Increased peripheral androgen activity in infertile Korean women with polycystic ovaries

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The present investigation was designed to determine whether infertile women with polycystic ovaries (PCO) have sonographic or endocrinological differences compared with women with PCO proven to be fertile. Sonographic morphology of the ovary was not significantly different between the groups. However, serum concentrations of \( 3\alpha \)-androstenediol glucuronide (\( 3\alpha\)-dioxG) were significantly higher in infertile women with PCO than in those proven to be fertile. Furthermore, a significant positive correlation was noted between \( 3\alpha\)-dioxG and luteinizing hormone (LH) in infertile women, but not in those who were fertile. The higher correlation between serum \( 3\alpha\)-dioxG concentrations and serum LH concentrations seen in infertile women with PCO, with the lack of a significant difference in LH concentrations between infertile and fertile women, suggests that serum \( 3\alpha\)-dioxG may be a leading cause of subfertility in women with PCO. Accordingly, our evidence for increased \( 3\alpha\)-dioxG in the infertile group needs to be confirmed by further studies, including direct \( 5\alpha\)-reductase assay, adrenal function tests and evaluation of hepatic conjugation activity.

Key words: \( 3\alpha\)-dioxG/gonadotrophin/infertility/polycystic ovary/sonography

Introduction

Infertility occurs in association with various disorders. Polycystic ovaries (PCO) may be a factor in infertility or recurrent miscarriage, as was first suggested by Sagle et al. (1988) who found ultrasonic evidence of PCO in 46 out of 56 women (82%) with recurrent miscarriage (Sagle et al., 1988). However, the typical features seen on ovarian ultrasonography (increased numbers of follicles and stromal hyperplasia) have also been reported in 20% of normal women (Polson et al., 1988). The presence of PCO does not, therefore, necessarily indicate the full-blown clinical syndrome of polycystic ovarian disease (Conway et al., 1985). It has been suggested recently that high concentrations of luteinizing hormone (LH) during the follicular phase may impair fertilization (Stanger and Yovich, 1985; Howles et al., 1986) by prematurely activating oocyte maturation factor (Jacobs, 1987). In addition, high concentrations of androgen in the follicular phase could impair intrafollicular control mechanisms (Yen, 1980). In none of these reported cases, however, has it been possible to determine unequivocally which circulating hormones cause infertility in PCO. Some patients with PCO were infertile, but others were not. Defining the causes of infertility in such cases might, therefore, be valuable for their management. The aim of this study was to investigate the hypothesis that infertility in women with PCO may be caused by subtle follicular phase abnormalities, including excess secretion of LH or androgen.

Materials and methods

Study subjects

The study group comprised 86 women with PCO, none of whom had thyroid or prolactin abnormalities or other systemic medical diseases. They were referred for infertility, menstrual disturbances (oligomenorrhea or amenorrhea), hirsutism, or abnormal uterine bleeding. The criteria for diagnosing PCO with vaginal ultrasound were those of Adams et al. (1985), namely the presence of multiple cysts (>10) 2–8 mm in diameter arranged peripherally around a dense core of stroma (Adams et al., 1985). Patients were classified according to whether or not they had a history of infertility. The numbers of subjects in each group were as follows: 52 women with PCO proven to be fertile and 34 with PCO who were infertile. Among the latter, infertility was not explicable by other apparent factors and they had tried to conceive over the past 2 years. Among this group of 34, 15 had never been pregnant (primary infertility), whereas eight had suffered miscarriages after giving birth to one to three children, and the remaining 11 women had failed to achieve pregnancy again after giving birth to one to three children (secondary infertility). Control data were obtained from 12 normal women with regular menses, no hirsutism, normal body mass index (BMI) (range, 17.3–23.4 kg/m2; mean, 19.8), and normal ovarian morphology. They had previously delivered one to four children and were comparable with the study group with respect to age and BMI. The clinical data for these women are shown in Table I. The mean (SD) length of infertility was 4 (0.2) years. The mean (SD) age of the control group was 29.9 (6.7) years. A ‘regular cycle’ was defined as a cycle length of 22-36 days, with ovulatory luteal phase progesterone concentrations >30 nmol/l, and usually a biphasic basal body temperature (BBT) for at least four ‘normal’ cycles during the previous 6 months. Regular menses were reported by 16 of these 86 women with PCO. Functional oligomenorrhea was defined by exclusion of readily identifiable causes such as primary hypothalamic, pituitary or ovarian failure, ovarian and adrenal androgen-secreting tumours, or hyperprolactinaemia, thyroid or other extra-ovarian endocrine disorders, uterine abnormalities or pregnancy. Hair growth was assessed clinically using an adaptation of an established scoring system (Ferriman and Gallwey, 1961); hirsutism was defined as a Ferriman and Gallwey score of >7.
Sonographic examination
Ultrasound examinations were all performed by the same investigator (Dr J.B. Yoo), using an Acuson scanner (Acuson 128XP®, Acuson, Mountain View, CA, USA) with a 5 MHz vaginal endoprobe on cycle day 2, 3, 4, or 5. The number of follicles was established by scanning each ovary from the inner to the outer margin in longitudinal cross-sections (Robinson et al., 1992). Ovarian volume was estimated according to the following formula: 0.52 × D1 × D2 × D3 (Orsini et al., 1985), where D1 is the longitudinal diameter, D2 the anteroposterior diameter, and D3 the transverse diameter of the ovary. Small follicles were counted and their distribution pattern was examined. Mean follicle numbers and ovarian volume were calculated as the sum of left and right divided by two.

