Follicular and endocrine dose responses according to anti-Müllerian hormone levels in IVF patients treated with a novel human recombinant FSH (FE 999049)

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Objective To study the association between serum anti-Müllerian hormone (AMH) levels and follicular development and endocrine responses induced by increasing doses (5–2–12-1 µg/day) of a novel recombinant human FSH (rhFSH, FE 999049) in patients undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) in a GnRH antagonist protocol.

Design Secondary analysis of a randomized controlled trial with stratified randomization according to AMH (lower stratum: 5–0–14.9 pmol/l; higher stratum: 15.0–44.9 pmol/l).

Patients Infertile women of good prognosis (n = 265).

Measurements Follicular development and endocrine parameters during controlled ovarian stimulation (COS) with rhFSH.

Results Serum FSH levels increased with increasing rhFSH doses and steady-state levels for each dose were similar in both AMH strata. In the whole study population, significant (P < 0.001) positive dose responses were observed for the number of follicles ≥12 mm, and serum levels of oestradiol, inhibin B, inhibin A and progesterone at end of stimulation. In comparison with the higher AMH stratum, patients in the lower AMH stratum had significantly different slopes of the dose–response curves for these hormones, and no clear dose-related increase was observed for the number of follicles in these patients.

Conclusions Dose–response relationships between rhFSH and follicular development and endocrine parameters are significantly different for IVF/ICSI patients with lower and higher serum AMH levels at start of COS.

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Introduction

Recombinant follicle-stimulating hormone (rFSH) preparations indicated for controlled ovarian stimulation (COS) in women undergoing treatment of infertility were introduced nearly two decades ago.1 However, dose–response studies were not included during their clinical development as the potency of daily rFSH preparations in humans was assumed to be similar to those of urinary FSH preparations.2 Therefore, the dose responses of rFSH in patients undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment, especially of low and high responder patients, have been poorly described in the literature and are mainly extrapolated from randomized clinical trials (RCTs) comparing two dosages of FSH preparations in normal responders.3 Furthermore, optimal starting doses in various patient populations have not been established prospectively.

The aim of COS is to obtain an adequate number of competent oocytes with minimum safety risks for patients.4 However, a large variability in the ovarian and endocrine responses is a well-known phenomenon in regular IVF/ICSI patients given a fixed standard dose of FSH,5 because the functional ovarian reserve, that is the pool of FSH-recruitable follicles, reflects the woman’s reproductive age and ovarian ageing differs markedly between individuals.6 It is therefore crucially important to individualize COS regimens, with the aim of eliminating iatrogenic risks, such as ovarian hyperstimulation syndrome (OHSS) due to an excessive response, and minimizing the risk of cycle cancellation due to poor response.7 For this reason, several biomarkers of ovarian reserve have been evaluated for their ability to accurately predict ovarian response to exogenous FSH stimulation.8–10 Anti-Müllerian hormone (AMH) is currently considered as the most robust measure of ovarian reserve11,12 and is a better predictor of the number of oocytes retrieved compared with other serum markers such as menstrual cycle
day-3 FSH and basal levels of inhibin B and oestradiol, or ultrasound assessment of antral follicle count (AFC). Recently, a novel rFSH (rhFSH) has been expressed for the first time in a human cell line (PER.C6® Crucell, Leiden, The Netherlands), while existing rFSH preparations (i.e. follitropin-α and follitropin-β) are derived from Chinese hamster ovary (CHO) cell lines. The amino acid sequences of the rhFSH molecule are identical to the sequences of natural human FSH, but compared with CHO-derived rFSH, the sialic acid content of rhFSH is higher having both α2,3 and α2,6 sialylation. Accordingly, it resembles natural human FSH more closely. Phase 1 studies in healthy women of reproductive age indicated that rhFSH and follitropin-α, each dosed in equivalent international units (IU), displayed significantly different pharmacokinetic and pharmacodynamic properties. Therefore, a phase 2 dose–response trial of rhFSH was initiated in IVF/ICSI patients using a broad range of dosages, and it was demonstrated prospectively that a significant AMH-dependent dose–response relationship existed between FSH exposure and the number of oocytes retrieved. More interestingly, because the patients’ AMH levels were used to stratify the randomization of potential low and high responders in each rhFSH dose group, the dose responses could be compared between patients with estimated lower and higher ovarian reserve.

