Ovarian sensitivity to follicle stimulating hormone is blunted in normo-ovulatory women with Down’s syndrome

Rosa Maria Cento, Letizia Ragusa, Caterina Proto, Antonino Alberti, Gaetano Fiore, Fabio Colabucci and Antonio Lanzone

OASI Institute for Research, Troina, Enna, Italy

1To whom correspondence should be addressed at: Obstetrics and Gynecology Unit, OASI Institute of Research, Via Conte Ruggero 73, 94018 Troina, Enna, Italy

Ovarian sensitivity to follicle stimulating hormone (FSH) during the early follicular phase of the human menstrual cycle was studied in six post-menarchal patients with Down’s syndrome and 12 normo-ovulatory women. Pure FSH (75 IU) was given i.v. to six controls and six Down’s syndrome patients, while saline was administered to the remaining six controls. Plasma concentrations of luteinizing hormone (LH), FSH, oestradiol, testosterone and growth hormone (GH) in samples collected for a period of 26 h after the injection were assayed. In control patients FSH injection increased oestradiol stimulated area under the curve (AUC). This value was significantly higher than that found in Down’s syndrome patients (P < 0.02), who exhibited an oestradiol-stimulated AUC equivalent to saline-treated controls. In Down’s syndrome, GH plasma concentrations were significantly lower than in the control group (P < 0.05). These results indicate that the ovarian sensitivity to FSH in patients with Down’s syndrome is blunted. Lower GH plasma concentrations found in this group may in part account for this biological feature.

Key words: Down’s syndrome/early follicular phase/FSH/oestradiol

Introduction

Previous studies have reported that women with Down’s syndrome (DS) often have gonadal dysfunction (Hasen et al., 1980; Hsiang et al., 1987). Menarche may occur at a later average age in women with DS than in controls (Bellone et al., 1980). Moreover, although the length of menstrual cycles in DS is within the normal range, a reduction of the reproductive function is generally accepted. Various authors have found an increased frequency of anovulation or defects of luteal function (Tricomi et al., 1964; Scola et al., 1992); however, in other reports, several reproductive events were documented (Rani et al., 1990).

In our recent paper, we demonstrated, in a selected regularly menstruating group of DS patients, a significantly higher incidence of anovulation and luteal defects than in controls; furthermore, both oestradiol and progesterone plasma concentrations were greater in controls than in women with DS in all phases of the cycle and in the mid-luteal phase respectively (Cento et al., 1996). These findings suggest that in DS patients, primary dysfunction of follicular maturation may lead to anovulation or impairment of luteal function.

In our previous study, we investigated the existence of different sensitivity of the ovaries to follicle stimulating hormone (FSH) in the various stages of the human follicular phase: early stages of follicular growth are the most responsive to the elevation of circulating FSH concentrations, whereas the ovarian sensitivity spontaneously decreases as follicular maturation progresses (Caruso et al., 1993).

Since in the early follicular phase the physiological increase of circulating FSH is related to the follicular recruitment and dominance, the present study was designed to investigate the effect on oestradiol production of increased circulating concentrations of FSH in the early stages of follicular development in a group of normo-ovulatory DS women. For this purpose, we administered in a single dose to selected DS subjects pure urinary FSH. The results are compared with those obtained from a group of healthy women at reproductive age.

Materials and methods

Six women with Down’s syndrome, age 16–29 years, with at least 2 years of gynaecological history, followed at the OASI Institute, Enna, Italy, as outpatients, were chosen for the study.

None of the subjects had clinical signs of acne or hirsutism; normal adrenal and thyroid function had been previously tested. Obesity was defined as a value of body mass index (BMI) >25 kg/m².Normally ovulatory cycles were documented by the biphasic pattern of basal body temperature and by mid-luteal plasma progesterone concentrations >8 ng/ml for three previous consecutive cycles.

The study was approved by the Ethical Committee of OASI, Institute for Research. This study was designed to improve the quality of life of subjects suffering from DS. In fact, some of these patients, due to their handicap, are not able to avoid sexual abuse in their environment, resulting in unwanted pregnancies. Therefore, it is important to increase our knowledge on ovarian function of DS patients in order to alert the parents to their potential reproductive problems. With regard to informed consent, all parents were clearly informed about the aim of the study and all signed the acceptance of the protocol.

