 Fully human glyco-optimized recombinant FSH (follitropin epsilon) - a randomized, comparator-controlled phase II clinical trial

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Published in:
Reproductive BioMedicine Online

Link to article, DOI:
10.1016/j.rbmo.2019.09.003

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

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ARTICLE

Fully human glyco-optimized recombinant FSH (follitropin epsilon) – a randomized, comparator-controlled phase II clinical trial

KEY MESSAGE
In this phase II trial, follitropin epsilon, a novel human recombinant FSH, induced a similar ovarian response to daily 150 IU follitropin alfa over a wide dose range in women undergoing ovarian stimulation for IVF. Use of follitropin epsilon should be tested in a phase III trial.

ABSTRACT
Research question: The study aimed to determine the standard treatment dose of follitropin epsilon for ovarian stimulation in the context of IVF treatment.

Design: A total of 247 women aged 18-37 years were treated with either 52.5, 75, 112.5 or 150 IU follitropin epsilon daily, or 150 IU every other day, or 150 IU follitropin alfa daily in a long gonadotrophin-releasing hormone agonist protocol. The study was performed as a randomized, assessor-blinded, comparator-controlled, six-armed phase II trial in eight fertility clinics in two European countries.

Results: The primary results were as follow. First, none of the doses of follitropin epsilon showed superiority for the main outcome measure, i.e. number of follicles ≥12 mm in size. Follitropin epsilon 75 IU produced results most similar to those of follitropin alfa 150 IU. In terms of secondary results, stronger effects of follitropin epsilon 112.5 IU compared with follitropin alfa 150 IU were seen for secondary outcome measures such as hormone concentrations (oestradiol, inhibin B and progesterone) and oocyte number.

Conclusions: Follitropin epsilon 75 IU daily results in a similar ovarian response to a standard dose of 150 IU follitropin alfa. This dose could be tested in a phase III trial.

KEYWORDS
Dose-finding
Follitropin epsilon
Human glyco-optimized recombinant FSH
Ovarian stimulation
Recombinant FSH
INTRODUCTION

Follitropin epsilon is a recombinant FSH (rFSH) produced in a cell line of the human expression system GlycoExpress (Glycotope, Germany) for application in assisted reproductive technology (ART), in particular for follicle maturation in women during IVF stimulation protocols. This novel follitropin was designed as a recombinant, fully human molecule with optimized human glycosylation as an alternative to the marketed rFSH products produced in Chinese hamster ovary (CHO) cells and urine-derived FSH.

Human pharmacological characteristics of follitropin epsilon, as investigated in healthy young women aged 18–40 years, have recently been reported (Abd-Elaziz et al., 2017). That study demonstrated higher pharmacodynamic responses for follitropin epsilon in terms of serum oestradiol and inhibit B concentrations, as well as greater follicular growth (number and size of follicles) after administration of even smaller bioactive doses (IU), based on the Steelman-Pohley in-vivo rat assay (Steelman and Pohley, 1953) compared with urine-derived FSH (Bravelle, Ferring, Germany) and CHO-derived rFSH (Gonal-f, Merck Serono, Germany).

This report presents data from the first study with follitropin epsilon in women undergoing ovarian stimulation for assisted reproduction. The primary objective of the study was to determine the recommended standard treatment dose of follitropin epsilon as assessed by follicular growth dynamics in women between 18 and 37 years of age undergoing intracytoplasmic sperm injection (ICSI) treatment. Secondary objectives were to compare the efficacy, safety and tolerability of the different follitropin epsilon treatment arms compared with the CHO-derived rFSH follitropin alfa.

MATERIALS AND METHODS

Study design
This prospective, assessor-blinded, randomized, controlled, parallel-group, multicentre (n = 8), six-arming phase II study was conducted to compare the effectiveness, safety and tolerability of various doses and treatment regimens of follitropin epsilon with a CHO-derived rFSH (follitropin alfa; Gonal-f, Merck Serono) in women undergoing IVF with ICSI. The study was designed, conducted, recorded and reported in compliance with the principles of Good Clinical Practice guidelines (EMA, 2006). Reporting follows the recommendations of the CONSORT 2010 Statement (Schulz et al., 2010). The study was conducted at eight sites in Germany and Hungary between January and August 2013. The trial registration number was EudraCT No. 2012-003006-27 (registration date 22 June 2012; date of first patient enrolment 8 January 2013).

Institutional review board approval was obtained from all sites (Germany: ref. no. 12/0459-ZS EK 13 from 8 January 2013; Hungary: ref. no. 47376-0/2012-EKL from 14 November 2012) before starting the trial. Before any study-specific procedures were performed, written informed consent was obtained from all participants. The patients who participated in the trial did not receive any compensation as an inducement to participate.

Patients eligible for this study were infertile women with an indication for ICSI treatment who met the following criteria: age 18–37 years at screening; serum FSH concentrations ≤12 IU/l (day 1–5 of a spontaneous menstrual cycle); anti-Mullerian hormone (AMH) concentration 1–4 ng/ml assessed by the local laboratory, and fertilization by ICSI was guided transvaginal retrieval. Metaphase II (MII) oocytes were classified as apoptotic, non-MII, arrested at metaphase I (M1) or at anaphase II. Oocytes were classified as having no evidence of fertilization or undergoing apoptosis.

