A prospective randomized clinical trial comparing recombinant follicle stimulating hormone (Puregon) and human menopausal gonadotrophins (Humegon) in non-down-regulated in-vitro fertilization patients

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A randomized clinical trial was performed comparing recombinant follicle stimulating hormone (rFSH, Puregon, n = 54) and human menopausal gonadotrophin (HMG, Humegon, n = 35) in infertile women undergoing in-vitro fertilization without the use of a gonadotrophin-releasing hormone (GnRH) agonist. Most patients had a tubal or idiopathic infertility, the latter always longer than 4 years’ duration. Patients with sperm abnormalities were excluded. None of the between-group differences in treatment outcome was statistically significant. In the rFSH group, a mean number of 11.2 oocytes was retrieved compared with 8.3 in the HMG group. Ongoing pregnancy rates per started cycle were higher in the rFSH group (22.2%) than in the HMG group (17.1%). Implantation rates were 27.5% in the rFSH group in comparison with 16.7% in the HMG group. In the rFSH group, a mean total dose of 1410 IU during 6.2 days was administered compared with 1365 IU in 6.0 days in the HMG group. Oestradiol concentrations on the day of human chorionic gonadotrophin administration were 3889 pmol/l in the rFSH group and 3145 pmol/l in the HMG group. In 15 subjects (rFSH: n = 9, 16.7%; HMG: n = 6, 17.1%) luteinizing hormone concentrations higher than 10 IU/l were seen during stimulation. In two of them, both from the rFSH group, ongoing pregnancies were achieved. The results indicate that rFSH (Puregon) is at least as efficacious as HMG and that acceptable pregnancy rates can be achieved without the use of a GnRH agonist.

Key words: FSH/HMG/IVF/Puregon/trial

Introduction

Most assisted reproduction technologies make use of ovarian stimulation by gonadotrophins prior to oocyte harvesting. Gonadotrophin-releasing hormone (GnRH) down-regulation prior to follicle stimulation by GnRH agonists (GnRH-a) has become prevalent in these treatments. The main reason for this is the acclaimed superiority in results and the augmented convenience of the treatment. A meta-analysis of prospective randomized studies comparing down-regulated and non-down-regulated treatment has revealed a significant difference in pregnancy rates between the two stimulation protocols (Hughes et al., 1992). However, it was the poor results in a number of the studies in the non-analogue arm that led to the significance rather than the superior results in the analogue arm. It was clear from some of the studies in the meta-analysis as well as other studies that results in the non-down-regulated cycles can be close or similar to those in which analogues have been used. In addition, it has been published that with the use of GnRH-a the risk of complications, especially ovarian hyperstimulation syndrome, has been increased (Hughes et al., 1992).

Commonly applied preparations include human menopausal gonadotrophins (HMG) and urinary follicle stimulating hormone (uFSH). These preparations are derived from post-menopausal urine and most of them contain more than 95% contaminating proteins. Batch-to-batch inconsistency and unreliable supply are well-known disadvantages of HMG and uFSH (Loumaye et al., 1995). Recently, more than 99% pure FSH preparations have become available by means of recombinant DNA technology. The genes encoding for both FSH subunits have been transfected in a Chinese hamster ovary cell line that subsequently produces FSH in the culture medium (Olijve et al., 1996). Extensive purification procedures ensure a highly purified product that, owing to well-controlled manufacturing conditions, exhibits a high batch-to-batch consistency.

It may be questioned whether the presence of luteinizing hormone (LH) activity in HMG and even in uFSH is necessary for adequate (multi)follicular development. A small-scale study on in-vitro fertilization (IVF) indicated that the use of recombinant FSH (rFSH), which did not contain any LH activity, did not hamper the development of follicles or compromise steroidogenesis (Devroye et al., 1994), even when potent GnRH-a was being used.

Given the considerations regarding the use as a first choice of GnRH-a prior to follicle stimulation, we performed a prospective, randomized, assessor-blind comparison between rFSH (Puregon) and HMG (Humegon) without the use of GnRH agonist.

Materials and methods

Patients

Between March 1992 and February 1994, infertile female subjects were recruited in a single centre. The aim was to include 100 patients. Inclusion criteria were as follows: at least 18 years and at most 39 years of age at the time of screening; cause of infertility potentially solvable by IVF; maximum of three previous IVF or other assisted reproduction techniques in which oocytes were collected at least once; normal ovulatory cycles with a mean length of between 24 and
35 days and an intra-individual variation of plus or minus 3 days (but never outside the 24–35 days range); good physical and mental health; and a body weight 80–130% of the ideal body weight (adapted from the Metropolitan Life Insurance Company Tables).

