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A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer

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Objective: To compare the efficacy and safety of highly purified menotropin (hphMG) and recombinant FSH (rFSH) for controlled ovarian stimulation in a GnRH antagonist cycle with compulsory single-blastocyst transfer.
Design: Randomized, open-label, assessor-blind, parallel groups, multicenter, noninferiority trial.
Setting: Twenty-five infertility centers in seven countries.
Patient(s): Seven hundred forty-nine women.
Intervention(s): Controlled ovarian stimulation with hphMG or rFSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer on day 5 in one fresh or subsequent frozen blastocyst replacement in natural cycles initiated within 1 year of each patient's start of treatment.
Main Outcome Measure(s): Ongoing pregnancy (primary end point) and live birth rates, as well as pharmacodynamic parameters.
Result(s): The ongoing pregnancy rate after a fresh cycle was 30% with hphMG versus 27% with rFSH for the per-protocol (PP) population and 29% versus 27% for the intention-to-treat (ITT) population. Noninferiority of hphMG compared to rFSH was established. Considering frozen cycles initiated within 1 year, the cumulative live birth rate for a single stimulation cycle was 40% and 38% for women treated with hphMG and rFSH, respectively (both PP and ITT). Significant differences in pharmacodynamic end points were found between the two gonadotropin preparations.
Conclusion(s): Highly purified hMG is at least as effective as rFSH in GnRH antagonist cycles with compulsory single-blastocyst transfer.
Clinical Trial Registration Number: NCT00884221. (Fertil Steril® 2012;97:561–71. ©2012 by American Society for Reproductive Medicine.)
Key Words: Highly purified menotropin, recombinant FSH, GnRH antagonist, single-blastocyst transfer

The long GnRH agonist and the fixed GnRH antagonist protocols with either menotropins or recombinant FSH (rFSH) preparations are the most widely used protocols for controlled ovarian stimulation (COS) for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). A differential follicular and endocrine response to stimulation with menotropins, containing FSH and LH activity, compared with rFSH preparations, containing only FSH activity, has been well characterized in patients undergoing the long GnRH agonist protocol (1–3). The availability of several comparative randomized controlled trials (RCTs) using the long GnRH agonist protocol has enabled establishing of the effect of the different profiles of these gonadotropins on treatment outcome. According to recent meta-analyses, the use of menotropins for COS provides higher live birth rates than rFSH in the long GnRH agonist protocol (4, 5).

On the other hand, the amount of comparative data between menotropins and rFSH preparations in GnRH antagonist cycles is limited to a single-center RCT allowing transfer of up to three cleavage-stage embryos (6). Further and larger comparative studies are required to establish if there is

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a differential response to ovarian stimulation and on treatment outcome between these preparations. Such comparative studies should incorporate some of the latest advancements and new technologies in the field of assisted reproductive technologies (ART) as well as the clinical policies that attempt to optimize efficacy and increase patient convenience while minimizing the risks. These include mild starting dose of gonadotropins and laboratory advancements in embryo culture, selection, and cryopreservation procedures, as well as single-embryo transfer policies in both fresh and frozen cycles. Blastocyst transfers have been associated with higher live birth rates compared with transfers of cleavage-stage embryos (7, 8), and single-embryo transfer is increasingly advocated as a way to reduce multiple pregnancies and their associated risks while maintaining an acceptable success rate after a fresh cycle and after cumulative cryopreserved cycles (9, 10). Regarding cryopreservation of blastocysts, vitrification has been reported to increase the post-thawing survival rate compared with slow freezing (11–13). Regarding luteal phase support, further studies on the consequences of using shorter duration of progesterone supplementation have been advocated (14), because prolongation into early pregnancy has been shown to not provide further beneficial effects on treatment outcome (15, 16). This has the additional benefit of increasing patient convenience, just as replacement of thawed blastocysts in a natural cycle is also associated with fewer pharmacologic interventions.

The present trial was designed to compare efficacy of menotropin and rFSH in GnRH antagonist cycles while taking these clinical policies and recent developments into consideration. It is the first large RCT comparing gonadotropin preparations in cycles where all patients underwent compulsory single-embryo transfer at the blastocyst stage.

MATERIALS AND METHODS

The Menopur in GnRH Antagonist Cycles with Single Embryo Transfer (MEGASET) study was a randomized, open-label, assessor-blind, parallel-groups, multicenter trial designed to document noninferiority regarding ongoing pregnancy rates of highly purified menotropin (hphMG) versus a marketed rFSH preparation used for COS following a GnRH antagonist protocol. It involved 25 centers in seven countries (Belgium, Czech Republic, Denmark, Poland, Spain, Sweden, and Turkey). The trial was initiated in September 2009 and the posttrial follow-up was completed in November 2011. The trial was assessor blinded, and all investigators, central assessors, laboratory personnel, and sponsor staff involved in analyzing and interpreting data were kept blinded to the treatment allocation throughout the trial. The trial was carried out in accordance with the declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulatory requirements. The trial protocol (no. FE 999906 CS08) was approved by both the local regulatory authorities and the independent ethics committees covering all participating centers. Written informed consent was provided by each patients before any trial-related examinations were initiated. The fresh stimulation cycle and the frozen-blastocyst replacement cycles initiated within 1 year after randomization were provided to the patients free of charge.

Trial Population

The main inclusion criteria were the following: women aged 21–34 years with a body mass index (BMI) of 18–25 kg/m^2; primary diagnosis of infertility being unexplained infertility or mild male factor; eligible for ICSI according to the investigator; infertile for $\geq$12 months before randomization; regular menstrual cycles of 24–35 days, presumed to be ovulatory; hysterosalpingography, hysteroscopy, or transvaginal ultrasound documenting a uterus consistent with expected normal function; transvaginal ultrasound documenting presence and adequate visualization of both ovaries without evidence of abnormality; the trial cycle being the first or second COS cycle ever or the first or second COS cycle after having achieved ongoing pregnancy in a previous COS cycle; early follicular-phase serum levels of FSH of 1–12 IU/L; and early follicular-phase total antral follicle (diameter 2–10 mm) count $\geq$10 for both ovaries combined. The main exclusion criteria were: women with polycystic ovaries or endometriosis stage I-IV; poor response in a previous COS cycle, defined as either >20 days of gonadotropin stimulation, cancellation due to limited follicular response, or development of fewer than four follicles $\geq$15 mm; severe ovarian hyperstimulation syndrome (OHSS) in a previous COS cycle; history of recurrent miscarriage; current or past (up to 12 months before randomization) abuse of alcohol or drugs; intake of more than 14 units of alcohol per week during the past month or smoking more than ten cigarettes per day within 3 months before randomization.

