Livestock Studies 2025; 65(2), 66 -70
https://doi.org/10.46897/livestockstudies. 1847056

LIVESTOCK STUDIES

RESEARCH ARTICLE

Effect of eCG Injection Timing on Reproductive Performance in Anatolian Merino Ewes During the Non-Breeding Season

Mesut KIRBAŞ[1,*], Neffel Kürşat AKBULUT[2]

[1]Bahri Dağdaş International Agricultural Research Institute. Karatay, Konya, Türkiye
[2]Necmettin Erbakan University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology. Ereğli, Konya
*Corresponding Author

Article History
Received: 24 Nov 2025
Accepted: 17 Dec 2025

Corresponding Author
E-mail: mesutkirbas@gmail.com

Keywords
Ewe
Equine chorionic gonadotropin
Reproductive parameters
Out of the breeding season

Abstract

The aim of this study is to determine how the timing of eCG administration affects reproductive parameters such as estrus rate, conception rate, pregnancy rate, single, twin and triplet lambing rates, and litter size in Anatolian Merino ewes whose estrus cycles are induced with intravaginal progesterone outside the breeding season. For this purpose, 187 Anatolian Merino ewes were randomly divided into two groups, and a sponge containing 20 mg flugeston acetate was inserted into both groups on day 0 for estrus induction. Group 1 (n=93) received 480 IU eCG intramuscularly (IM) on day 8 (one day before sponge removal), while Group 2 (n=94) received it on day 9 (sponge removal day). On the 9th day, the sponges were removed from both groups, and estrus was monitored. Differences between groups in reproductive parameters such as estrus rate, conception rate, pregnancy rate, single, twin and triplet birth rates, and litter size were tested using chi-square analysis. The analysis revealed no significant differences between groups in terms of reproductive parameters (P>0.05). Consequently, it was observed that eCG administration 24 hours before and at the time of sponge removal could yield similar results in Anatolian Merino ewes induced with intravaginal progesterone outside the breeding season

Introduction

The cornerstone of sheep farming is to achieve a higher level of production without creating an economic burden and to maximise reproductive success (Akbulut and Kutlu 2024). To improve sheep fertility and control reproductive processes, natural methods and various hormone applications are used in addition to technological developments (Özyurtlu and Bademkıran 2010). To enable more productive lamb production throughout the year, hormonal treatments in ewes are essential. These treatments are reproductive tools used to synchronise or induce estrus in natural mating or artificial insemination programmes. Although various protocols exist for synchronising and/or triggering estrus, applications based on progestogens and eCG yield more satisfactory results in terms of estrus activity and fertility (Barrett *et al*., 2004; Quintero-Elisea *et al*., 2022). One of the most common methods of estrus synchronisation in ewes involves the use of synthetic progestogens (fluorogestone acetate, medroxyprogesterone acetate, etc.) or progesterone-impregnated devices inserted into the vagina. This method is effective both during the breeding season and outside of it (Eldomany *et al*., 2023). The 12-14 day progesterone-impregnated devices application simulates the normal luteal phase length while also allowing sufficient time for spontaneous corpus luteum lysis before the sponge is removed. At the end of this protocol, estrus is detected in 75-100% of ewes within 24-72 h following sponge removal (Olivera-Muzante *et al*., 2011; Pérez-Clariget *et al*., 2021). Short-term treatments (e.g., 6 days) have been effectively utilized for estrus induction, particularly during the non-

breeding season. (Ungerfeld and Rubianes 1999). The use of eCG is also of great importance in addition to progesterone treatment. This eCG injection causes faster pituitary endocrine responses and estradiol secretion, mediating follicular growth and stimulating estrus and ovulation (Abecia *et al*. 2012; Akbaş and Köse 2017). eCG is a glycoprotein hormone secreted in high amounts in the bloodstream during days 40-130 of a mare's pregnancy. A single molecule of this hormone possesses the biological activity of both FSH (Follicle-Stimulating Hormone) and LH (Luteinising Hormone) (Somanjaya *et al*., 2021).

The success of estrus synchronisation may depend on the duration of treatment with the progesterone agent used (Martinez-Ros *et a*l., 2018), and it has been reported that the timing of eCG hormone administration may also be effective (Quintero-Elisea *et al*., 2022). It has been suggested that, in addition to progesterone administration, the day of injection of eCG hormone may affect reproductive performance also outside the breeding season (Kırbaş *et al* 2025). In this study, we aimed to determine how the administration of eCG one day prior to and at the time of sponge removal, following a 9-day intravaginal progesterone treatment in Anatolian Merino ewes outside the breeding season, affected reproductive parameters such as estrus rate, conception rate, pregnancy rate, single, twin and triplet lambing rates, and litter size.

