CLINICAL ASSISTED REPRODUCTION

Altered Balance Between the 5α -Reductase and Aromatase Pathways of Androgen Metabolism During Controlled Ovarian Hyperstimulation With Human Menopausal Gonadotropins¹

CRISTIN C. SLATER,^{2,3} LILLY CHANG,² FRANK Z. STANCZYK,² and RICHARD J. PAULSON²

Submitted: December 13, 2000

Accepted: June 15, 2001

Purpose: To evaluate androgen production and metabolism during controlled ovarian hyperstimulation.

Methods: Five women, aged 33–42, were studied. All participants were undergoing controlled ovarian hyperstimulation with gonadotropin-releasing hormone agonist and human menopausal gonadotropins. Serum estradiol, estrone, androstenedione, testosterone, 3α -androstenediol glucuronide, and sex hormone-binding globulin levels were measured at 6 time points during the cycle.

Results: The levels of all steroids increased significantly from baseline during controlled ovarian hyperstimulation. Mean total testosterone levels increased from 0.29 ± 0.05 ng/mL to 0.58 ± 0.07 ng/mL after gonadotropin stimulation. Sex hormone-binding gonadotropin levels increased from 50 ± 16 nM to 73 ± 12 nM after gonadotropin stimulation. Estrone/androstenedione and estradiol/testosterone ratios, reflecting the aromatase pathway, increased whereas 3α -androstenediol glucuronide/androstenedione and 3α -androstenediol glucuronide/testosterone ratios, reflecting 5α -reductase activity, decreased.

Conclusions: Controlled ovarian hyperstimulation with human menopausal gonadotropins results in increased serum testosterone and androstenedione levels. Whereas there is an enhancement in androgen metabolism by aromatase, 5α -reductase activity with regard to androgen metabolism is diminished.

KEY WORDS: androgen metabolism; aromatase; controlled ovarian hyperstimulation; 5α -reductase.

INTRODUCTION

The ovary is a major site of androstenedione (A) and testosterone (T) production. Approximately 50% of the total A production and 70% of the total production of T in a premenopausal woman is derived from the ovary. During controlled ovarian hyperstimulation with human menopausal gonadotropins (hMG) and, to a lesser extent, with purified follicle-stimulating hormone (FSH), there is increased secretion of not only estrogens, but also androgens, namely, A and T (1–3). After hMG administration (on the day of hCG administration), serum T and A levels as high as 124 ng/dL and 411 ng/dL have been reported (1,4).

Androgens are cleared from the circulation by two major pathways. One pathway involves the enzyme, 5α -reductase, which converts A and T to the reduced metabolites, 5α -androstenedione and dihydrotestosterone (DHT), respectively. The other pathway involves transformation of A and T to estrone (E_1) and estradiol (E_2), respectively, through the action of the

¹ Presented at the 46th Annual Meeting of the Pacific Coast Fertility Society, April 22–26, 1998, Indian Wells, California.

² Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Keck School of Medicine of the University of Southern California, Los Angeles, California.

³ To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Women's and Children's Hospital, Room 8K9, 1240 North Mission Road, Los Angeles, California 90033; e-mail: coulam@hsc.usc.edu.

enzyme, aromatase. Aromatase is located in the granulosa cells and its activity is known to be enhanced by FSH (5–8). However, there is little information about 5α -reductase activity during ovarian hyperstimulation. It is also not clear how an increase in both estrogens and androgens would combine to influence androgen action. Estrogens would be expected to increase sex hormone-binding globulin (SHBG) and high luteal progesterone levels would be expected to compete with T and A for 5α -reductase. It is not clear how the net effect of these changes would affect androgen metabolism.

Therefore, the objective of the present study was to determine whether 1) the 5α -reductase pathway is altered with respect to T metabolism during ovarian hyperstimulation, and 2) changes in circulating SHBG levels occur during treatment.

MATERIALS AND METHODS

Five women, aged 33–42, were studied. All were undergoing controlled ovarian hyperstimulation using a gonadotropin-releasing hormone (GnRH) agonist and hMG stimulation in preparation for in vitro fertilization (IVF). All had regular menses and no evidence of polycystic ovaries. Leuprolide acetate (Lupron®, TAP Pharmaceuticals, Deerfield, IL), 1.0 mg/day, was administered subcutaneously for 10 days beginning on day 21 of the menstrual cycle. After 10–14 days of GnRH-agonist treatment, serum levels of E_2 were measured. If serum E_2 levels were <30 pg/mL, hMG was administered intramuscularly daily, and the GnRH-agonist dose was decreased to 0.5 mg/day. After 5 days of hMG administration, follicular development was monitored in all patients by transvaginal ultrasound and serum E_2 levels. Follicular stimulation and monitoring continued until follicle maturity criteria (follicle diameter = 18 mm) were reached. At that time, human chorionic gonadotropin (hCG) 10,000 IU was administered intramuscularly. Oocyte recovery was performed 34–36 h after hCG administration. Embryo transfer was performed 3 days after follicle aspiration.