Treatment Regimen

The use of oral contraceptives for programming of the trial cycle was not allowed. On day 2–3 of the menstrual cycle, patients were randomized in a 1:1 ratio to treatment with either hphMG (Menopur; Ferring Pharmaceuticals) or rFSH (follitropin beta; Puregon; MSD). Randomization was performed with the use of a computer-generated randomization list stratified by center. The list was prepared by an independent statistician in the biometrics department at Ferring. Patients were randomized through the electronic case report form system which sequentially assigned patients to the lowest available number. Randomization was handled by a study nurse to maintain blinding of the investigators. The block size was concealed during the trial and after code break was revealed to be six.

The hphMG preparation was provided as a multidose vial with powder and two prefilled syringes with solvent. After reconstitution, each vial delivered 1,200 IU FSH activity and 1,200 IU LH activity at 600 IU/mL. The rFSH preparation was provided as pen and cartridge with 900 IU FSH activity/1.08 mL solution for injection.

The gonadotropin starting dose was fixed at 150 IU for the first 5 days. Follicular development was monitored by transvaginal ultrasound after 5 days of treatment and thereafter at least every 2 days. From stimulation day 6 onwards, dosing could be changed by 75 IU per adjustment and not more frequently than every 4 days. The GnRH antagonist (ganirelix acetate; Orgalutran; MSD) was initiated on stimulation day 6 at a daily dose of 0.25 mg and continued

throughout the gonadotropin treatment period. A single injection of 250 μg hCG (choriogonadotropin alpha; Ovitrelle; Merck Serono) was administered to induce final follicular maturation as soon as three follicles of ≥17 mm were observed, i.e., the day of reaching the hCG criterion or the next day. The target for ovarian stimulation was to obtain eight to ten oocytes. Oocyte retrieval took place 36 ± 2 hours after hCG administration. The cycle was canceled in case of fewer than three follicles with a diameter ≥12 mm on day 14 of stimulation or in case of more than 25 follicles with a diameter ≥10 mm. The maximum allowed gonadotropin dose was 375 IU daily, and patients could be treated with gonadotropin for a maximum of 20 days. Coasting was not allowed.

All oocytes retrieved were assessed for cumulus mass appearance and maturity stage. Metaphase II oocytes were inseminated using partner sperm by ICSI at 4 ± 1 hours after retrieval. All oocytes, embryos, and blastocysts were assessed daily by the local embryologists until 5 days after oocyte retrieval, unless they were discarded at an earlier time point. Fertilization was assessed at 19 ± 1 hours after insemination, and embryos with two pronuclei continued culture. Embryo quality was assessed at 26 ± 1, 44 ± 2, 68 ± 2, and 92 ± 2 hours after insemination. On day 5 (116 ± 2 and 120 ± 2 hours after insemination), the quality evaluations of blastocysts consisted of expansion and hatching status, inner cell mass grading, and trophectoderm grading (17). Blastocyst expansion and hatching status (grade 1–6), inner cell mass (grade A–C), and trophectoderm (grade A–C) were evaluated by using the definitions described by Gardner and Schoolcraft (17). On day 5 after oocyte retrieval, a single blastocyst of the best quality available was transfered and all remaining blastocysts were frozen by vitrification. Vaginal progesterone capsules (600 mg/d; Utrogestan; Seid) were provided for luteal phase support from the day after oocyte retrieval to the day of the serum β-hCG test (13–15 days after embryo transfer) or menses; prolonged luteal phase support beyond this time point was not allowed. Clinical pregnancy was confirmed by transvaginal ultrasound 5–6 weeks after embryo transfer, and ongoing pregnancy was confirmed by transvaginal ultrasound 10–11 weeks after embryo transfer.

Blood samples for analysis of circulating concentrations of endocrine parameters (FSH, LH, E_2, and P) were assessed throughout the stimulation period (i.e., until the day of reaching the hCG criterion). In addition, antimüllerian hormone (AMH) was analyzed in the samples on stimulation day 1 and inhibin B was analyzed in the samples on days 1 and 6. The serum samples were analyzed at a central laboratory (Laboratory for Clinical Research, Kiel, Germany). FSH, LH, E_2, and P were analyzed by electrochemiluminescence immunoassay (Roche Diagnostics). AMH and inhibin B were analyzed by enzyme-linked immunosorbent assay (Beckman Coulter). The assays used for FSH, LH, E_2, P, AMH, and inhibin B had a sensitivity of <0.10 IU/L, 0.10 IU/L, 18.4 pmol/L, 0.095 nmol/L, 0.08 ng/mL, and 2.6 pg/mL, respectively, and total imprecision (% coefficient of variation) of <4.5, <2.2, <13.0, <4.8, <7.7, and <6.82, respectively. Premature LH surge (LH concentration ≥10 IU/L accompanied by P concentration ≥3.18 nmol/L [1.0 ng/mL]) was also evaluated.

Patients with no ongoing pregnancy at the end of the trial period and with a surplus of blastocysts could undergo frozen-embryo replacement cycles with compulsory single-blastocyst transfer within 1 year after their date of randomization. Replacement of thawed reexpanded blastocysts occurred on day of LH peak + 7 in natural cycles, with the use of a urinary kit (Clearblue Ovulation Test, SPD Swiss Precision Diagnostics) to determine the LH peak. All patients with an ongoing pregnancy in the fresh cycle or the 1-year post-randomization frozen-blastocyst replacement cycles were followed until delivery and live birth data were collected.

Trial End Points

The primary end point was ongoing pregnancy rate, defined as presence of at least one intrauterine pregnancy with a viable fetus 10–11 weeks after embryo transfer in the fresh cycle. Secondary end points included positive β-hCG rate and clinical pregnancy rate (transvaginal ultrasound showing at least one intrauterine gestational sac with fetal heart beat 5–6 weeks after transfer), follicular development, endocrine profile, oocytes retrieved, fertilization rate, embryo quality, endometrial profile, and safety assessments. The incidence of treatment-emergent adverse events (onset after start of stimulation), OHSS, pregnancy loss, and patient self-assessed local tolerability were also investigated. Cases of OHSS were categorized according to Golan et al.'s classification system (18) and were defined as early OHSS if the onset was ≤9 days after hCG administration and late OHSS if the onset was >9 days after hCG administration. Apart from the criterion for cancellation of the cycle (more than 25 follicles ≥10 mm), measures to treat or prevent OHSS was according to local clinical practice. Early pregnancy loss was defined as a positive β-hCG test but no ongoing pregnancy at 10–11 weeks after embryo transfer, and late pregnancy loss was defined as a confirmed ongoing pregnancy but no live birth. Local tolerability parameters (redness, pain, itching, swelling, and bruising) were assessed by the patients 1 hour and 24 hours after the gonadotropin injections on stimulation days 1–5 and recorded in a diary supplied for that purpose. The intensity of each reaction was evaluated as either none, mild, moderate, or severe.

