Suppression and recovery of gonadotropin and steroid secretion by a gonadotropin-releasing hormone receptor antagonist in healthy women with normal ovulation versus women with polycystic ovary syndrome in the early follicular phase

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Objective: To evaluate the effects of the GnRH antagonist cetrorelix on the gonadal axis in patients with polycystic ovary syndrome (PCOS).

Design: Observational clinical study.

Setting: Academic research center.

Patient(s): Ten patients with PCOS and 10 controls with normal ovulation.

Intervention(s): Patients received a daily cetrorelix injection (0.25 mg SC at 9:00 AM) for 6 days, starting from day 3 of the menstrual cycle.

Main Outcome Measure(s): Serum gonadotropin, E2, T, 17-OH-P, and androstenedione plasma levels were evaluated at baseline and at 12 and 24 hours after each daily injection. These hormones were also assayed at days 10, 12, and 14 of the menstrual cycle.

Result(s): We observed in patients with PCOS a significantly higher suppression of FSH and LH for the entire length of therapy; LH recovery secretion was significantly higher in the PCOS group. Regarding androgens, we found a greater suppression of T. Androstenedione and 17-OH-P showed a trend toward a higher suppression in PCOS.

Conclusion(s): Gonadotropin and androgen suppression by GnRH antagonist is more effective in PCOS than in controls, suggesting a higher sensitivity of GnRH receptors in PCOS to this drug. (Fertil Steril 2009;91: 1857–63. ©2009 by American Society for Reproductive Medicine.)

Key Words: GnRH antagonist, cetrorelix, polycystic ovary syndrome, GnRH receptors

Polycystic ovary syndrome (PCOS) affects approximately 6% to 15% of women at reproductive age (1). It is a very heterogeneous disorder with a wide spectrum of clinical and biochemical manifestations. The main characteristics are chronic anovulation and hyperandrogenism, especially increased levels of T and androstenedione (A) (2). Moreover, the effective androgen levels are further increased by a decrease in sex hormone–binding globulin (SHBG) (3–6). Although the etiology of PCOS is puzzling, perturbation of gonadotropin secretion is one of the hallmarks of this disorder (7). In fact in PCOS, FSH level is typically in the lower follicular range; conversely, plasma LH level is commonly increased, and its pulse frequency (and by inference GnRH pulse frequency) is persistently rapid, with approximately one pulse per hour (7). GnRH plays a pivotal role in the control of female reproduction and is secreted by hypothalamic neurons in a pulsatile way (8). It binds to specific receptors on pituitary gonadotrophs, followed by the secretion of the gonadotropins, LH and FSH, which regulate steroidogenesis and gametogenesis in the ovary (8).

The main difficulties of ovulation induction in PCOS are represented by multifollicular development, partially due to the negative consequences of chronic, tonic, high serum levels of LH. Heightened pituitary sensitivity to exogenous GnRH in patients with PCOS accounts for this exaggerated pulsatile LH release (9). Two kind of drugs are available to prevent premature LH surge and to lower the interferences with the hypothalamo-hypophysial-ovarian axis during ovarian stimulations: GnRH agonists and GnRH antagonists. GnRH agonists are not commonly used in standard protocols for ovulation induction in PCOS because of the higher amount of gonadotropins required to achieve ovulation, the greater prevalence of multiple follicle development, and consequently a higher risk of ovarian hyperstimulation syndrome.
and multiple pregnancies. On the contrary, GnRH antagonists, competitive inhibitors of GnRH receptor, might bring several advantages in ovulation induction therapy for this kind of patients. These drugs lead to a rapid suppression of LH release by blocking receptors without any intrinsic effect and by inhibiting their microaggregation; therefore, the post-receptor mechanisms are not triggered. Thanks to this mechanism and to the lack of “flare-up effect” that is typical of agonists, the result is that their management is easier. The first antagonist was synthesized more than 20 years ago (10). However, its clinical application was hampered by a high histaminergic potential. Recently, new substances lacking the histaminergic side effects (11) have been developed. One of these drugs is cetrorelix, which recently has been introduced into ovarian stimulation protocols to prevent premature LH surges.

In this study we wanted to compare the effect of such a drug on the hypothalamic-pituitary axis in PCOS with that in women with normal ovulation at their follicular phase. The aim was to use a GnRH antagonist as a tool to study and to further elucidate the dynamic source and the role of exaggerated LH production in the syndrome.

