Individualized ovarian stimulation for in vitro fertilization: a multicenter, open label, exploratory study with a mixed protocol of follitropin delta and highly purified human menopausal gonadotropin

Francois Bissonnette, M.D.,[a,b] Jaume Minano Masip, M.D.,[a,b] Isaac-Jacques Kadoch, M.D.,[a,b] Clifford Librach, M.D.,[c] John Sampalis, Ph.D.,[d] and Albert Yuzpe, M.D.[e]

[a] Clinique Ovo, Montreal, Quebec; [b] Obstetrics and Gynecology Department CHUM, Montreal, Quebec; [c] CReATe Fertility Centre, Toronto, Ontario; [d] McGill University, Montreal, Quebec; [e] Olive Fertility Centre, Vancouver, British Columbia; and REI Division, Department of Obstetrics and Gynecology, Faculty of Medicine, University of British Columbia, Kelowna, British Columbia, Canada

Objective: To evaluate the safety profile and the number of usable blastocysts on day 5 and on day 6 after treatment with an individualized dosing regimen of a follitropin delta and highly purified human menopausal gonadotropin (HP-hMG) for controlled ovarian stimulation.
Design: Multicenter, open label, exploratory study.
Setting: Reproductive medicine clinics.
Patient(s): A total of 110 patients (aged 18–40 years).
Intervention(s): Follitropin delta coadministered with HP-hMG, with follitropin delta dose fixed according to an established algorithm and HP-hMG dose at 75 IU when the follitropin delta starting dosage was <12 μg; 150 IU when follitropin delta dosage was 12 μg and weight <100 kg, and 225 IU when follitropin delta dosage was 12 μg and weight ≥ 100 kg (dosage adjustments confined to HP-hMG only).
Main Outcome Measure(s): Mean number of good-quality blastocysts obtained at day 5 and day 6 as well as the proportion of women with ovarian hyperstimulation syndrome (OHSS).
Result(s): A cohort study was compared with the follitropin delta group from the Evidence-based Stimulation Trial with Human Recombinant Follicle-Stimulating Hormone in Europe and Rest of World 1 (ESTHER-1) study. Even when stratified by age, a statistically significantly higher mean in the number of oocytes retrieved and number of good-quality blastocysts was observed in this study compared with the ESTHER-1 trial in which follitropin delta was used alone. The rate of patients triggered with a gonadotropin-releasing hormone agonist was statistically significantly higher in our Menopur and Rekovelle Combined Study (MARCS) cohort (43%) when compared with the rates reported in the follitropin delta cohort in the ESTHER-1 study (2.3%). Incidence of any grade of OHSS was 9.3% in the present study compared to 2.6% in follitropin delta group from ESTHER-1 trial. No cases of moderate or severe OHSS were observed in our study compared with 1.4% in the follitropin delta group of ESTHER-1.
Conclusion(s): Optimizing the ovarian response during in vitro fertilization employing a mixed protocol of individualized dosing of follitropin delta and HP-hMG resulted in a statistically significant number of usable blastocysts on days 5 and 6 with an increased risk of mild OHSS, which did not require medical intervention or hospitalization.

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Reprint requests: Francois Bissonnette, M.D., Clinique Ovo, 8000 Decarie Blvd, H4P 2S4, Montreal, Quebec, Canada (E-mail: f.bissonnette@cliniqueovo.com).

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Key Words: Follitropin delta, good-quality blastocyst, HP-hMG, ovarian response, OHSS

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The ovarian response to stimulation with exogenous gonadotropins during in vitro fertilization (IVF) is a key factor affecting the success of the procedure (1, 2). However, standardizing an effective and safe ovarian stimulation dosage has been difficult due to the substantial heterogeneity in patients' ovarian response to the same dose of gonadotropin. The limited prognostic value of patient characteristics—including age, follicle-stimulating hormone (FSH), and antral follicle count (AFC)—and their inconsistent association with ovarian response to gonadotropins, as well as the lack of validated dosing algorithms (3, 4), further complicate the matter.

Advances in translational research such as genomics, proteomics, or metabolomics have shown how the patient's genetic and phenotypic profile can affect disease presentation and progression as well as response to treatment. This has led to the introduction of personalized medicine, whereby treatment regimens are adjusted according to the patient's profile to optimize the effectiveness and safety of the treatment (5).

Follitropin delta is a novel recombinant human FSH that is uniquely expressed in a human fetal retinal cell line by recombinant DNA technology. The amino acid sequences of the two FSH subunits α and β are identical to the endogenous human FSH sequences. Its mechanism of action is mediated by binding to the FSH receptors present in the ovary which triggers intracellular mechanisms that initiate several hormone and cellular events responsible for regulating the maturation of Graafian follicles and granulosa cell estrogen production (6). The major difference between follitropin alfa and follitropin delta is the difference in their glycosylation profile. Follitropin delta has a higher proportion of tri- and tetra-sialylated glycans than follitropin alfa and also has both α2,3- and α2,6-linked sialic acid whereas follitropin alfa only has α2,3-linked sialic acid (7). These differences drive follitropin delta to present higher exposure and lower serum clearance. Consequently, follitropin delta induces a higher ovarian response in humans than follitropin alfa when administered at equal doses of biological activity in international units (IU) (8). Also a recent observation that a daily dose of 10 μg of follitropin delta is equivalent to the conventional dose of 150 IU/day of follitropin alfa indicates that follitropin delta provides a higher ovarian response in humans when administered not only at equal units of biological activity (7) but also at the same microgram weight dose (9). Its dosage can be individualized based on a validated algorithm that is a function of the patient's basal antimüllerian hormone (AMH) level and body weight (Supplemental Table 1, available online).

