Irregular cycles and steroid hormones in polycystic ovary syndrome

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BACKGROUND: This cross-sectional study was undertaken to evaluate the factors that relate to menstrual status (oligo-amenorrhoea versus eumenorrhoea) in polycystic ovary syndrome (PCOS). METHODS: A total of 234 women with clinical and biochemical features suggestive of PCOS underwent metabolic and hormonal evaluation. A forward stepwise logistic regression model was created based on the results to determine variables related to ovulatory status. RESULTS: Only follicular phase progesterone and estradiol (E₂) were retained in the final model. This model correctly classified 80% of PCOS women by ovulatory status. Univariate analysis revealed no difference in progesterone between ovulatory groups but E₂ was higher in anovulatory groups. This suggested interaction between progesterone and E₂ and the single interaction variable (progesterone/E₂) also classified 80% of women by ovulatory status correctly. CONCLUSION: The results suggest that a low ratio of progesterone to E₂ is associated with menstrual irregularity and ovulatory status in PCOS.

Key words: estradiol/ovulation/polycystic ovaries/progesterone

Introduction

After pubertal maturation in females, the hypothalamus serves as an autonomous pacemaker, with a pulse frequency that is modulated by ovarian signals (Buffet and Bouchard, 2001) and an inherent maximal firing frequency of approximately one pulse per hour in the absence of ovarian restraint (Marshall et al., 1991). Studies have shown that while GnRH regulates follistatin gene expression (Kirk et al., 1994), this is mediated via modulation of GnRH pulse frequency. Rapid-frequency GnRH pulses stimulate follistatin gene expression. Follistatin binds to and inactivates activin and this is then associated with reduced FSHβ mRNA and FSH secretion (Miller et al., 2002). Slowing of the GnRH pulse frequency (less than one pulse per hour) is therefore required for FSH secretion (Mather et al., 2001) and FSH in turn is mandatory for follicular maturation and hence ovulation. Irregular cycles and anovulation in women with polycystic ovary syndrome (PCOS) is characterized by arrested growth of antral follicles, and it has been thought that a relative lack of FSH contributes to the persistence of this problem (Franks, Mason and Willis, 2000; Doi et al., 2005). Since ovarian restraint and thus slowing of GnRH pulse secretion appears to be effected by progesterone acting to enhance hypothalamic opioid activity, it has been postulated (Marshall et al., 1992; Chabbert-Buffeta et al., 2000) that PCOS may ultimately reflect abnormalities of the estradiol–progesterone/opioid/GnRH neuron feedback mechanisms, with failure to establish slowing and the resulting persistent high frequency GnRH stimulus, decreasing FSH with consequent abnormal follicular maturation.

Contrary to this viewpoint, however, are data that suggest that a lack of FSH may not really be the problem in PCOS. Although there is evidence of decreased sensitivity of the GnRH pulse generator in PCOS to the inhibitory feedback action of estradiol (E₂) and progesterone (Pastor et al., 1998), this seems to be mediated by androgens (Eagleson et al., 2000) which are elevated even in ovulatory PCOS. Furthermore, increased androgens are associated with increased GnRH pulse amplitude or high LH rather than pulse frequency or low FSH (Patel et al., 2004) and serum FSH levels in PCOS are not significantly different from those during the early follicular phase of the normal cycle (Fauser et al., 1991). It is possible therefore that rather than failure of hypothalamic restraint, either an inhibitor of FSH receptor signal transduction or a defect in the FSH signalling mechanism exists in PCOS granulosa cells (Erickson et al., 1992). The former seems more likely given that FSH is able to markedly stimulate granulosa cell mitosis and the formation of pre-ovulatory follicles in PCOS ovaries (Schoemaker et al., 1978). We therefore studied the metabolic and clinical differences of PCOS subsets, with and without anovulatory symptoms, in an attempt to further our understanding of
the mechanisms associated with failure of follicular maturation and thus anovulation in this syndrome.

