Clinical evidence for an LH ‘ceiling’ effect induced by administration of recombinant human LH during the late follicular phase of stimulated cycles in World Health Organization type I and type II anovulation

Ernest Loumaye1, Patrick Engrand1,4, Zeev Shoham2, Stephen G.Hillier3 and David T.Baird3 on behalf of the Recombinant LH Study Group*

1Serono International, Reproductive Health Clinical Development Unit, Geneva, Switzerland, 2Department of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, Israel and 3Department of Obstetrics and Gynaecology, University of Edinburgh Centre for Reproductive Biology, Edinburgh, UK

BACKGROUND: The objective of these studies was to test the hypothesis that over-dosing with recombinant human LH (rLH) during the late follicular phase would suppress the development of follicles. METHODS: Two double-blind studies were conducted. In study A, WHO I anovulatory patients received treatment with rFSH and rLH. When at least one follicle reached a mean diameter of 10±13 mm, patients were randomized using a computer-generated randomization list (stratified by centre) to rFSH and rLH (225 IU/day) (n = 8) or rLH alone (n = 6) or rFSH alone (n = 6). In study B, WHO II anovulatory patients with a hyper-responsive FSH were randomized to rLH (225 IU/day) (n = 4) or rLH 450 IU/day (n = 8) or placebo (n = 5). RESULTS: Study A: the mean number of follicles >11 mm was 4.2 ± 0.3 in the rFSH group, 1.5 ± 0.7 in the rLH group and 6.0 ± 2.3 in the rFSH/rLH group (P = 0.07). 0/8 patients presented follicular growth arrest in the rFSH group, but 4/6 in the rLH group and 1/6 in the rFSH/rLH did. Study B: 5/12 patients presented follicular growth arrest in the rLH groups, but none in the placebo group. The mean number of follicles >11 mm was 4.6 ± 1.8 for the placebo group, 2.5 ± 1.9 for the rLH 225 IU group and 4.2 ± 1.4 in the rLH 450 IU group (not significant). CONCLUSIONS: Results of this pilot study suggest that rLH alone can trigger follicular growth arrest in a significant number of patients, suggesting the existence of an ‘LH ceiling’ during late follicular maturation.

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

Introduction

Ovarian follicular maturation from the small antral follicle stage to a pre-ovulatory graafian follicle requires the combined actions of two gonadotrophins, i.e. FSH and LH. At the beginning of each menstrual cycle, follicle recruitment and growth depends on a particular serum level of FSH, below which follicular development will stop (Brown, 1978; Scheele et al., 1993; Fauser and Van Heusden, 1997). This has been defined as the ‘FSH threshold’. The FSH threshold evolves during follicular growth, with the FSH requirement being highest at the early antral stage before declining during the late antral stage (Scheele et al., 1993; Fauser and Van Heusden, 1997).

Although follicular growth can be induced by FSH in the total absence of LH, the resulting follicles have developmental deficiencies such as abnormally low production of estradiol (E2) and an inability to luteinize and rupture in response to an hCG stimulus [Couzinet et al., 1988; Glasier et al., 1988; Shoham et al., 1991b; Shoham et al., 1993; Schoot et al., 1994; European Recombinant Human LH Study Group, 1998; Loumey and O’Dea, 2002; Hemsey et al., 2001]. Optimal follicular development is therefore also dependent on a minimal exposure to LH or ‘LH threshold’. Extensive clinical testing in patients suffering from severe deficiency in LH and FSH has demonstrated that serum LH levels of ≥1.2 IU/l are necessary to provide adequate LH support to FSH-induced
fOLLICULAR DEVELOPMENT (Hemsey et al., 2001; Loumaye and O’Dea, 2002) when endogenous LH secretion is absent. Sufficient LH supply can be delivered by a daily injection of 75 IU rLH (European Recombinant Human LH Study Group, 1998).

Pre-clinical evidence has also accumulated suggesting that developing follicles have finite requirements for exposure to LH, beyond which normal maturation ceases (Hillier, 1994). This has given rise to the concept of an ‘LH ceiling’, which defines an upper limit of stimulation. The physiological relevance of the ability of LH to arrest follicular growth is potentially 2-fold. The most obvious is the response to the mid-cycle LH surge. Within hours after the onset of the surge, granulosa cell mitosis stops, the oocyte resumes meiosis and cumulus oophorus cells undergo functional and morphological changes in advance of ovulation (Shoham et al., 1995). The pathological relevance of this effect has been demonstrated in patients undergoing IVF, who had undergone an attenuated LH surge prior to the time of oocyte recovery. This leads to low fertilization rate and reduced embryo viability (Howles et al., 1986; Howles, 1990; Loumaye, 1990).

