Title:

Randomized, assessor-blinded trial comparing highly purified human menotropin and recombinant follicle-stimulating hormone in high responders undergoing intracytoplasmic sperm injection

Authors:

Craig A. Witz, M.D., Gaurang S. Daftary, M.D., M.B.A., Kevin J. Doody, M.D., John K. Park, M.D., M.S., M.Sc., Yodit Seifu, Ph.D., Vladimir I. Yankov, M.D., and Patrick W. Heiser, Ph.D.,

Journal:

Fertil Steril 2020; 114(2):321-330.

Randomized, assessor-blinded trial comparing highly purified human menotropin and recombinant follicle-stimulating hormone in high responders undergoing intracytoplasmic sperm injection

Craig A. Witz, M.D.,[a] Gaurang S. Daftary, M.D., M.B.A.,[b] Kevin J. Doody, M.D.,[c] John K. Park, M.D., M.S., M.Sc.,[d] Yodit Seifu, Ph.D.,[b] Vladimir I. Yankov, M.D.,[b] and Patrick W. Heiser, Ph.D.,[b] on behalf of the Menopur in GnRH Antagonist Cycles with Single Embryo Transfer – High Responder (MEGASET-HR) Trial Group

[a] Houston Fertility Institute, Houston, Texas; [b] Ferring Pharmaceuticals, Inc., Parsippany, New Jersey; [c] Center for Assisted Reproduction, Bedford, Texas; and [d] Carolina Conceptions, Raleigh, North Carolina

Objective: To evaluate the efficacy and safety of highly purified human menotropin (HP-hMG) and recombinant follicle-stimulating hormone (rFSH) for controlled ovarian stimulation in a population of patients predicted to be high responders.
Design: Randomized, open-label, assessor-blinded, parallel-group, noninferiority trial.
Setting: Fertility centers.
Patient(s): A total of 620 women with serum antimüllerian hormone (AMH) $\geq$5 ng/mL.
Intervention(s): Controlled ovarian stimulation with HP-hMG or rFSH in a GnRH antagonist assisted reproductive technology (ART) cycle. Fresh transfer of a single blastocyst was performed unless ovarian response was excessive, in which all embryos were cryopreserved. Subjects could undergo subsequent frozen blastocyst transfer within 6 months of randomization.
Main Outcome Measure(s): Ongoing pregnancy rate (OPR) after fresh transfer (primary endpoint), as well as cumulative live birth, ovarian hyperstimulation syndrome (OHSS), and pregnancy loss rates.
Results: OPR/cycle start after fresh transfer was 35.5% with HP-hMG and 30.7% with rFSH (difference: 4.7%, 95% CI −2.7%, 12.1%); noninferiority was established. Compared to rFSH, HP-hMG was associated with significantly lower OHSS (21.4% vs. 9.7% respectively; difference: −11.7%, 95% CI −17.3%, −6.1%) and cumulative early pregnancy loss rates (25.5% vs. 14.5% respectively; difference: −11.0%, 95% CI −18.8%, −3.14%). Despite 43 more transfers in the rFSH group, cumulative live birth rates were similar with HP-hMG and rFSH at 50.6% and 51.5% respectively (difference: −0.8%, 95% CI −8.7%, 7.1%).
Conclusion(s): In high responders, HP-hMG provided comparable efficacy to rFSH with fewer adverse events, including pregnancy loss, suggesting its optimized risk/benefit profile in this population.
Clinical Trial Registration Number: NCT02554279 (clinicaltrials.gov). (Fertil Steril® 2020;114:321–30. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Highly purified menotropin, Menopur, recombinant FSH, high responders, GnRH antagonist

Received November 26, 2019; revised March 2, 2020; accepted March 19, 2020; published online May 13, 2020.
C.A.W. was an investigator in the MEGASET-HR trial. G.S.D. is a full-time employee of Ferring Pharmaceuticals, Inc. K.J.D. was an investigator in the MEGASET-HR trial. J.K.P. was an investigator in the MEGASET-HR trial. Y.S. was employed by Ferring Pharmaceuticals, Inc. at the time that the MEGASET-HR trial was conducted. V.I.Y. is a full-time employee of Ferring Pharmaceuticals, Inc. P.W.H. is a full-time employee of Ferring Pharmaceuticals, Inc.
Supported by Ferring Pharmaceuticals, Inc. USA Parsippany, NJ.
The results of this trial were previously presented, in part, at the American Society for Reproductive Medicine 2017 Scientific Congress and Expo, San Antonio, Texas, October 28-November 1, 2017; at the Pacific Coast Reproductive Society annual meeting; Indian Wells, California, March 21-25, 2018; and at the American Society for Reproductive Medicine 2018 Scientific Congress and Expo, Denver, Colorado, October 7-10, 2018.
Reprint requests: Patrick W. Heiser, Ph.D., 100 Interpace Parkway, Parsippany, NJ 07054 (E-mail: Patrick.Heiser@ferring.com).

Fertility and Sterility® Vol. 114, No. 2, August 2020 0015-0282

https://doi.org/10.1016/j.fertnstert.2020.03.029

Patients undergoing assisted reproductive technologies (ART) are heterogeneous in their response to gonadotropin stimulation. Further improvement in efficacy, safety, and cycle efficiency requires greater personalization of gonadotropin treatment. Newer technologies have provided an opportunity to achieve such personalization by tailoring gonadotropin dose to ovarian potential. Serum antimüllerian hormone (AMH) levels and ultrasound-derived antral follicle count (AFC) have both been shown to be robust predictors of ovarian response. However, AFC lacks methodological standardization and is dependent on ultrasound-operator skill, making AMH the preferred biomarker, particularly in the context of multicenter clinical trials (1, 2). AMH levels have successfully guided gonadotropin dosing in ART, and therefore can be used to more accurately define and select specific study populations (3).

High responders exhibit good overall prognosis with robust follicular response, serum estradiol (E_2) levels, and oocyte yields (>15) (4, 5). However, these gains are tempered by a high risk of developing ovarian hyperstimulation syndrome (OHSS) and/or cancellation of fresh embryo transfer to prevent it, causing an undesirable delay to pregnancy and the possibility of increased cost. Current strategies for such patients are reactive, delay time to pregnancy, and focus on mitigation of complications instead of proactive benefit (6–8) Thus, there is an unmet need to proactively identify patients at risk for hyper response prior to the start of stimulation, and to develop new treatment approaches to improve care in this patient population.

Serum AMH measurements have been successfully used to predict likely high responders (2, 3, 9). Moreover, a recent retrospective study indicated that there is an opportunity to personalize treatment of this cohort based not just on dose but also upon the type of gonadotropin used to drive controlled ovarian stimulation. Baseline serum AMH testing of samples collected in a post hoc analysis of two phase 3 randomized controlled trials were used to define patient quartiles. Both trials compared outcomes in patients treated with either human-derived HP-hMG (a gonadotropin mixture of follicle-stimulating hormone [FSH] and human chorionic gonadotropin [hCG]–derived luteinizing hormone [LH] activity) or recombinant follicle stimulating hormone (rFSH) produced in Chinese hamster ovarian cells. In addition to these constituent differences in the gonadotropin preparations, there is also species-related diversity in tertiary protein structure that informs their biological activity and clearance (10–12). The analysis found that HP-hMG treatment of potential high responders, defined as patients with baseline AMH levels in the highest quartile (>5.2 ng/mL), was associated with a lower median number of retrieved oocytes, significantly lower incidence of high response (>15 oocytes), fewer interventions for OHSS, and increased live birth rate compared with rFSH in GnRH agonist or antagonist protocols (13–15).

