Circulating immunoreactive and bioactive follicle stimulating hormone concentrations in anovulatory infertile women and during gonadotrophin induction of ovulation using a decremental dose regimen

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Our purpose was to determine whether decreased follicle stimulating hormone (FSH) activity, either systemic or at the follicular level, is involved in impaired follicle growth associated with normogonadotrophic anovulation. To differentiate between the possible levels of disturbance, bioactive (BIO-FSH; using the in-vitro rat granulosa cell aromatase bioassay) and immunoreactive (IRMA-FSH) FSH serum concentrations of three groups of subjects were compared: (i) 172 normogonadotrophic anovulatory infertile women during baseline conditions, (ii) 22 clomiphene-resistant polycystic ovary syndrome patients undergoing ovulation induction by exogenous gonadotrophins using a decremental dose regimen, and (iii) nine regularly cycling controls. BIO-FSH [13.2 (range 0.8–29.5) IU/l] and IRMA-FSH [4.4 (range 1.2–9.3) IU/l] concentrations in anovulatory women during baseline conditions were significantly lower than maximum concentrations reached during the follicular phase in controls [18.7 (13.2–23.4) and 6.4 (5.7–10.0) IU/l respectively], but were not significantly different from initial concentrations in controls [10.4 (7.2–19.6) and 4.8 (2.8–8.2) IU/l respectively]. Moreover, concentrations of IRMA-FSH and BIO-FSH were negatively correlated (r = −0.25, P = 0.01, and r = −0.24, P = 0.02 respectively) with the interval between last vaginal bleeding and blood sampling. Maximum concentrations of IRMA-FSH [7.6 (3.9–10.9) IU/l] during ovulation induction by gonadotrophins were not significantly different from maximum [6.4 (5.7–10.0) IU/l] concentrations in controls, whereas maximum BIO-FSH concentrations [13.5 (8.7–17.4) versus 18.7 (13.2–23.4) IU/l] were significantly lower. Our findings suggest that (i) circulating FSH does not reach concentrations that are sufficient to induce normal follicle development in anovulatory women during baseline conditions, and (ii) the FSH threshold for ovarian stimulation of this patient group is not different from normal.

Key words: anovulation/FSH/gonadotrophins/hyperandrogenemia/polycystic ovary syndrome

Introduction

Pituitary follicle stimulating hormone (FSH) plays an essential and complex role in the ovarian reproductive process, including enhancement of aromatase enzyme activity, induction of luteinizing hormone (LH) receptor formation and increase in FSH receptor number on granulosa cells and stimulation of general cell functions such as DNA and protein synthesis (Hsueh et al., 1989). Despite the growing knowledge with regard to FSH action in vitro, the in-vivo relationship between FSH and ovarian pathophysiology is still incompletely understood.

Although ‘normogonadotrophic’ anovulation by definition suggests that abnormal gonadotrophin secretion by the pituitary can be excluded as an important aetiologic factor, augmented LH concentrations (and increased LH/FSH ratios) are frequently observed and are considered a crucial criterion for diagnosis of polycystic ovary syndrome (PCOS) (Fauser and De Jong, 1993). Considering the crucial role of FSH in normal follicular development, it may be postulated that decreased FSH activity (either systemic or at the follicular level) is involved in impaired follicle growth associated with chronic normogonadotrophic anovulation. Immunoreactive FSH concentrations do not necessarily reflect bioactivity of circulating FSH, since FSH consists of multiple isohormones with different carbohydrate contents, each with its own half-life and bioactivity. The distribution of various isoforms determines bioactivity of circulating FSH. Due to considerable methodological difficulties regarding the assessment of FSH bioactivity in vivo, only a few studies applying in-vitro bioassay systems have been performed during the normal menstrual cycle and they have yielded conflicting results (Jia et al., 1986; Padmanabhan et al., 1987; Reddi et al., 1990). Preliminary studies have shown bioactive FSH serum concentrations (Fauser et al., 1991) and follicular fluid concentrations (Erickson et al., 1992; Fauser, 1994) to be within the normal range in anovulatory women with or without PCOS.

