Original Article

A Multicenter Phase IV Study to Investigate the Immunogenicity of Recombinant Human Follicle-Stimulating Hormone and Its Impact on Clinical Outcomes in Females Undergoing Controlled Ovarian Stimulation

Abha Majumdar, Le Hoang[1], Ly T. Loc[2], Padma Srivastava[3], Chitra Ramamurthy[4], Ratnabali Chakravorty[5], Yogeshwar S. Nandanwar[6], Rashmi M. D[7], Rahul V. Mayekar[6], Jayashree Sridhar[8], Ganesh H. Divekar[9], James John[9]

Department of Obstetrics and Gynaecology, Sir Ganga Ram Hospital, New Delhi, [3]Department of Obstetrics and Gynaecology, Inamdar Multispeciality Hospital, Pune, [6]Department of Obstetrics and Gynaecology, Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai, [9]Department of Clinical Research and Pharmacovigilance, Bharat Serums and Vaccines Limited, Navi Mumbai, Maharashtra, [4]Department of Obstetrics and Gynaecology, Apollo Hospitals, Bengaluru, [7]Department of Obstetrics and Gynaecology, Apollo BGS Hospitals, Mysore, Karnataka, [5]Department of Obstetrics and Gynaecology, ILS Hospitals, Kolkata, West Bengal, [8]Department of Obstetrics and Gynaecology, Choithram Hospital and Research Centre, Indore, Madhya Pradesh, India, [1]Center for Assisted Reproduction, National Hospital of Obstetrics and Gynecology, Hanoi, [2]Infertility Department, Hung Vuong Hospital, Ho Chi Minh City, Vietnam

Abstract

Context: Therapeutic proteins can cause immune responses, which may have clinical implications. **Aims:** The aim of the study was to assess the immunogenicity of recombinant human follicle-stimulating hormone (r-hFSH), when used for controlled ovarian stimulation (COS). **Settings and Design:** Prospective, multicenter study conducted at reproductive medicine clinics in India and Vietnam. **Materials and Methods:** A total of 285 women, aged 20–40 years, undergoing 354 COS cycles for either intrauterine insemination (IUI) or *in vitro* fertilization (IVF) were studied. The primary outcome measure was the incidence of development of anti-drug antibodies (ADA) and their neutralization potential. Other outcome measures were follicle development, dose and duration of r-hFSH, positive serum pregnancy test, clinical pregnancy, cycle cancellation, and adverse events (AEs). **Statistical Analysis Used:** A sample size of 250 was planned. Descriptive statistics are presented. **Results:** Four patients tested positive for ADA after r-hFSH administration at different time points; all of them tested negative, subsequently. None were found to have neutralization potential. The mean dose and duration of r-hFSH were 816 IU and 8.1 days in IUI and 2183 IU and 9.5 days in IVF, respectively. The serum and clinical pregnancy rates were 12.4% and 11.6% in IUI and 32.7% and 29.9% in IVF cycles, respectively. Seven AEs were reported, including two cases of ovarian hyperstimulation syndrome; two AEs were judged to be serious. **Conclusions:** The tested r-hFSH has very low immunogenic potential and did not lead to the development of neutralizing antibodies. The overall efficacy and safety of the drug were in-line with existing literature data, and no specific clinical impact of immunogenicity could be identified.

Keywords: *Antidrug antibody, assisted reproductive technique, immunogenicity, in vitro fertilization, infertility, intrauterine insemination*

Address for correspondence: Dr. Rahul V. Mayekar, Department of Obstetrics and Gynaecology, Lokmanya Tilak Municipal Medical College and General Hospital, Mumbai - 400 022, Maharashtra, India.
E-mail: dr.rvmayekar123@gmail.com

Received: 06-03-2019 **Revised:** 25-06-2019
Accepted: 01-11-2019 **Published:** 17-12-2019

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/jhrs.JHRS_33_19

How to cite this article: Majumdar A, Hoang L, Loc LT, Srivastava P, Ramamurthy C, Chakravorty R, *et al.* A multicenter phase iv study to investigate the immunogenicity of recombinant human follicle-stimulating hormone and its impact on clinical outcomes in females undergoing controlled ovarian stimulation. J Hum Reprod Sci 2019;12:303-9.

