

Induction of Multiple Follicular Development by a Single Dose of Long-Acting Recombinant Follicle-Stimulating Hormone (FSH-CTP, Corifollitropin Alfa) for Controlled Ovarian Stimulation before *in Vitro* Fertilization

P. DEVROEY, B. C. FAUSER, P. PLATTEAU, N. G. BECKERS, M. DHONT, AND B. M. MANNAERTS

Center for Reproductive Medicine (P.D., P.P.), Dutch-Speaking Brussels Free University, 1090 Brussels, Belgium; Center of Reproductive Medicine (B.C.F., N.G.B.), Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands; University Hospital Gent (M.D.), B-9000 Gent, Belgium; and Clinical Development Department (B.M.M.), NV Organon, 5340 BH Oss, The Netherlands

In a first feasibility study, the efficacy and safety of a single dose of recombinant long-acting FSH (FSH-CTP) were investigated in *in vitro* fertilization (IVF) patients undergoing controlled ovarian stimulation with a flexible GnRH antagonist protocol. Eligible subjects were randomized to receive a single dose of 120 μg ($n = 25$), 180 μg ($n = 24$), or 240 μg ($n = 25$) corifollitropin alfa (FSH-CTP) or to start daily fixed doses of 150 IU recombinant FSH (rFSH) ($n = 24$, reference). Subjects who received a single dose of FSH-CTP continued 1 wk after injection (treatment d 8) with fixed daily doses of 150 IU rFSH (Puregon/Follistim) until the day of triggering final oocyte maturation.

The terminal half-life of FSH-CTP was, on average, 65 h and dose independent. Cycle cancellation before human chorionic gonadotropin (hCG) administration occurred in only three subjects treated with FSH-CTP. The median duration of stimulation was 10.0 d in each FSH-CTP group and 9.0 d in the daily rFSH group. The total number of follicles at least 11 mm at stimulation d 8 and at the day of hCG administration tended to increase with dose of FSH-CTP, although a significant dose-response relationship was revealed only for the number of

follicles at least 15 mm on the day of hCG ($P = 0.03$). Serum estradiol levels and inhibin-B levels were not significantly different between the four groups on d 8 and on the day of hCG. In total, 12 subjects (17.6%) in the FSH-CTP groups and two subjects (8.3%) in the rFSH group experienced a premature LH rise (defined as LH ≥ 10 IU/liter) before the start of the GnRH antagonist (P value not significant between groups). This relatively high incidence of women demonstrating an early LH rise in the FSH-CTP groups may be related to the higher initial rises of serum estradiol and the use of a flexible GnRH antagonist protocol. The mean number of oocytes recovered per started cycle was higher in FSH-CTP-treated subjects compared with rFSH-treated subjects (significant at $P = 0.03$ for the 240- μg FSH-CTP group), but no difference could be noted between the number of good quality embryos (range of means, 3.8–4.8 per attempt), and equal numbers of embryos were available for embryo transfer. In summary, FSH-CTP appeared to be a potent inducer of multiple follicular growth; additional research will be needed to select the optimal FSH-CTP dose and treatment time interval. (*J Clin Endocrinol Metab* 89: 2062–2070, 2004)

CLINICAL PROTOCOLS FOR induction of multifollicular development in women undergoing conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) commonly rely on daily FSH injections. The availability of a longer-acting FSH preparation with a comparable biopotency might allow for the development of new treatment regimens requiring fewer injections. Long-acting FSH may be created by additional glycosylation of the FSH molecule (1) or by coupling the carboxyl-terminal part (CTP) of the β -subunit of human chorionic gonadotropin (hCG) to the FSH β -subunit (2, 3). The very first report on the design of a long-acting FSH agonist was by Boime and coworkers (3),

Abbreviations: AUC, Area under the serum concentration-time curve; C_{max} , peak serum concentration; CTP, carboxyl-terminal part; E_2 , estradiol; EIA, enzyme immunoassay; ET, embryo transfer; FSH-CTP, long-acting recombinant FSH; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; OHSS, ovarian hyperstimulation syndrome; P, progesterone; rFSH, recombinant FSH.

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who used site-directed mutagenesis and gene transfer techniques to develop FSH-CTP, which is produced and secreted by a Chinese hamster ovary cell line and contains four N-linked carbohydrate chains ($\alpha 52$, $\alpha 78$, $\beta 7$, and $\beta 24$) and four O-linked carbohydrate chains at the CTP ($\beta 115$, $\beta 121$, $\beta 126$, and $\beta 132$), the latter causing a 3- to 4-fold increased *in vivo* half-life as compared with wild-type recombinant FSH (rFSH).

Preclinical research has indicated that FSH-CTP has an *in vitro* pharmacological activity comparable to rFSH and an anticipated half-life that is 2- to 3-fold longer compared with rFSH. Because of its long half-life, standardization of FSH-CTP by means of the classical Steelman-Pohley bioassay (4) does not provide a reliable estimate of its *in vivo* bioactivity from a pharmacological or clinical perspective. Therefore, dosages of pure recombinant FSH-CTP are expressed in mass (micrograms) instead of international units.

A general concern for all therapeutic biopharmaceuticals, especially for designed analogs with deviating amino acid sequence or carbohydrate site chains (5), is their potential immunogenicity induced by repeated injections or long-term treatment (6). Therefore, the first human study of FSH-CTP

was performed in hypogonadotropic hypogonadal male volunteers who received four single sc injections of FSH-CTP at 4-wk intervals (6). Repeated FSH-CTP administration appeared to be safe and well tolerated and did not give rise to antibody formation. In these hypogonadotropic men, serum FSH-CTP levels peaked on average 46 h after injection, whereas the average elimination half-life was 95 h. In a second FSH-CTP study in healthy pituitary-suppressed female volunteers, a single injection of 120 μ g FSH-CTP induced multiple follicle growth (7) comparable to that induced by daily 150 IU rFSH for 7 d using the same study model (8). Pharmacokinetic analysis revealed that peak serum FSH-CTP concentrations were reached at about approximately 36 h after injection and that the terminal half-life was between 60 and 75 h.

