Ovarian response to recombinant human follicle-stimulating hormone: a randomized, antimüllerian hormone–stratified, dose–response trial in women undergoing in vitro fertilization/intracytoplasmic sperm injection

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Objective: To evaluate the dose–response relationship of a novel recombinant human FSH (rhFSH; FE 999049) with respect to ovarian response in patients undergoing IVF/intracytoplasmic sperm injection treatment; and prospectively study the influence of initial antimüllerian hormone (AMH) concentrations.
Design: Randomized, controlled, assessor-blinded, AMH-stratified (low: 5.0–14.9 pmol/L [0.7–<2.1 ng/mL]; high: 15.0–44.9 pmol/L [2.1–6.3 ng/mL]) trial.
Setting: Seven infertility centers in four countries.
Patient(s): Two hundred sixty-five women aged ≤37 years.
Intervention(s): Controlled ovarian stimulation with either 5.2, 6.9, 8.6, 10.3, or 12.1 μg of rhFSH, or 11 μg (150 IU) of follitropin alfa in a GnRH antagonist cycle.
Main Outcome Measure(s): Number of oocytes retrieved.
Result(s): The number of oocytes retrieved increased in an rhFSH dose–dependent manner, from 5.2 ± 3.3 oocytes with 5.2 μg/d to 12.2 ± 5.9 with 12.1 μg/d. The slopes of the rhFSH dose–response curves differed significantly between the two AMH strata, demonstrating

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that a 10% increase in dose resulted in 0.5 (95% confidence interval 0.2–0.7) and 1.0 (95% confidence interval 0.7–1.3) more oocytes in the low and high AMH stratum, respectively. Fertilization rate and blastocyst/oocyte ratio decreased significantly with increasing rhFSH doses in both AMH strata. No linear relationship was observed between rhFSH dose and number of blastocysts overall or by AMH strata. Five cases of ovarian hyperstimulation syndrome were reported for the three highest rhFSH doses and in the high AMH stratum.

Conclusion(s): Increasing rhFSH doses results in a linear increase in number of oocytes retrieved in an AMH-dependent manner. The availability of blastocysts is less influenced by the rhFSH dose and AMH level.

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Key Words: Antimüllerian hormone, in vitro fertilization, recombinant FSH, ovarian response, oocyte, blastocyst

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The purpose of controlled ovarian stimulation (COS) with gonadotropins for IVF/intracytoplasmic sperm injection (ICSI) is to obtain an adequate number of competent oocytes with the minimum risks for the woman (1). The magnitude of the ovarian response and the number of embryos/blastocysts available for transfer is generally considered to be a function of the gonadotropin dose (2), the type of stimulation protocol (3–6), and the patient's profile (7–9).

Individual variability in ovarian response to a given dose of gonadotropins is well recognized, and significant efforts have been made to identify clinical parameters that can predict ovarian response, as well as lead to improved efficacy and safety outcomes (10–13). The use of biomarkers of the functional ovarian reserve (14) in combination with prospectively designed gonadotropin dose–response studies should define more patient-tailored dosing regimens that fulfill both the clinical efficacy and safety objectives for COS. The initial serum concentration of antimüllerian hormone (AMH) is increasingly being recognized as the preferred biomarker of ovarian response to COS compared with patient age, day-3 FSH, inhibin B, and E_2 (15), and to be as good as antral follicle count (AFC) assessed by ultrasound (16, 17), but with less intra- and intercycle variation than AFC (18). Moreover, AMH assessments can be applied to all IVF clinics, independent of observers' bias.

The present investigation evaluated the dose–response relationship of a novel recombinant human FSH (rhFSH; FE 999049) with respect to ovarian response in patients undergoing COS for IVF/ICSI and studied the influence of initial AMH concentrations on the dose–response curve. Recombinant human FSH is expressed from a cell line of human fetal retinal origin (PER.C6) with an amino acid sequence identical to the native human FSH sequence and existing recombinant FSH preparations derived from Chinese hamster ovary (CHO) cell lines (i.e., follitropin alfa and beta). The human cell line was chosen to resemble the glycosylation profile of native human FSH. In fact, the sialic acid content of rhFSH is different and more complex, with both α2,3 and α2,6 sialylation compared with the CHO-derived FSH products, which only contain α2,3 sialylation (19). The composition of the carbohydrate moieties can modulate the in vivo activity of FSH by impacting its clearance and, potentially, by the binding properties to the FSH receptor. Previous studies in healthy women showed that administration of identical bioactive doses (international units [IU] based on the Steelman-Pohley in vivo rat assay) of rhFSH and follitropin alfa resulted in slower clearance for rhFSH and significantly higher pharmacodynamic responses with rhFSH in terms of serum E_2 and inhibin B, as well as more follicles (20). Because the translation of biological activity in IU from a rat assay to humans is not the same for rhFSH and CHO-derived FSH products, a dose–response characterization of the novel rhFSH was needed in patients undergoing COS for IVF/ICSI treatment.

MATERIALS AND METHODS

This was a randomized, controlled, open-labeled, parallel-group phase 2 trial conducted at seven centers in four countries (Belgium, Czech Republic, Denmark, and Spain) from September 2011 through May 2013. The trial was assessor-blinded, and all investigators, embryologists, central laboratory personnel, and sponsor staff involved in analyzing and interpreting data were kept blinded to treatment allocation throughout the trial. The study was performed in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulatory requirements. The study protocol was approved by the local regulatory authorities and the independent ethics committees covering all participating centers. All patients provided written, informed consent.

Trial Population

Women diagnosed with tubal infertility, unexplained infertility, infertility related to endometriosis stage I/II, or with partners diagnosed with male factor infertility were eligible for the trial. Additional main inclusion criteria were as follows: age 18–37 years; body mass index 18.5–32.0 kg/m^2; infertility for at least 1 year before randomization; regular menstrual cycles of 24–35 days, presumed to be ovulatory; hysterosalpingography, hysteroscopy, or transvaginal ultrasound documenting a uterus consistent with expected normal function; transvaginal ultrasound documenting presence

and adequate visualization of both ovaries, without evidence of significant abnormality; early follicular phase FSH serum concentration of 1–12 IU/L and total antral follicle (diameter 2–10 mm) count ≥6 and ≤25 for both ovaries combined; serum AMH concentration of 5.0–44.9 pmol/L (0.7–6.3 ng/mL); willing to accept transfer of one blastocyst in the fresh cycle; and willing to accept transfer of one blastocyst in frozen embryo replacement cycles initiated within 6 months after randomization. The main exclusion criteria were as follows: known polycystic ovary syndrome associated with anovulation; known endometriosis stage III–IV; three or more COS cycles for IVF/ICSI; poor ovarian response in a previous COS cycle using an average daily FSH dose ≥150 IU, defined as development of fewer than four follicles ≥15 mm or cycle cancellation due to limited follicular response; excessive ovarian response in a previous COS cycle using an average daily FSH dose <225 IU, defined as >25 oocytes retrieved or cycle cancellation due to excessive ovarian response, including risk of ovarian hyperstimulation syndrome (OHSS); severe OHSS in a previous COS cycle; history of recurrent miscarriage; current or past (up to 1 year before randomization) abuse of alcohol or drugs; and intake of more than 14 units of alcohol per week during the past month or smoking more than 10 cigarettes per day within 3 months before randomization.