Sample Size and Statistical Analysis

The trial was powered to demonstrate noninferiority of hphMG versus rFSH regarding ongoing pregnancy rate in the fresh cycle, assuming a noninferiority margin of −10% (absolute). Thus, noninferiority could be claimed if the lower limit of the two-sided 95% confidence interval (CI) of the difference in ongoing pregnancy rates between hphMG and rFSH was above −10%. Assuming an ongoing pregnancy rate of 30% in both treatment groups (7), 660 patients were required to obtain 80% power of demonstrating noninferiority. With a sample size of 660 patients and an observed ongoing pregnancy rate in the rFSH group of 30%, the observed ongoing pregnancy rate in the hphMG group had to be at least 27% to claim noninferiority with a −10% margin. For robustness, noninferiority was to be documented for both the intention-to-treat (ITT) population, defined as all randomized

and exposed patients, and the per-protocol (PP) population, defined as all randomized and exposed patients except those excluded as a result of major protocol deviations. The screen failure rate and the rate of protocol deviations were assumed to be 5%–10%. Therefore approximately 800 screened patients were expected to lead to 720–760 randomized and exposed patients (ITT) and to 648–722 patients in the PP analysis set.

The 95% confidence intervals for the primary end point and the closely related secondary end points (positive β-hCG and clinical pregnancy) were based on the normal approximation. Categoric variables (e.g., echogenicity) were compared between treatment groups by using the chi-square test. Binary variables (e.g., endometrial triple layer structure) were evaluated by using the Fisher exact test. The endocrine parameters were compared on the log scale by using analysis of covariance models with treatment as factor and log-transformed baseline value as covariate. Exposure to gonadotropin, follicular development, endometrial thickness, oocytes retrieved, and embryo development were compared between treatment groups by using the Wilcoxon rank sum test. The impact of P at the end of stimulation and the number of oocytes retrieved on ongoing pregnancy were assessed by using a logistic regression model. All tests were two sided; P values of $<.05$ were considered to be significant, and P values in the range of $\geq.05$ to $<.1$ were considered to be indicative of a trend. No adjustments for multiplicity were applied. Data are presented as mean $\pm$ SD across patients unless otherwise specified.

RESULTS

Baseline

A total of 810 patients signed informed consents and were screened for eligibility, after which 749 patients were randomized and exposed to treatment: 374 to hphMG and 375 to rFSH (Fig. 1). Before the trial was unblinded, 73 patients were excluded from the ITT population owing to major deviations from the protocol, of which the most frequent were inclusion/exclusion criteria not met (4%), hCG administered outside criterion (1%), and incorrect timing of start of GnRH antagonist (1%). Thus, the PP population included 676 patients (343 for hphMG and 333 for rFSH). The two treatment groups were similar regarding demographics, fertility history, and baseline characteristics at the time of starting stimulation (Table 1).

Controlled Ovarian Stimulation

After 5 days of exposure to gonadotropins at a daily dose of 150 IU, there were significant differences in follicular development and endocrine profile between the two treatment groups. The serum FSH concentration was higher ($P<.001$) in the hphMG group than in the rFSH group, and more follicles ≥ 12 mm ($P=.011$) as well as higher levels of E_2 ($P=.003$) and inhibin B ($P<.001$) were detected on day 6 of stimulation in the rFSH group than in the hphMG group (Table 2). Furthermore, mean levels of P on stimulation day 6 were higher with rFSH ($P=.025$) and associated with a wider range, as indicated by the high SD (Table 2). The LH concentration on day 6 of stimulation was similar between groups. Regarding dose adjustments on day 6, the daily dose of gonadotropin was maintained at 150 IU on day 6 of stimulation in 67% and 73% of the patients receiving hphMG and rFSH, respectively, increased to 225 IU for 31% and 25%, and decreased to 75 IU in 1% and 2%.

At the end of stimulation, no significant differences between groups were noted in the number of follicles ≥ 17 mm or 15–16 mm, but significantly ($P=.024$) more follicles with a diameter of 12–14 mm were observed in the rFSH group (Table 2). The serum concentrations of FSH, LH, and E_2 at the end of stimulation were significantly ($P<.001$) higher in the hphMG group. Throughout the stimulation period, premature LH surge was observed in 6% of the patients in both treatment groups. The average serum P level and the proportion of patients with serum P concentrations above 4 nmol/L at the end of stimulation (16% in the hphMG group and 14% in the rFSH group) were similar between the treatment groups.

The vast majority of patients maintained the initial dose level throughout the entire stimulation period (58% in the hphMG group and 65% in the rFSH group) or only adjusted once (40% in the hphMG group and 34% in the rFSH group). The total amount of gonadotropin used was significantly ($P=.009$) higher with hphMG (Table 2), with an average difference of 80 IU, corresponding to 10 IU more per day than with rFSH.

