

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 FSH β 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.S.). To qualify as a PCOS index case, a woman had to have a free androgen index (FAI) $>5\%$ and hirsutism or oligomenorrhoea, after exclusion of other causes of hirsutism and oligomenorrhoea. 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 (Escobar-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, hypertensive 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 (oligo-amenorrhoeic; group 1) and those with regular menstrual cycles (group 2). Since nine out of 10 women who have oligo-amenorrhoea 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-amenorrhoea (Haddad, 1984) while anovulation does not occur in eumenorrhoeic 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 amenorrhoea. A regular menstrual cycle was defined as a cycle with an intermenstrual 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 Gallwey 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/m^2 , since with $\text{BMI} < 30 \text{ kg/m}^2$, 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] $>27 \text{ kg/m}^2$ (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) $\times 100$.

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 amenorrhoea. 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 ($<6.2 \text{ IU/l}$) and FSH ($<8.8 \text{ IU/l}$) were measured by coated tube immunoradiometric assay (IRMA) from Diagnostic Products Corp. (CA, USA). Sex hormone-binding globulin (SHBG, $>20 \text{ nmol/l}$) was measured by a non-competitive 'liquid phase' IRMA from Orion Diagnostica (Espoo, Finland). Prolactin ($<406 \text{ mIU/l}$) was measured by coated tube IRMA (DiaSorin, s.r.l., Saluggia, Italy). Androstenedione ($<9.2 \text{ nmol/l}$) was measured by radioimmunoassay (Diagnostic Systems Laboratories Inc., Texas, USA). Serum testosterone ($<3 \text{ nmol/l}$) and estradiol (E_2 , $<285 \text{ pmol/l}$) were measured by coated tube radioimmunoassay (Orion Diagnostica). Serum dehydroepiandrosterone sulphate (DHEA-S, $<10.3 \mu\text{mol/l}$), 17HP ($<7.9 \text{ nmol/l}$), progesterone ($<4.5 \text{ nmol/l}$) and fasting insulin (FI, $<22 \text{ mIU/l}$) levels were measured by a coated tube radioimmunoassay from Diagnostic Products Corp. Serum insulin-like growth factor I (IGF-I) 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 χ^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 χ^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-amenorrhoeic 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/m² (IQR 26.6–36.8) and 29.2 kg/m² (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 and 28% (33/119) of PCOS women in group 1 and 13% (10/75) of PCOS women in group 2 had acanthosis nigricans in the posterior neck (χ^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 E₂ 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 E₂ and progesterone differences, they were stratified by BMI. This revealed a non-significant trend towards a higher E₂ and lower progesterone in group 1 compared to group 2 women (Table Ib). However, progesterone relative to E₂ 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 (E₂, 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 E₂ and progesterone were retained in the final step (Table II). The interaction variable progesterone/E₂ was created since univariate analysis did not reveal a difference in progesterone between groups (Table Ia). This interaction variable was

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

	Group 1		Group 2		<i>P</i>	Percentage above ULN Group 1/group 2
	Valid <i>n</i>	Median (IQR)	Valid <i>n</i>	Median (IQR)		
Steroid hormones						
DHEA-S $\mu\text{mol/l}$	132	6.9 (4.8–9)	91	8 (5.9–10.2)	< 0.05	18/22
FAI %	136	13.97 (9.8–20.9)	98	12.1 (7.3–17)	< 0.01	100/100
E ₂ pmol/l	119	144 (102–181)	93	110 (80–152)	< 0.001	3/4
Progesterone nmol/l	47	4.60 (3.8–5.5)	36	5.2 (4.1–7.3)	NS	51/64
Testosterone nmol/l	136	2.7 (2.1–3.4)	98	2.5 (1.9–3.1)	NS	37/34
Androstenedione nmol/l	131	11.1 (8.1–14.3)	93	10.2 (7.7–13.6)	NS	70/66
Progesterone/E ₂ (nmol/nmol)	46	27.7 (22.4–36.7)	36	48 (38.3–80.6)	< 0.0001	A ratio of 38 seems the optimal threshold
Glucose- and insulin-related variables						
HOMA%S	74	37.5 (23.2–67.9)	48	54.5 (38.1–76)	< 0.05	All $< 100\%$ except 1
FI mIU/l	80	17.6 (9.9–28.5)	56	13.4 (9.2–17.6)	< 0.05	41/21

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; E₂ = estradiol; HOMA%S = homeostatic model assessment, sensitivity; FI = fasting insulin; NS = non-significant.

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

	Group 1					Group 2				
	Obese		Lean		P	Obese		Lean		P
	n	Median (IQR)	n	Median (IQR)		n	Median (IQR)	n	Median (IQR)	
E ₂ (pmol/l)	57	155 (111–194)	54	140 (88–167)	NS	39	115 (84–152)	45	104 (80–152)	NS
Progesterone (nmol/l)	20	4.4 (3.8–5.1)	25	4.8 (3.8–5.7)	NS	14	4.4 (3.7–5.2)	19	6.7 (4.3–8)	NS
Progesterone/E ₂ ratio	19	23.9 ^a (21.6–29.1)	25	31.1 ^b (25.2–37.1)	<0.05	14	39.7 ^a (27.6–48)	19	65.8 ^b (45–96)	<0.05

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

^aP = 0.001;

^bP < 0.001.

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 IIb).

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 in 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

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

	B ^a	Significance level for each variable	Exp(B)	95.0% CI for Exp(B)	
				Lower	Upper
Estradiol	- 0.028	0.000	0.973	0.959	0.986
Progesterone	0.407	0.008	1.502	1.111	2.031
Constant	1.413	0.200	4.109		

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.

^aNegative 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: $\chi^2 = 9.3$ (df = 8; P = 0.32).

CI = confidence interval.

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

	Predicted:		Percentage correct
	Group 1	Group 2	
Observed:			
Group 1	38	8	82.6
Group 2	8	28	77.8
Overall percentage			80.5

The threshold value is 0.50.

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

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 coordinately 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 17 α -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; Ficiocioglu *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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