Does the addition of recombinant LH in WHO group II anovulatory women over-responding to FSH treatment reduce the number of developing follicles? A dose-finding study

J.N.Hugues1,7, J.Soussis2, I.Calderon3, J.Balasch4, R.A.Anderson5 and A.Romeu6 on behalf of the Recombinant LH Study Group*

1Center of Reproductive Medicine, Hôpital Jean Verdier, Bondy, France, 2IVF & Genetics, Athens, Greece, 3Lin Professional Clinic, Haifa, Israel, 4Hospital Clinic de Barcelona, Barcelona, Spain, 5Edinburgh Royal Infirmary, Edinburgh, UK and 6Hospital Universitario La Fe, Valencia, Spain

7To whom correspondence should be addressed at: Center of Reproductive Medicine, Hôpital Jean Verdier, Av du 14 Juillet, Bondy, 93143, France. E-mail: jean-noel.hugues@jvr.ap-hop-paris.fr

BACKGROUND: In anovulatory women undergoing ovulation induction, addition of recombinant human LH (rLH) to FSH treatment may promote the dominance of a leading follicle when administered in the late follicular phase. The objective of this study was to find the optimal dose of rLH that can maintain the growth of a dominant follicle, whilst causing atresia of secondary follicles.

METHODS: Women with infertility due to anovulation and over-responding to FSH treatment were randomized to receive, in addition to 37.5 IU recombinant human FSH (rFSH), either placebo or different doses of rLH (6.8, 13.6, 30 or 60 \( \mu g \)) daily for a maximum of 7 days. The primary efficacy endpoint was the proportion of patients who had exactly one follicle \( \geq 16 \) mm on hCG day.

RESULTS: Among 153 enrolled patients, the five treatment groups were similar in terms of baseline characteristics. The proportion of patients with exactly one follicle \( \geq 16 \) mm ranged from 13.3% in the placebo group to 32.1% in the 30 \( \mu g \) rLH group (\( P = 0.048 \)). The pregnancy rate ranged from 10.3% in the 60 \( \mu g \) group to 28.6% in the 30 \( \mu g \) rLH group. Adverse events were similar between groups.

CONCLUSIONS: In patients over-responding to FSH during ovulation induction, doses of up to 30 \( \mu g \) rLH/day appear to increase the proportion of patients developing a single dominant follicle (\( \geq 16 \) mm). Our data support the ‘LH ceiling’ concept whereby addition of rLH is able to control development of the follicular cohort.

Key words: anovulation/follicular growth/recombinant human FSH/recombinant human LH

Introduction

Ovarian follicular development in the late stage of folliculo-genesis requires the combined actions of two gonadotrophins: FSH and LH. The pivotal role of FSH for follicular recruitment and growth has been well demonstrated. In clinical practice, a minimal FSH requirement, defined as the ‘FSH threshold’, must be surpassed to initiate follicular growth (Brown, 1978; Scheele et al., 1993; Fauser and Van Heusden, 1997). This concept has been applied to the stimulation of WHO group II anovulatory women through a stepwise administration of FSH, the so-called ‘chronic-low-dose step-up protocol’ (Seibel et al., 1984; Buvat et al., 1989; Hamilton-Fairley et al., 1991).

However, although follicular growth can be induced by FSH in the total absence of LH, there is also some evidence that optimal follicular development requires a minimal exposure to LH to ensure adequate estradiol (E2) production, full oocyte maturity, follicular rupture and the ability for granulosa cells to be luteinized in response to hCG (Couzinot et al., 1988; Glasier et al., 1988; Shoham et al., 1991; Balasch et al., 1995). As demonstrated in patients suffering from severe deficiency in LH and FSH, daily injections of low doses of recombinant human LH (rLH; 75IU) are required to ensure optimal follicular maturation (European Recombinant Human LH Study Group, 1998).
LH receptors appear on granulosa cells that have been adequately stimulated by FSH and the developing follicle becomes increasingly dependent on LH (Richards et al., 1987). In the granulosa cells of the pre-ovulatory follicle, aromatase is functionally coupled to the LH receptors such that LH directly regulates follicular estrogen secretion (Zeleznik and Hillier, 1984; Zeleznik, 2001). Consequently, substitution of FSH by LH therapy in the late follicular phase is able to maintain adequate steroidogenesis (Sullivan et al., 1999) and even final stages of follicular maturation (Balasch and Fabregues, 2003).

