A randomized, double-blind, multicentre clinical trial comparing starting doses of 150 and 200 IU of recombinant FSH in women treated with the GnRH antagonist ganirelix for assisted reproduction

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BACKGROUND: Studies with the GnRH antagonist ganirelix in assisted reproduction have indicated that compared with traditional GnRH agonist downregulation protocols, slightly fewer oocytes are retrieved. In this study it was investigated whether an increase in the starting dose of recombinant FSH (rFSH) could compensate for this loss. METHODS: A randomized, double-blind, multicentre clinical trial comparing a starting dose of 150 and 200 IU of rFSH (follitropin β), in women undergoing treatment with the GnRH antagonist ganirelix. RESULTS: In total, 257 women were treated with rFSH, of whom 131 received 150 IU and 126 women 200 IU. Overall, 10.3 oocytes were retrieved in the 150 IU group and 11.9 in the 200 IU group (P = 0.051). This difference became significant when women with cycle cancellation before HCG administration were excluded. Nearly 500 IU of additional rFSH was given in the high-dose group (2014 versus 1541 IU). In the low-dose group, 4.6 high-quality embryos were obtained compared with 4.5 in the high-dose group. Vital pregnancy rates were similar (31 and 25% in the 150 and 200 IU-treated women, respectively). Serum concentrations of FSH, estradiol and progesterone were significantly higher in the high-dose group at day 6 of rFSH treatment and on the day of HCG administration. In the high-dose group, serum LH concentrations were higher at day 6 of rFSH treatment but lower at the day of HCG administration. CONCLUSION: By increasing the starting dose from 150 to 200 IU of rFSH, slightly more oocytes can be retrieved in GnRH antagonist protocols for assisted reproduction. However, because this did not translate into a higher number of high quality embryos, the clinical relevance of such a dose increase may be questioned.

Key words: follitropin β/ganirelix/GnRH antagonist/RCT/recombinant FSH

Introduction

Recently, GnRH antagonists such as cetrorelix and ganirelix have become available as alternatives to GnRH agonists for controlled ovarian stimulation in assisted reproduction (Reismann et al., 2000; Out and Mannaerts, 2002). These compounds are efficient in the prevention of premature LH surges. Compared with GnRH agonists they offer the advantage of more convenience because of significantly shortened treatment periods (European Orgalutran Study Group, 2000; European and Middle East Orgalutran Study Group, 2001; North American Ganirelix Study Group, 2001). In these three studies, 8.7, 11.7 and 7.9 oocytes were retrieved in the ganirelix-treated women, respectively, compared with 9.7, 14.1 and 9.6 in the GnRH agonists groups. However, this significantly lower oocyte yield was not reflected in a lower number of high-quality embryos available for transfer.

It is relevant to ask whether the lower number of oocytes retrieved in GnRH antagonist-treated women can be overcome by increasing the dose of gonadotrophins used for ovarian stimulation. In GnRH agonist-downregulated cycles, such an increase is known to lead to more follicles and, hence, more oocytes are available for fertilization (Latin American Puregon IVF Study Group, 2001; Out et al., 2001). This could result in more frozen embryos and, ultimately, higher cumulative pregnancy rates per single stimulation cycle (Jones et al., 1997). One study addressed this issue in GnRH antagonist-treated women (Wikland et al., 2001). In this open-label, randomized clinical trial, 120 women received either 150 or
225 IU of recombinant FSH (rFSH, follitropin $\alpha$) and 0.25 mg of cetrorelix. In the low-dose group 9.1 oocytes were retrieved, and 11.0 oocytes were recovered in the high-dose group ($P = 0.024$).

We investigated, in a double-blind fashion and in a larger group of patients ($n = 257$), the effects of a starting dose of either 150 or 200 IU of another rFSH preparation (follitropin $\beta$) and a different GnRH antagonist (ganirelix).

Materials and methods

Study design

This was a prospective, randomized, double-blind, multicentre study performed between June 2000 and December 2001 in six infertility centres in the UK. The aim was to include 260 patients, with 130 patients in each treatment group (see Sample size considerations). The study was approved by the Anglia & Oxford Multi-centre Research Ethics Committee (MREC) and Local Research Ethics Committees (LREC) of the individual hospitals involved. Each subject gave written informed consent before participating in the study.

Selection of patients

Inclusion criteria were as follows: females of infertile couples for whom controlled ovarian stimulation and IVF with or without ICSI was indicated; age at least 18 years but not more than 39 years at the time of screening; normal regular menstrual cycles with a range of 24–35 days; body mass index between 18 and 29 kg/m$^2$; and body weight between 50 and 90 kg.