Intra-ovarian modification of granulosa cell function is considered important for regulation of follicular development (Hillier, 1991). As granulosa cells of PCOS patients show normal (Erickson et al., 1979) or even elevated (Mason et al., 1994) FSH-induced aromatase activity in vitro, it may be postulated that locally produced factors rather than defective granulosa cells are involved in arrested follicle growth in...
these patients. Such factors supposedly lead to a higher FSH threshold (Brown, 1978), which in turn implies that supraphysiological FSH concentrations may be needed to induce ovulation successfully. However, data on FSH concentrations during gonadotrophin ovulation induction are scarce (Healy and Burger, 1983). Data from patients undergoing gonadotrophin induction of ovulation with a decreasing dose regimen (Schoot et al., 1995; van Santbrink et al., 1995a) are of particular interest since these schedules more closely mimic physiological circumstances (decreasing follicular phase FSH serum FSH concentrations), thus providing insight into the sensitivity of ovaries to FSH stimulation. In order to elucidate mechanisms underlying anovulation further and to distinguish between abnormalities at the pituitary or ovarian level as a possible cause of impairment of follicle growth, serum FSH concentrations were studied using immunoradiometric assays (IRMA) and the in-vitro rat granulosa cell aromatase bioassay (BIO) in three groups of subjects: (i) normogonadotrophic anovulatory infertile patients, (ii) patients undergoing gonadotrophin ovulation induction with a decreasing dose regimen and (iii) regularly cycling controls.

Materials and methods

Subjects and protocol

The study was performed according to the guidelines of the declaration of Helsinki and was approved by the Ethics Review Committee of the Dijkzigt University Hospital, Rotterdam, The Netherlands. Informed consent was obtained from all participants involved.

Three groups of patients participated in this study. The first group comprised 172 women attending our fertility clinic because of oligomenorrhoea (intervals between uterine bleeding exceeding 42 days, n = 127) or amenorrhoea (no menstrual periods for at least 6 months, n = 45). For their clinical, endocrine and sonographic characteristics, see Table 1. Patients with abnormal immunoreactive FSH (normal range 1.0–10.0 IU/l), thyroid-stimulating hormone (normal range 0.2–4.2 mU/l) or prolactin concentrations (normal range <15 μg/l) were excluded. Blood sampling was scheduled at day 3–5 of a spontaneous or progesterone-induced withdrawal bleeding in 77 patients. In the remaining 95 patients, blood samples were drawn at random at the time of the intake visit (median 10 (range 1–84) days since the last menstrual bleeding). Transvaginal ultrasound examination was performed at the time of blood withdrawal. A subset of PCOS patients was arbitrarily defined according to a combination of clinical, endocrine and sonographic criteria that have been published previously (Fauser et al., 1991). The diagnosis of PCOS required positive scoring in at least three out of four of the following criteria: (i) polycystic appearance of ovaries upon transvaginal ultrasound examination (Pache et al., 1992), (ii) hirsutism (Ferriman and Gallway (1961) score >8), (iii) elevated androgen concentrations as assessed by an elevated free androgen index (testosterone concentration × 100 divided by sex hormone-binding globulin concentration (SHBG)) >5, and/or dehydroepiandrosterone sulphate (DHEAS) >10 μmol/l, or (iv) obesity (BMI, weight divided by square length) >26 kg/m².

