Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists—a randomized study

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BACKGROUND: Highly purified hMG (hp-hMG) has recently shown better cycle outcome than the recombinant FSH (rFSH) when compared in GnRH agonist long protocol cycles. However, they have not yet been compared in GnRH antagonist cycles. METHODS: A RCT comparing the ongoing pregnancy rate (primary end-point) in 280 patients undergoing IVF/ICSI after stimulation with hp-hMG or rFSH controlled with a GnRH antagonist. RESULTS: No significant differences were observed between hp-hMG and rFSH in terms of the ongoing pregnancy rate per started cycle (35.0 versus 32.1%, respectively; \(P = 0.61\)); relative risk: 1.09 (95% confidence interval: 0.78–1.51; risk difference: 2.9%). No differences were observed for implantation, clinical pregnancy and pregnancy loss rates. More oocytes were obtained from patients receiving rFSH than hMG (14.4 ± 8.1 versus 11.3 ± 6.0, respectively; \(P = 0.001\)). Estradiol was higher at the end of stimulation in the hp-hMG group (\(P = 0.02\)), whereas progesterone was higher in patients stimulated with rFSH (\(P < 0.001\)). CONCLUSIONS: A similar outcome was observed for hp-hMG and rFSH when used for stimulation in GnRH antagonist cycles. However, some differences were found in ovarian response in terms of oocyte yield and hormonal profile. Clinical Trials.gov Trial registration number: NCT00669786.

Keywords: GnRH antagonists; ovarian stimulation; FSH; hMG; RCT

Introduction

Highly purified hMG (hp-hMG) and recombinant FSH (rFSH) have been widely and successfully used for ovarian stimulation in infertile women undergoing treatment for IVF/ICSI and embryo transfer. Comparing the effectiveness of both compounds has been the object of large debate since the development of embryo transfer (Van Wely et al., 2003). Several studies comparing the outcome of rFSH and hMG have been reported, most of which were performed in women undergoing pituitary down-regulation with a GnRH agonist long protocol (Ng et al., 2001; Westergaard et al., 2001; The European and Israeli Study Group on highly purified hMG versus rFSH, 2002; Balasch et al., 2003; Goldfarb and Desai, 2003; Kilani et al., 2003; Rashidi et al., 2005; Andersen et al., 2006; Hompes et al., 2007). Recently, two meta-analyses showed a better outcome in terms of the live birth rate when hp-hMG was used for ovarian stimulation compared with rFSH in the GnRH agonist long protocol (Al-Inany et al., 2004; Merviel et al., 2004; Bosch et al., 2005), nor those that compared success rates when LH was administered or not (Cédrin-Durnerin et al., 2004; Griesinger et al., 2005) have been able to address the role, if any, that LH administration plays during the follicular phase of a stimulated cycle for IVF-ET under pituitary suppression with this kind of compound.

Given this background, it seems appropriate to investigate whether providing multiple follicular development with hp-hMG in GnRH antagonist cycles causes a clinically relevant improvement in cycle outcome in terms of ongoing pregnancy rates when compared with the well-known protocol with rFSH and a GnRH antagonist in the daily dose regimen.

Materials and Methods

Study population

Two hundred and eighty patients undergoing their first IVF/ICSI treatment in our center were randomized to follow ovarian stimulation with hp-hMG (\(n = 140\)) or rFSH (\(n = 140\)). An Institutional Review Board approval was obtained and the study was conducted from...
January 2003 to December 2006, and was continued by a follow-up period to collect pregnancy outcome data.

Patients were selected if they met all the following inclusion criteria: women with good physical and mental health, aged 18–37 years; regular menstrual cycles ranging from 25 to 35 days; BMI < 30 kg/m²; normal basal serum FSH (≤ 10 IU/l) and estradiol (E₂) (≤ 75 pg/ml) levels determined on Day 3 of the cycle previous to controlled ovarian stimulation (COS); no uterine (fibroids, adenomyosis, Mullerian malformations), ovarian (endometriosis, polycystic ovaries) or adnexa (hydrosalpinx) abnormalities assessed by vaginal ultrasound. The exclusion criteria were: patients with a history of recurrent pregnancy loss; any significant systemic disease, endocrine or metabolic disorder; having concomitant medication interfering with the purposes of the study; patients who have received any ovulation induction drug within one month before their inclusion in the study.

