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## Effect of different short term synchronization protocols on estrus and fertility in non-pregnant ewes during the breeding season

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**ABSTRACT.** The aim of this study was to investigate the efficacy of different short term synchronization protocols on estrus and pregnancy rates in merino ewes that had not become pregnant after at least three matings during the breeding season. Three different protocols were used as follows: Group I: Ewes (n=30) were inserted intravaginal progesterone sponge (florogestan asetate; 30 mg) for 6 days plus PGF2 $\alpha$  (125  $\mu$ g, i.m.) at the time of sponge removal, Group II: Ewes (40) were treated same as in group 1 plus eCG (250 IU, i.m.) at time of sponge removal, and Group III: Ewes (n=38) were only injected with PGF2 $\alpha$  at the same time with the ewes in the other two groups. Estrus was detected by rams (n=4), 24 hours after PGF2 $\alpha$  and ewes detected in estrus were mated. Ten rams were used for mating. Pregnancy was diagnosed 60 days after mating by ultrasonography. Estrus, conception and pregnancy rates were: for Group I 100%, 73.3% and 73.3%, for Group II 92.5%, 89.2% and 82.5% and for Group III 81.6%, 100% and 81%. In Group III, pregnancy loss after pregnancy diagnosis was significantly higher (35.4%) compared with the other groups (Group I: 13.6% and Group II: 15%)

**Keywords:** sheep, season, synchronization, pregnancy, lambing rates

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## INTRODUCTION

This study was designed to evaluate fertility of ewes that were not pregnant after at least three matings. The main aim was to reintroduce those ewes to the flock and increase the lambing ratio. In the sheep breeding industry, ewes that do not become pregnant in the breeding season are usually culled off. However, the sheep population has been constantly decreased and good quality ewes with specific characteristics of any breeds, such as those in this study, are especially important. Non-pregnant ewes must be given more chances to become pregnant and stay in the breeding stock.

Estrus synchronization is an important tool in the reproduction management of cow and ewes. Synchronizing the time of breeding and lambing in the flock is highly beneficial for farmers (Gonzalez-Bulnes et al., 2005). Mainly, two different strategies have been developed to synchronize estrus in ewes: prostaglandin based and progestagen based protocols (Gordon, 1975; Timurkan and Yıldız, 2005; Hashim et al., 2013). Short (7 days) and long term (14-day) synchronization protocols are used in sheep and goat. Progesterone applications and eGG at the time of progesterone agent removal or PGF<sub>2α</sub> administrations 48 hours earlier are usually used (Karaca and Kilboz, 2010). Estrus was detected in 96.7% and in 96.0% of ewes after progesterone application for 14 or 7 days respectively (Tekin et al., 1992, Cox et al., 2012). Prostaglandin based protocols work through reducing the length of the estrous cycle by regression of the active corpus luteum and progestagen based protocols extend the cycle via administration of exogenous progesterone (Kusina et al., 2000). PGF<sub>2α</sub> is the main prostaglandin agent used for estrus synchronizations and leads to estrus and ovulation (Menchaca et al., 2004). Progestagens are commonly used in sheep for estrus synchronizations (Killian et al., 1985). Although these strategies have high fertility rates at first service, they also have some weakness in the outcome. According to literature, prostaglandin based protocols leads to lower first services pregnancy rates compared to progestagen based methods due to disrupted follicular dynamics and inconsistency in the ovulation time (Boland et al., 1978; Godfrey et al., 1997; Barret et al., 2002). Progestagens compared to prostaglandins induce low-quality preovulatory follicles leading to poor luteal function and embryo viability (Gonzalez-Bulnes et al., 2005). However, using these protocols in ewes that have not conceived could give another chance for pregnancy and fertility. Furthermore, these methods achieve high

estrus and ovulation rates in a short time of period. This study was performed close to the end of the breeding season and inseminating these ewes as soon as possible was of critical importance.