On the other hand, the concept of an ‘LH ceiling’ was initially based upon clinical observations that follicles exposed to inappropriately high concentrations of LH enter atresia or become prematurely luteinized, and oocyte development may be compromised (Howles et al., 1986; Chappel and Howles, 1991; Jacobs, 1991). The dose dependence of this LH effect was illustrated by studies in vitro: ‘low-dose’ treatment with LH serves generally to enhance steroidogenesis without inhibiting DNA synthesis, but ‘high-dose’ LH causes enhanced synthesis of progesterone, suppression of aromatase activity and inhibition of cell growth (Overs et al., 1992; Yong et al., 1992). Thus, developing follicles appear to have finite requirements for stimulation by LH, beyond which normal development ceases.

Whereas each follicle has a threshold beyond which it must be stimulated by FSH to initiate pre-ovulatory development (Brown, 1978), it may also have a ‘ceiling’ within which it should be stimulated by LH, above which normal development is terminated (Hillier, 1993). During the second half of the follicular phase, as plasma FSH concentrations decline, the LH-dependent phase of pre-ovulatory follicular development only proceeds normally if LH is present at concentrations beneath this ceiling value (Hillier, 2000). When the ceiling is exceeded at the mid-cycle surge of LH, further division of granulosa cells would cease as luteinization proceeds (Hillier, 1994).

In this respect, the potential effect of LH administration in controlling the number of developing follicles deserves clinical investigation. Recently, Loumaye et al. (2003) provided evidence that rLH alone can trigger follicular growth arrest in WHO group I or II anovulatory patients. Therefore, rLH may be of particular use in treatment regimens that aim to achieve mono-ovulation for conception in vivo. However, the optimal dose of rLH required to induce atresia of secondary follicles is still not established.

The objective of this prospective, randomized, double-blind, multicentre study was to evaluate the effects of four different doses of rLH administered in the late follicular phase and to establish the minimal effective dose required to promote maturation and ovulation of a single follicle while inducing atresia in secondary follicles.

Materials and methods

Patients

This study was conducted in WHO Group II anovulatory women fulfilling the following criteria: (i) pre-menopausal women aged between 18 and 39 years, wishing to conceive; (ii) infertility due to ovulatory dysfunction as based on WHO group II criteria (normogonadotropic oligo-ovulation or anovulation attested by low (<10 nmol/l) progesterone value 20–25 days after spontaneous or progestogen-induced menses and undergoing treatment for ovulation induction; (iii) body mass index (BMI) between 18 and 35 kg/m²; (iv) at least one tube patent and a normal uterine cavity, as documented by hysterosalpingography, laparoscopy or hysteroscopy within the past 5 years; (v) male partner with semen quality compatible with conception following natural intercourse or intrauterine insemination.

Study design

The study was conducted according to Good Clinical Practice guidelines, and ethical committee approval was obtained to conduct the study in all participating centres.

To be eligible, patients were to have been stimulated with urinary FSH (uFSH) or recombinant FSH (rFSH) for ovulation induction and have started FSH treatment within 5 days of spontaneous or induced menstruation. The starting dose of FSH was chosen to achieve follicular recruitment without exceeding a daily dose of 150 IU according to the BMI. Women were enrolled in the study if they were experiencing an excessive follicular response to FSH stimulation treatment attested by the presence on ultrasound of at least three follicles 11–15 mm in diameter, but no follicles > 15 mm in diameter at any point during stimulation with FSH. Patients were then randomized to receive rLH or placebo in combination with 37.5 IU of rFSH daily from the day of randomization (S1) until the criteria for hCG administration were reached, or for a maximum of 7 days (S7). The treatment period could be interrupted before completion if obvious regression of all follicles was recorded or if the patient was at risk of developing ovarian hyperstimulation syndrome (OHSS). A single injection of hCG (5000 IU) was given within 36 h of the last rLH or placebo/rFSH injection if monitoring by ultrasound showed a maximum of two follicles with a mean diameter ≥16 mm and if the total follicular number and the serum E₂ levels were not indicative of OHSS risk.