Study design
This was a randomized, open-label, single-center controlled study to compare hp-hMG (Menopur®, Ferring Pharmaceuticals, Copenhagen, Denmark) and rFSH (Gonal F®; Serono, Geneva, Switzerland) in patients undergoing ovarian stimulation for IVF/ICSI-ET controlled with a GnRH antagonist. Patients were randomized 1:1 to receive hp-hMG or rFSH according to a computer-generated list. This list was arranged into four blocks of 70 patients. Patients were allocated to one group of two when they were scheduled to start ovarian stimulation. The randomization list was centralized at the institution’s call center, and the center was contacted to allocate each randomized patient into a group.

The primary end-point was the ongoing pregnancy rate per randomized patient (intention-to-treat analysis, ITT). The ongoing pregnancy rate was defined as the presence of at least one viable fetus beyond week 20 of pregnancy on ultrasound. A started cycle was considered when patients had their first injection of gonadotropins. Delivery rate was also recorded. Secondary outcome end-points were a clinical pregnancy rate defined as the presence of at least one viable fetus beyond week 20 of pregnancy on ultrasound. The luteal phase was supplemented with a GnRH antagonist. Patients were randomized 1:1 to receive 0.25 mg of the GnRH antagonist Cetrorelix (Cetrotide®; Serono) was administered when at least three follicles reached 18 mm in diameter, and oocyte retrieval was scheduled 36 h later. Standard IVF or ICSI procedures were applied, as previously described (Gil-Salom et al., 1995). A maximum of three embryos were transferred on Day 3 of the cleavage state. Ultrasound guidance was used for all embryo transfers. The luteal phase was supplemented with 400 mg/day of vaginal micronized progesterone (Progestif®; Effik, Madrid, Spain). A pregnancy test (serum β-hCG determination) was done 13 days after embryo transfer.

Statistical analysis
The sample size was calculated using a one-sided significance level of 0.05 and a power of 80% to detect a major clinically relevant difference of 1.5 relative risk (RR) in the ongoing pregnancy rate between groups (30–45%), favoring hp-hMG administration. According to these parameters, 128 patients were required for each treatment group. As 10% losses after starting the stimulation per group were predicted, a total of 280 patients (140 in each group) were randomized. This sample size provides a statistical power of 70% in a two-sided hypothesis.

Data are expressed as the mean ± SD for continuous variables, and as the mean and 95% confidence interval (95% CI) for categorical variables. Normality of distribution of continuous variables was assessed with the Kolmogorov–Smirnov test. Differences between groups of normally distributed variables were assessed with the Student’s t-test, while not normally distributed variables were compared with the Mann–Whitney U test. Non continuous variables were compared with the χ² test with Yates correction when appropriate.

Results
Demographic and baseline characteristics of the study participants are shown in Table I. No relevant between-group differences were observed for any of the compared parameters. Distribution of infertility causes was similar between groups, as was the basal hormonal profile.

Two hundred and ninety three patients were initially recruited for the study. Of these, 13 were excluded by randomization for various different reasons, which represents a participation rate of 95.6% (Fig. 1). Of the 280 patients initially included, 32 did not reach the oocyte retrieval procedure [14 patients receiving rFSH (10.0%) and 18 having hp-hMG (12.9%); (P = 0.45)]. Nine patients (6.4%) were cancelled because of the low response in both groups. In the rFSH

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<th>Table I. Demographic and baseline hormonal profile characteristics of the study participants.</th>
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<td><strong>Baseline parameters</strong></td>
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Values are expressed by mean ± SD for continuous variables and number (%) for categorical variables. r-FSH, recombinant FSH; hp-HMG, highly purified hMG; E₂, estradiol.
group, 5 (3.6%) patients were cancelled for being at risk of OHSS, whereas cancellations for this reason in the hp-hMG were 9 (6.4%); (P = 0.27) (Fig. 1).