Introduction

Exogenous proteins, including therapeutic ones, are immunogenic and have the potential to cause antibody formation; the tendency of a protein to mount such a response is referred to as immunogenicity, which can be long-term and can lead to immunological memory. Development of such antibodies may lead to failure of a drug's development during clinical studies or may interfere with efficacy or lead to serious complications in the postmarketing phase.[1,2]

Preparations of recombinant human follicle-stimulating hormone (r-hFSH) are used for controlled ovarian stimulation (COS) in infertile females prior to the performance of intrauterine insemination (IUI) or *in vitro* fertilization (IVF). FSH preparations are, in general, considered to have low immunogenic potential.[3] However, biological drugs are complex and variable in structure, and their manufacture involves complex biotechnological processes, making them quite sensitive to changes in manufacturing processes. Another contributing factor is that different manufacturers use different molecular clones and cell banks and may have different fermentation and purification processes.[4] Thus, different preparations of the same biological drug may vary in terms of purity, potency, and immunogenicity. The present study was envisaged as a prospective, multi-center clinical study to assess the immunogenicity of a r-hFSH preparation in patients with infertility, when used for COS as part of one, two, or three successive cycles of either IUI or IVF.

Materials and Methods

Study design

This was a prospective, multicenter, open-label, controlled study to assess the immunogenicity of an r-hFSH preparation (Foligraf®, manufactured by Bharat Serums and Vaccines Limited, Mumbai, India). Although the choice of gonadotropin (only r-hFSH) and the minimum and maximum dose of r-hFSH was fixed, the choice of IUI/IVF and other treatment protocols was at the investigator's discretion. The study was conducted at 12 centers (ten centers in India and two centers in Vietnam).

The study protocol was approved by the Indian and Vietnamese drug regulatory authorities and the institutional ethics committees of all the participating centers. The study was registered on Clinical Trials Registry-India (CTRI/2014/08/004886). The study was performed in accordance with the principles of the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulatory requirements. All participants provided written informed consent.

Study participants

Premenopausal women aged 20–40 years with infertility requiring COS as a part of one, two, or three successive cycles, of either IUI or IVF, were eligible for the study. Additional main inclusion criterion was the presence of normal reproductive tract anatomy compatible with pregnancy. The main exclusion criteria were history of receiving injectable gonadotropins within the past 3 months; severe endometriosis; pelvic pathology or chronic systemic disease that would compromise pregnancy; pregnancy, lactation, or contraindication to pregnancy; history of abuse of alcohol or drugs; history of tumors of the ovary, breast, adrenal gland, pituitary, or hypothalamus and malformation of sexual organs incompatible with pregnancy; and history of hypersensitivity to any gonadotropin. A minimum of 250 patients was planned to be included in the study.

