

RESULTS: Women with PCOS had a significantly higher weight, BMI, fasting insulin and HOMA-IR values. Bivariate analysis indicated that all parameters were strongly associated with BMI. After adjusting for BMI, patients with PCOS were found to have significantly lower basal metabolic rate (BMR) compared to controls ($p=0.016$). In addition, lean body mass and skeletal muscle mass was also lower than controls ($p=0.016$). Interestingly, skeletal muscle mass decrease was significant in the legs but not in the trunk or arms.

CONCLUSION: Patients with PCOS have lower basal metabolic rate (BMR) even when controlled for BMI. This may be due to the lower lean body mass and skeletal muscle mass of the legs which have been associated with resting metabolic rates. Therefore, PCOS patients may be at risk of lower metabolism that can lead to obesity related to PCOS.

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PREVALENCE OF ANDROGENIC ALOPECIA (AGA) IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME (PCOS) AND CHARACTERIZATION OF ASSOCIATED CLINICAL AND BIOCHEMICAL FEATURES. M. Quinn,^a L. Pasch,^a K. Shinkai,^b L. Kuzmich,^a M. Cedars,^a H. Huddleston.^a ^aDepartment of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco School of Medicine, San Francisco, CA; ^bDepartment of Dermatology, University of California San Francisco School of Medicine, San Francisco, CA.

OBJECTIVE: As a clinical manifestation of PCOS, AGA has received less attention in the literature compared with hirsutism or acne. Our goal is to describe the prevalence of AGA in patients with a diagnosis of PCOS and to characterize associated features.

DESIGN: Retrospective observational cohort at subspecialty clinic.

MATERIALS AND METHODS: Patients being evaluated between 2007-2012 in a multi-disciplinary clinic for PCOS were consecutively enrolled in a research database. PCOS was diagnosed by Rotterdam Criteria and all subjects were systematically evaluated by a reproductive endocrinologist, dermatologist and psychologist. Objective findings of hyperandrogenism, laboratory parameters suggestive of hyperandrogenemia or metabolic dysfunction, ultrasound measurements, and subjective report of mental health were analyzed in patients with and without AGA. Statistical calculations included chi-square and t-test where appropriate.

RESULTS: Two hundred fifty four subjects met criteria for diagnosis of PCOS. 56 of 254 patients with PCOS (22%) had clinical evidence of AGA. Subjects with PCOS and AGA were more likely to have acne or hirsutism than those without AGA (96.3% vs 70.6%, $p<0.001$). Subjects with AGA were more likely to report concern with hair loss (70.4% vs 37.7%, $p<0.001$); however, their rating of their own health and score on mini-Beck depression inventory were no different from subjects without AGA. There was no difference between subjects with and without AGA in age, biochemical hyperandrogenism, oligo-anovulation, polycystic appearing ovaries by ultrasound, body mass index (BMI), fasting insulin, fasting glucose or waist to hip ratio.

CONCLUSION: This study demonstrates that AGA is prevalent within PCOS. AGA associates with other manifestations of clinical hyperandrogenism, but does not appear to be associated with greater risk of biochemical or metabolic abnormalities than PCOS alone. These findings suggest that development of AGA may be mediated by local rather than systemic androgen levels.

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ACTIVATION OF NUCLEAR FACTOR κ B IN RESPONSE TO CREAM INGESTION IS RELATED TO OVARIAN ANDROGEN HYPERSECRETION IN POLYCYSTIC OVARY SYNDROME. O. A. Abdelhadi, M. K. Shepard, C. L. Sia, T. J. Garrett, F. González. Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN.

OBJECTIVE: We examined the effect of cream ingestion on NF κ B activation in women with Polycystic Ovary Syndrome (PCOS) compared with ovulatory controls; and the relationship of this effect with the ovarian androgen response to HCG administration.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: Fifteen women with PCOS (7 lean and 8 obese) between ages 18-40, diagnosed on the basis of oligo- or amenorrhea and hyperandrogenemia, and 13 ovulatory controls (8 lean; 5 obese) of similar age were selected for study. All subjects ingested 100 ml of dairy cream and received a single 5000 IU IM injection of HCG within 5-8 days

of menses. NF κ B activation was quantified by electrophoretic mobility shift assay in mononuclear cells isolated from blood samples drawn while fasting and 2 hours after cream ingestion. Androgens were measured from blood samples drawn 0, 24, 48 and 96 hours after HCG administration. Insulin sensitivity was derived by IS_{OGTT}.

