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Reproductive and economic evaluation of sexual stimulation during the anestrous period in a commercial farm with neonatal lamb losses

[*Avaliação reprodutiva e econômica da estimulação sexual durante o período antrópico em uma fazenda comercial com perdas neonatais de cordeiros*]

A. *Takci*[1], M.B. *Kivrak*[1*], H. *Murat*[2], S.U. *Cizmeci*[3]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey
[2]Department of Livestock Economics and Management Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Konya Selcuk University, Konya, Turkey

ABSTRACT

This study was performed during the anestrous, involving 140 Akkaraman Kangal ewes whose lambs had died in the neonatal stage due to pneumonia and enteritis. Intravaginal sponge containing progesterone was placed to the animals (Group 1, n = 70) on day 0 and removed after 7 days, following which 263 µg PGF2α and 500 IU eCG were administered to the sheep. Ram introduction was performed for 7 days (days 8–14), starting from the day after the removal of the intravaginal sponge (day 8). The animals in Group 2 (n = 70) were not exposed to any treatment. Ram introduction was performed simultaneously in both the groups. To determine the reproductive response, reproductive parameters such as estrous, pregnancy, multiple pregnancy, and embryonic mortality rates, number of births, number of offspring, and fertility, as well as their economic implications, were compared between groups. Each reproductive parameter exhibited a statistical difference between groups. An economically positive trend was observed in the study group compared with the control group. It was concluded that in case of lamb losses in commercial farms that derive profit from lambing, pregnancy of ewes can be achieved via sexual stimulation without waiting for the next breeding season.

Keywords: anestrous, Kangal, sheep, neonatal death, synchronization

RESUMO

Este estudo foi realizado durante o anestrous, envolvendo 140 ovelhas Akkaraman Kangal cujos cordeiros haviam morrido no estágio neonatal devido a pneumonia e enterite. A esponja intragaginal contendo progesterona foi colocada aos animais (Grupo 1, n = 70) no dia 0 e removida após 7 dias, após os quais 263 µg PGF2α e 500 UI eCG foram administrados às ovelhas. A introdução do carneiro foi realizada por 7 dias (dias 8-14), a partir do dia seguinte à remoção da esponja intravaginal (dia 8). Os animais do Grupo 2 (n = 70) não foram expostos a nenhum tratamento. A introdução do carneiro foi realizada simultaneamente em ambos os grupos. Para determinar a resposta reprodutiva, foram comparados entre os grupos parâmetros reprodutivos, tais como estrogênio, gravidez, gravidez múltipla e taxas de mortalidade embrionária, número de nascimentos, número de descendentes e fertilidade, bem como suas implicações econômicas. Cada parâmetro reprodutivo exibia uma diferença estatística entre os grupos. Uma tendência economicamente positiva foi observada no grupo de estudo em comparação com o grupo de controle. Concluiu-se que no caso de perdas de cordeiros em fazendas comerciais que obtêm lucros com a parição, a gravidez de ovelhas pode ser obtida através de estimulação sexual sem esperar pela próxima estação de reprodução.

Palavras-chave: anestrous, Kangal, ovelhas, morte neonatal, sincronização

*Corresponding author: mbkivrak@cumhuriyet.edu.tr
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INTRODUCTION

The neonatal stage is a critical stage for lambs because of the high morbidity and mortality during this stage. Lamb mortality in the neonatal stage is a major economic loss in animal husbandry (Aydogdu, 2016). Lamb deaths that occur during this period substantially reduce business inputs (Nash *et al.*, 1997; Aydogdu, 2016). Lamb deaths limit the development of the sheep breeding industry globally, occurring at a rate of 15%–20% presently (Flinn *et al.*, 2020). The annual economic burden of lamb deaths and the strategies to reduce these deaths is 540 million dollars in Australia (Flinn *et al.*, 2020).

More than 65% of lamb mortality in the neonatal period has multifactorial etiology (Radostits *et al.*, 2006). Diseases such as watery mouth disease, diarrhea, pneumonia, and umbilical cord inflammation are the main infectious diseases engendering lamb deaths in the early postpartum period (Scott, 2001).

Today, sheep farming is a primary source of income, especially in developing countries, which have harsh climates and poor soil conditions. With the effective use of estrous synchronization protocols, the reproductive performance of sheep can be enhanced, thereby increasing lambing and the profitability of sheep breeders (Abecia *et al.*, 2011; Amiridis and Cseh, 2012; Ghasemi-Panahi *et al.*, 2016).