Exclusion criteria were: history of/or current endocrine abnormality such as polycystic ovary syndrome or evidence of ovarian dysfunction; elevated early follicular phase (menstrual cycle day 2–7) circulating FSH and/or LH concentrations according to cut-off levels used in the local laboratory; any clinically significant abnormal laboratory value; any ovarian and/or abdominal abnormality that would interfere with adequate ultrasound investigation of at least one ovary; only one ovary; contra-indications for the use of gonadotropins; use of hormonal preparations within 1 month prior to the date of signing consent; alcohol or drug abuse, or history thereof, within the 12 months preceding signing informed consent; or administration of investigational drugs within 3 months prior to screening. When all inclusion criteria and none of the exclusion criteria were met, the subject was considered to be eligible.

Study drugs and study procedures

rFSH (follitropin $\beta$, Puregon®; NV Organon, Oss, The Netherlands) was supplied as solution for injection (0.5 ml) in vials containing 100, 150 or 200 IU in vivo bioactivity. Ganirelix (Orgalutran®; NV Organon) was supplied as solution for injection in syringes containing 0.25 mg of ganirelix per 0.5 ml. HCG (Pregnyl®; NV Organon) in doses of 5000 IU per ampoule was supplied to trigger ovulation. For i.m. or s.c. injection of HCG, two ampoules were reconstituted with 1 ml of solvent.

During the admission visit, demographic and other subject variables were obtained. In addition, a general medical and gynaecological history was obtained, a general medical and gynaecological examination was performed, and endocrinological, biochemical and haematological analyses of blood were performed. All general medical, biochemical and haematological measurements were performed according to routine procedures of the individual study centres.

Eligible subjects were randomized by receiving a subject number from a randomization list corresponding with patient boxes in which the medication was kept. The 150 and 200 IU rFSH vials were indistinguishable. The randomization was done in blocks of four and was computer-generated using random numbers.

On day 2 or 3 of the menstrual period, rFSH was started and remained fixed for the first five rFSH treatment days. On treatment day 6, ganirelix treatment was started by daily s.c. administration in the morning up to and including the day of HCG administration. The last rFSH dose was administered on the day of HCG injection. During ganirelix treatment, the dose of rFSH could be adjusted downwards to 100 IU daily based on the clinical judgment of the investigator. For this purpose separate vials containing 100 IU were made available. Increments of the rFSH dose were not permitted. The duration of rFSH treatment was maximally 14 days. When at least three follicles $\geq 17$ mm were observed, HCG was administered to trigger ovulation. Oocyte pick-up procedures were carried out according to local protocols. After oocyte retrieval, both IVF and ICSI were allowed. Luteal phase support was given by daily progesterone starting at the latest at the day of embryo transfer for at least 2 weeks, or up to menses.

Serum concentrations of estradiol ($E_2$), progesterone, FSH and LH were measured at the first rFSH treatment day, the sixth treatment day and at regular intervals afterwards up to and including the day of HCG administration using local assays. On these days, vaginal ultrasound investigations were also performed to monitor follicle development.

Study end-points

The primary end-point was the number of cumulus–oocyte complexes (hereafter referred to as oocytes) retrieved. Secondary parameters included: the duration of rFSH and ganirelix treatment; the number of follicles on the day of HCG administration; the number of mature oocytes in women undergoing IVF and number of metaphase II oocytes in women undergoing ICSI; the number of good-quality embryos; endocrine parameters including FSH, LH, progesterone and $E_2$ throughout stimulation and on the day of HCG administration; clinical and vital pregnancy rates; and implantation and miscarriage rates. The daily doses to be given were fixed as per protocol and the total dose was therefore not to be considered an end-point.

Classification of oocytes as either mature or immature, and embryo quality as type 1, 2, 3 or 4, was performed according to previously published criteria (Staessen et al., 1989). In oocyte classification no distinction was made between women who underwent IVF or ICSI, and the maturity was based on the appearance of the cumulus cells, the corona radiata and the nuclear status. Type 1 and 2 embryos were considered to be of good quality.

The implantation rate was defined as the number of gestational sacs seen on transvaginal ultrasound examination divided by the total number of embryos replaced. Vital pregnancies were those pregnancies where a fetal heartbeat was observed under ultrasound investigation. No strict definition of the ovarian hyperstimulation syndrome (OHSS) was given. In the analysis of the occurrence of OHSS, its incidence and severity were based on the fact that the investigator reported it as such.