Materials and methods

Patient population

Women with PCOS were recruited from the practices of the authors (S.D.K.K.S.). To qualify as a PCOS index case, a woman had to have a free androgen index (FAI) >5% and hirsutism or oligomenorrhea, after exclusion of other causes of hirsutism and oligomenorrhea. This definition includes those women with regular menstrual cycles, despite the fact that hirsutism with regular menstrual cycles is frequently labelled as idiopathic hirsutism. However, it is now obvious that the majority of these women also have polycystic ovaries by ultrasound and at least one endocrine abnormality to support the diagnosis of polycystic ovary syndrome (Adams et al., 1986; Jahanfar and Eden, 1993). Further studies also reveal the same abnormalities of ovarian steroidogenesis in these women as in classic PCOS, suggesting that mild PCOS is much more common than idiopathic hirsutism (Carmina and Lobo, 1999; Carmina and Lobo, 2001). Indeed, about half of the hyperandrogenic sisters of probands with chronic hyperandrogenic anovulation themselves have ovulatory menstrual cycles (Legro et al., 1998) and so-called idiopathic hirsute women with normoandrogenaemia still have subtle increases in ovarian secretion of 17α-hydroxyprogesterone (17HP) and a minimally increased adrenal (delta 4) 17,20-lyase activity, suggesting that even these patients themselves might represent the mildest forms of PCOS (Escober-Morreale et al., 1997).

We therefore reserve the term idiopathic for those hirsute women with both regular menstrual cycles and normal serum androgens (FAI) despite the presence of hirsutism and this might represent the mildest form of PCOS.

Secondary causes of hirsutism and anovulation, such as non-classical 21-hydroxylase deficiency, hyperprolactinaemia, or androgen-secreting tumours, were excluded by appropriate tests. Confounding medications that affect the metabolic criteria examined were excluded and these include oral contraceptive agents, hypotensive medications, and insulin-sensitizing medications. Other confounding reproductive conditions, including pre-menarche, pregnancy, lactation, hysterectomy or menopause were also excluded. These patients were then divided into two groups based on their menstrual history: those with irregular menstrual cycles (oligomenorrheic; group 1) and those with regular menstrual cycles (group 2). Since nine out of 10 women who have oligo-amenorrhea will be anovulatory (Doldi et al., 1998) and a similar number will have polycystic ovaries (Adams et al., 1986), this symptom serves as a marker of ovulatory status in PCOS women. The reverse is also true to some extent since 80% of anovulatory women have oligo-amenorrhea (Haddad, 1984) while anovulation does not occur in eumenorrheic women (Malcolm and Cumming, 2003).

Study procedures and laboratory methods

All subjects (n = 234) were examined between cycle days 1 and 7 after spontaneous menstruation or after prolonged amenorrhea. A regular menstrual cycle was defined as a cycle with an inter-menstrual interval of 21–35 days and the variation of cycle length from one period to another was ≤7 days. A cycle was considered oligomenorrhoeic if the intermenstrual interval was ≥36 days (less than nine cycles per year) and amenorrhoeic if the intermenstrual interval was >6 months. Hirsutism was graded using a modification of the Ferriman and Gallway scoring system (Ferriman and Gallwey, 1961) based on a study by Derksen et al. (1993). A woman was considered hirsute if the score was ≥4 counted from only five (instead of nine) hormone-sensitive areas (i.e. lip, chin, chest, upper abdomen and lower abdomen). Written informed consent was obtained from all subjects for use of their data and this study was approved by the local ethics committee at both our institutions.