Using data from the recent RCT, the aim of this study was to study the association between the patients’ serum AMH levels and the rhFSH-induced follicular development and endocrine responses including serum levels of FSH, LH, oestradiol, progesterone, inhibin A and inhibin B.

Materials and methods

This study is a secondary analysis of data prospectively collected in a randomized, open-labelled, parallel-group, dose–response phase 2 trial conducted at seven centres in four countries (Belgium, Czech Republic, Denmark and Spain) from September 2011 through May 2013 (ClinicalTrials.gov Identifier: NCT01426386). The trial was assessor-blinded, and all investigators, central laboratory personnel and sponsor staff involved in analysing and interpreting data were kept blinded to treatment allocation throughout the trial. The trial was performed in accordance with the Declaration of Helsinki, the International Conference on Harmonisation 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 centres. All patients provided written informed consent.

Trial population and treatment regimen

The trial enrolled good-prognosis patients, and the population, design, methods and main outcome results are described in detail elsewhere. The main inclusion criteria were as follows: age 18–37 years; tubal infertility, unexplained infertility, infertility related to endometriosis stage I/II, or partners diagnosed with male factor infertility; FSH 1–12 IU/l; AFC ≥6 and ≤25 for both ovaries combined; AMH 5–44.9 pmol/l (0.7–6.3 ng/ml); body mass index (BMI) 18.5–32.0 kg/m²; and regular menstrual cycles 24–35 days. Women with known polycystic ovary syndrome associated with anovulation, known endometriosis stage III–IV, poor ovarian response in a previous COS cycle using an average daily FSH dose ≥150 IU, or excessive ovarian response in a previous COS cycle using an average daily FSH dose <225 IU were excluded. Screening was performed within 3 months of stimulation day 1 (randomization) for assessment of compliance with inclusion and exclusion criteria, including AMH assessment.

On days 2–3 of the menstrual cycle, the patients were randomly assigned at equal ratios to receive fixed daily subcutaneous injections during the stimulation period of either 5, 6, 8, 8-6, 10-3 or 12-1 μg rhFSH (FE 999049; Ferring Pharmaceuticals, Saint-Prex, Switzerland), or 11 μg (150 IU) follitropin-α (Gonal-F filled by mass; Merck Serono, Darmstadt, Germany) which was included as a reference group. The rhFSH dose is expressed in mass (μg), as the IU’s assessed by the pharmacopeial rat bioassay (Steelman–Pohley in vivo bioassay) do not fully reflect the response of rhFSH in humans. Randomization was stratified according to the patients’ serum concentration of AMH at screening [lower stratum: 5–0–14.9 pmol/l (0.7–<2.1 ng/ml); higher stratum: 15–0–44.9 pmol/l (2.1–6.3 ng/ml)]. On stimulation day 6, a GnRH antagonist (ganirelix acetate, Orgalutran, MSD) was initiated at a daily dose of 0.25 mg and continued throughout the stimulation period. As soon as ≥3 follicles with a diameter ≥17 mm were observed, triggering of final follicular maturation was undertaken with 250 μg recombinant hCG (choriogonadotropin alfa, Ovitrelle; Merck Serono) if there were <25 follicles ≥12 mm, or with 0.2 mg GnRH agonist (tiaprelen acetate, Decapeptyl; Ferring Pharmaceuticals) if there were 25–35 follicles ≥12 mm. If there were >35 follicles ≥12 mm, the cycle was cancelled; coasting was not allowed. If <3 follicles ≥10 mm were observed on stimulation day 10, the cycle could be cancelled. Oocyte retrieval took place 36 ± 2 h after triggering of final follicular maturation, and the oocytes could be inseminated by IVF or ICSI.

Assessment of follicular development

Transvaginal ultrasound of the left and right ovary was performed at stimulation days 1, 4, 6 and end of stimulation to count the number of follicles and measure the size of the follicles.

Hormone assays

The serum concentration of AMH (1 pmol/l = 0.140 ng/ml; 1 ng/ml = 7.143 pmol/l) at screening was assessed by a central laboratory (ICON Central Laboratories, Dublin, Ireland) using the Beckman Coulter Gen II ELISA assay. The specimens were kept at ambient temperature for 1–5 days prior to analysis (i.e. during storage at the trial centre and shipment to the central laboratory) to avoid possible complement interference. Blood samples for endocrine monitoring were sampled at stimulation day 1 prior to the start of stimulation, and ≥8 h after the previous administration at days 4 and 6 and end of stimulation; end of stimulation reflects all patients receiving hCG when three fol-
icles ≥17 mm were reached and two patients who had excessive ovarian response leading to triggering with GnRH agonist. The serum specimens for endocrine parameters (except AMH) were stored individually at −18 °C at the trial centres before shipment in frozen containers to the central laboratory and subsequent analysis in batches. An overview of the validated analytical methods, sensitivity and precision is given in Table S1.