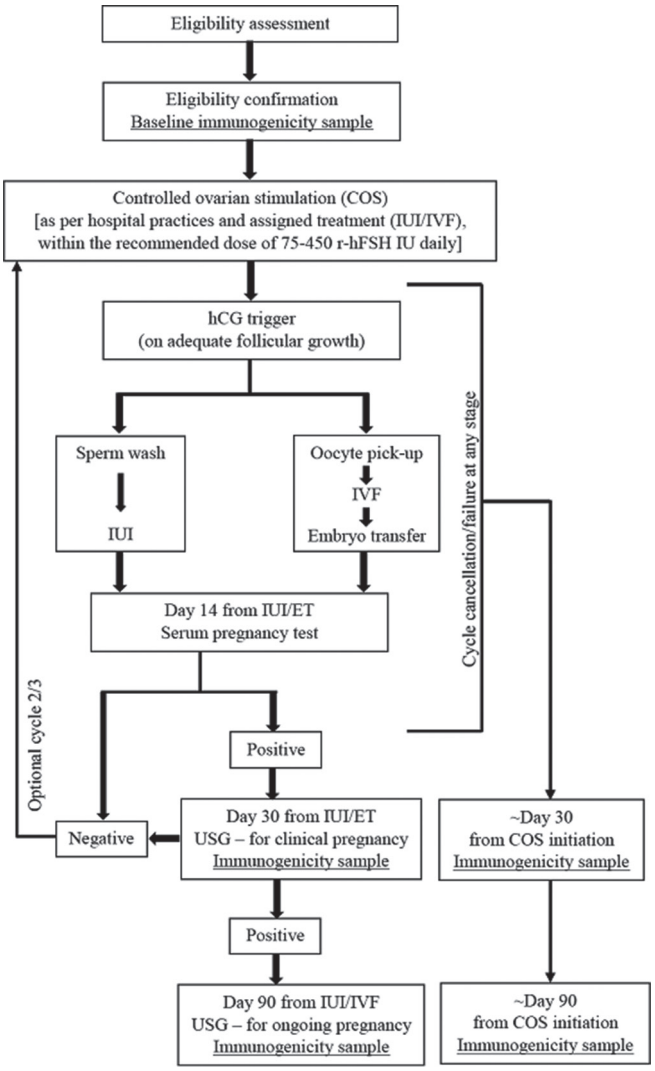

Figure 1: Order of study activities. IUI = Intrauterine insemination, IVF = *In vitro* fertilization, ET = Embryo transfer, USG = Ultrasonography

Study flow

The order of the study activities is depicted in Figure 1.

Study outcomes

The primary outcome measure was the incidence of development of anti-drug antibodies (ADA) and their neutralization potential. The secondary outcome measures included follicles >16 mm, total dose and duration of r-hFSH, biochemical (serum β-human chorionic gonadotropin [hCG] test) pregnancy rate, clinical pregnancy rate, cycle cancellation rate, and incidence of adverse events (AEs). The efficacy outcomes were analyzed separately for patients assigned to IUI and IVF treatments.

ADA testing method

1. Detection of ADA – A radioimmunoprecipitation assay was developed at Bioanalytical Laboratory, Syngene International Limited, Bengaluru, India, for the detection of ADA in human serum. The method was validated following the current regulatory guidelines[5-7] by the United States Food and Drug Administration and European Medicines Agency (EMA). The assay cutpoint (for binding percentage) was determined statistically from the level of binding seen with the use of negative controls during assay validation and was based on a targeted false-positive rate of 5%

The assay involved the following:

- Screening assay – Here, the aim was to determine the presence of antibodies based on their ability to recognize the relevant antigenic determinants in the therapeutic protein.[8] It involved incubation of positive control antibodies/ADA prepared in human serum with Iodine-125 (^{125}I)-labeled r-hFSH. Precipitation of the antigen–antibody complex was accomplished by addition of polyethylene glycol (PEG) to precipitate the ^{125}I-labeled FSH/anti-FSH antibody complexes. The radioactivity in the bound fraction was measured using a gamma counter as counts per minute, and the results were expressed as a binding percentage. The binding percentage was directly proportional to the concentration of anti-FSH antibodies in the sample
- Confirmatory assay – This step aimed to minimize false-positive results from the initial screening.[8] It followed the same principle as the screening assay, but with an additional competitive step, in which the positive controls and study samples were retested following prior incubation with and without the excess drug (r-hFSH). Due to the prior incubation, the ADA was no longer freely available. These mixtures were further incubated with ^{125}I-labeled r-hFSH. After precipitation of the antibody complexes with PEG, the radioactivity in the bound fraction was measured using a gamma counter. As compared to positive controls, which were not incubated with the drug, positive controls and test samples incubated with the drug showed reduction in binding percentage. Test samples with values above the assay cutpoint were confirmed positive

2. Antibody titer check – The samples, which were confirmed positive with the detection assay, were then checked for antibody titer. The reciprocal of the highest dilution of the sample with a response that remained above the assay cutpoint was considered as the antibody titer
3. Assessment of neutralization potential of ADA – The ADA-positive samples are required to be further characterized to check if they are capable of neutralizing the biological function of the drug.[8] In many cases, the ADA raised against a drug may not neutralize the biological activity of the drug, and such ADA does not interfere with the efficacy of the drug. Hence, it is essential to evaluate the ADA-positive samples for neutralizing potential in a functional assay.

   The positive ADA samples were further characterized for binding of ADA to the drug receptor and to evaluate if the ADA were neutralizing or nonneutralizing. This was achieved by a cell-based radioimmunoassay, developed and validated at Chelatec SAS, France. This competitive binding assay used transfected Chinese Hamster Ovary cells expressing a receptor for FSH and ^{125}I-labeled r-hFSH as a tracer and was aimed to evaluate the clinical relevance of any persistent ADA response. The assay was validated for various assay parameters as per the international guidelines before testing the samples tested positive for ADA.[5]

   The presence of neutralizing antibodies in the test serum samples was expected to decrease the binding of the ^{125}I-labeled r-hFSH to the receptor, giving rise to reduction in the radioactivity. The binding and neutralization of radiolabeled drug and the neutralization assay were optimized using a polyclonal anti-FSH antibody raised in rabbits.

