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Similar ovulation rates, but different follicular development with highly purified menotrophin compared with recombinant FSH in WHO Group II anovulatory infertility: a randomized controlled study

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BACKGROUND: The contribution of the LH activity in menotrophin preparations for ovulation induction has been investigated in small trials conducted versus FSH preparations. The objective of this study was to demonstrate non-inferiority of highly purified urinary menotrophin (HP-HMG) versus recombinant FSH (rFSH) with respect to the primary outcome measure, ovulation rate. METHODS: This was a randomized, open-label, assessor-blind, multinational study. Women with anovulatory infertility WHO Group II and resistant to clomiphene citrate were randomized (computer-generated list) to stimulation with HP-HMG (n = 91) or rFSH (n = 93) using a low-dose step-up protocol. RESULTS: The ovulation rate was 85.7% with HP-HMG and 85.5% with rFSH (per-protocol population), and non-inferiority was demonstrated. Significantly fewer intermediate-sized follicles were observed in the HP-HMG group ($P < 0.05$). The singleton live birth rate was comparable between the two groups. The frequency of ovarian hyperstimulation syndrome and/or cancellation due to excessive response was 2.2% with HP-HMG and 9.8% with rFSH ($P = 0.058$). CONCLUSIONS: Stimulation with HP-HMG is associated with ovulation rates at least as good as a rFSH in anovulatory WHO Group II women. LH activity modifies follicular development so that fewer intermediate-sized follicles develop. This could have a positive impact on the safety of ovulation induction protocols.

Key words: anovulation/highly purified menotrophin/ovulation induction/polycystic ovary syndrome/recombinant FSH

Introduction

Monofollicular development and subsequent mono-ovulation and singleton pregnancy are the aims of ovulation induction therapy. FSH alone is sufficient to stimulate follicular development, even in women with hypogonadotrophic hypogonadism (Shoham *et al*., 1993; Balasch *et al*., 1995), although in these patients LH activity is required for adequate steroidogenesis, fertilization and implantation (Shoham *et al*., 1991; Balasch *et al*., 1995). It has been hypothesized that LH activity may be of clinical relevance in ovulation induction cycles in anovulatory women as it could promote monofollicular development (Loumaye *et al*., 2003). Exposure to LH activity during the follicular phase could facilitate selective follicular growth, decrease the number of intermediate-sized follicles and increase the proportion of women who develop one mature follicle. The LH activity in menotrophin preparations could be used to promote mono-ovulation in ovulation induction protocols. This could lead to a reduction in the risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies and its associated complications. There have been some controversies regarding the use of preparations with LH activity in women with polycystic ovary syndrome (PCOS), since these women generally have elevated LH levels. There is, however, extensive clinical documentation with menotrophins, supporting its use in clomiphene

citrate-resistant women with PCOS (Seibel *et al*., 1985; Homburg *et al*., 1988; Abdel Gadir *et al*., 1990; Larsen *et al*., 1990; McFaul *et al*., 1990; Sagle *et al*., 1991). A meta-analysis of these small studies suggests similar ovulation and pregnancy rates between menotrophins and urinary FSH-only preparations in women with PCOS undergoing ovulation induction (Nugent *et al*., 2000). The present investigation is the first clinical trial comparing highly purified menotrophin (HP-HMG) and recombinant FSH (rFSH) in women with World Health Organization (WHO) Group II anovulatory infertility resistant to clomiphene citrate. The study aimed to demonstrate non-inferiority of HP-HMG versus rFSH with respect to ovulation rate.

