we have suggested (Acién et al. 2004) the following classification of malformations of the female genital tract which would include the ASRM classification in class 3:

1. Agenesis or hypoplasia of a whole urogenital ridge. There will be absence of kidney, functioning ovary, tube, hemivagina and hemivaginal (undetectable) in that same side. It can also associate with vertebral and/or auditory anomaly.

2. Mesonephric anomalies with absence of the Wolfian duct opening to the urogenital sinus and of the ureretal bud sprouting. There will be renal agenesis and ipsilateral bladder vaginism, and usually, ureter anomaly due to the absence of the “inductor” function of the injured mesonephric duct on the Müllerian duct (uterine duplicity with without interseptal or interurterine communication). If there is an ectopic sprout of the ureretal bud, there could then be renal hypoplasia and ectopic urerter opening into the blind vagina. These are the most complex malformations and can appear:
   A) With large hematosalpinx in the blind hemivagina.
   B) With Gartner’s pseudocysts in the anterolateral wall of the permeable vagina.
   C) With partial resorption of the intervaginal septum seen as a buttonhole on the anterolateral wall of the permeable vagina.
   D) With complete unilateral vaginal or cervico-vaginal agenesis.

3. Isolated müllerian anomalies (uterine anomalies, ASRM classification), can affect:
   A) Paramesonephric or Müllerian ducts: uterine and/or tibal anomalies, sometimes segmentary. Unicornuate, bicornuate, didelphys, septe uterus and others.
   B) Müllerian tubercle: vaginal (or cervico-vaginal) agenesis or atresia and segmentary atresias as the transversal vaginal septum.
   C) Both Müllerian tubercle and ducts: Mayer-Rokitansky-Kuster-Hauser Syndrome (uni or bilateral).
   D) Other Müllerian anomalies as discrepancy in the fusion and resorption processes, segmentary defects, affectation of the Müllerian tubercle and müllerian remnants.


5. Malformative combinations: mesonephric anomaly on one side and müllerian on the contralateral side and eventual associated anomaly of the urogenital sinus.

O-207 Laparoscopic assisted creation of neo-vagina in case of vaginal agenesis

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Objective: The most common condition associated with vaginal agenesis is the MRKH-syndrome, but it is also seen in AIS. Occurring in 1:5000 live female births the MRKH-syndrome should be known by gynecologists and adequate diagnostics and therapy should be chosen. The development of a new or well modified surgical method with new and improved instruments for the creation of a neo-vagina, also in the presence of renal malformations and to evaluate surgical and technical complications, duration of surgery, traction and hospital stay with fewer surgical and no technical complications, but better functional results could be achieved. It is therefore a safe, simple, short, effective and less traumatic procedure.

O-208 A slim PCOS-phenotype, characterized by heterozygous-normal/low genotype on the FMR1 (fragile X) gene

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Introduction: We previously defined the normal number of CGG repeats on the FMR1 (fragile X) gene in regards to ovarian function, independent of race/ethnicity, at 26-32. Defining women as normal (norm, both alleles in range) heterozygous (het, one outside of normal) and homozygous (hom, both outside) further established distinct aging patterns of ovarian reserve, based on anti-Müllerian hormone (AMH) levels. het patients can, however be norm/low (< 26) or norm/high (>32). In this study we investigated the het-norm/low genotype further.

Materials: We selected from our Center’s research data base 36 consecutive lean (BMI < 25) patients with either FMR1 norm (n = 20) or het-norm/low (n = 16) genotypes who initially presented for ovulation induction, including young egg donors (n = 4) and infertility patients (n = 32). All had, within 30 days from initial presentation, undergone evaluations of antral follicular counts (AFC) on cycle days-2/3, random AMH assessments and determination of CGG repeat counts on the FMR1 gene. A generalized linear model was created to compare the association of presence of CGG counts < 26 on one allele (genotype, het-norm/low) with equally lean women with norm genotype (both alleles at CGG counts 26-32), adjusted for age and BMI on ovarian reserve (OR), reflected in AMH levels.

Results: In cross-sectional analysis significantly distinct OR patterns in AMH and AFCs were observed, based on genotype, in women under age 38 years:
het-norm/low women demonstrated significantly more AFCs at young ages (p = 0.015) but this difference was lost above age 38. At any AMH level het-norm/low genotypes demonstrated approximately 4-times the AFC of norm women. AMH per antral follicle (AMH/AFC) was, therefore significantly lower with het-norm/low genotype (p = 0.02) but this difference was lost above age 38 years.