Significant exclusion criteria included more than two previously unsuccessful IVF or ICSI cycles (defined as no embryo transfer or no biochemical or clinical pregnancy achieved), more than two miscarriages, stage III or IV endometriosis, intramural fibroid and uterine cavity abnormalities, a previous poor response (<3 oocytes), a history of or current polycystic ovary syndrome, a history of ovarian hyperstimulation syndrome (OHSS) or endocrine abnormalities.

Eligibility for randomization at the end of down-regulation required an absence of ovarian cysts, a serum oestradiol concentration <50 pg/ml and a negative pregnancy test.

Treatment
Eligible patients underwent down-regulation using subcutaneous treatment with once-daily 100 µg triptorelin acetate (gonadotrophin-releasing hormone agonist; Decapeptyl or Gonapepty, Ferring, Germany), starting on day 20–22 of a spontaneous menstrual cycle.

Eight to 25 days after starting down-regulation, a pregnancy test was performed to exclude patients who were pregnant. At the same time, transvaginal ultrasonography was performed, and oestradiol concentration was determined to confirm down-regulation. Thereafter patients were randomized to one of the six FSH treatment arms to receive either 52.5, 75, 112.5 or 150 IU follitropin epsilon once daily, 150 IU follitropin epsilon every second day, or 150 IU follitropin alfa once daily.

In addition to ongoing triptorelin acetate treatment, participants started FSH treatment within 8 days after confirmation of down-regulation. The fixed dose of FSH treatment assigned to each patient was administered every 24 ± 2 h (daily administration) or 48 ± 2 h (2-day scheme) in the evening for a maximum duration of 18 days. As soon as one follicle had a diameter of ≥20 mm (the human chorionic gonadotrophin [HCG] criterion), FSH treatment was stopped. Triptorelin acetate was administered until (and including) the day of HCG injection. Administration of 250 µg recombinant HCG took place in the evening of the same day that the HCG criterion was fulfilled (24–48 h after the last FSH treatment) to induce final oocyte maturation.

At 32–36 h after HCG administration, oocytes were recovered by ultrasound-guided transvaginal retrieval. Metaphase II (MII) oocytes were classified as defined by the European Society of Human Reproduction and Embryology (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011) by the local laboratory, and fertilization by ICSI was performed in all cases. A maximum of two embryos was transferred 2–3 days after oocyte recovery. Progesterone gel 90 mg (Crinone 8%, Merck Serono, Germany) was given once daily vaginally with embryo transfer as luteal phase...
support, starting on day 2 after oocyte recovery.

All patients were followed up at a safety visit, 14–20 days after oocyte retrieval, including confirmation of pregnancy for patients who had undergone an embryo transfer. In case of confirmed pregnancy, progesterone treatment was continued until a total duration of 30 days had been achieved from progesterone initiation on day 2 after oocyte pick-up. A final examination/en of study visit was performed 4–6 weeks after the last FSH treatment for all randomized patients.

Randomization to one of the six treatment arms was performed per centre using computer-generated, randomly permuted blocks with an undisclosed fixed block size of six. Randomization was performed after successful down-regulation by use of an interactive web-based randomization system.

FSH treatment was administered to the participants using assessor-blinded procedures, i.e. neither the investigator nor any other study personnel involved in study-related assessments knew which treatment was being administered. Only the patient and the dispensing study team member were not blinded, owing to technical reasons so that the correct dose could be administered.

Assessments
On day 1 of FSH treatment, transvaginal ultrasound sonography was performed to assess the number of basal antral follicles by counting each follicle with a diameter within the range 2.0–10.0 mm. During stimulation, ultrasound assessments were performed at least every second day to count the number and size of follicles from stimulation day 2 onwards up to the day on which one follicle had reached ≥20 mm.

Blood sampling for analysis of oestradiol and inhibin B serum concentrations was performed at screening (oestradiol only), to check down-regulation, on days 1 and 2 of FSH treatment, and at least every second day thereafter until one follicle reached ≥20 mm in size.

Throughout the study, patients recorded daily adverse events and concomitant medication usage in a patient diary. Any change in the patient’s clinical assessment compared with previous visits was considered an adverse event and was reported as such. The investigator examined the injection sites for FSH and triptorelin separately at each visit.

Patients with an ongoing pregnancy at the final visit were followed up over an observational post-study period until the pregnancy outcome was reached.

Statistical analysis and sample size
The intention-to-treat population (including all the randomized participants) constituted the denominator for the efficacy analyses. Safety analysis was performed on all the patients who had had at least one dose of triptorelin. A sample size of 37 in each group was calculated to have 80% power to detect a difference of 3.0 in the mean number of follicles, assuming that the common SD was 4.5 and using a two group t-test with a 0.05 two-sided significance level.

The primary efficacy variable was the number of follicles with a diameter of ≥12 mm on the day when the HCG criterion were met, after stimulation with follicitopin epsilon compared with follicitropin alfa. Follicle number instead of number of oocytes retrieved was used as the primary end-point because the number of follicles represents the most immediate response of the ovary to the drug, which is essential for a phase II dose-finding study.