Statistics

According to the EMA guidelines[9] on r-hFSH, the immunogenicity of r-hFSH is low, and neutralizing antibodies have not been reported. Hence, a pragmatic sample size of 250 was planned for the study. Qualitative data are presented in the form of frequency,

and percentage and quantitative data are represented in the form of mean ± standard deviation (SD) or median (interquartile range) as per the distribution of the variable, with the former used in case of data with normality and the latter in the absence of the same.

Results

Patient characteristics and disposition

The study was conducted between September 26, 2014, and April 28, 2016. A total of 293 women were screened across the 12 sites, of whom 285 were enrolled and exposed to the study drug. Out of these, 180 were assigned to IUI, and 105 were assigned to IVF treatment. A total of 234 patients underwent one treatment cycle, while 33 and 18 patients underwent two and three treatment cycles, respectively. Thus, the total number of treatment cycles included in the study was 354. The mean (± SD) age and body mass index of patients were 30.1 ± 4.54 years and 23.1 ± 4 kg/m^2, respectively. Seventeen percent of the patients were aged above 35 years, and 27% of the patients had a previous history of failed COS-infertility treatment cycle (s). The mean duration of infertility was 5 ± 3.8 years. The participant flow is depicted shown in Figure 2. Demographics and baseline characteristics are provided in Table 1.

Immunogenicity results

Four patients tested positive for anti-r-hFSH antibodies after the administration of r-hFSH at different time points; all of these subsequently turned negative [Table 2]. None of these samples tested positive for neutralizing anti-r-hFSH antibodies. None of these patients developed any hypersensitivity reaction or were judged to have reduced response to r-hFSH.

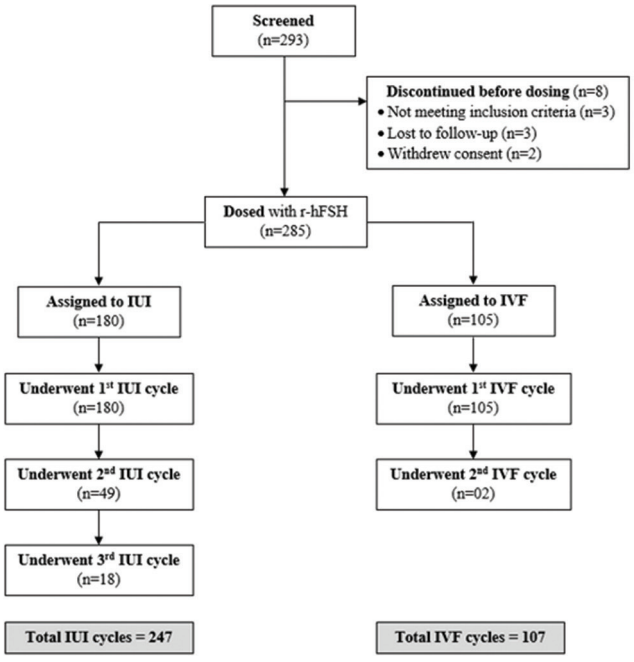

Figure 2: Participant flow in the study. r-hFSH = Recombinant human follicle-stimulating hormone, IUI = Intrauterine insemination, IVF = *In vitro* fertilization

The clinical course and time points in the patients who seroconverted during the study are summarized in Table 3.

Efficacy results

The efficacy results in the IUI and IVF cycles are summarized in Table 4. The mean total dose and duration of r-hFSH treatment used for ovarian stimulation in IUI cycles were 816 (±543) IU and 8.1 (±3.6) days, respectively. The average number of follicles with diameter >16 mm at the end of ovarian stimulation was 1.7 (±1.6). In 202 (83.5%) of the cycles, there was development of at least 1 follicle >16 mm in diameter. Thirty (12.4%) IUI cycles showed biochemical pregnancy test (serum β-hCG test) positive on day 14 after IUI procedure, while 28 (11.6%) of these showed clinical pregnancy result in positive identified by ultrasonography (USG) at day 30 after IUI procedure.

