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Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial

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*Menotrophin versus Recombinant FSH *in vitro* Fertilisation Trial

BACKGROUND: LH activity may influence treatment response and outcome in IVF cycles. METHODS: A randomized, assessor-blind, multinational trial compared ongoing pregnancy rates (primary end-point) in 731 women undergoing IVF after stimulation with highly purified menotrophin (HP-hMG) (n = 363) or recombinant FSH (rFSH) (n = 368) following a long GnRH agonist protocol. Patients received identical pre- and post-randomization interventions. One or two embryos were transferred on day 3. RESULTS: More oocytes were retrieved ($P < 0.001$) after rFSH treatment (11.8) compared with HP-hMG treatment (10.0), but a higher proportion developed into top-quality embryos ($P = 0.044$) with HP-hMG (11.3%) than with rFSH (9.0%). At the end of stimulation, lower estradiol (E_2) ($P = 0.031$) and higher progesterone ($P < 0.001$) levels were found with rFSH, even after adjusting for follicular response. The distribution of hypo-, iso- and hyper-echogenic endometrium showed a significant ($P = 0.023$) shift towards the hyperechogenic pattern after rFSH treatment. The ongoing pregnancy rate per cycle was 27% with HP-hMG and 22% with rFSH [odds ratio (95% confidence interval): 1.25 (0.89–1.75)]. CONCLUSION: Superiority of HP-hMG over rFSH in ongoing pregnancy rate could not be concluded from this study, but non-inferiority was established. Pharmacodynamic differences in follicular development, oocyte/embryo quality, endocrine response and endometrial echogenicity exist between HP-hMG and rFSH preparations, which may be relevant for treatment outcome.

Key words: embryo quality/highly purified menotrophin/IVF/pregnancy/recombinant FSH

Introduction

The impact of different gonadotrophin preparations used in ovarian stimulation, such as menotrophins and recombinant FSH (rFSH) preparations, on treatment outcome in women undergoing controlled ovarian hyperstimulation for assisted reproduction technologies (ARTs) has been widely debated. A recent meta-analysis evaluating the outcome of truly randomized controlled trials (van Wely *et al*., 2003) found a borderline significant difference of a 5% higher clinical pregnancy rate in women stimulated with menotrophins (27%) compared with rFSH (22%). No separate analysis was performed for IVF and ICSI cycles, but the difference in pregnancy rates is primarily found in IVF cycles (Westergaard *et al*., 2001; Platteau *et al*., 2004). The meta-analysis concluded that additional large randomized trials were needed to precisely estimate any difference between menotrophins and rFSH (van Wely *et al*., 2003).

Not just more, but also more stringent trials should be conducted, and these should avoid the major deficiencies of previous efficacy trials in ART (Daya, 2003; Vail and Gardener, 2003; Arce *et al*., 2005). The large comparative trials between menotrophins and rFSH have included both IVF and ICSI cycles (Westergaard *et al*., 2001; The European and Israeli Study Group (EISG) on highly purified hMG versus rFSH, 2002), which may reflect two somewhat distinct populations, and this may therefore not constitute an optimal approach from neither a methodological nor a clinical point of view. Most of the studies conducted so far have not accounted for several potential sources of variability among the participating centres in pre- and post-randomization procedures. Among the studies

comparing menotrophins versus rFSH preparations, differences among patients in type and dose of GnRH agonist and hCG preparations, stimulation goal and adjustment policy, criterion and timing for hCG administration, number of embryos transferred, day of embryo transfer and type, dose and duration of luteal support could affect the estimate of the difference between the interventions applied (Arce *et al*., 2005).

Furthermore, there is a need for a better understanding of the differential effects of different gonadotrophin preparations and characterization of the impact of administration of LH activity during controlled ovarian hyperstimulation in down-regulated cycles on follicular dynamics, endocrine response, endometrial development and embryo quality. Only small studies have attempted to evaluate the pharmacodynamic differences associated with stimulation with or without LH activity (Filicori and Cognigni, 2001). No large study has been adequately designed to characterize the role of LH activity during ovarian stimulation and its impact on pharmacodynamic and treatment outcome endpoints. We report the results of a stringent, comprehensive, assessor-blind comparative study designed to evaluate whether a major difference in ongoing pregnancy rate (i.e. corresponding to an absolute difference of 10% between 22 and 32%) could be demonstrated between menotrophins and rFSH in a single trial conducted in IVF cycles. Documentation of the pharmacodynamic differences between gonadotrophin preparations with and without LH activity should help clinicians to optimize treatment outcome.

Materials and methods

Study population

Seven hundred and thirty-one patients with indication for IVF were randomized at 37 fertility clinics in 10 countries (eight in Belgium, three in France, three in Finland, three in Czech Republic, three in Poland, four in Denmark, three in Sweden, five in Israel, two in Slovenia and three in Spain) to controlled ovarian hyperstimulation with highly purified menotrophin (HP-hMG) ($n = 363$) or rFSH ($n = 368$). The study was carried out in accordance with the Declaration of Helsinki on good clinical practice, and ethical committee approval was obtained in all participating centres. The study was conducted from February 2004 to December 2004, followed by a follow-up period covering collection of pregnancy outcome. No protocol amendments were issued during the study.