For the second group, 28 of the above-mentioned 172 anovulatory women who underwent induction of ovulation by daily i.m. administration of exogenous gonadotrophins were asked to participate in this study. Six patients remained anovulatory and were excluded from further analysis. Ovulation was induced successfully in 22 patients, as shown by ultrasound evidence of follicular growth and rupture, and elevated mid-luteal progesterone concentrations (mean ± SD 148 ± 101 nmol/l). Mean age was 28 ± 2 years and mean BMI 27.6 ± 4.3 kg/m². All women suffered from PCOS, as described above, and had failed to ovulate despite previous treatment with increasing doses of clomiphene citrate up to 150 mg per day from cycle day 3–7. A decremen tal dose regimen of gonadotrophins was applied. After pretreatment for 3 weeks with a gonadotrophin-releasing hormone (GnRH) agonist (Buserelin®, Hoechst, Amsterdam, The Netherlands) to avoid premature luteinization, randomly chosen human menopausal gonadotrophins (HMG, Humegon®, N.V. Organon, Oss, The Netherlands), or purified FSH (Metrodin®, Serono, Amsterdam, The Netherlands) were started at an initial dose of 2 ampoules (150 IU FSH) i.m. per day. Single batches were used for both HMG and purified FSH. If at least one follicle exceeded a diameter of 9 mm, the dose was decreased to 1.5 ampoule (=112.5 IU) per day for a fixed period of 3 days. A further decrease to 1 ampoule (75 IU) per day was continued until the mean diameter of the leading follicle exceeded 18 mm (Schoot et al., 1995). At this time, administration of buserelin and exogenous gonadotrophins was discontinued and 10 000 IU of human chorionic gonadotrophin (HCG, Pregnyl® Organon) was administered i.m. as a bolus injection. Daily blood sampling and ultrasound scanning were performed from the start of gonadotrophin treatment until 1 day after HCG administration.

The third group consisted of nine regularly cycling young healthy women who served as controls. They entered the study through poster advertisement and were paid for participation. Mean age was 26 ± 5 years and mean cycle length was 28 ± 1 days. Mean BMI was 22.7 ± 2.3 kg/m². No endocrine disease was present, and no medication had been taken for at least 6 months. Starting 2–3 days before expected menses (estimation based on length of preceding cycles), daily blood withdrawal and transvaginal ultrasound examination was performed every other day until a single follicle exceeding 9 mm diameter was observed, at which time selection of the dominant follicle was presumed to occur (Pache et al., 1990; van Santbrink et al., 1995b). Thereafter, daily sampling and ultrasound scanning took place. Ovulatory cycles were demonstrated in all subjects by ultrasound detection of ovulation and confirmed by elevated mid-luteal progesterone concentrations (mean ± SD 50 ± 17 nmol/l).

Hormone assays

Blood was centrifuged within 30 min after withdrawal and serum was stored at −20°C until assayed. Immunoradiometric assay (IRMA) kits (Medigenix, Fleurus, Belgium) were used to measure immunoreactive FSH and LH concentrations, as previously described (Fauser et al., 1991). Data are expressed in terms of the Medical Research Council (MRC) 78/549 reference preparation for FSH, and the MRC 68/40 for LH. Intra- and interassay coefficients of variation were <3 and 8% for FSH and <5 and 15% for LH respectively. Cross-reactivity with α-subunit was 0.06% for the FSH IRMA and 0.4% for the LH IRMA. Bioactive FSH concentrations in serum samples were measured using the in-vitro rat granulosa cell aromatase bioassay (Dahl and Hseau, 1989). The reference preparation (human pituitary gonadotrophin I-3; FSH potency, 3100 IU/mg by the HCG-augmentation bioassay) was obtained using the Second Reference Preparation of human menopausal gonadotrophin standards from the National Hormone and Pituitary Distribution Program, NIADDK (Bethesda, MD, USA). As shown previously, LER-907 was parallel to I-3 (Dahl and Hseau, 1988) and the IRMA standard (78/549) (Fauser et al., 1990). Intra- and interassay variations were 9 and 12%. Samples from a given patient were run in the same assay. All steroids were measured by radioimmunoassay. Testosterone measurements have been described previously (van Landeghem et al., 1981). SHBG, oestradiol, androstenedione and DHEAS kits were
concentration divided by the IRMA-FSH concentration on an individual basis. The bio-to-immuno ratio (B/I ratio) was defined as BIO-FSHmin and IRMA-FSHmin and compared to the highest BIO-FSH and IRMA-FSH concentrations in controls and ovulation induction, concentrations at treatment day 1 of ovulation induction (usually the day of HCG administration). The lowest BIO-FSH and IRMA-FSH concentrations subjects during ovulation induction were considered to be the FSHmax. The lowest BIO-FSH and IRMA-FSH concentrations in patients during the stimulation phase of ovulation induction (usually the day of HCG administration). The bio-to-immuno ratio (B/I ratio) was defined as the BIO-FSH concentration divided by the IRMA-FSH concentration on an individual basis.