Randomization and medications used

Either urinary FSH (Metrodin® or Metrodin HP®, Serono, Switzerland) or rFSH (Gonal F®, Serono, Switzerland) were used for the initial part of the ovarian stimulation cycle, prior to randomization. Doses of rLH (Luveris®; Serono) were selected for each patient in a randomized fashion, without any stratification per treatment group. Subcutaneous administration of 37.5 IU rFSH in addition to the rLH or placebo injection was performed in every case, whatever the amount of FSH used during the stimulation period prior to randomization.

rLH/rFSH administration was started on the day of randomization (S1), once eligibility had been confirmed and the patient had given her informed consent for entry into the study. Patients were randomized to one of the following treatments, all administered daily by subcutaneous injection: (i) placebo; (ii) 6.8 µg (150 IU) rLH; (iii) 13.6 µg (300 IU) rLH; (iv) 30 µg (660 IU) rLH; (v) 60 µg (1325 IU) rLH, rLH or placebo treatment assigned to each patient was determined according to a computer-generated randomization list stratified by centre. A single injection of 5000 IU hCG (Profasi®; Serono) was given when the criteria for triggering ovulation were met, as described above.

Monitoring

Monitoring was primarily performed by vaginal ultrasonography. The examinations were performed weekly prior to
the administration of rLH/placebo and daily during the treatment period. All follicles with a mean diameter (i.e. the mean of the two longest perpendicular diameters) >10 mm were recorded according to their size. Whenever possible, the individual growth over time of each follicle with a diameter ≥11 mm was recorded.

Blood samples were taken for local E2 measurements during the whole period and for retrospective analysis of E2, progesterone, LH and inhibin A and B, prior to administration of rLH/placebo and on a daily basis up to the day of hCG administration (or the last day of gonadotrophin treatment if no hCG was administered). The luteal phase was assessed and monitored by measuring progesterone serum values between day 6 and day 8 post-hCG.

**Hormone assays**

All assays were performed at a central laboratory: LCG Bioscience Clinical (Science Department, Bourn Hall Clinic, Bourn, Cambridge, UK). Serum FSH and LH were measured using a validated, commercially available immunoradiometric assay (IRMA) method (MAIAClone; Serono). The limit of quantification for serum FSH and LH was 1 IU/l. Serum E2, progesterone, inhibin A and inhibin B were measured using validated commercially available immunoassays. Centralized immediate safety biochemistry and haematology analyses were performed by Covance CLS (Covance Centre for Safety and Clinical Research, Bridgend, UK). Serum FSH and LH were measured using a validated, commercially available immunoradiometric assay (IRMA) method (MAIAClone; Serono). The limit of quantification for serum FSH and LH was 1 IU/l. Serum E2, progesterone, inhibin A and inhibin B were measured using validated commercially available immunoassays. Centralized immediate safety biochemistry and haematology analyses were performed by Covance CLS (Covance Centre for Safety and Clinical Research, Bridgend, UK).

**Study endpoints and sample size calculation**

The primary efficacy endpoint of the study was the proportion of patients who received hCG and who had exactly one follicle ≥16 mm in diameter. The main secondary efficacy endpoints were: (i) proportion of patients who received hCG according to the protocol criteria; (ii) mean number of pre-ovulatory follicles (≥16 mm in diameter) on the day of hCG; (iii) mean number of medium-sized follicles (between 11 and 15 mm in diameter); (iv) difference between the number of baseline follicles compared with the number of pre-ovulatory follicles on the day of hCG; (v) longitudinal follicular growth (where possible); (vi) frequency of ovulation (luteal phase progesterone levels) and inhibin A and B, prior to administration of rLH/placebo and on a daily basis up to the day of hCG administration (or the last day of gonadotrophin treatment if no hCG was administered). The luteal phase was assessed and monitored by measuring progesterone serum values between day 6 and day 8 post-hCG.

The primary efficacy endpoint of the study was the proportion of patients who received hCG and who had exactly one follicle ≥16 mm in diameter. The main secondary efficacy endpoints were: (i) proportion of patients who received hCG according to the protocol criteria; (ii) mean number of pre-ovulatory follicles (≥16 mm in diameter) on the day of hCG; (iii) mean number of medium-sized follicles (between 11 and 15 mm in diameter); (iv) difference between the number of baseline follicles compared with the number of pre-ovulatory follicles on the day of hCG; (v) longitudinal follicular growth (where possible); (vi) frequency of ovulation (luteal phase progesterone levels ≥25 nmol/l); (vii) clinical pregnancy rate and outcome.

