

# A randomized, controlled, first-in-patient trial of choriogonadotropin beta added to follitropin delta in women undergoing ovarian stimulation in a long GnRH agonist protocol

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**STUDY QUESTION:** Does addition of choriogonadotropin beta (recombinant CG beta) to follitropin delta increase the number of good-quality blastocysts following ovarian stimulation in a long GnRH agonist protocol?

**SUMMARY ANSWER:** At the doses investigated, the addition of CG beta reduced the number of intermediate follicles and related down-stream parameters including the number of oocytes and blastocysts.

**WHAT IS KNOWN ALREADY:** CG beta is a novel recombinant hCG (rhCG) molecule expressed by a human cell line (PER.C6<sup>®</sup>) and has a different glycosylation profile compared to urinary hCG or rhCG derived from a Chinese Hamster Ovary (CHO) cell line. In the first-in-human trial, the CG beta pharmacokinetics were similar between men and women. In women, the AUC and the peak serum concentration ( $C_{max}$ ) increased approximately dose proportionally following single and multiple daily doses. In men, a single dose of CG beta provided higher exposure with a longer half-life and proportionately higher testosterone production than CHO cell-derived rhCG.

**STUDY DESIGN, SIZE, DURATION:** This placebo-controlled, double-blind, randomized trial (RAINBOW) was conducted in five European countries to explore the efficacy and safety of CG beta as add-on treatment to follitropin delta in women undergoing ovarian stimulation in a long GnRH agonist protocol. Randomization was stratified by centre and age (30–37 and 38–42 years). The primary endpoint was the number of good-quality blastocysts (Grade 3 BB or higher). Subjects were randomized to receive either placebo or 1, 2, 4, 8 or 12 µg CG beta added to the daily individualized follitropin delta dose during ovarian stimulation.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** In total, 620 women (30–42 years) with anti-Müllerian hormone (AMH) levels between 5 and 35 pmol/l were randomized in equal proportions to the six treatment groups and 619 subjects started treatment. All 619 subjects were treated with an individualized dose of follitropin delta determined based on AMH (Elecys AMH Plus Immunoassay) and body weight. Triggering with rhCG was performed when 3 follicles were  $\geq 17$  mm but no more than 25 follicles  $\geq 12$  mm were reached.

**MAIN RESULTS AND THE ROLE OF CHANCE:** The demographic characteristics were comparable between the six treatment groups and the overall mean age, body weight and AMH were  $35.6 \pm 3.3$  years,  $65.3 \pm 10.7$  kg and  $15.3 \pm 7.0$  pmol/l, respectively. The incidence of cycle cancellation (range 0–2.9%), total follitropin delta dose (mean 112  $\mu$ g) and duration of stimulation (mean 10 days) were similar across the groups. At stimulation Day 6, the number and size of follicles was similar between the treatment groups, whereas at the end-of-stimulation dose-related decrease of the intermediate follicles between 12 and 17 mm was observed in comparison to the placebo group. In contrast, the number of follicles  $\geq 17$  mm was similar between the CG beta dose groups and the placebo group. A reduced number of intermediate follicles (12 to 17 mm) and fewer oocytes (mean range 9.7 to 11.2) were observed for all doses of CG beta compared to the follitropin delta only group (mean 12.5). The mean number of good-quality blastocysts was 3.3 in the follitropin delta group and ranged between 2.1 and 3.0 across the CG beta groups. The incidence of transfer cancellation was higher in the 4, 8 and 12  $\mu$ g group, mostly as no blastocyst was available for transfer. In the group receiving only follitropin delta, the ongoing pregnancy rate (10–11 weeks after transfer) was 43% per started cycle versus 28–39% in CG beta groups and 49% per transfer versus 38–50% in the CG beta groups. There was no apparent effect of CG beta on the incidence of adverse events, which was 48.1% in the placebo group and 39.6–52.3% in the CG beta dose groups. In line with the number of collected oocytes, the overall ovarian hyperstimulation syndrome incidence remained lower following follitropin delta with CG beta (2.0–10.3%) compared with follitropin delta only treatment (11.5%). Regardless of the dose, CG beta was safe and well-tolerated with low risk of immunogenicity.

**LIMITATIONS, REASONS FOR CAUTION:** The effect of the unique glycosylation of CG beta and its associated potency implications in women were not known prior to this trial. Further studies will be needed to evaluate optimal doses of CG beta for this and/or different indications.

**WIDER IMPLICATIONS OF THE FINDINGS:** The high ongoing pregnancy rate in the follitropin delta group supports the use of individualized follitropin delta dosing in a long GnRH agonist protocol. The addition of CG beta reduced the presence of intermediate follicles with the investigated doses and negatively affected all down-stream parameters. Further clinical research will be needed to assess the optimal dose of CG beta in the optimal ratio to follitropin delta to develop this novel combination product containing both FSH and LH activity for ovarian stimulation.

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## Introduction

Choriogonadotropin beta (CG beta, FE 999302) is a novel recombinant hCG (rhCG) expressed by a human cell line (PER.C6<sup>®</sup>). Like natural hCG, CG beta is a glycoprotein molecule consisting of an  $\alpha$ -subunit of 92 amino acids which is identical to that present in human FSH, LH and thyroid-stimulating hormone, and a  $\beta$ -subunit of 145 amino acids that confers biological specificity. HCG and LH bind to the same receptor due to high 80% homology of the  $\beta$ -subunit with an important difference being that hCG carries a 24-amino acid C-terminal extension, the so-called C-terminal peptide (CTP) (Pierce and Parsons, 1981; Ascoli et al., 2002; Casarini et al., 2018). At least one-third of the mass of CG beta is made up by eight carbohydrate moieties, of which six are attached to the  $\beta$ -subunit and two are attached to the  $\alpha$ -subunit hCG. The four N-linked carbohydrate chains are attached to Asn52 and Asn78 of the  $\alpha$ -subunit and Asn13 and Asn30 of

the  $\beta$ -subunit, whereas the O-linked oligosaccharides are attached to Ser121, Ser127, Ser132 and Ser 138 on the CTP.

The major difference between CG beta and other hCG preparations is related to glycosylation, which is highly complex with a wide range of O-linked and N-linked structures and including combinations of bi-, tri- and tetra-antennary glycans across the four different attachment sites (Cole, 2009, 2010). The glycosylation of CG beta is unique and reflects the glycosylation capabilities of the human cell line. The glycosylation profiles of the expressed hCG protein depend on the specificity of glycosyl transferases in different cell lines; as a result, each cell line produces unique rhCG products (Cottingham et al., 2011). The expressed CG beta glycosylation carries a higher overall negative charge, as demonstrated by more acidic isoforms on gel analysis, due to a combination of both  $\alpha$ 2,3-linked sialic acid and  $\alpha$ 2,6-linked sialic acid attached to N-linked glycosylation due to the activity of the respective glycosyl transferases. CG beta glycosylation and degree of

sialylation increase the biological activity of CG-beta in humans in contrast to choriogonadotropin alfa (CG alfa), produced by a Chinese Hamster Ovary (CHO) cell line; the latter contains less sialylation overall and no N-linked  $\alpha$ 2,6-linked sialic acid. Moreover, N-linked  $\alpha$ 2,6-linked sialic acid may influence the biological clearance differently among species and that may extend the circulating half-life in humans (Koechling *et al.*, 2017).

Phase I trial 000220 confirmed that the glycosylation of CG beta indeed results in different pharmacokinetic and pharmacodynamic profiles (longer half-life and higher potency) in comparison to CG alfa (Broksø Kyhl *et al.*, 2021). The purpose of this first-in-human trial was to examine the safety, pharmacokinetics and pharmacodynamics of CG beta in healthy women and men. Single- and multiple-dose pharmacokinetics of CG beta were evaluated in women and single-dose pharmacokinetics and pharmacodynamics of CG beta were evaluated in men in comparison to CG alfa. CG beta was safe and well-tolerated in all 84 healthy volunteers. In women, the AUC and the peak serum concentration ( $C_{max}$ ) increased approximately dose proportionally following single and multiple doses of CG beta. The apparent clearance (CL/F) was  $\sim$ 0.5 l/h, the mean terminal half-life  $\sim$ 45 h and the apparent distribution volume ( $V_z/F$ ) was  $\sim$ 30 l. After single administration in men, the mean AUC was 1.5-fold greater for CG beta than for CG alfa. Mean  $C_{max}$  and  $V_z/F$  were comparable for the two preparations. In accordance with the differences in AUC, the CL/F was lower for CG beta (CL/F 0.5 versus 0.8 l/h), explained by a longer terminal half-life (47 versus 32 h). In men, serum testosterone levels induced by a single dose CG beta reflected the pharmacokinetic profiles with a slight delay, resulting in 59% higher AUC for CG beta. The pharmacokinetic parameters for CG beta were comparable in men and in women and the PK of CG alfa were consistent with the CG alfa data in the literature (Trinchard-Lugan *et al.*, 2002; Bagchus *et al.*, 2017).