Table II shows the ovarian stimulation parameters outcome. Patients of both groups received a similar total dose of gonadotropins. Serum E2 levels on the day of rhCG administration were higher in the hp-hMG group, whereas progesterone was higher in patients who received rFSH. Ovarian response was significantly higher in the rFSH group in terms of the number of COCs collected and metaphase II oocytes obtained (ICSI cycles). The fertilization rate, the number of transferred embryos and the embryos available for cryopreservation were comparable between both groups.

Eleven patients did not have embryo transfer: 5 patients (4.1%) in the hp-hMG group and 6 (4.8%) in the rFSH group. All the embryos of two patients of each group were cryopreserved because of clinical OHSS. Seven patients (three in the hp-hMG group and four in the rFSH group) did not present embryo transfer because of the low quality of their embryos (Fig. 1). Therefore, embryo transfer was performed in 117 patients receiving hp-hMG and 120 patients receiving rFSH. The cycle outcome is detailed in Table III.

In short, no significant differences were observed between study groups in terms of implantation, pregnancy, ongoing pregnancy and delivery rates per randomized patient. One patient of each group had a pregnancy loss beyond week 20. Figure 2 shows the RR and 95% CI of each outcome end-point for hp-hMG cycles in relation to the rFSH group.

Discussion

In this study, hp-hMG and rFSH have been compared for the first time when used in ovarian stimulation with a GnRH antagonist for IVF/ICSI-ET. The ongoing pregnancy and delivery rates per randomized patient (ITT analysis) were similar between both groups, with an RR of 1.09 (95% CI: 0.78–1.51) which reflects a non-significant difference between both groups. When other cycle outcome parameters were compared, e.g. implantation and clinical pregnancy rates, similar results were observed. Because of a low response or low risk of OHSS, cancellation rates were comparable between both groups, as were the number of cycles without embryo transfer and the pregnancy loss rate.

The comparison of hMG and rFSH effectiveness in ovarian stimulation for IVF/ICSI-ET has been the object of a number of studies. They have been compared in non down-regulated cycles (Jansen et al., 1998), in down-regulated cycles with a GnRH agonist short protocol (Strehler et al., 2001), but mainly in down-regulated cycles through a GnRH agonist long protocol (Ng et al., 2001; Westergaard et al., 2001; Diedrich et al., 2002; The European and Israeli Study Group on highly purified hMG versus rFSH, 2002; Balasch et al., 2003; Goldfarb and Desai, 2003; Kilani et al., 2003; Rashidi et al., 2005; Andersen et al., 2006; Hompes et al., 2007). Nevertheless, very few data have been reported when both compounds were compared in GnRH antagonist cycles (Al-Inany et al., 2008). The different hormonal profile observed in the follicular phase when hMG and rFSH are used, especially as far as the serum LH levels are concerned (Huine et al., 2007), makes the comparison of hp-hMG and rFSH more interesting.
employed was similar between both groups, the number of agonist long protocol trials. Albeit the dose of gonadotropins used for the down-regulation, there was a smaller difference in the 95% CI observed (0.78–1.51) is consistent with those reported in the GnRH cycles.

The largest body of evidence available on the comparison of these two compounds is derived from two recent meta-analyses (Al-Inany et al., 2008; Coomarasamy et al., 2008) which include 2179 and 2937 cycles, respectively. Both studies observed a significantly better live birth rate when hp-hMG was employed for ovarian stimulation, with a relative increase of 18% (Coomarasamy et al., 2008) to 20% (Al-Inany et al., 2008). In the present study, in which a GnRH antagonist was used for ovarian stimulation, with a relative increase of 9%. Nevertheless, the 95% CI observed (0.78–1.51) is consistent with those observed in two recent single multicenter studies (Andersen et al., 2006; Hompes et al., 2007).

In this study, IVF and ICSI cycles were included indiscriminately. Some authors have suggested that differences between hp-hMG and rFSH are mainly observed in the IVF cycles (Westergaard et al., 2001; Platteau et al., 2004). In the present study, the majority of cycles were ICSI as the male factor was the most frequent indication. A separated analysis was not performed because of the low number of pure IVF cycles.

