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A multicenter, randomized, phase III study comparing the efficacy and safety of follitropin alpha biosimilar and the original follitropin alpha

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ABSTRACT

Objective: The aim of the present study was to investigate the therapeutic equivalence between the follitropin alpha biosimilar and the reference medication in women undergoing assisted reproductive technologies (ART).

Study design: This multicenter, randomized (1:1), embryologist-blinded, parallel-group, comparative phase III study involved 110 women aged 20–35 years old with tubal and/or male factors of infertility. All of the subjects underwent controlled ovarian hyperstimulation (COH) using a gonadotropin-releasing hormone antagonist (GnRH-ant) protocol. Over the 5-day fixed-dose regimen, the women received 150 IU/day of follitropin alpha biosimilar (n = 55) or original follitropin alpha (n = 55), followed by dose adaptation. The primary endpoint for assessing the therapeutic equivalence was the number of retrieved oocytes using a pre-determined clinical equivalence margin of ± 3.4 oocytes.

Results: Similar numbers of oocytes were retrieved in both groups: 12.16 ± 7.28 in the follitropin alpha biosimilar group and 11.62 ± 6.29 in the original follitropin alpha group, with mean difference of 0.546 ± 1.297 oocytes (95% confidence interval [CI]: -2.026, 3.116), p = 0.002 (intention-to-treat [ITT] population). Additionally, no statistically significant differences were found for secondary endpoints: the onset of biochemical (34.7% and 36.7%, p = 0.883), clinical pregnancy (26.5% and 32.7%, p = 0.507), delivery (26.5% and 24.5%, p = 0.817) and take-home baby rate (28.6% and 26.5%, p = 0.816) for the follitropin biosimilar and original follitropin groups (per-protocol [PP] population). Ovarian hyperstimulation syndrome was observed in subjects with a positive pregnancy test in 0% and 3.64% of cases and after triggering ovulation in 7.27% and 3.64% for the follitropin biosimilar and original follitropin groups, respectively.

Conclusions: This study demonstrated similar therapeutic equivalence and safety profiles between the follitropin alpha biosimilar and the reference follitropin in women who underwent COH in GnRH-ant cycles.

Abbreviations: ART, assisted reproductive technology; IVF, in vitro fertilization; r-hFSH, recombinant human follicle-stimulating hormone; IU, International Units; OPU, ultrasound-guided follicular aspiration; AFC, antral follicle count; GnRH-ant, gonadotropin-releasing hormone antagonist; GnRH-a, gonadotropin-releasing hormone agonist; FSH, follicle-stimulating hormone; LH, luteinizing hormone; NIBSC, The National Institute for Biological Standards and Control; AMH, anti-Müllerian hormone; ITT, intent-to-treat; PP, per-protocol; CI, confidence interval; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; COH, controlled ovarian hyperstimulation; SC, subcutaneous; OHSS, ovarian hyperstimulation syndrome; ET, embryo transfer; PCOS, polycystic ovary syndrome; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; PN, pronucleus; EMA, European Medicinal Agency; Neu5Gc, N-glycolyl neuraminic acid; CHO, Chinese hamster ovary.

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Background

As is known, biosimilars are not exact copies of the reference product in particular due to dissimilarities in host cell lines and genetic vectors used for modification, and the manufacturing and purification processes are also different [1]. Therefore, the manufacturer of r-hFSH biosimilars is required to conduct phase I and III randomized, controlled trials aiming to demonstrate that the changes in research and development, as well as manufacturing processes, do not affect the chemical identity, purity, potency and safety of the finished product. According to the European Medicinal Agency (EMA) guidelines for r-hFSH, biosimilar products should be estimated only in comparison with the original r-hFSH in pre-clinical, pharmacokinetics and pharmacodynamics studies [2].

The r-hFSH biosimilar Primapur® (IVFarma LLC, Russia) has demonstrated similar physicochemical properties with the original follitropin alpha Gonal-F® (Merck Serono S.p.A., Italy) and NIBSC standard in comparative pre-clinical studies [3,4]. Results of a phase I, randomized crossover study of the safety and tolerability of a single 300 IU subcutaneous dose (SC) in healthy young volunteers revealed no significant differences in pharmacokinetics parameters between Primapur® and Gonal-F® [5], detailed clinical protocol and results: NCT03857230 (ClinicalTrials.gov)

The objective of this study was to research the therapeutic equivalence of the follitropin alpha biosimilar to the original follitropin alpha in controlled ovarian hyperstimulation for convenient in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) using a GnRH-ant protocol. The number of oocytes retrieved is the recommended primary endpoint as stated by EMA [2] since it is known that the number of oocytes retrieved is well correlated with the efficiency of ART [6,7]. The secondary endpoints included the number of mature and fertilized oocytes, the number of days of stimulation, the total dose of r-hFSH injected, embryo transfers on days 3 and 5 and the occurrence rate of biochemical and clinical pregnancies. Live-birth delivery and take-home baby rates were also assessed. The safety end-points included descriptions of adverse effects documented during the study.

