Elevated FSH concentrations in imminent ovarian failure are associated with higher FSH and LH pulse amplitude and response to GnRH

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Imminent ovarian failure (IOF) in women is characterized by regular menstrual cycles and elevated early follicular phase FSH. This study explored underlying neuroendocrine causes of elevated FSH concentrations on day 3 of the menstrual cycle. The characteristics of episodic secretion of FSH and LH, the pituitary response to gonadotrophin-releasing hormone (GnRH), plasma oestradiol, and dimeric inhibin A and inhibin B on day 3 were compared in 13 women with elevated FSH concentrations (>10 IU/l) and 16 controls. FSH amplitudes were higher in the IOF group than in the controls (P < 0.0001). The FSH pulse frequency did not differ between groups. The FSH response to GnRH was higher in the IOF patients than in the controls (P < 0.0001). Mean LH, LH amplitude and LH response to GnRH were higher in the IOF group, but LH pulse frequency did not differ between the groups. Concentrations of inhibin A and inhibin B were lower in the IOF group, while oestradiol showed no differences. We concluded that in women with IOF, the pituitary is more sensitive to GnRH. This leads to higher FSH and LH pulse amplitudes which underlie the elevated FSH concentrations in the early follicular phase.

Key words: gonadotrophin pulsatility/imminent ovarian failure/inhibin/reproductive ageing

Introduction

Early follicular phase FSH concentrations are often used to predict the outcome of IVF (Muasher et al., 1988; Scott et al., 1989; Licciardi et al., 1995; Scott and Hofmann, 1995). Elevated day 3 FSH concentrations are well correlated with a poor response in ovarian stimulation and low pregnancy rates (Scott and Hofmann, 1995). These patients are often referred to as having imminent or incipient ovarian failure (IOF) (Jones et al., 1986; Cameron et al., 1988; Buckler et al., 1991).

It is generally assumed that early follicular phase FSH, at the time of recruitment of follicles, is elevated due to diminished ovarian feedback of steroids and inhibins (Sherman and Korenman 1975; Lee et al., 1988; MacNaughton et al., 1992). Inhibins are dimeric proteins that selectively inhibit FSH secretion. Inhibin A is composed of a common α subunit and a βA subunit, and inhibin B consists of an α subunit combined with a βB subunit. In recent years, studies on the differential secretion of inhibin A and inhibin B in the menstrual cycle (Groome et al., 1996) and in-situ hybridization studies (Roberts et al., 1993) have shown that inhibin A appears to be primarily secreted by the mature follicle and corpus luteum. Inhibin B appears to be secreted by smaller pre-ovulatory follicles. Decreased concentrations of both inhibin A and inhibin B can theoretically contribute to high FSH concentrations in the early follicular phase.

It is not fully clear, however, what compounds, attributing to the rate of pituitary FSH secretion, are altered in patients with IOF. Hypothalamic causes for elevated FSH concentrations could induce an altered release mode (more pulses) of pulsatile gonadotrophin-releasing hormone (GnRH) as in mothers of dizygotic twins (Lambalk et al., 1998). At the level of the pituitary a higher sensitivity to the GnRH pulses might explain the elevation of FSH. FSH and LH are released in a pulsatile manner. Studying episodic gonadotrophin release enables observation of dynamic changes in the FSH release of patients with IOF. Since pulsatile LH is considered to be a good representation of the episodic activity of the hypothalamic GnRH pulse generator, a detailed analysis of its episodic gonadotrophin secretion in combination with information on the LH and FSH response to a GnRH challenge, will potentially reveal responsible mechanisms.

The aim of this study was to evaluate the hypothalamic and pituitary contribution to the elevated FSH concentrations in women with IOF. Therefore, the pulsatile release of FSH and LH on day 3 of the menstrual cycle was examined and the subsequent pituitary response to GnRH in patients with IOF and controls was evaluated. The role of the ovary as a potential cause of the differences in gonadotrophin secretion was also evaluated by concomitant measurement of the concentrations of oestradiol, inhibin A and inhibin B.

