Original Research (XXXX) X(X); X - XX
https://doi.org/10.22103/VCBR.2026.26053.1101

Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

ORIGINAL ARTICLE

Evaluation of Newly Produced Equine Chorionic Gonadotropine (eCG/PMSG) with Effects on Immature Rat Ovaries: A Method Article

Mohammad Javad Jahedi , Seyed Morteza Aghamiri * , Mansooreh Miri Moghaddam

Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Online ISSN: 3060-7663 **https://doi.org/10.22103/VCBR.2026.26053.1101**

***Correspondence**

Author's Email:
aghamirimorteza@uk.ac.ir

Article History

Received: *05 October 2025*
Revised: *19 January 2026*
Accepted: *13 June 2026*
Published: *11 July 2026*

Keywords

Pregnant Mare Serum Gonadotropin (PMSG)
Ovarian Weight
Bioassay
Gonadotropin Potency
Reproductive Biotechnology

Abstract

Equine Chorionic Gonadotropin (eCG), also known as Pregnant Mare Serum Gonadotropin (PMSG), is widely applied in reproductive biotechnology due to its dual follicle-stimulating hormone (FSH)- and luteinizing hormone (LH)-like activities and prolonged half-life. The classical rat ovarian weight bioassay remains one of the standard methods for assessing PMSG potency. This study aimed to compare the biological activity of PMSG preparations produced by Farnood Dam Kara (Iran) and Syntex (Argentina) in immature Wistar rats. Forty-nine immatures female Wistar rats (~50 g) were randomly assigned into six experimental groups, receiving PMSG at 10, 20, or 40 IU (Farnood or Syntex), and one control group receiving saline. A single subcutaneous injection was administered, and ovarian weights were measured 68 hours later. Both Farnood and Syntex PMSG significantly increased ovarian weight compared with controls (0.063 ± 0.006 g). A dose-dependent response was observed in treatment groups: Farnood increased ovarian weight from 0.090 ± 0.013 g (10 IU) to 0.139 ± 0.019 g (40 IU), while Syntex increased it from 0.087 ± 0.011 g (10 IU) to 0.135 ± 0.020 g (40 IU). Both preparations demonstrated comparable dose-dependent effects, confirming their potency. Newly manufactured PMSG (Farnood) may serve as a reliable and cost-effective alternative to imported formulations.

How to cite this article: Mohammad Javad Jahedi, Seyed Morteza Aghamiri, Mansooreh Miri Moghaddam. Evaluation of Newly Produced Equine Chorionic Gonadotropine (eCG/PMSG) with Effects on Immature Rat Ovaries: A Method Article. *Veterinary and Comparative Biomedical Research*, XXXX X(X): X – XX. https://doi.org/10.22103/VCBR.2026.26053.1101

© **The Author(s), 2026.** This open-access article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0), permitting non-commercial use, distribution, and reproduction in any medium, provided the original author(s) and source are properly credited. No commercial use or modifications are allowed without prior permission. Third-party material is included under the same license unless otherwise stated. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc/4.0/.

Introduction

Equine chorionic gonadotropin (eCG), also known as pregnant mare serum gonadotropin (PMSG) .The hormone eCG (or PMSG) is exclusively produced by the fetal trophoblast cells of the horse embryo in pregnant mares. Its secretion into the mare's bloodstream begins around days 35-40 of pregnancy and continues until approximately days 100-120. The key difference between eCG and other gonadotropins lies in its origin: it is produced by the placenta, rather than the pituitary gland. The primary commercial source for collecting this hormone is the serum of pregnant mares during this specific period. It is a glycoprotein hormone composed of two distinct glycosylated subunits, α and β, which are linked non-covalently—a structural characteristic shared by all gonadotropins (1). The α-subunit is common among gonadotropins, while the β-subunit confers biological specificity. The slow dissociation rate of eCG is attributed to the β-subunit's unique "seatbelt" structure, which wraps around the α-subunit, stabilizing the heterodimer and prolonging its half-life in circulation. This structural mechanism is also observed in human chorionic gonadotropin (hCG) and contributes to the hormone's prolonged biological activity (2).

