The onset of the initial rise in follicle-stimulating hormone during the human menstrual cycle

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BACKGROUND: The rise in FSH (FSHr) that leads to the recruitment of a cohort of follicles during the menstrual cycle occurs during the luteal–follicular transition, however, it is unclear whether it consistently occurs on one particular day, or is subjected to reproductive ageing. METHODS: We determined the FSHr in 836 complete menstrual cycles from 102 women with regular menses using an algorithm, and additionally compared the relative variation in FSH during the last 14 days of the cycle. Possible effects of reproductive ageing on the onset of FSHr were also investigated. RESULTS: The day of FSHr follows a normal distribution with a median value of 24 (relative to first day of menses), mean 24.1 and SD 2.1. Analysis of the relative changes in FSH during the last 14 days of the cycle revealed the first significant rise on day 24 (P < 0.0033), coinciding with the first significant drop in estrogens (P < 0.0002). No effect of chronological age, or initial FSH levels, on FSHr was found, however, there was an inverse relationship between total follicular phase length (from day of FSHr to LH peak) and initial FSH levels (P < 0.0001). CONCLUSIONS: The initial FSH rise in the cycle occurs consistently 4 days before menses, is related to a drop in estrogen levels, and is not affected by reproductive ageing.

Key words: estrogens/follicular phase/follicle-stimulating hormone/menstrual cycle/urinary hormones

Introduction

Although the onset of menses is the reference for the beginning of the reproductive cycle in the woman, the endocrine processes leading to follicular development and ovulation start earlier. The first hormonal event in the cycle is the rise in FSH level, which is responsible for the recruitment of a new cohort of follicles. This FSH rise occurs late in the luteal phase (Ross et al., 1970; Vermesh and Kletzky 1987) and is often defined as the luteal–follicular transition, however, it is not yet established whether it consistently occurs on one particular day, or is affected by the process of reproductive ageing. These issues have profound implications, both for our understanding of ovarian physiology as well as the clinical applications that might derive for the treatment of infertility and contraception.

The length of the menstrual cycle is not stable throughout reproductive life. One of the first manifestations of reproductive ageing is a reduction in the length of the follicular phase (Sherman and Koreman 1975; Lenton et al., 1984; Klein et al., 1996). Because FSH levels rise in parallel to this reduction of the follicular phase, an attractive explanation for this phenomenon is that the higher FSH accelerates follicular development (Lenton et al., 1988; Klein et al., 1996, 2002; Miro et al., 2004a). On the other hand, others have suggested that the reduction in length might instead be the result of earlier onset of FSH rise during the luteal–follicular transition (van Zonneveld et al., 2003).

During the luteal phase of the human menstrual cycle, the corpus luteum inhibits follicular development by secreting the hormones estradiol and inhibin A, known to suppress FSH production (Ross et al., 1970; Groome et al., 1996). In this way, the rise in FSH might well be related to a drop in the production of these suppressive hormones.

The main aim of this study is to determine whether the FSH rise during the luteal–follicular phase transition occurs on a particular day. Additionally we intended to determine the possible relationship with levels of luteal hormones, and to investigate potential changes in the onset of FSH rise due to reproductive ageing. For this purpose, we use a large database consisting of daily variations in urinary hormonal markers in 102 women during several cycles. For identifying the day of FSH rise, we employed two different analytical approaches. First, we developed an algorithm that retrospectively detects the first sustained rise in FSH during the cycle. Second, we compared the variation in mean FSH levels during the last 14 days of the cycle.
Materials and methods

Subjects and sampling

This study is based on hormonal data from 836 complete cycles from 102 healthy women (median nine complete cycles per case) with regular menstrual cycles (mean length 27.7 days, SD = 3.5) aged 19–52 years (median 36.5, SD = 7.2). None of the participants were suffering from any known endocrine disease, were breast feeding, receiving any fertility medication or were taking hormone-based contraceptives during the study period. The participants had no known fertility problem and none of the cycles investigated ended in pregnancy.