Computations

Body mass index (BMI) was calculated as the ratio of weight (kg)/height (m)². Obesity was defined by the conventional threshold of 30 kg/m², since with BMI < 30 kg/m², there is a greater incidence of inappropriate gonadotrophin secretion (Arroyo et al., 1997) and less hyperinsulinaemia (since insulin sensitivity decreases significantly in humans [without PCOS] >27 kg/m²² (Campbell and Gerich, 1990)). The homeostasis model assessment (HOMA) was applied for insulin sensitivity analysis. This has been widely used in clinical research to assess insulin sensitivity (Radziuk, 2000; Mather et al., 2001). It is a structural computer model of the glucose-insulin feedback system in the homeostatic (overnight-fasted) state. The model consists of a number of non-linear empirical equations describing the functions of organs and tissues involved in glucose regulation and can be calculated from a computer program (Levy et al., 1998) available freely from the Diabetes Trials Unit of the University of Oxford. These are solved numerically to predict glucose, insulin and C-peptide concentrations in the fasting steady state for any combination of pancreatic β-cell function and insulin sensitivity (or resistance). These predictions allow the deduction of β-cell function (HOMA%B) and insulin sensitivity (HOMA%S; normal = 100%) from pairs of fasting glucose and insulin (or C-peptide) measurements. Unlike fasting insulin (FI) and the insulin/glucose ratio (IGR), the HOMA calculation compensates for fasting hyperglycaemia (Quon, 2001). The HOMA value correlates well with clamp techniques and has been frequently used to assess changes in insulin sensitivity after treatment (Mather et al., 2000). FAI was calculated as total testosterone (nmol/l) divided by SHBG (nmol/l) × 100.

Assays

All hormone assays had been determined from a single sample after an 8 h overnight fast. This sample was collected between 08:00 and 11:00 in the first week of the cycle or after ≥1 month amenorrhea. Serum steroid hormone levels were determined as follows (upper or lower limit for healthy follicular phase women given by <or> respectively in parenthesis): Serum LH (<6.2 IU/l) and FSH (<8.8 IU/l) were measured by coated tube immunoradiometric assay (IRMA) from Diagnostic Products Corp. (CA, USA). Sex hormone-binding globulin (SHBG, >20 nmol/l) was measured by a non-competitive ‘liquid phase’ IRMA from Orion Diagnostica (Espoo, Finland). Prolactin (<406 mIU/l) was measured by coated tube IRMA (DiaSorin, s.r.l., Saluggia, Italy). Androstenedione (<9.2 nmol/l) was measured by radioimmunoassay (Diagnostic Systems Laboratories Inc., Texas, USA). Serum testosterone (<3 nmol/l) and estradiol (E₂, <285 pmol/l) were measured by coated tube radioimmunoassay (Orion Diagnostica). Serum dehydroepiandrosterone sulphate (DHEA-S, <10.3 μmol/l), 17HP (<7.9 nmol/l), progesterone (<4.5 nmol/l) and fasting insulin (FI, <22 mIU/l) levels were measured by a coated tube radioimmunoassay from Diagnostic Products Corp. Serum insulin-like growth factor 1 (IGF-1) levels were measured using a two-site IRMA from Diagnostic Systems Laboratories, Inc. The inter- and intra-assay variations of these assays were <10 and <5% respectively. All assays were highly specific with <1.4% cross-reactivity to structurally related hormones. None of the hormone assays included an extraction step to separate it from its binding protein in serum.
### Statistical analysis

Exploratory analyses were conducted by use of binomial logistic regression analysis. Biochemical variables were used to identify factors likely to predict binary group membership of the women with PCOS. All variables were continuous. The analysis was carried out initially in a multivariate fashion by inserting one variable in the model and then using forward stepwise regression to obtain a final model. The Wald statistic was used to test the significance of individual logistic regression coefficients for each independent variable (that is, to test the null hypothesis in the logistic regression that a particular logit (effect) coefficient is zero). An overall test of model adequacy was obtained by the Hosmer and Lemeshow goodness-of-fit test, with an adequately fitted model being indicated by a non-significant $\chi^2$-value. Odds ratios (OR) and 95% confidence intervals (95% CI) for all individual variables in the final model were estimated. Continuous variables were also summarized as median and interquartile range (IQR) and a two-sample Wilcoxon and rank-sum (Mann–Whitney $U$) test was used to determine the differences in all steroid values, fasting insulin and gonadotrophin parameters between groups. The $\chi^2$-statistic was used to determine differences between groups for categorical variables. All multivariate analyses were performed using SPSS version 12 (SPSS Inc., Chicago, Illinois, USA) and univariate analyses were done using Epi-info version 6 (CDC, Atlanta, Georgia, USA).

