4.3 ± 5.4) secretory phase. Microscopic results for HOXA-10 expression were: stromal rsg lesion (2.8 ± 1.6) in secretory and rsg muscular (0.7 ± 1.1) secretory phase. There was no expression of HOXA-10 in glandular or proliferative phase. Morphometric results were: rsg lesion (1.2 ± 3.0) and rsg muscular (1.3 ± 2.0) proliferative phase; rsg lesion (0.7 ± 1.0) and rsg muscular (0.8 ± 1.1) secretory phase. PR-B was more expressed in the secretory phase (p = 0.02) and positively associated to HOXA-10 expression (r = 0.008).

Limitations, reason for caution: There is an improbable possibility of computer software error when analyzing glandular and stroma in the rectosigmoid muscular lesions. In this situation, PR-B might be expressed only in rectosigmoid lesion and not in the muscular tissue.

Wider implications of the findings: Different from previous studies, our data support a strong expression of PR-B in endometriosis. HOXA-10 is expressed in rec- tosigmoid endometriosis, which confirms its common expression in different endometriotic lesions. PR-B is present in rsg lesions and absent in rsg muscular, and it is directly associated to HOXA-10 expression in rectosigmoid disease. These findings suggest that progesterone may have a role in imparting the developmental identity to deep endometriosis, an action mediated by the HOXA-10 gene.

Study funding/competing interest(s): None

Trial registration number: Not applicable

P-236 Estrogen receptor beta (ER-β) is strongly expressed in rectosigmoid endometriosis and its adjacent muscular tissue independently of the phase of the menstrual cycle

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Study question: To evaluate the expression of ER-β protein in rectosigmoid endometriotic lesion (rsg lesion) and its adjacent muscular tissue (rsg muscular), during both proliferative and secretory phases of the menstrual cycle.

Summary answer: ER-β is strongly expressed in rsg lesion and rsg muscular layer in both phases. Its expression is independent of the phase and site of expression (lesion or adjacent muscular tissue) in the rectosigmoid.

What is known already: ER-β is expressed in ovarian and peritoneal endometriosis. Its presence in rectosigmoid endometriosis, a typical model of deep disease, was not evaluated before. It is suggested that high levels of ER-β contributes to the estrogen-rich environment and its mitogen action on endometriosis foci. Besides, high levels of ER-β in relation to its counterpart, ER-α, could mediate the estrogen action of suppressing progesterone receptor expression and induce progesterone resistance.

Study design, size, duration: Retrospective analysis of pathological specimens from 18 patients (9 in proliferative and 9 in secretory phases) who underwent laparoscopic resection of endometriosis, including rectosigmoidectomy, from March 2003 to October 2007.

Participants/materials, setting, methods: Surgeries were performed in Sao Paulo, Brazil. Samples were included in tissue microarray (TMA) construction and immunostaining for the protein. Morphometric analysis was performed by specific computer software. Mean and SD values were estimated. Bonferroni’s comparisons were used to evaluate differences in expressions between sites and menstrual cycle phases.

Main results and the role of chance: ER-β was expressed in rsg lesion (8.9 ± 3.9) and rsg muscular (7.8 ± 5.4) in the proliferative phase. ER-β was also expressed in rsg lesion (10.8 ± 8.3) and rsg muscular (6.9 ± 8.7) in the secretory phase. ER-β expression was independent of cycle phase (p = 0.92) and the site of the rectosigmoid (p = 0.13).

Limitations, reason for caution: Immunostaining analyzes are semi-quantitative and subjected to technical variability. To overcome observer variability, it was used a quantitative method (morphometric analysis), nevertheless technical flaws related to the morphometric method may not have been excluded.

Wider implications of the findings: These findings confirm previous results of strong ER-β expression in endometriosis. This is the first study in which rectosigmoid endometriotic lesions were used exclusively as a model for studying deeply infiltrative endometriosis as they accurately represent deep endometriosis. The finding or ER-β expression in the rectosigmoid muscular also suggests that estrogen may have a role in smooth muscle metaplasia that surrounds deep endometriotic lesions.

Study funding/competing interest(s): None

Trial registration number: Not applicable

P-237 Impact of endometriosis and endometriomas on pelvic inflammatory disease after follicular aspiration

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Study question: To assess the influence of endometriomas and endometriosis on the risk of pelvic inflammatory disease after oocyte aspiration.

Summary answer: In the presence of endometrioma larger than 2 cm the pelvic inflammatory risk is 12 times increased compared with the general population, and 5 times increased in endometriosis cases without endometrioma > 2 cm.

What is known already: Pelvic inflammatory disease is a rare complication of IVF. The presence of a previous endometrioma/endometriosis could be a risk factor for PID, but few large series are available.
Study design, size, duration: During a 2 year period a prospective study was designed to analyze the influence of endometrioma/ endometriosis on the PID rate after oocyte aspiration. Among endometriosis patients two subgroups were performed: women with endometrioma > 2 cm (n = 47) and those with smaller endometriomas or with endometriosis but without endometriomas (n = 118). The control group was constituted by 570 consecutive women assisted at the same unit during the same period of time, without endometriosis and without other PID risks (PID history, adnexal mass) (n = 570).

Participants/materials, setting, methods: All the patients were subjected to similar ovarian stimulation protocol and oocyte pick up methodology. Immediately before oocyte aspiration the vagina was washed with large amounts of sterile saline solution, Endometriomas were not subjected to puncture or aspiration. One hour after oocyte aspiration, 1 single dose of azithromycin (1 g) was given. 

Main results and the role of chance: The rate of PID in women with endometrioma > 2 cm was 2.12% (1/47), and of 0.9% (1/111) in women with endometriosis with no endometrioma larger than 2 cm, compared with 0.17% (1/ 570) in the control population. Although the differences were not statistically different PID rate was 12 times higher among the patients with endometriomas > 2cm. On the other hand patients with smaller endometrioma or endometriosis without endometrioma had a 5 time increased risk

Limitations, reason for caution: Although a modest number of participants (n = 38) was investigated, a larger number of patients should be tested. Confounders such as hormonal treatment and regularity of the menstrual cycle were accounted for by stratifying examination of endometriological biopsies. Larger test cohorts are needed to validate endometriosis-associated altered PR expression patterns.

Wider implications of the findings: This study provides a proof-of-concept that endometriotic lesions are in a PR-A dominant state. In addition we found that endometriosis from women with endometriosis is also in a PR-A dominant state. We propose that retrograde menstrual efflux of a PR-A-dominant endometrial tissue into the peritoneal cavity may predispose the development of endometriosis and the subsequent progression of the disease.

Study funding/competing interest(s): This study was supported by grants from the Learner Research Fund to the Division of Reproductive Endocrinology and Infertility, University Hospitals of Cleveland.

Trial registration number: Not applicable

P-238 Abundance and localization of progesterone receptor isoforms in endometrium, peritoneal endometriosis and endometrioma cyst wall

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Study question: What is known already: Progesterone receptor (PR) isoforms, PR-A and PR-B, in endometrium from women with and without endometriosis, and in peritoneal and ovarian endometriotic lesions?

Study design, size, duration: We conducted a cross-sectional study of the PR isoforms in endometrium and endometriotic lesions.

Main results: We found that PR-A and PR-B are abundant in endometrium from women with and without endometriosis, and in peritoneal and ovarian endometriotic lesions. PR-A is the dominant isoform in endometrium, while PR-B is more abundant in endometriotic lesions.

Summary answer: Levels of PR-A and PR-B in endometrium from women with endometriosis differ from levels in women without endometriosis. In all cases of endometriosis, PR-A was dominant, whereas in ovarian endometriosis, PR-B was dominant. These findings provide new insights into the role of PR isoforms in endometriosis and ovarian endometrioma.

P-239 The role of salpingoscopy in assessing the inner fallopian tubes of infertility patients with ovarian endometriom(a)s

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Study question: Can an endometrioma alone affect pregnancy rates for endometriosis-related infertility, or must it be combined with a condition in the inner fallopian tubes?

Summary answer: This study provides evidence that endometriomas may affect pregnancy rates in women with endometriosis.

Study design, size, duration: We recruited 157 infertility patients with unilateral or bilateral endometriomas for the present study. Patients underwent laparoscopic ovarian cystectomy and salpingoscopy. Post-operative patients started infertility treatment as soon as possible, except for ART treatment.

Main results: The average age of the patients was 33.9 ± 3.2 years and 33.9 ± 1.8 months, respectively, and there were no significant differences between the endometrioma and control groups. The average F score for the endometrioma group was 0.40 ± 0.07, with a range of 0 to 3, which was significantly lower than that of the control group (0.96 ± 0.17, p < 0.01). The percentage of the patients with F scores of 0 in the endometrioma group (75.9%) was significantly higher than that of the patients in the control group (125/235 = 53.2%, p <
0.05). The pregnancy rate after conventional treatment for the endometrioma group was 21.7%, and all pregnant patients achieved an F score of less than 2.

Limitations, reason for caution: This was a retrospective analysis.

Wider implications of the findings: In our previous report, the patients with no or small abnormal results in fallopian tubes more easily achieved pregnancy with conventional infertility treatment by comparison with those having many abnormal results inside the fallopian tubes. If salpingoscopy shows that the patients with endometrioma(s) also have abnormal results inside their fallopian tubes, they might not expect to achieve pregnancy via conventional treatment, and should receive ART treatment instead.

Study funding/competing interest(s): The authors have received no funding for this study, and they have no financial interest in any companies. Therefore, there are no competing interests.

Trial registration number: This study dose not have RCT status, and, therefore, it did not receive a trial registration number.

P-240  Intrauterine application of diluted seminal plasma in in vitro fertilization does not improve pregnancy rates - a placebo controlled double blinded randomized trial

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Study question: Does intrauterine application of diluted seminal plasma (SP) at the time of ovum pickup improve pregnancy rates in In vitro Fertilisation (IVF) treatment?

Summary answer: Intrauterine instillation of diluted SP at the time of ovum pickup does not change pregnancy rates in IVF.

What is known already: SP modulates endometrial function in vitro and in animal models. In addition sexual intercourse around the time of embryo transfer increased the number of viable embryos. In contrast, high gonadotropin stimulation affect endometrial function. A previous randomized double blind study demonstrated increased, however not significant, pregnancy rates following intrauterine application of undiluted SP. As this study was not conclusive and as it could have been affected by sexual intercourse, we analysed the intrauterine application of diluted SP.

Study design, size, duration: Monocentric, prospective, double-blinded, placebo controlled randomized trial with 279 women undergoing in vitro fertilization from April 2007 until Februar 2012 at an University Department of Gynaecological Endocrinology and Reproductive Medicine.

Participants/materials, setting, methods: 279 women were randomly grouped to either receive intrauterine diluted seminal plasma from the patients partner (n = 138) or placebo (n = 141) at the time of ovum pickup. The two groups were not different for age, duration of infertility, number of prior in vitro fertilisation, as well as prior implantation failure.

Main results and the role of chance: Of the 279 study participants 40 (14.3%) were excluded, as embryo transfers did not take place, either due to fertilisation failure or ovarian hyperstimulation syndrome and rescue kryopreservation. Of the remaining participants, intrauterine instillation of 138 women with 20% seminal plasma, diluted with saline chloride and of 141 with placebo (sodium chloride) was performed. The clinical pregnancy rates showed no significant difference with 31.1% (38/122) per embryo transfer in the study group and 35.0% (41/117) per embryo transfer in the control group, respectively.

Limitations, reason for caution: According to the previous study, using intracervical SP, a total number of 422 patients would have been needed to prove a significant difference in pregnancy rate of 13%. This number of patients was not analysed as an interim analysis did not reveal any increase in pregnancy rate. A bias in patient recruitment and treatment can be excluded due to the double blind design of the study.

