Hormonal Effects of Flutamide in Young Women with Polycystic Ovary Syndrome

VINCENZO DE LEO, DANILA LANZETTA, DONATO D'ANTONA, ANTONIO LA MARCA, AND GIUSEPPE MORGANTE

Department of Obstetrics and Gynecology, University of Siena, Siena 53100, Italy

ABSTRACT

Anovulation in women with polycystic ovary syndrome (PCOS) is the direct effect of high local androgen concentrations on the ovary. Antiandrogens are substances that prevent androgens from expressing their activity on target tissues. Flutamide is a nonsteroid antiandrogen that has been found effective in hirsute patients, although its mechanism of action is unclear.

Eight girls, ranging in age from 16–19 yr, with moderate to severe hirsutism and menstrual irregularities were enrolled in this study. The basal hormonal pattern showed anovulatory cycles; increased concentrations of LH, androstenedione, and testosterone; and increased LH/FSH ratio. A baseline ultrasound scan revealed polycystic ovaries in all patients. All were given 250 mg flutamide twice a day for 6 months. LH, FSH, androstenedione, testosterone, estradiol, and progesterone were evaluated before treatment, every 4 days during the third month of treatment, and on day 24 of the sixth month of treatment. Hirsutism improved, androgen levels dropped, and ovulatory cycles were restored in all subjects. Ultrasonographic examination in follicular phase showed a significant reduction in ovarian volume and ovaries of normal appearance with one dominant follicle. The most important result of the present study was that flutamide restored ovulation in anovulatory PCOS patients. This finding supports the hypothesis that flutamide reduces androgen synthesis through restoration of ovulation, although a direct block of the steroidogenic enzymes of androgen biosynthesis in ovarian thecal cells cannot be excluded. (*J Clin Endocrinol Metab* **83:** 99–102, 1998)

POLYCYSTIC ovary syndrome (PCOS) is the commonest cause of menstrual disorders. It is characterized by infertility, oligomenorrhea or amenorrhea, hirsutism, and acne or seborrhea. About half the women with this syndrome are obese, and some have diabetes mellitus (1, 2). Insulin resistance is a common disorder in PCOS patients (3, 4). Both infertility and the clinical disorders have been ascribed to high levels of LH or a high LH/FSH ratio and increased ovarian androgen production (5, 6). The polycystic ovary is enlarged and pearly white, with a thick capsule and many subcapsular follicles (7, 8). Hyperandrogenism associated with increased serum LH concentrations and ultrasonographic evidence of polycystic ovaries are the usual diagnostic criteria for PCOS (9), although some researchers also include oligomenorrhea and hirsutism (10, 11).

The most widely accepted theory of the pathogenesis of the PCOS is that it results from a complex cycle of events centering on overproduction of androstenedione (A) and its peripheral conversion to estrone (9). According to a recent theory, the pathogenesis of PCOS involves heterogeneous disorders that directly increase one of the three variables: the LH/FSH ratio in plasma, the plasma androgen/estrogen ratio, or the process of follicular atresia. Several findings have suggested that the common denominator in women with hyperandrogenic anovulation, regardless of whether they have typical PCOS, is functional ovarian hyperandrogenism (12).

In PCOS patients, high local androgen concentrations are responsible for anovulation by a direct effect on the ovary (13). Androgen-induced follicular atresia is thought to occur by entry of androgens into the granulosa cells of preantral follicles, where they bind to receptors and cause cell death (14). Androgens cause deterioration of follicles by increasing the number of pycnotic granulosa cells and degenerating oocytes. In rat follicles, 5α-dihydrotestosterone not only inhibits aromatase activity and stimulates progesterone (P) production in granulosa cells, but also induces atresia in preantral and antral follicles (15, 16). Antiandrogens are substances that prevent androgens from expressing their activity on target tissues. Flutamide is a nonsteroid antiandrogen of known efficacy in hirsute patients, but it is not clear whether it acts only by blocking androgen receptors or also by inhibiting the biosynthesis of androgens.

