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Effect of additional progesterone treatment during intravaginal progesterone priming in anestrous ewes

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Summary

Oestrus induction with intravaginal progesterone is the most preferred method in anoestrus sheep. In previous studies it was argued that the decrease in the progesterone level before the sponge is removed reduces the efficiency. This study aimed to test the hypothesis that additional progesterone treatment during the progesterone priming in anestrous ewes could increase the reproductive efficiency of the induction. The study conducted on 261 Kangal sheep, which were pregnant in autumn and gave birth in spring and breastfed their lambs for about 60-75 days. All animals included in the study were randomly divided into 3 different groups as P-7 (n = 86), P-8 (n = 80) and control (n = 95). At the beginning of the study a progesterone impregnated sponge was placed to all ewes (Day 0). During sponge treatment, an injection of progesterone was given to P-7 and P-8 group 7 and 8 days after the was sponge placed, respectively. As for control group, no additional progesterone was given during sponge treatment. All groups received 500 IU pregnant mare serum gonadotropin at the sponge withdrawal. Ram was introduced to all ewes the 10th days of the study. There was no difference between the groups in terms of estrus rate (P = 0.28), pregnancy rate (P = 0.32) and multiple pregnancy rates (P = 0.38) (P = 0.28). We considered that the main reason for unexpected low progesterone concentration at the end of the treatment might be the result of high feed intake and metabolic rate. On the other hand, this additional dose could be caused by an excessive increase in progesterone levels in the sheep, which could be the reason for the decrease in reproductive parameters. Additional progesterone support during progesterone-based synchronization yielded acceptable results. However, progesterone can be administered in injection form in the earlier days of synchronization or before sponge insertion to obtain better results.

Keywords: anestrus, additional progesterone, ewe

Oestrus induction with intravaginal progesterone is the most preferred method in anoestrus sheep. Almost all of these methods are based on a 12-14 day progesterone treatment followed by an equine chorionic gonadotropin (eCG) injection at the end of the progesterone treatment. The induction of estrus out of breeding season aims to imitate the physiological estrus cycle (6). Although there is follicular development in the anoestrus period, ovulation is not observed, so CL is not formed (9, 13). Oocyte quality decreases when there is no functional and progesterone-secreting CL. This causes a decrease in reproductive efficiency. Sponges implemented for oestrus induction gradually release the progesterone, creating an artificial luteal phase (14).

In ewes, the luteal period lasts for an average of 14-15 days and a follicular wave lasts for an average of 4-5 days. Therefore, progesterone treatment should be at least 6-7 days and at most 12-14 days. Long-term progesterone treatments, as well as inadequate progesterone treatments, negatively affect efficiency (8). After ovulation, the level of progesterone rises rapidly, increasing the circulating concentration above 1 ng/ml. With the release of prostaglandin, it falls below 0.5 ng/ml in the follicular period. Therefore, similarly the circulating progesterone concentration should rapidly increase above 1 ng/ml during progesterone treatment and remain above this level until the sponge is removed. If the progesterone level remains higher for a while after the progesterone treatment is

terminated, or more importantly, if the progesterone level falls below 1 ng/ml before the sponge is removed, it causes a failure in estrus induction (1).

In some studies (2, 7, 12) conducted on anoestrus and measuring progesterone levels, it is argued that progesterone level decreases on the 4th-5th days of the application, causing a decrease in the pregnancy rates. In the light of these studies, additional progesterone support could increase reproductive efficiency of the estrus induction. Therefore, our study aimed to test the hypothesis that additional progesterone treatment during the progesterone priming in anestrous ewes could increase the reproductive efficiency of the induction.

