Article

LH improves early follicular recruitment in women over 38 years old

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Abstract

Although the capacity of recombinant FSH alone to induce folliculogenesis is undisputed, many believe that follicular recruitment in women over 38 years old could be improved by supplementing rFSH with human menopausal gonadotrophin (HMG). The present study sought to determine whether recombinant LH could reproduce the effect of HMG in women over 38 years during ovulation induction. Fifty-eight patients received rFSH (225 IU/day) supplemented with one ampoule of HMG (75 IU of FSH/75 IU of LH/HCG per day) for 5 days. Another 36 patients received rFSH (300 IU/day) supplemented with one ampoule of LH (75 IU/day), also for 5 days. Both groups of patients received similar amounts of rFSH (1500 IU), LH/HCG (375 IU) and rLH (375 IU) and recruited a similar number of follicles as counted on day 6 (4.07 ± 3.1 in the HMG group versus 3.7 ± 3.2 in the LH group respectively) or on the day that human chorionic gonadotrophin (HCG) was indicated (6.5 ± 2.7 versus 5.8 ± 2.5 respectively). Ovarian stimulation was shorter, but not significantly so, in the group of patients receiving rFSH + HMG (10.5 ± 1.7 days) than in the group of patients treated with rFSH ± rLH (12 ± 1.8 days). Significantly more MII oocytes were seen in the group treated with rFSH + rLH than in the group treated with rFSH + HMG (93.1 versus 75.3%, P < 0.05). With respect to pregnancy rates, 14/54 (26%) patients receiving rFSH + HMG and 16/34 (47%) patients receiving rFSH + rLH had a positive serum HCG. No significant difference in the number of miscarriages was observed between the two groups. In conclusion, the present results seem to indicate that rLH could be the HMG component that aids early follicular recruitment.

Keywords: follicular recruitment, HMG, recombinant LH, women over 38 years

Introduction

Urinary-derived human menopausal gonadotrophin (HMG) has been the source of human gonadotrophins for ovulation induction since 1962 (Lunenfeld, 2004; Basset and Driebergen, 2005), and has proven to be very effective and well tolerated. Nevertheless, concerns related with its biological origin, the possibility of viral contamination (Matorras and Rodriguez-Escudero, 2003) and the presence of miscellaneous urinary proteins (Giudice et al., 1994; Rodgers et al., 1995) has led to HMG being progressively questioned and substituted by recombinant FSH (rFSH).

Although the capacity of rFSH to induce folliculogenesis on its own is undisputed, many believe that follicular recruitment in women over 38 years old could be improved by supplementing rFSH with HMG (Segal and Casper, 1990; Csemiczky et al., 1994; De Placido et al., 2001; Loutradis et al., 2003). Exactly what the component is in HMG that counteracts the subtle defects that seem to appear in an age-dependent manner in follicular recruitment (Rodgers et al., 1995) is difficult to ascertain, since HMG is a mixture of gonadotrophins (FSH and LH/HCG) with miscellaneous undefined urinary proteins.

In fact, as revealed by electrophoresis, HMG is composed of gonadotrophins (75 IU of FSH and 75 IU of LH/HCG), with the bulk being undefined proteins, such as tumour necrosis factor binding protein-I, transferrin, urokinase, Tamm–Horsfall glycoprotein and epidermal growth factor, as well as other components (Giudice et al., 1994; Rodgers et al., 1995) that...
can affect the mitotic and differentiation programme of the granulosa cells.

In respect to gonadotrophins present in HMG (FSH and LH/HCG) as co-factors that assist rFSH in early follicular recruitment, the combination may reflect, to some extent, the glycosylation profile of the circulating FSH in the follicular phase (Flack et al., 1994; Anobile et al., 1998; Uloa-Aguirre et al., 1999). As the follicular phase progresses, the FSH secreted by the pituitary goes from being more acidic in the beginning (as in HMG) to gradually becoming more basic (as in rFSH) in the late follicular phase (Andersen et al., 2004; Basset and Driebergen, 2005). In any event, both of the rFSHs currently available are able to promote folliculogenesis independently of their glycosylation and sialylation profiles (Rafferty et al., 1995; Basset and Driebergen, 2005).