RESULTS: The change from baseline (%) in NF κ B activation (% Δ NF κ B) following cream ingestion was higher ($p<0.01$) in lean and obese women with PCOS (23.5 \pm 4.6, 19.5 \pm 3.3) and obese controls (12.2 \pm 2.9) compared with lean controls (-3.0 \pm 3.2). Compared with weight-matched controls, women with PCOS exhibited a greater area under the curve (AUC) following HCG administration for testosterone (lean: 7280 \pm 739 vs. 4009 \pm 340, $p<0.008$; obese: 8064 \pm 1429 vs. 3878 \pm 325, $p<0.003$), androstenedione (lean: 488 \pm 30 vs. 317 \pm 26, $p<0.0001$; obese: 527 \pm 12 vs. 294 \pm 19, $p<0.0001$) and 17OHP (lean: 13230 \pm 1129 vs. 8528 \pm 747, $p<0.009$; obese: 15008 \pm 1824 vs. 9098 \pm 963, $p<0.004$). The % Δ NF κ B was negatively correlated with IS_{OGTT} ($r=-0.61$; $p<0.002$), and positively correlated with the AUC for testosterone ($r=0.53$; $p<0.008$), androstenedione ($r=0.58$; $p<0.003$) and 17OHP ($r=0.42$; $p<0.04$).

CONCLUSION: In PCOS, NF κ B activation in response to cream ingestion is independent of obesity. We speculate that in PCOS, lipid-induced inflammation promotes insulin resistance and ovarian androgen production.

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THE INFLUENCE OF NON-ESTERIFIED FATTY ACIDS (NEFA) ON THE OVARIAN PRODUCTION OF ANDROGENS IN A CONTEXT OF IN VITRO FERTILISATION (IVF) - PRELIMINARY RESULTS. A. Gervais,^a M.-C. Battista,^a B. Carranza-Mamane,^b H. Lavoie,^c J.-P. Baillargeon.^a ^aEndocrinology, Sherbrooke University, Sherbrooke, QC, Canada; ^bGynecology-obstetrics, Sherbrooke University, Sherbrooke, QC, Canada; ^cProcrea Cliniques, Montréal, QC, Canada.

OBJECTIVE: Polycystic ovary syndrome (PCOS) is the main cause of anovulatory infertility and is characterised by hyperandrogenism. To assess the role of lipotoxicity (lipid induced cellular toxicity) in PCOS hyperandrogenesis, we aimed to: 1) correlate follicular fluid (FF) levels of NEFA with androgens and parameters of insulin resistance and inflammation; and 2) compare FF levels of NEFA and other variables between women with or without PCOS.

DESIGN: Cross-sectional controlled study with women undergoing an IVF procedure.

MATERIALS AND METHODS: Women were recruited during their IVF protocol in the PCOS or non-PCOS group based on AE-PCOS criteria. FF was collected during oocyte aspiration for later measurement of the above-mentioned parameters. Correlations were performed using Spearman non parametric tests. Our sample size gives 80% power to detect a correlation $r\geq 0.45$ in the non-PCOS group. Variables were compared between the two groups using Mann-Whitney non parametric tests.

RESULTS: The average BMI for PCOS ($n=5$) and non-PCOS ($n=32$) women were 34 and 25.0 Kg/m². In the non-PCOS group, we found significant positive correlations between NEFA levels and testosterone ($r=0.41$, $p=0.03$) and IL-6 ($r=0.34$, $p=0.09$); and a negative correlation between NEFA and leptin levels ($r=-0.53$, $p=0.005$), after correction for BMI. In comparison with non-PCOS women, PCOS women had higher FF levels of testosterone (14.6 vs 1.3 nM, $p=0.001$) and leptin (104.7 vs 44.1 μ M, $p=0.01$); similar FF level of NEFA (0.27 vs 0.27 mM), glucose (2.97 vs 3.09 uM) and triglycerides (0.34 vs 0.17 nM); and slightly increased FF level of TNF- α (1.7 vs 1.4 pM, $p=0.09$) and IL-6 (15.2 vs 13.4 pM, $p=0.03$).