Statistical methods

The dose–response relationships at end of stimulation were evaluated using analysis of covariance (ANCOVA) models. For each endocrine parameter, the observed values were log-transformed and the model included log(dose) and log(baseline) as covariates and AMH stratum as factor. The ANCOVA model for number of follicles ≥12 mm included log(dose) as covariate and AMH stratum and trial site as factors. To evaluate the potential difference in dose response between the two AMH strata, an interaction term (AMH and trial site as factors. To evaluate the potential difference in dose

Results

Patient population

The study randomized 265 patients of whom 117 patients (44%) had serum AMH <15 pmol/l (lower stratum between 5-0 and 14-9 pmol/l) and 148 patients (56%) had AMH ≥15 pmol/L (higher stratum between 15-0 and 44-9 pmol/l). Two hundred and twenty-two patients received one of the five doses of rhFSH (42, 45, 44, 44 and 47 patients allocated to the 5-2, 6-9, 8-6, 10-3 and 12-1 µg/day groups, respectively), and 43 patients received 11 µg/day of follitropin-α. There were no clinically relevant differences between the six treatment groups at baseline with respect to age, body weight, BMI, AFC and AMH levels; the overall mean (±SD) values for the trial population were 32.7 (±3.0) years, 62.6 (±9.2) kg, 23-1 (±3-1) kg/m², 13-9 (±4-4) and 18-4 (±9-7) pmol/l, respectively. The patients randomized in the lower and higher AMH strata had mean (±SD) AMH levels of 9-6 (±2-6) and 25-3 (±7-5) pmol/l, respectively.

Follicular development during stimulation

Fig. 1 shows the follicular development. The total number of follicles ≥12 mm observed at stimulation day 4, 6 and end of stimulation in all patients is shown in Fig. 1a, and in the lower and higher AMH strata in Fig. 1b,c, respectively. The mean number of total follicles ≥12 mm increased with the daily dose of rhFSH administered. At end of stimulation, a significant (P < 0.001) dose-dependent increase in follicles ≥12 mm was observed (Fig. 1d); the mean number (±SD) of follicles ≥12 mm was lowest (6-7 ± 3-2) in the 5-2 µg group and highest (11-7 ± 4-5) in the 12-1 µg group. The mean number of follicles ≥12 mm in the follitropin-α group was 10-9 ± 4-6. During rhFSH treatment, the slopes of the time–response curves with respect to number of follicles ≥12 mm were steeper in the patients in the higher AMH stratum than in the lower AMH stratum (Fig. 1b,c). At end of stimulation, the slopes of the dose–response curves were significantly (P = 0.044) different between the two AMH strata (Fig. 1e). The more marked follicular responses at end of rhFSH stimulation in patients with higher AMH were also apparent at different size categories (Figure S1).

Serum hormone levels during stimulation

Fig. 2 shows the serum hormone levels during stimulation for all patients in each rhFSH dose group. Prior to the first FSH injection on stimulation day 1, the mean endogenous FSH levels ranged from 6-4 to 7-4 IU/l in the different treatment groups; patients with lower AMH had significantly higher FSH levels than patients with higher AMH (7-7 ± 2-8 IU/l vs 6-6 ± 1-8 IU/l, P < 0-001). Up to stimulation day 6, the FSH level increased in a linear dose-dependent manner, and thereafter, it remained at steady state until the end of stimulation for all rhFSH doses (Fig. 2a). At end of stimulation, the mean FSH level ranged from 8-2 to 17-1 IU/l in the 5-2–12-1 µg rhFSH dose groups, respectively, and was 10-1 IU/l in the follitropin-α group. Within each rhFSH dose level, there was no difference in the FSH levels during stimulation between the lower and higher AMH strata (Fig. 3a,b). The dose–response relationship between the administered dose of rhFSH and the FSH level in serum was significant (P < 0-001) at stimulation day 6 and at end of stimulation, overall (Fig. 2a) and in both AMH strata (Fig. 3c,d).