Materials and methods

Subjects

Patients, referred to our infertility clinic, were all screened on day 3 of the menstrual cycle for possible elevated FSH for a period of 3 years between January 1995 and January 1998. All patients with a FSH value of ≥10 IU/l were asked to participate in the study. In our IVF clinic, patients with basal FSH values ≥10 IU/l show very
poor outcome with respect to the number of oocytes retrieved and pregnancy rate. Controls were either patients referred to our clinic for reversal of tubal ligation or volunteers recruited by advertisement, with cycle day 3 FSH values of <10 IU/l. There was no attempt to match controls and patients for age, weight or other demographic characteristics. Patients in both the study population and the control group had regular menstrual cycles of 21–35 days, no hot flushes and no medical or hormonal treatment during at least 3 months prior to the study. Controls had no history of infertility. The local ethics committee approved the study. All subjects gave informed consent.

**Study design**

Serial blood samples were collected on day 3 of a later menstrual cycle (study cycle). An indwelling catheter was placed in a forearm vein for 6 h. Blood was drawn into heparinized tubes every 10 min. Sampling started between 08:00 and 09:00. Immediately after the last sample a GnRH challenge with an i.v. injection of 100 µg GnRH (HRF; Wyeth, Hoofddorp, The Netherlands) was given and three additional blood samples were taken after 30, 60 and 90 min. The basal body temperature (BBT) of all subjects was measured during the study cycle.

**Hormone measurements**

LH and FSH were measured in duplicate by commercially available immunometric assays (Amerlite; Amersham, Bucks, UK). The lower limit of detection was 0.3 IU/l for LH and 0.5 IU/l for FSH. The assays were calibrated against the 1st International Reference Preparation (IRP) 68/40 and 2nd IRP 78/549 for LH and FSH respectively. Of each individual, all samples were analysed in the same run for each hormone. The inter- and intra-assay coefficients of variation (CV) were <9 and 5% for LH and FSH.

Inhibin A and inhibin B were measured in duplicate by ultra-sensitive two-site enzyme immunoassays (Serotec, Oxford, UK). The development of these commercially available assays was based on the work of Groome et al. (Groome et al., 1994, 1996). The lower limit of detection was 3 pg/ml for inhibin A and 15 pg/ml for inhibin B. The inter-assay CV was <9% for both inhibin A and inhibin B. Oestradiol was measured by radioimmunoassay (Sorin Biomedical, Saluggia, Italy) with a lower limit of detection of 18 pmol/l and an inter-assay CV of <11%. For data analysis, values below the lower limits of detection were assigned the value of assay sensitivity.

**Pulse analysis**

Pulse analysis was carried out with a computerized version of a previously developed and validated method (Lambalk et al., 1985; Scheele et al., 1987). The algorithm is valid for replicate repeated measurements of LH and FSH with a chance of <5% to indicate non-existing pulses as a pulse in series of 100 samples taken from pooled serum. This method is particularly of value in detecting episodic secretion of hormones with relatively long half-lives because pulses are indicated when a significant rise occurs without the requirement of a subsequent decline. Nadirs preceding the pulses are indicated as marker points in the hormone patterns rather than the pulses themselves.

**Statistical analysis**

The patients had to have elevated day 3 FSH in the screening as well as in the study cycle. The controls had normal FSH concentrations in both cycles. For each subject, the mean concentrations of LH and FSH, the mean pulse amplitude over the 6 h period and the frequency of LH and FSH pulses per 6 h were calculated. The maximal gonadotrophin increment was taken as a parameter for the response to the GnRH challenge. The non-parametric Mann–Whitney U-test was used for differences between groups. $P < 0.05$ was considered to be statistically significant. Spearman rank correlations were calculated for overall relation between mean FSH concentrations and inhibin A and inhibin B.

**Results**

**Baseline characteristics**

There were 13 patients in the IOF group, 16 in the control group. Age, body mass index (BMI), exposure to smoking in pack years (number of years of daily intake of 20 cigarettes), and current smoking were similar for the groups (Table I). All controls were ovulatory as evidenced by biphasic BBT charts. One patient in the IOF group had a monophasic BBT. Menstrual cycle length and follicular phase length were significantly shorter in the IOF group compared to controls.

**Gonadotrophins**

Figure 1 shows examples of the secretory gonadotrophin patterns and responses to GnRH from each group.

**FSH**

The characteristics of pulsatile FSH secretion are summarized in Figure 2. The mean FSH was significantly higher in the IOF group ($15.4 \pm 5.3$ IU/l), than in the control group ($4.4 \pm 1.1$ IU/l, $P < 0.0001$). The FSH pulse amplitude was higher ($P < 0.0001$) in the IOF group ($1.20 \pm 0.56$ IU/l) compared with the controls ($0.37 \pm 0.11$ IU/l). The IOF group showed a significant ($P < 0.0001$) increase in FSH response to GnRH ($10.3 \pm 5.5$ IU/l) compared with the control group ($2.3 \pm 1.3$ IU/l). The pulse frequencies did not differ.