Exclusion criteria were: infertility caused by endocrine abnormalities such as hyperprolactinaemia, polycystic ovary syndrome, and absence of ovarian function; male infertility as defined by abnormalities such as hyperprolactinaemia, polycystic ovary syndrome, and absence of ovarian function; male infertility as defined by spermatozoa/ml and/or <40% normal forms and/or <40% normal motility; any ovarian and/or abdominal abnormality that would interfere with adequate ultrasound investigation; hypertension (sitting diastolic blood pressure >90 mm Hg and (or) systolic blood pressure >150 mm Hg); chronic cardiovascular, hepatic, renal, or pulmonary disease; a history of (within 12 months) or current abuse of alcohol or drugs; administration of non-registered investigational drugs within 3 months prior to screening. When all criteria were met, the subject was considered to be eligible. The study was approved by the Ethics Committee of the hospital. All subjects gave written informed consent. This investigation was performed according to the declaration of Helsinki and the European Community note on Good Clinical Practice for trials on medicinal products in the European Community (CPMP Working Party on Efficacy of Medicinal Products, 1990).

Study design

This was a randomized, assessor-blind, prospective, single-centre study comparing recombinant FSH (rFSH, Org 32489, follitropin beta, Puregon; NV Organon, Oss, The Netherlands, batch numbers CP 091077, 091134 and 092146) and HMG (Humegon; NV Organon, batch numbers CP 091151 and 093133). The objective of the study was to assess the safety and efficacy of rFSH in relation to HMG for induction of ovarian stimulation in infertile women treated by IVF without the use of a GnRH agonist. Eligible subjects were randomized by receiving a subject number from a randomization list corresponding with patient boxes in which the medication was kept. Subject boxes were assigned to either 150 or 225 IU of rFSH or HMG i.m. for the first 4 days. Afterwards, the dose was adjusted according to follicular development as assessed by ultrasound scanning. Since, for technical reasons, rFSH was supplied in vials and HMG in ampoules, a double-blind design was not feasible. Instead, an assessor-blind design was chosen in which preparation and administration of the medication was done by a study coordinator who took no part in any decision concerning the dose during treatment. When at least two follicles ≥15-mm diameter were present, 10 000 IU of human chorionic gonadotrophin (HCG, Pregnyl; NV Organon) was given i.m. to induce ovulation. Oestradiol concentrations were not taken into account in deciding upon administering HCG. After oocyte retrieval and fertilization, a maximum of three embryos were transferred. Luteal support consisted of a minimum of three i.m. injections of 1500 IU HCG or 100 mg progesterone intravaginally three times per day.

End-points

The primary outcome variable was the number of oocytes retrieved. Secondary variables included number of follicles ≥15-mm diameter at the day of HCG administration, length of treatment, total dose, serum concentrations of oestradiol on the day of administering HCG, number of mature oocytes recovered, number of high quality embryos, implantation rate, clinical and ongoing pregnancy rates per started cycle and transfer. The implantation rate was defined as the number of gestational sacs on ultrasound per 100 embryos transferred. The definition of a clinical pregnancy included miscarriages with or without proof of a vital fetus. An ongoing pregnancy was defined as a vital pregnancy at least 12 weeks after embryo transfer. In addition, the fertilization rate [number of oocytes with two pronuclei (PN) 12–16 hours after incubation divided by the number of oocytes incubated] and the number of subjects with polyspermy (at least 3 PN) were assessed.

The main safety parameters were the incidence of ovarian hyper-stimulation syndrome and the development of anti-FSH antibodies. Also, common laboratory parameters were compared before and after treatment. These parameters included routine blood biochemistry as sodium, potassium, chloride, bicarbonate, phosphorus, calcium, glucose, urea, creatinine, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, lactate dehydrogenase, total bilirubin, total protein albumin; haematology parameters included haemoglobin, haematocrit, erythrocytes, leukocytes plus differentiation; and urinalysis included quantitative estimation of pH and qualitative estimations of protein, acetone, glucose, and haemoglobin.

Assessments

At screening, a medical history was obtained and a physical examination was performed. Routine blood biochemistry, haematology and urinalysis were performed and the following endocrinological parameters were measured: serum oestradiol, FSH, LH, progesterone, testosterone, prolactin, and dehydroepiandrosterone sulphate. An ultrasound scan was performed to exclude ovarian abnormalities. Sperm analysis of the patient took place and was repeated at the time of fertilization.

Serum FSH, LH, oestradiol and progesterone were measured on the first day of treatment and on the day of HCG administration. In between, assessments of serum oestradiol and LH were performed on a regular basis. Frequent ultrasound scans were made to monitor follicular growth.