In the United States, most clinicians use both HP-hMG and rFSH concurrently as mixed stimulation protocols. However, there is an absence of high-quality evidence demonstrating the advantages of such an approach over stimulation with either gonadotropin preparation alone. Furthermore, there is tremendous heterogeneity in the ratios of HP-hMG:rFSH as applied in current clinical practice, which makes it difficult to select meaningful consensus ratios for evaluation in a trial. Finally, any difference attributable to specific gonadotropin preparations can be determined only when their use as single agents is compared.

Therefore, the aims of the current noninferiority trial were as follows: to prospectively evaluate the efficacy and safety of HP-hMG vs. rFSH treatment, aligned with previous studies, as a proof-of-concept in a predefined population of patients predicted to be high responders; to characterize differences first observed in the earlier analysis; and to offer a novel strategy for therapeutic personalization.

MATERIALS AND METHODS

Trial Design

MEGASET-HR was an open-label, parallel-group trial conducted in 31 U.S. centers between August 2015 and February 2018 in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and applicable regulatory requirements. The first subject was randomized and enrolled on October 15, 2015. The trial was assessor blinded to individuals performing the ultrasound monitoring, embryologists, and central laboratory personnel.

Trial Population

Eligible subjects included women aged 21–35 years with menstrual cycles of 21–45 days, body mass index (BMI) 18–30 kg/m^2, infertility for ≥1 year, day 2 or 3 serum FSH levels of 1–12 IU/L, total testosterone, prolactin, and thyroid stimulating hormone within normal limits, and serum AMH ≥5 ng/mL at screening. All AMH measurements were determined at a single reference laboratory (ReproSource, Inc.), using materials and reagents from Beckman Coulter-DSL. As an additional step to ensure assay consistency, this laboratory used a reference serum sample to normalize AMH measurements. Women with stage III–IV endometriosis; history of recurrent miscarriage; previous ART failure from poor response; AFC (diameter 2–10 mm) <10 for both ovaries combined and/or use of hormonal birth control <3 months prior to screening were excluded. Full inclusion and exclusion criteria are listed in Supplemental Table 1.

Trial Procedures

Randomization lists were generated by the study statistician prior to the first subject's initial visit. Subjects were randomized 1:1 to controlled ovarian stimulation with HP-hMG (Menopur, Ferring Pharmaceuticals) or rFSH (Gonal-f; EMD-Serono) immediately prior to administration of trial drug. Randomization numbers were allocated sequentially to the subjects in the order in which they were randomized. Treatment was initiated on day 2 or 3 of the menstrual cycle at a dose of 150 IU HP-hMG or rFSH for the first 5 days (Supplemental Fig. 1). Starting at day 6, the dose could be adjusted daily by 75 IU. The maximum daily dose was 300 IU/d and the maximum treatment duration was 20 days. Coasting was not permitted. Once the lead follicle measured $\geq$14 mm and/or serum E_2 levels were $\geq$300 pg/mL, 0.25 mg/d GnRH antagonist (Ganirelix acetate injection; Ganirelix; Merck) was administered. When three follicles of $\geq$17 mm were observed by transvaginal ultrasound, oocyte maturation was induced by 250 μg hCG (Ovidrel; EMD-Serono). Oocytes were retrieved ~36 hours after hCG administration and inseminated by intracytoplasmic sperm injection (ICSI) 4 $\pm$ 1 hours after retrieval. DNA was prepared from trophectoderm biopsy samples of all blastocysts in the trial. Preimplantation genetic testing for aneuploidy (PGT-A) was used as an independent, objective indicator of embryo quality. At the time of trial design, the clinical utility of PGT-A in different patient cohorts based on age was less understood. Biopsied cells were designated euploid (46, XX or 46, XY) or aneuploid by real-time polymerase chain reaction analysis as previously described (16). During the trial, the assay displayed a higher error rate than previously reported due to a reagent issue, with no reason to believe that assay variances differed for either trial arm.

Patients receiving an hCG trigger underwent fresh transfer of a single blastocyst of best quality by morphology on day 5, following ICSI. All remaining blastocysts were frozen using vitrification. Genetics results were not available to inform blastocyst selection for fresh transfer. Starting on the evening of the day after retrieval, 100 mg two times per day of vaginal progesterone (P_4) (Endometrin; Ferring Pharmaceuticals) was administered until the serum hCG test (10–14 days after transfer). Vaginal P_4 was continued for a maximum of 10 weeks with confirmed pregnancy.

In cases of excessive ovarian response (>30 follicles of $\geq$12 mm each and/or estradiol [E_2] levels $\geq$5,000 pg/mL), a GnRH agonist (4 mg leuprolide acetate) was administered $\geq$12 hours after the last GnRH antagonist dose, fresh transfer was canceled, and all blastocysts were biopsied; viable blastocysts were frozen for use in a subsequent transfer cycle.

Pregnancy outcomes from fresh transfer were collected in a post-trial follow-up. In addition, for subjects with no ongoing pregnancy in the fresh cycle, single frozen blastocyst transfers could be initiated within 6 months of the date of randomization, in which genetic results could be used to select a euploid blastocyst for transfer; there was no trial limitation to the number of transfers that could be performed during this period. Pregnancy outcomes for these frozen blastocyst transfers were also collected in the post-trial follow-up.

Trial Endpoints and Assessments

In the core trial, the primary endpoint was ongoing pregnancy rate, defined as the presence of one or more intrauterine pregnancies with a fetal heartbeat at 10–11 weeks' gestation (8–9 weeks after fresh blastocyst transfer). Secondary endpoints included the following: cumulative ongoing pregnancy, early pregnancy loss (two positive β-hCG tests but no ongoing pregnancy at 10–11 weeks' gestation), OHSS frequency, follicular development, endocrine profile, endometrial development, number of oocytes retrieved, and blastocyst quality. Endocrine parameters were centrally assessed on blood samples throughout the stimulation period; a description of assay platforms and their associated limits of quantitation is provided in Supplemental Table 2. Day 5 blastocyst quality was locally assessed by the Gardner and Schoolcraft scale (17).

Safety was assessed based on vital signs, laboratory results, and reports of treatment-emergent adverse events (TEAEs) that occurred from the time of first administration of trial drug to the last end-of-treatment visit. OHSS was classified using the Golan classification system (18).

Post-trial endpoints included the live birth rate after fresh or frozen transfer and cumulatively, neonatal health, and pregnancy loss rates from frozen blastocyst transfer cycles.

Sample Size and Statistical Analysis

The trial was powered to demonstrate noninferiority in ongoing pregnancy rate for fresh cycles treated with HP-hMG vs. rFSH based on a prespecified margin of −12% for the lower limit of the two-sided 95% confidence interval (CI) for the difference in ongoing pregnancy rates consistent with the exploratory objectives of the trial. An estimated 275 randomized subjects per treatment group were needed to achieve $\geq$80% power, assuming an ongoing pregnancy rate of 50% for both groups and a one-sided significance level of 0.025 (19).

The 95% CIs for the difference between HP-hMG and rFSH in ongoing pregnancy, positive β-hCG, clinical pregnancy, and live birth rates as well as other binary outcomes were established based on an asymptotic normal distribution. Treatment group differences in continuous outcomes were calculated by the Hodges–Lehmann estimates with 95% CIs calculated by the Moses method. Categorical data were summarized using numbers and percentages, with percentages based on the total number of subjects within the given analysis set. Continuous data were summarized with descriptive statistics. All statistical tests were performed using a two-sided test at a 5% significance level. No adjustments were made for multiple tests. Comparability of baseline and clinical characteristics between treatment groups was assessed by the Wilcoxon–Mann–Whitney test using χ^2 statistic (degrees of freedom = 1) for continuous variables and the two-sided Fisher's exact test for categorical variables.