Based on these findings, FSH serum concentrations in the present study were classified as initial (FSHini), maximum (FSHmax) and minimum (FSHmin) concentrations in controls and ovulation induction patients. In controls, FSHini was defined as levels found at the first day of a regular menstrual period. In patients undergoing gonadotrophin ovulation induction, concentrations at treatment day 1 (before initiation of treatment) were considered initial levels. The highest BIO-FSH and IRMA-FSH concentrations in controls and subjects during ovulation induction were considered to be the FSHmax. The lowest BIO-FSH and IRMA-FSH concentrations (usually occurring 2–3 days before the LH peak) in controls were defined as BIO-FSHmin and IRMA-FSHmin and compared to the lowest concentrations found in patients during the stimulation phase of ovulation induction (usually the day of HCG administration). The bio-to-immuno ratio (B/I ratio) was defined as the BIO-FSH concentration divided by the IRMA-FSH concentration on an individual basis.

### Data analysis

Data are presented as mean ± SD if distributed normally, and as median and range (in parentheses) if distributed otherwise. Box-and-whisker plots (SPSS for Windows, SPSS Inc.) were used to depict data that were not normally distributed. Comparisons of means or medians between groups were performed using Student’s unpaired t-test or the Mann-Whitney test respectively. The correlation coefficients between initial, maximum and minimum B/I ratios of regularly cycling controls were 0.03 (0.02; 0.04; 0.001; 0.01). Part of inclusion criteria (no statistical evaluation).

### Results

#### Bioactive and immunoreactive serum FSH concentrations in regularly cycling women

Daily IRMA- and BIO-FSH concentrations and FSH B/I ratios of controls are shown in Figure 1. Median IRMA-FSHini, IRMA-FSHmax and IRMA-FSHmin concentrations in regularly cycling controls were 4.8 (2.8–8.2), 6.4 (5.7–10.0) and 2.2 (0.7–4.7) IU/l respectively (Figure 2). Median BIO-FSHini, BIO-FSHmax and BIO-FSHmin concentrations in regularly cycling controls were 10.4 (7.2–19.6), 18.7 (13.2–23.4) and 6.3 (4.2–12.4) IU/l respectively. Median initial, maximum and minimum B/I ratios of regularly cycling controls were 2.5 (1.5–3.0), 2.4 (2.2–3.3) and 2.3 (1.8–8.6) respectively. Significant differences (P < 0.05) were found for IRMA-FSH and BIO-FSH between FSHini and FSHmax, FSHmax and FSHmin, and FSHini and FSHmin. With regard to initial, maximum and minimum B/I ratios in controls, no two groups were significantly different at the 0.05 level. Correlation coefficients between initial, maximum and minimum IRMA-FSH and BIO-FSH concentrations were 0.77 (P = 0.02), 0.63 (not significant) and 0.46 (not significant) respectively. The median decrease from maximum to minimum concentrations of IRMA-FSH and BIO-FSH concentrations and the number of days of decrease in controls are shown in Table II.