The data related to ovarian stimulation outcome obtained in this study are comparable to those reported in the GnRH agonist long protocol trials. Albeit the dose of gonadotropins employed was similar between both groups, the number of COCs retrieved was significantly lower in the hp-hMG group, showing a mean difference of –3.1 oocytes. This finding coincides with the Merit study (Andersen et al., 2006) in which 1.8 oocytes less were obtained in the hp-hMG group, and with that by Hompes et al. (2007) with a mean difference of 2.8 oocytes, thus favoring rFSH. The lower number of COCs obtained when hp-hMG is employed for COS could reflect a LH effect during the follicular phase which induces an atresia of a number of follicles. This has also been observed when hMG and rFSH have been compared in ovulation induction in World Health Organization Group II anovulatory infertility patients (Platteau et al., 2006), where a lower number of intermediate follicles were present in patients receiving hp-hMG. Moreover, it has been demonstrated that the number of oocytes retrieved in GnRH antagonist cycles tends to diminish as serum LH levels increase throughout the follicular phase (Bosch et al., 2005).

In close relationship with this finding, the serum E2 levels were significantly higher at the end of the follicular phase in the hp-hMG group, despite the lower number of COCs finally retrieved, which reflects that E2 production per mature follicle significantly increased when hp-hMG was administered. This was also observed in the Merit study (Smitz et al., 2007), and was clearly related to LH activity. In GnRH antagonist cycles (Bosch et al., 2005), the patients who showed higher serum LH levels throughout the follicular phase also presented significantly higher serum E2 concentrations on the day of hCG administration.

The administration of hp-hMG has also led to a higher follicular production of E2 if compared with rFSH (Smitz et al., 2007). This could explain the higher proportion of top-quality embryos per obtained oocyte observed in patients treated with hp-hMG compared with those who received rFSH (Ziebe et al., 2007). In fact in the present study, the number of embryos available for transfer (embryos transferred plus embryos cryopreserved) was equal between groups despite the lower number of COCs obtained in the hp-hMG group.

On the other hand, serum progesterone concentrations were significantly higher in the rFSH group. Increased progesterone levels have been already related to FSH administration, in either GnRH antagonist cycles (Bosch et al., 2005) or GnRH agonist long protocol cycles (Andersen et al., 2006). This increase is explained by FSH activity, as demonstrated by several authors (Adonakis et al., 1998; Filicori et al., 2002;
Smidt et al., 2007), and which may stimulate granulosa cell activity, while LH may induce progesterone catabolism to androgens at the level of theca cells. In this study, the mean progesterone concentration on the day of rhCG administration was $0.99 \pm 0.48$ ng/ml in the rFSH group, versus $0.73 \pm 0.42$ ng/ml in the hp-hMG patients ($P < 0.001$). However, this difference was not of clinical relevance as progesterone concentration was shown to be detrimental in GnRH antagonist cycles at concentrations $>1.2$ ng/ml (Bosch et al., 2003).

In conclusion, hp-hMG and rFSH displayed a similar outcome when used in ovarian stimulation with a GnRH antagonist for IVF/ICSI-ET in terms of the ongoing pregnancy and delivery rate per started cycle. No differences were observed for implantation, clinical pregnancy and pregnancy loss rates. Although this is the first study comparing both gonadotropins in the GnRH antagonist protocol, these findings are limited because of the small number of patients included. Moreover, the statistical power of the present study in a two-sided test is 70%, as sample size calculation was initially performed for a one-sided hypothesis, expecting a better outcome when hp-hMG was used. According to the results hereby presented, only the absence of a large difference between hp-hMG and rFSH groups in cycle outcome can be concluded, and two-sided tests are recommended if further studies are developed. The significant detection of a relative difference of 10% requires the analysis of more than 3000 patients per group. Therefore, large multicenter analyses or meta-analyses including several single studies are necessary to determine differences of this magnitude.

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Submitted on December 16, 2007; resubmitted on April 30, 2008; accepted on May 7, 2008