A second possible physiological role for LH is through its contribution to follicle dominance and mono-ovulation. During the early follicular phase, multiple follicles are present that are able potentially to develop into pre-ovulatory follicles under the influence of peri-menstrual increase in circulating FSH. The dominance of only one such follicle is attributed to the declining levels of FSH during the follicular phase, which falls below the threshold of secondary follicles. In addition to this, the rising serum LH level during the late follicular phase could contribute to this selection process by inducing secondary follicle growth arrest. If effective, this LH property could be exploited clinically to promote mono-ovulation when inducing ovulation in anovulatory women. However, no clinical data are available to support such a role for LH.

Ovulation induction with gonadotrophins is the first line therapy for anovulatory patients who have failed to ovulate or conceive after clomiphene citrate treatment. These treatments are extremely effective in both World Health Organization (WHO) group II/polycystic ovary syndrome (PCOS) patients and in WHO group I/hypogonadotropic hypogonadal patients (WHO, 1973; Balen et al., 1994). However, a significant adverse outcome of these treatments is multiple follicular development resulting in multiple ovulation and multiple pregnancy (Levene et al., 1992). ‘Soft’ protocols have been developed to minimize this response such as ‘chronic low dose FSH’, in which the dose of FSH is gently titrated upwards, attempting to cross the FSH threshold level for a single dominant follicle (Seibel et al., 1984; Buvat et al., 1989; Hamilton-Fairley et al., 1991; Meldrum, 1991; Shoaham et al., 1991a; Homburg and Howles, 1999; Marci et al., 2001). In the ‘step-down FSH’ protocol the process is reversed to take similar advantage of differences in FSH thresholds between the dominant follicle and secondary follicles (Fauser and Van Heusden, 1997; van Santbrink and Fauser, 1997). These regimens of administration lead to a mono-ovulation rate of ~65% and a reduction in multiple pregnancy rates from 25 to ~10% (White et al., 1996; Homburg and Howles, 1999).

The objective of the studies reported below was to test in a clinical setting the hypothesis that over-dosing with rLH during the late follicular phase would provide a means of suppressing the development of secondary follicles and promoting final maturation and ovulation of a single pre-ovulatory follicle.

Materials and methods

Two double-blind, placebo-controlled, multicentre pilot studies were conducted. Study A was conducted in hypogonadotrophic hypogonadal women (WHO group I anovulation) (WHO, 1973) with severe deficiency in LH and who were willing to conceive. Study B was conducted in WHO group II anovulatory patients (PCO) willing to conceive and who over-responded to FSH therapy. Both studies were conducted according to Good Clinical Practice (GCP) standards, including written informed consent prior to enrolment in the study. Both protocols were approved by the centres’ IRB or Ethics Committee.

Study A (WHO I) design

This was a double-blind, placebo-controlled, randomized, parallel group, pilot study, carried out in pre-menopausal women aged 18–39 years with a clinical history of hypogonadotrophic hypogonadism (WHO group I type anovulation) who had stopped any treatment with gonadotrophins or steroid replacement therapy >1 month before screening, with a negative progesterone challenge test during screening and hormonal values within prescribed limits within 6 months of study entry, i.e. serum FSH <5.0 IU/l, LH <1.2 IU/l, TSH <0.5 µU/ml, free testosterone between 11 and 24 pmol/l, testosterone <3.5 nmol/l and prolactin <520 mIU/l.

Patients were to have no clinically significant abnormal haematology, clinical chemistry or urinalysis finding in the 6 months before the study and a body mass index (BMI) between 18.4 (10th percentile for age 18 years) and 31.4 kg/m² (90th percentile for age 38 years).

In the initial open phase of treatment, patients received treatment with both rFSH (Gonal-F®; Serono, Aubonne, Switzerland) (starting dose 112.5 IU/day; if necessary this could be increased at 7 day intervals to a maximum dose of 262.5 IU/day) and rLH (Lupriven®, fixed dose of 225 IU/day; Serono). The open phase could continue for up to 28 days. When at least one follicle reached a mean diameter of 10–13 mm, patients were randomized to one of three blinded treatments: (i) continued treatment with both drugs (rFSH/rLH group), (ii) rLH alone (placebo substituted for rFSH; rLH placebo group) or (iii) rFSH alone (placebo substituted for rLH; rLH placebo group). rLH was given at the same fixed dose as in the open phase (i.e. 225 IU/day) and rFSH at the dose the patient was receiving at the end of the open phase. The blinded phase could continue for up to 7 days.