Methods

This was multicenter, randomized (1:1), embryologist-blinded, parallel-group, therapeutic equivalence study of two solutions of r-hFSH for subcutaneous administration - Primapur® and Gonal-f®, conducted in the Russian Federation in 3 specialized IVF centres: "AltraVita" Human Reproduction Clinic (Moscow); Perinatal Medical Center (Moscow); and Lapino Clinical Hospital (Moscow Region). The study was conducted in accordance with the clinical study protocol and the following international documents: GCP Guideline, according to Principles of the World Medical Association Declaration of Helsinki (Fortaleza, Brazil, 2013); ICH Harmonized Tripartite Guideline (ICH E6).

Study population

Inclusion criteria. The inclusion criteria were: women aged 20–35 years old with a regular menstrual cycle (duration: 21–35 days). Established causes of infertility: tubal and/or male factors, first or second attempt at IVF/ICSI; $18 \leq$ BMI ≤ 30 kg/m^2; FSH 10< IU/l and oestradiol level <50 pg/ml (cycle day 2–5); AMH ≥ 1.0 ng/ml; $4 \leq$ AFC ≤ 15. A signed informed consent form that confirmed in writing the patient's consent to participate in this clinical study was required.

Exclusion criteria. The exclusion criteria were: women with established contraindications to the use of ART methods; hypersensitivity to follitropin alpha or excipients; history of 2 or more cycles of IVF/ICSI; history of severe OHSS; PCOS; endometriosis; uterine cavity pathology; history of poor or excessive response to stimulation with a r-hFSH. Male infertility factors: severe oligoasthenoteratozoospermia; azoospermia.

Treatment administration

Randomization into two groups was carried out using centralized treatment allocation with an Interactive Web Response System (IWRS), based on the WinPepi random number generator programme, version 11.50 (module ETCETERA 3.26) [8], at a ratio of 1:1 for reference and the study drug. Starting on day 2–3 of the menstrual cycle, a fixed daily SC dose of 150 IU of Primapur®/Gonal-F® was administered each day as shown at Fig. 1. A double-blind design was not feasible due to the use of two unique pen-injector devices for each drug. A single-blind design was assigned for the embryology lab from the day of randomization until the end of the trial: all patients were recorded in internal medical documentation as receiving an international non-patented name drug (follitropin alpha). Clinicians carrying out the ultrasound-guided follicular aspiration were blinded, physicians performing ultrasound and deciding dose adjustment were not blinded. To achieve adequate follicular development, doses could be corrected after day 5 to a maximum of 450 IU per day, based on ultrasound examinations (Fig. 1). A GnRH-ant, ganirelix acetate (Orgalutran®, MSD, Netherlands) 0.25 mg was added daily, starting when the leading follicle reached a mean diameter of 14 mm. Less than 37 h after the intramuscular administration of 5000-10 000 IU of hCG (Pregnyl®, MSD, Netherlands) or 0.2 mg of GnRH-a (GnRH agonist) (Decapeptyl®, Ferring Pharmaceuticals, Switzerland) in women at high risk for OHSS development, transvaginal oocyte retrieval was performed, followed by IVF or ICSI according to the centre's standard procedures. The hCG trigger criteria: leading 2–3 follicles < 18 mm; GnRH-a criteria in women at risk of OHSS: growth of more than 15 follicles < 14 mm on the day of trigger. Transfer of a maximum of 2 embryos or blastocysts was allowed after oocyte retrieval. Luteal phase support was provided after oocyte retrieval at the investigator's discretion. Evaluation of biochemical pregnancy occurred on days 12–17 after embryo transfer with a positive test for beta hCG $\geq$ 25 mIU/ml. Clinical pregnancy defined as gestational sac with a fetal heart activity occurred at the 10th week after embryo transfer (Fig. 1).

Outcome measurements

The primary endpoint was the number of oocytes retrieved. Secondary endpoints included: the number of follicles $\geq$ 16 mm on the day of hCG (or GnRH-a) administration; oocyte quality outcome (MII stage); fertilization rate (zygotes with two pronuclei, 2 P N);

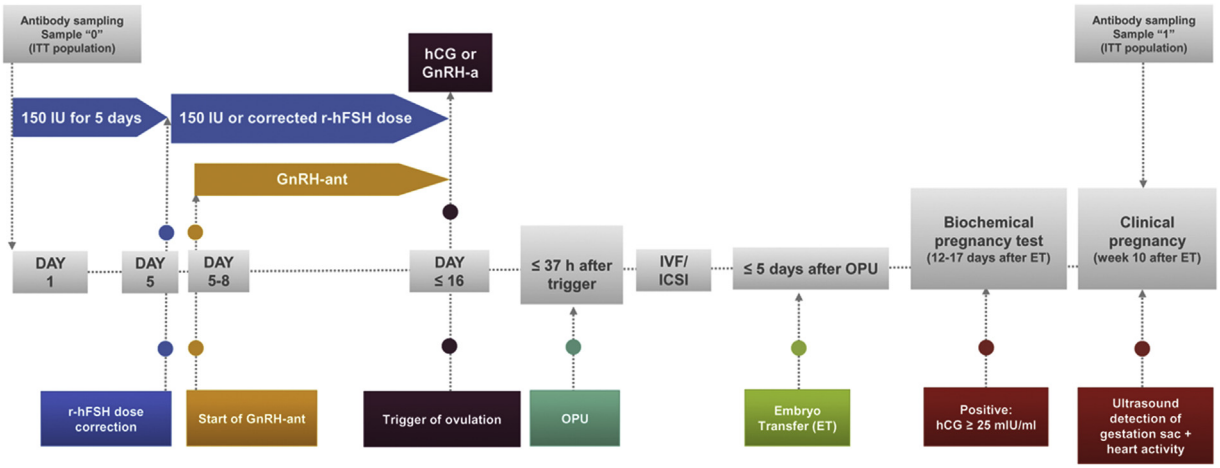

Fig. 1. Study design.