The studies were performed in the early follicular phase (days 4–7 of the cycle) after spontaneous menses. At 10.00 a.m., the subjects received an i.v. injection of pure FSH (Metrodin, Serono, Italy), 75 IU dissolved in 1 ml saline solution. Blood samples were collected during a period of 26 h, at time 0 (basal value) and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26 h after FSH injection. Samples were centrifuged and plasma stored at −20°C until assayed.

Luteinizing hormone (LH), FSH, prolactin (PRL), oestradiol, pro-
gesterone, testosterone, androstenedione, dihydroepiandrostenedione sulphate (DHEAS) and growth hormone (GH) were assayed in basal plasma samples (time 0); in the other plasma samples LH, FSH, oestradiol, testosterone and GH were assayed. All samples of the same patients were assayed simultaneously. All hormones were evaluated using commercial radioimmunoassay kits (Radim, Pomezia, Italy). LH, FSH, PRL and GH were assayed by double antibody technique, all steroids were determined using the dextran–charcoal technique. Intra and inter-assay coefficient of variations and sensitivity were respectively: LH, 4 and 5%, 0.2 mIU/ml; FSH, 4 and 6%, 0.2 mIU/ml; oestradiol, 3 and 6%, 8 pg/ml; GH, 3 and 6%, 0.04 ng/ml. Cross-reaction for oestradiol assay with other steroids and for GH assay with PRL was <1%.

Ultrasound examination of the ovaries was also performed during the cycle of study in order to exclude the presence of ovarian cysts and confirm that ovulation had occurred. Ovarian volume (evaluated by the formula of a prolate ellipsoid) and follicle development were recorded.

Data from DS patients were compared with those obtained from 12 normo-ovulatory women (18–30 years of age) with tubal factor sterility observed at the same stage of menstrual cycle. Six of them received i.v. 75 IU of pure FSH (FSH–control group); the other six subjects received i.v. 1 ml saline solution bolus (saline–control group). Plasma samples were collected with the same modalities described above. No patient of either group had taken medication known to affect plasma sex steroid concentrations for at least 3 months before the observation.

The results were analysed as the area under the 26 h curve (AUC) for each hormone assayed and calculated by the trapezoidal rule. Specifically, to evaluate the true increase of steroids after FSH regardless of their basal plasma secretion, results were expressed as follows: (i) absolute increase (stimulated AUC), calculated by the difference between AUC and basal AUC (area under the curve theoretically because of the basal unstimulated secretion and calculated assuming as constant values during the 26 h period those at time of FSH or saline injection); (ii) percentage increase (ΔAUC = stimulated AUC/baseline AUC×100).

Statistical analysis was performed by non-parametric Wilcoxon test. A P-value < 0.05 was considered to be the limit of significance.

Results

Table I shows the clinical and endocrine characteristics in DS and control patients: no difference for BMI and baseline hormone concentrations was found between the two groups. Only GH baseline plasma concentrations were found to be significantly lower in DS group with respect to those observed in controls (P < 0.05).

Table I. Clinical and baseline endocrine characteristics of study groups.

<table>
<thead>
<tr>
<th>Patients (number)</th>
<th>FSH DS</th>
<th>C–FSH</th>
<th>C–saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>24.3±4.1</td>
<td>23.1±2.6</td>
<td>24.5±3.3</td>
</tr>
<tr>
<td>6</td>
<td>2.7±1.2</td>
<td>2.3±0.6</td>
<td>3.5±1.6</td>
</tr>
<tr>
<td>6</td>
<td>2.0±0.5</td>
<td>1.7±0.4</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>6</td>
<td>0.9±0.2</td>
<td>1.0±0.3</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>6</td>
<td>1.1±0.7</td>
<td>2.3±0.9</td>
<td>2.1±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SD.

<table>
<thead>
<tr>
<th>FSH DS compared with C–FSH, P &lt; 0.05.</th>
<th>FSH DS compared with C–saline, P &lt; 0.05.</th>
</tr>
</thead>
</table>

Conversion factor for SI values: LH = 1.0; androstenedione = 3.49; DHEAS = 2.714; GH = 1.0.

FSH = folliclic stimulating hormone, BMI = basal metabolic index, LH = luteinizing hormone, DHEAS = dihydroepiandrostenedione sulphate, GH = growth hormone.