The mean serum LH levels at stimulation day 1 were similar between the rhFSH groups and ranged from 4-5 to 5-1 IU/l prior to the first rhFSH injection. At stimulation day 4, LH levels had declined in all five dose groups to mean values of 1-3–2-2 IU/l, overall (Fig. 2b) and in both AMH strata (Fig. 4a, b); thereafter, mean LH levels rose on stimulation day 6 in the two highest dose groups, especially in the higher AMH stratum. A significant (P = 0-020) dose-dependent increase in LH was noted in this group of patients at stimulation day 6, while there was no dose–response relationship in patients with lower AMH (Fig. 4c). Following the administration of GnRH antagonist starting at stimulation day 6, LH levels decreased in all rhFSH treatment groups up to end of stimulation, when the levels ranged from 1-3 IU/l in the lowest dose group to 1-0 IU/l in the highest dose group. Within each rhFSH dose group, the mean LH level was similar in the two AMH strata and no dose–response relationships were noted (Fig. 4d). The mean LH level at end of stimulation was 1-0 IU/l in the follitropin-α group.

Fig. 2c shows serum oestradiol levels during stimulation for all patients in each rhFSH dose group. The oestradiol level increased in a dose-related manner, and a significant (P < 0-001) dose-related increase was observed at the end of stimulation, at which time the mean levels ranged from 2780 to 5651 pmol/l in the rhFSH dose groups, and was 4780 pmol/l in the follitropin-α group. At end of stimulation, oestradiol was considerably higher in the higher AMH stratum than in the lower AMH stratum at all rhFSH doses, with the exception of the lowest dose of 5-2 µg (Fig. 5a,b). The slopes of the rhFSH
The total number (mean ± SE) of follicles ≥12 mm observed at stimulation day 4, day 6 and the end of stimulation in all patients (a), and in subsets of patients with lower anti-Müllerian hormone (AMH) (5–14·9 pmol/l) (b) or higher AMH (15–44·9 pmol/l) (c), during stimulation with fixed daily doses of rhFSH. The number of follicles ≥12 mm at end of stimulation in the five rhFSH dose groups is shown for all patients (d) and by AMH stratum (e); P values reflect the dose–response relationship. The number of follicles ≥12 mm at the end of stimulation following a fixed daily dose of 11 µg follitropin-α is added as a reference.

Fig. 1 The total number (mean ± SE) of follicles ≥12 mm observed at stimulation day 4, day 6 and the end of stimulation in all patients (a), and in subsets of patients with lower anti-Müllerian hormone (AMH) (5–14·9 pmol/l) (b) or higher AMH (15–44·9 pmol/l) (c), during stimulation with fixed daily doses of rhFSH. The number of follicles ≥12 mm at end of stimulation in the five rhFSH dose groups is shown for all patients (d) and by AMH stratum (e); P values reflect the dose–response relationship. The number of follicles ≥12 mm at the end of stimulation following a fixed daily dose of 11 µg follitropin-α is added as a reference.
Fig. 2 Serum hormone levels (mean ± SE) measured at stimulation day 4, day 6 and the end of stimulation in all patients. Insert panels show the levels at end of stimulation in the five rhFSH dose groups; P values reflect the dose–response relationship. The hormone levels at the end of stimulation following a fixed daily dose of 11 μg follitropin-α are added as a reference.

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dose–response curves were significantly \((P = 0.008)\) different between the AMH strata (Fig. 5e).

The serum progesterone levels tended to decline in all rhFSH dose groups during the first days of stimulation followed by gradual rises after stimulation day 4. At end of stimulation, small, but significant \((P < 0.001)\), dose-related increases were observed in the whole rhFSH population (Fig. 2d) and in the higher AMH stratum (Fig. 5f), although the mean progesterone levels remained below 1-0 ng/ml in all dose groups. At end of stimulation, the slope of the dose–response curve in the lower AMH stratum was significantly \((P = 0.012)\) lower than that of the higher AMH stratum, and no dose-related increase in progesterone was observed in the women with lower AMH (Fig. 5f). In the follitropin-α group, the mean serum progesterone level at end of stimulation also remained below 1-0 ng/ml.