Each woman collected daily first morning void urine samples (Lasley et al., 1994; Santoro et al., 2003a) for several successive cycles. Samples were kept in universal specimen bottles containing sodium azide as preservative (0.1% volume). Volunteers were asked to keep the samples refrigerated until delivered to the laboratory (on a weekly basis). On arrival, the samples were aliquoted and stored at 4°C until the hormonal analyses were performed.

Hormonal analyses

All samples were analysed for follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrone 3-glucuronide (E1G) and pregnadiol 3-glucuronide (PdG) by immunoassay, using AutoDelfia® (Perkin Elmer Life Sciences, Cambridge, UK) following in house established and validated protocols, as previously described (Miro et al., 2004a, b).

Data preparation and analyses

The concentrations of FSH, LH and E1G were adjusted in order to compensate for urine volume fluctuations as previously described (Miro et al., 2004b). Briefly, the ln(PdG) profiles were smoothed using cubic spline, and the residuals were used to adjust the concentrations of the other three hormones. The advantage of the smoothing is that it has the effect of reducing local (2–3 days) fluctuations with no bias towards particular shapes over a long time interval. This was achieved by using the SAS/IML Splinec routine with a smoothing parameter of 10 (Littell et al., 1996; Miro et al., 2004b).

FSH results are expressed as international units/litre (IU/l) and E1G as ng/ml.

Parameters, algorithms and statistics

The onset of FSH rise (FSHr) was determined by an algorithm applied to smoothed-adjusted FSH values. The algorithm retrospectively detects the first day when a sustained increase in FSH concentration takes place, based on the change in FSH slope relative to the current FSH level. This change in slope is measured by the parameter 'surge size' (S) for each day within the interval between LH peaks in successive cycles, excluding the last 10 days.

Right slope (RS) is the slope of the line joining the current day with the day 3 days later, and left slope (LS) is the slope of the line joining the current day with the day 2 days earlier.

\[ S = |RS - \text{maximum}(LS, 0)|/\text{maximum}(FSH\text{level}, 0.1) \]

The division by maximum (FSH level, 0.1) gives greater weight to slope changes at low [FSH], provided these exceed 0.1 IU/l to avoid undue sensitivity. In this way FSHr is defined as the first day in the cycle for which S > 0.85 × maximum (S).

FSHr found by the algorithm is defined in relation to the first day of menses, which is regarded as day 1.

Comparison of relative variation in FSH and E1G during the luteal phase

To determine the proportional changes in FSH and E1G during the luteal–follicular phase transition, relative changes in FSH and E1G during the last 14 days of the cycle were analysed. To do this, concentrations of FSH and E1G during this interval were divided by the maximum value occurring on each cycle. Consecutive values were compared in order to determine the first significant rise (for FSH) and fall (for E1G).

FSH rise and reproductive ageing

To study the possible effect of reproductive ageing on FSHr, we analysed consistent variation of FSHr with two different parameters related to reproductive ageing: chronological age and initial levels of FSH (iFSH) in the cycle (mean value on days 1–5 of the cycle).

In addition we investigated the relationship between increasing levels of iFSH and follicular phase length, and compared with the relationship between increasing iFSH and FSHr. To do this, we distributed the cycles into three categories according to iFSH as: ≤5; > 5 to ≤10 and >10 IU/L. In a previous study, we found a progressive reduction in follicular phase length with increasing FSH based on these three categories, thus, we regard them an appropriate reference for this study (Miro et al., 2004a). Follicular phase length was considered as the interval from day of FSH rise to the day of the LH peak.

Statistical comparisons

To determine the effect of chronological and reproductive age on FSHr, regression analysis with ANOVA was used. All other comparisons were based on one way ANOVA.

Results

Distribution of the parameter day of FSH rise (FSHr)

The algorithm to determine FSHr was applied to a total of 836 complete menstrual cycles. Figure 1 shows examples of the application of the algorithm to two real cycles. The parameter FSHr adjusts to a normal distribution, and relative to the first day of menses, the mean value was −4.2, the median −4, and the SD 1.89. Day −4 was also the modal value, with a frequency of 25.21% (Figure 2 and Table 1).