Materials and methods

Study population

Anovulatory WHO Group II women, who failed to ovulate or conceive on clomiphene citrate, were recruited at 29 fertility clinics (eight in Belgium, nine in Denmark, five in Sweden and seven in the UK). The inclusion criteria were: (i) women with good physical and mental health, aged 18–39 years who failed to ovulate with clomiphene citrate doses of at least 100 mg/day for at least 5 days or failed to conceive after three cycles of ovulation induction with clomiphene citrate; (ii) WHO Group II infertility with chronic anovulation (amenorrhoea or oligomenorrhoea, or anovulatory cycles based on progesterone levels in women with cycle lengths of 21–35 days); (iii) infertility for ≥1 year before randomization; (iv) BMI 19–35 kg/m^2 at the time of randomization; (v) at least one patent tube documented within 3 years prior to screening; (vi) normal pelvis documented by a transvaginal ultrasound with respect to uterus, Fallopian tubes and ovaries within 3 months prior to screening; (vii) early follicular phase serum FSH levels 1–12 IU/l and levels of prolactin and total testosterone not suggestive of hyperprolactinaemia or androgen-secreting tumours; (viii) a male partner with semen analysis showing acceptable values for intrauterine insemination, or semen from a donor; and (ix) signed informed consent form, prior to screening. The exclusion criteria included: (i) a history of ≥12 unsuccessful ovulation induction cycles; (ii) persistent ovarian cysts (≥15 mm) for >1 cycle or ovarian endometrioma on ultrasound; (iii) any significant systemic disease, endocrine or metabolic abnormalities (pituitary, thyroid, adrenal, pancreas, liver or kidney); (iv) 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); (v) treatment with clomiphene citrate, metformin, gonadotrophins or GnRH analogues within 1 month prior to randomization; (vi) pregnancy, lactation or contraindication to pregnancy; (vii) current or past (last 12 months) abuse of alcohol or drugs; (viii) a history of chemotherapy (except for gestational conditions) or radiotherapy (ix) undiagnosed vaginal bleeding; (x) tumours of the ovary, breast, adrenal gland, pituitary or hypothalamus; malformation of sexual organs incompatible with pregnancy; and (xi) hypersensitivity to any trial product.

Study design

This was a randomized, open-label, assessor-blind, parallel-group, multicentre, multinational, non-inferiority ovulation induction study comparing HP-HMG (MENOPUR, Ferring Pharmaceuticals A/S, Copenhagen, Denmark) and rFSH (follitropin alfa, GONAL-F, Serono, Geneva, Switzerland) with respect to ovulation rates using a low-dose step-up protocol. 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 May 2003 to June 2004. Eligible subjects were randomized 1 : 1 to HP-HMG or rFSH at the time of starting stimulation, based on a computer-generated randomization list prepared by an independent statistician. The block size was concealed. All investigators and sponsor study staff were blinded to treatment allocation throughout the study, and the treatment code was not unblinded for any subject during the study. Gonadotrophin distribution was handled by study nurses.

Stimulation treatment was started 2–5 days after a spontaneous or progesterone-induced menstrual bleed. The starting dose of HP-HMG or rFSH was 75 IU daily, which was maintained for 7 days. After the first 7 days, the gonadotrophin dose was evaluated according to individual response. The dose was maintained at 75 IU if one follicle was ≥10 mm, and the dose was increased by 37.5 IU if there were no follicles ≥10 mm. The dose could be maintained or adjusted every 7-day period (on day 7, 14, 21, etc), according to this scheme. The maximum allowed daily dose was 225 IU, and subjects were treated with gonadotrophin for a maximum duration of 6 weeks. Compliance was assessed by self-reported diaries of gonadotrophin use. Gonadotrophin stimulation was maintained until at least one of the following criteria for HCG administration were met: one follicle with a diameter of ≥17 mm or two to three follicles with diameters of ≥15 mm. Subjects were not given HCG in either of the following situations: no follicular response after 6 weeks of gonadotrophin treatment or ≥4 follicles with diameters of ≥15 mm. Subjects who reached the HCG criteria received a single subcutaneous or intramuscular injection of HCG (PROFASI, Serono) at a dose of 5000 IU to trigger ovulation. Subjects given HCG were recommended sexual intercourse or were planned for intrauterine insemination according to the standards at the investigational site; luteal support was prohibited. At least one blood sample was taken during the midluteal phase (6–9 days after HCG administration) and analysed for progesterone by a central laboratory. A quantitative pregnancy test (serum β-HCG) was taken 12–16 days after HCG administration. In case of pregnancy, a transvaginal ultrasound was performed 7 ± 2 weeks and 12 ± 2 weeks after HCG administration to confirm clinical and ongoing pregnancy, respectively. All pregnancies were followed up to delivery.

Study end-points

The primary objective of the study was to demonstrate non-inferiority of HP-HMG compared with rFSH with respect to ovulation rate after one cycle of gonadotrophin treatment. Ovulation was defined as a midluteal serum progesterone concentration of ≥25 nmol/l (≥7.9 ng/ml). Measurement of midluteal progesterone was performed by a central laboratory using a competitive immunoassay using direct chemiluminometric technology with a sensitivity of 0.48 nmol/l (Quest Diagnostics Limited, Heston, UK). The protocol also allowed that subjects with a clinical pregnancy documented via transvaginal ultrasound would be counted as subjects with ovulation in the absence of a midluteal progesterone sample; however, this was not the case for any subject in the study.

