Introduction

At present, many women are experiencing age-related infertility owing to social trends that lead to deferred childbearing and to the current age of the ‘baby boom’ generation (Practice Committee of the American Society for Reproductive Medicine, 2002). Although there is no strict definition of advanced reproductive age in women, infertility becomes more pronounced after 35 years of age. Thus, experts have stressed that evaluation and treatment of infertility should not be delayed in women 35 years of age or older (Spéroff, 1993; Practice Committee of the American Society for Reproductive Medicine, 2002).

Abstract

Women undergoing intracytoplasmic sperm injection (ICSI) for male factor infertility were randomly assigned to receive ovarian stimulation in a long agonist protocol with a combination of recombinant human FSH (r-hFSH; Gonal-F®) and recombinant human LH (r-hLH; Luveris®) (n = 212) starting on day 6 of FSH stimulation until human chorionic gonadotrophin (HCG) at a daily fixed dose of 150 IU r-hLH, or with r-hFSH alone (n = 219). There was no significant difference in the number of metaphase II oocytes retrieved (10.3 versus 10.4) in patients treated with r-hFSH and r-hLH versus r-hFSH alone; however, more embryos were transferred in the LH-supplemented group (2.9 versus 2.8, \(P = 0.037\)). Overall, the implantation rates were 22.9 versus 27.0% in patients treated with r-hFSH and r-hLH versus with r-hFSH alone respectively (NS). The respective numbers of MII oocytes retrieved in patients <35 or \(\geq\)35 years were 11 versus 8.3 (\(P = 0.010\)) for patients treated with r-hFSH alone, and 10.7 versus 9.3 (NS) for those given supplemental r-hLH (150 IU) from day 6. Implantation rates in patients <35 years treated with r-hFSH were higher (30.7%) than those receiving r-hFSH and r-hLH, (23.5%) (\(P = 0.068\)). In patients \(\geq\)35 years, the implantation rates were 21.7% for those patients supplemented with 150 IU r-hLH from day 6 of stimulation versus 15.7% when treated with FSH alone (NS). Younger patients therefore do not seem to benefit from an LH-supplemented ovarian stimulation protocol, but women \(\geq\)35 years undergoing assisted reproduction may benefit from using r-hLH in addition to r-hFSH.

Keywords: age, ovarian stimulation, recombinant FSH, recombinant LH
Treatment options for age-related infertility include ovarian stimulation with intrauterine insemination (IUI) and IVF. The presence of male factor, tubal disease, or endometriosis supports proceeding directly to IVF in women of advanced reproductive age (Practice Committee of the American Society for Reproductive Medicine, 2002). Pregnancy rates from IVF are generally higher than those from IUI, but these rates also decrease significantly with female age. This age-related decline in IVF success is related to both decreased ovarian responsiveness to gonadotrophins and a marked decrease in embryo implantation rates (Practice Committee of the American Society for Reproductive Medicine, 2002).

The change in ovarian responsiveness with increasing age is recognized by the common practice of increasing the daily dose of gonadotrophins administered, mainly in the form of LH-containing preparations, in association with the use of ‘micro’-dose gonadotrophin-releasing hormone (GnRH) agonist protocols. Such protocols reduce both excessive pituitary suppression and direct effects on the ovaries by the agonist (Olivennes et al., 1996; Lamli et al., 1999; Phelps et al., 1999; Surrey and Schoolcraft, 2000; Lisi et al., 2001; De Moustier et al., 2002; Meo et al., 2002; Practice Committee of the American Society for Reproductive Medicine, 2002). This clinical practice thus represents an attempt to increase FSH but also LH effects on the ovaries, acting through both exogenously administered and endogenous gonadotrophins.