Hormonal assays
We analysed serum samples from 86 women with PCO and 12 controls. Each woman was studied during the early follicular phase of her menstrual cycle (2–5 days after the onset of a spontaneous or induced cycle). At 0800 h, after an overnight fast, a baseline blood sample was drawn for separating serum from all blood samples and frozen at −20°C until analysed. To avoid interassay variation, all samples for a given patient were analysed in duplicate in the same assay. For all serum samples, the following measurements were obtained: follicle stimulating hormone (FSH), LH, testosterone (Diagnostic Products Corporation), 17β-estradiol (Diagnostic Systems Laboratories Inc., Webster, TX, USA), androstenedione (Diagnostic Systems Laboratories Inc., Webster, TX, USA; DHEAS: ICN Biomedicals, Costa Mesa, CA, USA). Intra- and interassay coefficients of variation were 6.4–10% for testosterone, 4.3–7.6% for androstenedione and 9.0 and 9.5% for DHEAS respectively. Serum 3α-diolG was measured using a radioimmunoassay kit supplied by Diagnostic Systems Laboratories, using a previously published method (Horton et al., 1984); intra- and interassay coefficients of variation were 7.5 and 6.7% respectively.

Separate hormonal assays were applied to the primary and secondary infertility groups within the PCO infertile group. No significant difference was found and so they were therefore treated as a single group for the purposes of this study.

Statistical analysis
Each result is expressed as mean and SD. All statistical analyses were performed using SAS for Windows V 6.12; P < 0.05 was considered significant. Student’s two-tailed unpaired t-test or the Mann–Whitney rank sum test was used to demonstrate whether any differences in sonographic findings existed between the two groups with PCO. The χ² test was used to compare percentages. Analysis of variance (ANOVA) was used to demonstrate whether any differences in endocrine parameters existed between the three groups and then Duncan’s procedure, which is a multiple comparison technique, was used to specify significant differences between each group. Correlation analysis was performed using Pearson’s correlation coefficient.

Results
On the basis of prior obstetric history, women with PCO were classified into two groups: (i) PCO and infertility for at least the previous two years; and (ii) PCO and proven fertility (>1 live birth) during the past 3 years. Of the 86 women studied, 52 were proven to be fertile, and 34 were infertile. There was no significant difference in age, cycle regularity or BMI between the groups (Table I). Among the infertile women, 25 (74%) had oligomenorrhoea, while 45 (87%) of fertile women suffered from this. Of 34 infertile women with PCO, only one was hirsute, while three women proven to be fertile had abnormal hair growth (not significant) (Table I). Sonographic features (mean ovarian volume, mean number of follicles, follicle distribution pattern, bilaterality) were evaluated in the two subgroups with PCO (Table II). Mean ovarian volume

Table I. Clinical features of women with PCO, grouped according to whether they were infertile or fertile

<table>
<thead>
<tr>
<th></th>
<th>Infertile (n = 34)</th>
<th>Fertile (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)†</td>
<td>29.2 ± 3.4</td>
<td>27.2 ± 5.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)†</td>
<td>22.4 ± 3.2</td>
<td>23.3 ± 5.4</td>
</tr>
<tr>
<td>Menstrual cycle (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>regular</td>
<td>9 (26)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>irregular/oligo*b</td>
<td>21 (62)</td>
<td>34 (65)</td>
</tr>
<tr>
<td>amenorrhoea</td>
<td>4 (12)</td>
<td>11 (21)</td>
</tr>
<tr>
<td>DUB* (n)</td>
<td>2 (5.9)</td>
<td>4 (7.7)</td>
</tr>
<tr>
<td>Hirsutism (n)</td>
<td>1 (2.9)</td>
<td>3 (5.8)</td>
</tr>
<tr>
<td>Infertility (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary</td>
<td>15 (44)</td>
<td></td>
</tr>
<tr>
<td>secondary</td>
<td>19 (56)</td>
<td></td>
</tr>
</tbody>
</table>

*Results are given as mean ± standard deviation.
†Oligomenorrhoea.
‡Dysfunctional uterine bleeding.