Previous comparative studies applying urinary hCG during ovarian stimulation have suggested that increased hCG levels during ovarian stimulation in down-regulated women are positively associated with good-quality embryos and/or implantation rates (Andersen *et al.*, 2006; Ziebe *et al.*, 2007; Thuesen *et al.*, 2012; Arce and Smits, 2013). This first patient trial explores the impact of CG beta when added to follitropin delta for ovarian stimulation in a long GnRH agonist protocol which ensured that endogenous LH levels remained low during the whole stimulation period.

## Materials and methods

### Study design

This was a Phase 2, multicentre, double-blind, randomized, parallel-group, dose-range trial to investigate the efficacy and safety of CG beta (FE 999302) as add-on treatment to follitropin delta in women undergoing ovarian stimulation following a long GnRH agonist protocol. The trial was conducted in five countries (Belgium, Czech Republic, Denmark, Spain and UK). The trial protocol (number 000289) was approved by the local regulatory authorities and the independent ethics committees covering all participating centres. The first subject was randomized 13 June 2018 in Spain and the last subject completed the trial 8 January 2020. The trial was performed in accordance with the principles of the Declaration of Helsinki, the

International Conference on Harmonization Guidelines for Good Clinical Practice and local regulatory requirements. All participants provided written, informed consent.

### Study participants

Women (age 30–42 years) who were undergoing their first or second IVF/ICSI cycle due to unexplained infertility, tubal infertility, endometriosis stage I/II or with partners diagnosed with male factor infertility, were eligible for the trial. Additional main inclusion criteria were BMI 17.5–32.0 kg/m<sup>2</sup>, regular menstrual cycles of 24–35 days and anti-Müllerian hormone (AMH) levels at screening of 5.0–35.0 pmol/l as measured by Elecsys<sup>®</sup> AMH Plus Immunoassay at the central laboratory. The main exclusion criteria were poor or excessive ovarian response in a previous COS cycle, endometriosis stage III–IV, history of recurrent miscarriage and use of hormonal preparations (except for thyroid medication) during the last menstrual cycle before randomization.

### Randomization and treatment

Women were screened within 90 days prior to start of desensitization with the GnRH agonist (triptorelin acetate, Gonapeptyl<sup>®</sup>) 0.1 mg/day subcutaneously in the mid-luteal phase of their menstrual cycle. Ovarian stimulation was started after 14 days if pituitary desensitization was confirmed (serum oestradiol <50 pg/ml or 180 pmol/l; local laboratory), and treatment with GnRH agonist was continued until the end of the stimulation period. Down-regulated subjects were randomized to receive a fixed daily dose of 1, 2, 4, 8 or 12  $\mu$ g of CG beta or placebo. Based on its specific bioactivity in rats and its comparative potency in men (Broksø Kyhl *et al.*, 2021), the lowest dose of 1  $\mu$ g FE 999302 was estimated to have a potency in women close to a therapeutic dose of 225 IU LH or 30 IU hCG bioactivity. All randomized subjects received an individualized fixed daily dose of follitropin delta, determined on the basis of their AMH level at screening and their body weight at stimulation Day 1, throughout the stimulation period. Women came daily to the clinic for their CG beta dose injection which had five different injection volumes (50, 100, 200, 400 and 600  $\mu$ l, respectively) and therefore five placebo groups with different injection volumes were used to ensure that the injection volume did not reveal whether a subject was randomized to CG beta or placebo. In total, 620 subjects were randomized to one of the five CG beta dose groups or to one of the five placebo groups using a 5:1 ratio for each injection volume. The randomization was performed centrally through the electronic case record form using a computer generated randomization list, and was stratified by centre and age (30–37 and 38–42 years). The randomization number was allocated to the subject together with the treatment allocation. When a subject was randomized to the trial, she was assigned to the lowest available randomization number.

### Trial procedures

During stimulation, subjects were monitored by transvaginal ultrasound on stimulation Days 1 and 6 and hereafter at least every second day. When the leading follicle reached a diameter of  $\geq$ 15 mm, transvaginal ultrasound was performed daily. Triggering of final follicular maturation was done as soon as  $\geq$ 3 follicles with a diameter of  $\geq$ 17 mm were observed. If there were <25 follicles with a diameter  $\geq$ 12 mm, a single dose of rhCG (Ovitrelle<sup>®</sup>, 250  $\mu$ g) was administered, but if there were

$\geq 25$  follicles with a diameter  $\geq 12$  mm, the cycle had to be cancelled. If it was judged by the investigator that  $\geq 3$  follicles with a diameter  $\geq 17$  mm could not be reached, but 1 or 2 follicles with a diameter  $\geq 17$  mm were observed, the cycle was either cancelled due to poor follicular development or triggering of final follicular maturation was induced, as judged by the investigator.

Oocyte retrieval took place 36 h ( $\pm 2$  h) after triggering and the maturity of each oocyte was assessed and each metaphase II oocyte was inseminated by ICSI. All inseminated oocytes were incubated in an EmbryoScope<sup>®</sup> (Vitrolife) for time-lapse monitoring and embryo development was recorded up to the day of transfer. Assessment of blastocyst quality on Day 5 after oocyte retrieval was done locally and centrally and consisted of assessment of three parameters: blastocyst expansion and hatching status (Grade 1–6), blastocyst inner cell mass grading (Grade A–D) and trophoctoderm grading (Grade A–D) (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Blastocysts were scored by using the system of Gardner and Schoolcraft (1999) with the addition of D-categories for inner cell mass and trophoctoderm. For all subjects, blastocyst transfer was to be performed on Day 5 after oocyte retrieval. Single blastocyst transfer was mandatory for subjects  $\leq 37$  years. In subjects  $\geq 38$  years, the transfer policy was dependent on the quality of the available blastocysts, i.e. single blastocyst transfer if they had at least one good-quality blastocyst, and transfer of maximum two blastocysts if they had no good-quality blastocysts. The remaining blastocysts could be cryopreserved and used by the subject after completion of the trial, in accordance with local guidelines and regulations. Vaginal progesterone tablets (Lutinus<sup>®</sup>) 100 mg three times daily were provided for luteal phase support from the day after oocyte retrieval and until the ongoing pregnancy visit. A serum  $\beta$ hCG test was performed 13–15 days after transfer, clinical pregnancy was confirmed by transvaginal ultrasound 5–6 weeks after transfer, and ongoing pregnancy was confirmed by transvaginal or abdominal ultrasound 10–11 weeks after transfer. The safety endpoints collected included (serious) adverse events (S)AEs, incidence of ovarian hyperstimulation syndrome (OHSS) immunogenicity and injection site reactions. The grading (1, 2, 3, 4 or 5) and categorization of mild, moderate and severe OHSS were based on Golan's classification system (Golan et al., 1989). Local tolerability was assessed three times daily (immediately, 30 min, and 24 h after each IMP injection) by the subject and recorded in a diary.

## Bioanalysis

Blood samples were collected at screening, before and after stimulation to evaluate by central laboratories serum hormones, clinical chemistry and haematology parameters, as well as anti-CG beta antibodies. The serum concentration of AMH was measured at screening using the Elecsys<sup>®</sup> AMH Plus immunoassay from Roche Diagnostics (Rotkreuz, Switzerland).

Serum CG beta was measured using a sandwich immunoassay, using a biotin-labelled monoclonal mouse anti-hCG Beta 2 as capture antibody and a ruthenium-labelled monoclonal mouse anti-hCG Holo C3 as detection antibody with electrochemiluminescence (ECL) detection (Meso Scale Discovery system). The analytical standard used was CG beta for quantification of CG beta, and the analytical range was 0.0250–3.20 ng/ml serum (64.0 ng/ml with extended dilution). Inter-assay precision was  $\leq 5\%$  (as calculated using ANOVA) and the inter-

assay bias was within  $\pm 6\%$ , in the main method validation. Serum FSH and LH were measured by ADVIA automated immunoassays (Siemens) and serum concentrations of oestradiol by the Elecsys<sup>®</sup> automated assay (Roche), whereas measurement of all other steroids was performed by means of validated LC-MS/MS methods. Inhibin A was assessed with the Beckmann Coulter Access assay and inhibin B with an EIA/chemiluminescence assay (AnshLite). The analyses for antibodies against rhCG were performed using a validated bioanalytical method and the method validations followed the principles of Shankar et al. (2008) and the regulatory guideline (EMA-CHMP, 2017). The method was a semi-homogenous bridging assay using ECL as detection method. The trial samples were analysed using a tiered approach. All samples (study samples and controls) were analysed as duplicates and the mean signal was used for determination of results.