In sheep breeding, increasing fertility parameters to provide better productivity without increasing inputs or cost is preferred. Accordingly, the breeding process and reproductive performance of sheep can be controlled and increased, respectively, via natural methods and various hormonal therapies (Özyurtlu and Bademkiran, 2010; Texeira *et al.*, 2016).

This study aimed to determine the effectiveness of sexual stimulation on the reproductive and economic parameters of sheep and commercial farms, respectively, during the early anestrous period of dams that lost their lambs in the neonatal period.

MATERIAL AND METHOD

The study was conducted at a sheep farm (coordinates, 38.842174779662926, 37.28949932275392; altitude, 1640) in Kalederesi Village, Gürün District, Sivas/TÜRKİYE Province.

This study included 140 Akkaraman Kangal ewes that became pregnant in autumn, gave birth in spring, and lost their lambs because of pneumonia and enteritis in approximately the first month after birth (neonatal stage). Number of sheep per shepherd was 200. The pretreatment mean body weight was 44 ± 5 kg, and the Body Condition Score (BCS) was between 2.5 and 3.25. Blood samples were collected before the treatment to confirm that all sheep were in anestrous by measuring the progesterone levels. The sheep were between 55 and 70 days postpartum. BCS was determined according to the method described by Ferguson *et al*. (1994) and the scores ranged from 1 to 5. Because the study was performed in anestrous, 14 Akkaraman Kangal rams aged 3–6 (4.1±1.4) years (mean weight, 97±5.6kg; BCS, 3.5±0.3) were used for mating during sexual stimulation.

When examining farm records it was found that pregnancy was not achieved in previous years despite the introduction of rams during the anestrous period. This supports the consideration that Akkaraman Kangal sheep is a nonprolific breed.

The rams were separated from the herd 30 days before the treatment onset and placed in a different compartment in the same shelter. Therefore, the ram effect was not utilized. In total, 140 sheep that experienced neonatal lamb losses were divided into two equal groups. Simultaneously separating rams from the herd, the ewes were grazed only at night for 45 days, and exposure to daylight was reduced by keeping the animals in their shelters during daytime. In addition, postpartum physiological processes involving uterine involution, endometrial regeneration, and bacterial elimination of the uterus was expected to occur within the specified period.

The synchronization protocol is shown in Figure 1. An intravaginal sponge (Chronogest®, fluorogestone acetate, 20 mg, MSD Animal Health, Ankara, Türkiye) was inserted in the animals in Group 1 (n = 70) containing progesterone on day 0. After 7 days, the vaginal sponges were removed and 263µg PGF2α

(Estrumate®, d-cloprostenol, MSD Animal Health, Ankara, Türkiye) and 600 IU eCG (PMSG) (Chorono-Gest/PMSG, PMSG, Intervet International BV, Boxmeer, Netherlands) hormones were administered to the sheep. Ram introduction was performed 1 day after the removal of the intravaginal sponge. After keeping the rams with the sheep for 7 days, the rams were separated from the herd. The sheep in Group 2 (n = 70) were not administered any treatments; however, the ram effect was induced by introducing ram simultaneously with Group 1.