Secondary efficacy variables were as follows: number of retrieved cumulus–oocyte complexes (COC); number of MII oocytes, number of two pronuclei (2PN) oocytes 1 day after follicle puncture; biochemical pregnancy (yes/no), defined as a positive B-HCG test; clinical pregnancy (yes/no), defined as the presence of gestational sac with a heartbeat; implantation rate (the number of fetal sacs on sonography divided by the number of embryos transferred per embryo transfer); the number of doses and total dose of FSH; and pharmacodynamic parameters – serum concentrations of oestradiol and inhibin B.

Safety variables included: incidence of adverse events, OHSS, as defined by Papanikolaou and colleagues (Papanikolaou et al., 2006) and Navot and co-workers (Navot et al., 1992), and anti-drug antibodies; clinical laboratory evaluations (haematology, biochemistry and urinalysis); vital signs (blood pressure and heart rate); BMI; electrocardiogram measurements; and overall tolerability (physical assessments and injection site reactions).

The statistical analysis (SAS version 9.1.3, SAS Institute, Cary, NC, USA) was conducted using sequential (hierarchical) testing for pairwise comparison of the four follitropin epsilon daily dosing regimens against follitropin alfa 150 IU/day. Each of these comparisons was performed using an analysis of covariance (ANCOVA) model, which included treatment (two levels) and site as factors, and age as a covariate. Given the one-sided hypotheses to be tested, the analysis of treatment effects was performed as a one-sided test at a significance level of 0.025, which is equivalent to the two-sided test at the significance level of 0.05 specified by the protocol. Because the testing was sequential, a type I error rate of α = 0.05 was maintained for the entirety of the primary end-point. Failure of any stage in the sequence implied automatic failure of all subsequent stages.

As part of an exploratory analysis, all pairwise comparisons between the treatment arms were performed using the above-described ANCOVA model without further adjustments for multiple comparisons. These exploratory comparisons also included the treatment arm ‘follitropin epsilon 150 IU every second day’. For the least square mean, 95% confidence intervals of treatment differences based on the ANCOVA model were provided. As a further sensitivity analysis, Wilcoxon rank sum tests were performed on the pairs of treatment arms specified by the set of hypotheses of the primary analysis.

Pairwise comparisons similar to those specified for the primary analysis were performed for the following variables: number of retrieved COC, number of MII oocytes, and number of 2PN oocytes 1 day after follicle puncture.

RESULTS

Patient disposition and basal characteristics
A total of 258 patients were found to be eligible and started down-regulation. Of these, 247 patients were randomized to one of the six treatment arms, and 243 followed the protocol without violations. A patient flow chart is given in Figure 1.
The patients’ characteristics per treatment cohort are shown in Table 1. The patient population that entered the study was well balanced between the treatment groups with respect to age, weight, BMI, smoking, alcohol drinking habits and basal FSH, AMH and AFC.

Overall, 243 patients (98.4%) underwent follicle puncture. Four patients did not undergo follicle puncture because of either hyper-responsiveness (three patients) or protocol deviations (one patient). Of the patients who underwent follicle puncture, 241 had at least one MII oocyte retrieved.

**Ovarian response to stimulation**

Outcome results for all randomized patients are presented in Table 2.

The primary efficacy end-point of the study was the number of follicles with a diameter of ≥12 mm on the day when the HCG criterion was met. The sequential (hierarchical) testing for the pairwise comparison of the four follitropin epsilon daily dosing regimens with follitropin alfa 150 IU did not yield any statistically significant differences. In comparison with follitropin alfa 150 IU, the differences in number of follicles ≥12 mm of the individual follitropin epsilon dose groups were in the range -1.2 (follitropin epsilon 52.5 IU) to +1.5 (follitropin epsilon 112.5 IU), with the follitropin epsilon 150 IU every second day (+0.4 follicles) and follitropin epsilon 75 IU (+0.6 follicles) groups showing similar results. As this main statistical approach failed to demonstrate a difference of three follicles or more between the two compounds as assumed in the power calculation, all further statistical analyses were performed on a descriptive level, and reported P-values should be taken as descriptive P-values (Abt, 1987).

The number of follicles increased with increasing doses of follitropin epsilon (Figure 2A, Table 2). This increase resulted in statistically significant differences between the lowest follitropin epsilon group (52.5 IU) and the higher follitropin epsilon once-daily dose groups (P = 0.0479 versus 75 IU, P = 0.0033 versus 112.5 IU, and P = 0.0097 versus 150 IU once daily), whereas no significant differences occurred between any of the other follitropin epsilon groups.

During the first 9 days of stimulation, follitropin epsilon doses of ≥112.5 IU led to higher follicle numbers than doses of 150 IU follitropin alfa (112.5 IU, P = 0.0064; 150 IU once-daily, P = 0.0014; 150 IU every second day, P = 0.0013). No difference was observed between 75 IU follitropin epsilon and 150 IU follitropin alfa (P = 0.0813), and even 52.5 IU produced an equal number of follicles during the first 8 days, indicating a stronger potency of follitropin epsilon (Figure 2B).