**LH**

Figure 3 summarizes the results for pulsatile LH secretion. Mean LH in the IOF group ($5.7 \pm 2.0$ IU/l) was significantly higher ($P < 0.0001$) than in the control group ($2.5 \pm 0.9$ IU/l). The LH pulse amplitudes in the IOF group ($1.77 \pm 0.71$ IU/l) were significantly higher ($P < 0.001$) than in the control group ($0.92 \pm 0.39$ IU/l). There were no differences in the LH pulse frequency. As with FSH, the maximal LH increment to GnRH was higher ($P < 0.001$) in the IOF group ($19.9 \pm 9.1$ IU/l) than in the controls ($9.5 \pm 3.4$ IU/l).
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Figure 1. Examples of LH (dashed lines) and FSH (solid lines) secretion patterns and responses to gonadotrophin-releasing hormone (GnRH) in a woman with imminent ovarian failure (IOF) (upper panel) and a control patient (lower panel) on cycle day 3. ▲ = start of an FSH pulse, △ = start of an LH pulse.

Figure 2. Means and SD of different parameters of episodic FSH secretion on cycle day 3 in imminent ovarian failure (IOF) patients compared with controls. *P < 0.0001.

Figure 3. Means and SD of different parameters of episodic LH secretion on cycle day 3 in imminent ovarian failure (IOF) patients compared with controls. *P < 0.001; **P < 0.0001.

Figure 4. Means and SD of oestradiol, inhibin A and inhibin B on cycle day 3 in imminent ovarian failure (IOF) patients compared with controls. *P < 0.05; **P < 0.01.

Oestradiol and inhibins

Figure 4 shows the results of oestradiol, inhibin A and inhibin B measurements. Patients in the IOF group showed oestradiol concentrations similar to the control group. In the IOF group, serum concentrations of inhibin A and inhibin B were significantly lower than in the control group. Mean inhibin A in the IOF group was 5.5 ± 6.1 pg/ml and in the control group 7.9 ± 3.2 pg/ml, P < 0.01. Mean inhibin B in the IOF group was 52.9 ± 44 pg/ml, and in the control group 81.8 ± 40 pg/ml, P < 0.05. There was an inverse correlation between inhibin A and FSH (r = −0.71, P < 0.001). An inverse correlation was also found (r = −0.63, P < 0.001) for inhibin B and FSH.

Discussion

Elevated FSH in women with IOF can be explained by the presence of higher FSH pulses. These larger FSH pulses result from an increase in pituitary response to GnRH. Moreover, there appears to be a hitherto unreported, subtle concomitant
rise of LH on day 3 of the cycle. The secretory dynamics of LH appear to be a copy of FSH with similar higher pulse amplitudes and responses to GnRH.

These findings contrast with a previous study of the episodic FSH secretion in mothers of hereditary dizygotic twins. In those we found elevated FSH concentrations in association with an increased number of FSH pulses, without changes in the response to GnRH and without alterations in LH and feedback (Lambalk et al., 1998). This indicates that in twin mothers the origin of elevated early follicular phase FSH is pituitary or supra-pituitary, whereas elevated FSH in imminent ovarian failure is of ovarian origin. These contrasting findings, in seemingly identical conditions, i.e. elevated early follicular phase FSH, underscore the importance of investigating the dynamics of the episodically secreted gonadotrophins.

Only a few others have studied the dynamics of gonadotrophin secretion in relation to the luteal follicular transition (Hall et al., 1992) and in reproductive ageing (Wilshire et al., 1995; Klein et al., 1996b; Reame et al., 1996). The increase in LH pulse frequency in this stage of the menstrual cycle, due to increased GnRH pulsatility, shows the importance of GnRH action in the increase of FSH in the early follicular phase (Hall et al., 1992).