When one follicle reached a mean diameter of ≥18 mm and there were no more than two other follicles ≥11 mm in diameter, 10 000 IU urinary hCG was administered s.c.

The trial was designed to recruit 24 women who were anovulatory and wishing to conceive. A total of 24 patients was enrolled. Twenty of these 24 patients were qualified to start the blinded phase: six patients received rFSH alone, six patients received rLH alone and eight patients received both rFSH and rLH

Study B (WHO II) design

Study B was a double-blind, placebo-controlled, randomized, parallel group, pilot study, carried out in pre-menopausal women aged 18–39 years who had failed to ovulate or conceive after clomiphene citrate treatment. These treatments were extremely effective in both World Health Organization (WHO) group II/polycystic ovary syndrome (PCOS) patients and in WHO group I/hypogonadotropic hypogonadal patients (WHO, 1973; Balen et al., 1994). However, a significant adverse outcome of these treatments is multiple follicular development resulting in multiple ovulation and multiple pregnancy (Levene et al., 1992). ‘Soft’ protocols have been developed to minimize this response such as ‘chronic low dose FSH’, in which the dose of FSH is gently titrated upwards, attempting to cross the FSH threshold level for a single dominant follicle (Seibel et al., 1984; Buvat et al., 1989; Hamilton-Fairley et al., 1991; Meldrum, 1991; Shoaham et al., 1991a; Homburg and Howles, 1999; Marci et al., 2001). In the ‘step-down FSH’ protocol the process is reversed to take similar advantage of differences in FSH thresholds between the dominant follicle and secondary follicles (Fauser and Van Heusden, 1997; van Santbrink and Fauser, 1997). These regimens of administration lead to a mono-ovulation rate of ~65% and a reduction in multiple pregnancy rates from 25 to ~10% (White et al., 1996; Homburg and Howles, 1999).
and have started the FSH treatment within 5 days of spontaneous or induced menstruation. In addition, they were required to be euthyroid, and to have a BMI of <35 kg/m².

Patients were recruited from a population undergoing routine ovulation induction with FSH in each of the participating clinical units. The distinctive eligibility criteria were a hyper-response to FSH treatment defined as the presence of ≥4 follicles that were ≥8 mm and <13 mm in diameter, no larger follicles and an endometrial thickness of ≥8 mm. If a patient satisfied these eligibility criteria, she was informed about the study on the same day and was asked if she wanted to participate. If affirmative, she was asked to sign the informed consent form. She then received a unique study number. Her FSH treatment was stopped and she was given the study drug allocated to this identification number randomizing her to one of three blinded treatments, i.e. (i) a daily s.c. injection of rLH (Luveris) at a dose of 225 IU/day s.c. or (ii) rLH at a dose of 450 IU/day s.c. or (iii) placebo. The rLH treatment phase was ≤7 days. The 7-day rLH/placebo treatment period could also be interrupted before completion if obvious regression of all follicles was recorded or if the patient was at risk of developing ovarian hyperstimulation syndrome. According to the protocol, when the follicular response was judged to be adequate (at least one follicle ≥18 mm and a total number of follicles ≥11 mm was ≥3), ovulation was to be triggered by one single s.c. injection of 5000 IU of hCG. No luteal support was administered.

The trial was originally designed to recruit 36 women who were anovulatory and wishing to conceive. Of these 36 patients, 12 were to be allocated to each of the three treatment arms. However, due to the slow patient recruitment and study shelf-life limitation, a total of 17 patients was enrolled. Of these, five patients were allocated to and received placebo, four patients were allocated to and received rLH 225 IU/day and eight were allocated to and received rLH 450 IU/day.

Selecting the LH doses
Mean serum LH levels are quite variable in the WHO group II PCOS anovulation population. Using an immunoradiometric assay (IRMA) assay (LH MAIA clone), in a population of 180 WHO group II anovulatory patients stimulated with FSH only (urinary human FSH or rFSH), the mean basal level of LH was 7.4 IU/l (SD 5.7; range 0.5–57). On the day of hCG administration, in 387 cycles, the mean serum LH level was 7.7 IU/l (SD 8.4, range 0.5–61) (Serono study GF 5642: data on file). If an average increase of 50% of the mean serum LH level is desired in this population, a dose of rLH resulting in a mean LH Cmax, after each daily injection of 3.5 IU/l should be used. According to the pharmacokinetic characteristics of rLH, this daily dose is 450 IU rLH s.c. For study A it was decided to investigate a dose of 225 IU rLH/day; and for study B, to investigate two doses of rLH, i.e. 225 and 450 IU/day.

