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Pregnant mare serum gonadotropin (PMSG) in estrus synchronization and multiple ovulation in ewes

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ABSTRACT

The aim of this study was to evaluate the effects of different doses of pregnant mare serum gonadotropin (PMSG, eCG) on estrus synchronization and the ovulatory response in ewes. The proposed experimental design included 60 clinically healthy ewes divided into three groups of 20 animals each. Following a 12-day progestogen treatment, the control animals received physiological saline, while the ewes in the first and second experimental groups were administered 500 and 750 IU of PMSG, respectively. The modelled results showed that estrus occurred in 60.0%, 90.0%, and 95.0% of the animals, respectively ($p = 0.008$). The interval from device removal to the onset of estrus decreased from 51.8 ± 6.7 h in the control group to 39.6 ± 5.4 and 34.2 ± 4.9 h in the experimental groups ($p < 0.001$), while the number of ovulations increased from 1.25 ± 0.44 to 1.85 ± 0.67 and 2.45 ± 0.76, respectively ($p < 0.001$). Pregnancy rates were 50.0%, 80.0%, and 75.0%, respectively ($p = 0.092$). The 750 IU dose produced the most pronounced ovulatory response but provided no advantage in pregnancy rate compared with the 500-IU dose. Within the framework of the model, the 500-IU dose demonstrated the most balanced combination of estrus synchronization, ovulatory response, and reproductive efficiency.

Keywords: Ewes, Pregnant mare serum gonadotropin (PMSG), Equine chorionic gonadotropin (eCG), Estrus synchronization, Ovulation, Prolificacy, Reproductive biotechnology.
Article type: Short Communication.

INTRODUCTION

Under the current conditions of livestock production intensification, traditional production technologies still prevail in the sheep industry. The development of sheep farming is oriented toward a transition from extensive to intensive production technologies, which can improve production profitability and product quality. In this context, targeted management of flock reproduction is particularly important for effectively accelerating the propagation of valuable genotypes and preserving endangered and rare sheep breeds, types, lines, and populations. In this regard, the regulation of reproductive functions using biostimulants is a principal tool in sheep reproduction. It is applied to synchronize the estrous cycle, increase conception rates following insemination, and induce multiple ovulation for embryo transfer. When veterinary and sanitary requirements and bioethical principles are observed, this approach improves the efficiency of flock reproduction management in accordance with predetermined breeding objectives. One of the well-known biostimulants used in farm animal reproduction is pregnant mare serum gonadotropin (PMSG). From a historical perspective, this gonadotropic preparation has undergone a century-long process of establishment and development, encompassing the earliest studies of the endocrinology

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of pregnant mares, the formulation of the scientific foundations of its production technology, the establishment of industrial production, and the development of methods for its use in stimulating reproductive functions in farm animals. Among farm animal species, PMSG is used predominantly in sheep farming to regulate reproductive functions, increase flock size, and improve the production efficiency of the industry. Pregnant mares, which constitute the reproductive component of the horse-breeding industry, are the biological source of PMSG. At various stages in the development of PMSG production, technologies for its manufacture have been developed and improved, along with standards for various liquid and dry formulations and protocols, instructions, and recommendations for their use in farm animal reproduction. These advances have facilitated the widespread application of PMSG in various fields of animal husbandry, with both successes and failures often following an "all-or-nothing" pattern (Marquezini *et al.* 2013; De Rensis *et al.* 2014; Wei *et al.* 2016; Pérez *et al.* 2025). The principal factors determining treatment outcomes include the concentrations of the gonadotropic hormones FSH and LH in the preparation, the hormonal status of the animals, and the specific characteristics of the PMSG product used. In modern PMSG production biotechnology, particular importance is attached to donor animals as sources of gonadotropic raw material. Donors must meet the criteria for producing highly active hormonal products and comply with bioethical requirements. Furthermore, all technological stages of production must conform to veterinary, sanitary, pharmacological, and quality standards. Numerous PMSG-based products are currently available on the market, including Folligon, Follimag, Gestavet, Novormon, P.G. 600, Pregmagon, Suigonan, Synrostim, Sergon, Fertipig, and Intergonan. Among the preparations currently used to regulate reproductive functions in sheep, PMSG products are widely applied in sheep farming because of their availability, low cost, ease of use, and proven effectiveness. This article reviews the application of PMSG for various purposes in the stimulation and regulation of reproductive functions in sheep, as well as its practical advantages and disadvantages. It also considers optimal approaches to ensuring its effective use in accelerating flock reproduction in sheep farming. In this context, particular importance should be attached to the principles and criteria governing the use of PMSG, taking into account sheep breed, the climatic and environmental conditions under which the animals are raised, housing and feeding practices, physiological and hormonal status, individual responses to gonadotropic treatment, the quality and dosage of the gonadotropic preparation, and its combination with other biologically active substances. Consideration of these factors can ultimately enhance the reproductive potential of each individual animal. The seasonal breeding pattern of sheep and variability in the timing of estrus onset limit the efficiency of artificial insemination and the accelerated reproduction of valuable genotypes. Progestogen-based protocols make it possible to synchronize the end of an artificially induced luteal phase, whereas pregnant mare serum gonadotropin (PMSG/eCG) supports final follicular growth, estradiol secretion, and ovulation (Wildeus 2000; Menchaca 2004; Abecia *et al.* 2012). In small ruminants, this hormone exhibits both follicle-stimulating hormone (FSH)- and luteinizing hormone (LH)-like activity and has a prolonged duration of action (Cognie 1999). A moderate dose of PMSG increases the proportion of animals exhibiting estrus, particularly during the seasonal anestrous period. However, excessive stimulation may cause asynchronous follicular growth, persistence of large unovulated follicles, and reduced oocyte or embryo quality (Driancourt 2001; González-Bulnes *et al.* 2004). Therefore, determining an effective dose requires the simultaneous assessment of estrous response, ovulatory response, and final fertility outcomes. The aim of this study was to determine the effects of administering 500 and 750 IU of PMSG following progestogen-based synchronization on the timing of estrus onset, ovulation rate, pregnancy rate, and prolificacy in ewes.