RESULTS

Trial Subjects

Of 1,258 screened patients, 620 were randomized and 619 were treated (modified intent to treat [mITT]). One subject

randomized to HP-hMG spontaneously became pregnant prior to the start of ovarian stimulation and was the sole mITT population exclusion. A total of 99.5% completed stimulation day 6, 96.8% received trigger (hCG or GnRH agonist), 96.5% underwent oocyte retrieval, and 63.2% completed fresh blastocyst transfer (Supplemental Fig. 2). Overall, 91 (14.7%) subjects (37 [11.9%] HP-hMG subjects and 54 [17.5%] rFSH subjects) had the hCG trigger replaced with GnRH agonist. Baseline characteristics did not significantly differ between groups. Mean subject age was 30.2 years, body mass index was 24.3 kg/m^2, AMH was 7.7 ng/mL, and AFC was 30.7 (Table 1).

Stimulation Response

The mean number of oocytes retrieved was 15.1 and 22.2 in HP-hMG–treated and rFSH-treated subjects respectively. This difference of approximately seven fewer retrieved oocytes per HP-hMG–treated subject culminated in three fewer all-quality (5.6 vs. 8.5) and similar numbers of morphologically excellent quality blastocysts (3.0 vs. 3.9). The percentage of blastocysts subdivided by either morphological or genetic (euploidy) quality parameters was similar between groups; HP-hMG was associated with a longer treatment duration (10.9 vs. 9.3 days) and greater total administered dose (2114.5 vs. 1498.9 IU) (Table 2). Additional stimulation and blastocyst transfer parameters are summarized in Supplemental Table 3.

Efficacy and Safety Outcomes

The primary endpoint, ongoing pregnancy rate/cycle start after the fresh IVF cycle, was 35.5% in HP-hMG–treated compared to 30.7% in rFSH-treated subjects (difference: 4.7%; 95% CI, −2.7%, 12.1%) (Fig. 1A), thereby meeting the predefined noninferiority objective. Live birth rates were 52.2% and 48.7% respectively in HP-hMG–treated and rFSH-treated subjects after fresh blastocyst transfer. The live birth rates were 63.4% and 50.8% in HP-hMG–treated and rFSH-treated subjects respectively, after frozen blastocyst transfer within 6 months of randomization (Fig. 1B, C).

Cumulative live birth rates per cycle start were 50.6% and 51.5% in HP-hMG–treated and rFSH-treated patients (difference: −0.8%, 95% CI −8.7%, 7.1%) (Fig. 1D). However, subjects treated with rFSH underwent more transfers (334 vs. 291) (Fig. 1E) with greater numbers of transfers involving multiple embryos (39 vs. 17: considered protocol deviations; no subject received a transfer of more than two blastocysts) compared to those treated with HP-hMG (Fig. 1F). Of the 56 cycles in which more than one embryo was transferred, 53 occurred in frozen cycles. A complete summary of the number of blastocysts transferred per cycle and treatment group is provided in Supplemental Table 4.

Early pregnancy loss rates were lower in HP-hMG–treated compared to rFSH-treated cohorts in both fresh and frozen cycles (Fig. 1G). Cumulatively, the aggregate pregnancy loss in fresh and frozen cycles was significantly lower at 14.5% after HP-hMG compared to

TABLE 1

Baseline demographic and clinical characteristics (mITT).

Parameter	HP-hMG (n = 310)	rFSH (n = 309)	Total (n = 619)	*P* value[a]
Age, y	30.0 ± 3.08	30.4 ± 3.02	30.2 ± 3.05	.0894
Weight, kg	66.1 ± 10.12	65.8 ± 10.96	65.9 ± 10.54	.5888
BMI, kg/m^2	24.4 ± 3.29	24.3 ± 3.39	24.3 ± 3.34	.5167
Duration of infertility, mo	36.7 ± 25.79	37.1 ± 28.38	36.9 ± 27.09	.6614
Cause of infertility, n (%) [b]				
Oligo-ovulation	50 (16.1)	56 (18.1)	106 (17.1)	.5238
Endometriosis	20 (6.5)	25 (8.1)	45 (7.3)	.4439
Male factor	136 (43.9)	129 (41.7)	265 (42.8)	.6262
Tubal factor	44 (14.2)	43 (13.9)	87 (14.1)	1.0000
Idiopathic	105 (33.9)	112 (36.2)	217 (35.1)	.5561
Other	28 (9.0)	29 (9.4)	57 (9.2)	.8904
AFC	30.5 ± 15.47	31.0 ± 12.24	30.7 ± 13.94	.2130
AMH, pmol/L (ng/mL)	56.1 ± 25.96 (7.8 ± 3.61)	53.9 ± 17.47 (7.5 ± 2.43)	55.4 ± 22.15 (7.7 ± 3.08)	.7988
≥35.7 (≥5.0)	310 (100)	309 (100)	619 (100)	
≥42.9 (≥6.0)	217 (70.0)	216 (69.9)	433 (70.0)	
≥50.0 (≥7.0)	143 (46.1)	141 (45.6)	284 (45.9)	
≥57.1 (≥8.0)	92 (29.7)	96 (31.1)	188 (30.4)	
LH, U/L	6.9 ± 4.04	6.4 ± 3.49	6.6 ± 3.78	.0543
FSH, U/L	6.4 ± 1.55	6.2 ± 1.55	6.3 ± 1.55	.2750
E_2, pmol/L (pg/mL)	136.95 ± 70.00 (37.3 ± 19.1)	144.59 ± 84.02 (39.4 ± 22.9)	140.7 ± 77.2 (38.3 ± 21.0)	.0671
P_4, nmol/L (ng/mL)	0.6 ± 1.82 (0.2 ± 0.6)	0.7 ± 2.46 (0.2 ± 0.8)	0.6 ± 2.16 (0.2 ± 0.7)	.4218
Total testosterone, nmol/L (ng/dL)	1.0 ± 0.52 (28.8 ± 15.0)	1.0 ± 0.42 (28.8 ± 12.1)	1.0 ± 0.47 (28.8 ± 13.6)	.7734

Note: Unless otherwise noted, data are presented as mean ± standard deviation or n (%). AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index; df = degrees of freedom; E_2 = estradiol; FSH = follicle-stimulating hormone; HP-hMG = highly purified human menotropin; LH = luteinizing hormone; mITT = modified intent-to-treat; P_4 = progesterone; rFSH = recombinant follicle-stimulating hormone.

[a] *P* values were calculated from the Wilcoxon–Mann–Whitney test using chi-square statistic (df = 1) for continuous variables and from the two-sided Fisher exact test for categorical variables.

[b] Subjects may have had more than one cause of infertility.

Witz. HP-hMG vs. rFSH in high responders. Fertil Steril 2020.

TABLE 2

Stimulation, oocyte retrieval, and embryo development (mITT).

