JBRA Assisted Reproduction 2021;**25**(4):524-532
doi: 10.5935/1518-0557.20210023

Original Article

Efficacy, safety, and immunogenicity of a biosimilar recombinant human follicle-stimulating hormone (Folitime®) vs. Gonal-f® in women undergoing ovarian stimulation for IVF: A randomized, multicenter, evaluator-blinded, non-inferiority study

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ABSTRACT

Objective: We compared the efficacy, safety, and immunogenicity of a biosimilar recombinant human follicle-stimulating hormone (Folitime®) with Gonal-f® in women undergoing ovarian stimulation for in-vitro fertilization.

Methods: This randomized (1:1), multicenter, assessor-blinded, non-inferiority, parallel-group, controlled study conducted at four infertility clinics in Argentina included infertile normogonadotropic women with ages below 39 years, with menstrual cycles of 25/35 days and a body mass index of 18–32 kg/m2 undergoing assisted reproductive technology therapy. During a 5-day fixed-dose phase, the women received 225 IU/day of Folitime® (n=49) or Gonal-f® (n=44), followed by a dose-adaptation phase up to a maximum of 450 IU/day. The non-inferiority margin for oocyte retrieval was estimated at -4 oocytes (one-sided test). Immunogenicity was investigated on days 9 and 84, following the start of treatment.

Results: The mean number of oocytes retrieved was 12.6 (SD 7.4) in the Folitime® group and 13.4 (SD 6.9) in the Gonal-f® group (per protocol analysis, 95% confidence interval = -3.82; 2.33), within the non-inferiority margin. Pregnancy rate at week 10 was 24.4% among subjects treated with Folitime® and 19.5% for subjects treated with Gonal-f®. One serious adverse drug reaction—late mild ovarian hyper stimulation syndrome and deep venous thrombosis in the left deep jugular vein—occurred in a subject treated with Folitime®. None of the subjects developed antibodies against the study drugs. There were no unexpected safety findings.

Conclusions: Folitime® is non-inferior to Gonal-f®, with no differences in the safety profile and has been approved as a biosimilar in Argentina.

Keywords: biosimilar, recombinant FSH, Folitime®, Gonal-f®, *in-vitro* fertilization

INTRODUCTION

Assisted reproduction technologies (ART) such as in-vitro fertilization (IVF) require controlled ovarian hyper stimulation to increase the number of female gametes, and hence the chances of successful treatment outcomes (Palermo *et al*., 1992). Currently, this is achieved by gonadotropin treatment with human menopausal gonadotropins and/or follicle stimulating hormone (FSH). The first generation of recombinant FSH (rFSH) was developed in the 1990s and two products came in the market, namely follitropin beta (Puregon; NV Organon, Oss, The Netherlands) and follitropin alpha (Gonal F; Ares-Serono, Geneva, Switzerland) (Meniru, 1999). These products were comparable with regard to oocyte yield and delivery rates.

Recombinant-hFSH (follitropin alpha for injection) is a human FSH preparation of recombinant DNA origin, which consists of two non-covalently linked, non-identical glycoproteins designed as the alpha- and beta-subunits. These alpha- and beta-subunits are encoded by separate genes and contain 92 and 111 amino acids, respectively. Their primary and tertiary structures are indistinguishable from those of human FSH. Like other glycoprotein hormones, FSH displays a high degree of structural heterogeneity owing to differences in the amount and/or composition of the carbohydrate residues, particularly sialic acid (de Leeuw *et al.*, 1996). rFSH is produced with genetically modified Chinese hamster ovary cells cultured in bioreactors. Purification by immunochromatography using an antibody that specifically binds to FSH results in a highly purified preparation, with a consistent FSH isoform profile and high specific activity (Olijve *et al*., 1996). Based on data derived from physicochemical tests and bioassays, follitropin alpha, follitropin beta, and another rFSH product are comparable at inducing follicular growth in women who do not have primary ovarian failure. FSH, the active component of r-hFSH, is the primary hormone responsible for the recruitment and development of follicles. To produce the final maturation of follicles and ovulation in the absence of an endogenous luteinizing hormone surge, human chorionic gonadotropin (hCG) or a GnRH agonist must be given following the administration of r-hFSH, when monitoring of the patient indicates that sufficient follicular development has occurred.