A mean number of 14.5 COC was retrieved from participants treated with follitropin epsilon 112.5 IU, compared with a mean value of 11.1 for the follitropin alfa 150 IU group (P = 0.0068). In addition, statistically significant differences were shown for the comparisons follitropin epsilon 112.5 IU versus follitropin epsilon 52.5 IU (P = 0.0032), and follitropin epsilon 150 IU every second day versus follitropin epsilon 52.5 IU (P = 0.0372) (Figure 2C).

Similar results were seen for the number of MII oocytes, with a mean of 10.7 MII oocytes retrieved from patients treated with follitropin epsilon 112.5 IU and 8.6 MII oocytes retrieved from those treated with follitropin alfa 150 IU (P = 0.0283). Additionally, the mean number of MII oocytes showed a statistically significant difference between the follitropin epsilon 112.5 IU group and the follitropin epsilon 52.5 IU group (10.7 and 7.9 MII oocytes, respectively, P = 0.0077), and between the follitropin epsilon 150 IU every second
day group and the follitropin epsilon 52.5 IU group (101 and 79 MII oocytes, respectively, P = 0.0313).

Assessment of fertilization outcome was performed 16–20 h after the ICSI procedure and included a determination of the number of injected oocytes with two pronuclei. ANCOVA showed no statistically significant differences between treatment groups in the mean numbers of 2PN oocytes 1 day after follicle puncture.

A maximum of two embryos was transferred per patient. No clinically relevant differences were observed in the mean values of the number of embryos being transferred, with values ranging from 1.7 for the follitropin epsilon 52.5 IU group to 1.9 for the follitropin epsilon 150 IU once-daily and follitropin epsilon 150 IU every second day groups, compared with 1.8 for the follitropin alfa group.

Pregnancy and live birth rates were evaluated as secondary outcome parameters and are reported in Table 2.

### Endocrine assessments
Serum concentrations of oestradiol and inhibin B were measured as a pharmacodynamic assessment of FSH treatment.

Oestradiol and inhibin B concentrations increased during treatment in all groups (independent samples median test, P = 0.024 and P = 0.035, respectively). All doses of follitropin epsilon, except the 52.5 IU dose, led to higher oestradiol serum concentrations than 150 IU follitropin alfa (75 IU, P = 0.0094; 112.5 IU, 150 IU once daily, and 150 IU every second day, P < 0.0001), and higher inhibin B concentrations during the first 8 days of stimulation (75, 112.5 and 150 IU once-daily, and 150 IU every second day, all P < 0.0001) (Figure 3A, B).

On the day of HCG administration, the mean oestradiol concentration ranged between 8577 pmol/l (52.5 IU) and 13,693 pmol/l (112.5 IU) in the follitropin epsilon groups, whereas it reached 8281 pmol/l in the follitropin alfa 150 IU group, mostly corresponding with the number of follicles (Table 2). Within the follitropin epsilon groups, only the 52.5 IU group showed a significantly lower oestradiol concentration (median) compared with the other dose groups (75 IU, P = 0.008; 112.5 and 150 IU once-daily, P < 0.001; 150 IU every second day, P = 0.004), whereas oestradiol concentration was significantly lower in the follitropin alfa 150 IU group compared with the 112.5 IU (P = 0.004) and the 150 IU every second day (P = 0.014) follitropin epsilon groups (Figure 4A).

The results for inhibin B differed slightly from those for oestradiol. On the day of HCG administration, mean inhibin

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**TABLE 1** DEMOGRAPHICS AND BASELINE CHARACTERISTICS OF THE 247 PATIENTS, ACCORDING TO TREATMENT GROUP

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Folitropin epsilon QD</th>
<th>Folitropin epsilon QAD</th>
<th>Folitropin alfa QD</th>
</tr>
</thead>
<tbody>
<tr>
<td>52.5 IU</td>
<td>75 IU</td>
<td>112.5 IU</td>
<td>150 IU</td>
</tr>
<tr>
<td>No. of patients</td>
<td>41</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>Characteristics, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.5 (3.8)</td>
<td>31.9 (3.3)</td>
<td>31.9 (3.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>641 (92)</td>
<td>62.7 (91)</td>
<td>64.2 (102)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7 (3.0)</td>
<td>22.7 (2.7)</td>
<td>23.1 (2.7)</td>
</tr>
<tr>
<td>Non-smoker, n (%)</td>
<td>40 (97.6)</td>
<td>34 (81.0)</td>
<td>36 (90.0)</td>
</tr>
<tr>
<td>Non-alcohol drinker, n (%)</td>
<td>25 (61.0)</td>
<td>26 (61.9)</td>
<td>28 (70.0)</td>
</tr>
<tr>
<td>Maternal history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (years)</td>
<td>1.3 (1.5)</td>
<td>1.1 (1.6)</td>
<td>1.4 (1.7)</td>
</tr>
<tr>
<td>Cause, n (%)</td>
<td>8 (19.5)</td>
<td>10 (23.8)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Previous ART, n (%)</td>
<td>14 (34.1)</td>
<td>15 (35.7)</td>
<td>11 (27.5)</td>
</tr>
<tr>
<td>Endocrine basal profile, median (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>3.7 (1.2–71)</td>
<td>3.7 (19–98)</td>
<td>3.6 (19–60)</td>
</tr>
<tr>
<td>Oestradiol (pmol/l)</td>
<td>67 (37–129)</td>
<td>59.5 (37–204)</td>
<td>58 (37–344)</td>
</tr>
<tr>
<td>Inhibin B (UI/l)</td>
<td>14.7 (11.3–53.2)</td>
<td>16.4 (12–125.1)</td>
<td>15.6 (10.2–179.4)</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>2.2 (1–4)</td>
<td>2.0 (1–3.7)</td>
<td>2.1 (1–11.4)</td>
</tr>
<tr>
<td>AFC, n, mean (SD)</td>
<td>13.2 (2.2)</td>
<td>131 (2.5)</td>
<td>13.0 (2.3)</td>
</tr>
</tbody>
</table>

