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Effectiveness of highly purified human menopausal gonadotropin vs. recombinant follicle-stimulating hormone in first-cycle in vitro fertilization–intracytoplasmic sperm injection patients

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Objective: To compare the effectiveness of highly purified hMG with recombinant FSH (rFSH) in IVF–intracytoplasmic sperm injection patients who were treated with a GnRH agonist.
Design: An open-label, prospective, randomized comparison of fixed gonadotropin regimens.
Setting: Eighteen Dutch IVF centers.
Patient(s): Six hundred twenty-nine patients who were selected for IVF–intracytoplasmic sperm injection.
Intervention(s): Patients were randomized to receive either highly purified hMG or rFSH in a fixed dosage of 150 IU/d after GnRH-agonist suppression (long protocol).
Main Outcome Measure(s): Ongoing pregnancy rate per started cycle. Difference between the two treatment groups was tested by using odds ratios, including the 95% confidence limits (intention-to-treat sample), and by using the Fisher's exact test (per-protocol sample).
Result(s): The ongoing pregnancy rates per started cycle were 26.3% and 25.2% for highly purified hMG and rFSH, respectively (no statistically significant difference). Treatment with highly purified hMG resulted in statistically significantly fewer oocytes (n = 7.8) than did treatment with rFSH (n = 10.6). There were no differences with respect to fertilization rates and implantation rates. Cycles with highly purified hMG were statistically significantly less often canceled as a result of ovarian hyperresponse (2.0% vs. 6.0% for highly purified hMG and rFSH, respectively).
Conclusion(s): Compared with rFSH, highly purified hMG did not result in superiority in ongoing pregnancy rates in first-cycle IVF–intracytoplasmic sperm injection patients who were treated with a fixed dosage of 150 IU of gonadotropin per day. Compared with rFSH, treatment with highly purified hMG resulted in retrieval of fewer oocytes, a lower incidence of hyperresponse, and comparable pregnancy rates. (Fertil Steril® 2008;89:1685–93. ©2008 by American Society for Reproductive Medicine.)

Key Words: Highly purified hMG, rFSH, IVF, ICSI, pregnancy rate

At present, different gonadotropin preparations such as human menopausal gonadotropins (hMG or menotropins), including both LH and FSH activity, and recombinant FSH (rFSH) preparations are used in pituitary-suppressed women who are undergoing controlled ovarian stimulation for IVF procedures. In the last decade, however, conflicting opinions on the value of exogenous LH in the process of follicular development and oocyte maturation have led to much debate (1). Studies elsewhere have shown that gonadotropin preparations containing little LH activity combined with pituitary desensitization by a GnRH agonist (GnRH-a) are effective in controlled ovarian stimulation, suggesting that in most women, the remaining endogenous LH levels in these suppressed cycles are sufficient for folliculogenesis. However, several prospective, randomized trials, comparing the effect of FSH-only and hMG preparations in IVF by using a long GnRH-a protocol, have shown that severe suppression of serum LH levels (<1 IU/L) may occur in about half of the FSH-treated subjects (2). These very low LH levels may result in relatively low E_2 serum levels, possible alterations in the physiology of the maturing follicles (2), and less favorable reproductive outcome (3, 4). In addition, it is well known that controlled ovarian stimulation in patients with hypogonadotropic hypogonadism with preparations that do not contain LH activity will not result in ongoing pregnancies (5, 6). In a Cochrane meta-analysis (7), the investigators pooled the data of four truly randomized trials comparing hMG vs. rFSH in a long GnRH-a protocol, including the study of the European and Israeli Study Group (EISG) (8). In this analysis, hMG treatment resulted in more ongoing pregnancies and live births than did rFSH, but the difference was not statistically significant. The clinical pregnancy rate (PR) per woman did reach borderline significance in favor of hMG. Considering the role of LH in the follicular steroidogenesis (9) and the

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[†] FIRM = First IVF-ICSI Cycle Recombinant FSH vs. Menotropin.
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just-mentioned data, it has been suggested that the lowered basal LH secretion that results from the use of GnRH-a, together with the absence of exogenous LH in rFSH preparations, may contribute to a decreased PR in IVF-ICSI.