For decades, PMSG has been widely employed in reproductive biotechnology, particularly in superovulation and ovulation induction protocols across multiple mammalian species, including cattle, sheep, pigs, and laboratory rodents (3). Its efficacy in stimulating follicular development and ovulation makes it indispensable in assisted reproductive technologies (ART), embryo transfer programs, and livestock breeding. To ensure consistent and reproducible treatments, the in vivo potency of PMSG must be precisely assessed, as variations in bioactivity can significantly impact treatment outcomes (4).

The strong biological activity of eCG stems from two key factors: its unique combination of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) activity in non-equine species, and its extended circulatory half-life, which enhances its therapeutic effectiveness (5). Unlike other gonadotropins, eCG exhibits prolonged FSH-like activity due to its resistance to metabolic clearance, allowing for sustained stimulation of ovarian follicles with minimal administration frequency. This dual activity and prolonged half-life make eCG particularly valuable in protocols requiring sustained gonadotropic stimulation, such as superovulation in cattle or estrus synchronization in sheep (6).

Recent research has explored alternative methods for assessing PMSG potency, such as receptor-binding assays and in vitro bioassays using granulosa or Leydig cell cultures (7). However, these methods have not yet fully replaced the in vivo assay, as they may not completely capture the hormone's complex in vivo interactions. Meanwhile, advances in recombinant DNA technology have enabled the production of recombinant PMSG subunits, which offer potential improvements in purity and consistency over traditional extraction methods utilizing pregnant mare serum (5). Further developments in recombinant hormone production and alternative bioassays are expected to refine PMSG standardization, thereby enhancing its applications in reproductive medicine and animal breeding (8).

This study aimed to evaluate and compare the effects of eCG (PMSG) produced by Farnoud Dam Kara Company (Iran) and Syntex Company (Argentina) on ovarian weight in immature Wistar rats.

Materials and Methods

Experimental Animals and Grouping

A total of 49 immature female Wistar rats (body weight approximately 50 g; estimated age 4-5 weeks) were obtained from the animal breeding facility at Kerman University of Medical Sciences, Kerman, Iran. During the experiment, the animals were housed in standard plastic cages under controlled environmental conditions (22–25 °C; 12 h light/12 h dark cycle) with free access to water and a commercial rodent diet (Javaneh Khorasan Co., Mashhad, Iran). The rats were assigned to seven groups, consisting of six experimental groups, each with n = 7 rats and one control group (n = 7), as follows:

Group A: Received PMSG (Novormon, Syntex, Argentina) at a dose of 40 IU.

Group B: Received PMSG (Novormon, Syntex, Argentina) at a dose of 20 IU.

Group C: Received PMSG (Novormon, Syntex, Argentina) at a dose of 10 IU.

Group D: Received PMSG (Farnood Dam Kara, Iran) at a dose of 40 IU.

Group E: Received PMSG (Farnood Dam Kara, Iran) at a dose of 20 IU.

Group F: Received PMSG (Farnood Dam Kara, Iran) at a dose of 10 IU.

Control group: Received only a normal saline solution.

Animals were randomly assigned to experimental groups using a simple manual randomization method. Ovarian excision and weighing were performed by an investigator blinded to treatment allocation, and all data were coded prior to statistical analysis to minimize potential assessment bias.

Drug Administration

The PMSG used in this study was obtained from two commercial sources: Syntex, Argentina (Novormon®; Batch No.: NV0324; Exp. date: MAR/2026), and Farnood Dam Kara, Iran (newly manufactured batch, without an assigned lot number at the time of the experiment; Exp. date: NOV/2026). Both hormone preparations were stored under refrigerated conditions (2–8 °C) until reconstitution and use, following manufacturer recommendations. Hormonal treatment was administered as a single-dose (0.1 mL) injection at time point t = 0. PMSG vials were reconstituted in sterile saline, and dosing solutions were prepared to deliver 10, 20, or 40 IU, with a constant injection volume of 0.1 mL per animal under sterile conditions. All injections were administered subcutaneously to ensure appropriate absorption and stable drug availability, thereby enabling evaluation of the hormonal effects within the designated timeframe.