According to current concepts of the roles of FSH and LH in folliculogenesis, LH plays an essential role in the final stages of follicular maturation (Hillier, 2001; Zeleznik, 2001). Once an appropriate (i.e. LH-responsive) stage of follicular development has been achieved in response to treatment with FSH, granulosa cells become receptive to LH stimulation and LH becomes capable of exerting its actions on both theca cells and granulosa cells. In fact, at non-saturating concentrations of FSH and LH, the responses are additive; indeed, it has been postulated that the maturing follicle reduces its dependence on FSH by acquiring LH receptors (Hillier, 2001; Zeleznik, 2001). Thus, LH may play an essential role in determining oocyte maturity and developmental potential in IVF cycles. However, exposure of the developing follicle to inappropriately high concentrations of LH may interfere with follicular and oocyte maturation and thus adversely affect the reproductive process (Hillier, 1994; Daya, 2001; Shoham, 2002).

Now that recombinant human LH (r-hLH) is available as a stand-alone product, the present study was undertaken to investigate the usefulness of r-hLH supplementation in women treated with recombinant human FSH (r-hFSH) under pituitary suppression for assisted reproductive techniques. The specific aim of the study was to test whether the addition of r-hLH can be useful in the subgroup of women of advanced reproductive age while being not deleterious for the remaining assisted reproduction population when administered from stimulation day 6.

It is well established that the best possibility of success is in the first cycle of IVF treatment and that there is a significant negative effect with increasing number of attempts thereafter (Templeton et al., 1996). Secondly, Daya (2003) has recently advocated the enrolment of subjects into studies who are being treated for the very first time, so as to reduce any potential bias resulting from previous treatments. For these reasons this study also analysed naive patient data, as in Westergaard et al. (2001).
Safety

Adverse events were recorded at each visit according to standard procedures. All patients who received at least one dose of study medication were included in the safety evaluation. Ovarian hyperstimulation syndrome (OHSS) was defined according to the classification proposed by Golan et al. (1989).

End-points

The primary efficacy variable was number of metaphase II oocytes retrieved. Secondary efficacy variables included: number of fertilized oocytes; number of pregnancies (total, biochemical and clinical); implantation rates; and number of live births.

Subgroup analyses

Additional analyses were performed with the patient population stratified according to age (≥35 years versus <35 years) and number of previous cycles (first ICSI cycle versus those who had undergone one or more previous cycles).

Sample size and statistical methods

It was calculated that a sample size of 280 patients would give 80% power to detect the expected difference in the primary end-point, the number of metaphase II oocytes retrieved (based on previous studies of r-hFSH versus HMG) of 8.2 percentage points in favour of r-hFSH alone (Imthurn et al. 1996). Allowing for withdrawals from treatment, the planned sample size was 296 patients, 148 in each group. The total number of patients recruited eventually reached 431 in total and all patients irrespective of any protocol deviation were included in this analysis (intention to treat (ITT) population).

For the primary efficacy end-point, the 95% confidence interval of the difference between treatment groups was calculated using an ANOVA model that included effects for treatment group and centre. Secondary efficacy variables were analysed using ANOVA for continuous variables and logistic regression, the Cochran–Mantel–Haenzsel test or exact test for categorical variables. In addition, a sensitivity analysis based on a logistic regression model was employed where required.

Results

Patient disposition and demographics

The ITT population included a total of 431 patients who were enrolled and treated; 212 were randomized to the combination treatment and 219 to r-hFSH alone. The two groups were well matched for baseline demographic characteristics (Table 1). The combined r-hFSH + r-hLH group included 60 women aged ≥35 years and in the r-hFSH group there were 56. Basal serum LH concentrations did not differ significantly between the two groups (Table 1).

Primary end-point and stimulation characteristics

The majority of patients successfully completed ovarian stimulation and received HCG (97% in the combination group and 93% in the r-hFSH group). Most patients also underwent oocyte retrieval (96 and 92% respectively). Considering the ITT population as a whole, there was no significant difference in the number of metaphase II oocytes retrieved (10.4 ± 6.3 versus 10.3 ± 5.9) for the FSH alone versus LH-supplemented groups. There were no differences between the overall treatment groups for length of treatment, ampoules of r-hFSH used, follicular development and oestradiol on the day of HCG administration (Table 2).