Figure 1 shows the FSH concentrations after saline or FSH injection in DS and control patients. FSH baseline concentrations (time 0 h) were slightly, but not significantly higher in DS group. In patients receiving pure FSH, the FSH AUC was significantly higher than those receiving saline alone. No difference was recorded among FSH-treated patients. The highest FSH plasma values were obtained 2 h after pure i.v. FSH injection and remained significantly higher as compared to the ones found in saline-treated patients until 14 h after the injection. No significant changes in LH values were observed during the study (data not shown).

GH plasma concentrations did not change during the period of study, thus, the AUC-GH values in controls were significantly higher than in DS patients (AUC-GH: FSH–control group: 3900 ± 1404 ng/ml×26 h; saline–control group: 3588 ± 1248 ng/ml×26 h; DS group: 1716 ± 1092 ng/ml×26 h; P < 0.05).

Table II and Figure 2 compare the oestradiol and testosterone responses to i.v. pure FSH in all studied groups. FSH administration elicited a significant increase in oestradiol plasma concentration in the FSH–control group when compared with those found in control patients receiving saline. In DS patients FSH administration increased peripheral oestradiol plasma concentrations. In contrast, this increase was significantly lower than in the FSH–control group, but of the same order of magnitude when compared to saline–control group. In five DS patients the Δ increase of oestradiol was very low (range 0–18%; median 8), while in one patient there was a response (Δ increase: 40%) comparable to those observed in FSH–control group (Δ increase: range 21–57%; median 34). No relationship between responses to FSH and clinical and endocrine characteristics in DS patients was found.

Saline or FSH i.v. injection did not produce any significant increase of testosterone plasma concentrations in all studied groups.

No difference among the groups was observed for the ovarian volume as well as for the number and growth of follicles developed in the cycle studied (data not shown).

Discussion

The present study indicates that in a group of normo-ovulatory patients affected by DS, the ovarian sensitivity to exogenous FSH administration is blunted as compared with the response elicited by exogenous FSH in healthy women in terms of oestradiol production.

Previous studies have reported in DS an increased incidence of abnormalities in sexual development (Benda, 1969); in addition, puberal development is delayed in men and women (Bellone et al., 1980) and ovarian hypoplasia has been described as a constant finding (Benda, 1969). Based on these data, it was believed that oligoamenorrhea or chronic
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Figure 1. In the study protocol three groups of patients were selected: six patients with Down’s syndrome, FSH DS (■—■) received pure follicle stimulating hormone (FSH) (75 IU) i.v.; the remaining six controls, C–saline (△—△), received saline alone. Plasma samples were collected for a period of 26 h. Values of FSH are expressed as means ± SD and area under curve (AUC). *C–saline compared with FSH DS, $P < 0.05$. **C–saline compared with FSH DS, $P < 0.01$. +, C–saline compared with C–FSH, $P < 0.05$. ++, C–saline compared with C–FSH, $P < 0.01$. Conversion factor in SI value: FSH 5.1. Conversion factor in SI value: FSH 5.1.

Table II. In-vivo response to i.v. FSH in patients with Down’s syndrome and in control groups

<table>
<thead>
<tr>
<th></th>
<th>Plasma oestradiol (pg/ml × 26 h)</th>
<th>$\Delta$ AUC (%)</th>
</tr>
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<tbody>
<tr>
<td>Down’s syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH 75 IU (n = 6)</td>
<td>8500 ± 7500$^a$</td>
<td>18 ± 10$^a$</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (n = 6)</td>
<td>13 393 ± 11 090$^b$</td>
<td>11 ± 9$^b$</td>
</tr>
<tr>
<td>FSH 75 IU (n = 6)</td>
<td>49 785 ± 22 857</td>
<td>36 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SD.

$^a$Control FSH compared with control saline and Down’s syndrome FSH, $P < 0.02$.

$^b$Control FSH compared with control saline, $P < 0.01$.

$^c$Control FSH compared with Down’s syndrome FSH, $P < 0.05$.