Blood samples were studied at 6 time points during the cycle: 1) prior to GnRH agonist in the midluteal phase (baseline); 2) after GnRH suppression (serum $E_2 < 30$ pg/mL); on day 1 of hMG; 3) in the follicular phase during hMG stimulation, 2 days prior to hCG administration; 4) on the day of, but prior to, hCG administration; 5) on the day of aspiration; and 6) 2 weeks postaspiration. The blood samples were

allowed to clot, then were centrifuged, and stored at -15°C until analyzed.

Serum levels of A, T, E_1 , E_2 , SHBG, and 3α -androstenediol glucuronide, a distal metabolite of DHT and a specific marker of 5α -reductase activity (9), were quantified by specific radioimmunoassays (RIAs) as described previously (10–13). Prior to quantification of A, T, E_1 , and E_2 by RIA, steroids were first extracted with ether acetate:hexane, and the extracts were subjected to Celite-column partition chromatography. 3α -Androstenediol glucuronide and SHBG were measured by highly specific direct RIAs using commercial kits (Diagnostic Systems Laboratories, Webster, TX).

Aromatase activity was inferred from the ratios of E_1/A and E_2/T , whereas 5α -reductase activity was calculated as 3α -androstenediol glucuronide/T and 3α -androstenediol glucuronide/A. Steroid ratios have been used as a measure of enzyme activity, e.g., for 3β -hydroxysteroid dehydrogenase and 17,20-desmolase, when an effective assay does not exist to measure enzyme activity (14–17).

Significance of differences for each analyte over the time points tested was determined by analysis of variance. Data are shown as mean $\pm$ standard deviation.

RESULTS

The mean A, T, E_1 , E_2 , 3α -androstenediol glucuronide, and SHBG levels during the controlled ovarian hyperstimulation cycles are shown in Fig. 1(a)–(c).

During the hMG cycle, mean serum E_1 and E_2 levels increased significantly between baseline and day of aspiration ($P = 0.002$, $P = <0.0001$, respectively). Mean SHBG levels also followed this trend and increased significantly between these time points ($P = 0.009$).

Mean serum androgen levels, including T and A, and 3α -androstenediol glucuronide, also increased between baseline and day of aspiration ($P = 0.001$, $P = 0.003$, and $P = 0.1$, respectively).

The extent of aromatization of A and T to E_1 and E_2 can be assessed by the ratios of E_1/A and E_2/T , respectively. Both E_1/A and E_2/T increased dramatically from approximately 100 to 500 and 300 to 5000, respectively, between the time of GnRH suppression and the day of follicle aspiration (Fig. 2(a)). These changes were statistically significant ($P = 0.008$, $P < 0.0001$, respectively). Figure 2(b) shows the mean ratios of 3α -androstenediol glucuronide/T and

Fig. 1. (a) Mean serum estrone (E₁) and estradiol (E₂) levels during the hMG cycle. Blood samples were studied at 6 time points during the cycle: 1) midluteal phase; 2) after GnRH suppression; 3–4) follicular phase; 5) day of aspiration; and 6) 2 weeks postaspiration. ANOVA was done between time points 1 and 5 for E₂ and E₁ (*P < 0.0001, *P = 0.002, respectively). (b) Mean serum testosterone (T), androstenedione (A) and 5α-androstane-3α,17β-diol glucuronide (3α-diol G) levels during the hMG cycle. Blood samples were studied at 6 time points during the cycle: 1) midluteal phase; 2) after GnRH suppression; 3–4) follicular phase; 5) day of aspiration; and 6) 2 weeks postaspiration. ANOVA was done between time points 1 and 5 for T, A, and 3α-diol G (*P = 0.001, *P = 0.003, *P = 0.1, respectively). (c) Mean serum sex hormone-binding globulin (SHBG) levels during the hMG cycle. Blood samples were studied at 6 time points during the cycle: 1) midluteal phase; 2) after GnRH suppression; 3–4) follicular phase; 5) day of aspiration; and 6) 2 weeks postaspiration. ANOVA was done between time points 1 and 5 (*P = 0.009).

