Purified urinary follicle stimulating hormone induces different hormone profiles compared with menotrophins, dependent upon the route of administration and endogenous luteinizing hormone activity

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The effects of treatment of patients with gonadotrophin-releasing hormone analogue (GnRHa) combined with purified follicle stimulating hormone (FSH) for in-vitro fertilization (IVF) were investigated in detail to determine the influences of different administration routes and the degree of suppression of luteinizing hormone (LH). Responses to exogenous gonadotrophins were studied in infertile women (n = 60) with normal menstrual rhythm whose endogenous gonadotrophin activity was suppressed using a GnRHa in a long protocol. They were randomized to receive i.m. administration of human menopausal gonadotrophins (HMGim, Pergonal) or purified follicle stimulating hormone (FSH, Metrodin High Purity) administered either i.m. (MHPim) or s.c. (MHPsc). Responses were assessed by measuring plasma FSH, LH, oestradiol, testosterone and progesterone. After stimulation day 4, the MHPsc group showed significantly higher circulating concentrations of FSH than either the MHPim or HMGim group. However, the HMG group showed significantly higher oestradiol concentrations after stimulation day 5 than either MHP group. The differences in circulating oestradiol concentrations in the MHP-treated patients appeared to be strongly influenced by the mean circulating concentrations of LH in the follicular phase. The patients who showed mean follicular phase LH concentrations of <1 IU/l showed longer follicular phases, lower circulating oestradiol and testosterone concentrations and also lower follicular fluid concentrations of oestradiol and testosterone, indicating a reduction in the normal follicular metabolism of progesterone to androgens and oestrogens under these conditions. This group of patients also showed longer follicular phases, which may have consequences for future clinical management.

Key words: hormone profile/LH/menotrophins/purified urinary FSH

Introduction

Purified follicle stimulating hormone (FSH), derived from human urine, has recently become available for the induction of follicular growth in women. Its specific bioactivity is greatly increased compared with the menotrophins previously available, with a concomitant reduction in contaminating urinary products, including luteinizing hormone (LH) (Giudice et al., 1994).

The specific activity is similar to that of recombinant products whose pharmacokinetic and pharmacodynamic qualities have been described in detail for both i.m. and s.c. administration (Le Cotonnec et al., 1993, 1994a). These data suggest that there is little difference in activity (as measured by ovarian responses) of the new preparations, or in the profiles seen when the compounds are administered by i.m. or s.c. routes. Comparative pharmacodynamic and pharmacokinetic data for purified FSH (Metrodin High Purity, MHP) have not been published. However, a recent report has shown that human menopausal gonadotrophin (HMG) exhibits different pharmacodynamic profiles when administered in a bolus dose using the s.c. route compared with the i.m. route (Dobbs et al., 1994). It is possible that minor differences will be exaggerated when the gonadotrophins are used in higher daily doses, as is often the case in stimulation for in-vitro fertilization (IVF), and influences upon the dynamics of ovarian responses have not been assessed.

Women with hypogonadotrophic hypogonadism treated with FSH alone show reduced oestradiol output during the follicular phase compared with those treated with conventional menotrophins containing both FSH and LH (Couzinet et al., 1988). This is strong circumstantial evidence supporting the two cell–two gonadotrophin hypothesis of follicular oestradiol biosynthesis and secretion, in which LH stimulates follicular theca cell androgen production which is then converted to oestradiol by granulosa cells under the influence of FSH (Baird, 1983).

Preparations like MHP have no LH activity, and reduced oestradiol secretion is likely to be a consequence of their use in patients with negligible endogenous LH activity. The degree of endogenous LH activity required for normal oestradiol production is known to be small since in rats, administered amounts that are not detectable in the circulation may be sufficient (Hillier et al., 1995). The concentrations required in the human are also unclear, but monkeys treated with a GnRH antagonist and with an absence of LH showed not only reduced oestradiol secretion, but also slower rates of follicular growth, with similar cohort sizes (Zelinsky-Wootten, 1996).

