Evaluating ovarian reserve: follicle stimulating hormone and oestradiol variability during cycle days 2–5

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A prospective measurement of follicle stimulating hormone (FSH) and oestradiol between cycle days 2 and 5 was conducted to investigate the intra- and inter-cycle variability in a healthy population of women with regular menstrual intervals. Daily serum samples were obtained from 44 women for a total of 66 cycles on cycle days 2, 3, 4 and 5. FSH concentrations were consistent on all cycle days measured. Oestradiol concentrations on cycle day 2 were not different from cycle day 3, but concentrations on cycle day 4 and cycle day 5 were statistically different from both cycle day 2 and cycle day 3 by analysis of variance (P = 0.05). Evaluation of functional ovarian reserve by cycle day 3 FSH measurement has become the standard in most assisted reproductive technology programmes. The recent change in FSH standardization coupled with the inflexibility of cycle day 3 testing has led to a re-evaluation of testing protocols. Cycle day 3 appears to have emerged as a dictum because most ovulation induction protocols are initiated on cycle day 3, 4 or 5. Flexibility of sampling day can be introduced as suggested by these results. The additional information ascertained from oestradiol testing as applied to evaluation of ovarian reserve warrants further investigation.

Key words: follicle stimulating hormone/oestradiol/ovarian reserve/reproductive potential

Introduction
The concept of ovarian reserve as assessed by follicle stimulating hormone (FSH) measurement has proven useful in predicting pregnancy outcome (Scott and Hoffman, 1995). Determination of cycle day 3 FSH has evolved as the standard for predicting oocyte quality and the likelihood of conception in assisted reproductive technology programmes. The information obtained from cycle day 3 FSH testing is invaluable in counselling patients as to their chances of achieving a pregnancy and deciding on options for stimulation protocols (Scott et al., 1989). Thus, considerable deliberation for appropriate management may result from evaluation of a single timed laboratory determination (Hershlag et al., 1992).

Muasher et al. (1988) demonstrated that basal cycle day 3 concentrations of FSH reflected the reproductive potential of that menstrual cycle. This could be applied further to discriminate between patients who would be more likely to respond to ovarian stimulation and those who would not (Toner et al., 1991). Cycle day 3 testing has emerged as a dictum from these studies because most stimulation protocols were initiated on cycle day 3, 4 or 5 (Jones et al., 1984; Marrs et al., 1984). The validity of testing on other days has not yet been explored.

The balance between early follicular gonadotrophin-releasing hormone (GnRH) and ovarian steroidogenesis may be another indicator of ovarian reserve, helpful in discerning the potential for ovarian stimulation in a specific cycle or patient (Hodgen, 1989). Early follicular phase oestradiol concentrations may reflect the stage of follicular development, with higher concentrations associated with asynchrony of follicular development. An abrupt early rise of oestradiol may be a subtle sign of the shortened follicular phase often seen prior to menopause.

The purpose of this study was to evaluate the intra- and inter-cycle variability of serum values of FSH and oestradiol in the early follicular phase (cycle days 2–5), utilizing an assay based on the new FSH standard [World Health Organization (WHO), 2nd International Reference Preparation (2nd IRP)], to determine if greater flexibility in testing days was possible.

Materials and methods
A total of 44 healthy women (median age 33; mean age 34.5; range 24–49 years) with regular menstrual intervals were recruited. Daily serum samples were obtained on menstrual cycle days 2, 3, 4 and 5 during the time period between March, 1994 and January, 1995. In all, 66 cycles were available for evaluation, but only 39 had determinations on all 4 days evaluated (29 patients, mean age 31.9 ± 5.6 years). FSH serum concentrations were determined through a solid phase FSH-coated immunoradiometric assay (FSH Coat-a-Count, IRMA, DPC, Los Angeles, CA, USA). This assay was standardized in terms of the WHO 2nd IRP of pituitary FSH (78/549). Oestradiol determinations were performed using a commercially available radioimmunoassay (RIA) kit (Pantex, Santa Monica, CA, USA) with inter- and intra-assay variabilities of 5.0 and 2.2% respectively. Each sample was centrifuged and stored as serum. Samples were batched and analysed three times per week (Monday, Wednesday, Friday). Duplicates were run to obtain information on inter-cycle and intra-cycle variability. Statistical analysis was performed using Systat Statistical Software (Evanston, IL, USA). Analyses were performed using analysis of variance or Pearson
Evaluation of ovarian reserve

Table I. Mean follicle stimulating hormone (FSH) and oestradiol concentrations between cycle days 2 and 5

<table>
<thead>
<tr>
<th>Cycle day</th>
<th>FSH (mIU/ml)</th>
<th>Oestradiol (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>FSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mIU/ml</td>
<td>5.7 ± 1.9</td>
<td>5.9 ± 2.1</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>172.4 ± 81.0</td>
<td>172.0 ± 77.2</td>
</tr>
</tbody>
</table>

*Significantly different from cycle days 2 and 3; \( P < 0.05 \).