Some studies have suggested that flutamide reduces androgen synthesis and/or increases its metabolism to inactive androgens (17, 18). Assessment of endocrine data in women during flutamide therapy has been controversial because the study populations have consisted of women with different menstrual cycle characteristics and clinical symptoms.

The hypothesis of this study was that flutamide could antagonize the high intraovarian levels of androgens in PCOS. Accordingly, we evaluated the effect of flutamide treatment on ovulatory function, hirsutism, and hormonal patterns in eight young hirsute women with irregular cycles.

Subjects and Methods

Patients and methods

Eight girls, ranging in age from 16–19 yr, with moderate to severe clinical signs of hyperandrogenism were enrolled in the study. The study was approved by the ethical committee of the University Medical School, and informed consent was obtained from each patient. None of the girls required contraception or was receiving hormone therapy during the

study period or in the previous 6 months. The clinical diagnosis of PCOS was based on hyperandrogenism and chronic anovulation. Menstrual bleeding occurred every 45–60 days. No patient had virilization or congenital adrenal hyperplasia (on the basis of normal levels of 17-hydroxyprogesterone). Five patients were obese, with a body mass index

TABLE 1. Hormonal values in blood samples obtained on day 8 of the menstrual cycle before therapy and of the third month of flutamide treatment show significant reductions ($P < 0.01$) in LH, A, T, fT, and SHBG during therapy and normalization of the LH/FSH ratio

	Before flutamide	Third month of flutamide treatment
LH (mlU/mL)	14.2 ± 2.3	8.6 ± 2.2
FSH (mlU/mL)	4.9 ± 1.0	6.1 ± 0.9
LH/FSH ratio	2.8 ± 0.3	1.5 ± 0.2
A (pg/mL)	3120 ± 570	1840 ± 230
T (pg/mL)	1012 ± 180	645 ± 90
fT (pg/mL)	4.1 ± 0.8	2.2 ± 0.3
SHBG (nmol/L)	36 ± 16	68 ± 19
E_2 (pg/mL)	85 ± 12	72 ± 9
P (pg/mL)	720 ± 90	470 ± 55

Values are the mean $\pm$ SD.

of 25 or more. The degree of hirsutism was evaluated according to the criteria of the modified Ferriman and Gallwey method (19), and the mean score was 16.4 ± 3.2 (mean $\pm$ SD). The basal hormonal pattern [LH, FSH, A, T, estradiol (E_2), P, free T (fT)] was evaluated every 4 days from days 8–40 of the cycle in the month before flutamide therapy was started and in the third month of therapy. Before treatment, basal hormone values revealed anovulatory cycles, increased serum concentrations of LH, an increased LH/FSH ratio, and A and testosterone (T) levels at the upper limits of the normal range. Serum P levels were also determined in luteal phase (day 24 of menstrual cycle) during the sixth month of therapy.

A baseline ultrasound scan with a 5-MHz abdominal probe (Sonoline SL-2, Siemens, Milan, Italy) when the patient had a full bladder was performed to evaluate the uterus and ovaries. Ovarian volumes were calculated from the maximum longitudinal, antero-posterior, and transverse diameters. Ultrasonographic diagnosis of polycystic ovaries was based on the presence of 10 or more follicles (2–10 mm in diameter) in 1 or both ovaries.