During controlled ovarian stimulation, daily administration of exogenous FSH overrides the normal selection process of a single dominant follicle by fulfilling the FSH threshold window requirements for multiple precursor follicles (9, 10). When a single dose of long-acting FSH-CTP is given at a suprathreshold dose, multiple follicular development will be induced that will persist as long as serum FSH-CTP levels remain above the threshold requirements of these follicles. In case FSH-CTP levels decline below the threshold, FSH-sensitive follicles cease to develop and become atretic. During stimulation, this follicular atresia could be prevented by switching in a timely manner to the treatment with daily rFSH. Very recently, the first successful treatment of a patient with a 7-yr history of primary infertility was reported after treatment with a single dose of 180 μ g FSH-CTP followed by three injections of 150 IU rFSH (11).

The current study was performed to explore a new treatment regimen for controlled ovarian stimulation using a single dose of long-acting FSH-CTP to support 7 d of multiple follicular growth followed by the daily administration of rFSH to complete the stimulation cycle up to the day of hCG administration. For that purpose, subjects scheduled for IVF or ICSI received either 120, 180, or 240 μ g FSH-CTP followed by daily injections of 150 IU rFSH. The primary endpoint of this trial was the total dose of rFSH, as it was assumed that increasing doses of FSH-CTP would require less rFSH to reach the same criteria of hCG. Apart from an evaluation of clinical outcome parameters, the number and size of follicles as well as hormonal responses were compared with those of subjects who received daily rFSH administration.

Subjects and Methods

Subjects

In total, 99 subjects were randomized: 25 subjects to the 120- μ g FSH-CTP group, 25 subjects to the 180- μ g FSH-CTP group, 25 subjects to the 240- μ g FSH-CTP group, and 24 subjects to the 150-IU rFSH group. All subjects underwent ovarian stimulation for conventional IVF or ICSI. Subjects were between 18 and 39 yr of age and had a regular menstrual cycle (24–35 d) and normal body weight (body mass index 18–29 kg/m²). This study was approved by the local ethical committees of all three participating centers and is in agreement with the Declaration of Helsinki for Medical Research Involving Human Subjects.

Study design

This study was an open-label randomized four-arm trial. Subjects in the FSH-CTP groups started ovarian stimulation on cycle d 2 or 3 with

a single sc dose of FSH-CTP (Org 36286, corifollitropin alfa, NV Organon, The Netherlands) of 120, 180, or 240 μ g followed 1 wk later (treatment d 8) with a fixed daily sc dose of 150 IU rFSH (rFSH/Puregon/Follistim, NV Organon) up to and including the day of hCG. In the control group, subjects started on cycle d 2 or 3 with a fixed daily sc dose of 150 IU rFSH up to and including the day of hCG. To prevent premature LH surges, a GnRH antagonist (ganirelix; 0.25 mg in 0.5 ml daily, Orgalutran/Antagon, NV Organon) was administered sc starting on the day that the leading follicle had reached 14 mm. When at least three follicles greater than or equal to 17 mm were observed by transvaginal sonography, hCG (10,000 IU Pregnyl, NV Organon) was administered sc for the induction of final oocyte maturation. Approximately 30–36 h later, oocyte retrieval followed by conventional IVF or ICSI was performed. At embryo transfer (ET), 2–5 d after oocyte pick-up, no more than three embryos were replaced. All subjects received luteal-phase support by means of vaginal micronized progesterone (600 mg/d) or im progesterone (50 mg/d) starting on the day of embryo transfer, at the latest.

Assessments

Before the start of ovarian stimulation, pregnancy was excluded by means of an hCG test. A blood sample was taken for hormone assessments, and ultrasound was performed. The subject returned to the clinic for transvaginal ultrasound and blood sampling on d 3, 5, and 7 and daily thereafter up to and including the day of hCG. Blood sampling was performed before ganirelix and FSH administration. Additional blood samples of all subjects were taken on the day of oocyte pick-up, the day of ET, and at 2 wk after ET.

Serum FSH-CTP levels were determined by enzyme immunoassay (EIA) with a coefficient of variation (CV) of less than 20% and a lowest detection limit of 0.079 ng/ml. Antibodies against FSH-CTP were assessed using an RIA based on the formation of an immune complex between a specific antibody and ¹²⁵I-labeled FSH-CTP. These samples were assessed with a coefficient of variation of less than 20% [the assays for FSH-CTP and for antibodies against FSH-CTP are also described by Bouloux *et al.* (6)]. Serum FSH, LH, estradiol (E₂), and progesterone (P) levels were determined by time-resolved fluoroimmunoassay (AutoDELFLIA, Wallac Oy, Finland) with a coefficient of variation of less than 20%. Lowest detection limits were 0.25 IU/liter, 0.6 IU/liter, 13.6 pg/ml, and 0.31 ng/ml for FSH, LH, E₂, and P, respectively. All measurements were performed at NV Organon using validated assays.

Serum inhibin-B levels were determined by EIA (Oxford Bio-Innovation Ltd., Oxford Shire, UK) with a coefficient of variation of less than 20% and a lowest detection limit of 15.6 pg/ml. The inhibin-B levels were assessed by a central laboratory (AAL, Neu-Ulm, Germany) using a validated assay.

Statistical methods

The aim of this study was to measure the efficacy and efficiency of the various FSH-CTP doses; thus, the precision of the estimates, rather than the power of the study, was of interest. The primary endpoint of the study was the rFSH dose needed from treatment d 8 onwards, as it was assumed that with higher doses of FSH-CTP less rFSH would be needed to reach the same criteria for hCG administration. With 25 subjects treated in each group, the total dose of rFSH could be estimated with a precision (SE) of approximately 70 IU (based on a SD of 350 IU). This means that a two-sided 95% confidence interval of the total dose of rFSH has a width of 280 IU (*i.e.* mean total dose \pm 140 IU). With a daily fixed rFSH dose of 150 IU, this coincides with a width of approximately 2 d for the confidence interval of the mean treatment duration (*i.e.* mean treatment duration \pm 1 d).