Parameter	HP-hMG (n = 310)	rFSH (n = 309)	Difference (95% CI)[a]
Stimulation			
Total dose of gonadotropin, IU	2,114.5 ± 798.85	1,498.9 ± 417.36	525.00 (450.00, 600.00)
Dose increase (%)	181 (58.4)	71 (23.0)	
Dose maintained (%)	114 (36.8)	209 (67.6)	
Dose decreased (%)	13 (4.2)	29 (9.4)	
Duration of stimulation, days	10.9 ± 2.40	9.3 ± 1.42	1.00 (1.00, 2.00)
Day of hCG trigger			
Estradiol, pmol/L (pg/mL)	10,311.00 ± 6,543.75 (2,808.8 ± 1,782.6)	11,750.46 ± 7,353.34 (3,200.9 ± 2,003.1)	−1211.46 (−2276.08, −146.85)
Progesterone, nmol/L (ng/mL)	2.2 ± 2.75 (0.7 ± 0.9)	3.3 ± 3.02 (1.0 ± 0.9)	−0.89 (−1.14, −0.63)
Oocyte retrieval			
No. of oocytes retrieved/subject	15.1 ± 10.12	22.2 ± 11.54	−7.00 (−8.00, −5.00)
No. of MII oocytes/subject	10.1 ± 7.18	15.9 ± 9.01	−5.00 (−7.00, −4.00)
No. of 2PN oocytes/subject	8.2 ± 5.90	12.9 ± 7.38	−4.00 (−5.00, −3.00)
Fertilization rate[b]	55.09 ± 21.73	59.07 ± 18.73	−3.57 (−6.76, 0.00)
Embryo development (day 5)			
No. of blastocysts/subject	5.6 ± 4.31	8.5 ± 5.68	−3.00 (−3.00, −2.00)
No. of excellent blasts/subject	3.0 ± 2.77	3.9 ± 3.73	0.00 (−1.00, 0.00)
Blastocyst morphology			
Excellent[c]	28.3%	30.7%	
Good[d]	26.5%	22.5%	
Neither[e]	45.1%	45.8%	
Missing	0.1%	1.0%	
Blastocyst karyotype			
Aneuploidy rate[f]	24.5% ± 25.14	27.4% ± 26.25	
No. of subjects with fresh transfer (%)	201 (64.8)	191 (61.8)	3.03 (−4.56, 10.62)
No. of subjects with frozen transfer (%)	82 (26.5)	130 (42.1)	−15.62 (−23.00, −8.24)
Ongoing pregnancy/cycle start (%)	35.5	30.7	4.7 (−2.7, 12.1)
Live birth rate (fresh transfer)	52.2	48.7	3.6 (−6.4, 13.4)
Live birth rate (frozen transfer)	63.4	50.8	12.7 (−0.9, 26.2)

Note: Unless otherwise noted, data are presented as mean ± standard deviation. CI = confidence interval; HP-hMG = highly purified human menotropin; MII = metaphase II; mITT = modified intent-to-treat; PN = pronuclei; rFSH = recombinant follicle-stimulating hormone.

[a] Differences between HP-hMG and rFSH were calculated by the Hodges-Lehmann estimates with 95% CIs calculated by the Moses method for continuous outcomes; the 95% CIs for binary outcomes were based on asymptotic normal distribution.

[b] Calculated as the number of 2PN oocytes divided by the number of oocytes retrieved.

[c] Defined as those with blastocyst expansion and hatching status 4–6, inner cell mass grade A, and trophectoderm grade A or B.

[d] Defined as those with blastocyst expansion and hatching status 3–6, inner cell mass grade B, and trophectoderm grade A or B.

[e] All other blastocysts.

[f] Calculated as number of aneuploid blastocysts per number of blastocysts with known ploidy.

Witz. HP-hMG vs. rFSH in high responders. Fertil Steril 2020.

25.5% after rFSH treatment (difference: −10.97%, 95% CI −18.8%, −3.14%).

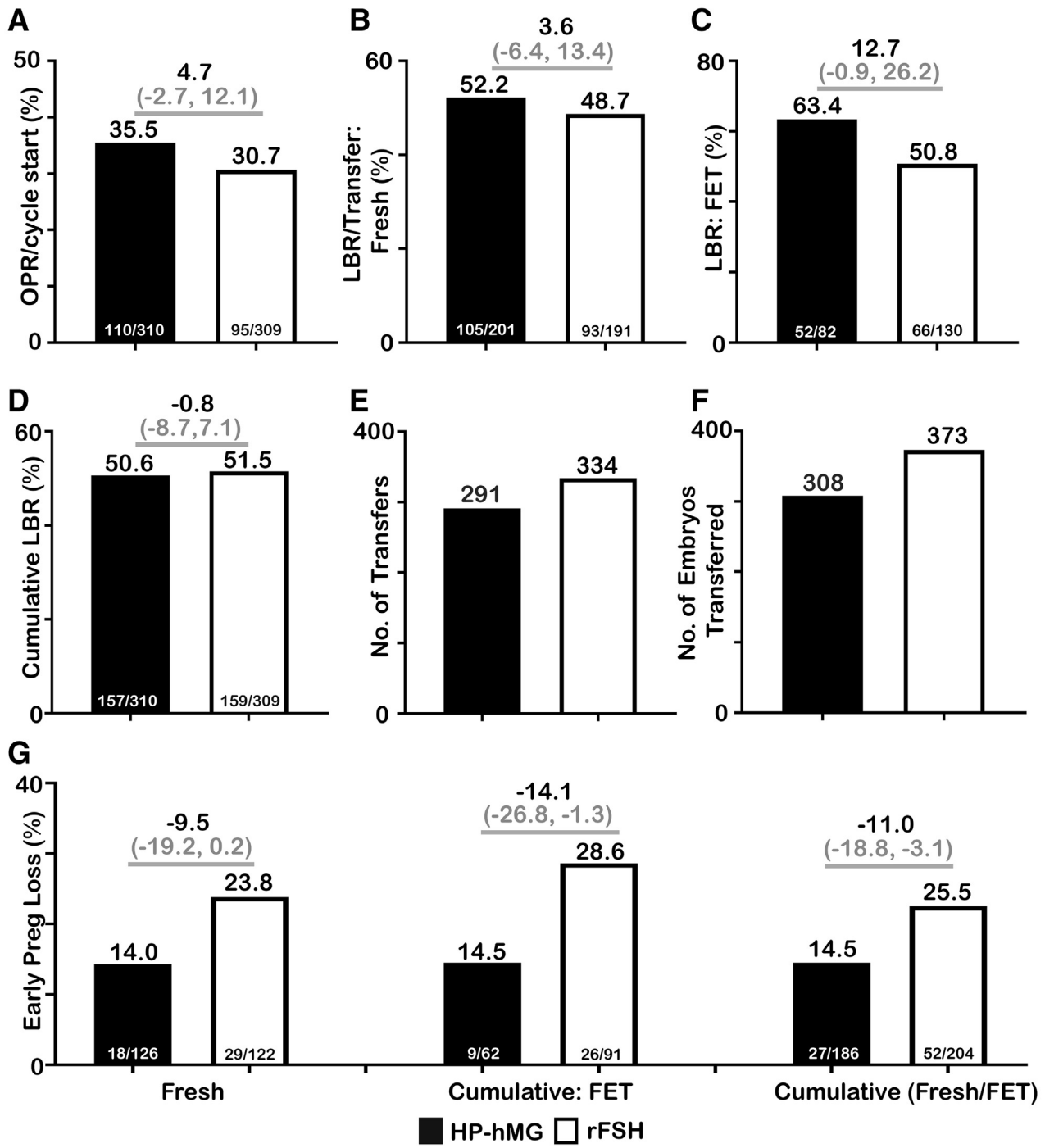
The overall incidence of treatment-emergent adverse events (TEAEs) was 57.7% with HP-hMG and 70.6% with rFSH. The incidence of OHSS was significantly lower with HP-hMG compared with rFSH treatment (9.7% vs. 21.4%; difference: −11.7%, 95% CI −17.3%, −6.1%); (Table 3). Most OHSS cases were mild or moderate in intensity in both groups. Cycle cancellations due to excessive response were reported for three subjects treated with HP-hMG and for six subjects treated with rFSH. Early OHSS rates were significantly lower in those treated with HP-hMG compared with rFSH (6.1% vs. 17.5%: difference: −11.3%, 95% CI −16.4%, −6.3%) (Table 3). Severe early OHSS was reported in 3 (1.0%) subjects in the HP-hMG group and 7 (2.3%) subjects in the rFSH group.