Spare serum samples for the determination of anti-FSH were taken before and after treatment. These samples were sent to NV Organon, Oss, The Netherlands, for central determination using a previously described assay (Out et al., 1995). Routine blood biochemistry, haematology and urinalysis were repeated as soon as possible after treatment had ended.

Classification of oocytes as either mature or immature and embryos as type 1, 2, 3, or 4 was performed according to previously published criteria (Staessen et al., 1989). Type 1 and 2 were considered to be high quality embryos.

Statistical analysis

For ordinal data, analyses of variance (ANOVA) were performed and, if not applicable, the Wilcoxon test, or equivalently, the Mantel–Haenszel test was used. For binary data the Mantel–Haenszel test statistic was used.

For the number of oocytes retrieved, assuming a SD of six oocytes and that 85 subjects out of 100 treated women would have an oocyte retrieval, and based on the corresponding r-distribution, the minimum detectable difference between the two groups was 3.7 oocytes (power of 80% and two-sided 5% significance level). Similarly, with respect to pregnancy rates, it was assumed that the pregnancy rate with HMG per started cycle was 15%. On the basis of 100 started cycles (with a 3:2 randomization ratio) an ongoing pregnancy rate with recombinant FSH less than 0.5% or larger than 40.5% could be detected.

All analyses were performed on an intent-to-treat basis, including all subjects who received at least one ampoule of rFSH or HMG. The main advantages of this rule were that more patients were available for final analysis of efficacy and that it more closely reflected how physicians evaluated a therapeutic agent in the clinical setting, outside an experimental control.
Puregon versus Humegon in IVF

Figure 1. Percentage of patients who were randomized, treated, had an oocyte retrieval and embryo transfer in a prospective randomized comparison of recombinant follicle stimulating hormone (rFSH) and human menopausal gonadotrophins (HMG).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>rFSH</th>
<th>HMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>32.0</td>
<td>31.1</td>
</tr>
<tr>
<td>Mean body mass index (kg/m²)</td>
<td>22.4</td>
<td>22.6</td>
</tr>
<tr>
<td>Mean duration of infertility (years)</td>
<td>4.3</td>
<td>3.5</td>
</tr>
<tr>
<td>No. (%) of subjects with primary infertility</td>
<td>25 (46.3)</td>
<td>21 (60.0)</td>
</tr>
<tr>
<td>No. (%) of subjects with secondary infertility</td>
<td>29 (53.7)</td>
<td>14 (40.0)</td>
</tr>
<tr>
<td>No. (%) of subjects with cause of infertility:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tubal</td>
<td>31 (57.4)</td>
<td>17 (48.6)</td>
</tr>
<tr>
<td>endometriosis</td>
<td>1 (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>tubal/endometriosis</td>
<td>1 (1.9)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>idiopathic</td>
<td>21 (38.9)</td>
<td>17 (48.6)</td>
</tr>
</tbody>
</table>

Table II. Results on primary and secondary variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>rFSH</th>
<th>HMG</th>
<th>Difference</th>
<th>SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of oocytes retrieved</td>
<td>11.2</td>
<td>8.3</td>
<td>2.9</td>
<td>2.01</td>
<td>−1.1 to 6.8</td>
</tr>
<tr>
<td>Number of follicles ≥15 mm²</td>
<td>5.5</td>
<td>5.4</td>
<td>0.1</td>
<td>0.73</td>
<td>−1.3 to 1.5</td>
</tr>
<tr>
<td>Serum oestradiol (pmol/l) at human chorionic gonadotrophin administration</td>
<td>3889</td>
<td>3145</td>
<td>745</td>
<td>566</td>
<td>−365 to 1855</td>
</tr>
<tr>
<td>Number of mature oocytes</td>
<td>10.6</td>
<td>7.5</td>
<td>3.1</td>
<td>1.94</td>
<td>−0.7 to 6.9</td>
</tr>
<tr>
<td>Total dose (IU)</td>
<td>1410</td>
<td>1365</td>
<td>45</td>
<td>49.5</td>
<td>−52.5 to 142.5</td>
</tr>
<tr>
<td>Number of treatment days</td>
<td>6.2</td>
<td>6.0</td>
<td>0.2</td>
<td>0.21</td>
<td>−0.2 to 0.6</td>
</tr>
<tr>
<td>Number of high quality embryos</td>
<td>3.1</td>
<td>3.0</td>
<td>0.1</td>
<td>0.97</td>
<td>−1.8 to 2.0</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>27.5</td>
<td>16.7</td>
<td>10.8</td>
<td>6.9</td>
<td>−2.8 to 24.4</td>
</tr>
<tr>
<td>Clinical pregnancy rate per cycle (%)</td>
<td>24.1</td>
<td>22.9</td>
<td>1.2</td>
<td>9.27</td>
<td>−16.9 to 19.3</td>
</tr>
<tr>
<td>Clinical pregnancy rate per embryo transfer (%)</td>
<td>33.3</td>
<td>29.6</td>
<td>3.7</td>
<td>11.75</td>
<td>−19.3 to 26.7</td>
</tr>
<tr>
<td>Ongoing pregnancy rate per cycle (%)</td>
<td>22.2</td>
<td>17.1</td>
<td>5.1</td>
<td>8.77</td>
<td>−12.1 to 22.2</td>
</tr>
<tr>
<td>Ongoing pregnancy rate per embryo transfer (%)</td>
<td>30.8</td>
<td>22.2</td>
<td>8.5</td>
<td>11.24</td>
<td>−13.4 to 30.5</td>
</tr>
</tbody>
</table>