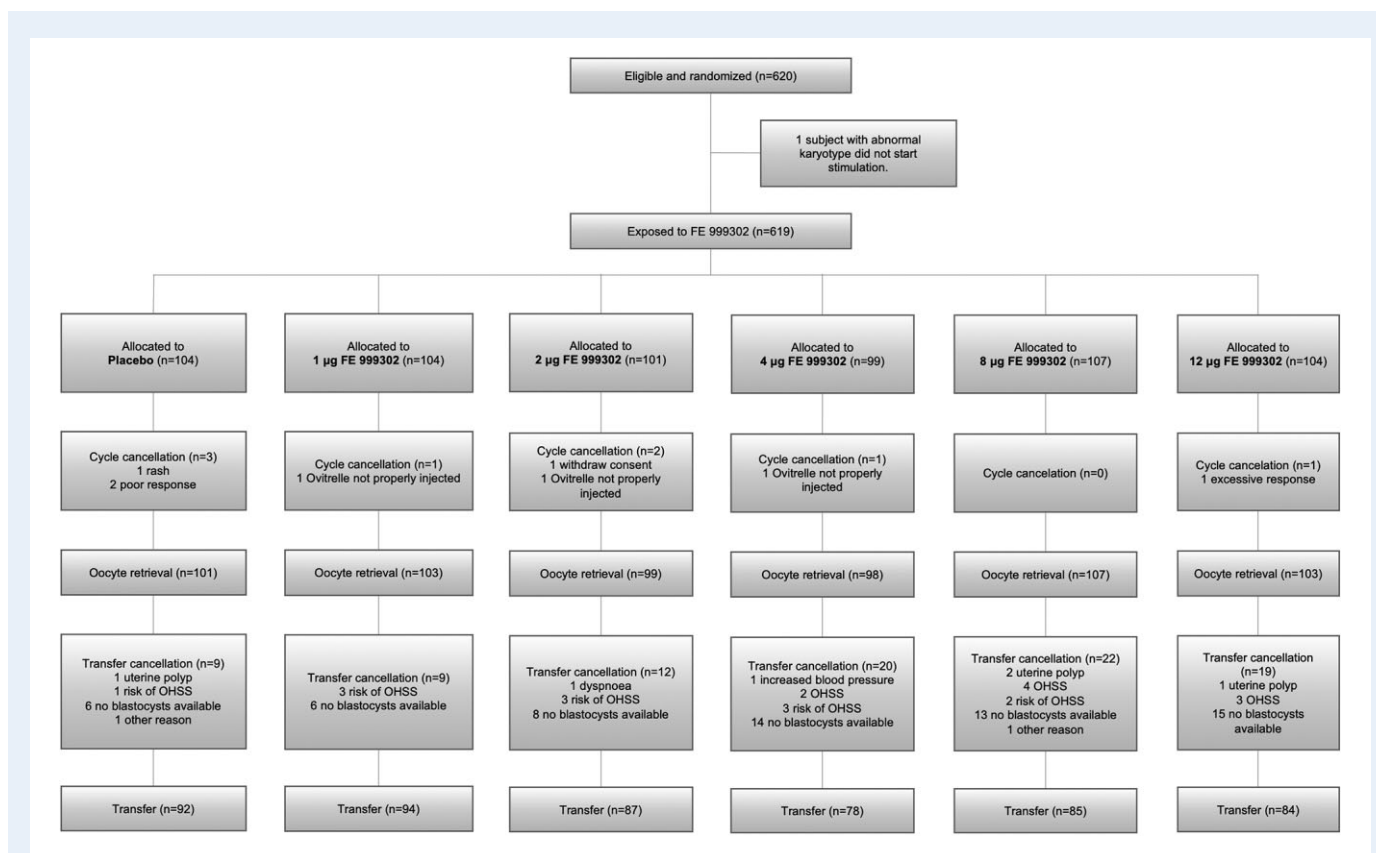
## Statistical analysis

All statistical analyses were based on the Full Analysis Set, including all randomized subjects that started treatment with CG beta or placebo. The number of good-quality blastocysts on Day 5 after oocyte retrieval was compared between treatments using a negative binomial regression model with treatment, age stratum (30–37 or 38–42 years) and AMH at screening ( $< 15$  pmol/l or  $\geq 15$  pmol/l) as factors. A logarithmic link function was used for the mean in the model. Pairwise comparisons between the mean ratio for each CG beta dose versus placebo were derived from the model together with the corresponding 95% CI and *P*-value, testing the hypothesis of no difference between the dose and placebo. Number of follicles  $\geq 12$  mm at the end-of-stimulation visit, follicles  $\geq 17$  mm at the end-of-stimulation visit, oocytes retrieved, metaphase II oocytes, fertilized oocytes, embryos Day 3, and blastocysts Day 5 were analysed using the same method. Serum hormones at the end-of-stimulation visit were compared between treatments using a multiplicative (i.e. log-transformation of dependent variable and covariate) analysis of covariance model with treatment, age stratum and AMH at screening as factors, and the concentration before start of stimulation as a covariate. Pregnancy endpoints were compared between treatments using a logistic regression model with treatment, age stratum and AMH at screening as factors. Dose–response relationships were investigated using the above models but with  $\log(\text{dose})$  as a covariate instead of treatment as a factor (and excluding the placebo treatment). Based on a previous trial (Nyboe Andersen and Nelson et al., 2017), the number of good-quality blastocysts on Day 5 in subjects  $\geq 30$  years treated with follitropin delta were well described by a negative binomial distribution with mean 1.77 and overdispersion 0.88. Assuming similar mean and overdispersion in this trial, a sample size of 100 subjects per group would give 80% power to detect a true increase of 58% in number of good-quality blastocysts. With a placebo mean of 1.77, this corresponds to a mean difference of 1.03 good-quality blastocysts.

## Results

### Disposition and baseline characteristics

A total of 620 eligible women were randomized, of whom 619 started stimulation: 515 women were exposed to CG beta and 104 subjects



**Figure 1.** Flow chart showing disposition of women participating in the trial and reasons for cycle cancellation.

were exposed to placebo. The disposition of women from start of stimulation until blastocyst transfer is presented by treatment group in Fig. 1. The incidence of cycle cancellation was low and similar across treatment groups (0–2.9%), whereas the incidence of transfer cancellation was 8.9% in the placebo group and 8.7%, 12.1%, 20.4%, 20.6% and 18.4% in the 1, 2, 4, 8 and 12 µg dose groups, respectively. The main reason for transfer cancellation was that there were no blastocysts available for transfer on Day 5 after oocyte retrieval.

The demographic characteristics and body weight measurements were comparable between the six treatment groups. Overall, the mean age was  $35.6 \pm 3.3$  years, the mean body weight was  $65.6 \pm 10.7$  kg and the mean BMI was  $23.9 \pm 3.5$  kg/m<sup>2</sup>. Most of the subjects (64.3%) had normal weight (BMI 18.5 to <25.0 kg/m<sup>2</sup>) but the trial population also included 1.9% underweight subjects (BMI <18.5 kg/m<sup>2</sup>), 25.7% overweight subjects (BMI 25.0 to <30.0 kg/m<sup>2</sup>) and 8.1% obese subjects (BMI  $\geq 30.0$  kg/m<sup>2</sup>). The proportion of subjects with primary infertility was 59.3% and the mean duration of infertility was  $32.6 \pm 23.5$  months. The most common primary reasons for infertility were unexplained infertility (54.8%) and infertility related to male factor (33.2%). The mean AMH at screening was 15.3 pmol/l, 53.2% of the subjects had AMH <15 pmol/l and 46.8% of the subjects had AMH  $\geq 15$  pmol/l.

### Desensitization and ovarian stimulation

All 619 subjects who started ovarian stimulation were down-regulated and the average duration of triptorelin treatment was  $25.3 \pm 2.6$  days.

The duration of stimulation was on average  $10.2 \pm 1.5$  days and similar between the groups and the total dose of follitropin delta was on average  $112 \pm 23.8$  µg (average daily dose:  $11.0 \pm 1.6$  µg). In total, 64% of women received the maximum daily dose of 12 µg follitropin delta.