The Evidence-based Stimulation Trial with Human Recombinant Follicle-Stimulating Hormone in Europe and Rest of World 1 (ESTHER-1) trial was a noninferiority, randomized, clinical trial that compared an individualized fixed dosage of follitropin delta determined by an algorithm based upon each woman's AMH and body weight with a conventional adjustable follitropin alfa dosage in patients undergoing their first IVF treatment. The results of the study demonstrated that despite the lack of dose adjustment an individualized follitropin delta dosage was noninferior to conventional follitropin alfa stimulation with respect to ongoing pregnancy rate and ongoing implantation rate. However, the incidence of ovarian hyperstimulation syndrome (OHSS) and the measures taken to prevent OHSS were statistically significantly lower in the patients treated with the personalized dose regimen (10).

In humans, FSH and luteinizing hormone (LH) work in concert to stimulate folliculogenesis and ovulation. They are used for controlled ovarian stimulation (COS) to increase the number of oocytes produced in IVF. Determination of a stimulation protocol and FSH dosage is generally established by the physician on the basis of the patient's age, body weight, and ovarian reserve profile. Although in vitro and animal models provide evidence of hormone-specific mechanisms of action, the choice of the optimal gonadotropin combination to be used in COS is not well standardized and is highly subjective, depending on the clinician's decision (11).

The effect of adding LH during COS has been widely studied. Some improvements have been reported in assisted reproduction outcomes, especially in patients with decreased ovarian reserve or in patients with inadequate response in a previous cycle (12). Both LH and human chorionic gonadotropin (hCG) act on the same receptor, activating different signal transduction pathways. Human chorionic gonadotropin is thought to enhance angiogenesis, becoming crucial in follicular development. Studies have shown that supplementation with highly purified human menopausal gonadotropin (HP-hMG), which contains hCG-driven LH bioactivity, generates a significant increase in proapoptotic cell gene expression in the stratum granulosum layer, which suggests a lead role in the developmental competence of the oocyte (13).

Owing to this, the FSH-LH combination concept, also referred to as "mixed" protocols (concomitant hMG and FSH), has been widely used for ovarian stimulation for over a decade. Indeed, COS protocols that use both HP-hMG (a source of both FSH- and hCG-driven LH activity) and FSH, also known as "blended" protocols, are commonly used in an attempt to obtain better-quality oocytes and embryos and thus higher pregnancy rates compared with the use of recombinant FSH alone. The Menopur Mixed Protocol (COMBINE) trial compared the fertilization rate between a combination of HP-hMG (Menopur) and FSH (Bravelle) mixed in the same syringe with Menopur alone with no difference found between the arms (14). However, to date no data exist

on combining an hMG preparation with follitropin delta, the new recombinant FSH.

Our current study, the Menopur and Rekovelle Combined Study (MARCS), explored a new regimen that takes into consideration each patient's weight and AMH concentration to determine the suitable doses of gonadotropins for COS. This novel approach is beneficial for patients because it increases the number of mature oocytes and good-quality blastocysts without compromising the patient's safety. The primary end point of this study was the number of usable blastocysts available on days 5 and 6 of embryo culture. The secondary end points were the ovarian response, embryology, and safety of this personalized regimen.

MATERIALS AND METHODS

Design

This was a multicenter, open-label, single–cohort with external (synthetic) control study assessing the efficacy and safety of a personalized dosage of follitropin delta in combination with HP-hMG. Patients were enrolled at four research sites in Canada. The study protocol was approved by local regulatory authorities as well as independent ethics committees. The study was performed in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulatory requirements. All participants provided written and informed consent before undergoing any study-related procedures.

Participants

Women enrolled in the study were between 18 and 40 years of age, had a confirmed diagnosis of infertility (including stage I/II endometriosis and tubal factor infertility), and were undergoing their first IVF/intracytoplasmic sperm injection (ICSI) cycle. Additional inclusion criteria were regular menstrual cycles of 24 to 35 days, presence of both ovaries, use of ejaculated sperm (fresh or frozen) for insemination, and an early follicular phase FSH serum concentration of <10 IU/L. The main exclusion criteria were stage III/IV endometriosis, high risk for OHSS (AMH ≥ 35 pmol/L), history of recurrent miscarriages (defined as ≥ 3 consecutive pregnancy losses), and use of hormone treatment (except for thyroid medication) during the last menstrual cycle before enrollment. The study medication was provided to the participants.

Procedures

All patients participating in the study were treated with a fixed personalized daily subcutaneous dose of follitropin delta, determined by an established algorithm based on body weight and serum AMH level (Supplemental Table 1). All AMH measurements were performed at a central laboratory (CReATe Fertility Centre) using the automated Elecsys AMH immunoassay (Roche), and were determined within the last 12 months before the start of ovarian stimulation (15).

Two separate syringes were used to coadminister HP-hMG with follitropin-delta to all patients at personalized doses according to the patient's body weight and the calculated dose of follitropin delta. More specifically, the initial dose of HP-hMG was 75 IU when the follitropin delta dosage was <12 μg; 150 IU when the follitropin delta dosage was 12 μg and body weight was <100 kg; and 225 IU when the follitropin delta dosage was 12 μg and body weight was ≥ 100 kg.

Gonadotropin therapy was initiated on day 2 of the menstrual cycle. A gonadotropin-releasing hormone (GnRH) antagonist was initiated on day 6 of stimulation and continued throughout the remainder of stimulation. The response to stimulation was monitored via serum estradiol (E_2) levels and vaginal sonography. Any adjustment in stimulation was confined to HP-hMG and was allowed only starting from day 6 of stimulation. The HP-hMG dose could be adjusted up or down according to the E_2 level on day 6 of stimulation regardless of follicular development, with 225 IU as the maximum daily dose. The HP-hMG dose adjustments were done according to the schedule described in Supplemental Table 2 (available online).