DISCUSSION

In this randomized, open-label, assessor-blinded, parallel-group, multicenter noninferiority U.S. trial, we have established that per transfer, HP-hMG treatment was associated with similar live birth and significantly lower pregnancy loss rates in fresh and frozen blastocyst transfer cycles in predicted high-responder subjects undergoing ART. Consequently, 43 more transfer cycles and 65 more transferred embryos were required in the rFSH arm to achieve similar cumulative live birth rates.

This is the first comparator trial to show that HP-hMG was associated with lower pregnancy loss rates after both fresh and frozen transfers. HP-hMG–treated subjects also had lower peak estradiol levels, lower trigger-day progesterone levels, and a significantly diminished incidence of OHSS, an iatrogenic complication. It is possible that these differences in response to gonadotropin stimulation could have affected the endometrium, altering the likelihood of achieving an ongoing pregnancy after fresh transfer. However, this is unlikely to be the primary cause of the difference in pregnancy loss rate observed between treatment groups. Trial criteria mandated leuprolide trigger and freeze-all for extreme hyper response. Importantly, the differential pregnancy loss

FIGURE 1



MEGASET-HR: efficacy outcomes. (A) Ongoing pregnancy rate per cycle start defined as the presence of one or more intrauterine pregnancies with a fetal heartbeat at 10–11 weeks' gestation or 8–9 weeks after fresh blastocyst transfer. (B) Live birth rate in subjects with fresh blastocyst transfer. (C) Live birth rate in subjects with frozen blastocyst transfer. (D) Cumulative live birth rate. (E) Total number of blastocyst transfers (fresh and frozen). (F) Total number of blastocysts transferred. (G) Pregnancy loss occurring in fresh cycles, any frozen cycle, and cumulative (defined as the ratio of all patients with early pregnancy loss in fresh and frozen cycles, i.e., before 11 weeks' gestation) to outcomes of all patients with two consecutive serum β-hCG tests. HP-hMG = highly purified human menotropin; rFSH = recombinant follicle-stimulating hormone; OPR = ongoing pregnancy rate; LBR = live birth rate; FET = frozen embryo transfer.

Witz. HP-hMG vs. rFSH in high responders. Fertil Steril 2020.

TABLE 3

Summary of safety outcomes.

Parameter	HP-hMG (n = 310)	rFSH (n = 309)	Total (n = 619)
TEAE[a]	179 (57.7%)	218 (70.6%)	397 (64.1%)
Procedural pain	71 (22.9%)	71 (23.0%)	142 (22.9%)
Nausea	37 (11.9%)	40 (12.9%)	77 (12.4%)
Abdominal distension	25 (8.1%)	35 (11.3%)	60 (9.7%)
Constipation	22 (7.1%)	36 (11.7%)	58 (9.4%)
Headache	29 (9.4%)	22 (7.1%)	51 (8.2%)
Abdominal pain	21 (6.8%)	24 (7.8%)	45 (7.3%)
Vaginal hemorrhage	21 (6.8%)	22 (7.1%)	43 (6.9%)
OHSS[b]	30 (9.7%)[c]	66 (21.4%)[c]	96 (15.5%)
Mild	7 (2.3%)	18 (5.8%)	25 (4.0%)
Moderate	15 (4.8%)	39 (12.6%)	54 (8.7%)
Severe	8 (2.6%)	9 (2.9%)	17 (2.7%)
Serious TEAEs	8 (2.6%)	11 (3.6%)	19 (3.1%)
AE-related discontinuation	2 (0.6%)	4 (1.3%)	6 (1.0%)
Deaths	0	0	0

Note: Unless otherwise noted, data are presented as n (%). AE = adverse events; HP-hMG = highly purified human menotropin; OHSS = ovarian hyperstimulation syndrome (early OHSS was defined as having an onset ≤9 days after triggering final follicular maturation, before trigger, or during stimulation when trigger was not used; late OHSS was defined as onset >9 days after trigger); rFSH = recombinant follicle-stimulating hormone; TEAEs, treatment-emergent adverse events.

[a] TEAEs with an incidence of at least 5% by MedDRA System Organ Class and Preferred Term (safety analysis set).

[b] Classification of grade was determined using Golan's classification system (18).

[c] Difference in OHSS incidence: 11.7%, (confidence interval –17.3, –6.1%).

Witz. HP-hMG vs. rFSH in high responders. Fertil Steril 2020.

rate was observed even in frozen embryo transfer cycles in which earlier stimulation response would presumably have had no impact on the endometrium. Instead, an improvement in embryo quality associated with use of HP-hMG could account for the early lower pregnancy loss rate observed in that trial arm. However, embryo quality as determined by conventional (morphology) and state-of-the-art (chromosome number) parameters was similar in the two groups. Therefore, HP-hMG stimulation may have had a beneficial impact on the oocyte that was not reflected by currently established embryo assessment technologies.

In contrast to two fewer retrieved oocytes observed in prior studies, HP-hMG–treated subjects in this trial, limited to predicted high-responders, had a mean of seven fewer oocytes retrieved; this difference in yield narrowed with advancing embryo development (13, 15). Differential gonadotropin response observed here could result from inclusion of patients with polycystic ovary syndrome (PCOS); 106 oligo-ovulatory subjects (17.1%) with elevated serum AMH level were likely to have PCOS pathology, which was not explicitly excluded. The inherent heterogeneity of PCOS means that in real-world clinical practice, such subjects contribute significantly to the overall pool of predicted high responders. Luteinizing hormone activity–driven atresia of small/medium follicles could have diminished oocyte numbers in HP-hMG–treated subjects (20, 21). Reduced follicular recruitment in the HP-hMG arm is likely to have been a contributor to the lower OHSS rate that was also observed in these patients, a key benefit identified in this trial.

Currently, there are no consensus criteria that identify potential high responder patients before starting therapy. Instead, hyper response is diagnosed while patients are in treatment, and reactive measures to mitigate complications are then instituted, usually resulting in a delay in time to pregnancy. This is the first trial to use serum AMH (≥5 IM/mL) as a marker to prospectively identify potential hyper responders and to offer a potential strategy to prevent complications while enabling efficient progression to embryo transfer and opportunity for pregnancy. Mean serum AMH levels were 7.5 IU/mL in patients treated with rFSH and 7.8 IU/mL in those treated with HP-hMG. As expected, the incidence of OHSS among trial subjects was high, which validated the prognostic significance of AMH as a marker for identification of hyper responder patients prior to treatment start. Moreover, despite clinical consequences for individual patients, OHSS is often underdiagnosed and underreported in the literature (26). A combination of including only high-risk patients, in a trial setting, with standardized application of diagnostic, monitoring, and follow-up criteria, is another reason for the high rate of OHSS in this study. However, the starting gonadotropin dose of 150 IU/d in the trial is aligned with the indicated dose of rFSH and lower than that of HP-hMG when endogenous gonadotropins are suppressed. Moreover, on average, this dose is lower than that in current stimulation protocols in the United States.

OHSS incidence was mostly mild/moderate in intensity. Nevertheless, these findings suggest that there is scope for further dosage reduction, particularly for rFSH, to diminish the incidence of freeze-all and delayed embryo transfer. Although the trial design did mandate leuprolide acetate trigger for extreme high response based on best clinical evidence at the time, the intervention could be applied to patients with more modest levels of hyper response.