Mounting evidence indicates that LH or hCG activity during ovarian stimulation treatment is capable of modulating folliculogenesis by reducing the number of smaller (10) or intermediate-sized (11) follicles. Most of the LH activity of hMG preparations is derived from the hCG content (12), which is included by external spiking in some preparations (13) or is present as a natural component of the urine of postmenopausal women in Menopur (which was used in this trial) (Menopur; Ferring GmbH, Kiel, Germany). Therefore, it may be that the hCG content in hMG preparations plays an important role in optimizing controlled ovarian stimulation by modulating the folliculogenesis, improving the endometrial receptivity, and reducing the potential risks such as ovarian hyperstimulation syndrome (OHSS) (10). The positive effects of exogenous LH or hCG activity derived from hMG on folliculogenesis, embryo quality, and endometrial receptivity have been addressed recently in large randomized trials (11, 14, 15).

The aim of the present randomized study was to prove superiority (in terms of ongoing PRs) of highly purified hMG vs. rFSH in a relevant clinical setting. Normogonadotropic women undergoing their first IVF-ICSI cycle in a long GnRH-a protocol were treated with a fixed dose of 150 IU/d, taking ongoing PR as the primary endpoint. Fixation of the dose allows an unbiased comparison of the stimulation characteristics of highly purified hMG and rFSH. Results were discussed in the light of recent insights on the effects of LH activity on folliculogenesis, embryo quality, and endometrial development.

MATERIALS AND METHODS

Subject Population

On the basis of power calculations, >600 women were randomized who were scheduled for IVF or ICSI according to the criteria of the participating center. The main selection criteria were the following: healthy females, ≥18 and ≤39 years of age, with no endocrine abnormality such as polycystic ovary syndrome, hypogonadotropic hypogonadism, or day 3 FSH levels of >12 IU/L. As treatment differences in reproductive outcome become more pronounced in first-cycle attempts (16), the patient selection was limited to women who were undergoing their first IVF-ICSI treatment.

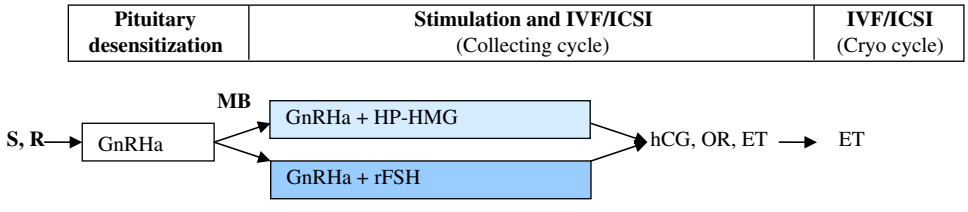
Study Design

This was an open-label, randomized, multicenter study to compare the effectiveness of highly purified hMG vs. rFSH in first-cycle IVF-ICSI patients. The study was approved by the institutional review board of the Vrije Universiteit Medical Centre Amsterdam and by the independent ethics committee of each participating center, and it was conducted according to the Declaration of Helsinki (October 2000 version).

Figure 1 presents an overview of the study treatments and procedures. All patients were informed about the purpose and possible hazards of this study both orally and in writing and gave their written consent, after which they were randomized in a 1:1 ratio to treatment with highly purified hMG or with rFSH. A central randomization procedure was followed by using an interactive voice response system. Permuted blocks of random size that were stratified on center were used. Treatment with GnRH-a (daily SC injections of either 1 mg leuprorelin-acetate [Lucrin; Abbott Laboraties B.V., Zwolle, The Netherlands] or 0.1 mg triptorelin [Decapeptyl; Ferring GmbH]) was started in the midluteal phase of the menstrual cycle and continued until the day of hCG injection. After occurrence of the menstrual bleeding after the pituitary desensitization, gonadotropin treatment was started, but only if ultrasound showed no formation of a major (diameter of >2 cm) or functional (serum E_2 of <200 pmol/L) cyst. If such a cyst appeared persistent during the treatment of the GnRH-a, and E_2 levels did not drop below 200 pmol/L within 2 weeks after the menstrual bleeding, the patient was excluded from the trial. Highly purified hMG (Menopur) or rFSH (either Gonal-F [Serono S.A., Aubonne, Switzerland] or Puregon [N.V. Organon, Oss, The

FIGURE 1

Study design. HP-hMG = highly purified hMG.