Triggering of final follicular maturation was performed when ≥ 3 follicles reached 17 mm in diameter. The choice of the triggering medication was based on serum E_2 levels before trigger: hCG 5,000–10,000 IU if E_2 $<10,000$ pmol/L; and GnRH agonist 0.2 mg (triptorelin acetate, Decapeptyl; Ferring Pharmaceuticals) if E_2 $\geq$ 10,000 pmol/L. In case of excessive response, defined as serum levels of E_2 $\geq 15,000$ pmol/L on the day of trigger or ≥ 20 follicles of ≥ 12 mm, a freeze-all strategy was employed. In the case of poor follicular development, defined as <3 follicles of ≥ 17 mm reached by day 20, the cycle was cancelled.

Oocyte retrieval was scheduled 36 ± 2 hours after triggering of final follicular maturation. Oocytes were inseminated by IVF or ICSI (on a case-by-case basis) using ejaculated sperm from a partner or donor. All embryos were cultured up to day 5 or 6. All fresh embryo transfers were performed on day 5 of culture, and all were single-embryo transfers. Surplus blastocysts were cryopreserved for future use when the grade was 3BB or higher according to the Gardner classification (16). Daily intramuscular progesterone was prescribed for luteal phase support. Adverse events were recorded from the time of signing the informed consent until the end of the study, defined as day-6 blastocyst formation. If any adverse event was reported during the study visits, follow-up calls were done by the research team until the resolution of the adverse event.

Outcomes

The primary end point of the study was the number of good-quality (>3BB) blastocysts, also referred to as "usable blastocysts," at day 5 or 6. The secondary end points were the ovarian response, embryology, and safety of the personalized regimen. Ovarian response and embryology included duration of stimulation, total doses of follitropin delta, percentage of patients with dose adjustments, percentage of patients triggered with GnRH agonist, the mean number of mature oocytes obtained, the mean number of embryos on day 3, and the percentage of day-3 embryos that evolved into a usable blastocysts on day 5 or 6, defined as the attrition rate between

day-3 embryos and blastocysts. Safety was assessed by evaluating the incidence and severity of early and late onset moderate to severe OHSS, classified according to Golan's system (Supplemental Table 5, available online) (17).

Statistical methods

This study employed a single-cohort, open-label design with an external (synthetic) control as a comparator. The patients enrolled in the ESTHER-1 trial who were treated with personalized doses of follitropin delta were used as the comparators. The primary end point analysis was based on the comparison of the mean number of good-quality usable blastocysts on days 5 and 6 using Student's *t*-test for independent samples. The same method was used to compare MARCS and the ESTHER-1 follitropin delta cohort with respect to the secondary end points.

Patient demographics and baseline characteristics were reported for the patients enrolled in MARCS and those enrolled in the follitropin delta arm of the ESTHER-1 trial. Between-cohort differences with respect to patient demographics and baseline characteristics were assessed for statistical significance with appropriate bivariate statistics. Baseline variables, for which statistically significant differences were observed between the MARCS study and the ESTHER-1 follitropin delta cohort, were considered as potential confounders. The associations of potential confounders with the primary end point—specifically the number of good-quality usable blastocysts at day 5 or 6—were assessed with linear regression for continuous scale confounders and analysis of variance (ANOVA) for categorical confounders. As a sensitivity analysis, confounders for which a statistically significant association was detected with the primary end point were included as independent variables in a multivariate linear regression model used to adjust the differences between the MARCS study and the ESTHER-1 follitropin delta cohort with respect to the primary end point.

The effect of age on treatment efficacy was assessed by the differences in the mean number of good-quality usable blastocysts at days 5 and 6 between patients classified in the following age groups: 18.0–34.9, 35.0–36.9, and 37.0–40.0 years old. We used ANOVA to assess the difference between the different age cohorts with respect to the primary end point.

A required sample size of 150 patients for the current study was based on an expected effect size of 25%, pooled standard deviation of 2.2, statistical significance of 5% (α–0.05), and a power of 80%. Even if 47 patients were excluded for protocol deviations, the sample size was still large enough to show statistically significant differences in terms of number of good-quality usable blastocysts.

RESULTS

Comparison between MARCS and ESTHER-1

Baseline Characteristics. The study was conducted between May 2018 and February 2019, and included women aged 18–40 years undergoing their first IVF-ICSI cycle. Patients were enrolled from four different centers; two centers enrolled 50 patients each, one center enrolled 10 patients, and another center enrolled 47 patients. However, the 47 patients enrolled from one center were excluded from the analysis because of the statistically significantly high losses to follow-up and several incidents of noncompliance with the dose regimen proposed in the MARCS study. The demographic and baseline characteristics profile of these 47 patients was similar to the profile of the remaining 110 patients who were included in the final analysis.

The results in Table 1 show that no differences were observed in the mean age of the patients enrolled in the current study and patients enrolled in the ESTHER-1 follitropin delta group ($P=.076$). However, a statistically significantly higher proportion of patients between 38 and 40 years was found ($P=.013$). When compared with the ESTHER-1 follitropin delta cohort, the MARCS study patients also had a lower mean AMH ($P<.001$) and lower proportion of male factor related infertility ($P<.001$) but a higher mean body weight ($P<.001$). There were no differences between the MARCS study and the ESTHER-1 follitropin delta cohort with respect to the proportion of patients with primary infertility, duration since infertility diagnosis, mean AFC, and median FSH at baseline.

Primary end point. The results of the primary efficacy analyses are summarized in Table 2. The mean and standard deviation in the number of good-quality usable blastocysts at days 5 to 6 was 4.9 (±3.9) for the MARCS study compared with 2.0 (±2.2) for the ESTHER-1 follitropin delta cohort ($P<.001$).