Serum FSH-CTP levels were analyzed using the nonlinear mixed-effects model program NONMEM. A one-compartment model with first-order absorption with the subject's weight as covariate on total serum clearance and volume of distribution gave an accurate description of the serum FSH-CTP concentrations in time. A fixed value for absorption rate k_a of 0.102 h⁻¹ was assumed (as estimated in a previous trial with FSH-CTP), because no information was available in this trial on the absorption phase of the pharmacokinetic profile. For this model, both population (mean) and individual parameter estimates were obtained.

Individual estimates of exposure to FSH-CTP (AUC and C_{max}) were calculated from individual serum FSH-CTP levels.

In addition to summary statistics, for continuous parameters such as the duration of stimulation, a treatment-group comparison was performed using ANOVA. If the *P* value of the ANOVA model was ≤ 0.05 , Dunnett's *t* test was performed to compare the three FSH-CTP groups with the Puregon group; if the *P* value of the ANOVA model was > 0.05 , no further comparison between treatment groups was performed. The dose-response relationship of FSH-CTP with respect to the number of follicles was assessed by using a linear regression model with dose as the covariate. The frequency of incidence of premature LH rises was analyzed using the exact χ^2 method.

Results

Subject characteristics and disposition

A total of 98 subjects were randomized and treated: 25 with 120 μg FSH-CTP, 24 with 180 μg FSH-CTP, 25 with 240 μg FSH-CTP, and 24 with daily 150 IU rFSH. Demographic and fertility characteristics at screening and the number of subjects who started treatment and had oocyte pick-up and ET are included in Table 1. There were no differences between the four treatment groups with respect to age ($P = 0.06$), body mass index ($P = 0.79$), incidence of primary infertility ($P = 0.67$), duration of infertility ($P = 0.22$), or cause of infertility ($P = 0.38$).

Four subjects started stimulation but did not receive hCG: two subjects because of an excessive response (180 μg FSH-CTP and 240 μg FSH-CTP, respectively) and two subjects because of a too-low response (240 μg FSH-CTP and 150 IU rFSH, respectively). Three subjects who received hCG did not continue with oocyte pick-up because of absence of sperm (120- μg group) or because of too few preovulatory follicles (120 and 180 μg , respectively). In total, six subjects in the FSH-CTP dose groups who had oocyte retrieval did not proceed with ET because of fertilization failure or the recovery of no or too few embryos.

Stimulation characteristics

There were no statistical differences between the four treatment groups with respect to the duration of stimulation or the total amount of rFSH administered from d 8 onwards. The median duration of stimulation was 10.0 d in each FSH-CTP dose group and 9.0 d in the rFSH reference group. Thus,

after a single injection of 120, 180, or 240 μg FSH-CTP, an additional 3 d of treatment with rFSH (on average, 450 IU in each dose group) were needed to reach the criteria for hCG administration. The total amount of FSH needed in the reference group was 1350 (1200–1950) IU. The median starting day of ganirelix was on stimulation d 7.0 in all four treatment arms.

Pharmacokinetic evaluation

Serum samples were analyzed both in the FSH-CTP immunoassay and in the FSH Delfia. Figure 1 displays a correlation between the FSH-CTP concentrations (after logarithmic transformation) measured by means of both assays. A linear model was applied to quantify this relationship and yielded a coefficient of correlation of 0.98. Mean serum FSH-CTP concentrations and derived pharmacokinetic parameters are given in Table 2. For all three FSH-CTP doses tested, the mean elimination half-life ($t_{1/2}$) was approximately 65 h. Furthermore, the dose-normalized AUC and C_{max} were similar across doses, implying that the serum concentrations of FSH-CTP were proportional to the dose within the dose range tested. Total serum clearance was 0.237 liters/h and the volume of distribution was 22.1 liters at the mean weight in the population (62.2 kg).

Follicular dynamics

There were no large differences between the four treatment groups with respect to the total number of follicles of at least 11 mm that developed from d 1 up to the day of hCG (Fig. 2 and Table 3). The initial follicular response (Fig. 2) was highest in the 120- μg group, although on d 5, the numbers of follicles at least 11 mm, at least 15 mm, or at least 17 mm were not significantly different among the four treatment groups. On d 8, subjects treated with 120 μg who received hCG had fewer follicles of at least 15 mm ($P = 0.05$) and fewer follicles of at least 17 mm ($P = 0.02$) compared with those treated with rFSH. In the 180- μg group, only the number of follicles at least 17 mm were significantly lower ($P = 0.04$), whereas on the day of hCG, no significant differences were noted between the numbers of follicles of different size classes.

By means of a linear regression model, a significant dose-response relationship was revealed only for the number of

TABLE 1. Subject characteristics and disposition per treatment group

	FSH-CTP			rFSH, 150 IU
	120 μg	180 μg	240 μg	
Age (yr)	30.4 \pm 3.8	31.5 \pm 3.8	33.4 \pm 4.1	32.1 \pm 4.3
Body mass index (kg/m ²)	23.2 \pm 2.8	22.9 \pm 3.5	22.6 \pm 2.7	23.4 \pm 2.8
Primary infertility (%)	56	46	56	42
Main causes of infertility (%)				
Tubal	28	4	32	33
Male	40	42	44	38
Tubal and male	0	17	0	4
Unknown	20	25	20	17
Endometriosis	8	4	0	0
Duration of infertility (yr)	4.2 \pm 3.1	4.9 \pm 3.6	5.6 \pm 4.3	4.0 \pm 3.2
Subjects (n)				
Start stimulation	25	24	25	24
hCG	25	23	23	23
Oocyte pick-up	23	22	23	23
ET	20	21	21	23

Linear Model ($y=ax+b$) : $\ln(\text{FSH})= 0.85 \cdot \ln(\text{FSHCTP}) + 2.198$

