Ovarian response patterns to human menopausal gonadotropin in mixed hyperandrogenemia

Miklos Hamori, Attila Török, Manfred Zwirner and Hans-Rudolf Tinneberg

Department of Obstetrics and Gynecology, University of Tübingen, FRG, and Department of Obstetrics and Gynecology, University of Pécs, Hungary

Abstract. Twenty-eight hyperandrogenemic women suffering from infertility owing to chronic anovulation were treated with hMG. Only 7 patients exhibited the typical polycystic ovarian appearance of multiple subcortical cysts, however, a wide range (6-15 cm³) of ovarian volume was observed. The LH/FSH ratio was consistently lower than 2.5 and circulating androgens of both ovarian and adrenal origin were elevated. The 4 days dexamethasone suppression test showed more than 80% suppression of dehydroepiandrosterone-sulphate and a variable (40-60%) reduction of testosterone and androstenedione levels. Two different patterns of follicular development were observed in response to hMG. Sixteen patients exhibited polycystic ovarian reaction, whereas 12 women had a follicular growth pattern similar to that seen in hMG-stimulated normo-ovulatory subjects. Patients with polycystic ovarian reaction showed a significantly increased androstenedione response to hMG when compared with the other group. Moreover, the non-stimulated ovarian volume was found to be markedly greater than in subjects without polycystic reaction. Thus, ovarian stimulation of patients with mixed hyperandrogenemia may elucidate the presence of borderline polycystic ovaries; furthermore the increased accumulation of androstenedione may suggest an inherent ovarian failure.

Chronic anovulation is frequently associated with hyperandrogenemia of various origin (1). Polycystic ovarian syndrome (PCOS) is recognized as the commonest cause of anovulation, though the exact pathomechanism of the disorder is still not clearly defined (2). Elevated ovarian androgens arise from the tonic, LH-dependent stimulation of the thecal and stromal tissue (3,4), producing an unfavourable intrafollicular accumulation of testosterone (T) and androstenedione (5). This mechanism does not explain the androgen increase in PCOS with normal immunoreactive LH level (6,7). In these cases either the raised LH bioactivity (8) or local, non-steroidal factors (9) may be responsible for the defect of normal follicular development.

The characteristic sonographic picture in PCOS (10) and the frequently elevated LH/FSH ratio are of great importance, but in borderline cases the diagnosis cannot be set up at the first instance. Subcapsular small cysts may shrink owing to long-lasting amenorrhoea, furthermore they are not so manifest in ovarian hyperthecosis, a subgroup of ovarian hyperandrogenemia (11).

Mixed hyperandrogenemia has been reported in 30-40% of all patients with androgen overproduction, secreting increased amounts of T, androstenedione and dehydroepiandrosterone-sulphate (DHEA-S) (12,13). The dexamethasone suppression test is widely used to differentiate the adrenal from the ovarian source, usually achieving a rapid reduction (over 80%) of DHEA-S in non-tumorous adrenal hyperfunction (14). The androgens T and androstenedione are secreted by the adrenals and ovaries in about equal amounts (15), approximately 50% of T is produced by peripheral conversion of androstenedione (16). The double source of T and androstenedione makes the evaluation of mixed hyperandrogenemia difficult, even by the use of the dexamethasone suppression test.
This study presents the results of hMG stimulation in clomiphene-resistant anovulatory women who had a long history of infertility and mixed, not LH-dependent hyperandrogenemia. We found that ovarian stimulation may detect borderline PCOS cases and that patients with PCOS produce an exaggerated increase in androstenedione in response to ovulation induction with hMG.

Subjects and Methods

Twenty-eight women (26 to 39 years of age) complaining of infertility for more than 3 years were enrolled in the study. The selection criteria were mixed hyperandrogenemia, normal LH/FSH ratio, and the presence of normal or only slightly enlarged (<15 cm²) ovaries. Only 7 patients exhibited multiple subcortical cysts of borderline-sized ovaries. Thus the true, classic form of PCOS was excluded from the study and attention was focused on the investigation of mixed hyperandrogenemia associated with chronic anovulation.

Gynecological follow-up excluded other potential infertility factors as tubal blockage and hyperprolactinemia. Infertility was associated with oligo-amenorrhea, in 70% of the subjects a mild or moderate hirsutism was present (17). None of the women had elevated cortisol or 17α-OH progesterone levels consistent with Cushing's syndrome or late-onset adrenal hyperplasia. Anovulation was confirmed by basal body temperature chart and progesterone determinations. Basal endocrine parameters were obtained repeatedly, the mean of 3 determinations served as the non-stimulated baseline level. Only women with an LH/FSH ratio below 2.5 were included in the study; the relation of the 2 gonadotropins was controlled several times. The upper normal level of serum androgens were 9.0 μmol/l for DHEA-S, 8.0 nmol/l for androstenedione, 2.8 nmol/l for T, and 10.5 pmol/l for free testosterone (FT). A 4 days dexamethasone suppression test was performed with daily administration of 3-4 mg Fortecortin (Ferring, Kiel, FRG), achieving a sufficient (>80%) reduction of the DHEA-S secretion.