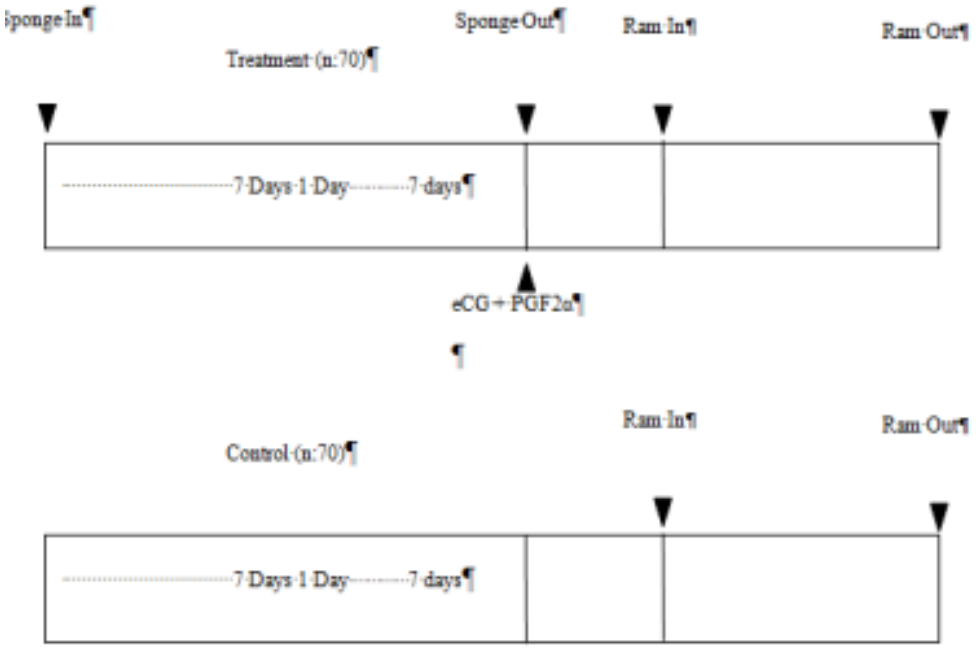

Fig. 1 Treatment schedule. PGF2α: prostaglandin F2α (131.5 µg cloprostenol, Estrumate); eCG: equine chorionic gonadotropin (600 IU; Chronogest® PMSG 6000[1]); sponge; 20 mg fluorogestone acetate, (Chronogest®[1])

Following the introduction of rams, pregnancy examination was performed twice via the transrectal route (day 38) and transabdominal route (day 68) using a B-mode, linear-array ultrasonography device containing a 5.0–7.5 MHz rectal probe (Mindray Bio-Medical Electronics Co., Ltd. DP50 VET, Shenzhen, China). Transrectal examinations were performed in the supine position. During the transabdominal examination, the hairless area just above the breast, ventral to the right paralumbar fossam was preferred for probe placement. In case of pregnancy-related findings, complete breast examination in the dorsocaudal direction was performed. Following the detection of the gestational sac, the sheep were considered pregnant owing to the presence of embryo/fetal membranes, amniotic fluids, fetus heartbeat, and placentome based on the gestation period as observed during the ultrasonography examination. The birth of the lambs was followed up and recorded.

The reproductive parameters, including the estrous, pregnancy, multiple pregnancy, and embryonic death rates and the number of births and offspring, after the administration of the treatment were compared between the groups to determine the reproductive response to the treatments.

After the blood samples were stored at room temperature for half an hour, they were centrifuged at 3,000 rpm for 5 min. Then, the serum samples were collected into 1-ml tubes and stored at −80 °C till analysis. Chemiluminescence microparticle immunoassay method using Progesterone Chemiluminescence

test kit (7K77) and fully automatic device (ARCHITECT-i2000SR Immunoassay Analyzer Abbott Illinois, USA) was employed. The analytical sensitivity of progesterone was ≤0.1 (0.1–36.0) ng/ml. The range of the intra-assay coefficient of variation was 3.4%–5.5% and 1.6%–2.2% for low and high progesterone concentrations, respectively. Analyses of the serum (serum and blood collected in serum separator tubes) and plasma (with Na heparin, Li heparin, and K EDTA anticoagulants) samples were validated. No other anticoagulants other than the specified anticoagulants were used for the validation.

The findings obtained in the study were analyzed to calculate the economic difference between the study and control groups to determine the economic benefits of the treatment using the following formula:

economic difference;

$$EF = \sum M_1 - \sum M_2$$

total income of the experimental group;

$$\sum M_1 = (\sum Kg_1 - \sum P_1)$$

total income of the control group;

$$\sum M_2 = (\sum Kg_2 - \sum P_2)$$

where **EF** is income difference, $\mathbf{M_1}$ is the total economic income obtained from the animals in the experimental group, $\mathbf{M_2}$ is the total income from the animals in the control group**,** $\sum$**Kg** is (total lamb production × market value*)/current dollar rate**, and $\sum$**P** is (applied treatment cost*** + travel expenses + veterinarian practice fee + labor cost****)/current dollar rate.

*The current market price for lambs was included in the calculation separately as male and female.

** The Central Bank of the Republic of Türkiye, 2021.

***Progesterone sponge + 500 IU (international unit) eCG hormone + 263 µg PGF2α hormone + consumables required per unit animal (injector, cotton, alcohol, etc.) costs were included in the calculation.

****Calculation was made based on the total working hours for the minimum wage in 2020 (Ministry Of Family, Labour And Social Services Of The Republic Of Türkiye 2020).