Treatment with GnRHa does not usually result in total elimination of LH, and there has been little discussion as to whether this is a biological or methodological phenomenon. However, ovarian responses, assessed by oestradiol and follicular diameters, have been shown to be similar when FSH is used instead of HMG as the stimulant when women with normal menstrual rhythm are treated with GnRHa for IVF.
(Sagle et al., 1991; Hull et al., 1994). There appears to be a range of LH concentrations obtained in patients treated with GnRHa, and these can be maintained for a considerable duration (Cedars et al., 1990); with the new FSH preparations containing no LH activity it is possible that there may be a subgroup with low LH concentrations in which oestradiol secretion patterns, and perhaps other ovarian responses, are influenced.

The aim of this study was to compare hormone profiles during treatment with MHP using either i.m. or s.c. routes of administration with those produced by menotrophins (HMG), in women with normal menstrual rhythm treated with a GnRHa for ovulation induction for IVF. Ovarian responses in patients treated with MHP were also examined by reference to the degree of LH suppression, to determine whether GnRHa treatment can suppress LH concentrations to the degree where ovarian responses may be affected by the absence of LH in the stimulant formulation.

### Materials and methods

#### Patients and treatment

A total of 60 patients with tubal infertility, aged between 28 and 40 years, with normal menstrual rhythm were randomized to i.m. treatment with HMG (HMGim, n = 20, Pergonal, Serono UK Ltd, Welwyn Garden City, UK) or purified FSH [Metrodin-HP (Serono UK Ltd)] administered by either the i.m. route (MHPim, n = 20), or the s.c. route (MHPsc, n = 20) in the abdominal wall.

All patients were treated using the long GnRHa protocol (buserelin, 5×150 µg/day, intranasally, Hoechst Roussel UK Ltd, Uxbridge, UK) starting on day 21 of the previous menstrual cycle. The gonadotrophin treatment was started (day S1) on cycle day 3–5, provided that there was no cyst present in the ovaries at ultrasound scan. The initial gonadotrophin dose (225 IU/day) was maintained for at least 7 days, whereupon titration of the dose was effected depending on responses. Patients were stimulated until the criteria for administration of human chorionic gonadotrophin (HCG, Profas, 10000 IU; Serono UK Ltd) were obtained, i.e. a minimum of three follicles with diameter >16 mm, with a minimum circulating oestradiol concentration of 1000 pg/ml (3600 pmol/l).

#### Hormone measurements

Blood plasma samples were taken daily, between 08.00 and 10.00 h and prior to the gonadotrophin injection, throughout the follicular phase, and assayed for oestradiol, LH and FSH using direct fluoroimmunoassays (DELFIA, Turku, Finland). Samples were also assayed for FSH using a radioimmunoassay (Miaclone; Serono Diagnostics, Turku, Finland) Samples were also assayed for oestradiol, LH and FSH using direct fluoroimmunoassays (DELFIA, Turku, Finland). Samples were also assayed for oestradiol, LH and FSH using direct fluoroimmunoassays (DELFIA, Turku, Finland). Samples were also assayed for oestradiol, LH and FSH using direct fluoroimmunoassays (DELFIA, Turku, Finland). Samples were also assayed for oestradiol, LH and FSH using direct fluoroimmunoassays (DELFIA, Turku, Finland). Samples were also assayed for oestradiol, LH and FSH using direct fluoroimmunoassays (DELFIA, Turku, Finland).

Direct assays of the gonadotrophin preparations showed that the fluoroimmunoassay for FSH gave a B/I ratio of ~2.5/1, whilst the corresponding ratio for the radioimmunoassay was ~2/1, where the 'B' represents biological activity obtained in the in-vivo bioassay used for all FSH assay systems, and the 'I' represents the immunoassay value.