days of stimulation and total r-hFSH administered; number of embryo transfers on days 3 and 5; biochemical and clinical pregnancy rates; and delivery and take-home baby rates. The percentage of patients who needed a r-hFSH dose adjustment on the days 5–8 of COH, number of patients who abandoned the IVF/ICSI programme in the process of stimulation and number of non-responders to stimulation were analyzed as secondary endpoints as well. Adverse events were recorded as a secondary safety endpoint, including OHSS during COH and upon positive pregnancy test [9].

Anti-FSH antibodies were assessed as a secondary safety endpoint in all of the randomized patients at 2 points: baseline (sample "0″) before COH, and week 10 after ET on the day of the clinical pregnancy test (sample "1″) and for women with negative tests for biochemical pregnancy (Fig. 1). Serum samples were analyzed at 1 central laboratory for the presence of anti-FSH antibodies using a human anti-follicle-stimulating hormone (FSH) antibody ELISA Kit, cat # CSB-E16516 h (Wuhan Huamei Biotech Co., China) [10,11]. According to the manufacturer's instruction, ratio of optical density (OD) sample/OD negative control $\geq$ 2.1 was assumed to be positive (presence of anti-FSH antibodies), and a ratio less than 2.1 was assumed to be negative.

Statistical methods

The aim of this study was to evaluate the therapeutic equivalence of Primapur® compared with Gonal-F® in terms of the number of oocytes retrieved. To date, two clinical studies of FSH biosimilars compared with Gonal-F® have been published and used GnRH-a as an endogenous LH peak suppressor with the prespecified oocyte equivalence margins of 2.9 and 3 oocytes [12,13]. For the present study to specify the equivalence margin for oocytes retrieved, analysis of existing COH data for normal responders in IVF cycles with GnRH-ant and r-hFSH with the starting dose 150 IU was performed. Analysis of ART programmes with GnRH-ant and r-hFSH with 150 IU as the starting dose revealed a minimum of 8.2 and a maximum of 18.1 oocytes aspirated [14]. Analysis of published data on COH in women with normal responses to stimulation revealed the range of 4 to 15 oocytes retrieved [15]. Alignment of these two intervals led to the interval of the mean quantity of oocytes retrieved in GnRH-ant cycles in normal responders with a starting dose of r-hFSH of 150 IU of 8.2 to 15 oocytes (or 11.6 $\pm$ 3.4 oocytes). Standard deviation (SD) about $\pm$6.05 of oocyte retrieved in IVF cycles with r-hFSH was extracted from meta-analysis [14]. The required number of patients in equal parallel groups was calculated according to [16]. H0 (null hypothesis): $\mu A - \mu B \leq -d$, $\mu A - \mu B \geq +d$; H1 (alternative hypothesis): $-d \leq \mu A - \mu B \leq +d$, where μA and μB are the selective average number of oocytes retrieved with the study and reference drugs, respectively. Thus, to provide a study power of at least 80% at a significance level of $\alpha = 0.05$ (two sided) and with an equivalence margin of 3.4 oocytes, SD $\pm$ 6.05 the required sample size was 55 subjects per group and 110 subjects in total. As shown in GnRH-ant cycle with r-hFSH and normal-responder women the probability of live birth in the fresh cycle the same for patients with high (>15 oocytes retrieved) versus normal (10–15 oocytes), or normal versus suboptimal (4–9 oocytes) responders [6]. Thus, an equivalence margin of 3.4 retrieved oocytes assumed in the present study can be referred as clinically acceptable between two therapies in terms of live birth rate in the fresh IVF cycle.

The primary endpoint was evaluated in all randomized patients (ITT population), as well as some secondary endpoints: number of follicles on the day of trigger injection; mature (MII) and fertilized oocytes (2 P N); the total dose of r-hFSH injected; and the number of days of stimulation. The safety analysis included all randomized patients who received at least one r-hFSH dose. Comparative analysis of qualitative and categorical variables was performed using the chi-square test and Fisher criteria. Because of the non-normality of the distribution of the primary and secondary endpoints (proved by the Shapiro-Wilk test), the Mann-Whitney *U* test was performed. $p<0.05$ was considered statistically significant.

Results

Subject disposition

Of 118 women screened, 110 were randomized into the trial (ITT population) to receive Primapur® (N = 55) or Gonal-F® (N = 55), between 08.02.2017 and 17.08.2018 (Fig. 2). The distribution of randomized patients by IVF centers: AltraVita – 50; Perinatal Medical Center– 41; and Lapino Hospital – 19. Demographic and clinical characteristics were comparable between the treatment groups. No appreciable differences were observed in baseline FSH, oestradiol, AMH, or AFC between the groups (Table 1). PP population consists of 98 patients with fresh ET, 49 patients in each group (Fig. 2). In 12 patients embryo transfers were not performed: 6 in the Primapur® group (4 – risk of OHSS, 1 – no embryos to transfer, 1 - no embryos with 2 P N); and 6 in the Gonal-F® group (3 – risk of OHSS, 1 – no embryos to transfer, 2 – due to family reasons).