### Results

#### Clinical parameters in each group

All women were hyperandrogenic (FAI $\geq 5\%$) based on our inclusion criteria; of these, most were hirsute (90%, $n = 211$). Just over half (58%, $n = 136$) were oligo-amenorrheic while the rest had regular cycles. Two-thirds of the women were aged $< 27$ years with the median age of the women in group 1 being 21 years (IQR 18–27) versus 20 years (IQR 18–25) in group 2 (not significant). The BMI of these PCOS women was high (median 30.5 kg/m$^2$ (IQR 26.6–36.8) and 29.2 kg/m$^2$ (IQR 25.2–33.6) in groups 1 and 2 respectively; not significant), but ranged from normal to high. A total of 194 women had data on acanthosis nigricans (10/75) of PCOS women in group 2 had acanthosis nigricans, and 28% (33/119) of PCOS women in group 1 and 13% (26/195) of PCOS women in group 2 had acanthosis nigricans in the posterior neck ($\chi^2$: $P < 0.01$). The hirsutism score was 7 (IQR 4–10) in group 1 and 10 (IQR 6–13) in group 2 ($P < 0.01$).

#### Gonadotrophins

The median LH was 6.6 IU/l (IQR 3.3–9.5) in group 1 and 2.7 IU/l (IQR 1.9–5.5) in group 2 ($P < 0.001$). Median FSH levels were the same in both groups (5.5 IU/l, IQR 4.5–6.9; not significant).

#### Fasting glucose and insulin levels and homeostasis model assessment

The fasting glucose level was not different in the two groups [median 5.3 mmol/l (IQR 5.1–5.7) in group 1 versus 5.2 (IQR 4.9–5.6) in group 2]. The fasting insulin level in group 1 was higher than that in group 2 (Table I). Although there was no difference in BMI in group 1 compared to group 2, the median HOMA%S in group 1 was significantly lower than that in group 2 (Table I).

#### Follicular phase hormone profile versus group status

DHEA-S was significantly lower whereas FAI and E$_2$ were significantly higher in group 1 as opposed to group 2 women (Table Ia). There were no differences in prolactin, 17HP, androstenedione, testosterone or IGF-I. To assess the influence of obesity on between-group E$_2$ and progesterone differences, they were stratified by BMI. This revealed a non-significant trend towards a higher E$_2$ and lower progesterone in group 1 compared to group 2 women (Table Ib). However, progesterone relative to E$_2$ was significantly higher in both lean as compared to obese women as well as group 2 compared to group 1 women (Table Ib).

Six steroid hormones (E$_2$, DHEA-S, testosterone, androstenedione, progesterone and 17HP) BMI and HOMA%S were then entered as independent variables into a forward stepwise logistic regression analysis versus group status. Of the three variables selected by univariate analysis, only E$_2$ and progesterone were retained in the final step (Table II). The interaction variable progesterone/E$_2$ was created since univariate analysis did not reveal a difference in progesterone between groups (Table Ia). This interaction variable was.

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**Table Ia.** Median and interquartile ranges (IQR) for variables in regular and irregularly cycling women in the basal state

<table>
<thead>
<tr>
<th>Steroid hormones</th>
<th>Group 1</th>
<th>Group 2</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valid $n$</td>
<td>Median (IQR)</td>
<td>Valid $n$</td>
</tr>
<tr>
<td>DHEA-S $\mu$mol/l</td>
<td>132</td>
<td>6.9 (4.8–9)</td>
<td>91</td>
</tr>
<tr>
<td>FAI %</td>
<td>136</td>
<td>13.97 (9.8–20.9)</td>
<td>98</td>
</tr>
<tr>
<td>E$_2$ $\mu$mol/l</td>
<td>119</td>
<td>144 (102–181)</td>
<td>93</td>
</tr>
<tr>
<td>Progesterone nmol/l</td>
<td>47</td>
<td>4.60 (3.8–5.5)</td>
<td>36</td>
</tr>
<tr>
<td>Testosterone nmol/l</td>
<td>136</td>
<td>2.7 (2.1–3.4)</td>
<td>98</td>
</tr>
<tr>
<td>Androstenedione nmol/l</td>
<td>131</td>
<td>11.1 (8.1–14.3)</td>
<td>93</td>
</tr>
<tr>
<td>Progesterone/E$_2$ (nmol/nmol)</td>
<td>46</td>
<td>27.7 (22.4–36.7)</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glucose- and insulin-related variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valid $n$</td>
<td>Median (IQR)</td>
<td>Valid $n$</td>
</tr>
<tr>
<td>HOMA%S</td>
<td>74</td>
<td>37.5 (23.2–67.9)</td>
<td>48</td>
</tr>
<tr>
<td>FI mIU/l</td>
<td>80</td>
<td>16.7 (9.9–28.5)</td>
<td>56</td>
</tr>
</tbody>
</table>