None of the observed differences between the recombinant follicle stimulating hormone (rFSH) or human menopausal gonadotrophin (HMG) treatment groups were statistically significant.

Results

Patients
A total of 109 patients (rFSH: n = 66; HMG: n = 43) was randomized; 89 (rFSH: n = 54; HMG: n = 35) started gonadotrophin treatment. This discrepancy was owing to the fact that the study had to be truncated prematurely due to non-medical factors outside our control. Twenty-three subjects (rFSH: n = 15; HMG: n = 8) started treatment but did not have an embryo transfer. Thus, of all patients randomized, 59.1% in the rFSH group had an embryo transfer, compared with 62.8% in the HMG group (see Figure 1).

Demographic and infertility characteristics are given in Table I. Age and body mass index were comparable. Most patients had had tubal (57.4 and 48.6% in the rFSH and HMG groups, respectively) or idiopathic infertility for a minimum of 4 years (38.9% and 48.6%, respectively). There was no dominance of first IVF cycles in the treated population (data not shown).

Primary and secondary end-points
Results are given in Table II and III. The mean number of oocytes retrieved in the rFSH group was 11.2 and 8.3 in the HMG group. The treatment difference of 2.9 was not statistically significant. There were no statistically significant differences between rFSH and HMG in the outcome of all secondary variables. In the rFSH and HMG groups respectively, the number of follicles ≥15-mm diameter was 5.5 and 5.4, the number of treatment days 6.2 and 6.0, and the total dose given 1410 IU and 1365 IU. The oestradiol concentration on the day of HCG administration was 3889 pmol/l in the rFSH group, and 3145 pmol/l in the HMG group.

Median FSH and LH concentrations on the day of HCG administration were 14.1 IU/l and 3.8 IU/l respectively, in the rFSH group, and 13.7 IU/l and 4.1 IU/l respectively, in the HMG group. The median progesterone concentration was 1.9 nmol/l in the rFSH group, compared with 1.8 nmol/l in the HMG group.
Although the median normal fertilization rate was lower for rFSH (46.2%) than for HMG (61.8%), the mean numbers of transferable embryos were similar (4.1 in the rFSH and 4.4 in the HMG group). Of 13 pregnancies in the rFSH group, one aborted (8%), compared with two out of eight pregnancies in the HMG group (25%). In addition, one ectopic pregnancy was seen (in the rFSH group).

The ongoing pregnancy rate per started cycle was higher in the rFSH group (22.2%) than in the HMG group (17.1%). In the calculated pregnancy rate in the HMG group, one pregnancy was included of a patient randomized to receive HMG, but who erroneously received rFSH. If this pregnancy was counted as a rFSH pregnancy, the ongoing pregnancy rates were 23.6% and 14.7% (treatment difference: 8.9, SE: 8.8, 95% confidence interval: –8.3 to 26.2, not significant). The implantation rate similarly analysed was 22/80 (27.5%) for rFSH, and 10/60 (16.7%) for the HMG group (not significant).

In total, five pairs of twins (rFSH: n = 3; HMG: n = 2) and two sets of triplets (both in the rFSH group) were reported. It is noteworthy that one of these triplets occurred after transfer of only two embryos.

None of the treated patients developed an ovarian hyperstimulation syndrome. There was no development of anti-FSH antibodies after treatment with rFSH. No clinically relevant shifts from baseline in laboratory variables were noted.