Materials and Methods

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of the Experimental Animal Production and Research Centre, Faculty of Veterinary Medicine, Selçuk University (SÜVDAMEK) under decision number 2025/71.

Animal

The study was conducted at a private sheep enterprise located in the Karatay district of Konya province. A total of 187 Merino ewes aged at 2-5 years, which had become pregnant and lambed during the previous mating season and were found to have no health problems upon examination, were used. The ewes, with an average body weight of 60–65 kg, were housed in indoor pens, fed according to NRC (2007) guidelines, and provided with ad libitum access to water. For mating, 20 rams of known fertility were utilized.

Estrus Induction Protocol and Study Groups

187 Anatolian Merino ewes were randomly divided into two groups, and a sponge containing 20 mg flugestone acetate (Chronogest CR, MSD) was inserted into both groups on day 0 for estrus induction. Group 1 (n=93) received 480 IU eCG (Chrono-gest PMSG, MSD) intramuscularly (IM) on day 8 (1 day before sponge removal), while Group 2 (n=94) received it on day 9 (sponge removal day). On day 9, the sponges were removed from both groups, and estrus was monitored.

Estrus Monitoring and Mating

Estrus monitoring commenced 24 hours (h) after the sponge was removed and was carried out twice daily for four days. The rams were introduced to the flock in pairs for mating, and the ewes were removed from the flock after mating.

Determination of Pregnancy

The pregnancy examination was performed approximately 30-35 days after mating. A linear probe (7.5 MHz) ultrasound device (Hasvet WED 3000, Türkiye) was used to detect pregnancy via transrectal ultrasound. The diagnosis of pregnancy was made by visualising fluid in the uterus, the placenta, foetal movement and the foetal heartbeat (Tekin and Köse 2022).

Reproductive Parameters

Reproductive parameters were calculated according to the formulas (Kutlu and Akbulut, 2025) given below.

$$Estrous\ rate = \frac{the\ number\ of\ ewes\ showing\ estrus\ behaviours}{the\ number\ of\ ewes\ receiving\ sponge}\ x\ 100$$

$$Conception\ rate = \frac{the\ number\ of\ pregnant\ ewes}{the\ number\ of\ mated\ ewes}\ x\ 100$$

$$Pregnancy\ rate = \frac{the\ number\ of pregnant\ ewes}{the\ number\ of\ ewes\ receiving\ sponge}\ x\ 100$$

$$Lambing\ rate = \frac{the\ number\ of\ lambing\ ewes}{the\ number\ of\ pregnant\ ewes}\ x\ 100$$

$$Single\ lambing\ rate = \frac{the\ numberof\ single\ lambing\ ewes}{the\ number\ of\ lambing\ ewes}\ x\ 100$$

$$Twin\ lambing\ rate = \frac{the\ numberof\ twin\ lambing\ ewes}{the\ number\ of\ lambing\ ewes}\ x\ 100$$

$$Triplets\ lambing\ rate = \frac{the\ numberof\ triplets\ lambing\ ewes}{the\ number\ of\ lambing\ ewes}\ x\ 100$$

$$Litter\ size = \frac{the\ number\ of\ total\ lambs}{the\ number\ of\ lambing\ ewes}\ x\ 100$$

Statistical Analysis

The statistical analyses were performed using SPSS version 23. Data normality was assessed using the Shapiro–Wilk test. Differences between groups in reproductive parameters such as estrus rate, conception rate, pregnancy rate, single, twin and triplet lambing rates, and litter size were tested using chi-square analysis. Differences were considered significant at $P < 0.05$.

Results and Discussion

In our study, we examined the effect of eCG administration 24 h before and at the time of sponge removal on reproductive parameters such as estrus rate, conception rate, pregnancy rate, single, twin and triplet lambing rates, and litter size in Central Anatolian Merino ewes with induced estrus outside the breeding season. The reproductive parameters obtained in the groups are presented in Table 1.

When estrus rates were examined in the first service, 82 out of 93 ewes (88.18%) in the eCG -1 group showed signs of estrus, while 79 out of 94 ewes (84.04%) in the eCG 0 group showed estrus. In the

Table 1: Reproductive parameters in groups.