#### Bioactive and immunoreactive serum FSH concentrations in normogonadotrophic anovulatory patients

The endocrine characteristics of 172 anovulatory patients are shown in Table I. Significantly lower BIO-FSH, but not IRMA-FSH, concentrations were found in amenorrhoeic as compared to oligomenorrhoeic subjects, but no significant difference was found with regard to B/I ratio. LH, androstenedione and testosterone concentrations were significantly higher in amenorrhoeic as compared to oligomenorrhoeic women. The
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IRMA-FSH, BIO-FSH and LH concentrations of PCOS patients were not significantly different from those of non-PCOS patients, whereas androstenedione concentrations were significantly higher ($P = 0.01$). A significant correlation was found between IRMA-FSH and BIO-FSH concentrations in all anovulatory subjects ($r = 0.41, P < 0.001$, data not shown). A negative correlation was found between the number of days between the vaginal bleeding and blood withdrawal (bleeding-sampling interval) and IRMA-FSH ($r = -0.25, P = 0.01$) or BIO-FSH ($r = -0.24, P = 0.02$) concentrations (Figure 3). IRMA-FSH and BIO-FSH concentrations were not found to be correlated with LH, testosterone, androstenedione, or DHEAS concentrations (data not shown). Neither did we find a correlation between IRMA-FSH or BIO-FSH concentrations and the total number of ovarian follicles in anovulatory subjects. No correlation was found between bleeding-sampling interval and androstenedione and testosterone concentrations (data not shown).

Median IRMA-FSH concentrations in anovulatory patients [4.5 (1.2–9.3) IU/l] did not differ significantly from IRMA-FSHini concentrations in controls, whereas they were significantly lower ($P = 0.001$) than IRMA-FSHmax and significantly higher ($P = 0.001$) than IRMA-FSHmin concentrations in controls (Figure 2). Median BIO-FSH concentrations in anovulatory patients [13.0 (0.80–27.5) IU/l] were not significantly different from BIO-FSHini in regularly cycling controls, whereas they were significantly lower ($P = 0.01$) than BIO-FSHmax and significantly higher ($P = 0.01$) than BIO-FSHmin concentrations in regularly cycling controls (Figure 2). Median FSH B/I ratios of anovulatory patients [2.7 (0.1–64.0)] showed no significant differences as compared to initial, maximum or minimum values in controls.

**Bioactive and immunoreactive serum FSH concentrations in normogonadotrophic anovulatory patients during gonadotrophic induction of ovulation**

Daily IRMA- and BIO-FSH concentrations and FSH B/I ratios of patients undergoing gonadotrophin induction of ovulation using a decremental dose regimen and controls are shown in Figure 1. Initial, maximum and minimum concentrations of IRMA-FSH and BIO-FSH during induction of ovulation as compared to controls are shown in Figure 2. In ovulation

**Figure 1.** Daily follicular phase IRMA-FSH and BIO-FSH serum concentrations and B/I ratios in polycystic ovary syndrome (PCOS) patients undergoing ovulation induction by gonadotrophins using a decreasing dose regimen (left panel) and in regularly cycling controls (right panel). Values represent mean ± SEM and are grouped according to the day of human chorionic gonadotrophin (HCG) injection during ovulation induction and the spontaneous luteinizing hormone (LH) surge in controls.
Figure 2. IRMA-FSH and BIO-FSH serum concentrations in 172 normogonadotrophic anovulatory infertile women (left panel) as compared to the initial, maximum and minimum concentrations in 22 patients undergoing ovulation induction using a decremental dose regimen (middle panel) and nine regularly cycling controls (right panel). Boxes indicate the 25th and 75th percentiles, with a solid line within the box showing the median value; whiskers show the largest and smallest observed value that is not an outlier. Outliers are shown by open circles in the plot and are defined as values more than 1.5 box-lengths from the 25th or 75th percentile.

Table II. Median values for decrease in serum follicle stimulating hormone (FSH) from maximum to minimum concentrations in polycystic ovary syndrome (PCOS) during gonadotrophin ovulation induction with a decremental dose regimen and in unstimulated regularly cycling controls. Ranges are given in parentheses

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n = 22)</th>
<th>Controls (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRMA-FSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days between FSHmax and FSHmin</td>
<td>4 (1–9)</td>
<td>7 (6–13)</td>
</tr>
<tr>
<td>Daily decrease (IU/l)</td>
<td>0.6 (0.1–2.1)</td>
<td>0.5 (0.1–0.9)</td>
</tr>
<tr>
<td>Daily decrease (% of FSHmax)</td>
<td>9 (2–29)</td>
<td>7 (2–13)</td>
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<tr>
<td>BIO-FSH</td>
<td></td>
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<tr>
<td>Days between FSHmax and FSHmin</td>
<td>3 (0–8)</td>
<td>8 (6–12)</td>
</tr>
<tr>
<td>Daily decrease (IU/l)</td>
<td>1.5 (0.5–6.6)</td>
<td>1.4 (0.9–1.9)</td>
</tr>
<tr>
<td>Daily decrease (% of FSHmax)</td>
<td>11 (4–56)</td>
<td>8 (5–11)</td>
</tr>
</tbody>
</table>