Euthanasia and Necropsy

Ovaries were collected 68 hours after PMSG administration (9). The rats were euthanized by exposure to carbon dioxide (CO_2) in a specialized chamber. Following euthanasia, the ovaries from each rat were carefully dissected, trimmed of surrounding fat and connective tissue, and weighed together as a pair using a calibrated digital analytical balance (Sartorius TE 153S, Kern & Sohn, Germany) with a precision of 0.001 g. The paired ovarian weight (expressed in grams) was recorded for each animal.

Statistical Analysis

Data were first evaluated for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. When the assumptions for parametric testing were met, comparisons among groups were performed using one-way ANOVA. In cases where ANOVA indicated significant differences, Tukey's post-hoc test was used for multiple pairwise comparisons. All values are presented as mean ± SD, and the number of animals in each group (n) is indicated in both Table 1 and Figure 1. Statistical significance was set at $P < 0.05$. All analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

Results

The reported values represent the total paired ovarian weight for each animal. Mean paired ovarian weights (mean ± SD) for each experimental group are presented in Table 1. A comparison between the two drugs showed a comparable dose-dependent effect on ovarian weight for both Farnood and Syntex PMSG products (Table 1, Figure 1). The results indicated that administration of either Farnood or Syntex at various doses (10, 20, and 40 IU) significantly increased ovarian weight in rats compared to the control group. In the control group, the mean ovarian weight was 0.063 ± 0.006 g, whereas in the treated groups, ovarian weight increased in a dose-dependent manner. For Farnood, ovarian weight increased from 0.090 g at 10 IU to 0.139 g at 40 IU. Similarly, Syntex increased ovarian weight from 0.087 g at 10 IU to 0.135 g at 40 IU; consequently, no significant differences were observed between the two PMSG products (Table 1). These findings suggest that both compounds effectively stimulate ovarian growth in a dose-dependent manner.

Table 1. Paired ovary weights in control and each experimental group (n = 7 per group) of immature Wistar rats in the Syntex (Argentina) and Farnood (Iran) eCG products. Values are presented as mean ± SD.

	Syntex (IU)			Farnood (IU)			Control
	10	**20**	**40**	**10**	**20**	**40**	
Ovarian weight (g)	0.087	0.121	0.135	0.090	0.111	0.139	0.063
Standard Deviation	0.011	0.024	0.020	0.013	0.018	0.019	0.006

Discussion

The standard in vivo bioassay for PMSG, developed over 75 years ago by Cole and Erway (10), measures the dose-dependent increase in ovarian weight in prepubertal rats. This assay remains the gold standard for potency testing due to its reliability and reproducibility. The method involves a single subcutaneous injection of PMSG, followed by ovarian weight assessment after a standardized period (typically 72–96 hours) (9). The increase in ovarian weight correlates directly with the hormone's biological activity, providing a quantitative measure of potency (11). Despite advancements in in vitro assays, the rat ovarian weight bioassay remains widely used by both academic researchers and pharmaceutical manufacturers to evaluate crude and purified PMSG preparations, and to ensure batch-to-batch consistency for clinical and veterinary applications (12).