The inclusion criteria were (i) women with good physical and mental health, aged 21–37 years with regular menstrual cycles of 21–35 days and presumed to be ovulatory; (ii) tubal or unexplained infertility, including endometriosis stage I/II and mild male factor, eligible for IVF treatment; (iii) infertility for ≥1 year before randomization, except for proven bilateral tubal infertility; (iv) BMI of 18–29 kg/m^2 at the time of randomization; (v) hysterosalpingography, hysteroscopy or transvaginal ultrasound documenting a uterus consistent with expected normal function (e.g. no clinically interfering uterine fibroids) within 3 years before randomization; (vi) transvaginal ultrasound documenting the presence of both ovaries, without evidence of abnormality (e.g. no endometrioma) and normal adnexa (e.g. no hydrosalpinx) within 6 months before randomization; (vii) early follicular phase serum FSH levels of 1–12 IU/l; (viii) willing to accept transfer of one or two embryos; (ix) male partner with sperm quality compatible with fertilization via IVF procedure (results obtained within 12 months before randomization) or previous clinical pregnancy; (x) confirmation of down-regulation before randomization, defined as either menstrual bleeding and transvaginal ultrasound showing a shedded endometrium with a thickness of <5 mm and no ovarian cysts or serum estradiol (E_2) levels of <50 pg/ml (local laboratory) and transvaginal ultrasound showing no ovarian cysts and (xi) signed informed consent form before screening. The exclusion criteria included (i) polycystic ovarian syndrome, endometriosis stage III/IV or severe male factor requiring ICSI; (ii) more than three previously consecutive unsuccessful IVF cycles; (iii) previous poor response in an IVF cycle, defined as >20 days of gonadotrophin stimulation, cancellation due to limited follicular response or less than four follicles of ≥15 mm diameter; (iv) previous IVF cycle with unsuccessful fertilization, defined as fertilization of ≤30% of the retrieved oocytes; (v) history of recurrent miscarriage; (vi) severe ovarian hyperstimulation syndrome (OHSS) in a previous IVF cycle; (vii) any significant systemic disease, endocrine or metabolic abnormalities (pituitary, thyroid, adrenal, pancreas, liver or kidney); (viii) use of any non-registered investigational drug during the 3 months before screening or previous participation in the study and any concomitant medication that would interfere with the evaluation of the study medication (non-study hormonal therapy, except thyroid medication, anti-psychotics, anxiolytics, hypnotics, sedatives and need for continuous use of prostaglandin inhibitors); (ix) treatment with clomiphene citrate, metformin, gonadotrophins or GnRH analogues within 1 month before randomization; (x) pregnancy, lactation or contraindication to pregnancy; (xi) current or past (3 months) smoking habit of >10 cigarettes per day; (xii) current or past (last 12 months) abuse of alcohol or drugs; (xiii) a history of chemotherapy (except for gestational conditions) or radiotherapy; (xiv) undiagnosed vaginal bleeding; (xv) tumours of the ovary, breast, adrenal gland, pituitary or hypothalamus and malformation of sexual organs incompatible with pregnancy and (xvi) hypersensitivity to any trial product.

Study design

This was a randomized, open-label, assessor-blind, parallel-group, multicentre, multinational study comparing HP-hMG (MENOPUR, Ferring Pharmaceuticals A/S, Copenhagen, Denmark) and rFSH (follitropin alfa, GONAL-F, Serono, Geneva, Switzerland). Eligible patients were randomized 1:1 to HP-hMG or rFSH, based on a computer-generated randomization list prepared by an independent statistician not involved in the study. Randomization was stratified by age (<35 years, 35–37 years) in each centre. Sealed envelopes were used to conceal the treatment allocation until randomization. The randomization envelopes were sequentially numbered. Randomization took place after confirmation of down-regulation and immediately before start of gonadotrophin treatment in order to reduce post-randomization withdrawals. The block size was not disclosed during the study. All investigators, embryologists, laboratory personnel and sponsor staff, including the statistician responsible of the statistical analysis, were blinded to treatment allocation throughout the study. All handling of study medication, at site and during interaction with the patients, was done by the study nurses, and precaution was taken to ensure that the treatment assignments were not available to the investigators. Patients were instructed (in writing in the patient information document and orally by the study nurse) not to discuss their drug assignment with the investigator. All information regarding treatment assignments was kept in a locked cupboard that was not accessible by the investigator. All randomization envelopes as well as all emergency code envelopes available at each clinic and at Ferring Pharmaceuticals were inspected and accounted for before breaking of the blind.

The study was initiated based on the results seen in the EISG (Platteau *et al*., 2004), incorporating recent methodological recommendations for efficacy trials in ART (Daya, 2003; Arce *et al*., 2005). All patients in all centres and countries received identical type and dose of concomitant fertility treatments, i.e. GnRH agonist for

down-regulation, hCG for triggering final maturation and progesterone for luteal support. All drugs were purchased centrally and imported to the countries participating in the trials, ensuring standardized origin and formulation. Pituitary down-regulation was done using triptorelin acetate, 0.1 mg/day s.c. (DECAPEPTYL, Ferring Pharmaceuticals A/S) started 5–7 days before estimated start of next menses and continued until end of gonadotrophin administration. GnRH agonist was administered 10–28 days before start of gonadotrophin treatment. Gonadotrophins (i.e. HP-hMG or rFSH) were administered s.c. and the injections could be done by the patient, her partner or a nurse. The starting dose of HP-hMG or rFSH was 225 IU s.c. for the first 5 days, followed by individual adjustments according to the patient's follicular response. Both gonadotrophin preparations were provided in packages of 75 IU. Doses >225 IU were administered by two injections, each with gonadotrophin dissolved in 1-ml solvent. The starting dose was within the labelling recommendations for rFSH and HP-hMG and identical to that used by the European and Israeli Study Group on highly purified hMG versus rFSH (2002). Follicular development was monitored by ultrasound at least every 2 days after the first 5 days. The dose could be changed by 75 IU per adjustment and not more frequent than every 4 days. The target for the ovarian stimulation was set to be 7–15 oocytes at retrieval, as this would yield a sufficient number of embryos of adequate quality for transfer without a major increase in the risk of OHSS (Arce *et al*., 2005). The maximum allowed dose was 450 IU daily and patients were treated with gonadotrophin for a maximum of 20 days. Choriongonadotropin alfa, 250 μg s.c., (OVITRELLE, Serono) was administered to induce final follicular maturation within 1 day of observing three or more follicles of ≥17 mm diameter. Oocyte retrieval took place 36 h (±2 h) after hCG administration. Follicular fluid from one or two follicles of ≥17 mm diameter was collected at oocyte retrieval. Blood sampling for endocrine parameters (FSH, LH, hCG, E_2, progesterone, androstenedione, total testosterone and sex hormone-binding globulin) and assessment of the endometrium (endometrial thickness, triple-layer pattern and echogenicity) were done on day 1, day 6, last stimulation day and day of oocyte retrieval. A complete presentation of the collection and analytical aspects as well as discussion of the endocrine data in serum and follicular fluid will be reported separately. All oocytes were followed individually from retrieval to transfer/freezing on day 3 after retrieval or until they were disregarded. Assessments of cumulus mass appearance were done at oocyte retrieval followed by insemination via IVF procedures at 3 h (±1 h) after oocyte retrieval. Fertilization and embryo quality were assessed by the local embryologists using the inverted microscope at 20 h (±1 h), 28 h (±1 h), 44 h (±1 h) and 68 h (±1 h) after oocyte retrieval. The embryo quality evaluation consisted of assessment of cell number and five parameters of embryo morphology: degree of fragmentation, localization of fragments, blastomere uniformity, multinucleation and cytoplasmic appearance. At 28 h (±1 h), 44 h (±1 h) and 68 h (±1 h), the local embryologists took a representative picture of the embryos for subsequent evaluation by a panel of central embryologists. A top-quality embryo was defined as four to five cells on day 2, seven or more cells on day 3, equally sized blastomeres and ≤20% fragmentation on day 3 and no multinucleation. The data presented in this manuscript are based on the evaluation by the local embryologist. Transfer of one or two embryos of minimum quality, defined as four or more cells with no cleavage arrest (i.e. cleavage must have occurred within the last 24 h) and ≤20% fragmentation, was done on day 3 after oocyte retrieval. Vaginal progesterone gel 90 mg/day 8% (CRINONE, Serono) for luteal support was given from the day of embryo transfer till confirmation of clinical pregnancy (5–6 weeks after embryo transfer) or negative serum βhCG test (13–15 days after embryo transfer). Ongoing pregnancy was determined 10–11 weeks after embryo transfer. All pregnancies were followed up to delivery. In addition, frozen embryos derived from the study are currently followed until 3 years after study completion.