MB= menstrual bleeding; ET= embryo transfer; OR= oocyte retrieval; R= randomization; S= screening and informed consent procedure

Hompes. Effectiveness of hMG vs. rFSH in IVF-ICSI. Fertil Steril 2008.

Netherlands]) was injected SC in a fixed dosage of 150 IU/d. This dosage reflects the starting dosage in common practice in Europe. The purification process of highly purified hMG allows administration of this preparation SC, with a local tolerability profile that is comparable with that of rFSH (8). Because the appearance of these commercially available formulations differs, blinding was not performed. Investigators were instructed to keep the daily dosage at 150 IU unless a hyperresponse (too many follicles) or poor response (too few follicles) occurred, according to the judgment of the participating center. In these cases, one could cancel the stimulation cycle or decide to decrease or respectively increase the daily dose. Patients were documented as having "hyperresponse" and "poor response", respectively. Minimum criteria for hCG injection were at least three follicles with a diameter of $\geq$16 mm, but with the stimulation phase not exceeding 14 days (in poor responders).

The day after the last gonadotropin injection, hCG was administered (10,000 IU, either SC or IM).

Oocytes were retrieved 32–42 hours after hCG administration. In vitro fertilization–ICSI procedures and embryo transfer were performed according to the clinic's standard practice. No more than two embryos were transferred. Vaginal administration of P (Progestan [N.V. Organon], 3 $\times$ 200 mg per d) was given as luteal support. In frozen embryo transfer cycles, procedures also were performed according to local clinical practice.

Assessments

Primary efficacy endpoint was the ongoing PR per started collecting cycle. The start of a cycle was defined as the start of gonadotropin treatment. Ongoing pregnancy was defined as positive heart action, confirmed $\geq$10 weeks after embryo transfer (ET) by ultrasound examination.

Other outcome parameters assessed were total highly purified hMG/rFSH dose (IU), days of gonadotropin stimulation, serum E_2 and LH values on the day of hCG, number of oocytes retrieved, fertilization rate (number of fertilized oocytes per number of retrieved oocytes in %), number of embryos (transferred and cryopreserved), implantation rate (number of embryonic sacs observed by ultrasound per number of transferred embryos), and (ongoing) PRs per embryo transfer. In addition, the incidence of adverse events was evaluated.

Because the success rate of IVF-ICSI also depends on the results obtained with the cryopreserved embryos, cumulative outcome data were determined during 1 year after the embryo transfer in the collecting cycle. Using these cumulative data, final delivery rate (percentage of subjects who delivered) and live-birth rate (percentage of subjects who delivered a live-born baby) were assessed.

Statistical Analysis

For the PRs, both the intention-to-treat (ITT) analysis and per-protocol (PP) analysis have been performed. The ITT analysis is based on all randomized patients who started highly purified hMG or rFSH treatment, and the PP analysis is based on all patients with embryo transfer who did not incur any major protocol violations likely to bias the efficacy evaluation and who did not change the gonadotropin dosage. For all secondary efficacy parameters, PP analyses were performed, based on all PP patients for whom that specific parameter was evaluated. Baseline characteristics were analyzed for all subjects who were randomized, and the adverse events, for all subjects who were treated (subjects actually treated or ITT subjects).

Summary statistics (mean, SD, median, minimum, and maximum) were evaluated for all parameters. The two-tailed Fisher's exact test was used for the treatment effect on (ongoing) PRs, final delivery, and live-birth rates. For the other efficacy results, the difference between two treatment groups was assessed with the Wilcoxon test. Treatment effect on the incidence of adverse events and the number of adverse events per patient was analyzed with the two-tailed Fisher's exact test and the Wilcoxon test, respectively. In addition, 95% confidence limits were calculated for all outcome parameters. In a post hoc ITT analysis of ongoing PR (described in the statistical analysis plan section "efficacy results"), logistic regression on treatment effect was assessed with determination of noninferiority on the basis of the odds ratio and its 95% confidence intervals (CIs). The noninferiority limit of highly purified hMG vs. rFSH was prespecified as 0.650.