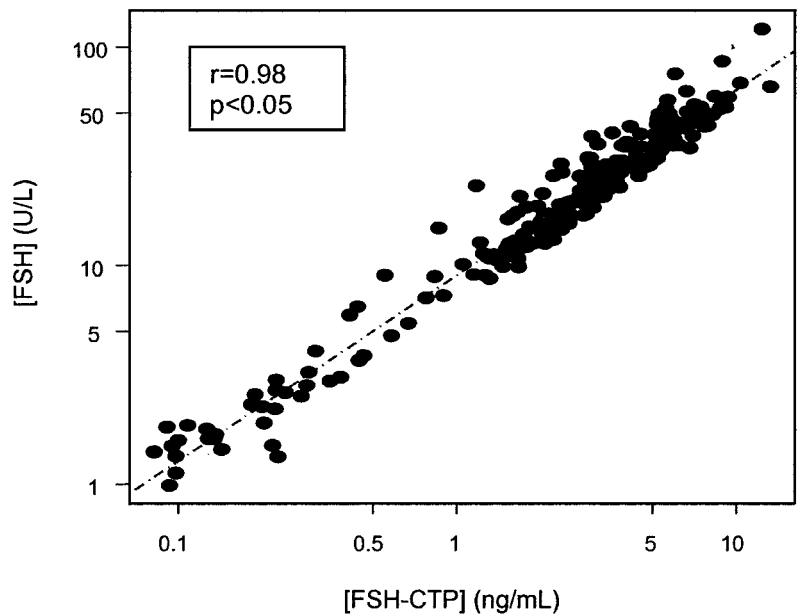


FIG. 1. Correlation plot of serum samples measured by the FSH-CTP immunoassay and the FSH Delfia.

TABLE 2. Mean (\pm SD) serum FSH-CTP levels and derived pharmacokinetic parameters

	Units	Dose of FSH-CTP		
		120 μg	180 μg	240 μg
d 3	ng/ml	3.5 ± 1.2	5.5 ± 1.8	7.3 ± 2.2
d 5	ng/ml	2.3 ± 0.7	3.6 ± 1.1	4.8 ± 1.3
d 7	ng/ml	1.3 ± 0.4	2.2 ± 0.7	2.8 ± 0.7
d ET	ng/ml	0.13 ± 0.05	0.18 ± 0.12	0.29 ± 0.12
$T_{1/2}$	H	64.1	65.6	64.9
T_{max}	H	24.6	24.8	24.7
C_{max}	ng/ml	4.3	6.6	8.9
Dn C_{max}	$\mu\text{g/ml}/\mu\text{g}$	0.0355	0.0367	0.0369
$\text{AUC}_{0-\infty}$	ng·h/ml	511.7	815.2	1080.0
Dn $\text{AUC}_{0-\infty}$	ng·h/ml/ μg	4.3	4.5	4.5

Dn, Dose normalized.

follicles at least 15 mm comparing the three FSH-CTP dose groups ($P = 0.03$, data not shown).

Hormones during the follicular and luteal phase

Serum concentrations of FSH, LH, E_2 , inhibin-B, and P measured at regular intervals during the follicular and luteal phase up to 2 wk after ET are presented in Fig. 3.

During the first days after FSH-CTP administration, serum FSH immunoreactivity measured by Delfia increased with the dose given (Fig. 3A). Thereafter, this immunoreactivity declined to median values of 8.1, 11.1, and 16.1 IU/liter on d 8 and 9.0, 11.2, and 13.9 IU/liter on the day of hCG in the 120-, 180-, and 240- μg groups, respectively. Because of daily administration of rFSH, serum FSH increased from 6.6 IU/liter (predose d 1) to 8.2 IU/liter on d 8 and to 8.8 IU/liter on the day of hCG. On the day of ET and 2 wk after ET, serum FSH levels were similar in all treatment FSH-CTP groups.

During the first days of stimulation, serum LH levels declined in all four treatment groups (Fig. 3B). Median serum LH values increased from d 5 onwards in all treatment

groups, except for subjects treated with 240 μg who showed increasing LH levels from d 3 onwards. Because of initiation of ganirelix treatment (on average on d 7 in each treatment group), serum LH declined again in all treatment groups, resulting, on the day of hCG, in serum LH levels of 0.9, 0.9, 1.0, and 1.8 IU/liter in the 120-, 180-, and 240- μg FSH-CTP and rFSH group, respectively. There were no differences in the serum LH levels during the luteal phase.

The serum E_2 profile during the follicular and luteal phase was similar for all treatment groups (Fig. 3C). Initial rises of E_2 , reflected by serum values on d 5, were higher in the 120- μg group ($P = 0.06$) and 240- μg group ($P = 0.03$) compared with those in the rFSH group. Initial rises of serum E_2 levels were lower in the 180- μg group, but at d 8 and at the day of hCG administration, no significant differences were found between the four treatment arms.

Inhibin-B levels largely varied between subjects within each treatment group, and no statistical difference was found between the four groups on d 5, d 8, or the day of hCG as shown in Fig. 3D. Median values were clearly highest in subjects treated with 120 μg , who showed a small temporary decrease of inhibin-B between d 7 and 8.

At all time points, except for the end of the luteal phase, serum P levels were similar between the treatment groups (Fig. 3E). Serum P levels of subjects treated with 120 μg FSH-CTP and rFSH had returned to normal early follicular phase values, whereas subjects treated with 180 and 240 μg FSH-CTP showed P values that were still raised in an apparent dose-dependent manner. However, these differences did not reach statistical significance in the 180- μg group ($P = 0.08$) and the 240- μg group ($P = 0.07$) in comparison with rFSH.

LH rises

In total, 12 subjects experienced an early LH rise after administration of FSH-CTP on d 1 but before the start of

