containing 5.64 M ethyleneglycol (EG) + 5% (w/v) PVP + 0.5 M sucrose was used for vitrification. For URV, ovarian sections loaded on Cryosupport which consist of 3 fine needles were immersed in liquid nitrogen (LN,) directly. For conventional vitrification, the sections were packaged in 0.25 mL straw and the straws were immersed in LN2. The cryoprotectants used in slow freezing were 1.5 M propanediol and 0.1 M sucrose. After warming, morphologies of follicles were analyzed using light microscopy and transmission electron microscopy.

Results: The proportion of morphologically-normal follicles vitrified ultra-rapidly (93%) was higher (p < 0.05) than those of follicles vitrified in a straw (63%) and frozen by slow freezing (59%). When the ovarian cortex was vitrified ultra-rapidly and vitrified in straw; the surface ratio of lysosomes per oocyte cytoplasm (1.3%) were lower (p < 0.05) than slow freezing (2.6%) and similar to that in non-frozen ovaries (1.1%). Recovery of the hormone cycle after non-thermalisation of 124 days was confirmed in all three cynomolgus monkeys with heterotopic autotransplantation to the omentum of ovaries cryopreserved by URV, and fertilized ova were successfully obtained.

Conclusions: During this study, we successfully established a new method for cryopreservation of ovarian tissue and heterotopic autotransplantation in primates (cynomolgus monkeys). Based on these results, we obtained approval from the Ethics Committee of St. Marianna University School of Medicine to apply this technique clinically to humans.

O-172 Metformin improves pregnancy and live birth rates in women with polycystic ovary syndrome - a multicentre placebo-controlled randomised trial

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Introduction: Polycystic ovary syndrome (PCOS) is characterized by chronic anovulation and hyperandrogenism with clinical manifestations of irregular menstrual cycles, infertility, hirsutism or acne. The condition is the most common endocrinopathy in women affecting approximately 10 percent of women of reproductive age. The aim of this review is to determine if the use of metformin, a insulin sensitising agent, improves live birth and pregnancy rates in PCOS patients in in vitro fertilization (IVF) treatment. The use of insulin-sensitizing agents, such as metformin, in women with PCOS undergoing ovulation induction or IVF cycles has been widely studied. There is physiologic rationale for believing that supression of insulin levels with metformin might reduce hyperinsulinaemia and hyperandrogenism on ovarian response. As a consequence it improves both pregnancy, and live birth rates.

Objective: The objective of this study is to determine the effectiveness of metformin co-treatment during IVF or ICSI in achieving pregnancy or live birth in women with PCOS.

Material and Methods: Search strategy: We searched the Menstrual Disorders and Subfertility Group’s trials register, Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, LILACS, meta Register of Controlled Trials and reference lists of articles.

Selection criteria: Types of studies: randomized controlled trials (RCTs) comparing metformin treatment with placebo in PCOS women who underwent IVF/ICSI treatment.

Types of participants: women of reproductive age with anovulation due to PCOS (ESHRE/ASRM 2003) with or without co-existing infertility factors.

Types of interventions: metformin versus placebo before and during IVF/ICSI treatment.

Types of outcome measures: live birth rate, pregnancy and clinical pregnancy rates, fertilization rate, number of oocytes retrieved, total dose of FSH, number of days of gonadotrophin treatment, cycle cancellation rate, miscarriage rate, incidence of ovarian hyperstimulation syndrome (OHSS), incidence of patient reported side effects, serum estradiol level on day of hCG trigger, serum androgen level and fasting insulin and glucose levels.

Data collection & analysis: Two reviewers extracted the data independently according to the protocol. Method of the randomization, characteristics of the studied groups and allocation concealment were evaluated.

Results: Six studies were included and a total of 483 PCOS women were analysed. The meta-analysis demonstrates that there is difference between comparison groups, favouring metformin, but not statistically significant in the following outcomes: live birth rate - metformin group (39/136 - 28.7%) and placebo/no treatment group (33/136 - 24.3%) (OR 0.77; 95% CI 0.27 to 2.18) and clinical pregnancy rate - metformin group (71/216 - 32.9%) compared to the placebo/no treatment group (56/210 - 26.7%) (OR 0.71; 95% CI 0.39 to 1.28). However, the meta-analysis of the six RCTs shows that metformin significantly reduces the risk of OHSS at IVF/ICSI cycles (5.7% versus 21.2%, OR 0.27; 95% CI 0.11 to 0.43).