Study end-points

The primary end-point of the study was ongoing pregnancy rate per started cycle (i.e. randomized patient). Patients only underwent one treatment cycle in the study. Ongoing pregnancy was defined as the presence of at least one viable fetus 10–11 weeks after embryo transfer documented by transvaginal ultrasound, and ongoing implantation rate was defined as viable fetuses per embryos transferred. Secondary clinical parameters included clinical pregnancy rate (transvaginal ultrasound showing at least one intrauterine gestational sac with fetal heart beat 5–6 weeks after embryo transfer), endometrial status, follicular development, oocytes retrieved, fertilization rate, embryo quality, endocrine profile in serum and follicular fluid and treatment efficiency. The major safety end-points were the incidence of treatment-emergent adverse events (onset after start of stimulation), moderate/severe OHSS and early pregnancy loss. Cases of OHSS were categorized according to Golan's classification system (Golan *et al*., 1989) and were defined as early OHSS if onset was within 9 days of hCG administration and as late OHSS if onset was >9 days after hCG administration. Early pregnancy loss was defined as a positive βhCG test analysed by the local laboratory but no ongoing pregnancy. A live birth cycle was defined as a cycle that resulted in at least one live born neonate, regardless of the number of other neonates and whether they were live born or stillborn.

Statistical analysis

The sample size calculation was based on the comparison of two binomial proportions on the log odds scale using a two-sided significance level, α, of 0.05 and a power of 80%. The study was powered to detect an odds ratio (OR) of one treatment versus the other treatment of 1.67 in ongoing pregnancy rate, and assuming ongoing pregnancy rates of 32 and 22% with the two treatments, at least 304 patients in each treatment group were required for this study. The sample size calculation was based on comparative trial data with these two gonadotrophins in IVF cycles (Platteau *et al*., 2004).

Test for superiority of one preparation over the other in terms of ongoing pregnancy rate was based on the likelihood ratio test in a logistic regression analysis. The main treatment effect was estimated using a model adjusting for age strata. The difference between treatments and the effect of age was expressed as ORs with 95% confidence intervals (CIs) (likelihood ratio based). The study protocol described a possible conversion from superiority to non-inferiority, including specification of the non-inferiority limit. Non-inferiority between HP-hMG and rFSH with respect to ongoing pregnancy rate was addressed based on the pre-defined non-inferiority limit of 0.65 for the OR of HP-hMG versus rFSH (corresponding to limits of –6.5 to –7.8% in the difference in proportion scale with an overall ongoing pregnancy rate of 22–28%). The study was designed and conducted in line with regulatory guidance justifying a switch to non-inferiority [European Medicines Agency (1998), ICH E9; European Medicines Agency (2000), CPMP/EWP/482/99; European Medicines Agency (2001), ICH E10; European Medicines Agency (2005), EMEA/CPMP/EWP/2158/99].

All analyses presented are based on the intention-to-treat (ITT) population (all randomized patients according to the actual treatment received). Additionally, ongoing pregnancy rate was calculated for the per-protocol (PP) population for robustness of findings. Secondary end-points were analysed in the same way as the primary end-point for

binary data, and for continuous data, analysis of variance (ANOVA) models were used to compare treatment groups. The impact of progesterone at the end of stimulation on several clinical parameters was addressed in an exploratory manner using logistic regression and ANOVA models. All analyses were adjusted for age stratum in line with the design of the study. No adjustment for multiplicity was performed. Data are presented as mean ± standard deviation (SD), unless otherwise specified.

Results

Baseline

A total of 821 patients were screened for eligibility, of whom 781 started down-regulation with triptorelin 0.1 mg daily (Figure 1). Of these, 731 patients proceeded to randomization at the end of down-regulation: 363 were treated with HP-hMG and 368 were treated with rFSH. The main reasons for screening failure were failure to down-regulate, spontaneous pregnancy and sperm quality not compatible with IVF. Demographics, baseline characteristics and the endocrine profile at the time of starting stimulation were comparable between the two treatment groups (Table I).

Controlled ovarian hyperstimulation

On day 6 after starting ovarian stimulation, there were significantly more follicles of ≥10 mm diameter (P = 0.007) and higher E_2 levels (P = 0.004) in patients in the rFSH group