Fig. 2. (a) Mean ratios of estrone (E_1) to androstenedione (A) (E_1/A) and estradiol (E_2) to testosterone (T) (E_2/T) during the hMG cycle. Blood samples were studied at 6 time points during the cycle: 1) midluteal phase; 2) after GnRH suppression; 3–4) follicular phase; 5) day of aspiration; and 6) 2 weeks postaspiration. ANOVA was done between time points 1 and 5 for E_1/A (* $P = 0.008$, * $P < 0.0001$, respectively). (b) Mean ratios of 3α -androstanediol glucuronide (3α -diol G) to androstenedione (A) (3α -diol G/A) and 3α -diol G to testosterone (T) (3α -diol G/T) during the hMG cycle. Blood samples were studied at 6 time points during the cycle: 1) midluteal phase; 2) after GnRH suppression; 3–4) follicular phase; 5) day of aspiration; and 6) 2 weeks postaspiration. ANOVA was done between time points 1 and 5 for 3α -diol G/A and 3α -diol G/T (* $P < 0.05$, * $P = 0.09$, respectively).

3 α -androstenediol glucuronide/A, which represent the 5 α -reduction pathways during exogenous hMG stimulation. There was a statistically significant decrease for 3 α -androstenediol glucuronide/A ($P < 0.05$) from 2.6 to 1.5 between the time of GnRH suppression and the day of aspiration. 3 α -androstenediol glucuronide/T decreased from 9.6 to 6.2 ($P = 0.09$), between the time of GnRH suppression and the day of aspiration.

DISCUSSION

We found that controlled ovarian hyperstimulation was associated with an increase in serum levels of all of the steroids measured. As expected, mean E₁ and E₂ levels were suppressed during down-regulation with the GnRH agonist, and increased during the follicular phase with exogenous gonadotropin stimulation (Fig. 1(a)). After aspiration, the levels decreased to baseline values. T and A levels were suppressed during GnRH suppression and increased during follicular exogenous gonadotropin stimulation, peaking at mean levels of 0.58 ng/mL and 2.8 ng/mL, respectively (Fig. 1(b)). This elevation in ovarian androgens is not surprising. Levels of T and A as high as 1.24 ng/mL and 4.11 ng/mL, which are approximately twice as high as the mean levels in our study, have been reported (1). Free T levels in conjunction with total T levels, have also been reported to rise during controlled ovarian hyperstimulation, despite increased SHBG levels (1). The elevation of both free and total T most likely results from ovarian stimulation by the LH component of urinary gonadotropin preparations (18). It is intriguing that T and A are increased in spite of the short half-life of LH in hMG, and how little LH is needed to stimulate folliculogenesis. Gelety and Surrey (3) reported that GnRH agonists used prior to controlled ovarian hyperstimulation may result in incomplete suppression of circulating progesterone and androgens. These relative elevations, occurring early in the development of the follicular cohort, did not appear to affect IVF cycle outcome (3). Interestingly, we found that 3 α -androstenediol glucuronide levels increased during GnRH suppression, and decreased prior to increasing during follicular exogenous gonadotropin stimulation (Fig. 1(b)). We speculate that this lag in response is due to the 5 α -reduction of T and A prior to subsequent suppression of androgen levels and the long half-life of 3 α -androstenediol glucuronide.

As anticipated, SHBG levels were found to decrease during GnRH suppression, and increase dur-

ing follicular exogenous gonadotropin stimulation (Fig. 1(c)). It was of interest to note the elevation of SHBG levels during controlled ovarian hyperstimulation, in the face of elevations in both the androgens and estrogens. Androgens and estrogens have opposing individual effects on SHBG levels, with androgens causing a decrease and estrogens stimulating SHBG production (19,20). The increase in SHBG levels suggests that SHBG production is more dependent on estrogen than on androgen stimulation. We found that SHBG levels continued to increase after aspiration, despite decreasing E₂ levels. This continued rise in SHBG may well act to decrease the amount of bioavailable T at the time of implantation, since SHBG preferentially binds androgens over E₂.

Although the sample size is small, all of the individual patients had similar hormone trends. The coefficient of variation was <30% at each time point studied. Despite the disparity in age range between the patients (9 years), all of the individual hormone curves followed the same trend. Therefore, although the older participants generally had lower hormone levels, their hormone and pathway curves all followed the same trend as with the younger participants curves.

Of the 5 patients studied, 2 achieved pregnancy and 3 did not achieve pregnancy. There was no difference in hormone and/or pathway curves between the conception and nonconception cycles.

We did not find any correlation with androgen levels and oocyte quality. All of our androgen levels were within the physiologic range of premenopausal women; however, the highest testosterone concentration was 65 ng/dL.