Other clinical parameters evaluated were clinical pregnancy rate (transvaginal ultrasound showing at least one intrauterine gestational sac with fetal heart beat 7 ± 2 weeks after HCG administration), ongoing pregnancy rate (transvaginal ultrasound showing at least one viable fetus 12 ± 2 weeks after HCG administration), live birth rate, singleton live birth rate, number of follicles according to size, number of subjects with monofollicular (one follicle ≥17 mm and no follicles of 15–16 mm)

and bi-/multifollicular (≥2 follicles ≥15 mm) development, endometrial thickness at the time of HCG administration, and efficiency in terms of total gonadotrophin dose administered, duration of gonadotrophin treatment and threshold dose (i.e. the last gonadotrophin dose prior to reaching the HCG criteria). The major safety end-points were the incidence of OHSS [categorized as mild, moderate or severe according to Golan's classification (Golan *et al*., 1989)], multiple gestations and the number of cancellations due to risk of over-response. In addition, the local tolerability (injection site reactions in terms of redness, pain, itching, swelling and bruising) was self-assessed 1 h and 24 h after administration throughout the stimulation phase.

Statistical analysis

The sample size calculation was based on comparison of two binomial proportions with a two-sided significance level of 0.05 and a power of 80%. The overall expected ovulation rate was 80%, and the non-inferiority limit for the difference between treatments (HP-HMG – rFSH) was prespecified at –20%. A 20% reduction in ovulation rate would be expected to translate to a decrease in live birth rate of 5%, which would be considered to be a clinically relevant difference between treatments. On the basis of these conditions, 126 women (63 per group) were needed for the study. The estimate of the difference in ovulation rate between treatment groups with corresponding two-sided 95% confidence interval (CI) was calculated using the SAS procedure GENMOD using a binomial distribution with the identity link function. Subjects with no information on ovulation were defaulted to a negative response. The analyses on ovulation rate were made on both the per-protocol (PP) and intention-to-treat basis (ITT; all randomized subjects), with the PP analysis specified as the primary. Secondary end-points were analysed in the same way as the primary end-point for binary data, and for continuous data two-sample *t*-tests and Wilcoxon test were used to compare treatment groups. The secondary end-points are presented for the ITT analysis set. The ITT population for analysis of efficacy end-points included subjects according to planned randomization, while the safety population included subjects according to the actual treatment received (Figure 1).

No adjustment for multiplicity was performed, as there was only one primary end-point, and all other end-points were considered secondary. The main analysis of efficacy and safety end-points was unadjusted. Efficiency analyses were adjusted for BMI, failure to ovulate on clomiphene citrate and diagnosis of polycystic ovaries.

Results

A total of 229 subjects were screened for eligibility, and of these, 184 were included in the study: 91 were randomized to HP-HMG and 93 were randomized to rFSH (Figure 1). Demographic and baseline characteristics were comparable between the two treatment groups, with the exception of the following two aspects (Table I): obese subjects (BMI ≥30 kg/m^2) accounted for 33.0% of the subjects in the HP-HMG group and for only 15.1% in the rFSH group; and 53.8% of the subjects in the HP-HMG group had earlier failed to ovulate on clomiphene citrate compared with 37.6% in the rFSH group. The endocrine profile at the time of starting stimulation (including levels of LH, FSH, estradiol, progesterone, prolactin, androstenedione, total testosterone, sex hormone-binding globulin, glucose and insulin and the LH/FSH ratio) was comparable among subjects in the two groups (Table II). Subjects with major protocol violations were excluded from the PP analysis (Figure 1). The most common reasons for protocol violation were incorrect dose adjustment and HCG administered despite not meeting or exceeding the HCG criteria. There were more protocol violations in the HP-HMG group; however, a careful evaluation of the reasons for each violation indicated no pattern except for the finding that the dose-adjustment scheme was violated more frequently in the HP-HMG group.

