

## PROSPECTIVE STUDY OF SERUM ANDROGEN LEVELS STRATIFIED BY INCREASED OR NORMAL SERUM FOLLICLE-STIMULATING HORMONE IN INFERTILE WOMEN WITH REGULAR MENSES

Jerome H. Check, M.D., F.A.C.E., and Mark Peymer, B.A.

### ABSTRACT

**Objective:** To evaluate serum androgen levels in infertile perimenopausal women who were still euestrogenic and had regular menses.

**Methods:** We compared 48 women who had early follicular serum follicle-stimulating hormone (FSH) levels of  $\leq 10$  mIU/mL (group 1) with 50 women who had early follicular serum FSH levels of  $>15$  mIU/mL (group 2).

**Results:** No differences were detected between groups 1 and 2 in early follicular mean levels of dehydroepiandrosterone sulfate (DHEAS) (157 versus 167  $\mu$ g/dL). Mean serum testosterone, free testosterone, and androstenedione levels, however, were significantly lower in the group with higher FSH levels (group 2) than in group 1 (27.6 versus 35.8 ng/dL, 1.3 versus 2.1 pg/mL, and 130 versus 164 ng/dL, respectively;  $P < 0.05$ ). No differences were detected between groups 1 and 2 in the frequency of increased DHEAS, testosterone, free testosterone, or androstenedione levels as well as the frequency of low serum DHEAS levels. The group with FSH levels of  $>15$  mIU/mL, however, had a significantly greater frequency of patients with low levels of testosterone, free testosterone, and androstenedione than did the group with lower FSH levels: 54% versus 33%; 26% versus 10%; and 26% versus 10%, respectively ( $P < 0.05$ ).

**Conclusion:** We found no evidence that infertility problems in perimenopausal women are complicated by increasing androgen levels. The mechanism for lower androgen levels is not apparent. (*Endocr Pract.* 1996; 2:375-378)

### INTRODUCTION

An important source contributing to serum and ovarian testosterone levels in premenopausal women is provided by conversion of androstenedione to testosterone by mature follicles; the primary source of androstenedione is the theca cells under the influence of pituitary luteinizing hormone (LH). Interestingly, the concentration of ovarian testosterone in postmenopausal women has been reported as only slightly less than that in premenopausal women despite the absence of conversion of androstenedione to testosterone in mature follicles (1-7). This finding may be

related to higher LH levels generating higher levels of androgens (for example, androstenedione) but with less efficient conversion to testosterone.

A priori, one might hypothesize that perimenopausal women, who have higher LH levels than do premenopausal women but still have mature follicles, may have higher serum testosterone levels related to increased production of androstenedione with conversion to testosterone in the mature follicles.

In the current study, we compared serum LH and androgen levels in infertile perimenopausal women (on the basis of follicular phase serum follicle-stimulating hormone [FSH] levels) with serum levels in premenopausal infertile women with normal early follicular phase serum FSH levels (1).

### MATERIALS AND METHODS

The study patients were recruited from the outpatient infertility clinic of the Cooper Hospital/University Medical Center and ranged in age from 22 to 44 years. Patients were required to have regular menses, as determined by patient history; thus, no one was in overt menopause. Cycles could be no shorter than 25 days or longer than 33 days; the menses could not vary by 4 or more days each cycle. For inclusion in the study, each patient had to generate a minimal serum estradiol level of 150 pg/mL at midcycle. From the initiation of the study, the first 50 women with early follicular phase serum FSH levels of  $>15$  mIU/mL were included, as were the first 50 infertile women whose FSH levels were  $\leq 10$  mIU/mL.

The patients were categorized into two groups on the basis of menstrual cycle day 3 serum FSH levels: group 1 = serum FSH  $\leq 10$  mIU/mL and group 2 = serum FSH  $>15$  mIU/mL (considered the perimenopausal group). Blood specimens were obtained only on day 3; if day 3 was a Sunday, a blood sample was not obtained and assessment of the patient was deferred until the next cycle. Determination of the serum FSH level was repeated during the next cycle, and if the level was not in the same category, the patient was excluded from the study. Originally, each group had 50 patients. The final numbers were 48 patients in group 1 (2 patients were excluded because the second FSH determination was  $>10$  mIU/mL) and 50 patients in group 2 (all serum FSH levels were  $>15$  mIU/mL for both determinations). No patient refused to participate in the study.

