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RESEARCH ARTICLE

ASSURING THE QUALITY OF HUMAN MENOPAUSAL GONADOTROPIN (MENOTROPHIN) BATCHES BY ESTABLISHING THEIR FSH AND LH ACTIVITIES BEFORE USE IN TREATMENT OF INFERTILITY

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ABSTRACT

Human menopausal gonadotrophin (HMG) or menotrophin causes growth and maturation of the ovarian follicle in women who do not have primary ovarian failure by mimicking the action of endogenous LH and FSH and in men it promotes sperm formation if testosterone level and FSH levels are low. Therapeutic menotrophins are preparations of gonadotrophins having both FSH and LH activity, extracted from the urine of postmenopausal women, which have undergone additional steps for purification and then used as fertility treatment. In this study, we are reporting the evaluation of 23 batches of therapeutic menotrophin as per US pharmacopeia, for establishing their identity as well as Follicle Stimulating Hormone (FSH) activity and Lutenizing Hormone (LH) activity before batch release into the market for use in patients in India. 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 23 batches were identified to be menotrophin and their estimated potencies of both FSH activity and LH activity were found to be within the specification limits as per the US Pharmacopeia.

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INTRODUCTION

Human Menopausal Gonadotrophin (HMG) or menotrophin are natural hormones necessary for human reproduction as they are important in females for the development of follicles produced by ovaries and in males for the development of sperms. HMG causes growth and maturation of the ovarian follicle in women who do not have primary ovarian failure by mimicking the action of endogenous LH and FSH. In men, HMG promotes sperm formation if testosterone level and FSH levels are low. The LH component stimulates the production of testosterone and FSH promotes the formation of sperm. Menotrophin is a treatment option for infertile women if their pituitary gland fails to stimulate ovulation, as it directly stimulates the ovaries thus promoting fertility. It is also one of the ovulation drugs often used in Assisted Reproductive Techniques (ART) and may be effective in promoting fertility in women with ovarian dysfunction or endometriosis. In men with primary or secondary pituitary hypofunction, the drug causes spermatogenesis when administered along with Chorionic gonadotrophin. Therapeutic menotrophins are preparations of gonadotrophins having both FSH and LH activity, extracted from the urine of postmenopausal women, which have undergone additional steps for purification and then used as fertility treatment. HMG initially extracted from urine by researchers in 1949, became commercially available around a decade later and was successfully introduced in clinical use in 1961 by Bruno Lunenfeld (Lunenfeld, B. 2004). The initial preparations of HMG contained varying levels of FSH, LH, and hCG. However, in 1999 further improvement in the purification process led to production of highly purified HMG, with FSH and LH activities standardized at 75 IU for each type of gonadotrophin, as measured using a standard in-vivo bioassay (Rogerio de Barros F. Leao & Sandro C.

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Esteves, 2014). In 1980s, further improvements in the purification methods also led to production of purified FSH and then highly purified urinary FSH (HP-hFSH) in 1993 (Lunenfeld, B. 2004). Lately, the recombinant DNA technology has helped in the production of highly pure FSH or LH products which are not contaminated by other proteins that may be present after urinary extraction. There are many studies which have compared Urinary HMG versus highly purified or recombinant FSH and while some studies seem to suggest that pure FSH gives better results than HMG (Bagratee *et al.,*1998) others claim that recombinant FSH is more efficient and reduces costs (Daya *et al.* 2001).

A Cochrane Collaboration analysis did not reveal major differences in clinical outcomes when comparing urinary versus recombinant FSH (Van Wely *et al.* 2011). It may be concluded that both urinary gonadotrophins, mainly HMG preparations and recombinant FSH have similar efficacy in terms of achieving a pregnancy or live birth per treatment cycle. While some of these studies were in favour of HMG preparations, albeit the lower confidence limits were 1% or less, others reported no differences in pregnancy outcomes between the two treatments. Furthermore, no significant differences were noted for spontaneous abortion, multiple pregnancy, cycle cancellation and OHSS rates. (Rogerio de Barros F. Leao and Sandro C. Esteves, 2014). As per the British and US Pharmacopoeias, Menotrophin is a preparation containing glycoprotein gonadotrophins possessing follicle stimulating and lutenising activities and the ratio of IU of Lutenising hormone (LH) activity to IU of follicle stimulating hormone (FSH) activity is approximately one. When necessary, Chorionic Gonadotrophin obtained from the urine of pregnant women may be added to induce a desired level of LH-like biological activity.

