Original Article

Presynchronization in Sheep Ensures Synchronization of Next Estrus and Improves Fertility

Bülent BÜLBÜL[1*] Mehmet KÖSE[2] Mesut KIRBAŞ[3] Kenan ÇOYAN[4]
Mehmet Bozkurt ATAMAN[4] Emine Aysun BEKÇİ[3] Seyit ÜMÜTLÜ[3]
Uğur DEMİRCİ[3] Bumin Emre TEKE[3]

Abstract

In this study, fertility was evaluated either in the first hormonally synchronized or in the subsequent natural estrus among 202 sheep during the breeding season. Intramuscular (im) $PGF_{2\alpha}$ was administered 11 days apart in both the PG (n=50) and Pre-PG (n=50) groups, while im $PGF_{2\alpha}$ was given at the removal of an intravaginal progesterone sponge (after 11 days) in the Sponge (n=51) and Pre-Sponge (n=51) groups. Estrus was monitored following the last hormonal application (day 0) in all groups and starting from day 16 in the Pre-PG and Pre-Sponge groups for 5 days. Ewes were mated during the first hormonally synchronized estrus (PG and Sponge groups) or in the next (natural) estrus (Pre-PG and Pre-Sponge groups). The estrus rate and litter size in the Pre-Sponge group were higher than those in the other groups (P<0.05). In the Pre-Sponge group, pregnancy and lambing rates were higher than in the PG and Sponge groups, and fecundity was higher than in the PG group (P<0.05). It was concluded that progesterone and $PGF_{2\alpha}$-based estrus synchronization may negatively affect fertility. Additionally, the next estrus is also synchronized after using progesterone and $PGF_{2\alpha}$-based synchronization protocols. Fertility loss caused by the application of exogenous hormones can be mitigated by mating at the next natural estrus. Furthermore, the next natural estrus can be utilized in situations where hormone use is inappropriate but synchronization is desired.

Keywords: Estrus synchronization, Ewe, Fertility, Presynchronization, Progesterone, Prostaglandin F_{2a}

[1]*Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, TR-35890 İzmir, TÜRKİYE*
[2]*Dicle University, Faculty of Veterinary Medicine, Department of Gynecology and Obstetrics, TR-21280 Diyarbakır, TÜRKİYE*
[3]*Bahri Dağdaş International Agricultural Research Institute, Department of Animal Research, TR-42020 Konya, TÜRKİYE*
[4]*Selçuk University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, TR-42003 Konya, TÜRKİYE*
***Correspondence**: bulent.bulbul@deu.edu.tr* (B. Bülbül)
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Introduction

Reproductive efficiency is the basis of the creation and sustainability of profitability in intensive livestock breeding, as it is the guarantee of the preservation and improvement of the herd existence and productivity level (Nan *et al*., 2021). In order for the criteria determining reproductive efficiency to be within optimal limits, the success level of reproductive herd management, which consists of components such as the determination of estrus of females, their mating / artificial insemination (AI), early pregnancy diagnosis, protection of pregnancy health, management of the birth season, is very important (Letelier *et al*., 2011; Beltman, 2013). One of the most frequently used reproductive herd management practices in sheep breeding to improve fertility in recent years is the synchronization of the estrus cycles of the sheep in the herd (Hameed *et al*., 2021).

Since estrus synchronization in goat and sheep breeding enables estrus to occur at the planned time, it provides an opportunity to improve estrus detection efficiency, to tighten the breeding season, thus lambing season, and the production of products at the desired time, and to use production resources such as breeding material, feed, and labor effectively. In addition, estrus synchronization is also widely used for the more efficient and easier implementation of biotechnological applications such as artificial insemination, embryo transfer, treatment attempts to decrease embryonic deaths, and scientific studies (Köse *et al*., 2012; Yu *et al*., 2018).

Estrus synchronization in sheep breeding is mostly done with exogenous hormone applications. Many estrus synchronization methods have been developed for sheep, in which hormones such as GnRH, progesterone (P4), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), melatonin, eCG, etc. used either alone or combined with other hormones (Wildeus, 2000). The basis of these methods is to control the duration of the luteal phase of the estrus cycle, and the time of occurrence of estrus can be planned by extending or shortening the duration of the luteal phase. In prolonging the duration of the luteal phase, the formation of new estrus is suppressed by creating a progestative effect with P4 and its analogues. In shortening the duration of the luteal phase, the life of the corpus luteum is shortened and the transition to the follicular phase is accelerated by creating a luteolytic effect with $PGF_{2\alpha}$ and its analogues (Wildeus, 2000; Fierro *et al*., 2013). However, no matter which estrus synchronization method is applied, it is important and desired for the breeders that the synchronization method applied is cheap, easy to apply, tightens the estrus of the sheep in the herd and does not have negative side effects on the general health status and most importantly their fertility (Wildeus, 2000).