The choice of the sample size was based on the ITT analysis of the primary endpoint, for example, the ongoing PR per started collecting cycle. A study published elsewhere (16), comparing the PRs of hMG vs. rFSH in IVF-ICSI, reported an ongoing PR rate of 33% for rFSH and of 45% for hMG in first-cycle patients on the basis of a PP analysis. Assuming that the ITT analysis will contain about 10% more patients than the PP analysis, all without ongoing pregnancy, we anticipated that the ongoing PR for rFSH is about 30% and that the PR for hMG would be about 11% higher. On the basis of these percentages, it has been calculated that the sample size needed for 80% power is 293 patients per group. So, rounded off, a total of 600 randomized patients was needed.

RESULTS

Subjects

Disposition and data sets analyzed In total, 18 Dutch IVF centers participated, with the number of subjects randomized per center ranging from 5 to 77. Table 1 presents the disposition of subjects. A total of 629 were randomized. A major violation of entry criteria (highly purified hMG, n = 13; rFSH, n = 11) or withdrawal of consent (highly purified hMG, n = 6; rFSH, n = 4) before the start of study medication were reasons to exclude these patients from the full analysis set. Therefore, the remaining group of 595 subjects who started gonadotropin treatment was defined as the ITT population.

A total of 76 subjects discontinued before hCG injection. The main reason for discontinuation was poor response; this included 29 (9.9%) and 24 (7.9%) ITT cases for the highly

TABLE 1

Disposition of subjects and data sets analyzed.

Analysis set	HP-hMG, n (%)	rFSH, n (%)
Randomized subjects (= ASR)	312 (100)	317 (100)
All subjects with study treatment (= ITT)	293 (93.9)	302 (95.0)
PP subjects with hCG injection	222 (71.2)	238 (75.1)
PP subjects who had OR	220 (70.5)	238 (75.1)
PP subjects who had IVF and/or ICSI	219 (70.2)	236 (74.4)
PP subjects with ET in collecting cycle	198 (63.5)	209 (65.9)
After IVF	122	123
After ICSI	76	88[a]

Note: HP = highly purified; ET = embryo transfer; OR = oocyte retrieval; ASR = all subjects who were randomized.
[a] Two PP subjects with ET received both IVF and ICSI.

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purified hMG and rFSH group, respectively (P=.4722). In the remaining group of 519 ITT subjects receiving hCG, a total number of 46 subjects were excluded from PP analysis because of the change of dose during the stimulation period in case of a poor ovarian response or hyperresponse. In addition, a total of 13 major protocol violations (not included in the PP analysis) were recorded in the highly purified hMG (n = 7) and rFSH (n = 6) group, respectively.

Total number of poor-response subjects The total number of subjects with poor response consists of the ones who discontinued because of poor response plus the ones who continued on a higher dose, irrespective of whether the latter subjects completely finished the IVF-ICSI cycle (excluded from PP analysis). The total number of poor response subjects, with or without dose increase, was 47 (16.0%) in the highly purified hMG group and was 31 (10.3%) in the rFSH group (P<.05).

Characteristics Baseline characteristics are presented in Table 2, which shows that both treatment groups were comparable with regard to the main demographic characteristics and gynecological anamnesis. The numbers of subjects who had a standard IVF treatment were 155 and 156 in the highly purified hMG and rFSH group, respectively. The numbers of ICSI cycles were 90 and 101, respectively. In the rFSH group, three subjects had a combined IVF-ICSI procedure. All subjects were first–IVF-ICSI cycle patients and gave written informed consent.

Ongoing PR (Primary Endpoint)

The ongoing PRs per started stimulation (ITT) were 26.3% in the highly purified hMG group vs. 25.2% in the rFSH group (Table 3). For the ongoing PR per started stimulation, the odds ratio of highly purified hMG vs. rFSH is 1.054 (95% CI, 0.778–1.428). Therefore, superiority of highly purified hMG could not be demonstrated. However, in a post hoc analysis, noninferiority of highly purified hMG was established, because the lower odds ratio limit is clearly above the prespecified noninferiority limit.