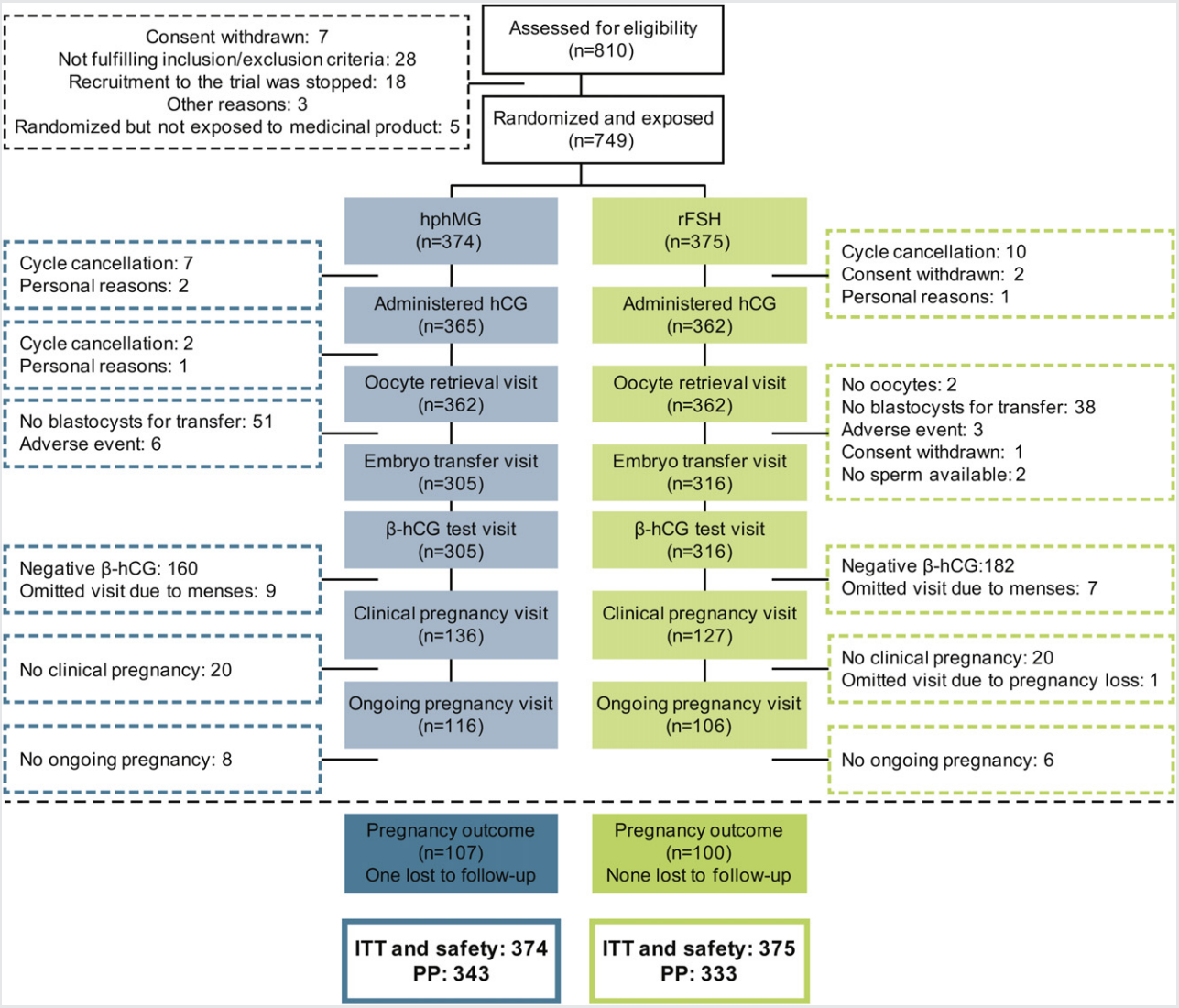
Oocyte Retrieval and Embryo Transfer

Oocyte retrieval was performed for 97% of the patients in both groups. Cancellations due to excessive response were reported for two patients in the rFSH group and no patients in the hphMG group, and due to poor response in seven patients in the hphMG group and six patients in the rFSH group. The number of oocytes retrieved was significantly higher ($P<.001$) in the rFSH group than in the hphMG group (Table 3). The proportion of patients with fewer oocytes than the prespecified target was 45% and 36% in the hphMG and rFSH groups, respectively, whereas 15% and 24%, respectively, had 15 or more oocytes retrieved.

There were no significant differences between groups in the percentage of metaphase II oocytes or fertilization rate. There were significantly ($P=.005$) more embryos on day 3 in the rFSH group, but the number of blastocysts on day 5 were not significantly different between groups. Early cleavage rate, embryo quality on day 3, and blastocyst quality on day 5 were similar in the hphMG and rFSH groups (Table 3). The treatment groups were also similar regarding the percentage of patients with blastocysts on day 5 (82% and 85% in the hphMG and rFSH groups, respectively) and patients with at least one surplus frozen blastocyst (55% and 57%, respectively).

Embryo transfer was performed for 82% of the patients in the hphMG group and 84% of the patients in the rFSH group. Patients underwent single-blastocyst transfer on day 5 after oocyte retrieval. Three of the transfers deviated from the protocol, involving one transfer of a morula on day 5 in the rFSH

FIGURE 1



Trial flow chart and disposition of patients by trial visit. ITT = intention-to-treat; PP = per-protocol.

Devroey. SBT outcome with hphMG and rFSH. Fertil Steril 2012.

group and two transfers of a cleavage-stage embryo on day 2 in each of the treatment groups. The main reasons for cancellation of embryo transfer were lack of a blastocyst for transfer (14% in the hphMG group and 10% in the rFSH group) and early OHSS (1% in both treatment groups). Patients with no blastocyst for transfer had significantly lower mean AMH ($P<.001$), lower mean inhibin B ($P<.001$), and higher mean basal FSH ($P<.001$) on day 1 of stimulation, as well as fewer oocytes at retrieval ($P<.001$) and fewer embryos on day 3 ($P<.001$). Of the patients with no blastocyst on day 5, only 13% had embryos of top quality and 38% had no embryos on day 3. Thus, embryo transfer on day 3 would maximally have led to 7% more transfers if all patients with embryos underwent transfer on day 3, or lower if a prespecified embryo quality criterion for transfer on day 3 had been applied. Difficult transfers were reported in 6% of the patients in both groups and eventualities were reported for 6% and 3% of the patients in the hphMG and rFSH groups, respectively. The majority of the transfers were ultrasound guided (72% in the hphMG group and 71% in the rFSH group) and done with soft catheters (95% and 94%, respectively). At the endometrial level, there were no significant differences in thickness, triple-layer structure, or echogenicity pattern between the two treatment groups at any time during stimulation or at the day of embryo transfer (Table 2).