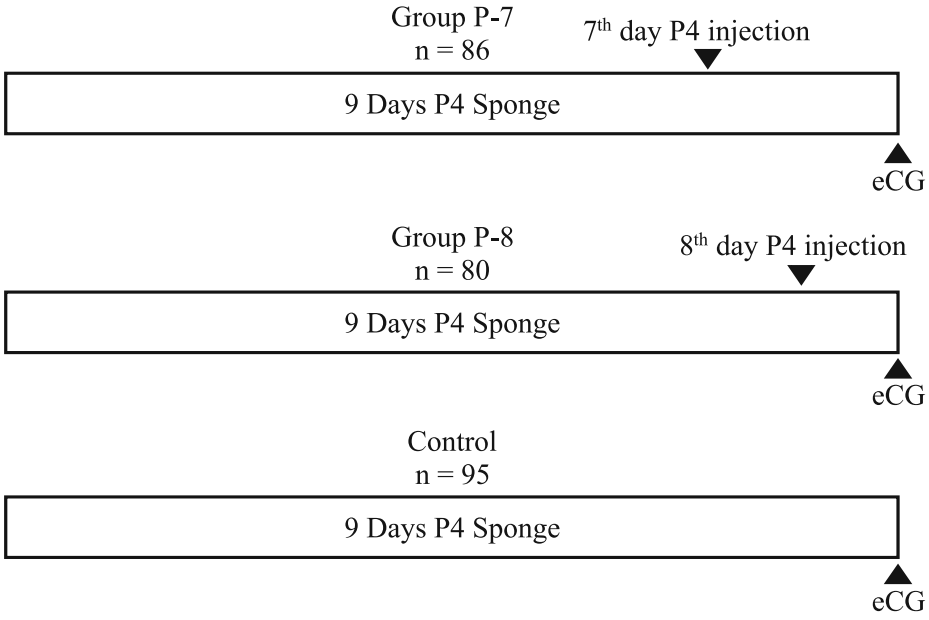

Fig. 1. Treatment scheme
Explanations: eCG – equine chorionic gonadotropin (600 IU; Chronogest® PMSG 6000); P4 Sponge – fluorogestone acetate (Sponge; 40 mg fluorogestone acetate, Chrono-gest)

Material and methods

Location. The study was carried out in a sheep farm at 39.83371433796894 and 36.34688098838113 coordinates and at an altitude of 1290 meters in Ortaklar Village of Yıldızeli District of Sivas Province, Türkiye. Its pasture is located in a geography where the steppe is dominant among the high mountains.

Animals and treatment schedule. This study was approved by the Animal Research Ethics Committee of Cumhuriyet University with the decision date and no11.11.2020/362.

The material of the study consisted of 261 Kangal sheep, which were pregnant in autumn and gave birth in spring and breastfed their lambs for about 60-75 days. The study was carried out in May when sheep were physiologically in the anoestrus in the northern hemisphere. Healthy sheep with no disease in the last two months were included in the study. All animals included in the study were randomly divided into 3 different groups as P-7 (n = 86), P-8 (n = 80) and control (n = 95). Prior to oestrus induction, the animals included in the study were weighed and body condition score (BCS) scores were determined. The body condition scoring method explained by Ferguson et al. (1994) was used, and the BCS values of these ewes were scored from 1 to 5 (Ferguson et al., 1994). The treatment scheme has been displayed in Figure 1. In the study, a sponge containing 20 mg of flugeston acetate (Chronogest® CR, MSD, Turkey) containing progesterone was applied to all animals on day zero of the study. On the 9th day of the application, intravaginal sponges were removed and 131.5 µg cloprostenol sodium (PGF2α) (PGS, Alke, Turkey) and 500 IU pregnant mare serum gonadotropin (eCG) (Chronogest/PMSG®, MSD, Turkey) were applied. The animals in Group 1 were administered 50 mg of the additional Progesterone hormone I.M. (PROGESTAN 50, KoçakFarma, Turkey) one day before the vaginal sponge was removed (on day 8), and the animals in Group 2 were administered 50 mg of the hormone I.M. (intra-muscular; intramuscular) 2 days before the vaginal sponge was removed (on day 7), while the animals in Group 3 were not administered any additional application and were regarded as the control group (3). Rams were introduced to the ewes 1 day after the last application (10th day), and matings were followed and recorded for 5 days (10th-15th days). Pregnancy was diagnosed ultrasonographically twice: 28-30 days after mating of ewes and 78-80 days after.

Statistical analysis. Statistical analysis of the data obtained in the study SPPS 25 (IBM Corp. Released in 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was conducted using the statistical package program. First, the data's normality and homogeneity were checked by performing the Shapiro-Wilk and Levene tests. In the animals determined to be pregnant, the rate of estrus, pregnancy rate, single and multiple pregnancy rates among the groups were evaluated by chi-square analysis. In the study, the data were expressed in percentages. The significance level was accepted as $p < 0.05$ and $p < 0.01$.

Results and discussion

At the beginning of the application, the average live weight of the sheep was 55.2 ± 5.4 kg and the average live weight of the rams was 102.3 ± 5.7 kg. The mean body condition score was 3.31 ± 0.57 in ewes and 3.6 ± 0.50 in rams. There was no statistical difference between the groups in terms of live weight and body condition score.

The findings obtained in the study are shown in Table 1. Statistically, there was no difference between the groups in terms of estrus rate (P = 0.28), pregnancy rate (P = 0.32) and multiple pregnancy rates (P = 0.38) (P = 0.28).