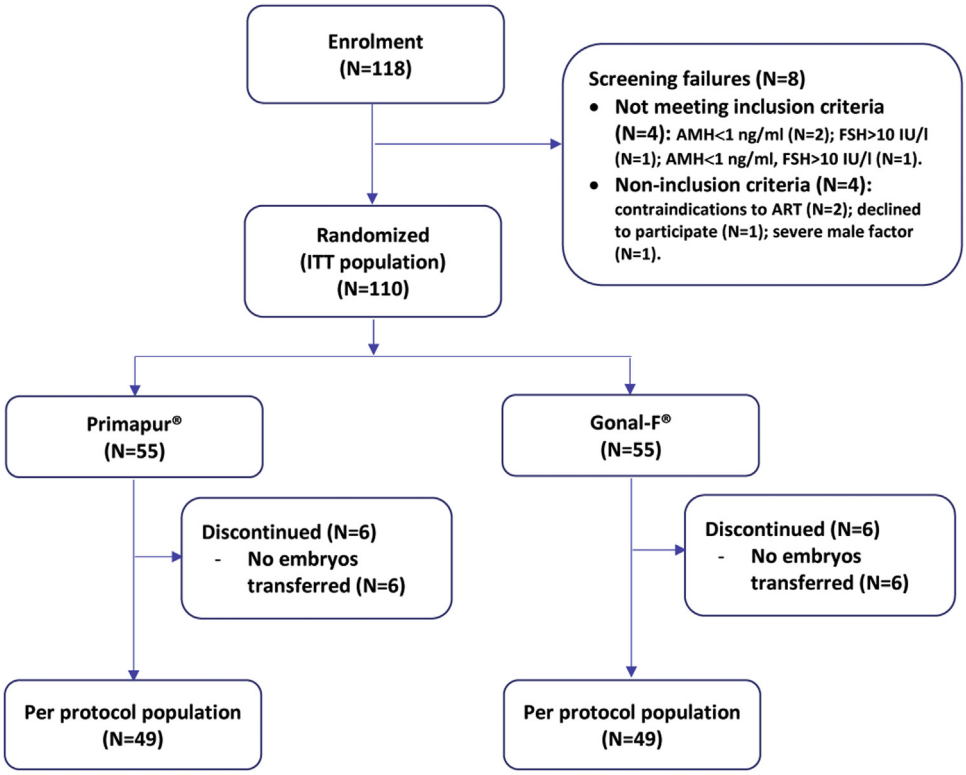

Fig. 2. Patient disposition scheme.

Primary endpoint

Primapur® treatment resulted in a number of aspirated oocytes that was statistically equivalent to that of the patients treated with Gonal-F® (Table 2). The treatment difference was $\Delta = 0.546 \pm 1.297$ oocytes [95% CI: -2.026, 3.116] with a p-value for equivalence of p = 0.002 (ITT population), demonstrating equivalence as pre-defined in the equivalence hypothesis (±3.4 oocytes).

Secondary endpoints

The mean differences in the number of follicles (≥16 mm) on the day of hCG or GnRH-a injection was 0.709 ± 1.067 [95% CI: -1.405, 2.824]. An alternative trigger of ovulation (GnRH-a) was received in 18 patients at risk of OHSS: 9 patients in the Primapur® group (with 9 fresh ET) and 9 patients in the Gonal-F® group (8 fresh ET, 1 -freeze all). The mean differences in mature oocytes at the MII stage of development 0.218 ± 1.129 [95% CI: -2.455, 2.019] and zygotes with two pronuclei 0.636 ± 1.190 [95% CI: -2.995, 1.723] were statistically indistinguishable (p < 0.05) (Table 3). The mean total r-hFSH dose and duration of treatment were similar in both groups with p = 0.488 and 0.629, respectively (Table 3). The proportions of patients requiring dose adaptation were 23.6% in the Primapur® group and 20.0% in the Gonal-F® group (p = 0.644). The mean dose adjustments were 50.0 ± 0.0 IU (Primapur®) and 42.0 ± 15.1 IU (Gonal-F® group) (p = 0.063). There were no patients with inefficient responses to r-hFSH treatment or premature termination of COH in either group. According to the study design, a maximum of 2 embryos was allowed for transfer, and the mean numbers of transferred embryos were 1.2 ± 0.43 in the Primapur® group and 1.3 ± 0.47 in the Gonal-F® group. ET on day 3 (and day 5) after OPU was implemented in the Primapur® and Gonal-F ® groups: 22.4 (77.6)% and 18.4 (81.6)%, respectively (Table 4). There were no significant differences in biochemical and clinical pregnancy rates observed between the treatment groups (Table 5). No ectopic pregnancies were observed during the study. Two multiple pregnancies (twins) occurred during the study, one in each treatment group: 2.04% (PP population). One hundred percent of clinically pregnant patients went on to have full-term live births in

Table 1
Demographic and clinical characteristics for ITT population and [PP population].