AFC, antral follicle count; AMH, anti-Müllerian hormone; ART, assisted reproduction technology; BMI, body mass index; QAD, every second day; QD, once a day.
TABLE 2 DURATION OF STIMULATION, ENDOCRINE PROFILE AND FOLLICLES ≥12 MM ON THE DAY OF HCG ADMINISTRATION, OOCYTES RETRIEVED AND OUTCOME OF ART ACCORDING TO TREATMENT GROUP

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Follitropin epsilon QD</th>
<th>52.5 IU</th>
<th>75 IU</th>
<th>112.5 IU</th>
<th>150 IU</th>
<th>Follitropin epsilon QAD</th>
<th>150 IU</th>
<th>150 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td></td>
<td>41</td>
<td>42</td>
<td>40</td>
<td>43</td>
<td>42</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>No. of FSH doses</td>
<td></td>
<td>11 (7–17)</td>
<td>10 (7–15)</td>
<td>10 (8–16)</td>
<td>10 (6–14)</td>
<td>5 (4–7)</td>
<td>11 (8–18)</td>
<td></td>
</tr>
<tr>
<td>Total FSH dose (IU)</td>
<td></td>
<td>5775 (3675–892.5)</td>
<td>750 (525–1125)</td>
<td>1125 (900–1800)</td>
<td>1500 (900–2100)</td>
<td>750 (600–1050)</td>
<td>1650 (1200–2700)</td>
<td></td>
</tr>
<tr>
<td>Total FSH dose (IU)</td>
<td></td>
<td>609.5 (114.9)</td>
<td>787.5 (117.4)</td>
<td>1209.4 (196.9)</td>
<td>15279 (264.4)</td>
<td>821.4 (100.7)</td>
<td>1684.6 (269.8)</td>
<td></td>
</tr>
<tr>
<td>Days to HCG</td>
<td></td>
<td>11.7 (2.2)</td>
<td>10.5 (1.4)</td>
<td>10.8 (1.8)</td>
<td>10.3 (1.6)</td>
<td>10.6 (1.3)</td>
<td>11.3 (1.8)</td>
<td></td>
</tr>
<tr>
<td>HCG, no. of patients</td>
<td></td>
<td>41</td>
<td>41</td>
<td>40</td>
<td>41</td>
<td>42</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td></td>
<td>7.40 (1.69)</td>
<td>9.75 (2.19)</td>
<td>14.02 (3.47)</td>
<td>18.24 (4.30)</td>
<td>8.99 (1.83)</td>
<td>14.94 (3.41)</td>
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</tr>
<tr>
<td>Oestradiol (pmol/l)</td>
<td></td>
<td>8577 (9739)</td>
<td>11,559 (7947)</td>
<td>13,693 (8946)</td>
<td>12,987 (9683)</td>
<td>11,991 (5699)</td>
<td>8281.4 (5699)</td>
<td></td>
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<tr>
<td>Inhibin B (IU/l)</td>
<td></td>
<td>901 (629)</td>
<td>904 (466)</td>
<td>874 (429)</td>
<td>657 (356)</td>
<td>999 (497)</td>
<td>671 (399)</td>
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<tr>
<td>Progesterone (nmol/l)</td>
<td></td>
<td>2.4 (1.3)</td>
<td>2.9 (1.4)</td>
<td>4.1 (3.5)</td>
<td>3.9 (1.9)</td>
<td>2.7 (1.3)</td>
<td>2.6 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Patients with progesterone &gt;4.77 nmol/l</td>
<td></td>
<td>3 (7.32)</td>
<td>4 (9.76)</td>
<td>10 (25.00)</td>
<td>13 (31.71)</td>
<td>1 (2.38)</td>
<td>1 (2.63)</td>
<td></td>
</tr>
<tr>
<td>Follicles ≥12 mm</td>
<td></td>
<td>11.2 (4.6)</td>
<td>13.0 (4.2)</td>
<td>13.9 (4.4)</td>
<td>13.7 (4.9)</td>
<td>12.8 (3.8)</td>
<td>12.4 (5.14)</td>
<td></td>
</tr>
<tr>
<td>COC retrieved</td>
<td></td>
<td>10.2 (7.0)</td>
<td>12.6 (5.0)</td>
<td>14.5 (5.8)</td>
<td>12.6 (5.7)</td>
<td>13.4 (5.7)</td>
<td>11.1 (5.4)</td>
<td></td>
</tr>
<tr>
<td>MII oocytes</td>
<td></td>
<td>79 (5.0)</td>
<td>94.8 (4.6)</td>
<td>10.7 (4.9)</td>
<td>8.8 (4.6)</td>
<td>10.1 (4.0)</td>
<td>8.6 (4.4)</td>
<td></td>
</tr>
<tr>
<td>ICSI, no. of patients</td>
<td></td>
<td>40</td>
<td>41</td>
<td>39</td>
<td>41</td>
<td>42</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>2PN oocytes</td>
<td></td>
<td>6.0 (4.6)</td>
<td>7.3 (4.0)</td>
<td>7.5 (4.0)</td>
<td>6.6 (3.7)</td>
<td>7.5 (4.6)</td>
<td>6.2 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Embryos</td>
<td></td>
<td>4.5 (4.4)</td>
<td>4.4 (2.9)</td>
<td>4.3 (3.7)</td>
<td>4.7 (3.1)</td>
<td>4.1 (3.4)</td>
<td>4.6 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Embryo transfer, no. of patients</td>
<td></td>
<td>38</td>
<td>40</td>
<td>38</td>
<td>41</td>
<td>41</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Positive β-HCG</td>
<td></td>
<td>23 (56.1)</td>
<td>17 (40.5)</td>
<td>23 (57.5)</td>
<td>16 (37.2)</td>
<td>16 (38.1)</td>
<td>22 (56.4)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td></td>
<td>18 (43.9)</td>
<td>14 (33.3)</td>
<td>20 (50.0)</td>
<td>9 (20.9)</td>
<td>12 (28.6)</td>
<td>21 (53.8)</td>
<td></td>
</tr>
<tr>
<td>Live birthsb</td>
<td></td>
<td>14 (34.1)</td>
<td>11 (26.2)</td>
<td>18 (45)</td>
<td>8 (18.6)</td>
<td>10 (23.8)</td>
<td>18 (46.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Values are median (range); all other values are mean (SD).