Outcome of ICSI and pregnancy

Results for the efficacy end-points of the ITT population are presented in Table 3. None of the differences between groups was statistically significant, except that the mean number of embryos transferred was higher in the r-hFSH + r-hLH group (2.9 ± 0.6 versus 2.8 ± 0.7, P = 0.037). The number of high quality (A and B grade) embryos transferred was 2.4 ± 1.0 in the FSH + LH group and 2.2 ± 1.1 in the FSH alone group. Overall implantation rates were 22.9 and 27.0% respectively for the groups treated with r-hFSH + LH and r-hFSH alone (NS).

Table 1. Baseline characteristics of the study population. Values are mean ± SD unless otherwise stated.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group</th>
<th>Total patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-hFSH + r-hLH</td>
<td>r-hFSH alone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 212)</td>
<td>(n = 219)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.4 ± 3.8</td>
<td>31.9 ± 3.7</td>
<td>32.2 ± 3.8</td>
</tr>
<tr>
<td>BMI</td>
<td>24.3 ± 4.7</td>
<td>24.8 ± 5.6</td>
<td>24.6 ± 5.1</td>
</tr>
<tr>
<td>Smoker (%)a</td>
<td>13 (6.1)</td>
<td>16 (7.3)</td>
<td>29 (6.7)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.9 ± 3.1</td>
<td>3.7 ± 2.6</td>
<td>3.8 ± 2.9</td>
</tr>
<tr>
<td>Basal FSH (mIU/ml)</td>
<td>5.1 ± 1.9</td>
<td>5.0 ± 1.9</td>
<td>5.1 ± 1.9</td>
</tr>
<tr>
<td>Basal LH (mIU/ml)</td>
<td>3.8 ± 2.7</td>
<td>3.7 ± 2.5</td>
<td>3.7 ± 2.6</td>
</tr>
<tr>
<td>Basal oestradiol (pmol/l)</td>
<td>80.0 ± 152.5</td>
<td>99.5 ± 469.0</td>
<td>89.8 ± 348.8</td>
</tr>
<tr>
<td>Previous assisted reproduction cycle(s) (%)</td>
<td>56 (26.4)</td>
<td>60 (27.4)</td>
<td>116 (26.9)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
aDefined as consuming less than 11 cigarettes/day; NS = not significant.
Twenty-two patients were protocol violators (15 in the r-hFSH + r-hLH and 7 in the FSH alone group), as they had more than three embryos replaced. In order to estimate the impact of this imbalance, the analysis of clinical pregnancy was repeated twice: on the set of all patients randomized (ITT data set, \(n = 431\)) adjusted with the number of embryos transferred using a logistical regression model, and on the set of patients excluding those patients who did not fulfil the protocol (\(n = 409\)). As the ITT population was the primary population of interest, and the results for the two populations were very similar, only the results for the ITT population are presented here. For the ITT population, there were no statistically significant differences between groups for the clinical pregnancy rate (Table 3). Live birth rates are not available for the whole study population because of incomplete follow-up.

**Table 2. Gonadotrophin treatment and ovarian response. Values are mean ± SD unless otherwise stated.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group</th>
<th>Total patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-hFSH alone ((n = 212))</td>
<td>r-hFSH + r-hLH ((n = 219))</td>
<td></td>
</tr>
<tr>
<td>Days to ovarian arrest</td>
<td>13.2 ± 3.3</td>
<td>13.2 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>9.4 ± 1.8</td>
<td>9.5 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Ampoules of FSH</td>
<td>29.5 ± 10.3</td>
<td>30.2 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>Ampoules of LH</td>
<td>4.4 ± 5.1</td>
<td>0.0 ± 0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Follicles &gt;10 mm on HCG day</td>
<td>13.1 ± 6.4</td>
<td>13.3 ± 6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Follicles &gt;15 mm on HCG day</td>
<td>9.3 ± 4.7</td>
<td>9.4 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Oestradiol (pmol/l) on HCG day</td>
<td>8720 ± 5012</td>
<td>8435 ± 4853</td>
<td>NS</td>
</tr>
<tr>
<td>Patients with follicular puncture (%)</td>
<td>406 (94.2)</td>
<td>202 (92.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>14.1 ± 7.5</td>
<td>13.8 ± 7.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
NS = not significant.

