analyzed by Affymetrix gene microarrays. Validation of single gene expression was performed by qPCR.

Main results and the role of chance: Analysis of the subpopulations of IMCs revealed a significant enrichment (over 2-fold, $P < 0.01$) of the Ly6G^{med}/Ly6C^{high} monocytic IMC fraction in tumor derived CD45$^+$ hematopoietic cells compared to placenta, paralleled by a concomitant, more than 2-fold decrease ($P < 0.01$) of the Ly6G^{high}/Ly6C^{med} granulocytic IMC subpopulation. In gonadotropin stimulated ovaries we observed a ~2.5 fold increase in Ly6Ghigh/Ly6C^{med} granulocytic IMCs compared to unstimulated controls. Tumor derived- and gonadotropin stimulated ovaries derived Ly6G^{med}/Ly6C^{high} IMCs expressed low levels of Cx3CR1 compared to the same cell population in placentas and unstimulated ovaries. Decreased expression of Cx3CR1 within IMCs has been shown to delineate a cellular population that actively contributes to tumor progression.

We next assessed the global transcriptional signature of tumor derived IMCs (T-IMCs) compared to placental IMCs (P-IMCs). Analysis of the top overexpressed genes in T-IMCs revealed several key players in tumor angiogenesis including Sema3a, and matrix metalloproteinases such as Mmp2, Mmp3, Mmp13, and Mmp14, as well genes that are involved in cancer progression and cell proliferation. Of note, various genes that were up-regulated in P-IMCs were shown to play a role in reproductive tissue angiogenesis, including Serpine1, Arg1, and Flt1.

Limitations, reasons for caution: This is an animal experiment and its findings need to be further validated also in humans.

Wider implications of the findings: IMC subpopulations diverge in tumor versus reproductive tissues, favoring monocytic IMCs in the former and granulocytic IMCs in the latter. This divergence is associated with unique expression of proangiogenic genes. Selective targeting of these genes may thus be further investigated as selective angiogenic therapies for cancer, placental disease, and ovarian-hyperstimulation.

Trial registration number: NA

P-447 Current but not former use of combined contraception is associated with glucose metabolism disorders in premenopausal women: a prospective population-based cohort study

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Study question: Is the former or current use of combined hormonal (CHC) and progestin-only (POC) contraceptives associated with abnormal glucose metabolism in premenopausal women?

Summary answer: Former CHC or former/current POC use was not associated with prediabetes/type 2 diabetes (T2DM), but the association was significant in current CHC users.

What is known already: Combined contraceptive use has been associated with impaired glucose tolerance (IGT) and increased insulin resistance, which are risk factors for prediabetes and T2DM. To date there are only few population-based studies with large enough population and with adequate adjustments. The results concerning hormonal contraceptive use and prediabetes/T2DM are inconsistent and no clear association between them has been demonstrated in general populations, but some prospective population-based studies have revealed an increased risk in current CHC users.

Study design, size, duration: In a prospective follow-up of a large national birth cohort ($n = 5889$) the women were clinically examined at the age of 46 and asked for their former and current use of hormonal contraceptives. The glucose metabolism indices were compared between current CHC ($n = 194$) or POC ($n = 1090$) and current non-hormonal contraceptive users ($n = 1204$), and on the other hand between former CHC ($n = 1884$) or POC ($n = 72$) users and women with no history of hormonal contraceptive use ($n = 358$).

Participants/materials, setting, methods: At age 46, a questionnaire was sent to 5123 women. Of them, 3708 (72.4%) answered, 3280 (64.0%) participated in clinical examinations and 2780 in a 2-h oral glucose tolerance test (OGTT). Prediabetes (impaired fasting glucose, IFG and/or IGT) and newly diagnosed T2DM (nT2DM) were diagnosed by OGTT. Diagnosis for previously diagnosed T2DM (pT2DM) was assessed from postal questionnaires and confirmed from hospital discharge and national drug registers.

Main results and the role of chance: Current CHC, POC and non-hormonal contraceptive users as well as former CHC and POC users and women who had never used hormonal contraception did not differ regarding BMI and waist circumference.

Current CHC use was significantly associated with prediabetes (odds ratio, OR: 2.1, 95% confidence interval, 95% CI: 1.3–3.4), nT2DM (OR: 3.4, 95% CI: 1.4–10.1), prediabetes/nT2DM (OR: 2.2, 95% CI: 1.5–3.5) and prediabetes/nT2DM/pT2DM (OR: 1.9, 95% CI: 1.3–2.9), but not with pT2DM compared with use of non-hormonal contraception.

Current POC use was not significantly associated with pre-diabetes, nT2DM or pT2DM compared with use of non-hormonal contraception.

Compared with current POC use, current CHC use was significantly associated with prediabetes (OR: 2.0 95% CI: 1.2–3.1) and prediabetes/nT2DM (OR: 2.0, 95% CI: 1.3–3.1), but not with pT2DM. Former CHC use was not significantly associated with prediabetes or nT2DM/pT2DM compared with former POC use or never use of hormonal contraception.