Pregnancy and Live Birth in the Fresh Cycle

Information on pregnancy was obtained for all patients, with none lost to follow-up. The ongoing pregnancy rate per started cycle was 30% with hphMG and 27% with rFSH for the PP population (Fig. 2A) and 29% and 27%, respectively, for the ITT population (Fig. 2B). The treatment difference in ongoing pregnancy rates was 3.0% (95%

TABLE 1

Clinical profile of patients in the trial prior to start of stimulation.

	hphMG (n = 374)	rFSH (n = 375)
Clinical characteristics		
Age (y)	30.8 ± 2.8	30.4 ± 2.6
Weight (kg)	60.6 ± 6.8	59.9 ± 7.0
Body mass index (kg/m^2)	22.1 ± 1.9	21.9 ± 2.0
Waist:hip ratio	0.8 ± 0.08	0.8 ± 0.06
Primary infertility	80%	75%
Primary cause of infertility		
Unexplained infertility[a]	38%	40%
Mild male factor[b]	62%	60%
Duration of infertility (y)	3.2 ± 1.8	3.1 ± 1.7
First IVF/ICSI cycle	75%	75%
Previous IUI cycles	49%	52%
Previous IUI cycles with gonadotropins	28%	31%
Menstrual cycle length (d)	28.5 ± 1.7	28.7 ± 2.1
Day 1 (before start of stimulation)		
Mean ovarian volume (cm^3)	5.8 ± 2.8	5.9 ± 2.8
Antral follicles (≥2 mm)	15.6 ± 5.3	15.7 ± 5.8
Endometrial thickness (mm)	3 ± 2	3 ± 2
LH (IU/L)	6.2 ± 2.3	6.2 ± 2.1
FSH (IU/L)	7.5 ± 2.3	7.4 ± 2.4
E_2 (pmol/L)	180 ± 106	177 ± 100
P (nmol/L)	2.2 ± 1.1	2.2 ± 1.1
Inhibin B (ng/L)	87 ± 40	85 ± 35
AMH (pmol/L)	27 ± 19	27 ± 20

Note: Values are mean ± SD unless otherwise specified. AMH = antimüllerian hormone; ICSI = intracytoplasmic sperm injection; IUI = intrauterine insemination.

[a] Unexplained infertility was defined as follows: regular menstrual cycles of 24–35 days presumed to be ovulatory, patency of fallopian tubes documented by hysterosalpingography, hysterosalpingo-contrast sonography, or laparoscopy, and sperm sample with concentration of spermatozoa ≥20 million/mL, total volume ≥2 mL, and ≥50% motility.

[b] Mild male factor was defined as follows: sperm sample with total spermatozoa count ≥5 million, sperm with motility, and not fulfilling the sperm sample criteria established for unexplained infertility.

Devroey. SBT outcome with hphMG and rFSH. Fertil Steril 2012.

TABLE 2

Clinical parameters from stimulation phase to embryo transfer.

	hphMG (n = 374)	rFSH (n = 375)	*P* value
Day 6 of stimulation			
Follicles ≥12 mm	3.6 ± 2.8	4.2 ± 3.1	.011[a]
E_2 (pmol/L)	2,626 ± 1,405	2,973 ± 1,702	.003[b]
P (nmol/L)	2.2 ± 1.9	2.8 ± 10.8	.025[b]
LH (IU/L)	4.9 ± 5.0	5.5 ± 6.0	.558[b]
hCG (IU/L)	1.7 ± 0.6	–	–
FSH (IU/L)	14.1 ± 3.5	12.2 ± 3.1	<.001[b]
Inhibin B (ng/L)	604 ± 324	722 ± 424	<.001[b]
Endometrial thickness (mm)	8 ± 2	9 ± 2	.328[a]
Echogenicity (hypo/iso/hyper)	54%/38%/8%	54%/39%/7%	.960[c]
Endometrial triple layer structure	91%	91%	.899[d]
End of stimulation			
Follicles ≥12 mm	10.9 ± 4.7	11.8 ± 4.9	.025[a]
Follicles 12–14 mm	3.3 ± 2.6	3.8 ± 2.9	.024[a]
Follicles 15–16 mm	2.7 ± 2.2	2.7 ± 2.3	.728[a]
Follicles ≥17 mm	4.9 ± 2.2	5.2 ± 2.4	.285[a]
E_2 (pmol/L)	8,797 ± 6,030	7,022 ± 4,945	<.001[b]
P (nmol/L)	3.1 ± 3.4	3.1 ± 3.3	.630[b]
LH (IU/L)	2.8 ± 2.8	2.1 ± 1.6	<.001[b]
hCG (IU/L)	2.1 ± 0.8	–	–
FSH (IU/L)	15.7 ± 4.1	12.6 ± 3.7	<.001[b]
Endometrial thickness (mm)	11 ± 2	11 ± 2	.883[a]
Echogenicity (hypo/iso/hyper)	38%/52%/10%	39%/52%/9%	.857[c]
Endometrial triple layer structure	98%	98%	.603[d]
Total dose of gonadotropin (IU)	1,433 ± 371	1,353 ± 296	.009[a]
Total duration of stimulation (d)	8.8 ± 1.6	8.5 ± 1.3	.077[a]
Day of blastocyst transfer			
Endometrial thickness (mm)	11 ± 2	11 ± 2	.786[a]
Echogenicity (hypo/iso/hyper)	6%/18%/76%	6%/18%/76%	.945[c]
Endometrial triple layer structure	53%	54%	.873[d]

Note: Numbers are mean ± SD unless otherwise indicated.

[a] Wilcoxon test.

[b] Test for treatment difference based on log-transformed values.

[c] χ^2 test.

[d] Fisher exact test.

Devroey. SBT outcome with hphMG and rFSH. Fertil Steril 2012.

CI −3.8 to 9.8) and 2.2% (95% CI −4.2 to 8.6) for the PP and ITT populations, respectively, both in favor of hphMG. The lower limit of the 95% CI for the difference in ongoing pregnancy rate was well above the preestablished noninferiority margin of −10% for both the PP and the ITT populations. Furthermore, the direction of the findings was similar for ongoing pregnancy rates among patients with transfer: 36% for hphMG and 32% for rFSH in the PP population (Fig. 2C) and 35% for hphMG and 32% for rFSH in the ITT population (Fig. 2D).

Information on pregnancy outcome was obtained for all patients with an ongoing pregnancy, with no patients or fetuses lost to follow-up. Late pregnancy loss occurred for two patients in the hphMG group and four patients in the rFSH group, all during the second trimester. All deliveries resulted in live-born neonates, and there were no stillbirths. The live birth rate after the fresh cycle was 29% with hphMG and 26% with rFSH for the PP population and 28% and 26%, respectively, for the ITT population.