There were no significant differences in age, body mass index, prolactin, 17-hydroxyprogesterone or insulin-like growth factor-I. ULN = upper limit of normal; DHEA-S = dehydroepiandrosterone sulphate; FAI = free androgen index; E$_2$ = estradiol; HOMA%S = homeostatic model assessment, sensitivity; FI = fasting insulin; NS = non-significant.
the only variable associated with group differences in a further logistic regression (not shown). When the regression model was used to predict group membership of the 82 women for whom both E2 and progesterone data were available (threshold $P = 0.5$), >80% of them were correctly classified with more correct allocations in group 1 compared to group 2 (Table Ib).

When two subsets were made of the women (Table Ib) by BMI levels, it was apparent that obese women had a lower progesterone/E2 ratio than lean women. However, even obese women had the same differential between groups 1 and 2 as lean women, adding to the robustness of these results.

### Discussion

In this study we evaluated the hormone and metabolic profile of 234 women with PCOS in order to determine variables that might relate to their ovulatory symptoms. An exploratory logistic regression model retained only E2 and progesterone in the model after forward stepwise selection and FSH was not retained. This suggests that changes in the follicular phase serum E2 and progesterone relate to menstrual status independently of FSH levels. Since FSH is mandatory for follicular maturation but was similar in both groups and since failure of follicular development beyond the antral stage is overcome by exogenous FSH (Doi et al., 2005), there is the possibility that changes in follicular phase levels of E2 and progesterone reflect changes in FSH receptor signal transduction in PCOS. Group 1 included both those with increased and normal LH levels, unlike group 2 whose LH levels were consistently normal, in accordance with the results of others (Adams et al., 2004). However, normal LH did not predict menstrual status and thus cycle regularity seemed to be unrelated to changes in LH. We also found no independent relationship between LH or androgens with menstrual status in this study.

Our results suggest that it is not absolute E2 values, but rather a higher E2 relative to progesterone, that is associated with failure of follicular maturation as manifested by irregular menstrual cycles. Progesterone was not significantly different in either group of PCOS women for a univariate analysis (Table Ia), suggesting that it is indeed an interaction between both steroids rather than individual levels that is associated with irregular cycles and thus anovulation. Only the single interaction variable (progesterone/E2) correctly classified 80% of women by menstrual status in a further logistic regression model. This ratio might then reflect follicular development in the follicular phase of the cycle.

This line of reasoning is supported by studies showing that E2 itself is not of importance to human folliculogenesis (Palter et al., 2001) and may even lead to anovulation in primate studies (Richardson et al., 1992), whereas follicular development and ovulation are facilitated by progesterone (Batista et al., 1992; Graham and Clarke, 1997). Nevertheless, there are also several reports stressing that progesterone adversely affects oocyte maturation and fertilization, and is harmful to endometrial receptivity, and a high progesterone/E2 ratio, termed premature luteinization, has

### Table Ib. Median and interquartile ranges (IQR) for estradiol (E2) and progesterone levels stratified by body mass index (BMI)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obese</td>
<td>Lean</td>
<td>$P$</td>
<td>Obese</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Median (IQR)</td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>$E_2$ (pmol/l)</td>
<td>57</td>
<td>155 (111–194)</td>
<td>NS</td>
<td>39</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>20</td>
<td>4.4 (3.8–5.1)</td>
<td>NS</td>
<td>14</td>
</tr>
<tr>
<td>Progesterone/E2 ratio</td>
<td>19</td>
<td>23.9a (21.6–29.1)</td>
<td>&lt;0.05</td>
<td>14</td>
</tr>
</tbody>
</table>

Lean are BMI <30 kg/m² and obese are BMI ≥30 kg/m².