Treatment Regimen

On day 2–3 of the menstrual cycle, patients were randomly assigned, in a 1:1:1:1:1:1 ratio, to receive fixed daily SC injections of either 5.2 μg, 6.9 μg, 8.6 μg, 10.3 μg, or 12.1 μg of rhFSH (FE 999049; Ferring Pharmaceuticals), or 11 μg (150 IU) of follitropin alfa (Gonal-F filled by mass; Merck Serono). The rhFSH dose is expressed in micrograms because the determination of potency by the pharmacopoeial rat bioassay (Steelman-Pohley in vivo bioassay, IU) does not fully reflect the response of this rFSH preparation from a human cell line when administered to humans (20). Follitropin alfa was included as the reference arm for validation purposes of the ovarian response. Randomization was stratified according to the patients' serum concentration of AMH at the screening visit (low stratum: 5.0–14.9 pmol/L [0.7–<2.1 ng/mL]; high stratum: 15.0–44.9 pmol/L [2.1–6.3 ng/mL]) and by trial site using a block size of six. An independent statistician in the Department of Biometrics at Ferring Pharmaceuticals generated the randomization list using SAS version 9.2 (SAS Institute). Randomization was performed centrally through the electronic Case Report Form system by assigning the lowest randomization number available within stratum.

On stimulation day 6, a GnRH antagonist (ganirelix acetate; Ganirelix Acetate Injection/Orgalutran, Merck/MSD) was initiated at a daily dose of 0.25 mg and continued throughout the stimulation period. When three or more follicles with a diameter ≥17 mm were observed, triggering of final follicular maturation was done with 250 μg recombinant hCG (choriogonadotropin alfa; Ovidrel/Ovitrelle, EMD Serono/Merck Serono) if there were <25 follicles ≥12 mm, or with 0.2 mg GnRH agonist (triptorelin acetate; Decapeptyl, Ferring Pharmaceuticals) if there were 25–35 follicles ≥12 mm. In case of >35 follicles ≥12 mm, the cycle was to be cancelled; coasting was not allowed. In case of fewer than three follicles ≥10 mm observed on stimulation day 10, the cycle could be cancelled.

Oocyte retrieval took place 36 ± 2 hours after triggering of final follicular maturation, and the oocytes could be inseminated by IVF or ICSI. For patients who underwent triggering with hCG, a single blastocyst-stage embryo of the best quality according to the criteria of Gardner and Schoolcraft (21) was transferred on day 5 after oocyte retrieval while surplus blastocysts could be cryopreserved. For patients who underwent triggering with GnRH agonist, no transfer took place in the fresh cycle, and the blastocysts were instead cryopreserved. Vaginal progesterone tablets (Endometrin/Lutinus, Ferring Pharmaceuticals) 100 mg three times daily were provided for luteal-phase support from the day after oocyte retrieval until the clinical pregnancy visit. Patients with cryopreserved blastocysts could undergo cryopreserved replacement cycles, initiated within 6 months of randomization, with compulsory single-blastocyst transfer in a natural cycle.

Trial Endpoints and Trial Assessments

The primary endpoint was the number of oocytes retrieved. The secondary endpoints included duration of stimulation, fertilization, blastocyst number and quality, pregnancy, live birth, and OHSS. The serum concentration of AMH (1 ng/mL = 7.143 pmol/L) at screening was assessed by a central laboratory using the Beckman Coulter Gen 2 ELISA assay, after being stored at ambient temperature between 1 and 5 days to avoid possible complement interference. Circulating levels of FSH were assessed by a central laboratory using an electrochemiluminescence immunoassay. A serum β-hCG test was performed 13–15 days after transfer. Clinical pregnancy, defined as at least one intrauterine gestational sac with fetal heart beat, was confirmed by ultrasound 5-6 weeks after transfer. All pregnancies in fresh and cryopreserved cycles were followed until live birth.

Sample Size and Statistical Analysis

The trial was dimensioned to have 90% power of demonstrating a dose–response relationship of rhFSH with respect to oocytes retrieved at the two-sided 5% significance level. The power calculation assumed an increase in number of oocytes retrieved of ≥3.5 when doubling the dose and a standard deviation of 5.6, yielding 200 subjects equally distributed across the five rhFSH groups and thus a total of 240 subjects for the six treatment groups, including the reference arm.

All summaries and analyses are based on the intention-to-treat population grouped according to treatment received. The primary endpoint was modeled using analysis of covariance (ANCOVA), with center and AMH stratum as factors and log(dose) as covariate including an interaction between AMH stratum and log(dose). Six rhFSH-treated subjects who cancelled the cycle because of poor response were included in the dose–response modeling as having one oocyte retrieved. One subject who discontinued for personal reasons

was excluded from the dose–response modeling. The robustness was verified by repeating the analysis for observed data. The analysis was repeated within each AMH stratum. Dose–response for continuous variables was evaluated by ANCOVA using a similar model structure as for the primary endpoint. Dose–response for binary endpoints was evaluated by logistic regression including AMH stratum and log(dose) in the model. Pregnancy and live birth rates were compared across rhFSH treatment groups and between AMH strata using χ^2 tests. All statistical analyses were performed in SAS version 9.2. The reference arm (follitropin alfa, 11 μg) was included for external validity, and no statistical comparisons were contemplated.

RESULTS

Trial Population

Details of the patients participating in the study are shown in Supplemental Figure 1 (available online). Of the 334 patients who were screened, 265 eligible patients were randomized, with a distribution of 56% (n = 148) and 44% (n = 117) in the high and low AMH stratum, respectively. The majority of the subjects where white (259 of 265) and not Hispanic or Latino (262 of 265). The distribution of initial AMH values in the trial population is shown in Supplemental Figure 2. A total of 222 patients received one of the five doses of rhFSH, and 43 patients received 11 μg follitropin alfa. Demographics and baseline characteristics were not significantly different between the treatment groups (Supplemental Table 1), whereas significantly higher mean AFC values ($P<.001$) and lower median basal FSH values ($P<.001$) were observed for patients with high AMH compared with low AMH.

COS and Oocyte Retrieval

The mean duration of stimulation decreased significantly ($P<.001$) with increasing doses of rhFSH; however, at each dose level, the duration of stimulation was similar between the AMH strata (Table 1). Cycle cancellation before oocyte retrieval occurred in seven patients; one because of personal reasons and six because of poor response, of whom five were in the low AMH stratum (Supplemental Fig. 1).