The realization of important advantages expected in herd management with estrus synchronization practices is directly related to the rate of ewes conceived in synchronized estrus (Fierro *et al*., 2013). However, when the results of the comparative studies are examined, it is seen that the fertility results obtained are quite variable and there are quite different predictions and determinations in terms of the causes (Fierro *et al*., 2011; Letelier *et al*., 2011). In addition, the increasing awareness of consumers about individual and environmental health in recent years and the legal regulations require that the products produced and offered for consumption in sheep breeding should be clean, natural and comply with ethical rules, and they encourage and force breeders to produce in this direction (Martin and Kadokawa, 2006). Therefore, within the scope of sustainable livestock activities aiming to protect human and animal health and reduce environmental pollution, studies aiming to reduce or even completely abolish hormonal practices in sheep continue increasingly (Dursun, 2022). It is known that P4 and its derivatives accumulate especially in adipose tissue in humans and animals and may cause environmental pollution. Therefore, it is stated that the use of $PGF_{2\alpha}$ may be a more appropriate choice for synchronization of the estrus cycle. In addition, the advantages of $PGF_{2\alpha}$ and its analogues over P4 are that the costs are quite low, their application is easier and their negative effects on animal welfare are lower (Letelier *et al*., 2011). However, since prostaglandins are effective through regression of the functional corpus luteum, they are not effective in the follicular, early and late luteal periods of the estrus cycle, as in the seasonal anestrus period in which there is no cyclic activity in sheep. During the injection of prostaglandins, it is not possible to know which stage of the estrus cycle the sheep are in, so a single injection is not sufficient to synchronize the estrus. For this reason, in order to synchronize the estrus of the sheep in the herd, they should be injected twice with an interval of 9-10 (at least 7) days (Fierro *et al*., 2013).

Central Anatolian Merino (80% German Meat Merino x 20% Akkaraman) is a breed that is well adapted to Central Anatolian conditions and is highly preferred by breeders for pure and crossbreeding purposes (Behrem, 2021). It has a large body and its body is covered with a uniform white fleece except the face and lower part of the legs. However, it is preferred by breeders for meat production rather than fleece due to its deep, long and wide body and full and meaty hip structure, good fattening performance and reproductive ability superior to native breeds in the regions it is adapted to (Kırbaş *et al*., 2022).

In this study, it was aimed to determine the effect of mating on some fertility parameters in the first or second estrus after two synchronization protocols based on $PGF_{2\alpha}$ and P4, which are widely used in practice during the breeding season in Central Anatolian Merino sheep.

Materials and Methods

Ethical Statement: This study was conducted in accordance with the guidelines of the Animal Experiments Local Ethics Committee and with an experimental protocol approved by the ethics committee for the use of animals in research and experimentation of the Bahri Dağdaş International Agricultural Research Institute, Turkey (Approval no: 22.07.2013/8).

Materials: The study was carried out in Bahri Dağdaş International Agricultural Research Institute located at

37°51'44'' north latitude and 32°33'32'' east longitude and an average altitude of 1005 m above sea level in the middle part of Turkey. In the study, 202 ewes, 2-5 of age and 60-70 kg of body weight, and 20 rams with proven fertility of Central Anatolia Merino were used in August which is accepted as breeding season in Turkey. All ewes were housed in the same half-open pen. They were grazed in artificial pasture in daytime. Also, each ewe was offered 0.5 kg of concentrate mixture daily. Water and mineral salts were available ad libitum.

Methods: Before the study was started, blood samples were collected by jugular vein into heparinized tubes twice with 10 d apart (on d -21 and just before $PGF_{2\alpha}$ or sponge applications on d -11) for P4 analyzes, and samples were centrifuged at 1500×g for 10 min to separate plasma. Plasma samples were harvested in eppendorf tubes and stored at -20°C until analyzed. Ewes having concentrations of plasma P4 greater than 1 ng/ml in at least one of two samples were involved in the study. On the same day of the second blood sample, ewes were randomly divided into four groups as described below, and the estrus synchronization protocols were started with the first PG injection (PG and Pre-PG groups) or sponge application (Sponge and Pre-Sponge groups) in all groups (*Fig. 1*).

