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RESEARCH ARTICLE

EVALUATION OF THERAPEUTIC RECOMBINANT FOLLICLE STIMULATING HORMONE BATCHES FOR THEIR IDENTITY AND POTENCY BEFORE USE IN PATIENTS

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ABSTRACT

Follicle stimulating hormone (FSH) is a gonadotropic hormone produced by the anterior lobe of the mammalian pituitary gland which regulates the normal female and male gamete growth and maturation and normal gonadal steroid production. Deficient endogenous production of FSH is a known cause of infertility and therapeutic preparations of FSH are widely used in the treatment of this condition. In this study, 27 batches of therapeutic rh-FSH preparations were evaluated for their identity and potency before the batch release for use in patients, by using rat ovary weight gain assay as described in the European Pharmacopoeia. The results were calculated by using the Parallel-line assay; COMBISTATS v 4.0 software from EDQM. Based on the findings, it was established that all the 27 batches of rh-FSH were identified to be Follicle Stimulating Hormone and their estimated potencies were found to be within the specification limits as per the European Pharmacopeia.

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INTRODUCTION

Follicle stimulating hormone (FSH) is a gonadotropic hormone produced by the anterior lobe of the mammalian pituitary gland. It regulates the normal female and male gamete growth and maturation and normal gonadal steroid production. In particular, it stimulates the maturation of germ cells and thus is involved in spermatogenesis and folliculogenesis. Deficient endogenous production of FSH is a known cause of infertility and administration of therapeutic preparations of FSH are widely used in the treatment of this condition. Naturally derived FSH, manufactured from the urine of post-menopausal women, has been available for over 30 years. However, recombinant DNA technology has now made manufacturing of FSH possible without the need for the collection of large volumes of urine and also provides a highly pure product devoid of infectious or pharmacological contaminants such as luteinising hormone (LH). It has been widely assumed that recombinant products have better batch to batch consistency compared with human urine derived products and show more consistent clinical response (Pierce and Parson, 1981). The first recombinant hFSH, GONAL-f (r-hFSH, follitropin alfa for injection), was licensed in 1995 for marketing in the European Union (Lunenfeld, B., 2004). Recombinant FSH is identical to the pituitary or urinary FSH in amino acid sequence, glycosilation site, receptor binding capacity and in vitro biologic activity (Shoham and Insler, 1998). Recombinant FSH has been reported to produce more oocytes with a lower total dose per cycle in assisted reproductive technologies compared with highly purified human menopausal gonadotrophin (Lehart *et al*., 2010). In females, therapeutic FSH products are used in assisted reproduction technology mainly for Induction of ovulation when a single healthy oocyte is required, induction of superovulation to maximize efficiency when assisted reproductive technologies are used that allow replacement of a fixed number of embryos and for stimulation of spermatogenesis in males (Mathew *et al*., 2000). However, there is a narrow dose-range for use of FSH between a threshold level required to stimulate growth of a follicle(s) and the maximal dose (ceiling) above which overstimulation can occur (Ben-Rafael *et al*., 1995). Thus there

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Table 1. Comparison between the Potency Values (%) determined by the in vivo bioassay by the manufacturer and NIB

S. No.	Strength (Label Claim) (IU/ml) or (IU)	Sample Packing	Potency (%) estimated by Manufacturer	Potency (%) estimated by NIB	rh-FSH content as determined by NIB (IU/ml)
1	75 IU	Vial	99	101.9	76.425
2	225 IU/0.5 ml	PFS	104	103.4	465.3
3	150 IU/0.3 ml	PFS	100	97.5	487.5
4	150 IU	Vial	109	104.4	156.6
5	75 IU/0.15 ml	PFS	106	100.5	502.5
6	300 IU/0.6 ml	PFS	102	100.3	501.5
7	150 IU/0.3 ml	PFS	104	104.5	522.5
8	150 IU	Vial	105	104.3	156.45
9	75 IU	Vial	104	99.5	74.625
10	75 IU/0.15 ml	PFS	104	105.4	527
11	225 IU/0.45 ml	PFS	105	105.8	529
12	300 IU/0.6 ml	PFS	101	103.5	517.5
13	150 IU/0.3 ml	PFS	103	102	510
14	300 IU/0.6 ml	PFS	100	99.8	499
15	75 IU	Amp.	94	98.8	74.1
16	150 IU	Vials	104	101.5	152.25
17	300 IU/0.6 ml	PFS	102	100.3	501.5
18	225 IU/0.45 ml	PFS	103	105.1	525.5
19	100 IU/0.5 ml	Amp.	102	100.8	201.6
20	50 IU/0.5 ml	Amp.	111.6	100.5	100.5
21	75 IU	Vials	104	103.8	77.85
22	75 IU	Vials	103	103.3	77.475
23	50 IU/0.5 ml	Vial	106	100.6	100.6
24	100 IU/0.5 ml	Vial	100	99.4	99.4
25	75 IU	Vial	109	96.9	72.675
26	600 IU/0.72 ml	Vial	109.2	96.3	802.5
27	300 IU/0.36 ml	Vial	103.2	100.4	836.67