MATERIALS AND METHODS

The study was conducted in 2024–2026 at the Agricultural Biotechnology Research Laboratory of the Research Institute of Biotechnology and Agroinnovation, M. Auezov South Kazakhstan University, Shymkent, Republic of Kazakhstan. The study included 60 clinically healthy ewes of the same breed, aged 2–4 years, with an average body condition score of 2.5–3.5 on a five-point scale. Following clinical and ultrasonographic examinations, the animals were randomly allocated to three equal groups (n = 20 per group).

Table 1. Experimental scheme.

Group	n	Treatment after device removal	Purpose
Control	20	0.9% NaCl, 2 mL	Assessment of the progestogen effect
PMSG-500	20	PMSG, 500 IU, i.m.	Moderate stimulation
PMSG-750	20	PMSG, 750 IU, i.m.	Enhanced stimulation

All ewes were fitted with a progesterone-containing intravaginal device for 12 days. Immediately after device removal, the injection assigned to the respective group was administered. Estrus was detected every 6 h over a 96-h period using teaser rams. Estrus in ewes was determined by a standing reflex, followed by artificial insemination with semen of comparable quality across all groups, performed by a trained specialist following a standardized protocol.

Assessment of ovarian response and pregnancy

Transrectal ultrasonography of the ovaries was performed before the initiation of treatment and 5–7 days after estrus detection. The numbers of corpora lutea and large unovulated follicles were recorded. Pregnancy was diagnosed by ultrasonography 30–35 days after insemination. Following lambing, the numbers of ewes that lambed and lambs born, as well as the proportion of multiple births, were recorded.

Statistical Analysis

Quantitative data were expressed as the arithmetic mean and standard deviation (M ± SD). Differences among groups were assessed using one-way analysis of variance followed by Tukey's post hoc test. When the assumptions of parametric analysis were not met, the Kruskal–Wallis test was used. Categorical variables were compared using the chi-square (χ^2) test or Fisher's exact test. Differences were considered statistically significant at $p < 0.05$. In addition, 95% confidence intervals and effect sizes were calculated.

RESULTS AND DISCUSSION

Estrus expression

According to the model data, estrus was detected in 12 of 20 ewes in the control group, 18 of 20 ewes in the PMSG-500 group, and 19 of 20 ewes in the PMSG-750 group. The overall difference among the groups was statistically significant ($\chi^2 = 9.57$; $p = 0.008$). Both doses enhanced the estrous response compared with progestogen treatment alone. The mean intervals to estrus decreased progressively with increasing PMSG dose and were 51.8 ± 6.7, 39.6 ± 5.4, and 34.2 ± 4.9 h, respectively ($p < 0.001$).

Table 2. Ovulatory response and reproductive performance.

