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The Effects of Carazolol Injection During Mating Following Progestagen Administration on Conception Rate in Lactating Lacaune Ewes

Metehan Kutlu[1,a,*], Neffel Kürşat Akbulut[1,b]

[1]*Necmettin Erbakan University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 42310, Konya, Türkiye*
[*]*Corresponding author*

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ABSTRACT

The aim of the present study was to determine the the effects of carazolol injection during mating following progestagen + eCG administration on conception rate in breeding season Lacaune ewes. Sixty-two Lacaune ewes were used in the study. On day 0, an intravaginal sponge containing 20 mg flugestone acetate was inserted and left in place for 10 days. On day 10, the sponges were removed, and each ewe received an intramuscular injection of eCG at a dose of 480 IU. Ewes in estrus were mated with a proven rams (ewe:ram ratio of 5:1). Fifty-nine mated ewes were randomly assigned into two groups: control and treatment. In the carazolol group (n = 30), ewes received an intramuscular injection of carazolol (0.01 mg/kg; Simpanorm, Fatro, Italy) on the day of estrus, post-mating. In the control group (n = 29), ewes were not administered any treatment. Pregnancy diagnosis was conducted in all ewes on days 30 and 60 post-mating using transabdominal ultrasonography with a Hitachi EUB-405 device equipped with a 3.5 MHz convex probe. The estrus rate, conception rate, total pregnancy rate, and early fetal death rate were evaluated across all groups. The study results indicated no statistically significant differences between the control group and the carazolol group regarding conception rates (62.1% vs. 66.7%) and total pregnancy rates (79.3% vs. 83.3%). In addition, as a result of repeated ultrasound examinations, no early fetal death was found in the study. In conclusion, carazolol injection during mating following progestagen + eCG administration did not increase on fertility in breeding season Lacaune ewes.

[a] *metehankutlu2@hotmail.com* *https://orcid.org/0000-0002-1782-583X* | [b] *nkakbulut@gmail.com* *https://orcid.org/0000-0003-3853-9960*

Introduction

During the breeding season and outside the breeding season, estrus synchronization and estrus stimulation protocols are used to ensure that sheep become pregnant, allowing births to occur at the desired time. With these protocols, litter size can be increased, production can be scheduled according to periods of increased demand for meat and milk, and continuous production can be maintained throughout the year with three lambing in two years (Alaçam, 1993; Fındık, 2017; Takcı et., 2023; Uçar & Özyurtlu, 2012). Various hormone protocols are used in estrus synchronization/stimulation in order to control reproduction in ewes (Arıkan et., 2021). According to a meta-analysis study, it was reported that the progestagen +eCG protocols was the most effective method in ensuring estrus synchronization and desired pregnancy rates during the breeding season, and the pregnancy rate was 90.3% on average (Arıkan et., 2021).

The pregnancy rate can be increased by adding different hormones/drugs to these protocols. In order to prevent embryonic losses and increase reproductive performance in ewe, GnRH or hCG (Hashem et., 2015; Khan et., 2007; Kutlu & Dinç, 2021; Lashari & Tasawar, 2013; Olfati & Moghaddam, 2013; Sirjani et., 2012; Walker et., 1989) can be injected at various times after removal of the sponge. It is also reported that NSAIDs can be used after mating in farm animals to prevent luteolytic PGF2α release as a result of insufficient IFN-τ secretion (Aké-López et., 2005; Binelli et., 2001; Erdem & Guzeloglu, 2010). Successful results are not always obtained after additional hormone/drug application used in studies to increase reproductive efficiency. Mefepronic acid injection in addition to the progestagen + eCG protocol did not have an enhancing effect on reproductive parameters in ewes (Kutlu et., 2023). Further studies need to be continued in order to reach 100% pregnancy rate with estrus synchronization in ewes.

Carazolol (l-[carbazol-4-yloxy]-3-isopropylamino-2-propanol) is a beta-adrenergic antagonist that closely resembles adrenaline in structure. Following intramuscular administration, it is rapidly absorbed and exhibits a high clearance rate (Abshagen & Möllendorff, 1980). Carazolol has a strong affinity for both beta-1 and beta-2 adrenergic receptors (Bartsch et., 1981) and maintains its blocking effect for approximately 12 hours (Wengert, 1978). Carazolol has been reported to be used in many animal species, especially pigs, to treat stress-induced circulatory disorders; to reduce fetal deaths resulting from weak uterine contractions; to shorten the duration of parturition in multiple litters and thus reduce litter loss; to artificially inseminate females (Ballarini & Guizzardi, 1981; Enginler et., 2024; Retzer, 1987). Carazolol increases uterine contractions when used by iv injection in pigs (Rudloff & Bostedt, 1984).

Internal and external stress factors negatively affect many physiological events in ewes, and there is a close relationship between them and infertility (Gürbulak et., 2016). Intravaginal sponge application and estrus synchronization in ewes provide a significant advantage in grouping lambing periods. However, it should not be forgotten that these methods may create some stress factors. In particular, the presence of people in the barn during intravaginal sponge application and hand-mating method may cause stress on the animals (Acharya et., 2022).