CONCLUSION: Our results suggest that NEFA might be implicated in the androgenesis of non-PCOS women by a mechanism linked to inflammation. PCOS women have similar NEFA levels but higher levels of androgen and IL-6, suggesting that NEFA induce more lipotoxicity in these women or that NEFA-induced lipotoxicity may have more impact on androgenesis in PCOS.

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HIGHER ANDROGEN AND ESTROGEN OUTPUT AND LESS FOLLICULAR ATRESIA CAN BE INDUCED IN HEALTHY OVARIES BY CULTURING THEM WITH SERA FROM PCOS PATIENTS: IS PCOS REALLY OF INTRAOVARIAN ORIGIN? O. Oktem,^{a,b} Y. Guzel,^b C. Taskiran,^b U. Ince,^c E. Palaoglu,^d B. Urman.^{a,b} ^aObstetrics and Gynecology, Koc University School of Medicine, Istanbul, Turkey; ^bWomen's Health Unit, American

Hospital, Turkey; ^cPathology, Acibadem University, Istanbul, Turkey; ^dClinical Laboratory, American Hospital, Istanbul, Turkey.

OBJECTIVE: Polycystic ovarian syndrome (PCOS) has distinct ovarian features such as high androgen output, higher aromatase activity (higher estrogens) and less follicular atresia. It is unclear whether these changes are related to disturbed intraovarian environment or is due to some exogenous factors in the circulation. We aimed to answer this question.

DESIGN: An in vitro study.

MATERIALS AND METHODS: Ovarian cortical pieces obtained from 10 healthy donors undergoing operations for benign pathologies were cultured using conventional techniques for 6 days in α MEM culture media supplemented with 10% donors' own sera or sera from 5 PCOS patients obtained at early follicular phase. Half of the culture media was replenished every other day and assayed for E2 and fT and follicle survival was compared.

RESULTS: The mean age of controls and PCOS patients were comparable (31.4 ± 2.1 vs. 32.6 ± 1.4 , respectively).

Ovarian samples cultured with PCOS sera produced significantly higher amounts of fT and E2 than those cultured with their own sera. Comparable amounts of both hormones measured in culture media at baseline (day 0) ruled out the possibility that their higher levels are derived from PCOS sera. FSH (2.3 ± 0.4 vs. 3.4 ± 0.5 , $p > 0.05$) and LH levels (0.3 ± 0.09 vs. 0.2 ± 0.01 , $p > 0.05$) were also similar in control vs PCOS culture media at baseline. After 6 day culture period significantly higher number of primordial follicles survived in samples cultured with PCOS sera compared to those treated with their own sera (3.5 ± 1.6 vs. 2.6 ± 1.6 $p < 0.01$).

E2 and fT productions from ovaries cultured with donors's (control) sera vs. PCOS sera.

	Day 0	Day 2	Day 4	Day 6
E2 pg/mL (Control sera)	11.1 ± 2.3	388.7 ± 121	519.2 ± 162	581.9 ± 372
E2 pg/mL (PCOS sera)	10.9 ± 0.8	$1013 \pm 893^*$	$1451 \pm 654^*$	$1239 \pm 854^*$
fT pg/mL (Control sera)	0.4 ± 0.01	9.2 ± 7.2	6.7 ± 2.9	16.2 ± 6.2
fT pg/mL (PCOS sera)	0.5 ± 0.02	9.6 ± 5.4	$21.7 \pm 12.7^{**}$	$25.6 \pm 12.3^{**}$

* $p < 0.01$; ** $p < 0.05$.

CONCLUSION: These results suggest that ovarian dysfunction in PCOS might be at least in part can be accounted for by some factors in the serum.

STEM CELLS

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PRIMORDIAL GERM CELLS (PGCs) DERIVED FROM PATIENTS WITH TURNER SYNDROME AND KLINEFELTER SYNDROME OFFER INSIGHT TO THE MECHANISM OF PREMATURE GONADAL FAILURE. P. Yango, E. Altman, H. Nguyen, M. Zamah, M. I. Cedars, N. D. Tran. Obstetrics and Gynecology, UCSF Medical Center, San Francisco, CA.

OBJECTIVE: Premature primary gonadal failure is the hallmark of Turner (TS) and Klinefelter syndromes (KS). However, the mechanism of germ cell exhaustion in these patients is unknown. We aim to investigate this mechanism by examining primordial germ cells (PGCs) generated from patients with these syndromes.