Induction patients, significant differences (P < 0.05) were found between IRMA-FSHini and IRMA-FSHmax, IRMA-FSHmax and IRMA-FSHmin, and IRMA-FSHini and IRMA-FSHmin. Also, BIO-FSHini and BIO-FSHmin were each significantly different from BIO-FSHmax (P < 0.05), whereas there was no significant difference between BIO-FSHini and BIO-FSHmin. No significant correlation was found between IRMA-FSH and BIO-FSH concentrations in patients during ovulation induction (initial: \( r = 0.42, P = 0.05 \); maximum: \( r = 0.04, P = 0.85 \); minimum: \( r = 0.41, P = 0.06 \)). Significant differences were found between ovulation induction patients and controls with regard to IRMA-FSHini concentrations [3.6 (0.9–6.3) versus 4.8 (2.8–8.2) IU/l, \( P = 0.03 \)], and IRMA-FSHmin concentrations [4.7 (2.7–8.2) versus 2.2 (0.7–4.7) IU/l, \( P < 0.001 \)]. IRMA-FSHmax concentrations showed no significant difference [7.6 (3.9–10.9) versus 6.4 (5.7–10.0) IU/l]. Comparing BIO-FSH concentrations of ovulation induction patients to controls, significantly lower \( P = 0.002 \) initial [6.6 (1.0–10.8) versus 10.4 (7.20–19.6) IU/l, \( P = 0.002 \)] and maximum [13.5 (8.7–17.4) versus 18.7 (13.2–23.4) IU/l, \( P = 0.002 \)] concentrations were found in ovulation induction patients, whereas minimum concentrations were not found to be significantly different [9.9 (4.2–13.2) and 6.3 (4.2–12.4) IU/l].
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The median decrease from maximum to minimum concentrations of IRMA-FSH and BIO-FSH concentrations and the number of days of decline in patients undergoing ovulation induction are shown in Table II. No significant differences were found between patients undergoing ovulation induction and controls with regard to daily decline of FSH concentrations. The initial B/I ratio in ovulation induction patients [1.9 (0.9–5.7)] was not significantly different from controls, whereas the maximum [1.3 (0.5–2.5)] and minimum [1.8 (0.8–3.8)] B/I ratios were significantly lower (P < 0.001 and P = 0.04 respectively).

Discussion

Since FSH plays a crucial role in the stimulation of follicle development, investigation of ovarian function and immuno-reactive and bioactive FSH serum concentrations in patients suffering from normogonadotrophic anovulation during baseline conditions and during gonadotrophin induction of ovulation might yield valuable information. Our data confirm that the spontaneous menstrual cycle is characterized by an increase of IRMA-FSH concentrations in the early follicular phase, followed by a gradual decrease in the late follicular phase (Yen, 1991). BIO-FSH concentrations throughout the spontaneous menstrual cycle are less well established, partly due to methodological differences in the assays used. Padmanabhan et al. (1987) reported that, using an in-vitro Sertoli cell aromatase bioassay system, late follicular B/I ratios were significantly higher as compared to those of the early follicular or luteal phase. This finding seems in keeping with the appearance of more basic and biopotent isofoms of FSH at midcycle (Wide and Bakos, 1993). Using an in-vitro rat granulosa cell assay, Reddi et al. (1990) reported that circulating bioactive FSH concentrations are maximal 12–13 days before the onset of the midcycle LH surge, which is the time of recruitment of the cohort of developing follicles of which a single follicle will gain dominance and ovulate. In the present study, we found no significant differences between initial, maximum and minimum B/I ratios, which is in line with data presented by Jia et al. (1986) and Kessel et al. (1988), who also found a more equal distribution of B/I ratios throughout the cycle using a similar granulosa cell aromatase assay.