The mean total dose and duration of r-hFSH treatment used for ovarian stimulation in IVF

Table 1: Demographics and relevant baseline characteristics

Parameter	Value
Age (years), mean±SD	30.1±4.5
Weight (kg), mean±SD	55.7±10.4
Height (cm), mean±SD	155.3±6.4
BMI (kg/m^2), mean±SD	23.0±4.2
Infertility duration (years), mean±SD	5.0±3.8
Reason of infertility, *n* (%)	
Unexplained	145 (51)
Female factor	85 (30)
Male factor	48 (17)
Male + female factor	5 (2)
History of failed COS cycles, *n* (%)	77 (27)

SD=Standard deviation, BMI=Body mass index, COS=Controlled ovarian stimulation

Table 2: Immunogenicity results

Particular	Value
Total individuals (*n*)	285
Individuals with at least one postdrug ADA detection, *n* (%)	4 (1.4)
Individuals with ADA detection at day 30	2
Individuals with ADA detection at day 90	2
Individuals with ADA with neutralizing potential	0

ADA=Antidrug antibody

Table 3: Clinical course in individuals with antidrug antibody

S No.	Demography and medical history	Immunogenicity findings (ADA: Positive/negative)	Clinical course
1	22-year-old female	Baseline: Negative	Had 2 follicles >16 mm by day 6
	Infertility for 4 years	Day 30: Positive	Received hCG trigger on day 6
	History: 1 IUI cycle with CC; No gonadotropin use	Day 90: Negative	Underwent IUI on day 8
	Assigned to IUI		Serum pregnancy test negative
	r-hFSH 75 IU/day for 5 days (cumulative: 375 IU)		No adverse or local reactions
2	25-year-old female	Baseline: Negative	IUI cancelled due to surgery for endometrial polyp and uterine adhesions
	Infertility for 1 year	Day 30: Negative	
	History: No IUI or IVF; No gonadotropin use	Day 90: Positive	No adverse or local reactions
	Assigned to IUI	Follow-up (Day 270): Negative	
	r-hFSH 75 IU/day for 4 days (cumulative: 300 IU)		
3	34-year-old female	Baseline: Negative	Had 1 follicle >16 mm by day 8
	Infertility for 3 years	Day 30: Negative	Received hCG trigger on day 8
	History: 2 failed IUIs with CC; No gonadotropin use	Day 90: Positive	Underwent IUI on day 10
	Assigned to IUI	Follow-up (Day 228): Negative	Menses on day 22
	r-hFSH 75 IU/day for 8 days (cumulative: 600 IU)		No adverse or local reactions
4	31-year-old female	Baseline: Negative	1st cycle
	Infertility for 5.5 years	Day 30 (1st cycle): Negative	Had 1 follicle >16 mm by day 10
	History: 6 failed IUIs with CC; No gonadotropin use	Day 30 (2nd cycle): Positive	Spontaneous ovulation on day 12
	Assigned to IUI	Day 90 (1st cycle): Negative (Day 60 of 2nd cycle)	Underwent IUI on day 12
	1st cycle		Serum pregnancy test negative
	r-hFSH 150 IU/day for 10 days (cumulative: 1500 IU)		No adverse or local reactions
	2nd cycle		2nd cycle
	r-hFSH 150-225 IU/day for 12 days (cumulative: 2175 IU)		No follicle development
			No adverse or local reactions

ADA=Antidrug antibody, CC=Clomiphene citrate, IUIs=Intrauterine inseminations, IVF=*in vitro* fertilization, r-hFSH=Recombinant-human follicle-stimulating hormone, hCG=Human chorionic gonadotropin

cycles were 2183 (±869) IU and 9.5 (±2.2) days, respectively. The average number of follicles with diameter >16 mm at the end of ovarian stimulation was 5.1 (±3.5). Thirty-five (32.7%) IVF cycles showed biochemical pregnancy test (serum β-hCG test) positive at day 14 after embryo transfer (ET), while 32 (29.9%) of these showed clinical pregnancy result in positive identified by USG at day 30 after ET.