Randomization and drug used
Blinded vials of rLH/placebo were packed individually for each patient. rLH (Luveris) was supplied in vials containing 75 IU of LH and 47.75 mg of sucrose, phosphate buffer and Tween 20 in a lyophilized form. Matching placebo vials containing only sucrose, phosphate buffer and Tween 20 were also supplied. The rLH or placebo treatment assigned to each patient was determined according to a computer-generated randomization list stratified by centre. When a patient had signed the informed consent form and had been found eligible for the study, she received a unique patient identification number in sequential, chronological order.

Monitoring
Monitoring was primarily done by vaginal ultrasonography. The examinations were performed prior to the administration of rLH/placebo and at 1–2 day intervals (as clinically indicated) during LH treatment. All follicles with a mean diameter (= the mean of the two longest perpendicular diameters) ≥10 mm (i.e. ≥11 mm) were recorded according to their size.

Blood sampling was taken for retrospective analyses of E₂, progesterone, androstenedione, LH and FSH prior to the administration of rLH/placebo and at each occasion the patient came to the centre for ultrasound. For study A, blood samples were collected just before starting rLH and rFSH stimulation (S1), on the day of randomization to rFSH placebo, rFSH and rLH or rLH placebo and on the day of hCG administration (or the last day of gonadotrophin treatment if no hCG was administered). For study B, blood samples were collected just before the day of randomization to placebo, rLH 225 IU or rLH 450 IU and on a regular 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 between days 6 and 9 post-hCG, and the day of spontaneous menstruation was recorded. Investigating the safety of the rLH for potential antibodies, one pre- and one post-treatment sample was assayed in a central laboratory.

Statistical methods
These were pilot studies, which were not designed on statistical grounds. Results are reported as mean ± SD, median and range, unless otherwise specified. The statistical methods used were directly related to the nature of the variable analysed, i.e. Fisher’s exact test, analysis of variance, and analysis of covariance. P < 0.05 was considered to be statistically significant.

Hormone assays
All assays were performed in a central laboratory, SCL Bioscience Services, Bourn Hall Clinic, Cambridge, UK. Serum FSH and serum LH were measured using a validated, commercially available IRMA method (MAIAClone assay). The limit of quantification for serum LH and serum FSH was 1.0 IU/l. Serum E₂, progesterone and androstenedione were measured using validated, commercially available immunoassays.

Data collection
The study was performed and monitored according to the sponsor’s Standard Operating Procedures for clinical trials, and data were collected on case report forms. The case report forms were completed by the study co-investigator(s), checked and signed by the responsible principal investigator, and then checked against the source documents and verified for consistency prior to retrieval from the centre.

A double data entry was done using CLIMED (Simed, Paris, France) as data entry screens. After comparison of the two entries, computerized checks were carried out by the database monitor and statistician, and data inconsistencies thus detected were queried and then corrected in the database according to the investigator’s answers.

Results
Demographics
In study A, six participating centres enrolled a total of 24 patients. Twenty of these 24 patients were qualified to start the blinded phase: six patients received rFSH alone, six patients received rLH alone and eight patients received both rFSH and rLH. Patients’ distribution, and demographic characteristics are summarized in Table I.
The mean number of follicles ≥11 mm on day of hCG administration (or on the last day of treatment if hCG was not administered) was 4.2 ± 0.3 in the rFSH/placebo group, 1.5 ± 0.7 in the rLH/placebo group and 6.0 ± 2.3 in the rFSH/rLH group. Differences were statistically significant between the rFSH placebo and rLH placebo groups (P = 0.017) and between the rLH placebo and rFSH/rLH groups (P = 0.003). The mean number of follicles ≥14 mm on the last day of stimulation was 2.7 ± 0.5 in the rFSH/placebo group, 0.5 ± 0.3 in the rLH/placebo group and 2.0 ± 0.9 in the rFSH/rLH group. Difference was borderline for statistical significance between the rFSH placebo and rLH placebo groups (P = 0.052). Moreover, the differences in the overall number of follicles by diameter are illustrated in Figure 1. The majority of patients from the rFSH placebo and rFSH/rLH groups had more than three follicles ≥11 mm on day of hCG (or the last day of treatment): 5/6 (83.3%) for rFSH placebo and 4/8 (50.0%) for rFSH/rLH. In contrast, the majority of patients who received rLH placebo had either no follicles ≥11 mm or only one follicle ≥11 mm [total 4/6 (66.6%)] on day of hCG or the last day of stimulation. The distribution of patients with 0, 1, 2, 3 or >3 follicles ≥11 mm on day of hCG (or the last day of treatment) differed significantly between the rFSH placebo and rLH placebo groups (P = 0.028). The distribution of patients with 0, 1, 2, 3 or >3 follicles ≥14 mm on day of hCG (or the last day of treatment) differed significantly between rFSH placebo and rLH placebo (P = 0.015).

