Luteal phase progesterone excretion in ovulatory women with polycystic ovaries

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BACKGROUND: Various studies have reported a prevalence of polycystic ovaries (PCO) of ∼20% in the ‘normal’ population. Our aim was to investigate the frequency of ovulation and pattern of luteal phase progesterone secretion in a group of women with PCO who reported regular cycles and in whom ovulation had been established on the basis of previous investigations. METHODS: Subjects collected early morning urine samples for pregnanediol-3-glucuronide measurement from day 10 of the cycle to day 1 of their next menses. Results in three consecutive cycles from women with PCO (group 1, n = 10 and 29 for patients and cycles respectively) were compared with results from two groups with normal ovaries; with either infertility (group 2, n = 10 and 30) or proven fertility (group 3, n = 6 and 19). RESULTS: There were considerable variations in cycle length. The median (range) was group 1: 28 (23–47); group 2: 26 (21–36) and group 3: 27 (25–38) days with more short cycles in both infertile groups. There was more variation in pregnanediol:creatinine in the normal-ovary infertile and PCO groups than in the fertile controls. Levels were higher in the early luteal phase in the fertile normal group than in either infertile group, and the mid-luteal phase peak was lower in the infertile women with normal ovaries. In summary, there was greater variability in luteal phase pregnanediol:creatinine ratios in the PCO and infertile normal-ovary groups than in women with normal ovaries and proven fertility. CONCLUSION: Women with PCO did not have more variation in cycle length than fertile women with normal ovaries, but there were significantly lower levels of progesterone in the early luteal phase. This may contribute to the delay in conception in these patients.

Key words: luteal phase/menstrual cycles/ovulation/polycystic ovaries/progesterone

Introduction

Polycystic ovarian syndrome has long been recognized as the most common cause of anovulatory infertility (Yen, 1980; Franks, 1996). The ability to detect polycystic ovaries (PCO) by ultrasound, however, has revealed that the obese, anovulatory, hirsute woman represents just one end of a spectrum of presentation which has, at the other end, those who appear to have no other clinical manifestation of the syndrome (Polson et al., 1988). The high prevalence of PCO in the normal population has been demonstrated by several studies and has been found to be in the region of 20% (Polson et al., 1988; Clayton et al., 1992; Farquhar et al., 1994).

The significance of PCO, in terms of fertility, in the population of asymptomatic women is unknown, but it seems reasonable to postulate that such women have a significant number of anovulatory cycles despite reporting regular menses. PCO were found to be present in >50% of ovulatory women presenting with infertility who had a primary diagnosis of tubal, male factor or unexplained infertility (Kousta et al., 1999). This was twice the expected prevalence and implies an effect of PCO on the fertility of these women.

The aim of this study was to investigate the frequency of ovulation in a group of women with PCO who presented with infertility, but who reported regular cycles and to assess the pattern of luteal phase progesterone secretion in these women.

Materials and methods

Patients

Patients were recruited into one of three groups. The first two groups comprised patients attending a single out-patient clinic who had presented with infertility, but who had reported regular menstrual cycles, i.e. <4 days variation in the length of consecutive cycles; group one were found to have PCO by ultrasound scanning and group 2 had normal ovaries. All had undergone full investigation and had been given a primary diagnosis of tubal, male factor or unexplained infertility. In each subject, evidence of ovulation had been established.
Table I. Clinical details of the three study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
<th>No. of subjects</th>
<th>No. of cycles</th>
<th>Age (years) median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Infertile PCO</td>
<td>10</td>
<td>29</td>
<td>34 (23–39)</td>
</tr>
<tr>
<td>2</td>
<td>Infertile-normal</td>
<td>10</td>
<td>30</td>
<td>34 (30–40)</td>
</tr>
<tr>
<td>3</td>
<td>Fertile-normal</td>
<td>6</td>
<td>19</td>
<td>33 (25–40)</td>
</tr>
</tbody>
</table>

using cycle monitoring by ultrasound and by the finding of a mid-luteal progesterone >30 nmol/l. These were compared with a control group (group 3) who had regular cycles and normal ovaries and who were of proven fertility in that they had at least one completed pregnancy and had no self-perceived delay in conception. Approval for the study was granted by the local ethics committee. A detailed menstrual cycle and reproductive history was taken from each subject. Exclusion criteria were: day 8 FSH >10 IU (checked twice), age >40 years, hormonal medication and breast feeding within the previous four months.