Table II. Inter-cycle variability of serum follicle stimulating hormone (FSH) and oestradiol concentrations

<table>
<thead>
<tr>
<th>Cycle day</th>
<th>FSH (mIU/ml)</th>
<th>Oestradiol (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cycle 1</td>
<td>Cycle 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.5 ± 3.3</td>
<td>6.4 ± 2.8</td>
</tr>
<tr>
<td>3</td>
<td>6.3 ± 2.6</td>
<td>6.1 ± 2.3</td>
</tr>
<tr>
<td>4</td>
<td>7.1 ± 3.9</td>
<td>5.9 ± 2.0</td>
</tr>
<tr>
<td>5</td>
<td>5.7 ± 1.5</td>
<td>5.9 ± 1.2</td>
</tr>
</tbody>
</table>

correlation coefficient as indicated. Corrections for multiple comparisons were employed where appropriate. A \( P \) value of < 0.05 was considered to be statistically significant. The data are reported with means and SD.

Results

Mean FSH and oestradiol values obtained on cycle days 2–5 are given in Table I. No significant difference was noted among FSH concentrations during this time period. Oestradiol values, however, were not constant through the early follicular phase. Serum oestradiol concentrations began to increase by cycle day 4 such that the mean value on cycle day 4 (202.9 ± 99.3 pmol/ml) was significantly greater than on cycle day 2 (172.4 ± 81.0 pmol/ml, \( P = 0.005 \)) or cycle day 3 (172.0 ± 77.2 pmol/ml, \( P = 0.026 \)). In addition, the mean value on cycle day 5 (229.5 ± 125.5 pmol/ml) was significantly greater than that on cycle day 4 (202.9 ± 99.3 pmol/ml, \( P = 0.004 \)).

No correlation between oestradiol and FSH values was found on cycle day 2 and cycle day 3. As oestradiol concentrations began to rise on cycle day 4, a negative correlation between oestradiol and FSH was found, though this reached statistical significance only on cycle day 5 (\( r = -0.38, P = 0.011 \)).

FSH values increased with increasing age (Figure 1). This positive correlation was statistically significant (\( P < 0.05 \)) on all days measured. No correlation between age and early follicular oestradiol concentrations was identified (Figure 2, A–D). Oestradiol concentrations varied independent of patient age. Investigation was performed on a subset of 33 cycles of women <40 years of age (mean age 30.0 ± 3.4; median 31.5 ± 3.7 years) in whom all cycle day data points were available for evaluation. There was no difference in FSH values on all 4 cycle days evaluated. Oestradiol concentrations were noted to increase starting on cycle day 4. Again, no correlation between oestradiol concentrations and age was noted. In this subset FSH values correlated with oestradiol values only on cycle day 5 (\( P = 0.29, r = 0.349 \)).

A total of 19 subjects (mean age, 33.4 ± 6.2 years) had more than one cycle available for analysis. Of these 19 women, 14 had data from two cycles, three had data from three cycles and two had data from four cycles. When the inter-cycle variability of oestradiol and FSH concentrations for two cycles was considered, no difference in either oestradiol or FSH concentrations on cycle day 2 through cycle day 5 was noted. These data are shown in Table II. In those subjects with data from more than two cycles available, it also appeared that no significant inter-cycle variability occurred, though the sample size (\( n = 5 \)) was too small to reach statistical significance.

The FSH assay utilized was standardized in terms of WHO 2nd IRP of pituitary FSH (78/549) with intra- and inter-assay
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Figure 2. Correlation between patient age and oestradiol (E₂) concentrations on cycle days 2–5. E₂ levels varied independently of patient age.

The recent introduction of a new FSH standard (WHO, 2nd IRP) based on pituitary FSH concentrations rather than menopausal urinary FSH values requires re-evaluation of testing results, especially since the value curve has been compressed. According to the manufacturer as well as our own parallel testing with both assays on each specimen (unpublished), the conversion factor is 0.6 (1 mIU/ml, 2nd IRP = 1 mIU/ml, 1st IRP × 0.6). New threshold values for ovarian reserve need to be assigned beyond which few viable pregnancies are likely to occur (Scott and Hofmann, 1985; Pearlstone et al., 1992). Intra- and inter-assay variability needs to be carefully controlled since a single test may dictate a patient’s stimulation protocol or determine the cancellation of not only that cycle but also of further treatment (Hershlag et al., 1992).