Frozen-Blastocyst Replacement Cycles

A total of 116 patients (31%) in the hphMG group and 122 patients (33%) in the rFSH group had blastocysts thawed, of whom 107 (29%) and 115 (31%), respectively, had blastocyst transfer in a frozen cycle. Thus, of the patients who had blastocysts thawed, 8% in the hphMG group and 6% in the rFSH group did not undergo transfer. Patients underwent up to 6 transfer cycles, with an average of 1.6 ± 0.9 transfer cycles in the hphMG group and 1.6 ± 1.0 in the rFSH group, using a total of 228 and 243 blastocysts, respectively. Information on ongoing pregnancy and pregnancy outcome was obtained for all frozen blastocyst replacement cycles, and no patients or fetuses were lost to follow-up. Considering the fresh and frozen cycles initiated within 1 year from start of treatment, the cumulative ongoing pregnancy rate per patient was 41% for hphMG and 39% for rFSH for the PP population and 40% and 39%, respectively, for the ITT population. The cumulative live birth rate per patient for a single stimulation cycle with single-blastocyst transfer in

TABLE 3

Oocyte retrieval and embryo development data.

	hphMG (n = 374)	rFSH (n = 375)	*P* value[a]
Day of oocyte retrieval			
No. of oocytes retrieved	9.1 ± 5.2	10.7 ± 5.8	< .001
Metaphase II oocytes/ oocytes retrieved	77 ± 23%	78 ± 19%	.798
Day 1			
Fertilization rate (2 PN/metaphase II)	75 ± 23%	76 ± 22%	.969
2 cells at 26 h/2PN	30 ± 32%	32 ± 33%	.303
Day 3			
No. of embryos available	4.0 ± 3.0	4.8 ± 3.7	.005
Top-quality embryos/2PN	31 ± 30%	31 ± 28%	.546
Day 5			
No. of blastocysts	2.7 ± 2.5	3.1 ± 3.0	.125
Blastocyst 1/2PN	7 ± 17%	7 ± 16%	.406
Blastocyst 2/2PN	7 ± 15%	5 ± 11%	.232
Blastocyst 3/2PN	10 ± 16%	10 ± 15%	.412
Blastocyst 4/2PN	18 ± 22%	20 ± 26%	.438
Blastocyst 5/2PN	7 ± 16%	6 ± 13%	.390
Blastocyst 6/2PN	0	0	–
Blastocyst 4AA/2PN	5 ± 13%	5 ± 14%	.958
Blastocyst 5AA/2PN	2 ± 7%	2 ± 7%	.954

Note: Numbers are mean ± SD. 2PN = 2 pronuclei. Blastocyst expansion and hatching status: 1 = early blastocyst, blastocele volume less than one-half of that of the embryo; 2 = blastocyst with blastocele volume at least one-half of that of the embryo; 3 = blastocyst with a blastocele completely filling the embryo; 4 = expanded blastocyst with a blastocele volume larger than that of the early embryo, with a thinning zona; 5 = hatching blastocyst with the trophectoderm starting to herniate through the zona; 6 = hatched blastocyst, in which the blastocyst has completely escaped from the zona; AA = inner cell mass A (tightly packed, many cells) and trophectoderm A (many cells forming a cohesive epithelium).

[a] Wilcoxon test.

Devroey. SBT outcome with hphMG and rFSH. Fertil Steril 2012.

both the fresh and the frozen cycle was 40% after stimulation with hphMG and 38% after stimulation with rFSH for both PP and ITT populations.

Safety

The overall incidence of treatment-emergent adverse events during the fresh cycle was similar for the two treatments, with 39% of the patients in the hphMG group and 37% of the patients in the rFSH group reporting at least one adverse event. The most frequently reported adverse events were as follows in the hphMG and rFSH groups, respectively: abortion (10% and 9%), headache (7% and 5%), vaginal hemorrhage (no events reported during the stimulation period; 6% and 3%), pelvic pain (5% and 4%), and nausea (5% and 4%).

OHSS was experienced by 3% (10 patients) in each treatment group. Early-onset OHSS was reported in 2% of the women in both the hphMG group (seven patients) and the rFSH group (eight patients) being of moderate/severe grade for 1.6% (six patients) in each treatment group. The following interventions done because of excessive ovarian response were noted during the trial: cycle cancellation due to excessive response, paracentesis, and albumin administration. The percentage of patients with interventions associated with excessive response or to prevent early OHSS was significantly higher ($P=.025$) in the rFSH group than in the hphMG group. These interventions covered cycle cancellation due to excessive response (no patients with hphMG, two patients with rFSH), paracentesis (five patients with hphMG, nine patients with rFSH), and intravenous albumin administration (seven patients with hphMG, 17 patients with rFSH). Late OHSS was experienced by 1% (two cases of moderate and two of severe intensity) in the hphMG group and 0.5% (two cases of mild intensity) in the rFSH group. One patient in the hphMG group experienced both early and late OHSS. All moderate/severe late OHSS were reported in patients who eventually had an ongoing pregnancy.

At least one injection site reaction was reported by 48% of the patients in the hphMG group and 53% in the rFSH group, but only 5% and 6%, respectively, had reactions of moderate intensity and only 0.3% and 1%, respectively, had reactions of severe intensity. Moderate/severe injection site reactions after 1 hour or 24 hours were reported as follows for the hphMG and rFSH groups, respectively: bruising (2% and 4%), itching (0.3% and 0.5%), pain (3% and 2%), redness (0.5% and 0.8%), and swelling (0.5% and 0.3%).

Regarding pregnancy outcome, four monochorionic diamniotic twins were born in the fresh cycle after stimulation with hphMG, and there were no twins in the rFSH group. Congenital malformations were reported for one fetus in the hphMG group (a congenital bladder anomaly leading to termination) and for two fetuses and three neonates in the rFSH group (a single umbilical artery and a trisomy 21 leading to elective terminations, cleft palate, polydactyly, and congenital cerebral cyst). In the frozen cycles with replacement of a single blastocyst after vitrification and thawing, no monozygotic twins were observed, and congenital malformations were reported for three neonates in the hphMG group (polydactyly, hypospadia, and atrial septal defect) and for none in the rFSH group.