Similar biotherapeutic products (SBPs) or biosimilars are defined as biotherapeutic products that are similar in terms of quality, safety, and efficacy to an already licensed reference biotherapeutic product (WHO Expert Committee on Biological Standardization, 2009). The European Medicines Agency (EMA) has released several guidelines related to the development of biosimilar products (EMA, 2014a; 2014b) and a specific guideline for the development of SBPs containing rec-hFSH (EMA, 2013a). As of date, two r-hFSH biosimilar products have been approved by the EMA, Ovaleap® (Teva Pharma B.V., Netherlands) (EMA, 2013b), and Bemfola® (Finox AG, Switzerland)

Received August 25, 2020
Accepted March 01, 2021

(EMA, 2014c). Since the time of its approval, the efficacy and safety of r-hFSH biosimilar products have been matters of discussion (Orvieto & Seifer, 2016; Strowitzki *et al*., 2016a). A systematic review and meta-analysis comparing an r-hFSH biosimilar to the original r-hFSH in women undergoing IVF/intracytoplasmic sperm injection cycle (ICSI) was recently published (Longobardi *et al*., 2019). This article reviewed three randomized controlled trials (RCTs) (Strowitzki *et al*., 2016b; Rettenbacher *et al*., 2015; NCT01687712, 2017) comparing an r-hFSH biosimilar to the original drug. The primary endpoint was live birth and secondary endpoints included clinical pregnancy and moderate to severe ovarian hyperstimulation syndrome (OHSS). The live birth rate associated with the biosimilar product was significantly lower when compared to that obtained using the original r-hFSH (RR 0.82, 95% CI: 0.70–0.97), and the clinical pregnancy rate was also significantly lower (RR 0.83, 95% CI: 0.71–0.96). With the exception of the two r-hFSH biosimilar products approved in Europe, there is currently no fully developed r-hFSH biosimilar for ovarian stimulation available in Latin America.

This phase III study aimed at comparing the efficacy, safety, and immunogenicity of an r-hFSH biosimilar product and the original r-hFSH in women undergoing ovarian stimulation for IVF.

METHODS

Study design

A randomized, multicenter, assessor-blinded, non-inferiority, parallel-group, controlled study (Clinicaltrials.gov trial registration number: NCT02454556) was conducted at four private infertility clinics in four different states of Argentina. The main study was carried out for 10 weeks after the administration of hCG for subjects not achieving pregnancy and for up to 44 weeks after the administration of hCG for pregnant subjects. A pharmacokinetics (PK) sub-study was performed with 24 subjects already included in the trial. This paper reports the results from the main study, which includes the primary endpoint study.

This was an assessor-blinded, randomized clinical trial (RCT) because both drugs were delivered by subcutaneous injection using different devices. A blinded assessor was in charge of evaluating the efficacy endpoints. Patients were instructed not to comment about the format of the medication to the assessor.

Patients

Women aged between 18 and 38 years, with a body mass index (BMI) ranging between 18 and 32 kg/m^2, regular menstrual cycles of 25–35 days, basal FSH under 10 IU/L (cycle day 2–5), and undergoing their first or second cycle in the present series of IVF were included. Other key inclusion criteria were: total antral follicle count of ≥8 to ≤18 follicles, with a follicle diameter <10 mm (sum of both ovaries) in the early follicular phase; documented history of infertility due to tubal factor, male factor, or unexplained infertility; presence of both ovaries and a normal uterine cavity as confirmed by transvaginal ultrasound within three months of randomization. The key exclusion criteria were: more than two previous unsuccessful IVF retrieval cycles; history of ovarian hyperstimulation syndrome (OHSS); moderate to severe endometriosis; presence of hydrosalpinx; history of poor ovarian response to gonadotropin treatment (defined as fewer than five oocytes retrieved in a previous attempt); any hormonal treatment within one month of the start of the FSH treatment (with the exception of levothyroxine); intrauterine leiomyomas ≥5 cm; ovarian cyst or enlargement of undetermined origin; otherwise clinically relevant disease; and history of recurrent miscarriage (three or more, even when unexplained) or extrauterine pregnancy in the previous three months. These criteria are similar to those used in the trial that established the efficacy of the original r-hFSH when compared with human menopausal gonadotropins.

Randomization

The subjects enrolled at the investigation sites were randomly assigned to groups in a 1:1 ratio to one cycle treatment with an r-hFSH biosimilar product or original r-hFSH. The computer-generated sequence was concealed until interventions were assigned. The randomization sequence was not stratified.

Objectives and endpoints

The primary efficacy outcome was the number of oocytes retrieved in the per-protocol set, following the EMA guidelines. The hypothesis to be tested was that the r-hFSH biosimilar is non-inferior to the original r-hFSH, with a non-inferiority margin of four oocytes. This margin was established based on the original trials that led to the approval of the original r-hFSH (Bergh *et al*., 1997). When the cycle was cancelled during stimulation, the number of retrieved oocytes was counted as zero.

The secondary efficacy measures were: total dose; number of days of r-hFSH stimulation; patients needing adjustments in the dose of r-hFSH; cycle cancellations and their reasons; number of fertilized oocytes; fertilization rate; number of good quality embryos; number of embryos transferred; implantation rate; biochemical pregnancy rate; clinical pregnancy rate 10 weeks post embryo transfer; and pregnancy outcome. Embryo quality was defined according to the ESHRE Istanbul Consensus criteria (Alpha Scientists in Reproductive Medicine & ESHRE Special Interest Group of Embryology, 2011). Investigators were recommended to transfer up to two embryos to diminish the risk of multiple gestation.