MATERIALS AND METHODS

Patient Population
The study was approved by the Institutional Board of our department, and an informed consent was obtained from all studied subjects. Ten patients with PCOS and 10 controls were observed. The control group consisted of 10 healthy volunteer women who had normal ovulation and normal basal hormonal profiles (Table 1). The two groups were matched for age and body mass index (BMI).

Both populations had been free of any drug treatment in the last 3 months before our study. Diagnosis of PCOS was based on Rotterdam consensus criteria represented by the presence of at least two of three elements: oligo-ovulation or anovulation; clinical and/or biochemical signs of hyperandrogenism (plasma androgen levels at the upper limit of or above the normal range A, 0.5–2.5 ng/mL [1.745–8.725 nmol/L]; T, 0.2–0.6 ng/mL [0.6934–2.080 nmol/L]); and the presence of bilaterally polycystic ovaries on ultrasonography (12), associated with an ovarian stroma/total area ratio >0.34, as previously published (13). A normal LH/FSH ratio was not considered an exclusion criterion (13). To exclude patients with other endocrine disorders (thyroid dysfunction, adrenal dysfunction, hyperprolactinemia) a basal hormonal evaluation was made at the beginning of the study; in particular serum levels of LH, FSH, P, E2, T, A, 17-OH-P, SHBG, DHEAS, PRL, TSH, free T3, free T4, fasting glucose, and fasting insulin were evaluated. Regarding the menstrual patterns of PCOS we had five patients with amenorrhea and five with anovulatory oligomenorrhea; the latter started our protocol at day 3 of a spontaneous menstrual cycle whereas the patients with amenorrhea started the therapy randomly, after the same basal hormonal evaluation and an ultrasound pelvic examination that allowed us to exclude ovarian activation. This was decided to avoid any possible influence of progesterin on gonadotropins. The presence of a late-onset adrenal enzyme

<table>
<thead>
<tr>
<th>Basal hormone profile</th>
<th>PCOS (n = 10)</th>
<th>Controls (n = 10)</th>
<th>αP value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mUI/L)</td>
<td>6.9 ± 3.52</td>
<td>4.06 ± 0.51</td>
<td>.02</td>
</tr>
<tr>
<td>FSH (mUI/L)</td>
<td>4.98 ± 1.33</td>
<td>6.0 ± 0.91</td>
<td>NS</td>
</tr>
<tr>
<td>A (ng/mL)</td>
<td>2.91 ± 0.94</td>
<td>1.26 ± 0.98</td>
<td>.02</td>
</tr>
<tr>
<td>17-OH-P (ng/mL)</td>
<td>1.1 ± 0.86</td>
<td>0.7 ± 0.25</td>
<td>NS</td>
</tr>
<tr>
<td>T (ng/mL)</td>
<td>0.65 ± 0.05</td>
<td>0.41 ± 0.12</td>
<td>.01</td>
</tr>
<tr>
<td>E2 (pg/mL)</td>
<td>47.6 ± 12.83</td>
<td>55.6 ± 35.74</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol (ng/mL)</td>
<td>119.12 ± 39.38</td>
<td>109.4 ± 14.18</td>
<td>.008</td>
</tr>
<tr>
<td>DHEAS (ng/mL)</td>
<td>2,720.71 ± 773.27</td>
<td>2,412.0 ± 200.67</td>
<td>NS</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>32 ± 8.84</td>
<td>61.4 ± 19.13</td>
<td>.008</td>
</tr>
<tr>
<td>TSH (μUI/mL)</td>
<td>2.61 ± 1.02</td>
<td>2.24 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Free T3 (pg/mL)</td>
<td>3.21 ± 0.15</td>
<td>3.45 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Free T4 (pg/mL)</td>
<td>11.8 ± 1.26</td>
<td>11.04 ± 1.94</td>
<td>NS</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>18.02 ± 6.42</td>
<td>20.48 ± 9.59</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (μUI/mL)</td>
<td>19.07 ± 9.83</td>
<td>6.42 ± 2.74</td>
<td>.018</td>
</tr>
<tr>
<td>Glucose (mg/mL)</td>
<td>89.22 ± 13.27</td>
<td>81 ± 9.85</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Values are expressed as means ± SD. NS = not significant.
αStatistical analysis was performed with use of ANOVA followed by the Wilcoxon–Mann-Whitney test. Values with P < .05 were considered statistically significant.

defect was excluded by an ACTH test (250 μm IV; Synacthen, Ciba-Geigy, Basel, Switzerland), according to the criteria reported by New et al. (14).