There were no significant differences between the groups. Values in parentheses are percentages.

PCO = polycystic ovaries.

Infertile women with polycystic ovaries

Table II. Comparison of sonographic features of women with PCO, grouped according to whether they were infertile or fertile

<table>
<thead>
<tr>
<th></th>
<th>Infertile (n = 34)</th>
<th>Fertile (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ovarian volume (ml) ab</td>
<td>9.9 ± 3.8</td>
<td>10.9 ± 5.8</td>
</tr>
<tr>
<td>Mean number of follicles a</td>
<td>15.7 ± 9.8</td>
<td>16.4 ± 9.6</td>
</tr>
<tr>
<td>Follicle distribution (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C + P</td>
<td>6 (18)</td>
<td>9 (17)</td>
</tr>
<tr>
<td>P</td>
<td>28 (82)</td>
<td>43 (83)</td>
</tr>
<tr>
<td>Unilateral (n)</td>
<td>5 (15)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Bilateral (n)</td>
<td>29 (85)</td>
<td>49 (94)</td>
</tr>
</tbody>
</table>

C + P = central and peripheral distribution of follicles; P = peripheral distribution of follicles; PCO = polycystic ovaries.

aResults are given as mean ± standard deviation.
bMean ovarian volume in the control group was 7.4 ± 5.1 ml.
There were no significant differences between the groups. Values in parentheses are percentages.
Table III. Follicular phase concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), androstenedione, testosterone, sex hormone binding globulin (SHBG), free androgen index, dehydroepiandrosterone sulphate (DHEAS) and 3α-androstenediol glucuronide (3α-diolG) in the two PCO groups, and controls

<table>
<thead>
<tr>
<th></th>
<th>PCO</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infertile (n = 34)</td>
<td>Fertile (n = 52)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>10.9 (8.3)b</td>
<td>10.6 (8.2)c</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>6.1 (2.0)</td>
<td>5.6 (2.0)</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>1.7 (1.3)b</td>
<td>1.9 (1.3)c</td>
</tr>
<tr>
<td>Androstenedione (ng/ml)</td>
<td>2.3 (1.0)b</td>
<td>1.9 (1.0)c</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.4 (0.3)</td>
<td>0.3 (0.3)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>65.3 (37.1)</td>
<td>63.3 (24.8)</td>
</tr>
<tr>
<td>Free androgen index</td>
<td>3.0 (3.0)</td>
<td>1.9 (2.7)</td>
</tr>
<tr>
<td>DHEAS (µg/dl)</td>
<td>187.3 (110.7)</td>
<td>214.3 (96.0)</td>
</tr>
<tr>
<td>3α-diolG (ng/ml)</td>
<td>2.0 (1.1)c</td>
<td>1.2 (0.5)ac</td>
</tr>
</tbody>
</table>

Results are shown as mean (SD).

aInfertile versus fertile: P < 0.01.
bInfertile versus control: P < 0.05.
cFertile versus control: P < 0.01.

was significantly greater in the two PCO groups than in controls (7.4 ± 5.1 ml, P < 0.05). Mean ovarian volume and number of follicles per ovary were similar in both groups with PCO. There was no difference in the distribution pattern of small follicles and bilaterality. Table III shows the endocrine findings for the two PCO groups and controls. Those with PCO had significantly higher basal serum LH concentrations and a greater LH/FSH ratio than controls (P = 0.0026; P = 0.0021 respectively). Concentrations of the precursor androgen, androstenedione, were significantly higher in the two PCO groups than in controls (P = 0.0062). More striking was the difference in the 5α-reduced androgen metabolite, 3α-diolG. The concentration of this was nearly twice as high in the PCO groups as in controls, and was also significantly higher in the infertile group compared with the fertile one (P = 0.0001). No differences in total testosterone, DHEAS, SHBG, or free androgen index were noted between the three groups. The relationship between serum 3α-diolG and LH concentrations is shown in Figure 1. Serum 3α-diolG concentrations were significantly correlated with LH concentrations (r = 0.4554, P = 0.008, Figure 1A) in the infertile PCO group, but not in those PCO women who were fertile (r = -0.0587, P = 0.679, Figure 1B). However, there was no significant relationship between 3α-diolG and androstenedione, testosterone, free androgen index or DHEAS.