Bivariate analyses showed that among the variables for which a significant difference was observed between the MARCS study and ESTHER-1 follitropin delta cohort, only patient age ($P=.025$) and AMH ($P<.001$) were also associated with the number of good-quality usable blastocysts at days 5 and 6 and thus should be considered as potential confounders. Antral follicle count was also statistically significantly associated ($P<.001$) with the number of good-quality blastocysts at days 5 and 6 and was included in the adjusted analyses because the difference between the studies for this variable was clinically important (Supplemental Table 3, available online). Therefore, the sensitivity analysis was based on a multivariate model that included patient's age, AFC, and AMH as covariates to adjust for the difference between the MARCS study and ESTHER-1 follitropin delta cohort with respect to the primary end point (Supplemental Table 4, available online).

After adjusting for potential confounders, as previously described, the difference between the studies remained statistically significant. In fact, the adjusted difference was higher than that observed in the unadjusted analyses (Table 2).

Secondary end points. Table 2 shows that the MARCS study patients had a longer duration of stimulation ($P<.001$), a higher total dose of follitropin delta ($P<.001$), a higher proportion requiring triggering with GnRH agonist ($P<.001$), a higher number of oocytes retrieved ($P<.001$), a higher number of metaphase II (MII) oocytes ($P<.001$), a higher number of embryos on day 3 ($P<.001$), and higher attrition rates between day-3 embryos and blastocysts ($P<.001$). The rate of

TABLE 1

Demographics and baseline characteristics by cohort.

Characteristic	Cohort		*P* value
	MARCS (n = 110)	ESTHER-1 (n = 665)[a]	MARCS vs. ESTHER-1[a]
Age (y)	34.05 ± 3.47	33.4 ± 3.9	.076
Age group (y)			
<35	55 (50.0%)	394 (59.2%)	.013
35–37	24 (21.8%)	161 (24.2%)	
38–40	31 (28.2%)	110 (16.5%)	
Body weight (kg)	71.65 ± 14.6	64.7 ± 10.7	<.0001
Primary infertility	80 (72.7%)	470 (70.7%)	.667
Reason for infertility			
Unexplained	52 (47.3%)	281 (42.3%)	<.001
Male factor	26 (23.6%)	268 (40.3%)	
Female factor[b]	18 (16.4%)	114 (17.1%)	
Mixed	14 (12.7%)	NA	
Other	0	2 (0.3%)	
Infertility duration (y)	2.94 ± 1.97	2.94 ± 2.03	.999
AFC, 2–10 mm (n)	16.1 ± 16.1	14.7 ± 6.9	.371
AMH (pmol/L)	13.6 (9–20.5)	16.3 (9–24.8)[d]	<.001
AMH (ng/mL)	1.9 (1.26–2.87)	2.28 (1.26–3.47)	<.001
FSH[c]	7.31 (6.25–8.37)	7.5 (6.2–9.2)	NS

Note: Values are mean ± standard deviation or number and percentage unless otherwise indicated. AFC = antral follicle count; AMH = antimüllerian hormone; ESTHER-1 = Evidence-based Stimulation Trial with Human Recombinant Follicle-Stimulating Hormone in Europe and Rest of World 1; FSH = follicle-stimulating hormone; MARCS = Menopur and Rekovelle Combined Study; NA = not available (ESTHER-1 recorded all reasons for infertility, and "mixed" was not an option); NS = not statistically significant.

[a] Personalized follitropin delta dose (ESTHER-1).

[b] The sum of tubal factor infertility and endometriosis in the ESTHER-1 study are considered a unique category, "female factor," in MARCS study in terms of reason for infertility.

[c] Median (interquartile range).

[d] Data from clinical trial report.

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TABLE 2

Primary and secondary end points by cohort.

Characteristic	Cohort		*P* value
	MARCS (n = 110)[a]	ESTHER-1 (n = 665)[b]	MARCS vs. ESTHER-1[b]
Primary end points			
Good-quality usable blastocysts at day 5–6[c]	4.9 ± 3.9[d]	2.0 ± 2.2	<.001
Sensitivity analysis			
Good-quality usable blastocysts at day 5–6	5.39 ± 2.0[d]	2.0 ± 2.2	<.001
Secondary end points			
Duration of stimulation (d)	11.33 ± 1.29[e]	8.9 ± 1.9	<.001
Follitropin delta total dose (μg)	123.72 ± 21.75[e]	90.0 ± 25.3	<.001
Women with investigator-requested gonadotropin dose adjustments	67 (39.1%)	221 (33.2%)[f]	<.001
Poor response leading to cycle cancellation	3 (2.7%)	25 (3.8%)	.289
Triggering with GnRH agonist	46 (43%)[e]	10 (1.5%)	<.001
Oocytes retrieved CCOO	14.55 ± 7.58[e]	10.0 ± 5.6	<.001
Mature oocytes retrieved (MII)	11.28 ± 5.76[e]	7.4 ± 4.3[g]	<.001
Embryos on day 3	8.30 ± 5.05[d]	5.4 ± 3.7	<.001
Attrition rates between day 3 and blastocyst	59.03%[d]	37.03%	<.001

Note: Values are mean ± standard deviation or number and percentage unless otherwise indicated. CCOO = cumulus oophorous; ESTHER-1 = Evidence-based Stimulation Trial with Human rFSH in Europe and Rest of World 1; GnRH = gonadotropin-releasing hormone; MARCS = Menopur and Rekovelle Combined Study; MII = metaphase II.

[a] Where N is the number of participants taken into account for each variable differs in the cases noted by *d* and *e*).

[b] ESTHER-1 (P) group with personalized follitropin delta dose.

[c] Observed/unadjusted estimates.

[d] N = 106 patients. Four patients did not obtain usable embryo on day 5 or 6 (see Supplemental Fig. 1).

[e] N = 107 patients. Three patients obtained a poor response leading to cycle cancellation.

[f] Although an adjustment was requested by an investigator, no adjustment was done in the ESTHER-1 (P) group as per protocol.

[g] Data from clinical trial report.

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patients triggered with a GnRH agonist was statistically significantly higher in the MARCS study (43%) when compared with the rate reported in the ESTHER-1 follitropin delta cohort (2.3%) ($P<.001$).