In addition to the above calculation, the estimated added economic value of the new lambs in the farm was calculated using the following formula:

$$\sum MA^* = A_d + A_{ey} + D_s + E_s + E_d$$

A_d: It was predicted that 80% of the ewes could reach breeding quality. The economic value of dams is obtained by multiplying their number and market value.

A_{ey}: It was predicted that the ewes that reached breeding quality could give birth to a single lamb for a year, with a 50% probability of the lambs being rams or ewes. It was predicted that all the lambs obtained will be sold at the age of 6 months, thereby bringing in revenue to the farm. The economic revenue is calculated by multiplying the market sales value of the female and male animals in this category and the units sold. When calculating A_{ey}, the breeding values of the newborns were ignored.

D_s: It was predicted that 20% of the ewe lambs will be sold at the age of 6 months. The economic revenue is obtained by multiplying the units sold and the market price.

E_s: It is predicted that 80% of the ram lambs will be sold to the market. The economic value is obtained by multiplying the male units sold and the market value.

E_d: It was predicted that 20% of males can reach breeding quality. The economic value of breeding rams was obtained by multiplying their number and market value.

*When calculating the A value, all expenses, including feed, maintenance, utility bills, veterinary/health, were calculated and subtracted from the economic value.

The formulas used in the economic analysis were adapted from the economic analysis methods used by Murat (2011, 2020).

STATISTICAL ANALYSIS

Statistical analysis of all the study data was evaluated using SPPS 25 statistical package program (IBM - International Business Machines Corporation. Armonk, NY, USA.). The variables were tested for normality and homogeneity using Shapiro–Wilk and Levene tests, respectively. Single effect of the treatments on estrous, pregnancy, fertility, and multiple pregnancy rates were analyzed using chi-square test. Fisher's exact test was used when one of the expected cell values was <5. Litter size data were log-transformed to attain normal distribution and analyzed using independent sample t-test. For all the analyses, $P < 0.05$ was considered statistically significant.

RESULTS

On examining the progesterone levels, no ewe with a P4 (progesterone) value above the luteal level (≥1 ng/ml) was found in both the groups,

with the P4 value of the ewes in both groups being at the sub-basal level (<1 ng/ml).
The parameters used in the interpretation of reproductive outcomes were established as follows:

Estrous Rate, number of estrous animals in the group/total number of animals in the group
Pregnancy rate, number of pregnant animals in the group/total number of animals in the group
Multiple pregnancy rate, number of multiple pregnant animals in the group/total number of pregnant animals in the group
Number of births, number of animals that completed their pregnancy and gave birth among pregnant animals
Number of lambs, number of offspring born from pregnant animals in the group

A total of 46 lambs (24 female and 22 male) were obtained from 32 births in Group 1, and a total of 5 lambs (3 male and 2 female) were obtained from 5 births in Group 2.

Economic analysis calculated $\sum M_1$ as $4.471,96 and $\sum M_2$ as 615.01 $. The study group provided an extra $3856.95 compared with the control group. Hence, it was determined that the study group showed 88.15% better economic outcomes than the control group. Considering the economic sustainability of livestock husbandry, this revenue has significant importance.

In the pregnancy examination performed on day 60 following the synchronization, it was observed that the pregnancy did not continue in one of the animals in the study group. The effect of synchronization protocol on the estrous, pregnancy, and fertility rates are presented in table 1. In the treatment group 32 ewes delivered 46 lambs while 5 ewes delivered 5 lambs in the control group. During the second ultrasonographic examinations 1 embryonic death was determined. There was no embryonic death in the control group. The estrous and pregnancy rates in the study group were significantly higher than control group (P>0.05). The differences in the fertility rate between groups were not statistically significant due to the small number of lambing in the control group. The litter size in the study group was 1.42±0.11, showing a similar fertility rate as the control group with no significant differences between groups.

Table 1. Estrous (mated/all), pregnancy (pregnancies/all), and fertility (pregnant/mated) rates and the number of pregnant versus nonpregnant ewes on Day 30 post mating (pregnant/nonpregnant)

		Groups		Total
		Treatment	Control	
Estrous rate	Positive	51[a] (72,9%)	7[b] 10,0%	58
	Negative	19[a] (27,1%)	63[b] (90,0%)	82
Total		70	70	140
Pregnancy rate	Positive	33 [a] (47.1%)	5[b] (7,1%)	38
	Negative	37 [a] (52.9%)	65[b] (92,9%)	102
Total		70	70	140
Fertility rate	Positive	32[a] (62,7%)	5[a] (71,4%)	38
	Negative	18 [a] (37.3%)	2[a] (28,6%)	20
Total		51	7	58

a,b: Varied characters in the same row are different (P < 0.05).

