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RESEARCH ARTICLE

The Effects of Different Doses of eCG Injection on Reproductive Parameters in Nulliparous Merino Ewes During the Non-Breeding Season

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Abstract

In this study, the effects of adding 400, 500, and 600 IU eCG to progesterone treatment on rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size in nulliparous Merino ewes the out of breeding season were investigated. A total of 90 nulliparous Merino ewes were utilized in the study. On day 0, a vaginal sponge containing 60 mg medroxyprogesterone acetate was inserted for a seven-day period. Ewes were randomly divided into three groups at the time of sponge removal. Group I (G-400), comprising 30 ewes, received 400 IU of eCG. Group II (G-500) received 500 IU of eCG (n=30), while Group III (G-600) received 600 IU of eCG (n=30). The statistical analysis revealed that the conception rates between the groups were statistically higher in the G-600 group than in the G-400 and G-500 groups (G-400 vs. G-600, P=0.091; G-500 vs. G-600, P=0.080). Pregnancy rates between the groups were found to be statistically higher in the G-600 group than in the G-400 group (P<0.021). The administration of a high dose of eCG (600 IU) in conjunction with progesterone synchronization yielded favorable outcomes with respect to reproductive parameters, particularly an elevated conception and pregnancy rates.

Introduction

In sheep breeding, the primary objectives are to achieve greater efficiency without increasing costs and to enhance reproductive performance (Özyurtlu and Bademkıran, 2010). The profitability of sheep farming is closely linked to the number of lambs produced annually (Bulbul *et al*., 2014). Exogenous hormones are the leading biotechnological products used in animal husbandry, especially to improve fertility. During the breeding season, progesterone and and prostaglandin F2 alpha (PGF2α) can be used for estrus synchronization and equine chorionic gonadotropin (eCG) to induce ovulation in sheep farming (Köse *et al*., 2022). The protocols entail the administration of progesterone to the ewe, whether short- or long-term, with the objective of stimulating the luteal phase of the cycle. Once this period has elapsed, the source of the progesterone is removed and eCG is applied (Cline *et al*., 2001).

eCG is a glycoprotein hormone secreted by trophoblastic epithelial cells of fetal origin that form the equine endometrial cups (Manteca Vilanova *et al*., 2019). eCG has been observed to exhibit high affinity for follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-related receptors. This affinity has been associated with increased estradiol before ovulation (Rostami *et al*., 2011) and increased progesterone after ovulation in dairy cows (Rowe *et al*., 2019). In these treatments, the estrus response and onset of estrus may be affected by factors such as the dose and the time of administration of eCG, breed and season, body condition and geographical region (Quintero-Elisea *et al*., 2022).

In estrous synchronization protocols, low doses of eCG generally reduce the time to onset of estrus.

Additionally, evidence indicates that low doses of eCG typically enhance the number and size of large follicles, though it may exert minimal influence on the ovulation rate (Moakhar *et al*., 2012). The effect of low-dose eCG in non-breeding season ewes is of great interest. The effect of eCG on the dynamics of the follicular wave in ewes during the non-breeding season is well known (Roshan *et al*., 2023). A deeper understanding of this mechanism could facilitate the development of more effective methods for regulating sheep breeding (Barrett *et al*., 2004).

The number of progesterone and eCG-based studies on nulliparous ewes is insufficient, and the available information is limited (Santos-Jimenez *et al*., 2022). In studies conducted out of the breeding season, there are reports indicating that multiparous ewes have show a better response to these hormones compared to nulliparous ewes (Santos-Jimenez *et al*., 2022), as well as studies reporting no significant difference (Ungerfeld and Rubianes, 1999). In a study in ewe lambs, higher pregnancy and conception rates were found in the groups using eCG compared to the control group (Dias *et al*., 2020). It can be posited that the utilisation of eCG in ewe lambs may facilitate the attainment of a greater number of lambs.

It is thought that increasing the dose of eCG may induce estrus by stimulating follicular development and improve some reproductive parameters. It is hypothesized that the response to progesterone and different doses of eCG may vary non-breeding season in nulliparous Merino ewes. In this study, the effects of adding 400, 500, and 600 IU eCG to progesterone treatment on rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size in nulliparous Merino ewes out of the breeding season were investigated.

Materials and Methods

The present study was conducted with approval from Selçuk University Animal Experiments Local Ethics Committee, Konya, Türkiye (2024/084).

Animals

This study was conducted in a commercial sheep farm (Lat: 37° 86’ 44.06» N, Long: 34° 16’ 33.55» E and Alt: 1.020 m) in Konya province, Türkiye during the non-breeding season in 2024. A total of 96 nulliparous (1 year old, 45-50 kg) Merino ewes, clinically healthy with no abnormalities in the perineum, were used. Six ewes whose sponge had been fell were excluded from the study. The study continued with 90 ewes. The ewes were permitted to graze on pasture for a duration of 12 hours each day and were not provided with any compound feed. They had ad libitum access to water. Prior to mating, the animals were not subjected to any nutritional flushing.