Despite seven fewer oocytes per subject, the difference in the mean number of excellent-quality blastocysts was reduced to 0.9. Moreover, the supernumerary oocytes retrieved in rFSH-treated subjects did not appear to confer an appreciable clinical benefit in the trial. An open question is whether collection of live birth data from frozen transfers for longer periods of time could have revealed differences. Embryo quality generally establishes the hierarchy of transfer order. In this trial, embryos of highest morphological grade were transferred fresh, and mostly euploid embryos of highest morphological grade were transferred in up to three consecutive subsequent frozen transfers; such practice trends would argue against the possibility of significant gains in cumulative live birth rates over a longer period of time.

Current U.S. clinical practice trends favor maximizing ovarian response. Relative unfamiliarity with the inherent differences in ovarian response between the two gonadotropins may have accounted for upward revision of dosage after day 6 in the HP-hMG group in this assessor-blinded trial, resulting in higher total doses of HP-hMG administered vs. rFSH. However, there is no robust evidence that mid-cycle gonadotropin dose changes provide benefit in follicle recruitment, progression, or ultimate maturity. Health economic outcomes research is required to assess the projected financial impact of the higher drug use of HP-hMG observed in the trial relative to other efficacy and safety parameters.

Another emerging practice trend is a preference for elective frozen embryo transfer over fresh transfer. However, recent

clinical trials did not find increased live birth rates after frozen transfer in non-PCOS patients (22–25). Significant differences in the live birth rate were not observed between fresh and frozen transfer in the MEGASET-HR trial, even though PGT results informed euploid blastocyst transfer in frozen transfers. These findings are significant, as high-responder patients typically have all embryos frozen (26). A lower incidence of excessive response and accompanying OHSS suggests a reduced imperative to freeze all embryos, allowing a greater opportunity for fresh transfer. The shorter time to birth that results would likely translate to increased convenience, patient satisfaction, and lower costs, although these parameters remain to be analyzed, particularly considering the differences in the total gonadotropin dose administered and the duration of stimulation. Several studies have found evidence of worse maternal and neonatal outcomes associated with fresh vs. frozen transfer cycles, suggesting that the supraphysiological environment created by ovarian stimulation protocols may be detrimental to both embryo implantation and placentation (27–31). Response to stimulation with HP-hMG as a single agent is more moderate compared with protocols containing rFSH, which could mitigate such risks in fresh transfer cycles.

There is an increased need to provide patient-tailored care across all medical disciplines (32–34). One way to personalize controlled ovarian stimulation is through choice of gonadotropin (13, 15, 35). In high responders, HP-hMG treatment was consistently associated with a moderate stimulation profile, lower incidence of complications, lower pregnancy loss, and corresponding higher probability of ongoing pregnancy and live birth per transfer in fresh or frozen cycles. The optimized risk/benefit profile with HP-hMG presents an opportunity to individualize the treatment of high responders and suggests that choice of gonadotropin has a role in protocol personalization.

Acknowledgments: We thank all trial participants (including patients, investigators, and support staff); J. Ruman for contributions to trial design; K. O'Brien for trial management; A. Wynn for data management; and Everest Clinical Research Corporation, DSP Clinical Research, and ReproSource.

REFERENCES

1. Nelson SM, Klein BM, Arce JC. Comparison of antimüllerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. Fertil Steril 2015;103:923–30.
2. Nelson SM, Yates RW, Lyall H, Jamieson M, Traynor I, Gaudoin M, et al. Anti-Müllerian hormone-based approach to controlled ovarian stimulation for assisted conception. Hum Reprod 2009;24:867–75.
3. Nyboe Andersen A, Nelson SM, Fauser BC, García-Velasco JA, Klein BM, Arce JC, et al. Individualized versus conventional ovarian stimulation for in vitro fertilization: a multicenter, randomized, controlled, assessor-blinded, phase 3 noninferiority trial. Fertil Steril 2017;107:387–96.
4. Broer SL, Dólleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update 2011;17:46–54.
5. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. Hum Reprod Update 2014;20:124–40.
6. Youssef MA, Van der Veen F, Al-Inany HG, Mochtar MH, Griesinger G, Nagi Mohesen M, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst Rev 2014, CD008046.
7. Toftager M, Bogstad J, Bryndorf T, Løssl K, Roskær J, Holland T, et al. Risk of severe ovarian hyperstimulation syndrome in GnRH antagonist versus GnRH agonist protocol: RCT including 1050 first IVF/ICSI cycles. Hum Reprod 2016; 31:1253–64.
8. Wang R, Lin S, Wang Y, Qian W, Zhou L. Comparisons of GnRH antagonist protocol versus GnRH agonist long protocol in patients with normal ovarian reserve: a systematic review and meta-analysis. PLoS One 2017;12: e0175985.
9. Yates AP, Rustamov O, Roberts SA, Lim HY, Pemberton PW, Smith A, et al. Anti-Mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod 2011;26:2353–62.
10. Wolfenson C, Groisman J, Couto AS, Hedenfalk M, Cortvrindt RG, Smitz JE, et al. Batch-to-batch consistency of human-derived gonadotrophin preparations compared with recombinant preparations. Reprod Biomed Online 2005;10:442–54.
11. Becker J, Walz A, Daube S, Keck C, Pietrowski D. Distinct responses of human granulosa lutein cells after hCG or LH stimulation in a spheroidal cell culture system. Mol Reprod Dev 2007;74:1312–6.
12. Kilani Z, Dakkak A, Ghunaim S, Cognigni GE, Tabarelli C, Parmegiani L, et al. A prospective, randomized, controlled trial comparing highly purified hMG with recombinant FSH in women undergoing ICSI: ovarian response and clinical outcomes. Hum Reprod 2003;18:1194–9.
13. Devroey P, Pellicer A, Nyboe Andersen A, Arce JC, Group MEGASET. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. Fertil Steril 2012;97:561–71.
14. Arce JC, Klein BM, La Marca A. The rate of high ovarian response in women identified at risk by a high serum AMH level is influenced by the type of gonadotropin. Gynecol Endocrinol 2014;30:444–50.
15. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum Reprod 2006;21:3217–27.
16. Treff NR, Tao X, Ferry KM, Su J, Taylor D, Scott RT. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril 2012;97:819–24.
17. Gardner D, Schoolcraft W. In vitro culture of human blastocysts. London, UK: Parthenon Publishing Group; 1999.
18. Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. Obstet Gynecol Surv 1989;44:430–40.
19. Yeh JS, Steward RG, Dude AM, Shah AA, Goldfarb JM, Muasher SJ. Pregnancy outcomes decline in recipients over age 44: an analysis of 27,959 fresh donor oocyte in vitro fertilization cycles from the Society for Assisted Reproductive Technology. Fertil Steril 2014;101:1331–6.
20. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Hum Reprod 1994;9:188–91.
21. Filicori M, Cognigni GE, Tabarelli C, Pocognoli P, Taraborrelli S, Spettoli D, et al. Stimulation and growth of antral ovarian follicles by selective LH activity administration in women. J Clin Endocrinol Metab 2002;87:1156–61.
22. Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, et al. Transfer of fresh versus frozen embryos in ovulatory women. N Engl J Med 2018;378:126–36.
23. Chen ZJ, Legro RS. Fresh versus frozen embryos in polycystic ovary syndrome. N Engl J Med 2016;375:e42.
24. Vuong LN, Dang VQ, Ho TM, Huynh BG, Ha DT, Pham TD, et al. IVF Transfer of Fresh or Frozen Embryos in Women without Polycystic Ovaries. N Engl J Med 2018;378:137–47.
25. Roque M, Haahr T, Geber S, Esteves SC, Humaidan P. Fresh versus elective frozen embryo transfer in IVF/ICSI cycles: a systematic review and meta-analysis of reproductive outcomes. Hum Reprod Update 2019;25:2–14.
26. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. Hum Reprod 2011;26:2593–7.