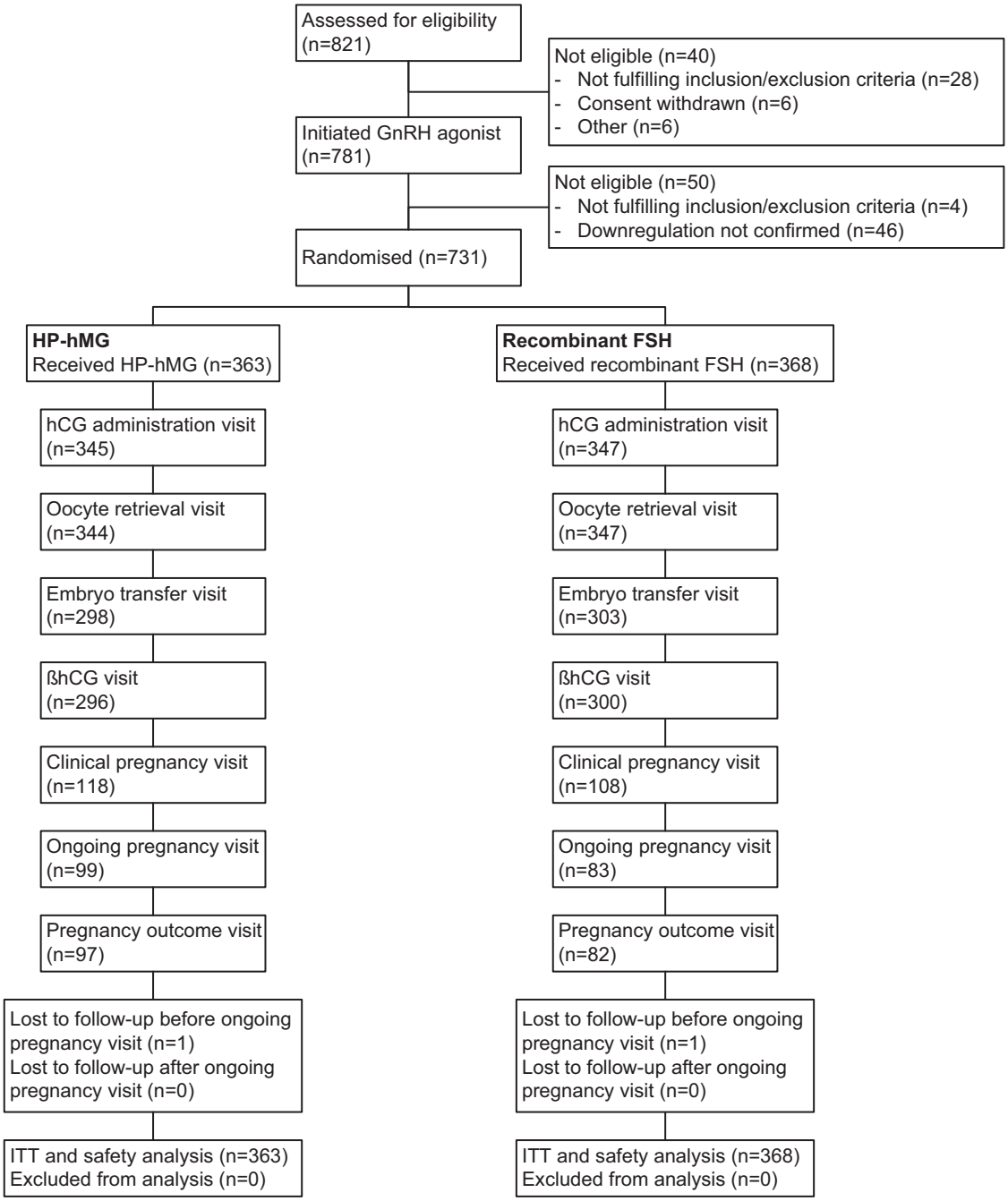

Figure 1. Study flow chart and disposition of patients by study visit. HP-hMG, highly purified menotrophin; ITT, intention-to-treat population.

Table I. Demographics and baseline characteristics of patients in the study

	HP-hMG (n = 363)	rFSH (n = 368)
Age (years)	30.8 ± 3.2	30.9 ± 3.3
<35	313 (86%)	306 (83%)
35–37	50 (14%)	62 (17%)
Weight (kg)	62.7 ± 8.5	61.0 ± 8.2
BMI (kg/m^2)	22.6 ± 2.7	22.1 ± 2.6
Cycle length (days)	28.3 ± 1.8	28.3 ± 1.6
Primary infertility	228 (63%)	234 (64%)
Primary cause of infertility		
Unexplained infertility	151 (42%)	166 (45%)
Tubal infertility	131 (36%)	125 (34%)
Mild male factor	46 (13%)	40 (11%)
Other (including endometriosis I/II)	35 (10%)	37 (9%)
Duration of infertility (years)	3.9 ± 2.3	3.9 ± 2.2
First treatment cycle	265 (73%)	246 (67%)
Duration of GnRH agonist before start of stimulation (days)	14.8 ± 4.1	14.8 ± 3.9
Mean ovarian volume at day 1 (cm^3)	5.2 ± 3.1	5.1 ± 3.4
Antral follicles at day 1	10.9 ± 6.4	10.8 ± 6.9
LH at day 1 (IU/l)	2.2 ± 1.4	2.3 ± 1.3
FSH at day 1 (IU/l)	3.9 ± 1.4	4.0 ± 1.5
Progesterone at day 1 (nmol/l)	1.3 ± 0.6	1.3 ± 0.6
Androstenedione at day 1 (nmol/l)	4.6 ± 1.8	4.4 ± 1.9
Total testosterone at day 1 (nmol/l)	0.71 ± 0.3	0.66 ± 0.3
SHBG at day 1 (nmol/l)	58 ± 25	58 ± 24
FAI at day 1	1.51 ± 1.1	1.36 ± 1.0

FAI, free androgen index; HP-hMG, highly purified menotrophin; rFSH, recombinant FSH; SHBG, sex hormone-binding globulin.

compared with the HP-hMG group. In the majority of patients (60% for HP-hMG and 66% for rFSH), the dose was maintained at 225 IU after day 6, whereas in 33% in the HP-hMG group and 25% in the rFSH group, the daily dose was increased to 300 IU. Less than 10% of the patients had the dose reduced to 150 IU on day 6 (7.2% in the HP-hMG group and 9.2% in the rFSH group).

At the last day of stimulation, the total number of follicles was significantly higher in the rFSH group (P = 0.013) (Table II). In line with this, there were significantly more follicles at all breakdowns according to size (≥10, ≥12, ≥15 and ≥17 mm) for patients in the rFSH group. Significantly lower E_2 levels (P = 0.031) and higher progesterone levels (P < 0.001) at the end of stimulation were observed in patients in the rFSH group compared with the HP-hMG group. The significant difference was also maintained after adjusting the E_2 and progesterone levels by the number of follicles observed, and for the subset of patients (135 with HP-hMG and 171 with rFSH) who maintained the dose of 225 IU throughout the study. A total of 126 patients had progesterone levels of >4 nmol/l (median 4.85 nmol/l) at the end of stimulation: 41 with HP-hMG and 85 with rFSH. Circulating FSH levels at the end of stimulation were significantly higher in the HP-hMG group compared with the rFSH group (P < 0.001), whereas LH levels were comparable. At the end of stimulation, a mean hCG level of 2.94 IU/l was found in the HP-hMG group.

