Clinical and hormonal effects of the combination gonadotrophin-releasing hormone agonist plus oral contraceptive pills containing ethinyl-oestradiol (EE) and cyproterone acetate (CPA) versus the EE–CPA pill alone on polycystic ovarian disease-related hyperandrogenisms

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The aim of this study was to compare the clinical and hormonal effects of the combination of a long-acting gonadotrophin-releasing hormone analogue (GnRH-a) plus an oral contraceptive (OC) pill containing ethinyl-oestradiol (EE) and cyproterone acetate (CPA) versus the EE–CPA pill alone in patients with polycystic ovarian disease (PCOD) and related hyperandrogenisms, in order to evaluate whether the addition of GnRH-a has any advantage. A total of 12 PCOD patients were treated with the EE–CPA pill alone for 10 consecutive cycles according to an OC standard regimen. A further 12 patients were treated with GnRH-a, one i.m. injection every 28 days for a total of eight injections, combined with the EE–CPA pill for 10 consecutive cycles. The latter was thus prolonged for two cycles more than GnRH-a. Clinical evaluations (symptoms, weight, Ferriman–Gallwey score) and hormonal and biochemical analyses were assessed before, during (at 3 or 6 months) and after treatment, either when spontaneous cycles had resumed or after 3 months of amenorrhea. There was a significant improvement in hirsutism, and a strong reduction in gonadotrophin, oestradiol, testosterone, androstenedione and 17-OH-progesterone concentrations in both treatment groups but with no significant differences between them, except in the gonadotrophin concentrations. Cortisol and triglyceride concentrations increased during treatment in both groups. The Ferriman–Gallwey score remained significantly decreased in both groups after treatment, as did androstenedione in the GnRH-a plus EE–CPA pill group, but there were no significant differences between the two groups. No changes were observed in prolactin, dehydroepiandrosterone sulphate (DHEA-S), insulin, glycaemia and cholesterol concentrations. However, when only the obese and more hirsute patients were considered, significant differences between the two groups were found during treatment in the Ferriman–Gallwey score and the gonadotrophin and DHEA-S concentrations (which increased during treatment in obese patients with the pill alone), and after treatment in the Ferriman–Gallwey score and the concentration of 17-OH-progesterone in the more hirsute patients, with the GnRH-a plus pill group having better results. In conclusion, a cyclic prolonged treatment with OC EE–CPA pills is not improved in most PCOD patients by the addition of GnRH-a, and is complicated and expensive. However, the addition of a long-acting GnRH-a may be recommended in obese and severely hirsute patients.

Key words: cyproterone acetate/GnRH analogues/hyperandrogenism/oral contraceptive pill/polycystic ovarian disease

Introduction

Several therapeutic approaches have been applied to polycystic ovarian disease (PCOD) patients, including diet (Guzick et al., 1994) and pharmacological agents. As the treatment goal, especially for related hirsutism, is a reduction in the production, bioavailability or binding of the androgens to their target organs, the traditional treatment has been the combination of oral contraceptives (OC) and anti-androgens, such as cyproterone acetate (CPA). The results of OC treatments have often been disappointing, not only because of the incomplete suppression of ovarian function, but also because the progestin component of most OC pills is derived from 19-nor-testosterone and therefore has some androgenic activity (Morcos et al., 1994). CPA has also been reported to be effective alone, associated with OC pills or in combination with oestrogens as an OC pill (Belisle and Love, 1986; Falsetti and Galbignani, 1990; Neumann, 1994). This combination [CPA + oestrogens (EE)] can simultaneously decrease androgen production and block androgen action, and it appears to be especially suited to the treatment of hirsutism, acne or both (Barbieri, 1992; Neumann, 1994). Spirolactone has also been used in the treatment of hirsutism with adequate results but with a significant incidence of dysfunctional uterine bleeding (Helfer et al., 1988). Subsequently, flutamide was shown to be even more effective (Marcondes et al., 1992; Cusan et al., 1994; Moghetti et al., 1995; Pucci et al., 1995), but because of its potential hepatotoxicity, it requires constant surveillance of liver function (Wysowski and Fourcroy, 1994; Moghetti et al., 1995). Finally, it has been reported that finasteride is useful in idiopathic hirsutism (Ciotta et al., 1995), and ketoconazole in PCOD patients (Gökmen et al., 1996).