DESIGN: In vitro derivation of PGCs from patient specific induced pluripotent stem cells (iPSCs).

MATERIALS AND METHODS: Fibroblasts from TS (2), KS (2), normal male (2), and female (2) were reprogrammed into iPSCs using exogenous Oct-3/4, SOX2, c-Myc, and Klf4. iPSCs were evaluated for pluripotency (Oct-3/4, NANOG, SOX2, Tra-1-81, Tra-1-60, and SSEA4) and multilineage differentiation. In vitro derived PGC from iPSCs were sorted by FACS, analyzed by qPCR and microscopy.

RESULTS: All iPSC lines demonstrated similar proliferation kinetics, expressed high levels of pluripotent genes, and were capable of multilineage differentiation. PGCs, defined by co-expression of SSEA1/c-Kit, were successfully derived from all iPSC lines. While efficiency of PGC derivation from XX and XY iPSCs was $2.85 \pm 0.4\%$ as expected, significantly more PGCs were derived from TS and KS iPSCs, $4.1 \pm 0.3\%$ and $3.7 \pm 0.3\%$ ($p < 0.003$), respectively. Although sorted PGCs from normal donors expressed both germ cell and meiotic markers (Stra8, Scp3, Msx1 and

Msx2), PGCs from TS and KS patients did not show evidence of meiotic initiation. Additionally, Bax and cleaved CASP3 were detected only in TS derived PGCs suggesting early apoptosis in these PGCs.

CONCLUSION: PGCs were successfully derived from patients with known germ cell defect from sex chromosome aneuploidy. Although PGC production was not compromised in patients with TS and KS, meiotic initiation was inhibited. Interestingly, early activation of the intrinsic apoptotic pathway was detected in TS PGCs, while this was not detected in KS PGCs, suggesting different mechanisms and timing of germ cell depletion. Thus, patient specific iPSCs serve as an effective model to study germ cell biology.

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ABSTRACT WITHDRAWN

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OOGONIAL STEM CELLS GENERATE MATURE OOCYTES IN AN AUTOLOGOUS RHEBUS MACAQUE TRANSPLANTATION MODEL. E. F. Wolff,^a L. L. Libfraind,^a R. P. Weitzel,^b J. Tilly,^c A. H. DeCherney,^a J. F. Tisdale.^b ^aProgram in Reproductive and Adult Endocrinology, NICHD, Bethesda, MD; ^bMCHB, NHLBI, Bethesda, MD; ^cVincent Center for Reproductive Biology, MGH, Boston, MA.

OBJECTIVE: Oogonial stem cell (OSC) transplantation has resulted in the birth of donor-derived offspring in mice (Nat Cell Biol 2009 11:631), and OSCs isolated from ovaries of women generate immature oocytes in human ovarian xenografts (Nat Med 2012 18:413). Here we tested if OSCs generate oocytes in primates.

DESIGN: Autologous OSC transplantation in adult Rhesus macaques.

MATERIALS AND METHODS: One ovary was removed from a Rhesus macaque, and the cortex was processed for FACS-based purification of OSCs (Nat Protoc 2013 8:966). After expansion in vitro for 11 weeks, cultured OSCs were engineered to express GFP. Lentiviral transduced cells were re-sorted to isolate the GFP+ fraction, from which 3×10^5 cells were injected into the cortex of the remaining ovary 4 days later. After 4 months, controlled ovarian stimulation (COH) was induced using hMG with Ganirelix, and hCG was given to induce follicular maturation. Laparoscopy was performed to aspirate oocytes, which were assessed for GFP by fluorescence and PCR.

RESULTS: Cultured OSCs expressed *PRDM1*, *IFITM3*, *DPPA3*, *SOX2* and *NANOG*, confirming their primitive germline identity. COH of the transplanted female resulted in peak serum estradiol levels of $2,178$ pg/ml, and a total of 5 oocytes were retrieved by follicular aspiration (4 metaphase-II, 1 immature). Fluorescence microscopy identified one of the metaphase-II oocytes as GFP-positive, which was confirmed by vector specific PCR-based detection of GFP transgene integration. The four remaining oocytes were negative for GFP expression by both direct fluorescence and PCR analyses.

CONCLUSION: Adult ovary-derived OSCs generate mature oocytes following autologous transplantation in non-human primates. Ongoing studies are assessing fertilization and developmental competency of these oocytes in this and another OSC-transplanted animal.

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