Safety results

A total of seven AEs were reported by six patients [Table 5]. Out of seven AEs, five were graded as mild, one was moderate, and one was severe. There were three AEs, which were reported as serious since they required hospitalization of the patients. These included one case of ovarian hyperstimulation syndrome (OHSS), ectopic pregnancy, and viral fever. The patient with ectopic pregnancy required endoscopic removal of the ectopic growth, which resulted in the removal of a section of her right fallopian tube. Two AEs (both OHSS) were judged to be related to the study treatment. One of these patients developed mild OHSS with mild abdominal pain and bloating which subsided with symptomatic medication. The other patient developed moderate OHSS with moderate abdominal pain and abdominal distension and was hospitalized and administered oral fluids and intravenous colloids for a day. All the AEs were resolved.

Discussion

Based on existing literature on immunogenicity of FSH (including r-hFSH), the anticipated incidence of ADA to r-hFSH was very low. The results reported here confirm the low immunogenic potential of the tested preparation of r-hFSH and provide further evidence for the efficacy and safety of this preparation.

Although not routinely investigated in clinical practice, an immune response could be responsible for unexpected clinical outcomes. The clinical manifestations may include loss of efficacy, neutralization of the natural counterpart, and immune effects such as allergy, anaphylaxis, or serum sickness.[2,10,11] The development of such antibodies is usually exposure dependent, and the risk increases in line

with several factors, such as time on treatment, changes in the drug formulation, alterations to the protein structure, and the manufacturing process, variations between batches of the drug, the route of administration, the dose level, and the frequency of dosing.[6]

In the present study, ADA was detected in only 1.4% (4 out of 285) of the enrolled patients after the administration of r-hFSH, with none of them identified to be neutralizing in nature. Notably, the development of these ADA was transient in all patients, and further testing showed the absence of ADA in all these patients. Further assessment of the patients with detected ADA in relation to their efficacy, and safety data also did not reveal any impact of the ADA. None of these patients developed any adverse effect or local injection site reactions. Three of the four patients, who completed COS, achieved the development of mature follicle for further IUI procedure; the cycle was canceled in the fourth patient for the performance of a surgery and could not be assessed for efficacy.

The present study, being a phase 4 study, did not have stringent controls on the regimen of r-hFSH used for COS, which was essentially left at the investigator's clinical judgment. The average success rate with IUI is considered to be around 13%.[12] A 2014 meta-analysis reported clinical pregnancy rates ranging from 7.6% to 22.6% per cycle in patients, who received r-hFSH for COS-IUI.[13] A retrospective study with 2019 IUI cycles from France reported a pregnancy rate of 14.8%.[14] A recent retrospective analysis of 800 IUI cycles from a tertiary center in India reported a clinical pregnancy rate of 14.1%.[15] The clinical pregnancy rate of 11.6% in IUI cycles observed in this study is in line with the literature-reported data. With respect to success rates in IVF, a 2011 Cochrane review of randomized controlled studies comparing r-hFSH versus urinary gonadotrophins in ART cycles reported clinical pregnancy rates ranging from 16.7% to 56% with r-hFSH, with most studies reporting a rate around 24%–27%.[16] The clinical pregnancy rate of about 30% with IVF in the present study is in conformity with these data.

OHSS (one mild and one moderate) was reported in only two (0.7%) patients assigned to IVF treatment. There was one case of ectopic pregnancy, another complication known to occur with higher frequency in those undergoing ART. The only other serious AE reported was a case of viral fever, which resolved with symptomatic treatment and was judged to be unrelated to the treatment procedure. There were three other AEs reported, all of which were nonserious and were deemed unrelated by the investigators.

We acknowledge that the fixed time points for immunogenicity assessment may fail to identify all patients who develop an antibody response, as there can be wide variations in the onset and duration of such a response; and continuing pharmacovigilance will be needed.

Table 4: Efficacy data

Parameters	Assigned treatment	
	IUI	IVF
Number of individuals (*n*)	180	105
Number of cycles (*n*)	242	107
Results		
Dose of r-hFSH (IU), mean±SD	817±542	2183±869
Duration of r-hFSH (days), mean±SD	8.1±3.6	9.5±2.2
Number of follicles >16 mm (*n*), mean±SD	1.7±1.6	5.1±3.5
Cycles with at least 1 follicle >16 mm, *n* (%)	202 (83.5)	*
Biochemical (serum) pregnancy positive, *n* (%)	30 (12.4)	35 (32.7)
Clinical pregnancy, *n* (%)	28 (11.6)	32 (29.9)
Cycle cancellation, *n* (%)	66 (26.7)	21 (19.6)