**Table 3. Oocyte retrieval and outcome of ICSI. Values are mean ± SD unless otherwise stated.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group</th>
<th>Total patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-hFSH alone ((n = 212))</td>
<td>r-hFSH + r-hLH ((n = 219))</td>
<td></td>
</tr>
<tr>
<td>Number of MII oocytes</td>
<td>10.3 ± 6.1</td>
<td>10.3 ± 6.1</td>
<td>NS</td>
</tr>
<tr>
<td>Number of 2PN oocytes</td>
<td>7.4 ± 4.6</td>
<td>7.4 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>54.1 ± 22.0</td>
<td>52.7 ± 22.1</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos cryopreserved</td>
<td>2.2 ± 3.5</td>
<td>2.2 ± 3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Patients with embryos transferred (%)</td>
<td>395 (91.6)</td>
<td>197 (90.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>2.8 ± 0.7</td>
<td>2.8 ± 0.7</td>
<td>0.037</td>
</tr>
<tr>
<td>Patients with clinical pregnancy (%)</td>
<td>181 (42.0)</td>
<td>91 (41.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
NS = not significant.

**Subgroup analyses**

There were no significant differences for the primary end-point between patient subgroups stratified by age and number of previous cycles. For the <35 and ≥35 age groups, the respective numbers of metaphase II oocytes retrieved were 11.0 versus 8.3 (\(P = 0.010\)) for patients treated with r-hFSH alone and 10.7 versus 9.3 (NS) for those given supplemental r-hLH from day 6. For patients aged under 35, the number of high quality embryos transferred was 2.2 ± 1.1 in the group receiving FSH alone and 2.4 ± 1.0 (NS) for those given supplemental r-hLH. For the ≥35 age group, corresponding values were 2.1 ± 1.1 and 2.4 ± 1.1, respectively. Implantation rates in patients <35 years treated with r-hFSH alone were higher than for those receiving r-hFSH + r-hLH (30.7 versus 23.5%, respectively).
In patients aged ≥35 years, implantation rates were 21.7% for those patients supplemented with 150 IU r-hLH from day 6 of stimulation, versus 15.7% for those treated with r-hFSH alone (NS) (Figure 1).

For the ITT and evaluable patient population, the clinical pregnancy rate was higher in women aged ≥35 years who received both r-hFSH and r-hLH, although this difference did not reach statistical significance (Table 4). However, the difference in clinical pregnancy rates was significant ($P < 0.03$) with an odds ratio (95% CI) of 3.13 (1.23–7.97) for the ITT population aged ≥35 years who were undergoing their first assisted reproduction cycle (Table 4). This subgroup of patients also had significantly more embryos transferred in the r-hFSH + r-hLH group (3.1 ± 0.6 versus 2.8 ± 0.7; $P = 0.04$). Using a sensitivity analysis on this ITT population aged ≥35 years based on a logistic regression model adjusted with the number of embryos transferred, the clinical pregnancy rates were 36.3% versus 19.6% (FSH + LH versus FSH, NS). Younger women, by contrast, showed a trend towards better outcomes when treated with r-hFSH alone for some of the secondary end-points, with the clinical pregnancy rate for the ITT population being 42.9 versus 45.4% (FSH + LH versus FSH, NS) and the adjusted clinical pregnancy rate being 39.8 versus 45.8% (FSH + LH versus FSH, NS).

The effect of age on clinical pregnancy rates was examined with and without adjustment for differences in numbers of embryos transferred. For the ITT population treated with r-hFSH alone, pregnancy rates were 74/163 (45.4%) for those aged <35 years and 17/56 (30.4%) for those aged ≥35 years ($P = 0.051$). Adjusted clinical pregnancy rates were significantly different (43.1 versus 26.8%, $P = 0.038$) in favour of the younger age group. For the r-hFSH + r-hLH group, there was no difference in clinical pregnancy rates between younger and older women for either the ITT (42.9 versus 41.5%, NS) or adjusted (41.9 versus 39.6%, NS) analyses. Results were similar for women undergoing their first ART cycle, except that for the r-hFSH group the difference in pregnancy rates was significant for the ITT analysis ($P = 0.03$) as well as the adjusted analysis.

