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Evaluation of mixed protocols with Bravelle® (human-derived FSH) and Repronex® (hMG) to assess clinical efficacy (EMBRACE) in women undergoing in vitro fertilization

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Objective: To compare the efficacy and safety of three different ratios of human-derived follicle-stimulating hormone/human menopausal gonadotropin (human-derived FSH:hMG, Bravelle and Repronex) mixed together in the same syringe and administered subcutaneously once daily, to in vitro fertilization (IVF) patients <34 years or 34 to 40 years of age.

Design: Two randomized, prospective, age stratified, IVF studies.

Setting: Twenty-one academic and private clinics with experience in IVF/embryo transfer (ET).

Patient(s): Infertile premenopausal women undergoing IVF-ET.

Intervention(s): Pituitary suppression with leuprolide acetate, randomization to one of three treatment groups, followed by gonadotropin stimulation (GS) for up to 15 days. The human-derived FSH:hMG ratios were the following: Group 1, a 1:1 ratio throughout; Group 2, a 3:0 ratio that was changed to 1:1 after GS day 5; Group 3, a 2:1 ratio that was increased to 3:1, 4:1, or 5:1 after GS day 5, as needed.

Main Outcome Measure(s): Mean number of oocytes retrieved; peak estradiol levels; dose and duration of stimulation; implantation rates; adverse events; injection site pain; and pregnancy and live birth rates.

Result(s): Overall, women <34 years had higher E_2 levels, more oocytes retrieved, and improved implantation and live birth rates compared with women 34 to 40 years old. Nonetheless, each ratio of human-derived FSH:hMG produced comparable implantation rates, and continuing pregnancy and take-home baby rates.

Conclusion(s): All three ratios of human-derived FSH:hMG in both age groups produced comparable pregnancy and live birth rates with similar safety results. (Fertil Steril® 2004;82:348–57. ©2004 by American Society for Reproductive Medicine.)

Key Words: Bravelle, Repronex, FSH, LH, hMG, urofollitropin, in vitro fertilization, subcutaneous, highly purified, human clinical trial, pregnancy

The discovery that follicle-luteinizing stimulating hormone (FSH) and (leuteinizing) hormone (LH) were necessary for follicular development and growth ushered in a new era in the treatment of infertility (1). In 1958, this culminated in the first use of human pituitary gonadotropin extracts to induce ovulation in anovulatory women (2). Lunenfeld (3) was the first to

report the use of human menopausal gonadotropin (hMG) for follicular stimulation that resulted in a pregnancy. The first commercially available gonadotropin in the United States for follicle stimulation, hMG, was approved in 1970. These early preparations contained approximately equal amounts of FSH and LH activity and were derived from the urine of postmenopausal women. From these beginnings, advanced technical developments and enhanced chromatographic techniques have been applied to produce an evolution of human-derived products of increasing consistency, purity, and predictable biological activity. As such, human-derived products have remained a standard treatment modality for infertile women (4–7).

Early advances in chromatographic separation techniques led to the development of an injectable, human-derived FSH preparation containing up to 1% LH activity (8) that was approved in the United States in 1986. A more highly purified version of FSH was then developed and appeared in 1996. Coincident with these developments in human-derived gonadotropins was the application of tools used in molecular biology. These tools made possible the production of recombinant FSH secreted from genetically engineered Chinese hamster ovary cells (9), which is devoid of LH activity. Despite identical amino acid sequences in both human-derived and recombinant FSH preparations, there are differences in the glycosylation patterns, which are known to influence the isoform profiles of these compounds (10). The clinical implications of differing isoform profiles for FSH products are unclear, and, at this time, there are no convincing data to indicate superiority of either human-derived or animal-derived FSH formulations for ovarian stimulation in terms of efficacy or safety (7, 11).

The impetus behind the development of FSH-only products was studies suggesting that elevated levels of follicular-phase serum LH had adverse effects on conception rates and outcomes in anovulatory women with polycystic ovary syndrome (12, 13) and even in women with regular cycles (14). This hypothesis was subsequently tested in randomized trials comparing hMG and FSH (15–18). These studies and others, despite their adequate design, were unable to identify a consistent statistical or clinical advantage in favor of either hMG or FSH.

Controlled ovarian hyperstimulation protocols that use both hMG and FSH, known as "mixed protocols," have become popular in an attempt to achieve higher pregnancy rates (19) and reduce the incidence of ovarian hyperstimulation syndrome (OHSS) over protocols that employ FSH alone (20). However, today a variety of mixed protocols are being used without the benefit of empiric data to guide physicians in choosing a ratio of FSH:LH activity for each woman.

The present study was designed to explore the efficacy and safety of three different ratios of human-derived FSH: hMG, in an attempt to identify any substantial differences in these regimens for ovulation induction in in vitro fertilization (IVF) procedures. Based on data from the Society for Assisted Reproductive Technology (SART) and the American Society for Reproductive Medicine report demonstrating that older women respond differently than younger women to ovarian stimulation protocols (4), we stratified our patients by age in order to examine their responses to the differing FSH:LH activity ratios.

MATERIALS AND METHODS

Study Design

These were two prospective, randomized, comparative, three-arm, open-label studies conducted in parallel at 21 academic and private IVF centers. Each center was a member of SART and obtained institutional review board approval for the study. All patients provided written informed consent before participation.