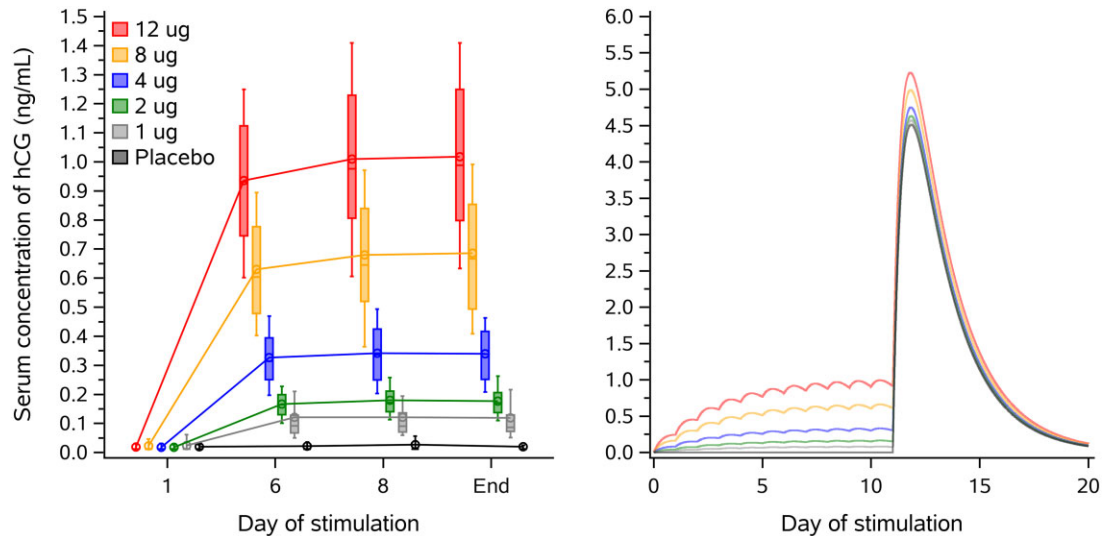
Serum FSH and LH concentrations were similar between the treatment groups at the start of stimulation and during stimulation. Mean serum FSH and LH were respectively  $\approx 4$  IU/l and  $\approx 1.5$  IU/l at the start of stimulation and within 6 days of stimulation, serum FSH increased to steady state levels of 14 to 15 IU/l, whereas serum LH slightly further decreased to  $\approx 0.9$  IU/l which low level was retained during the whole stimulation period.

Serum hCG concentrations increased dose proportionally with the CG beta dose and steady state concentrations were reached on stimulation Day 6 (see Fig. 2, left panel). Based on concentrations predicted from Phase I data, treatment with CG beta during the follicular phase had minor impact on hCG exposure during the luteal phase following triggering with Ovitrelle (see Fig. 2, right panel).

### Ovarian response and blastocyst quality

In Fig. 3, the number of follicles per size class at stimulation Days 6, 8 and end-of-stimulation is presented. At stimulation Day 6, little difference was noticed between the treatment groups, whereas at the end-of-stimulation, a CG beta dose related decrease of the intermediate follicles between 12 and 17 mm was observed in comparison to the placebo group (see also Table I). In contrast, the number of follicles





**Figure 2. Serum hCG concentrations.** Values are shown during stimulation (left) and predicted hCG concentrations (right) including hCG levels after triggering with 250  $\mu$ g Ovitrelle based on previous Phase I data (Broksø Kyhl et al., 2021). The box-and-whisker plot (left) indicates the mean (circle), 25%, 50% and 75% percentiles (box), and 10% and 90% percentiles (whiskers).

$\geq 17$  mm was similar between the CG beta dose groups and the placebo group. For follicles  $\geq 10$  mm,  $\geq 12$  mm and  $\geq 15$  mm in diameter at end-of-stimulation there was a statistically significant negative dose-response trend with increasing CG beta doses ( $P=0.0357$ ,  $0.0474$ ,  $0.0230$ , respectively).

During stimulation, serum oestradiol, progesterone, 17-OH progesterone, androstenedione and testosterone increased with increasing dose of CG beta. Dose-dependent increases were also observed at the day of oocyte retrieval, with the exception of serum progesterone which on this day decreased with increasing doses of CG beta (Fig. 4). When serum progesterone levels on the day of oocyte retrieval were corrected for the number and size of follicles at the end-of-stimulation, a CG beta-dose related decline of serum progesterone was still observed (data not shown). Increases of serum androstenedione, testosterone and oestradiol plateaued at a daily dose of 8  $\mu$ g CG beta. Serum inhibin B and inhibin A concentrations increased during stimulation in line with the number and sizes of growing follicles and at the end-of-stimulation, serum inhibin B and inhibin A levels decreased with increasing dose of CG beta (Fig. 4, see also Table I).

Mean endometrial thickness increased in the placebo group from 7.0 mm at stimulation Day 6 to 11.0 mm at the end-of-stimulation and the mean endometrium thickness in the CG beta dose groups at these days was between 7.2–7.5 mm and 10.2–11.0 mm, respectively. There were no differences in the endometrial triple-layer structure or endometrial echogenicity pattern in women treated with CG beta and women treated with placebo (data not shown).

Table I presents a summary of the statistical analyses of all end-points. The mean number of oocytes retrieved was lower in the CG beta dose groups (mean per group ranged between 9.7 and 11.2) compared to the placebo group (12.5) and accordingly the mean number of metaphase II oocytes were lower in the CG beta dose groups (mean per group ranged between 7.3 and 8.4) compared to the

placebo group (9.7). The mean number of embryos on Day 3 was lower in the CG beta dose groups (range between 5.1 and 6.1) compared to the placebo group (7.4).

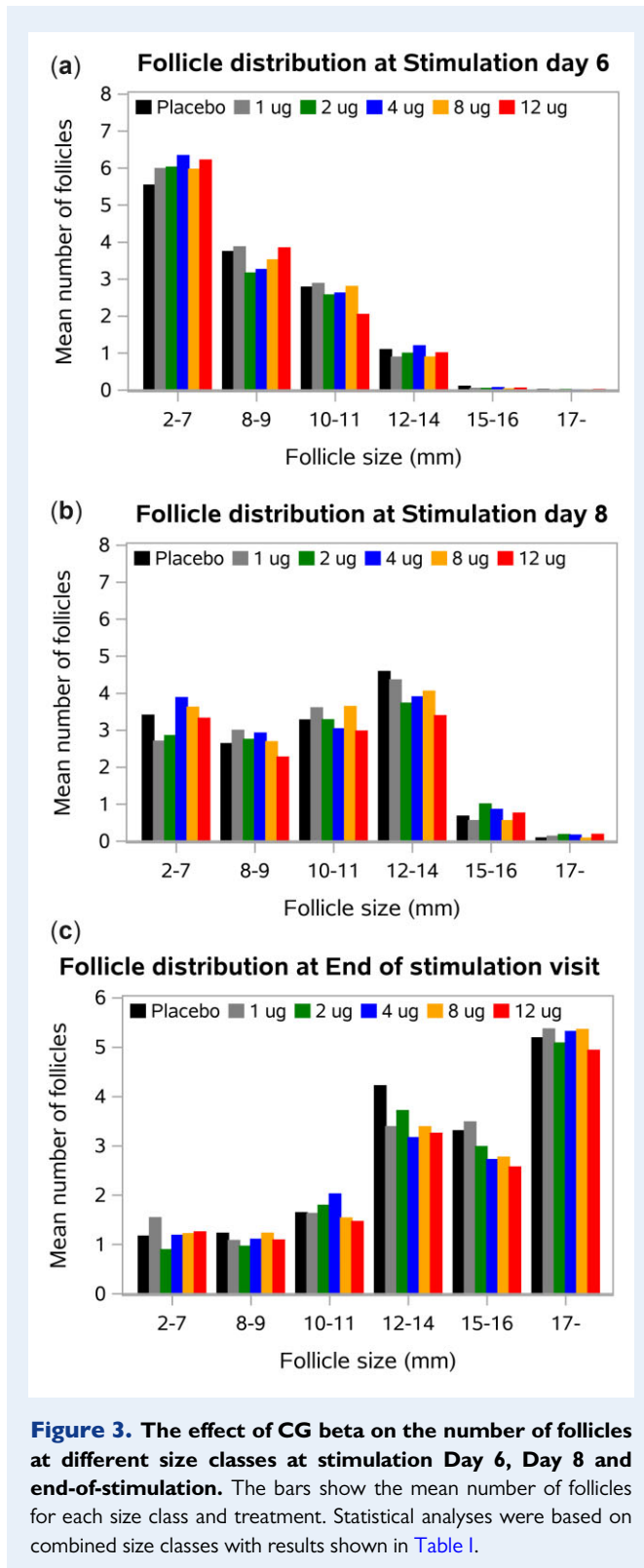
The blastocyst status on Day 5 after oocyte retrieval is illustrated in Fig. 5. The mean number of good-quality blastocysts was lower in the CG beta dose groups (ranged between 2.1 and 3.0) compared with the placebo group (3.3). The difference was statistically significant for the 1, 4, 8 and 12  $\mu$ g dose groups ( $P=0.0012$ ,  $0.0021$ ,  $0.0491$  and  $0.0005$ , respectively). No dose-response trend was observed ( $P=0.5455$ ). The results for local assessments confirmed the results of the central assessments.