**Progestosterone analysis**

Luteal phase progesterone excretion was assessed by analysis of urinary ratios of pregnanediol:creatinine in early morning urine samples collected on consecutive days commencing on day 10 of the cycle and proceeding to the first day of menses. Samples were collected in three consecutive cycles and stored at –20°C. Samples from completed cycles were delivered frozen to the laboratory where they were centrifuged and aliquoted for assay of pregnanediol-3-glucuronide (P-3-G) for 10 min at 1000 rpm, as previously described (Yong et al., 1992)

**Analysis of data**

Differences in cycle length between the groups was determined by the Mann–Whitney U-test. Progesterone metabolite results are expressed as P-3-G:creatinine ratios. Patterns of luteal phase P-3-G were normalized to the first day of the subsequent menstrual period for statistical analysis. Analysis of luteal phase P-3-G patterns was initially made by calculating mean area under curve (AUC) for each patient, with the baseline taken as the mean of the first (day–13) and last (day 0) result in each case. Differences between groups were then determined by analysis of variance followed by Tukey/Kramer post hoc test. Differences in day –10 and peak P-3-G:creatinine were analysed by the Mann–Whitney U-test. Throughout, significance was assumed when P < 0.05.

**Results**

**Patients**

There were no significant differences in age between groups. The clinical details of each group are described in Table I.

**Cycle length**

In each group there was considerably more variation in cycle length than expected from the subjects recalled history of ‘regular’ cycles. The median and range of cycle length was 28 days (range 23–47) for PCO, 26 days (range 21–36) for infertile women with normal ovaries and 27 (25–38) for the normally fertile group. These data are expressed graphically in Figure 1. There was a similar degree of variation in cycle length between the groups. The infertile women with normal ovaries and the women with PCO had a higher proportion of short cycles with 6/30 and 5/30 cycles in each group being <25 days, whereas there were none of this duration in the fertile women.

**P-3-G excretion**

To overcome the variation in cycle length and allow for valid comparisons, luteal phase P-3-G:creatinine ratios were normalized by comparing samples from the 14 days leading up to menstruation. The typical pattern of luteal phase P-3-G secretion showed a rise of 1.5–2.0 units to a peak at day –8 where day 1 is the first day of menses. Levels then fell to follicular phase concentrations by the commencement of menstruation.

Abnormal patterns of P-3-G:creatinine were seen in a number of patients, usually characterized by sudden falls or swings in excretion. Qualitative analysis of individual cycles revealed that there were 4/30 cycles in the PCO and 12/30 in the infertile normal group that showed a grossly abnormal pattern. Two of these cycles came from one patient in the PCO group and nine from three patients in the infertile normal group. A typical example of a single cycle from each group is shown in Figure 2.
Luteal phase progesterone in ovulatory women with PCO

Figure 2. Typical example of luteal phase daily pregnanediol-3-glucuronide:creatinine in a single cycle in one patient from each group. Profiles in infertile women were often characterized by erratic levels.

Collating data from all cycles from each group revealed a narrow range in the normal fertile group but a greater variation in the PCO and even greater variability for the infertile normal group. The median and range of daily P-3-G:creatinine is shown in Figure 3A. There were no significant differences between the PCO and the other two groups when the AUC was calculated, but the infertile women had lower values than the normally fertile women (*P* < 0.05). The range and median values for AUC in individual cycles in each group are shown in Figure 3B.