Statistical analysis

The number of oocytes retrieved was to be compared between treatment groups using analysis of variance (ANOVA) or the Cochran Mantel–Haenzel test, depending on the frequency of oocytes, in order to allow for centre to be included as a stratum. The observed frequencies were high enough for ANOVA to be used. The model therefore included terms for centre and treatment. The difference between treatments was calculated and presented along with its associated two-sided 95% confidence interval (CI). A separate
analysis was carried out omitting the first 10 patients treated in each individual centre to study the possibility of a learning effect.

Continuous secondary variables were analysed using the ANOVA methods described above, whilst binomial data (e.g. pregnancy rate) were analysed by the Cochran Mantel–Haenzel test. The statistical analysis was performed for all subjects who received at least one injection of rFSH.

Sample size considerations
With 130 subjects included in each treatment group and assuming a SD of 6.0 oocytes for the number of oocytes retrieved, a difference of 2.06 oocytes could be detected between the two treatment groups with a power of 80% and a significance threshold of 5%.

Results
Study population
A total of 264 women were randomized in six centres, of whom 257 were subsequently treated with rFSH (Figure 1). The number of patients treated per centre ranged between 15 and 76. The 150 IU daily dose treatment was given to 131 women (low-dose group), compared with 126 women in the 200 IU group (high-dose group).

Both groups had comparable demographic and infertility characteristics (Table I). The main causes of infertility were male and tubal infertility. Early follicular serum FSH concentrations were 6.3 IU/l (SD 1.8) and 6.1 IU/l (SD 1.6) in the low- and high-dose groups, respectively.

Cycle cancellations
In the 150 IU group, the cycle was cancelled prior to oocyte retrieval in four women (3.1%) because of insufficient ovarian response (n = 3) and premature luteinization (n = 1). In the high-dose group, three cycles were cancelled (2.4%) because of insufficient ovarian response (n = 1), risk of hyperstimulation (n = 1) and premature luteinization (n = 1). As a result, 127 and 123 women had an oocyte retrieval in the low- and high-dose groups, respectively. Of these, 154 underwent IVF and 96 women had ICSI. Fertilization failure was seen in one woman in the 150 IU treatment group (0.8%) and in six women in the 200 IU treatment group (4.9%). Six women in the low-dose group and eight in the high-dose group did not have a fresh embryo transfer because of perceived risk of ovarian stimulation. For one woman in the 200 IU group, the cycle was cancelled after fertilization for unknown reasons. Therefore, 120 women had an embryo transfer in the 150 IU group, compared with 108 women in the 200 IU group.

Primary end-point
The number of oocytes retrieved per woman ranged from 0 to 38. The overall and per-centre mean numbers of oocytes are given in Table II. Overall, 10.3 oocytes were retrieved in the 150 IU group and 11.9 in the 200 IU group. This difference approached statistical significance (P = 0.051). When women whose cycle was cancelled after initiating rFSH treatment were excluded (four in the low-dose group and three in the high-dose group), the difference became statistically significant (P = 0.047).

The mean number of oocytes per centre ranged from 8.5 to 14.4 in the 150 IU group and from 9.7 to 15.0 in the 200 IU group.

Secondary end-points
The mean total dose of rFSH used was 1541 IU (SD 224, range 900–2400) in the 150 IU group and 2014 IU (SD 279, range 1200–2600) in the 200 IU group. In only five women in the low-dose group and four in the high-dose group the dose was reduced to 100 IU, in both groups for an average duration of 2 days. Data on secondary end-points are given in Table III. Average duration of rFSH treatment was 10 days in both groups, and for a mean duration of 5 days ganirelix was co-administered. Although there were more follicles seen on ultrasound in the 200 IU group, this difference was not statistically significant. In the 150 IU group, 4.6 good-quality embryos were obtained, compared with 4.5 in the 200 IU group.

Table I. Demographics and infertility characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>150 IU (n = 131)</th>
<th>200 IU (n = 126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years (SD))</td>
<td>32.7 (3.6)</td>
<td>32.2 (3.5)</td>
</tr>
<tr>
<td>Mean weight (kg (SD))</td>
<td>62.8 (9.1)</td>
<td>63.3 (8.6)</td>
</tr>
<tr>
<td>Mean height (cm (SD))</td>
<td>163.4 (7.7)</td>
<td>164.0 (6.3)</td>
</tr>
<tr>
<td>Mean body mass index (kg/m² (SD))</td>
<td>23.5 (2.9)</td>
<td>23.5 (2.7)</td>
</tr>
<tr>
<td>Number (%) of women with cause of infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>40 (30.5)</td>
<td>28 (22.2)</td>
</tr>
<tr>
<td>Male factor</td>
<td>43 (32.8)</td>
<td>49 (38.9)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>7 (5.3)</td>
<td>15 (11.9)</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>28 (21.4)</td>
<td>27 (21.4)</td>
</tr>
<tr>
<td>Mixture of causes</td>
<td>13 (10.0)</td>
<td>7 (5.6)</td>
</tr>
<tr>
<td>Mean duration of infertility (years (SD))</td>
<td>4.6 (2.7)</td>
<td>4.6 (2.5)</td>
</tr>
<tr>
<td>Number (%) of women with primary infertility</td>
<td>67 (51.1)</td>
<td>78 (61.9)</td>
</tr>
<tr>
<td>Number (%) of women with secondary infertility</td>
<td>64 (48.9)</td>
<td>48 (38.1)</td>
</tr>
</tbody>
</table>
group. Vital pregnancy rates per started cycle in the low- and high-dose groups were 31% and 25%, respectively (not significant).