Discussion

The prevalence of polycystic ovarian morphology was high, but was accompanied by minimal clinical manifestations with apparently no deleterious effects on earlier fertility. An isolated finding of PCO may thus be a normal variation and should not necessarily imply altered fertility potential, even though PCO are not uncommon and the condition may be diagnosed as part of an infertility work-up. Many of the patients with PCO in this study showed menstrual disturbances. However, an earlier survey (Clayton et al., 1992) reported that 75% of women with PCO had irregular menses and a similar proportion with normal ovaries showed menstrual disturbances, so this was not a distinguishing feature for PCO. PCO has been previously reported in 18–23% of apparently healthy women (Polson et al., 1988; Sagle et al., 1988) without endocrine signs of PCO. It is reassuring that the finding of PCO in this sample of women did not appear to have always adversely affected fertility (Clayton et al., 1992). Thus, PCO morphology would appear to be fairly common and the biological relevance of this finding to the reproductive capability of the female population is of considerable importance.

The present report is an attempt to determine the sonographic and endocrinological differences in women with PCO according to their reproductive capability. It is difficult to determine why the frequency of infertility in our PCO subjects was not as high as in an earlier report (Sagle et al., 1988); however the latter report may have concerned a group of women with a clinical syndrome known as polycystic ovarian disease (28.3% of them had acne and 13% were hirsute). We chose increased mean number of follicles per whole ovary as the criterion for PCO. Since its introduction as the diagnostic criterion for PCO (Adams et al., 1985), follicle number has
been the most frequently used and investigated sonographic parameter. The value of stromal density in PCO diagnosis has been questioned in recent studies since it appears to be a subjective parameter (Dewailly et al., 1994). However, others (Kyei-Mensah et al., 1998) studied the relationship of ovarian stromal volume to serum androgen concentrations in patients with PCO and found a positive correlation of stromal volume with serum androstenedione concentration only in the PCO women with clinical symptoms (menstrual irregularity, infertility, obesity or hirsutism), but not in those without clinical symptoms. In the present study, we were not able to measure stromal volume.

Although there was no significant difference in the concentrations of androstenedione between the two PCO groups, the tendency for higher androstenedione concentrations in infertile PCO may suggest a difference of stromal volume between them. In a patient group with bilateral PCO, it was shown that those patients with a sonographic image revealing more follicles per ovary exhibited more biochemical disturbances (Takahashi et al., 1993). Since correlations between sonographic and endocrine characteristics in women with PCO have been described by various authors, it is to be expected that women with PCO who are fertile, and those who are infertile, could be distinguished on the basis of sonographic features. However, we were unable to demonstrate any differences in ovarian features between them, though there was a difference in 3α-diolG concentrations. In this study, median ovarian volumes of infertile PCO and of those proven to be fertile were also very similar. Sonographic changes in ovaries of women with PCO did not, therefore, reflect their reproductive capability.

The relationship between hypersecretion of LH and infertility is clearly more complex than can be accounted for simply by anovulation (Conway, 1996). It has been suggested that higher concentrations of LH may produce a non-viable factor for the ovum (Stanger and Yovich, 1985; Howles et al., 1986; Jacobs, 1987), and cause adverse endometrial changes for implanted blastocysts (Jeffcoate, 1984), even though the women concerned had a regular ovulatory cycle. It has therefore been suggested that high LH is a predictor of fertility problems. In this study, however, mean serum LH concentrations were not higher in infertile women with PCO than in those who were fertile. Thus, the finding of equal LH hypersecretion in PCO groups suggests that there is another factor leading to infertility with hypersecretion of LH. In addition, the basal concentrations of FSH were not different between the groups. A word of caution is warranted, however, since we and others (Regan et al., 1990; Tulppala et al., 1993) collected only a single blood sample, and this may have been insufficient to reveal true changes because of marked fluctuations in gonadotrophin concentrations.