The next candidate to be considered as a co-factor candidate in HMG is the LH/chorionic gonadotrophin (HCG) mixture in which HCG is added to make the 75 IU required for an appropriate response in the in-vivo rat bioassay when the amount of LH in HMG is too low (Rodgers et al., 1992; Stokman et al., 1993; Basset and Driebergen, 2005). During folliculogenesis, LH enhances follicular development and steroidogenesis in granulosa and luteal cells (Dufau et al., 1976; Hernández, 1995; Filicori, 1999; Howles, 2000; Hugues and Cedrin-Durnerin, 2000; Filicori and Cogigni, 2001; Acevedo et al., 2004) and, in conjunction with local growth factors, maintains oestradiol production and follicular differentiation (Hillier, 1994; Hernández, 1995). Moreover, after 14 days of pituitary desensitization, LH can be abnormally low, so LH is normally added to prevent this and to ensure adequate androgen production in theca cells. Notwithstanding, the LH requirements for ovulation induction are still controversial, with opinions against (Sills et al., 1999; Balasch et al., 2001; Peñarrubia et al., 2003) and in favour (Fauser, 1997; Fleming et al., 1998; Filicori et al., 2001; Westergaard et al., 2001; Acevedo et al., 2004) still being regularly expressed in scientific journals.

With this in mind, and since all the urinary-derived HMG (independently of the amount of LH it may contain) at the present time is supplemented with HCG (Rodgers et al., 1992; Stokman et al., 1993; van de Weijer et al., 2003) and contaminated with miscellaneous proteins (Giudice et al., 1994; Rodgers et al., 1995), it would be difficult to determine the specific role of LH in follicular recruitment. Since the only pure source of LH activity is recombinant LH (Baer and Loumaye, 2003), the present study, sought to determine whether rLH could reproduce the effect of HMG in women over 38 during ovulation induction. Accepting a trust level of 95% and a statistical power of 80% and based on experience from previous studies, the sample size for this study was established as 35 patients per group. The study was not powered to use pregnancy as an end-point.

To this end, 102 couples were consulted for permission to include them in this study. Eight refused and 94 infertile couples were recruited following the guidelines set by the Spanish Committee of Assisted Reproductive Techniques, in accordance with the Helsinki Declaration of 1975 on human research and with the approval of the IRB. Each woman received oral information in relation to the study and gave her written consent before initiating the study.

The main inclusion criteria were: women between 38 and 40 years old, normal basal serum concentration of FSH, LH (<12 IU/l), oestradiol (<50 pg/ml), a regular menstrual cycle (ranging from 25 to 32 days) and normal body mass index (between 19 and 25 kg/m²).

**Hormonal treatment**

Ovarian stimulation was performed in both groups with rFSH (Gonal F; Serono, Madrid, Spain) in combination with either HMG (HMG-Lepori; Pharma Lepori, Madrid, Spain) or rLH (Luveris; Serono).

All patients were pituitary down-regulated in their mid-luteal phase with Leuprorelin (Procrin; Abbott, Madrid, Spain), at a dose of 0.1 ml/day subcutaneously injected for 2 weeks. Pituitary desensitization was ascertained by vaginal ultrasound scanning and serum oestradiol concentrations (<50 pg/ml). When suppression was obtained, leuprorelin was reduced to 0.05 ml/day and ovarian stimulation with gonadotrophins started.

Patients were randomly assigned by a computer-generated list in the order of their enrolment. When the percentage of patients that could be lost during the course of the study was considered, it was decided to increase the number of initial included patients. For this reason, different numbers of patients were enrolled in each group. There was no concealment of treatment allocation. Fifty-eight patients received rFSH (225 IU/day) supplemented with one ampoule of HMG (75 IU of FSH/75 IU of LH/HCG per day). Thirty-six patients received rFSH (300 IU/day) supplemented with one ampoule of rLH (75 IU/day), since 75 IU of rLH seems to be effective in promoting optimal follicular development in LH and FSH deficient anovulatory women (European Recombinant LH Study Group, 1998) and in most women (Gordon et al., 2001). Both regimens were maintained constant during 5 days and administered as a single i.m. injection for rFSH + HMG or s.c. for rFSH + rLH on a daily basis following manufacturer’s indications.

On day 6 of stimulation, follicular recruitment was evaluated by vaginal ultrasound (follicles between 10 and 16 mm were counted) and, independently of the status of follicular recruitment, HMG and rLH were stopped. When follicle diameter of the majority of the follicles reached 20 mm.