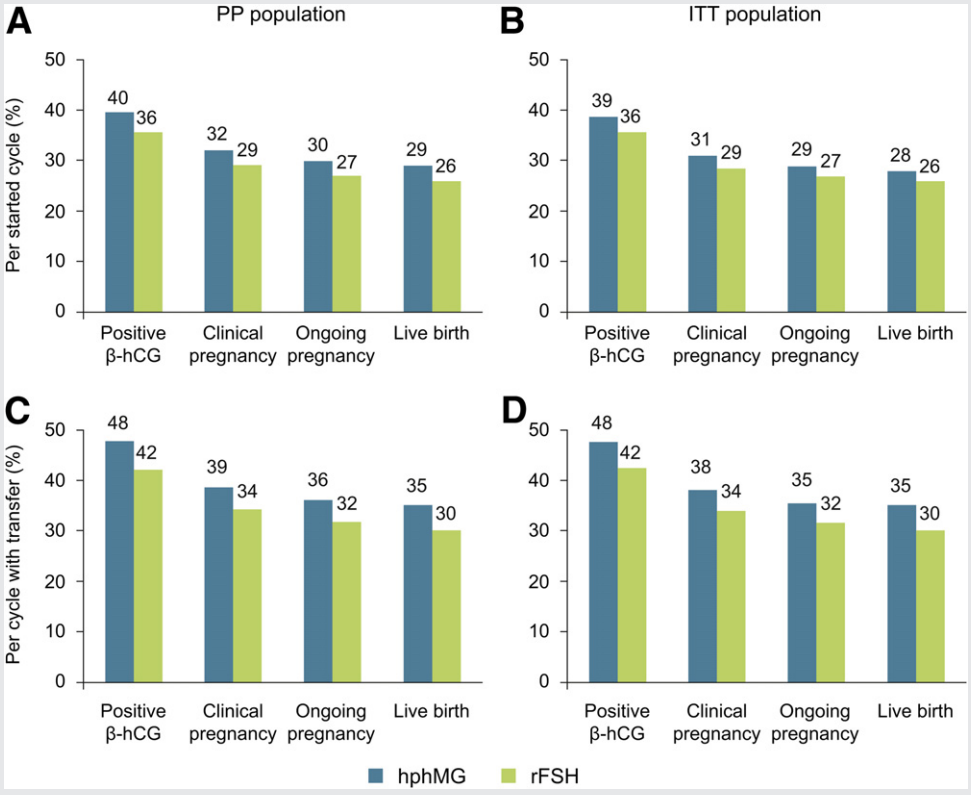
Exploratory Evaluation of Ongoing Pregnancy by Progesterone at End of Stimulation

Additional analyses of the ongoing pregnancy rates were performed according to P levels at the end of stimulation, including in combination with the ovarian response. Overall, there was a significant ($P=.011$) decrease in ongoing pregnancy rate with increased P levels at the end of stimulation. In the rFSH group, patients with an end-of-stimulation P level >4 nmol/L had a significantly ($P=.049$) lower ongoing pregnancy rate compared with those with P levels ≤4 nmol/L: 16% versus 29%, respectively. In contrast, the ongoing pregnancy rate in the hphMG group was 30% among patients with end-of-stimulation P levels >4 nmol/L, which was similar to that of 29% for those with P levels ≤4 nmol/L. A reduction in ongoing pregnancy rate similar to that in rFSH patients with P levels >4 nmol/L was observed in the hphMG group only when the P levels were >7 nmol/L. In general, the ongoing pregnancy rate was positively affected by the number of oocytes retrieved but negatively affected by the level of P at the end of stimulation (Fig. 3). However, in the rFSH group, the ongoing pregnancy rate for patients with P ≤4 nmol/L reached a plateau already in the 8–14 oocytes category.

DISCUSSION

The present trial provides evidence that hphMG is at least as effective as rFSH in GnRH antagonist cycles, and the

FIGURE 2



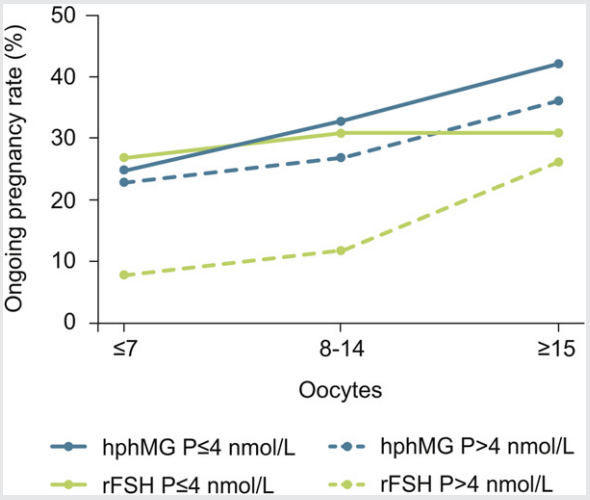
Pregnancy and live birth data for the fresh cycle per started cycle for (A) the per-protocol (PP) population and (B) the intention-to-treat (ITT) population. Pregnancy and live birth data for the fresh cycle per cycle with transfer for (C) the PP population and (D) the ITT population.

Devroey. SBT outcome with hphMG and rFSH. Fertil Steril 2012.

robustness of the conclusion is supported by the consistency of findings across all of the populations analyzed. All point estimates go in the same direction, and the live birth rates after the fresh cycle support the main conclusions based on the predefined end point of ongoing pregnancy rates. The findings are in line with the earlier trial comparing menotropins and rFSH in GnRH antagonist cycles (6). The pregnancy rates obtained in the present trial should be viewed in the context of a multicenter trial with a nonindividualized stimulation protocol, taking into consideration the number of oocytes retrieved and that single-blastocyst transfer was not elective but compulsory in all patients, regardless of their ovarian response and the embryo development or quality during the initial days of culture. The ongoing pregnancy rate in the rFSH group was in line with other controlled trials with patients, or a subgroup of patients, undergoing COS with rFSH in a GnRH antagonist cycle with single-embryo transfer (7, 19). Higher ongoing pregnancy rates per cycle with transfer could be expected when single-blastocyst transfer is elective (20) or when the decision of single-embryo or single-blastocyst transfer is made after stimulation, such as at oocyte retrieval or day 3. The compulsory culture to blastocyst stage in the present trial affected the number of cycles canceled due to lack of available blastocysts for transfer, and it is estimated that maximally 7% more patients in each of the hphMG and rFSH groups could have undergone transfer if transfer of embryos (without a prespecified quality criterion) had been allowed on day 3. It is unlikely that a cleavage-stage transfer would had increased the overall ongoing pregnancy rate in the present study, because blastocyst transfers have been associated with higher live birth rates compared with transfers of cleavage-stage embryos (7, 8).