**Safety results**

There was no difference between groups in the rate of adverse events; 45 patients in the combination group (21%) and 49 (22%) in the r-hFSH group experienced at least one adverse event. The most common adverse events were headache and OHSS, each occurring in 18 patients. Most adverse events were mild or moderate in severity. Three patients withdrew from the study because of risk of OHSS, two in the combination group and one in the r-hFSH group.

**Discussion**

It is well established that successful IVF and embryo transfer requires both stimulation of the ovary and suppression of the pituitary. Thus, exogenous gonadotrophins and GnRH analogues are the key hormones required to maximize IVF success. The long protocol of GnRH agonist administration is the most commonly adopted protocol for assisted reproduction cycles worldwide, mainly in young, normogonadotrophic women (Barbieri and Hornstein, 1999). GnRH agonists do not usually result in total elimination of LH and it is accepted that <1% of follicular LH receptors need to be occupied to elicit a maximal steroidogenic response. Accordingly, resting concentrations of

![Figure 1. Implantation rates per cycle in women of different age groups who underwent ovarian stimulation using recombinant human FSH with and without supplemental recombinant human LH.](image)

**Table 4.** Clinical pregnancy rates per started cycle in the intention to treat (ITT) group and evaluable patient population for women aged <35 and ≥35 years after ovarian stimulation with r-hFSH + r-hLH or r-hFSH alone.

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>Treatment group</th>
<th>P-value$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-hFSH + r-hLH</td>
<td>r-hFSH alone</td>
</tr>
<tr>
<td>All &lt;35</td>
<td>ITT (%)</td>
<td>63/147 (42.9)</td>
</tr>
<tr>
<td></td>
<td>Adjusted$^a$ (%)</td>
<td>39.8</td>
</tr>
<tr>
<td>All ≥35</td>
<td>ITT (%)</td>
<td>27/65 (41.5)</td>
</tr>
<tr>
<td></td>
<td>Adjusted$^a$ (%)</td>
<td>35.4</td>
</tr>
<tr>
<td>Aged ≥35 and undergoing first assisted reproduction cycle</td>
<td>ITT (%)</td>
<td>22/48 (45.8)</td>
</tr>
<tr>
<td></td>
<td>Adjusted$^a$ (%)</td>
<td>36.3</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

$^a$Predicted clinical pregnancy rates and $P$-value from a regression logistic model adjusted for the number of embryos transferred.

$^b$All $P$-values were not significant.
Article - Effect of adding r-hLH to r-hFSH in assisted reproduction treatment - R Marrs et al.

LH (1–10 IU/l) should be sufficient to provide maximal stimulation of thecal cells (Chappel and Howles, 1991). This is further supported by a recent study in down-regulated young oocyte donors showing that the inclusion of exogenous LH activity (in the form of HMG) in the ovarian stimulation protocol can have beneficial effects on oocyte yield and quality in those donors with LH concentrations <1 IU/l once pituitary suppression was achieved (Tesarik and Mendoza, 2002). In this study, however, a long acting depot agonist preparation was also used to achieve pituitary suppression (Tesarik and Mendoza, 2002). This is more convenient for patient use, but it is associated with more profound, sustained down-regulation than the short acting preparations (Yim et al., 2001). The use in such a study (Tesarik and Mendoza, 2002) of oral nonsteroidal preceding depot agonist administration may have induced an even more profound pituitary suppression. Some studies have also reported a significant reduction in FSH efficacy versus treatment with supplemental LH and reflected in more vials of FSH required (Balasch et al., 2003) when a subcutaneous or depot GnRH agonist was employed. This was not the case here as there was no difference in the stimulation characteristics in the two treatment groups. Caution should therefore be used when considering results from studies where depot agonists are used compared with the present report, using a ‘routine’ down-regulation scheme that is more commonly used in assisted reproduction practice.