Sixty-one couples had been treated for infertility by at least one other physician before our current study. No patient was taking gonadotropin-releasing hormone agonists, estrogens, or glucocorticoids before the measurement of early follicular phase hormone levels. Some

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From the University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, New Jersey.

Reprint requests to Dr. J. H. Check, 7447 Old York Road, Melrose Park, PA 19027. Copyright © 1996 AACE.

patients subsequently were treated with ethinyl estradiol from the day after therapy with clomiphene citrate was discontinued to ovulation time, in order to counteract the adverse effect of the medications on the cervical mucus (8).

The serum FSH and LH levels were measured by either double-antibody radioimmunoassay (RIA) (Amersham Corporation, Arlington Heights, IL) or enzyme-linked immunosorbent assay (Diagnostic Products Corporation [DPC], Los Angeles, CA). The correlation between assays was 0.977 for LH and 0.989 for FSH. The androgens measured were dehydroepiandrosterone sulfate (DHEAS) (RIA coated tube method; DPC); testosterone (RIA coated tube method; ICN Biomedicals Inc., Costa Mesa, CA); androstenedione (double-antibody method; Diagnostic Systems Laboratory, Webster, TX); and free testosterone (RIA coated tube method; DPC). The normal ranges were as follows: DHEAS—25 to 410  $\mu\text{g/dL}$ ; testosterone—20 to 80  $\text{ng/dL}$ ; androstenedione—80 to 300  $\text{ng/dL}$ ; and free testosterone—0.7 to 3.6  $\text{pg/mL}$ . All hormone levels are reported as mean  $\pm$  standard deviation.

Although all women had regular menstrual cycles, if they did not attain a mature follicle (defined as reaching a mean diameter of 18 to 24 mm in association with a serum estradiol level of  $\geq 200$   $\text{pg/mL}$ ) (9), they were treated with clomiphene citrate in a dosage of 50  $\text{mg/day}$  for 5 days, beginning on day 5 of the cycle. Follicle-maturing drugs were used only after at least one cycle of investigation. All day 3 hormonal levels were determined before clomiphene citrate was used; therefore, the medication should have had no influence on the androgen or gonadotropin levels. The data, however, were evaluated to determine whether the need for follicle-maturing medication was associated with higher or lower androgen levels on the basis of the serum FSH.

Because the androgen levels were not normally distributed, the Mann-Whitney  $U$  test was used to compare the androgen and LH levels in the two groups of patients. A  $t$  test was used to compare the mean age of the patients in the two groups, and  $\chi^2$  analysis was used to compare the

distribution of androgen levels in the two groups.  $P$  values of 0.05 or less were considered significant.

## RESULTS

The patients in group 1 (FSH  $\leq 10$   $\text{mIU/mL}$ ) ranged in age from 22 to 44 years (mean, 32.2; SD, 4.8; median, 33). The patients in group 2 (FSH  $> 15$   $\text{mIU/mL}$ ) also ranged from 22 to 44 years of age (mean, 35.0; SD, 4.6; median, 36). The patients in group 1 were significantly younger than the patients in group 2 ( $P = 0.004$ ). Overall, 29 (60%) of the 48 patients in group 1 and 30 (60%) of the 50 patients in group 2 were given follicle-maturing drugs during the follicular phase and progesterone supplementation during the luteal phase. An additional 11 patients in group 1 and 8 patients in group 2 were treated with progesterone supplementation because of out-of-phase endometrial biopsies. Eight group 1 patients and 7 group 2 patients had tubal factor. Five group 2 patients had male factor exclusively; 17 group 1 patients and 10 other group 2 patients had male factor in addition to either ovulation defects or tubal problems. No patient in this study had cervical factor problems unless subsequently treated with clomiphene citrate.

The 48 patients in group 1 had a mean serum FSH level of  $8.4 \pm 1.7$   $\text{mIU/mL}$  (median, 9.0). The 50 patients in group 2 had a mean serum FSH value of  $25.8 \pm 16.4$   $\text{mIU/mL}$  (median, 20.0).