The ongoing PRs (95% exact confidence limits) per embryo transfer for the PP sample were 34.8% (28.2%–41.9%) for highly purified hMG and 32.1% (25.8%–38.8%) for rFSH (Table 3). The difference was not statistically significant (P=.5994).

Table 3 also presents the ongoing PRs per transfer for IVF and ICSI separately. For none of these subgroups was the

TABLE 2

Demographic baseline data, main reason for infertility, and fertilization procedure.

Demographic baseline data	HP-hMG (n = 312)	rFSH (n = 317)
Age (y)	31.7 ± 3.8	32.0 ± 3.7
Body mass index (kg/m^2)	24.1 ± 4.2	24.1 ± 4.4
Duration of infertility (mo)	36.9 ± 19.2	37.0 ± 17.7
Age class		
<30 y	28.6	23.0
30–34 y	44.7	49.5
35–39 y	26.7	27.4
Main reason for infertility		
Male factor	182 (58.3)	185 (58.4)
Tubal factor	42 (13.5)	37 (11.7)
Combination	9 (2.9)	10 (3.2)
Unexplained	65 (20.8)	69 (21.8)
Anovulation	3 (1.0)	5 (1.6)
Other	11 (3.5)	11 (3.5)
Fertilization procedure		
IVF	155 (49.7)	153 (48.3)
ICSI	90 (28.8)	98 (30.9)
IVF and ICSI	0	3 (0.9)

Note: Data are mean ± SD, percentages, or n (%).
HP = highly purified.

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TABLE 3

Primary and secondary efficacy endpoints (PP, ITT).

Parameter	HP-hMG	rFSH	*P* value
Mean (PP)			
Total injected dosage (IU)	1,821.0	1,759.7	.0654
No. of stimulation days	12.1	11.7	.0654
Serum LH levels, day of hCG injection (IU/L)	1.88	1.86	.8690
Serum E_2 levels, day of hCG injection (pmol/L)	6,364.3	7,004.8	.0184[d]
Serum E_2 level/no. of oocytes retrieved (pmol/L)	820.1	663.3	—
No. of oocytes retrieved	7.76	10.56	<.0001[d]
No. of oocytes fertilized	4.16	5.61	.0007[d]
Fertilization rate (%)	63.1	60.5	.3471
No. of embryos transferred	1.67	1.69	.6330
No. of embryos cryopreserved	3.74	3.90	.9250
Implantation rate in fresh cycle (%)	29.3	25.8	.3261
Biochemical pregnancy rate in fresh cycle (%[a])			
ITT	31.7	29.8	.6570
PP	41.9	38.3	.4795
Ongoing pregnancy rate in fresh cycle (%[a])			
ITT	26.3	25.2	NS
PP	34.8	32.1	.5994
Ongoing pregnancy rate in fresh cycle, for IVF and ICSI separately (%[a])			
ITT[b]	IVF, 28.5; ICSI, 35.6	IVF, 27.4; ICSI, 33.7	
PP	IVF, 34.4; ICSI, 35.5	IVF, 29.3; ICSI, 36.4	
Cumulative[c] ongoing pregnancy rate (%[a])			
ITT	28.0	26.6	.7130
PP	37.4	33.8	.4702
Cumulative[c] delivery rate (%[a])			
ITT	28.0	26.2	.6450
PP	37.4	33.5	.4085
Cumulative[c] live-birth rate: ITT (%[a])	27.0	24.8	.5752

Note: HP = highly purified; NS = not statistically significant: odds ratio = 1.054 (95% CI, 0.778–1.428).

[a] ITT analysis: % of all subjects treated; PP analysis: % of subjects with embryo transfer.

[b] Percentages based on all ITT subjects who actually had an IVF or ICSI rather than on all subjects treated, as in the above-mentioned ongoing pregnancy rates.

[c] Including 1-y outcome of cryo cycles.

[d] Statistically significant at .05 level.

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difference between the highly purified hMG and rFSH group statistically significant. The most pronounced trend in difference in ongoing PR was observed for the PP sample after IVF. In this subgroup, the PRs (exact 95% confidence limits) were 34.4% (26.1%–43.6%) for highly purified hMG and 29.3% (21.4%–38.1%) for rFSH (*P*=.4127).