In recent years, gonadotrophin-releasing hormone agonistic analogues (GnRH-a) have been introduced for the evaluation and treatment of PCOD patients. These produce a state of complete, yet reversible, suppression of pituitary gonadotrophin
secretion. This results in the suppression of both ovarian functions, namely ovulation and steroidogenesis. Hence, in cases of ovarian hyperandrogenism, GnRH-a has been reported to be of great benefit (Chang et al., 1983; Andreyko et al., 1986; Steinhold et al., 1987; Falsetti and Pasinetti, 1994; Goni et al., 1994; Morcos et al., 1994). Long-acting GnRH-a can be used alone (Goni et al., 1994), although this causes hypo-oestrogenic-related symptoms and decreases bone mineral content, or in combination with an oestrogen and progestin replacement therapy (Morcos et al., 1994), with either standard OC pills (Carr et al., 1995; Elkind-Hirsch et al., 1995) or OC pills containing ethinyl-oestradiol (EE) and CPA (Falsetti and Pasinetti, 1994; Ciotta et al., 1996).

The aim of this study was to compare the clinical and hormonal effects of the combination of a long-acting GnRH-a plus OC pills containing EE and CPA, versus OC pills alone in PCOD patients so as to evaluate whether the addition of a GnRH-a is advantageous and, if so, in which situations.

Materials and methods

Subjects
A total of 24 patients with an average age of 23.2 years (range 14–32), diagnosed as having PCOD with hirsutism (96%), amenorrhoea or oligomenorrhoea (92%), obesity (61%), seborrhoea (37%) or acne (17%), were included in this study. A further five patients were included initially, but later excluded because of the absence of information in the third and sixth months of treatment. The diagnosis of PCOD was established from the patient’s clinical history, examination, relevant laboratory investigations [including elevated concentrations of serum androgens and an increased luteinizing hormone (LH)/follicle stimulating hormone (FSH) ratio] and ovarian transvaginal ultrasonographic criteria (Adams et al., 1986; Takahashi et al., 1994), including the presence of multiple (>10), small (2–8 mm) peripheral follicles bilaterally, without dominance, around a dense core of stroma. Diagnosis was also based on the exclusion of other PCOD-like syndromes, including adrenal dysfunction, Cushing’s syndrome, congenital adrenal hyperplasia, androgen-producing tumours and thyroid dysfunction. General biochemistry (including lipid concentrations) and concentrations of serum gonadotrophin, prolactin, insulin and ovarian, thyroid and adrenal hormones were assessed in all patients. An adrenocorticotropic hormone stimulation test (New et al., 1983) and an insulin resistance test to 100 g oral glucose load were performed in all patients. Other inclusion criteria were no other treatment for their disease in the past 3 months, no desire for pregnancy and the absence of other systemic diseases.

Study design
After the initial diagnosis of PCOD by clinical, hormonal and echographic methods, the included patients were re-evaluated either on days 5–10 of the menstrual cycle or at any time if they were amenorrhoeic or during a period of anovulation as documented by plasma progesterone concentrations. They were asked to fast and abstain from smoking for the 12 h preceding the evaluation. A vein catheter was inserted into an antecubital vein, and after 10 min of rest, a blood sample was obtained for haematological and biochemical analysis, including basal gonadotrophin, prolactin, oestradiol, progesterone, 17-OH-progesterone, cortisol, dehydroepiandrosterone sulphate (DHEA-S), testosterone, androstenedione and insulin concentrations. More recently, sex hormone-binding globulin and free testosterone indices were included. Additionally, a complete clinical examination was performed. Hirsutism was assessed by a global scoring system (Ferriman and Gallwey, 1961), in which a single observer (P.A.) assessed hair growth in 11 body areas on a scale of 0–4. Height, weight, body mass index (calculated as weight in kg/m²) and percentage of the ideal body weight (IBW; calculated using the Association of Life Insurance Directors and Actuarial Society of America tables) were also assessed.