Patients

A total of 111 women <34 years of age were recruited and screened by 10 centers: three women failed to down-regulate, and 108 were randomized to gonadotropin treatment. In the other study, a total of 128 women, 34 to 40 years of age, were recruited and screened by 11 centers: four women failed to down-regulate; three had positive pregnancy tests before leuprolide acetate (LA) administration; one developed ovarian cysts; and 120 were randomized to gonadotropin treatment.

Patients needed to be nonsmoking women with regular ovulatory menstrual cycles of 24 to 35 days and a documented history of infertility for at least 1 year attributed to or associated with either tubal factor infertility, endometriosis (American Fertility Society revised stage I or II only), or unexplained causes. In addition, the male partners needed to have had a semen analysis that was considered normal according to revised World Health Organization (WHO) standard values within 6 months of initiation of the study.

A minimum of one menstrual cycle, without any involvement in assisted reproductive techniques (ART), was required before being enrolled. Patients were excluded if there was evidence of any clinically relevant systemic disease or any surgical or medical condition, which, in the judgment of the investigator, would interfere with the absorption, metabolism, or excretion of gonadotropins or that would make an ensuing pregnancy unsafe. Patients were excluded from the study if they had a positive pregnancy test within the last 3 months; a body mass index >34; a history of abnormal uterine bleeding; history of chemotherapy; documented intolerance or allergy to any gonadotropin; or an active history of substance abuse.

Patients were not to be currently breast-feeding, receiving oral contraceptives during the cycle prior to the start of the study, or to have participated in any experimental drug study within the previous 60 days.

TABLE 1

Ratios of hFSH:hMG administered among the treatment groups.

Stimulation days	1–5	6–15[a]			
1:1 hFSH:hMG					
Total FSH:LH activity	225:112.5	225:112.5	300:150	375:187.5	450:225
Dose ratio hFSH:hMG	2:1	2:1	2:1	2:1	2:1
Vial ratio Bravelle:Repronex	1.5:1.5	1.5:1.5	2:2	2.5:2.5	3:3
hMG add-on					
Total FSH:LH activity	225:0	225:112.5	300:150	375:187.5	450:225
Dose ratio hFSH:hMG	3:0	2:1	2:1	2:1	2:1
Vial ratio Bravelle:Repronex	3:0	1.5:1.5	2:2	2.5:2.5	3:3
Low-dose hMG					
Total FSH:LH activity	225:75	225:75	300:75	375:75	450:75
Dose ratio hFSH:hMG	3:1	3:1	4:1	5:1	6:1
Vial ratio Bravelle:Repronex	2:1	2:1	3:1	4:1	5:1

[a] The dose of hFSH and hMG was allowed to be adjusted upward every other day, as needed, by 75 or 150 IU FSH while maintaining the assigned ratio starting on day 6 to achieve adequate follicular development; or it could be decreased at the investigators' discretion to minimize any occurrence of OHSS.

Keye. Mixed protocols in IVF patients. Fertil Steril 2004.

Methods

Eligible women received LA (0.5 mg/day, subcutaneous) beginning 7 days before the anticipated onset of the next menses and continuing for a maximum of 20 days until serum estradiol (E_2) levels of ≤45 pg/mL were achieved and an endometrial thickness of <6 mm was observed. Thereafter, LA was reduced to 0.25 mg/day and continued until the day before human chorionic gonadotropin (hCG) administration. Following successful down-regulation, women were randomized (blocks of 3; Target Health Inc., SAS Proc Plan, Cary, NC) to one of the three treatment groups. The human-derived FSH: hMG vial ratios of the three groups were as follows: the 1:1 human-derived FSH:hMG group initiated and maintained a 1:1 ratio throughout stimulation; the hMG add-on group initiated the cycle with human-derived FSH only (3:0 ratio) and then switched to a 1:1 ratio of human-derived FSH:hMG on stimulation day 6, that was then maintained until the end of stimulation; the low-dose hMG group initiated the cycle with a 2:1 ratio and then, as needed, beginning on gonadotropin-stimulation day 6, sequentially adjusted the ratio to 3:1, 4:1, or 5:1 (Table 1). For all groups, gonadotropin stimulation was initiated with and maintained at a total daily dose of 225 IU of human-derived FSH for the first 5 days of stimulation. Thereafter, doses could be increased every other day, starting on day 6, as needed, to a maximum of 450 IU human-derived FSH per day to induce optimal follicular development. Ovarian stimulation was not to exceed 15 days, and the gonadotropin dose could be decreased at any time for safety reasons at the discretion of the investigator.

In all treatment groups, human-derived FSH (Bravelle; Ferring Pharmaceuticals Inc., Suffern, NY) and hMG (Repronex; Ferring Pharmaceuticals Inc.) were reconstituted in 0.9% NaCl, mixed together in the same syringe and administered as a single, daily, 1.0 mL, subcutaneous injection, regardless of the dose. A previous study established that after reconstitution in 0.9% NaCl, the mixing of these two specific human derived gonadotropins (Bravelle and Repronex) in a single syringe did not alter the expected bioactivity of either agent (21). Patients were instructed to keep a daily record of the injection site and presence and intensity of any associated pain. Injection site pain was evaluated on a numerical scale of 1 (no pain) to 10 (severe pain), as previously reported (7).