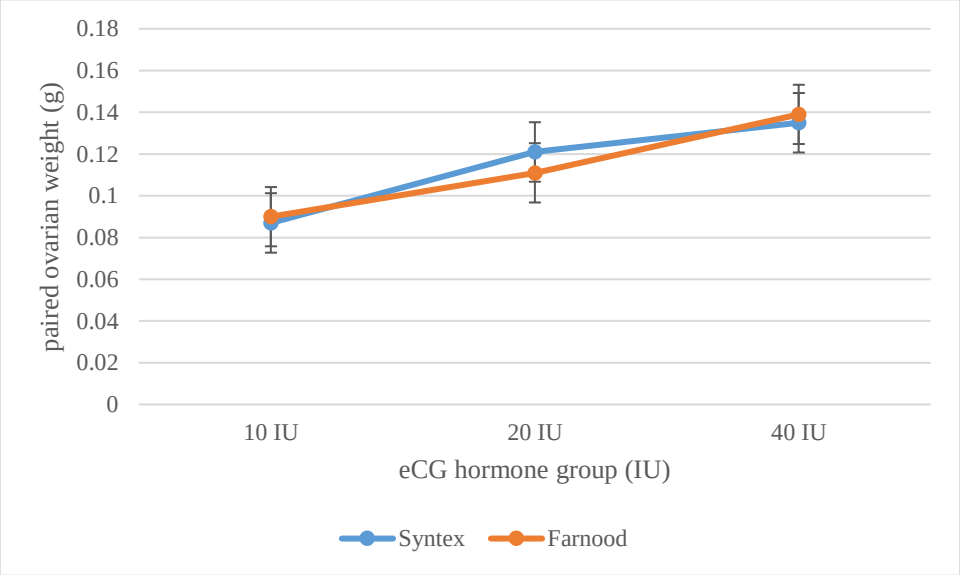

Figure 1. Changes in the paired ovarian weight of immature Wistar rats in groups receiving Syntex (Argentina) and Farnood (Iran) eCG hormone. *Asterisks denote statistical significance ($P < 0.05$); all comparisons were non-significant.

The present study demonstrated that administration of eCG (PMSG) preparations from both Farnood Dam Kara (Iran) and Syntex (Argentina) significantly increased ovarian weight in immature Wistar rats in a dose-dependent manner. This finding is consistent with the classical ovarian weight bioassay developed by Cole and Erway (10), which has long been recognized as the gold standard for in vivo assessment of gonadotropin potency. The observed increase in ovarian weight confirms the similar biological activity of both preparations and reflects their ability to stimulate follicular growth, likely through their combined FSH- and LH-like activities in non-equine species (5, 13).

The biological effectiveness of PMSG is largely attributed to its unique molecular structure, particularly its high degree of glycosylation and the β-subunit's "seatbelt" configuration, which slow its metabolic clearance (2, 6). This results in a prolonged half-life and sustained gonadotropic stimulation of ovarian follicles, explaining the clear dose–response relationship observed in this study (14). By demonstrating the equivalent activity of the locally manufactured product and the imported preparation, the results highlight the potential of Farnood PMSG as a reliable and cost-effective alternative for reproductive biotechnology programs. This is particularly relevant for livestock breeding and estrus synchronization protocols in which affordability and supply security are critical factors (3, 8).

Although the difference in ovarian weight between the two PMSG groups was non-significant at 20 IU, no meaningful difference was observed at the highest dose (40 IU), indicating that both products can achieve a similar stimulatory effect on ovarian growth. These findings align with previous reports highlighting dose-dependent increases in ovarian weight following PMSG administration in rodents (9, 10), reinforcing the robustness of the rat bioassay in detecting subtle differences between hormone preparations (15).

Despite the strengths of this study, certain limitations should be acknowledged. First, the experiment was conducted exclusively in immature female Wistar rats, which, although widely accepted for PMSG potency testing (6), may not fully replicate the hormone's activity in other species used in reproductive biotechnologies. Second, only ovarian weight was assessed as the outcome parameter, whereas additional endpoints such as histological evaluation of follicular development, hormonal profiling, or reproductive outcomes (e.g., ovulation rate and embryo yield), could provide a more comprehensive understanding of the biological effects (7). Furthermore, because equine-derived gonadotropins can occasionally trigger immunogenic reactions or variable pharmacokinetics in different species (4, 8), future studies should examine Farnood PMSG in target livestock species and compare it with recombinant or highly purified eCG products.

In conclusion, this study confirms that both Farnood Dam Kara and Syntex eCG hormone products effectively stimulate ovarian growth in a dose-dependent manner, with no significant differences observed between the products at the various doses tested. These findings highlight the reliability of the rat ovarian weight bioassay for potency assessment and suggest that newly manufactured PMSG preparations may serve as viable alternatives to imported

products for reproductive applications. Future studies incorporating broader outcome measures and cross-species validation are warranted to further establish the comparative efficacy, safety, and clinical utility of different PMSG sources.

Acknowledgements

The authors would like to thank the Farnood Dam Kara Company for its support of this study.

Authors' Contributions

Mohammad Javad Jahedi: Investigation, Methodology, Resources, Writing - Original Draft. **Seyed Morteza Aghamiri:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Project administration, Supervision. **Mansooreh Miri Moghaddam:** Visualization, Validation, Data curation. All authors read and approved the final manuscript.

Data Availability

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

Ethical Approval

All experimental procedures were performed in accordance with the national regulations for the care and use of laboratory animals and the ARRIVE 2.0 guidelines. Accordingly, the experimental protocol was reviewed and approved by the Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran, and all efforts were made to minimize animal discomfort throughout the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable.