Fig. 2e,f shows serum inhibin B and inhibin A levels during stimulation for all patients in each rhFSH dose group. The levels of both hormones increased with the rhFSH dose, and significant dose responses were observed at end of stimulation \((P < 0.001)\), at which stage the mean levels of inhibin B were 403, 664, 793, 720, 773 ng/l and mean levels of inhibin A 167, 234, 303, 301, 348 ng/l in the 5-2, 6-9, 8-6, 10-3, 12-1 μg rhFSH groups, respectively, and 726 and 278 ng/l, respectively, in the follitropin-α group. In both AMH strata, the increases in inhibin B flattened after day 6 in the higher rhFSH dose groups (Fig. 6a, b), whereas inhibin A levels continued to rise markedly in all dose groups (Fig. 6c,d). Compared to patients in the higher AMH stratum, no clear dose response was observed for inhibin B in patients in the lower AMH stratum (Fig. 6e). In contrast, marked significant \((P \leq 0.007)\) dose responses of inhibin A at
end of stimulation were observed in both AMH strata, although the magnitude of the inhibin A rise was lower in the lower AMH stratum (Fig. 6f).

**Discussion**

Until recently, there were no adequately powered dose–response studies of daily administration of gonadotrophins in IVF/ICSI patients to provide relevant information on the relation between FSH exposure and ovarian response during COS. In fact, this is the first randomized dose–response trial of daily recombinant FSH treatment in which the ovarian response was studied prospectively over a broad dose range. Moreover, the randomization was stratified for AMH with the aim of having a balanced distribution of patients with estimated lower and higher response in each dose group. The present study demonstrated significant positive relationships between the daily dose of rhFSH in the range of 5.2–12.1 μg and follicular development and endocrine responses. Subset analysis showed that these relationships were more apparent in patients with higher than with lower AMH levels.

In line with the pharmacokinetic properties of rFSH expressed by a CHO cell line, serum FSH levels increased in a linear dose-dependent manner following daily injections of rhFSH. Hence, the number of follicles ≥12 mm increased in all dose groups and a significant dose response was observed at end of stimulation, consistent with the primary end-point of this trial showing a highly significant relationship between the dose and the number of oocytes retrieved. In the present study, it is shown that the degree of ovarian response in patients with lower and higher AMH levels was clearly different. As increasing FSH

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*Fig. 4* Serum LH levels (mean ± SE) measured at stimulation day 4, day 6 and the end of stimulation in subsets of patients with lower anti-Müllerian hormone (AMH) (5–14.9 pmol/l) (a) or higher AMH (15–44.9 pmol/l) (b). The serum LH levels at day 6 (c) and at end of stimulation (d) in the five rhFSH dose groups are shown for each AMH stratum; *P* values reflect the dose–response relationship. Serum LH levels at end of stimulation following a fixed daily dose of 11 μg follitropin-α are added as a reference.
Fig. 5 Serum oestradiol levels (mean ± SE) measured at stimulation day 4, day 6 and the end of stimulation in subsets of patients with lower anti-Müllerian hormone (AMH) (5–14.9 pmol/l) (a) or higher AMH (15–44.9 pmol/l) (b), and serum progesterone levels (mean ± SE) in subsets of patients with lower AMH (c) or higher AMH (d), during stimulation with fixed daily doses of rhFSH. The serum oestradiol (e) and progesterone (f) levels at end of stimulation in the five rhFSH dose groups are shown for each AMH stratum; P values reflect the dose–response relationship. Serum oestradiol and progesterone levels at the end of stimulation following a fixed daily dose of 11 μg follitropin-α are added as a reference.
Fig. 6 Serum inhibin B levels (mean ± SE) measured at stimulation day 4, day 6 and the end of stimulation in subsets of patients with lower anti-Müllerian hormone (AMH) (5–14 pmol/l) (a) or higher AMH (15–44 pmol/l) (b), and serum inhibin A levels (mean ± SE) in subsets of patients with lower AMH (c) or higher AMH (d), during stimulation with fixed daily doses of rhFSH. The serum inhibin B (e) and inhibin A (f) levels at end of stimulation in the five rhFSH dose groups are shown for each AMH stratum; P values reflect the dose–response relationship. Serum inhibin B and inhibin A levels at the end of stimulation following a fixed daily dose of 11 µg follitropin-α are added as a reference.
levels induced a much steeper dose–response curve in terms of follicular development in patients with higher AMH levels than in patients with lower AMH levels, it may be concluded that the selection of the appropriate daily rhFSH dose is more critical for potential high responders. In addition, adjusting the starting gonadotrophin dose in a subsequent treatment cycle by the same magnitude will have a larger impact on the ovarian response of high responders than of low responders. Whereas lowering of the daily dose of rhFSH in the next cycle is essential in high responders, increasing the daily rhFSH dose in low responders may only be helpful as long as the maximal ovarian response is not yet reached. In clinical practice, low responders may often receive much higher gonadotrophin doses than required, even though clinical research has shown that such an approach will not improve the number of oocytes retrieved.20,21