Treatment groups, synchronization and mating protocols

On day 0, a vaginal sponge containing 60 mg of medroxyprogesterone acetate (Esponjavet®, Hipra, Spain) was inserted for a seven-day period. On day 7, the sponges were removed. The ewes were randomly divided into 3 groups at the time the sponges were removed and 400 IU of eCG (Oviser®, Hipra, Spain) was injected to ewes in Group 1 (G-400) (n=30), 500 IU of eCG to ewes in Group 2 (G-500) (n=30), and 600 IU of eCG was injected to the ewes in Group 3 (G-600) (n=30).

A teaser ram was employed twice daily for 1-hour sessions over 36 hours following the removal of sponges to detect estrus. Ewes identified as being in estrus were hand-mated with one of the proven fertile Merino rams, maintaining a ewe-to-ram ratio of 7:1.

Ultrasonography and calculation of reproductive parameters

In all ewes, transabdominal ultrasound examination (Hitachi EUB-405, Japan, 3.5 MHz convex probe) was performed to diagnose pregnancy on day 45 post-mating. Pregnancy (single/twin) were diagnosed when embryos were seen on ultrasound examination. The litter size was determined at parturition.

The rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size were calculated as reproductive parameters as follows;

$$Estrus\ rate = \frac{the\ number\ of\ ewes\ showing\ estrus\ behaviours}{the\ number\ of\ ewes\ receiving\ intravaginal\ sponge}$$

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

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

$$Fetal\ mortality\ rate = \frac{number\ of\ detected\ death\ fetus}{the\ number\ of\ pregnant\ ewes}\ x\ 100$$

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

$$Multiple\ birth\ rate = \frac{the\ number\ of\ multiple\ lambing\ ewes}{the\ number\ of\ pregnant\ ewes\ in\ each\ group}\ x\ 100$$

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

Statistical analysis

Statistical analyses were performed using SAS (Version 8.0). Reproductive parameters were assessed using the Chi-squared test, Fisher’s exact test (Selvi, 2024), and PROC GENMOD procedure. The results were reported as percentages. Statistical significance was defined as $p < 0.05$, and tendency was considered for $0.05 < p \leq 0.10$.

Results

Results for the rate of estrus, conception, pregnancy, fetal mortality, lambing, multiple birth and litter size are given in Table 1.

In the statistical analysis, differences between the groups in rate of estrus, fetal mortality, lambing, multiple birth and litter size were found to be insignificant (P > 0.05). Conception rates between groups were found to be higher in the G-600 group than in the G-400 and G-500 groups (G-400 vs G-600 P=0.091; G-500 vs G-600 P=0.080). Pregnancy rates between the groups were found to be statistically higher in the G-600 group than in the G-400 group (P<0.05).

Discussion

One method for increasing productivity in sheep is to obtain offspring during the anestrous period in Turkiye. During the non-breeding season, progestagens and eCG hormones are often used to stimulate functional activity in the ovaries. (Kaçar *et al*., 2008). eCG is known to be the most commonly used hormone to improve fertility in reproduction (Moakhar *et al*., 2012). The effectiveness of eCG is affected by many factors such as injection method, dose and source of eCG and season (Ali, 2007).

The objective of the presented study was to investigate the effects of different doses of eCG on reproductive parameters in nulliparous Merino ewes that had been synchronized with progesterone during the non-breeding season. In ewes, it has been reported that short term intravaginal progestagen treatment successfully induce and synchronize estrus in both breeding and non-breeding seasons (Ahmed Amer and Maher Hazzaa, 2009; Ataman *et al*., 2006). A study conducted non-breeding season demonstrated that the administration of eCG in conjunction with short-term progesterone resulted in a high estrus rate in both nulliparous and multiparous ewes (Ungerfeld and Rubianes, 1999). Santos-Jiménez (2022) observed that the estrus rate was higher in multiparous ewes than in nulliparous ewes during the non-breeding season. This result was attributed to the silent estrus of nulliparous ewes.

In some studies, conducted during the non-breeding season, estrus rates were observed to be high in ewes that had been synchronized with progesterone-based eCG applications at varying doses (Efe, 2010; Ungerfeld and Rubianes, 1999). The administration of 300, 500, and 700 IU of eCG following the intravaginal sponge application for 14 days during the seasonal anoestrus period resulted in estrus rates of 69.66% in all groups of Algerian Rembi sheep. (Bacha *et al*., 2014). Aköz *et al*. (2006) injected 300, 500 and 700 IU eCG after using intravaginal sponge containing 30 mg and 40 mg fluorogesterone acetate (FGA) in Merino x Akkaraman crossbred ewes during non-breeding season, and found the estrus rates to be 100%, 93.3% and 100% in the 30 mg FGA group, and 93.3%, 92.8% and 100% in the 40 mg FGA group, respectively. As with the aforementioned studies, it can be stated that the administration of varying doses of eCG does not result in statistically significant alterations in estrus rates, as observed in the presented study. It has been reported that eCG exhibits both FSH and LH activity and that these hormones are necessary for peri-ovulatory maturation of mammalian follicles (Murphy, 2018). Additionally, some studies have reported that eCG

Table 1. Reproductive parameters of nulliparous ewes.