The incidence of any grade OHSS in the MARCS study was 9.3% compared with 2.6% in the ESTHER-1 cohort treated with individualized follitropin delta dosing ($P=.001$). No cases of moderate or severe OHSS (0) were observed in the present study compared with 1.4% in the ESTHER-1 follitropin delta cohort ($P=.21$).

Comparison between age groups

Baseline Characteristics. The results summarized in Table 3 show that the patients in the 18.0- to 34.9-year-old age group had a statistically significantly lower mean body weight ($P=.047$), a higher mean AFC ($P<.001$), and a higher mean AMH ($P=.006$) when compared with the older age group patients. An inverse linear relationship was observed between increasing age and AFC ($\beta = -1.228$, $P<.001$).

Primary end point. Patients in the younger age group had a statistically significantly higher mean number of good-quality usable blastocysts at day 5–6 ($P=.006$). However, after adjusting for AMH and AFC, the differences between the age groups, although clinically important, were no longer statistically significant ($P=.244$) (Table 4). The mean number of good-quality usable blastocysts at days 5 and 6 in all age groups of the MARCS study was statistically significantly higher than that reported in the ESTHER–1 follitropin delta cohort ($P<.001$) (Table 2). The results of multivariate linear regression analysis showed that the most important factor contributing to the between–age-group difference with respect to the number of good-quality usable blastocysts at days 5 and 6 was the number of oocytes and MII oocytes (Supplemental Table 3).

Secondary end points

When compared with the older patients, patients in the younger age group received a lower mean dose of follitropin delta ($P=.054$) and HP-hMG ($P=.002$) and had a higher mean number of oocytes retrieved ($P<.001$), a higher mean number of MII oocytes ($P=.003$), and a higher mean number of embryos reaching day 3 of development ($P=.009$) (Table 4). There was a difference with respect to the type of trigger medication with a higher proportion of GnRH-agonist used in the younger age group and a higher proportion of hCG 10,000 IU used in the older age group ($P=.024$).

Safety

The incidence of OHSS was low and similar for all age groups in the MARCS study, although the younger age group had a higher rate of grade 1 OHSS compared with the older groups (11.1% vs. 7.4%, respectively), but the difference was not statistically significant ($P=.507$). The freeze-all strategy with frozen embryo transfer was applied in 63.6% of patients in the MARCS study. No statistically significant differences between the age groups were observed ($P=.164$) (Table 4).

DISCUSSION

The results of this study show that ovarian stimulation in IVF-ICSI cycles using a mixed protocol of follitropin delta and HP-hMG results in a statistically significantly higher number of good-quality usable blastocysts at days 5 and 6 when compared with cycles where follitropin delta was used as a monotherapy. Our finding is consistent with the results of a recent meta-analysis that showed that the number of mature

TABLE 3

Distribution data: demographics and baseline characteristics by age group.

	Age group (y)				
Characteristics	**18.0–34.9**	**35.0–36.9**	**37.0–40.0**	**Total**	***P* value**
N (%)	55 (50%)	24 (22%)	31 (28%)	110 (100%)	
Body weight (kg)	68.45 ± 12.06	76.88 ± 16.19	73.26 ± 16.42	71.65 ± 14.61	.047
Infertility history					
Primary	43 (78.2%)	17 (70.8%)	20 (64.5%)	80 (72.7%)	.382
Secondary	12 (21.8%)	7 (29.2%)	11 (35%)	30 (27.3%)	
Reason for infertility					
Unexplained	22 (40%)	13 (54.17%)	17 (54.84%)	52 (47.3%)	.360
Male factor	17 (30.91%)	5 (20.83%)	4 (12.9%)	26 (23.6%)	
Female factor	11 (20%)	2 (8.33%)	5 (16.13%)	18 (16.4%)	
Mixed	5 (9.09%)	4 (16.67%)	5 (16.13%)	14 (12.7%)	
Duration of infertility (y)	2.65 ± 1.58	2.79 ± 1.74	3.55 ± 2.59	2.94 ± 1.97	.119
AFC, 2–10 mm	19.65 ± 8.78	14.32 ± 9.50	11.42 ± 6.39	16.1 ± 9.02	< .001
Endocrine profile					
AMH (pmol/L)	17.71 ± 8.76	12.76 ± 8.28	12.16 ± 8.22	15.07 ± 8.84	.006
AMH (ng/mL)	2.48 ± 1.23	1.78 ± 1.16	1.7 ± 1.15	2.11 ± 1.24	.006
FSH	6.72 ± 1.48	8.48 ± 11.27	7.45 ± 3.23	7.31 ± 5.59	.437
E_2 (pg/mL)	1,759 ± 1,133	1,391 ± 907	1,563 ± 1,061	1,624 ± 1,068	.348
E_2 (ng/mL)	479 ± 309	379 ± 247	426 ± 289	442 ± 291	.348

Note: Values are mean ± standard deviation or number and percentage as applicable. AFC = antral follicle count; AMH = antimüllerian hormone; E_2 = estradiol; FSH = follicle-stimulating hormone.

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TABLE 4

Primary and secondary end points distributed by age group.