Our data are consistent with the enhancement of the aromatase pathway during controlled ovarian hyperstimulation, whereas the 5 α -reductase pathway was inhibited. This demonstrates that androgens are preferentially metabolized via the aromatase pathway during controlled ovarian hyperstimulation. Although it is not surprising that the aromatase pathway predominates, it is interesting that 5 α -reductase activity is suppressed with regard to androgen metabolism. This suggests that the actual conversion of A and T to DHT is not solely substrate dependent. It is possible that 5 α -reductase activity is modulated by competitive inhibitors, such as progesterone (21). Krieg *et al.* (22) investigated 5 α -reductase in benign prostatic hyperplasia and found the mean inhibitor constants of progesterone, E₁, and E₂, with regard to 5 α -reductase activity, to be 0.11, 15.5, and 5.1, respectively. Thus, if the mean inhibitor constants for 5 α -reductase in

ovarian tissue resemble those of prostatic tissue, supraphysiologic concentrations of progesterone, E₁, and E₂ obtained during controlled ovarian hyperstimulation could exceed the inhibitor constants for 5 α -reductase, thereby inhibiting the activity of this enzyme. A number of prior reports have substantiated 5 α -reductase modulation by sex steroids (21–26). Cassidenti *et al.* (21) have shown in vitro, that E₂, progesterone, and the 19-nor-progestins inhibit the conversion of T to DHT in genital skin at high doses. E₂, progesterone, levonorgestrel, and norethindrone demonstrated 40.8 $\pm$ 14.2%, 97 $\pm$ 5.3%, 47.9 $\pm$ 6.3%, and 59 $\pm$ 4.6% inhibition, respectively. In addition, the suggestion has been made that prenatal exposure to synthetic progestins contributes to an increased incidence of hypospadias (26). One potential mechanism for such an effect may be competitive inhibition of 5 α -reductase.

Exogenous progesterone administered in the luteal phase after IVF could also cause decreased conversion of androstenedione and testosterone to dihydrotestosterone. The clinical relevance of various androgen concentrations on the endometrium is not known.

We conclude that although androgen levels increase during controlled ovarian hyperstimulation, androgen metabolism engenders a less androgenic milieu. This is due to increased aromatase activity, and increased SHBG levels, which decrease free T levels. By contrast, androgen metabolism by 5 α -reductase is decreased, possibly as a result of the presence of high levels of other steroids.