27. Kalra SK, Ratcliffe SJ, Coutifaris C, Molinaro T, Barnhart KT. Ovarian stimulation and low birth weight in newborns conceived through in vitro fertilization. Obstet Gynecol 2011;118:863–71.
28. Shapiro BS, Daneshmand ST, De Leon L, Garner FC, Aguirre M, Hudson C. Frozen-thawed embryo transfer is associated with a significantly reduced incidence of ectopic pregnancy. Fertil Steril 2012;98:1490–4.
29. Li Z, Sullivan EA, Chapman M, Farquhar C, Wang YA. Risk of ectopic pregnancy lowest with transfer of single frozen blastocyst. Hum Reprod 2015;30:2048–54.
30. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril 2012;98:368–77.
31. Magdi Y, El-Damen A, Fathi AM, Abdelaziz AM, Abd-Elfatah Youssef M, Abd-Allah AA, et al. Revisiting the management of recurrent implantation failure through freeze-all policy. Fertil Steril 2017;108:72–7.
32. Fauser BCJM. Patient-tailored ovarian stimulation for in vitro fertilization. Fertil Steril 2017;108:585–91.
33. Paulson RJ. Introduction: contemporary approaches to alternative ovarian stimulation strategies for in vitro fertilization. Fertil Steril 2017;108:555–7.
34. Mega JL, Sabatine MS, Antman EM. Population and personalized medicine in the modern era. JAMA 2014;312:1969–70.
35. European and Israeli Study Group on Highly Purified Menotropin versus Recombinant Follicle-Stimulating Hormone. Efficacy and safety of highly purified menotropin versus recombinant follicle-stimulating hormone in in vitro fertilization/intracytoplasmic sperm injection cycles: a randomized, comparative trial. Fertil Steril 2002;78:520–8.

Ensayo aleatorizado, ciego para el evaluador, que compara la menotropina humana altamente purificada y la hormona estimulante del folículo recombinante en pacientes con alta respuesta sometidas a inyección intracitoplasmática de espermatozoides.

Objetivo: Evaluar la eficacia y la seguridad de la menotropina humana altamente purificada (HP-hMG) y la hormona estimulante del folículo recombinante (rFSH) para la estimulación ovárica controlada en una población de pacientes con alta respuesta prevista.

Diseño: Ensayo aleatorizado, abierto, ciego para el evaluador, grupo paralelo, de no inferioridad.

Entorno: Centros de fertilidad.

Paciente(s): Un total de 620 mujeres con un valor sérico de hormona antimülleriana (AMH) $\geq$5 ng/mL.

Intervención (es): estimulación ovárica controlada con HP-hMG o rFSH en un ciclo de técnica de reproducción asistida (TRA) con antagonista de la GnRH. Se realizó una transferencia en fresco de un solo blastocisto a menos que la respuesta ovárica fuera excesiva, en cuyo caso todos los embriones se criopreservaron. Los sujetos pudieron someterse a una transferencia de blastocisto congelado posterior dentro de los 6 meses desde la aleatorización.

Principales medidas de resultados: tasa de embarazo en curso (TEC) después de una transferencia en fresco (objetivo primario), así como la tasa acumulada de nacidos vivos, síndrome de hiperestimulación ovárica (SHO) y tasas de pérdida de embarazo.

Resultado(s): La TEC/ciclo iniciado después de una transferencia en fresco fue de 35.5% con HP-hMG y 30.7% con rFSH (diferencia: 4.7%, IC 95% -2.7%, 12.1%); se estableció la no inferioridad. En comparación con rFSH, HP-hMG se asoció con tasas significativamente más bajas de SHO (21.4% vs. 9.7%, respectivamente; diferencia: -11.7%, IC 95% -17.3%, -6.1%) y tasas acumuladas de pérdida gestacional temprana (25.5% vs. 14.5% respectivamente; diferencia: -11.0%, IC 95% -18.8%, -3.14%). A pesar de que hubo 43 transferencias más en el grupo de rFSH, las tasas acumuladas de nacidos vivos fueron similares con HP-hMG y rFSH de 50.6% y 51.5% respectivamente (diferencia: -0.8%, IC 95% -8.7%, 7.1%).

Conclusión(es): En pacientes con alta respuesta, HP-hMG proporcionó una eficacia comparable a la rFSH con menos eventos adversos, incluida la pérdida del embarazo, lo que sugiere su perfil optimizado de riesgo/beneficio en esta población.

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL TABLES

Supplemental Table 1. Inclusion and Exclusion Criteria

Inclusion criteria
1. Signed informed consent, prior to any study-related procedure.
2. Females aged 21 to 35 years with regular ovulatory menstrual cycles of 21 to 45 days, with a BMI between 18 and 30 kg/m^2 who desired pregnancy.
3. Patients were to be high responders, defined as having serum AMH ≥35.7 pmol/L (≥5 ng/mL) at screening.
4. Documented history of infertility (e.g., unable to conceive for ≤12 months or for ≤6 months if receiving donor sperm), with Day 2 or Day 3 serum FSH level between 1 and 12 IU/L (inclusive), the results of which were to be obtained ≤6 months prior to screening. The highest FSH result ≤6 months prior to screening was considered for inclusion. Patients with documented bilateral tubal occlusion established as a cause of infertility were eligible at diagnosis.
5. Male partner had semen analysis that was at least adequate for ICSI at screening or ≤6 months prior to the screening date. Sperm from partners with severe male factors requiring invasive or surgical sperm retrieval could not be used. Use of donor sperm was allowed.
6. Willing to accept transfer of one blastocyst per cycle.
7. At least one cycle with no fertility medication immediately prior to screening.
8. Hysterosalpingography, hysteroscopy, or saline hysterosonogram documenting uterine anatomy appropriate for ART at screening or ≤12 months prior to screening.
9. TVUS documenting presence and adequate visualization of ≤1 ovary, without evidence of abnormality (e.g., no endometrioma, no dermoid cysts) and normal adnexa (e.g., no hydrosalpinx) at screening.
10. Total testosterone, prolactin, and TSH within the normal limits for the clinical laboratory or considered not clinically significant by the Investigator at screening or ≤12 months prior to screening. (Patients with high TSH levels who received replacement therapy and were considered adequately controlled could have been enrolled at the discretion of the Investigator.)
11. Pap smear test results that were appropriate for ART, in the opinion of the Investigator, at screening or ≤24 months prior to screening.
12. Negative serum hepatitis B surface antigen, hepatitis C antibody, human immunodeficiency virus antibody, and rapid plasma reagin tests at screening or ≤6 months prior to screening.
13. Willing and able to comply with the protocol for the duration of the study.