Wider implications of the findings: The study has proven that intrauterine instillation of diluted seminal plasma at the time of follicle aspiration does not increase pregnancy rates. These results question the clinical relevance of an effect of seminal plasma on endometrial receptivity and implantation in humans as well as of sexual intercourse around the time of follicle aspiration in IVF.

Study funding/competing interest(s): no competing interests

Trial registration number: DRKS00004615

P-241  Involvement of hepatocyte growth factor-induced epithelial-mesenchymal transition in adenomyosis

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Study question: Is there any role of hepatocyte growth factor (HGF), an estrogenic growth factor, in epithelial-mesenchymal transition (EMT) in adenomyosis?

Summary answer: HGF may be involved in gland invagination deep into myometrium by inducing EMT at the endo-myoermal interface in adenomyosis.

What is known already: Estrogen is involved in EMT as a mechanistic basis for the metastasis of cancer cells and in the pathogenesis of diffuse adenomyosis.

Study design, size, duration: This is a case-controlled biological research with prospective collection of endometrial tissues/cells from control women and women with focal/diffuse adenomyosis and retrospective evaluation.

Participants/materials, setting, methods: Biopsy specimens were collected from 15 women with adenomyosis and 12 women without adenomyosis. Endometrial epithelial cells (EECs) were isolated in primary culture. The tissue expressions of HGF/E-cadherin/N-cadherin/vimentin and SLUG/SLAIN were examined by immunohistochemistry and confirmed at gene level. Effect of HGF on cellular change and cell migration was examined.

Main results and the role of chance: A dose-dependent cell separation effect of HGF on EECs was observed. The higher E-cadherin gene expression achieved with confluent EECs was suppressed after application of HGF. An inverse immunoexpression between HGF and E-cadherin was observed in gland cells of the basalis endometrium derived from women with both diffuse and ipsilateral side of focal adenomyosis. In contrast, vimentin expression was increased. This effect was lost in control women and in contralateral side of focal adenomyosis. Treatment with HGF changed phenotype of EECs into mesenchymal phenotype in a time-dependent fashion and induced migration of EECs. HGF also up-regulated gene and protein expression of SLUG and SLAIN, two transcriptional repressors of E-cadherin. In addition to estrogen, HGF-induced EMT may be involved in the pathogenesis of adenomyosis.

Limitations, reason for caution: Further multi-center studies with large number of samples, analysis of variable confounding factors, and study on the effect of estrogen suppressing agent on EMT are necessary to strengthen our current findings.

Wider implications of the findings: Still claiming as a multi-factorial disease, our findings may provide some new insights to understand the pathophysiology or pathogenesis of adenomyosis and may have new therapeutic potential.

Study funding/competing interest(s): This study was supported in part by Grants-in-aid for Scientific Research from the Japan Society for the Promotion of Science. There is related to this study.

Trial registration number: not applicable

P-242  Androgens modulate the morphological and biological characteristics of human endometrial stromal cells decidualized in vitro

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Study question: To investigate the direct effects of androgens on the endometrium, focusing the morphological changes and on the oxidative defense responses in decidualizing human endometrial stromal cells (HESCs).

Summary answer: Androgens enhance morphological changes associated with decidualization, including the development and expansion of cytoplasmic organelles and gap junctions, and the expression of prolactin (PRL), a decidual marker. Furthermore, androgens enhance cellular resistance to oxidative stress-induced apoptosis by induction of FOXO1-dependent ROS scavengers, such as SOD2.

What is known already: Endometrial androgen receptor (AR) expression is confined to HESCs. Tissue androgen levels and conversion of androstenedione to testosterone are higher in secretory than in proliferative endometrium. AR in decidualizing HESCs regulate a relatively small but distinct group of genes involved in cytoskeletal organization, cell motility and cell cycle progression.
Study design, size, duration: In vitro experiments on cultured primary HESCs. Participants/materials, setting, methods: HESCs isolated from hysterectomy specimens were decidualized with 8-bromo-cAMP (8-b-cAMP) and progesterone (P4) in the presence or absence of dihydrotestosterone (DHT) at various concentrations. Hydrogen peroxide was used as a source of reactive oxygen species (ROS).

Main results and the role of chance: PRL production was induced in HESCs in response to 8-b-cAMP and P4. DHT further enhanced the secretion of PRL in cells treated with 8-b-cAMP plus P4. The effect of DHT was blocked by the anti- androgen flutamide. Phase contrast image analysis demonstrated that DHT increases the shape index of decidualizing cells, which was reversed upon cotreatment with flutamide. Electron microscopy demonstrated that DHT enhances many of the ultrastructural changes induced by 8-b-cAMP and P4 in HESCs. Decidualizing cells are characterized by an abundant cytoplasm, multiple cell surface projections and, unlike undifferentiated HESCs, form two or more cell layers. DHT further stimulated cytoplasmic expansion, lipid droplet formation, the production of an abundant extracellular matrix, and gap junction formation in decidualized HESCs. DHT enhanced resistance to oxidative stress-induced apoptosis on decidualized HESCs. Moreover, DHT enhanced FOXO1 expression in parallel with the increased SOD2 protein, but not with SOD1.

Limitations, reason for caution: In vitro cell culture studies such as this endometrial stromal cells culture often attracts criticism for the fact that the environment is not a normal physiologic one. The cellular response of stromal cells may change in the absence of epithelial cells as occurred in vivo.

Wider implications of the findings: Androgens enhance the morphological and ultrastructural changes associated with decidualization and confer resistance to oxidative stress signals. Thus, androgen actions in the endometrium may be critical for embryo implantation and trophoblast invasion.

Study funding/competing interest(s): T.K. has nothing to disclose. J.J.B. has nothing to disclose. O.I. has nothing to disclose.

Trial registration number: none

P-243 The identification of specific immune endometrial profiles in patients with previous implantation failures after IVF-ET increases dramatically subsequent pregnancy rates

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Study question: Is the documentation of the local immune endometrial equilibrium, during the implantation window of non-conceptual cycles, in patients with previous implantation failures (IF) after IVF-ET, useful to improve subsequent pregnancy rates through personalized therapeutic strategies to optimize uterine receptivity?

Summary answer: Recruitment, maturation and activation of endometrial natural killer cells (eNK) were deregulated in 77% of the patients, with distinct profiles of uterine receptivity: either poor local reactivity or excessive immune reactivity. Personalization of reproductive therapeutic strategies according to the immune endometrial profile increases the potentiality of pregnancy.

What is known already: Mature eNK cells are essential for embryo implantation. However, in a pro-inflammatory environment, eNK cells may be transformed in lymphocytes activated killer cells, triggering potential cytotoxic activity and endometrial apoptosis. Local recruitment and maturation of eNK cells may be enhanced by an endometrial injury during the cycle preceding IVF and hCG supplementation during the luteal phase. On the opposite, corticosteroids and estrogens may decrease pro-inflammation cytokines production and progesterone acts as a natural immune-suppressor.

Study design, size, duration: This prospective observational cohort study included 175 patients with previous IF. An immune uterine check-up was performed following by recommendations for personalized therapeutic strategies in case of under- or over-endometrial activation. The outcome was the pregnancy rate occurring after the first embryo transfer following the evaluation.

Participants/materials, setting, methods: Endometrial biopsies were performed during the luteal phase of a non-conceptual cycle. Quantifications of eNK recruitment (CD56+ by immunochemistry) and mRNA expression of various cytokines (IL-15, IL-18, TWEAK, Fn-14, G-CSF) were performed by real time PCR. Endometrial activation disequilibrium was identified by an algorithm (EP 12177377.4-2404) including these local data.

Main results and the role of chance: Regarding the uterine profile, we documented an under-activation in 29% of the patients and an over-activation in 47.5%. In case of poor activation, we recommended to perform a local injury during the previous luteal phase, to apply a minimal ovarian stimulation for the attempt and to supplement the luteal phase with human Chorionic Gonadotrophin. In case of over activation, we recommended avoiding local injury in the previous cycle, prednisolone and vitamin E supplements from day 1 of the IVF cycle and high dose of progesterone and estrogen during luteal phase. Ongoing pregnancy rates (PR) following the first ET after the uterine check-up and adapted therapeutics were 50.9%, 42% and 15% for patients with respectively under, over and normal endometrial activation (p = 0.008).

Limitations, reason for caution: This proof of concept suggests that immune endometrial disequilibrium is present in 77% of IF patients. The observed PR after personalized therapeutic strategies according to the endometrial profile was two times higher than expected. Randomized controlled trials must be conducted to prove the current hypothesis.

Wider implications of the findings: Optimization of uterine receptivity seems possible through a strict characterization of the immune endometrial profile at the time of uterine receptivity prior to IVF attempt. Personalization of subsequent therapies increased dramatically the PR in these IF patients. Further documentation of the immune endometrial profile may be also useful for patient with unexplained infertility, unexplained recurrent miscarriages or before any IVF attempt to increase the PR.

Study funding/competing interest(s): MatriceLab Innove EP 12177377.4-2404

Trial registration number: 2013-A00072-43

P-244 Dysregulated sphingolipid metabolism promotes cell growth in endometriosis

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Study question: To examine the effects of sphingolipid metabolism and their dysregulation may be associated with enhanced cell growth and contribute to endometriosis

Summary answer: We found an accumulation of sphingolipids in the sera and peritoneal fluid (PF), and the up-regulation of specific endometrial sphingolipid metabolizing enzymes in the endometrium. Glucosylceramides (GlcCer) are implicated as a physiologically important sphingolipid by opposing effects against apoptosis-mediating ceramides, inducing cell proliferation and plausibly mediating endometriotic lesion growth.

What is known already: Sphingolipids play key roles in cell proliferation and cell death in many human diseases, and in experimental animal models and cellular work, elevated GlcCer has been shown to possess growth stimulatory effects. The association of glucosylceramide accumulation and hyperploidification and organomegaly seen in human and experimental diseases demonstrate the effect of dysregulated sphingolipid metabolism on tissue proliferation. Endometrial stromal cells originating from women with endometriosis have blunted responses to the apoptosis-inducing effects of ceramide.

Study design, size, duration: 62 women undergoing laparoscopic procedures for suspected endometriosis, sterilization procedures and/or pelvic pain formed the case-control study cohort. 38 patients were diagnosed as having endometriosis (according to revised AFS classification of endometriosis), and 24 women who did not have endometriosis or have benign gynecological presentations formed the control group.

Participants/materials, setting, methods: The serum, PF and endometrial tissues were analyzed using mass spectrometry-based lipidomics. Quantitative RT-PCR and immunohistochemistry was used to measure sphingolipid enzyme
levels. Ishikawa cells served as a model to test effects of glucosylceramides and ceramides on cell growth and proliferation (BrdU and flow cytometry analysis).

**Main results and the role of chance**: Unbiased mass spectrometry profiling of sphingolipids revealed significantly higher levels of serological and PF GlicCer ($P = 0.0004$ and 0.005 respectively) in women with severe endometriosis relative to controls. There was up-regulation of specific endometrial sphingolipid metabolizing enzymes, including GlicCer synthase ($P = 0.041$) which correlated with decreased apoptotic cells in the endometrium. Cells incubated with GlicCer alone showed enhanced cell proliferation (40-50% increase over vehicle; $P < 0.01$) and even in the presence of ceramide, GlicCer maintained its mitogenic properties by stimulating cell division and attenuating ceramide-induced apoptosis (40% increase over vehicle, $P < 0.005$). By flow cytometry analysis, we observed that co-incubation of cells with GlicCer and ceramides exhibited similar apoptosis rates as GlicCer alone or vehicle (4.56% versus 4.38% and 5.12% respectively; compared to 17.70% when incubated with ceramide).