A comparison of mean and median serum androgen and LH levels in the two study groups is presented in Table 1. The only androgen level that was not significantly different between the two groups was DHEAS ( $P = 0.558$ ). The levels of testosterone, free testosterone, and androstenedione were all lower in group 2 ( $P = 0.013$ , 0.010, and 0.003, respectively). LH levels, however, were significantly higher in the group with FSH levels  $> 15$   $\text{mIU/mL}$  in comparison with the LH values in the group with FSH levels  $\leq 10$   $\text{mIU/mL}$ .

The distribution of androgen levels on the basis of serum FSH values is shown in Table 2. The patients

**Table 1**  
Comparison of Androgen and LH Levels in Infertile Perimenopausal Women, Stratified by Follicle-Stimulating Hormone Level\*

Study subjects	DHEAS ( $\mu\text{g/dL}$ )	T ( $\text{ng/dL}$ )	A ( $\text{ng/dL}$ )	Free T ( $\text{pg/mL}$ )	LH ( $\text{mIU/mL}$ )
Group 1—FSH $\leq 10$ $\text{mIU/mL}$ (N = 48)					
Mean $\pm$ SD	167.2 $\pm$ 92.8	35.8 $\pm$ 19.6	164.3 $\pm$ 78.3	2.1 $\pm$ 1.4	7.8 $\pm$ 4.7
Median	134	29	144	1.7	7.0
Group 2—FSH $> 15$ $\text{mIU/mL}$ (N = 50)					
Mean $\pm$ SD	156.9 $\pm$ 85.4	27.6 $\pm$ 13.2	129.7 $\pm$ 74.8	1.3 $\pm$ 0.9	16.4 $\pm$ 10.7
Median	147	20	114	1.1	16.5
Mann-Whitney $U$ test					
P value	0.558	0.013	0.010	0.003	0.0001

\*A = androstenedione; DHEAS = dehydroepiandrosterone sulfate; FSH = follicle-stimulating hormone; LH = luteinizing hormone; SD = standard deviation; T = testosterone.

with increased FSH levels had a higher proportion of testosterone, free testosterone, and androstenedione levels that were below the normal range for the assay than did the patients with FSH levels of <10 mIU/mL.

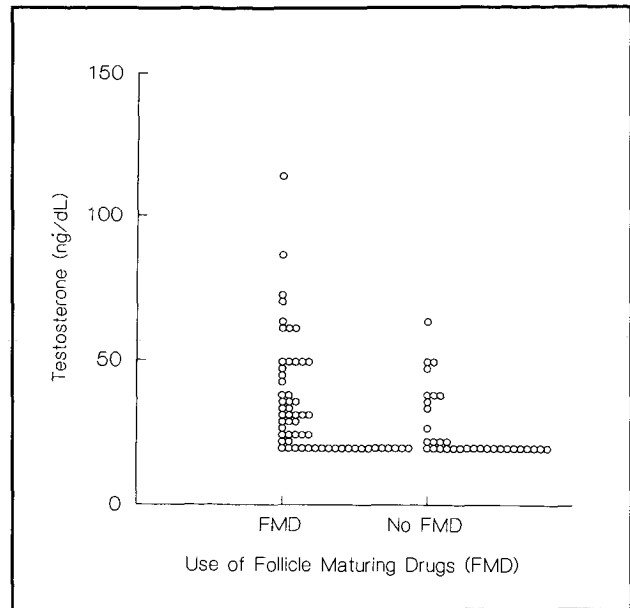
A comparison of androgen levels stratified by high or low early follicular serum FSH level and by the use of follicle-maturing drugs is presented in Table 3. In the group with FSH ≤10 mIU/mL, the median serum levels of androstenedione, free testosterone, and testosterone were higher in the patients who required follicle-maturing drugs than in those who did not. No statistically significant differences in androgen levels were noted, however, between groups taking or not taking clomiphene citrate when serum FSH levels were >15 mIU/mL. When the two groups that used follicle-maturing drugs were compared, the group with low FSH values had higher levels of androstenedione, free testosterone, and testosterone.

The frequency distribution of testosterone levels based on use or no use of follicle-maturing drugs is shown in Figure 1. In addition, a comparison of the distribution of testosterone values based on high or low baseline serum FSH levels is depicted in Figure 2.