A total of 258 women (97%) underwent oocyte retrieval, of whom 256 had triggering of final follicular maturation with hCG, and two patients in the high AMH stratum had excessive ovarian response leading to triggering with GnRH agonist (Supplemental Fig. 1). Among the women treated with rhFSH, the mean number (±SD) of oocytes retrieved increased from 5.2 ± 3.3 in the 5.2 μg group to 12.2 ± 5.9 in the 12.1 μg group, and was 10.4 ± 5.2 in the 11 μg follitropin alfa group (Table 1). A statistically significant ($P<.001$) dose–response relationship with respect to number of oocytes retrieved was established for rhFSH (Fig. 1A). This relationship remained significant ($P<.001$) within both AMH strata, whereas the slopes of the dose–response curves differed significantly between the two strata ($P=.025$). The dose–response model predicted that a 10% increase in rhFSH dose would result in an increase of 0.5 oocyte (95% confidence interval 0.2–0.7) in the low AMH stratum and 1.0 oocyte (95% confidence interval 0.7–1.3) in the high AMH stratum. A responder analysis of the primary endpoint showed that the proportion of patients with an adequate ovarian response, defined as 8–14 oocytes retrieved, was most frequent in the 8.6 μg (54%) and 12.1 μg groups (60%) in the high and low AMH stratum, respectively (Fig. 1B and C).

Fertilization Rate and Blastocyst Availability

The fertilization rate, as well as the probability that an oocyte developed to a blastocyst, decreased significantly with increasing rhFSH doses ($P<.001$) (Fig. 2, Table 1). The mean number of blastocysts and blastocysts of good quality was in the range of 2.3–3.2 and 0.9–1.4, respectively, across the rhFSH groups, with no significant linear dose–response relationship, either overall (Table 1) or within AMH strata (Fig. 3). In the high AMH stratum, there were no statistically significant differences among groups in the number of total or good-quality blastocysts. Nevertheless, in the low AMH stratum, the number of blastocysts and good-quality blastocysts were significantly ($P<.05$) higher in the 12.1 μg group compared with the other rhFSH dose groups combined.

In the ICSI cycles (81% of all cycles) in which nuclear maturation was determined before insemination, the findings were similar to those in the overall population, with a significant dose–response effect of rhFSH for number of oocytes retrieved ($P<.001$) and metaphase II oocytes ($P<.001$), but not for the number of blastocysts and good-quality blastocysts (Supplemental Fig. 3). The number of metaphase I oocytes increased significantly ($P<.001$) with rhFSH dose, from 0.3 ± 0.5 (5.6 μg) to 1.0 ± 1.2 (12.1 μg); and the number of germinal vesicle oocytes also increased significantly ($P<.001$) with rhFSH dose, from 0.5 ± 0.8 (5.6 μg) to 1.8 ± 2.6 (12.1 μg) (Supplemental Table 2). The probability that a metaphase II oocyte developed to a blastocyst decreased significantly with increasing rhFSH doses overall ($P<.001$) and in the high AMH stratum ($P<.001$), but not in the low AMH stratum (Supplemental Fig. 3).

Treatment Outcome

In the fresh cycle, no statistically significant relationships between the rhFSH dose and positive β-hCG, clinical pregnancy, and live birth rates were observed (Table 1). The cumulative live birth rate (the fresh blastocyst transfer cycle plus subsequent frozen–thawed blastocyst transfer cycles) was numerically higher in the high AMH stratum (46% vs. 36%, $P=.133$); most likely as a consequence of the significantly higher number of blastocysts, total (3.2 ± 2.7 vs. 2.2 ± 1.6, $P=.017$) and of good-quality (1.4 ± 1.6 vs. 0.9 ± 1.1, $P=.028$), in the high AMH stratum compared with the low AMH stratum. However, it should be noted that the trial was not powered to detect differences in pregnancy and live birth rates between the treatment groups. A total of five moderate/severe OHSS were reported, and all in the high AMH stratum: two early OHSS in patients exposed

TABLE 1

Stimulation and outcome variables of the patients, according to treatment group and AMH stratum.

Variable	rhFSH 5.2 μg (n = 42)	rhFSH 6.9 μg (n = 45)	rhFSH 8.6 μg (n = 44)	rhFSH 10.3 μg (n = 44)	rhFSH 12.1 μg (n = 47)	*P* value[a]	Follitropin alfa 11 μg (n = 43)
Duration of stimulation, d							
All patients	9.6 ± 2.2	9.0 ± 2.0	8.4 ± 1.6	7.9 ± 1.4	8.1 ± 1.8	<.001	8.6 ± 1.6
Low AMH stratum[b]	9.2 ± 2.3	8.6 ± 1.8	8.5 ± 1.4	7.9 ± 1.5	7.9 ± 2.4	.009	8.6 ± 1.4
High AMH stratum[c]	10.0 ± 2.2	9.3 ± 2.1	8.3 ± 1.9	7.9 ± 1.3	8.3 ± 1.2	<.001	8.6 ± 1.8
Oocytes retrieved, n							
All patients	5.2 ± 3.3	7.9 ± 5.9	9.2 ± 4.6	10.5 ± 7.0	12.2 ± 5.9	<.001	10.4 ± 5.2
Low AMH stratum	4.5 ± 2.2	6.3 ± 4.9	7.4 ± 3.8	6.9 ± 3.6	9.4 ± 4.9	<.001	7.8 ± 3.4
High AMH stratum	5.9 ± 3.9	9.1 ± 6.4	10.6 ± 4.8	13.6 ± 7.8	14.4 ± 5.8	<.001	12.4 ± 5.4
Fertilization rate, %							
All patients	66 ± 27	65 ± 19	53 ± 23	58 ± 28	56 ± 20	<.001	62 ± 23
Low AMH stratum	71 ± 20	66 ± 18	58 ± 27	61 ± 28	57 ± 21	.005	63 ± 21
High AMH stratum	62 ± 31	64 ± 20	49 ± 18	56 ± 28	56 ± 20	.006	61 ± 25
Blastocysts, n							
All patients	2.3 ± 1.7	3.1 ± 2.7	2.7 ± 2.0	2.8 ± 2.8	3.2 ± 2.2	.092	3.5 ± 2.5
Low AMH stratum	2.2 ± 1.3	2.4 ± 1.4	2.3 ± 1.5	2.0 ± 1.6	3.1 ± 2.0*	.266	3.0 ± 2.2
High AMH stratum	2.3 ± 1.9	3.6 ± 3.3	3.1 ± 2.4	3.5 ± 3.4	3.3 ± 2.4	.188	3.9 ± 2.7
Blastocysts/oocytes, %							
All patients	45 ± 27	40 ± 23	30 ± 18	32 ± 28	29 ± 19	<.001	35 ± 23
Low AMH stratum	50 ± 28	44 ± 25	33 ± 20	34 ± 30	36 ± 21	.038	38 ± 26
High AMH stratum	42 ± 27	38 ± 22	27 ± 16	30 ± 27	25 ± 17	<.001	33 ± 21
Good-quality blastocysts,[d] n							
All patients	0.9 ± 1.2	1.4 ± 1.7	1.2 ± 1.2	1.4 ± 1.5	1.3 ± 1.3	.178	1.6 ± 1.6
Low AMH stratum	0.9 ± 0.9	0.9 ± 1.1	0.6 ± 0.9	1.0 ± 1.1	1.5 ± 1.5*	.156	1.5 ± 1.5
High AMH stratum	1.0 ± 1.4	1.7 ± 2.0	1.6 ± 1.3	1.7 ± 1.7	1.2 ± 1.2	.487	1.7 ± 1.8
Outcome in fresh cycle,[e] n (%)							
Positive βhCG rate[f]							
All patients	18/42 (43)	23/45 (51)	18/44 (41)	17/44 (39)	24/47 (51)	.650	23/43 (53)
Low AMH stratum	8/19 (42)	8/19 (42)	9/20 (45)	9/20 (45)	9/21 (43)	.999	10/18 (56)
High AMH stratum	10/23 (43)	15/26 (58)	9/24 (38)	8/24 (33)	15/26 (58)	.271	13/25 (52)
Clinical pregnancy rate[f]							
All patients	15/42 (36)	18/45 (40)	16/44 (36)	11/44 (25)	19/47 (40)	.554	22/43 (51)
Low AMH stratum	6/19 (32)	6/19 (32)	7/20 (35)	5/20 (25)	7/21 (33)	.970	10/18 (56)
High AMH stratum	9/23 (39)	12/26 (46)	9/24 (38)	6/24 (25)	12/26 (46)	.538	12/25 (48)
Live birth rate[f]							
All patients	15/42 (36)	17/45 (38)	16/44 (36)	11/44 (25)	18/47 (38)	.670	20/43 (47)
Low AMH stratum	6/19 (32)	6/19 (32)	7/20 (35)	5/20 (25)	6/21 (29)	.970	8/18 (44)
High AMH stratum	9/23 (39)	11/26 (42)	9/24 (38)	6/24 (25)	12/26 (46)	.615	12/25 (48)
Cumulative live birth rate[f,g]							
All patients	17/42 (40)	22/45 (49)	18/44 (41)	15/44 (34)	21/47 (45)	.698	22/43 (51)
Low AMH stratum	7/19 (37)	8/19 (42)	7/20 (35)	6/20 (30)	8/21 (38)	.955	8/18 (44)
High AMH stratum	10/23 (43)	14/26 (54)	11/24 (46)	9/24 (38)	13/26 (50)	.816	14/25 (56)