Characteristic	Primapur® N = 55 [N = 49]	Gonal-F® N = 55 [N = 49]	Total N = 110 [N = 98]
Age (mean ± SD), years	31.3 ± 2.68 [31.2 ± 2.79]	30.0 ± 2.71 [30.1 ± 2.71]	30.65 ± 2.75 [30.64 ± 2.78]
BMI (mean ± SD), kg/m^2	22.0 ± 2.69 [22.0 ± 2.69]	22.3 ± 3.06 [22.6 ± 3.11]	22.15 ± 2.87 [22.28 ± 2.91]
Duration of infertility (mean ± SD), month	46.4 ± 32.4 [46.1 ± 30.0]	36.9 ± 26.6 [37.2 ± 23.1]	41.56 ± 28.22 [41.64 ± 27.03]
Antral follicle count (mean ± SD), n	11.2 ± 3.2 [10.8 ± 3.2]	12.4 ± 2.4 [12.4 ± 2.4]	11.80 ± 2.87 [11.61 ± 2.93]
AMH (mean ± SD), ng/ml	4.57 ± 2.96 [4.48 ± 3.08]	5.47 ± 3.82 [5.36 ± 3.95]	5.02 ± 3.43 [4.92 ± 3.55]
FSH (mean ± SD), IU/l	6.46 ± 1.86 [6.54 ± 1.88]	6.76 ± 1.89 [6.74 ± 1.95]	6.61 ± 1.87 [6.64 ± 1.91]
Oestradiol (mean ± SD), pg/ml	35.87 ± 12.67 [35.95 ± 12.90]	33.82 ± 12.45 [33.74 ± 12.79]	34.85 ± 12.54 [34.85 ± 12.83]
Causes of infertility, n (%)			
Tubal factor	21 (38.2%) [20 (40.8%)]	18 (32.7%) [16 (32.7%)]	39 (35.5%) [36 (36.7%)]
Male factor	21 (38.2%) [16 (32.7%)]	27 (49.1%) [26 (53.0%)]	48 (43.6%) [42 (42.9%)]
Tubal and male factors	13 (23.6%) [13 (26.5%)]	10 (18.2%) [7 (14.3%)]	23 (20.9%) [20 (20.4%)]

Table 2
Primary endpoint results: number of oocytes retrieved (ITT population).

Parameter	Primapur® (mean ± SD), N = 55	Gonal-F® (mean ± SD), N = 55	Mean difference (mean ± SD)	p-value	95% CI	
					Lower limit	Upper limit
Number of oocytes retrieved, n	12.16 ± 7.28	11.62 ± 6.29	0.546 ± 1.297	0.002	−2.026	3.116

Table 3
Secondary endpoint results: number of follicles ≥16 mm on the day of hCG or GnRH-a injection, mature (MII) and fertilized oocytes (2 PN), total dose of r-hFSH injected, and number of days of stimulation (ITT population).

Parameter	Primapur® (mean ± SD), N = 55	Gonal-F® (mean ± SD), N = 55	Mean difference (mean ± SD)	p-value	95% CI	
					Lower limit	Upper limit
Follicles ≥16 mm, n	12.09 ± 6.159	11.38 ± 4.965	0.709 ± 1.067	0.806	−1.405	2.824
Mature oocytes (MII stage), n	9.64 ± 6.27	9.86 ± 5.55	0.218 ± 1.129	0.617	−2.455	2.019
Fertilized oocytes (zygotes with 2 PN), n	8.13 ± 6.61	8.76 ± 5.85	0.636 ± 1.190	0.445	−2.995	1.723
Total r-hFSH dose, IU	1532.7 ± 267.2	1517.9 ± 255.2	14.9 ± 49.8	0.488	−83.9	113.6
Days of stimulation, n	9.75 ± 1.08	9.73 ± 1.03	0.018 ± 0.201	0.629	−0.379	0.416

Table 4
Secondary endpoint results: number of embryo transfers on days 3 and 5 (PP population).

Parameter	Primapur® N = 49	Gonal-F® N = 49	p-value
Embryo transfer (day 3), n (%)	11 (22.4%)	9 (18.4%)	0.623
Embryo transfer (day 5), n (%)	38 (77.6%)	40 (81.6%)	

the Primapur® group (13/13) and 75% in the Gonal-F® group (12/16), constituting 26.5% and 24.5% delivery rates, respectively (Table 5). Overall, a total of 27 live births occurred in the study after fresh ET, and the take-home baby rates, defined as the number of deliveries resulting in a live born neonate per PP population, were 28.6% (n = 14) and 26.5% (n = 13), respectively (Table 5).

Adverse event profiles

All adverse events occurring during the clinical study were classified according to the MedDRA 10.0 vocabulary and are summarized in Table 6. Severe OHSS developed in subjects with a positive pregnancy test in 0 (0%) in cases the Primapur® group and in 2 cases (3.64%) in the Gonal-F® group. OHSS during COH after hCG (or GnRH-a) trigger was: 4 (7.27%) cases in the Primapur® and 2 (3.64%) cases in the Gonal-F® group. Other frequently reported adverse effects were abdominal pain, miscarriage and vaginal bleedings.