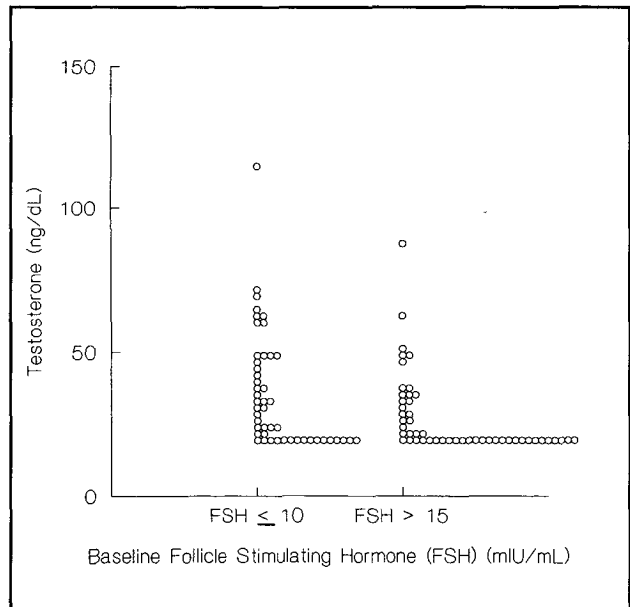
**Table 2**  
Distribution of Androgen Levels in Infertile Perimenopausal Women, Stratified by Follicle-Stimulating Hormone\*

Androgen	Group 1 (FSH ≤10 mIU/mL)		Group 2 (FSH >15 mIU/mL)	
	No.	%	No.	%
DHEAS (μg/dL)				
Low (<25)	1	2	0	...
Normal (25-410)	47	98	49	98
High (>410)	0	...	1	2
T (ng/dL)				
Low (<20)	16	33	27	54†
Normal (20-80)	31	65	22	44
High (>80)	1	2	1	2
A (ng/dL)				
Low (<80)	5	10	13	26†
Normal (80-300)	39	81	36	72
High (>300)	4	8	1	2
Free T (pg/mL)				
Low (<0.7)	5	10	13	26†
Normal (0.7-3.6)	37	77	35	70
High (>3.6)	6	12	2	4

\*A = androstenedione; DHEAS = dehydroepiandrosterone sulfate; FSH = follicle-stimulating hormone; T = testosterone.  
†P<0.05, χ<sup>2</sup> analysis.



**Fig. 1.** Comparison of distribution of testosterone levels in study subjects, stratified by use of follicle-maturing drugs (FMD).



**Fig. 2.** Comparison of distribution of testosterone levels in study subjects, stratified by baseline follicle-stimulating hormone (FSH) levels.

**DISCUSSION**

The data from this study demonstrate that perimenopausal women have lower levels of androgens than do nonperimenopausal women. The mechanism causing these lower levels is unknown. The possibility exists, however, that the increased FSH level allows more aromatization of androgens to estrogens. Further studies are needed to elucidate this mechanism. Thus, increased androgen levels do not seem to be a confounding variable that lowers rates of pregnancy in perimenopausal women.

**Table 3**  
**Comparison of Androgen Levels in Infertile Perimenopausal Women,**  
**Stratified by Baseline Follicle-Stimulating Hormone and Use of Follicle-Maturing Drugs\***

Androgen	FSH $\leq$ 10 mIU/mL		FSH $>$ 15 mIU/mL	
	No FMD (N = 16)	FMD (N = 32)	No FMD (N = 18)	FMD (N = 32)
DHEAS ( $\mu$ g/dL)	134.1 $\pm$ 84.2 (114)	186.1 $\pm$ 96.3 (164)	138.5 $\pm$ 58.0 (130)	171.4 $\pm$ 98.4 (164)
A (ng/dL)	133.5 $\pm$ 65.7 (123) <sup>†</sup>	180.8 $\pm$ 80.2 (150) <sup>†‡</sup>	123.4 $\pm$ 57.1 (114)	134.7 $\pm$ 86.4 (115) <sup>‡</sup>
Free T (pg/mL)	1.5 $\pm$ 1.1 (1.4) <sup>†</sup>	2.5 $\pm$ 1.5 (2.1) <sup>†‡</sup>	1.3 $\pm$ 0.7 (1.1)	1.4 $\pm$ 1.0 (1.1) <sup>‡</sup>
T (ng/dL)	27.7 $\pm$ 13.3 (20) <sup>†</sup>	40.6 $\pm$ 21.3 (36) <sup>†‡</sup>	26.1 $\pm$ 10.0 (20)	29.0 $\pm$ 15.2 (22) <sup>‡</sup>

\*Data are presented as mean  $\pm$  standard deviation (median). A = androstenedione; DHEAS = dehydroepiandrosterone sulfate;

FMD = follicle-maturing drugs; FSH = follicle-stimulating hormone; T = testosterone.