There was no significant difference in endometrial thickness and in the proportion of patients with endometrial triple-layer structure. However, at the end of stimulation, there was a statistically significantly shift in endometrial echogenicity towards more frequent hyperechogenic endometrium in the rFSH group compared with the HP-hMG group (P = 0.023). The same findings were observed in those patients who remained on the 225 IU dose throughout the study. At the end

Table II. Clinical parameters during stimulation and oocyte and embryo parameters from retrieval to transfer/freezing

	HP-hMG (n = 363)	rFSH (n = 368)	P-value[a]
Estradiol (E_2) (nmol/l), day 6	1.0 ± 0.9	1.1 ± 1.0	0.004
Total number of follicles, day 6	12.0 ± 5.7	12.4 ± 6.7	0.187
Follicles ≥10 mm, day 6	4.1 ± 4.5	4.9 ± 4.9	0.007
E_2 (nmol/l), day of hCG	7.2 ± 4.3	6.6 ± 4.0	0.031
Progesterone (nmol/l), day of hCG	2.6 ± 1.3	3.4 ± 1.7	<0.001
Patients with progesterone at the end of stimulation >4 nmol/l	41 (11%)	85 (23%)	<0.001
Follicles, day of hCG			
Total	14.8 ± 6.9	15.9 ± 7.6	0.013
≥10 mm	12.6 ± 5.8	13.7 ± 5.9	0.005
≥12 mm	11.2 ± 5.2	12.3 ± 5.4	0.003
≥15 mm	8.1 ± 4.0	8.8 ± 4.3	0.010
≥17 mm	5.3 ± 2.7	5.8 ± 3.5	0.050
Endometrial thickness (mm), day of hCG	10.7 ± 1.9	10.8 ± 2.0	0.780
Triple-layer structure, day of hCG	347 (96%)	355 (97%)	0.532
Echogenic pattern, day of hCG[b]			0.023
Hypoechogenic	150 (42%)	129 (36%)	
Isoechogenic	173 (48%)	176 (49%)	
Hyperechogenic	35 (10%)	56 (16%)	
Treatment duration (days)	10.4 ± 1.9	10.1 ± 1.7	0.017
Total dose (IU)	2508 ± 729	2385 ± 622	0.006
Average daily dose (IU)	238 ± 29	233 ± 27	0.013
Oocytes retrieved	10.0 ± 5.4	11.8 ± 5.7	<0.001
Fertilization rate (%)	51.6 ± 29.2	52.5 ± 28.2	0.650
Embryos on day 3, total	6.3 ± 4.7	7.4 ± 5.0	0.002
Embryos cryopreserved	1.8 ± 2.8	1.9 ± 2.9	0.463
Top-quality embryos (local)	1.1 ± 1.6	1.1 ± 1.6	0.937
Top-quality embryos/oocytes retrieved (%) (local)	11.3 ± 16.1	9.0 ± 13.0	0.044
Proportion of patients with top-quality embryos (%) (local)	50%	47%	0.508

[a]Adjusted for age strata.
[b]Trend comparison, Wilcoxon–Mann–Whitney test.

of stimulation, a significantly ($P < 0.001$) higher proportion of patients in the rFSH group had a hyperechogenic endometrium when the progesterone levels were >4 nmol/l compared with those with levels of ≤4 nmol/l (Table III).

Regarding efficiency of treatment, patients were treated with HP-hMG for 0.3 days (8 h) more on average compared with rFSH ($P = 0.017$). The total dose of gonadotrophin used was significantly higher with HP-hMG ($P = 0.006$), and the average daily dose for the entire treatment period was 5 IU more per day in the HP-hMG group ($P = 0.013$).

Oocyte retrieval and emmbryo transfer

Oocyte retrieval was performed for 95% of the patients in the HP-hMG group and 94% in the rFSH group. In the HP-hMG group, 19 patients discontinued the study before oocyte retrieval for the following reasons (number of patients in parenthesis): inability to reach the hCG criterion (12), >25 follicles with a diameter of ≥10 mm (3), ovarian hyperfunction (1), increased serum E_2 (1), spontaneous pregnancy (1) and cycle converted to ICSI (1). In the rFSH group, 21 patients did not attend the oocyte retrieval visit for the following reasons: inability to reach the hCG criterion (10), >25 follicles with a diameter of ≥10 mm (8), ovarian hyperfunction (1), OHSS (1) and pelvic inflammatory disease (1).

The goal set for the ovarian stimulation of 7–15 oocytes was reached by 60% of the patients in the HP-hMG group who underwent oocyte retrieval and 57% in the rFSH group (Figure 2). The number of oocytes retrieved was significantly ($P < 0.001$) higher in the rFSH group compared with the HP-hMG group (Table II and Figure 2). The fertilization rate was similar among treatment groups. The number of top-quality embryos per patient did not differ between treatment groups, but the proportion of top-quality embryos by oocytes retrieved was significantly ($P = 0.044$) higher in the HP-hMG group than in the rFSH group. The mean number of embryos cryopreserved per patient with oocyte retrieval was similar among groups [1.8 ± 2.8 for HP-hMG and 1.9 ± 2.9 for rFSH ($P = 0.463$)].

A total of 601 patients had embryo transfer, corresponding to 82% of the randomized patients in both the HP-hMG and rFSH groups. The main reason for cancellation of embryo transfer was lack of transferable embryos on day 3. This was mainly due to fertilization failure. All transfers except for one patient took place on day 3 after oocyte retrieval. The mean number of embryos transferred was 1.7 ± 0.5 in both groups. Single embryo transfer was done in 32% of the transfers in the HP-hMG group and 31% in the rFSH group. Embryo transfer was done by clinical touch for 66% of the patients in the HP-hMG group and 63% in the rFSH group. A soft catheter was used for 92 and 91% of the patients in the HP-hMG and rFSH groups, respectively.

Ongoing pregnancy

Information on ongoing pregnancy was obtained for all patients in the study with the exception of two patients (one in each group) who were lost to follow-up after confirmation of clinical pregnancy. The ongoing pregnancy rate was 27% in the HP-hMG group and 22% in the rFSH group (Table IV). The OR of ongoing pregnancy was 1.25 in favour of HP-hMG. However, the 95% CI was 0.89–1.75 and therefore superiority of HP-hMG with respect to ongoing pregnancy was not shown ($P = 0.204$). Non-inferiority of HP-hMG compared with rFSH was shown with a margin of 0.89, which was well above the pre-specified non-inferiority limit of 0.65. For the PP population ($n = 624$), the OR of ongoing pregnancy was 1.20 with a 95% CI of 0.83–1.73. The most frequent major protocol violations in this study were transfer of embryos not meeting the defined minimum criteria (3.4%), cycle conversion to ICSI (1.9%), late timing of hCG administration (1.9%) and hCG administered outside criteria (1.8%). There was no indication of heterogeneity of treatment effect across age strata. Among the patients with embryo transfer, the ongoing pregnancy rate was 33% in the HP-hMG group and 27% in the rFSH group.