Characteristics	Age group (y) 18.0–34.9	35.0–36.9	37.0–40.0	Total	*P* value
N (%)	55 (50%)	24 (22%)	31 (28%)	110 (100%)[a]	
No. of good-quality blastocysts					
Day 5–6 (unadjusted)	6.08 ± 4.23	4.22 ± 3.49	3.45 ± 2.84	4.91 ± 3.87[c]	.006
Day 5–6 (adjusted)[b]	5.36 ± 0.49	4.03 ± 0.72	4.24 ± 0.62	NA	.244
Secondary end points					
Follitropin delta dose/d	10.48 ± 2.06	11.35 ± 1.69	11.38 ± 1.7	10.92 ± 1.92[d]	.054
HP-hMG dose/d	120 ± 39.79	146.88 ± 41.25	147.58 ± 36.14	133.64 ± 41.12[d]	.002
Days of stimulation	11.23 ± 1.12	11.57 ± 1.50	11.32 ± 1.42	11.33 ± 1.29[d]	.581
No. of follicles					
<15 mm[a]	11.58 ± 9.64	6.87 ± 7.10	4.33 ± 4.81	8.51 ± 8.56[d]	<.001
≥15 mm[a]	10.72 ± 5.38	7.57 ± 3.27	7.77 ± 3.69	9.20 ± 4.76[d]	.004
Endometrium thickness (mm)	11.18 ± 2.11	11.11 ± 2.56	10.54 ± 2.04	11.05 ± 2.24[d]	.296
E_2 at day 6 of stimulation	1,760 ± 1,134	1392 ± 907	1,563 ± 1,060	1,624 ± 1,068[d]	.348
pg/mL	479 ± 309	379 ± 247	426 ± 289	442 ± 291	
Final E_2	11,090 ± 5,278	9,742 ± 5,970	8,952 ± 4,558	10,181 ± 5,276[d]	.183
pg/mL	3,021 ± 1,438	2,654 ± 1,626	2,438 ± 1,242	2,773 ± 1,437	
HP-hMG dose adjustment					
No change	33 (60%)	17 (70.83%)	17 (54.84%)	67 (60.9%)	.410
225 IU	13 (23.64%)	7 (29.17%)	11 (35.48%)	31 (28.2%)	
150 IU	3 (5.45%)	0 (0)	0 (0)	3 (2.7%)	
75 IU	2 (3.64%)	0 (0)	2 (6.45%)	4 (3.6%)	
37.5 IU	4 (7.27%)	0 (0)	1 (3.23%)	5 (4.5%)	
Withdrawal of HP-hMG	0 (0)	0 (0)	0 (0)	0 (0)	
Trigger medication					
Decapeptyl 0.2 mg	29 (54.72%)	6 (26.09%)	11 (35.48%)	46 (43%)	.024
hCG 5,000 IU	15 (28.30%)	5 (21.74%)	8 (25.81%)	28 (26.2%)	
hCG 10,000 IU	9 (16.98%)	12 (52.17%)	12 (38.71%)	33 (30.8%)	
No. of CCOO	17.79 ± 8.23	12.39 ± 5.11	10.61 ± 5.32	14.55 ± 7.58[d]	<.001
No. of oocytes (MII)	13.13 ± 6.24	10.17 ± 5.02	8.94 ± 4.34	11.28 ± 5.76[d]	.003
No. of embryos at day 3	9.66 ± 5.48	7.96 ± 4.51	6.23 ± 3.92	8.30 ± 5.05[c]	.009
Attrition rates between day 3 and blastocyst	62.94%	53.02%	55.37%	59.16%[c]	.411
Safety outcomes					
OHSS[b]					
None	48 (88.89%)	21 (91.30%)	29 (93.55%)	98 (90.7%)	.771
Mild grade 1	6 (11.11%)	2 (8.70%)	2 (6.45%)	10 (9.3%)	
Mild grade 2	0 (0)	0 (0)	0 (0)	0 (0)	
Transfer type[b]					
No transfer (no blastocysts available)	0 (0)	2 (8.70%)	2 (6.45%)	4 (3.7%)	.164
Fresh embryo transfer	17 (32.08%)	10 (43.48%)	8 (25.81%)	34 (32.7%)	
Freeze all	36 (67.92%)	11 (47.83%)	21 (67.74%)	68 (63.6%)	

Note: Values are mean ± standard deviation or number and percentage unless otherwise indicated. CCOO = cumulus oophorous; E_2 = estradiol; HP-hMG = highly purified human menopausal gonadotropin; MII = metaphase II; OHSS = ovarian hyperstimulation syndrome.

[a] N = The number of participants taken into account for each variable differs in the cases marked with *c* and *d*.

[b] Least square mean ± standard error of the mean, adjusted for antral follicle count, and antimüllerian hormone level.

[c] N = 106 patients. Four patients did not obtain usable embryo on day 5 or 6 (see Supplemental Fig. 1).

[d] N = 107 patients. Three patients obtained a poor response leading to cycle cancellation.

Bissonnette. Follitropin delta mixed protocol for IVF. Fertil Steril 2020.

oocytes and embryos as well as implantation rate are higher when HP-hMG is used in combination with recombinant FSH instead of recombinant FSH alone (13). However, another recent publication comparing the use of recombinant FSH alone versus in combination with HP-hMG, found no statistically significant differences in the number of MII oocytes or embryos obtained between the two groups; however, a statistically significant difference in implantation rates in favor of the use of combination regimen was still observed (18). Another retrospective cohort study also concluded that adding HP-hMG to recombinant FSH may not improve the embryo profile (19). The inconsistent results observed in these studies indicate that more research is needed to elucidate the potential benefits of the mixed protocols.

Our study also has some limitations. Given its exploratory nature, we have focused on the number of good-quality usable blastocysts at days 5 and 6 instead of implantation and pregnancy rates. The use of a historical control group instead of a control obtained by randomization is another disadvantage. Even though the eligibility criteria of both studies were quite similar, we still found differences in the baseline characteristics: in MARCS, patients were older and presented with

higher BMI and lower AMH. To address this limitation, we have used a sensitivity analysis to account for potential co-founders as suggested by some investigators (20). Another constraint of using an external (synthetic) control study design is the fact that inclusion and exclusion criteria cannot be modified. This has prevented us from evaluating the safety of our dosing regimen in patients at high risk of OHSS (with AMH $\geq$35 pmol/L).