Parameters	Groups					
	1st service		2th service		Total	
	eCG -1	eCG 0	eCG -1	eCG 0	eCG -1	eCG 0
Estrus Rate (%)	88.18 (82/93)	84.04 (79/94)	36.96 (17/46)	42.30 (22/52)	88.18 (82/93)	84.04 (79/94)
Conception Rate (%)	57.32 (47/82)	53.16 (42/79)	100 (17/17)	100 (22/22)	78.05 (64/82)	81.01 (64/79)
Pregnancy Rate (%)	50.54 (47/93)	44.68 (42/94)	36.96 (17/46)	42.30 (22/52)	68.82 (64/93)	68.09 (64/94)
Lambing Rate (%)	85.11 (40/47)	92.86 (39/42)	100 (17/17)	100 (22/22)	89.06 (57/64)	95.31 (61/64)
Single lambing rate (%)	45.0 (18/40)	38.46 (15/39)	88.24 (15/17)	77.27 (17/22)	57.89 (33/57)	52.46 (32/61)
Twin lambing rate (%)	55 (22/40)	53.85 (21/39)	11.76 (2/17)	27.73 (5/22)	42.1 (24/57)	42.62 (26/61)
Triplets lambing rate (%)	0 (0/40)	7.69 (3/39)	0 (0/17)	0 (0/22)	0 (0/57)	4.91 (3/61)
Litter size	1.55 (62/40)	1.69 (66/39)	1.12 (19/17)	1.23 (27/22)	1.42 (81/57)	1.52 (93/61)

*Statistical analysis showed no significant difference between the groups (*P*>0.05)

second service, estrus rates were 36.96% in the eCG -1 group and 42.30% in the eCG 0 group (P>0.05). Zeleke *et al*. (2005) conducted a study on Dorper ewes in May-June and found that 300 IU eCG administered 24 h before, during, and 24 h after sponging resulted in estrus responses ranging from 94.6% to 98.3% (Zeleke *et al*., 2005). Hemmati *et al*. (2020) conducted a study on Farahani ewes during the non-breeding season, administering 400 IU eCG 24 and 48 h prior to CIDR removal, and found estrus responses of 54% and 82%, respectively (Hemmati *et al*., 2020). Quintero-Elisea *et al*. (2022) administered 200 and 300 IU eCG 24 h before and during sponge removal in ewes and found estrus rates of 85.3% and 78%, respectively. (Quintero-Elisea *et al*., 2022). Quintero-Elisea *et al*. (2011) conducted a separate study on Blackbelly and Pelibuey ewes that exhibit estrus throughout the year. They administered eCG 24 and 48 h before and after sponge removal and found estrus rates of 78.1%, 96.8%, and 100%, respectively (Quintero-Elisea *et al*., 2011). Doğruer *et al*. (2015) conducted an non-breeding season study on ewes, administering eCG 48 h prior to sponge removal and at removal of sponge finding estrus rates of 68.40% and 89.40%, respectively (Doğruer *et a*l., 2015). In these studies, it was observed that eCG administration 24 or 48 h prior to sponge removal caused a similar or greater estrus response than eCG administration at the time of sponge removal. Doğruer *et al*. (2015) reported that this difference may be due to the earlier formation of the hormonal effects of eCG on the follicular wave and follicles when eCG is injected before the end of progestagen treatment (Doğruer *et a*l., 2015). The absence of a difference in estrus rates suggests that early eCG administration did not allow sufficient time for the follicular wave to reach the optimal stage for ovulation, thereby limiting the hormonal effects.

In our study, the pregnancy rates in the eCG-1 and eCG 0 groups were found to be 50.54% and 44.68%, respectively, while the conception rates were 57.32% and 53.16%, respectively. Although studies investigating the effect of eCG administration timing on conception and pregnancy rates generally have not reported significant differences, there are studies indicating higher rates when eCG is administered prior to sponge removal (Zeleke *et al*., 2005; Doğruer *et al*., 2015), as well as studies observing higher rates in groups treated on the day of sponge removal (Gözer *et al*., 2023) In studies conducted on ewes outside the breeding season, Doğruer *et al*. (2015) reported a pregnancy rate of 76% and a conception rate of 85.20% when eCG was administered 48 h before sponge removal, while Gözer *et al*. (2023) reported a pregnancy rate of 52.77% and a conception rate of 79.16% when eCG was applied at the time of sponge removal. It has been proposed that eCG administration before progesterone withdrawal may reduce the population of small follicles while increasing that of medium and large ones, in addition to shortening estrus duration and inducing earlier preovulatory LH peaks and ovulations (Zeleke *et al*., 2005; Quintero-Elisea *et al*., 2011; Gözer *et al*.,2023).