A transvaginal ovarian ultrasonography was performed. Each patient was then allocated one of the two treatment protocols: (i) an OC pill containing 0.035 mg EE and 2 mg CPA (Diane 35; Schering España SA, Madrid, Spain), from the beginning of the next spontaneous or progesterone-induced cycle, administered for 10 consecutive cycles according to an OC standard regimen (pill only group); or (ii) an i.m. injection of the long-acting GnRH-a, triptoreline, δ-Trp-6-luteinizing hormone-releasing hormone microcapsules (Decapeptyl 3.75; Lasa Laboratories, Barcelona, Spain), every 28 days, from day 1 of the next spontaneous or induced cycle, and sometimes during the second half of the studied cycle, up to a total of eight injections. In combination with Decapeptyl 3.75, the same OC pill containing EE and CPA (Diane 35) was administered from day 5 of the cycle for 10 consecutive cycles according to an OC standard regimen, thus prolonging administration of the pill for two cycles more than GnRH-a (GnRH-a plus pill group). Subsequently, patients were asked to attend the Gynecological Endocrine Clinic at trimonthly intervals for clinical evaluation (hirsutism, weight, hypo-oestrogenic-related symptoms, psychological status, etc.). Hormonal and biochemical screenings were repeated randomly at 3 or 6 months of treatment, and also after the second spontaneous menstruation or at 3 months of amenorrhoea after withdrawing the treatment. In all, 12 patients were treated with the pill Diane 35 alone for 10 months (pill only group). Five of them had hormonal screening repeated during the third or fourth cycle of treatment, while the remaining seven were checked during the sixth or seventh cycle. Another 12 patients were treated with the long-acting GnRH-a, Decapeptyl 3.75, plus the Diane 35 pill (GnRH-a plus pill group). Six underwent further hormonal screening during the third or fourth cycle of treatment, and the remaining six during the sixth or seventh cycle. There were no significant differences in the values obtained at these time points, such that all values were classed together as ‘during treatment’. Only nine patients from the pill only group and 10 from the GnRH-a plus pill group returned for their clinical evaluation and hormonal and biochemical analyses in the third month after withdrawing from treatment. The remaining patients from each group decided to continue taking the pill as a contraceptive method without interruption.

Assays
Before, during and after treatment, hormonal screening for FSH, LH, prolactin, oestradiol, cortisol, 17-OH-progesterone, testosterone, androstenedione, DHEA-S and insulin was carried out. Glycaemia, cholesterol, high density lipoproteins (HDL)-cholesterol and triglycerides were also assessed. FSH, LH and prolactin concentrations were measured by an immunoenzymatic assay (MELA; Abbot); oestradiol and testosterone by a double-antibody radioimmunoassay (ICN Biomedicals Inc., Costa Mesa, CA, USA); Δ-4-androstenedione by a solid-phase radioimmunoassay (Coat-a-Count; Diagnostic Products Corporation, Los Angeles, CA, USA); and insulin by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden) using commercially available kits.

Basal parameters
The basal clinical, hormonal and biochemical parameters of the PCOD patients included in each treatment group are shown in Table
Triglycerides (mg %) 40–128 (CF
HDL-Cholesterol (mg %) 30–70 (CF
Glycaemia (mg %) 70–105 (CF
Insulin (µIU/ml) 3–20 (CF
DHEA-S (µg/ml) 0.3–0.8 (CF
Body mass index (kg/m²) Ideal body weight (%)
Weight (kg) 67.2
Height (m) 1.58
Insulin (µIU/ml) 3–20 (CF
Testosterone (ng/ml) 0.3–0.8 (CF
Ferriman–Gallwey score 9.0
Ferriml stimulating hormone (mIU/ml) 2.6–1.2³
Luteinizing hormone (mIU/ml) 2.9 ± 2.8³
Oestradiol (pg/ml) 21.3 ± 3.9³
17-OH-Progesterone (ng/ml) 0.9 ± 0.8³
Testosterone (ng/ml) 0.3 ± 0.2³
Androstenedione (ng/ml) 3.3 ± 1.4³
DHEA-S (µg/ml) 3.1 ± 1.3
Insulin (µIU/ml) 13.3 ± 8.7