Conclusions: This Cochrane review with meta-analysis has found no evidence that metformin treatment before or during ART (assisted reproductive techniques) cycles improves live birth or pregnancy rates. Moreover the risk of OHSS in PCOS patients undergoing IVF/ICSI cycles was reduced with metformin. Further large RCTs are necessary to definitively answer if the use of metformin in PCOS women undergoing ART improves live birth and pregnancy rates.
An intent-to-treat analysis (with a Kaplan–Meier estimate) showed that metformin significantly improved PR (49.7 % vs. 34.9%, p = 0.01) and LBR (46.5% vs. 30.5%, p = 0.005) vs. placebo.

The rate of miscarriage was low and did not differ between the two groups (metformin 13.2% vs. placebo 17.0%, p = 0.8).

In Cox regression analysis including BMI, waist-hip ratio, serum testosterone and free androgen index, the hazard rate (HR) for metformin remained significant for pregnancy (HR = 1.75, CI 95% 1.19–2.55). Kaplan–Meier estimation suggested that BMI was the most important determining factor after metformin: the PR was 56.6% and LBR 54.4% in the non-obese women on metformin, 43.6% and 38.7% in the non-obese women on placebo (p = 0.12 and p = 0.08, NS, between the metformin and placebo groups); 41.4% and 36.5% in the obese women on metformin, and 26.0% and 22.1% in the obese women on placebo (p = 0.04 between the metformin and placebo groups), respectively.

Conclusions: The miscarriage rate was low in this population and metformin did not decrease it. Metformin alone or combined with other infertility treatments improved PR and LBR in women with PCOS and the most beneficial effect was observed in obese women. We conclude that women with PCOS suffering from anovulatory infertility, especially obese ones, benefit from combining metformin with “traditional” infertility treatment.

O-173 Effect of metformin on follicular anti-Müllerian hormone concentrations in women with PCOS undergoing IVF treatment

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Introduction: Our recent randomised controlled trial (Tang et al., 2006) demonstrated that co-treatment with metformin for patients with PCOS undergoing IVF/ICSI improves treatment outcomes and reduces the risk of developing ovarian hyperstimulation syndrome (OHSS). These findings were substantiated in a recent Cochrane review (2009). Recent studies revealed that women with PCOS have a significantly higher serum level of Anti-Müllerian hormone (AMH) compared with women with normal ovaries. Furthermore, follicular AMH concentrations were found to be positively correlated with embryo implantation and pregnancy rates. We wished to ascertain the effect of metformin on follicular AMH levels in women with PCOS undergoing IVF cycles.

Materials and Methods: Patients with PCOS who underwent IVF/ICSI treatment using a long GnRH agonist protocol were randomised to receive either metformin 850mg or placebo tablets twice daily at the start of down-regulation until the day of egg collection. Follicular fluid was collected from the follicles (diameter between 17 and 19mm) from which the eggs were retrieved. The fluid was frozen within 4 hours for future analysis. AMH was analyzed in duplicate using ELISA (Beckman-Coulter, France).

Results: Sixty-nine subjects undergoing 77 IVF/ICSI cycles were randomised to receive metformin (n = 39) or placebo (n = 38). There were no significant differences in the baseline demographic data between the two groups with a mean age (MET = 31.8 years old, PLA = 31.7 years old, P = 0.433), BMI (MET = 27.7kg/m², PLA = 27.4kg/m², P = 0.789) and median cycle length (MET = 35days, PLA = 35days, P = 0.431). Baseline FSH concentrations were also similar (MET = 4.49IU/L, PLA = 5.15 IU/L).

