Tuesday, October 23, 2001
3:45 P.M.

O-162

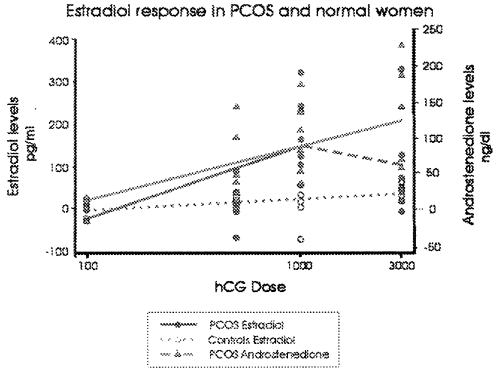
In PCOS estradiol secretion is dose-dependent and hypersensitive to the LH analog hCG. D. F. Rychlik, R. B. Barnes, E. J. Bieber, R. L. Rosenfield. Univ of Chicago, Chicago, IL.

Objective: To determine if the LH analog, hCG, stimulates estradiol secretion in a dose-dependent fashion in normal and women with polycystic ovary syndrome (PCOS).

Design: A randomized clinical study.

Materials/Methods: We studied 24 mid-follicular phase normal women and 22 with PCOS, defined by oligo-ovulation and an elevated plasma free testosterone (T) not suppressed by dexamethasone (Dex). Subjects were randomized to 100, 500, 1,000, and 3,000 IU hCG. Dex was given throughout the study to suppress adrenal steroids. Estradiol and androstenedione (AD) levels were measured at zero and 24 hours after hCG administration. Differences between normal and PCOS dose-response slopes were determined by multiple linear regression for each steroid.

Results: In normal women the estradiol dose-response slope was significant from 100 to 3000 IU of hCG ($p = 0.015$). The PCOS women demonstrated a significant estradiol dose-response between 100-1000 IU of hCG ($p = <0.001$) and had a significantly steeper slope compared to normals ($p = <0.0001$). Only PCOS subjects had a significant dose-response of AD ($p = 0.023$).



Conclusions: To our knowledge, this is the first evidence in humans of a dose-response of estradiol to the LH analog hCG. The PCOS ovary had a steeper estradiol dose-response curve and reached a maximum response at a lower hCG dose. This suggests that the normal ovary is less sensitive to hCG and to down-regulation by hCG compared to the PCOS ovary. There was a significant dose-response of AD only in PCOS women which suggests an alteration of thecal cell function. Possible causes of the estradiol dose-response are 1) thecal cell secretion of estradiol precursors or factors that stimulate aromatase such as IGF-1, 2) direct thecal cell secretion of estradiol in response to hCG or, 3) granulosa cell LH receptors increase aromatase activity in response to hCG. Our findings indicate overproduction of estrogen in the PCOS ovary is in part caused by an increased sensitivity to stimulation by LH.

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Tuesday, October 23, 2001
4:00 P.M.

O-163

Effect of exogenous gonadotropins on in vivo epidermal growth factor receptor (EGF-R) expression in murine ovarian follicles throughout the estrous cycle. B. Scoccia, G. Uncu, K. Elter, J. V. Ilekis. Univ of Illinois at Chicago, Chicago, IL.

Objective: EGF-R is expressed in the murine ovary and ligand (EGF, TGFa) activation plays a role in normal folliculogenesis. In this study, we investigated the effect of ovulation induction with exogenous gonadotropins on in vivo EGF-R expression in rat ovarian follicles during the estrous cycle.

Design: Prospective, randomized, controlled murine study, approved by the animal IRB, at an academic institution.

Materials/Methods: Adult female Wistar rats were housed on a 12-hour light/12-hour dark schedule. Estrous cycles were monitored by vaginal smears. Animals were separated into two groups: rats in the study group were injected intraperitoneally with 10 IU PMSG in early proestrus followed by 10 IU hCG 56 hours after PMSG; rats in the control group were injected with 0.9% saline only. Three animals in each group were euthanized according to their vaginal smears at late proestrus, estrus and metestrus. The ovaries were cryopreserved. Immunohistochemistry was performed using a monoclonal anti-EGF-R antibody (Clone no. 29.1, mouse IgG) and a peroxidase-labeled goat anti-mouse IgG, as the secondary antibody. A chromogenic reaction was developed by incubation with a solution of 3-Amino-9-ethylcarbazole for 15 minutes. All sections were counterstained with Mayer's hematoxylin and evaluated by light microscopy. The intensity of EGF-R immunostaining in the ovarian follicles was assessed by using a computer-assisted image analysis and results were expressed as "pixels per area (ppa)". Statistical analysis was done with the Wilcoxon test and ANOVA with post-hoc tests, where appropriate. P value of <0.05 was considered significant.

Results: In late proestrus, there was a weak EGF-R expression in the control group, while moderate staining was seen in the study group ($P = 0.06$). During estrus, EGF-R expression significantly increased in the control group ($p < 0.05$ vs proestrus), while it decreased in the PMSG stimulated group ($P = NS$ vs proestrus). EGF-R staining was lower during estrus in the PMSG stimulated group when compared to that in the control group ($p < 0.05$). Following ovulation, during metestrus, EGF-R expression decreased in the control group, although not significantly ($P = NS$, vs estrus), and it increased in the study group ($p < 0.05$, vs estrus). However, the EGF-R staining during metestrus was comparable between control and study groups ($P = NS$).

Conclusions: Exogenous gonadotropin administration to cycling rats significantly modulates EGF-R expression in ovarian follicles in vivo when compared to cycling controls. We postulate that changes in EGF-R expression in the granulosa and theca cells during gonadotropin stimulation may modulate ligand (EGF and TGFa) tissue effects during folliculogenesis.

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Tuesday, October 23, 2001
4:15 P.M.

O-164

Detection of differential gene expression profiles between in-vitro and in-vivo developed graafian follicles by DNA microarray. H. Liu, Z. He, Z. Rosenwaks. Ctr for Reproductive Medicine & Infertility, Weill Medical Coll of Cornell Univ, New York, NY.

Objective: To study differential gene expression profiles of in-vitro and in-vivo developed Graafian follicles in order to identify essential factors involved in folliculogenesis.

Design: A novel Dig-chem-link method was developed to label total mRNAs instead of reverse-transcribed cDNAs. After hybridization, gene expression in the in-vitro and in-vivo developed Graafian follicles were scanned and compared.

Materials/Methods: Mechanically isolated preantral follicles (Group A) transformed into Graafian follicles after in-vitro culture with gonadotropins for 10 days (Group A) were compared with Graafian follicles which achieved a similar anatomic and developmental stages after 2 days of in-vivo PMSG stimulation (Group B). Total RNAs extracted from Graafian follicles of both groups were labeled with a novel Dig-chem-link method and then hybridized with Clontech Atlas® mouse cDNA expression arrays. After hybridization, the labeled probes on the arrays were detected, scanned, and analyzed for comparison.

Results: Of the 588 known studied genes, 62 and 84 were detected in both Group A (in-vitro) and Group B (in-vivo), respectively. Thirty-three were expressed in both groups, 29 were expressed only in Group A, and 51 were expressed only in Group B. When compared with Group A, more transcription activators (12 vs. 6), receptors (17 vs. 9), growth factors and cytokines (8 vs. 5), and kinase related proteins (10 vs. 3) were expressed in Group B.