It was planned to enrol a total of 150 evaluable patients (30 patients per group). This number was based upon the results obtained from previous studies carried out in the same patient population, assuming 80% power and 5% significance level (two-sided) for the proportion of patients/cycle with exactly one follicle ≥16 mm in diameter.

**Statistical methods**

The statistical method used depended on the nature of the variable analysed. As one of the main objectives of the study was to find the minimal effective dose of rLH, the primary analysis carried out was that of a dose relationship, with a linear modelling using treatment received as a factor for comparison. A parametric model (logistic regression) was computed in order to perform the dose relationship and subsequent pairwise comparisons. Paired comparisons were carried out between placebo and each of the doses of rLH and then between each rLH dose and each of the subsequent dosing regimes. All secondary endpoints were analysed using the same methods of statistical analysis described above. Other statistical tests such as analysis of variance, logistic regression, exact logistic regression, Cochran–Mantel–Haenszel or Fisher’s exact test were used as appropriate. Due to observed differences between treatment groups in BMI and dose of FSH or rLH, the analysis of the primary endpoint was adjusted for these parameters in order to better evaluate treatment effect.

**Results**

**Demographic characteristics**

Twenty-two participating centres enrolled a total of 153 patients, all of whom were allocated to one of the treatment groups (31 to placebo; 32 to 6.8 μg rLH; 29 to 13.6 μg rLH; 30 to 30 μg rLH; 31 to 60 μg rLH). Six were excluded from the evaluable patient (per-protocol) analysis due to major protocol deviations that may have had a clinically significant impact on the assessment of the efficacy of the study drug. The proportion of these patients was balanced between groups (one each from the placebo and 13.6 μg rLH groups, and two each from the 30 and 60 μg rLH groups).

The main demographic parameters of the 147 analysed patients were statistically balanced between treatment groups as regards past medical history, current medical condition, associated infertility factors, obstetric history, and baseline hormonal parameters. A total of 132 (89.8%) patients had been previously stimulated with clomiphene citrate and/or gonadotrophins (Table I). The proportion of previously

<table>
<thead>
<tr>
<th>Table I. Demographic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Type of infertility</td>
</tr>
<tr>
<td>Primary</td>
</tr>
<tr>
<td>Secondary</td>
</tr>
<tr>
<td>Missing</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
</tr>
<tr>
<td>Duration of anovulation (years)</td>
</tr>
<tr>
<td>Infertility-associated factors (%)</td>
</tr>
<tr>
<td>Male factor infertility</td>
</tr>
<tr>
<td>Endometriosis</td>
</tr>
<tr>
<td>One patent tube</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Previous stimulation (%)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

BMI = body mass index; rLH = recombinant human LH.
stirulated cycles (mean of 3.2 clomiphene citrate and mean of 2.0 gonadotrophin cycles) was also balanced between treatment groups.

**Ovarian stimulation with FSH prior to randomization**

The duration of FSH treatment prior to randomization ranged from 3 to 28 days, with a mean duration of 9.7 days. The dose of FSH administered ranged from 200 to 3300 IU, with a mean dose of 985 IU (Table II). Although not statistically significant, some differences were observed between treatment groups \((P = 0.28 \text{ and } 0.22 \text{ respectively})\). At the time of randomization, the number of follicles with a mean diameter between 11 and 15 mm ranged from 3 to 15, with a mean of 5.1 follicles. No follicle >15 mm in diameter was seen on the ultrasound of any of the patients, confirming that all patients were eligible for study entry. These parameters were considered by the statistical analysis to be balanced between treatment groups.

**Efficacy results**

The efficacy data set included all randomized patients who completed the scheduled rLH administration without major deviation from the protocol \((n = 147)\). The mean duration of treatment was 2.9 days, with a range from 1 to 7 days. Overall, the mean duration did not differ statistically between treatment groups. Of the 147 patients who received rLH/placebo treatment, 94 (64%) received hCG according to the protocol criteria. The other 53 (36%) patients withdrew before receiving hCG and did not complete the study: 43 failed to meet the protocol criteria for hCG administration (due to the risk of multiple pregnancy), nine presented follicular regression and one withdrew on her own decision. The proportion of patients who discontinued was statistically balanced between treatment groups.