## Clinical outcome

Pregnancy rates in the CG beta dose groups were negatively affected by the reduced number of blastocysts. The pregnancy outcomes were all numerically lower in the CG beta dose groups. The positive  $\beta$ hCG rate ranged from 34.4% to 47.9% in the CG beta dose groups versus 49.8% in the placebo group, the vital pregnancy rate ranged from 28.4% to 41.2% versus 42.9% in the placebo group, and the ongoing pregnancy rates from 28.4% to 39.2% compared with 42.9% in the placebo group. In women with embryo transfer, the ongoing pregnancy rate was 48.9% in the placebo group and 31.9%, 34.5%, 50.0%, 47.1% and 38.1% in the 1, 2, 4, 8 and 12  $\mu$ g dose groups, respectively.

## Safety outcome

The incidence of adverse events was 48.1% in the placebo group and ranged between 39.6% and 52.3% in the CG beta dose groups. There was no apparent effect of CG beta on the incidence of adverse events. The 20 most frequent adverse events based on total frequency are shown by preferred term in Fig. 6, together with a risk difference



comparison between the 12 µg dose group and placebo. There were no other substantial differences between the treatment groups and no other statistically significant differences between the 12 µg dose group and placebo.

A total of 11 SAEs were reported including three SAEs in the placebo group, one SAE in each of the 1, 2 and 4 µg dose groups, three SAEs in the 8 µg dose group and two SAEs in the 12 µg dose group. The most common SAE was OHSS reported twice in the placebo group and once in the 4, 8 and 12 µg groups, respectively. In total, 41 women experienced OHSS of which 25 subjects were suffering from early OHSS. There were 12 cases (11.5%) in the placebo group (3 mild, 6 moderate and 3 severe) and between 2 and 6 cases (2.0–10.3%) in each CG beta dose group (11 mild, 12 moderate and 6 severe). The incidence of OHSS was numerically lower in the CG beta dose groups compared with the placebo group and the risk of OHSS was statistically significantly lower in the 12 µg dose group compared with the placebo group. Most subjects had at least one injection site reaction of mild intensity (54.8% in the placebo group and 35.6–71.2% in the CG beta groups). Most injection site reactions were of mild intensity (54.8% in the placebo group and 44.2–71.2% in the CG beta groups) and the most common types of injection site reactions were pain, redness and bruising. Overall, the incidence of redness and pain were similar across all treatment groups, while bruising increased with increasing CG beta dose. Moderate injection site reactions were reported in 6%, and severe injection site reactions in 0.6% of subjects, without any dose related trends.

A total of 14 women had treatment-induced anti-CG beta antibodies; 1.0% in the placebo group, 1.0% in the 1 µg group, 2.0% in the 2 µg group, 4.0% in the 4 µg group, 3.7% in the 8 µg group and 1.9% in the 12 µg group. All women had post-dosing titres  $\leq 1$  and none of the antibodies were of neutralizing capacity.

## Discussion

This is the first trial in which patients undergoing ovarian stimulation with recombinant FSH (follitropin delta) also concomitantly received a recombinant hCG (CG beta) treatment in daily dosages of 1 to 12 µg. All women were treated with the individualized dosing algorithm of follitropin delta using a long GnRH agonist protocol to minimize the effect of endogenous LH. Following desensitization, endogenous LH levels were low ( $\approx 1.5$  IU/l) in all groups and during the first 5 days of stimulation; serum LH decreased slightly to mean levels below 1 IU/l, and remaining constant thereafter. Whereas post-down-regulation, endogenous LH concentrations were very low in all dose groups, serum CG beta concentrations increased proportionally with the CG beta dose and steady state levels were reached on stimulation Day 6, which is in agreement with the pharmacokinetics of CG beta established in female volunteers (Broksø Kyhl *et al.*, 2021). All women exposed to CG beta were treated with the individualized follitropin delta regimen; 64% of women received a fixed dose of daily 12 µg follitropin delta, whereas 36% of women received a lower daily dose. Since the therapeutic window of CG beta was unknown, the trial was designed to investigate the efficacy and safety of a broad range of CG beta doses to enable the selection of the optimal CG beta dose or a range of doses for further development. However, regardless the CG beta dose, a reduction in the number of intermediate follicles available at end-of-stimulation was observed impacting all down-stream parameters, including the number of good-quality blastocysts and pregnancy rates. These findings are in contrast to a small clinical trial in which urinary hCG in daily doses of 50, 100 and 150 IU was added to a daily

**Table 1** Results of statistical analyses of primary and secondary endpoints.

Endpoint	Placebo N = 104	1 µg N = 104	2 µg N = 101	4 µg N = 99	8 µg N = 107	12 µg N = 104
Follicles ≥ 12	12.7	11.8	11.6	11.0*	11.4*	10.6*
Ratio vs. placebo		0.93	0.91	0.86	0.90	0.83
95% CI		(0.84; 1.03)	(0.83; 1.01)	(0.78; 0.96)	(0.81; 0.99)	(0.75; 0.92)
P-value		0.1504	0.0812	0.0045	0.0324	0.0004
Follicles ≥ 17	5.2	5.3	5.1	5.3	5.3	4.9
Ratio vs. placebo		1.01	0.97	1.01	1.03	0.94
95% CI		(0.90; 1.14)	(0.86; 1.10)	(0.90; 1.14)	(0.91; 1.15)	(0.84; 1.06)
P-value		0.8779	0.6533	0.8604	0.6747	0.3436
FSH (IU/l)	14.9	14.5	15.1	14.7	14.8	15.0
Ratio vs. placebo		0.98	1.02	0.99	0.99	1.02
95% CI		(0.92; 1.03)	(0.96; 1.08)	(0.94; 1.05)	(0.94; 1.05)	(0.96; 1.07)
P-value		0.4039	0.5442	0.7983	0.8476	0.6936
LH (IU/l)	0.9	1.0	1.0	1.0	1.0	0.9
Ratio vs. placebo		1.06	1.07	1.10	1.07	0.95
95% CI		(0.93; 1.21)	(0.93; 1.22)	(0.97; 1.26)	(0.94; 1.23)	(0.83; 1.08)
P-value		0.3746	0.3276	0.1474	0.3032	0.4131
Oestradiol (pmol/l)	5959	7874*	8758*	9185*	11151*	9750*
Ratio vs. placebo		1.32	1.47	1.54	1.87	1.64
95% CI		(1.15; 1.52)	(1.27; 1.70)	(1.34; 1.78)	(1.62; 2.16)	(1.42; 1.89)
P-value		0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Progesterone (nmol/l)	1.3	1.3	1.4	1.6	2.2*	2.5*
Ratio vs. placebo		0.98	1.06	1.17	1.65	1.82
95% CI		(0.83; 1.16)	(0.90; 1.26)	(0.98; 1.39)	(1.39; 1.96)	(1.54; 2.16)
P-value		0.8069	0.4818	0.0754	<0.0001	<0.0001
17-OH-Progesterone (nmol/l)	3.6	4.6*	5.3*	6.3*	9.2*	9.8*
Ratio vs. placebo		1.27	1.48	1.75	2.55	2.70
95% CI		(1.09; 1.47)	(1.27; 1.72)	(1.50; 2.04)	(2.19; 2.96)	(2.32; 3.14)
P-value		0.0025	<0.0001	<0.0001	<0.0001	<0.0001
Androstenedione (nmol/l)	5.2	7.3*	8.1*	9.2*	11.5*	11.3*
Ratio vs. placebo		1.41	1.57	1.78	2.21	2.17
95% CI		(1.27; 1.56)	(1.41; 1.74)	(1.60; 1.98)	(2.00; 2.45)	(1.96; 2.41)
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Testosterone (nmol/l)	1.1	1.5*	1.6*	1.9*	2.5*	2.4*
Ratio vs. placebo		1.42	1.48	1.76	2.37	2.25
95% CI		(1.25; 1.60)	(1.31; 1.67)	(1.56; 1.99)	(2.10; 2.68)	(1.99; 2.54)
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Inhibin B (ng/l)	686	657	600*	598*	577*	471*
Ratio vs. placebo		0.96	0.88	0.87	0.84	0.69
95% CI		(0.84; 1.09)	(0.77; 1.00)	(0.77; 0.99)	(0.74; 0.96)	(0.60; 0.78)
P-value		0.5204	0.0468	0.0417	0.0084	<0.0001
Inhibin A (ng/l)	365	344	335	331	335	288*
Ratio vs. placebo		0.94	0.92	0.91	0.92	0.79
95% CI		(0.83; 1.07)	(0.81; 1.04)	(0.80; 1.03)	(0.81; 1.03)	(0.70; 0.89)
P-value		0.3474	0.1652	0.1168	0.1567	0.0001
Oocytes retrieved	12.5	10.6*	10.7*	10.6*	11.3	9.7*
Ratio vs. placebo		0.84	0.85	0.84	0.90	0.78
95% CI		(0.75; 0.96)	(0.75; 0.97)	(0.74; 0.96)	(0.80; 1.02)	(0.69; 0.88)
P-value		0.0075	0.0130	0.0081	0.0910	<0.0001