The criteria for hCG administration were that the woman had at least three follicles ≥16 mm, calculated as the mean of three perpendicular planes by transvaginal ultrasound, along with an acceptable E_2 level for the number of follicles, as judged by the investigator. The next day, patients received Novarel™ (10,000 IU, intramuscular; Ferring Pharmaceuticals Inc.) to complete oocyte maturation; oocytes were retrieved 34 to 36 hours later.

Standard center-specific IVF culture conditions were allowed, but intracytoplasmic sperm injection (ICSI) or assisted hatching were not. A maximum of four embryos were transferred into the uterus on day 3 to 5 after oocyte retrieval. The day of transfer depended on the usual embryologic and clinical decision characteristics used by each investigator. Patients received progesterone (progesterone in oil, 50 mg, intramuscular, daily; Schein Pharmaceuticals, Florham Park, NJ) for luteal-phase support starting on day 2 or 3 after oocyte retrieval; it was continued until either a negative pregnancy test was obtained or a positive fetal heart motion in an intrauterine pregnancy was observed. In the case of the latter, it was up to the discretion of the physician whether or not to continue progesterone supplementation. Throughout the study, investigators recorded the presence and nature of any adverse events.

A serum pregnancy test (β-hCG) was performed 14 days after embryo transfer, and, if positive, a second β-hCG measurement was performed 2 days later. With two positive results, transvaginal ultrasound was performed 2 weeks later to confirm a clinical pregnancy and repeated 1 week later to document a continuing pregnancy.

In keeping with the traditional measure of gonadotropin efficacy, the total number of oocytes retrieved was the primary outcome measure. Secondary efficacy measures were the dose and duration of gonadotropin stimulation; mean daily E_2 levels, calculated as the mean of all E_2 levels measured from the start of gonadotropin stimulation to the day of hCG administration; peak serum E_2 levels; the percentage of cycles with oocyte retrieval; and the percentage of cycles with embryo transfer and implantation rates. In addition, chemical, clinical, and continuing pregnancy rates as well as live birth rates were evaluated. All study analyses are based on the intention-to-treat population—that is, all patients randomized to receive gonadotropin treatment.

Statistical Analysis

The sample size calculations were based on the clinical assumption that 14 oocytes per woman would be retrieved. Power calculations were based on an α level of 0.05 with 80% power to detect a 30% difference with 35 evaluable women per group.

In assessing the primary efficacy measure, the number of oocytes retrieved per cycle, the difference between pairs of treatment groups was assessed by one-way analysis of variance (ANOVA). Dunnett's procedure was used to determine whether these comparisons were statistically significant. After examination of the bivariate relationships, analysis of covariance (ANCOVA) was used to assess the effect of treatment after adjustments for pretreatment subject characteristics, in this case baseline age and body mass index. From this data, 95% confidence intervals (CI) were calculated for the efficacy outcomes for each treatment group and compared. Chi-square tests were used to evaluate the incidence of adverse events, to make among-group and pairwise comparisons of the number of women with at least one adverse event and with at least one mild to moderate adverse event. Pain at the injection site was initially evaluated using a one-way ANOVA analysis. Subsequently, a linear mixed model was used to make comparisons between treatment groups. All statistical analyses were generated by SAS version 6.12 (SAS Institute).

RESULTS

Women <34 Years of Age

One hundred and eight women were randomized to receive the 1:1 human-derived FSH:hMG ratio (n = 35), hMG add-on (n = 39), or low-dose hMG (n = 34) treatment protocol. Combined demographic data for all patients <34 years of age are presented in Table 2. Patients were well-matched in terms of age (mean years ± SD): 30.0 ± 2.2, versus 30.0 ± 2.3, versus 30.5 ± 2.0, in the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups (P = .527), respectively. Similarly, body mass index was well-matched at 24.2 ± 3.7, versus 24.2 ± 4.4, versus 24.3 ± 4.7 (P = .997), in the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups, respectively. There were no among group differences in the infertility diagnoses of patients <34 years of age in the three dosing regimens.

TABLE 2

Baseline demographic characteristics for the 108 women who were <34 years of age and the 120 women who were 34 to 40 years of age.

	Women <34 years of age (n = 108)	Women 34 to 40 years of age (n = 120)	*P* value
Age (mean years)	30.1	36.6	<.0001
Weight (mean pounds)	144	147	.432
Height (mean inches)	64.5	64.7	.655
Body mass index (mean)	24.2	24.7	.444
Primary Infertility Diagnosis (%)			
Tubal factor	52.9	48.4	.503
Endometriosis	18.3	9.2	.040
Unexplained	28.8	42.4	.030
Baseline Serum[a]			
FSH (mIU/mL)	6.2	6.4	.478
LH (mIU/mL)	4.8	4.1	.034
E_2 (pg/mL)	39.0	43.7	.061
Day 1 Serum[b]			
FSH (mIU/mL)	4.3	4.2	.680
LH (mIU/mL)	4.4	3.6	.025
E_2 (pg/mL)	24.9	28.0	.020

[a] Immediately prior to initiation of leuprolide acetate (mean values).
[b] Immediately prior to the first dose of gonadotropin stimulation (mean values).