Interventions
The patients received a daily cetrorelix injection (0.25 mg SC at 9:00 AM) for 6 days, starting at day 3 of the menstrual cycle or randomly in the nonmenstruating women with PCOS.

Main Outcome Measures
Serum gonadotropin (FSH and LH), E\textsubscript{2}, T, A, and 17-OH-P levels were evaluated beyond baseline and at 12 and 24 hours after each daily cetrorelix injection. These hormones were also assayed at days 10, 12, and 14 of the menstrual cycle to evaluate the recovery of their serum levels. Data are expressed as mean ± SD for each time point investigated and as percentage in respect to the baseline value set equal to 100.

Moreover we also analyzed the suppression area under the 144-hour curve (AUC) assayed for each hormone and calculated by the trapezoidal rule (15). More specifically, to evaluate the real suppression of gonadotropins and steroids after cetrorelix, regardless of basal plasma secretion, results were expressed as absolute inhibition (suppressed AUC [sAUC]), calculated by the difference between AUC and basal AUC (area under the virtual curve, which describes the basal unsuppressed secretion calculating by assuming the values—measured at the time of the first cetrorelix injection—as constant values during the 144-hour period). Cetrorelix acetate (Cetrotide) was purchased from Serono Biotech and Beyond (Merck Serono International SA, a division of Merck KGaA, Darmstadt, Germany).

Tolerability
Single SC administration of cetrorelix did not induce any systemic or local adverse events of clinical note. Transient local discomfort was reported by six subjects at the injection time.

Statistical Analysis
Statistical analysis was performed with use of analysis of variance (ANOVA) followed by the Wilcoxon–Mann-Whitney test for comparison of data derived from two groups. Values with $P<.05$ were considered statistically significant.

RESULTS
Basal Evaluation
Table 1 shows the baseline endocrine characteristics of the studied groups. As expected, mean LH, T, and A plasma levels were significantly higher in patients with PCOS; conversely SHBG level was significantly lower in the PCOS group.

Similarly, insulin basal levels were higher in PCOS; these data are in accordance with the metabolic alterations typical of PCOS. No differences were found for the other assayed hormones between the two groups.

Age and BMI were not significantly different between control and PCOS groups; mean age was 27 years for PCOS and 26.71 years for control ($P=.88$), and mean BMI was 26.89 ± 4 for PCOS and 25.45 ± 4 for controls ($P=.73$). In both groups (PCOS and control) we evaluated, for each examined point, the mean serum hormonal levels and their suppression, expressed as a percentage in respect to the basal value.

Gonadotropins
Figure 1a shows mean FSH levels at each studied time. As is already known, in patients with PCOS we also found basal FSH level in the lower range. Mean FSH levels were not significantly different between the two groups at the baseline and 12 hours after the first drug injection. They became significantly lower in the PCOS group 24 hours after the first drug injection ($P<.01$), and this behavior lasted for the entire length of cetrorelix therapy, until 24 hours after the last drug injection.

As expected, mean LH levels were significantly higher in the PCOS group at the baseline ($P<.05$) (Fig. 1b). The difference with the control group disappeared 12 hours after the first drug injection until the day of the last drug injection. On days 8, 10, and 12 LH recovery secretion was significantly higher in the PCOS group ($P<.05$). Conversely, on day 14 LH serum levels were significantly higher in controls because of the preovulatory LH peak in the group with normal ovulation.

Steroids
Mean T levels were classically higher in the PCOS group at the baseline ($P<.01$), whereas during antagonist treatment they became not significantly different when compared with controls (data not shown). This result can be explained by the greater suppression of androgens in PCOS. In fact the higher sensitivity of androgens to cetrorelix treatment is further demonstrated in the same figure demonstrated where T values are expressed as a percentage in respect to the baseline for each examined point of the treatment. The difference between the two groups became statistically significant every 12 hours after each drug injection ($P<.05$). It is interesting to underline the fact that T behavior is related to that of LH, in agreement with acute regulation of T secretion by LH.

Regarding A and 17-OH-P plasma levels, we observed a trend toward a higher suppression in PCOS that became statistically significant only in a few examined points (data not shown). Concerning E\textsubscript{2} suppression, no differences were found between the two groups during the treatment; as expected, after the last drug injection, we observed a preovulatory increase of E\textsubscript{2} in the group with normal ovulation (data not shown).