The common practice is to submit ewes in the breeding season to natural mating under controlled conditions following detected estrous. In the flock at which this study was performed, about 5-6% of the ewes did not become pregnant after at least three matings with - rams of proven fertility. These ewes were submitted to a variety of commonly used synchronization protocols (Gordon, 1975; Timurkan and Yıldız, 2005). In the current study, ewes that were not pregnant after at least three matings at natural estrous during the breeding season were submitted to short term synchronization protocols. Progesterone was administered for 6 days and all treatments were performed on the day of the progesterone source removal to reduce the work force and stress (injection, aggregation). Planning the breeding program with lower labor cost and animal care is always desirable (Oyediji et al., 1990; Simonetti et al., 1999). These short term synchronization protocols could also have beneficial effects on regulating the estrous cycle of those ewes that would otherwise be culled off from the flock and submitted to the slaughterhouse.

## MATERIAL AND METHODS

### Animal Material

This study was performed in the Bahri Dağdaş International Agricultural Research Institute in Konya, Turkey. Anatolian Merino ewes (n=108), housed in this Institute, were used. Mean age of ewes was 5 years and mean body weight was 65 Kg. Breeding season started in early July (the year 2010) and ewes were submitted to mating with rams of proven fertility. The mating date was recorded for each ewe and pregnancy diagnosis was performed by ultrasonography 30-45 days after the mating, if the ewe did not come into estrus again. Non-pregnant ewes were again submitted to mating. This procedure was repeated until each ewe was allowed to mate three times and thereafter still non-pregnant ewes were included in the present study.

### Experimental procedure

Three different protocols were used as follow (Fig 1): Group I: Ewes (n=30) were inserted intravaginal progesterone sponge (florogestan acetate, 30 mg; Chronogest® CR/Sünger, İntervet, İstanbul, Türkiye

) for 6 days and injected (i.m.)  $\text{PGF}_{2\alpha}$  (tiaprost, 125  $\mu\text{g}$ ; İliren, Intervet, İstanbul, Türkiye) at the time of sponge removal, Group II: Ewes (40) were treated same as in Group I and were also injected (i.m.) with eCG (250 IU; Chronogest/eCG, Intervet, İstanbul, Türkiye) at the time of sponge removal, and Group III: Ewes ( $n=38$ ) were only injected  $\text{PGF}_{2\alpha}$  at the same time with the ewes in the other two groups. Estrus was detected twice a day (06:00h and 18:00h, for 4 day) by aproned rams ( $n=4$ ), 24 hours after  $\text{PGF}_{2\alpha}$ . All ewes detected in estrus were mated. Ten rams of proven fertility were used for mating. Pregnancy was diagnosed 60 days after mating by ultrasonography, as the routine farm management procedure of the latest pregnancy diagnosis of the breeding season, before possible culling.

### Statistical analysis

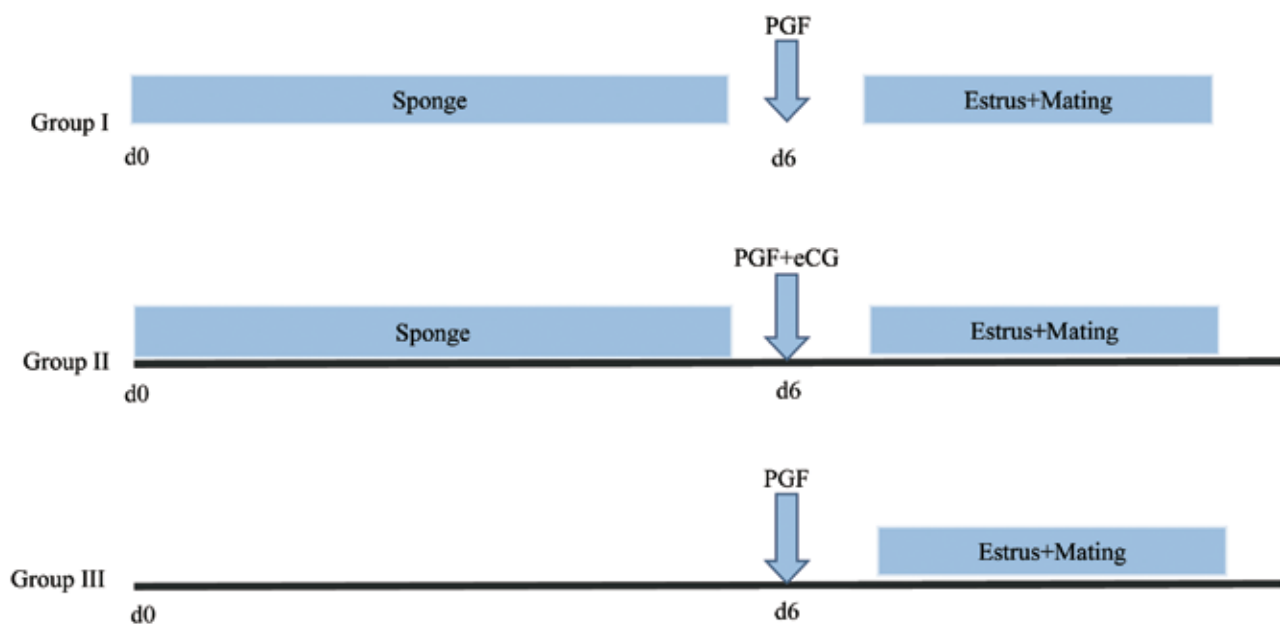
Data were analyzed using z-test ratio comparison with Minitab version 16 statistics program. P value < 0.05 was considered to be significant.