Comparison of the mean of daily values for each group revealed that the early luteal phase values were significantly higher in women of proven fertility than in the two infertile groups (day –10: normal versus PCO, *P* = 0.02; normal versus infertile, *P* = 0.006). Although the early luteal mean P-3-G was lower in the PCO group than in the normal women, the peak value attained was not different. In contrast, the peak in the infertile women was significantly lower than in both of the other groups (infertile versus PCO, *P* = 0.01; infertile versus normal, *P* = 0.006). These data are illustrated in Figure 3C.

That the early luteal phase values show the most difference is emphasised when comparing the mean data for the early (days –13 to –7 inclusive) or late (days –6 to 0) luteal phase between groups, as shown in Figure 4. There were no significant differences between the groups in the late luteal phase, but both infertile groups had lower values than the fertile women in the early luteal phase; normal fertile versus PCO or infertile normal, *P* < 0.002. These differences remained significant even if the data from one patient in the PCO group and the three in the infertile normal group who had been found to have very abnormal progesterone profiles were omitted (*P* < 0.04).

Discussion
The most striking finding of this study was the degree of cycle irregularity in women reporting regular cycles. Despite the

Figure 3. (A) Median (■) and ranges of daily urinary pregnanediol-3-glucuronide:creatinine levels in each group. (B) Median (■) and range of area under the curve for urinary pregnanediol-3-glucuronide:creatinine for individual cycles in each group. There was no difference between PCO and the other two groups, but the profile in infertile women with normal ovaries was significantly different (*P* = 0.03, ANOVA followed by Tukey/Kramer post hoc test). (C) Mean and SE of daily urinary P-3-G:creatinine. Levels were higher in the early luteal phase in the normal fertile women (day –10 *: normal versus PCO, *P* = 0.02; normal versus infertile, *P* = 0.006, Mann–Whitney U-test). The peak (+) was higher in the PCO group than in the infertile women with normal ovaries (infertile versus PCO *P* = 0.01, infertile versus normal *P* = 0.006, Mann–Whitney U-test).

history of no more than 4-day cycle-to-cycle variation, it is clear that many of the subjects recruited to this study did not fulfill these criteria on prospective examination. Interestingly, there was also a notable degree of variation in normally fertile women. Previous studies have reported a similar degree of variability in cycle length in the general population with a prevalence of 6–15% depending on the definition of regularity and the age of the group in question (Arey 1939; Chiazze et al., 1968; Bachmann and Kommann, 1981). As most of these studies were performed prior to the onset of routine ultrasound scanning of the ovaries, the cause of the cycle irregularity was not examined. Recent data have revealed that a significant proportion of these women would be expected to
Figure 4. Comparison of mean (SE) urinary pregnanediol-3-glucuronide:creatinine in the early or late luteal phase. a versus c and b versus c, P < 0.002, Mann–Whitney U-test.

The degree of menstrual variation in women with normal ovaries on ultrasound has not been determined separately, but only one woman out of 116 with normal ovaries reported irregular cycles in the study by Polson et al. (1998).

Interestingly, the increased variability in cycle length in both of the infertile groups was due in part to a high prevalence of short cycles, 16% being of <25 days duration. No cycles of this length were documented in the women of proven fertility. Short cycles have been reported in several of the studies investigating cycle length in the general population, but the majority of these were found in perimenopausal women (Trelor et al., 1967; Sherman and Korenman, 1975). A gradual decrease in cycle length of 2–3 days was observed between the ages of 20 and 40 years with an increase in the number of short cycles from the age of 40 years to the time of menopause. The short cycles found in two groups in our study could not be attributed to differences in age because the age was similar in the three groups and perimenopausal women were excluded. A later study of >2000 menstruating women of all ages reported that 24% had cycles of ≤25 days, data which are similar to those presented here (Wood et al., 1979). The significant proportion of women with short and long cycles in this group led the investigators to conclude that normal cycle length should be considered to be between 21 and 35 days. Applying these criteria to our data we concluded that only three cycles (one in each group) could be considered abnormal in length.