The above notwithstanding, there seems to be a range of LH concentrations obtained in patients treated with GnRH agonists. With the use of urinary FSH preparations containing negligible LH activity it is possible that there may be a subgroup of patients with low LH concentrations in whom ovarian responses are influenced (Fleming et al., 1996, 1998; Westergaard et al., 2000). This can become especially relevant considering the following points. First, such women cannot be identified in advance by measuring LH concentrations after down-regulation (Loumaye et al., 1997; De Placido et al., 2000). Second, oocyte maturity and fertilization rate in assisted reproduction may be influenced by the particular hormonal stimulation that preceded oocyte retrieval (Pieters et al., 1991).

It has recently been suggested that, in a subset of patients, a suboptimal ovarian response to the long GnRH agonist protocol associated with the use of FSH-only gonadotrophin preparations may be due to low LH activity caused by low serum concentrations of LH and/or low LH bioactivity (De Placido et al., 2001). The following facts support this possibility. First, the ovarian response to ovarian stimulation with gonadotrophins is often reduced in patients receiving long-term down-regulation (Marcus and Edwards, 1994; Fàbregues et al., 1998) or in those who required prolonged GnRH agonist treatment to achieve down-regulation, with a subsequent profound suppression of endogenous gonadotrophins (Fleming et al., 1998; Ravhon et al., 2000). Although pharmacological doses of FSH alone are capable of stimulating ovarian follicular development, LH is strictly necessary to achieve final follicular maturation and oocyte fertilization (Couzinet et al., 1988; Balasch et al., 1995). Second, one alternative approach in low-responders to the prolonged down-regulation associated with the long protocol consists of the ‘flare’ regimens that involve follicular phase initiation of GnRH agonist with minimal delay before commencing ovarian stimulation with gonadotrophins. In such regimens a direct effect of GnRH agonist enhancing LH secretion exists (Surrey and Schoolcraft, 2000). Finally, although immunoreactive LH and bioactive LH concentrations are related, differences are often observed (Schoor et al., 1999), and this may explain the lack of correlation reported between the immunoreactive concentrations of LH and the requirement for LH during folliculogenesis (De Placido et al., 2000).

In recent years, a number of studies have provided new information about the role of LH in the process of ovulation induction. In particular, Sullivan et al. showed that r-LH can substitute for FSH in supporting the later stages of follicular growth (Sullivan et al., 1999). Filicori and Cognigni have advocated the use of a ‘biphasic’ regimen for ovarian stimulation providing mainly FSH activity in the early to mid-follicular phase and mainly LH activity in the later stages of ovarian stimulation (Filicori and Cognigni, 2001). Based on these data it might be argued that treatment with r-LH at a higher dose or starting earlier in the cycle might have given better results for the combined treatment group as a whole. However, at the time this study was designed, protocols involving the separate administration of r-hFSH and r-hLH were rare. The rationale for using a dose of 150 IU/day was based on the concentrations of LH measured in women down-regulated with GnRH agonists (de Cotonec et al., 1998). This dose of r-LH achieved a Cmax of 1.2 IU/l, a concentration found to be important in hypogonadotrophic women supplemented with r-hFSH alone (O’Dea et al., 2000). Concentrations of LH <1.2 IU/l were associated with insufficient oestradiol concentrations and a failure to become pregnant. There appears to be a therapeutic ‘window’ of LH concentrations, because high concentrations of LH have been associated with atresia of developing follicles in women with hypogonadotrophic hypogonadism or polycystic ovary disease (Shoham, 2002; Loumaye et al., 2003).