Immunogenicity profile

No evidence of new anti-FSH antibody development in either group was seen (Table 7). A total of 109 serum samples had anti-FSH levels less than the prespecified positive limit (OD sample/OD negative control ≥ 2.1) before and after COH, apart from one serum sample in the Gonal-F® group that was positive before (2.24) and after (2.86) treatment.

Discussion

Normogonadotrophic patients enrolled in this study were representative, showing the ability of exogenous r-hFSH to the stimulate development of multiple follicles in women without endocrine and ovarian disturbances during COH. The FSH dosing regimen with the 150 IU/day starting dose, followed by its adjustment on the basis of the ovarian response, was used, and GnRH-ant protocol was recommended for normal responder patients enrolled in the present study [17–19]. The population analyzed in this study might not be completely representative of the general population undergoing IVF/ICSI and further post-authorization studies should be implemented to evaluate the efficacy of Primapur® in patients undergoing ART in GnRH-a cycles and with other causes of infertility, e.g. endometriosis, PCOS and poor response to COH.

Our phase III study found Primapur® to be equivalent to Gonal-F® in the primary endpoint of the number of oocytes retrieved during COH in women undergoing ART. In addition, the secondary endpoints of the study were: number of follicles ≥16 mm on the day of trigger injection, mature and fertilized oocytes, r-FSH doses, and days of COH and were similar between the treatment groups.

Analysis of delivery and take-home baby rates did not reveal significant differences, but there were more pregnancy losses in the Gonal-F® group after 10th week following ET. Such differences are not relevant to COH at IVF centers and could have several reasons, such as nutritional supplement usage [20], issues of maternity care [21] and genetic aspects of miscarriage [22], which were not controlled for or analyzed in the present study.

Table 5
Secondary endpoint results: the occurrence rates of biochemical and clinical pregnancies; and live-birth delivery and take-home baby rates (PP population).

Parameter	Primapur® N = 49	Gonal-F® N = 49	Mean difference [95% CI]	p-value X^2
Biochemical pregnancy (hCG≥25 mIU/ml, 12-17 days after ET), n (%)	17 (34.7%) [21.4; 48.0%]	18 (36.7%) [23.2; 50.2%]	−2.0% [-21.0; 17.0%]	0.833
Clinical pregnancy (ultrasound detection of gestational sac, 10 weeks after ET), n (%)	13 (26.5%) [14.1; 38.9%]	16 (32.7%) [19.6; 45.8%]	−0.62% [-24.3; 11.9%]	0.507
Delivery rate, n (%)	13 (26.5%) [14.1; 38.9%]	12 (24.5%) [12.5; 36.5%]	2.00% [-15.3; 19.3%]	0.817
Take-home baby rate, n (%)	14 (28.6%) [16.0; 41.3%]	13 (26.5%) [14.1; 38.9%]	2.10% [-15.6; 19.8%]	0.816

Table 6
Serious adverse events and other adverse events (ITT population).

Serious adverse events	Primapur® N=55	Gonal-F® N=55
Total, n (%)	2 (3.64%)	2 (3.64%)
Injury, poisoning and procedural complications		
Iatrogenic injury, n (%)	1 (1.82%)	0 (0)
Pregnancy, puerperium and perinatal conditions		
Threatened miscarriage, n (%)	1 (1.82%)	0 (0)
Reproductive system and breast disorders		
Ovarian hyperstimulation syndrome*, n (%)	0 (0)	2 (3.64%)
Other adverse events		
Total, n (%)	21 (38.18%)	13 (23.64%)
Cardiac disorders		
Palpitations, n (%)	1 (1.82%)	0(0)
Sternum pain, n (%)	1 (1.82%)	0(0)
Investigations		
Laboratory investigations, n (%)	1 (1.82%)	0(0)
Musculoskeletal and connective tissue disorders		
Fatigue, n (%)	1 (1.82%)	0(0)
Nervous system disorders		
Drowsiness, n (%)	1 (1.82%)	0(0)
Pregnancy, puerperium and perinatal conditions		
Abnormal vaginal bleeding, n (%)	0 (0)	1(1.82%)
Early toxicosis during pregnancy, n (%)	0(0)	1(1.82%)
Lower abdominal pain, n (%)	2(3.64%)	4(7.27%)
Spontaneous miscarriage, n (%)	0(0)	2(3.64%)
Spotting, n (%)	1(1.82%)	1(1.82%)
Reproductive system and breast disorders		
Abnormal vaginal bleeding, n (%)	3(5.45%)	1(1.82%)
Decrease in breast sensitivity, n (%)	2(3.64%)	0(0)
Lower abdominal pain, n (%)	3(5.45%)	1(1.82%)
Menses, n (%)	0(0)	1(1.82%)
Ovarian hyperstimulation syndrome**, n (%)	4(7.27%)	2(3.64%)
Pain in lower abdomen, n (%)	1(1.82%)	0(0)
Spotting, n (%)	2(3.64%)	0(0)

* Severe OHSS in subjects with a positive pregnancy test.
** OHSS after hCG (or GnRH-a) trigger.