Estrus rate was calculated by the number of ewes in estrus / number of ewes in the group x 100, Conception rate by the number of pregnant ewes / number of ewes mated in heat x 100, Pregnancy rate by the number of pregnant ewes / number of ewes in the group x 100, Fetal loss by the number of pregnant ewes on day 60 that did not give birth / number of pregnant ewes x 100, Lambing ratio by the number of ewes that gave birth / number of ewes in the group x 100, Prolificacy by the number of ewes / number of ewes that gave birth, Twinning rate by the number of ewes with twins / number of ewes in the group x 100.

### RESULTS

The results of this study are presented in Table 1. Estrus detection rates, conception and pregnancy rates, lambing rates, number of alive lambs and prolificacy were similar in all three groups. Pregnancy loss after pregnancy diagnosis (d60) was higher ( $P < 0.05$ ) in Group III (35.4%) compared to Group I (13.6%) and Group II (15%). Furthermore, a total of 84 lambs were born from 67 ewes.

**Figure 1.** Experimental model of study. (Sponge=Florogestan acetate 30 mg,  $\text{PGF}$ =  $\text{PGF}_{2\alpha}$  125  $\mu\text{g}$ , eCG=250 IU).



**Table 1.** Estrus detection, conception and pregnancy rate, lambing ratio, single and twin birth rate, number of alive lambs and prolificacy after short term estrous synchronization protocols.

Group	N	Estrus detection % (n)	Conception % (n)	Pregnancy % (n)	Fetal loss % (n)	Lambing ratio % (n)	Single birth % (n)	Twin birth % (n)	Number of alive lambs (n)	Prolificacy
I	30	100 (30/30)	73.3 (22/30)	73.3 (22/30)	13.6 (3/22)	86.4 (19/22)	84.2 (16/19)	15.8 (3/19)	22	1.16 (22/19)
II	40	92.5 (37/40)	89.2 (33/37)	82.5 (33/40)	15 (5/33)	85 (28/33)	75 (21/28)	25 (7/28)	35	1.25 (35/28)
III	38	81.6 (31/38)	100 (31/31)	81 (31/38)	35.4 * (11/31)	64.6 (20/31)	65 (13/20)	35 (7/20)	27	1.35 (27/20)
Total	108	90 (98/108)	87.8 (86/98)	79.6 (86/108)	22 (19/86)	78 (67/86)			84	

\*Significantly different in the column

Group I (n=30): intravaginal progesterone sponge for 6 days plus PGF<sub>2α</sub> at the time of sponge removal,

Group II (n=40) same as in group 1 plus eCG at the time of sponge removal

Group III (n=38) only PGF<sub>2α</sub> at the same time with the ewes in the other two groups.