Changes in the onset of FSH rise in relation to reproductive aging

Results from these analyses are shown in Figure 3. Analysis of the variation in the initial levels of FSH (iFSH) and FSHr for each cycle showed no significant correlation (R² = 0.002, P = 0.1924) for chronological age-FSHr, and (P = ) for iFSH–FSHr.

To determine the possible effect of chronological age on the onset of FSH rise at the beginning of the cycle, mean FSHr value was estimated for each subject, and the possible correlation was analysed. There was no significant correlation between both parameters (R² = 0.016, P = 0.2075).

Cycles were divided into three categories according to iFSH (≤5; > 5 to ≤10 and >10 IU/L). Total follicular phase length (from FSHr to day of LH peak) was compared between the three categories. A consistent reduction in follicular phase length with increasing iFSH category was found: 18.1, 17.2 and 16.6 days respectively, P < 0.0001.
In contrast, there was no significant difference in FSHr between the same three iFSH categories: $-4$, $-4$ and $-3.99$ days, $P = 0.9947$ Figure 4).

**Figure 2.** Distribution of the parameter ‘day of first sustained rise in FSH’ (FSHr) during the luteal-follicular transition in 836 cycles, from 102 women with regular menstrual cycles (median = 9 cycles per case). Day 1 corresponds to the first day of menses.

**Table I.** Some of the most relevant statistical parameters on the distribution of FSH rise during the menstrual cycle. (Data from 102 women, 836 cycles, median nine cycles per case.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>75% quartile</td>
<td>−3.00</td>
</tr>
<tr>
<td>Median</td>
<td>−4.00</td>
</tr>
<tr>
<td>25% quartile</td>
<td>−5.00</td>
</tr>
<tr>
<td>Mean</td>
<td>−4.1</td>
</tr>
<tr>
<td>Upper 95% mean</td>
<td>−3.98</td>
</tr>
<tr>
<td>Lower 95% mean</td>
<td>−4.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.05</td>
</tr>
<tr>
<td>$n$</td>
<td>836</td>
</tr>
</tbody>
</table>

In contrast, there was no significant difference in FSHr between the same three iFSH categories: $-4$, $-4$ and $-3.99$ days, $P = 0.9947$ Figure 4).

**Relative changes in FSH and estrogens during the last 14 days of the cycle**

FSH and E1G values during the last 14 days of the cycle (days $-14$ to $-1$) were divided by the maximum value found in each cycle. The mean value of each hormonal marker ratio, from day $-14$ to $-1$ are shown in Figure 5. From day $-14$, FSH values followed a negative slope, reaching a minimum value on day $-6$. From this day on, the first significant rise in FSH was found on day $-4$ ($P = 0.0033$). E1G levels too follow a negative slope for the first few days, stabilizing on day $-10$ through day $-5$ at 0.46, afterwards, values drop, with the first significant shift on day $-4$ ($P = 0.0002$).

**Discussion**

In spite of its relevance on follicular recruitment, the FSH rise during the luteal–follicular transition has been the object of very few quantitative studies, and the few existing reports are based on very few cases, and focus on issues other than consistency of the rise, or age-related variability. Our results indicate that in the normal cycle, the onset of FSH rise occurs consistently 4 days before menses, is directly related with a drop in estrogens levels, and is not affected by reproductive ageing.

An early study comparing variation in circulating levels of FSH during the luteal–follicular transition in conception and non-conception cycles, shows a distinct change to positive slope in the levels of circulating FSH about 11–12 days after the LH peak in non-conception cycles (Lenton et al., 1982). A similar, more recent, study found that in non-conception cycles, FSH profiles, both in serum and urine, show consistently a shift to positive slope around day 11 after the LH peak, or 4 days before menses (Qiu et al., 1997). Others reported a rise in FSH around 3 days before menses in non-conception cycles (Welt et al., 1997). Finally, a more recent study based on 35 young healthy women locates the FSH rise about 11–12 days after the shift in body basal temperature (van Zonneveld et al., 2003).