a Number (% of the intention-to-treat population).2PN, 2 pronuclei; ART, assisted reproductive technology; COC, cumulus-oocyte complex; HCG, human chorionic gonadotrophin; ICSI, intracytoplasmic sperm injection; MII, metaphase II; QD, once a day; QAD, every second day.

B values within the follitropin epsilon groups were similar, ranging between 871 IU (112.5 IU) and 999 IU (150 IU) every second day, with exception of the 150 IU once-daily group (657 IU/l), which showed the lowest value (table 2). A comparison of the medians showed statistically significant differences between the 150 IU once-daily group and the groups receiving 75 IU (P = 0.048) and 150 IU every second day (P = 0.004). In the follitropin alfa 150 IU group, inhibin B concentration (median) was significantly lower than in the 75 IU (P = 0.011), 112.5 IU (P = 0.028) and 150 IU every second day (P = 0.002) follitropin epsilon treatment groups (figure 4b).

Progesterone was assessed only on the day of HCG administration. Its concentrations (median) showed a dose-dependent increase within the follitropin epsilon dose groups and were higher in the 112.5 IU (P = 0.006) and 150 IU once-daily (P < 0.001) groups compared with the 150 IU follitropin alfa group (table 2; figure 4c). In addition, the number of patients with progesterone concentrations >4.77 nmol/l (>1.5 ng/ml) increased with increasing doses, up to almost 32% in the 150 IU QD dose group (P = 0.0009). Interestingly, only one patient (2.63%) reached this level in the follitropin alfa group (table 2).

The rate of patients with progesterone concentrations >4.77 nmol/l who did not become pregnant differed between the dose groups, being highest in the 150 IU once-daily group (52.5 IU, 1/3; 75 IU, 2/4; 112 IU, 4/10; 150 IU, 9/13; 150 IU every second day, 0/1; follitropin alfa 150 IU, 0/1).

Safety results

In general, follitropin epsilon was safe and well tolerated. In total, 38.0% of all participants treated with follitropin epsilon reported treatment-emergent adverse events (TEAE), compared with 28.2% in the follitropin alfa group. However, for follitropin epsilon there was no evidence of a dose-dependent increase in TEAE (table 3).

The most frequently observed TEAE observed in all 247 patients were: nervous system disorders (41 patients [16.6%]), including headache (36 patients [14.6%]); gastrointestinal disorders (39 patients [15.8%]), including abdominal pain (16 patients [6.5%]); and general disorders and administration site conditions (25 patients [10.1%]), including injection site erythema (13 patients [5.3%]) and injection site pain (11 patients [4.5%]).
The TEAE reported most often was headache, which was reported in four patients (10.3%) in the follitropin alfa 150 IU group, and in total in 32 of 208 patients (15.4%) in the follitropin epsilon treatment groups; however, there was no apparent relationship to follitropin epsilon dose. In no case was the causality of headache assessed as ‘related’ to the administration of FSH.

The majority of TEAE were mild in intensity (67 patients [27.1%]), with a similar frequency in all treatment groups. Severe TEAE were reported for four patients (1.6%). Headache, the most frequently reported TEAE, was severe in two patients (0.8%). In addition, severe OHSS with ascites, and a severe injection site reaction (one patient each, 0.4% each) were recorded.