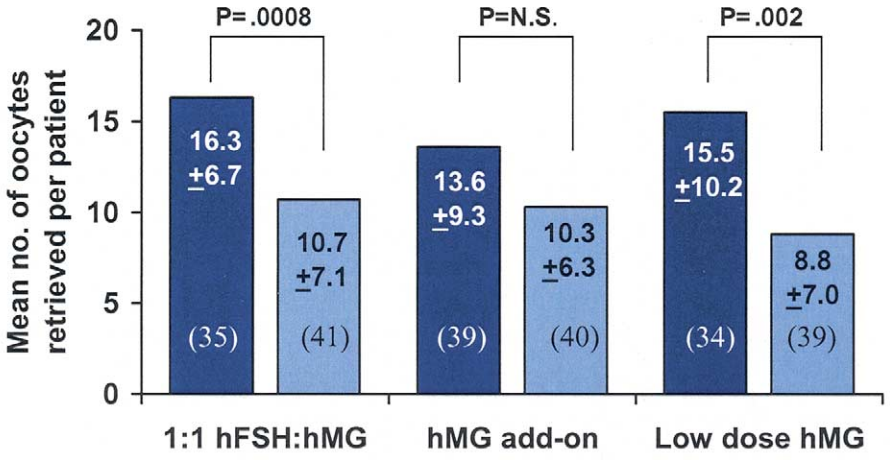
Keye. Mixed protocols in IVF patients. Fertil Steril 2004.

The primary efficacy measure of this study was the total number of oocytes retrieved from each woman. In women <34 years of age, there were numerically more oocytes retrieved from women in the 1:1 human-derived FSH:hMG group than the other two groups, although the difference did not attain statistical significance (Fig. 1).

When secondary efficacy parameters were examined, there was a statistically significant difference (P <.015) in the total dose (IUs) of human-derived FSH used among the treatment groups. A pairwise comparison showed that the women in the 1:1 human-derived FSH:hMG group used significantly less (P = .005) human-derived FSH (2168 ± 691) than those in the low-dose hMG group (2616 ± 687). Similarly, women in the hMG add-on group (2319 ± 551) used less (P = .052) human-derived FSH compared to the low-dose hMG group. There was no statistical difference in the total dose of human-derived FSH used between the 1:1

FIGURE 1

Mean number of oocytes retrieved from women in the <34 years of age (*dark blue*) and the women 34 to 40 years of age (*light blue*). The mean number of oocytes (± SD) is indicated along with the number of women (in parentheses). Women in the 1:1 human-derived FSH:hMG group and in the low-dose hMG group who were <34 years of age had significantly higher numbers of oocytes retrieved than women 34–40 years of age in the same dose ratio groups. There were no statistically significant differences among the treatment groups within either age range.



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human-derived FSH:hMG group and the hMG add-on group. Overall, the mean duration of gonadotropin stimulation (days) did not differ among the women in the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups, 9.4 ± 1.7, versus 9.4 ± 1.2, versus 9.9 ± 1.4 (P = .272), respectively.

Mean daily E_2 levels (Fig. 2A) and mean peak serum E_2 levels (see Fig. 2B) are presented for treatment group comparisons. Women <34 years of age in the 1:1 human-derived FSH:hMG group achieved significantly higher (P <.001) mean daily E_2 levels (see Fig. 2A), than either the hMG add-on or low-dose hMG groups. In addition, the 1:1 human-derived FSH:hMG group had significantly higher mean peak E_2 levels as compared to either the hMG add-on (P <.001) or the low-dose hMG groups (P <.009) (see Fig. 2B).

There were no statistically significant differences between the hMG add-on and the low-dose hMG groups in either the mean daily E_2 or mean peak serum E_2 levels.

The number of women who had embryo transfer was similar (P = .650) among the three treatment groups: 32/35 (91.4%) in the 1:1 human-derived FSH:hMG group; 34/39 (87.2%) in the hMG add-on group; and 32/34 (94.1%) in the low-dose hMG group. Ninety-four percent of the women (101/108) <34 years of age had a day-3 embryo transfer. The mean numbers of embryos transferred in women <34 years of age was similar (P = .547) among the three treatment groups: 2.7 ± 1.0 in the 1:1 human-derived FSH:hMG group; 2.5 ± 1.2 in the hMG add-on group; and 2.7 ± 1.0 in the low-dose hMG group. The chemical, clinical, and continuing pregnancy rates and live birth rates are presented in Figure 3. The pregnancy rates and live birth rates were comparable across the dosing regimens in women <34 years of age, and there were no statistically significant differences in any of the outcome parameters. Implantation rates (number of intrauterine gestational sacs divided by the number of embryos transferred) were not statistically significantly different among treatment groups (P = .804), 28.1% versus 32.0% versus 28.3% for the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups, respectively.