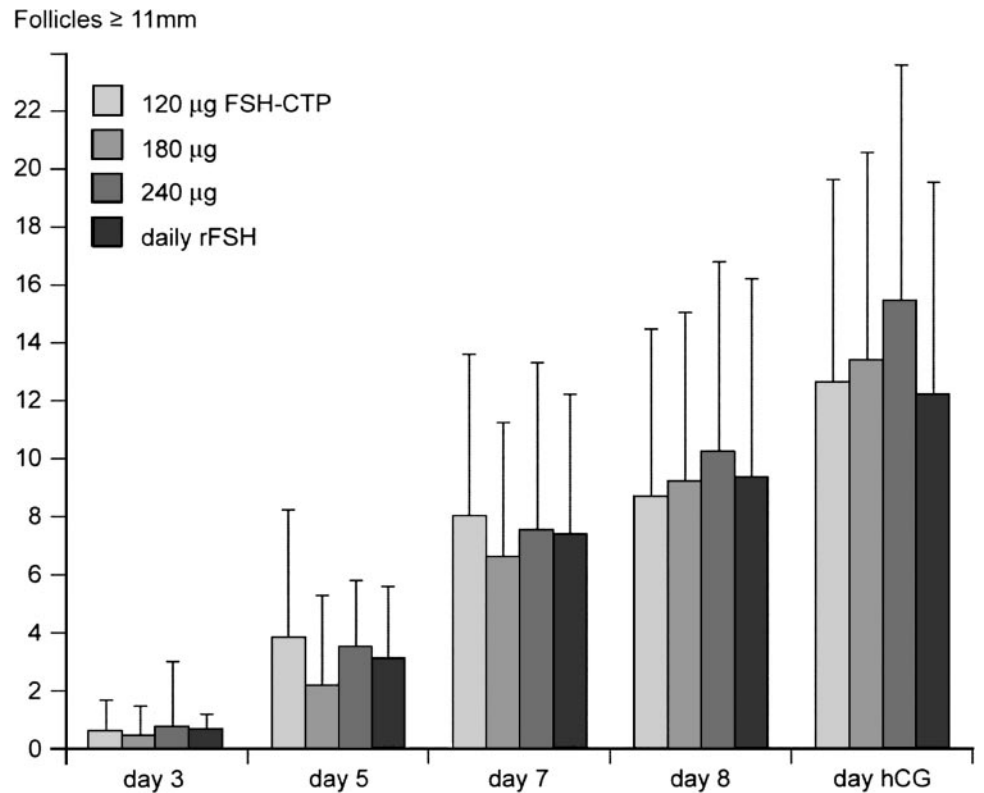


FIG. 2. The total number of follicles at least 11 mm measured on the various days of stimulation restricted to subjects with hCG administration.

TABLE 3. Mean (\pm SD) number of follicles of different size classes on d 8 and on the day of hCG, restricted to subjects with hCG administration

		Treatment groups				ANOVA <i>P</i> value
		120 μ g	180 μ g	240 μ g	rFSH	
d 8	≥ 11 mm	8.8 \pm 5.6	9.3 \pm 5.7	10.3 \pm 6.6	9.4 \pm 6.9	0.87
	≥ 15 mm	2.5 \pm 2.0 (<i>P</i> = 0.05)	2.5 \pm 2.0 (<i>P</i> = 0.06)	3.8 \pm 3.1 (<i>P</i> = 0.93)	4.2 \pm 2.4	0.03
	≥ 17 mm	0.8 \pm 1.1 (<i>P</i> = 0.02)	0.9 \pm 1.1 (<i>P</i> = 0.04)	1.4 \pm 1.5 (<i>P</i> = 0.51)	1.9 \pm 1.7	0.03
Day of hCG	≥ 11 mm	12.7 \pm 6.8	13.5 \pm 7.1	15.5 \pm 8.3	12.3 \pm 7.3	0.46
	≥ 15 mm	5.9 \pm 2.5	6.6 \pm 3.1	7.8 \pm 3.1	6.3 \pm 2.4	0.10
	≥ 17 mm	3.3 \pm 0.9	3.5 \pm 1.2	4.0 \pm 2.0	3.7 \pm 1.3	0.29

Treatment group comparison was performed using ANOVA, and in case *P* < 0.05, Dunnett's *t* test was performed to compare the three FSH-CTP groups.

ganirelix treatment: five subjects in the 120- μ g group, four subjects in the 180- μ g group, and three subjects in the 240- μ g group. One subject (240- μ g group) had an LH rise during ganirelix treatment. Six of these 13 premature LH rises were accompanied by concomitant P rises (>3.2 nmol/liter). Twelve subjects had oocyte pick-up (mean number of oocytes recovered was 11.1 per attempt), and 10 subjects had ET (mean number of good quality embryos was 3.3 per attempt), of which two subjects became pregnant. Two subjects treated with rFSH experienced a premature LH rise before the start of ganirelix, one subject was discontinued because of a too-low response, and one subject became pregnant. The incidence of premature LH rises was not significantly different between the four groups (*P* = 0.80 by using the exact χ^2 test).

Clinical outcome

The clinical outcome is presented in Table 4. The mean number of recovered oocytes was comparable (mean range, 11.0–12.0) for subjects treated with FSH-CTP and tended to

be lower for those women treated with daily rFSH (mean, 7.9). The incidence of metaphase II oocytes recovered in ICSI patients was not different among the groups (*P* = 0.84), *i.e.* 87, 74, and 74% in the 120-, 180-, and 240- μ g dose groups, respectively, and 84% in the daily rFSH group. Whereas the mean number of oocytes recovered per started cycle tended to be higher in three FSH-CTP treatment groups compared with the daily rFSH group, the number of good quality embryos was not statistically different between the treatment groups (range of means, 3.8–4.8 per attempt). Moreover, equal numbers of embryos were available for ET, which was performed on mean (sd) d 3.70 (0.92), 3.57 (0.87), and 3.28 (0.71) in the 120-, 180-, and 240- μ g dose groups, respectively, and on d 3.56 (0.84) in the daily rFSH group. In total, 15 ongoing pregnancies were obtained in the FSH-CTP groups and 10 pregnancies in the rFSH reference group (*P* value not significant). Ongoing pregnancies included three twins in the FSH-CTP groups and one twin in the reference group. In nonpregnant subjects, menses occurred after hCG adminis-