**Materials and methods**

**Study design and patients**

This study was a prospective randomized cohort study performed to determine whether recombinant LH could reproduce the effect of HMG in terms of follicular recruitment in women over 38 years during ovulation induction for IVF–embryo transfer.
Oocyte retrieval, intracytoplasmic sperm injection (ICSI)–embryo transfer

Oocyte retrieval was performed 34 h post-HCG under mild sedation. ICSI was routinely performed in all fertilization procedures. In the Centre, ICSI is the normal procedure in all cases. In any event, with respect to the potential harm of ICSI, it has been found that with more than 600 cycles/year, the major fetal malformation rate is similar to that of natural pregnancies. Fertilization was achieved when two pronuclei were observed. Embryos were cultured until the day of transfer (day 3) in IVF media (Vitrolife, Möln达尔svägen, Göteborg, Denmark) and graded as per Veeck’s criteria (Veeck, 1998). A maximum of three normally developed embryos were transferred to the uterine cavity on day 3 of culture using a Wallace embryo transfer catheter. In addition, any embryo with multinucleated blastomeres was excluded from transfer.

Luteal phase support

The luteal phase was routinely supplemented (in all patients in both groups) with 600 mg/day of natural progesterone (Utrogestan; Seid, Madrid, Spain).

Pregnancy outcomes

A pregnancy test (HCG; BioMerieux, Marcy-L’Etoile, France) was performed 15 days after embryo transfer. If positive, an ultrasound scan was scheduled for 2 weeks later to determine the number and status of the implanted embryos. The co-existence of a positive HCG and embryo/s with a positive heartbeat (seen by ultrasound) was defined as a clinical pregnancy; otherwise it was considered to be a biochemical pregnancy. Implantation rates were calculated by dividing the total number of embryos transferred by the number of embryos implanted.

Six cycles were cancelled, all due to low response (fewer than two follicles ≥20 mm on the day HCG was to be administered). Four of these cycles were in the rFSH + HMG group and two in the other group.

No hyperstimulation syndrome or early luteinization occurred in either group.

Blood sampling

Hormonal control assays were determined with a commercial enzyme immunoassay kit (Vidas; Biomerieux, Barcelona, Spain). The detection limit and the inter-assay coefficient of variation were 0.05 IU/l and 3.2\%, 0.05 IU/l and 3.2\%, 0.05 IU/l and 3.2\% and 0.05 IU/l and 3.2\% for FSH, LH, oestradiol and HCG respectively.

Statistical analysis

Data were expressed as the mean ± SD. All continuous variables were normally distributed and were compared with Student’s t-test. The chi-squared test and Fisher’s test were used to compare clinical outcome between the two groups. Results were considered significant at the 5\% level (P-value <0.05).

Results

This study sought to determine whether recombinant LH could reproduce the effect of HMG in terms of follicular recruitment in women over 38 years of age during ovulation induction for IVF–embryo transfer. Since all the commercially available urinary-derived HMG preparations are supplemented with HCG and contaminated with unknown substances, this study compared rFSH + HMG with rFSH + rLH and considered rLH as a pure source of LH that was free of contaminants.

To this end, 58 patients received rFSH + HMG and 36 received rFSH + rLH, as described in Materials and methods. As seen in Tables 1 and 2, no significant differences were observed between groups related to the causes or origin of their infertility, age, body mass index or menstrual period duration. Equally, FSH, LH and oestradiol values determined on day 3 of their menstrual cycle were not significantly different between patients treated with FSH ± HMG or with rFSH ± rLH respectively.

In respect of the total amount of gonadotrophins used in the first 5 days to induce follicular recruitment, both groups of patients received similar units of rFSH (1500 IU), LH/HCG (375 IU) and rLH (375 IU). Nevertheless, and independently of the ovulation induction protocol, both groups of patients treated with either FSH + HMG or FSH + rLH recruited a similar number of follicles (≥10 mm but ≤16 mm in diameter) as counted on day 6 (4.07 ± 3.1 versus 3.7 ± 3.2 respectively) or on the day that HCG was indicated (6.5 ± 2.7 versus 5.8 ± 2.5 respectively). Ovarian stimulation period was shorter, but not significantly so, in the group of patients receiving rFSH + HMG (10.5 ± 1.7 days) than in the group of patients treated with rFSH + rLH (12 ± 1.8 days; Table 3).