The primary efficacy endpoint of the study was the proportion of patients who received hCG and who had exactly one follicle \(\geq 16\) mm in diameter on that day. Of the 147 randomized patients, 34 (23.1%) met these two criteria. Within the treatment groups, the proportion of patients meeting these criteria ranged from 13.3% in the placebo group to 32.1% in the 30 \(\mu\)g rLH group (Table II). After controlling for BMI and the dose of FSH, pairwise comparison of the 30 \(\mu\)g rLH group versus placebo showed a significant difference \((P = 0.048)\).

Table II shows the secondary efficacy endpoints for the 94 patients who received hCG according to the protocol criteria (maximum of two follicles with a mean diameter \(\geq 16\) mm). The proportion of these patients was slightly higher in the 30 \(\mu\)g rLH-treated group than in the 60 \(\mu\)g rLH-treated group. The number of follicles \(\geq 16\) mm in diameter on the day of hCG ranged from one to two with 1.6 \(\pm 0.5\) follicles in all the rLH groups and 1.8 \(\pm 0.4\) follicles in the placebo group. This difference did not achieve significance. Furthermore, the number of small follicles (4–10 mm) was not significantly different between treatment groups.

If we consider the hormonal parameters at the time of hCG administration (Table III), the statistical analysis showed no significant difference for E2 values but significantly higher mean levels for LH in the 60 \(\mu\)g rLH group compared with placebo \((P = 0.007)\). Inhibin A and B mean values on the day of hCG administration were not significantly different between groups. However, significant differences in inhibin B were detected 2 days before hCG administration, due to a high inhibin B level in the placebo group \((1223.3 \pm 1185.5 \text{ pg/ml})\), compared with 537.3 \(\pm 460.1 \text{ pg/ml}\) in the 6.8 \(\mu\)g group \((P = 0.013\) versus placebo), \(557.5 \pm 459.8 \text{ pg/ml}\) in the 30 \(\mu\)g group \((P = 0.031)\) and \(500.0 \pm 561.5 \text{ pg/ml}\) in the 60 \(\mu\)g group \((P = 0.012)\).

A treatment effect for progesterone values was detected, due to high mean levels in the 60 \(\mu\)g rLH group, and the difference was highly significant \((P < 0.001\) at S4). Among the 94 patients who received hCG, 74 (79%) had a luteal phase progesterone level \(\geq 25 \text{ nmol/l}\) and the proportion of patients did not differ significantly between treatment groups.

The pregnancy rate was evaluated in the overall population. Twenty-seven of the 147 (18.4%) patients had at least one serum hCG >10 IU/l. Within treatment groups, the proportion of patients ranged from 10.3% in the 60 \(\mu\)g rLH group to 28.6% in the 30 \(\mu\)g rLH group, while being 20% in the placebo group, 18.8% in the 6.8 \(\mu\)g rLH group and 14.3% in the 13.6 \(\mu\)g rLH group. The difference between treatment groups was statistically significant.