Serum FSH, LH, E2, progesterone and androstenedione levels are summarized in Table III. As expected, serum FSH remained constant in patients who continued FSH administration whereas it dropped sharply in patients who stopped FSH...
administration. Serum LH remained low and most often below the limit of quantification (1 IU/l) in all three groups. Serum E2 did not further increase in the FSH-alone group, declined in the rLH-alone group and increased in the rFSH/rLH group. Serum progesterone remained low in all three groups during rLH administration. It is noteworthy that one patient developed a spontaneous LH surge and luteinized in the rFSH/rLH group. Androstenedione increased in all three groups. Two pregnancies occurred, both in the rFSH/rLH group.

**Study A safety results**

Treatment with rLH was well tolerated. There were no serious or significant adverse events during the study. No patient developed anti-LH antibodies.

**Study B efficacy results**

Seventeen patients started the blinded treatment. Ten of those 17 received hCG and seven were cancelled prior to hCG administration for the following reasons: one patient for excessive response in the placebo group; two for follicular regression in the 225 IU rLH group and three for follicular regression and one excessive response in the 450 IU rLH group. rLH/placebo treatment lasted on average 4.5 ± 0.5 days with no significant difference between treatment groups.

At the end of this treatment phase, in 5/12 patients from the LH groups no follicular growth was recorded on ultrasound, resulting in the cancellation of hCG administration (two in the 225 IU/day LH group, and three in the 450 IU LH group). This contrasts with no patient (0/5) presenting similar lack of...
### Table III. Study A: serum hormone levels measured at T1 (first day of the blinded phase), day of hCG (or last day of stimulation) and on day of hCG + 6–9 (patients who received hCG)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>T1 (first day of stimulation)</th>
<th>Day of hCG or last day of stimulation</th>
<th>Post hCG day 6–9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM Median Range</td>
<td>Mean ± SEM Median Range</td>
<td>Mean ± SEM Median Range</td>
</tr>
<tr>
<td><strong>FSH (IU/l)</strong></td>
<td>rFSH placebo</td>
<td>8.6 ± 1.3 8.1 5–14</td>
<td>8.5 ± 1.4 7.0 5–12</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH placebo</td>
<td>12.4 ± 2.8 9.9 9–26</td>
<td>3.3 ± 0.9 3.0 1–6</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rFSH/rLH</td>
<td>9.7 ± 1.2 10.2 4–15</td>
<td>3.9 ± 0.9 9.6 4–13</td>
<td>NA</td>
</tr>
<tr>
<td><strong>LH (IU/l)</strong></td>
<td>rFSH placebo</td>
<td>1.1 ± 0.1 1.0 1–1</td>
<td>1.0 ± 0.0 1.0 1–1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH placebo</td>
<td>1.3 ± 0.2 1.0 1–3</td>
<td>1.9 ± 0.6 1.3 1–5</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rFSH/rLH</td>
<td>1.6 ± 0.3 1.1 1–3</td>
<td>1.6 ± 0.3 1.4 1–3</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Estradiol (pmol/l)</strong></td>
<td>rFSH placebo</td>
<td>691 ± 301 474 160–2171</td>
<td>726 ± 443 302 163–2483</td>
<td>749 ± 6 749–749</td>
</tr>
<tr>
<td></td>
<td>rLH placebo</td>
<td>669 ± 197 630 129–1311</td>
<td>116 ± 42 100 33–316</td>
<td>394 ± 294 394–100–687</td>
</tr>
<tr>
<td></td>
<td>rFSH/rLH</td>
<td>1416 ± 589 650 187–4885</td>
<td>3453 ± 1453 1537 251–11257</td>
<td>2020 ± 610 1722–1011–4331</td>
</tr>
<tr>
<td><strong>LH (IU/l)</strong></td>
<td>rFSH placebo</td>
<td>1.1 ± 0.2 1.1 1–2</td>
<td>1.6 ± 0.4 1.3 1–3</td>
<td>73.0 ± 3 73–73</td>
</tr>
<tr>
<td></td>
<td>rLH placebo</td>
<td>2.1 ± 0.4 1.6 1–4</td>
<td>1.9 ± 0.6 1.2 1–5</td>
<td>17.6 ± 16.5 17.6–1–34</td>
</tr>
<tr>
<td></td>
<td>rFSH/rLH</td>
<td>2.3 ± 0.5 2.0 1–5</td>
<td>22.9 ± 20.0 2.8 2–143</td>
<td>65.4 ± 18.4 64.0–27–130</td>
</tr>
<tr>
<td><strong>Estradiol (pmol/l)</strong></td>
<td>rFSH placebo</td>
<td>4.9 ± 1.0 3.7 3–10</td>
<td>4.1 ± 1.2 3.2 2–8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH placebo</td>
<td>5.9 ± 1.0 3.4 3–9</td>
<td>5.6 ± 1.0 5.2 3–10</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rFSH/rLH</td>
<td>7.7 ± 1.3 7.3 3–14</td>
<td>10.6 ± 2.2 10.8 3–22</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not applicable.