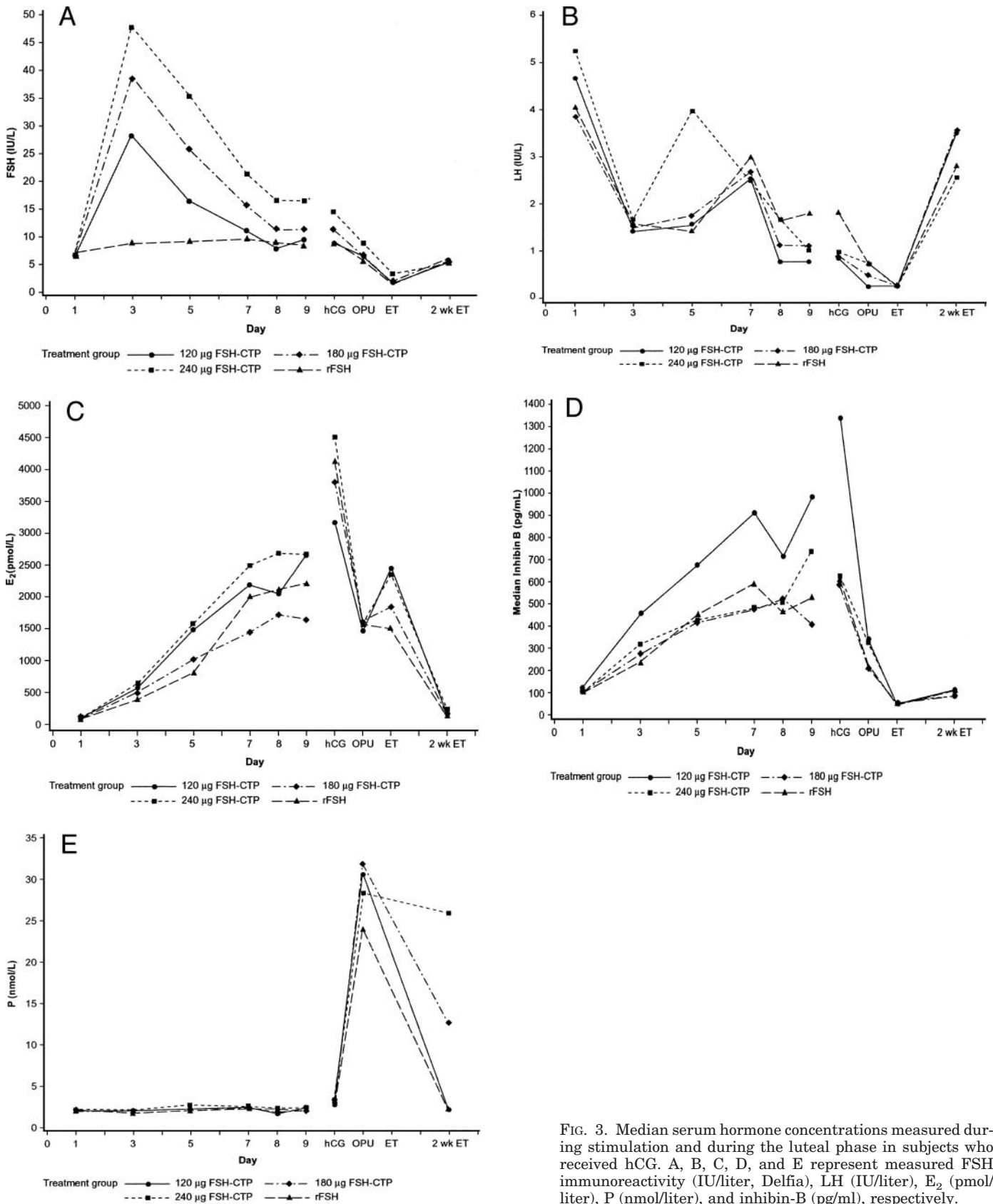


FIG. 3. Median serum hormone concentrations measured during stimulation and during the luteal phase in subjects who received hCG. A, B, C, D, and E represent measured FSH immunoreactivity (IU/liter, Delfia), LH (IU/liter), E₂ (pmol/liter), P (nmol/liter), and inhibin-B (pg/ml), respectively.

TABLE 4. Clinical outcome (mean \pm SD) of a randomized comparison between three single doses of FSH-CTP and daily rFSH for IVF or ICSI

	FSH-CTP, 120 μ g (n = 25)	FSH-CTP, 180 μ g (n = 24)	FSH-CTP, 240 μ g (n = 25)	rFSH, 150 IU/d (n = 24)
No. of oocytes per started cycle	11.0 \pm 7.1	11.1 \pm 7.5	12.0 \pm 7.3	7.9 \pm 4.1
Metaphase II oocytes in ICSI	10.9 \pm 6.9 (n = 11)	8.5 \pm 6.3 (n = 14)	9.1 \pm 5.5 (n = 15)	8.6 \pm 3.0 (n = 11)
Fertilization rate	73 \pm 27%	68 \pm 31%	67 \pm 31%	74 \pm 15%
No. of embryos obtained				
Total	8.5 \pm 5.5	6.6 \pm 4.9	7.3 \pm 5.9	5.3 \pm 3.2
Good quality	4.8 \pm 5.0	3.8 \pm 3.3	3.9 \pm 4.1	3.8 \pm 3.4
Transferred	2.0 \pm 0.2	2.0 \pm 0.5	1.9 \pm 0.5	2.0 \pm 0.3
Ongoing pregnancies per started cycle	4/25	5/24	6/25	10/24

tration on d 16.9 \pm 1.2 (mean \pm SD) in the 120- μ g group, on d 17.8 \pm 3.5 in the 180- μ g group, on day 17.9 \pm 3.8 in the 240- μ g group, and on d 16.8 \pm 2.0 in the rFSH group.

Safety data

Ovarian hyperstimulation syndrome (OHSS) occurred in two subjects after treatment with 120 μ g FSH-CTP (grade II and III) and in two subjects after treatment with 240 μ g FSH-CTP (grade I and II). Two of these four subjects were pregnant. After treatment with daily rFSH, two subjects developed OHSS grade I and II, respectively, and both appeared to be pregnant.

In total, six serious adverse events were reported for five subjects. These reports included bleeding after oocyte retrieval (120 μ g), ectopic pregnancy (120 and 180 μ g), and three of the six cases of OHSS. In total, eight subjects (32.0%) in the 120- μ g group, five subjects (20.8%) in the 180- μ g group, eight subjects (32.0%) in the 240- μ g group, and four subjects (16.7%) in the rFSH group had at least one adverse event, of which headache was the most frequently reported. No antibodies against FSH-CTP were detected. In general, FSH-CTP was well tolerated in terms of the assessed safety parameters, and no relevant differences between treatment groups were observed.