Table I: Basal clinical, hormonal and biochemical parameters of the polycystic ovarian disease patients in the two treatment groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal values</th>
<th>Pill only group (n = 12)</th>
<th>Mean ± SD (range)³</th>
<th>GnRH analog plus pill group (n = 12)</th>
<th>Mean ± SD (range)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.7 ± 4.0 (17–32)</td>
<td>22.4 ± 5.3 (14–32)</td>
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<tr>
<td>Height (m)</td>
<td>1.58 ± 0.06 (1.47–1.69)</td>
<td>1.61 ± 0.06 (1.51–1.72)</td>
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<tr>
<td>Weight (kg)</td>
<td>67.2 ± 13.7 (48–90)</td>
<td>78.1 ± 18.6 (53–106)</td>
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<tr>
<td>Ideal body weight (%)</td>
<td>&lt;110</td>
<td>122.1 ± 25.2 (93–170)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>&lt;25</td>
<td>26.9 ± 5.4 (21–38)</td>
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<tr>
<td>Ferriman–Gallwey score</td>
<td>&lt;8</td>
<td>12.0 ± 4.9 (5–21)</td>
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<tr>
<td>Insulin resistance</td>
<td>0</td>
<td>2/12 (16.7%)</td>
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<td>Follicle stimulating hormone (mIU/ml)</td>
<td>3–14 (CF × 1)</td>
<td>4.4 ± 2.1 (1.2–9.3)</td>
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<tr>
<td>Luteinizing hormone (mIU/ml)</td>
<td>3–20 (CF × 1)</td>
<td>8.9 ± 4.6 (1.9–18.5)</td>
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<tr>
<td>Prolactin (ng/ml)</td>
<td>2–20 (CF × 1)</td>
<td>15.2 ± 12.4 (5.2–45.0)</td>
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<tr>
<td>Oestradiol (pg/ml)</td>
<td>10–90 (CF × 3.671)</td>
<td>66.7 ± 25.6 (15–119)</td>
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<tr>
<td>Cortisol (µg/dl)</td>
<td>5–25 (CF × 27.5)</td>
<td>21.8 ± 3.8 (16.6–28.5)</td>
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<tr>
<td>17-OH-Progesterone (ng/ml)</td>
<td>0.3–3.0 (CF × 3.026)</td>
<td>1.63 ± 0.60 (0.75–2.50)</td>
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<tr>
<td>Testosterone (ng/ml)</td>
<td>0.3–0.8 (CF × 3.47)</td>
<td>1.01 ± 0.40 (0.47–2.10)</td>
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<tr>
<td>Androstenedione (ng/ml)</td>
<td>0.4–4.5 (CF × 3.49)</td>
<td>4.5 ± 1.4 (2.2–7.6)</td>
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<tr>
<td>DHEA-S (µg/ml)</td>
<td>0.7–3.9 (CF × 3.49)</td>
<td>3.6 ± 1.8 (1.2–8.3)</td>
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<tr>
<td>Insulin (µIU/ml)</td>
<td>8–25 (CF × 1)</td>
<td>14.6 ± 8.5 (5.7–35.0)</td>
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<td>Glycemia (mg %)</td>
<td>70–115 (CF × 0.056)</td>
<td>90.0 ± 9.7 (70–105)</td>
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<td>Cholesterol (mg %)</td>
<td>120–220 (CF × 0.0259)</td>
<td>174.4 ± 40.0 (93–252)</td>
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<tr>
<td>HDL-Cholesterol (mg %)</td>
<td>30–70 (CF × 0.0259)</td>
<td>50.0 ± 13.3 (25.5–73.0)</td>
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<tr>
<td>Triglycerides (mg %)</td>
<td>40–128 (CF × 0.01g/l)</td>
<td>98.5 ± 50.0 (37–200)</td>
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DHEA-S = dehydroepiandrosterone sulphate; CF = conversion factor; HDL = high density lipoproteins; SD = standard deviation.