### Table IV. Study B: serum hormone levels measured at first day of the blinded phase, day of hCG (or last day of stimulation) and on day of hCG + 6–9 (patients who received hCG)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Day of rLH placebo start</th>
<th>Day of hCG or last day of stimulation</th>
<th>Post hCG day 6–9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM Median Range</td>
<td>Mean ± SEM Median Range</td>
<td>Mean ± SEM Median Range</td>
</tr>
<tr>
<td><strong>FSH (IU/l)</strong></td>
<td>Placebo</td>
<td>122 ± 2.5 9.5 8–21</td>
<td>6.5 ± 1.7 7.9 2–11</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH 225 IU/day</td>
<td>125 ± 3.4 11.4 6–21</td>
<td>7.4 ± 1.5 6.1 6–12</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH 450 IU/day</td>
<td>111 ± 1.4 9.8 8–19</td>
<td>6.9 ± 0.7 6.3 5–10</td>
<td>NA</td>
</tr>
<tr>
<td><strong>LH (IU/l)</strong></td>
<td>Placebo</td>
<td>7.8 ± 2.8 4.8 3–18</td>
<td>6.1 ± 1.2 6.9 2–8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH 225 IU/day</td>
<td>5.3 ± 1.3 6.2 1–7</td>
<td>6.8 ± 2.2 6.4 2–12</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>rLH 450 IU/day</td>
<td>4.6 ± 1.6 3.4 1–13</td>
<td>6.7 ± 1.6 4.8 3–15</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Estradiol (pmol/l)</strong></td>
<td>Placebo</td>
<td>4032 ± 1682 3612 598–10 017</td>
<td>4781 ± 2063 3540 313–11 040</td>
<td>7171 ± 3073 4418–3788–13 306</td>
</tr>
<tr>
<td></td>
<td>rLH 225 IU/day</td>
<td>1492 ± 817 8512 384–3880</td>
<td>2560 ± 2358 227 153–9633</td>
<td>9932 ± 9747 9932–185–19 678</td>
</tr>
<tr>
<td></td>
<td>rLH 450 IU/day</td>
<td>1377 ± 335 1315 123–2809</td>
<td>1967 ± 942 297 133–7269</td>
<td>1281 ± 551 1143–226–2613</td>
</tr>
<tr>
<td><strong>Progesterone (nmol/l)</strong></td>
<td>Placebo</td>
<td>4.6 ± 0.8 4.3 3–7</td>
<td>8.9 ± 4.8 4.5 2–28</td>
<td>95.3 ± 22.0 103.0–54–129</td>
</tr>
<tr>
<td></td>
<td>rLH 225 IU/day</td>
<td>3.1 ± 0.5 3.1 2–4</td>
<td>2.7 ± 0.6 2.5 2–4</td>
<td>121.1 ± 119.0 121.1–240</td>
</tr>
<tr>
<td></td>
<td>rLH 450 IU/day</td>
<td>2.5 ± 0.4 2.3 1–4</td>
<td>2.9 ± 0.6 2.3 1–6</td>
<td>68.8 ± 37.4 48.5–27–176</td>
</tr>
</tbody>
</table>

NA = not applicable.
folicular growth in the placebo group. In addition, 1/5 patients had excessive follicular development in the placebo group and 1/12 in the rLH treatment group. The distribution of number of follicles by diameter per patient is presented in Figure 2.