Discussion

This study is the first to demonstrate, in IVF patients, that a single dose of the long-acting FSH-CTP, a chimeric recombinant glycoprotein hormone, is able to induce multifollicular growth and to maintain the growth of these follicles during an entire week. Although peak serum FSH-CTP levels were reached 2 d after injection and declined thereafter up to d 8 when daily rFSH was started, multiple follicles continued to grow up to a size comparable to those induced by daily rFSH injection. In the current study, all patients started rFSH treatment 1 w after the FSH-CTP injection. Apparently, serum FSH-CTP levels remained above the critical threshold value for substantial follicle development during the first week in all three dose groups, because the cancellation rate before the day of hCG was very low. In addition, the total dose of rFSH required to reach the hCG criteria was similar between the treatment groups, together indicating that the lowest effective dose of FSH-CTP may be lower than the doses tested. Depending on the dose of FSH-CTP and the individual variability in response, some patients may benefit from a lower dose with a shorter time interval up to the start

of rFSH, whereas others may be able to reach the criteria for hCG without any additional rFSH.

To date, the bioactivity of circulating FSH-CTP and its threshold to induce multifollicular growth are difficult to explore. Serum FSH-CTP levels are most reliably measured in a specific FSH-CTP immunoassay, which does not cross-react with rFSH. However, future investigators or prescribers of FSH-CTP will not be able to use such a specific assay and may be tempted to measure circulating FSH activity by means of their own FSH EIA. Although this study demonstrates that FSH-CTP cross-reacts in the FSH EIA (Delfia) in a linear dose-related fashion, its immunoreactivity in this assay cannot be used as an absolute quantitative value because monoclonal antibodies induced against rFSH have a different affinity to FSH-CTP than to rFSH, and the recognition of FSH-CTP is interfered with by endogenous and exogenous FSH. Together this implies that the FSH-CTP (threshold) levels need to be reassessed in clinical experiments and cannot be extrapolated from FSH EIA measurements. In this IVF study, the pharmacokinetics of FSH-CTP appeared to be dose proportional within the dose range of 120–240 μ g, and the terminal half-life (\sim 65 h) was dose independent. In a previous FSH-CTP study using pituitary-suppressed female volunteers (7), the pharmacokinetic properties were almost identical, indicating that these properties are unlikely to be affected by hormonal status or other fertility drugs.

Overall, after a single dose of FSH-CTP, on average only three additional doses of rFSH were needed to reach the hCG criteria, and the total duration of treatment was comparable to daily rFSH treatment. Regardless of the FSH-CTP dose given and whether additional rFSH was needed, the incidence of OHSS was low in this study and did not differ among the treatment groups. Moreover, as in healthy female volunteers (7), the incidence of side effects in IVF patients was low, and none of the subjects developed antibodies against this chimeric long-acting recombinant FSH agonist.

In this FSH-CTP study, regularly cycling subjects were treated with a GnRH antagonist protocol, which implies that the injected FSH-CTP dose adds up to the natural early follicular FSH rise. In comparison with down-regulated subjects, treatment with daily rFSH in a low-dose GnRH antagonist protocol requires less FSH to reach the same criteria for hCG administration (12–14). Like rFSH, a certain FSH-CTP dose might be equally effective in a GnRH antagonist and GnRH agonist protocol, although the duration of treatment

may become longer after pretreatment with a GnRH agonist. In the current study, applying a flexible initiation of GnRH antagonist administration, subjects in the 240- μ g group showed increased median LH levels as early as stimulation d 3 and in all other treatment groups from stimulation d 5 onwards. Comparable rises of endogenous LH have recently been observed in a flexible protocol of GnRH antagonist (at least one follicle of 15 mm) compared with patients who started the GnRH antagonist after 6 d of daily stimulation with rFSH (15). It was reported by the authors that these patients have a significantly lower chance for ongoing pregnancy [8.8% (6 of 68) in the flexible group *vs.* 23.9% (11 of 46) in the fixed group]. In the current study, patients treated with a flexible protocol and stimulated daily with rFSH showed a similar average increase of LH before the start of the GnRH antagonist treatment. Nevertheless, the implantation rate and pregnancy rate (10 of 24) in this treatment group was extremely high. Using a flexible protocol of GnRH antagonist (started when one follicle reaches a diameter of 14 mm) seems to increase the incidence of premature LH rises (LH > 10 IU/liter) before the start of the antagonist as compared with a fixed GnRH antagonist protocol (started d 6 of stimulation). Premature LH surges were observed on stimulation d 5 or 7 with an overall incidence of 17.6% in the FSH-CTP-treated subjects and of 8.3% in daily rFSH-treated subjects. Using a fixed protocol, these incidences were 4.3% (12) and 2.7%, respectively (13), in two studies with a starting dose of 150 IU rFSH and 15% in one study using a starting dose of 225 IU rFSH daily (14). Because approximately 50% of these early rises come along with progesterone rises, implying premature luteinization, a timely start of GnRH antagonist treatment in patients who start in the early follicular phase with a relatively high starting dose of rFSH or FSH-CTP seems to be essential.

To reach the same criteria for hCG administration, subjects treated with FSH-CTP required 1 d more of rFSH treatment compared with subjects treated with a fixed daily dose of rFSH. Although the initial ovarian response to FSH-CTP was appropriate, especially in the lowest-dose group, at d 8, the number of follicles at least 15 mm and at least 17 mm was lower in FSH-CTP-treated subjects. However, the difference was minor and not significant for the 240- μ g group. In the 120- μ g group, the growth of follicles tended to progress less between d 7 and 8, when a small decrease of inhibin B was also noted. Previous IVF studies (16, 17) and the current study have shown that inhibin B levels during daily rFSH administration increase up to d 8 and thereafter plateau. In this study, the decrease of inhibin B levels during stimulation may have been a first indication that serum FSH-CTP levels declined to suboptimal levels to maintain multiple follicular development. On the day of hCG, no differences were noted between the four groups for the number of follicles of the different size classes, indicating that treatment with rFSH reduced the differences noted on d 8 to a large extent in all three FSH-CTP dose groups.