**Table II. Efficacy results**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>6.8 (\mu)g rLH</th>
<th>13.6 (\mu)g rLH</th>
<th>30 (\mu)g rLH</th>
<th>60 (\mu)g rLH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of FSH treatment (days)</td>
<td>9.0 (5.1)</td>
<td>9.1 (3.7)</td>
<td>9.0 (4.3)</td>
<td>11.1 (6.2)</td>
<td>10.6 (5.1)</td>
<td>9.7 (5.0)</td>
</tr>
<tr>
<td>prior to randomisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH dose (IU) prior to randomisation</td>
<td>889.7 (558.6)</td>
<td>870.7 (409.9)</td>
<td>931.3 (522.2)</td>
<td>1099.6 (708.1)</td>
<td>1149.1 (646.4)</td>
<td>984.6 (578.0)</td>
</tr>
<tr>
<td>Randomized patients (n)</td>
<td>30</td>
<td>32</td>
<td>28</td>
<td>28</td>
<td>29</td>
<td>147</td>
</tr>
<tr>
<td>Patients receiving hCG (%)</td>
<td>17 (56.7)</td>
<td>22 (68.8)</td>
<td>18 (64.3)</td>
<td>21 (75.0)</td>
<td>16 (55.2)</td>
<td>94 (63.9)</td>
</tr>
<tr>
<td>Patients with only one follicle (\geq 16) (%)</td>
<td>4 (13.3)</td>
<td>8 (25.0)</td>
<td>7 (25.0)</td>
<td>9 (32.1(^a))</td>
<td>6 (20.7)</td>
<td>34 (23.1)</td>
</tr>
<tr>
<td>No. of follicles (day of hCG) (SD) (\geq 16) mm</td>
<td>1.8 (0.4)</td>
<td>1.6 (0.5)</td>
<td>1.6 (0.5)</td>
<td>1.6 (0.5)</td>
<td>1.6 (0.5)</td>
<td>1.6 (0.5)</td>
</tr>
<tr>
<td>11–15 mm</td>
<td>3.4 (3.4)</td>
<td>2.9 (1.8)</td>
<td>3.7 (3.5)</td>
<td>3.9 (2.7)</td>
<td>3.2 (3.8)</td>
<td>3.4 (3)</td>
</tr>
<tr>
<td>4–10 mm</td>
<td>12 (9.8)</td>
<td>11.8 (6.4)</td>
<td>15.8 (10.4)</td>
<td>12.1 (10.8)</td>
<td>13.9 (10.4)</td>
<td>13 (9.5)</td>
</tr>
<tr>
<td>Clinical pregnancy (%)</td>
<td>5 (16.7)</td>
<td>5 (15.6)</td>
<td>3 (10.7)</td>
<td>8 (28.6)</td>
<td>2 (6.9)</td>
<td>23 (15.6)</td>
</tr>
<tr>
<td>OHSS (%)</td>
<td>0</td>
<td>2 (6.3)</td>
<td>1 (3.4)</td>
<td>1 (3.3)</td>
<td>1 (3.2)</td>
<td>5 (3.3)</td>
</tr>
</tbody>
</table>

Values are mean (\% or SD).
\(^a\)P < 0.05.

hCG = human chorionic gonadotrophin; rLH = recombinant human LH.
Although the proportion of patients who had two fetal sacs to the number of sacs was balanced across treatment groups. (4.3%) had three fetal sacs. Distribution of patients according sound scan, while five (21.7%) had two fetal sacs, and one patients, 17 (73.9%) had exactly one fetal sac seen on ultrasound scan, while five (21.7%) had two fetal sacs, and one patients, 17 (73.9%) had exactly one fetal sac seen on ultrasound scan. These data provide new evidence that, in clinical practice, high-dose LH supplementation in the late follicular phase may be beneficial to reduce the size of the follicular cohort, consistent with the LH ceiling hypothesis. The clinical relevance of this observation should be stressed considering that, even with careful stepwise administration of FSH, the rate of mono-ovulation is only ~65% in stimulated cycles (White et al., 1996; Homburg and Howles, 1999). Therefore, the safety of step-up protocols has still to be improved.

Our data extend the ‘LH ceiling’ concept to clinical practice and confirm the results of a recently published pilot study (Loumaye et al., 2003). In that study, WHO group II anovulatory patients were given placebo, or 225 or 450 IU rLH, without addition of any FSH support, if they presented an over-response to FSH, defined by the presence of four or more follicles that were ≥8 and <13 mm in diameter. Follicular growth arrest was only observed in patients treated with rLH (5/12), as attested by a reduction in the adjusted mean number of follicles ≥14 mm in diameter. Moreover, while placebo treatment was not associated with significant changes in serum E2 levels, administration of rLH alone led to a further increase in serum E2 levels, as previously described by Sullivan et al. (1999). However, some patients with follicle regression presented a sharp decline in serum E2 levels, which could be related to the absence of FSH support during the late follicular phase.