Three patients (1.2%) experienced serious TEAE (one patient in each of these treatment groups: follitropin epsilon 112.5 IU, OHSS and ascites assessed as severe and leading to significant intervention; follitropin alfa 150 IU, OHSS of moderate intensity leading to hospitalization; follitropin epsilon 150 IU QD, ectopic pregnancy leading to hospitalization). For one patient (0.4%) in the follitropin epsilon 75 IU treatment group, the drug was permanently withdrawn due to TEAE (non-serious OHSS that was assessed as related).

During the study, nine patients (3.6%) experienced OHSS according to the central assessment. These included three patients (7.7%) treated with follitropin alfa and six patients (2.9%) treated with follitropin epsilon.

**DISCUSSION**

This is the first clinical study in infertile patients testing the novel recombinant human FSH follitropin epsilon for ovarian stimulation. The primary insight of this study is that all doses between 52.5 and 150 IU were effective with respect to the primary outcome – variable number of follicles ≥12 mm. The increase in number of follicles between the lowest and the highest dose is smaller than expected, at <3 follicles. In comparison with the reference compound follitropin alfa and its standard dose of 150 IU, all doses of follitropin epsilon showed a similar outcome with respect to the primary end-point. Differences in follicle number between 150 IU follitropin alfa and all individual follitropin epsilon doses were in the range of −1.2 (52.5 IU follitropin epsilon) to +1.5 (follitropin epsilon 112.5 IU) follicles.

In consequence, the primary hypothesis that doses of follitropin epsilon ≥75 IU would result in a higher number of follicles compared with follitropin
TABLE 3  
TEAE OBSERVED IN ≥2% OF THE 247 PATIENTS, DISTRIBUTED ACCORDING TO TREATMENT GROUP

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Follicitropin epsilon QD</th>
<th>Follicitropin epsilon QAD</th>
<th>Follitropin alfa QD</th>
<th>Totala</th>
</tr>
</thead>
<tbody>
<tr>
<td>52.5 IU</td>
<td>41</td>
<td>42</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>75 IU</td>
<td>16 (39.0)</td>
<td>13 (31.0)</td>
<td>16 (40.0)</td>
<td>15 (34.9)</td>
</tr>
<tr>
<td>112.5 IU</td>
<td>9 (22.0)</td>
<td>6 (14.3)</td>
<td>8 (20.0)</td>
<td>4 (9.3)</td>
</tr>
<tr>
<td>150 IU QD</td>
<td>9 (22.0)</td>
<td>5 (11.9)</td>
<td>6 (15.0)</td>
<td>4 (9.3)</td>
</tr>
<tr>
<td>150 IU every second day</td>
<td>8 (19.5)</td>
<td>3 (7.1)</td>
<td>9 (22.5)</td>
<td>5 (11.6)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>4 (9.8)</td>
<td>1 (2.4)</td>
<td>2 (5.0)b</td>
<td>3 (7.0)b</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (2.4)</td>
<td>0 (0.0)</td>
<td>4 (10.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>3 (7.3)</td>
<td>2 (4.8)</td>
<td>1 (2.5)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>5 (12.2)</td>
<td>5 (11.9)</td>
<td>4 (10.0)</td>
<td>6 (14.0)</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>1 (2.4)</td>
<td>0 (0.0)</td>
<td>4 (10.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (7.3)</td>
<td>2 (4.8)</td>
<td>1 (2.5)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>General disorders and administration site conditions</td>
<td>5 (12.2)</td>
<td>5 (11.9)</td>
<td>4 (10.0)</td>
<td>6 (14.0)</td>
</tr>
</tbody>
</table>

a Percentages are calculated in the intention-to-treat population. None of the T1 patients starting down-regulation but not randomized experienced an adverse event. The majority of events were mild.

b One event of moderate intensity in each group.

c Central assessment: not a case of OHSS, one in each group.

d Central assessment, severe OHSS (central assessment = second assessment by two experts). OHSS, ovarian hyperstimulation syndrome; QD, once a day; QAD, every second day; TEAE, treatment-emergent adverse event.
FIGURE 4 Serum concentrations (medians and quartiles) of oestradiol (E2) (A), inhibin B (B) and progesterone (C) on the day of HCG administration analysed by treatment arm (Mann–Whitney U-test). QAD, every second day; QD, once-daily.

number of follicles ≥12 mm in size. This increase attenuated above the dose of 112.5 IU follitropin epsilon, indicating a non-linear dose–effect relation in the population treated. Although the difference in follicle number seen between the 52.5 IU dose and the three higher doses showed statistical significance, this is probably...
of minor clinical relevance. Whereas a similar pattern can be observed in the subsequent development stages of COC and mature (MII) oocytes, the variation between the follitropin epsilon dose groups becomes smaller after fertilization according to 2PN numbers. With a range of 6–75 2PN oocytes, all the follitropin epsilon dose groups tested achieved a good basis for successful embryo development.

The optimal number of oocytes retrieved for successful IVF/ICSI treatment in fresh cycles is often considered to be in the range 8–15 (Magnusson et al., 2018). The highest proportion of patients with 8–15 oocytes was found in the 75 IU (58.5%) and 112.5 IU (57.5%) groups, compared with 52.6% in the follitropin alfa 150 IU group. However, in the 150 IU every second day group, 50.0% of patients also fell within this range (FIGURE 2D).