Plasma samples on S1, S6, and on the day of HCG administration (SHCG) were also assayed, using radioimmunoassays (DPC Ltd) for testosterone and also progesterone on SHCG.

The steroid hormones were assayed in a minimum of three follicular fluid samples, uncontaminated by blood or aspiration medium, and a mean value was calculated for each patient. Cases (n = 7) where fewer than three follicular fluid samples were collected were excluded from the calculations.

The mean circulating FSH and LH concentrations in the follicular phase were recorded for each patient by calculating the mean of four values between stimulation days 5 and 8, inclusive. These days were chosen because 'steady state' values of FSH were not obtained prior to this and dose changes and were excluded from day 8. These data were used to investigate the effects of the different circulating concentrations of FSH and LH on ovarian function.

The role of the low concentrations of circulating LH in patients treated with MHP was investigated by dividing the patients into groups whose mean follicular phase LH (days S5–S8 inclusive) was either <1.0 IU/l (MHP<LH group) or >0.99 IU/l (MHP>LH) respectively. Below 1.0 IU/l the interassay variation for LH was increased above the 8% shown for values above 1.0 IU/l, and it was deemed that a cut-off below this value would be relatively unreliable. Ultrasound assessments of follicular size were made frequently during the follicular phase.

#### Statistics

Comparisons of responses were made using two sample t-tests and Mann–Whitney test for non-parametric data. Contingency table data were compared using χ² analyses.

#### Results

##### Randomization

The block randomization resulted in three groups of patients with similar ages (HMG, 32.4 ± 2.8; MHPim, 33.7 ± 3.9; MHPsc, 33.3 ± 3.6 years) and body mass index values (HMG, 23.26 ± 2.05; MHPim, 24.27 ± 2.84; MHPsc, 22.37 ± 2.61).

##### Comparisons of the three treatment groups

Figure 1 shows that the circulating mean concentration of FSH rose in all three groups to reach a 'steady state' level from day S5 onwards. From day 4 after treatment started (S4), the MHPsc group showed significantly (P < 0.005) higher concentrations compared with either the MHPim or HMG group. Figure 1 also shows that the oestradiol concentration in the HMG group rose more rapidly and to significantly higher values between days S5 and S8 (S5 and S6, P < 0.01; S7 and S8, P < 0.005) compared to either MHP-treated group. Doses of gonadotrophin were changed in some patients after day 7, so further comparison after this point was inappropriate.

Circulating concentrations of immunoassayable LH were similar in the two MHP groups (not shown), with mean values (S5–S8 inclusive) of 1.32 IU/l (MHPim) and 1.19 IU/l (MHPsc), while the HMG group showed significantly higher (P < 0.01) concentrations, with a mean of 1.81 IU/l (SD = 0.83).

Table I shows that, at the time of HCG administration, there was no difference in the oestradiol concentrations between the groups, which all showed large standard deviations, and the numbers of mature sized follicles were also similar. These were both constitutive parameters in the criteria for HCG administration, so significant differences would not be expected. The lengths of the follicular phases (treatment days), a criterion with considerable subjectivity because the criteria for HCG administration were essentially minimum values,
Figure 1. Profiles of mean follicle stimulating hormone (FSH) and mean oestradiol concentrations seen in the first week of treatment with human menopausal gonadotrophin administered i.m. (HMGim; \( n = 20 \)), or Metrodin High Purity administered i.m. (MHPim; \( n = 20 \)) or s.c. (MHPsc; \( n = 20 \)). Despite the MHPsc group showing higher FSH concentrations from day 4 after treatment started (S4), the HMGim patients showed higher oestradiol values (*\( P < 0.01 \)) over the same period.