Pregnancy rate per cycle was defined as the number of gestational sacs with fetal heart activity at 10 weeks per total cycles started. Multiple gestation rate was calculated as the number of multiple gestations per total clinical pregnancies.

Pharmacodynamic measurements included number and size distribution of follicles at the start of the treatment; number and size distribution of follicles on the day of hCG injection; number of follicles over 14 mm on the day of hCG injection; serum levels of estradiol, luteinizing hormone, and serum progesterone on the day of hCG injection; number of mature oocytes retrieved; and number of good quality oocytes.

Safety endpoints included: systemic adverse drug reactions (ADR) (including OHSS and its severity) and local ADR, such as pain, bruising, redness, itching, and swelling. Frequent ADRs were defined as ADRs occurred in more than 2% of subjects Safety data were collected from patient diaries and medical records during the subjects' visits to investigation sites. Local pain at the injection site was evaluated by the participants through a visual analog scale (VAS), in which zero indicates no pain and 10 indicates maximum pain.

Immunogenicity

Immunogenicity was evaluated based on the presence of antidrug antibodies (ADAs) and neutralizing antibodies (NAbs) in blood samples during screening, and on days 9 and 84 after the start of r-hFSH treatment. Women receiving more than one treatment cycle of ART were studied at each treatment cycle, allowing the assessment of repeated doses effect on immunogenicity. Antidrug antibodies (ADAs) and NAbs were measured at the Centro de Diagnóstico Molecular S.A., Buenos Aires. Human anti-follicle-stimulating hormone (FSH) antibody levels were

measured with ELISA kits (Human Anti Follicle Stimulating hormone FSH antibody- Mybiosource, San Diego, USA).

Treatments

The subjects were administered the r-hFSH biosimilar with pre-filled multidose syringes. The original r-hFSH was administered with RFF Redi-ject injection pens. After a baseline transvaginal ultrasound (US) performed in cycle day 2 or 3, therapy was initiated at a fixed daily dose of 225 IU, until sufficient follicular development was attained. According to investigator's criteria, adjustments of 75-150 IU were considered after 5 days based on patient response as seen in US monitoring. From then on, dose adjustments could be done every 3 days not exceeding a maximum daily dose of 450 IU.

GnRH antagonist Cetrorelix (Cetrotide® Merck S.A., Ciudad Autónoma de Buenos Aires, Argentina) was administered subcutaneously, at a dose of 0.25 mg/day, from stimulation day 6 until the day of uHCG administration. Urinary HCG (Pregnyl®, MSD Argentina S.R.L., Ciudad Autónoma de Buenos Aires, Argentina) 10,000 IU was administered to complete oocyte maturation when three or more follicles with a mean diameter of 17 mm or greater were attained. The administration of uHCG was withheld to reduce the risk of developing OHSS when the ovaries were abnormally enlarged. Micronized progesterone (Utrogestan® Ferring Argentina S.A., Ciudad Autónoma de Buenos Aires, Argentina) was administered starting on the morning after oocyte retrieval at a daily dose of 600 mg until week 7 of pregnancy or menses. In accordance with local regulations, all medications were provided for free by the sponsoring company.

Other concomitant treatments were allowed (such as enoxaparin) when infertility related to antiphospholid antibodies or thrombophilia were diagnosed, according to local site standards.

Sample size and statistical analysis

The non-inferiority margin for oocyte retrieval was estimated at -4 oocytes, with a power of 80%. With a significance level of 5% (one-sided test), and a 10% dropout rate, at least 44 randomized subjects per group were required to reach the sample size for the primary endpoint (Tajer, 2011).

Statistical data analysis was performed using SPSS (IBM version 16.0). For primary efficacy analyses, the one-sided difference in means test was used. A 95% confidence interval (95% CI) was provided for the estimates. The last observation carried forward approach was selected for missing data in the intention-to-treat (ITT) analysis. The safety parameters were reported using descriptive statistics. Secondary endpoints were not adjusted for multiplicity. Description of categorical variables was made with distribution of absolute and percent frequencies; numerical variables were described based on means, standard deviations and ranges. Student's t test was applied to study numerical variables; the Chi-squared test and Cochran's Q test were applied to study categorical variables.

Ethical considerations

The study was conducted according to the Declaration of Helsinki, the Council for Harmonization Good Clinical Practices Guidelines, and national and state Argentinean regulations. Each study site received approval from its respective institutional ethics committee. The study protocol, research sites, and investigators were approved by the regulatory authority in Argentina, the National Administration of Drugs, Foods, and Medical Devices (ANMAT). Written informed consent was obtained from all patients.