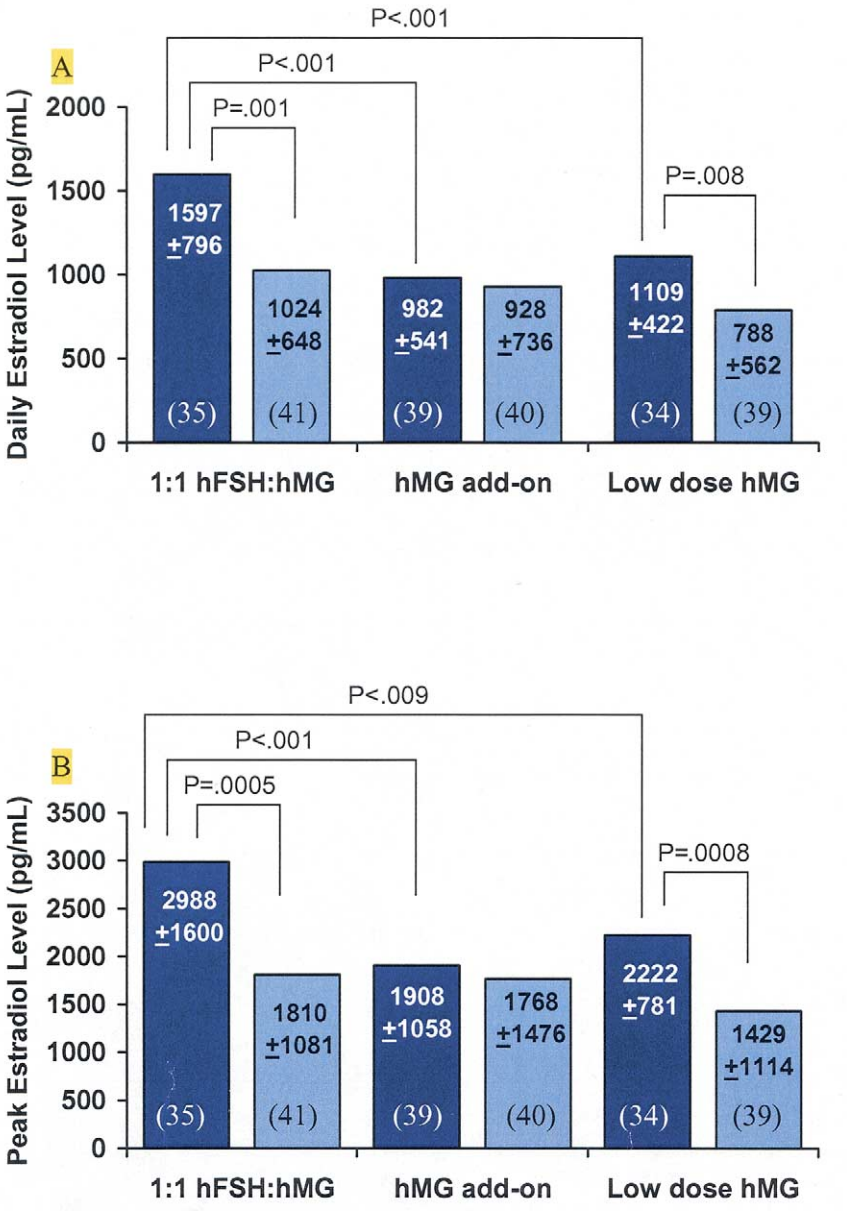
Daily diary injection-site pain scores were modestly higher (P = .044) in women in the 1:1 human-derived FSH:hMG group (3.8) as compared with the hMG add-on group (2.9) or the low-dose hMG group (2.7). There was no statistically significant among-group differences in the nature or frequency (P = .084) of adverse events across the three dosing regimens (Table 3).

Women 34 to 40 Years of Age

One hundred and twenty women were randomized to receive the 1:1 human-derived FSH:hMG ratio (n = 41), hMG add-on (n = 40), or low-dose hMG (n = 39) treatment protocol. Combined demographic data for all patients 34 to 40 years of age are presented in Table 2. Patients were well-matched in terms of age (mean years ± SD): 36.3 ± 2.0, versus 36.8 ± 1.9, versus 36.8 ± 1.9 in the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups (P = .435), respectively. Similarly, body mass index