³No significant differences in any analysed parameters between the two groups (unpaired t-test).

Table II: Ferriman–Gallwey score and hormonal analysis during and after treatment with pill only (Diane 35) or gonadotrophin-releasing hormone analogue (GnRH-a; Decapeptyl 3.75) plus pill in patients with polycystic ovarian disease-related hyperandrogenism

I. There were no significant differences in any clinical or hormonal parameters between the two groups; however, the patients in the GnRH-a plus EE–CPA pill group were slightly more obese, hirsute and insulin-resistant, with the corresponding hormonal parameters slightly more altered compared with the EE–CPA pill alone group.

Statistical analysis

Statistical analyses were performed using a computer statistical package (R-Sigma; Horus Hardware, 1990). The paired t-test was used to compare the results ‘before’ and ‘during’ treatment, and ‘before’ and ‘after’ treatment. The unpaired t-test for independent variables was used to compare the two treatment groups and their ‘before’, ‘during’ and ‘after’ results. Values in tables are expressed as means ± SD. To compare percentages of subjective improvement, the Mann–Whitney U-test was used. The pretreatment value of each parameter in each studied case was taken to be 100%, and the corresponding percentage was applied to the results during and after treatment, being represented in each figure as the mean of these percentages. Likewise, the unpaired t-test was applied between these ‘during’ and ‘after’ percentages for both treatment groups. Differences were considered to be statistically significant if P < 0.05 but, given that there were few patients in each treatment group, the possibility of a type II error is acknowledged.

Results

From the clinical viewpoint, there were no significant modifications in body weight, although there was a tendency for this to increase during treatment in both groups. The Ferriman–Gallwey scores decreased significantly with both treatments (P < 0.001), and remained low after treatment with no significant differences between the two groups. The subjective indices of improvement (seborrhoea, quality of hair, psycho-
logical status) were better for the GnRH-a plus EE–CPA pill group than for the pill alone group (91.7 versus 58.3% of ‘good’ improvement during treatment), but these differences did not reach statistical significance. None of the patients reported hot flushes, vaginal dryness or other hypo-oestrogenic-related symptoms.

The responses of the Ferriman–Gallwey score and hormonal parameters to both treatment protocols are shown in Table II. FSH, LH, oestradiol, 17-OH-progesterone, testosterone and androstenedione concentrations decreased significantly and cortisol concentrations increased significantly during treatment in both groups. Triglyceride concentrations in the Diane 35
GnRH-a and oral contraceptive pill in PCOD

Figure 2. (A and B) Mean percentages of reduction in the values for the Ferriman–Gallwey score and 17-OH-progesterone (17-OH-P) during treatment (DT) and after treatment (AT) compared with the pretreatment values (BT; 100%) in those patients with a Ferriman–Gallwey score $\geq 12$. (C and D) Mean percentages of reduction in the values for the Ferriman–Gallwey score and dehydroepiandrosterone sulphate (DHEA-S) during treatment (DT) and after treatment (AT) compared with the pretreatment values (BT; 100%) in obese polycystic ovarian disease patients (IBW $\geq 120\%$). (×) Treatment with Diane 35 only. (*) Treatment with gonadotrophin-releasing hormone analogue, Decapeptyl 3.75, plus Diane 35. Values significantly different between the two treatment groups are indicated.

The pill only group also increased significantly during treatment. Differences between the concentrations during treatment in the two groups were significant only for FSH and LH, which were more decreased in the GnRH-a plus pill group. After treatment, differences between parameters and their pretreatment values ceased to be significant, except for the androstenedione which remained significantly decreased in the GnRH-a plus pill group, but with no significant difference between the two treatment groups. There were no significant variations during or after treatment in either group for prolactin, DHEA-S, insulin, glycaemia, cholesterol or HDL-cholesterol.