All patients were given 250 mg flutamide (Eulexin, Schering Plough, Milan, Italy) as tablets twice a day uninterruptedly for 6 months. Hirsutism was evaluated by a modified form of the Ferriman and Gallwey method that has been used in our clinic for many years. A score was assigned to patients before and after 6 months of flutamide treatment. A score greater than 8 is indicative of hirsutism. Before starting the study, multiscreen blood chemistry was performed with liver function tests, lipid profiles, renal parameters, and blood counts.
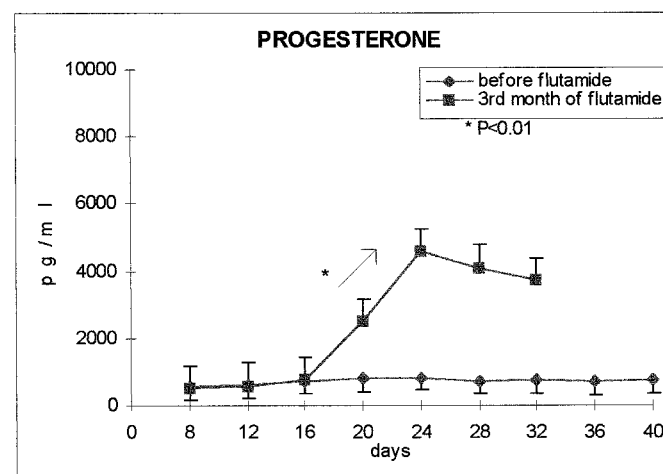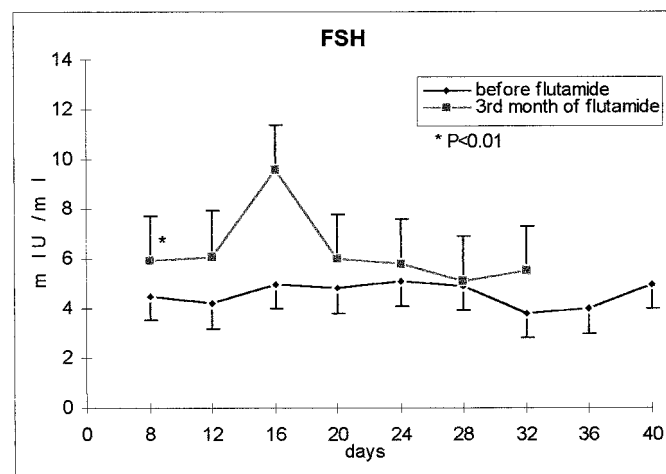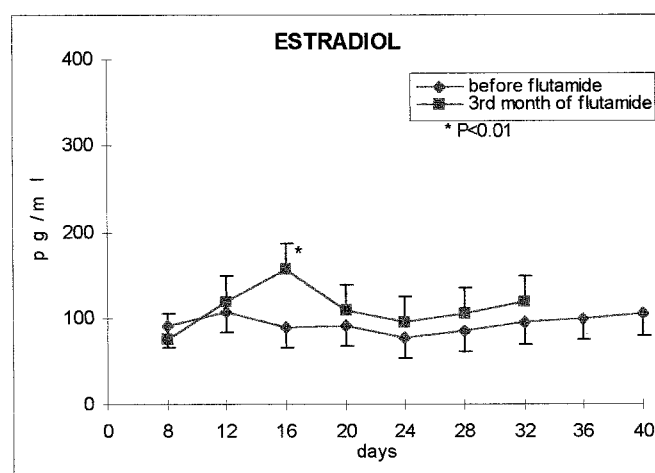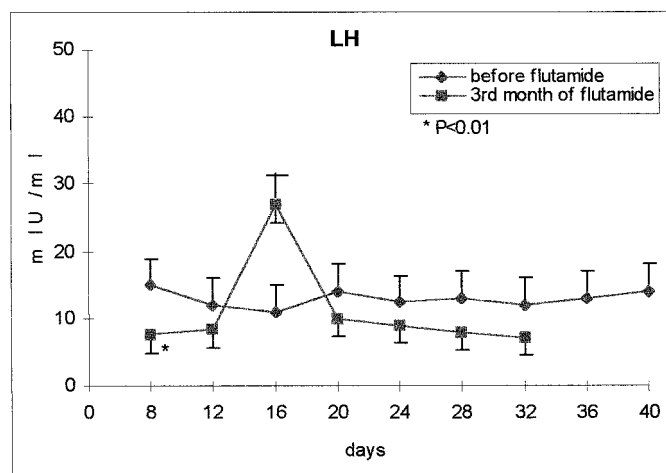

Fig. 1. Comparison of plasma LH and FSH levels in PCOS patients before therapy and during the third month of flutamide treatment.