As with every treatment, the risks of employing more aggressive protocols must be balanced against the efficacy benefits. Safety concerns regarding OHSS must be considered seriously when gonadotropin combinations (mixed protocols) are employed (21-23)

Data have shown a relationship between the number of oocytes retrieved and live-birth rates. Beyond 15 oocytes there appears to be no increase in live-birth rates (1, 2), but the risk of OHSS increases exponentially (1). Moreover, when 15 or more oocytes are retrieved, an increase in abdominal discomfort as determined by visual analogue scale assessments is observed in most patients. In the present study, 17.79 $\pm$ 8.23 oocytes were obtained in the 18.0–34.9 years group; 12.39 $\pm$ 5.11 in the 35.0–36.9 years group, and 10.61 $\pm$ 5.32 in 37.0–40.0 years group. The youngest group seemed to respond excessively to the combined treatment. However, patients over 35 years of age seemed to reach the desired response. This observation is in agreement with the fact that fertility declines in mid-30s partially due to the associated decline in ovarian reserve and in part to the degradation of oocyte quality (24). These results suggest that women aged older than 35 years would experience the greatest benefit in terms of oocytes retrieved from the combined dosing protocol assessed in the current study. They also suggest that a refinement in the HP-hMG dosing and the impact of the patient's age deserve further evaluation.

The follitropin delta total dose and the duration of stimulation in the MARCS study were statistically significantly higher than those reported in the ESTHER-1 follitropin delta cohort. These observed differences could be explained by the differences in the baseline characteristics, such as the increased mean body weight of the patients in the MARCS study; pharmacokinetic data have demonstrated that the follitropin delta serum FSH level is inversely related to the body weight and directly related to the follitropin delta dose. It is believed that this association is due to the fact that follitropin delta is distributed within the extracellular fluid space. Hence, the volume of distribution of follitropin delta increases with the increase in body weight, which must be considered when dosing follitropin delta to optimize the ovarian response (10).

As this was an exploratory study, adjustments in the medication regimen were expected, so it is remarkable that no change in HP-hMG dosage was required for over 60% of patients. Furthermore, no HP-hMG dose reductions were required in the patients between 35 and 36 years of age, indicating that the proposed combined regimen seems suitable for this age group.

The proportion of patients triggered with GnRH agonist was statistically significantly higher in the MARCS study (43%) when compared with that reported in the ESTHER-1 follitropin delta cohort (2.3%). The exploratory nature of our study established a threshold of 10,000 pmol/L of E_2, beyond which a GnRH agonist trigger was used instead of hCG and could explain this finding. As a result, a low number of fresh transfers was performed in MARCS study (32.7%). It is logical to expect that the combination of two gonadotropins may stimulate a higher ovarian response, especially in younger patients and those patients with higher AMH levels. The increased number of oocytes inevitably entails an increase in abdominal distension and discomfort during treatment, but the present study shows that no other adverse events such as nausea or diarrhea were reported.

The strength of this study is that it is the first study to report using a combination (mixed protocol) of follitropin delta and HP-hMG that shows an increased number of good-quality blastocysts in patients over 35 years old with an increase in the risk of only mild OHSS, which has not required medical intervention or hospitalization.

The findings of this study have important implications. It has shown that the number of good-quality, usable blastocysts obtained by day 5 or 6 of embryo culture can be statistically significantly increased by using this mixed protocol without compromising safety. This is most obvious in the $\geq$35-year-old patients. The number of usable embryos becomes a very important criteria especially in areas where there is inadequate or no funding for IVF.

Further refinement in the HP-hMG dosing is necessary and should lead to a reduction in undesired effects without jeopardizing the benefits of the mixed protocol. Also, research using a randomized cohort design is necessary to determine whether the results from our study can be extrapolated to other populations, such as women older than 40 years. In addition, this mixed protocol must be compared with other mixed protocols using other recombinant FSH preparations in a study that also evaluates pregnancy and live-birth rates.

CONCLUSION

Optimizing ovarian response in IVF by employing a mixed protocol of individualized dosing of both follitropin delta and HP-hMG results in a statistically significant increase in the number of good-quality usable blastocysts on day 5 or 6 with an increased risk of only mild OHSS, which has not required medical intervention or hospitalization. The subgroup of 35- to 36-year-old and 37- to 40-year-old patients are the ones that appear to benefit the most from the application of this combination regimen, although refinement in the HP-hMG dosage selection could result in improvement in the patients younger than 35 years as well. More research needs to be done to corroborate our findings in other patient populations.