Table 7
Anti-FSH antibody levels before and after treatment with r-hFSH (ITT population).

Antibody sampling	Primapur® (mean OD Sample/OD Negative control ± SD) N = 55	Gonal-F® (mean OD Sample/OD Negative control ± SD) N = 54*
Sample "0"	0.20 ± 0.18	0.18 ± 0.19
Sample "1"	0.19 ± 0.18	0.18 ± 0.26

* One serum was positive: Sample "0" (OD sample/OD negative control = 2.24) and Sample "1" (2.86) were suggested to be positive.

OHSS was the most common adverse effect during treatment. The overall OHSS rate (all forms) was similar and consisted of 7.27% in each treatment group, in line with the reported frequency of OHSS for normal responder patients [23] and similar to that for existing biosimilars [12,13].

In the present study no newly raised anti-FSH antibodies were detected in either group. As was reported for r-hFSH biosimilars representing solutions with purity of more than 99%, the probability of anti-FSH antibody formation is low [12,13] and the immunogenicity risk has not clearly been identified for follitropin alpha to date [24]. With high probability, antibodies to FSH can appear mainly in reaction to nonhuman sialic acid - N-glycolyl neuraminic acid (Neu5Gc), which can exist in r-hFSH due to the usage of CHO host cells [25]. The comparable total content of Neu5Gc and its acetylated derivative Neu5Gc9Ac were detected earlier for Gonal-F® (0.46%) and Primapur® (0.41%) [3]. As was shown, pre-existing Neu5Gc-reactive antibodies in the serum of patients undergoing IVF could be detected before r-hFSH treatment and did not affect the efficacy or safety of ART [26].

The level of endogenous FSH changes during the menstrual cycle, not only quantitatively but also structurally with predominant glycosylation patterns peculiar to the appropriate phase of the menstrual cycle [27,28]. As is known, r-hFSH biosimilars can slightly differ in their glycosylation and sialylation profiles [1]. The rise in biosimilars should suggest not only a lower cost for infertile couples undergoing COH but also usefulness for healthcare professionals due to similar but not identical glycosylation patterns of r-hFSH preparations, which could have beneficial therapeutic effects in a given patient [29].

Ethics approval and consent to participate

The study protocol and informed consent were approved by the Russian Ministry of Health (RCT 754 dated 26.10.16) and the independent interdisciplinary ethics committee for ethical review of clinical studies (protocol 17 dated 28.10.2016).

Consent for publication

Not applicable.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contribution

Execution of the study and acquisition of the data: ZB, LV, YF, NM, MO, MT, YS, AK, AM, GK, TT, IZ, and NB. Data analysis and interpretation: TT, LS, RS, VA, and SY. Study design and data interpretation: ZB, LV, MO, EM, IZ, and MP. All of the authors made substantial contributions to the revising of the article and provided final approval of the version to be published.

There were no conflicts of interest. MP is a stockholder in IVFarma LLC; TT, LS and RS served as principal investigators in the study. IZ is now a Head of Research Infertility at NovaClinic (Moscow, Russia). NB is now a gynecologist at the European Medical Center (Moscow, Russia).

Declaration of Competing Interest

There were no conflicts of interest. MP is a stockholder in IVFarma LLC; TT, LS and RS served as principal investigators in the study. IZ is now a Head of Research Infertility at NovaClinic (Moscow, Russia). NB is now a gynecologist at the European Medical Center (Moscow, Russia).

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References

[1] Orvieto R, Seifer DB. Biosimilar FSH preparations - are they identical twins or just siblings? Reprod Biol Endocrinol 2016;14(1):32.

[2] Committee for Medicinal Products for Human Use. Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human follicle stimulating hormone (r-hFSH). EMA 2012;2013:597110.

[3] Vorob'ev II, Proskurina OV, Khodak YA, Gosudarev AI, Semikhin AS, Byrikhina DV, et al. Physicochemical properties, toxicity, and specific activity of a follitropin alpha biosimilar. Pharm Chem J 2017;50(11):753–60, doi:http://dx.doi.org/10.1007/s11094-017-1525-3.

[4] Orlova NA, Kovnir SV, Khodak YA, Polzikov MA, Nikitina VA, Skryabin KG, et al. High-level expression of biologically active human follicle stimulating hormone in the Chinese hamster ovary cell line by a pair of tricistronic and monocistronic vectors. PLoS One 2019;14(7):e0219434, doi:http://dx.doi.org/10.1371/journal.pone.0219434.

[5] Tyul'kina EE, Gordeev IG, DYu Grebenkin, Kazei VA, Tsikarishvili MM, Luchinkina EE, et al. Randomized crossover comparative study of safety, tolerance and pharmacokinetics of primapur vs. Gonal-f upon single-dose subcutaneous administration in healthy volunteers. Eksperimental'naya i Klinicheskaya Farmakologiya 2017;80(4)13–7, doi:http://dx.doi.org/10.30906/0869-2092-2017-80-4-13-17 (in Russian).

[6] Drakopoulos P, Blockeel C, Stoop D, Camus M, de Vos M, Tournaye H, et al. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? Hum Reprod 2016;31:370–6.