Fig. 2. Comparison of plasma E_2 and P levels in PCOS patients before therapy and during the third month of flutamide treatment.

Hormone assays

Plasma FSH, LH, E$_2$, P, A, T, fT, and sex hormone-binding globulin (SHBG) levels were assayed by double antibody RIA using commercial kits from Radim (Rome, Italy) for FSH, LH, and T; from Sorin (Saluggia-VC, Italy) for E$_2$, P, A, and SHBG; and from DSL Wherter (TX) for fT. Samples were assayed in duplicate at two dilutions. Samples from a given subject were analyzed in the same assay for each hormone to avoid interassay variations. Quality control pools at low, normal, and high LH, FSH, E$_2$, P, A, T, fT, and SHBG levels were present in each assay. The detection limit of the assay was 0.20 mIU/mL for LH, 0.18 mIU/mL for FSH, 5 pg/mL (18 pmol/L) for E$_2$, 50 pg/mL (0.16 nmol/L) for P, 30 pg/mL for A, 80 pg/mL for T, 0.15 pg/mL for fT, and 2.5 nmol/L for SHBG. Intra- and interassay variations were 7.8% and 8.2% for LH, 6.2% and 6.5% for FSH, 4.2% and 4.9% for E$_2$, 8.5% and 10.8% for P, 5.6% and 6.4% for A, 3.4% and 4.6% for T, 3.2% and 3.4% for fT, 5.6% and 4.6% for SHBG.

Statistical analysis

Plasma hormone levels (as absolute values, mean ± SD) were expressed as milliinternational units per mL (FSH and LH), picograms per mL (P, E$_2$, A, T, and fT), and nanomoles per L (SHBG). Student's *t* test for paired and unpaired data was used, as appropriate, to analyze hormonal changes. $P < 0.01$ was considered significant.

Results

Clinical effects

All women showed an improvement in menstrual cyclicity. Menstrual bleeding returned to once per month within 30–34 days. Cycles were ovulatory, with typical P levels and a reduction in pretreatment levels of T and A. Routine blood chemistry did not reveal any significant changes. A slight, not clinically significant increase in serum transaminases and lipid profile was evident after 6 months of therapy. The Ferriman and Gallwey score for hirsutism decreased significantly from 16.4 ± 3.2 to 6 ± 1.5 after 6 months of flutamide treatment.