Tab. 1. Estrous ratio, pregnancy rate and multiple pregnancy rate obtained in P-7 (n = 86), P-8 (n = 80) and control (n = 95) after additional P4 administration on different days simultaneously with intravaginal progesterone priming in anestrus ewes

Groups	Oestrus Ratio (Positive Negative)	Pregnancy Rate (Positive-Negative)	Multiple Pregnancy Rate (Single-Multiple)
P-7	84.9% (73-13)	53.5% (46-40)	47.8% (22-24)
P-8	82.5% (66-14)	56.3% (45-35)	66.7% (30-15)
Control	83.2% (79-16)	60.0% (57-38)	57.9% (33-24)

In our study, additional progesterone support applied during progesterone-based synchronization in anestrus did not make a significant difference in estrus rate, pregnancy rate and multiple pregnancy rate parameters.

In the study conducted by Takcı (19) on primiparous Kangal ewes in anestrus, the pregnancy rate was determined to be 40% in the highest group after progesterone-based synchronization. In the study conducted by Cizmeci et al. (2) on Kangal ewes in the anoestrus, 36% of pregnancies were obtained in the highest group after intravaginal progesterone administration. Demiral et al. (5) found that the pregnancy rate was 75% in the intravaginal progesterone group. In our study, 83.2% of the animals showed oestrus in the highest group and the pregnancy rate was 60% in the same group. When the previous studies on Kangal sheep were evaluated, we concluded that the estrus and pregnancy rates we obtained after our application was acceptable.

In our study, the support of additional progesterone was expected to increase reproductive efficiency. One of the reasons for not seeing the expected increase may be that the progesterone level decreased earlier than expected. In the study conducted by Takcı (19) on Kangal sheep, the average level of progesterone was determined as 0.25 ng/ml after 9 days of intravaginal progesterone administration. In the study conducted by Cizmeci et al. (2) on Kangal ewes, it was observed that the progesterone level was below 1 ng/ml, although GnRH was applied for progesterone support before progesterone-based synchronization in the anoestrus. Similarly, in the study conducted by Swellum et al. (18), it was found that the progesterone level was below 1 ng/ml on the day the sponge was removed. However, Letelier et al. (12) found that progesterone levels decreased below 1 ng/ml on the 2nd day of the application after intravaginal sponge application containing twice the dose of progesterone. Therefore, in our study, additional progesterone was applied 1 and 2 days before the sponge was removed. However, progesterone measurement was not performed during the applications. The reason why the pregnancy rates were similar in all groups may be that the progesterone level fell below the threshold level very early, as in the study by Letelier et al. (12).

On the other hand, there are studies in which progesterone level is above 1 ng/ml for a long time in ewes with the sponge application. In the study conducted by Kaşıkçı et al. (10) on Tahirova ewes, half sponge (estimated 10 mg flugestone acetate) progesterone level remained above 1.22 ng/ml even after 13 days of treatment. In the study conducted by Gök et al. (6) on Purple Karaman, Gokceada, and Kivircik ewes, it was observed that the progesterone level was still above 2 ng/ml after 10 days of intravaginal sponge application. In the study conducted by Kaya et al. (11) on ewes of the Tuj breed, it was stated that the level of progesterone was above 1 ng/ml after 14 days of intravaginal progesterone administration. Considering the mentioned studies, it is observed that different results are obtained in various breeds of ewes. This may be due to the fact that Kangal breed ewes are larger compared to the breeds in the other studies. In our study, the average live weight of the sheep was 55.2 ± 5.4 kg. There is an inverse relationship between the concentration of feed intake and peripheral concentrations of plasma progesterone in ewes (15-17). Hence, we considered that the main reason for unexpected low progesterone concentration at the end of the treatment might be the result of high feed intake and metabolic rate.

It is a well-known phenomenon that progestagen concentration above at least 1 ng/ml followed by its rapid withdrawal is a necessary prerequisite for acceptable fertility. In our study, additional progesterone support was given for injection. When injectable forms of progesterone are administered as IM at a dose of 25 mg, its level remains above 0.5 ng/ml for 48 hours after reaching peak concentration in plasma within 2 hours (3). In our study, 50 mg of progesterone was administered to the ewes as a single injection. When given as an injection, its effectiveness increases depending on the dose (4). This additional dose could be caused an excessive increase in progesterone levels in the sheep, which could be the reason for the decrease in reproductive parameters. Therefore, progesterone injection 2 or 3 days before mating with a ram may have prevented the rapid decrease of progesterone before ram mating as desired.

In conclusion, additional progesterone support on the last days of progesterone-based synchronization did not increase or decrease reproductive efficiency. However, progesterone can be administered in injection form in the earlier days of synchronization or before sponge insertion. This could be prevented by excessive increase of progesterone and allows rapid decrease with sponge removal. Thus, better results can be achieved. Therefore, further investigations are needed to shed light on this matter.

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