Table III. Endometrial status and pregnancy rates associated with progesterone levels at the end of stimulation

	HP-hMG			rFSH		
	Progesterone at the end of stimulation ≤4 nmol/l ($n = 305$)	Progesterone at the end of stimulation >4 nmol/l ($n = 41$)	*P*-value[a]	Progesterone at the end of stimulation ≤4 nmol/l ($n = 268$)	Progesterone at the end of stimulation >4 nmol/l ($n = 85$)	*P*-value[a]
Endometrial thickness (mm)	10.8 ± 1.9	10.6 ± 2.2	0.514	10.7 ± 1.9	11.2 ± 2.2	0.069
Triple-layer structure	97%	95%	0.446	98%	98%	0.937
Echogenic pattern[b]			0.092			0.001
Hypoechogenic	43%	35%		38%	31%	
Isoechogenic	49%	48%		51%	42%	
Hyperechogenic	9%	18%		11%	27%	
Progesterone (nmol/l), day of hCG (median)	2.30	4.80	–	2.70	4.90	–
Oocytes retrieved	9.7 ± 5.0	12.9 ± 7.3	<0.001	11.0 ± 5.3	14.1 ± 6.4	<0.001
Top-quality embryos	0.9 ± 1.3	1.7 ± 3.0	0.007	1.0 ± 1.7	1.1 ± 1.5	0.696
Top-quality embryos/oocytes retrieved	10.9%	11.3%	0.973	9.4%	8.1%	0.446
Ongoing pregnancy rate/cycle started	28%	22%	0.522	26%	15%	0.035
Ongoing pregnancy rate/embryo transfer	33%	26%	0.526	30%	18%	0.034
Ongoing implantation rate	24%	19%	0.506	23%	11%	0.025

[a]Adjusted for age strata.
[b]Trend comparison, Wilcoxon–Mann–Whitney test.

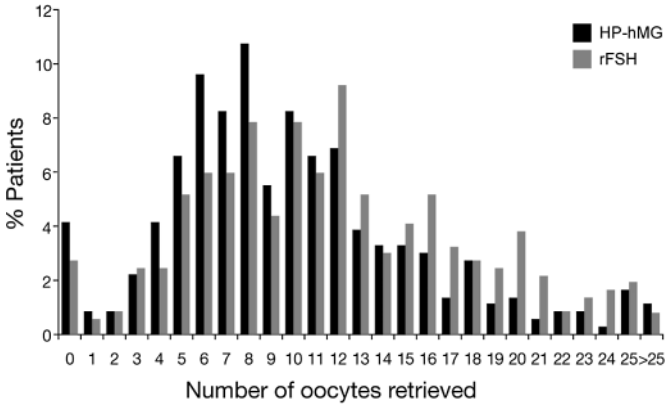

Figure 2. Graph showing percentage of patients versus number of oocytes retrieved following ovarian stimulation using HP-hMG or rFSH.

The OR of ongoing pregnancy was 1.26 (95% CI: 0.89–1.80) for HP-hMG compared with rFSH among patients with embryo transfer.

The ongoing pregnancy rate among women <35 years was 30% for HP-hMG compared with 24% for rFSH, which did not reach significant difference ($P = 0.082$). There were no significant differences between treatment groups in ongoing pregnancy rate in the stratum of patients 35–37 years of age ($P = 0.188$), for whom the ongoing pregnancy rate was 8% in the HP-hMG group and 16% in the rFSH group. Patients with poor ovarian response, here defined as 0–3 oocytes (36 patients in the HP-hMG group and 35 patients in the rFSH group), had an ongoing pregnancy rate of 8% with HP-hMG and 6% with rFSH ($P = 0.667$). In patients with at least four oocytes retrieved, the ongoing pregnancy rate was 29 and 24% for HP-hMG and rFSH, respectively ($P = 0.211$). The ongoing pregnancy rate was not significantly different ($P = 0.828$) among patients with one embryo transferred with 24% for HP-hMG and 26% for rFSH and neither for patients with two embryos transferred ($P = 0.097$) with 36% for HP-hMG compared with 28% for rFSH. Three patients (one in the HP-hMG group and two in the rFSH group) had three embryos transferred, but none had an ongoing pregnancy. The distribution of singleton and multiple pregnancies was similar for HP-hMG and rFSH. Singleton pregnancies accounted for 77% and 74%, respectively, of the ongoing pregnancies in the HP-hMG and rFSH groups. There were two pregnancies in the HP-hMG group with three viable fetuses; both women had two embryos transferred.

An exploratory analysis suggested a reduced ongoing pregnancy rate in patients with high progesterone levels at the end of stimulation (Table III). In the rFSH group, a significantly ($P = 0.035$) lower ongoing pregnancy rate per started cycle of 15% was noted for patients with progesterone levels of >4 nmol/l compared with 26% in patients with progesterone levels of ≤4 nmol/l. This was not associated with a reduced embryo quality.

Safety

There were no clinically relevant differences between treatment groups regarding the safety profile. The overall incidence of adverse events was 51 and 49% in the HP-hMG and rFSH groups, respectively. Besides vaginal bleeding as a result of menses, the most frequently reported adverse events were as follows in the HP-hMG and rFSH groups, respectively: abortion (covering spontaneous abortion, missed abortion, complete abortion and incomplete abortion) (9 versus 10%), pelvic pain (6 versus 6%), headache (5 versus 5%), post-procedural pain (3 versus 4%), OHSS (4 versus 3%), nausea (2 versus 4%) and abdominal distension (2 versus 3%).

OHSS was experienced by 23 patients in the study: 13 patients (4%) in the HP-hMG group and 10 patients (3%) in the rFSH group. Moderate/severe early OHSS was recorded for five patients treated with HP-hMG and six patients treated with rFSH, and moderate/severe late OHSS occurred for three and two patients, respectively (Table IV). All moderate/severe late OHSS were in patients with clinical pregnancy and in three instances in patients with twin pregnancies (two with HP-hMG and one with rFSH). The total number of patients experiencing early pregnancy losses (ectopic pregnancy, biochemical pregnancy, complete abortion, incomplete abortion and missed abortion) was 33 (26%) in the HP-hMG group and 39 (32%) in the rFSH group. This included two patients in the HP-hMG

Table IV. Pregnancy and safety outcome

	HP-hMG ($n = 363$)	rFSH ($n = 368$)	P-value[a]
Embryos transferred	1.7 ± 0.5	1.7 ± 0.5	0.710
Clinical pregnancy/cycle started	100/363 (28%)	87/368 (24%)	0.263
Ongoing pregnancy/cycle started	97/363 (27%)	82/368 (22%)	0.204
<35 years	93/313 (30%)	72/306 (24%)	0.082
35–37 years	4/50 (8%)	10/62 (16%)	0.188
Ongoing pregnancy rate/embryo transfer	97/298 (33%)	82/303 (27%)	0.193
1 embryo transferred	23/94 (24%)	24/93 (26%)	0.828
2 embryos transferred	74/203 (36%)	58/208 (28%)	0.097
Live birth/cycle started	96/363 (26%)	82/368 (22%)	0.236
Singleton live birth/cycle started	76/363 (21%)	63/368 (17%)	0.231
Ongoing implantation rate	119/503 (24%)	102/515 (20%)	0.247
Moderate/severe early OHSS	5/363 (1.4%)	6/368 (1.6%)	1.000
Moderate/severe late OHSS	3/363 (0.8%)	2/368 (0.5%)	0.773
Early pregnancy loss	33/129 (26%)	39/122 (32%)	0.296

OHSS: ovarian hyperstimulation syndrome.
[a]Adjusted for age strata.

group, who lost one fetus but continued the pregnancy with one remaining viable fetus.