	G-400 (*n*=30)	**G-500 (*n*=30)**	**G-600 (*n*=30)**	***P***
Estrus rate (%)	73.3 (22/30)	90.0 (27/30)	90.0 (27/30)	0.135
Conception rate (%)	59.1^{c} (13/22)	53.3^{c} (16/27)	81.5^{d} (22/27)	**
Pregnancy rate (%)	43.3^{a} (13/30)	53.3^{ab} (16/30)	73.3^{b} (22/30)	*
Fetal mortality rate (%)	7.6 (1/13)	18.8 (3/16)	13.6 (3/22)	0.679
Lambing rate (%)	92.3 (12/13)	81.2 (13/16)	86.3 (19/22)	0.679
Multiple birth rate (%)	16.7 (2/12)	23.1 (3/13)	42.1 (8/19)	0.261
Number of Lambs	14	16	28	
Single	10	10	11	
Twin	2 (4)	3 (6)	7 (14)	
Triplets	-	-	1 (3)	
Litter Size	1.17 (14/12)	1.23 (16/13)	1.47 (28/19)	0.244

Notes: Values with different superscripts (a, b) in the same row are significant differences among the groups (P < 0.05)
Values with different superscripts (c, d) in the same row are tendency differences (0.05 < P ≤ 0.1) among the groups.
* Pregnancy rate P value *G-400 vs G-600 P=0.021*
**Conception rate P value *G-400 vs G-600 P=0.091*; *G-500 vs G-600 P=0.080*
The numbers in parentheses are the formulation of the reproductive parameter.

causes an increase in progesterone concentrations (Baruselli *et al*., 2009). The findings of this study indicate that the administration of 600 IU eCG may enhance luteal activity, thereby improving pregnancy rates. While numerical differences were observed in certain examined characteristics, no statistically significant differences were identified. Takcı *et al*. (2023) reported that different eCG doses did not have a significant effect on reproductive parameters in primiparous ewes during the early anoestrus period. Köse *et al*. (2022) reported that different doses (400 and 600 IU) of eCG did not result in any differences in reproductive performance in ewes during the breeding season. Efe (2010) stated that although the use of 500 - 600 and 700 IU eCG after progesterone during the anoestrus period in ewes caused higher pregnancy rates than the control group (without eCG) and the dose difference did not affect the pregnancy rates. In a study conducted on Merino ewes during the non-breeding season, Doğanay (2011) used 400 and 600 IU eCG in addition to 14-day intravaginal progesterone application and found no statistical difference in pregnancy rates. Koyuncu *et al*. (2001) stated that the use of 0, 500 and 700 IU eCG in ewes synchronized with progesterone and found the litter size to be 1.21, 1.58 and 1.96, respectively on Kıvırcık ewes during the breeding season. In studies on the effects of eCG on reproductive parameters in ewes, it is seen that many factors are effective and therefore the results of the studies are not consistent (Köse *et al*., 2016). In order to eliminate this confusion in pregnancy rates, the optimum eCG dose needs to be determined. (Sharif *et al*., 2023). For this purpose, Sharif *et al*. (2023) used 300 and 600 IU eCG excluding the control group (without eCG) in Beetal goats at the beginning of the breeding season, and reported an increase in pregnancy rates in the group receiving 600 IU eCG compared to the other groups. Moaktar *et al*. (2012) in their study on Chall sheep during the natural breeding season, used 0, 450, 550, 650, 750, and 850 IU eCG and as a result, obtained higher pregnancy rates in the groups that received 550 and 650 IU eCG. Based on the studies conducted, it has been observed that different reproductive outcomes occur during breeding and non-breeding season. Many authors have argued that these results are due to the different responses of sheep breeds to eCG doses (Quintero-Elisea *et al*., 2022; Roshan *et al*., 2023). In our study, the Merino ewes used are capable of exhibiting reproductive activity for most of the year and have good fertility rates and, it is thought that the reproductive outcomes obtained, compared to other studies, to be due to the breed-specific characteristics.

Conclusion

In conclusion, it was determined that high dose eCG (600 IU) used in addition to synchronization with intravaginal progesterone during non-breeding season in nulliparous Merino ewes had positive results on reproductive parameters, especially causing an increase in pregnancy and conception rates.

Conflict of Interest

The authors declare that there is no conflict of interest.

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