Figure 1 shows the mean percentages of the values for the studied parameter during and after treatment, with respect to the pretreatment values (defined as 100%), separately for both treatment groups. This enables a graphic representation of the comparative effects of the treatments on the Ferriman–Gallwey score and the hormonal parameters. Although the Ferriman–Gallwey score decreased to a greater extent with GnRH-a plus pill (66.5 versus 72.3%), the differences were not significant. There were significant differences between the two groups only in FSH and LH concentrations during treatment, the percentage of reduction again being higher for those receiving the GnRH-a plus pill. There were no differences in the remaining parameters between the two treatment groups. Differences between determinations at 3 or 6 months were also not statistically significant, although some parameters appeared to be slightly lower at 6 months. However, if only those patients with moderate or severe hirsutism were considered (Ferriman–Gallwey score $\geq 12$; seven patients during treatment and five after treatment in the pill only group; 10 and nine patients respectively in the GnRH-a plus pill group), the decrease in that score was significantly greater (68.0 versus 79.5%) and after treatment (69.0 versus 87.2%) in the GnRH-a plus pill group than in the Diane 35 pill only group (Figure 2A). However, there were no significant differences in the hormonal and biochemical parameters, except for gonadotrophins during treatment and for 17-OH-progesterone which increased after the pill alone treatment (Figure 2B).

Similarly, if only those obese patients with an IBW $\geq 120\%$
were considered (Figure 2C and D; five patients during and after treatment in the pill only group; eight patients during and six after treatment in the GnRH-a plus pill group), the differences in the percentages of the values between the two treatment groups were significant during treatment for the Ferriman–Gallwey score and the FSH, LH and DHEA-S concentrations, with the GnRH-a plus EE–CPA pill being the more effective treatment. After treatment, this protocol remained effective (decreasing testosterone and androstenedione), but the differences were not statistically significant. Interestingly, in these obese patients the androstenedione and DHEA-S concentrations increased slightly during treatment with the EE–CPA pill alone.

Discussion
In accordance with previous studies (Belisle and Love, 1986), a cyclical prolonged treatment (10 months) with OC pills containing EE and CPA (Diane 35) was effective in reducing gonadotrophic and androgen concentrations and improving hirsutism, acne and subjective perceptions (seborrhoea, psychological status and quality of new hair) in PCOD patients. The addition of a long-acting GnRH-a in the form of triptoreline (Decapeptyl 3.75), one i.m. injection every 28 days for eight cycles, improved the clinical results slightly (Ferriman–Gallwey score and subjective perceptions), although the differences were not statistically significant. The only significant difference resulting from the addition of GnRH-a to the OC pill was the greater reduction in gonadotrophins (especially LH) during treatment. After treatment, the Ferriman–Gallwey score and androgen concentrations (testosterone and androstenedione) in the GnRH-a plus pill group remained lower than in the group with OC pill alone, but again the differences were not statistically significant. Carr et al. (1995) and Elkind-Hirsch et al. (1995) observed similar results during treatment using a standard OC pill alone, GnRH-a alone or a combination of the two. However, Ciotta et al. (1996) observed a reduction of hirsutism after the administration of GnRH-a plus EE–CPA pills to severely affected women with PCOD who had been unresponsive to the OC EE–CPA pill alone.

When GnRH agonist depots alone were used in long treatments for hirsutism and PCOD-related hyperandrogenism (Falsetti and Pasinetti, 1994; Goni et al., 1994), they precipitated a menopausal-like status secondary to a profound pituitary gonadotrophin suppression, resulting in a parallel significant fall in gonadotrophin and oestradiol concentrations. This medical castration led, as expected, to the simultaneous inhibition and therapy. The authors wish to thank Dr J.J.Prieto from the Histology Department, School of Medicine, University of Alicante, Spain, for reviewing the English, and Susana Guixot for typing the manuscript.

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