There was a narrow range of subject-to-subject variation in total urinary P-3-G:creatine ratio across the luteal phase in fertile women. This range was increased in the PCO group and was wider still in the infertile women with normal ovaries. Analysis of individual cycles revealed that a few patients were responsible for the majority of these abnormal cycles. Three patients in the infertile normal group failed to produce a normal P-3-G profile in any of the three cycles studied. The reason for the apparent fluctuation in P-3-G during the luteal phase in these patients is not clear, but is unlikely to be a methodological problem as there were no similar examples in the normal fertile group. There was no apparent link between cycle length and the profile of the P-3-G:creatinine ratio.

The higher P-3-G concentrations in the fertile women were most apparent in the first half of the luteal phase. Similar data were found in two previous studies, one investigating serum progesterone levels in women presenting with infertility (Lenton et al., 1978) and a second comparing levels in cycles in which conception occurred with those from non-conception cycles (Lenton et al., 1982). In the first study, progesterone levels were found to be lower specifically in the first half of the luteal phase in infertile women who were judged to be ovulatory compared with a control group of women who were of proven fertility. Although it was assumed that the deficiency of progesterone was implicated in the infertility of these women, increasing plasma progesterone levels by a variety of medical interventions had no impact on fertility. The ovarian morphology of these groups was not investigated, but in our study similar results were obtained in women with infertility regardless of ovarian morphology.

The main factor distinguishing the cycles of women with PCO from those of the infertile women with normal ovaries, was the peak level of P-3-G:creatinine reached during the mid-luteal phase. Although levels in both infertile groups were lower initially, P-3-G in the women with PCO eventually rose to be similar to those seen in the fertile women. However, this peak was reached 1 day later. In the second study (Lenton et al., 1982), progesterone levels in conception cycles were clearly higher than in non-conception cycles as early as day 3–8 after the LH surge (equivalent to our day –12 to –6). This was presumably too early to be a result of corpus luteum rescue due to conception. A total of 15–20% of values in the non-conception group were low. The lower mean early luteal and peak levels in the infertile groups in the current study are a reflection of the number of cycles in which progesterone levels were variable throughout the luteal phase. This may be due to abnormal follicular development in these patients, resulting in inadequate corpus luteum function.

Poor follicular development was suggested to be the cause of infertility in another study in which steroid levels were assessed throughout the menstrual cycle in women with previously unexplained infertility (Dodson et al., 1975). As with our data, close examination of individual cycles revealed that a minority of the group was responsible for most of the abnormal cycles. Poor progesterone secretion followed significantly lower levels of pre-ovulatory estradiol in these patients.

Although the most aberrant P-3-G secretion profiles were seen in the infertile women with normal ovaries, there was no apparent correlation between cycle length and luteal phase P-3-G values. This suggests that in this study at least, an abnormal cycle length was not a good predictor of poor corpus luteum function.

In summary, we have found that women with PCO who report regular cycles do not have more variation in cycle
length than women with normal ovaries. There was, however, a greater degree of variability in luteal-phase progesterone production, but this was also evident in infertile women with normal ovaries. The most apparent difference between fertile and infertile women, regardless of ovarian morphology, was the level of progesterone metabolite in the early luteal phase. In both infertile groups the lower levels were mainly due to a few patients who appeared to have consistently abnormal progesterone secretion which may be implicated in their infertility. However it is likely that, in most patients, other factors are also involved. That ovulatory PCO may associated with sub-fertility, is demonstrated by a recent study to determine the prevalence of PCO in regularly-cycling women with infertility of various causes (Kousta et al., 1999). Interestingly, this morphology was over-represented in women with tubal disease, sperm dysfunction and unexplained infertility compared with a control group of parous volunteers. The implication of this association is not clear, but our study suggests, that in the majority of patients, impaired fertility in ovulatory women with PCO is not primarily due to inadequate progesterone production.

References

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