During the process of hand-mating with rams, the animal responds to the noise, rough behavior or strain etc., in the barn with an increase in adrenaline secretion. Thus, as a result of the stimulation of beta 2-adrenoreceptors in the myometrium by adrenaline, the effect of oxytocin, which provides uterine contractions, is eliminated and the uterine tone weakens, as a result of which the passage time of spermatozoids through the genital canal is prolonged and they age. It has been determined that if fertilization occurs with old and fertile spermatozoids, the resulting zygote cannot continue its life and dies (Kırşan et., 1998). In addition, frequent ejaculation in rams leads to a significant decrease in volume density and number of sperm per ejaculate. Therefore, excessive use of rams in hand mating could negatively impact sperm volume and fertility outcomes (Jennings & McWeeney, 1976).

This study aimed to assess the impact of carazolol injection during mating on some reproductive parameters in Lacaune ewes following progestagen and eCG treatment during the breeding season.

Materials and Methods

Animals and Management

This study was conducted in a commercial sheep farm (Lat:37°49'15.31" N, Long: 34°016'25.49" E and Alt: 1.043 m) in Konya province in Türkiye during the breeding season (November) in 2024.

A total of sixty-two Lacaune lactating ewes, clinically healthy, 2‑5 years old with 50‑60-kg body weight having 120-130 days postpartum, were used. The ewes were milked twice a day, at 07:00 in the morning and 18:00 in the evening. The average milk yield was 1 kg per ewe per day. All ewes were fed a balanced diet consisting of 60% roughage and 40% concentrate feed. The roughage portion included equal parts wheat straw and alfalfa straw (50:50). Diets were formulated to meet the energy and protein requirements of the ewes using OptiTMR Pro 4.0.33, with *ad libitum* access to water.

Synchronization, Mating Protocols and Treatment Groups

An intravaginal sponge containing 20 mg flugestone acetate (Chronogest® CR, MSD, France) was inserted for ten days (n= 62). On day 10, all ewes received an intramuscular injection of 480 IU eCG (Chronogest/PMSG, MSD, France). Estrus detection was carried out using a teaser ram, which was introduced to the ewes twice daily for 1-hour sessions over 24 hours following sponge removal. Ewes showing signs of estrus were hand-mated with one of the proven rams at a ewe-to-ram ratio of 5:1. Fifty-nine mated ewes were randomly assigned into two groups: control and treatment. In the carazolol group (n = 30), ewes received an intramuscular injection of carazolol (0.01 mg/kg; Simpanorm, Fatro, Italy) on the day of estrus, post-mating. In the control group (n = 29), ewes were not administered any treatment.

Ultrasonographic Examination

Ewes were considered pregnant if the presence of gestational sac, embryo/fetus, offspring fluids and placentomes were observed in the transabdominal pregnancy examination performed on the 30th day and 60th day after mating. In ultrasonographic (USG) examinations, a real-time B-mode ultrasound device (Hitachi EUB-405, 3.5 MHz convex probe) was used by the same person.

Determination of Reproductive Performance

Estrus rate (ER), conception rate (CR), total pregnancy rate (TPR) and early fetal death rate (EFDR) were calculated as reproductive parameters as follows;

$$\text{ER} = \frac{\text{NEB}}{\text{the number of total ewes}} \times 100$$

NEB: The number of ewes showing estrus behaviors

$$\text{CR} = \frac{\text{the number of pregnant ewes}}{\text{the number of mated ewes}} \times 100$$

$$\text{TPR} = \frac{\text{NP}}{\text{the number of mated ewes}} \times 100$$

NP: the number of pregnant ewes at 1st service, 2nd service and 3rd service

$$\text{EFDR} = \frac{\text{number of detected death fetus}}{\text{the number of pregnant ewes}} \times 100$$

Statistical Analysis

For analyzing the calculated reproductive parameters, Chi-squared test, Fisher's exact test using the Proc GENMOD function of SAS 9.8 software. Results were reported as percentages. Statistical significance was defined as $P < 0.05$.

Results

Results for estrus rate, conception rates, total pregnancy rate and early fetal death rate are presented in Table 1. Treatment did not affect any parameter ($P > 0.05$).