A total of 406 oocytes from the 54 patients treated with rFSH + HMG and 174 from the 34 patients treated with rFSH + rLH were collected. Significantly more MII oocytes were seen in the group of patients treated with rFSH + rLH than in the group of patients treated with rFSH + HMG (93.1 versus 75.3\%; P < 0.05). Consequently, more MII oocytes were present in the group of patients treated with rFSH + HMG (11.3\%) than in the group of patients treated with rFSH + rLH (2.2\%) (Table 4).

Fertilization rates were equal in both groups (76.3 versus 75\%). In connection to morphology, there were no statistical differences between two groups (Table 4).

In respect to pregnancy rates, 14/54 (26.0\%) patients receiving HMG and 16/34 (47.1\%) patients receiving rLH had a positive serum HCG. Consequently, the rLH patients had a significantly higher pregnancy/transfer rate than the HMG patients (P < 0.05). Nevertheless, no significant differences in the number of clinical pregnancies (85.7 versus 100\%) or miscarriages (16.6 versus 12.5\%) were observed between the two groups. All clinical pregnancies are ongoing.
Table 1. Causes of infertility in the two treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>rFSH + HMG</th>
<th>rFSH + rLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>58</td>
<td>36</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>4 (6.9)</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>Insemination failure</td>
<td>16 (27.6)</td>
<td>8 (22.2)</td>
</tr>
<tr>
<td>Male factor</td>
<td>23 (39.7)</td>
<td>14 (38.9)</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>15 (25.9)</td>
<td>11 (30.6)</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups. r = recombinant; HMG = human menopausal gonadotrophin.

Table 2. Basal parameters of the patients in the two treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>rFSH + HMG</th>
<th>rFSH + rLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 ± 0.7</td>
<td>38.8 ± 1.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.3 ± 0.4</td>
<td>21.8 ± 0.6</td>
</tr>
<tr>
<td>Menstrual period duration (days)</td>
<td>28.5 ± 1.3</td>
<td>27.7 ± 0.9</td>
</tr>
<tr>
<td>FSH (IU/ml)</td>
<td>6.9 ± 2.5</td>
<td>7.8 ± 3.1</td>
</tr>
<tr>
<td>LH (IU/ml)</td>
<td>4.7 ± 2.1</td>
<td>4.3 ± 1.8</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>43.2 ± 26</td>
<td>48.5 ± 34</td>
</tr>
</tbody>
</table>

All values are means ± SD. There were no statistically significant differences between the two groups. r = recombinant; HMG = human menopausal gonadotrophin; BMI = body mass index.

Table 3. Ovarian stimulation and response for the two treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>rFSH + HMG</th>
<th>rFSH + rLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units of FSH</td>
<td>2589 ± 896</td>
<td>2767 ± 751</td>
</tr>
<tr>
<td>Units of LH/HCG</td>
<td>375 ± 0</td>
<td>375 ± 0</td>
</tr>
<tr>
<td>Units of rLH</td>
<td>375 ± 0</td>
<td>375 ± 0</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>10.5 ± 1.7</td>
<td>12 ± 1.8</td>
</tr>
<tr>
<td>No. follicles (≥10 mm, ≤16 mm) day 6</td>
<td>4.07 ± 3.1</td>
<td>3.7 ± 3.2</td>
</tr>
<tr>
<td>No. follicles ≥16 mm on day of HCG</td>
<td>6.5 ± 2.7</td>
<td>5.8 ± 2.5</td>
</tr>
<tr>
<td>Oestradiol concentration (pg/ml) on the HCG day</td>
<td>2134 ± 1889</td>
<td>1986 ± 1496</td>
</tr>
</tbody>
</table>

All values are means ± SD. There were no statistically significant differences between the two groups. r = recombinant; HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

Table 4. Outcome for patients in the two treatment groups. Values are numbers with percentages in parentheses unless otherwise stated.