FIGURE 2

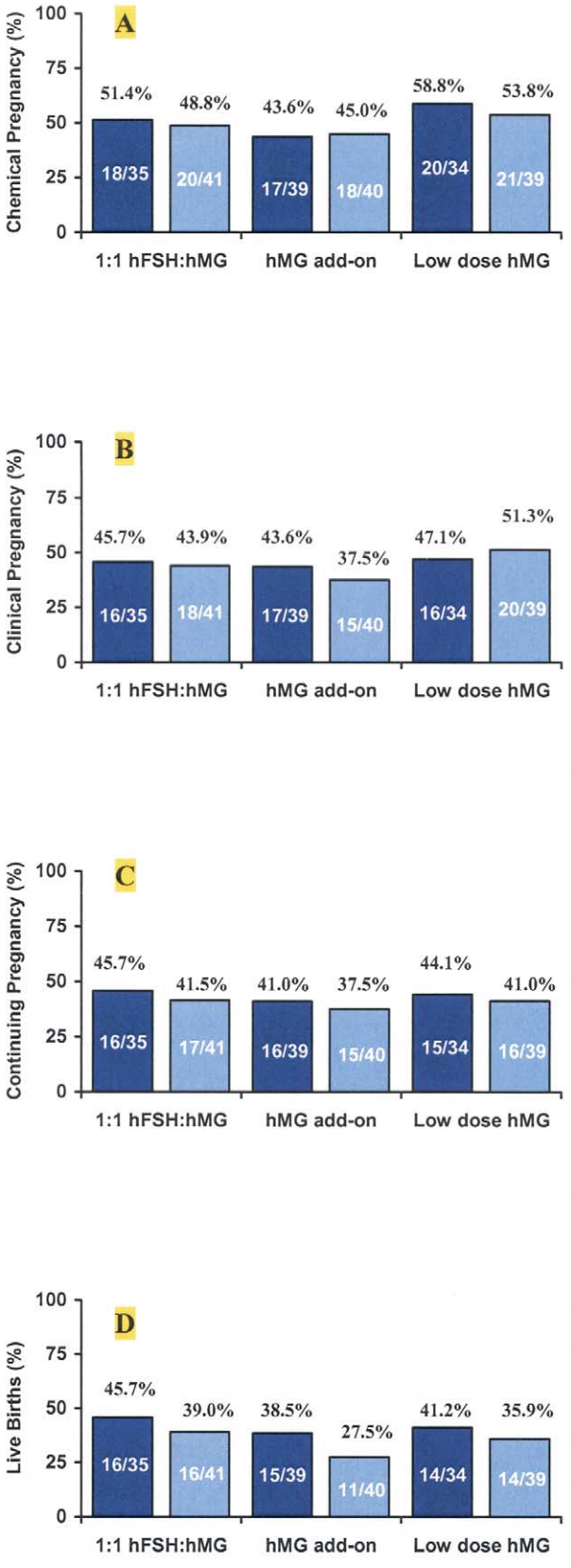
Mean daily and mean peak serum estradiol levels in women <34 years of age (*dark blue*) and women 34 to 40 years of age (*light blue*), in the three dosing ratio regimens, along with numbers of women evaluated (in parentheses). Values expressed are mean ± SD. (**A**) Mean daily serum estradiol levels. Women <34 years of age in the 1:1 human-derived FSH:hMG group had significantly higher levels than same aged patients in either the hMG add-on group (P <.001) or the low-dose hMG group (P <.001). The women in the 1:1 human-derived FSH:hMG group <34 years of age also had significantly higher (P = .001) levels than the women in the 1:1 human-derived FSH:hMG group who were 34 to 40 years of age. Women in the low-dose hMG group who were <34 years of age had significantly higher (P = .008) levels than women in the low-dose hMG group who were 34 to 40 years of age. (**B**) Mean peak serum estradiol levels. Women <34 years of age in the 1:1 human-derived FSH:hMG group had significantly higher levels than same-aged patients in the hMG add-on group (P <.001) and the low-dose hMG group (P <.009). The women in the 1:1 human-derived FSH:hMG group <34 years of age also had significantly higher (P = .0005) levels than the women in the 1:1 human-derived FSH:hMG group who were 34 to 40 years of age. Women in the low-dose hMG group who were <34 years of age had significantly higher (P = .0008) levels than the women in the low-dose hMG group who were 34 to 40 years of age.



Keye. Mixed protocols in IVF patients. Fertil Steril 2004.

FIGURE 3

Percentage of women with (**A**) chemical, (**B**) clinical, and (**C**) continuing pregnancy rates and (**D**) live birth rates. There was no statistically significant among-group difference in either pregnancy or live birth rates across the three dose ratios, in either age group. When women <34 were compared with women 34 to 40 years of age, treated with the same dose ratio, there were no between-group differences. Dark blue: Women <34 years of age. Light blue: Women 34 to 40 years of age.



Keye. Mixed protocols in IVF patients. Fertil Steril 2004.

was well-matched at 24.9 ± 4.3, versus 24.9 ± 4.9, versus 24.2 ± 4.4 ($P = .751$), in the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups, respectively. There were no among-group differences in the infertility diagnoses of patients 34 to 40 years of age in the three dosing regimens.

The primary efficacy measure of this study was the total number of oocytes retrieved from each woman. There were no statistically significant differences in the number of oocytes obtained between treatment groups (see Fig. 1). When secondary efficacy parameters were evaluated, no statistically significant differences ($P = .893$) were observed in the total dose of human-derived FSH (IU) used among groups, 2489 ± 909, 2557 ± 906, and 2465 ± 840 for the 1:1 human-derived FSH:hMG, hMG add-on, and low-dose hMG groups, respectively. Overall, the mean duration of gonadotropin stimulation (days) did not differ between the 1:1 human-derived FSH:hMG, hMG add-on, and the low-dose hMG groups: 9.5 ± 1.8 versus 9.5 ± 2.0 versus 9.2 ± 1.9 ($P = .603$), respectively.

Mean daily E_2 levels (see Fig. 2A) and mean peak serum E_2 levels (see Fig. 2B) are presented for treatment group comparisons. There were no statistically significant differences observed in either mean daily E_2 levels or mean peak serum E_2 levels between the 1:1 human-derived FSH:hMG, hMG add-on and low-dose hMG groups (see Fig. 2). The number of women who had embryo transfer was similar among the three treatment groups ($P = .435$): 37/41 (90.2%) in the 1:1 human-derived FSH:hMG group; 32/40 (80.0%) in the hMG add-on group; and 34/39 (87.2%) in the low-dose hMG group. Eighty-one percent of the women (97/120) 34 to 40 years of age had a day-3 embryo transfer. The mean numbers of embryos transferred in women 34 to 40 years of age was similar ($P = .518$) among the three treatment groups: 2.4 ± 1.2 in the 1:1 human-derived FSH:hMG group; 2.2 ± 1.4 in the hMG add-on group; and 2.1 ± 1.2 in the low-dose hMG group. The chemical, clinical, and continuing pregnancy rates and live birth rates are presented in Figure 3. The pregnancy rates and live birth rates were comparable across the dosing regimens in women 34 to 40 years of age, and there were no statistically significant differences in any of the outcome parameters. Implantation rates (number of intrauterine gestational sacs divided by the

TABLE 3

Summary of adverse events (%) for women <34 years of age and women 34 to 40 years of age in the 1:1 hFSH:hMG, hMG add-on and low-dose hMG dosing regimens.