Note: Plus-minus values are means ± SD.

[a] *P* values reflect the dose–response relationship. For duration of stimulation, oocytes retrieved, number of blastocysts, and number of good-quality blastocysts the *P* values are based on ANCOVA models with center and AMH stratum as factors and log(dose) as covariate. *P* values for fertilization rate and blastocyst-to-oocyte rate are based on logistic regression models with AMH stratum as factor and log(dose) as covariate. *P* values for outcome variables are based on χ^2 tests.

[b] The low AMH stratum is a serum AMH concentration in the range from 5.0 to 14.9 pmol/L (0.7 to <2.1 ng/mL).

[c] The high AMH stratum is a serum AMH concentration in the range from 15.0 to 44.9 pmol/L (2.1 to 6.3 ng/mL).

[d] Blastocysts with a score of at least 3BB (i.e., expanding blastocysts with inner cell mass and trophectoderm gradings of A or B), according to the morphology grading system by Gardner and Schoolcraft (21).

[e] Per started cycle.

[f] n/total n (%).

[g] Including patients with cryopreserved blastocyst transfer cycles initiated within 6 months after start of COS.

* *P*< .05 vs. the other dose groups.

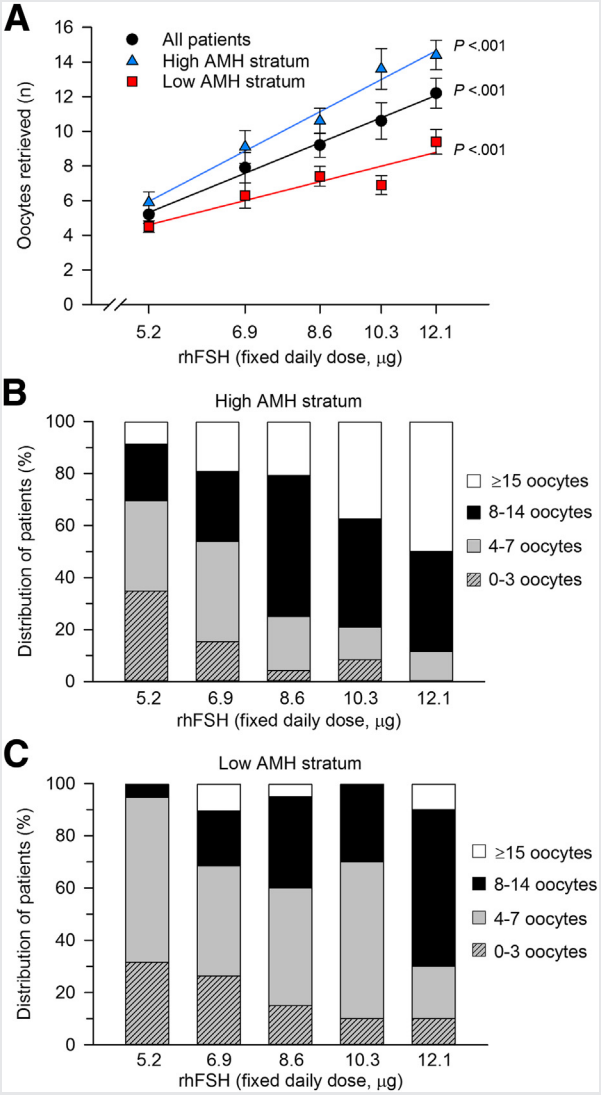
Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.

to the highest rhFSH dose groups (10.3 and 12.1 μg, respectively), and three late OHSS (one in the 8.6 μg group and two in the 12.1 μg group).