The mean number of follicles \( \geq 11 \) mm adjusted for the total number of follicles \( \geq 8 \) mm at baseline was 4.6 \( \pm \) 1.8 for the placebo group, 2.5 \( \pm \) 1.9 for the rLH 225 IU group and 4.2 \( \pm \) 1.4 in the rLH 450 IU group (\( P \) = not significant for all pairwise comparisons). The adjusted mean number of follicles \( \geq 14 \) mm was 3.3 for the placebo group, 1.4 for the rLH 225 IU group and 1.7 in the rLH 450 IU group. Although this represents a reduction for LH treatment, no significant differences were observed (\( P = 0.23 \) and 0.27 for LH 225 IU and LH 450 IU respectively versus placebo).

Mean serum FSH was elevated on the day of starting the rLH/placebo treatment. A significant decrease was recorded after stopping FSH administration in all three groups (Table IV). Serum LH levels were also elevated on the day of starting the treatment phase and remained elevated during rLH/placebo treatment. No statistically significant difference in serum LH was recorded between the placebo group and the rLH groups. Serum E2 was higher in the placebo group than in the rLH treatment groups. This is consistent with the higher number of follicles at baseline in the placebo group. Both mean and median serum E2 levels in the placebo group indicate that E2 secretion was maintained during placebo administration. Contrasting with this pattern, in the rLH treatment groups, median serum E2 levels underwent a large fall. Serum P was low at baseline in all three groups and remained low, including patients receiving rLH. In patients receiving hCG, mid-luteal phase serum E2 remained elevated whereas serum progesterone very significantly increased, consistent with proper luteinization. Two pregnancies were confirmed, both in the placebo group.

**Study B safety results**

The rLH treatment was well tolerated. There were no significant adverse events reported during the study. No patient developed anti-LH antibody.

**Discussion**

These two pilot studies were designed to assess the impact of rLH administration on follicular growth during the late follicular phase. The ultimate therapeutic aim would be to use rLH for minimizing the number of ovulatory follicles in order to reduce multiple pregnancy rates. Doses of 225 and 450 IU rLH were administered daily from the moment follicles reached a diameter of 10–13 mm up to the time of hCG injection.

These two studies have in common the administration of rLH alone during the late follicular phase. As a control, the study conducted in WHO group II anovulatory patients (study B) had a placebo group in which no gonadotrophin was administered. The study conducted in WHO group I anovulatory patients (study A) did not have a placebo-only group, since the absence of endogenous gonadotrophin would have led to rapid follicular regression.

By protocol, all patients who entered these studies had some growing follicles, but none \( \geq 13 \) mm in diameter. The assessment of follicular growth with ultrasound during the study showed a similar and consistent pattern of response to rLH in both studies. (i) Administration of rLH alone prevented follicles from growing, and of reaching a late antral stage in a significant number of patients (see Figures 1 and 2). A total of 9/26 patients had follicular growth arrest in the rLH-alone groups contrasting with 0/11 in the control groups (i.e. rFSH alone in study A, and placebo alone in study B) (Fisher’s exact \( P = 0.036 \)). This illustrates the capability of rLH to arrest follicular growth at the dose used, in one-third of this patient population. (ii) In patients with continuing follicular growth, an additional effect of LH was noted. More patients had only one dominant follicle (defined as a follicle with a diameter \( \geq 14 \) mm) in the rLH groups (8/17) than in the control groups (2/11) (Fisher’s exact \( P = 0.226 \)). This observation suggests that LH may facilitate selective follicular growth.

This effect of rLH is different from the concept of coasting (stopping FSH administration) (Dhont et al., 1998). In study A, no coasting was performed in the control group and the comparison is with FSH-only treatment. In study B, coasting was performed in the control group but this was not associated with either follicular growth arrest or clear-cut emergence of a dominant follicle.

The hormone profiles during treatments with rLH were also informative. Stopping rFSH administration led, as expected, to a consistent decline in serum FSH levels in both populations. Within a few days, serum FSH levels dropped to \( \sim 25\% \) of initial levels in WHO group I patients. This is compatible with the rFSH terminal half-life of \( \sim 35 \) h (Porchet et al., 1994). By contrast, in WHO group II patients, serum FSH decline was limited to 50\% of initial levels, reflecting the contribution of endogenous FSH in this population.