As documented for the long GnRH agonist protocol (1, 2), the present trial using the GnRH antagonist protocol found pharmacodynamic differences between hphMG and rFSH in follicular development and endocrine profile. Already by day 6 of stimulation, more follicles ≥ 12 mm and higher inhibin B, E_2, and P levels were observed with rFSH compared with hphMG, pointing toward a more pronounced initial effect on follicular growth and steroidogenesis. By the end of stimulation, the follicular response still differed between the two preparations, but the difference was limited to around one additional follicle in the size group 12–14 mm, with no difference in larger-size follicles. Despite the slightly lower follicle number with hphMG, E_2 levels were substantially higher after treatment with hphMG compared with rFSH. This finding has already been reported in both long GnRH agonist and GnRH antagonist cycles (1, 6) and is attributed to the continuous exposure to the LH activity in hphMG, which induces higher levels of aromatizable

FIGURE 3



Ongoing pregnancy rate in the fresh cycle by oocytes retrieved according to treatment group and end-of-stimulation P levels ≤4 nmol/L and >4 nmol/L (intention-to-treat population).

Devroey. SBT outcome with hphMG and rFSH. Fertil Steril 2012.

androgens leading to higher E_2 concentrations in the second half of the follicular phase (2). Alternatively, the difference in E_2 levels could be explained by different elimination kinetics of the FSH isoforms in the two gonadotropin preparations. Despite the fact that the serum LH concentration at the end of stimulation was significantly higher in the hphMG group than in rFSH group, the magnitude of the difference appeared to be clinically irrelevant and not associated with P elevations, and no difference was observed in the frequency of premature LH surges between the groups.

Elevation of P at the end of stimulation has been associated with clinical consequences (21), possibly by a potential premature advancement of the endometrium leading to a consequent mismatch in the embryo-endometrium dialog (22, 23) if transfers are made at cleavage stage but not at blastocyst stage (24, 25). In the present trial, the mean P concentration at the end of stimulation was similar for hphMG compared with rFSH, and P concentrations >4 nmol/L were observed in a similar proportion of patients in both treatment groups. It has previously been reported that a P increase at the end of stimulation in both long GnRH agonist and GnRH antagonist protocols is mainly related to FSH activity rather than to LH activity (1, 6, 26) and related to FSH exposure as well (21, 26). The lower starting dose of 150 IU/d in the present trial may partly explain the lower incidence of patients with P elevations at the end of stimulation with rFSH compared with earlier studies (1, 27). To illustrate the differences in exposure, the total gonadotropin consumption in the present trial was ~1,400 IU, which is close to one-half of the 2,400–2,600 IU/d reported in the earlier trials where higher starting doses were used (1, 6, 27).

The data suggest that the ongoing pregnancy rate for day 5 blastocyst transfers is affected by the elevations of P levels in both treatment groups, but that the threshold for impact appears to be lower in the rFSH group than in the hphMG group. The outcome in the hphMG patients appears to be less influenced by elevations of P, and higher levels are required to observe a detrimental effect in pregnancy rates. The effect of P elevations is observable across oocyte yield. Nevertheless, patients with high oocyte yield (i.e., 15 or more oocytes) after stimulation with rFSH but with P ≤4 nmol/L had a lower ongoing pregnancy rate than could be expected. The role of the endocrine environment and/or other factors during the stimulation affecting the pregnancy rate in this subgroup of patients requires further exploration.

Given the fairly young population included in the trial, cycle cancellations for poor or excessive response was limited to <3% in both groups. On average, 1.5 more oocytes were recovered in the rFSH group than in the hphMG group, despite similar mean AMH levels at the start of stimulation. Fewer oocytes retrieved after stimulation with hphMG than with rFSH have also been reported in studies following the long GnRH agonist protocol (1) and the antagonist protocol (6) and could be related either to differences between gonadotropins in the FSH component or to the presence of LH activity in the hphMG preparation. The higher number of oocytes retrieved in the rFSH group did not lead to higher pregnancy and live birth rates in the fresh cycle nor higher cumulative rates after 1 year of frozen replacement cycles compared with the hphMG group. Actually, subgroup evaluations suggested an apparent negative impact on pregnancy rates, with larger oocyte yield, in fresh cycles in rFSH-exposed patients, a finding supported by a large observational study (28). Regarding early OHSS, the overall incidence was similar between groups. However, the observed incidence of early OHSS is affected by preventive interventions to minimize or eliminate the risk of this side effect of COH (i.e., cycle cancellation, paracentesis, and albumin administration). Because more patients in the rFSH group had excessive ovarian response, it is not surprising that significantly more preventive interventions were reported in that group, although the overall incidence in the trial was driven by policies at individual clinics.

Regarding embryo development, it should be noted that besides the culture media having to be commercially available, this aspect was not further standardized. In contrast, the method of fertilization was standardized to ICSI, with homogeneous assessment time points relative to insemination for all oocytes, thereby enabling some evaluation of comparative embryo quality. Several reports have suggested that LH activity could influence embryo quality in patients undergoing COS for IVF/ICSI (3, 29). Higher embryo quality based on morphologic parameters, as observed with hphMG in an IVF cycle using a GnRH agonist protocol and starting doses of 225 IU (1), was not observed in this trial. The lower starting dose of hphMG of 150 IU in the present trial (therefore a lower LH activity exposure), the use of the GnRH antagonist protocol, or the ICSI procedure may have conditioned these observations.

Both preparations were well tolerated as well as associated with good general and local tolerability. Pregnancy losses, positive β-hCG, ongoing pregnancy, and live birth rates were in line with what has been observed in the long GnRH agonist protocol with progesterone support up to clinical

pregnancy (1). This supports the notion that extension of P into early pregnancy may not have a clinical effect in an unselected population and may represent unnecessary drug exposure (15, 16). All cases of monozygotic twinning were reported in the fresh cycle and clustered in the hphMG group. The overall frequency of monozygotic twinning in the trial was 2%, which is in line with the ~2% incidence rate reported in other individual IVF/ICSI studies with blastocyst transfer and in a meta-analysis (30–33). The monozygotic twinning rate in the hphMG group was 4%, with the 95% confidence interval including the incidence rates reported in the literature.

In conclusion, treatment with hphMG is at least as effective in achieving pregnancy as treatment with rFSH when used for COS in a GnRH antagonist cycle. The present trial provides further evidence that these gonadotropin preparations are associated with differential follicular response and serum endocrine profile during the stimulation phase. Cryopreserved cycles initiated over a 1-year period contribute to increase the cumulative live birth rates, which are in line with the findings from fresh cycles.

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