DISCUSSION

Lamb losses in the neonatal stage are among the leading causes of economic loss for sheep breeders worldwide (Nash *et al.*, 1997). The lack of reproductive yield in sheep during anestrous is the most critical issue that reduces farm operating efficiency and profitability (Knights *et al.*, 2001). Generally, it is aimed to obtain offsprings twice a year or thrice in 2 years via the

administration of various hormones to increase fertility and achieve higher offspring rates in sheep (Smith, 2006). The follicular dynamics of the ovaries of ewes throughout the anestrous period is not fully stagnant and may remain partially active. During this period, because of the continuation of follicular stimulating hormone (FSH) synthesis, fluctuations in follicular development parallel with FSH fluctuations has been demonstrated (Bartlewski *et al.*, 1998), and thus, the reproduction system can be stimulated to obtain pregnancies by interventions to ovulation since the follicular activity continues during the anestrous period (Simões, 2015). Estrous induction methods outside the breeding season are based on the combination of progesterone and its analogs with eCG. It is known that the number of pregnancies obtained only using P4 in anestrous period is less than that of the pregnancies obtained with the combination of synchronization and P4 during the breeding season (Wildeus, 2000). By using progesterone for sexual synchronization, the negative feedback effect of progesterone on gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) is utilized, and when the P4 concentration drops suddenly, the LH concentration increases by 30 times, with the dominant follicle becoming sensitive to LH, thereby inducing ovulation (Kaçar *et al.*, 2016). The study aimed to stimulate estrous and induce ovulation in the early anestrous period by utilizing the effect of progesterone on GnRH and LH, or to provide ovulation in a shorter period by combining it with gonadotropins and PGF2α. Accordingly, without waiting for the next breeding season, the sexual stimulation of the dams who lost their lambs for various reasons was achieved. The reproductive and economic aspects of the administration were then evaluated.

The use of progesterone and its analogs in synchronization protocols is based on the principle of controlling the release of LH by progesterone to mimic the function of corpus luteum in the estrous cycle, and thus inducing estrous and ovulation (Abecia *et al.*, 2012). To enable more effective progesterone administration, sufficient levels of gonadotropin should be available and exogenous FSH and endogenous gonadotropin levels should be increased to initiate the preovulation process. Accordingly, eCG hormone, which is obtained from pregnant mare serum and has LH and FSH-like effects, should be administered while progesterone-containing materials are removed (Powell *et al.*, 1996). To minimize progesterone exposure, it has been demonstrated that estrous and ovulation can be synchronized by reducing the duration of progesterone-containing devices in the vagina (Knights *et al.*, 2001). For this reason, intravaginal device (sponge) containing progesterone was kept in the vagina for as short duration as possible (7 days). To achieve successful sexual stimulation, it was aimed to provide shorter ovulation period by combining the eCG (PMSG) and PGF2α hormones during the removal of the sponge. In a study investigating the impact of eCG applied at different doses on ovarian functions, it has been shown that eCG >750 IU increased the ovulation rate but decreased the pregnancy rate. With eCG administered at doses of 550–650 IU, pregnancy rates were found to be higher with multiple pregnancies (Kermani *et al.*, 2012). In addition, D-cloprostenol, a synthetic form of PGF2α, has been effectively used for estrous induction and synchronization in sheep, providing efficient sexual stimulation by terminating progesterone activity (Ramírez *et al.*, 2018). Accordingly, 600 IU of eCG/PMSG and 263µg of PGF2α hormone in the form of D-cloprostenol were administered after the removal of the intravaginal sponge in the present study. Moakhar *et al*. (2012) achieved a pregnancy rate of 75% using 550 IU and 650 IU eCG/PMSG, whereas the pregnancy and multiple pregnancy rates were 47% and 33% (11/33), respectively, in this study group. The difference may be due to the fact that the sheep in the abovementioned studies were conducted on prolific breeds (Kermani *et al.*, 2012).