Endocrine effects

Table 1 shows hormone levels in blood samples obtained during the follicular phase before and during the third month of flutamide treatment. Significant reductions ($P < 0.01$) were observed in levels of LH, A, T, and fT and in the LH/FSH ratio. In the same samples, SHBG showed a significant increase. Figure 1 shows the patterns of LH and FSH over a period of days of the menstrual cycle before and during the third month of treatment. Before flutamide administration, basal LH levels were high and without a peak, with a mean value of 14.2 ± 2.3 mIU/mL for all cycles. The LH/FSH ratio was 2.8 ± 0.3. During therapy, LH showed a reduction in basal levels, and the LH/FSH ratio decreased. An ovulatory peak around day 16 was evident in all patients. Before flutamide therapy, E$_2$ was practically constant, with a slight increase in basal levels after day 20 and a mean value of 108 ± 12 pg/mL. P did not show any variations; its mean value was 900 ± 200 pg/mL or less, which was typical of anovulatory cycles in all periods of observation. During flutamide treatment, E$_2$ and P patterns changed to those of ovulatory cycles, with mean luteal phase P levels in ranging from 4500–5800 pg/mL (Fig. 2). Plasma androgen levels were high before therapy. During flutamide treatment, T, fT (Fig. 3), and A (Fig. 4) plasma levels showed a significant reduction ($P < 0.01$). Plasma levels of P evaluated on day 24 of the menstrual cycle of the sixth month of flutamide treatment (range, 5200–6300 pg/mL) confirmed the occurrence of a luteal phase.
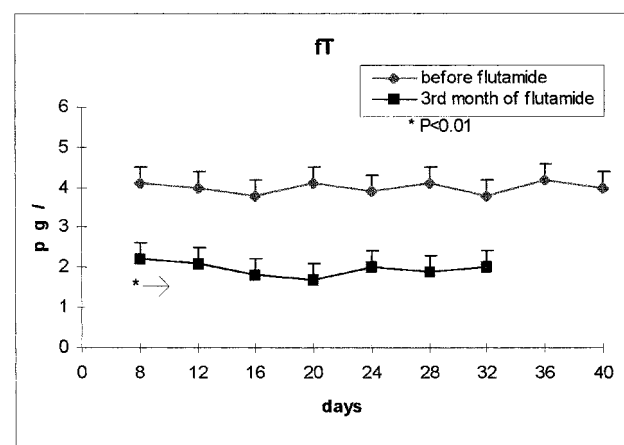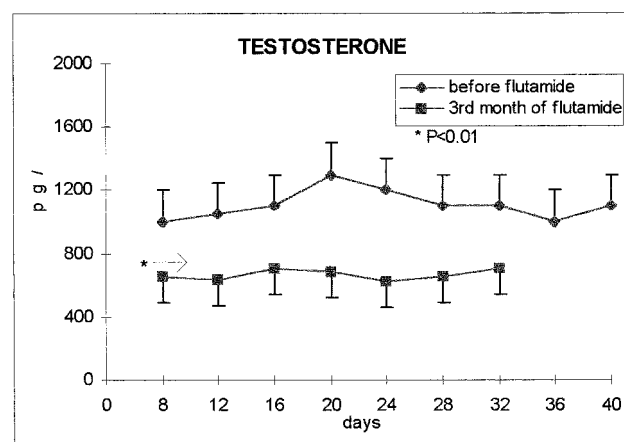

FIG. 3. Comparison of plasma T and fT levels before therapy and during the third month of flutamide treatment.
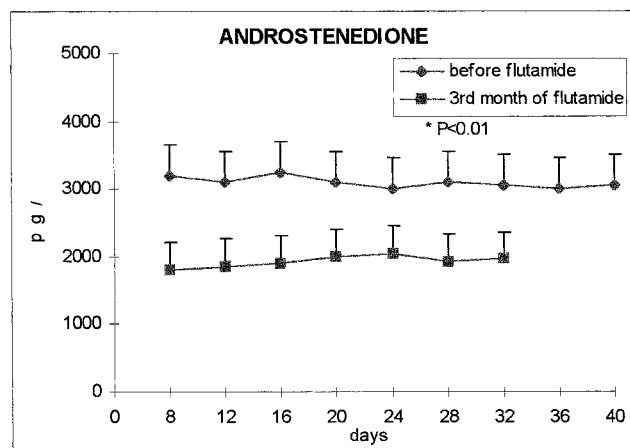

FIG. 4. Comparison of plasma A levels before therapy and during the third month of flutamide treatment.

Discussion

The present study demonstrates the efficacy of flutamide in the treatment of hirsutism and confirms the results of other studies in which flutamide was given alone or in combination with an oral contraceptive (20–23). The important result

of the present study was that flutamide restored ovulation in anovulatory PCOS patients and reduced plasma levels of androgens. Flutamide treatment has been reported not to affect gonadotropin, E_2, and P levels and, therefore, does not alter the mechanism of ovulation (24). This is why it is usually administered together with an oral contraceptive. However, some researchers (25) have found that flutamide produced amenorrhea in women with regular cycles and in women with PCO. Reductions in T and, to a lesser extent, A have been reported after flutamide therapy (20, 21).

In our study we observed a significant reduction in plasma androgen levels and restoration of ovulatory cycles. These findings support the hypothesis that flutamide reduces androgen synthesis through restoration of ovulation (26), although a direct block of the steroidogenic enzymes of androgen biosynthesis in ovarian thecal cells cannot be excluded. In rat testicular cells *in vitro*, flutamide has been reported to inhibit 17–20-lyase activity of cytochrome P450 (17). This enzyme activity has been detected in human thecal cells stimulated with LH and is high in polycystic ovaries (26). Flutamide may reduce plasma androgen levels by inhibiting this enzyme activity.