Our study was set up to establish the minimal effective dose required to induce atresia of secondary follicles, whilst supporting the growth of a dominant follicle to pre-ovulatory conditions. To facilitate this, a low dose of FSH was added to the rLH/placebo treatment. According to our data, 30 µg rLH seems to be the most appropriate dose to reduce the size of the developing cohort. Indeed, the proportion of patients who received hCG and who had exactly one follicle ≥16 mm in diameter was significantly higher among patients treated with 30 µg rLH compared with the placebo group. No statistically significant dose effects were detected for these secondary outcome measures, possibly because of the relatively small number of patients in each group. Even if we cannot exclude the possibility that the reduction in the FSH dose may have contributed to follicular atresia, it seems to be unlikely due to the pharmacokinetic and pharmacodynamic properties of FSH preparations (Le Cotonnec et al., 1994). Therefore, our data suggest that 30 µg rLH is the minimal effective dose required to induce arrest of large follicle development.

Another report on the consequences of LH activity on the dynamics of folliculogenesis has been previously reported by Filicori et al. (2002). In that study, administration of

<table>
<thead>
<tr>
<th>n</th>
<th>Placebo (6.8 µg rLH)</th>
<th>13.6 µg rLH</th>
<th>30 µg rLH</th>
<th>60 µg rLH</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (pmol/l)</td>
<td>1692 (1440)</td>
<td>1431 (877)</td>
<td>1698 (1682)</td>
<td>1881 (1897)</td>
<td>1515 (1347)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>10.3 (11.6)</td>
<td>8.4 (8.3)</td>
<td>6.9 (3.9)</td>
<td>8.3 (3.7)</td>
<td>14.1 (7.6)</td>
</tr>
<tr>
<td>Inhibin A (pg/ml)</td>
<td>74.8 (73.0)</td>
<td>53.8 (41.9)</td>
<td>79.9 (72.3)</td>
<td>80.6 (63.6)</td>
<td>60.5 (65.8)</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>574 (797)</td>
<td>338 (247)</td>
<td>458.8 (493.8)</td>
<td>547 (708)</td>
<td>402 (1030)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

Discussion

Ovarian stimulation for chronic anovulation is a challenge for clinicians due to the high risk of over-response, multiple pregnancy and OHSS. There is still a need for a better understanding of the control of follicular growth and for developing new protocols for clinical use. This multicentre, dose-finding study shows that, in patients over-responding to FSH during ovulation induction, doses of up to 30 µg rLH/day are well tolerated in the late follicular phase and appear to increase the proportion of patients developing a single dominant follicle without inducing any premature luteinization. These data provide new evidence that, in clinical practice, high-dose LH supplementation in the late follicular phase may be beneficial to reduce the size of the developing follicular cohort, consistent with the LH ceiling hypothesis. The clinical relevance of this observation should be stressed considering that, even with careful stepwise administration of FSH, the rate of mono-ovulation is only ~65% in stimulated cycles (White et al., 1996; Homburg and Howles, 1999). Therefore, the safety of step-up protocols has still to be improved.

Our data extend the ‘LH ceiling’ concept to clinical practice and confirm the results of a recently published pilot study (Loumaye et al., 2003). In that study, WHO group II anovulatory patients were given placebo, or 225 or 450 IU rLH, without addition of any FSH support, if they presented an over-response to FSH, defined by the presence of four or more follicles that were ≥8 and <13 mm in diameter. Follicular growth arrest was only observed in patients treated with rLH (5/12), as attested by a reduction in the adjusted mean number of follicles ≥14 mm in diameter. Moreover, while placebo treatment was not associated with significant changes in serum E2 levels, administration of rLH alone led to a further increase in serum E2 levels, as previously described by Sullivan et al. (1999). However, some patients with follicle regression presented a sharp decline in serum E2 levels, which could be related to the absence of FSH support during the late follicular phase.

Our study was set up to establish the minimal effective dose required to induce atresia of secondary follicles, whilst supporting the growth of a dominant follicle to pre-ovulatory conditions. To facilitate this, a low dose of FSH was added to the rLH/placebo treatment. According to our data, 30 µg rLH seems to be the most appropriate dose to reduce the size of the developing cohort. Indeed, the proportion of patients who received hCG and who had exactly one follicle ≥16 mm in diameter was significantly higher among patients treated with 30 µg rLH compared with the placebo group. No statistically significant dose effects were detected for these secondary outcome measures, possibly because of the relatively small number of patients in each group. Even if we cannot exclude the possibility that the reduction in the FSH dose may have contributed to follicular atresia, it seems to be unlikely due to the pharmacokinetic and pharmacodynamic properties of FSH preparations (Le Cotonnec et al., 1994). Therefore, our data suggest that 30 µg rLH is the minimal effective dose required to induce arrest of large follicle development.