Former use of POC was not associated with prediabetes or pT2DM/nT2DM compared with never use of hormonal contraception.

The results did not change after adjustment for socio-economical status, alcohol consumption, smoking, number of deliveries and use of cholesterol lowering medication.

Limitations, reasons for caution: Use of hormonal and non-hormonal contraception was based on self- reporting which may have led to information bias. We excluded women reporting CHC use at any time of their life from the group of former POC users, which could have distorted the results.

Wider implications of the findings: Current CHC use in premenopause associates with a significant risk for prediabetes and T2DM, supporting active screening for these disorders in premenopausal CHC users. These findings also raise the question of whether it is advisable to recommend CHC use in older premenopausal women and in women with adverse metabolic profile.

Trial registration number: NA

P-448 CBL gene is a central gene overexpressed in cumulus cells of obese Polycystic Ovary Syndrome (PCOS) women without clinical insulin resistance compared to non-obese PCOS

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Study question: Which genes of the insulin pathway are relevant in cumulus cells of obese PCOS women when undergoing to an IVF cycle?

Summary answer: The gene CBL is overexpressed and acts as central gene of insulin signaling pathway in the cumulus cells of obese PCOS women.

What is known already: Insulin plays a central role on PCOS women and can be responsible for worst IVF outcomes on those patients. The precise mechanism of insulin action on cumulus cells and the consequences to oocyte maturation is not completely elucidated in obese or even in non-obese PCOS patients. PCOS women have oocytes of poorer quality even without clinical evidence of insulin resistance. The gene expression profiles from insulin pathway in cumulus cells could provide new insights, mainly in obese patients, where hyperinsulinemia should be deleterious and may explain the

worst IVF outcomes. New therapy strategies would be possibly offered to those patients.

Study design, size, duration: Cross-sectional study to evaluate gene expression at the insulin pathway in *cumulus* cells of patients submitted to IVF treatment from January 2013 to October 2014. Fifteen PCOS patients according to Rotterdam criteria were subdivided in non-obese ($n = 9$, BMI <25, Control-group) and obese ($n = 6$, BMI >30, Obese-group). Both groups demonstrated a normal insulin resistance index according to the homeostasis model assessment insulin resistance (HOMA-IR).

Participants/materials, setting, methods: PCOS infertile patients were submitted to IVF with standard ovarian stimulation protocol, oocytes were recovered to IVF and cumulus cells were removed for gene expression analysis. RNA purification was carried out and quantitative PCR array analysis of gene expression profile (RT^2 Profiler™ PCR Array Human Insulin Signaling Pathway- PAHS-030ZC-Qiagen, USA) was done according with manufacturer instructions. We considered genes up- or down-regulated in obese-group compared to control-group, those presenting fold-change ≥3 or ≤3 and $p < 0.05$.

Main results and the role of chance: Among the 84 genes of insulin pathway analyzed, nine genes were statistically significant overexpressed in obese patients compared to non-obese. There were no down-regulated genes in obese-group compared to controls. The up-regulated genes were BCL2L1 (fold=4.7; $p = 0.021$), BRAF (fold=3.8; $p = 0.031$), DOK1 (fold=4.6; $p = 0.040$), FBP1 (fold=5.3; $p = 0.011$), FRS2 (fold=4.1; $p = 0.044$), PCK2 (fold=4.4; $p = 0.041$), RPS6KA1 (fold 4.2; $p = 0.044$), SORBS1 (fold=3.6; $p = 0.014$) and CBL (fold=6.2; $p = 0.019$). The mentioned genes are mainly involved on glucose uptake and cell proliferation, regulation of insulin sensitivity and promoting gluconeogenesis, diminishing available pyruvate. All actins could damage the energetic balance in early stages of embryo development. The bioinformatics tool (SABiosciences – GNCPro™ –Qiagen, USA) was used to evaluate the network and interaction among up-regulated genes. That analysis showed that 75% of the overexpressed genes have a strong relationship to CBL gene. CBL is a gene involved in cell signaling and protein ubiquitination, which inhibits intracellular signal transduction by targeting some tyrosine kinases. There is growing evidence that CBL acts inhibiting systemic insulin resistance promoted by macrophage pro-inflammatory cytokines, which are secreted in adiposity tissue of obese women.

Limitations, reasons for caution: A small sample size was included to screen the 84 genes. However, the sample power was calculated and higher than 80%. Although insulin resistance was not clearly demonstrated, insulin may play different effects in obese patients. Also ovarian stimulation might produce damages in the cumulus cells and oocyte competence.

Wider implications of the findings: CBL is a central gene to the perfect cumulus cells signaling function. In obese PCOS patients, where insulin resistance leads to hiperinsulinemia and may selective overexpress the CBL activity, which could promote detrimental effects on the follicle development.