Figure 2. Profiles of mean oestradiol concentration in patients during the first week of treatment with human menopausal gonadotrophin (HMG, \( n = 20 \)) or with Metrodin High Purity (MHP) grouped according to their mean follicular phase luteinizing hormone (LH) concentrations. The MHP<LH group showed mean circulating LH values <1.0 IU/L (\( n = 17 \)), while the MHP>LH showed mean values ≥1.0 IU/L (\( n = 23 \)). Differences between the two MHP groups were significant (\( P < 0.05 \)) on days 5, 6 and 7 after treatment started (S5, S6, S7), as were those between the HMG and MHP<LH groups. The HMG group did not show any significant difference from the MHP>LH group.

Table I. Responses of the three treatment groups on the day of human chorionic gonadotrophin (HCG) administration. Values are means with SD in parentheses

<table>
<thead>
<tr>
<th></th>
<th>No of follicles &gt;16 mm diameter</th>
<th>Concentration on day of HCG administration</th>
<th>No. of treatment days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oestradiol (pg/ml)</td>
<td>Progesterone (mg/ml)</td>
</tr>
<tr>
<td>HMGim</td>
<td>5.50 (2.31)</td>
<td>2235 (1131)</td>
<td>1.41 (0.50)</td>
</tr>
<tr>
<td>MHPim</td>
<td>5.55 (2.82)</td>
<td>2508 (2424)</td>
<td>1.26 (0.62)</td>
</tr>
<tr>
<td>MHPsc</td>
<td>5.45 (3.02)</td>
<td>2076 (1009)</td>
<td>1.67 (1.03)</td>
</tr>
</tbody>
</table>

*Duration of treatment was significantly shorter in the HMG-treated group than in the MHP-treated patients \( (P < 0.05) \)

HMGim = human menopausal gonadotrophin administered i.m.; MHPim and MHPsc = purified follicle stimulating hormone, i.e. Metrodin High Purity, administered i.m. or s.c.

were longer in the MHP patients by >2 days \( (P < 0.05) \). There was no difference in the plasma progesterone or testosterone concentrations seen on the day of HCG administration.

The role of low follicular phase concentrations of LH in MHP treatment

The impact of low follicular phase LH concentrations was explored (Figure 2 and in Tables II and III) by measuring LH values in patients treated with MHP. The discriminator value for mean LH concentration to separate the patients was <1.0 IU/L. Suppressed LH was observed in 17 patients treated with MHP, of whom nine were from the MHPsc group, indicating that the route of FSH administration was unrelated to the circulating LH concentration. The effect of suppressed LH in the MHP-treated patients on oestradiol secretion during the first week of stimulation (days S1–S7) is shown in Figure 2. The patients treated with HMG were divided with respect to circulating LH concentrations, since it was presumed that the LH within the HMG preparation would influence ovarian responses, irrespective of the circulating concentration observed 24 h after administration. There was no difference

Table II. Responses to stimulation in the MHP<LH (mean LH <1.0 IU/L) and MHP>LH (mean LH ≥1.0 IU/L) groups and in the HMG-treated patients. Values are means (SD) or percentages

<table>
<thead>
<tr>
<th></th>
<th>MHP&lt;LH</th>
<th>MHP&gt;LH</th>
<th>HMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>17</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Oestradiol at HCG (pg/ml)</td>
<td>1919 (1095)</td>
<td>2564 (2222)</td>
<td>2235 (1131)</td>
</tr>
<tr>
<td>Testosterone at day S6* (pg/ml)</td>
<td>334 (203)</td>
<td>335 (127)</td>
<td>490 (239)</td>
</tr>
<tr>
<td>Testosterone at HCG (pg/ml)</td>
<td>480 (177)</td>
<td>602 (266)</td>
<td>663 (223)</td>
</tr>
<tr>
<td>No. of days of treatment</td>
<td>14.8 (3.5)</td>
<td>13.3 (4.3)</td>
<td>11.6 (3.1)</td>
</tr>
<tr>
<td>Maximum follicle diameter (mm) at SS*</td>
<td>12.3 (3.7)</td>
<td>13.4 (4.4)</td>
<td>15.1 (3.8)</td>
</tr>
<tr>
<td>No. of follicles with diameter &gt;16 mm at HCG</td>
<td>6.06 (3.3)</td>
<td>5.09 (2.5)</td>
<td>5.5 (2.3)</td>
</tr>
<tr>
<td>No of oocytes retrieved</td>
<td>7.5 (4.7)</td>
<td>7.5 (4.1)</td>
<td>8.05 (3.5)</td>
</tr>
<tr>
<td>Normal fertilization rate (%)</td>
<td>61.3</td>
<td>68.9</td>
<td>62.4</td>
</tr>
</tbody>
</table>