**Separate analysis omitting the first 10 patients treated**

When the first 10 women who were treated were excluded from the analysis, an average of 11.0 oocytes were retrieved in the 150 IU group (\(n = 103\)), compared with 11.6 in the 200 IU group (\(n = 97\); not significant). Vital pregnancy rates were 32% and 26% in the low- and high-dose groups, respectively (not significant).

**Rises of serum LH concentrations**

During ganirelix treatment, two women had a serum LH concentration >10 IU/l in the low-dose group on treatment days 10 and 12, respectively. In both cases, the cycle was completed and there was no pregnancy. In the high-dose group, there were no subjects with serum LH concentrations >10 IU/l during ganirelix administration.

Before the first administration of ganirelix, eight women in the low-dose group (6.1%) and 19 women in the high-dose group (15.1%) had increased LH concentrations (>10 IU/l) as measured on day 6 of rFSH administration. In two cases (one in each group), the cycle was cancelled because of premature luteinization. In the low-dose group, four out of the eight women (50%) with raised serum LH concentrations became pregnant, compared with three (15.8%) in the high-dose group, of which one subsequently had a miscarriage.

**Serum FSH, LH, E2 and progesterone concentrations**

Endocrinological parameters (means) during stimulation are given in Figure 2. Statistically significant differences between the two groups were found for circulating FSH, E2, LH, and progesterone on day 6 of rFSH stimulation and on the day of HCG administration. In the high-dose group, serum LH concentrations were higher at the start of ganirelix treatment (day 6: 5.25 versus 3.94 IU/l; \(P = 0.014\)) and lower on the day of HCG administration (1.31 versus 1.67 IU/l; \(P = 0.046\)), compared with the low-dose group. Serum FSH concentrations were significantly higher at beginning of ganirelix treatment and on the day of HCG administration compared with the low-dose group in both groups, the administration of ganirelix did not result in a decrease of the serum FSH concentrations: in the 150 IU group these concentrations were 11.0 and 10.8 IU/l at start of ganirelix treatment and the day of HCG administration, respectively, compared with 14.1 and 13.9 IU/l in the high-dose group.

Both E2 and progesterone concentrations were also significantly higher in the high-dose group at day 6 and on the day of HCG administration compared with the low-dose group.
Safety

OHSS was reported in eight women receiving the 150 IU dose (6.2%) and in 10 women in the 200 IU group (8.0%). Of these, three and two, respectively, were severe and required hospitalization. There were three ectopic pregnancies (one in the 150 IU group and two in the 200 IU group). In the 200 IU group, there was a hospitalization because of deep venous thrombosis.

Discussion

This is the first double-blind trial investigating the impact of two different rFSH doses in women undergoing assisted reproduction using ganirelix to prevent premature LH surges. It shows that on average an additional 1.6 oocytes can be retrieved using an extra dose of nearly 500 IU of rFSH in a 200 IU daily rFSH regimen compared with 150 IU per day \((n = 11.9 \text{ versus } 10.3, \text{ respectively})\). The variation in collected oocytes was large between the six centres: in one centre 9.7 oocytes were retrieved with the high-dose regimen, compared with 14.4 in another centre using the low-dose regimen. The centre that recruited the highest number of women had the smallest difference in oocyte yield between the high- and low-dose groups, and also the highest overall number of oocytes retrieved per patient.