The question of the significance of inter-cycle variability of FSH has been raised before. According to Scott et al. (1990), inter-cycle variability in basal FSH was more marked in patients with values ≥15 mIU/ml (1st IRP) but did not indicate changes in ovarian response to stimulation. One cycle was not more propitious for pregnancy than another based on a lower FSH value. Our paired data showed no significant variability from cycle to cycle in 19 subjects. Data on pregnancy rates with the new FSH standard are currently being gathered to determine if differences exist from previously established threshold criteria.

Sherman and Korenman (1975) and Sherman et al. (1976) introduced the concept of a menopausal transition period in which menses continued at regular (or irregular) intervals, but hormonal concentrations became increasingly variable, and patterns of hormonal regulation changed. Elevated or normal

Discussion

The importance of cycle day 3 FSH for evaluation of ovarian reserve and subsequent pregnancy potential has recently been emphasized in women >35 years during the infertility work-up (Scott and Hofmann, 1995). Basal FSH values have been utilized to decide on treatment protocols and to counsel patients as to their potential pregnancy success (Toner et al., 1991). Many authors attest to the importance of cycle day 3 testing (Fenichel et al., 1989; Pearlstone et al., 1992; Tanbo et al., 1992). The emphasis on cycle day 3 testing seems to have evolved in part from convenience, based on the cycle day 3 start of most stimulation protocols (Jones et al., 1984; Marres et al., 1984). According to Hodgen’s work (1989), early follicular growth and recruitment occur in the beginning of the cycle prior to cycle days 5–7. By day 7 the one follicle destined to ovulate has been selected. Since the objective in ovarian hyperstimulation for assisted reproductive technology is to recruit more than one dominant follicle, stimulation must be initiated prior to the loss of this multipotentiality of the follicles. Before the widespread use of (GnRHa), stimulation protocols traditionally began on cycle day 3 or 4. Thus, basal testing had to be performed by cycle day 3. According to our data, testing for FSH on any of cycle days 2–5 will give equivalent results, regardless of patient age.
FSH concentrations with both decreased or elevated oestradiol concentrations during the cycle were noted. Distinct menstrual patterns were seen in the peri-menopausal state with both short follicular or luteal phases resulting in shortened cycle lengths explaining the diminished cycles which had been noted peri-menopausally by Treloar et al. (1967). The dissociation of early FSH from oestradiol values seen in older women remains to be explained, but a loss of inhibin production by the granulosa cells has been postulated as being responsible (McLachlan et al., 1987). The patients with occult ovarian failure described by Cameron et al. (1988) fall into this category.

Oestradiol plays a key role in folliculogenesis, necessary for granulosa cell mitotic divisions, synthesis of FSH receptors on granulosa cells, and, with FSH, induction of luteinizing hormone (LH) receptors (Hodgen, 1989). Basal oestradiol values may impart information on ovarian activity when increased, suggesting that the recruitment phase is already completed with selection of the dominant follicle having occurred. The lack of correlation between oestradiol and FSH concentrations on cycle days 2 and 3 became a negative relationship by cycle day 4, with statistical significance reached on cycle day 5. The relative effect of oestradiol suppression of FSH versus the inhibit effect in an individual cycle remains unknown.

A single determinant of basal FSH, established previously (Scott et al., 1989, 1995) serves as a predictive assessment of ovarian reserve. Ovarian response to controlled stimulation can be evaluated by the clomid challenge test (Scott and Hofmann, 1995) or the recently described exogenous FSH ovarian reserve test (EFORT) (Fanchin et al., 1994). These dynamic tests may be more accurate in predicting ovarian response and pregnancy outcome than a single basal determinant.

In many practices, evaluation of ovarian reserve prior to ovulation induction, especially in patients >35 years old, is becoming routine. Rigid adherence to cycle day 3 collections no longer seems necessary. As demonstrated here, FSH values are equivalent on cycle days 2–5. Oestradiol, however, begins to rise on cycle day 4. These findings will add greater flexibility for both patients and physicians in evaluation of the infertile couple. The added information of early oestradiol concentrations in conjunction with FSH needs to be evaluated with regard to pregnancy outcome. Finally, there remains the task of determining a new threshold value of FSH using the 2nd IRP; above which one can anticipate that there would be few ongoing pregnancies.

References

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