Conclusions: The het-norm/low genotype of the FMR1 gene is associated with a slim PCOS phenotype, characterized by active follicular recruitment but low AMH levels per antral follicle at younger ages. Low AMH values per antral follicle in these patients may be a reflection of follicular environment and oocytes quality, and deserve further exploration as to their relevance on treatment outcomes.

O-209 Unfavorable hormonal and metabolic alterations in PCOS maintain after menopausal transition

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Introduction: Women with PCOS are known to suffer from hyperandrogenism as well as impaired glucose tolerance often combined with metabolic syndrome. Previous studies have also indicated increased risk for cardiovascular disease (CVD) as well as events related to this health problem. According to our previous studies the ovarian androgen production capacity is enhanced in women with PCOS remaining high until late reproductive age. However, it is not well documented whether these hormonal and metabolic alterations maintain in menopause. The possibly persisting hyperandrogenism in women with PCOS together with metabolic disturbances may enhance health risks with advancing age in these women. Therefore glucose tolerance and ovarian steroid secretion capacity were studied by means of oral glucose tolerance test (OGTT) and human chorionic gonadotrophin (hCG) test in control women and women with PCOS during menopausal transition.

Materials and Methods: Thirty control women [aged 45-62 yr; body mass index (BMI) 20.8-29.7 kg/m²] and 16 women diagnosed with PCOS (aged 43-59 yr; BMI 25.9-36.9 kg/m²) were recruited. All study women with PCOS fulfilled Rotterdam’s criteria. The subjects were divided in premenopausal (pre: n = 11 control, n = 9 PCOS) and postmenopausal (post: n = 19 control, n = 6 PCOS) groups. After 12h fasting blood samples for serum glucose, insulin and C-peptide measurements were drawn before OGTT and at 30, 60 and 120 min. To evaluate ovarian steroid secretion capacity the subjects were given 5000 IU of hCG i.m., and serum samples for 17-hydroxyprogesterone (17-OHP), androstenedione (A), testosterone (T) and estradiol (E) were collected before and 24, 48, 72, 96 h after the injection. Basal serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex hormone-binding globulin (SHBG), inhibin-B and high sensitive CRP (hs-CRP, reflecting risk for CVD) were also measured.

Results: In OGTT, the area under the curve of insulin (AUC) was increased in PCOS both in pre- and postmenopausal control women compared to controls, and the same difference was observed in AUCins after menopausal transition. Serum insulin levels tended to be higher in PCOS women than in control women both in premenopause and postmenopause (pre: 9.8 mU/L vs. 4.8 mU/L, p = NS; post: 10.2 mU/L vs. 5.0 mU/L, p = NS). Insulin sensitivity index (2h) as well as the whole-body insulin sensitivity, calculated as described by Matsuda and DeFronzo were lower in PCOS in both age groups (insulin sensitivity index, as the whole-body insulin sensitivity index (BMI) 20.8-29.7 kg/m²) were lower and free androgen index (FAI) was higher in PCOS in both age groups.

Conclusions: The results indicate that impaired glucose metabolism, chronic inflammation and enhanced ovarian androgen secretion observed in premenopausal women with PCOS maintain after menopausal transition emphasizing life-long health risks related to this syndrome.

O-210 Polycystic ovary morphology is a frequent finding in healthy postmenarchial adolescents during the second decade of life

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Introduction: A high prevalence of polycystic ovarian morphology (PCOM) in healthy adolescents has been described. However, the significance of this finding and the prevalence of PCOM in adolescents of different ages is not known. A physiologic decline in ovarian volume (OV) and follicle number (FN) with age has been described during adulthood. We hypothesize that a decline in the prevalence of PCOM occurs as the adolescent ages.

The aim of this study is to determine the prevalence of PCOM in healthy adolescents of different ages, and to study the relationship of PCOM with steroid, gonadotropins and insulin levels.

Material and Methods: We recruited 72 healthy adolescents (age: 12-19.9 years), who were not hyperandrogenic (Ferriman-Gallwey score (F-G) lower than 7, without severe acne) and had normal menstrual cycles. The girls were divided in three groups according to their age: G1 (12-14.9 years old), G2 (15 - 16.9 years old) and G3 (17 -19.9 years old). A complete physical exam was performed. Body mass index (BMI), FG, acne and waist-to-hip ratio (WHR) was determined.