The only statistically significant differences between follitropin epsilon and follitropin alfa 150 IU were observed in the 112.5 IU dose group for number of COC and MII oocytes (COC, +3.4; MII oocytes, +2.1). Differences between these two groups were also found for the endocrine parameters oestradiol, inhibin B and progesterone. We interpret these observations as a hypothesis that 112.5 IU follitropin epsilon shows a higher bioactivity, which should be followed up in further studies with larger cohorts, including patients with a broader ovarian response spectrum.

Dosing with 150 IU follitropin epsilon did not lead to a further numerical increase in follicles ≥12 mm or retrieved COC compared with the 112.5 IU dose group and even resulted in the lowest number of pregnancies and live births. It needs further clarification whether this outcome is a consequence of higher gonadotrophin dosing, as controversially discussed in the literature (Van Blerkom and Davis, 2001; Roberts et al., 2005; Kok et al., 2006).

Of special interest is the outcome in the 150 IU every second day group. The every second day administration results in an outcome for nearly all measures that was comparable to that seen for 75 IU follitropin epsilon given daily. The every second day administration of a double dose of follitropin epsilon is therefore potentially a valid approach that is worth further assessing in future studies.

Considering the endocrine pharmacodynamics, all doses of follitropin epsilon ≥75 IU displayed an earlier onset and stronger responses in terms of oestradiol and inhibin B increase, as well as for follicular growth with doses >75 IU during the first 9 days of treatment compared with 150 IU follitropin alfa. The serum hormone concentrations observed in participants treated with follitropin alfa were initially slightly reduced but reached the same level at a later time-point, probably due to the similar follicle numbers and sizes when the HCG criterion was eventually reached.

The dose-dependent increase in progesterone is in accordance with reported findings that a higher daily dose of FSH is the factor most related to the occurrence of an elevation in serum progesterone (Bosch et al., 2010). Several publications have previously described the negative impact of premature progesterone elevation on the outcome of ART (Bosch et al., 2003, Venetis et al., 2013). The impact of elevated progesterone values on pregnancy rates in this study remains unclear. The frequency of clinical pregnancy in patients with elevated progesterone values differed between the dose groups and was not directly related to the FSH dose. However, it could have contributed to the small number of pregnancies in the 150 IU group.

Owing to the very small number of participants with elevated progesterone in this subset, no definite conclusions can be drawn. It is important to emphasize that the sample sizes in the individual dosage groups are generally too small, and the sampling error too high, to allow the drawing conclusions on a binary outcome such as pregnancy occurrence. It is also noteworthy that the incidence of progesterone elevation was exceptionally low in the control arm of the trial (2.63%), while the incidence in the 75 IU once-daily follitropin epsilon dosage group (9.76%) was well within the expected incidence of progesterone elevation of >4.77 nmol/l (17.2%), as previously reported in a large systematic review (Venetis et al., 2012).

The results of this study confirm the findings from the previous study in young female volunteers, in which a stronger bioactivity of follitropin epsilon compared with urinary FSH and CHO-derived rFSH was observed (Abd-Elaziz et al., 2017).

The outcome of this study is aligned with the findings on the recombinant human FSH molecule follitropin delta, which is produced in the human cell line PER.C6, and has glyc.osylation properties different from those of follitropin epsilon (Olsson et al., 2014; Arce et al., 2014). Stronger pharmacodynamic activity and a higher output of oocytes at equivalent doses compared with a CHO-derived rFSH were also reported for this human rFSH. Thus, the conclusion is substantiated that a fully human FSH with optimized glycosylation results in improved activity of the gonadotrophin.

With respect to the standard treatment dose of follitropin epsilon, it can be concluded that daily 112.5 IU results in stronger effects than daily 150 IU follitropin alfa, whereas daily 75 IU is most similar to daily 150 IU follitropin alfa. This would translate in a 2.1 bioactivity ratio of follitropin epsilon to follitropin alfa. This needs to be substantiated in subsequent studies.

Finally, follitropin epsilon at all doses showed a good safety and tolerability profile. The potential higher bioactivity did not give evidence of a higher risk of OHSS.

ACKNOWLEDGEMENTS

The authors wish to acknowledge all the work of the staff from the participating study centres: Fertility Center Berlin, Reproductive Care, Berlin, Germany; Praxisklinik Sydow, Reproduction Institute, Berlin, Germany; Bielefeld Fertility Center, Private Reproduction Institute, Bielefeld, Germany; Kaali Institute, Reproduction Institute, Budapest, Hungary; Interdisziplinäres Kinderwunschzentrum, Private Reproductive Care Unit, Düsseldorf, Germany; University Hospital Heidelberg, Gynaecologic Endocrinology and Fertility Disorders, Heidelberg, Germany; Universitäres Kinderwunschzentrum Lübeck und Manhagen, Luebeck, Germany; and PANNON, Reproduction Institute, Tapolca, Hungary.
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Received 18 June 2019; received in revised form 2 September 2019; accepted 7 September 2019.