### Table IIa. Results of logistic regression using variables selected at the final step of a forward stepwise logistic regression run

<table>
<thead>
<tr>
<th></th>
<th>Significance level for each variable</th>
<th>Exp(B)</th>
<th>95.0% CI for Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td>−0.028</td>
<td>0.000</td>
<td>0.973</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.407</td>
<td>0.008</td>
<td>1.502</td>
</tr>
<tr>
<td>Constant</td>
<td>1.413</td>
<td>0.200</td>
<td>4.109</td>
</tr>
</tbody>
</table>

Variables in the initial model are given in the Results section. Variables with $P<0.05$ were included in the initial model in a stepwise manner, starting with the variable with the lowest $P$-value. A standard logistic regression was then run on the selected variables.

$^a$Negative B indicates that eumenorrhoea was lower in those with an increased steroid hormone value; positive B indicates that eumenorrhoea was higher in those with an increased value. B = logistic regression coefficient and EXP(B) is the odds ratio. Hosmer and Lemeshow goodness-of-fit test: $X^2 = 9.3$ (df = 8; $P = 0.32$).

### Table IIb. Classification table for menstrual group by binomial logistic regression model with two variables (estradiol, progesterone)

<table>
<thead>
<tr>
<th></th>
<th>Predicted:</th>
<th>Percentage correct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Observed:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>Group 2</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td>Overall percentage</td>
<td>80.5</td>
<td></td>
</tr>
</tbody>
</table>

The threshold value is 0.50.
been thought to adversely affect reproduction. This, however, is probably a result of a negative impact on endometrial receptivity rather than on oocyte or embryo quality (Shulman et al., 1996). Indeed successful pregnancy does occur with high basal levels of progesterone (Furuhashi et al., 2002), and premature luteinization—as based on elevated serum progesterone concentration—has actually been shown to reflect healthy follicular development with increased pregnancy rates in oocyte donors (Legro et al., 1993). Progesterone may therefore act to offset a negative effect of E2 so that a low ratio of progesterone to E2 might reflect E2 interference with folliculogenesis.

In the follicular phase of the human menstrual cycle, serum progesterone is mainly of adrenal origin (Judd et al., 1992; De Geyter et al., 2002) albeit under ovarian regulation since ovarian suppression with ethinyl estradiol decreases adrenal progesterone production (De Geyter et al., 2002). In addition, in a comparison of cultured human theca cells from PCOS and normal women, it has also been demonstrated that there is increased conversion of ovarian progesterone to androstenedione (Gilling-Smith et al., 1994). If this conversion also occurs within the adrenals, then a common ovarian factor that drives this conversion could account for the relative progesterone deficiency in PCOS. Further in vitro studies have demonstrated that in theca cells from women with PCOS, the capacity to secrete progesterone when challenged with a maximally effective dose of FSH and/or IGF-I was markedly reduced (8–10-fold) compared to normal granulosa cells (Erickson et al., 1992). This is in sharp contrast to the E2 responses which were much the same for polycystic ovaries and normal granulosa cells (Erickson et al., 1992). Furthermore, human granulosa luteal cells from PCOS ovaries, when incubated with follicular fluid from PCOS patients, showed a lower increase of progesterone production with respect to normal ovaries (Andreani et al., 1996), reflecting an abnormally decreased capacity to synthesize progesterone in vivo and in vitro (Doldi et al., 1998). This suggests that in PCOS women an ovarian factor might exist that stimulates conversion of progesterone to androstenedione rather than impairing progesterone production.