Secondary Endpoints

Table 3 presents a survey of the treatment effects on all secondary endpoints. A statistically significant difference was found between E_2 levels at day of hCG injection with highly purified hMG vs. rFSH (*P*=.0184).

The mean number of oocytes retrieved was lower in the highly purified hMG group compared with the rFSH group, with a difference (from rFSH) of −2.80 oocytes (95% CI, −4.23 to −1.36). The mean number of oocytes fertilized was also lower in the highly purified hMG group compared with the rFSH group, with a difference of −1.45 oocytes (95% CI, −2.41–−0.50). No significant treatment differences were seen in terms of LH levels at day of hCG injection, fertilization rate, number of embryos transferred and cryopreserved, and implantation rate.

The biochemical PRs both per started stimulation and per transfer were slightly higher in the highly purified hMG

group, although there was no statistically significant difference between both treatments. The number of subjects with embryo transfer in cryo cycle 1 and 2 was 19 and 1 in the highly purified hMG group and was 31 and 4 in the rFSH group. The numbers of ongoing pregnancies in these cryo cycles were 5 of 20 in the highly purified hMG group and were 4 of 35 in the rFSH. No statistically significant differences were observed for the ongoing PRs in the cryo cycles. The same applies to the cumulative ongoing PRs, cumulative delivery rate, and cumulative live-birth rate (combining results of collecting cycle and 1-y outcome of cryo cycles). The multiple-birth rate for the collecting cycle (PP analysis) was 7.1% (95% CI, 3.9%–11.6%) in the highly purified hMG group, vs. 6.2% (95% CI, 3.4%–10.4%) in the rFSH group. No significant difference was detected (P=.8427).

Safety

The incidence of adverse events, evaluated in all subjects treated (ITT), is summarized in Table 4. The only adverse event resulting in discontinuation of treatment was ovarian hyperresponse. The number of subjects discontinuing because of ovarian hyperresponse was 6 (2.0%) with highly purified hMG and was 18 (6.0%) with rFSH. The difference is significant (P=.0204). The percentages of adverse events indicated as possibly or probably related to highly purified hMG and rFSH treatment were 8.9% and 13.9%, respectively. The most frequently recorded adverse events were ovarian hyperresponse, OHSS (Table 4), missed abortion (5 with highly purified hMG and 3 with rFSH), and headache (3 with highly purified hMG and 5 with rFSH). A difference between treatment groups was observed for the adverse events "ovarian hyperresponse and/or OHSS," which were more frequently reported with rFSH treatment (13.2%), compared with the case of highly purified hMG treatment (6.1%). The difference was statistically significant (P=.0036). A total of 6 (of 10) cases of OHSS were considered to be serious, 1 with highly purified hMG and 5 with rFSH. Three of these serious OHSS cases were considered to be related to study treatment (all rFSH).

DISCUSSION

The present study is the first large randomized trial, comparing highly purified hMG vs. rFSH, that used a consistent long GnRH-a protocol and a fixed low gonadotropin dose in first-cycle IVF-ICSI patients, using ongoing pregnancy as the primary endpoint. Superiority of highly purified hMG over rFSH in terms of higher ongoing PRs could not be demonstrated with this fixed-dose regimen. In the Cochrane meta-analysis (7) on the effectiveness of hMG and rFSH in IVF-ICSI cycles, it became evident that hMG treatment resulted in a higher clinical PR and in higher ongoing pregnancy and live-birth rates than did rFSH, but the latter difference was of borderline significance. However, the heterogeneous pituitary suppression regimens and the flexible gonadotropin dosages used in these studies limited the potential for discriminating the features of these two gonadotropin preparations. A more recent study (17) did use more uniform procedures, such as a fixed gonadotropin dosage (150 IU/d). However, because small numbers of patients were used (50 patients per treatment arm), no statistically significant difference could be found in reproductive outcome. The importance of using a fixed gonadotropin dose was confirmed by the authors of the Cochrane review of 2003 (7). This approach had never been followed before in a comparative

TABLE 4

Adverse events (ITT group).