Conversion factor to SI values: oestradiol 3.671.

anovulation represented the most frequent pattern of menstrual cycle during the reproductive age of women with DS. However, a variable incidence of ovulatory cycles as well as authenticated cases of pregnancy associated with DS have been recently reported (Rani et al., 1990). Scola and Pueschel (1992) found that ~90% of women with DS have a biphasic pattern of basal body temperature; Tricomi et al. (1964) observed that no clinical sign of ovulation was documented in 30% of cases, while in the remaining subjects, 40% had true ovulation and 30% uncertain ovulation. In our recent series, based solely on DS patients with regular menses, we found a higher incidence of anovulation and luteal phase defects (Cento et al., 1996). Overall, these results could suggest that in patients with DS a primary dysfunction of follicular maturation may occur more frequently and may lead to anovulation or impairment of luteal function.

It is well known that in early follicular stages of menstrual cycles, the process of follicular recruitment and selection takes place in a way which is dependent on FSH activity (Zeleznik, 1981; Zeleznik and Kubik, 1986). In fact, in this phase FSH plasma concentrations are slightly elevated, whereas oestradiol concentrations are low (Messinis and Templeton, 1990). In previous studies, we demonstrated that ovarian sensitivity to FSH significantly changes during the different stages of the follicular phase. During the early follicular phase, the ovary increased its oestradiol production in response to FSH injection in a dose-dependent manner. Conversely, already at the mid-follicular phase a 2-fold to 4-fold elevation of circulating FSH concentrations did not evoke any further increase of oestradiol secretion as compared to that observed in untreated cycles (Caruso et al., 1993). These data are of particular interest considering that the group of DS patients studied has been selected on the basis of their normal cyclicity, which excluded all those patients with oligomenorrhoea, who generally account for a significant percentage of all post-menarchal DS subjects. Moreover, only one patient had a response of oestradiol to FSH injection in the range of that found in controls. Thus, although the number of patients studied is small, the difference between the two groups was highly significant.
It is well known that women with DS are frequently affected by obesity and GH deficiency (Cronk et al., 1988). Obesity is known negatively to affect ovarian function, and the restoration of normal weight generally increases the ovulation frequency in these patients (Bates and Whitworth, 1982). In our study no difference in BMI was observed among the two study groups. This suggests that excessive weight cannot be involved in the impairment of ovarian response to FSH.

It was recently suggested that GH may be involved in the regulation of ovarian physiology; although its exact role is still controversial, recent findings indicate that the ovary is one of the sites of GH reception and action (Katz et al., 1993). It is well known that isolated GH deficiency causes delayed puberty in children; the treatment of these patients with GH induces sexual maturation (Tanner and Whithous, 1975; Kulin et al., 1981). Moreover, in patients relatively resistant to gonadotrophin treatment, concurrent provision of GH may reduce the amount of gonadotrophin required to induce ovulation (Mason et al., 1990; Lanzone et al., 1992). In in-vitro studies, the GH treatment augments several FSH-induced activities such as the formation of LH/human chorionic gonadotrophin (HCG) receptors, the enhancement of aromatase function and progesterone biosynthesis in rat granulosa cells (Jia et al., 1986; Homburg et al., 1988), as well as the maturation of follicle and cumulus-enclosed rat oocytes (Apa et al., 1994). More recently, it was suggested that ovaries could vary in their sensitivity to GH action in relation to the stage of the human menstrual cycle.

One hypothesis is that GH could enhance ovarian sensitivity by augmenting or stimulating FSH receptors in the early stages of the follicular phase, where a GH involvement in the sensitive feedback relationship between oestradiol and FSH could be possible. In our study, the baseline circulating GH concentrations were significantly lower in DS patients than in control women. Furthermore, neither FSH, nor oestradiol plasma circulating concentrations at baseline were different in respect to controls. Thus, it is conceivable to suggest that the decreased GH secretion may in part account for the blunted sensitivity of ovary to FSH observed in DS patients. However, further studies are needed to elucidate this interesting point. In fact, since it is well known that several endocrino–metabolic features such as insulin secretion or GH/insulin-like growth factor (IGF)/insulin-like growth factor binding protein (IGFBP) axis activity may affect ovarian function, it would be of interest to explore in certain clinical situations, as is the case in DS, the impact of these abnormalities on ovarian function.

In conclusion, the present study confirms and extends the contention that in DS the ovarian function is impaired. In the light of our results, this dysfunction could be in part ascribed to a blunted ovarian sensitivity to gonadotrophins.

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