With regard to the primary end-point, the ovulation rate observed in the PP population was 85.7% for the HP-HMG group and 85.5% for the rFSH group (difference 0.2; 95% CI [–11.0; 11.3]). For the ITT population, the ovulation rate was 83.5 and 84.9% for the HP-HMG and rFSH groups, respectively (difference –1.4%; 95% CI [–12.0; 9.1]). Thus, non-inferiority of HP-HMG versus rFSH with respect to ovulation rate was demonstrated with a margin of –11.0% and –12.0% for the PP and ITT populations, respectively. The sensitivity of the primary analysis of ovulation rate was investigated by adjusting for age and BMI in a logistic regression analysis. The results were consistent and similar to the corresponding unadjusted analysis.

Regarding follicular development (ITT population), subjects in the HP-HMG group had on average significantly fewer intermediate-sized follicles (12–16 mm) at the end of stimulation than those in the rFSH group (1.04 and 1.91, respectively, $P = 0.009$). The mean number of follicles <12 mm was 18.1 in the HP-HMG group compared with 14.2 in the rFSH group, but this was not significantly different. There was no difference between groups in the mean number of follicles ≥17 mm. As there was a difference between treatment groups with respect to small/intermediate-sized follicles, the follicles of 10–16 mm were investigated further. Women stimulated with HP-HMG had a significantly lower number of follicles with a diameter of 15–16 mm ($P < 0.05$) and a trend for fewer follicles of 10–14 mm ($P = 0.095$) (Figure 2). Development of a single dominant follicle (one follicle ≥17 mm and no follicles 15–16 mm) was achieved for 63.7% in the HP-HMG group versus 54.8% in the rFSH group. Bi-/multifollicular development (≥2 follicles ≥15 mm) tended to be less frequent in the HP-HMG group than in the rFSH group (29.7 and 43.0%, respectively, $P = 0.060$).

The mean treatment efficiency parameters are presented in Table III. There were no statistically significant differences between the HP-HMG and rFSH groups with respect to the duration of gonadotrophin treatment, total gonadotrophin dose or threshold gonadotrophin dose. Owing to a skewed distribution among subjects with respect to duration of gonadotrophin therapy (1–42 days), it is relevant to consider the median data. The median treatment duration was 13 days in the HP-HMG group versus 11 days in the rFSH group. The median total gonadotrophin dose was 1088 and 825 IU in the HP-HMG and rFSH groups, respectively. The median threshold dose was 75 IU in both groups.

One subject (1.1%) in the HP-HMG group and three subjects (3.2%) in the rFSH group reported OHSS. The percentage of subjects who had OHSS or cycle cancellation owing to an excessive response was 2.2% with HP-HMG and 9.8% with rFSH ($P = 0.058$). The treatment outcome, in terms of clinical and ongoing pregnancy rates, was comparable between groups.