This indicates that apart from the gonadotrophin dose used, other more important and centre-specific factors like oocyte pick-up routines play a role in determining the overall oocyte yield. However, in the current trial it is difficult to imagine that local protocols would favour one gonadotrophin regimen above the other. The current study confirms the results of Wikland et al. (2001), who found, in a similar but smaller and non-blinded study, that nearly two extra oocytes could be retrieved using 225 IU of follitropin \(\alpha\) compared with 150 IU in cetrorelix cycles. Despite the increased number of oocytes, neither Wikland et al. (2001) nor us found a statistically significantly higher number of high-quality embryos. This result is in accordance with the findings of comparative studies between ganirelix and GnRH agonists (European Orgalutran Study Group, 2000; European and Middle East Orgalutran Study Group, 2001; North American Ganirelix Study Group, 2001). Therefore, the value of increasing the total number of retrieved oocytes should not be overemphasized.

Overall vital pregnancy rates per started cycle (31% and 25% in the 150 and 200 IU group, respectively) were good, and higher than usually reported within the UK where there is a maximum of three embryos transferred. In the period from April 2001 until March 2001, the live birth rate in IVF (including ICSI) per started cycle was 25.1% in women <38 years of age (Human Fertilisation and Embryology Authority, 2002). This may indicate that the initial fears that pregnancy rates would be lower using GnRH antagonists compared with GnRH agonists (Al-Inany and Aboulghar, 2002) are not justified in practice. It has been speculated that a learning curve may be a relevant factor explaining these slightly lower pregnancy rates. This could not be confirmed in the current study, where the results did not differ when the first 10 patients in the various centres were excluded. This might be due to the fact the many of the centres participating in this trial already had experience with GnRH antagonist protocols.

The endocrine assessments as effected in the current investigation pointed towards a number of interesting findings. After 6 days of rFSH stimulation during which ganirelix was not administered, serum LH concentrations declined to a significantly lower value in the 150 IU group and two in the 200 IU group. In the 200 IU group, there was a hospitalization because of deep venous thrombosis.
comitantly, serum E₂ concentrations were also significantly higher on day 6 of the rFSH stimulation. Serum LH concentrations >10 IU/l on that day were found in 15.6% of the 200 IU women compared with 6.1% women in the 150 IU group. In the North American ganirelix trial (North American Ganirelix Study Group, 2001), these rises were not associated with an adverse outcome. Although our study was too small to make firm conclusions on this issue, it seems reasonable not to cancel cycles with pre-ganirelix LH ‘surges’, as four out of the eight women in the low-dose group with these raised LH concentrations had a subsequent vital pregnancy.

Serum FSH concentrations in the high-dose group were, not surprisingly, significantly higher than in the low-dose group after 6 days of stimulation (14.1 versus 11.0 IU/l). Interestingly, these concentrations did not change after initiation of ganirelix administration, as shown by unaltered concentrations at the day of HCG administration (13.9 versus 10.8 IU/l) with only five women in the low-dose group and four in the high-dose group whose daily dose was decreased. This indicates that the overwhelming proportion of circulating FSH derives from exogenous administration, and that endogenous pituitary FSH secretion is minimized by the negative feedback effect of rising E₂ concentration. Consequently, it appears illogical to increase the daily rFSH dose at the moment of GnRH administration to compensate for an apparently non-existent drop in serum FSH concentrations. However, the fact that the serum LH concentrations in the high-dose group were slightly, but significantly, lower on the day of HCG administration compared with the low-dose group might indicate that feedback mechanisms still play a minor role.

Although not statistically significant, the implantation and pregnancy rates in the high-dose group were ~7% lower than in the low-dose group. Recently, it has been suggested that the early follicular phase endocrine environment may affect endometrium receptivity (Kolibianakis et al., 2003). In that study, implantation rates were markedly lower in the group of women in whom the GnRH antagonist was started at the moment a large follicle was present as compared with a fixed start on day 6 of rFSH stimulation. In the ‘flexible group’, higher concentrations of LH and an earlier rise of E₂ were start on day 6 of rFSH stimulation. In the ‘flexible’ group, higher concentrations of LH and an earlier rise of E₂ were observed on day 8 of stimulation, reflecting the later start of the GnRH antagonist administration in that group. Although purely speculative, it could be that in our study the significantly increased serum LH and E₂ concentrations at day 6 of rFSH stimulation prior to the start of ganirelix administration seen in the high-dose group, contributed to the lower implantation rate in that group. However, more research is needed to confirm that early follicular endocrine parameters influence pregnancy rates in GnRH antagonist cycles.

In conclusion, this study has shown that a higher daily rFSH dose of 200 IU increased the number of oocytes retrieved compared with a starting dose of 150 IU per day. However, this increased oocyte yield was modest (1.6 oocytes), and its interpretation should be seen in the perspective of the extra gonadotrophin consumption (nearly 500 IU). Moreover, the fact that the number of good-quality embryos was the same in the low- and high-dose groups indicates that the clinical relevance of the higher daily dose is limited.

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