RESULTS

Patient disposition and baseline characteristics

The recruitment period started on April 28, 2015, and the last patient visit was recorded on September 6, 2017. A total of 106 subjects were screened, and 100 were randomized—50 to each arm. Although the estimated sample size was 88 subjects, 100 subjects were randomized to reach the sample size necessary for the pK sub-study. One subject allocated to the r-hFSH biosimilar group did not receive the allocated intervention and two subjects allocated to the original r-hFSH group did not receive the allocated intervention because of spontaneous pregnancy, lack of compliance with protocol inclusion criteria on treatment cycle (n=2), and withdrawal of the informed consent (n=2).

Seven subjects discontinued their participation in the study; none did so for poor tolerance to study drugs. Two subjects treated with the r-hFSH biosimilar (one subject due to oocyte fertilization failure and the other due to informed consent withdrawal) and five subjects treated with original r-hFSH (four due to informed consent withdrawal and one due to failed embryo transfer) dropped out of the study. These subjects completed the stimulation treatment and oocyte retrieval was performed before discontinuation, and for this reason the primary endpoint could be evaluated.

Four subjects treated with the r-hFSH biosimilar and three treated with the original r-hFSH were excluded from primary per-protocol efficacy analysis because of protocol deviations (one case of deviation from the protocol inclusion criteria and the remaining cases underwent dose adjustments during the fixed dose period). Consequently, the population selected for primary endpoint per-protocol, secondary endpoint, and pharmacodynamics analyses included 86 subjects, 45 treated with r-hFSH biosimilar and 41 given original r-hFSH (Figure 1). ITT analysis included all 100 randomized subjects. Safety analysis was performed based on the data of 93 subjects who received at least one dose of the study drugs—49 received the r-hFSH biosimilar and 44 received the original r-hFSH. Immunogenicity was evaluated in the same population.

Both groups were comparable with respect to age, BMI, and duration of infertility. Male factor was the most common cause of infertility in both groups (Table 1).

Efficacy outcomes

The primary efficacy endpoint was the number of oocytes retrieved. In the per-protocol population, the number of oocytes retrieved was 12.6 (SD 7.4, range 2-36) in the r-hFSH biosimilar arm, compared to 13.4 (SD 6.9, range 3-28) in the original r-hFSH arm. The difference was -0.8 (95% CI= -3.82; 2.33), which was within the predetermined non-inferiority margin of -4 oocytes.

The results were confirmed in the ITT population, in which 11.9 (SD 7.5, range 0–36) and 11.6 (SD 7.7, range 0–28) oocytes were retrieved in the r-hFSH biosimilar and the original r-hFSH arms, respectively, with a difference of 0.3 (95% CI = -2.74; 3.30) (Figure 2).

Secondary efficacy endpoints

Ongoing pregnancy at week 10 was achieved by 24.4% (11/45) of the subjects treated with r-hFSH biosimilar and by 19.5% (8/41) of the subjects treated with original r-hFSH (difference: 4.9%, p=0.582). Twenty-one healthy babies were delivered from 19 pregnancies. Two patients had twins, one in each group (9.1% of the subjects in the r-hFSH biosimilar group and 12.5% of the individuals in the original r-hFSH group); there were no high order multiple gestations or ectopic pregnancies.