	No. (%) of women					
	1:1 hFSH:hMG		hMG add-on		Low-dose hMG	
Age (years)	<34 (n = 35)	34–40 (n = 41)	<34 (n = 39)	34–40 (n = 40)	<34 (n = 34)	34–40 (n = 39)
Any adverse event	19 (54.3)	18 (43.9)	19 (48.7)	23 (57.5)	25 (73.5)	19 (48.7)
Serious/severe adverse events[a]	2 (5.7)	3 (7.3)	4 (10.3)	4 (10.0)	2 (5.9)	0 (0.0)
Most frequently reported adverse events						
Abdominal cramps	5 (14.3)	2 (4.9)	1 (2.6)	5 (12.5)	3 (8.8)	3 (7.7)
Nausea	5 (14.3)	3 (7.3)	0 (0)	1 (2.5)	4 (11.8)	6 (15.4)
Headache	2 (5.7)	0 (0)	4 (10.3)	3 (7.5)	3 (8.8)	5 (12.8)
OHSS	4 (11.4)	0 (0)	2 (5.1)	3 (7.5)	2 (5.9)	2 (5.1)
Vaginal bleeding/spotting	1 (2.9)	3 (7.3)	2 (5.1)	0 (0)	4 (11.8)	1 (2.6)
Abdominal pain	4 (11.4)	1 (2.4)	2 (5.1)	1 (2.5)	1 (2.9)	0 (0)
Self-assessment injection-site pain score (mean ± SD)	3.8 (0.3)	2.5 (0.2)	2.9 (0.3)	2.8 (0.2)	2.7 (0.3)	3.0 (0.3)

[a] In the <34 year age group, one woman in the 1:1 hFSH:hMG group developed moderate OHSS and another developed a right subclavian thrombosis: both required hospitalization and resolved without sequelae. In the hMG add-on group, one woman developed OHSS and another experienced pelvic pain: both required hospitalization and resolved without sequelae. In the low-dose hMG group, one woman developed an ectopic pregnancy which resolved with methotrexate. In the 34 to 40 year old group, one woman in the 1:1 hFSH:hMG group developed an ectopic pregnancy which resolved with surgery. In the hMG add-on group, two women developed OHSS; both were hospitalized and both events resolved without sequelae.

Keye. Mixed protocols in IVF patients. Fertil Steril 2004.

number of embryos transferred) were not statistically significantly different among treatment groups ($P = .629$), 31.0%, 25.0%, and 30.1%, for the 1:1 human-derived FSH:hMG, hMG add-on, and low-dose hMG groups, respectively.

Daily diary injection-site pain scores were not statistically significantly different ($P = .365$) among the 1:1 human-derived FSH:hMG, hMG add-on, and low-dose hMG groups, 2.5 versus 2.8 versus 3.0, respectively. There was no statistically significant among-group differences ($P = .472$) in the nature and frequency of adverse events across the three dosing regimens (see Table 3).

Comparisons Between Age Groups

This analysis represents a comparison of the two different age groups of patients. The number of oocytes retrieved (primary efficacy measure) was statistically significantly different ($P = .0008$) between women <34 years of age (16.3 ± 6.7) and women 34 to 40 years of age (10.7 ± 7.1) in the 1:1 human-derived FSH:hMG group. Also, there was a statistically significant difference ($P = .002$) in the number of oocytes retrieved between women <34 years of age (15.5 ± 10.2) and women 34 to 40 years of age (8.8 ± 7.0) in the low-dose hMG group. The difference in oocytes retrieved between age groups in the hMG add-on group did not attain statistical significance ($P = .07$), but the trend was in favor of women <34 years of age (see Fig. 1).

Between-study secondary efficacy parameters were also evaluated. The mean duration of gonadotropin stimulation (days) and the mean total dose of human-derived FSH (IU) used was not statistically significantly different between age groups for any of the three dosing regimens. Women <34 years of age in the 1:1 human-derived FSH:hMG group and in the low-dose hMG group achieved significantly higher mean daily E_2 levels as compared to the women 34 to 40 years of age in the same dose ratio groups (see Fig. 2A). In addition, women <34 years of age in the 1:1 human-derived FSH:hMG group and in the low-dose hMG group achieved significantly higher mean peak E_2 levels as compared with the women 34 to 40 years of age in the same dose ratio groups (see Fig. 2B). There were no statistically significant differences in either mean daily E_2 levels or mean peak serum E_2 levels between women <34 years of age and women 34 to 40 years of age in the hMG add-on group. There were no statistically significant differences obtained between age groups in any dosing regimen for the percentage of women who underwent embryo transfer. Similarly, no statistically significant differences in pregnancy rates or live birth rates were observed between age groups in the three dosing regimens (see Fig. 3).