(continued)



Table I Continued

Endpoint	Placebo N = 104	1 µg N = 104	2 µg N = 101	4 µg N = 99	8 µg N = 107	12 µg N = 104
MII oocytes	9.7	8.2*	8.3*	8.0*	8.4*	7.3*
Ratio vs. placebo		0.85	0.86	0.83	0.87	0.75
95% CI		(0.74; 0.97)	(0.74; 0.98)	(0.72; 0.95)	(0.76; 1.00)	(0.65; 0.86)
P-value		0.0203	0.0269	0.0078	0.0440	<0.0001
Fertilized oocytes	7.4	6.0*	6.1*	5.5*	5.9*	5.1*
Ratio vs. placebo		0.81	0.82	0.75	0.80	0.69
95% CI		(0.69; 0.95)	(0.70; 0.97)	(0.63; 0.88)	(0.68; 0.94)	(0.58; 0.82)
P-value		0.0104	0.0209	0.0006	0.0069	<0.0001
Embryos day 3	7.4	5.9*	6.1*	5.5*	5.9*	5.1*
Ratio vs. placebo		0.80	0.82	0.75	0.80	0.69
95% CI		(0.68; 0.94)	(0.70; 0.97)	(0.63; 0.88)	(0.68; 0.94)	(0.58; 0.81)
P-value		0.0070	0.0183	0.0005	0.0069	<0.0001
Blastocysts day 5	5.3	4.0*	4.6	3.6*	4.2*	3.5*
Ratio vs. placebo		0.75	0.86	0.68	0.79	0.65
95% CI		(0.61; 0.92)	(0.70; 1.06)	(0.55; 0.85)	(0.64; 0.97)	(0.53; 0.81)
P-value		0.0068	0.1553	0.0005	0.0235	<0.0001
Good-quality blastocysts day 5	3.3	2.2*	3.0	2.2*	2.6*	2.1*
Ratio vs. placebo		0.65	0.89	0.66	0.78	0.63
95% CI		(0.50; 0.85)	(0.69; 1.15)	(0.51; 0.86)	(0.60; 1.00)	(0.48; 0.82)
P-value		0.0012	0.3822	0.0021	0.0491	0.0005
Positive hCG test (%)	49.8	34.4*	39.1	45.4	47.9	41.2
Odds ratio vs. placebo		0.53	0.65	0.84	0.93	0.71
95% CI		(0.30; 0.93)	(0.37; 1.13)	(0.48; 1.47)	(0.54; 1.60)	(0.41; 1.23)
P-value		0.0264	0.1293	0.5392	0.7899	0.2208
Vital pregnancy (%)	42.9	28.4*	30.1	41.3	40.3	35.3
Odds ratio vs. placebo		0.53	0.57	0.93	0.90	0.73
95% CI		(0.30; 0.94)	(0.32; 1.02)	(0.53; 1.64)	(0.52; 1.56)	(0.41; 1.28)
P-value		0.310	0.0589	0.8116	0.7012	0.2665
Ongoing pregnancy (%)	42.9	28.4*	29.1*	39.2	37.4	30.4
Odds ratio vs. placebo		0.53	0.54	0.86	0.80	0.58
95% CI		(0.29; 0.94)	(0.30; 0.98)	(0.49; 1.51)	(0.46; 1.39)	(0.33; 1.03)
P-value		0.0307	0.0410	0.5934	0.4221	0.0650

Mean values estimated from statistical analyses (see Statistical analysis section for details) adjusting for age stratum and AMH group (and pre-stimulation concentrations for serum hormones).

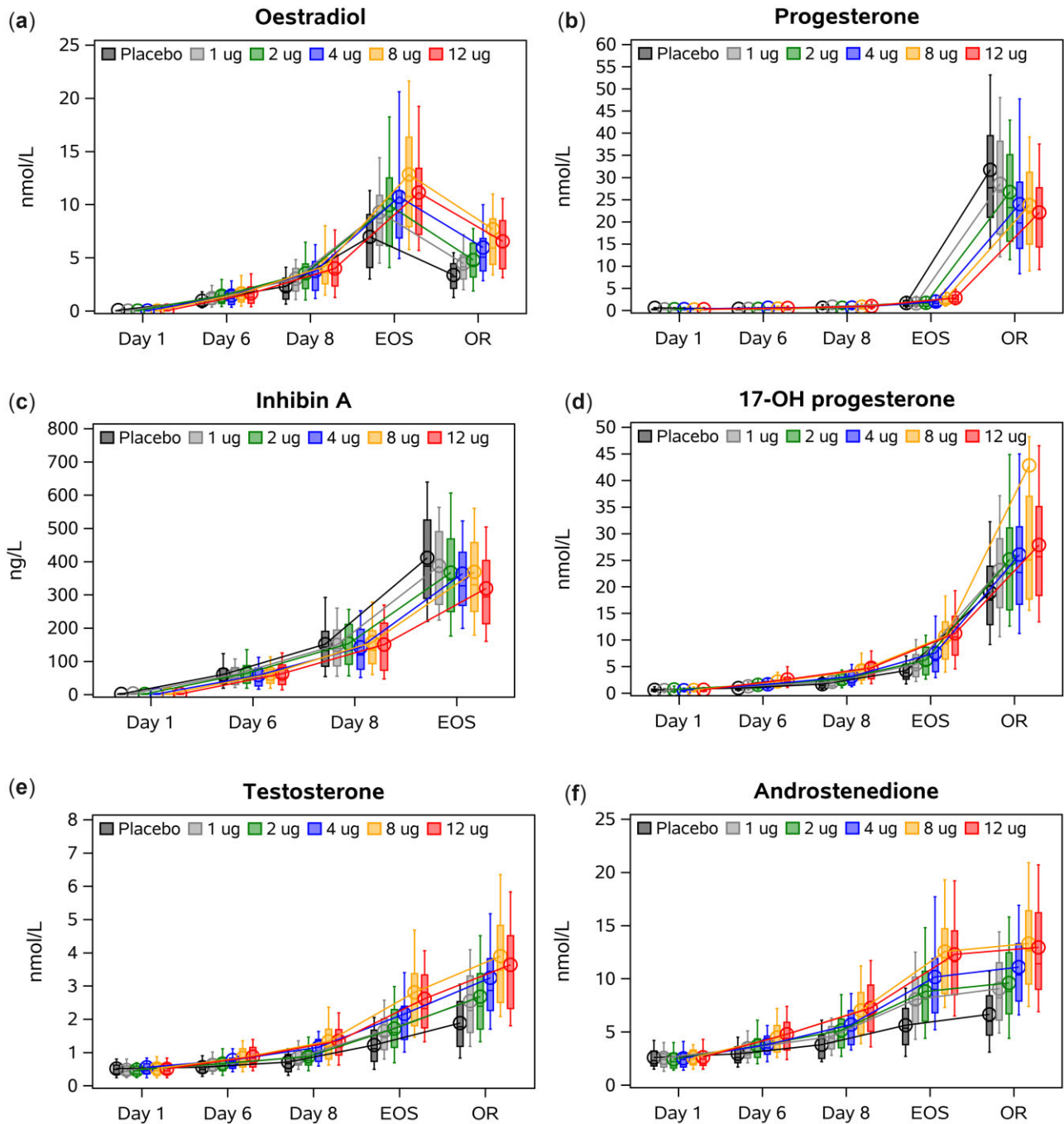
\*denotes statistically significant difference versus placebo ( $P < 0.05$ ). Results for follicles and serum hormones are the values at the end of stimulation visit.

dose of 150 IU rFSH, although in that study also the number of follicles 11–14 mm declined, although the difference was not statistically significant and did not result in lower numbers of oocytes (Thuesen *et al.*, 2012).