Table 1. Reproductive performance parameters of ewes

	Control Group (n = 29)	Carazolol Group (n = 30)
Estrus rate (%)	95.2 (59/62)	
Conception rate at 1st service on 30 post-mating day (%)	62.1 (18/29)	66.7 (20/30)
Conception rate at 1st service on 60 post-mating day (%)	62.1 (18/29)	66.7 (20/30)
Total pregnancy rate (%)	79.3 (23/29)	83.3 (25/30)
Early fetal death rate (%)	0 (0/18)	0 (0/20)

* There were no significant differences between groups (P>0.05)

Discussion

Intravaginal sponges containing progestagen with eCG are still the most widely used tools for initiating estrus stimulation in sheep flocks (Wildeus, 2000). In the presented study, the estrus rate was found to be 95.2% in ewes included in this study. Synchronization studies using different doses of progestagen+eCG report estrus rates between 64% and 100% in ewes (Hameed et., 2021). However, the success rate of this hormone protocol depends on many factors including breed, care and feeding conditions, season, lactation status, etc., and very different success rates are obtained in the studies carried out (Kutlu & Dinç, 2021).

Embryonic or fetal deaths in ewes cause major economic losses. According to research, embryonic and fetal death rates are approximately 30% in ewes (Dixon et., 2007; Fthenakis et., 2012). There is limited information on early fetal deaths in ewes, with rates ranging from 3.5% to 12% (Jones et., 2016). In the presented study, no early fetal death was observed in any animal during repeated ultrasonographic examinations on the 30th and 60th days after mating. Köse and Tekin (2022) reported the early fetal death rate as 6.1% in their study in which they performed two repeated examinations on the 35th day and the 50th day. In the presented study, it is thought that there was no early fetal death due to high animal welfare.

Pregnancy rate is a key indicator of reproductive performance and plays a crucial role in determining the economic profitability of farms. In the present study, it was found that conception rates and total pregnancy rate did not differ between in groups (P>0.05), indicating that carazolol had no effect on pregnancy. According to a meta-analysis study, with progestagen + eCG protocols, pregnancy rates in ewes in Turkey are 90.3% during the breeding season and 59.3% out of the breeding season (Arıkan et al., 2021). The conception rate and total pregnancy rate in the control group were acceptable. Carazolol is a β-adrenoceptor blocking drug that is structurally an analogue of adrenaline from the catecholamines (Borchard, 1998; Januszewski, 1985). Beta-adrenoreceptor blocking drugs interact with β-adrenoreceptors to form a drug-receptor complex, and it has been reported that they prevent endogenous catecholamines secreted especially under stress conditions from combining with β2-adrenoreceptors, and that this combination occurs in the form of competitive antagonism (Bademkıran & Horoz Kaya, 2006; Borchard, 1998; Costin et., 1983). Carazolol binds reversibly to beta receptors without causing adrenergic effects and blocks the action of catecholamines released during stress because their action sites are now saturated. Practically, when carazolol is applied, the negative effects caused by stress in the animal are reduced and the parameters are returned to normal physiological limits (Güneşli, 2018). In previous studies Hammer and Rüsse (1990) reported that a study involving 1.171 cows demonstrated that administering carazolol via injection into the cranial epigastric vein improved conception and pregnancy rates by approximately 10% in herds experiencing stress-related infertility. Kırşan et al (1998) reported that they applied carazolol administration via the vena coccygea to cows just before artificial insemination that uterine tonus increased in the carazolol group and the conception rate increased by 19% in the carazolol group compared to the control group. However, Pancarcı et al (2008) reported that although carazolol administration via the jugular vein five minute prior to timed artificial insemination increased uterine tonus in synchronized cows, it did not lead to any significant differences in conception rates. Gündüz et al (2010) in their study in which they inseminated Kıvırcık ewes with natural service, frozen or fresh semen, and in all three service methods, the lambing rate was 56% for control and 63% for carazolol, with no statistical difference between the groups. They reported that 0.5 mg carazolol intramuscular injection before natural mating or insemination did not improve the lambing rate. While these results obtained in the presented study are compatible with those reported in some studies (Gündüz et., 2010; Pancarcı et., 2008), they are also inconsistent with others (Hammerl & Rüsse, 1990; Kırşan et., 1998). These different results may be due to differences in species and breeds.

Conclusion

In conclusion, the present study showed that carazolol injection during mating following progestagen + eCG administration did not increase pregnancy in breeding season Lacaune ewes. Further studies with different breeds, different dosages and different administration methods are necessary to evaluate this findings.

Declarations

Ethical Approval Certificate

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of Selcuk University (November 2024 - 176/2024).

Author Contribution Statement

Conceptualization: Metehan Kutlu, Neffel Kürşat Akbulut; data curation: Metehan Kutlu; formal analysis and investigation: Metehan Kutlu; funding acquisition: Metehan Kutlu; investigation: Metehan Kutlu; methodology: Metehan Kutlu, Neffel Kürşat Akbulut; project administration: Metehan Kutlu, Neffel Kürşat Akbulut; resources: Metehan Kutlu; Software: Metehan

Kutlu; supervision: Metehan Kutlu; Neffel Kürşat Akbulut; validation: Neffel Kürşat Akbulut, Metehan Kutlu; visualization: Metehan Kutlu; writing—original draft preparation: Metehan Kutlu; writing—review and editing: Metehan Kutlu, Neffel Kürşat Akbulut.

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Conflict of Interest

The authors declare no competing interests.

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