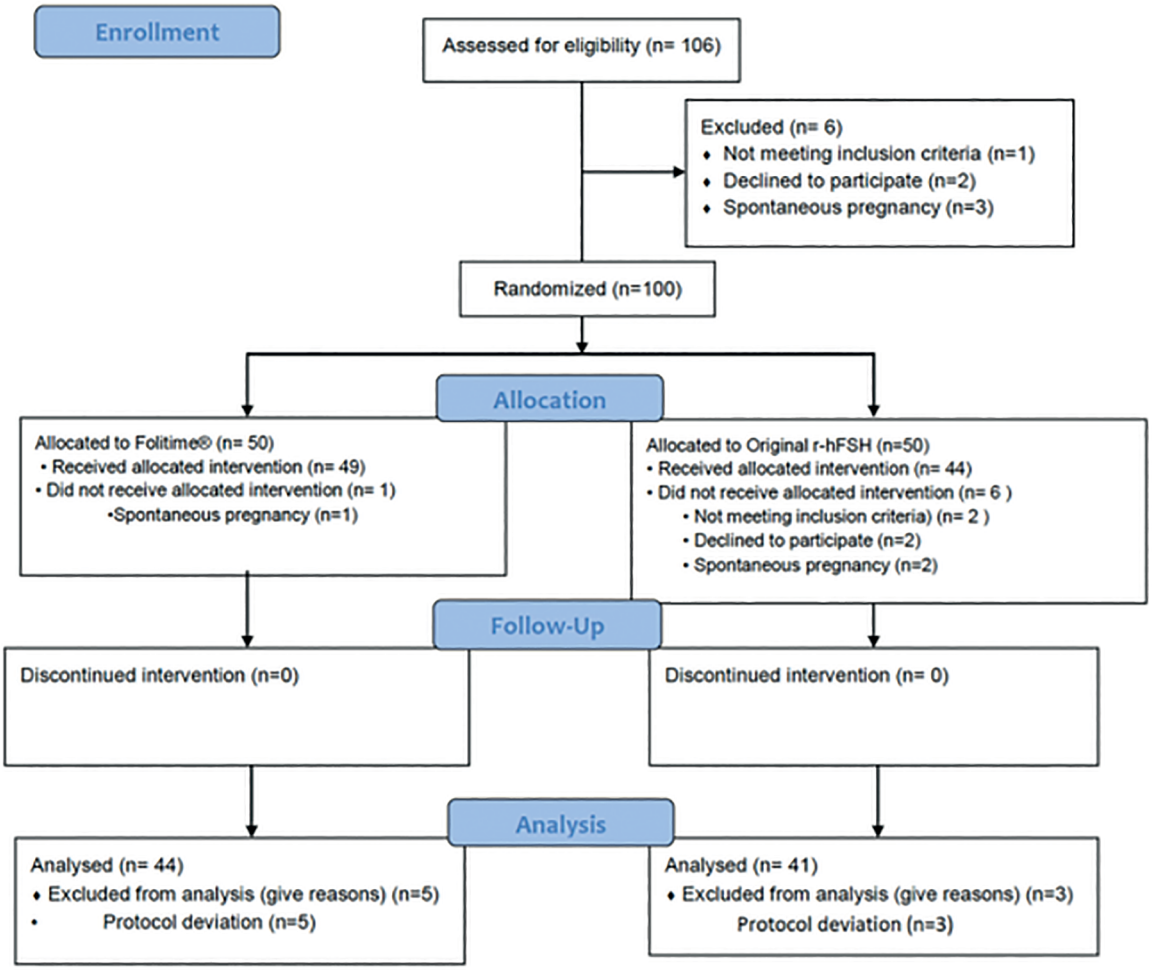

Figure 1. Patient Disposition.

Table 1. Demographic characteristics (subjects given least one dose of the study drugs).

	r-hFSH-BS n=49	**Original α r-hFSH n=44**
Age (y) - Mean (SD); Range	32.9 (3.0); 25-37	32.7 (3.2); 23-37
Weight (kg)- Mean (SD); Range	60.7 (8.2); 48.0-78.3	62.4 (10.5); 41.0-85.0
BMI (kg/m2)- Mean (SD); Range	23.0 (3.1); 18.1-30.4	23.9 (3.2);18.1-31.8
Main cause of infertility- % (n) Unexplained Tubal Male Other	 36.8% (18) 18.4% (9) 40.8% (20) 4.0% (2)	 25% (11) 9.1% (4) 54.5% (24) 11.4% (5)
Previous IVF cycles - % (n) None One-two	 63.3% (31) 36.7% (18)	 77.3% (34) 22.7% (10)
Duration of infertility (months)	51.2 (36.3) 12-216	47.7 (29.9) 0-132(2)

BMI=body mass index; FSH=follicle-stimulating hormone; %= percentage; n=number; SD=Standard Deviation.

There were no statistically significant differences when the following were compared: total dose; mean dose; days of treatment; and proportion of subjects needing dose adjustments (Table 2).

The number of subjects requiring dose increases in the r-hFSH biosimilar group was slightly higher than in the group treated with original r-hFSH (24.4% *vs*. 14.6%); the proportion of subjects requiring dose decreases in the group treated with r-hFSH biosimilar was lower than in the group treated with original r-hFSH (13.3% *vs*. 22%). None of these differences were statistically significant.

The proportion of non-transferred subjects, the mean number of fertilized oocytes, the number of good quality embryos, and the number of transferred embryos were similar in both groups. Table 3 shows the number of transferred embryos per group. The fertilization rate was