The earliest studies indicating that hCG may affect the number of follicles and/or oocytes in a dose-dependent manner were performed in rats and mice. In 1994, the effect of recombinant FSH and urinary hCG on follicular growth and atresia was described in immature hypophysectomized rats (Mannaerts *et al.*, 1994). The number of antral follicles was increased in an FSH dose-dependent manner between doses of 2.5 IU to 10 IU, and when this was supplemented with increasing amounts of hCG, the ovarian weight was augmented in an

hCG dose-dependent fashion. Relatively low doses of hCG caused a gradual shift of small follicles to large, preovulatory follicles. Furthermore, supplementation of 8 IU FSH with low dosages of hCG (0.2 or 0.5 IU) reduced the incidence of atresia in antral follicles of all size classes, whereas high dosages of hCG (2 or 5 IU) increased the number of atretic follicles. Similarly, an *in vivo* mouse model demonstrated that optimal follicular development required a combination of 20 IU FSH and 1 to 10 IU hCG (Yding Andersen *et al.*, 1999), whereas higher doses of 50 or 100 IU significantly reduced the number of pre-embryos.

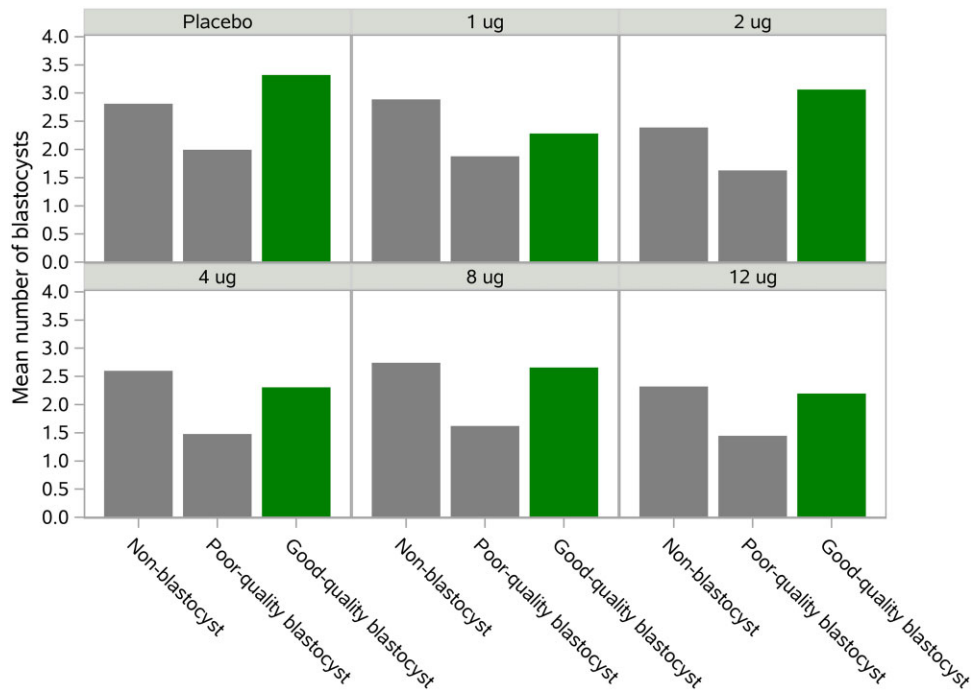
Regardless of the effect of CG beta on intermediate follicles, the incidence of cycle cancellations prior to triggering was low in this trial



**Figure 4.** The effect of CG beta on serum hormones concentrations of oestradiol, progesterone, inhibin A, 17-OH-progesterone, testosterone and androstenedione are shown during stimulation, at the end-of-stimulation (EOS) and at oocyte retrieval (OR). Descriptive data are shown with box-and-whisker plots for each treatment, indicating the mean (circle), 25%, 50% and 75% percentiles (box), and 10% and 90% percentiles (whiskers). Statistical analyses were based on data from the end of stimulation visit with results shown in Table 1.

and was similar across treatment groups. CG beta also did not affect the duration of stimulation which was on average 10 days in all treatment groups with no difference in the number of follicles  $\geq 17$  mm in diameter at end-of-stimulation. However, since the total number of follicles was lower at the end-of-stimulation, serum inhibin B and

inhibin A concentrations slightly decreased, with increasing doses of CG beta. In contrast, serum oestradiol, progesterone, 17-OH-progesterone, testosterone and androstenedione increased with the CG beta dose during stimulation, as also observed in a previous small dose-response study of urinary hCG added to rFSH for ovarian stimulation



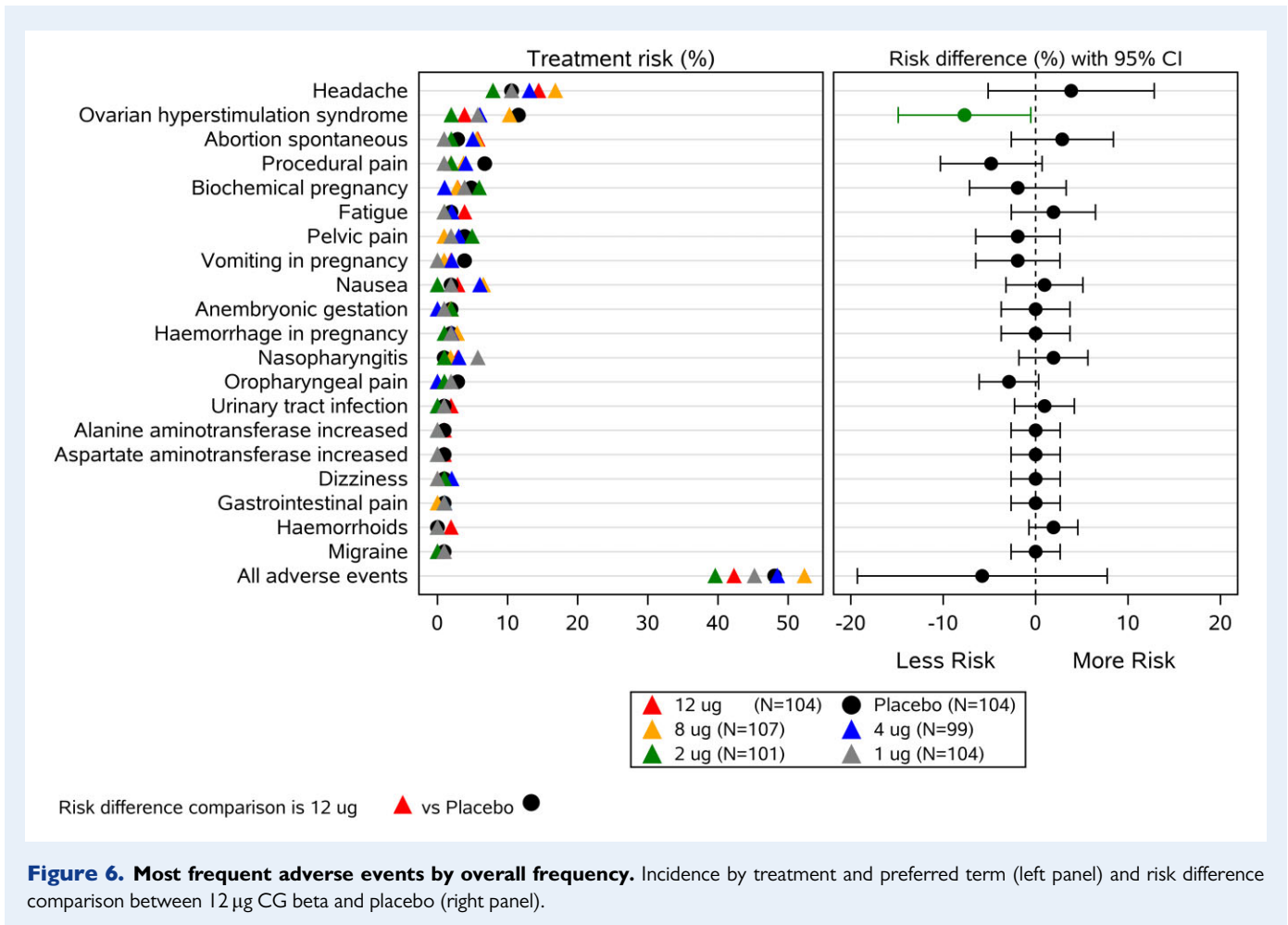
**Figure 5. Blastocyst status Day 5 by central assessment.** The bars show the mean number of blastocysts classified by quality for each treatment. Statistical analyses were based on total number of blastocysts and number of good-quality blastocysts, with results shown in Table I. Green bars: good-quality blastocysts; Grey bars: non-blastocysts and blastocysts that are not good-quality, respectively.