The ovarian volume was calculated from the 3 diameters (10) by transvaginal ultrasound. Ovarian enlargement (<15 cm³) and subcapsular polymicrofollicular structure were considered as typical sonographic signs of the advanced, classic PCOS.

After progesterone-induced withdrawal bleeding, 2 ampoules (150 IU) hMG (Humecon, Organon, Oberschleissheim, FRG) were administered daily from day 3; the dose was later adjusted to the individual response. Control of follicular development comprised transvaginal ultrasound and monitoring of serum E₂ and LH levels. An injection was given of 10⁶ IU hCG (Predalon, Organon, Oberschleissheim, FRG) when the dominant follicle(s) exceeded 16 mm and the E₂ level reached 700 pmol/l per large follicle.

Radioimmunoassays

In order to evaluate the hormonal patterns and especially the androgen response to hMG stimulation, DHEA-S, androstenedione, T and FT were determined together with sex hormone binding globulin (SHBG). All assays used ¹₂⁵I for the labelling of the tracer. LH and FSH were measured by the MAIAclone immunoradiometric assay (IRMA) from Serono Diagnostics (Freiburg, FRG). A direct radioimmunoassay (RIA) of Baxter Merz & Dade AG (Düdingen, Switzerland) was used for the determination of E₂; the hormone was measured in undiluted serum without extraction. The antibody had 0.02% cross-reactivity to other estrogens and a negligible (<0.001%) cross-reactivity against androgen compounds. The RIA for androstenedione measurement was the product of Diagnostic Systems Laboratories (Webster, TX); the hormone was measured in undiluted serum without extraction. The antibody in the assay showed 0.06% cross-reactivity against T and even a lower percent of cross-reactivity against any other serum androgen. The T RIA kit was purchased from ICN Biomedicals (Carson, CA); the determination was carried out in undiluted serum without extraction. The antibody cross-reacted with 0.56% against androstenedione and <0.01% against estrogens, progesterone and dehydroepiandrosterone at 50% binding of the 0 nmol/l standard. The kit for DHEA-S RIA was supplied also by ICN Biomedicals, whereas progesterone was measured using the COATRIA kit of bioMe rieux (Marcy l'Etoile, France). Monoclonal SHBG-IRMA and Coat-a-count FT kits were purchased from Diagnostic Product Corporation (Los Angeles, CA). The intra- and inter-assay variations of all kits were between 3 and 9%.

Two groups of patients were classified on the basis of follicular growth patterns (polycystic ovarian reaction versus normal follicular development). Statistical analysis of the hormonal parameters in the 2 groups was performed using the Student's t-test.

Results

The only selection criterion in the analysis of hyperandrogenemic patients was the development of follicles in the response to hMG. Polycystic ovarian reaction was defined as the presence of more than 10 follicles over 10 mm in each ovary. Sixteen patients exhibited a polyfollicular stimulatory pattern. This group (Group A) was compared with the remaining 12 hyperandrogenemic women (Group B) who presented a so-called "normal" follicular growth, similar to that observed in normo-ovulatory patients stimulated by hMG for in vitro fertilization.
Clinical characteristics of Groups A and B are depicted in Table 1, together with 2 generally accepted diagnostic criteria of PCOS, the LH/FSH ratio and the ovarian size. Whereas the difference for the LH/FSH ratio did not reach a significant level between the 2 groups, the ovarian volume of patients with polycystic reaction was significantly \((p<0.05)\) greater even before the initiation of hMG stimulation.

Concerning the ovarian response to ovulation induction, neither basal, nor peak \(E_2\) levels showed a significant difference between Group A and B.

The basal \(E_2\) concentrations were consistent with the early follicular phase of the normal cycle and demonstrate a sufficient estrogenization of the women. The mean peak \(E_2\) level is slightly but not significantly higher in Group A, but considering the number of large follicles, this increase must be evaluated as a reduced response to the comparable dose of administered hMG (Table 2). The ovarian volume at the time of hCG administrations accounts partly for the development of severe hyperstimulation (ovarian enlargement \(>8\, cm^3\) with ascites) in patients with polyfollicular reaction. Premature LH surge occurred also in Group A only.