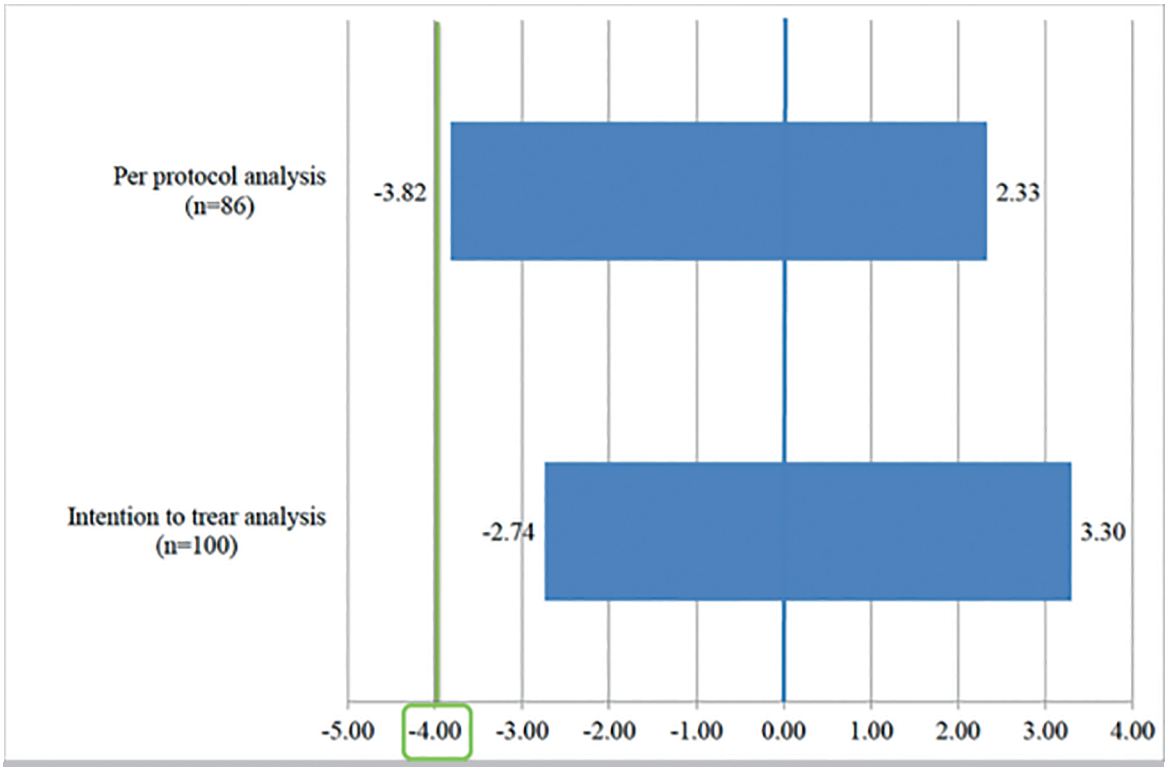

Figure 2. IC 95% for the difference in the mean number of oocytes retrieved by treatment arm. n=number.

Table 2. Secondary efficacy endpoints (per-protocol population).

Secondary Endpoint	r-hFSH-BS n=45	original α r-hFSH n=41	Difference	*p*
Total r-hFSH dose (IU) - Mean (SD); Range	2034 (439); 1200-3400	1965 (373); 1350-3000	69	0.431*
Mean r-hFSH dose (IU) - Mean (SD) Range	230 (20); 169-283	225 (19); 169-273	5	0.277*
Duration of r-hFSH stimulation (days) - Mean (SD) Range	8.8 (1.5); 6-13	8.7 (1.3); 6-11	0.1	0.708*
Total dose adaptation - % (n)	37.8% (17)	36.6% (15)	1.2%	0.909†
Dose increase - % (n)	24.4% (11)	14.6% (6)	8.4%	0.254†
Dose decrease- % (n)	13.3% (6)	22.0% (9)	-8.7%	0.293†
Fertilization rate - % (n)	69.2% (243/351)	73.7% (252/342)	-4.5%	0.100*
Number of fertilyzed oocytes - Mean (SD); Range	5.4 (4.0); 0-16	6.2 (3.7); 0-15	-0.8	0.375*
Good quality embryos (ESHRE) - Mean (SD) Range	0.38 (0.7); 0-2	0.22 (0.7); 0-3	0.16	0.276*
Number of embryos/blastocysts transferred - Mean (SD), Range	1.51 (0.7); 0-2	1.61 (0.7); 0-3	-0.10	0.515*
Implantation rate - % (n)	20.6% (14/68)	15.2% (10/66)	5.4%	0.275*
Biochemical pregnancies - % (n)	8.9% (4)	12.2% (5)	-3.3%	0.617†

**t* Student † Cochran
FSH=follicle-stimulating hormone; SD=Standard Deviation; %=percentage; n=number

slightly higher in the group treated with the original r-hFSH (73.7% *vs*. 69.2%) and the implantation rate was slightly better in the group treated with the r-hFSH biosimilar (20.6% *vs*. 15.2%). These differences were not statistically significant.

Pharmacodynamic results are shown in Table 4. Statistically significant differences were not observed.

Safety

The most common ADRs were headache, abdominal pain, and ovarian pain (Table 5). Twenty-three subjects (46.9%) treated with the r-hFSH biosimilar and 13 (29.5%) treated with the original r-hFSH had frequent systemic ADRs. The mean number of ADRs per subject was 0.9 and 0.7 in the groups treated with r-hFSH biosimilar and original r-hFSH, respectively.

Table 3. Number of transferred embryos (per-protocol population).

Number of transferred embryos	r-hFSH-BS n=45	original α r-hFSH n=41	Difference	*p*
0 (Cycle cancellations)	8.9% (4)	9.8% (4)	-0.9%	0.815*
1	31.1% (14)	24.4% (10)	6.7%	0.650*
2	60.0% (27)	61.0% (25)	-1.0%	0.343*
3	0	4.9% (2)	-5.9%	0.434*

*Chi-squared test.