There exists a growing body of evidence in women with PCO that links androgen excess to impaired intrafollicular control mechanisms (Yen, 1980; Apter and Vihko, 1990), leading to infertility. To our knowledge, however, there are no comparable data on androgen concentrations in women with PCO, whether or not they are infertile. Eden et al. (1989) studied 68 subfertile women with PCO and found these patients had higher concentrations of testosterone than normal women (Eden et al., 1989). In addition, among this subfertile group, 16 had regular cycles and no other identifiable cause of infertility; these women also had higher follicular-phase free androgen index than normal women. Carmina et al. (1997) studied 15 ovulatory women and 25 anovulatory women with PCO, and found anovulation in PCO was associated with higher concentrations of androgens (testosterone, androstenedione, DHEAS) (Carmina et al., 1997). However, no significant difference was found between ovulatory women with PCO and controls. They suggested that ovulatory women with PCO may form part of the spectrum of patients with polycystic ovarian disease. In our study, PCO groups had higher follicular-phase androstenedione than did controls. This discrepancy may arise from selection differences of study subjects. Free androgen index is a convenient marker for PCO, especially in a group with oligomenorrhea (Eden et al., 1988), although we failed to find a significant difference in free androgen index between PCO groups and controls.

Instead, 3α-diolG was significantly higher in PCO groups, especially in the infertile group. However, we were not able to detect significant hirsutism in the infertile group. This could be explained by racial differences in the number of hair follicles per unit area of skin and receptor sensitivity, as oriental women are rarely hirsute despite high concentrations of androgen. On the other hand, the 3α-diolG concentration may have been too low to lead to hirsutism in this study group. 3α-diolG may be produced primarily in androgen target tissues via intracellular conversion of weak androgens such as DHEA (dehydroepiandrosterone) and androstenedione, in either the testosterone or the 5α-androstenediene pathway (Horton and Lobo, 1984). Thus, serum 3α-diolG concentrations reflect an increased activity of 5α-reductase in the peripheral compartment and are also a good marker of peripheral androgen action (Horton and Lobo, 1984; Kirschner et al., 1987). Furthermore, 3α-diolG has a relatively slow metabolic clearance rate and thus, serum concentrations may not change significantly throughout the day (Greep et al., 1986). However, it has to be considered that 3 α-diolG also reflects hepatic conjugation activity (Rittmaster, 1993). Serum 3α-diolG concentrations have been shown to decrease after adrenal suppression with glucocorticoids, implying an important adrenal contribution to its formation (Meikle and Odell, 1986). Also, differences in DHEAS concentrations cannot account for differences in 3α-diolG between infertile and fertile women with PCO; the fact that the only significant difference between the infertile and fertile PCO women was in 3α-diolG concentration suggests that infertility is not only a function of circulating androgen concentrations, but may also be determined by androgen production in peripheral tissues and the adrenal gland.

The higher correlation between LH and 3α-diolG in infertile PCO women may indirectly reflect bioactivity of LH. Such hypersecreted LH, directly or indirectly through increased androgen and/or increased 5α-reductase activity, has an effect on oocytes, or endometrium. Increased serum 3α-diolG concentrations might, therefore, be due to an increase in bioavailable androgen resulting from increased LH bioactivity rather than to the effect of peripheral 5α-reductase on its activity. The
present study seems to suggest that increased 3α-diolG concentrations, as another factor related to a high LH concentration or a high LH/FSH ratio, might reflect fertility problems with PCO. It has been reported (Carmina et al., 1995) that serum 3α-diolG was significantly correlated with free testosterone \((r = 0.56; P < 0.01)\) and with androstenedione \((r = 0.48; P < 0.01)\) but not with adrenal androgens. Significantly greater peak responses of ovarian androgen secretion to human chorionic gonadotrophin in women with functional ovarian hyperandrogenism, compared with the response in normal women, indicates that this abnormality is LH-dependent (Levrant et al., 1997). In this present study, the higher correlation between 3α-diolG and LH in the infertile group also suggests that 3α-diolG might be affected by LH activity. Since the two PCO groups had significant differences in 3α-diol G concentrations but not in LH concentrations, it seems that the infertile group may respond to LH with greater ovarian androgen secretion if the source of the increased 3α-diol is from the ovary. Thus, the infertile group belongs to the spectrum of functional ovarian hyperandrogenism, as previously demonstrated (Levrant et al., 1997). The higher correlation between serum 3α-diolG and LH concentrations in the infertile group, together with the lack of significant difference in LH concentrations between the two groups with PCO, suggest that serum 3α-diolG determination could be a reliable index for predicting reproductive capability in women with PCO (multivariate regression analysis, \(P = 0.0018\), adjusted \(R^2 = 0.2160)\).

In summary, this study suggests that the increased androgenic activity seen in women with PCO negatively affects reproductive capability. The relevance of these findings to the genesis or aggravation of fertility problems in women with PCO remains to be determined. Accordingly, our evidence for increased 3α-diolG in infertile women with PCO needs to be confirmed by further studies, including 5α-reductase assay, adrenal function tests and evaluation of hepatic conjugation activity.

References


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