Clear changes in the activity of the gonadotrophin-releasing hormone pulse generator occur during the luteal–follicular transition, with an abrupt increase on day 11 after the LH peak (Hall et al., 1992; Welt et al., 2003), and the corpus luteum produces at least two hormones (inhibin A and estradiol) known to suppress FSH secretion. The relative effect of
these FSH inhibitory hormones has been investigated thoroughly.

In non-human primates injection of inhibin A during the early follicular phase suppresses FSH (Molskness et al., 1996), however, the effects were weak, and the concentrations used considerably high. Other studies using more physiological concentrations of inhibin A failed to exert any significant suppressive effect on FSH during the luteal–follicular transition (Fraser and Tsonis, 1994). Studies in humans suggest an absent or trivial role for inhibin A on FSH secretion during the luteal–follicular transition (Le Nestour et al., 1993; Lahlou et al., 1999; Welt et al., 2003) and that estradiol is the only significant regulator of FSH during the luteal–follicular transition (Hall et al., 1992; Le Nestour et al., 1993; Lahlou et al., 1999; Welt et al., 2003). Interestingly, we found that the first significant rise in FSH and drop in E1G occur on the same day, suggesting a close association between both episodes.

A reduction in the length of the follicular phase is one of the early known effects of reproductive ageing. Since this reduction occurs in parallel to an elevation in the initial Figure 3. Changes in day of FSH rise (FSHr) in relation to reproductive ageing. Regression analysis with ANOVA was applied between FSHr and (A) initial FSH levels in the cycle (iFSH), n = 796 cycles, Fr = 2.34; (B) chronological age, n = 102 women, Fr = 1.61.

Figure 4. Relationship between initial FSH levels in the cycle (iFSH) and (A) FSH rise day (FSHr); (B) total follicular phase length (FPL). All cycles were distributed into three categories according to the iFSH levels (≤ 5, n = 385; >5 to ≤ 10, n = 279; and >10, n = 132). Total FPL for each cycle is the interval between from FSHr to day of LH peak.

Figure 5. Relative variation of FSH and E1G levels during the last 14 days of the menstrual cycle. Daily levels of each hormonal marker were divided by the cycle’s maximum value. Figure shows the mean relative value for all cycles of each hormonal parameter. Changes in the relative values for consecutive days were compared, the dashed line indicates the first day when a significant shift (fall in E1G, P = 0.0002; rise in FSH, P = 0.0033) was detected.
levels of FSH, it has been hypothesized that an acceleration in follicular development occurs due to the higher FSH levels (Lenton et al., 1988; Klein et al., 2002; Miro et al., 2004a). Further support to the connection between reduction in follicular phase length, accelerated follicular development and higher FSH has been provided by studies showing comparatively higher mitotic index in granulosa cells from hyper-stimulated women, as well as those over 40 years of age (Gougeon and Testart, 1990; Gougeon, 1998).

An alternative interpretation to the accelerated follicular development hypothesis has been proposed, whereby the reduction in length is the result of earlier onset of FSH rise (van Zonneveld et al., 2003). In support of this view is a study reporting that follicular growth starts earlier in older women (Santoro et al., 2003b). We found no effect of chronological age, or initial FSH levels on the onset of FSH rise, moreover, there was an inverse relationship between initial levels of FSH and total follicular phase length (from day of FSH rise to LH peak). An explanation for these discrepancies might be that the above study compared statistical changes in the magnitude of daily FSH levels, and thus, the shift is found first in the group with the higher FSH (i.e. the older one). Our approach, though, is based on changes in the slope, and therefore, not subjected to this effect.

Since the age-related acceleration in follicular growth appears to affect events involved in the early follicular phase (Klein et al., 2002; Miro et al., 2004a), including follicle recruitment and selection, these results are clinically relevant. For instance, further research is needed to determine whether FSH levels prior to menses provide a better indicator of ovarian reserve than the conventional day 3 post-menses approach, or whether hyperstimulation treatment for infertility might benefit from starting 4 days before the onset of menses. Finally, it seems worth investigating the contraceptive potential of manipulating this stage of the cycle.

References


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