In terms of serum LH levels, at screening, prior to any LH administration, most patients in study A had a value below the limit of quantification (1.0 IU/l). The mean value was 1.1 IU/l and the median value was 1.0 IU/l. During the open phase when 225 IU rLH was administered daily, a minimal increase in serum LH level was recorded with a mean value of 1.4 IU/l and a median value of 1.0 IU/l. This is compatible with an rLH terminal half-life of 10–12 h, and a minimal accumulation after repeated administration (Le Cotonnec et al., 1998). In this population, stopping LH administration led to a return of serum LH mean levels to 1 IU/l, while continuing with 225 IU/day resulted in a slight increase with a median value of 1.3 and 1.4 IU/l (see Table III). This observation indicates that the impact on follicular development summarized above is obtained with quite minor variations in serum LH levels. This also concurs with a previous observation that the threshold dose of 75 IU rLH does lead to a very significant pharmacodynamic effect without measurable variations in serum LH levels (European Recombinant Human LH Study Group, 1988). In study B, prior to any LH administration, serum LH levels were significant with a mean value of 5.8 \( \pm \) 1.2 IU/l, which is compatible with WHO II PCO patients. In patients receiving 450 IU/day of rLH, the mean and median serum LH levels were increased by \( \sim 40\% \). However, this was not statistically significant. In the...
other groups, a less predictable sequence of serum LH levels was recorded. Large individual value variations due to fluctuations of endogenous LH as well as lack of strict timing imposed for sampling the patients may account for this observation.

In study A, the E2 pattern was consistent with previous reports in which stimulation was performed in hypogonadotropic hypogonadism with FSH only or with an association of FSH and LH (Couzin et al., 1988; European Recombinant Human LH Study Group, 1998). FSH alone only maintained levels recorded at the end of the open phase treatment. In patients treated with rFSH and rLH, serum E2 continued to increase up to the day of hCG administration. In patients receiving rLH only, a sharp drop in E2 was recorded. Thus, in this group, E2 production parallels the follicular growth arrest documented with ultrasound. In WHO group II anovulation or assisted reproduction patients, E2 levels are known to drop when coasting is performed in ovulatory patients stimulated with FSH (Dhont et al., 1998). In study B, placebo treatment was not associated with significant changes in serum E2 levels. Administration of rLH alone led to further increases in serum E2 levels as previously reported by Sullivan et al. (1999). However, some patients with follicle regression presented a sharp decline in serum E2 levels, as illustrated in the median values (Table IV). From these two studies, it can be concluded that LH alone can support E2 secretion as long as follicular growth arrest does not occur and as long as some FSH is present, whether exogenous (study A) or endogenous (study B) in origin.

Progesterone levels remained low (<6 nmol/l) in all patients in both studies, including patients who received 450 IU rLH per day (the only exception was one patient who underwent a premature LH surge with full luteinization before hCG administration). Thus doses of rLH up to 225 IU/day given alone or in association with rFSH, or up to 450 IU/day given alone, do not trigger luteinization. Importantly, this observation also suggests that the follicular growth arrest induced by LH at this stage of follicular development is distinct from the peri-ovulatory luteinization phenomenon.

Finally, some patients treated with rLH alone did not show a normal luteinization response when exposed to hCG, contrasting with patients treated with rFSH and rLH in study A or patients treated with placebo only in study B, all of whom attained serum progesterone >25 nmol/l during mid-luteal phase.

The impact of LH on oocyte fertilizability and capacity to lead to a viable embryo cannot be directly assessed from these studies in which conception was intended to occur in vivo. Two pregnancies were recorded in study A and both were in the rFSH + rLH group. Two pregnancies were recorded in study B, and both were in the placebo group. Although the groups were small and establishing a pregnancy depends on numerous other parameters, this suggests that administering LH without FSH may be too detrimental for oocyte quality, including the dominant follicle. In other words, a specific balance between LH and FSH may be required.

In conclusion, these two studies provide preliminary clinical evidence for the existence of a development-related ‘LH ceiling’ during the late pre-ovulatory follicular maturation, which, when breached, results in a spectrum of effects ranging from complete follicular growth arrest, to selective follicle growth arrest, to impaired ability to luteinize. Stopping exogenous FSH administration completely appears to be too detrimental, especially in WHO group I patients. Maintaining a low FSH supply during the administration of LH results in increased E2 levels with pregnancies, promoting development of a single dominant follicle in 45% of cases. Although no premature luteinization was recorded using LH in these patients, the possible impact of such treatment on oocyte quality remains unknown and requires further evaluation. Thus the results of this pilot study suggest a potential clinical benefit of the usage of rLH in ovarian stimulation regimes to promote mono-ovulation.

References


Submitted on July 7, 2002; accepted on October 18, 2002