Currently, both conventional HMG and highly purified HMG (HP-HMG) are commercially available. Identification of menotrophins may be established if it caused enlargement of the ovaries of immature female rats and increases the weight of seminal vesicles and prostrate gland of immature rats when administered in a bioassay. 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 menotrophin is determined using the bioassays for FSH by ovarian weight gain assay in female rats (Steelman and Pohley 1953) and for LH by seminal vesicle weight gain assay in male rats (Hell *et al.,* 1964, British Pharmacopeia 2014, US Pharmacopeia 37). National Institute of Biologicals (NIB) responsibly assures and reviews 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 Gonadotrohpin, Menotrophin (Human Menopausal Gonadotropin), Follicle Stimulating Hormone (Conventional and recombinant). In this study, we are reporting the evaluation of 23 batches of therapeutic Menotrophin from different manufacturers, for their identity and FSH and LH activity before batch release into the market for use in patients in India.

MATERIALS AND METHODS

Hormone Preparations: Twenty three Menotrophin preparations were analyzed for their identity and potency in this study. The 5th International Standard from National Institute for Biological Standards and Control (NIBSC, South Mimms, UK) with code 10/286 having FSH activity of 183IU per ampoule and LH activity of 177 IU per ampoule have been used as reference standard for the biological assay. Animals: Sexually immature 20-23 days old 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 assays. 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 Menotrophin both the FSH and LH activity were determined. For FSH activity, rats were assigned to 6 equal groups of 6 rats and for LH activity also the groups were made in same way i.e 6 groups of 6 rats each. However, as a commitment towards 3R's, 2 to 3 batches of Menotrophin 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.

Biological Assay

Follicle stimulating hormone activity: The potency of Menotrophin with respect to its follicle stimulating hormone activity was estimated by comparing its effect in enlarging the ovaries of immature female rats with that of Standard Reference Preparation of human urinary FSH and human urinary LH in the biological assay as described in the US pharmacopeia. The three doses of the Standard Preparation and that of the Menotrophin batch to be examined were chosen in geometric progression i.e 0.5 IU, 1.0 IU and 2.0 IU in 0.2 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 Gonadotrophin to ensure complete luteinization. Phenol (0.4% w/v) 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 Follicle stimulating hormone activity of the therapeutic menotrophin batches relative to that of the Standard Preparation was determined by parallel line assay based on the weights of the combined ovaries for each treatment group.

Lutenising hormone activity: The potency of Menotrophin with respect to its luteinizing hormone activity was estimated by comparing its effect in increasing the weight of seminal vesicles of immature male rats with that of Standard Preparation of human urinary FSH and human urinary LH as described in the US Pharmacopoeia. The three doses of the Standard Preparation and that of the Menotrophin batch to be examined were chosen in geometric progression i.e 1.75 IU, 3.5 IU and 7.0 IU in 0.2 ml phosphate albumin buffered saline (pH-7.2) which was injected subcutaneously over four consecutive days leading to total dose of 7.0 IU, 14 IU and 28 IU/rat. Phenol (0.4% w/v) was added as antimicrobial preservative. About 24 hrs after the last injection, the rats were

euthanized and the seminal vesicles from each rat were removed and weighed immediately. The Luteinizing hormone activity of the therapeutic menotrophin batches was determined relative to that of the Standard Preparation by parallel line assay based on the weights of the seminal vesicles 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 23 batches of different strengths of therapeutic menotrophin from different manufacturers were evaluated for their identity and potency with respect to their FSH and LH activity as per the US Pharmacopoeia.