*Not suitable as efficacy endpoint for IVF cycles. Percentages have been calculated with number of cycles in each assigned treatment group as denominator. IUI=Intrauterine insemination, IVF=*In vitro* fertilization, r-hFSH=Recombinant-human follicle-stimulating hormone, SD=Standard deviation

Conclusions

The results of this study support the low immunogenic potential of the r-hFSH, consistent with existing immunogenicity data for r-hFSH. The overall safety and

Table 5: Safety data

Adverse event		Number of events
System organ class*	Preferred term*	
Skin and subcutaneous tissue disorders	Swelling face	1
Endocrine disorders	OHSS	2
Infections and infestations	Viral infection	1
Pregnancy, puerperium, and perinatal conditions	Ectopic pregnancy	1
Gastrointestinal disorders	Abdominal distension	1
Respiratory, thoracic, and mediastinal disorders	Dyspnea	1
Total		7

*As per MedDRA. OHSS=Ovarian hyperstimulation syndrome, MedDRA=Medical dictionary for regulatory authorities

efficacy profile for the tested preparation is also in line with that available in the literature, and no new safety concerns were identified.

Financial support and sponsorship

This study was financially supported by Bharat Serums and Vaccines Limited.

Conflicts of interest

Ganesh H. Divekar and James John are employees of Bharat Serums and Vaccines Limited, India.

References

1. Brinks V, Jiskoot W, Schellekens H. Immunogenicity of therapeutic proteins: The use of animal models. Pharm Res 2011;28:2379-85.
2. Schellekens H. Factors influencing the immunogenicity of therapeutic proteins. Nephrol Dial Transplant 2005;20 Suppl 6:vi3-9.
3. Morte C, Celma C, De Geyter C, Urbancsek J, Coroleu Lletget B, Cometti B. Assessment of the immunogenicity of gonadotrophins during controlled ovarian stimulation. Am J Reprod Immunol 2017;78:1-5.
4. Mason J. Introduction of biosimilars: Not to be confused with generics. Prescriber 2013;24:7-8.
5. FDA/CDER/CBER. Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products – Guidance for Industry. FDA; 2016.
6. European Medicines Agency. Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins (EMEA/CHMP/BMWP/14327/2006); 2007.
7. FDA. Guidance for Industry Assay Development for Immunogenicity Testing of Therapeutic Proteins Guidance for Industry Assay Development for Immunogenicity Testing of Therapeutic Proteins. Draft Guid; 2009.
8. Wadhwa M, Knezevic I, Kang HN, Thorpe R. Immunogenicity assessment of biotherapeutic products: An overview of assays and their utility. Biologicals 2015;43:298-306.
9. 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). Guideline on Non-Clinical and Clinical Development of Similar Biological Medicinal Products Co. UK Eur Med Agency; 2011. p. 1-7.
10. Gunn GR 3rd, Sealey DC, Jamali F, Meibohm B, Ghosh S, Shankar G. From the bench to clinical practice: Understanding the challenges and uncertainties in immunogenicity testing for biopharmaceuticals. Clin Exp Immunol 2016;184:137-46.
11. Valor L, De La Torre I. Understanding the immunogenicity concept. Reum Clin 2013;9:1-4.
12. Bahadur G, Homburg R, Muneer A, Racich P, Alangaden T, Al-Habib A, *et al.* First line fertility treatment strategies regarding IUI and IVF require clinical evidence. Hum Reprod 2016;31:1141-6.
13. Luo S, Li S, Jin S, Li Y, Zhang Y. Effectiveness of gnRH antagonist in the management of subfertile couples undergoing controlled ovarian stimulation and intrauterine insemination: A meta-analysis. PLoS One 2014;9:e109133.
14. Dinelli L, Courbière B, Achard V, Jouve E, Deveze C, Gnisci A, *et al.* Prognosis factors of pregnancy after intrauterine insemination with the husband's sperm: Conclusions of an analysis of 2,019 cycles. Fertil Steril 2014;101:994-1000.
15. Wadhwa L, Fauzdar A, Wadhwa SN. An intrauterine insemination audit at tertiary care hospital: A 4½ years› retrospective analysis of 800 intrauterine insemination cycles. J Hum Reprod Sci 2018;11:279-85.
16. 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.