Post-study follow-up

Post-study follow-up information was collected for all 179 patients with ongoing pregnancy and all 224 viable fetuses. No patients with ongoing pregnancy and no fetuses were lost to follow-up. The frequency of patients with a cycle resulting in live birth was 26% for HP-hMG and 22% for rFSH. One patient in the HP-hMG group had a pregnancy loss before delivery. She had three viable fetuses at the ongoing pregnancy visit, and following elective termination of two fetuses, the third fetus was lost at a miscarriage. There was one stillborn child in the rFSH group, but as the child was part of a twin pregnancy and the remaining twin was a live born child, this constituted a live birth cycle. Four fetuses were lost in the HP-hMG group: one patient had elective termination of two fetuses and subsequent miscarriage of the third fetus, and one patient had an elective termination of one fetus in a twin pregnancy due to trisomy 21. In the rFSH group, one patient had a miscarriage of one fetus and one patient had a still birth of one fetus; both cases were originally twin pregnancies. The proportion of started cycles resulting in live birth of a singleton was 21% in the HP-hMG group and 17% in the rFSH group. There were 19 sets of twins in both treatment groups. There were no apparent differences between HP-hMG and rFSH groups with respect to neonatal health among the live born infants. The frequency of boys among the live born infants was 51% in the HP-hMG group and 56% in the rFSH group. The mean gestational age at delivery was 264 and 265 days in the HP-hMG and rFSH groups, respectively. The incidence of preterm birth (gestational age below 37 completed weeks) was 32% with HP-hMG and 30% with rFSH. On average, infants in the HP-hMG group had a birth weight of 2918 g, whereas infants in the rFSH group weighed 2877 g. Birth weight of at least 2500 g was recorded for 74 and 72% of the infants in the HP-hMG and rFSH groups, respectively. Among singletons, preterm birth occurred for 5% in the HP-hMG group and 13% in the rFSH group, and birth weight of <2500 g was reported at an incidence of 5 and 8%, respectively. For twins, the gestational age was on average 246 and 251 days in the HP-hMG and rFSH groups and the average birth weight was 2230 and 2274 g, respectively.

Discussion

In this study, the ongoing pregnancy rates were 27% with HP-hMG and 22% with rFSH, representing a non-significant relative difference of about 25%, but superiority was not concluded. Non-inferiority could be claimed as the lower limit of the 95% CI for the treatment difference in ongoing pregnancy was well above the pre-specified non-inferiority limit for both the ITT and PP populations. The ongoing pregnancy rate of 22% in the rFSH group was exactly as expected and defined in the study protocol and very similar to the 23% reported after rFSH treatment in the latest meta-analysis (van Wely *et al*., 2003). The findings of the present large study are in agreement with the results of the most recent systematic review of all truly randomized controlled trials (n = 1214) in women undergoing controlled ovarian hyperstimulation for IVF/ICSI (van Wely *et al*., 2003). This meta-analysis reported a borderline statistically significant difference of 5% in absolute terms in clinical pregnancy rates and a non-significant difference of 4% in ongoing pregnancy/live birth rates in favour of menotrophins (27%) over FSH-only preparations (23%) (van Wely *et al*., 2003). Although systematic reviews can address the sample size deficiencies of the individual trials in this area, they accumulate more variability as they include trials with diverse design and varying clinical practice. The present efficacy study, which minimized many of the potential sources of trial variability, resulted in pregnancy and live birth rates of similar level, difference and direction as the cumulative evidence from comparative trials available in the literature (van Wely *et al*., 2003). As a statistically significant better outcome was not documented in this IVF study, the concept of differentiated impact of different gonadotrophins depending on fertilization method as initially hypothesized still remains to be further explored. Given the magnitude of the effects and the feasibility limitations for large efficacy trials, this topic might be addressed by either even larger efficacy trials or perhaps more realistically by a meta-analysis.

This study included only patients eligible for IVF, and not patients requiring ICSI, for both methodological and clinical considerations. The large sample size and the comparability of criteria through all pre- and post-randomization procedures, including embryo transfer criteria and procedures, provide reassurance for the interpretation of pregnancy results. The percentage of patients not undergoing transfer must be seen in context of the strict protocol criteria before embryo transfer. These included cancellation criteria because of development of too many large follicles or inadequate response, minimum quality criteria for which embryos were allowed to be transferred and discontinuation from the study in case of insufficient sperm count necessitating conversion to ICSI. In both groups, the percentage of patients with embryo transfer was 82%, which is within expectations for an IVF (non-ICSI) study according to the European registry database (ESHRE, 2006). The mean number of embryos transferred is much lower than in previous large comparative trials between menotrophins and rFSH (Westergaard *et al*., 2001; The European and Israeli Study Group on highly purified hMG versus rFSH, 2002). In this study, almost one-third of the transfers were single embryo transfers; however, the study did not gather information about whether the single embryo transfer was elective or not. Regulatory restrictions (i.e. national policy for first cycles), clinical judgement and patient preference influenced single embryo transfer, and therefore a meaningful comparison between groups on single embryo transfers is limited. Some differential trends on ongoing pregnancy rates were present in the largest subgroups in the study, which were double embryo transfers (68% of the study population) and the patients below 35 years of age (85% of the study population).

This study also provides detailed information about the pharmacodynamics during ovarian stimulation with preparations containing FSH only or FSH/LH activity combined. Although the differences in pregnancy rates did not reach

statistical significance, the data indicate that LH activity plays a role in ovarian stimulation and show that there are major pharmacodynamic differences between these preparations in follicular development, endocrine response, embryo quality and endometrial status. After administering the same dose of 225 IU for 5 days in both groups to all patients, follicular response appeared to be more pronounced with rFSH. This was also accompanied by significantly higher E_2 levels at day 6 of stimulation in the rFSH group. Significantly higher levels of FSH were observed at day 6 with HP-hMG, which could be explained either by different elimination kinetics of the FSH isoforms in the gonadotrophin preparations, with a longer half-life for the FSH in menotrophins, or by epitope masking due to the variable carbohydrate chains of different isoforms. No significant difference between groups in LH concentration was observed at day 6 of stimulation. The hCG component in HP-hMG provides most of the LH activity in HP-hMG (Wolfenson *et al*., 2005).

