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Ovarian hyperstimulation augments adrenal dehydroepiandrosterone sulfate secretion*†

Peter R. Casson, M.D.‡§ Sandra A. Carson, M.D.§
Sonja B. Kristiansen, M.D. John E. Buster, M.D.§
Edward Umstot, M.S.

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, Tennessee

Objective: To determine if factor(s) secreted by the ovaries during hyperstimulation potentiate basal and ACTH-stimulated adrenal androgen secretion.

Design: Retrospective and prospective clinical study.

Setting: University tertiary care center infertility clinic.

Participants: Two hundred thirteen hyperstimulation cycles in endocrinologically normal women were identified from 92 patients with ovulatory infertility, aged 25 to 45 years. Further, seven endocrinologically normal infertile women, aged 22 to 37 years, who were undergoing empiric ovarian hyperstimulation for infertility were identified and studied.

Interventions: In the previously performed cycles, basal and peak serum DHEAS and cortisol (F) levels were assayed and compared with each other and to the extant E_2 levels. Additionally, at the baseline and the peak of ovarian hyperstimulation cycles, a standard ACTH test was performed and serum was assayed for DHEAS, DHEA, and F.

Main Outcome Measure: Basal and ACTH-stimulated serum DHEAS, DHEA (prospective part only), and F concentrations. Where applicable, mean peak values were generated and compared between the baseline and the peak of stimulation with or without a correction for intrapatient variability in F secretion.

Results: Basal serum DHEAS levels rose with ovarian hyperstimulation independent of F. Post-ACTH mean peak value concentrations rose with ovarian hyperstimulation for DHEAS but not DHEA or F.

Conclusions: Ovarian hyperstimulation potentiates basal and ACTH perturbed adrenal DHEAS secretion. This implies the existence of a humoral ovarian factor(s) that mediate this ovarian-adrenal cross-talk. Fertil Steril 1996;65:950–3

Key Words: DHEAS, ovarian hyperstimulation, adrenal gland

Dehydroepiandrosterone and DHEAS, the major products of adrenal steroidogenesis, may have roles in the prevention of obesity (1), oncogenesis (2), and postmenopausal osteoporosis (3), as well as cardioprotective (4) and insulin-sensitizing effects (5). Circulating DHEAS is also a substrate for ovarian sex steroidogenesis (6).

Adrenal secretion of cortisol (F) and androgens is under overall control of ACTH. There exist, however, physiologic and pathophysiologic circumstances in which secretion of DHEAS and F diverge. During adrenarche, serum DHEAS rises independent of F (7). Conversely, with aging, although F secretion remains constant, serum DHEAS drops markedly (8). Dehydroepiandrosterone sulfate levels rise variably in polycystic ovary disease (PCOD) (9), and fall with both physiologic (10) and supraphysiologic hyperinsulinemia (11). These observations imply existence of a mechanism that selectively modulates ACTH-stimulated adrenal androgen secretion.

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‡ Reprint requests and present address: Peter R. Casson, M.D., Department of Obstetrics and Gynecology, Baylor College of Medicine, 6550 Fannin, Suite 801, Houston, Texas 77030 (FAX: 713-798-8431).

§ Present address: Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas.

There also is compelling evidence that the ovary may modulate adrenal androgen secretion. Ovarian failure accelerates the age-related decline in adrenal androgens (12). Estrogen has been suggested as the mediator of this ovarian-adrenal interaction, as it appears to increase menopausal DHEAS levels (13), although this has not been confirmed (14, 15). Estrogen also is thought to be partially responsible for elevated DHEAS in PCOD (16). In vitro (in fetal adrenal cortical cells), such an action of estrogen requires supraphysiologic concentrations and is postulated to be due to a partial 3β-ol-hydroxysteroid dehydrogenase block (17). Other humoral ovarian factors also may play a role. A follicular peptide has been identified in rats that enhances adrenal F secretion (18). Whether similar or alternate modulators of adrenal steroidogenesis exist in humans is as of yet unknown.

We hypothesized, therefore, that ovarian hyperstimulation augments basal and ACTH-stimulated adrenal androgen secretion. Accordingly, in endocrinologically normal infertile women in whom gonadotropin-induced ovarian hyperstimulation was performed, we measured serum DHEAS concentrations, both in the basal state and after perturbation with ACTH. We now report our findings.

MATERIALS AND METHODS

University Institutional Review Board approval was obtained before commencement. The study was performed in two parts. In the first part, basal serum DHEAS and F were measured at beginning and peak of empiric ovarian hyperstimulation cycles in an ovulatory, endocrinologically normal cohort of infertile women with previous diagnoses of unexplained infertility, male factor, or endometriosis. In the second part, a similar group of women undergoing empiric ovarian hyperstimulation had a standard ACTH stimulation test performed, at the beginning and peak of the cycle. Ovarian stimulation in both parts of the study were initiated with basal $E_2 < 100$ pg/mL (367.1 pmol/L), and time of hCG administration was timed to the development of multiple ovarian follicles of average diameter > 16 mm.