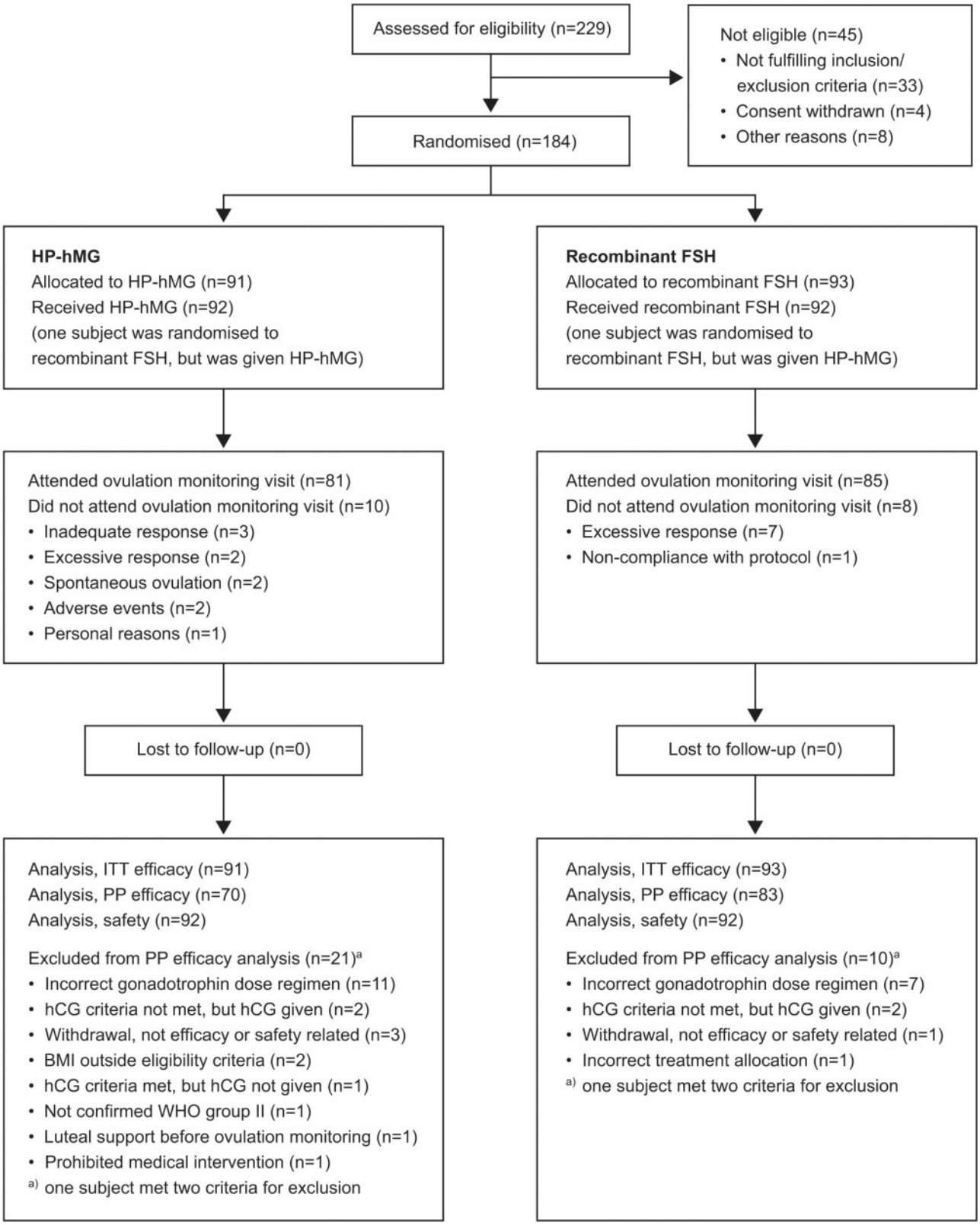

Figure 1. Study flow chart and disposition of subjects.

No multiple pregnancies were reported with HP-HMG, while two of the 16 (12.5%) pregnancies with rFSH were multiple gestations (Table III). One case of pregnancy loss between clinical pregnancy and ongoing pregnancy was reported in each group, and there were no losses after confirmed ongoing pregnancy (Table III). All ongoing pregnancies resulted in live birth cycles, and all viable fetuses at the ongoing pregnancy visit ended in delivery of live infants. The proportion of started cycles resulting in live birth of a singleton was 14.3% in the HP-HMG group and 15.1% in the rFSH group (Table III). The

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

Characteristic	HP-HMG (n = 91)	rFSH (n = 93)
Age (years)	29.0 ± 4.2	29.2 ± 3.8
Body weight (kg)	73.0 ± 16.0	68.9 ± 12.5
BMI (kg/m^2)	26.5 ± 5.2	25.0 ± 4.2
Subjects with BMI, *n* (%)		
<25 kg/m^2	42 (46.2)	54 (58.1)
≥25 kg/m^2 and < 30 kg/m^2	19 (20.9)	25 (26.9)
≥30 kg/m^2	30 (33.0)	14 (15.1)
Subjects with primary infertility, *n* (%)	52 (57.1)	60 (64.5)
Duration of infertility (years)	2.9 ± 1.8	3.0 ± 2.1
Previous cycles of ovulation induction (all)	4.6 ± 2.5	4.9 ± 2.5
Previous cycles of ovulation induction (with clomiphene citrate)	3.9 ± 2.5	4.1 ± 2.5
Clomiphene citrate non-responders, *n* (%)		
Failure to ovulate on clomiphene citrate[a]	49 (53.8)	35 (37.6)
Failure to conceive on clomiphene citrate[b]	42 (46.2)	58 (62.4)
Menstrual status, *n* (%)		
Amenorrhoea	19 (20.9)	19 (20.4)
Oligomenorrhoea	48 (52.7)	48 (51.6)
Anovulatory cycles (21–35 days)	24 (26.4)	26 (28.0)
Mean ovarian volume (cm^3)	8.5 ± 4.4	8.2 ± 4.2
Number of antral follicles >2 mm	25.1 ± 18.0	23.0 ± 15.3

HP-HMG, highly purified HMG; rFSH, recombinant FSH. All data are mean ± standard deviation except where stated.
[a]At least 100 mg/day for at least 5 days.
[b]After three cycles.