DISCUSSION

The present study demonstrates for the first time prospectively a significant AMH-dependent dose–response relationship between exogenous FSH and number of oocytes retrieved for IVF/ICSI. Different slopes of the dose–response curves were established according to AMH levels. Across all five tested rhFSH doses (5.2–12.1 μg), three to eight additional oocytes were obtained in the women presenting with high initial AMH concentrations compared with the low AMH women when the same rhFSH doses were administered. Cycle cancellation due to poor response was mainly clustered in the low AMH stratum and distributed across the rhFSH dose groups. Excessive response leading to changes in clinical

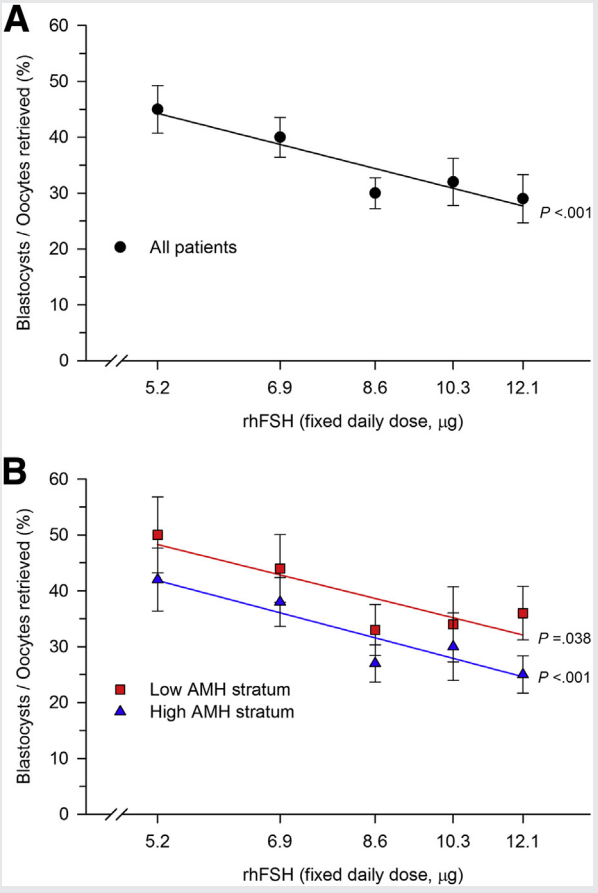
FIGURE 1



(**A**) Mean number of oocytes retrieved for patients in the five rhFSH dose groups, overall and by AMH stratum. The *vertical bars* represent standard errors. *P* values reflect the dose–response relationship. (**B**, **C**) Relative proportions of patients with different categories of number of oocytes retrieved by rhFSH dose group and AMH stratum (**B**, high AMH; **C**, low AMH).

Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.

FIGURE 2



Mean rate of blastocysts to oocytes retrieved for patients in the five rhFSH dose groups, (**A**) overall and by (**B**) AMH stratum. The *vertical bars* represent standard errors. *P* values reflect the dose–response relationship.

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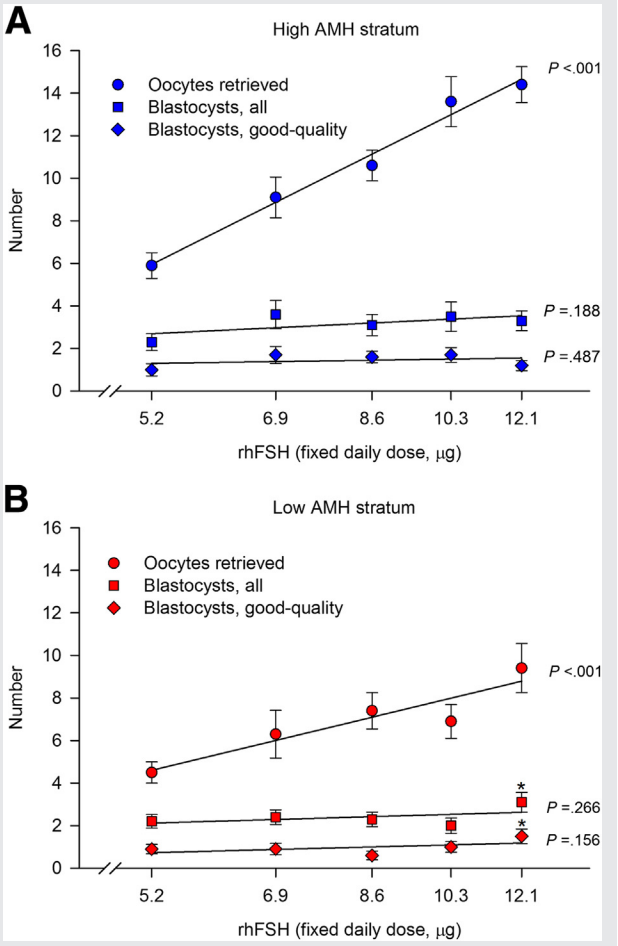
management, such as administration of a GnRH agonist bolus for triggering of final follicular maturation, was observed only in patients in the high AMH stratum. Additionally, the five cases of moderate/severe OHSS occurred only in the high AMH patient stratum and at the three highest doses of rhFSH. These findings reinforce the concept that the choice of an optimal gonadotropin dose for a given woman should be guided by an ovarian reserve marker, such as the initial serum level of AMH. Administration of a standard dose to all patients without taking into consideration their functional ovarian follicle reserve has been proven to cause major differences in degree of ovarian responses and safety issues, and furthermore, dose adjustments on stimulation day 6 to compensate the initial responses have been reported as inefficient (22, 23). The present study included women up to 37 years of age. Antimüllerian hormone has been shown to be a better predictor of the ovarian response than age in studies including women older than 37 years; see the review by La Marca et al. (15). The follitropin alfa 11 μg (150 IU) reference group had an ovarian response in line with expectations in GnRH antagonist cycles (24) and provided additional external validity to the dose–response curve for rhFSH.

The present study also suggests an inverse relationship between the rhFSH dose and fertilization and blastocyst-to-oocyte rates, for women in both the high and low AMH stratum. It can be speculated that the decrease in fertilization rate observed with higher doses of rhFSH may partly be a consequence of somewhat shorter stimulation periods affecting the nuclear and cytoplasmic maturation. However, because the duration of stimulation depends on the dose, the decrease in fertilization rate could be a consequence of higher doses,

FIGURE 3



Number of oocytes retrieved and blastocysts by rhFSH dose group (**A**, high AHM; **B**, low AMH). The *vertical bars* represent standard errors. *P* values reflect the dose–response relationship. * $P<.05$ vs. other dose groups combined.

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either directly or indirectly. The increase in number of oocytes retrieved obtained with higher rhFSH doses was not associated with a similar increase in the numbers of total blastocysts or good-quality blastocysts available for fresh transfer or cryopreservation. On the contrary, an apparent plateau regarding the number of available blastocysts was achieved with all rhFSH doses except the lowest in the high AMH stratum, whereas in the low AMH stratum only the women given the highest dose had equal numbers of blastocysts available for transfer as the women in the high AMH stratum. When administering a standard dose of gonadotropins in women undergoing COS, a relatively good association between the numbers of oocytes retrieved and blastocysts available for transfer would be expected because the response would be consistent with the ovarian reserve (i.e., the AMH level). Nevertheless, in the present study the average AMH level in all rhFSH dose groups was similar (Supplemental Table 1) owing to the AMH stratification at randomization, and therefore facilitates the interpretation of the effect of increasing gonadotropin doses and ovarian response potential on blastocyst availability.