is a significant risk to health due to the iatrogenic induction of ovarian hyper-stimulation syndrome or multiple pregnancies. Different physiological and clinical states can affect the levels of the threshold and ceiling for FSH treatment. Thus careful dose adjustment and monitoring of FSH levels and ovarian responses are required, particularly for patients with polycystic ovary syndrome. This cannot be achieved without accurate and reproducible calibration of therapeutic products. Further, the end point used for patient response to therapeutic preparations should also be carefully considered (Mathew *et al*., 2000).

Determining the potency of therapeutic FSH products is therefore, one of the important requirements as it measures the capability of a particular quantity of the product to produce a biological effect in patients. The biological activity i.e. potency of a product is directly linked to its clinical efficacy and that is why potency tests are performed as part of product release, comparability studies and stability testing. The biological activity of FSH is the sum of a complex combination of processes: release from the pituitary, survival in the circulation, transport to the site of action (i.e. the gonad), binding to the receptor, and activation of signal transduction pathways (Mathew *et al*., 2000). The assay developed in 1953 by Steelman and Pohley based on the stimulation of ovarian weight in gonadotropin (LH) - treated immature rats, has proved to be a robust specific in vivo bioassay for FSH activity and this assay remains the basis of pharmacopeia monographs for the statutory determination of the FSH potency of therapeutic preparations (Steelman and Pohley, 1953; European Pharmacopoeia, 2014). National Institute of Biologicals (NIB) has a mandate to responsibly assure and review the quality of a number of Biological products available through domestic manufacturers or imports. The Enzymes and Hormones Laboratory of NIB is primarily involved in Quality control evaluation of various human therapeutic hormones used in infertility treatments viz. Human Chorionic Gonadotropin, Menotropin (Human Menopausal Gonadotropin), Follicle Stimulating Hormone (Conventional and recombinant). In this study, we are reporting the evaluation of 27 batches of therapeutic recombinant human Follicle Stimulating Hormone for their identity and potency before batch release into the market for use in patients in India.

MATERIALS AND METHODS

Hormone Preparations

Twenty seven rh-FSH preparations were analyzed for their identity and potency in this study. The International Standard of rhFSH-WHO 92/642 obtained from the National Institute for Biological Standards and Control (NIBSC, South Mimms, UK) and innovator product Gonal F (Merck Serono S.P.A) have been used as reference standards for the biological assay.

Animals

Sexually immature 20-23 days old female Sprague Dawley rats, differing in age by not more than 3 days and weight within the range 10 g of each other were used for assay. Rats were housed in sterilized cages on a 12:12 hour light/dark cycle with food and water provided ad libitum. For each batch of rh-FSH, rats were assigned to 6 equal groups of at least 5 rats. However, as a commitment towards 3R's, 2 to 3 batches of recombinant follicle stimulating hormone were tested with common reference standard whenever possible. All the procedures were conducted after approval by Institutional Animal Ethics Committee (IAEC) in accordance with the regulatory guidelines provided by CPCSEA.