## DISCUSSION

In the flock at which this study was performed, the ratio of non-pregnant ewes was about 5-6 % in the breeding season. Since this is a valuable purebred breed, the genetical and economical value of each ewe is important. Therefore, culling off the non-pregnant ewes from the flock was not a desirable option. Thus, short term synchronization protocols were used to remain as many ewes as possible into the flock. Controlled breeding program and hormonal intervention to possibly temporary infertility problem (apart from pathological problems or other situations e.g. inadequate management) might be useful. Two main synchronization strategies are commonly used in sheep reproduction; prostaglandin and progestagen based protocols. In this research institute, these two strategies were previously used during the breeding season on the same breed and pregnancy rates were about 66% (Kirbaş et al., 2012). Pregnancy rate in the present study was higher than that reported previously by Kirbas et al. (2012). Although pregnancy rates were high, the pregnancy loss was an important problem, because it was between 13.6 and 35.4 %. It has been reported that the embryonic loss in ewes after synchronization of estrus and ovulation reaches up to 30% (Wilmot et al., 1986; Nancarrow, 1994); however, multiple pregnancies are common in ewes and loss of an embryo does not cause a loss of pregnancy, since the other embryo continues to grow. In this study, pregnancy diagnosis was performed after day 60 (day 0: mating). Therefore, there is no clue whether

non-pregnant ewes had also experienced an embryonic or fetal loss. The pregnancy loss was significantly higher in the group III (only PGF<sub>2α</sub>). Protocols based on PGF<sub>2α</sub> result in good estrus and ovulation synchronization; however fertility was poor (Menchaca et al., 2004, Quintero-Elisea et al., 2011). Fierro et al. (2011) demonstrated that even double dose of PGF<sub>2α</sub> 7 days apart created lower progesterone environment leading to low fertility. In the present study, estrus detection rate was about 81% and conception rate was 100% in PGF<sub>2α</sub> group, which were similar to progesterone based groups. However, lambing rate was numerically lower than progesterone based synchronization protocols (64.6% vs 86.4 and 85%), probably because of higher fetal loss in the PGF group. This loss could be due to long term effect of lower progesterone environment or because preovulatory follicle was not developed properly (Fierro et al., 2011).

The results of this study are similar to earlier reports in different breeds of ewes. Estrus detection rates were between 70 to 95% after progesterone based protocols in different studies (Gonzalez-Bulnes et al., 2005; Koyuncu and Ozis, 2010; Ozyurtlu et al., 2016; Akbaş and Köse, 2017). In this study estrus detection rate was >90%. Furthermore, pregnancy rates on day 60 were 73.3 and 82.5% for the progestagen treated groups. These results clearly showed that ewes in our study had no problems in terms of fertility and they did not become pregnant after three matings in the breeding season possibly because of the management

strategies (early starting season, possible heat stress and vitamin/mineral deficiency). Similarly, ewes synchronized with prostaglandins resulted in even greater pregnancy rate compared to an earlier study in the same farm (81% vs 68% Köse et al., 2016). However, fetal mortality ratio in this group was clearly higher. This is a common problem with synchronization protocols utilizing prostaglandins, as the embryo quality is somehow lower as shown by Zonturlu et al. (2011). Poor quality embryos after prostaglandin protocol, could explain the pregnancy loss in our study.

Regardless of the synchronization programs used, 86 ewes out of 108 became pregnant (as diagnosed on day 60) in this flock and 67 of them produced a total of 84 lambs. Originally, the aim of this study was to reevaluate ewes that did not become pregnant during the breeding season after at least three matings at natural estrus, in order to minimize the culling of otherwise valuable ewes from the breeding livestock.

This has also economic value in terms of reintroducing the ewes back to the flock for the next breeding season and having extra lambs to sell. This aim was mostly achieved, since almost 80% of pregnancy rate (as diagnosed on day 60) was obtained and 78% of the pregnant ewes gave birth to 84 lambs. Instead of culling 108 ewes from the flock, only 22 ewes that did not show estrous were removed from the flock. The most immediate economic benefit, profit (sale of lambs) / total cost of synchronization was 40 folds (67000 TL from lamb sale vs 1600 TL cost for drugs used). In conclusion, valuable ewes could be subjected to short term synchronization protocols at the end of breeding season to prevent the loss of the purebred breeds and to sustain the productivity of the sheep industry. All protocols investigated in this study could be used in farms with similar problems.

#### CONFLICT OF INTEREST

None declared.

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