MHP = Metrodin High Purity; LH = luteinizing hormone, HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

*Days after treatment started
b\( P < 0.01 \) comparing MHP<LH with MHP>LH and also with HMG group.

c\( P < 0.01 \) comparing MHP<LH with HMG group.
between the MHP-treated patients with LH ≥ 1.0 IU/l (MHP>LH, n = 23) and the HMG-treated patients. However, the MHP-treated patients with LH < 1.0 IU/l (MHP<LH, n = 17) showed significantly (P < 0.05) lower oestradiol output from day 5 onwards compared with the MHP>LH group as well as with the HMG group. These profiles suggest that the differences in the MHP-treated patients seen in Figure 1 (upper panel) are due to the low concentrations of LH seen in a proportion of the patients.

Table II shows that the MHP<LH patients did not show significantly lower circulating concentrations of oestradiol or testosterone at the time of HCG administration than either the HMG or the MHP>LH groups. Similarly, no differences in the numbers of large follicles (follicular diameter >16 mm), or in the numbers of oocytes aspirated at oocyte retrieval were observed, indicating that the degree of follicular growth was similar in all three groups. However, the duration of the follicular phase in the MHP<LH groups was significantly (P < 0.01) longer than in the HMG group, and also the size of the leading follicle when scanned at day 7/8 of stimulation was smaller (P < 0.01) in the MHP<LH group compared with the HMG groups. The MHP>LH group was not different from the HMG group in either of these parameters, indicating that rates of follicular growth may have been compromised in patients with circulating follicular phase LH < 1.0 IU/l who were treated with pure FSH. However, the fertilization rate of the oocytes retrieved was not significantly lower in the MHP<LH patients than in MHP>LH or HMG patients.

The reproductive steroid hormone concentrations seen in the follicular fluids are shown in Table III. Both oestradiol and testosterone were significantly lower in the MHP<LH group compared with either of the other two groups (P < 0.05 and P < 0.01, see Table III). There was no difference in the concentrations of progesterone between any of the groups.

Discussion

These results indicate that the new purer, monotherapeutic FSH preparations yield different responses compared with ‘traditional’ HMG treatment, depending upon both the route of administration and also the endogenous LH activity.

Higher steady-state circulating concentrations of immunoassayable FSH were seen with MHPsc administration than with either formulation administered i.m. Despite this difference in FSH concentrations, there was no difference in oestradiol response during the first week of treatment with MHPim, and both MHP groups showed lower oestradiol secretion than the HMG group. In fact, the raised FSH concentrations observed in the MHPsc group showed no benefit in the responses of either oestradiol secretion or increased follicular recruitment. However, the major determinant of oestradiol secretion amongst the MHP-treated patients appeared to be the endogenous LH activity. Other markers of ovarian response, e.g. follicular growth and androgen production, were also influenced by the LH concentration more than by the route of administration of FSH. Detection of differences in fertilization rates, however, would demand a much larger study, since significant differences were not shown with these relatively small numbers. More detailed investigations are required to determine the nature and degree of such effects.