Despite the fact that the subjects received a similar total dose of gonadotropins (MET = 1200, PLA = 1300, P = 0.442), significantly more oocytes were retrieved in the metformin group (17.0 vs 14.1, P = 0.038). The overall fertilisation rates (MET = 57.7%, PLA = 61.5%) and the median number of embryos transferred (MET = 2, PLA = 2) were no different between the two groups. Both the clinical pregnancy rate per cycle (MET = 58.8%, PLA = 36.85, P = 0.08) and the live birth rate (MET = 48.7%, PLA = 18.4%, P = 0.01) were higher in those treated with metformin. The incidence of moderate and severe OHSS was lower in the metformin group (5.1% vs 13.2%).

A significantly higher mean follicular AMH concentration was observed in the metformin group compared with the placebo group (37.1 pmol/l vs 26.2 pmol/l, P = 0.008). Both serum (coefficient = -0.299, P = 0.02) and follicular (coefficient = -0.322, P = 0.009) vascular endothelial growth factor (VEGF) concentrations were negatively correlated with follicular AMH levels. These findings were still significant after being adjusted for the number of follicles on the day of egg collection. Furthermore, after adjustment for the number of follicles, metformin reduced the serum oestradiol concentration on the day of hCG administration (coefficient = -0.137, P = 0.028, R² = 0.249).

Conclusions: Our results suggest a direct effect of metformin on folliculogenesis. The production of AMH is mainly observed in the granulosa cells in the pre-antral and antral follicles. The level significantly declined once the follicle reached a diameter of 8-10 mm. AMH concentrations are also low in the atretic follicles. Recent evidence suggests that metformin exerts an anti-apoptotic effect on luteinised granulosa cells. This may explain why follicular AMH levels are found to be higher in the metformin group. An increase in viability of granulosa cells could improve the quality of oocytes which may improve the pregnancy outcomes. Furthermore, AMH has been shown to decrease FSH sensitivity. Therefore, this could explain the negative correlations between follicular AMH and VEGF concentrations. In addition, this may partially explain the protective effect of metformin on OHSS since VEGF is a key mediator of OHSS. Further in vitro studies are needed to investigate the effects of metformin on AMH production and on metabolism of the granulosa cells.

Reference:

O-174 Kisspeptin, leptin and retinol-binding protein-4 in women with polycystic ovary syndrome

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Introduction: The polycystic ovary syndrome (PCOS) is a common heterogeneous disorder, and its complex pathogenesis involves increased LH levels, increased resistance to insulin and compensatory hyperinsulinaemia, which has been associated with enhanced androgen production and anovulation. Kisspeptin (metastatin), the product of the gene Kiss-1, identified as an important component in initiating GnRH secretion at puberty, recently appears to regulate the secretion of luteinizing hormone and promote ovulation. Leptin and retinol-binding protein-4 (RBP-4) are associated with obesity and insulin resistance. The aim of this study was to compare serum kisspeptin-54 (metastatin), leptin and retinol-binding protein-4 (RBP-4) levels in women with and without PCOS.

Methods: A total of 95 women were included in this study. Fifty-four had PCOS according to Rotterdam criteria and 41 were healthy women with regular menstrual cycles who served as control subjects. Blood samples were collected between day 3 and day 8 of menstrual cycle.

Results: Serum kisspeptin, leptin, and RBP4 levels were significantly higher in PCOS group compared to control group (10.55 ± 6.14, 6.98 ± 3.57 ng/mL, P < 0.001, 9.10 ± 6.62, 6.43 ± 5.41 ng/mL, P = 0.024, 23.33 ± 9.46, 15.48 ± 4.68 ng/mL, P < 0.001). Serum leptin and RBP4 levels were significantly higher in obese and overweight women with PCOS than those in non-obese PCOS women and controls (14.96 ± 7.33 vs. 6.29 ± 3.93 vs. 5.56 ± 2.58 ng/mL, P < 0.001; 27.95 ± 7.76 vs. 21.34 ± 9.21 vs. 15.20 ± 4.27 ng/mL, P < 0.001, respectively).

Conclusions: These findings in this study may indicate that kisspeptin, leptin and RBP-4 appear to be involved in the pathogenesis of PCOS.