Exclusion criteria

1. Known stage III-IV endometriosis (30).
2. Oocyte donor or embryo recipient; gestational or surrogate carrier.
3. History of recurrent miscarriage not followed by a live birth (recurrent was defined as ≥2 consecutive miscarriages).
4. Previous IVF or ART failure due to a poor response to gonadotropins. Poor response was defined as development of ≤2 mature follicles or history of 2 previous failed cycle cancellations prior to oocyte retrieval due to poor response.
5. Inadequate number of oocytes, defined as <5 oocytes retrieved in previous ART attempts.
6. Early follicular phase total antral follicle count (diameter 2-10 mm) <10 for both ovaries combined (results obtained at screening or ≤12 months prior to screening).
7. Patients's male partner had obvious leukospermia (>2 million white blood cells/mL) or signs of infection in semen sample ≤6 months of the patients's screening. If either of these conditions existed, the male was to be treated with antibiotics and retested prior to the patient's randomization.
8. Known abnormal karyotype of patient or her partner.
9. The use of hormonal birth control ≤3 months prior to screening.
10. Use or planned use any of the following medications during the pretreatment and treatment phase: hormonal drug products (including estrogen, androgen supplementation [i.e., dehydroepiandrosterone, androgen patch]), progesterone creams, progesterone in oil injections, hydrocortisone and other steroid drug products, and fertility modifiers such as insulin sensitizers. Occasional use of inhaled or topical corticosteroids may have been permitted.
11. The presence of any uncontrolled systemic disease.
12. Currently breastfeeding, pregnant, or had a contraindication to pregnancy that would have precluded participation in the trial.
13. Presence of abnormal uterine bleeding of undetermined origin.
14. Findings at the gynecological examination that precluded gonadotropin therapy, in the opinion of the Investigator.
15. History of chemotherapy (except for gestational conditions) or radiotherapy.
16. Current or recent substance abuse, including alcohol.
17. Current or recent (3 months prior to screening) smoking > 3 cigarettes per day.

18. Documented intolerance or allergy to any of the medications used, including the study medication.
19. Participation in any experimental drug study ≤30 days prior to screening.
20. Refusal or inability to comply with the requirements of the protocol for any reason, including scheduled clinic visits and laboratory tests.
21. Known mental incapacity or language barrier precluding adequate understanding of the informed consent information and the study activities.
22. Clinic staff member directly involved in the conduct of the study. Any other staff member interested in participating must have obtained IRB approval prior to participation.

ART, Assisted Reproductive Technology; AMH, anti-Müllerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; IRB, Institutional Review Board; IVF, in vitro fertilization; TVUS, transvaginal ultrasound; TSH, thyroid stimulating hormone.

Supplemental Table 2: Endocrine Parameter Assessment

Analyte	**Assay manufacturer**	**Method**	**Lower Limit of Quantitation**	**Upper limit of Quantitation**
AMH	Beckman Coulter, GenII	ELISA	0.1 ng/mL	20 ng/ml
βhCG	Beckman Coulter, Dxl 600	ELISA	0.5 mIU/mL	1350 mIU/mL
Estradiol	*N/A*	2D HPLC MS/MS Analysis	1.0 pg/mL	500 pg/mL
FSH	Meso Scale Discovery (MSD)	ELISA	0.017mIU/mL	99 mIU/mL
LH	Meso Scale Discovery (MSD)	ELISA	0.005 mIU/mL	100 mIU/mL
Progesterone	*N/A*	LC-MS/MS Analysis	10 ng/dL	5000 ng/dL
Testosterone	*N/A*	LC-MS/MS Analysis	2.5 ng/dL	5000 ng/dL

ELISA, enzyme-linked immunosorbent assay; 2D HPLC two dimensional high performance liquid chromatography with tandem mass spectrometry; LC-MS/MS liquid chromatography with tandem mass spectrometry; N/A, not applicable

Supplemental Table 3. Stimulation and Blastocyst Transfer (mITT)

Parameter	HP-hMG (n = 310)	rFSH (n = 309)
Day 6 of stimulation		
Follicular development[a]		
Follicle size, mm	5.95 ± 1.68	7.45 ± 2.04
Average size of 3 largest follicles, mm	9.69 ± 3.56	12.2 ± 2.59
Follicles ≥17 mm	16 (5.2%)	30 (9.7%)
Endocrine profile, change from baseline		
E_2, pmol/L (pg/mL)	1,183.8 ± 1,213.0 (322.5 ± 330.4)	3,232.6 ± 2,270.6 (880.6 ± 618.5)
P_4, nmol/L (ng/mL)	–0.2 ± 1.6 (–0.06 ± 0.5)	0.1 ± 2.8 (0.03 ± 0.9)
End of stimulation		
Follicular development[b]		
Follicle size, mm	12.2 ± 3.3[b]	13.3 ± 2.8
Average size of 3 largest follicles, mm	21.4 ± 2.5	21.1 ± 2.7
Follicles ≥17 mm	278 (100%)	296 (100%)
Endocrine profile, change from baseline		
LH, U/L	–3.7 ± 3.9	–4.4 ± 3.1
FSH, U/L	9.5 ± 4.0	6.0 ± 3.8
E_2, pmol/L (pg/mL)	10,071.7 ± 6,648.8 (2,743.6 ± 1,811.2)	11,822.2 ± 7,517.6 (3,220.4 ± 2,047.8)
P_4, nmol/L (ng/mL)	1.6 ± 3.3 (0.5 ± 1.0)	2.6 ± 3.8 (0.8 ± 1.2)
Testosterone, nmol/L (ng/dL)	1.7 ± 1.2 (49.0 ± 34.6)	1.2 ± 1.1 (34.6 ± 31.7)
Time of fresh blastocyst transfer		
Endometrial development[c]		
Endometrial thickness, mm	11.5 ± 2.8	11.3 ± 2.5
>9mm	123 (39.7%)	113 (36.6%)
Trilaminar	97 (31.3%)	71 (23.0%)

Echogenicity		
Hyper-echogenicity	67 (21.6%)	86 (27.8%)
Hypo-echogenicity	98 (31.6%)	70 (22.7%)

Unless otherwise noted, data are presented as mean ± standard deviation.

[a]HP-hMG, n = 309; rFSH, n = 310.

[b]HP-hMG, n = 278; rFSH, n = 296.

[c]HP-hMG, n = 164; rFSH, n = 155.

mITT, modified intent-to-treat; HP-hMG, highly purified human menotropin; rFSH, recombinant follicle stimulating hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; E_2, estradiol; P_4, progesterone.

Supplemental Table 4

FRESH CYCLE	HP-hMG (N=201)	rFSH (N=191)	Total (N=392)
Number of blastocysts transferred per subject n (%)			
1	199 (99.0%)	190 (99.5%)	389 (99.2%)
>1	2 (1.0%)	1 (0.5%)	3 (0.8%)
Mean (SD)	1.0 (0.10)	1.0 (0.07)	1.0 (0.09)
Median	1.0	1.0	1.0

FROZEN CYCLE 1	HP-hMG (N=82)	rFSH (N=130)	Total (N=212)
Number of blastocysts transferred per subject n (%)			
1	69 (84.1%)	98 (75.4%)	167 (78.8%)
>1	13 (15.9%)	32 (24.6%)	45 (21.2%)
Mean (SD)	1.2 (0.37)	1.2 (0.43)	1.2 (0.41)
Median	1.0	1.0	1.0

FROZEN CYCLE 2	HP-hMG (N=8)	rFSH (N=12)	Total (N=20)
Number of blastocysts transferred per subject n (%)			
1	6 (75.0%)	7 (58.3%)	13 (65.0%)
>1	2 (25.0%)	5 (41.7%)	7 (35.0%)
Mean (SD)	1.3 (0.46)	1.4 (0.51)	1.4 (0.49)
Median	1.0	1.0	1.0

FROZEN CYCLE 3	HP-hMG (N=0)	rFSH (N=1)	Total (N=1)
Number of blastocysts transferred per subject n (%)			
1	0	0	0
>1	0	1 (100.0%)	1 (100.0%)
Mean (SD)		2.0 (NA)	2.0 (NA)
Median		2.0	2.0

n = number of subjects with specified transfer.
N = number of subjects in the treatment group for the given cohort.
SD, standard deviation; NA, not applicable