Table II. Endocrine profile at the start of stimulation

	HP-HMG (n = 91)	rFSH (n = 93)
LH (IU/l)	7.2 ± 4.8	7.7 ± 4.3
Subjects with LH levels > 10 IU/l (%)	21.1	22.6
FSH (IU/l)	4.9 ± 1.4	5.4 ± 2.5
LH : FSH ratio	1.6 ± 1.2	1.6 ± 1.1
Prolactin (μg/l)	13 ± 13	11 ± 6
Androstenedione (nmol/l)	8.06 ± 4.78	7.12 ± 3.34
Total testosterone (nmol/l)	1.8 ± 0.7	1.7 ± 0.6
Sex hormone-binding globulin (nmol/l)	54 ± 37	62 ± 43
Free androgen index	5.55 ± 5.58	4.36 ± 3.66
Estradiol (pmol/l)	162 ± 96	164 ± 82
Glucose (mmol/l)		
Fasting	5.2 ± 0.6	5.1 ± 0.6
Non-fasting	5.2 ± 0.8	5.1 ± 0.8
Insulin (pmol/l)		
Fasting	100.6 ± 74.8	79.0 ± 104.8
Non-fasting	131.4 ± 134.4	113.1 ± 87.6

HP-HMG, highly purified HMG; rFSH, recombinant FSH. All data are mean ± standard deviation except where stated.

overall incidence of preterm birth (gestational age <37 completed weeks) was 27.8% (5/18) in the rFSH group, primarily owing to the twin pregnancies, while all infants in the HP-HMG group were born at term.

Serum FSH concentrations at the end of gonadotrophin treatment were significantly higher in the HP-HMG group versus the rFSH group, while LH and estradiol concentrations were

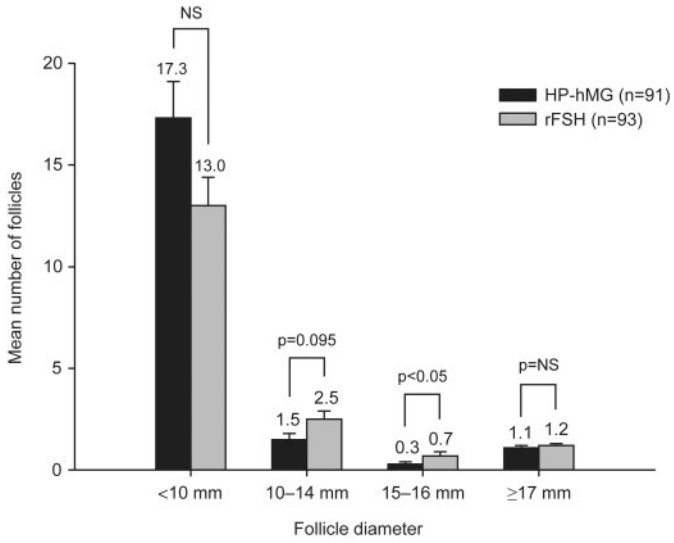

Figure 2. Distribution of follicles at the end of stimulation. Data are mean ± standard error of the mean; NS, non-significant.

not significantly different (Table III). There was no difference in endometrial thickness at the end of stimulation between groups (Table III). The mean circulating HCG level prior to administration of exogenous HCG was 1.08 IU/l in the HP-HMG-treated subjects.

The frequency of subjects with adverse events was similar in the two treatment groups (41.3% in the HP-HMG group and 40.2% in the rFSH group), and the adverse event profile was also similar. The most frequently reported adverse events were (HP-HMG versus rFSH): pelvic pain (7.6 versus 8.7%), nausea (4.3 versus 9.8%), vaginal bleeding (8.7 versus 3.3%) and headache (5.4 versus 6.5%). The local tolerability was comparable for HP-HMG and rFSH, with the majority of subjects reporting no local reactions and with most of the local reactions that were reported classified as mild. Bruising was reported by 42.4 and 37.0% of the subjects with HP-HMG and rFSH, respectively, reflecting the actual injection procedure rather than the preparation injected. Among injection site pain/reaction, pain at the injection site was reported by 27.2 and 28.3% of the subjects, redness by 15.2 and 21.7%, itching by 12.0 and 4.3% and swelling by 8.7 and 8.7% for the HP-HMG and rFSH groups, respectively.