The higher circulating concentrations of FSH seen after daily administration when the gonadotrophin was administered s.c. compared with the traditional i.m. route was an unexpected finding, since recombinant FSH, purified to a similar degree, showed bioavailability and clearance characteristics similar to those of the urinary product (Metrodin, Serono UK Ltd; Le Cotonnet et al., 1994b). The observations were confirmed by repeating the assay using a different immunoassay which slightly increased numerical values but which maintained the differences between the groups (unpublished data); the absence of differences in body mass index between the groups indicates that it is a physiological phenomenon. This observation suggests that, in practice, the s.c. route of administration yields an increased bioavailability of FSH which may be exploited in the future by adapted dosage schedules.

The oestradiol profiles showed lower concentrations in the MHP-treated patients during the first week of treatment, irrespective of the route of administration. A priori, this could be due to reduced oestradiol secretion by the recruited follicles, a reduced rate of follicular recruitment, a reduced cohort of follicles, or a combination of these phenomena. Similar numbers of follicles on the day of HCG administration indicate that total follicular recruitment was similar in all groups, suggesting that follicular recruitment and growth were similar in all groups. Although the duration of treatment was longer in the MHP patients, the overall outcome appeared to be little influenced by the route of administration or the purity of the FSH. The raised concentrations of FSH seen in the MHPsc group indicate that there is no more recruitment to be obtained above a certain threshold value.

On the other hand, the circulating LH activity appeared to have considerable influence on responses, irrespective of the route of FSH administration. This was demonstrated by the MHP<LH patients who showed reduced oestradiol secretion throughout the first week of treatment compared with both other groups. This is fully in accord with the observations in hypogonadotrophic hypogonadal women treated with FSH (Couzinet et al., 1988). The reduced plasma concentrations of oestradiol were reflected in lower follicular fluid concentrations of both testosterone and oestradiol in these patients, which may be explained by reduced thecal androgen biosynthesis leading to low oestradiol production and secretion. However,
the longer follicular phases and the smaller leading follicle
diameters seen at the first scan in these patients also suggest
that the combined effect of fully suppressed LH and mono-
therapy may also result in a reduced rate of follicular growth.

It is possible that this could have an impact at the level of
the oocyte, although similar fertilization rates were observed
in all groups. Whilst it is understood that fertilization rates
can be misleading, further investigations, including of embryo
development, should be done in order to confirm whether or
not an environment that has such profound influence on steroid
biosynthesis influences the biology of oocyte maturation and
embryo development. Abnormal steroid biosynthesis in LH
suppression induced by GnRH-antagonists has been shown to
be associated with different responses at the level of oocyte/embryo development in primates (Zelinski-Wooten et al., 1996).

The arguments over the actual level of LH activity, its
immunoassayable equivalent, and the duration of exposure
required during follicular growth have not yet developed to
any sophistication (Hillier et al., 1995), and the sequelae of
depression may be more profound than mere oestradiol production. The system of GnRHa suppression used in this
work is widely used in IVF programmes and has shown few
problems when used with HMG. However, its use with purified
FSH may have to be adapted to reduce the proportion of
patients showing effectively suppressed LH concentrations
(represented by <1.0 IU/l in the assay used in this study).
Alternatively, additional LH activity may be required to achieve
satisfactory follicular growth in all patients.

The absence of LH from the MHP preparations did not lead
to any reduction in the circulating progesterone concentrations
seen on the day of HCG administration. This is an important
observation that indicates that the LH component of HMG
had negligible luteinizing influence on the maturing follicles
prior to HCG administration. However, the apparently reduced
oestradiol secretion per follicle during the follicular phase,
although yielding similar concentrations on the day of HCG
administration, indicates that the monitoring of responses, and
the criteria by which HCG is administered, may require
amendment in many centres when purified FSH preparations
are used.

In summary, the higher FSH activity achieved by s.c.
administration of MHP showed negligible impact on follicular
recruitment and development, but the concentration of circulat-
ing LH activity showed considerable influence on steroid
biosynthesis and on other parameters of follicular development.

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