A transabdominal ultrasonographic study was performed during follicular phase (days 1-7). PCOM was defined by either 12 or more follicles measuring 2 – 9 mm in mean diameter and/or an ovarian volume (OV) greater than 10 ml in one or both ovaries. A fasting blood sample was obtained at the time of the ultrasonographic study. The following hormones were studied: luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), 17-hydroxyprogesterone (17OHP), dehydroepiandrosterone sulphate (DHEA-S), androstenedione (A), total testosterone (T), sex hormone binding globulin (SHBG), glucose and insulin. HOMA IR and free androgen index (FAI) were calculated.

Statistical analysis: Results are reported as mean and SEM or proportions (%). Data was analyzed using ANOVA, Mann-Whitney’s U test, Pearson’s χ², and Pearson’s correlation coefficient.

Results: PCOM was observed in 29.2% of the girls, and a similar prevalence of this finding was present in the three groups: 31.3; 34.8; and 28.6% in G1, G2 and G3, respectively (P = 0.7). Mean OV (MOV) was similar in the three group: G1 (6.6 ± 0.6 ml), G2 (7.4 ± 0.9 ml) and G3 (6 ± 0.6 ml; P = 0.3). Similarly, mean follicle number (MN) was similar in the three groups: 6.8 ± 0.6; 7 ± 0.7 and 6.5 ± 0.7 ml, in G1, G2 and G3, respectively (P = 0.5).

Girls with PCOM had similar levels of FG, BMI and WHR, androgens, FAI, SHBG, estradiol, insulin and HOMA IR and prevalence of mild- moderate acne compared to girls with normal ovarian morphology. However, lower FSH were observed in girls with PCOM compared to those with normal ovarian morphology (5.5 ± 0.3 ml vs 6.2 ± 0.2ml, respectively, P = 0.01). An inverse correlation of MOV, but not MN, with FSH was observed (r = -0.25 p = 0.03) and a positive correlation of MOV with FAI was observed (r = 0.5 p = 0.005). MOV and MN did not correlate with BMI, waist-to-hip ratio, FG. MN did not correlate with FSH or FAI.

Conclusions: We observed a high prevalence of PCOM in healthy adolescents throughout the second decade of life. PCOM was not associated with higher androgen levels or HOMA-IR levels, suggesting that PCOM appears not to be associated with hyperandrogenism or insulin-resistance in healthy girls. Lower levels of FSH were observed in girls with PCOM compared with girls with normal morphology. This study suggests that PCOM should be considered a prevalent condition during early adolescence.

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O-211 Antimullerian hormone as a predictor of ovarian reserve after laparoscopic ovarian drilling

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Background: PCOS is a common reproductive endocrinological disorder in females. Although LOD is one of treatments of choice for clomiphene citrate resistant PCOS woman, Consideration of substantial damage to ovarian tissue has been always mentioned too.
Objective: To measure ovarian reserve by AMH and TVS in women with PCOS before and after LOD.

Design: cross-sectional study.

Subjects: 86 women between 20 and 35 years old diagnosed to have PCOS.

Methods: TVS and AMH level were done before and after LOD.

Results: ovarian volume was 5.34±2.43 before LOD and became 5.17±2.12 mm after (NS). AFC before LOD was 20.4±4.1 and became 17.6±3.2 after (HS). AMH was 2.2±1.4 before and became 1.6±1.1 after (HS). No statistical significant correlation between AMH versus age and BMI before or after LOD. There was a statistical significant positive correlation between AMH and ovarian volume before and after LOD. But no correlation between AMH and E2, FSH, LH, TSH, AFC or prolactin before or after LOD.

Conclusion: LOD increased AFC, indicating that LOD is a good treatment for PCOS. However, decreased AFC after LOD shows its destructive effect on ovarian tissue.

O-212 Positive effect of melatonin on lactate production by human granulosa cells exposed to a range of concentrations of insulin

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Introduction: Melatonin is a pineal hormone which is subject to diurnal variation, with higher circulating levels at night. A physiological role for melatonin in the ovary is suggested by the presence of melatonin receptors on granulosa cells (GC) and levels of the hormone in follicular fluid that are higher than those observed in serum. Reduced melatonin, through pinealectomy in rats, leads to an ovarian morphology similar to that found in the polycystic ovary syndrome (PCOS). This syndrome is often associated with increased insulin resistance both generally and at the level of the GC. Because melatonin is helpful in maintaining insulin sensitivity, we hypothesised that melatonin might have a positive effect on insulin action on GC with regard to lactate production which constitutes an important source of 3-carbon units for oocyte development.