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REFERENCES

1. Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, et al. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: an analysis of 256,381 in vitro fertilization cycles. Fertil Steril 2014;101:967–73.
2. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod 2011;26:1768–74.
3. Fauser BC, Diedrich K, Devroey P. Evian Annual Reproduction (EVAR) Workshop Group 2007. Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation. Hum Reprod Update 2018;14:1–14.
4. Boothroyd C, Karia S, Andreadis N, Rombauts L, Johnson N, Chapman M. Consensus statement on prevention and detection of ovarian hyperstimulation syndrome. Aust NZ J Obstet Gynaecol 2015;55:523–34.
5. Majchrzak-Celińska, Baer-Dubowska A, Pharmacoepigenetics W. an element of personalized therapy? Expert Opin Drug Metab Toxicol 2017; 13:387–98.
6. Lunenfeld B, Bilger W, Longobardi S, Alam V, D'Hooghe T, Sunkara SK. The development of gonadotropins for clinical use in the treatment of infertility. Front Endocrinol (Lausanne) 2019;10:429.
7. Olsson H, Sandström R, Grundemar L. Different pharmacokinetic and pharmacodynamic properties of recombinant follicle-stimulating hormone (rFSH) derived from a human cell line compared with rFSH from a non-human cell line. J Clin Pharmacol 2014;54:1299–307.
8. Koechling W, Plaksin D, Croston GE, Jeppesen JV, Macklon KT, Andersen CY. Comparative pharmacology of a new recombinant FSH expressed by a human cell line. Endocr Connect 2017;6:297–305.
9. Arce JC, Larsson P, García-Velasco JA. Establishing the follitropin delta dose providing a comparable ovarian response as 150 IU/day follitropin alfa for controlled ovarian stimulation. Reprod Biomed Online 2020;41:616–22.
10. De Placido G, Alviggi C, Perino A, Strina I, Lisi F, Fasolino A, et al. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. Hum Reprod 2005;20:390–6.
11. Grøndahl ML, Borup R, Lee YB, Myrhøj V, Meinertz H, Sørensen S. Differences in gene expression of granulosa cells from women undergoing controlled ovarian hyperstimulation with either recombinant follicle-stimulating hormone or highly purified human menopausal gonadotropin. Fertil Steril 2009;91:1820–30.
12. Nyboe Andersen A, Nelson SM, Fauser BC, Garcia-Velasco JA, Klein BM, Arce JC for the ESTHER-1 Study Group. 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.
13. Santi D, Casarini L, Alviggi C, Simoni M. Efficacy of follicle-stimulating hormone (FSH) alone, FSH + luteinizing hormone, human menopausal gonadotropin or FSH + human chorionic gonadotropin on assisted reproductive technology outcomes in the "personalized" medicine era: a meta-analysis. Front Endocrinol (Lausanne) 2017;8:114.
14. Scobey MJ, Raike E, Marshall DC. Mixed protocols: multiple ratios of FSH and LH bioactivity using highly purified, human-derived FSH (BRAVELLE) and highly purified hMG (MENOPUR) are unaltered by mixing together in the same syringe. Reprod Biol Endocrinol 2005;3:61.
15. Arce JC, Klein BM, Erichsen L. Using AMH for determining a stratified gonadotrophin dosing regimen for IVF/ICSI and optimizing outcomes. In: Seifer DB, Tal R, editors. Anti-müllerian hormone. Hauppauge, NY: Nova Science; 2016:83–102.
16. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26:1270–83.
17. 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.
18. Kan O, Simsir C, Atabekoglu CS, Sonmezer M. The impact of adding hp-hMG in r-FSH started GnRH antagonist cycles on ART outcome. Gynecol Endocrinol 2019;35:869–72.
19. Tabata C, Fujiwara T, Sugawa M, Noma M, Onoue H, Kusumi M, et al. Comparison of FSH and hMG on ovarian stimulation outcome with a GnRH antagonist protocol in younger and advanced reproductive age women. Reprod Med Biol 2015;14:5–9.
20. Viele K, Berry S, Neuenschwander B, Amzal B, Chen F, Enas N, et al. Use of historical control data for assessing treatment effects in clinical trials. Pharm Stat 2014;13:41–54.
21. American Society of Reproductive Medicine. Ovarian hyperstimulation syndrome. Fertil Steril 2008;90(Suppl 3):S188–93.
22. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, et al. Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. Hum Reprod 2014;29:2099–113.
23. Kol S, Humaidan P. GnRH agonist triggering: recent developments. Reprod Biomed Online 2013;26:226–30.
24. Liu K, Case A. Advanced reproductive age and fertility. J Obstet Gynaecol Can 2011;33:1165–75.

Estimulación ovárica individualizada para fecundación in vitro: un estudio multicéntrico, abierto, exploratorio con un protocolo combinado de folitropina delta y gonadotropina menopáusica humana altamente purificada.

Objetivo: Evaluar el perfil de seguridad y el número de blastocistos utilizables en día 5 y día 6 tras el tratamiento con un régimen de dosis individualizadas de folitropina delta y gonadotropina menopáusica humana altamente purificada (HP-hMG) para estimulación ovárica.

Diseño: Estudio multicéntrico, abierto, exploratorio.

Entorno: Clínica de medicina reproductiva.

Paciente(s): Un total de 110 pacientes (edades 18-40 años).

Intervención(es): Folitropina delta coadministrada con HP-hMG, con la dosis de folitropina delta fijada según un algoritmo establecido y la de HP-hMG en 75 IU cuando la dosis de folitropina delta fue $<$12 ug; 150 IU cuando la dosis de folitropina delta fue 12 ug y el peso $<$ 100kg y 225 IU cuando la dosis de folitropina delta fue 12ug y el peso $\geq$ 100 kg (ajustes de dosis confinados a la HP-HMG).

Medida(s) de Resultado(s) Principal(es): Media de blastocistos de buena calidad obtenidos en día 5 y día 6, así como la proporción de mujeres con síndrome de hiperestimulación ovárica (OHSS).

Resultado(s): Un estudio de cohortes fue comparado con el grupo de folitropina delta del estudio Evidence-based Stimulation Trial with Human Recombinant Follicle-Stimulating Hormone in Europe and Rest of World 1 (ESTHER-1). Incluso tras estratificar por edad, una media mayor estadísticamente significativa de número de ovocitos obtenidos y número de blastocistos de buena calidad se observó en este estudio comparado con el ensayo ESTHER-1 en el que se usó sólo folitropina delta. La tasa de pacientes en las que se desencadenó la ovulación con un agonista de hormona liberadora de gonadotropina fue estadísticamente significativamente mayor en la cohorte de nuestro estudio Menopur and Rekovelle Combined Study (MARCS) (43%) comparada con las tasas comunicadas en la cohorte de folitropina delta el el estudio ESTHER-1 (2.3%). La incidencia de OHSS de cualquier grado fue de 9.3% en el presente estudio, comparada con 2.6% en el grupo de folitropina delta del ensayo ESTHER-1. No se observó ningún caso de OHSS moderado o severo en nuestro estudio, comparado con 1.4% en el grupo de folitropina delta del ESTHER-1.

Conclusión: La optimización de la respuesta ovárica durante la fecundación in vitro mediante un protocolo combinado de dosificación individualizada de folitropina delta y HP-hMG resultó en un número estadísticamente significativo de blastocistos utilizables en días 5 y 6 con un mayor riesgo de OHSS leve, que no requirió intervención médica ni hospitalización.