Basal DHEAS Levels

Empiric gonadotropin cycles, performed on women with normal ovulatory function over an 18-month period, were identified retrospectively. These cycles were performed for either unexplained or male factor infertility or minimal or mild endometriosis. The 8:00 A.M. pretreatment serum samples (from day 2 of the menstrual cycles) were retrieved as were those sera from 8:00 A.M. on the day of peak stimulation. Sera were assayed for DHEAS and F using direct iodinated double-antibody assays (Pantex Limited, Santa Monica, CA). All samples from the same patient were performed in the same assay. The DHEAS assay uses an initial dilution of 1:500 to 1:2,000, and has a cross-reactivity of 68% with DHEA, 1.5% with androstenedione (A), 0.1% with T, and $<0.01\%$ with E_2. The intra-assay coefficient of variation (CV) is 8.3%. The F assay has a cross-reactivity of $<0.01\%$ with DHEA and E_2. The intra-assay CV is 10.4%. Estradiol values, extracted from the patient's chart, had all been performed in the same iodinated double-antibody assay system. Those patients who did more than one cycle of controlled ovarian hyperstimulation (COH) during this time interval had mean cycle baseline and peak DHEAS, F, and E_2 values calculated. Statistical comparison of baseline and peak DHEAS, F, and E_2 was performed using paired *t*-tests, and change in E_2 versus DHEAS values were analyzed by simple second order regression analysis.

Adrenal Response to ACTH Stimulation

Seven endocrinologically normal, nonsmoking, infertile women were recruited. At 8:00 A.M. on day 2 of their menses, a standard ACTH stimulation test was performed before gonadotropin administration. Adrenocorticotropic hormone (0.25 mg IV bolus cosyntropin) was given and sera was collected at 0, 30, 60, 90, and 120 minutes after administration. This test was repeated at 8:00 A.M. on the morning of peak ovarian stimulation. All sera were frozen for batch assay as noted above. In addition to DHEAS and F, which were assayed as noted above, DHEA was measured using a tritiated extraction assay (Wein Scientific, Succasunna, NY). This assay has a cross-reactivity of 0.5% with DHEAS, 1% with A, and $<0.5\%$ with E_2 and T. The intra-assay CV is 9%. Data were analyzed by expressing steroid concentrations as a percentage of the baseline values and then extracting the peak value attained after ACTH administration for each steroid. The mean peak values at baseline and peak of ovarian stimulation were then compared using paired *t*-tests. Data were corrected further for intrapatient variability in F response quotient to ACTH by multiplying the mean peak values for DHEA and DHEAS by a coefficient from the difference between the baseline and peak mean peak value for F; they were compared again using paired *t*-tests.

RESULTS

Basal DHEAS

Two hundred thirteen COH cycles in patients were analyzed, with an age range of 25 to 45 years (mean age: 30 to 40 years) and an E_2 peak of 1,173

Table 1 Mean DHEAS, F, and E_2 Levels at Baseline and Peak COH*

	Baseline	Peak
DHEAS (μg/dL)	154.4 ± 8.7	172.3 ± 9.4
F (ng/dL)	14.7 ± 0.5	15.4 ± 0.8
E_2 (pg/mL)	66.1 ± 2.8	1230.3 ± 78.7‡

* Values are means ± SEM; n = 92. Conversion factors to SI units are as follows: DHEAS, 0.02714; F, 27590; and E_2, 3.761.
† $P = 0.01$.
‡ $P < 0.001$.

Table 2 Subject Characteristics*

Age (y)	30.4 (22 to 37)
Gravidity	0.6 (0 to 2)
Baseline DHEA (ng/dL)	473 (348 to 586)
Baseline DHEAS (μg/dL)	205 (114 to 417)
Baseline E_2 (pg/mL)	54 (41 to 69)
Peak COH E_2 (pg/ml)	1394 (388 to 2,826)

* n = 7. Conversion factors to SI units are as follows: DHEA, 0.03467; DHEAS, 0.02714; and E_2, 3.761.

± 51 pg/mL (4,306 ±187 pmol/L; mean ± SEM; range: 230 to 4,068 pg/mL or 844 to 15,299 pmol/L). After correction for multiple cycles in single patients by averaging parameters to create mean cycle values, the total number of cycles was 92. The mean DHEAS, F, and E_2 concentrations at the start and peak of stimulation are presented in Table 1. Estradiol rose significantly as did serum DHEAS ($P = 0.01$). Further analysis by linear regression demonstrated a significant association between the change in DHEAS and the change in E_2 levels (Fig. 1; $P = 0.015$, $R^2 = 0.065$).