**Limitations, reason for caution**: The cell proliferation was performed in an endometrial epithelial cell line. Verification in a stromal cell line will translate into a general biological effect. Further, sphingolipid profiling in endometriotic lesions will add an additional layer of evidence of sphingolipid metabolism aberration in women with endometriosis.

**Wider implications of the findings**: Our top-down systems approach defines a novel paradigm in endometriosis pathophysiology of how endometriotic lesions may grow in an environment conducive for cellular proliferation. The net outcome of an imbalanced sphingolipid landscape reveals new mechanistical insights that may be exploited for potential therapeutics directed at the pathophysiological mechanisms of endometriosis - that GlicCer synthase inhibitors may be useful in reducing endometriotic lesion growth, to ultimately improve the quality of life of these women.

**Study funding/competing interest(s)**: This research was supported by the National Research Foundation Singapore through the Singapore MIT Alliance for Research and Technology’s BioSystems and Micromechanics Inter-Disciplinary Research programme.

**Trial registration number**: N.A.

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**P-245 Transvaginal ultrasound for diagnosis of deeply infiltrating endometriosis**

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**Study question**: The aim of this study is to implement and to determine the ability of the transvaginal ultrasound technique to detect the presence of deeply infiltrating endometriosis in different pelvic locations.

**Summary answer**: Transvaginal ultrasound with bowel preparation showed a high accuracy for predicting deeply infiltrating endometriosis at laparoscopy.

**What is known already**: Deeply infiltrating endometriosis is a prevalent gynecologic disease characterized by lesions that penetrate below the surface of the pelvic peritoneum. It constitutes a major concern in view of the greater severity of the symptoms associated with this form of the disease, and its therapeutic complexity. Currently, magnetic resonance imaging, transrectal ultrasonography and transvaginal ultrasonography, are considered appropriate diagnostics methods. Nevertheless, the last offers advantages in terms of accessibility, cost-effectiveness and tolerability.

**Study design, size, duration**: Observational analysis, transversal multicenter study of diagnostic test, conducted between September 2011 and September 2012 at the Instituto de Investigaciones Materno Infantil, of the Universidad de Chile and at the Centro de Reproducción Humana of the Universidad de Valparaíso. This study has the approval of the Ethics Committee.

**Participants/materials, setting, methods**: This prospective study included 57 consecutive patients, with suspected rectovaginal endometriosis, who underwent surgical laparoscopy. Before surgery, transvaginal ultrasound was performed by a single operator with prior bowel preparation. The presence, location, size and degree of infiltration of endometriotic lesions were evaluated. Ultrasonographic results were compared to surgical and histological findings.

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**Main results and the role of chance**: Of the 57 patients submitted to surgery, 24 had moderate cyclic pain (42%), 15 severe cyclic pain (26%), 16 moderate dyspareunia (28%) and 11 severe dyspareunia (19%). Endometriosis was confirmed histologically in 35/57 patients. Endometrioma and deeply infiltrating endometriosis (DIE) were present in 35 and 31 of the women, respectively. For the diagnosis of DIE, sensitivity was 93%, specificity 100%, positive predictive value 100%, negative predictive value of 90%, positive likelihood ratio was undetermined and negative likelihood ratio 0.062. Specifically, for the diagnosis of uterosacral endometriosis, the S, E, PPV and NPV were: 75%, 100%, 100% and 88.2% respectively. For the diagnosis of intestinal endometriosis, the S, E, PPV and NPV were 100%.

**Limitations, reason for caution**: The ultrasound explorations where performed by a single operator, so an universal extrapolation of this results can be questioned. Also, the operator was unmasked for the symptoms of the patient which can be a bias of the results obtained.

**Wider implications of the findings**: These findings suggest that TVUS is an adequate exam for determining the presence of deeply infiltrating endometriosis. This information is useful when the surgical decision and planification are performed. It is also important when deciding the type of resection. We believe that the implementation of this technique in gynecological centers will increase the ability to reproduce these results and the quality of the primary evaluation of patients with suspected endometriosis.

**Study funding/competing interest(s)**: This study was funded by the Instituto de Investigaciones Materno Infantil, of the Universidad de Chile.

**Trial registration number**: none

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**P-246 IVF infertility treatment outcomes are negatively influenced by the severity of diffuse adenomyosis established by ultrasound examination**

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**Study question**: How the severity of ultrasound diagnosed adenomyosis impacts pregnancy and early pregnancy loss rates in patients undergoing IVF treatment with GnRH antagonist ovarian stimulation?

**Summary answer**: Depending on the severity of ultrasound diagnosed diffuse adenomyosis (mild, moderate and severe) pregnancy rates was significantly reduced ( clinical pregnancy rate 33.3%, 22.9%, and 10.5% respectively) and early pregnancy loss rate was significantly increased (14.3%, 50% and 100% respectively) in patients undergoing GnRH antagonist stimulation for IVF treatment.

**What is known already**: The presence of ultrasound diagnosed adenomyosis was associated with a significant reduction in implantation in patients undergoing GnRH antagonist stimulation for IVF treatment. Information from clinical studies about the effectiveness of IVF treatment of infertility in patients with adenomyosis is still controversial and often concerns all cases of ultrasound diagnosed adenomyosis as a single group without differentiating by stage and severity. We clarify correlation of the severity of adenomyosis measured by sonography and IVF outcomes.

**Study design, size, duration**: Retrospective cohort study of 428 infertile patients who, between January 2008 and September 2011, underwent a transvaginal ultrasound before IVF. Diffuse adenomyosis and severity were established in 96 patients. Patients were allocated in 3 groups according to the sonographic criteria of mild, moderate or severe adenomyosis. Aged < 39 years.

**Participants/materials, setting, methods**: There were 96 infertile patients in a hospital IVF unit eligible to be included in the study. In group A there were 42 patients with mild; in group B - 35 patients with moderate and in group C - 19 patients with severe degree of ultrasound diagnosed diffuse adenomyosis. **Main results and the role of chance** Viable pregnancy rate in group A was 33.3% compared with 22.9% in group B and 10.5% in group C, after one IVF treatment attempt. Pregnant patients were followed up and the rate of early pregnancy loss was 14.3% in group A, in group B and C was 50% and 100% respectively. All patients were undergoing IVF-ET treatment with GnRH antagonist ovarian stimulation with comparable dose of injectable FSH, number of follicles > 16 mm in diameter on the day of hCG injection, number of mature oocytes and embryos and duration of infertility.

**Limitations, reason for caution**: It should be cautioned that even sonographic criteria for the diagnosis of adenomyosis exist, their specificity is still under
discussion among gynecologists. Therefore, it is possible to misclassify severity of adenomyosis in some cases. Wider implications of the findings: Results of this study show that outcome of IVF infertility treatment significantly impaired in patient with ultrasound diagnosed moderate and severe adenomyosis. Different treatment approach such as example as surrogate maternity could be recommended to patients in order to overcome infertility which in turn will protect them from unnecessary material costs of ineffective IVF treatment and psychological trauma that accompany each failed cycle. Study funding/competing interest(s): This study received no funding and there are no conflicts of interests to be declared. Trial registration number: Not applicable.

P-247 Is dienogest effective in postoperative management of endometriosis compared to GnRHa?

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Study question: Is Dienogest effective in postoperative management of endometriosis compared to GnRHa? 

Summary answer: Dienogest proved to be as effective as GnRH agonist in management of postoperative pain for women with endometriosis

What is known already: Few randomised controlled trials were published but no systematic review was conducted on dienogest

Study size, design, duration: Systematic review and meta-analysis of randomised controlled trials

Participants/materials, setting, methods: All randomised controlled trials comparing dienogest vs GnRHa in women with endometriosis were recruited and data were extracted. Data were plotted in Revman software and fixed effect model was used to estimate the effect of the intervention

Main results and the role of chance: There was no significant difference between interventions on the absolute reduction in pain on the visual analogue scale (MD -1.60, 95% CI -9.17 to 5.97; p 0.68) or the proportion of women who reported pain improvement (OR 1.26, 95% CI 0.27 to 5.80; p 0.77). Two trials reported a significantly higher difference in BMD (305 women) following Dienogest compared with GnRHa (MD 2.74, 95% CI 0.14 to 5.35; p 0.04), but there was marked heterogeneity among the trial results (I2 83%). In one trial (248 women), the mean urine calcium level was not significant difference between groups (MD 26.10, 95% CI -39.89 to 92.09; p 0.44). Regarding the safety profiles, two trials (503 women) reported significantly less headaches (OR 0.62, 95% CI 0.40 to 0.95; p 0.03; 12%); and one trial (248 women) reported significantly less sleep disorders (OR 0.20, 95% CI 0.04 to 0.93; p 0.04), with Dienogest. There was no significant difference in other reported safety outcomes.

Limitations, reason for caution: some heterogeneity was found between the studies

Wider implications of the findings: Dienogest can be used with relative safety for managing pain with endometriosis

Study funding/competing interest(s): none

Trial registration number: systematic review (not clinical trial)

P-248 Adopt: a new surgical classification for endometriosis-associated infertility: can we adopt it - a pilot study

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Study question: Can we develop a simple surgical staging for endometriosis associated infertility using laparoscopy?

Summary answer: We propose a new simple surgical staging of endometriosis associated infertility (ADOPT classification). It is based on the assessment of 5 parameters pertinent to the evaluation of infertility. These include: Assessment of Adhesions (A), Obliteration of pouch of Douglas (D), Ovarian involvement (O), Peritoneal involvement (P) and assessment of tubes (T).

What is known already: Assessment of the severity of endometriosis has been inadequate and unsatisfactory. Despite being the standard classification for decades, the revised American Society of Reproductive Medicine (rASRM) classification still has many drawbacks limiting its practical use.

Study design, size, duration: Cohort study: 40 infertile patients aged (20-35 years) with laparoscopically diagnosed endometriosis.

Laparoscopic assessment of endometriosis was performed using the standard rASRM classification and our new ADOPT classification. Cases were followed for 6 months to find how the outcome matched the staging of endometriosis.

Participants/materials, setting, methods: The study included 40 infertile women (20-35 years) with laparoscopically diagnosed endometriosis. Laparoscopic assessment of endometriosis was performed using rASRM classification and our new ADOPT classification. Cases were followed for 6 months to find how the outcome (clinical pregnancy) matched the staging of endometriosis.

Main results and the role of chance: ADOPT was found to be more descriptive than rASRM classification. According to rASRM classification (8, 3, 12, 17) cases were stages (I, II, III, IV) of which (75%, 33.3%, 58%, 52.9%) achieved clinical pregnancy respectively, while according to our “ADOPT” classification (7, 4, 20, 9) cases were stages (I, II, III, IV), of which (85.7%, 75%, 65%, 11%) achieved clinical pregnancy respectively. ADOPT was found more simple and practical than rASRM classification.

Limitations, reason for caution: Our new surgical classification needs to be validated on a larger number of patients and its reproducibility should be evaluated.

Wider implications of the findings: ADOPT classification is a simple, practical, descriptive and prospective surgical tool for assessment of endometriosis associated infertility that might solve problems encountered with other classification systems. We believe that ADOPT classification system has fulfilled most of the criteria of an ideal classification system for endometriosis associated infertility

Study funding/competing interest(s): Trial registration number No

P-249 Use of tanning beds, sun exposure and the incidence of endometriosis

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Study question: Is UV exposure associated with the risk of endometriosis?

Summary answer: These data suggest that intense artificial or recreational UVR exposure increases endometriosis risk, whereas ambient sunlight UVR exposure decreases risk.

What is known already: An association between endometriosis and cutaneous melanoma has been reported, and evidence suggests that these diseases may share constitutional risk factors. Exposure to ultraviolet radiation is the major environmental risk factor for melanoma; however, only one retrospective study explored its relation to endometriosis so far.