<sup>†</sup> $P < 0.05$ , Mann-Whitney *U* test, comparing use of FMD within FSH group.

<sup>‡</sup> $P < 0.05$ , Mann-Whitney *U* test, comparing FSH groups within each FMD group.

Although LH is known to enhance the synthesis of androgens, the higher concentrations of LH were found to be associated with lower levels of androstenedione, testosterone, and free testosterone. Thus, the hypothesis that increased secretion of LH leads to an increase in androgens—similar to the pattern observed in patients with polycystic ovarian syndrome—was refuted (10-12).

The data did show that the need for follicle-maturing medication was associated with increased early follicular phase serum androgen levels; possibly, the higher androgen levels contribute to a more severe ovulatory disturbance. Nevertheless, because the need for follicle-maturing drugs was equal in women with low and high FSH levels, this factor did not influence the difference in serum androgens on the basis of the early follicular serum FSH levels.

Because all subjects in this study were having difficulty conceiving, the possibility exists that the pattern of androgen levels based on serum FSH might differ from that in a "fertile population. We chose an infertile population not only because these subjects were available but also because demonstration of an increase in androgen levels in the perimenopausal group, as initially suspected, could have led to therapy aimed at reducing such androgen levels.

## REFERENCES

- Judd HL, Judd GE, Lucas WE, Yen SS. Endocrine function of the postmenopausal ovary: concentrations of androgens and estrogens in ovarian and peripheral vein blood. *J Clin Endocrinol Metab.* 1974;39:1020-1024.
- Abraham GE, Maroulis GB. Effect of exogenous estrogen on serum pregnenolone, cortisol, and androgens in postmenopausal women. *Obstet Gynecol.* 1975;45:271-274.
- Greenblatt RB, Colle ML, Mahesh VB. Ovarian and adrenal steroid production in the postmenopausal woman. *Obstet Gynecol.* 1976;47:383-387.
- Judd HL, Lucas WE, Yen SS. Effect of oophorectomy on circulating testosterone and androstenedione levels in patients with endometrial cancer. *Am J Obstet Gynecol.* 1974;118:793-798.
- Vermeulen A. The hormonal activity of the postmenopausal ovary. *J Clin Endocrinol Metab.* 1976;42:247-253.
- Calanog A, Sall S, Gordon GG, Olivo J, Southren AL. Testosterone metabolism in endometrial cancer. *Am J Obstet Gynecol.* 1976;124:60-63.
- Lloyd CW, Lobotsky J, Weisz J, Papkin M, Zanartu J, Puza J. Concentration of unconjugated estrogens, androgens and gestagens in ovarian and peripheral venous plasma of women: the normal menstrual cycle. *J Clin Endocrinol Metab.* 1971;32:155-166.
- Check JH, Adelson HG, Davies E. Effect of clomiphene citrate therapy on post-coital tests in successive treatment cycles including response to supplemental estrogen therapy. *Arch Androl.* 1994;32:69-76.
- Check JH, Nowroozi K, Wu CH, Adelson HG, Lauer C. Ovulation-inducing drugs versus progesterone therapy for infertility in patients with luteal phase defects. *Int J Fertil.* 1988;33:252-256.
- Calogero AE, Macchi M, Montanini V, et al. Dynamics of plasma gonadotropin and sex steroid release in polycystic ovarian disease after pituitary-ovarian inhibition with an analog of gonadotropin-releasing hormone. *J Clin Endocrinol Metab.* 1987;64:980-985.
- DeVane GW, Czekala NM, Judd HL, Yen SS. Circulating gonadotropins, estrogens, and androgens in polycystic ovarian disease. *Am J Obstet Gynecol.* 1975;121:496-500.
- Laatikainen TJ, Apter DL, Paavainen JA, Wahlstrom TR. Steroids in ovarian and peripheral venous blood in polycystic ovarian disease. *Clin Endocrinol (Oxf).* 1980;13:125-134.