[7] Venetis CA, Tilia L, Panlilio E, Kan A. Is more better? A higher oocyte yield is independently associated with more day-3 euploid embryos after ICSI. Hum Reprod 2019;34(1):79–83.

[8] Abramson JH. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov. 2011;8(1):1.

[9] Humaidan P, Nelson SM, Devroey P, Coddington CC, Schwartz LB, Gordon K, et al. Ovarian hyperstimulation syndrome: review and new classification criteria for reporting in clinical trials. Hum Reprod 2016;31(9):1997–2004.

[10] Mohammadi Yeganeh ML, Moini A, Hemmat M, Salman Yazdi R, Bagheri Lankarani N, Khodabakhshi S, et al. The association of different auto-antibodies against ovarian tissues and gonadotropins and poor ovarian response in intracytoplasmic sperm injection cycles. Hum Fertil (Camb) 2017;20(2):126–31.

[11] Lofgren JA, Dhandapani S, Pennucci JJ, Abbott CM, Mytych DT, Kaliyaperumal A, et al. Comparing ELISA and surface plasmon resonance for assessing clinical immunogenicity of panitumumab. J Immunol 2007;178(11):7467–72.

[12] Rettenbacher M, Andersen AN, Garcia-Velasco JA, Sator M, Barri P, Lindenberg S, et al. A multi-centre phase 3 study comparing efficacy and safety of bemfola® versus gonal-f® in women undergoing ovarian stimulation for IVF. Reprod Biomed Online 2015;30(5):504–13.

[13] Strowitzki T, Kuczynski W, Mueller A, Bias P. Randomized, active-controlled, comparative phase 3 efficacy and safety equivalence trial of ovaleap® (recombinant human follicle-stimulating hormone) in infertile women using assisted reproduction technology (ART). Reprod Biol Endocrinol 2016;14:1.

[14] van Wely M, Kwan I, Burt AL, Thomas J, Vail A, Van der Veen F, et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. Cochrane Database Syst Rev 2011;2:CD005354.

[15] La Marca A, Papaleo E, Grisendi V, Argento C, Giulini S, Volpe A. Development of a nomogram based on markers of ovarian reserve for the individualisation of the follicle-stimulating hormone starting dose in in vitro fertilisation cycles. BJOG 2012;119(10):1171–9.

[16] Julious SA. Sample sizes for clinical trials with normal data. Stat Med 2004;23(12):1921–86.

[17] Marci R, Caserta D, Lisi F, Graziano A, Soave I, Lo Monte G, et al. In vitro fertilization stimulation protocol for normal responder patients. Gynecol Endocrinol 2013;29(2):109–12.

[18] Hamdine O, Eijkemans MJ, Lentjes EW, Torrance HL, Macklon NS, Fauser BC, et al. Ovarian response prediction in GnRH antagonist treatment for IVF using anti-müllerian hormone. Hum Reprod 2015;30(1):170–8.

[19] Rombauts L. Is there a recommended maximum starting dose of FSH in IVF? J Assist Reprod Genet 2007;24(8):343–9.

[20] Nohr EA, Olsen J, Bech BH, Bodnar LM, Olsen SF, Catov JM. Periconceptional intake of vitamins and fetal death: a cohort study on multivitamins and folate. Int J Epidemiol 2014;43(1):174–84.

[21] Shuvalova MP, Yarotskaya EL, Pismenskaya TV, Dolgushina NV, Baibarina EN, Sukhikh GT. Maternity care in russia: issues, achievements, and potential. J Obstet Gynaecol Can 2015;37(10):865–71.

[22] Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Best Pract Res Clin Obstet Gynaecol 2000;14(5):855–65.

[23] Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, et al. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: an analysis of 256,381 in vitro fertilization cycles. Fertil Steril 2014;101(4):967–73.

[24] Baker MP, Jones TD, Chamberlain P. Immunogenicity of biologics. In: Vaughan T, Osbourn J, Jallal B, editors. Methods and principles in medicinal chemistry; Protein therapeutics, Vol. 71. Willey; 2017. p. 387–423.

[25] Ghaderi D, Zhang M, Hurtado-Ziola N, Varki A. Production platforms for biotherapeutic glycoproteins. Occurrence, impact, and challenges of non-human sialylation. Biotechnol Genet Eng Rev 2012;28:147–75.

[26] Chamberlain PD. Multidisciplinary approach to evaluating immunogenicity of biosimilars: lessons learnt and open questions based on 10 years' experience of the European Union regulatory pathway. Biosimilars 2014;4:23–43.

[27] Ulloa-Aguirre A, Midgley Jr AR, Beitins IZ, Padmanabhan V. Follicle-stimulating isohormones: characterization and physiological relevance. Endocrine Review 1995;16:765–87.

[28] Zambrano E, Olivares A, Mendez JP, Guerrero L, Díaz-Cueto L, Veldhuis JD, et al. Dynamics of basal and GnRH-releasable serum follicle-stimulating hormone charge isoform distribution throughout the human menstrual cycle. J Clin Endocrinol Metab 1995;80: 1647–165.

[29] Jean-Claude Emperaire. Ovulation stimulation with gonadotropins. Springer: Cham Print; 2015, doi:http://dx.doi.org/10.1007/978-3-319-18654-2.