Adrenal progesterone secretion, however, is not mandatory for follicular development since, pre-menopausal women with adrenal insufficiency may have undetectable serum levels of adrenal steroids but normal cycles (Gebre-Medhin et al., 2000), and this implies that progesterone is probably required only to offset the negative E2 effect. This sort of effect has been documented on in vitro maturation and subsequent IVF of pig oocytes where E2 inhibits both nuclear and cytoplasmic maturation which can be prevented by progesterone (Li et al., 2004).

In our study, oligo-amenorrhoeic women who were obese had higher levels of circulating E2 relative to progesterone than lean women in each group (Table Ib), whereas absolute progesterone and E2 levels were similar, suggesting that increasing BMI drives a coordinated increase in E2 production [possibly via peripheral E2 production (Nelson and Bulun, 2001)] with inhibition of adrenal progesterone production. The latter might be mediated via insulin resistance and it has been shown that anti-diabetic agents that improve insulin sensitivity and lower blood insulin levels are also beneficial in improving ovulatory dysfunction in PCOS patients (Sattar et al., 1998; Loverro et al., 2002; Seli and Duleba, 2002), this effect being independent of weight loss (Ehrmann et al., 1997; Hasegawa et al., 1999). Even though most studies have demonstrated no significant change in E2 levels after metformin (Ibanez et al., 2001; Ibanez et al., 2002), both metformin and hypocaloric dieting (Pasquali et al., 2000) have been associated with increases in progesterone levels (albeit non-statistically significant due to wide variability in the later study). It is possible that metformin treatment results in ovulation by both coordinate increasing progesterone and decreasing aromatase activity (la Marca et al., 2002), thus bringing back the progesterone/E2 ratio into a beneficial range. Indeed, metformin treatment significantly lowers adrenal 17α-hydroxylase activity, and adrenal 17,20-lyase activity, suggesting a mechanism for increased progesterone relative to E2 (la Marca et al., 1999). A positive effect on the progesterone/E2 ratio for various other treatments can also be deduced. For example, aromatase inhibitors have a similar effect with no apparent adverse effect on endometrial thickness or pattern at midcycle (Mitwally and Casper, 2001, Mitwally and Casper, 2002; Fisher et al., 2002). Also, inhibition of CYP17 activity with ketoconazole has been shown to improve clomiphene responsiveness in PCOS patients (Ali Hassan et al., 2001).

Again, this implies that an increase in progesterone might be present in these patients, as inhibition of CYP17 is expected to increase progesterone and decrease E2 production.

These findings may explain why anovulatory women who are lean (and have mainly ovarian E2) do well with ovarian E2-reducing therapies such as ovarian cauterization (Greenblatt and Casper, 1987; Gjonnaess, 1994; Duleba et al., 2003; Amer et al., 2004) while obese anovulatory women (with peripheral E2 production) usually fare better with FSH-increasing therapies via gonadotrophins (Vicino et al., 2000) or clomiphene (Lopez-Lopez et al., 1987; Ficicioglu et al., 1996). A recent study (van Wely et al., 2005) has pointed out that a high LH/FSH ratio is the single most important predictor of ovulation after ovarian cauterization, and we have shown that a high LH/FSH ratio is much more likely to occur in PCOS in lean women with increased ovarian E2 (unpublished data). Others have also shown that increased basal serum levels of androstenedione predicts ovulation after ovarian cauterization (Vicino et al., 2000), and this again can be related to an effect of E2 since we have again shown that increased androstenedione is associated with both increased E2 and increased LH in PCOS (unpublished data). This suggests that the mechanism of effect of ovarian cauterization on ovulation might indeed be a reduction in ovarian E2. On the other hand, while obese anovulatory women respond better to clomiphene, this effect is not associated with increased progesterone (Judd et al., 1992) but rather with increased FSH that might override the effect of E2 in these women.

In conclusion, and contrary to the conventional idea (Doi et al., 2005) that menstrual irregularity and possibly
anovulation in PCOS is determined by relatively low FSH, our results suggest that this is more likely to reflect a loss of the progesterone modulation of the E2 effect on the ovaries leading to inhibition of FSH receptor signal transduction. This model should be validated on another set of cases to reach full statistical credibility and, if confirmed, may open up new avenues for the treatment of infertility in PCOS.

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