Discussion

The present study provides evidence that the ovulation rate with an HP-HMG preparation is at least as good as that achieved with a rFSH preparation in anovulatory WHO Group II women who failed to ovulate or conceive with clomiphene citrate. However, the data suggest that the follicular dynamics resulting from ovarian stimulation with preparations that contain, or are deprived of, LH activity differs. It has been hypothesized that LH activity may be of clinical relevance in ovulation induction cycles in anovulatory women as it could facilitate selective follicular growth, decreasing the number of intermediate-sized follicles and increasing the proportion of

Table III. Overview of the clinical data

Clinical criteria	HP-HMG (n = 91)	rFSH (n = 93)
Subjects who met the HCG criteria, *n* (%)	83 (91.2)	82 (88.2)
Number of follicles according to size[a]		
≤11 mm	18.1 ± 1.80	14.2 ± 1.39
12–16 mm	1.04 ± 0.25*	1.91 ± 0.34
≥17 mm	1.12 ± 0.07	1.24 ± 0.07
Duration of gonadotrophin treatment (days)	15.3 ± 7.9	12.0 ± 5.0
Total gonadotrophin dose (IU)	1491 ± 1177	1022 ± 580
Threshold gonadotrophin dose (IU)	99.8 ± 32.2	86.4 ± 21.0
FSH level just prior to HCG administration (IU/l)	9.6 ± 2.1*	7.6 ± 2.6
LH level just prior to HCG administration (IU/l)	11.6 ± 10.9	13.5 ± 15.7
HCG level just prior to HCG administration (IU/l)	1.08 ± 0.36	—
Estradiol level just prior to HCG administration (pmol/l)	1319 ± 1284	1508 ± 1768
Endometrial thickness at the time of HCG administration (mm)	9.6 ± 2.1	9.1 ± 2.1
Subjects who received HCG, *n* (%)	82 (90.1)	84 (90.3)
Subjects who ovulated, *n* (%)	76 (83.5)	79 (84.9)
Subjects who experienced OHSS, *n* (%)	1 (1.1)	3 (3.2)
Subjects with a clinical pregnancy (7 ± 2 weeks after HCG), *n* (%)	14 (15.4)	17 (18.3)
Subjects with an ongoing pregnancy (12 ± 2 weeks after HCG), *n* (%)	13 (14.3)	16 (17.2)
Singleton pregnancy	13 (14.3)	14 (15.1)
Multiple pregnancy	0	2 (2.2)
Subjects with a live birth, *n* (%)	13 (14.3)	16 (17.2)
Singleton live birth	13 (14.3)	14 (15.1)
Multiple live birth	0	2 (2.2)
Preterm birth[b], *n* (%)	0	5 (27.8)
Admission to neonatal intensive care unit[b], *n* (%)	1 (7.7)	3 (18.8)
Birth weight[b] (g)	3560 ± 680	3174 ± 754

HP-HMG, highly purified HMG; rFSH, recombinant FSH. Data are number of subjects (percentages) or mean ± standard deviation (SD), except for [a]data on follicles which are mean ± standard error of the mean and [b]data on infants which are number of infants (percentages) or mean ± SD. OHSS, ovarian hyperstimulation syndrome.
*Significant ($P < 0.05$) difference between HP-HMG and rFSH.

women with one single dominant follicle (Loumaye *et al*., 2003). In the present study, the number of intermediate-sized follicles was reduced with HP-HMG compared with rFSH, and the proportion of women with bi-/multifollicular development tended to be lower. Despite the differences in follicular development observed in this study, the ovulation rates did not differ between HP-HMG and rFSH for either the PP or the ITT population. The different follicular dynamics between HP-HMG and rFSH was noticed after 7 days of stimulation, as the slower follicle growth observed with HP-HMG prompted the investigator (who was unaware of treatment allocation) to increase the dose at that early stage. Some of the HP-HMG subjects had the gonadotrophin dose increased despite observing follicles ≥10 mm, indicating that even though follicle development was apparent, the growth was not perceived as sufficiently rapid. The differential follicular dynamics is attributed to the LH activity rather than to the specific activity of FSH (IU/mg protein) in preparations with the same bioactivity (IU). Previous studies comparing FSH-only preparations with different specific activity of FSH, but providing the same FSH bioactivity, have suggested either lower (Yarali *et al*., 1999) or higher (Coelingh Bennink *et al*., 1998) number of follicles after stimulation with rFSH compared to urinary FSH in low-dose ovulation induction protocols. The opposite directions of these findings may reflect differences in patient characteristics between the treatment groups within the studies. None of the studies have identified a differential pattern for the intermediate-sized follicles between FSH-only preparations. The decrease in intermediate-sized follicles is associated with LH activity (Loumaye *et al*., 2003). FSH activity will promote the growth of all follicle sizes, while LH activity will affect intermediate-sized follicles which have LH receptors expressed in the granulosa cells. With respect to the treatment efficiency parameters, there were no significant differences between HP-HMG and rFSH, but because of the differential dynamics of follicular development with HP-HMG, it may take 2–3 days more to reach the HCG criteria.