Study design, size, duration: The Nurses’ Health Study II is a prospective cohort of 116,678 female US nurses aged 25-42 years at enrollment in 1989, with follow-up for >20 years since.

Participants/materials, setting, methods: We used Cox proportional hazards regression models to calculate multivariable relative risks (RR) and 95% confidence intervals (CI). During 621,742 woman-years of follow-up, 4705 cases of laparoscopically-confirmed endometriosis were reported among premenopausal Caucasian women.

Main results and the role of chance: Tanning bed use during high school through age 35 was associated with significantly increased endometriosis risk as frequency and age of exposure increased (compared with never tanning bed exposure; <2 times/year at any age: RR = 1.01; ≥3 times/year in high school/college only: RR = 1.05; ≥3 times/year at age 25-35 years only: RR = 1.17; ≥3 times/year both age periods: RR = 1.27; P(trend) = 0.0002). Endometriosis risk was increased linearly with higher number of sunburns at ages 15-20 years (P(trend) = 0.05) and percentage of time using sunscreen in summer (P(trend) = 0.02). In contrast, risk was decreased with UVB flux in state of birth (P(trend) = 0.04), in state lived most at age 15 years (P(trend) = 0.01) and in state lived most at age 30 years (P(trend) = 0.0002).

Limitations, reason for caution: Because it was not possible to identify a precise time point at which the disease process was initiated at a molecular or cellular level, a limitation of this study is the inability to differentiate the time of endometriosis diagnosis from the time of disease onset. Additional limitations include lack
of data on stage of endometriosis, the possibility of asymptomatic disease in the control population, and self-report of UV exposure data. **Wider implications of the findings:** UV exposure may be associated with endometriosis risk, and this association may differ according to the type and intensity of UV exposure. These novel findings need to be confirmed in other populations, and more research is needed to understand their underlying mechanisms before any recommendation can be made at the population level. **Study funding/competing interest(s):** This project was supported by NICHD grants HD48544 and HD52473, NIH grant CA50385. The NHS II is supported by the Public Health Service grant CA50385 from the National Cancer Institute, NIH, U.S. Department of Health and Human Services. M.K. is supported by a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme (PIIF-GA-2011-302078) and through the Young Researcher Prize from the Bettencourt-Schueller Foundation. The authors have to declare. **Trial registration number:** -

### P-250 Expression of genes SOD1, SOD2 and GPX4 in cumulus oophorus cells of infertile women with and without endometriosis undergoing ovarian stimulation for ICSI

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**Study question:** To compare SOD1, SOD2, and GPX4 gene expression in Cumulus oophorus cells (COC) of infertile women with and without endometriosis submitted to controlled ovarian stimulation (COS) for intracytoplasmic sperm injection (ICSI), and to assess the interaction of the expression of these genes with the occurrence of clinical pregnancy following ICSI. **Summary answer:** Only infertile women with endometriosis in advanced stages showed increased expression of the SOD1 gene in COS, with a positive interaction between the increased expression of this gene and the occurrence of clinical pregnancy, suggesting that this gene might be considered a biomarker of ICSI success. **What is known already:** Some studies have demonstrated lower fertilization and pregnancy rates in women with endometriosis undergoing COS for ICSI. Although the underlying mechanisms are unknown, it has been suggested that worsening of oocyte quality and oxidative stress may be involved. Superoxide dismutase and glutathione peroxidase are important antioxidant enzymes. COC protect oocytes from entering apoptosis induced by oxidative stress and gene expression analysis of COC might be used as a noninvasive biomarker of oocyte quality and ICSI. **Study design, size, duration:** Cross-sectional. From February 2009 to October 2010, 451 patients started COS. 248 were eligible for study, 216 signed the informed consent form, 158 underwent oocyte retrieval, 133 donated COC, 112 were included in the study (mean age ± SD, 37.4 ± 4.6 years); the median follow-up was 62 months (range, 48-104 months). No endometriotic nodule infiltrating the muscularis of the rectosigmoid was identified. **Limitations, reason for caution:** A limitation of the current study is the small sample size; however, the presence of infiltrated resection margins is a rare event in patients undergoing colorectal resection for endometriosis. **Wider implications of the findings:** The findings of this study should be considered in the counseling of patients with infiltrated resection margins at colorectal resection for endometriosis. These patients should be reassured that histological infiltration of the rings of tissue contained in the circular stapler is not associated with disease recurrence. **Study funding/competing interest(s):** None. **Trial registration number:** None.

### P-251 Radiological long-term follow-up of patients with histologically infiltrated resection margins at colorectal resection for endometriosis

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**Study question:** Which is the risk of disease recurrence in patients with infiltrated resection margins at the time of colorectal resection for endometriosis? **Summary answer:** The presence of infiltrated resection margins at the time of colorectal resection for endometriosis does not seem to be associated with recurrence of disease. **What is known already:** Previous studies suggested that infiltrated resection margins do not influence pain symptoms, intestinal complaints and quality of life. However, no previous study performed follow-up of these patients by using radiological instruments. **Study design, size, duration:** This was a cross-sectional study. It was performed between January and October 2012. **Participants/materials, setting, methods:** Inclusion criteria were: colorectal resection for endometriosis performed at least 4 years before the study; infiltration of the resection margins; reproductive age at the time of the study. Patients underwent magnetic resonance enteroclysis (MRE, 300 ml of ultrasonographic gel diluted with saline solution introduced in the rectosigmoid). **Main results and the role of chance:** 27 women were included in the study (mean age ± SD, 41.2 ± 4.6 years); the median follow-up was 62 months (range, 48-104 months). No endometriotic nodule infiltrating the muscularis of the rectosigmoid was identified. **Limitations, reason for caution:** A limitation of the current study is the small sample size; however, the presence of infiltrated resection margins is a rare event in patients undergoing colorectal resection for endometriosis. **Wider implications of the findings:** The findings of this study should be considered in the counseling of patients with infiltrated resection margins at colorectal resection for endometriosis. These patients should be reassured that histological infiltration of the rings of tissue contained in the circular stapler is not associated with disease recurrence. **Study funding/competing interest(s):** None. **Trial registration number:** None.

### P-252 Correlation of histomorphology and clinicopathology of peritoneal endometriosis

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**Study question:** Is it possible to characterize peritoneal endometriosis by macroscopy (lesion color) and microscopy? **Summary answer:** There are strong similarities between the different color categories. **What is known already:** Endometriosis affects 4-30% of women of reproductive age and is one of the most common benign gynecological diseases. Etiology and pathogenesis is poorly understood. Peritoneal endometriosis differs in color and structure. It remains unclear how, or whether different macroscopic types of...
peritoneal endometriosis can vary histologically and immunohistochemically and if a correlation with the macroscopy is possible.

**Study design, size, duration:** In this study we included 47 patients with peritoneal endometriosis between 2011 and 2012.

**Participants/materials, setting, methods:** We included nullipara with peritoneal endometrioses in laparoscopy. Exclusion criteria was any previous abdominal surgery. We classified 65 lesions in red, brown, black, white. In addition to the excision of the lesion we did the curettage of the cervix to obtain endometrium. Histologically we did hematoxylin-eosin staining and investigated immunohistochemistry using a panel of special stains (Mib-1 to examine the proliferation, caldesmon, desmin, and actin to represent smooth muscle tissue in the surrounding stroma, Berliner Blau for detection of hemosiderin, EvG as fiber staining, S100 as nerve staining at the detection of estrogen and progesterone receptors).

**Main results and the role of chance:** There are strong similarities between the different color categories. No significant difference could be documented in our collective regarding the proliferation activity in the different lesion colors. Different results regarding the color categories were documented for: gland content, gland form, PR expression in stromal/epithelial cells and elastic fiber content and smooth muscle metaplasia. It remains unclear whether our findings contradict the theory of an age-related or developmental color-shift in endometriotic lesions or whether histomorphology and immunohistochemistry are simply not the right tools for the characterization of endometriotic lesions. The analysis of endometriotic lesions by molecular means eg microarray techniques may provide further information whether peritoneal endometriosis per se can be subdivided in further subgroups.

**Limitations, reason for caution:** Limitation of the study is the small number of patients and lesions.

**Wider implications of the findings:** Our findings do not go in line with studies, that found significant differences in lesion colors in special categories. In some points we found differences, that have been described in literature.

**Study funding/competing interest(s):** No study funding

**Trial registration number:** -

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**P-253 Endometrial differential biomarkers of recurrent spontaneous abortion**

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**Study question:** Is there any endometrial immunological parameter which leads the diagnosis to patient with recurrent spontaneous abortion?

**Summary answer:** Woman with recurrent spontaneous abortion showed increased endometrial mRNA MICA levels and peripheral proportion of LB1 cells. Moreover, infertile patients with positive antiphospholipid antibodies showed increased endometrial Tissue Factor.

**What is known already:** Natural Killer (NK) cells are the main lymphocytes population in the endometrium during implantation and early pregnancy. These cells express NKG2D receptors capable of binding to endometrial MICA. This interaction can trigger NK cell activation to cytolytic activity or cytokine production such as IFN-y and VEGF. Furthermore, increased number of LB1 cells has been postulated in abortion, together with the role of Tissue Factor in the etiology of antiphospholipid syndrome. Control-case studies are still scarce.

**Study design, size, duration:** Diagnostic test. It was performed during 2010-2012.

The sample size was 14 fertile women and 10 patients with recurrent spontaneous abortions (two or more consecutive miscarriage).

**Participants/materials, setting, methods:** Endometrial and blood samples were obtained during implantation window. CD56+/CD16+/-, CD142+ + CD9+ and CD19+ CD5+ cells were measured by flow cytometry. Endometrial expression of MICA, IFN-y and VEGF were determined by QPCR and/or ELISA. ROC curve analysis was performed in order to establish normal values. Statistical analysis was performed by Student or Mann Withney and correlations by Pearson test.

**Main results and the role of chance:** Women with repeated spontaneous abortions have no differences in total endometrial NK cells as compared to fertile women. However, 70% of these patients showed significantly increased MICA mRNA expression as well as the 54% of the population showed a higher proportion of LB1 cells, over the cut off value calculated by ROC curve. Although there was no differences in IFN-y mRNA levels, we found a statistically significant positive correlation between IFN-y and MICA mRNA levels (r = 0.5672 p = 0.0347) and between VEGF mRNA levels and the absolute count of CD16-NK cells (r = -0.7312 p = 0.0081). Moreover, APA+ (antiphospholipid antibodies) patients showed increased CD142+ CD9+ cells (p = 0.0081).

**Limitations, reason for caution:** It is a descriptive study. We evaluate some endometrial and peripheral blood biochemical parameters potentially associated with recurrent spontaneous abortions. Increasing sample size is needed to confirm this results.

**Wider implications of the findings:** Although more research is needed in order to confirm these results, we postulate that these immunological factors should be useful parameters to study woman with recurrent spontaneous abortions and it would be tested before the decision of complimentary immunological treatment.

**Study funding/competing interest(s):** ANR I + D 2007 NA 023/07.

**Trial registration number:** It was presented to the national authority but is not codified yet.

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**P-254 The presence of an ovarian endometrioma affects iron metabolism in the developing follicle**


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**Study question:** The presence of an endometrioma affects the developing follicles, leading to poor embryo quality. The aim of this study was to evaluate whether the iron metabolism in the developing follicles was affected by the proximity of an endometrioma.

**Summary answer:** The ferritin H/L ratio was reduced in follicles isolated from ovaries with an endometrioma, as compared with follicles isolated from healthy ovaries. Accordingly, transferrin receptor mRNA expression was downregulated in granulosa cells in follicles affected by ovaries.