The identity of all the 23 batches was established by the increase in weight of ovaries of immature female rats and increase in the weight of seminal vesicles of immature male rats upon the injection of the batches of Menotrophin for three and four consecutive days respectively when compared with reference standard in similar conditions. As per the Pharmacopoeia, the estimated potency for each component of menotrophin i.e FSH activity and LH activity should not be less than 80% and not more than 125% of the stated potency. The results of FSH activity obtained by NIB and that obtained by the Manufacturer as stated in their Certificate of Analysis of said batches is compared and provided in Table 1 and Figure 1 & the results of LH activity obtained 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 2. The estimated potencies for both the FSH and LH activity of all the 23 batches of Menotrophin were found to be within the specification limits as per the US Pharmacopoeia.

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

S. No.	Vial Strength (Label Claim)	FSH Activity (%) estimated by Manufacturer	FSH Activity (%) estimated by NIB	FSH Activity (IU) estimated by NIB	LH Activity (%) estimated by Manufacturer	LH Activity (%) estimated by NIB	LH Activity (IU) estimated by NIB
1	150 IU	102	94.4	141.60	95	97.21	145.82
2	75 IU	120	97.9	73.43	105	96.2	72.15
3	75 IU	101	107.9	80.93	94	99	74.25
4	75 IU	96	107.7	80.78	89	99.2	74.40
5	150 IU	100	98.8	148.20	93	98.71	148.07
6	150 IU	85	110.2	165.30	104	94.6	141.90
7	75 IU	104	112.9	84.68	99	97.8	73.35
8	75 IU	86	94.1	70.58	95	106.7	80.03
9	150 IU	86	104.9	157.35	98	105.3	157.95
10	75 IU	90	101.3	75.98	93	101.3	75.98
11	75 IU	82	100	75.00	93	108.6	81.45
12	75 IU	112.1	98.7	74.03	99.3	99.5	74.63
13	75 IU	95.02	100.9	75.68	113.7	102.6	76.95
14	75 IU	121	95.4	71.55	84	94.2	70.65
15	150 IU	109	98.7	148.05	114	102.8	154.20
16	150 IU	94	105.7	158.55	84	97.7	146.55
17	1200 IU	99.58	104.9	1258.80	97.83	104.8	1257.60
18	1200 IU	102.33	105.3	1263.60	113.08	105.2	1262.40
19	600 IU	105.98	108	648.00	110.75	107.4	644.40
20	600 IU	111.2	102.6	615.60	122.2	98.9	593.40
21	600 IU	111.8	98.2	589.20	122.2	100.8	604.80
22	75 IU	101.6	101.18	75.89	102	98.6	73.95
23	75 IU	110.93	106.2	79.65	110.53	103.5	77.63

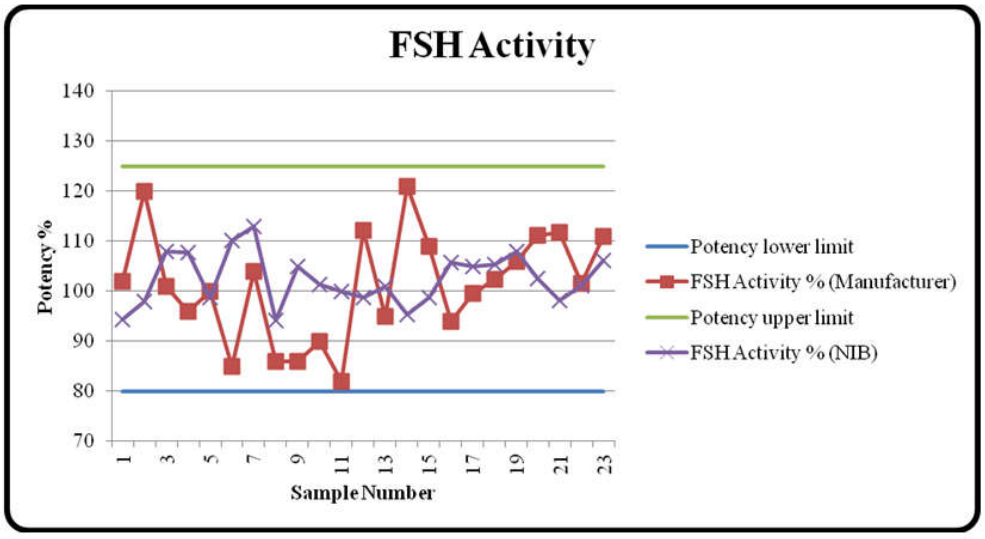

Figure 1. Comparison between the Potency values for FSH Activity of Menotrophin determined by manufacturer and NIB by in vivo bioassay