Another report on the consequences of LH activity on the dynamics of folliculogenesis has been previously reported by Filicori et al. (2002). In that study, administration of
increasing doses of hCG (from 50 to 200IU) from the mid-follicular phase can significantly reduce the number of small follicles without any change in the number of large follicles.

However, many discrepancies exist on the design of these experiments and may explain the opposite conclusion. First, the difference in the pharmacokinetic properties of the drugs used may account for the divergent effects. Indeed, the half-life of hCG is much longer than that of LH (~36 versus 11 h) (Trinchard-Lugan et al., 2002). Second, the design of the study performed by Filicori et al. differed from our own because a hypogonadotrophic state was induced prior to ovarian stimulation through a GnRH agonist administration. Therefore, the considerable difference in endogenous LH secretion resulting from these two protocols means that the effectiveness of exogenous LH supplementation cannot be strictly compared. Most importantly, in Filicori et al.’s study, administration of hCG preparations was started on a fixed day (day 7) of the ovarian stimulation, without any criteria of follicular growth. As LH receptors only appear on differentiated granulosa cells (Richards et al., 1987), it is uncertain whether the effects of hCG preparations on follicular growth were actually related to a direct LH effect on granulosa cells.

The purpose of our study was to address the issue of the direct effects of LH on follicular growth. Therefore, rLH was administered when follicles reached 11–15 mm in diameter (i.e. when LH receptors are constantly expressed on differentiated granulosa cells) (Richards et al., 1987). However, while this design allowed us to more specifically assess the direct effect of LH supplementation on follicular growth, we cannot exclude that the parallel stimulatory effect of LH on androgen secretion may also interfere with the control of follicular growth.

Another interesting finding from our study concerns the dose-related effect of LH on the luteinization process of granulosa cells. Our data clearly indicate that administration of rLH at a daily dose up to 30 µg does not induce premature luteinization. In contrast, a dose of 60 µg rLH was consistently associated with an increase in progesterone values. These findings provide evidence for a pivotal role of LH in the process of luteinization, and for an LH ceiling effect at >30 µg daily dose.

While other studies reported a relationship between the administered dose of FSH and follicular phase progesterone values (Ubaldi et al., 1996; Filicori et al., 2002), our results strongly suggest that LH plays a key role in this process. They also show that the dual effects of LH on the luteinization process and on the control of follicular growth are not equivalent, as the dose required to exert control upon follicular growth is slightly lower than that effective to induce luteinization. Our results contrast with the data provided by Filicori et al. (2002), who reported a slight but significant increase of progesterone values in the whole group of patients treated with hCG and did not observe any dose-dependent effect of hCG preparations. This discrepancy may be related to the different pharmacokinetic properties of both preparations. They also emphasize that conclusions drawn from studies using hCG preparations cannot be applied to those performed with rLH.

Finally, our study shows that, while the clinical pregnancy rate was slightly higher in patients treated with 30 µg rLH, the increase in progesterone secretion induced by a dose of 60 µg rLH may have accounted for the reduction in the pregnancy rate of that group. No clear benefit was observed on the risk of multiple pregnancy rate. This may be related to the small number of patients in each treatment group and further studies are required to investigate whether addition of LH bioactivity is actually effective to prevent the risk of multiple pregnancy.

In summary, this randomized, placebo-controlled, dose-finding study shows that, in patients over-responding to FSH during ovulation induction, doses of rLH up to 30 µg/day are well tolerated in the late follicular phase and appear to increase the proportion of patients developing a single dominant follicle. Our data support the ‘LH ceiling’ concept, whereby addition of a high dose of LH is able to control follicular growth by inducing atresia of developing follicles.

Acknowledgements
This study was set up and supported by Serono International SA, Geneva, Switzerland. We would like to thank E. Loumaye, C. Howles, T. Roztocil and G. Decosterd for their assistance, and P. Engrand and S. Larroque for their help in the statistical analyses.

References


Submitted on March 4, 2004; resubmitted on October 14, 2004; accepted on November 9, 2004