**What is known already:** The follicular fluid environment plays a crucial role in the development of a competent oocyte. Oocyte maturation is a multifactorial process partially dependent on the presence of reactive oxygen species and iron (Aleshireet al 1989). Data from different groups including ours indicate that embryos derived from oocytes isolated from endometrioma-affected ovaries are of poorer quality compared to embryos derived from oocytes isolated from healthy ovaries.

**Study design, size, duration:** Follicular fluids from individual follicles were collected during oocyte retrieval by transvaginal ultrasound-guided approach from women undergoing IVF cycles. A total of 36 follicles in contact with the endometrioma, 22 follicles not in contact with the endometrioma, and 14 follicles from healthy donors were analyzed.

**Participants/materials, setting, methods:** The levels of ferritin (H, heavy chain and L, light chain) in monofollicular fluid was measured by specific ELISAs. Granulosa cells from single follicles were isolated and gene expression analysis was performed by real-time PCR.

**Main results and the role of chance:** We found that levels of ferritin H were higher than levels of ferritin L in all the follicles analyzed (FH = 1.022 ng/mg of total proteins vs FL = 0.2952 ng/mg total protein, p = 0.023).

The content of ferritin L was significantly higher in the follicles in close contact with the endometrioma and increased when compared with the follicles from donors without endometriosis, suggesting a dysregulation of the iron metabolism. Also, a significant decrease in the ferritin H/L ratio was observed in the follicles from the ovaries with endometrioma.

Moreover, the expression of transferrin receptor, that is known to negatively correlate with the presence of iron, was reduced in granulosa cells from the follicles isolated from endometrioma-affected ovaries.

**Limitations, reason for caution:** Future analysis need to be performed to correlate these data with embryo quality and IVF outcomes. Furthermore, in order to
increase statistical significance, the analysis should be extended to a higher number of cases and controls.

Wider implications of the findings: Our data suggest that ferritin levels in the follicle are affected by the proximity with an endometrioma. This could have a detrimental impact on the oocyte development and therefore on embryo quality. Indeed ferritin L levels, which are strongly dependent on the labile iron pool, are increased in these follicles. Our results provide novel information that may contribute to resolve the controversy about the optimal treatment for fertility purposes in women with endometriomas.

Study funding/competing interest(s): This study was supported by Fondazione Giorgio Pardi, Milan, Italy.

Trial registration number: n.a.

P-255  Efficacy and safety of intrauterine insemination in patients with moderate to severe endometriosis: a 5 year cohort study, systematic review and meta-analysis

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Study question: What is the efficacy and safety of intrauterine insemination (IUI) with and without controlled ovarian hyperstimulation (COH) in patients with moderate to severe endometriosis with regard to the ongoing pregnancy and endometriosis recurrences rates?

Summary answer: IUI with COH results in higher cumulative pregnancy rates compared to IUI without COH. In six subsequent cycles the endometriosis recurrence rate increases in both groups.

What is known already: To date, performing IUI in moderate to severe endometriosis patients is not implemented in the international guidelines with regard to treatment of endometriosis related subfertility, as only limited data exist on efficacy and safety. Due to a fear of high endometriosis recurrence rates in combination with limited ongoing pregnancy rates clinicians are reluctant to perform IUI with COH in moderate to severe endometriosis patients. But again data are lacking.

Study design, size, duration: A retrospective cohort study is performed from January 2007 till July 2012 including 74 patients (292 cycles) with a follow-up to one year after the last insemination. A systematic review and meta-analysis including eight observational studies is performed to calculate the weighted mean pregnancy rate.

Participants/materials, setting, methods: All patients with moderate to severe endometriosis undergoing their first IUI treatment up to six subsequent cycles were included. Two treatment strategies were compared with regard to ongoing pregnancy and endometriosis recurrence rate; IUI with COH (n = 20) versus IUI without COH in the first three cycles (n = 54).

Main results and the role of chance: In the retrospective cohort, 17 couples had an ongoing pregnancy (5.8% per insemination; cumulative pregnancy rate 27.9%) and 25 (33.8%) patients had a recurrence of endometriosis. In patients undergoing IUI with COH versus IUI without COH in the first three cycles, life table analysis showed cumulative ongoing pregnancy rates in six subsequent cycles of 8.0%/1.9% (1), 35.0%/7.6% (2), 35.0%/9.8% (3), 45.8%/15.4% (4), 45.8%/21.2% (5), 45.8%/21.2% (6), respectively (p = 0.009). The cumulative endometriosis recurrence rate increased in six subsequent cycles from 5%/1.9% (1), 10.9%/7.6% (2), 35.2%/16.2% (3), 35.2%/18.8% (4), 35.2%/21.6% (5) to 83.8%/45.5% (6) in patients undergoing IUI with COH versus IUI without COH in the first three cycles (p = 0.06). Our meta-analysis demonstrated a weighted mean pregnancy rate of 19.2% per patient and 8.4% per cycle.

Limitations, reason for caution: The retrospective design of our study is the foremost limitation. However, this is the largest cohort so far supplemented by a systematic review and meta-analysis.

Wider implications of the findings: This study implies that hCG could up-regulated expression of StAR, Annexin IV and VEGF in human endometrial cells and what is the mechanism?

Study question: Could LH/hCG-R regulate the expression of steroidogenic acute regulatory (StAR), Annexin IV and vascular epithelial growth factor (VEGF) in human endometrial cells and what is the mechanism?

Summary answer: Up-regulated expression of StAR, Annexin IV and VEGF in endometrial cells was triggered by HCG(20U/d) with time-dependent and the up-expression of StAR by hCG was inhibited by PD98059 (the MAPK pathway blockers).

What is known already: LH/hCG-R was reported expressed in endometrium during menstruation cycle in human, but the functional of the receptor is unknown. StAR and Annexin IV were expressed in human endometrial cells and their up-expression by hCG was reversed by the blocker of PD98059 in RL95-2 cell lines in vitro in our previous reports.

Study design, size, duration: It’s an experimental study with human endometrial cells culture model in vitro. Seven endometrial specimens were collected by biopsies under HSP in department of gynecology of our hospital from March 2012 to October 2012.

Participants/materials, setting, methods: Endometrial cells was isolated by mechanical dissociation and centrifugation from endometrial tissue for the primary cells culture, which divided into three groups: Control group, HCG group and HCG/PD98059 group. The location of each protein were identified by immunohistochemistry. Quantity of proteins and mRNA were analyzed by Western Blot and RT-PCR semi-quantitative.

Main results and the role of chance: 1) The LH/hCG-R, StAR, Annexin IV or VEGF were detected in the cytoplasm of endometrium cells by immunohistochemistry. 2) The mRNA of StAR, Annexin IV, and VEGF were significantly up-regulated in HCG group compared with it in control group (P > 0.05) (WB: 0.957 ± 0.0373 vs 0.1724 ± 0.0554; 0.1332 ± 0.0679 vs 0.2931 ± 0.1711; 0.4069 ± 0.1897 vs 0.7781 ± 0.1615; Q-PCR:1.0000 vs 2.4267 ± 1.3718; 1.0000 vs 2.6785 ± 1.3592; 1.0000 vs 2.0132 ± 0.6024; receptivity: 3) The protein or mRNA of StAR were significantly down-regulated in the HCG/PD98059 groups compared with it in HCG group (P < 0.05); but the Annexin II did not showed changes. 4) Both the concentration of E2 and P4 in the culture supernatant increased significantly after intervention with HCG.

Limitations, reason for caution: This was mixed epithelial and stromal cells culture system, it could not distinguish the difference of the two cells. But the percentage of the two cell between the time of inoculation and HCG-intervention was no significant difference (P > 0.05); hCG triggered time-dependent experiment on Annexin IV and VEGF needs further study.

Wider implications of the findings: This study implies that hCG could up-regulated expression of StAR, Annexin IV And VEGF, which reflected the effection of hCG in the structural transform and function changes in endometrial cells. This will be useful for regulation endometrium function.

Study funding/competing interest(s): This study was supported by Natural Science Foundation of China(2008-30872762) and the Science Foundation of Guangdong Province(2009B030801022).

Trial registration number: None.

P-257  An in silico approach to biomarker and drug discovery in the endometrium

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Study question: Could LH/hCG-R regulate the expression of steroidogenic acute regulatory (StAR), Annexin IV and vascular epithelial growth factor (VEGF) in human endometrial cells and what is the mechanism?

Summary answer: Up-regulated expression of StAR, Annexin IV and VEGF in endometrial cells was triggered by HCG(20U/d) with time-dependent and the up-expression of StAR by hCG was inhibited by PD98059 (the MAPK pathway blockers).

What is known already: LH/hCG-R was reported expressed in endometrium during menstruation cycle in human, but the functional of the receptor is unknown. StAR and Annexin IV were expressed in human endometrial cells and their up-expression by hCG was reversed by the blocker of PD98059 in RL95-2 cell lines in vitro in our previous reports.

Study design, size, duration: It’s an experimental study with human endometrial cells culture model in vitro. Seven endometrial specimens were collected by biopsies under HSP in department of gynecology of our hospital from March 2012 to October 2012.

Participants/materials, setting, methods: Endometrial cells was isolated by mechanical dissociation and centrifugation from endometrial tissue for the primary cells culture, which divided into three groups: Control group, HCG group and HCG/PD98059 group. The location of each protein were identified by immunohistochemistry. Quantity of proteins and mRNA were analyzed by Western Blot and RT-PCR semi-quantitative.

Main results and the role of chance: 1) The LH/hCG-R, StAR, Annexin IV or VEGF were detected in the cytoplasm of endometrium cells by immunohistochemistry. 2) The mRNA of StAR, Annexin IV, and VEGF were significantly up-regulated in HCG group compared with it in control group (P < 0.05) (WB: 0.957 ± 0.0373 vs 0.1724 ± 0.0554; 0.1332 ± 0.0679 vs 0.2931 ± 0.1711; 0.4069 ± 0.1897 vs 0.7781 ± 0.1615; Q-PCR:1.0000 vs 2.4267 ± 1.3718; 1.0000 vs 2.6785 ± 1.3592; 1.0000 vs 2.0132 ± 0.6024; receptivity: 3) The protein or mRNA of StAR were significantly down-regulated in the HCG/PD98059 groups compared with it in HCG group (P < 0.05); but the Annexin II did not showed changes. 4) Both the concentration of E2 and P4 in the culture supernatant increased significantly after intervention with HCG.

Limitations, reason for caution: This was mixed epithelial and stromal cells culture system, it could not distinguish the difference of the two cells. But the percentage of the two cell between the time of inoculation and HCG-intervention was no significant difference (P > 0.05); hCG triggered time-dependent experiment on Annexin IV and VEGF needs further study.

Wider implications of the findings: This study implies that hCG could up-regulated expression of StAR, Annexin IV And VEGF, which reflected the effection of hCG in the structural transform and function changes in endometrial cells. This will be useful for regulation endometrium function.

Study funding/competing interest(s): This study was supported by Natural Science Foundation of China(2008-30872762) and the Science Foundation of Guangdong Province(2009B030801022).

Trial registration number: None.
Study question: Can a ‘Systems Biology’ approach be used to analyse transcriptomic datasets associated with different phases of the menstrual cycle to provide insights into relevant intra and extracellular events? Could this analysis be further applied to biomarker discovery and drug targeting in proliferative endometrial diseases such as endometriosis and endometrial cancer?

Summary answer: Biologically relevant differentially regulated networks were identified in both intracellular and extracellular compartments in ‘normal’ endometrium. Specific enzymes e.g. Superoxide Dismutase 2 (SOD2) were enriched in the dataset and represent potential targets of future ‘wet’ laboratory investigation.