Table 4. Pharmacodinamic endpoints (per-protocol population).

	r-hFSH biosimilar n=45	original α r-hFSH n=41	Difference	*p*
Number of follicles within 14.1 to 16 mm - Mean (SD); Range	2.8 (2.3); 0-9	2.9 (2.0); 0-9	-0.1	0.947*
Number of follicles >16 mm - Mean (SD); Range	6.0 (3.3); 2-16	5.8 (2.8); 2-14	0.2	0.746*
E2 levels - Mean (SD); Range	1927 (1420); 341-6065	1949 (1294); 619-7250	-22	0.941*
Number of metaphase II oocytes - Mean (SD); Range	8.9 (6.1); 1-29	9.7 (5.3); 3-24	-0.8	0.486*
Number of good quality oocytes - Mean (SD); Range	4.7 (6.1); 0-29	4.7 (4.5); 0-18	0.0	0.972*

*Student's t-test
SD=Standard Deviation

Table 5. Frequent Adverse Drug Reactions (more than 2% of subjects).

	r-hFSH biosimilar n=49	Original α r-hFSH n=44
Subjects % (n)	46.9% (23)	29.5% (13)
Reactions % (n)	42% (0.9)	29 (0.7)
Headache	14.3% (7) 13 reactions	4.5% (2) 2 reactions
Myalgia	4.1% (2) 2 reactions	2.3% (1) 1 reaction
Ovarian pain	14.3% (7) 9 reactions	20.5% (9) 14 reactions
Abdominal pain	16.3% (8) 8 reactions	11.4% (5) 6 reactions
Hematuria	0	2.3% (1) 1 reaction
Insomnia	2.0% (1) 1 reaction	0
Dizziness	4.1% (2) 2 reactions	2.3% (1) 1 reaction
Migraine	0	2.3% (1) 1 reaction
Nausea	4.1% (2) 2 reactions	0
Vulvar pruritus	2.0% (1) 1 reaction	0
Rash	2.0% (1) 1 reaction	0
Breast pain	0	4.5% (2) 3 reactions

n=number.

Most of the ADRs in both groups were mild. Three cases of severe headache were noted among subjects treated with r-hFSH biosimilar and no severe ADRs were observed in the subjects treated with original r-hFSH. One serious ADR occurred in a subject treated with r-hFSH biosimilar. This subject developed late onset mild OHSS on day 15–20 after the administration of hCG and had deep venous thrombosis in the left deep jugular vein on week 10 after administration, during her twin pregnancy. The patient was hospitalized for three days and treated with enoxaparin 120 mg/day until delivery on week 28. Both were live births.

Another subject treated with the r-hFSH biosimilar developed ovarian hyperstimulation on day 8 of treatment after the dose was decreased on day 6 from 225 to 75 IU/day; the embryo transfer was thus cancelled.

A total of 110 mild injection site reactions were observed in 29 subjects (59.2%, 2.2 ADRs per subject) treated with r-hFSH biosimilar, while 48 mild injection site reactions occurred in 20 subjects (45.5%, 1.1 local ADRs) treated with original r-hFSH. The most common injection site reactions were erythema and ecchymosis. These reactions did not cause the discontinuation of treatment.

Local pain was greater in subjects treated with original r-hFSH; however, this difference was not statistically significant (Table 6).

Immunogenicity

None of the subjects treated with r-hFSH biosimilar or original r-hFSH developed antibodies against the studied drugs.

DISCUSSION

To our knowledge, this is the first randomized clinical trial of an ovulation induction treatment with an r-hFSH biosimilar fully developed and manufactured in Latin America used in IVF procedures. In this non-inferiority trial, the primary endpoint confirmed that the r-hFSH biosimilar was non-inferior to the original r-hFSH. The established non-inferiority margin was -4 oocytes. While regulatory authorities recommend equivalence trials, non-inferiority trials may be performed, if previously justified (EMA, 2014a; FDA, 2015). In these studies, there is a risk of obtaining a superior result with the experimental drug in comparison to the reference one with an increase in ADRs. This risk is not possible with original r-hFSH, which has a wide therapeutic range.

Since the r-hFSH biosimilar was administered in prefilled syringes and the original r-hFSH was administered with RFF Redi-ject injection pens, a double-blind study was not feasible. For this reason, in accordance with EMA guidelines, blinded assessment of study outcomes that might be particularly affected by subjective factors, such as ultrasound examinations and parameters of oocyte/embryo quality, was carried out.

Clinically significant differences related to secondary efficacy endpoints were not found. Fertilization and implantation rates were comparable in both groups. According to EMA guidelines, ongoing pregnancy rate at 10 weeks after embryo transfer is also an acceptable primary non-inferiority demonstration endpoint. Pregnancy rates at week 10 and live birth rates were slightly higher with r-hFSH biosimilar. These differences were not statistically significant.