Funding

This study was not supported by any special funding.

References

1. Min KS, Shinozaki M, Miyazawa K, Nishimura R, Sasaki N, Shiota K, Ogawa T. Nucleotide sequence of eCG α-subunit cDNA and its expression in the equine placenta. *J Reprod Dev*. 1994; 40(4):301-305. https://doi.org/10.1262/jrd.40.301
2. Xing Y, Myers RV, Cao D, Lin W, Jiang M, Bernard MP, Moyle WR. Glycoprotein hormone assembly in the endoplasmic reticulum: IV. Probable mechanism of subunit docking and completion of assembly. *J Biol Chem*. 2004; 279(34):35458-368. https://doi.org/10.1074/jbc.M403055200
3. Ciller UA, Ciller IM, McFarlane JR. Equine chorionic gonadotrophin isoform composition in commercial products compared with isoform composition in pregnant mare plasma. *Reprod Fertil. Dev*. 2008; 20(9):77. https://doi.org/10.1071/SRB08Abs277
4. Murphy BD. Equine chorionic gonadotropin: an enigmatic but essential tool. *Anim Reprod*. 2018; 9(3):223-230.
5. Cole HH, Erway J. 48-hour assay test for equine gonadotropin with results expressed in international units. *Endocrinology*. 1941; 29(4):514-519. https://doi.org/10.1210/endo-29-4-514
6. Schuler G. Equines Choriongonadotropin: Biologie und veterinärmedizinische Bedeutung. *Tieraerztl Prax*. 2020; 48(05):344-354. https://doi.org/10.1055/a-1235-7973
7. Kuran M, Broadbent PJ, Hutchinson JM. Bovine granulosa cell culture for assessment of potency and specificity of antibodies to pregnant mares' serum gonadotrophin. *Eur J Endocrinol*. 1996; 134(4):497-500. https://doi.org/10.1530/eje.0.1340497
8. Rodríguez MC, Mussio PE, Villarraza J, Tardivo MB, Antuña S, Fontana D, Ceaglio N, Prieto C. Physicochemical characterization of a recombinant eCG and comparative studies with PMSG commercial preparations. *Protein J*. 2023; 42(1):24-36. https://doi.org/10.1007/s10930-023-10092-x
9. Combarnous Y, Mariot J, Relav L, Nguyen TM, Klett D. Choice of protocol for the in vivo bioassay of equine chorionic gonadotropin (eCG/PMSG) in immature female rats. *Theriogenology*. 2019; 130:99-102. https://doi.org/10.1016/j.theriogenology.2019.03.004
10. Christin-Maitre S, Vasseur C, Fauser B, Bouchard P. Bioassays of gonadotropins. *Methods*. 2000; 21(1):51-57. https://doi.org/10.1006/meth.2000.0974
11. Lapthorn A, Harris DC, Littlejohn A, Lustbader JW, Canfield RE, Machin KJ, Morgan FJ, Isaacs NW.

Crystal structure of human chorionic gonadotropin. *Nature.* 1994; 369(6480):455-461. https://doi.org/10.1038/369455a0

12. Murphy BD, Martinuk SD. Equine chorionic gonadotropin. *Endocr Rev.* 1991; 12:27-44. https://doi.org/10.1210/edrv-12-1-27
13. De Rensis F, López-Gatius F. Use of equine chorionic gonadotropin to control reproduction of the dairy cow: a review. *Reprod Domest Anim.* 2014; 49(2):177-182. https://doi.org/10.1111/rda.12268
14. Arce JC, Andersen AN, Fernández-Sánchez M, Visnova H, Bosch E, García-Velasco JA, Barri P, De Sutter P, Klein BM, Fauser BC. Ovarian response to recombinant human follicle-stimulating hormone: a randomized, antimüllerian hormone–stratified, dose–response trial in women undergoing in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril.* 2014; 102(6):1633-1640. https://doi.org/10.1016/j.fertnstert.2014.08.013
15. Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. The OECD program to validate the rat uterotrophic bioassay. Phase 2: dose-response studies. *Environ Health Perspect.* 2003; 111(12):1530-1549. https://doi.org/10.1289/ehp.5780