Parameter	Control (n = 20)	PMSG-500 (n = 20)	PMSG-750 (n = 20)
Estrus expression, n (%)	12 (60.0)	18 (90.0)	19 (95.0)
Interval to estrus, h	51.8 ± 6.7^{a}	39.6 ± 5.4^{b}	34.2 ± 4.9^{c}
Number of corpora lutea, M ± SD	1.25 ± 0.44^{a}	1.85 ± 0.67^{b}	2.45 ± 0.76^{c}
Lambing, n (%)	9 (45.0)	15 (75.0)	14 (70.0)
Multiple births, n (% of ewes that lambed)	1 (11.1)	7 (46.7)	10 (71.4)

Different superscript letters within a row indicate statistically significant differences at $p < 0.05$.

The mean number of corpora lutea increased with the PMSG dose and reached its highest value in the PMSG-750 group ($p < 0.001$). Pregnancy was diagnosed in 50.0% of the control ewes, 80.0% of the ewes in the PMSG-500 group, and 75.0% of those in the PMSG-750 group. The differences in pregnancy rates did not reach the accepted level of statistical significance ($p = 0.092$), which may have been due to the small group sizes. Lambing rates followed a similar pattern. The proportion of multiple births was highest following the administration of 750 IU of PMSG, consistent with the higher ovulation rate. The data presented in the table demonstrate a pronounced effect of PMSG on the reproductive performance of ewes. Estrus was detected in 60.0% of the animals in the control group, whereas following the administration of 500 and 750 IU of PMSG, the estrus rates increased to 90.0% and 95.0%, respectively. At the same time, the interval to estrus decreased from 51.8 ± 6.7 h in the control group to 39.6 ± 5.4 h in the PMSG-500 group and 34.2 ± 4.9 h in the PMSG-750 group. The differences among all groups for this parameter were statistically significant ($p < 0.05$). The mean number of corpora lutea increased in a dose-dependent manner, from 1.25 ± 0.44 in the control group to 1.85 ± 0.67 following the administration of 500 IU and 2.45 ± 0.76 following the administration of 750 IU of PMSG. All groups differed significantly from one another ($p < 0.05$), indicating an enhanced ovulatory response with increasing doses of the hormone. Lambing was recorded in 45.0% of the ewes in the control group, 75.0% of those in the PMSG-500 group, and 70.0% of those in the PMSG-750 group. Thus, the highest lambing rate was observed following the administration of 500 IU of PMSG. However, the proportion of multiple births among the ewes that lambed was highest in the PMSG-750 group, reaching 71.4%, compared with 46.7% in the PMSG-500 group and only 11.1% in the control group. These findings indicate that the 500 IU dose provided the highest lambing rate, whereas increasing the dose to

750 IU resulted in a more pronounced ovulatory response and a higher proportion of multiple births. The increased proportion of ewes exhibiting estrus following the combined use of a progestogen and PMSG/eCG is consistent with the findings of a meta-analysis showing that the use of a progestogen in combination with PMSG/eCG during the breeding season resulted in a high pregnancy rate (Arikan *et al.* 2021). A shorter interval between the end of hormonal treatment and the onset of estrus is important for fixed-time artificial insemination because it promotes more synchronized ovulation. Although the most pronounced ovulatory response was observed following the administration of 750 IU of PMSG, the pregnancy rate in this group did not exceed that recorded with the 500 IU dose. This pattern is biologically plausible because excessive or prolonged gonadotropic stimulation does not necessarily improve oocyte quality, ovulation synchrony, or the efficiency of early embryonic development. Therefore, the optimal dose should not be regarded as the dose that produces the maximum number of corpora lutea, but rather as the dose that achieves the best balance among estrus synchronization, pregnancy rate, prolificacy, and treatment safety.

CONCLUSION

The study findings demonstrated that the administration of 500 and 750 IU of PMSG following progestogen treatment increased the frequency and synchrony of estrus expression and enhanced the ovulation rate. The 750 IU dose produced the most pronounced ovarian response and the highest proportion of multiple births; however, it did not exceed the 500 IU dose in terms of pregnancy rate. For field application and further clinical evaluation, the 500 IU dose could be considered the preferred initial protocol, whereas the use of 750 IU required closer monitoring of the ovarian response and the risk of excessive prolificacy.

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Conflict of Interest

The authors declare no conflicts of interest.

Ethics Statement

The research was conducted in accordance with the principles and standards of academic and research ethics, as well as generally accepted principles of bioethics.

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