Interestingly, the number of oocytes recovered in all FSH-CTP dose groups was clearly higher compared with the number of oocytes recovered after daily rFSH injection. This observation might be related to the higher total amount of circulating FSH (FSH-CTP and rFSH) from d 8 onwards,

maintaining follicular growth up to the day of oocyte retrieval to a larger extent in the FSH-CTP-treated subjects compared with women treated with a fixed dose regimen of 150 IU rFSH. Because there was no statistically significant difference between the four groups with respect to the number of metaphase II oocytes recovered in ICSI patients or the number of good quality embryos, these high FSH levels at the end of the follicular phase may rescue smaller follicles, which finally deliver immature oocytes.

In this study protocol, the flexible time intervals between hCG injection and oocyte pick-up and between oocyte pick-up and transfer, reflecting the clinical practice of the study sites, may have induced additional variability with respect to clinical outcome of this relatively small study. Although not statistically significant, the cancellation rate was higher and the pregnancy rate was lower after treatment with FSH-CTP than after daily rFSH treatment. Whether these observations were made by chance or were related to the stimulation characteristics of FSH-CTP or its regimen remains uncertain until larger comparative trials have been performed.

In conclusion, a single dose of FSH-CTP administered during the early follicular phase of the menstrual cycle appeared to be a potent inducer of multiple follicular growth during a 7-d interval. The total amount of circulating FSH was much higher and declined from d 3 to 8 in subjects who received a single dose of FSH-CTP, but the follicle growth dynamics were very comparable to those induced by daily rFSH administration. Additional studies should establish the optimal FSH-CTP dose and regimen that will provide optimal outcome in different subsets of IVF patients.

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Address all correspondence and requests for reprints to: Bernadette Mannaerts, M.Sc., Clinical Development Department, PO Box 20, 5340 BH Oss, The Netherlands. E-mail: b.mannaerts@organon.com.

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References

1. Perlman S, van den Hazel B, Christiansen J, Gram-Nielsen S, Jeppesen CB, Andersen KV, Halkier T, Okkels S, Schambye HT 2003 Glycosylation of an N-terminal extension prolongs the half-life and increases the *in vivo* activity of follicle stimulating hormone. *J Clin Endocrinol Metab* 88:3227–3235
2. Klein J, Lobel L, Pollak S, Lustbader B, Ogden RT, Sauer MV, Lustbader JWJ 2003 Development and characterization of a long-acting recombinant hFSH agonist. *Hum Reprod* 18:50–56
3. Fares FA, Suganuma N, Nishimori K, LaPolt PS, Hsueh AJ, Boime I 1992 Design of a long-acting follitropin agonist by fusing the C-terminal sequence of the chorionic gonadotropin β subunit to the follitropin β subunit. *Proc Natl Acad Sci USA* 89:4304–4308
4. Steelman SL, Pohley FM 1953 Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology* 53:604–611
5. Schellekens H 2002 Bioequivalence and the immunogenicity of biopharmaceuticals. *Nat Rev* 1:457–462
6. Bouloux PMG, Handelsman DJ, Jockenhövel F, Nieschlag E, Rabinovici J, Frasa WL, de Bie JJ, Voortman G, Itskovitz-Eldor J 2001 First human exposure to FSH-CTP in hypogonadotrophic hypogonadal males. *Hum Reprod* 16:1592–1597
7. Duijkers IJM, Klipping C, Boerrigter PJ, Machielsens CSM, de Bie JJ, Voortman G 2002 Single dose pharmacokinetics and effects on follicular growth and serum hormones of a long acting recombinant FSH preparation (ORG 36286) in healthy pituitary suppressed females. *Hum Reprod* 17:1987–1993

8. **Voortman G, Mannaerts BMJL, Huisman JAM** 2000 A dose proportionality study of subcutaneously and intramuscularly administered recombinant human FSH (Follistim/RFSH) in healthy female volunteers. *Fertil Steril* 73:1187–1193
9. **Fauser BCJM, Heusden AM** 1997 Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev* 18:71–106
10. **Hohmann FP, Macklon NS, Fauser BCJM** 2003 A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for *in vitro* fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. *J Clin Endocrinol Metab* 88:166–173
11. **Beckers NGM, Macklon NS, Devroey P, Platteau P, Boerrigter PJ, Fauser BCJM** 2003 First live birth after ovarian stimulation using a chimeric long-acting human recombinant follicle-stimulating hormone (FSH) agonist (rFSH-CTP) for *in vitro* fertilization. *Fertil Steril* 79:621–623
12. **The European Orgalutran Study Group, Borm G, Mannaerts B** 2000 Treatment with the gonadotropin-releasing hormone antagonist ganirelix in women undergoing controlled ovarian hyperstimulation with recombinant follicle stimulating hormone is effective, safe and convenient: results of a controlled, randomized, multicentre trial. *Hum Reprod* 7:1490–1498
13. **The European and Middle East Orgalutran Study Group** 2001 Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing controlled ovarian hyperstimulation. *Hum Reprod* 16:644–651
14. **The North American Ganirelix Study Group** 2001 Efficacy and safety of ganirelix acetate (Antagon/Orgalutran) versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. *Fertil Steril* 75:38–45
15. **Kolibianakis EM, Albano C, Kahn J, Camus M, Tournaye H, Van Steirteghem A, Devroey P** 2003 Increased exposure of the genital tract to luteinizing hormone (LH) and estradiol (E2) in the early follicular phase is associated with a reduced chance of pregnancy in cycles stimulated with recombinant follicle stimulating hormone (FSH) and gonadotropin releasing hormone (GnRH) antagonists for *in-vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI). *Fertil Steril* 79:873–888
16. **Casper FW, Seufert RJ, Schaffrath M, Pollow K** 2001 Concentrations of inhibins and activin in women undergoing stimulation with recombinant follicle-stimulating hormone for *in vitro* fertilization treatment. *Fertil Steril* 75:32–37
17. **Fanchin R, Schonauer LM, Righini C, Frydman N, Frydman R, Taieb J** 2003 Serum anti-Mullerian hormone dynamics during controlled ovarian hyperstimulation. *Hum Reprod* 18:328–332

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