O-175 Modulatory effects of metformin on human endometrial stromal cell decidualisation and gene expression

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Introduction: Metformin is a well known anti-diabetic drug also used to induce ovulation in women with polycystic ovaries and anovulatory cycles, who wish to conceive. Its positive effect on the ovarian function has widely been demonstrated in vitro and in vivo. Furthermore in a randomized clinical trial a reduced abortion rate was found in women treated with metformin compared to women without metformin treatment. As this treatment also lead to an increase in endometrial thickness it was concluded that there might be a direct effect on the endometrium itself. We hereby investigate for the first time a potential effect of metformin on endometrial stromal cells in vitro, by looking at known decidualisation markers and some implantation markers. Furthermore addition of insulin was performed to imitate the feto-maternal communication in vivo.
Material and Methods: After informed consent (approved by Ethics protocol of Heidelberg University) five endometrial biopsies of the late proliferative phase (day 8-12) were taken from healthy, regularly cycling women (38.4 ± 1.83 years old) during laparoscopic surgery for benign reasons. Exclusion criteria were hormonal stimulation within the last three months, endocrinopathies, cancrosous lesions and irregular menstrual bleeding. Stromal cells were cultured and pased twice to assure purity, followed by decidualization with and without Metformin (1mmol). After decidualisation was assurred via prolactin expression in supernatant (ELISA), cells were treated with insulin (100ng) for 24 hours. Supernatant was then collected for IL-8 -protein analysis using ELISA and cells were frozen in liquid nitrogen for further analysis. TRIZOL RNA isolation was followed by RT-PCR using Tagman primers for IGFBP-1, IL-8, IL-1β and ICAM. Student test was used to assess statistical significance with a cut-off level of p < 0.05.

Results: The addition of metformin to the cell culture during the time of decidualisation led to a significant reduction of prolactin secretion of 3.1 fold at day 15. The effect of insulin was assessed on the mRNA level, where a marked reduction of IGFBP-1 was found after insulin exposure, which was attenuated by pre-treatment with metformin. Furthermore metformin co-treatment lead to a similarly low IGFBP-1 gene expression as if insulin had been added in the culture of control cells. Examining the implantation markers IL-8, IL-1β and ICAM, longterm, as short as 3 days treatment co-treatment lead to a significant upregulation of IL-8 and IL-1β mRNA expression, while ICAM was found unchanged. Nevertheless cells remained sensitive to insulin treatment and reacted with a profound reduction of IL-8 and IL-1β mRNA expression, similar to control cells. On the protein level, IL-8 excreted into the supernatant demonstrated a similar pattern of expression under metformin treatment.

Conclusion: We demonstrate for the first time an effect of metformin on protein and gene expression in human endometrial stromal cells in vitro, with a reduction of decidualization in stromal cells and a change in important implantation markers,IL-8 and IL-1β. The changes found in these cytokines may contribute to the reduced abortion rates found in “metformin takers”, since IL-8 is known to attract trophoblast cells into the endometrium and is found to be reduced in women with polycystic ovaries. IL-1β is also found to be reduced in IL-8 women with recurrent abortions and therefore the metformin effect may restore the expression level leading to a better microenvironment for the implanting trophoblast.

SELECTED ORAL COMMUNICATION SESSION

SESSION 46: ENDOMETRIAL FUNCTION DURING IMPLANTATION WINDOW

Tuesday 29 June 2010 17:00 - 18:00

O-176 Large-conductance calcium-activated potassium channels in human endometrium affect embryo implantation via altering the window of implantation factors

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Introduction: Recent studies showed that the functions of large-conductance calcium-activated potassium channels (BKCa channels) were beyond traditional ion channel. They were the important integrators in many physiological processes such as action potential repolarization; neurotransmitter release; hormone secretion; innate immunity; cell proliferation, apoptosis and onogenesis. In human, BKCa channels distribute widely in many different tissues. However, it remains unknown whether BKCa channels express in human uterine endometrial cells. In our present study, we have discovered BKCa channels expressed in uterine endometrium. Furthermore, we investigated the possible effect(s) of BKCa channels on embryo implantation.