What is known already: The microenvironment plays an important role in tissue homeostasis in health and disease. Within this microenvironment, the extra cellular matrix (ECM) proteins have been shown to play important roles in complex biological processes. Our understanding of the processes involved in the continual cycle of endometrial proliferation, degeneration and regeneration observed throughout a woman’s reproductive years is currently incomplete.

Study design, size, duration: A ‘Systems Biology’ approach utilised mRNA lists associated with ‘Normal Endometrium’ to analyse differential gene expression between the Early Secretory (ES), Mid Secretory (MS) and Late Secretory (LS) menstrual cycle phases. These differentially regulated genes were then used to identify enriched biological networks of interest in the different phases.

Participants/materials, setting, methods: mRNA lists were obtained using the NCBI GEO Datasets. ‘R’ Statistics package defined significantly differentially regulated genes (+2/-2 mean log fold change) in the ES, MS and LS Phases. The top ‘Networks’, ‘Biofunctions’ and ‘Canonical Pathways’ associated with the whole and extracellular datasets were identified using Ingenuity Pathways Analysis (IPA).

Main results and the role of chance: ‘Complement System’, ‘Wnt/B Cathecin signalling’ and ‘Inhibition of Matrix Metalloproteinases’ are important canonical pathways enriched to the extracellular datasets associated with both the MS and LS phases. ‘Superoxide Radicals Degradation’, the top canonical pathway enriched to the MS intracellular dataset (ratio ¼ 2.6), SOD2 is an oxygen-mediated gene of this pathway that is up regulated in the dataset.

‘Tryptophan Degradation to 2-amino-3-carboxy-2-muconate Semali Aldehyde’ is the top canonical pathway enriched to the LS dataset (ratio ¼ 3.7). Indoleamine 2,3-dioxygenase (IDO) from the LS dataset is an important enzyme in this pathway that is up regulated (mean log fold change 3.02).

False discovery rate was accounted for in the analysis

Limitations, reason for caution: Microarray analysis has limitations as not all mRNA transcribed results in protein synthesis meaning representation of gene expression can be overestimated. The dataset was refined extensively to ensure the data was representative of ‘normal endometrium’ however as biopsies were not collected by the investigators this cannot be completely ensured.

Wider implications of the findings: SOD2 may function in the endometrium to prevent oxidative stress during the implantation window and oxidative stress associated genes are involved in both endometriosis and endometrial cancer pathogenesis.

IDO has been previously identified within murine endometrium. It is important in tolerance of self, fetal and tumour antigens, contributing to tumour cell evasion of T-cell mediated rejection.

Further ‘wet’ laboratory work may elucidate the role of biomarkers identified in proliferative endometrial pathology e.g. Endometriosis and Endometrial Cancer

Study funding/competing interest(s): The authors declare no competing interests

Trial registration number: NA

Study question: Is the reduction in serum Anti-Mullerian Hormone (AMH) levels after conservative ovarian surgery dependent on the ovarian surgery in patients with endometriosis?

Summary answer: Median serum AMH levels decreased in all women with confirmed endometriosis after laparoscopic surgery, but the decrease was only significant in the group of women who underwent removal of endometriomas, both at three and six months after surgery.

What is known already: Previous studies have shown a reduction of AMH levels after surgery when endometriomas are removed. Women with endometriosis present often with diminished ovarian reserve. Serum AMH levels are currently the most reliable biochemical marker of ovarian reserve.

Study design, size, duration: Prospective cohort study between March 2011–March 2012. Women of reproductive age undergoing laparoscopic surgery for endometriosis (n = 35) with and without ovarian removal of endometriomas. Women (n = 14) without endometriosis undergoing laparoscopic sterilization served as control group. Eight patients were lost to follow up at six months.

Participants/materials, setting, methods: Thirty-five women (mean age 31.9yr) with endometriosis undergoing laparoscopic surgery; 26 with removal of endometriomas (mean 6.0cm) and 9 women with no ovarian surgery. A sterilization control group of 14 women. Serum AMH levels were estimated at baseline prior to surgery and at three- and six months after surgery.

Main results and the role of chance: Patients who underwent removal of endometriomas presented with a significant reduction in median AMH levels from 1.8 μg/L (range 0.2-7.9) to 1.0 μg/L (range 0.2-7.0) (n = 24) at 3 months (p = 0.008). Those levels further decreased at 6 months (1.0 μg/L, range 0.2-3.4) (n = 21) (p = 0.006). Women presenting with endometriosis but who did not require ovarian surgery also had decreased AMH levels at six months, although non-significantly (1.5 μg/L to 1.1 μg/L and 1.0 μg/L, (p = 0.213). Women who underwent laparoscopic sterilization showed a non-significant increase in serum AMH from 0.9 μg/L (range 0.22-6.5) to 1.3 μg/L at three months and 1.4 μg/L at six months.

Limitations, reason for caution: Several surgeons operated the patients. Although the laparoscopic technique for cyst surgery is standardized, there might be variation depending on the surgeons and their expertise.

Wider implications of the findings: Our results demonstrate a reduction of ovarian reserve estimated by serum AMH levels in patients with confirmed endometriosis. This reduction was more pronounced and statistically significant following surgery of ovarian endometriomas. Fertility is a major issue in young patients undergoing ovarian surgery, women planned for cystectomy should be counselled regarding their fertility potential, in particular those women presenting with an already reduced ovarian reserve and low levels of AMH at time of surgery.

Study funding/competing interest(s): Supported with grants from the local Research, Education and Development council, Department of Obstetrics and Gynaecology, Stockholm Sodersjukhuset and Department of Clinical Science, Intervention and Technology, CLINTEC, Karolinska Institutet.

Trial registration number: non applicable

P-259 The present of unmyelinated sensory C nerve fibers in menstrual blood tissues is useful for noninvasive diagnosis of endometriosis

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Study question: Whether unmyelinated sensory C nerve fibers present in menstrual blood tissues and it can be used as a novel way for noninvasive diagnosis of endometriosis

Summary answer: Confirmation of diagnosis remains to be a problem in women with endometriosis, since there are no noninvasive ways to diagnose endometriosis at moment. Certain methods has been used for making diagnosis of endometriosis, however, laparoscopy is required for confirmation. It indicates that improvement way to diagnose endometriosis is needed.

What is known already: It has been reported regarding the novel finding of multiple small unmyelinated sensory C nerve fibers in the functional layer of eutopic endometrium in women with laparoscopically proven endometriosis, while...
women without endometriosis do not have any nerve fibers in their eutopic endometrium functional layer.

**Study design, size, duration:** Diagnostic test study design included 24 subjects proven endometriosis and 6 subjects unproven endometriosis. The study was conducted within 7 months at dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia.

**Participants/materials, setting, methods:** The menstrual blood tissues were collected from subjects who underwent surgery for ovarian cyst and proven endometriosis by histopathology examination, and unproven endometriosis as control.

The paraffin embedded menstrual blood tissues of each sample was cut at 4 um and double stained with PGP9.5 antibody and hematoxylin-eosin as back-ground. All proven endometriosis subjects showed small unmyelinated sensory C nerve fibers in their menstrual blood tissues, meanwhile only 1 out of 6 unproven endometriosis subject showed this fibers.

Positive strong correlation was observed (p = 0.001) between the density of nerve fibers in menstrual blood tissue and the severity of endometriosis based on AFS score.

**Main results and the role of chance:** 20 (66.7%) subjects showed strong positive staining, 1 (3.3%) subject showed medium positive staining, and 3 (10%) subjects showed weak positive staining. Moreover, 6 (20%) subjects revealed negative result.

In addition, 11 (36.7%) subjects showed AFS score IV, 12 (40%) with AFS score III, 1 (3.3%) with AFS score II, and 6 (20%) with AFS score zero. All proven endometriosis subjects revealed small unmyelinated sensory C nerve fibers in their menstrual blood tissues, meanwhile only 1 out of 6 unproven endometriosis subject showed this fibers.

**Limitations, reason for caution:** Only 135 women were included. From a methodological perspective, additional properly randomised and reported long-term trials are needed to confirm the effectiveness of this method of therapy. The included trials lacked proper reporting on all aspects of potential biases thereby limiting the internal validity of the results. Furthermore, as most were short-term trials, it is not clear whether patient compliance over a prolonged period might affect the overall results. Even so, the consistent results presented by the trials regarding the decreased incidence of recurrence of painful periods is encouraging.

Finally, it should be noted that the majority of outcomes were not analysed due to unavailability of data, and even those that were analysed came from only three small trials that compared LNG-IUD insertion to no postoperative treatment or a GnRH analogue.

**Wider implications of the findings:** Further randomised controlled trials are encouraged to confirm these findings. If so, then local application of LNG-IUD should be adopted for pain associated endometriosis.

**Study funding/competing interest(s): None**

**Trial registration number:** registered in Cochrane databases

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**P-260 Postoperative application of LNG-IUD for symptomatic endometriosis**

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**Study question:** Does postoperative levonorgestrel intrauterine device (LNG-IUD) insertion in women with endometriosis improve pain and reduce symptoms compared with no treatment, placebo, or systemic therapy.

**Summary answer:** postoperative LNG-IUD use may reduce the recurrence of pain in women with endometriosis.

**What is known already:** Surgical treatment of endometriosis aims to remove visible areas of endometriosis. The aim of medical therapy is to inhibit growth of endometriotic implants by induction of a hypo-estrogenic state. Treatment with a hormone releasing intrauterine device, using levonorgestrel (LNG-IUD), has also been suggested but efficacy has to be tested.

**Study design, size, duration:** Systematic review of randomised controlled trials

**Participants/materials, setting, methods:** Randomized trials were included if they compared women who underwent surgical treatment for endometriosis within the preceding three months, to LNG-IUD insertion versus no treatment, placebo, or systemic therapy. Two reviewers independently selected studies for inclusion and extracted data to allow for an intention-to-treat analysis. For dichotomous data, the risk ratio (RR) and 95% confidence interval (CI) were calculated using the Mantel-Haenszel random-effects method. For continuous data, the mean difference (MD) and 95% CI were calculated using the inverse variance random-effects method.

**Main results and the role of chance:** there was a statistically significant reduction in the recurrence of painful periods in the LNG-IUD group compared with expectant management (RR 0.22, 95% CI 0.08 to 0.60, 95 women, I² 0%, Moderate strength of evidence). The proportion of women who were satisfied with their treatment was also higher in the LNG-IUD group, but did not reach statistical significance (RR 1.21, 95% CI 0.80 to 1.82, 95 women, I² 0%).

The number of women reporting a change in menstruation was significantly higher in the LNG-IUD group (RR 37.80, 95% CI 5.40 to 264.60, 95 women, I² 0%), however the number of women who failed to complete the allocated treatment did not differ between groups (RR 0.66, 95% CI 0.08 to 5.25, I² 43%).

**Limitations, reason for caution:** Only 135 women were included. From a methodological perspective, additional properly randomised and reported long-term trials are needed to confirm the effectiveness of this method of therapy. The included trials lacked proper reporting on all aspects of potential biases thereby limiting the internal validity of the results. Furthermore, as most were short-term trials, it is not clear whether patient compliance over a prolonged period might affect the overall results. Even so, the consistent results presented by the trials regarding the decreased incidence of recurrence of painful periods is encouraging.

Finally, it should be noted that the majority of outcomes were not analysed due to unavailability of data, and even those that were analysed came from only three small trials that compared LNG-IUD insertion to no postoperative treatment or a GnRH analogue.

**Wider implications of the findings:** Further randomised controlled trials are encouraged to confirm these findings. If so, then local application of LNG-IUD should be adopted for pain associated endometriosis.