Materials and Methods: Follicular aspirates were obtained at ovum collection for IVF according to an ethically approved procedure. Patients had apparently normal ovarian function with the cause of infertility ascribed to either tubal blockage or problems with the male partner. GC were prepared from aspirates by a method involving removal of blood cell contaminants, and established in culture. Replicate wells (with or without melatonin at 100 nM) were exposed to insulin over a concentration range (0, 1, 3, 10, 30, 100, 300, 1000 ng/ml). Lactate levels in culture media were estimated using a colorimetric method and plotted against insulin concentration for each preparation.

Results: Insulin stimulated lactate production by GC with a minimum effective dose of 1 ng/ml (P<0.05 vs zero insulin) rising to a maximum at approximately 300 ng/ml (P<0.001 vs zero insulin). Addition of melatonin to GC cultures (6 preparations from 6 individual patients) caused a trend towards increased lactate production at zero insulin, also at 1-100 ng/ml insulin. Analysis of the area under the curve (0-100 ng/ml) showed that this positive effect of melatonin was significant (P<0.031). There was no effect at the maximum response to insulin (300-1000 ng/ml).

Conclusion: Our observation that melatonin significantly enhanced lactate production in human GC over a range of insulin concentrations, supports our hypothesis and represents a novel finding. Lactate is an important 3-carbon unit within the follicle which may be transferred to the ovum possibly via interconversion with pyruvate. The possibility that melatonin enhances this process, may explain observed beneficial effects of melatonin on oocyte maturation. Previous work has shown that lack of melatonin is a problem for the ovary, pinealectomy causing morphological changes reminiscent of PCOS (see above). Our observation that melatonin is helpful in maintaining insulin responses in the ovary, suggests that there may be interaction between the effects of insulin and melatonin at the level of the ovary and may provide a potential explanation of why the morphological aspects of PCOS can be associated with insulin resistance in GC.

O-213 Reduced percentage of natural killer cells associated with impaired cytokine network in the secretory endometrium of PCOS women

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Introduction: Endometrial receptivity is a key determinant of the success of implantation. Changes in endometrial leukocytes subpopulations and most of all in the percentage of uterine natural killer cells (uNK), during the menstrual cycles play a pivotal role in the implantation process. uNK cells are identified by the expression of the NK marker CD56 at high concentrations (CD56 bright), but lack the expression of other typical NK markers such as CD16 responsible for NK mediated cellular cytotoxicity. In the secretory phase of the normal menstrual cycle there is a significant increase in the numbers of uNK cells. Chemokine ligand 10 (CXCL10), interleukins 15 (IL15) and 18 (IL18), are directly involved either in the recruitment of CD16-NK cells from PB or in the proliferation of the existing uNK cells. Since the expression of CXCL10, IL15 and IL18 is induced by sex hormones, an alteration of the hormonal balance may be associated with an abnormal endometrial cytokine network and leukocyte distribution. Infertility associated with PCOS derives from chronic anovulation and poor oocytes quality, but several lines of evidence suggest that the endocrinologic and metabolic abnormalities adversely affect endometrial function and implantation, leading in turn to subfertility and recurrent miscarriage. Dysregulated expression of some biomarkers of uterine receptivity, such as Glycodelin A, selectively over expressed by uNK cells, has been reported in the secretory endometrium of PCOS patients but whether in these patients the percentage and phenotype of uNK cells and of the other leukocytes may be altered, has not yet been investigated. The aim of the study was to evaluate, by flow cytometry, the percentage of uNK cells and other lymphocyte subsets in the endometrium from young infertile PCOS patients. We therefore evaluated the mRNA expression of IL15, IL18 and CXCL10 in the endometrium of the same patients. We compared the results with those obtained in fertile control women.

Materials and Methods: Endometrium was obtained from 28 infertile patients, aged 23-36 years, affected by PCOS and from 6 fertile control women aged 30-35 years, with normal menstrual cycles. Endometrial biopsies were performed using a 3 mm Novak’s curette connected to a 20 mL syringe. In 19 out of 28 PCOS patients and in 6 controls in which histological examination confirmed a late secretory endometrium, the percentage and phenotype of lymphocyte subsets were analyzed by flow cytometry. In the late secretory endometrium of 11 PCOS patients and 3 controls, the expression of interleukins 15 and 18 and of chemokine ligand 10 was analysed by PCR.