(Thuesen *et al.*, 2013). In contrast, following triggering of final follicular maturation with 250 µg rhCG, serum progesterone showed a dose-dependent decline at the day of oocyte retrieval. While this decline may be associated with the reduction of intermediate follicles, it was further observed that progesterone production per follicle declined in a CG-dose related manner. This observation may be related to too high and/or too long of exposure to CG beta that has been described in the literature to induce (temporarily) down-regulation of the LH receptor e.g. following the natural LH surge, following triggering of final follicular maturation, and following continuous exposure to pharmacological levels of hCG (Conti *et al.*, 1976; Cortvrint *et al.*, 1998; Menon *et al.*, 2004; Menon and Menon, 2012).

The ability of hCG or LH to inhibit multiple follicular development has been described before in women with World Health Organization type I and type II anovulation who received relatively high doses of recombinant LH during the late follicular phase (Loumaye *et al.*, 2003; Hugues *et al.*, 2005). Filicori *et al.* studied the impact of LH activity by applying hMG or urinary hCG in normal ovulatory women undergoing ovulation induction for IUI or ovarian stimulation for ART. The authors consistently reported a decrease of the number of small follicles (<10 mm) probably due to androgen-mediated follicle atresia (Filicori *et al.*, 2001, 2002, 2005). In previous studies the impact of LH/hCG treatment on follicular growth was studied following the induction of intermediate follicles with FSH/hMG, after which treatment was continued with high doses of LH/hCG in absence of FSH or a relatively low dose of FSH. Using this approach one study testing 100 to 400 IU hCG reported less numbers of follicles of 10–14 mm, although only

the highest dose of 400 IU hCG affected the oocyte and embryo yield (EP2292252B1). It has been well-described that the FSH Receptor (FSHR) and LH Receptor (LHR) expression in human granulosa cells is related to the diameter of the follicles (Jeppesen *et al.*, 2012). During ovarian stimulation, too high levels of androgens promote atresia (Hillier and Tetsuka, 1997) whereas FSH and oestrogen are essential for follicles to escape atresia and reach the preovulatory follicle stage (McGee and Hsueh, 2000). Such balance may be less delicate for large pre-ovulatory follicles producing sufficient amounts of oestradiol to prevent atresia, whereas in smaller and subsidiary, androgen dominant follicles, androgen action may contribute to follicle loss (Franks and Hardy, 2018).

The number of good-quality blastocysts on Day 5 after oocyte retrieval was the primary endpoint in this trial, as previous trials have suggested that urinary hCG supplementation during ovarian stimulation may improve embryo quality (Thuesen *et al.*, 2012). In the current trial, irrespective of the CG beta dose, a reduction in the number of good-quality blastocysts and the ongoing pregnancy rates per started cycle was observed due to CG beta mediated reduction in the number of intermediate follicles. Therefore, the overall lower pregnancy rate per started cycle in the CG beta groups was mainly due to the higher frequency of transfer cancellation because no good-quality blastocyst was available for transfer. The ongoing pregnancy rate per started cycle was highest and the blastocyst transfer cancellation rate was lowest in the placebo group. However, for women who had blastocyst transfer, the ongoing pregnancy rates in the placebo group and CG beta groups were more similar, indicating that CG beta treatment



did not affect implantation or the chance of pregnancy following blastocyst transfer. With respect to the impact of the CG beta dose, it is noted that a statistically significant dose–response was observed for number of follicles  $\geq 10$ ,  $\geq 12$  and  $\geq 15$  mm, but not for number of oocytes, good-quality blastocysts, or transfer cancellations, and not for any of the pregnancy endpoints. The lack of dose–response despite clear differences versus placebo indicates that all tested doses were on the top of the dose–response curve. Regarding the pregnancy endpoints, it is clear that the trial was not powered to detect differences in these endpoints, and that ongoing pregnancy results were very similar for the 1, 2 and 12 µg doses (28%, 29% and 30%) indicating no dose–response.

When this trial started, there was limited experience with individualized follitropin delta dosing in a long GnRH agonist protocol. Since a long GnRH agonist protocol does not allow triggering of final follicular maturation with a GnRH agonist, women with AMH  $>35$  pmol/l at screening were excluded from this trial to reduce the risk of cycle cancellation due to too high ovarian response. Based on historical data from studies comparing the GnRH antagonist protocol with a long GnRH agonist protocol, a higher ovarian response and number of oocytes retrieved was anticipated (European Orgalutran Study Group et al., 2000; Al-Inany et al., 2007). Overall, the clinical outcome in the placebo group was excellent, as the individualized follitropin delta

regimen in a long GnRH agonist protocol resulted on average in 12.5 oocytes, 3.3 good-quality blastocysts on Day 5 after oocyte retrieval and an ongoing pregnancy rate of 42.9% per started cycle, which were all higher than in a previous trial applying individualized follitropin delta dosing in a GnRH antagonist protocol (Nyboe Andersen and Nelson et al., 2017). Moreover, there were no cycle cancellations due to excessive ovarian response in the placebo group, although as a result of the higher ovarian response in this long GnRH agonist protocol, the incidence of OHSS tended to be higher in the placebo arm than in previous GnRH antagonist studies (Nyboe Andersen and Nelson et al., 2017; Fernández-Sánchez et al., 2019).

Concerning the safety profile of CG beta, all tested doses of CG beta were well-tolerated and the majority of injection site reactions were mild across dose groups. There was no apparent effect of CG beta on the incidence or severity of (serious) adverse events and adverse drug reactions. The most common adverse drug reactions were OHSS (2.0–9.3% across the CG beta dose groups) and headache (3.0–6.5% across the CG beta dose groups). Overall, the incidence of OHSS was lower in the CG beta dose groups compared with the placebo group, which is probably related to the lower number of oocytes retrieved in these dose groups. Treatment-induced anti-CG beta antibodies were detected with a low incidence of 1–4% across the dose groups and with an incidence of 1% in the placebo group. The titres of

the positive samples were either unquantifiable (<1) or very low ( $\leq 4$ ). None of the detected antibodies had neutralizing capacity, indicating that treatment with CG beta has a low risk of immunogenicity.

In conclusion, all tested CG beta doses were well-tolerated and no safety concerns were identified. The individualized dosing algorithm of follitropin alfa in a long GnRH agonist protocol, as reflected by the clinical outcome of placebo group, appeared to be most effective and safe resulting in on average 12.5 oocytes and an ongoing pregnancy rate of 43%. The clinical outcome of the CG beta treatment groups suggests that the tested doses of CG beta may have been too high. Both follitropin delta and CG beta are produced by the same human cell line and have shown to be more potent than FSH and hCG produced by CHO cell lines. Thus, re-establishing the optimal ratio of these two new recombinant gonadotrophins will be pivotal to develop a combination product containing both FSH and LH activity for ovarian stimulation.

## Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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## Authors' roles

M.F.S. (lead), H.V., C.B., A.P. and Y.K. were investigators of the trial. C.Y.A. and M.F. were respectively the chairman and clinical expert in the independent data monitoring committee. B.M. provided substantial contribution to the trial design and data interpretation and drafted the manuscript together with P.L. who performed the statistical analysis. All authors critically reviewed the manuscript for important intellectual content and approved the final version.

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## Conflict of interest

B.M. and P.L. are employees of Ferring Pharmaceuticals. M.F.S., H.V., C.Y.A., M.F., C.B., A.P. and Y.K. have received institutional clinical trial fees from Ferring Pharmaceuticals. C.B. has received payments for lectures from Organon, Ferring Pharmaceuticals, Merck A/S and Abbott. M.F.S. has received payment for lectures from Ferring Pharmaceuticals. Y.K. has received payment for lectures from Merck and travel support from Gedeon Richter. H.V. has received consulting fees from Oxo and Obseva and travel support from Gedeon Richter, Ferring Pharmaceuticals and Merck. C.Y.A. has received payment for lectures from IBSA, Switzerland. M.F. and C.Y.A. were reimbursed as members of the Data Monitoring Board in this trial. M.F. has an issued patent about unitary combination of FSH and hCG (EP1633389).

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