The result of dexamethasone suppression test shows that more than 80% of the DHEA-S secretion could be abruptly eliminated in 4 days (Table 3). However, regarding the androgens of mixed origin (T and androstenedione), only a variable (40-60%) suppression could be achieved. Table 4 demonstrates the peak levels of circulating androgens on the day of hCG administration (or spontaneous LH surge), as well as the increase in each androgen from baseline to stimulated values. As expected, gonadotropin therapy did not exert any influence on the adrenal function, thus the DHEA-S secretion remained unchanged. Androgens of both adrenal and ovarian source (androstenedione and T) increased in response to hMG treatment. Statistical difference was detected only for androstenedione; both the peak level and the incre-
Table 4.
Peak levels of dehydroepiandrosterone-sulphate (DHEA-S), androstenedione (A), testosterone (T), and free testosterone (fT) as well as their increase during hMG stimulation in Group A and B (values are mean ±so).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Group A (N=16)</th>
<th>Group B (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S (µmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>peak</td>
<td>11.7±1.8</td>
<td>11.5±1.6</td>
</tr>
<tr>
<td>increase</td>
<td>0.4±0.1</td>
<td>-0.5±0.1</td>
</tr>
<tr>
<td>A (nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>peak</td>
<td>19.0±5.0</td>
<td>14.2±3.5</td>
</tr>
<tr>
<td>increase</td>
<td>9.2±2.7</td>
<td>5.0±1.7</td>
</tr>
<tr>
<td>T (nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>peak</td>
<td>6.3±2.1</td>
<td>5.3±2.1</td>
</tr>
<tr>
<td>increase</td>
<td>1.8±0.7</td>
<td>1.5±0.7</td>
</tr>
<tr>
<td>fT (pmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>peak</td>
<td>30.1±13.3</td>
<td>31.5±12.6</td>
</tr>
<tr>
<td>increase</td>
<td>13.0±5.6</td>
<td>12.3±6.3</td>
</tr>
</tbody>
</table>

*The day of hCG administration or LH surge *p<0.05

ment rise from baseline during the hMG treatment were significantly higher in patients with polycystic ovarian reaction (Group A) than in Group B. The more potent androgen T and its free fraction increased in parallel, which was supported by the unchanged SHBG levels (data not shown).

Discussion

The classification of hyperandrogenemia is based on the evaluation of the source of increased androgens (14,16). The weak androgen DHEA-S is an excellent marker of the adrenal function, because it is secreted almost exclusively by the adrenal gland. Unfortunately, no similar relevant parameter exist for the control of ovarian androgen secretion. Therefore, the more potent androgens T and androstenedione are evaluated before and after dexamethasone suppression test, and the predominant source of excess androgens is calculated from the difference between basal and suppressed levels (12,15).

The present study was undertaken to evaluate the source of increased androgens in anovulatory, oligo-amenorrheic patients. Repeated gonadotropin determinations ruled out a raised (>3.0) LH/FSH ratio, frequently associated with the classic PCOS; moreover, multiple subcortical cysts (in not or only slightly enlarged ovaries) were seen only in one fourth of the subjects. The variable suppression (40-60%) of T and androstenedione levels obtained by the dexamethasone test proved a mixed androgen overproduction.

During hMG stimulation of the clomiphene-resistant patients an attempt was made to establish the major source of elevated androgen overproduction. Based exclusively on the follicular growth pattern, 16 out of 28 women showed with certainty the typical polycystic ovarian reaction soon after commencing the hMG administration. This follicular growth pattern was later confirmed during repeated (roughly comparable) stimulations. As PCOS subjects exhibit a wide heterogeneity, the explanation for this phenomenon may be complex, too. Ovarian hyperthecosis as a subgroup of ovarian hyperandrogenemia was reported to show slightly enlarged ovaries with rather dense structure (11). A near normal LH/FSH ratio was found in ovarian hyperthecosis (7,18) and the disorder was recently brought into connection with insulin resistance (9,19). Furthermore, long-lasting amenorrhea may cause atresia of the subcortical small cysts in PCOS, masking the sonographic characteristics of the disease.

The increased basal androstenedione level of the quiescent (not ovulating) ovaries can be explained either by a benign hyperplasia of androgen-producing ovarian tissues or by the arrested conversion of androstenedione to estrogens. However, the increased accumulation of androstenedione during hMG treatment may reflect the local failure of ovarian steroidogenesis in patients with PCOS (20,21). Partial aromatase deficiency is more likely than that of 17-ketosteroid reductase, because the T production from steroid precursors was unaffected. Compared with Group B no increased accumulation of T was observed during hMG stimulation in patients with polycystic ovarian reaction. A supposed preferential pathway of estrogen synthesis from androstenedione could explain the lack of T accumulation, but this is only a hypothesis. However, the suggested impaired mechanism of ovarian steroidogenesis implies only the development of similar follicular growth patterns, the individual hormonal response of which may vary greatly.

In summary, the ovarian stimulation by hMG proved to be useful in elucidating the predominance of androgen overproduction in mixed hyperandrogenemia. Patients with quiescent ovaries re-
sponded frequently with polyfollicular growth pattern, hyperstimulation, and premature LH surge. The excessive increase in androstenedione during hMG therapy may point to a local defect of ovarian steroidogenesis. It is concluded that the polycystic ovarian reaction (with the increase in androstenedione as a supplementary marker) can be used to elucidate the presence of (otherwise undetected) borderline PCOS.

References


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Dr Miklos Hamori,
Department of Obstetrics and Gynecology,
University of Tübingen,
Schleichstrasse 4,
FRG.