These results support the hypothesis that PCOS is a form of functional ovarian hyperandrogenism in which the central abnormality is elevated intraovarian androgen concentrations (27). Hypersecretion of androgens into the circulation stimulates the pilosebaceous unit and produces clinical manifestations, such as acne and hirsutism. The local androgen excess is responsible for anovulation by a direct effect on the ovary (28). An increased androgen to estrogen ratio is found in the follicular fluid of atretic follicles (29). Decreased estrogen production causes accumulation of androgens in the follicular fluid and suggests that androgens play a role in the process of atresia. Changes in follicular steroid levels are involved in the initiation of atresia. Androgens induce follicular atresia by entering the granulosa cells of preantral follicles. Cell death is induced by the binding of androgens to their own receptors (30). The mechanism of androgen-induced atresia is in antagonism to E_2-induced granulosa cell proliferation and development (28). When we administer a pure antiandrogen drug such as flutamide, the vicious circle is interrupted, and ovulation can be restored.

The present results show that besides blocking androgen receptors, flutamide also induces a significant reduction in plasma androgen levels by inhibiting the atretic effect of LH on thecal and granulosa cells. They imply that high intraovarian levels of androgens are a major pathogenetic factor in PCOS. The fact that flutamide does not alter the pituitary-ovarian axis (22) means that the problem of PCOS is primarily in the ovary, with secondary effects in the pituitary. As ovulatory cycles and fertility may be restored during flutamide therapy, contraceptive measures may need to be taken. Further studies are needed for insights into the effects of flutamide on androgen biosynthesis in the ovary and the adrenal gland.

References

1. **Adams J, Polson DW, Franks S.** 1986 Prevalence polycystic ovaries in women with anovulation and hirsutism. Br Med J. 293:355–359.

2. **Gilling-Smith C, Franks S.** 1993 Polycystic ovary syndrome. Reprod Med Rev. 2:15–32.

3. **Chang JR, Nakamura RM, Judd HL, Kaplan SA.** 1983 Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab. 57:356–359.

4. **Jalal I, Naiker P, Reddi K, Moodley J, Joubert SM.** 1987 Evidence for insulin resistance in nonobese patients with polycystic ovarian disease after inhibition of ovarian steroid secretion. Fertil Steril. 45:327–333.

5. **Waldestreicher J, Santoro NF, Hall JE, Filicori M, Crowley Jr WF.** 1988 Hyperfunction of the hypothalamic-pituitary axis in women with polycystic ovary disease: indirect evidence for partial gonadotroph desensitization. J Clin Endocrinol Metab. 66:165–172.

6. **Wajchenberg BL, Achando SS, Okada H, et al.** 1986 Determination of the source(s) of androgen overproduction in hirsutism associated with polycystic ovary syndrome by simultaneous adrenal and ovarian venous catheterization: comparison with the dexamethasone suppression test. J. Clin Endocrinol Metab. 63:1204–1210.

7. **Goldzieher JW, Green JA.** 1962 The polycystic ovary. Clinical and histologic features. J Clin Endocrinol Metab. 22:325–338.

8. **Farquhar CM, Birdsall MA, Manning P, Mitchell J, France JT.** 1994 The prevalence of polycystic ovaries on ultrasound scanning in a population of randomly selected women. Aust NZ J Obst Gynaecol. 34:67–72.

9. **Yen SSC.** 1980 The polycystic ovary syndrome. Clin Endocrinol (Oxf). 12:177–207.

10. **McKenna TJ.** 1988 Pathogenesis and treatment of polycystic ovary syndrome. N Engl J Med. 318:558–562.

11. **Barbieri RL.** 1991 Polycystic ovarian disease. Annu Rev Med. 42:199–204.