<table>
<thead>
<tr>
<th></th>
<th>rFSH + HMG (n = 54)</th>
<th>rFSH + rLH (n = 34)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. oocytes retrieved</td>
<td>406</td>
<td>174</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oocytes MII</td>
<td>306 (75.3)</td>
<td>162 (93.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Oocytes MI</td>
<td>46 (11.3)</td>
<td>4 (2.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Embryos G1</td>
<td>90 (39.8)</td>
<td>57 (47.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos G2</td>
<td>72 (31.8)</td>
<td>36 (30.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos G3</td>
<td>34 (20)</td>
<td>20 (16.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos G4</td>
<td>10 (4.4)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>Multinucleated embryos</td>
<td>10 (5.1)</td>
<td>4 (4.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>76.3</td>
<td>75.0</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancies per embryo transfer</td>
<td>1454/54 (26.0)</td>
<td>1634/34 (47.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total</td>
<td>12 (85.7)</td>
<td>16 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>2 (16.7)</td>
<td>2 (12.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

r = recombinant; HMG = human menopausal gonadotrophin; NS = not statistically significant.
Discussion

This study sought to determine the role of LH in early follicular recruitment in women >38 years of age.

Before proceeding further with the discussion, it should be pointed out that, independently of the results in this paper, this objective is not the comparison between rFSH/rLH and HMG, since this has been already determined to be almost equal in terms of parameters such as recruitment and pregnancy rates (Westergaard et al., 1996; Hernández, 2000; Strehler et al., 2001; Commenges-Ducos, 2002; Van Wely et al., 2003). Thus, this study was exclusively oriented towards determining whether LH is the HMG component that favours early follicular recruitment in women over 38 years. However, since all the commercially available urinary-derived HMG is supplemented with HCG (Rodgers et al., 1992; Stokman et al., 1993; van de Weijer et al., 2003) and contamined with unknown proteins (Giudice et al., 1994; Rodgers et al., 1995), which may have an unknown influence in follicular recruitment, the only available source of pure LH (Baer and Loumaye, 2003), rLH, was used for this study.

Fifty-eight patients were treated with a combination of FSH + HMG and 36 received rFSH + rLH, as described in Materials and methods, without significant differences in the origins of their infertility, age, FSH, LH and oestradiol concentrations.

For early folliculogenesis, both groups of patients received the same dose of gonadotrophins, one ampoule of HMG (75 IU of LH/HCG) or one ampoule of rLH (75 IU of LH) for patients treated with rFSH + HMG and rFSH + rLH respectively.

Ovarian stimulation was 2 days longer in the FSH + rLH than in the rFSH + HMG group, and the total amount of FSH increased with respect to the group of patients treated with rFSH + HMG, although not significantly so (Table 3). Nevertheless, and independently of the ovulation induction protocol, both groups of patients, rFSH + HMG and rFSH + rLH, recruited a similar number of follicles after 5 days of ovarian stimulation (4.07 ± 3.1 versus 3.7 ± 3.2), and these progressed up to 20 mm until the day HCG was indicated. This result suggests that rLH, always in the presence of FSH, was as effective as rFSH ± HMG in terms of early follicular recruitment in women over 38 years (Table 2). Furthermore, since rLH is devoid of any other gonadotrophin (HCG) or contamination with unknown proteins that may interfere in follicular recruitment, and since the patients treated with rLH recruited a similar number of follicles to the number recruited by the HMG patients, it is concluded that the LH/HCG fraction in HMG is the component that improves early follicular recruitment.

Although a significant increase in MII oocytes was seen in the group of patients treated with rFSH + rLH compared with the group of patients treated with rFSH + HMG (P < 0.05), at the present time no physiological clues exist to explain this difference; it is possible that antral follicle count in a basal cycle, not measured in this study, may provide an explanation. Nevertheless, there were no significant differences in fertilization rates, embryo quality, clinical pregnancies or miscarriages. This result agrees with previous studies (Sullivan et al., 1999; Filicori et al., 2001) reporting that rLH seems to promote better oocyte maturation than HMG; however, no explanation of this phenomenon has yet been elucidated.

In conclusion, the present results indicate that LH could be involved in early follicular recruitment since no differences in this regard were found between patients over 38 treated with either rFSH + HMG or rFSH + rLH. The mechanism as to how LH could help overcome subtle age-related recruitment follicular defects that seem to appear in older patients is unknown. However, growth factor peptides, such as insulin-like growth factor-I (Hillier, 1994; Hernández, 1995), are involved in the regulation of the cell cycle and synthesized in the theca-interstitial compartment in an LH-dependent manner and could influence this aspect.

Finally, since no differences between HMG and rLH in regard to recruitment or implantation rates were found, the question of which one to use (HMG or rLH) is more a personal choice than a technical one.

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