In the present study, lambing rate (single, twin, triplets) and litter size were similar in the groups (P>0.05). In the study conducted by Doğruer *et al*. (2015) the lambing rate in the group treated with eCG during sponging was 95.2%, the twin rate was 50%, and in the group treated with eCG 48 h before sponging, the lambing rate was 100% and the twin rate was 31%. In the same study, litter size was 1.50 in the group treated with eCG during sponge extraction, while it was 1.31 in the group treated with eCG 48 h prior to sponge extraction (Doğruer *et al*., 2015). In the study conducted by Quintero-Elisea *et al*. (2011) on ewes, the lambing, single lambing, and multiple lambing rates were 80.7%, 38.0%, and 62.0%, respectively, in the group administered eCG 48 h before sponge removal, 90.5%, 27.8%, and 72.2% in the group administered eCG 24 h before sponge removal, and 73.7%, 42.9%, and 57.1% in the group administered eCG at the time of sponge removal, respectively. The timing of eCG administration did not affect the overall lambing success or single/multiple lambing rates in ewes (Quintero-Elisea *et al*., 2011). Koyuncu and Altıcekic (2010), in their study on ewes, found lambing rates and multiple lambing rates to be 76.7% and 73.9%, respectively, in the group treated with eCG 24 h before sponge removal, while in the group treated with eCG during sponge removal, the rates were 86.2% and 61.9%. and 72.4% and 44%, respectively, in the group where eCG was administered 24 h after sponge removal. They stated that administering eCG 24 h before sponge removal shortened the time to ovulation and optimised follicle development, leading to more successful results (Koyuncu and Altıcekic, 2010). The absence of a significant difference in lambing rates and reproductive parameters, including litter size, suggests that the eCG application times in our study were close to each other.

Conclusion

In conclusion, eCG administration can be performed 24 h before sponge removal and at the time of sponge removal in Anatolian Merino ewes induced with intravaginal progesterone during the non-breeding season, yielding similar results. To minimise additional labour, eCG administration at the time of sponge removal is recommended.

Author Contributions

Mesut Kırbaş and Neffel K. Akbulut contributed equally to the conception and design of the study. Both authors were involved in data collection, analysis, and

interpretation. They also conducted the literature review, drafted the manuscript, and critically revised it. Both authors approved the final version of the manuscript.

Acknowledgements

The authors would like to thank the management and staff of Deresallar sheep farm for their contribution in providing the animals used in this study.

Conflict of Interest

The authors declare that they have no discernible commercial or financial interests that could have the potential to influence the findings of this study.

Funding Information

No funding was provided from any project.

References

Abecia JA, Forcada F, González-Bulnes A. Hormonal control of reproduction in small ruminants. Anim Reprod Sci 2012; 130: 173-179.

Akbaş ÖF, Köse AM (2017). Aşım sezonunda FGA ile senkronize edilen ivesi koyunlarında PGF2α ve PMSG uygulamasının bazı reprodüktif parametreler üzerine etkisi. Eurasian Journal of Veterinary Sciences, 33(2), 107-112.

Akbulut, N.K., Kutlu, M. (2024). The Effects of Different Doses of eCG Injection on Reproductive Parameters in Nulliparous Merino Ewes During the Non-Breeding Season. Livestock Studies 64: 51-55.

Barrett, D., Bartlewski, P., Batista-Arteaga, M., Symington, A., Rawlings, N. (2004). Ultrasound and endocrine evaluation of the ovarian response to a single dose of 500 IU of eCG following a 12-day treatment with progestogen-releasing intravaginal sponges in the breeding and nonbreeding seasons in ewes. Theriogenology 61: 311-327.

Dogruer, G., Ergun, Y., Karaca, F., Saribay, MK., Ates, CT., Akoz, M., Aydin, I. (2015). FGA tedavisi uygulanan anöstrüsteki koyunlarda eCG ve PGF2α'nın farklı zamanlarda uygulanmasının fertilite üzerindeki etkisi. Eurasian Journal of Veterinary Sciences 31: 158-162.