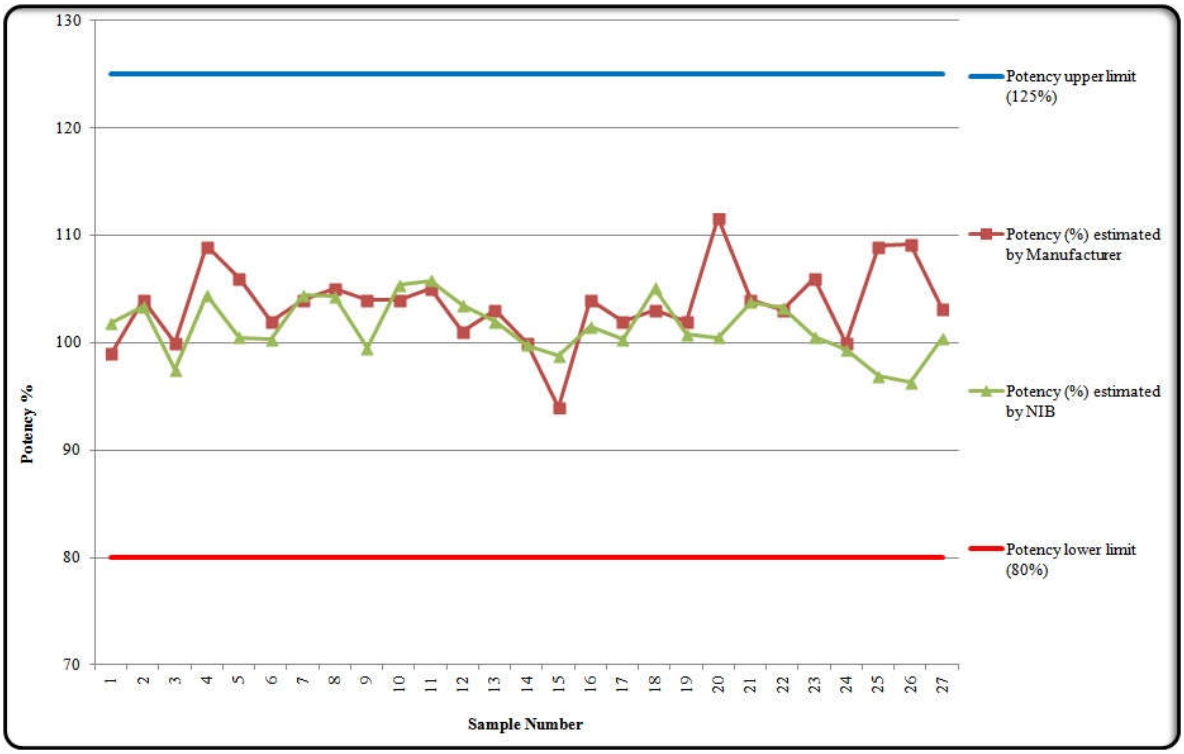

Figure 1. Comparison between the Potency values (%) determined by the in vivo bioassay by manufacturer and NIB

Biological Assay

The potency of batches of rh-FSH was determined by the rat ovary weight gain assay (Steelman and Pohley, 1953) as described in the European Pharmacopoeia. The three doses of the reference preparation and that of the rh-FSH batch to be examined were chosen in geometric progression i.e 0.5 IU, 1.0 IU, 2.0 IU in 0.5 ml phosphate albumin buffered saline (pH-7.2) which was injected subcutaneously over three consecutive days leading to total dose of 1.5 IU, 3.0 IU and 6.0 IU/rat. The buffer solution contained in daily dose not less than 14 IU of Chorionic Gonadotropin to ensure complete luteinisation. 0.4% w/v phenol was added as antimicrobial preservative. About 24 hrs after the last injection, the rats were euthanized and both the ovaries from each rat were removed and weighed immediately. The potency of the therapeutic rh-FSH batches relative to that of the reference agent was determined by parallel line assay based on the weights of the combined ovaries for each treatment group.

Statistical Methods

The results were calculated by using the Parallel-line assay; COMBISTATS v4.0 software from EDQM.

RESULT AND DISCUSSION

The 27 batches of different strengths of therapeutic rh-FSH available in form of vials, Prefilled Syringes (PFS) or ampoule were evaluated for their identity and potency as per the European Pharmacopoeia. As per European Pharmacopoeia specifications, the identity of all the 27 batches was established by the increase in weight of ovaries upon the injection of the batches of rh-FSH for three consecutive days when compared with reference standard in similar conditions. As per the Pharmacopoeia, the estimated potency of FSH should be not less than 80% and not more than 125% of the stated potency. The results of Potency assays performed by NIB and that obtained by the Manufacturer as stated in their Certificate of Analysis of said batches is provided in Table 1 and Figure 1. The estimated potencies of all the 27 batches of rh-FSH were found to be within the specification limits as per the European Pharmacopoeia.

Conclusion

Based on the findings of the present study, it was established that all the 27 batches of rh-FSH tested were identified to be Follicle Stimulating Hormone and their estimated potencies were found to be within the specification limits as per the European Pharmacopoeia.

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