The age-related increase in FSH concentration is associated with enhancement of pulsatile LH secretion, particularly in the LH pulse amplitudes as previously reported (Reame et al., 1996). This is in full agreement with the current observations and greater pituitary responsiveness to GnRH is probably responsible for this. In a smaller pulsatility study (Klein et al., 1996b), no differences were detected in the endocrinology of the early follicular phase in older and younger cycling women. Moreover, other authors (Wilshire et al., 1995) reported no differences in LH pulse amplitudes and number of pulses during the early follicular phase in younger versus older women. In a frequent sampling study of LH in the late follicular phase (Matt et al., 1998), a lower number of LH pulses was found in older women, which may indicate a slowing of the GnRH pulse generator in that phase. This is in line with our earlier observations in younger versus older post-menopausal women (Lambalk et al., 1997). Whether a slowing of the GnRH pulse generator demonstrated in the late follicular phase is responsible for a paradoxical increased concentration of FSH in the early follicular phase is highly questionable (Lambalk et al., 1989).

The GnRH challenge tests have been used by a few other authors to study gonadotrophin secretion dynamics in reproductive ageing. In contrast to our findings, one study (Fujimoto et al., 1996) described a lower gonadotrophin response to GnRH in older women. This apparent discrepancy may be explained by the fact that Fujimoto used age as a primary variable, while we compared the results of IOF patients with a control group. Muasher et al. found that the FSH/LH response to GnRH was correlated with results in IVF (Muasher et al., 1988). In this study, identical to our results, higher gonadotrophin responses in patients with elevated FSH were observed. Finally, an increased response of both LH and FSH to GnRH was described in a study on perimenopausal women but with irregular cycles of 10–90 days (Schmidt et al., 1996), while no differences in response to GnRH were observed in older subjects with a regular cycle.

Ovarian feedback seems to play a role in alterations of early follicular phase FSH secretion. There were no differences in oestriadiol concentration between the groups in our study. This is in agreement with previous observations (Sherman and Korenman, 1975; Lee et al., 1988; Buckler et al., 1991). In the current study, the elevated cycle day 3 FSH concentrations were found to be associated with lower concentrations of inhibin A and inhibin B. A number of studies have indicated an inverse relationship between inhibin B and calender age (Klein et al., 1996a) and a relationship between low inhibin B and poor outcome in assisted reproduction (Seifer et al., 1997). One study (Reame et al., 1998), also showed older cycling women to have lower follicular phase inhibin B. Recently, inhibin B was reported to be lower in the early follicular phase of older women (Welt et al., 1999), together with lower inhibin A on the day after the LH peak. In that study FSH concentrations were slightly higher in the early follicular phase of older (>=35 years) women. It is believed that lower inhibin B concentrations signify a decline in size of the available cohort of follicles, and increased early follicular phase FSH concentrations probably represent the same. By focusing on differentiation between high and normal FSH concentrations the results presented here clearly indicate a role of deficient inhibin A. Inhibin A is predominantly secreted in the luteal phase (Groome et al., 1996). Therefore, the early follicular phase rise of FSH may, at least in part, result from some luteal phase deficiency of the previous cycle (Danforth et al., 1998). These authors observed a good inverse relationship between luteal inhibin A concentrations and day 3 FSH values. In addition, it has been shown (Seifer et al., 1996) that cultured luteinised granulosa cells of women with high FSH concentrations produce less inhibin A.

Activins were not measured in this study. Inconsistent data are available on the role of activins in pituitary stimulation. Some authors (Ying et al., 1988) report that activins are capable of direct pituitary FSH stimulation, whereas others (Katayama and Conn, 1994) question this. Nevertheless, slightly higher activin A concentrations are found in older pre-menopausal women (Reame et al., 1998). These data were confirmed by another study (Santoro et al., 1999). So far, the role of activin A as a classical hormone involved in gonadal function has remained unclear (Harada et al., 1996).

Based on the current understanding of inhibin physiology (Hayes et al., 1998), it is not possible to explain fully the exaggerated GnRH-induced gonadotrophin response in patients with IOF. Inhibins selectively inhibit FSH secretion, so the decreased inhibin A and inhibin B may account for the increased FSH response, but not for the higher LH response. The concentrations of oestradiol and progesterone (data not shown) were not lower in IOF patients. Therefore, a decline in the activity of other ovarian regulators might be involved in the loss of negative feedback across the cycle. We speculate that a possible mediator of reproductive ageing is loss of gonadotrophin surge inhibiting factor (GnSIF) activity. This ovarian peptide keeps the pituitary in a low state of responsiveness to GnRH (de Koning, 1995; Fowler et al., 1996b; 1998).
References


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