Adrenocorticotropic Hormone Stimulation

The characteristics of the seven patients are outlined in Table 2. The raw and adjusted mean peak value data are presented in Figure 2. The mean peak value for DHEAS increased significantly with ovarian stimulation, both with and without correction for intrapatient variation in F secretion ($P = 0.03$ and 0.05, respectively) The mean peak values for F or DHEA response did not differ significantly between the baseline and peak of ovarian stimulation.

DISCUSSION

This study demonstrates that basal serum DHEAS concentrations and adrenal DHEAS response to ACTH both are elevated in patients undergoing COH. In humans, although estrogen at high levels appears to enhance DHEAS secretion by fetal adrenocortical cells in vitro (17), menopausal levels of estrogen replacement may (13) or may not (14, 15) increase DHEAS levels. Hyperestrogenemia in PCOD has been demonstrated to increase variably DHEAS levels (9), although other ovarian factors have been postulated to play a role (16). In this study, although the change in DHEAS levels is correlated with the change in E_2, the low R^2 value also implies that other as of yet unidentified ovarian factors also may be acting to elevate DHEAS levels.

The presence of augmented DHEAS response to ACTH stimulation in COH argues strongly that the elevation of the basal level of this hormone is adrenal in origin. The possibility that the DHEAS assay is measuring some other cross-reactive steroid of ovarian origin is remote, given that the antibody specificity of the assay is high and there is a large sample dilution preassay step. The observation that DHEAS secretion is augmented with ovarian hyperstimulation, although DHEA secretion does not change, im-

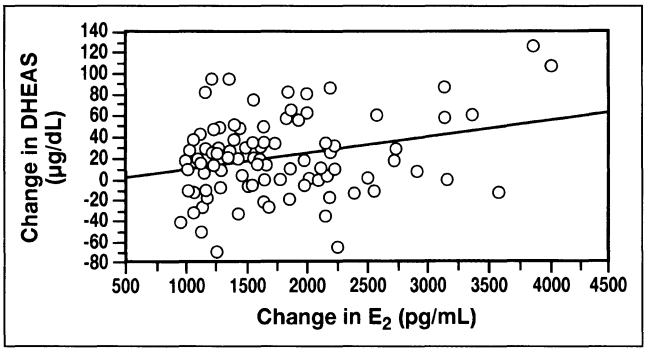

Figure 1 Linear regression performed between the change in E_2 and DHEAS concentrations before and at the peak of ovarian hyperstimulation (n = 92). A significant correlation between the two parameters exists, but the R_2 value implies that only 6.5% of the variation of DHEAS values are due to the change in E_2 levels. $P = 0.013$; $R^2 = 0.065$.

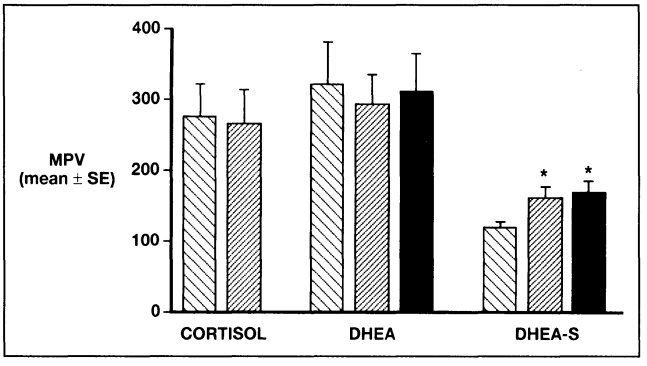

Figure 2 Mean peak values, expressed as percentage of baseline, for F, DHEA, and DHEAS at baseline (▨) and peak of COH (▧), demonstrating increased adrenal DHEAS response to ACTH with ovarian hyperstimulation (n = 7). This finding remained even after correction for intrapatient variability in F response (■).

plies a selective modulation of DHEAS compared with DHEA, perhaps at the level of sulfatase-sulfotranferase enzyme activity.

The existence of an ovarian factor or factors that potentiates adrenal androgen secretion is an appealing postulate, considering the ovary uses large amounts of circulating DHEAS as a prehormone for sex steroidogenesis (6). The possibility that this ovarian adrenal cross-talk exists is of some importance. Dehydroepiandrosterone and DHEAS decline with age (8), even in premenopausal women, and recently have been shown to have importance as immunomodulatory (19), insulin-sensitizing (5), and mood-enhancing agents (20). Ovarian failure or removal thus may result in more than just the adverse sequelae of hypoestrogenemia, but also a secondary adrenocortical androgen deficiency. If the existence of a mechanism of ovarian tropic support for adrenal androgen secretion is proven, and the promise of adrenal androgens in averting some age-related functional declines is fulfilled, menopausal hormonal replacement therapy may thus be optimized by the addition of low doses of adrenal androgens.

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