Eldomany, W., Abdel-Khalek, A.-K., Khalil, W., Yousif, A., El-Saidy, B., Hassan, M. (2023). Influence of long and short-term progesterone administration on estrous synchronization and reproductive performance in ewes during may season. Journal of Advanced Veterinary Research 13: 88-93.

Gözer, A., Saribay, M.K., Bahan, O., Doğruer, G., Parlaktaş, M. (2023). The Effect of Time of Equine Chorionic Gonadotrophin and Prostaglandin F2α Administration on Fertility Parameters in Akkaraman Yearlings Treated by Medroxyprogesterone Acetate in Non-breeding Season. Journal of Bahri Dagdas Animal Research 12 (2): 96-102,

Hemmati, M., Khodaei-Motlagh, M., Yahyaei, M. (2020). Effects of increased exposure time to eCG on reproductive performance in estrus-synchronized Farahani ewes. Journal of Livestock Science and Technologies 8: 15-20.

Kırbaş, M., Köse, M., Bülbül, B., Dursun, Ş., Demirci, U., Erduran, H., & Sari, D. (2025). Estrus synchronization using progesterone+ different gonadotropins or flushing+ ram effect in the breeding season in sheep. Revista MVZ Córdoba, 30(2), e3570.

Koyuncu, M., Altıcekic, S.O. (2010). Effects of progestagen and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breeding season. Asian-Australasian Journal of Animal Sciences 23: 308-311.

Kutlu, M., Akbulut, N. K. (2025). The effects of anti-galactagogue (sage, parsley extract) and anti-inflammatory (echinacea extract) feed supplements on prolactin levels and fertility in the re-pregnancy of lactating Merino ewes. Tropical Animal Health and Production, 57(2), 59.

Martinez-Ros, P., Rios-Abellan, A., Gonzalez-Bulnes, A. (2018). Influence of progesterone-treatment length and eCG administration on appearance of estrous behavior, ovulatory success and fertility in sheep. Animals 9: 9.

Olivera-Muzante, J., Fierro, S., López, V., Gil, J. (2011). Comparison of prostaglandin-and progesterone-based protocols for timed artificial insemination in sheep. Theriogenology 75: 1232-1238.

Özyurtlu, N., Bademkıran, S. (2010). Koyunlarda Östrus Senkronizasyonu ve Östrusu Uyarma Yöntemleri. Dicle Üniversitesi Veteriner Fakültesi Dergisi: 17-22.

Pérez-Clariget, R., López-Pérez, Á., Ungerfeld, R. (2021). Treatments with intravaginal sponges for estrous synchronization in ewes: length of the treatment, amount of medroxyprogesterone, and administration of a long-acting progesterone. Tropical Animal Health and Production 53: 345.

Quintero-Elisea, J.A., Macías-Cruz, U., Álvarez-Valenzuela, F.D., Correa-Calderón, A., González-Reyna, A., Lucero-Magaña, F.A., Soto-Navarro, S.A., Avendaño-Reyes, L. (2011). The effects of time and dose of pregnant mare serum gonadotropin (PMSG) on reproductive efficiency in hair sheep ewes. Tropical Animal Health and Production 43: 1567-1573.

Quintero-Elisea, J.A., Olguín-Arredondo, H.A., Velázquez-Morales, J.V., Garay-Martínez, J.R., Vázquez-Rocha, L., Limas-Martínez, A.G., Joaquín-Cancino, S. (2022). Effect of breed, breeding season, eCG dose, and eCG application time on the estrous cycle of hair ewe lambs. Agro Productividad 15: 187-194.

Somanjaya, R., Fuah, A., Rahayu, S., Setiadi, M., Abdullah, L. 2021. PMSG in ewes: A Practical and Efficient Step for Superovulation, IOP Conference Series: Earth and Environmental Science, IOP Publishing, p. 012010.

Tekin, T. C., Köse, A. M. (2022). Investigation of the effectiveness of ultrasonography in determining pregnancy and the number of fetuses on the 35th day of pregnancy in Awassi sheep. Journal of Advances in VetBio Science and Techniques, 7(2), 143-152.

Ungerfeld, R., Rubianes, E. (1999). Effectiveness of short-term progestogen primings for the induction of fertile oestrus with eCG in ewes during late seasonal anoestrus. Animal Science 68: 349-353.

Zeleke, M., Greyling, J., Schwalbach, L., Muller, T., Erasmus, J. (2005). Effect of progestagen and PMSG on oestrous synchronization and fertility in Dorper ewes during the transition period. Small ruminant research 56: 47-53.