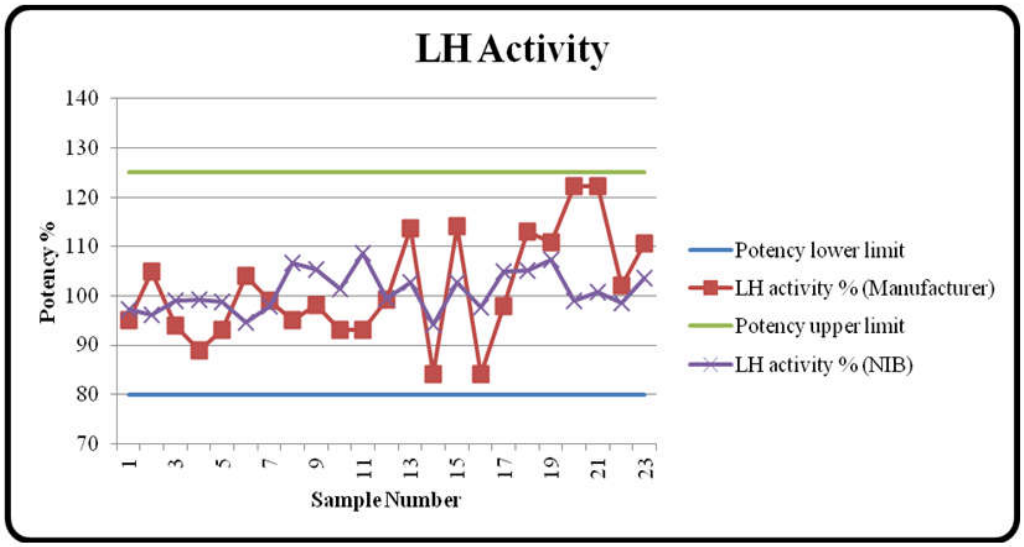

Figure 2. Comparison between the Potency values for LH Activity of Menotrophin determined by manufacturer and NIB by in vivo bioassay

Conclusion

Based on the findings of the present study, it was established that all the 23 batches of tested were identified to be Human Menopausal Gonadotrophin (HMG) and their estimated potencies of both FSH as well as LH activities were found to be within the specification limits as per the British Pharmacopoeia and US Pharmacopoeia.

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REFERENCES

Bagratee, J. S., Lockwood, G., López Bernal, A., Barlow, D. H. and Ledger, W. L. 1998. Comparison of highly purified FSH (metrodin-high purity) with pergonal for IVF superovulation. *Journal of Assisted Reproduction and Genetics,* 15 (2): 65–69.

British Pharmacopoeia 2014.

Daya, S., Ledger, W., Auray, J. P., Duru, G., Silverberg, K., Wikland, M., Bouzayen, R., Howles, C. M. and Beresniak, A. 2001. Cost-effectiveness modelling of recombinant FSH versus urinary FSH in assisted reproduction techniques in the UK. Human reproduction (*Oxford, England*) 16 (12): 2563–2569.

Hell V, R. Matthijsen R, Overbeek G.1964. Effects of human menopausal gonadotrophin preparations in different bioassay methods. Acta Endocrinol (Copenh). 47:409-18

Lunenfeld, B. 2004. Historical perspectives in Gonadotropin therapy. *Human Reprod Update*, 10: 453-67.

Rogerio de Barros F. Leao and Sandro C. Esteves 2014. Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech, CLINICS, 69 (4): 279-293.

Steelman, S.L. and Pohley, F.M. 1953. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology,* 53: 604–616.

United States Pharmacopoeia 37.

Van Wely, M., Kwan, I., Burt, A. L., Thomas, J., Vail, A., Van Der Veen, F. and Al-Inany, H. G. 2011. Van Wely, Madelon, ed. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. *The Cochrane Library* (2): CD005354

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