In a study, PMSG injection was administered at different times (24 hours before sponge removal, at the time of sponge removal, 24 hours after sponge removal, and none) during sexual stimulation with progesterone-containing intravaginal devices. It was determined that a better pregnancy rate was obtained at 24 hours before sponge removal (78%) and at the time of sponge removal, (%75) compared with 24 hours after sponge removal (70%) and none (60%) in the eCG/PMSG applied animals (Zeleke *et al.*, 2005). In the present study, eCG was administered to the animals in the study group during sponge removal, achieving a pregnancy rate of 47%. The lower pregnancy rate in our

study compared with the study by Zeleke *et al*. (2005) may be due to the fact that the infections that led to neonatal losses in the farm also affected the dams. Moreover, Zeleke *et al*. (2005) conducted the study on a profile breed and during the transition period from breeding season to anestrous. Kangal sheep is a nonprolific breed and has a short breeding period. In addition, the present study was conducted in early anestrous period.

In a study evaluating the effectiveness of synchronization in the early, middle, and late puerperal postpartum period on reproductive performance, pregnancy rates were 43.8%, 29.7%, and 47.1%, respectively (Ungerfeld and Sanchez-Davila., 2012). In the current study, conducted during the late postpartum stage, the reproductive results were similar.

Livestock including sheep breeding play a significant role in the economies of developing countries with large grazing areas. Ensuring controlled breeding and high breeding efficiency using breeding methods increase cost-effectiveness (Awel *et al.*, 2009). A successful economic management model should target high fertility rates with efficient synchronization protocols. Thus, the addition of new and healthy offsprings to the herd will ensure a sustainable and profitable livestock husbandry (Özyurtlu and Bademkiran., 2010; Texeira *et al.*, 2016). The above determinations are in line with the objectives and results of the present study.

Considering the economic aspects of the study, the study group provided extra $3856.95 revenue compared with the control group. The income per sheep was $55.09. The calculations showed that the study group achieved better results at a rate of 88.15% in terms of economic value than the control group. It has been determined that hormonal therapy increases reproductive performance and provides higher revenue. Swellum *et al*. (2018) stated that hormonal synchronization is a profitable intervention to increase reproductive performance, and consequently, profitability. This study also revealed similar results. Synchronization with controlled drug release is considered an effective method to improve reproductive performance and generate higher revenue (Swelum *et al.*, 2018).

Al-Karablieh and Abdelrahman (2000) found 22.1% higher mean fertility rate in the sheep treated with hormone therapy than in the control group. They reported that lambing rate increased from 64.9% to 111.0% and twinning rate increased from 3.13% to 29.9%. The increased net revenue due to hormone therapy was 12.5$/sheep. The economic efficiency results of study conducted were better than those of Al-Karablieh and Abdelrahman (2000). In the study conducted by Gizaw and Tegegne (2018), it was observed that the annual profit increased by 78.6% and 101.5%, respectively, in ewes subjected to synchronization by hormone therapy once and twice compared with ewes without hormone application. The synchronization application provided 188.8% and 223.7% greater profit, respectively, than by the breeding practices in rural areas (Gizaw *et al.*, 2018). The percentage profit values found by Gizaw and Tegegne (2018) were higher than the values in the present study. This result was associated with the fact that the dams in the present study were affected by respiratory infections that caused the death of neonatal lambs. The net profit obtained by Swelum *et al.* (2019) in their study was 17.6% higher in controlled internal device release (CIDR) treated animals than in the control group, which is lower than that in this study. In the present study, the profit obtained with the 7-day CIDR application was higher than the control group. This result is also consistent with the results of short-term CIDR application in some other studies (Swelum *et al.*, 2015, 2018, 2019).

Even if additional costs arise due to the single-use CIDR application in the study group, it is significant that this method contributes to establishing of more profitable operating model compared with the control group. This conclusion is consistent with the study of Swelum *et al*. (2019).

The variability of productivity and profit rates may be caused by numerous factors, such as geography, sheep breed, breeding characteristics, and differences in protocols.

CONCLUSION

Hence, performing sexual stimulation as soon as possible without waiting for the next breeding season in farms with postpartum lamb mortality is crucial to improve reproductive efficiency and agricultural economy. To form ovulating estrous in Akkaraman Kangal sheep during the anestrous period, sexual stimulation methods, including progesterone-containing intravaginal device, gonadotropin, and PGF2α, are needed.

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