Trial registration number: Not applicable

P-449 GnRH agonist triggering reduces pain symptoms during IVF/ICSI cycles

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Study question: To assess the dynamic of pain symptoms during IVF/ICSI cycles when final oocyte maturation is triggered by GnRH-agonist (GnRH-a) or hCG.

Summary answer: Using GnRHa for triggering ovulation limits the progression of pain symptoms following IVF/ICSI as compared to hCG triggering.

What is known already: Timely administration of GnRH-a has been shown to induce a surge of endogenous LH and FSH and trigger ovulation. The advent of antagonist COS protocols allows to use GnRH-a trigger, although endometrial alterations make deferring ET preferable. The shorter duration of LH effects on the growing follicles as compared to the long-lasting stimulation exerted by hCG reduces or suppresses the risk of OHSS. It therefore plausible that GnRH-a trigger might reduce the pain aggravation normally encountered in IVF/ICSI after ovulation induction and oocyte retrieval.

Study design, size, duration: This is a retrospective observational cohort study nested in a prospective published cohort (Santulli et al., 2015) conducted between 01/01/2014 and 31/12/2014 in a tertiary care university hospital. A total of 122 cycles from patients who underwent IVF or ICSI programs were analysed. Patients received an oral contraceptive synchronization treatment followed by ovarian stimulation with FSH and oocyte triggering with an injection of GnRHa or rhCG. Only non-pregnant women were retained for this study.

Participants/materials, setting, methods: Women were allocated to two groups: GnRHa triggering with a scheduled deferred embryo-transfer ($n = 70$) or hCG triggering with a fresh embryo transfer ($n = 57$). Pelvic pain scores were evaluated using a visual analogue scale. Total VAS score was defined as the sum of VAS scores of the different painful symptoms. Two evaluations were performed: during oral contraceptive synchronization treatment before ovarian stimulation and three weeks post-retrieval. Univariate and multivariate logistic regression models were conducted.

Main results and the role of chance: VAS average values for each symptom and for Total VAS score were comparable except for dysmenorrhea at final evaluation which was lower in "GnRHa triggering" group (4.81 ± 3.23 and 3.52 ± 3.23, $p = 0.046$). For both groups, pain increased during ART procedure. Trends for Total VAS change, calculated by subtracting the final VAS score evaluation from that at synchronization evaluation, revealed a significant lower pain increase in "GnRH-agonist triggering" group compared to "hCG triggering" group (3.77 ± 7.73 and 6.50 ± 6.57, $p < 0.05$, respectively). After multivariate logistic regression, GnRH-agonist triggering was associated with lower pain increase during the ART as compared to "hCG triggering group" (OR=0.31, IC95% 0.13–0.71, $p = 0.006$).

Limitations, reasons for caution: For comparing the dynamic of pain without biases from a developing pregnancy only non-pregnant women in "hCG triggering" group were analysed. Yet, we cannot rule out that selecting the non-pregnant sub-group of women did not introduce a bias of its own.

Wider implications of the findings: Finding that GnRH-a triggering is associated with less pain makes this the primary ART strategy for any woman fearing pain or at increased risk of pain, as for example in case of endometriosis. GnRH-a triggering is therefore a sound low pain option when ART related pain is best avoided.

Trial registration number: 0

P-450 Single human spermatozoon freezing technique for cryptozoospermia or non-obstructive azoospermia patients

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Study question: Is it possible to freeze a single spermatozoon and keep a high recovery rate to improve the clinical outcome of cryptozoospermia or non-obstructive azoospermia patients?

Summary answer: Our data indicates that it is highly possible to freeze a single spermatozoon with a high survival rate of about 80%.

What is known already: Clinical outcome of non-obstructive azoospermia or cryptozoospermia has increased after ICSI when using fresh spermatozoa. When oocyte collection cannot be performed on the same day of the Micro-TESE or in the case of cryptozoospermia, cryopreservation of collected spermatozoa becomes crucial. However, conventional freezing procedures are not appropriate for very low numbers of spermatozoa with poor motility.

Study design, size, duration: We performed a retrospective analysis of the clinical outcome of 79 ICSI cycles using our novel cryopreservation procedures for ejaculated spermatozoa from 27 cases of cryptozoospermia in 52 cycles and testicular spermatozoa from 20 cases of non-obstructive azoospermia in 27 cycles from January, 2012 to December, 2014.

Participants/materials, setting, methods: This study dealt with 27 men with cryptozoospermia and 20 men with non-obstructive azoospermia. Moving spermatozoa were carefully aspirated into a pipette one by one and put into a micro-drop of medium. About 2 µl of freezing medium was put on the tip of the CRYOTOP. 1–10 sperms were aspirated into an injection pipette and inserted into the medium and left in liquid nitrogen vapor for 2 min before being stirred in liquid nitrogen.