Both products showed similar safety profiles. The ADRs and serious ADRs observed in this trial were expected for these products and no new ADRs were reported. According to the label of Gonal-f® (EMD Serono, Inc., 2013), the most frequent ADRs observed in clinical trials in infertile women undergoing ART (IVF or ICSI) were headache in 18.6% and abdominal pain in 23.2% of the subjects. In the subjects administered r-hFSH biosimilar, headache was observed in 14.3% and abdominal pain in 16.3% of the subjects.

Two subjects (4.1%) given r-hFSH biosimilar had OHSS (one had late OHSS). No cases of OHSS were observed in the

Table 6. Local pain.

Pain	r-hFSH biosimilar	Original α r-hFSH	Difference	*p*
Day 1 Mean (SD) Range (n)	1.76 (3.2) 0-18 (n=49)	1.93 (3.6) 0-18 (n=44)	-0.17	0.801*
Day 2 Mean (SD) Range (n)	2.94 (7.4) 0-49 (n=49)	3.84 (10.0) 0-64 (n=44)	-0.90	0.621*
Day 3 Mean (SD) Range (n)	2.63 (4.1) 0-17 (n=49)	3.59 (10.0) 0-62 (n=44)	-0.96	0.540*
Day 4 Mean (SD) Range (n)	3.08 (6.7) 0-41 (n=49)	4.36 (12.7) 0-66 (n=44)	-1.28	0.53*
Day 5 Mean (SD) Range (n)	2.98 (5.7) 0-25 (n=49)	3.07 (7.1) 0-40 (n=44)	-0.09	0.947*
Day 6 Mean (SD) Range (n)	2.59 (6.0) 0-34 (n=46)	2.93 (11.2) 0-73 (n=42)	-0.34	0.857*
Day 7 Mean (SD) Range (n)	3.64 (9.9) 0-62 (n=42)	2.63 (10.5) 0-65 (n=38)	1.01	0.659*
Day 8 Mean (SD) Range (n)	1.87 (2.9) 0-12 (n=31)	4.14 (10.9) 0-51 (n=22)	-2.27	0.273*
Day 9 Mean (SD) Range (n)	1.93 (5.3) 0-20 (n=14)	1.58 (2.1) 0-7 (n=12)	0.35	0.835*
Day 10 Mean (SD) Range (n)	0.30 (0.5) 0-1 (n=10)	0.80 (0.8) 0-2 (n=5)	-0.50	0.161*
hCG Day Mean (SD) Range (n)	2.78 (7.8) 0-52 (n=49)	5.32 (14.3) 0-65 (n=44)	-2.54	0.284*

*Student's t-test; SD=Standard Deviation; N=number.

subjects treated with original r-hFSH. The label of Gonal-f® reports a rate of 4.6% for cases of ovarian hyperstimulation in clinical trials with infertile women undergoing ART. The same proportion is reported for Ovaleap® in the original clinical trial submitted to EMA authorities (CITA).

A total of 110 mild injection site reactions developed in 29 subjects (59.2%, 2.2 ADRs per subject) treated with r-hFSH biosimilar. It is important to take into account that the r-hFSH biosimilar was administered using pre-filled syringes and the original r-hFSH was administered with RFF Redi-ject injection pens. The original study performed with Bemfola® submitted to EMA authorities reported that 20.9% of the subjects had ecchymosis and 23.7% had erythema. Regarding immunogenicity, the r-hFSH biosimilar did not increase the risk of developing ADAs in comparison with original r-hFSH.

There are certain limitations to this study. The first is the small number of patients enrolled. Although the small sample size did not affect the evaluation of the efficacy of the r-hFSH biosimilar, only limited information about its safety could be obtained.

The second limitation is the homogeneity of the enrolled population, since the study was conducted entirely in Argentina. However, the study was performed in four different provinces with their own ethnic peculiarities. The Argentinian population includes a mixed-descent of European immigrants and Native Americans, making its population comparable with many other Latin American and European countries.

Since 2011, Argentina has had specific local regulation for registering biosimilar products (ANMAT, 2011). Comparability exercises and non-clinical data are required for each product. Clinical data may be required depending on the product to be approved. The r-hFSH biosimilar in question (Folitime®) has been approved by the Argentinean health authority based on analytical comparability and preclinical studies showing similar results for the r-hFSH biosimilar and the original r-hFSH and the data from phase III study described above.

In conclusion, based on the results of this RCT, the r-hFSH biosimilar administered in this study (Folitime®) is non-inferior to the original r-hFSH, and there are no apparent differences in their safety profiles. Our findings suggest that the capacity of third world countries in general and Latin-American nations in particular to fully develop biosimilar products and bring them to market might help remove the enormous inequity in the access to biological drugs that patients in these countries are very often confronted with, compared to individuals living in the developed world.

ACKNOWLEDGEMENT

We would like to thank Editage (www.editage.com) for English language editing**.**

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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