**Study funding/competing interest(s): None**

**Trial registration number:** registered in Cochrane databases

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**P-261 Follow-up of patients undergoing surgery for ovarian endometriosis: evaluation of ovarian reserve**

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**Study question:** To assess the impact of laparoscopic surgery for endometriotic cysts on the ovarian reserve evaluating pre and postoperative (12 months) levels of anti mullerian hormone (AMH) and the antral follicular count (AFC).

**Summary answer:** laparoscopic cystectomy of endometriosis causes a significant decrease in the levels of AMH and reduces the AFC, affecting the ovarian reserve.

**What is known already:** laparoscopic cystectomy of endometromias has been associated with concomitant excision of normal ovarian tissue resulting in significant follicle loss with possible subsequent reduction in ovarian reserve. Recently it has been shown that serum AMH level may be a valuable marker of ovarian reserve and that it appears to correspond well with AFC.

**Study design, size, duration:** This is a prospective study conducted from January to September 2011 at the Department of Obstetric and Gynecology of Università Cattolica del Sacro Cuore, Roma. We enrolled 36 consecutive patients who underwent laparoscopic excision of the endometriotic cyst; 30 completed the study and 6 were excluded for lack of compliance.

**Participants/materials, setting, methods:** Inclusion criteria: age 20-40 years, ultrasound diagnosis of endometrioma. All patients underwent laparoscopic cystectomy and were evaluated for AMH and AFC preoperative and after surgery. After excision of the cyst wall, the specimen was sent to the pathologist and, in the thickest section of the cyst, the follicles were counted.

**Main results and the role of chance:** the mean AMH level was 2.20 ng/ml prior to surgery and was reduced to 1.14 12 months post surgery; the rate of the decline of the hormone was 43%. In line with this findings the AFC decreased significantly after surgery. As expected, the patient age significantly correlates with the basal AMH level, the basal AFC and the number of follicles in the histologic section. Nevertheless there was no correlation between the AMH levels and AFC count after surgery and the number of follicles lost during the cystectomy. Interestingly...
the decrease observed in serum AMH levels and in AFC did not correlate with the cyst size.

Limitations, reason for caution: AMH and AFC are considered the most reliable markers of ovarian reserve, however both these tests reflect only the number of small antral follicles rather than the total follicle pool. Moreover the results need to be confirmed with a greater sample size.

Wider implications of the findings: In accordance with the literature this study shows that cystectomy for endometriomas causes significant damage to ovarian reserve with up to 40% fall in serum AMH concentration. The results suggest that the compromised ovarian reserve does not recover within 12 months. Despite the other literature findings we observed a significant reduction in AFC after the laparoscopic stripping of endometriomas and this data, along with the decrease in AMH, does not correlate with the cyst size.

Study funding/competing interest(s): The authors have no interests to disclose. This study was not financed.

Trial registration number: This is an observational study.

P-262 The Wnt/β-catenin signaling pathway might be involved in mechanisms regulating fibrosis in endometriosis: a study of in vitro and in vivo

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Study question: Is the Wnt/β-catenin signaling pathway involved in cellular and molecular mechanisms regulating fibrosis in endometriosis?

Summary answer: Inhibitors of Tcf/β-catenin complex significantly reduced contraction of endometriotic stromal cells and expression of fibrotic markers. Inhibitors of Tcf/β-catenin complex and β-catenin small interfering RNA significantly decreased TGF-β1 expression of fibrotic markers. Treatment with CGP049090 significantly reduced collagen synthesis in endometriotic implants in a mouse model.

What is known already: Our previous studies suggested that aberrant activation of Wnt/β-catenin signaling might be involved in the pathophysiology of endometriosis. We demonstrated that cellular mechanisms known to be involved in development of endometriosis were inhibited by targeting the Wnt/β-catenin pathway. Furthermore, recent studies demonstrated that the Wnt/β-catenin pathway might be a novel mediator of fibrosis and potential therapeutic target. Histologically, endometriosis is characterized by dense fibrous tissue surrounding the endometrial glands and stroma.

Study design, size, duration: This is a study of in vitro and in vivo. Endometrial and/or endometriotic tissues were obtained from patients with (n = 34) and without (n = 20) endometriosis. Endometrial and endometriotic stromal cell were isolated for the in vitro analysis. Endometrial tissues were implanted into a total of 40 nude mice.

Participants/materials, setting, methods: In vitro effects of inhibitors of Tcf/β-catenin complex (PKF 115-584 and CGP049090) on cell contraction and fibrotic markers were evaluated by collagen contraction assay, quantitative PCR and/or western blotting. In vivo effects of CGP049090 on fibrosis were evaluated by Sirius red staining. Statistical significance was defined as P < 0.05.

Main results and the role of chance: Endometriotic stromal cells mediated-contraction of collagen gels was significantly decreased by treatment with inhibitors of Tcf/β-catenin complex (PKF 115-584: 82.8 ± 10.5%; CGP049090: 78.1 ± 8.3% of initial surface area) compared to that of non-treated cells (21.6 ± 2.6%). Inhibitors of Tcf/β-catenin complex significantly decreased expression of fibrotic markers (collagen type I, alpha smooth muscle actin, fibronectin and connective tissue growth factor) in endometriotic stromal cells. TGF-β1 induced the expression of these fibrotic markers, which was attenuated by inhibitors of Tcf/β-catenin complex and β-catenin small interfering RNA. Wnt3a treatment significantly increased cell contraction and cell migration of endometriotic stromal cells. Score for Sirius red staining was significantly lower in treated mice compared to that of non-treated mice three weeks after implantation.

Limitations, reason for caution: Further pre-clinical studies are required to investigate whether treatment with inhibitors of Tcf/β-catenin complex could improve clinical symptoms such as pain.

Wider implications of the findings: The Wnt/β-catenin signaling pathway might represent a novel therapeutic target for treatment of endometriosis.

Study funding/competing interest(s): This study was supported in part by Karl Storz Endoscopy & GmbH (Tuttlingen, Germany). No competing interests are declared.

Trial registration number: N.A.

P-263 New insights into the mechanisms underlying menorrhagia: roles of smoothelin and calponin

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Study question: How does the vascular smooth muscle content and the stage of vascular smooth muscle cell (VSMC) differentiation in the endometrial blood vessels vary during the different phases of the menstrual cycle in normal women and is this pattern of variation altered in women with menorrhagia?

Summary answer: The vascular smooth muscle content (as a proportion of the vascular cross-sectional area) of endometrial blood vessels remained unchanged during the normal menstrual cycle and in menorrhagia; however, the expression of VSMC differentiation markers, smoothelin and calponin was reversed in the endometrial blood vessels in case of menorrhagia.

What is known already: Menorrhagia affects 30% of women of reproductive age and is the reason for two-thirds of all hysterectomies. Previous studies have suggested important structural and functional roles for endometrial blood vessels, including impaired vascular contractility. The differentiation stage of VSMC, from synthetic to contractile state, is associated with altered cellular phenotype contributing towards normal blood flow and pressure. This vascular maturation process has been little studied in the normal menstrual cycle or in menorrhagia.

Study design, size, duration: Endometrial biopsies were taken from hysterectomy specimens or from pipelle biopsies in controls, without endometrial pathology (proliferative 8, early-secretory 7, mid-secretory 5, late-secretory 9) and women with menorrhagia (proliferative 10, early-secretary 7, mid-secretory 4, late-secretory 6). Biopsies were formalin fixed and embedded in paraffin wax.

Participants/materials, setting, methods: Paraffin-embedded sections were immunostained for alpha smooth muscle actin (αSMA), myosin heavy chain (MyHC), h-caldesmon, desmin, smoothelin and calponin. Muscle was measured in αSMA-positive vascular cross-sections (n = 25/sample) as a ratio of muscular area/gross vascular cross-sectional area. VSMC differentiation was analysed by the presence/absence of differentiation markers compared with αSMA.

Main results and the role of chance: In normal endometrium, the proportion of blood vessels expressing αSMA increased from 63% in the proliferative phase to 79% in the late secretory phase, with a trend towards a decrease in the mid-secretory phase in menorrhagia (73% vs. 66%, P = 0.06). The overall arterial muscle content in both control and menorrhagia remained stable occupying 78-81% of a gross vascular cross-sectional area during the different menstrual cycle phases. The percentage muscle content vascular cross-sectional area did not differ between control and menorrhagia endometrium (P > 0.05) at any phase of the menstrual cycle. However, study of VSMC differentiation markers revealed an inverse relationship between the presence of calponin and smoothelin; in the late secretory phase calponin was decreased (P = 0.05) and smoothelin was increased (P = 0.02) in menorrhagia compared with normal controls.

Limitations, reason for caution: This study included both straight and spiral arterioles from only stratum functionalis of the endometrium. In the VSMC differentiation analysis, data are presented as the presence or absence of the differentiation markers in each field of view, corresponding with the vascular cross-sections included in the study of vascular muscle content.

Wider implications of the findings: This study contrasts with a previous study reporting reduced αSMA expression in the mid-secretory phase in menorrhagia, although measurement methodologies differed. Smoothelin and calponin have been widely implicated as important regulators of vascular tone, vascular contractility and rate of blood flow. Our results have uncovered a disparate pattern of smoothelin and calponin expression, potentially indicating a dysfunctional contraction mechanism in the endometrial blood vessels in menorrhagia.
P-264  Women with endometriosis have significantly more pain during intercourse than controls resulting in a severely impaired sexual functioning

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Study question: To compare sexual functioning of women with endometriosis and their partners with sexual functioning of a control group of women without the disease and their partners.

Summary answer: Women with endometriosis have increased dyspareunia and more often suffer from sexual dysfunction. Increased pain during intercourse is correlated with lower sexual functioning scores. Half of the women with endometriosis are afraid of losing their partner because of the effect of endometriosis on their sexual functioning.

What is known already: Women with endometriosis have a substantial increase in risk of deep dyspareunia in comparison to the general female population of corresponding age and there is growing evidence that the presence of dyspareunia plays a significant role in the perceived quality of life in endometriosis patients. Furthermore there are a few studies that indicate that women with endometriosis have decreased sexual functioning.

Study design, size, duration: In this cross-sectional study, sexual functioning of women with endometriosis (n = 38) and their partners (n = 35) was compared with sexual functioning of a control group of women attending the outpatient department for contraception (n = 28) and their partners (n = 20) in the period between June 2011 and December 2012.

Participants/materials, setting, methods: The study was questionnaire based and investigated the degree of dyspareunia using the visual analogue scale (0-100) (VAS), sexual functioning using the Female Sexual Functioning Index (FSFI) and quality of sexual functioning (VAS).

Main results and the role of chance: Women with endometriosis had higher VAS-scores for dyspareunia than controls (adjusted difference: 21.82, p = 0.001). Women with endometriosis considerably more often had sexual dysfunction than controls, based on the FSFI (44.7% versus 7.1%, adjusted odds ratio 12.10, p = 0.008). Women with endometriosis had lower quality of sexual functioning based on the VAS-score (adjusted difference: 23.35, p = 0.003). Lower VAS-scores for quality of sexual function were also found for male partners of women with endometriosis versus partners of controls; however this difference was not statistically significant. There was a strong correlation between increased pain during intercourse (VAS) and lower FSFI score (R² = 0.57, p < 0.001). Half of the women with endometriosis (50.0%, 95% CI 34.4%-65.6%) was afraid to lose their partner because of the effect of endometriosis on their sexual functioning.

Limitations, reason for caution: The sample size is relatively small. Women with endometriosis were younger than controls. Participants were not matched for BMI or age. Increased pain during intercourse with as a consequence a severe impaired sexual functioning. The results of this study need to be confirmed in a larger and preferably international population. Eventually, this information could be instrumental to develop coping strategies to improve sexual functioning.