By definition, IRMA-FSH concentrations in normogonadotrophic anovulatory subjects are within normal limits, which seems to suggest a limited role of FSH in the aetiology of this particular form of anovulation. However, the normal range is wide due to distinct changes in serum FSH concentrations during the follicular phase of the normal menstrual cycle. In addition, bioactivity of circulating FSH in anovulatory women may be different from that in regularly cycling subjects. Furthermore, single measurements of IRMA-FSH serum concentrations may be less powerful, due to the potential dynamics of FSH in anovulatory women. Very few data are available in the literature addressing these issues. Preliminary observations suggested that BIO-FSH concentrations (as measured by a rat Sertoli cell aromatase assay) in women with PCOS were within the range seen during the follicular phase of regularly cycling women (Padmanabhan et al., 1990). The amplitude of the BIO-FSH response to a GnRH agonist, however, has been shown to be markedly decreased in PCOS patients as compared to regularly cycling controls, suggesting a possible modification of FSH isofoms in this syndrome (Mechain et al., 1993).

Previously, Fauser et al. (1991) reported that in 35 anovulatory women as compared to normal controls no differences could be found with regard to IRMA-FSH, radioimmunoassay-FSH and BIO-FSH concentrations. Since the BIO-FSH and IRMA-FSH concentrations in controls were determined at cycle day 2 or 3 in this previous study, they can be considered comparable to the BIO-FSHini and IRMA-FSHini concentrations of controls in the present study. Both reports show that early follicular phase concentrations of BIO-FSH and IRMA-FSH are not significantly different in controls and anovulatory subjects. This observation still holds if a subgroup of anovulatory PCOS is made.

In addition, the present study shows for the first time that in regularly cycling women maximum and minimum concentrations of BIO-FSH and IRMA-FSH are reached during the course of the menstrual cycle that are significantly different from those in anovulatory subjects, i.e. although FSH concentrations in these anovulatory patients appear 'normal', a rise...
in FSH—which usually occurs during the luteo-follicular transition—is lacking. Moreover, we found a negative correlation between both IRMA-FSH and BIO-FSH concentrations in anovulatory subjects and the interval between blood sampling and last vaginal bleeding, suggesting that the abnormal steroid milieu causes a decrease of serum FSH concentrations over time. In addition, BIO-FSH and IRMA-FSH concentrations were found to be closely correlated in anovulatory women during baseline conditions and controls during the early follicular phase, whereas later during the follicular phase this correlation disappeared in controls. Amenorrheic patients showed higher IRMA-LH and androgen concentrations and lower BIO-FSH concentrations as compared to oligomenorrheic cases. Considering amenorrhea as the most extreme form of anovulation in normogonadotrophic women, this might indicate that the end point of persistent anovulation is a hyperandrogenic state with relatively low FSH (bio)activity.

Data on daily immunoreactive FSH serum concentrations during induction of ovulation using classical incremental dose regimens of exogenous gonadotrophins are largely lacking (Healy and Burger, 1983; Mallya et al., 1993), and no data on serum FSH bioactivity following administration of exogenous gonadotrophins have been published. In the case of normal ovarian response, FSH serum concentrations that mimic patterns occurring during the normal menstrual cycle should be able to induce ovulation. This does occur during pulsatile GnRH therapy in hypogonadotrophic anovulatory patients. The present study shows for the first time that FSH concentrations closely resembling physiological circumstances can establish an adequate ovarian response in PCOS patients, using a decremental ('step down') dose regimen of gonadotrophins. However, in the present study, blood withdrawal for serum FSH determinations was performed 24 h after the previous injection, and it may be suggested that maximum serum concentrations during the day are 20–30% higher (Mizumuna et al., 1990). Recently, B/I ratios for FSH have been described for various urinary gonadotrophin preparations and also within and between different batches of the same preparation (Rodgers et al., 1995). Since in our study single batches were used for both HMG and purified FSH, between-batch variation may be excluded. However, due to within-batch differences and the use of two FSH preparations, the actual amount of injected bioactive FSH may vary from day to day.