High gonadotropin doses, or excessive ovarian response to stimulation, have previously been suggested to have detrimental effects on embryo/blastocyst quality or chromosome abnormalities on the basis of animal studies (25, 26). However, in line with a previous study by Kok et al. (27), the present study actually indicates that increasing gonadotropin doses will not compromise the number of good-quality oocytes that will develop to blastocysts. The similar absolute numbers of blastocysts and good-quality blastocysts obtained with low and high doses of gonadotropins in the present study suggest that the larger oocyte cohorts retrieved after stimulation with high doses are composed of relatively few good-quality oocytes and many additional oocytes that do not either fertilize or develop to blastocysts. Furthermore, the inverse dose–response relationship concerning fertilization rate may not only be attributed to an increased proportion of nuclear immaturity (27) but also to more nuclear mature oocytes with compromised development potential. The proportion of MII oocytes that develop into blastocysts decreased with rhFSH dose. This was most pronounced in the high AMH stratum; it is hypothesized that more metaphase II oocytes with poor development potential were recruited with increasing rhFSH doses in patients in this stratum. These results would support the notion that only a small number of the oocytes per retrieval have the potential to develop into a competent embryo/blastocyst (28–32). Finally, within each AMH stratum the increase in the number of oocytes retrieved with increasing doses of rhFSH was not associated with higher clinical pregnancy or live birth rates in the fresh cycle or the cumulative fresh and cryopreserved mandatory single blastocyst transfer cycles. The numerically lower cumulative success rates in patients in the low AMH stratum compared with patients in the high AMH stratum seem to be a logical observation and are thought to be dependent on a diminished ovarian reserve in the patients with low AMH.

In conclusion, a significant positive relationship between the administered dose of rhFSH and the number of oocytes retrieved was demonstrated, with different slopes of the dose–response curves for women with low or high initial AMH levels, respectively. The women in the high AMH stratum had significantly more blastocysts available for transfer than women in the low AMH stratum, but in neither stratum did the increased oocyte yield at higher gonadotropin doses result in a similar increase in the numbers of blastocysts. It is suggested that there may exist a threshold level for the starting gonadotropin dose, related to the AMH level, above which more intense stimulation has a limited effect on increasing the number of competent oocytes. The data from this prospective trial provide further arguments for elaborating on more individualized gonadotropin-dosing regimens that fulfill quantitative and qualitative ovarian response objectives for IVF/ICSI.

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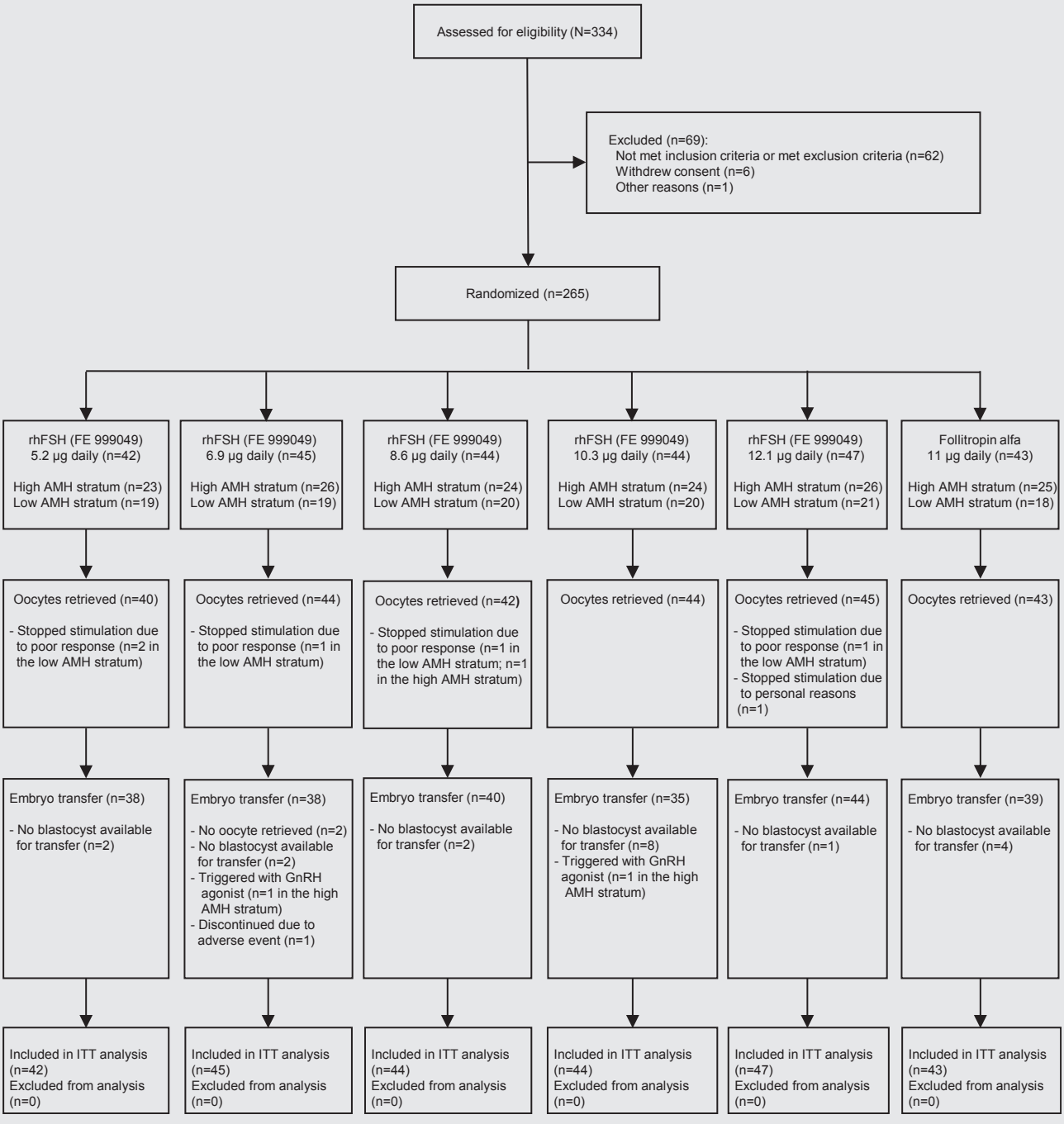
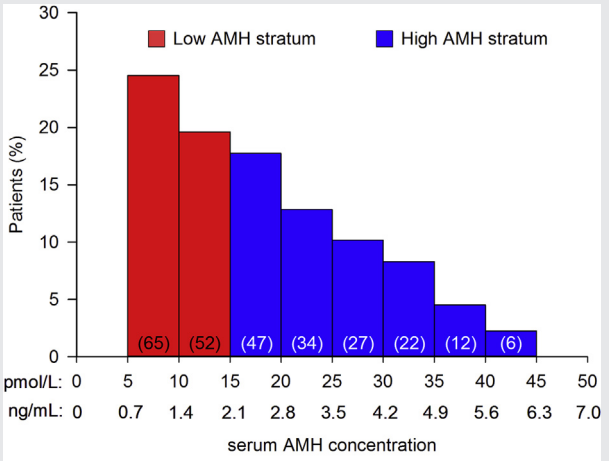
SUPPLEMENTAL FIGURE 1



Assignment, treatment, and analysis of patients. ITT = intention to treat.

Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.

SUPPLEMENTAL FIGURE 2



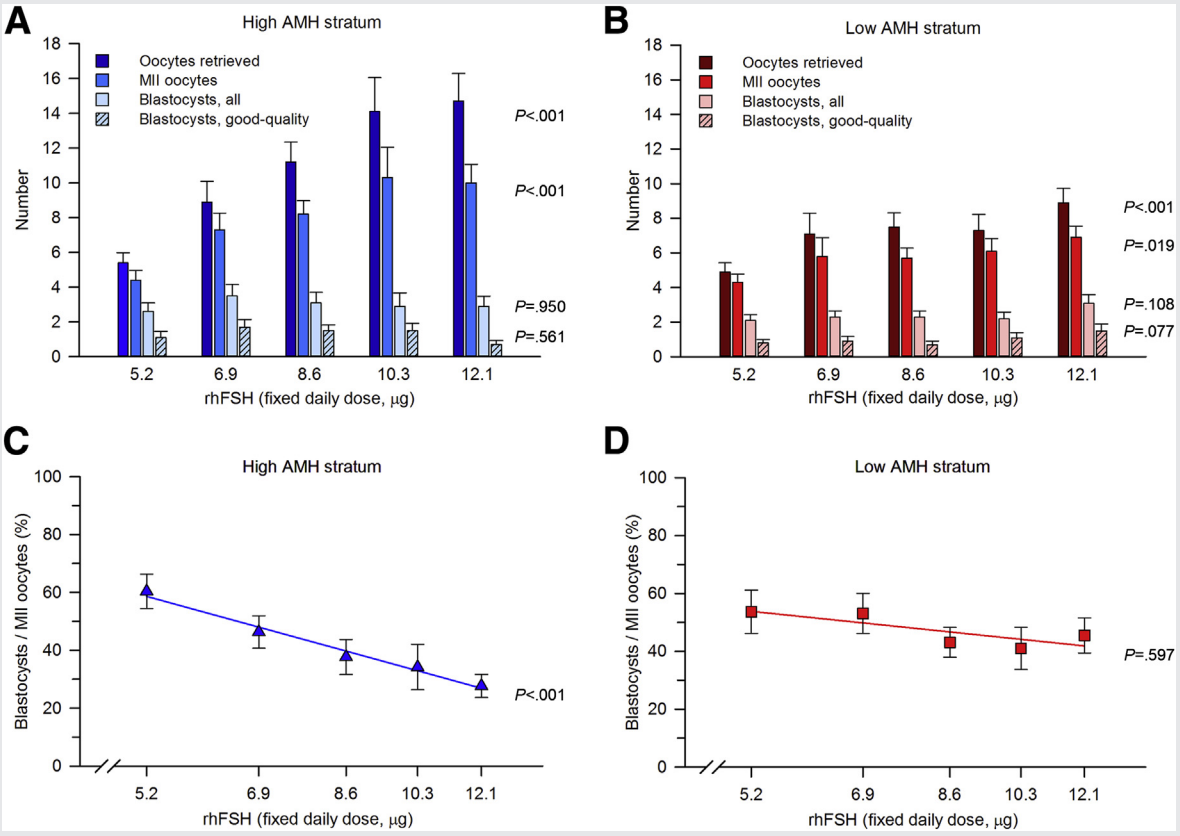
AMH percentiles (pmol/L)

	n	P5	P25	P50	P75	P95
Low AMH Stratum	117	5.8	7.4	9.4	11.7	14.4
High AMH Stratum	148	15.8	18.6	23.7	30.7	39.7

Distribution of initial AMH levels in the whole trial population.

Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.

SUPPLEMENTAL FIGURE 3



(**A**, **B**) Oocytes retrieved, metaphase II (MII) oocytes, and blastocysts by rhFSH dose group for the patients inseminated by ICSI (**A**, high AMH; **B**, low AMH). (**C**, **D**) Mean rate of blastocysts to MII oocytes for the ICSI patients (**C**, high AMH; **D**, low AMH). The *vertical bars* represent standard errors. *P* values reflect the dose–response relationship.

Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.

SUPPLEMENTAL TABLE 1

Demographic and baseline characteristics of the patients, according to treatment group.

Characteristic	rhFSH 5.2 μg (n = 42)	rhFSH 6.9 μg (n = 45)	rhFSH 8.6 μg (n = 44)	rhFSH 10.3 μg (n = 44)	rhFSH 12.1 μg (n = 47)	Follitropin alfa 11 μg (n = 43)	*P* value[a]
Age, y	33.6 ± 2.2	32.3 ± 3.5	32.8 ± 2.4	32.3 ± 3.2	32.6 ± 3.0	32.4 ± 3.0	.394
BMI, kg/m^2	23.0 ± 3.5	23.2 ± 3.2	23.2 ± 2.8	22.4 ± 2.6	22.3 ± 2.5	24.2 ± 3.6	.050
Infertility history							
Duration of infertility, y	3.3 ± 2.0	3.1 ± 2.2	3.4 ± 2.3	3.3 ± 2.3	3.4 ± 2.4	2.8 ± 1.4	.787
Primary infertility, n (%)	28 (67)	32 (71)	30 (68)	35 (80)	33 (70)	25 (58)	.427
Reason of infertility, n (%)							.915
Unexplained	24 (57)	21 (47)	23 (52)	23 (52)	23 (49)	20 (47)	
Tubal	5 (12)	4 (9)	2 (5)	1 (2)	5 (11)	6 (14)	
Mild male	6 (14)	7 (16)	10 (23)	6 (14)	9 (19)	9 (21)	
Moderate/severe male	7 (17)	11 (24)	8 (18)	12 (27)	8 (17)	7 (16)	
Endometriosis I/II	0 (0)	2 (4)	1 (2)	1 (2)	1 (2)	1 (2)	
Other	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	
Endocrine profile							
AMH[b]							
All patients							
pmol/L	16 (9–23)	18 (9–27)	16 (10–22)	16 (11–25)	16 (10–29)	19 (10–28)	.864
ng/mL	2.2 (1.3–3.2)	2.5 (1.3–3.8)	2.2 (1.4–3.1)	2.2 (1.5–3.5)	2.2 (1.4–4.1)	2.7 (1.4–3.9)	
Low AMH stratum[c]							
pmol/L	9 (7–11)	9 (7–12)	9 (7–11)	10 (8–13)	10 (7–11)	10 (9–12)	.740
ng/mL	1.3 (1.0–1.5)	1.3 (1.0–1.7)	1.3 (1.0–1.5)	1.4 (1.1–1.8)	1.4 (1.0–1.5)	1.4 (1.3–1.7)	
High AMH stratum[d]							
pmol/L	23 (17–29)	26 (19–29)	22 (19–29)	25 (21–34)	26 (19–31)	26 (19–31)	.684
ng/mL	3.2 (2.4–4.1)	3.6 (2.7–4.1)	3.1 (2.7–4.1)	3.5 (2.9–4.8)	3.6 (2.7–4.3)	3.6 (2.7–4.3)	
FSH (IU/L)							
All patients	6.4 (5.1–7.7)	7.0 (5.8–7.8)	6.6 (6.1–8.1)	6.9 (5.8–8.0)	7.0 (6.1–9.4)	6.8 (5.4–8.1)	.317
Low AMH stratum	6.1 (5.7–7.8)	7.2 (5.4–8.2)	7.9 (6.6–9.3)	7.6 (6.5–8.5)	8.0 (6.2–10.1)	6.9 (6.2–8.1)	.262
High AMH stratum	6.6 (4.9–7.3)	6.8 (5.8–7.3)	6.2 (5.3–6.7)	6.6 (5.3–7.6)	6.9 (6.1–8.1)	6.3 (5.1–8.1)	.338
AFC, n[e]							
All patients	13.7 ± 4.4	13.2 ± 4.7	13.5 ± 4.4	14.5 ± 4.4	14.3 ± 4.5	14.0 ± 4.2	.723
Low AMH stratum	11.6 ± 3.7	11.5 ± 2.9	11.6 ± 3.6	12.0 ± 3.2	13.3 ± 4.8	12.1 ± 2.7	.781
High AMH stratum	15.4 ± 4.3	14.5 ± 5.3	15.2 ± 4.4	16.7 ± 4.1	15.1 ± 4.2	15.3 ± 4.7	.686