Figure 2 shows the sAUC for each studied hormone in both groups. As clearly indicated, both gonadotropins and androgens showed a significantly greater sAUC in PCOS when
compared with controls. Conversely, E2 plasma levels were similarly suppressed in both groups.

DISCUSSION

This is the first study designed to analyze the effect of a GnRH antagonist on gonadotropin and ovarian steroid secretion in the early follicular phase in women with PCOS and in those with normal cycles. In fact all studies so far published investigated the effect of GnRH antagonists on the hypothalamus-pituitary-ovarian axis in stimulated cycles. The increased LH pulse frequency and release in PCOS is a well-known characteristic of the syndrome (16–19); in part this phenomenon seems due to a higher pituitary sensitivity to GnRH (16, 20, 21). The higher LH levels have represented a main problem in PCOS ovarian stimulation for the negative effect of this gonadotropin on follicular development. Lately, the use of GnRH agonists and antagonists in ovarian stimulation protocols has helped in preventing LH interference in follicular development; the antagonists have been better because, in addition to the rapid LH suppression, they lack a flare-up effect (10).
As expected, after GnRH antagonist administration we found higher LH and FSH suppression in PCOS than in controls; a similar behavior was observed for the androgens, mainly for T. Interestingly, for E2 suppression no difference was found between the two groups. This result can be explained by the fact that although androgen suppression was greater, the absolute values of both A and T did not differ among the two groups, being a similar substrate amount for aromatase activity; moreover this suggests that antagonist treatment has no direct effect on androgen conversion into estrogens. In any case the fact that, in PCOS, the use of GnRH agonists induces a significant gonadotropin and androgen reduction when compared with controls but not a similar E2 suppression can have important clinical implications for the crucial role of estrogens on endometrium and oocyte development (22). On the contrary, our previous studies (23) indicated that treatment with GnRH agonist induced a marked decrease of E2 secretion by the PCOS ovary, as a consequence of the dramatic reduction of A and T production. Also in that case a direct effect of treatment on aromatase activity has not been demonstrated.

Increased pituitary responsiveness to exogenous GnRH and to GnRH agonist in women with PCOS was already known (16, 20, 21, 24). The stronger suppression of gonadotropins we found in the PCOS group, by using the same drug dosage for all the studied subjects, suggests a higher sensitivity of these women to the GnRH antagonist as well. In addition to their classical pituitary location, specific GnRH receptors have been found in murine and human granulosa cells (25–27). However, because the consensus is that GnRH analogues (agonists and antagonists) have no impact on ovarian GnRH receptors (28–31), the higher responsiveness of women with PCOS is due purely to the higher sensitivity of their pituitary receptors. Furthermore, this better

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**FIGURE 2**

Follicle-stimulating hormone, LH, A, T, 17-OH-P, and E2 sAUC during treatment in PCOS and control groups. Values are calculated as indicated in Materials and Methods section. Statistical analysis was performed with use of ANOVA followed by the Wilcoxon–Mann-Whitney test for comparison of data derived from two groups (each one made up of 10 women). Values with $P<.05$ were considered statistically significant. CTR = control.
response may be due in part to E2 levels that are able to increase the fraction of gonadotrophs responding to GnRH (32–36). However, our data suggest that in PCOS the increased pituitary sensitivity is probably only in part estrogen mediated. In fact it was observed in the PCOS group also when their estrogen levels were suppressed.

We are aware of the existence of several published papers regarding the use and the effect of GnRH analogues in ovarian PCOS stimulation protocols. An interesting meta-analysis by Griesinger et al. elucidates the benefits of GnRH-antagonist protocols in patients with PCOS in terms of a shorter length of stimulation compared with GnRH agonist protocols plus the possibility of triggering ovulation by a GnRH analogue instead of hCG with a consequent lower risk of development of ovarian hyperstimulation syndrome (37). Furthermore the antagonist protocol represents a safer way to induce final oocyte maturation (38).

Starting from all these useful clinical observations we believe we offer a different point of view on the effect of this kind of drug on PCOS. In our opinion the originality of the present study is that for the first time a GnRH antagonist was used in unstimulated cycles in women with PCOS and controls. Our effort was to provide a better insight in the pathophysiology of gonadotropin and steroid secretion in women with PCOS. Our results confirm the excellent response of these patients to cetrorelix and lead us to believe that GnRH antagonist can represent the first-choice drug to control LH and androgen secretion in patients with PCOS who need a controlled ovarian hyperstimulation therapy.

REFERENCES