Results: In PCOS patients the percentage of CD56+ CD16- and of CD56brightCD16- cells was significantly lower (median and CI: 38% [31, 52.7] vs 63.7% [57.7, 69]; 17.4% [8, 41.6] vs 52 % [43, 60]; respectively; p<0.001) while the percentage of CD56+ was found significantly higher (45% [33.3, 64] vs. 26.1% [21.32]; p<0.001) as compared to controls. Accordingly, PCR analysis revealed that in the PCOS group, steady-state mRNA expression was reduced by about 64% (p = 0.0096), 42% (p = 0.0043) and 48% (p = 0.0011) as compared to the control group for IL15, IL18 and CXCL10, respectively.

Conclusions: The present study provides the first evidence that an abnormal endometrial lymphocyte pattern occurs in infertile women affected by PCOS, together with profound impairment of endometrial cytokine balance, even after normal ovulation. The poor reproductive potential observed in these patients could be partly related to a local dysregulation in the endometrial immune network during implantation.

Further investigations are needed to confirm these preliminary results obtained from untreated PCOS women, and to evaluate the influence of medical treatments on the leukocyte subset distribution in the endometrium of these patients as well as the consequences on their reproductive ability.

O-214 Cardiovascular risk factors in non-obese PCOS patients

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Introduction: Polycystic ovary syndrome (PCOS) is the most common endocrine disease related with infertility. Obesity is frequently present in women with PCOS and related with hyperinsulinemia, hypertension, and other genetic lipid profile. All of them are considered as cardiovascular risk factors. The adipose tissue produces adipokines like adiponectin, leptin and resistin. Adiponectin is an insulin sensitizer. Leptin is a product of the obesity and a marker of somatic energy storage and participates in a range of physiological actions being essential in the control of normal body weight. It crosses the haematencephalic barrier acting in the hypothalamus receptors depressing appetite and increasing thermogenesis. Resistin is related with insulin resistance. It is also known that an inflammatory ambient promotes the atherosclerosis. TNF-α is an inflammatory marker that increases the insulin resistance and promotes high blood pressure. The aim of this study was to evaluate if cardiovascular risk factors are increased in non-obese women with PCOS.

Material and Methods: Patients: Forty women diagnosed of PCOS according to the Rotterdam criteria were distributed as follows: Group 1 (n = 23) showing a body mass index (BMI) < 30 kg/m², group 2 (n = 21) showing a BMI >30 kg/m². These two groups were compared with 20 normal women (Group 3): BMI < 30 kg/m², normal ovulatory cycle and no hyperandrogenaemia.

Setting: Reference Assisted Reproduction Unit. University Hospital.

Interventions: Blood samples were collected between the days 2 and 5 of a spontaneous menstrual cycle. Serum levels of FSH, LH, prolactin, TSH, free testosterone, insulin, glucose, high sensitivity C reactive protein (hs-CRP), cholesterol, HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C), VLDL-Cholesterol (VLDL-C), triglycerides, adiponectin, leptin, resistin and tumour necrosis factor-α (TNF-α) were quantified. Blood pressure, height and weight, were also measured. Anova Test was used to find differences of parameters among groups. The significance level was established at p < 0.05.

Results: Group 2 presented significant higher systolic and diastolic blood pressure than Group 1 and Group 3 (p < 0.01). Basal insulin, LDL-C and triglycerides circulating levels, as well as HOMA index were significantly higher in Group 2 when compared with the other groups (p < 0.001). HDL-C levels were significantly reduced in group when 2 compared with groups 1 and 3 (p < 0.001). High sensitive CRP levels were significantly higher in group 2 compared to the remainder groups (p < 0.001). The levels of adiponectin were significantly higher in the group 3 (0.71 ± 0.20 ng/mL, 0.61 ± 0.16 ng/mL, and 0.80 ± 0.27 ng/mL, respectively in groups 1, 2 and 3, p = 0.023). The levels of leptin (1.51 ± 0.48 ng/mL, 1.96 ± 0.37 ng/mL, and 1.24 ± 0.30 ng/mL, respectively in groups 1, 2 and 3, p > 0.001) and TNF-α (0.14 ± 0.03 ng/mL, 0.16 ± 0.05 ng/mL, and 0.14 ± 0.03 ng/mL, respectively in groups 1, 2 and 3, p = 0.042) were higher in group 2 when compared with the other groups. Circulating levels of resistin showed no differences between groups.

Conclusions: Serum leptin level was higher in obese women with PCOS according with expected. Adiponectin is down regulated in women with PCOS. TNFα levels were higher in obese PCOS women. These results support the idea that alterations of adipose tissue metabolism and endocrine activities could play a role in the pathophysiology of PCOS. Women with PCOS present increased cardiovascular risk factors even if they do not have obesity. These patients have not only a fertility problem but also a cardiovascular problem.