Materials and Methods: Endometrial samples were collected from 60 women of reproductive age volunteered for this study. According to the Noyes pathological diagnosis they were divided into two groups: proliferative phase (44 cases), and mid-secretory phase (16 cases). Immunohistochemical, RT-PCR and Western blot assays were applied to examine the expression and distribution of BKCa channels in human uterine endometrium at proliferative phase or mid-secretory phase. Patch-Clamping analysis was used to verify the function of BKCa channels in human endometrial cells. Primary endometrial cells and Ishikawa cells were cultured in medium containing E2 and P4 in vitro. The expression of window of implantation (WOI) factors (integrin β3, claudin-4 and DKK-1) was examined by quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot after BKCa channels blocked with IbTX (a specific blocker for BKCa) or knock-down with siRNA in primary endometrial cells and Ishikawa cells. Additionally, we analyzed the effects of BKCa on the embryo adhesion by co-cultured ICR mice blastocysts and Ishikawa cells in vitro.

Results: (1) Immunohistochemical staining, RT-PCR and Western blot assay shown that both BKCa channels mRNA and protein were expressed in human endometrium. The location of BKCa channels were mainly in luminal and glandular epithelial cells of human endometrium. (2) The levels of BKCa mRNA and protein in human endometrium at the mid-secretory phase were significantly higher than that at the proliferative phase (P < 0.05, respectively). (3) Patch-Clamp experiment demonstrated that a voltage dependent current was detected in human endometrial cell. This current could be inhibited by treatment of cells with IbTX, a specific blocker for BKCa. (4) qRT-PCR and Western blot both showed the increased WOI factors expression induced by E2 and P4 in endometrium could be significantly attenuated by the blocking of BKCa or knock-down BKCa gene expression. (5) Embryo adhesion rate significantly decreased by the blocking of BKCa with IbTX in vitro.

Conclusions: BKCa channels express in luminal and glandular epithelial cells of human endometrium in a menstrual cycle dependent manner. BKCa channels may affect embryo adhesion via altering the production of WOI factors in endometrium.

O-177 Role of tweak in human endometrium

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Introduction: TWEAK (Tumor Necrosis Factor WEAK inducer of apoptosis) is a transmembrane protein highly expressed by several types of immune cells and in many tissues. It triggers multiple cellular responses including control of angiogenesis. It has been demonstrated TWEAK +/- mice have overabundant natural killer cells (NK). In addition, a recent study in our laboratory showed that TWEAK is weakly expressed in the immediate postimplantation period in CBA/J x DBA/2 mating (“abortion combination”) compared with CBA/J x BALB/c (the “control combination”); its neutralization resulted in a drastic enhancement of resorption rates in both cases. Thus, we supposed TWEAK could protect the embryo from resorption and act as a protective immunoregulator. In previous studies, we already observed that the expression of IL-15 and IL-18 was different in patients who failed to implant when compared with fertile controls and that this expression correlated with the local uNK (CD56+ ) cell recruitment. In turn, this led us to study TWEAK as a potential immunologic regulator of the effects of IL-15 and IL-18 on uNK cells recruitment/activity in the endometrium.

Material and Methods: The protocol enrolled 46 women, of whom 15 were fertile controls and 31 were women with repeated and unexplained implantation failure. Endometrial biopsies were performed during a monitored normal cycle 7 to 9 days after the ovulation surge with a standard Corner pipette. We localised the presence of TWEAK in human endometrium by immunohistochemistry. We documented, by real-time polymerase chain reaction (RT-PCR), among fertile women and patients who failed to implant, that TWEAK expression was increased in comparison to control samples. We also tested TWEAK neutralization by its neutralisation (TWEAK antibody) on IL-15 and IL-18 production by a new method of microhistoculture using collagen sponge as matrix for the endometrium biopsies. After 2 days of culture we realised ELISA test on culture supernatant and RT-PCR on biopsies.

Results: We observed that TWEAK is present both during the proliferative and the luteal phases with no major variation. In patients with a high IL-18...