12. **Barnes R, Rosenfield RL.** 1989 The polycystic ovary syndrome: pathogenesis and treatment. Ann Intern Med. 110:386–399.

13. **Ehrmann DA, Barnes RB, Rosenfield RL.** 1995 Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Endocr Rev. 16:322–353.

14. **Uilenbroek JTJ, Woutersen PJA, Van der Schoot P.** 1980 Atresia in preovulatory follicles: gonadotropin binding in steroidogenic activity. Biol Reprod. 23:219–229.

15. **Hillier SG, Ross GT.** 1979 Effect of exogenous testosterone on ovarian weight, follicular morphology and intraovarian progesterone concentration in estrogen-primed hypophysectomized immature female rats. Biol Reprod. 20:261–268.

16. **Azzolin GC, Saiduddin S.** 1983 Effect of androgens on the ovarian morphology of the hypophysectomized rat. Proc Soc Exp Biol Med. 172:70–73.

17. **Ayub M, Levell MJ.** 1987 Inhibition of rat testicular 17α-hydroxylase and 17,20-lyase activities by anti-androgens (flutamide, hydroxyflutamide, Ru23908, cyproterone acetate) *in vitro*. J Steroid Biochem. 28:43–47.

18. **Brochu M, Belanger A, Dupont A, Cusan L, Labrie F.** 1987 Effects of flutamide, and aminoglutethimide on plasma 5α-reduced steroid glucuronide concentrations in castrated patients with cancer of the prostate. J Steroid Biochem. 28:619–622.

19. **Hatch R, Rosenfield R, Kim MH, Tredway D.** 1981 Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 140:815–826.

20. **Cusan L, Dupont A, Gomez JL, Trembaly RR, Labrie F.** 1994 Comparison of flutamide and spironolactone in the treatment of hirsutism: a randomized controlled trial. Fertil Steril. 61:281–287.

21. **Fruzzetti F, De Lorenzo D, Ricci C, Fioretti P.** 1993 Clinical and endocrine effects of flutamide in hyperandrogenic women. Fertil Steril. 60:806–813.

22. **Moghetti P, Castello R, Negri C, et al.** 1995 Flutamide in the treatment of hirsutism: long-term clinical effects, endocrine changes, and androgen receptor behavior. Fertil Steril. 64:511–517.

23. **Simard J, Luthy I, Gay J, Belanger A, Labrie F.** 1986 Characteristics of interaction of the antiandrogen flutamide with the androgen receptor in various target tissues. Mol Cell Endocrinol. 44:261–270.

24. **Couzinet B, Pholsena M, Young J, Schaison G.** 1993 The impact of a pure anti-androgen (flutamide) on LH, FSH, androgens and clinical status in idiopathic hirsutism. Clin Endocrinol (Oxf). 39:157–162.

25. **Cesur V, Kamel N, Uysal AR, Erdogan G, Kaskal N.** 1994 The use of anti-androgen flutamide in the treatment of hirsutism. Endocr J. 41:573–577.

26. **Rosenfield RL, Barnes RB, Cara JF, Lucky AW.** 1990 Dysregulation of cytochrome P450c17α as the cause of polycystic ovary syndrome. Fertil Steril. 53:785–791.

27. **Hsueh AJW, Billing H, Tsafriri A.** 1994 Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr Rev. 6:707–724.

28. **McNatty KP.** 1980 Regulation of follicle maturation in the human ovary: a role for 5α-reduced androgens. In: Cumming IA, Funder JW, Mendelsohn FAO, eds. Endocrinology. Camberra: Australian Academy of Sciences; 1–51.

29. **Maxson WS, Haney AF, Schomberg DW.** 1985 Steroidogenesis in porcine atretic follicles: loss of aromatase activity in isolated granulosa and theca. Biol Reprod. 33:495–501.

30. **Uilenbroek JTJ, Woutersen PJA, Van der Shoot P.** 1980 Atresia in preovulatory follicles: gonadotropin binding in steroidogenic activity. Biol Reprod. 23:219–229.