Parameter	HP-hMG, n (%) (n = 293)	rFSH, n (%) (n = 302)	*P* values
Subjects with ≥1 adverse event (AE)	59 (20.1)	72 (23.8)	—
Subjects with ≥1 serious adverse event	9 (3.1)	16 (5.3)	—
Subjects with AEs (all, "ovarian hyperresponse") that resulted in cancellation	6 (2.0)	18[a] (6.0)	.0204[c]
Subjects with drug-related[b] AEs	26 (8.9)	42 (13.9)	—
Subjects with AE classifications			
Ovarian hyperresponse	16 (5.8)	32 (10.6)	—
OHSS	2 (0.7)	8 (2.6)	—
Ovarian hyperresponse and/or OHSS	18 (6.1)	40 (13.2)	.0036[c]
No. of adverse events per patient with ≥1 AE	1.1	1.26	.3391

Note: HP = highly purified.

[a] Including 1 subject who discontinued before ET in fresh cycle because of ovarian hyperresponse but who did have ET in cryo cycles.

[b] Related, as indicated by the investigator: probable or possible.

[c] Statistically significant at .05 level.

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study of this size. It has the advantage that it minimizes the potential for post-randomization variability. This is especially important in open studies, where investigators theoretically could introduce a bias by selectively adapting the dosage of a certain gonadotropin preparation.

However, as stated by Arce et al. (18), a strict fixed dose also has disadvantages and may not be ideal for evaluating PRs. The outcome is conditioned by the proportion of patients who respond to the dose selected. In this study, the fixed daily dose of 150 IU was insufficient or suboptimal for a considerable number of subjects, especially in the highly purified hMG group (16.0% in poor responders vs. 10.3% in the rFSH group; $P<.05$). If the dose had been increased upon observed response, more subjects may have responded normally, and a lower number of cases may have been canceled as a result of poor response, especially in the highly purified hMG group, which could have resulted in a higher PR. The significantly higher occurrence of poor responses in the highly purified hMG group could not be attributed to a higher proportion of older patients in this group, because the frequency distribution over age classes was similar in both treatment groups (Table 2). Further analysis on the influence of age was beyond the scope of this trial and will be discussed separately.

A higher fixed dose, however, will lead to more withdrawals as a result of ovarian hyperresponse and/or the occurrence of OHSS. Even with the relatively low daily dose of 150 IU used in our study, cycles were canceled because of ovarian hyperresponse, on advice of the participating center, in 2.0% and 6.0% of all started cycles for highly purified hMG and rFSH, respectively ($P<.05$). The incidence of OHSS was 0.7% and 2.6%, respectively. However, because there was no unequivocal definition of OHSS, these percentages do not allow firm conclusions about the incidence of OHSS. In two large studies performed elsewhere that compared highly purified hMG and rFSH in a long down-regulation protocol for IVF and/or ICSI, the OHSS incidences were similar in both treatment groups (8, 19). In these studies, however, the dosage could be individually adjusted after 5 days of treatment (225 IU/d). It is, however, noteworthy that a recent comparison of these preparations in ovulation induction cycles (11) revealed that the rate of OHSS and/or cancellation as a result of hyperresponse was 2.2% with highly purified hMG and was 9.8% with rFSH ($P=.058$), which is in line with our current findings.