The ovulation rates were in line with the assumption of 80% used in the sample size calculation, and similar to that found in previous studies with rFSH preparations reporting ovulation rates ranging from 64 to 88% with follitropin alfa (Loumaye *et al*., 1996; Yarali *et al*., 1999) and 76% with follitropin beta (Coelingh Bennink *et al*., 1998). The ongoing pregnancy rate in this study should be evaluated in the context of the type of population evaluated, the type of treatment protocol used (i.e. low-dose step-up) and the cancellation policy. Pregnancy rates were similar among groups, and in line with other reports of pregnancy rates of approximately 17% in clomiphene citrate-resistant women using low-dose protocols (Homburg and Howles, 1999; Yarali and Zeyneloglu, 2004). It has been suggested that the multiple pregnancy rate may be minimized by strict adherence to the criteria for administering HCG, and, therefore, it is important to note that the multiple pregnancies in the rFSH group occurred in subjects who did indeed adhere to the strict protocol criteria. The data from this study encourage further investigation if the incidence of multiple pregnancies could be reduced with the use of LH activity. The percentage of multiple pregnancies in ovulation induction cycles should be decreased to single digits, and if possible to be <5%, as there are substantial social, economic and health consequences of multiple pregnancies (Callahan *et al*., 1994; The ESHRE Capri Workshop Group, 2000).

An important issue to discuss is the perceived risk of OHSS in anovulatory patients receiving preparations with LH activity compared with FSH-only preparations. A meta-analysis of several studies of small sample size found no significant differences in pregnancy rate between urinary FSH preparations and menotrophins when used for ovulation induction in women with PCOS; however, a lower incidence of OHSS was reported in women receiving urinary FSH (Nugent *et al*., 2000). The findings from the present large study indicate that HP-HMG does not increase the risk of OHSS compared with rFSH preparations. Actually, the higher numbers of OHSS cases, cancellations due to excessive response and multiple pregnancies in the rFSH group could suggest that the LH activity could result in a safer and more controlled stimulation cycle.

Regarding the levels of circulating glycoproteins, the serum FSH concentration at the end of gonadotrophin treatment was on average higher in the HP-HMG group compared with the rFSH group, which was not explained by the differences in threshold dose between groups. These higher FSH levels could be explained either by different elimination kinetics of the FSH isoforms in the gonadotrophin preparations or by epitope masking due to the variable carbohydrate chains of different isoforms. The observation of similar LH levels between the subjects exposed to HP-HMG or rFSH is not surprising. The role of timing of sampling could contribute to this finding as LH has a very short half-life. In addition, HP-HMG has a low LH content, with most of the LH activity of this preparation derived from the HCG rather than LH content (Wolfenson *et al*., 2005).

In conclusion, the ovulation rate obtained with HP-HMG in WHO Group II anovulatory subjects resistant to clomiphene citrate is at least as good as that obtained with rFSH. A differential follicular response is observed in this study with the use of HP-HMG, resulting in fewer intermediate-sized follicles and less frequent bi-/multifollicular development. The use of LH activity in ovulation induction cycles might translate clinically to a lower risk of excessive response, OHSS and multiple pregnancies. Further studies are needed to confirm these findings.

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Conflict of interest

Peter Platteau, Anders Nyboe Andersen, Adam Balen and Paul Devroey have conducted clinical research sponsored by Ferring Pharmaceuticals, Serono and Organon. Per Sørensen, Lisbeth Helmgaard and Joan-Carles Arce are employees of Ferring Pharmaceuticals.

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