In the present study, the serum levels of oestradiol and inhibins during rhFSH treatment reflected the number and size of growing follicles. In line with an earlier study in normal responders,22 inhibin B levels increased markedly until stimulation day 6 after which the levels flattened or declined until end of stimulation. In contrast, oestradiol and inhibin A levels kept rising through the whole stimulation period and to much greater extents in the patients with higher AMH levels. The different time courses of the inhibin A and B levels were likely due to the fact that inhibin B is mainly secreted by pre-antral and small antral follicles, while inhibin A is predominantly produced by pre-ovulatory follicles.23,24 Hence, once the follicles reach pre-ovulatory sizes, the inhibin B production lowers, whereas the inhibin A–secreting capacity continues to increase during the late follicular phase. The noted discrepancy between the rises of inhibin B and inhibin A in both AMH strata fits well with the published literature on these levels in patients with and without developing symptoms of OHSS.25,26

Irrespective of the degree of ovarian stimulation, there were only slight differences between the rhFSH dose groups with regard to their average serum LH and progesterone levels. Initially, LH levels were decreased by rhFSH administration and subsequently by the administration of GnRH antagonist, but there was limited overall impact of increasing rhFSH doses on LH levels during stimulation. From stimulation day 4 onwards, progesterone levels rose gradually, and at end of stimulation, significant dose-related increases were observed in the whole population and in the higher AMH stratum; however, mean levels remained below 1-0 ng/ml in all dose groups, even for patients in the higher AMH stratum. Although the average LH and progesterone levels were not so different between treatment groups, further data are needed to establish the impact of elevated levels in individual patients following rhFSH treatment. Currently, a starting dose of 150 IU/day of rFSH is considered to be the lowest effective dose in regular IVF/ICSI patients undergoing their first treatment cycle using a GnRH antagonist protocol. However, this daily gonadotrophin dose may be too low or too high, leading to dose adjustments made half-way during the treatment cycle in an attempt to increase or decrease the number of growing follicles. However, the size of the follicle cohort that is recruited largely depends on the starting dose, while dose adjustments during stimulation are known to be less effective. Of the patients undergoing their first IVF cycle with a starting dose of 150 IU, over 50% need to alter the starting dose in the second treatment cycle, which has a significant impact on the ovarian response.27 Thus, ideally patients should receive in their first treatment cycle a daily FSH dose already tailored to their ovarian reserve to ensure the shortest time interval to a successful outcome. As supported by the present trial, follicular development and the number of retrieved oocytes are very different in patients with low and high ovarian reserve treated with the same rhFSH dose. To select the optimal daily dose for each patient, an individualized rhFSH dosing algorithm based on serum AMH levels might be helpful, especially in patients undergoing their first IVF cycle. Such an algorithm may prevent too low or too high ovarian responses in the majority of patients and ineffective overdosing in low responders, as well as dose adjustments during stimulation.14,28

In line with previous findings regarding oocyte yield, the data from this study suggest that the follicular and endocrine responses at the end of stimulation are higher with rhFSH than with follitropin-α when extrapolating the data to similar microgram doses of the two compounds. The apparently large differences between preparations in serum FSH levels (measured in this trial at least 8 h after the previous administration of gonadotropin) in relation to the magnitude of the pharmacodynamic responses are most likely due to the lower clearance and longer half-life of rhFSH as determined by its characteristic glycosylation profile.17

In conclusion, significant positive dose–response relationships were noted between administration of rhFSH and number of follicles, and serum levels of oestradiol, inhibin B, inhibin A and progesterone. These relationships were different for subsets of patients with lower or higher AMH at screening. This indicates the need for individualized rhFSH dosing based on a reliable biomarker of ovarian reserve, such as AMH, to obtain an adequate number of growing follicles and an endocrine environment that favours implantation in patients undergoing COS prior to IVF or ICSI.

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**Supporting Information**

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