Study funding/competing interest(s): Not applicable

Trial registration number: Not applicable

P-265  Non-invasive diagnosis of endometriosis: examination of cervical fluid with infrared spectroscopy

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Study question: Can cervical fluid be used to diagnose endometriosis using the technique of Fourier transform infrared spectroscopy (FT-IR)?

Summary answer: FT-IR has the potential to provide biochemical information about endometriosis from the cervical fluid. Our study showed that FT-IR spectroscopy based methods may be useful for the non-invasive diagnosis of endometriosis.

What is known already: There is no valid non-invasive technique to diagnose endometriosis, particularly for early staged cases. In the presence of endometriosis, eutopic endometrium presents a variety of difference when compared with endometrium from otherwise healthy women. Endometrial cells, proteins, lipids, nucleic acids, cytokines and some other structures are detected in cervical fluid.

Study design, size, duration: In a period of 12 months, total of 30 patients between 18-45 years undergoing laparoscopy were prospectively recruited. With regard to modified American Fertility Society scoring, patients were stratified as early (minimal-mild, n = 10) or late (moderate-severe, n = 10) endometriosis. Women without any visible lesion of endometriosis assigned to control group (n = 10).

Participants/materials, setting, methods: Cervical swab was obtained from the cervical canal with a cotton swab during early follicular and/or late luteal phase. The samples taken with the swap were diluted in 1 ml of saline and collected until the time of spectroscopy. Cervical smear for examination of cervical cytology was also taken.

Main results and the role of chance: In this study, we observed 14 significant peaks in the FT-IR spectra in patients with endometriosis and they were assigned to different biological molecules according to their bond structures. Although some variations were noticed particularly in lipid, protein and nucleic acid region, the most sensitive spectral region for the secondary protein structural components was the amide I band (1700-1600 cm⁻¹), which is due almost entirely to the C=O stretch vibrations of the peptide linkages. Relative intensity of peaks in endometriosis group were found to be increased in 1624 cm⁻¹ (Antiparallel β-sheet, and decreased in 1635 cm⁻¹ (Parallel β-sheet), 1670 cm⁻¹ (β-turn), 1682 cm⁻¹ (Antiparallel β-sheet) according to control group. The band area ratios of protein/DNA and protein/RNA increased in the endometriosis groups when compared with the control group.

Limitations, reason for caution: Since this is a preliminary study for determining the validity of cervical fluid in the diagnosis of endometriosis, the results should be confirmed with larger sample sized trials. There is also space for detecting the optimal technique to reveal cervical fluid in best conditions.

Wider implications of the findings: There is no universally accepted non-invasive method for the diagnosis of endometriosis. Although ultrasonography and other imaging techniques might be useful for the diagnosis of endometrioma and severe disease, a proper diagnostic test for early-moderate cases is absent. Once eutopic endometrium presents variation from the endometrium taken from otherwise healthy women, the examination of cervical fluid, which might reflect the status of endometrial cavity, is a good candidate for the diagnosis.

Study funding/competing interest(s): The project was funded by Hacettepe University, Research Project Institute (project number: 6028).

Trial registration number: Not a RCT.

P-266  Expressions of proliferating cell nuclear antigen (PCNA), cyclin D3, p27, p57 proteins and determination apoptotic cells in normal and dexamethasone-induced intrauterine growth restriction rat placentas

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Study question: Dexamethasone-induced intrauterine growth restriction rat placentas

Study funding/competing interest(s): Not applicable

Trial registration number: Not applicable
Study question: We determined the expressions of some cell cycle proteins and apoptotic cell number on normal and dexamethasone induced intra-uterine growth restricted (IUGR) rat placentas to see whether there are changes on the context of proliferation and apoptosis.

Summary answer: In parallel with reduced embryo and placenta weights in the dexamethasone-induced IUGR group we found reduced expression of PCNA and increased expressions of cyclin D3, p27 and p57 and higher TUNEL positive cell number in IUGR placentas compared to normal placentas.

What is known already: IUGR is a major clinical problem which causes perinatal morbidity and mortality and major etiologic factor is abnormal placentation. Despite the fact that placental development requires the coordinated action of trophoblast proliferation and differentiation, there are few studies on cell cycle regulators, which play the main roles in the coordination of these events and it is still not determined how mechanisms of coordination of proliferation and differentiation are influenced by dexamethasone-induced IUGR in the placenta.

Study design, size, duration: Female rats were mated with male rats, presence of sperm in vaginal smear accepted day 0 of pregnancy. Rats were injected 100 µg/kg dexamethasone on day 13, 200 µg/kg dexamethasone on days 14-19 of pregnancy. Control animals were injected saline solution. Six rats each group were sacrificed for each method.

Participants/materials, setting, methods: After Rattus norvegicus rats were sacrificed on day 20 of pregnancy, blood samples were taken, placentas were formaline fixed-paraffin embedded or snap-frozen. We applied Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), immunohistochemistry, Western blotting, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end-labeling (TUNEL), glucocorticoid assay and did transmission electron microscopic observations.

Main results and the role of chance: Although there was a statistically significant increase of glucocorticoid levels 60 minutes after dexamethasone injection, it was normal 180 minutes after the injection in IUGR group. Mean embryo and placenta weights of control rats were higher than IUGR group with statistically significant difference (p = 0.005) but dexamethasone didn’t affect the number of embryos. According to RT-PCR, immunohistochemistry and Western blotting results expression of PCNA was higher in control group than in IUGR group and it was statistically significant (p = 0.041), expressions of cyclin D3, p27 and p57 were higher in IUGR group. TUNEL positive cell numbers in IUGR group placentas were higher than control group placentas (p < 0.001). Electron microscopic observations was compatible with TUNEL results. Spongiotrophoblasts and labyrinth trophoblasts of IUGR placentas showed apoptotic cell characteristics.

Limitations, reason for caution: This study described decrease of proliferation, increase of apoptosis in dexamethasone injected IUGR rat placentas. Since dexamethasone is widely used to women having premature labor risk, reduces fetal growth and predisposes to increased risk of disease in later life, detailed studies should be done.

Wider implications of the findings: Our data suggests that glucocorticoid-induced restriction of fetal-placental growth is mediated, in part, via inhibition of cell cycle proteins and increase in apoptosis. Previous studies showed that dexamethasone caused a decrease in growth–promoting genes. Glucocorticoid metabolism during pregnancy is still debated. How dexamethasone acts in placental growth inhibition hasn’t been determined. Since one of the reasons for IUGR is abnormal placentation we are planning new research aiming to reveal increasing apoptosis rate in IUGR placentas.

Study funding/competing interest(s): There is s.

Trial registration number: No number.

Study question: Is it possible to evaluate precisely the impact of ovarian endometriotic cyst (EC), laparoscopic cystectomy, and age on follicle reserve in healthy ovarian tissues and in surgically resected cyst walls?

Summary answer: Ovarian endometriomas have a detrimental impact on follicle reserve in younger patients, further, laparoscopic cystectomy for EC may accelerate the rate of oocyte loss associated with aging.

What is known already: The rate of oocyte decline follows a biphasic pattern, characterized by acceleration between 32 to 38 years old. Ovarian reserve is also affected by external factors including ovarian disease and iatrogenic damage.

Study design, size, duration: This cohort and cross-sectional study. The follicles in normal ovarian tissue and resected cyst walls were morphologically graded using semi-quantitative scale. In normal ovarian tissue samples, the stromal area was measured, the number of follicles counted and the density calculated.

Participants/materials, setting, methods: Out of 110 patients who underwent laparoscopic ovarian cystectomy were recruited to the study after providing written informed consent. Based on histological assessment, 61 patients were found to have EC, whereas 42 patients had non-EC. Seven patients without normal ovarian stroma in the biopsy specimen were eliminated.

Main results and the role of chance: The density of follicles in ovarian tissues correlated with the patient age in both groups. In women aged < 35 years, the relative density of follicles in healthy ovarian tissues was lower in the EC group compared to the non-EC group, with a relative ratio at age 20, 30 and 35 years calculated to be 35.4%, 46.8% and 62.7%, respectively. There was no significant difference between the groups in patients aged > 35. The resection rate of normal ovarian tissue in cystectomy specimen of EC group was significantly higher than in non-EC group. According to the comparison between cases of cyst walls with and without normal ovarian tissues, the size of EC in the cases of cyst wall with ovarian tissue was significantly smaller than without ovarian tissue.

Limitations, reason for caution: Ovarian tissues were biopsied from visually healthy ovarian tissue expanded by ovarian cyst.

Wider implications of the findings: The rate of age-dependent follicle loss in the EC group was less pronounced than in the non-EC group, suggesting that persistent EC may elicit a protective response in adjacent ovarian tissue. The findings of our histological study support the notion that current practice of cystectomy of relatively small EC should be avoided before fertility treatment, such as IVF. On the other hand, if conservative surgery becomes indicated, aggressive cystectomy should be avoided as far as possible.

Study funding/competing interest(s): This study was supported by a grant from the Akdeniz University Research Fund (2005.02.0122.003), Antalya, Turkey. The authors declared they have no competing interests.

Trial registration number: No number.

P-267  Histological assessment of impact of ovarian endometrioma and laparoscopic cystectomy on ovarian reserve

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Study question: Is it possible to evaluate precisely the impact of ovarian endometriotic cyst (EC), laparoscopic cystectomy, and age on follicle reserve in healthy ovarian tissues and in surgically resected cyst walls?

Summary answer: Ovarian endometriomas have a detrimental impact on follicle reserve in younger patients, further, laparoscopic cystectomy for EC may accelerate the rate of oocyte loss associated with aging.

What is known already: The rate of oocyte decline follows a biphasic pattern, characterized by acceleration between 32 to 38 years old. Ovarian reserve is also affected by external factors including ovarian disease and iatrogenic damage.

Study design, size, duration: This cohort and cross-sectional study. The follicles in normal ovarian tissue and resected cyst walls were morphologically graded using semi-quantitative scale. In normal ovarian tissue samples, the stromal area was measured, the number of follicles counted and the density calculated.

Participants/materials, setting, methods: Out of 110 patients who underwent laparoscopic ovarian cystectomy were recruited to the study after providing written informed consent. Based on histological assessment, 61 patients were found to have EC, whereas 42 patients had non-EC. Seven patients without normal ovarian stroma in the biopsy specimen were eliminated.

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Limitations, reason for caution: Ovarian tissues were biopsied from visually healthy ovarian tissue expanded by ovarian cyst.

Wider implications of the findings: The rate of age-dependent follicle loss in the EC group was less pronounced than in the non-EC group, suggesting that persistent EC may elicit a protective response in adjacent ovarian tissue. The findings of our histological study support the notion that current practice of cystectomy of relatively small EC should be avoided before fertility treatment, such as IVF. On the other hand, if conservative surgery becomes indicated, aggressive cystectomy should be avoided as far as possible.

Study funding/competing interest(s): There is s.

Trial registration number: No number.

P-268  Surrogate mothers: background and motivations

K. Svitnev

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Study question: It’s a preliminary report to clarify motivations of intended surrogate mothers and “experienced” surrogates and, taking into consideration their background, specify if their main motive is an altruistic wish to help childless people to become parents, either it’s all about money or the surrogates are driven by combination of different motives.

Summary answer: There is no main driving force behind surrogacy and, although financial remuneration is essential for many surrogates, it’s almost always a mix of several motivations, including a sincere wish to help and a need to improve their own living conditions.

What is known already: Beyond money surrogates might have very different motivations, such as guilt over a past abortion, or just like to be pregnant.

Study design, size, duration: The study has been conducted for 6 months from August 2012 to January 2013. Only gestational surrogacy was studied as

Ethics and law

P-268  Surrogate mothers: background and motivations

K. Svitnev

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