Note: Plus-minus values are means ± SD. Hormone values are median (interquartile range). BMI = body mass index.

[a] *P* values are based on global tests to assess heterogeneity among treatment groups. *P* values for categorical variables are based on a two-sided χ^2 test. *P* values for continuous variables are based on analysis of variance of the van der Waerden normal scores (mean values) or the Kruskal-Wallis test (median values).

[b] The serum concentration of AMH was assessed by a central laboratory using Beckman Coulter Gen 2 ELISA.

[c] The low AMH stratum is a serum AMH concentration in the range from 5.0 to 14.9 pmol/L (0.7 to <2.1 ng/mL).

[d] The high AMH stratum is a serum AMH concentration in the range from 15.0 to 44.9 pmol/L (2.1 to 6.3 ng/mL).

[e] This measurement reports the total number of antral follicles with a diameter from 2 to 10 mm for both ovaries combined, assessed by transvaginal ultrasound at the day of starting COS.

Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.

SUPPLEMENTAL TABLE 2

Oocytes retrieved, metaphase II oocytes, and nonmature oocytes of the ICSI patients, according to treatment group.

Characteristic	rhFSH 5.2 μg (n = 33)	rhFSH 6.9 μg (n = 38)	rhFSH 8.6 μg (n = 35)	rhFSH 10.3 μg (n = 35)	rhFSH 12.1 μg (n = 31)	Follitropin alfa 11 μg (n = 35)	*P* value[a]
Oocytes retrieved							
All patients	5.1 ± 2.2	8.1 ± 5.3	9.3 ± 4.5	10.8 ± 7.3	11.7 ± 5.7	10.2 ± 4.9	<.001
Low AMH stratum[b]	4.9 ± 2.1	7.1 ± 4.8	7.5 ± 3.5	7.3 ± 3.8	8.9 ± 3.3	8.0 ± 3.4	<.001
High AMH stratum[c]	5.4 ± 2.4	8.9 ± 5.6	11.2 ± 4.7	14.1 ± 8.3	14.7 ± 6.2	12.0 ± 5.2	<.001
MII oocytes							
All patients	4.3 ± 2.1	6.7 ± 4.4	6.9 ± 3.1	8.3 ± 6.0	8.4 ± 3.7	7.9 ± 4.2	<.001
Low AMH stratum	4.3 ± 1.9	5.8 ± 4.3	5.7 ± 2.5	6.1 ± 3.0	6.9 ± 2.6	6.2 ± 3.0	.019
High AMH stratum	4.4 ± 2.3	7.3 ± 4.5	8.2 ± 3.2	10.3 ± 7.4	10.0 ± 4.1	9.4 ± 4.6	<.001
MI oocytes							
All patients	0.3 ± 0.5	0.6 ± 1.0	0.7 ± 0.8	0.9 ± 1.3	1.0 ± 1.2	1.1 ± 1.3	<.001
Low AMH stratum	0.4 ± 0.5	0.4 ± 0.6	0.6 ± 0.7	0.3 ± 0.6	0.5 ± 0.9	0.6 ± 0.9	.830
High AMH stratum	0.2 ± 0.4	0.6 ± 1.1	0.8 ± 0.8	1.4 ± 1.5	1.5 ± 1.3	1.5 ± 1.4	<.001
Germinal vesicle oocytes							
All patients	0.5 ± 0.8	0.8 ± 1.1	1.5 ± 1.7	1.4 ± 1.7	1.8 ± 2.6	0.9 ± 1.1	<.001
Low AMH stratum	0.2 ± 0.4	0.9 ± 1.2	1.2 ± 1.7	0.8 ± 1.2	1.4 ± 2.1	1.0 ± 1.2	.051
High AMH stratum	0.8 ± 1.0	0.7 ± 1.0	1.7 ± 1.6	1.9 ± 1.9	2.3 ± 3.0	0.8 ± 1.1	.001
Degenerated atretic oocytes							
All patients	0.0 ± 0.2	0.2 ± 0.4	0.3 ± 0.7	0.2 ± 0.5	0.4 ± 1.2	0.3 ± 0.8	.020
Low AMH stratum	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.3	0.2 ± 0.8	.060
High AMH stratum	0.1 ± 0.2	0.3 ± 0.6	0.6 ± 0.9	0.3 ± 0.6	0.8 ± 1.7	0.4 ± 0.8	.045

Note: Plus-minus values are means ± SD.
[a] *P* values for dose–response based on *F* test from an analysis of covariance model.
[b] The low AMH stratum is a serum AMH concentration in the range from 5.0 to 14.9 pmol/L (0.7 to <2.1 ng/mL).
[c] The high AMH stratum is a serum AMH concentration in the range from 15.0 to 44.9 pmol/L (2.1 to 6.3 ng/mL).

Arce. AMH and ovarian response to rhFSH. Fertil Steril 2014.