Fixation of the dose allows a direct comparison of the two gonadotropin preparations with respect to their effects on the ovaries. The results of the present trial demonstrate that highly purified hMG and rFSH induce a different stimulation profile. At an equal dose, highly purified hMG displays a milder stimulation pattern, reflected in a higher cancellation rate as a result of a poor ovarian response, a lower mean number of oocytes, lower E_2 serum levels, a lower incidence of ovarian hyperresponse, and a trend toward a smaller incidence of OHSS. The lower E_2 levels could be attributed to the lower mean number of follicles on the day of hCG in the highly purified hMG group. Comparison of the ratio of E_2 level to number of oocytes (as indicator of the number of follicles) provides a reverse result, that is, 820 pmol/L per oocyte vs. 663 pmol/L per oocyte for highly purified hMG and rFSH, respectively. This higher E_2 production per follicle observed with highly purified hMG also was observed in studies elsewhere that compared the same preparations. In those studies, higher E_2 serum levels (on day of hCG) were found in the highly purified hMG group, whereas the number of oocytes or follicles was similar (8) or slightly lower (19). The higher estrogen production per oocyte in the highly purified hMG group may be a result of the LH-like activity in highly purified hMG, resulting in both an induced formation of the androgen substrate in the smaller follicles, needed for conversion to estrogens, and a direct androgen turnover to estrogens in the granulosa cells of larger follicles also expressing LH or hCG receptors (10). The lower number of oocytes retrieved may be considered to be affected by either the additional influence of exogenous LH-like activity or by a difference among the preparations in FSH biopotency (11). The lack of differences in serum LH levels at the end of stimulation in the highly purified hMG vs. rFSH groups could be explained by the fact that highly purified hMG contains virtually no LH, because most of the LH activity is derived from the hCG content (12).

Despite the lower number of oocytes retrieved, highly purified hMG treatment resulted in a similar ongoing PR per started cycle and in a slightly higher ongoing PR per transfer (not reaching statistical significance), as compared with rFSH. This picture remains unaltered when the 1-year outcome of cryo cycles is included. The trend toward higher ongoing PRs per transfer for highly purified hMG compared with rFSH was more explicit after IVF than after ICSI. It has been suggested that the cumulus cells exposed to LH activity could play a role in oocyte and embryo development (19, 20). Recent identification of gene expression in cumulus cells during oocyte maturation (21) supports this hypothesis. However, because we did not use stratification for the fertilization method, this result only has an exploratory character. These results of the present trial are very similar to the results of the latest Cochrane meta-analysis (7) and to those of a recent trial comparing highly purified hMG with rFSH in IVF (19). A higher ongoing PR with highly purified hMG, compared with rFSH, was found but did not reach statistical significance, despite a lower number of oocytes retrieved. These results suggest that LH activity plays a role in improving the oocyte and embryo quality and/or the endometrial receptivity.

From that latest comparative trial (19), it indeed became evident that compared with rFSH, highly purified hMG resulted in a higher proportion of top-quality embryos per oocyte retrieved ($P<.05$). This is in line with results obtained elsewhere in macaques, revealing that LH activity may improve embryo viability (22). The similar LH serum levels in both highly purified hMG and rFSH at the end of stimulation in our trial are not surprising, because LH has a very short half-life, and the timing of the sampling is essential

for this finding. The most important LH activity in highly purified hMG appeared to be provided by the longer-acting hCG (11, 14, 15). It is noteworthy that increased concentrations of hCG on day 6 of stimulation in the highly purified hMG group were associated with a higher proportion of top-quality embryos and higher ongoing PRs in IVF cycles (19, 20). It also became evident that in the rFSH group, a higher proportion of patients had increased P levels (>4 nmol/L) at the end of stimulation, associated with an advanced hyperechogenic transformation of the endometrium and lower ongoing implantation and PRs ($P<.05$) (19, 23).

In addition, another recent study (11) that compared highly purified hMG and rFSH for ovulation induction demonstrated that the LH activity in highly purified hMG induces a more modulated folliculogenesis that is associated with a lower risk of excessive ovarian response, comparable to our findings, and an ovulation rate similar to that obtained with rFSH.

The data mentioned in the last three paragraphs support the suggestion that LH activity plays a role both in optimizing the quality and developmental potential of the oocytes obtained and in improving the endometrial receptivity. However, because no statistically significant difference could be found between the PRs that were obtained with highly purified hMG vs. rFSH stimulation, the significance of the different pharmacodynamic profiles of these two gonadotropins to the reproductive outcome should be further investigated by even larger efficacy trials or by a meta-analysis.

In conclusion, a fixed daily dose of 150 IU led to comparable ongoing PRs for rFSH and highly purified hMG. Nevertheless, there appears to exist a fundamental difference in the stimulation profile of these treatments. Highly purified hMG reveals itself as a milder preparation with fewer ovarian hyperstimulation phenomena and comparable pregnancy results at lower oocyte yields. The higher oocyte yield with rFSH does not result in more pregnancies, even when the results of cryo cycles are included.

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