**Premature LH surges**

In the rFSH group, nine subjects (16.7%) had LH concentrations higher than 10 IU/l during stimulation, compared with six subjects (17.1%) in the HMG group (see Table IV). In one subject (no. 13), no oocytes were recovered due to spontaneous ovulation. In all other subjects, oocytes were retrieved (range 1–30). No fertilization was seen in three subjects (nos. 3, 5 and 10). The number of type 1, 2, and 3 embryos ranged from 0 to 15. Two patients, both in the rFSH group (nos. 1 and 7), had a subsequent (triplet) ongoing pregnancy.

### Discussion

It is well accepted that GnRH analogues can prevent the occurrence of a premature LH surge, an event that occurs in about 15% of all non-down-regulated cycles. Thus, administering analogues to all first cycles means an ‘overkill’ in about 85%. Although analogues create an unequalled flexibility in the timing of aspiration, it could also be argued that their use conceals inaccurate follicle monitoring: HCG can be administered at a large range in follicular size without compromising the results. The disadvantage, however, is the increased expenditure as well as the added risks of the treatment. It is for these reasons that in our clinic the use of analogues is restricted to those patients who are at increased risk of a premature LH surge. In the majority of patients, the first cycle is performed without down-regulation.

This is the first randomized, controlled comparison between rFSH and HMG without using a GnRH agonist. Although a higher number of oocytes were retrieved and a higher ongoing pregnancy rate was observed in the rFSH group, the differences...
were not statistically significant. However, these results are in agreement with previously published comparisons between rFSH and uFSH (Hedon et al., 1995; Out et al., 1995). In fact, a combined analysis of these two studies plus the current investigation demonstrated a significantly improved ongoing pregnancy rate directly following the fresh transfer for rFSH (22.9% versus 17.9%, \(P = 0.039\); Out et al., 1997). Factors that might explain these higher pregnancy rates are the absence of contaminating, potentially FSH-inhibiting proteins in rFSH and a favourable isohormone composition, i.e. a predominantly relatively basic profile in contrast to the observed more acidic isohormone composition in post-menopausal urine-derived gonadotrophins (Lambert et al., 1995).

A relationship between increased LH concentrations and miscarriages has been suggested through its potential detrimental effect on oocyte quality (Balen et al., 1995). Since HMG contains an equal amount of FSH and LH activity, this might theoretically influence cycle outcome. However, the numbers of miscarriages in this study (one in the rFSH group and two in the HMG group) were obviously too low to allow firm conclusions. Also, such a negative role of exogenous LH seems unlikely because LH concentrations on the day of HCG administration were similar in both groups and fertilization data from patients with LH concentrations >10 IU/l did not show an apparent detrimental effect. In a recent comparison between highly purified uFSH and HMG using a GnRH agonist (Westergaard et al., 1996), the fertilization rate was even significantly higher in the HMG group.

The presence of LH activity in HMG did not result in higher oestradiol concentrations on the day of HCG administration in these patients. The interpretation of this finding should be put into the perspective that endogenous LH in these non-down-regulated patients was not suppressed. In contrast, in the study from Westergaard et al. (1996), it was shown that, on day 8 of the stimulation, oestradiol concentrations were significantly higher after stimulation with HMG than those after highly purified FSH. Unfortunately, oestradiol concentrations on the day of HCG administration were not given. However, it is unlikely that potential differences in oestradiol concentrations during stimulation cycles will lead to adverse endometrial receptivity, since the implantation rates were the same in that study.

It is noteworthy that fertilization rates in this study were lower than usually reported. This may be related to the stimulation without GnRH analogues on the one hand, and to the fact that in general no cycles were cancelled because of too low or too high follicle numbers, so that in a number of cases poorer quality oocytes were retrieved. This might also explain the relatively low proportion of patients with embryo transfer.

Although premature LH surges were seen in 15 subjects, in only one case were no oocytes obtained. Normal fertilization rates and the total number of transferable embryos in these patients were not jeopardised by these surges, and also two ongoing pregnancies (both in the rFSH group) were obtained. This demonstrates that a protocol without using a GnRH agonist can very well lead to acceptable outcomes. The apparent advantages of such a protocol are the diminished consumption of gonadotrophins (1365–1410 IU) during a relatively short stimulation period (± 6 days) and a consequently lower risk for the development of the ovarian hyperstimulation syndrome.

In conclusion, rFSH (Puregon) is at least as efficacious as HMG in non-suppressed infertile women undergoing IVF. Provided that adequate monitoring is taking place, there is no need to use GnRH agonists to reach pregnancy rates as seen in GnRH-agonist treated women. We firmly believe that follicle stimulation should become more individualized: instead of completely and indiscriminately down-regulating all patients, clinicians should search for selection criteria to distinguish those patients who really need GnRH analogues.

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References


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