Based on the proportion of patients with dose adjustments after the first 5 days of stimulation, the differences in ovarian response between treatments seem to have been observed clinically at an early stage. Although most of the patients in both groups stayed on the same dose after day 6, a few more patients increased the dose in the HP-hMG group compared with the rFSH group. The number of follicles at the end of stimulation, total as well as by size groups, was significantly higher with rFSH compared with HP-hMG. The difference between groups in total number of follicles was approximately one follicle. At the end of stimulation, mean FSH and E_2 concentrations were significantly higher with HP-hMG compared with rFSH, whereas LH concentrations were similar. The higher levels for these endocrine parameters were not explained by the increase in gonadotrophin dose in a proportion of the HP-hMG patients, as patients in the HP-hMG group with the same 225 IU dose throughout the study had similar results. The higher E_2 concentration in the HP-hMG group cannot be explained by the follicular response. Interestingly, progesterone at the end of stimulation was significantly higher with rFSH compared with HP-hMG, even after adjusting for ovarian response. The premature increase in progesterone has been historically considered to be attributed to an LH action in the context of premature LH surge. However, it has previously been reported that an increase in progesterone at the end of stimulation, and before hCG administration, is related to FSH activity rather than to LH activity (Filicori *et al*., 2002). Thus, the difference between HP-hMG and rFSH in progesterone could be hypothetically attributed to an FSH action in granulosa cells through paracrine signals that modify the enzymes involved in progesterone and androgen synthesis. In the relative absence of LH activity, the functionality of these enzymes may be affected resulting in higher levels of progesterone. The increase in progesterone levels negatively influenced endometrial status and pregnancy rates as discussed later.

The effect of stimulation with a particular gonadotrophin preparation result in a differentiated ovarian response, and this is reflected in the endometrial profile observed at the end of stimulation. In this study, there was a statistically significant shift in endometrial echogenicity towards more patients with hyperechogenic endometrium in the rFSH group. Endometrial hyperechogenicity has been associated with endometrial exposure to progesterone during the follicular phase (Fanchin *et al*., 1999). Advanced hyperechogenic transformation of the endometrium is associated with poor IVF outcome (Fanchin *et al*., 2000). Implantation and pregnancy rates have been shown to be poorer in women with higher endometrial echogenicity compared with those with lower echogenicity at the end of stimulation (Fanchin *et al*., 2000). In this study, pregnancy rates were lower among patients with preovulatory progesterone levels of >4 nmol/l compared with those with progesterone levels of ≤4 nmol/l (Table III). The data derived from this exploratory analysis suggest that even minor elevations in progesterone at the end of stimulation negatively affect implantation and ongoing pregnancy rates. This could be attributed to either a negative impact of progesterone on oocyte/embryo quality or on the endometrium. Patients with minor elevation of progesterone presented with higher ovarian response and availability of top-quality embryos for transfer were not compromised but more frequently had an advanced hyperechogenic endometrium (Table III). Treatment outcome in patients with minor progesterone elevations at the end of stimulation appears to be affected primarily by a detrimental effect on the endometrium.

An area of major debate is the contribution of LH activity to increase ovarian response to FSH stimulation. The relevance of obtaining a high number of oocytes at retrieval is to have as many embryos as possible of a quality suitable for transfer and cryopreservation. However, little is known about the quality of the oocytes retrieved and their developmental potential. In this study, more oocytes were obtained with rFSH than with HP-hMG, and this is in line with the findings of previous studies in IVF patients (Platteau *et al*., 2004). Despite the significant difference in oocytes retrieved, the proportion of patients with poor response (<4 oocytes) was identical in both groups (10%), but the proportion of patients with high response (>20 oocytes) doubled in the rFSH group (10%) compared with the HP-hMG group (5%). The significantly higher number of oocytes retrieved did not lead to more embryos frozen. Interestingly, the increased number of oocytes retrieved in the rFSH group was not accompanied by a higher number of top-quality embryos. Actually, the proportion of top-quality embryos available at the time of transfer was significantly higher in the HP-hMG group. These data would imply that LH activity plays a role in optimizing the quality or developmental potential of the oocytes obtained, in line with some non-clinical evidence (Weston *et al*., 1996). Limited data from randomized controlled trials are available in the clinical area regarding the impact of LH activity on embryo quality; however, a recent study (Lisi *et al*., 2005) reported a higher incidence of grade 1 and 2 embryos when supplementing LH activity to FSH stimulation in women undergoing a long agonist protocol. The mechanisms for the improved oocyte/embryo quality in IVF cycles after exposure to exogenous LH activity are not fully understood, but it has been hypothesized that it could materialize through cumulus cells when exposed to LH activity during stimulation (Platteau *et al*., 2004). Recent gene expression data supported this concept and provided some molecular evidence for a mediation of the cumulus cells in embryo development

(Assou *et al*., 2006). From a clinical perspective, further consideration should be given to investigating treatment protocols improving oocyte quality rather than maximizing the number of oocytes obtained.

No differences between groups were observed for any of the end-points related to safety. The exposure to exogenous LH activity during the ovarian stimulation phase did not affect the risk of OHSS. There was no difference in moderate/severe OHSS between preparations overall or when evaluated according to early and late presentation. There is no evidence from the present or previous studies that HP-hMG increases the risk of OHSS compared with rFSH preparations (The European and Israeli Study Group on highly purified hMG versus rFSH, 2002; Platteau *et al*., 2006). Neonatal safety was documented and there were no apparent differences between HP-hMG and rFSH with respect to the neonatal health of the live born children. As in the EISG study (Helmgaard *et al*., 2004), the incidence of preterm birth and low birth weight was related to singleton/multiple birth rather than to the type of gonadotrophin preparation.

In conclusion, non-inferiority with respect to ongoing pregnancy was demonstrated for HP-hMG compared with rFSH when used for controlled ovarian hyperstimulation following down-regulation with a GnRH agonist in a long protocol in women undergoing IVF. Pharmacodynamic differences are observed between HP-hMG and rFSH in follicular development and ovarian endocrine response, which potentially impacts on embryo quality and endometrial morphology, whereas the clinical safety profile of these preparations is comparable.

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Conflict of interest

Anders Nyboe Andersen has conducted clinical research sponsored by Ferring Pharmaceuticals, Serono, Organon, Novo Nordisk and Medicult. Paul Devroey has conducted clinical research sponsored by Ferring Pharmaceuticals, Organon and Serono. Joan-Carles Arce is employee of Ferring Pharmaceuticals.

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