REFERENCES

1. Martin KA, Hornstein MD, Taylor AE, Hall JE, Barbieri RL: Exogenous gonadotropin stimulation is associated with increases in serum androgen levels in in-vitro fertilization-embryo transfer cycles. *Fertil Steril* 1997;68:1011–1016
2. Adashi EY: The climacteric ovary as a functional gonadotropin-driven androgen-producing gland. *Fertil Steril* 1994;62:20–27
3. Gelety TJ, Surrey ES: Incomplete androgen and progesterone suppression following midluteal GnRHa prior to controlled ovarian hyperstimulation for IVF-ET. *J Assist Reprod Genet* 1997;14:525–530
4. Fanchin R, Righini C, Olivennes F, Taieb J, de Ziegler D, Frydman R: Premature plasma progesterone and androgen elevation are not prevented by adrenal suppression in in vitro fertilization. *Fertil Steril* 1997;67:115–119
5. Yong EL, Hillier SG, Turner M, Baird DT, Ng SC, Bongso A, Ratnam SS: Differential regulation of cholesterol side-chain cleavage (P450_{scc}) and aromatase (P450_{arom}) enzyme mRNA expression by gonadotrophins and cyclic AMP in human granulosa cells. *J Mol Endocrinol* 1994;12:239–249
6. Polan ML, Daniele A, Russell JB, DeCherney AH: Ovulation induction with human menopausal gonadotropin compared to human urinary follicle-stimulating hormone results in a significant shift in follicular fluid androgen levels without discernible differences in granulosa-luteal cell function. *J Clin Endocrinol Metab* 1986;63:1284–1291
7. Steinkampf MP, Mendelson CR, Simpson ER: Regulation by follicle-stimulating hormone of the synthesis of aromatase cytochrome P-450 in human granulosa cells. *Mol Endocrinol* 1987;1:465–471
8. Yong EL, Baird DT, Yates R, Reichert LE Jr, Hillier SG: Hormonal regulation of the growth and steroidogenic function of human granulosa cells. *J Clin Endocrinol Metab* 1992;74:842–849
9. Paulson RJ, Serafini PC, Catalino JA, Lobo RA: Measurements of 3 α ,17 β -androstenediol glucuronide in serum and urine and the correlation with skin 5 α -reductase activity. *Fertil Steril* 1986;46:222–226
10. Goebelsmann U, Horton R, Mestman JH, Arce JJ, Nagata Y, Nakamura RM, Thorneycroft IH, Mishell DR Jr: Male pseudohermaphroditism due to testicular 17 β -hydroxysteroid dehydrogenase deficiency. *J Clin Endocrinol Metab* 1973;36:867–879
11. Goebelsmann U, Arce JJ, Thorneycroft IH, Mishell DR Jr: Serum testosterone concentrations in women throughout the menstrual cycle and following hCG administration. *Am J Obstet Gynecol* 1974;119:445–452
12. Wong IL, Morris RS, Chang L, Spahn M-A, Stanczyk FZ, Lobo RA: A prospective randomized trial comparing finasteride to spironolactone in the treatment of hirsute women. *J Clin Endocrinol Metab* 1995;80:233–238
13. Goebelsmann U, Bernstein GS, Gale JA, Kletzky OA, Nakamura RM, Coulson AM, Korelitz JJ: Serum gonadotropin, testosterone, estradiol and estrone levels prior to and following bilateral vasectomy. In *Vasectomy: Immunologic and Pathophysiologic Effects in Animals and Man*, JH Lepow, R Crozier (eds.), New York, Academic Press, 1979, pp. 165–175
14. Stanczyk FZ: Steroid hormones. In *Mishell's Textbook of Infertility, Contraception, and Reproductive Endocrinology*, 4th edn., RA Lobo, DR Mishell Jr, RJ Paulson, D Shoupe (eds.), Malden, Blackwell Science, 1997, pp. 46–66
15. Liu CH, Laughlin GA, Fischer UG, Yen SS: Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: Evidence for a reduced 17,20-desmolase enzymatic activity. *J Clin Endocrinol Metab* 1990;71:900–906
16. Medina M, Herrera J, Flores M, Martin O, Bermudez JA, Zarate A: Normal ovarian function in a mild form of late-onset 3 β -hydroxysteroid dehydrogenase deficiency. *Fertil Steril* 1986;46:1021–1025
17. Chang YT, Zhang L, Alkaddour HS, Mason JJ, Lin K, Yang X, *et al.*: Absence of molecular defect in the type II 3 β -hydroxysteroid dehydrogenase (3 β -HSD) gene in premature pubarche children and hirsute female patients with moderately decreased adrenal 3 β -HSD activity. *Pediatr Res* 1995;37:820–824
18. Speroff L, Glass RH, Kase NG, eds.: Hormone biosynthesis, metabolism, and mechanism of action. In *Clinical Gynecologic Endocrinology and Infertility*, 6th edn., Philadelphia, Lippincott Williams & Wilkins, 1999, pp. 31–105

19. Holt JA: Reproductive hormones and their mechanisms of action. *In* Mishell's Textbook of Infertility, Contraception, and Reproductive Endocrinology, 4th edn., RA Lobo, DR Mishell Jr, RJ Paulson, D Shoupe (eds.), Malden, Blackwell Science, 1997, pp. 26–45
20. Toscano V, Balducci R, Bianchi P, Guglielmi R, Mangiantini A, Sciarra F: Steroidal and non-steroidal factors in plasma sex hormone binding globulin regulation. *J Steroid Biochem Mol Biol* 1992;43:431–437
21. Cassidenti DL, Paulson RJ, Serafini P, Stanczyk FZ, Lobo RA: Effects of sex steroids on skin 5 α -reductase activity in vitro. *Obstet Gynecol* 1991;78:103–107
22. Krieg M, Schlenker A, Voigt K: Inhibition of androgen metabolism in stroma and epithelium of the human benign prostatic hyperplasia by progesterone, estrone, and estradiol. *Prostate* 1985;6:233–240
23. Serafini PC, Catalino J, Lobo RA: The effect of spironolactone on genital skin 5 α -reductase activity. *J Steroid Biochem* 1985;23:191–194
24. Serafini P, Lobo RA: Prolactin modulates peripheral androgen metabolism. *Fertil Steril* 1986;45:41–46
25. Kuttenn F, Rigaud C, Wright F, Mauvais-Jarvis P: Treatment of hirsutism by oral cyproterone acetate and percutaneous estradiol. *J Clin Endocrinol Metab* 1980;51:1107–1111
26. Dean HJ, Winter JS: The effect of five synthetic progestational compounds on 5 α -reductase activity in genital skin fibroblast monolayers. *Steroids* 1984;43:13–24