The applied regimen has been shown to be a clinically useful method of induction of ovulation, with favourable success and complication rates (van Santbrink et al., 1995a). Moreover, the oestrogen response following initial gonadotrophin stimulation predicts treatment outcome (Schoot et al., 1995). Some differences between gonadotrophin-treated anovulatory patients and controls were noted. During ovulation induction, FSH B/I ratios were significantly lower as compared to controls, which suggests that the FSH isohormone profiles in serum during administration of urinary gonadotrophins (obtained from post-menopausal women) are different from those found during the normal menstrual cycle. Compared to controls, the increase of IRMA-FSH (but not BIO-FSH) concentrations from initial to maximum was more pronounced, whereas the decrease from maximum to minimum appeared to be less marked. The lower BIO-FSHini and IRMA-FSHini concentrations in women undergoing ovulation induction and the steeper rise of FSH concentrations may be explained by GnRH agonist administration 3 weeks prior to induction of ovulation in these patients. A less pronounced decrease from maximum to minimum serum FSH concentrations in ovulation induction patients seems to be largely attributable to a reduced number of days (4 versus 7) of declining FSH concentrations (see also Table II), since the daily decrease is similar (0.5–0.6 IU/l/day). However, the similarities between ovulation induction patients and controls seem to be of greater importance. IRMA-FSHmax concentrations of ovulation induction patients showed no difference as compared to controls, and BIO-FSHmax concentrations were even lower. This finding demonstrates that serum concentrations of IRMA-FSH which are similar to controls (and even lower BIO-FSH concentrations) are sufficient to induce ongoing growth and ovulation of follicles in the majority of clomiphene-resistant anovulatory patients, and that supraphysiological amounts of FSH are not needed for successful ovulation induction.

In summary, bioactive FSH concentrations in normogonadotropic anovulatory women, including those with PCOS, are within the normal range, excluding defective circulating FSH as an important aetiological factor. Although normal per se, the serum concentrations of BIO-FSH and IRMA-FSH in anovulatory women do not exhibit the rise which typically occurs during the early follicular phase in regularly cycling women. This intercycle rise (above the FSH threshold) is responsible for growth of a cohort of follicles from which the ovulatory follicle is selected. Instead, anovulatory women may exhibit a decrease in BIO-FSH and IRMA-FSH concentrations over time. The absence of a physiological rise of FSH following a menstrual bleeding suggests erroneously changed pituitary output probably due to altered steroid feedback. Based on dynamic changes of inhibin and FSH concentrations in serum following functional demise of the corpus luteum, it has been suggested that inhibin may be one of the ovarian signals for the inception of folliculogenesis (Roseff et al., 1989). However, data obtained from healthy volunteers receiving exogenous oestriadiol administration in the luteal phase of the menstrual cycle suggest that it is the decrease in plasma oestradiol rather than inhibin that is the triggering signal for the rise in plasma FSH during the luteo-follicular transition (Le Nestour et al., 1993). Both the immunoreactivity and bioactivity of circulating FSH in anovulatory women during gonadotrophin induction of ovulation using a decremental dose regimen resemble the early follicular phase in regularly cycling women. In particular, IRMA-FSHmax concentrations that were similar to those of the controls (and even lower BIO-FSHmax concentrations) were found to be sufficient to induce follicle growth in normogonadotropic clomiphene-resistant anovulatory women. This finding suggests that in these women a transient increase in serum FSH concentrations closely resembling the intercycle FSH rise during normal conditions can successfully induce follicular growth, indicating that the FSH threshold for ovarian stimulation in clomiphene-resistant normogonadotropic anovulatory women is not different from normal.
Acknowledgements

The authors wish to thank E. van Santbrink, M.D., for his assistance in collecting the data of the control subjects in this study. This study was financially supported by 'stichting Voorplantingsgeneeskunde Rotterdam'.

References


Received on August 21, 1995; accepted on October 16, 1995.