Ben-Amor and colleagues have published a preliminary report of a study where infertile women down-regulated with buserelin received either FSH (150 IU/day) or FSH (150 IU/day) plus a supplementation of r-hLH (75 IU/day) started when the leading follicle had reached 14 mm (mean diameter), until the day of HCG administration (Ben-Amor et al., 2000). Mean numbers of oocytes and metaphase II oocytes retrieved were similar between the two groups, suggesting a lack of benefit of r-hLH supplementation. Compared with the present study the patients received r-LH at a lower dose (75 IU/day) and for an average of 2 days only. The women also had a lower average age compared with those in the present study (30.4 years). In the light of current observations, their findings are consistent with the fact that r-hLH supplementation might be of benefit to older patients only.

One caveat in the present study is that significantly more embryos were transferred in the group that received LH supplementation (mean 2.9 versus 2.8, P = 0.04). Although this difference was not significant in the younger patient population, there was a trend in the older subgroup population (3.1 versus 2.8, P = 0.08) receiving LH. It seems unlikely that such a small difference, even if statistically significant, could be clinically meaningful. This was confirmed by adjusting the pregnancy rates to take account of the difference in embryos transferred (Table 4), showing no difference between unadjusted and adjusted outcomes.

Any effect of r-hLH supplementation on clinical outcomes may be most evident in patients of advanced reproductive age, who
are more prone to have a decreased response to ovarian stimulation and lower implantation rates (FIVNAT, 1999, 2000; Practice Committee of the American Society for Reproductive Medicine, 2002). The present study adds new data to the subject when showing that in patients receiving r-hFSH + r-hLH, pregnancy rates were similar in the younger and older age groups, i.e. there was no age-related fall in pregnancy rates with this regimen. This finding remained valid when the analysis was adjusted to allow for differences in the number of embryos transferred. However, in women receiving r-hFSH alone, there was a significant decline in pregnancy rates for women ≥35 years of age compared with those ≤35 years old (P = 0.05 for the ITT population and P = 0.038 adjusted for differences in number of embryos transferred).

Multiple novel roles of LH have been recently proposed and it has been postulated that LH may affect IVF results both by determining oocyte quality and by influencing uterine receptivity via ovarian oestradiol secretion or through direct effects on endometrium, myometrium, and uterine artery and vein (Rao, 2001; Shemesh, 2001; Tesarik and Mendoza, 2002). Therefore, it is tempting to postulate that r-hLH added to r-hFSH (from stimulation day 6 in the present investigation) improved oocyte developmental competence and/or implantation in an older patient population.

Finally, it is well known that patients aged ≥35 years have a lower likelihood of successful pregnancy after IVF compared with younger women (Templeton et al., 1996; Templeton and Morris, 1998). Also, irrespective of the woman’s age, there is a significant decline in the probability of conception with successive assisted reproduction cycles (Tan et al., 1994; de Mouzon et al., 1998; FIVNAT, 1999, 2000). Remarkably, the present study showed the greatest benefit of supplementary r-hLH compared with r-hFSH alone in older women in their first assisted reproduction cycle. This finding adds to the results reported by Lisi and colleagues (Lisi et al., 2001, 2002). In a preliminary study comparing r-hFSH alone with r-hFSH + r-hLH in patients who required >3000 IU r-hFSH on previous attempts, there were significant differences in implantation rate and ongoing pregnancy rate, favouring the r-hFSH + r-hLH group (Lisi et al., 2001). However, the number of patients (12) and cycles (17) involved was small. A larger study involving 122 cycles using r-hFSH + r-hLH and 331 using r-hFSH alone in unselected patients found no differences in endocrine, embryological or outcome measures (Lisi et al., 2002). There was a higher implantation rate in the group given added r-hLH among patients with low LH concentrations after down-regulation and among those who required large amounts of r-hFSH to complete ovarian stimulation. The present results complement those of Lisi et al. by focusing attention on a subgroup of patients who may benefit from supplementary r-hLH, and on the possible beneficial effects of supplementary r-hLH on implantation rates.

In conclusion, patients of older reproductive age undergoing assisted reproduction might benefit from the addition of r-hLH. Further studies are required to examine the possible effect of LH supplementation on age-related decline in pregnancy rate and the physiological mechanisms behind this observation.

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