When the two studies were compared for the incidence of adverse events, the difference in the percentage of women with an adverse event did not differ between age groups for the 1:1 human-derived FSH:hMG or the hMG add-on dosing regimens. However, more women in the <34 years age group receiving low-dose hMG reported adverse events (see

Table 3) compared with women 34 to 40 years of age in the same dosing regimen, 73.5% versus 50.0% ($P = .041$). In all treatment groups, most events were mild or moderate in severity, and there were no clinically meaningful differences in the nature of adverse events (see Table 3).

DISCUSSION

There has been little progress in improving the efficacy of controlled ovarian hyperstimulation (COH) protocols in infertility patients over the past decade. The increased use of donor oocytes, the introduction of ICSI, and improvements in the microenvironment for embryo culture have largely been responsible for the steadily improving pregnancy rates of IVF which have occurred since the mid-1990s (22). The introduction of recombinant gonadotropin preparations devoid of significant LH activity, once heralded as a major breakthrough in ovulation induction, have not had a clinically significant impact on pregnancy rates (14). In an attempt to improve the efficacy of ovulation induction protocols, many clinicians have recently employed protocols that combine hMG and highly purified or recombinant FSH, so-called "mixed protocols."

The use of mixed protocols has made it possible to vary the ratio of FSH to LH activity just as the pituitary does during the follicular phase of an unstimulated spontaneous menstrual cycle (23). While a variety of mixed protocols have been used by clinicians to improve the efficacy of COH, three mixed protocols were chosen for evaluation in this study. The first, referred to as the "1:1 human-derived FSH:hMG protocol," was chosen because it is commonly used in clinical practice, is simple to administer, and reduces the relative contribution of LH activity below that present in hMG. Using equal amounts of hMG and human-derived FSH, a constant ratio of FSH to LH activity of 2:1 was achieved. The second was referred to as the " hMG add-on" group because LH activity was introduced after 5 days of human-derived FSH alone. This protocol was chosen because of the concern that exposure to LH activity in the early follicular phase might suppress follicular development or cause premature luteinization of developing follicles. The third was called the "low-dose hMG" protocol, and it was chosen because it provided only small, permissive amounts of LH activity, likely to be below the theoretical LH-ceiling above which LH activity was hypothesized to be detrimental (24).

In spite of these differences in LH activity, the differences in efficacy were small and of little clinical significance. However, the 1:1 hMG protocol was associated with a trend toward the lowest dose of FSH required, the highest mean daily and peak estradiol concentrations, a greater number of oocytes retrieved, a higher fertilization rate, and a higher take-home baby rate than the other two dose ratios for both age groups. The other consistent observation was that fewer women in the 1:1 hMG group (47.4%) required an increase in the dose of gonadotropins as compared with the hMG add-on (60.3%) or the low-dose hMG (69.0%) groups. These findings are consistent with the theory that some women who receive a GnRH-agonist before the start of gonadotropin therapy may have profound suppression of their endogenous LH and thus may require continuous exogenous LH-activity for optimal follicular growth and oocyte maturation (25).

The benefit of youth was evident in this study. The younger women, who on average were 6 years younger than the older group, had higher daily and peak estradiol levels, a greater percentage met the criteria for hCG administration; a higher number of oocytes retrieved, and they had higher implantation and live birth rates. What is surprising is that these differences between age groups were small and often were not statistically significantly different with the exception of the mean total dose of FSH, and the daily and peak estradiol levels. One explanation for these findings is that the baseline FSH levels were not higher in the older women as compared with the younger women, suggesting there was no significant difference in ovarian reserve between the younger and older women. As such, this may not necessarily be representative of what is seen in an unselected group of women treated in most clinical practices. In addition, it is acknowledged that the studies were powered to detect differences among the dose ratio regimens and not specifically between age groups. Nonetheless, the LH activity in the mixed protocol may in some way be responsible for minimizing the age group differences.

One of the disadvantages of mixed protocols is the practice of splitting the doses into two injections, whereby the FSH is given at one time of the day and the hMG at another time of the day. In addition, this practice often requires a third injection for delivery of a GnRH agonist or antagonist. As a result, mixed protocols are somewhat inconvenient and often require more injections than protocols that use a single gonadotropin. The results from this study demonstrate that these two human-derived gonadotropin preparations can be conveniently mixed and administered together as a single daily injection, which produces adequate follicular growth, oocyte maturation, and excellent pregnancy rates. These clinical data confirm the results of previously conducted bioassay studies, which demonstrated the compatibility of mixing and administering these two human-derived gonadotropins as a single injection (21).

In summary, the results of this study demonstrate the efficacy and safety of three mixed protocol dosing regimens of human-derived FSH:hMG. While some differences were observed among the three dose ratios in patients <34 years of age, this study provides solid evidence that, in both young and older patients, the combination of human-derived FSH and hMG in COH yields excellent implantation and continuing pregnancy rates, as well as excellent take-home baby rates. The challenge ahead is to assess characteristics of individual women, and from this information be able to

determine in advance of an IVF attempt which COH protocol will produce the highest take-home baby rate.

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