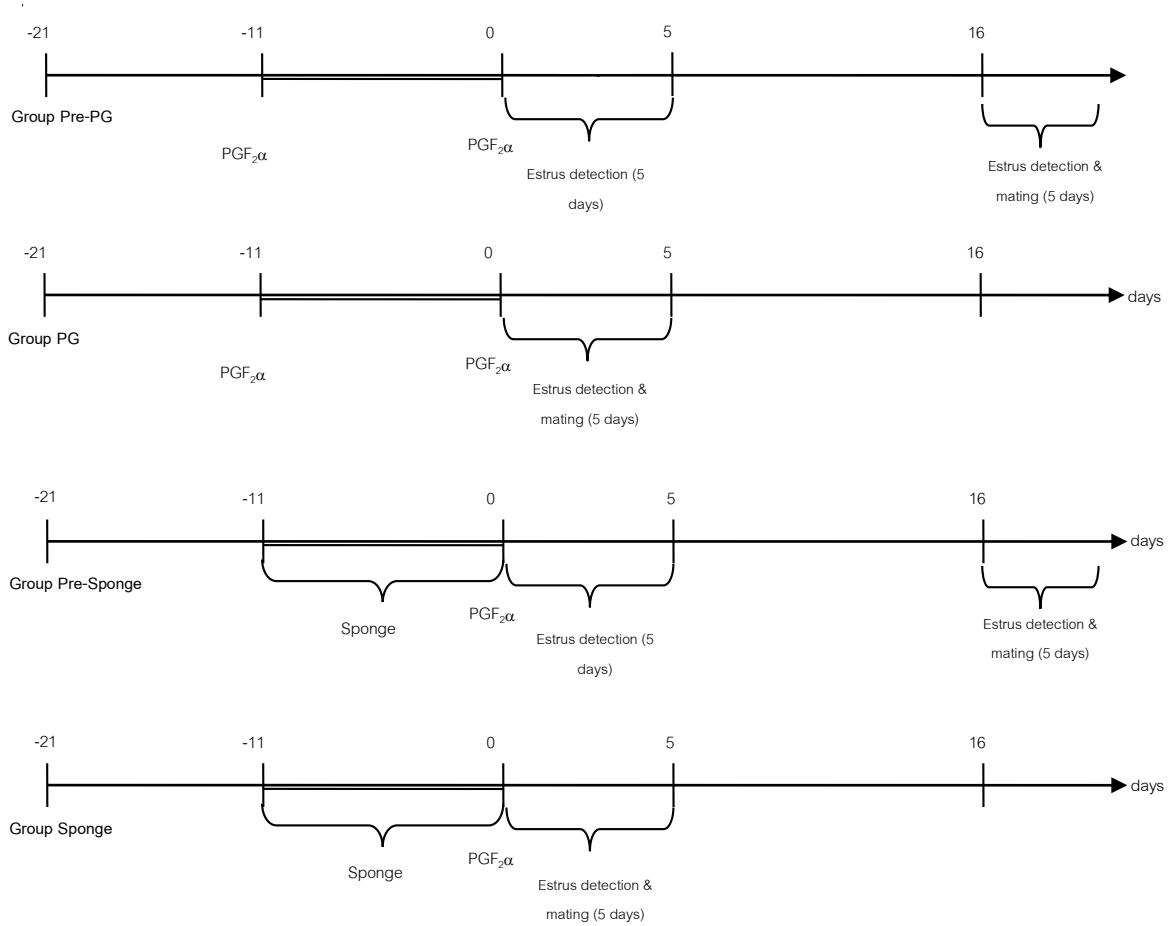

Figure 1 Estrus synchronization protocols in all groups

Two im injections of $PGF_{2\alpha}$ (125 µg d-cloprostenol, Minoprost, Provet, Turkey) 11 d apart were applied in PG (n=50) and Pre-PG (n=50) groups.

In Sponge (n=51) and Pre-Sponge (n=51) groups, intravaginal treatment of flugestone acetate (FGA) (40 mg flugestone acetate sponge, Chrono-Gest, Intervet, Turkey) for 11 d and an im injection of $PGF_{2\alpha}$ at the time of sponge removal were applied.

All ewes were checked for estrus twice at a 12-h interval in one day for five days after PG injection with teaser rams. Each ewe standing to be mounted by the teaser ram was considered to be in estrus, and was separated from the main flock. The onset of estrus behavior was recorded. Third blood samples were collected from all the animals at the second PG injection or sponge removal (on d 0), but fourth blood samples were collected before mating from ewes only in estrus. Plasma samples were harvested and stored as described previously. While those in estrus in PG and Sponge Group were mated in a separate paddock with the rams of Central Anatolia Merino breed previously known to be fertile, the ewes in Pre-PG and Pre-Sponge Group weren't mated. For 5 d starting from d 16 (16 d after the last $PGF_{2\alpha}$ injection), the ewes in Pre-PG and Pre-Sponge groups were checked for estrus as described previously, and the ewes in estrus at this time (first natural estrus) were mated with the rams. Ewes in all groups were checked for estrus for 5

d starting from day 16 after the first mating and recorded to calculate non-return rate (NRR).

Pregnancy diagnoses were performed 30 d after the mating by ultrasonography using a 6/8 MHz transrectal linear array transducer (Pie Medical 480, 100 LC, Holland). The number of birth and the number of each lamb were recorded at lambing. The following parameters were calculated with the formula given in parentheses for each group:
Last application-estrus interval (h) (the interval of the last $PGF_{2\alpha}$ injection and estrus detection),
Estrus rate (%) (number of ewes showing estrus behavior/treated ewes, at first estrus following hormonal synchronization in PG and Sponge groups and at subsequent/first natural estrus in Pre-PG and Pre-Sponge groups),
Non-return rate (%) (number of ewes not in estrus 16-21 d after mating/mated ewes)
Pregnancy rate (%) (number of pregnant ewes/treated ewes),
Lambing rate (%) (number of lambing ewes/treated ewes),
Fecundity (number of lambs born/treated ewes),
Litter size (number of lambs born/lambing ewes),
Multiple birth rate (%) (number of multiple lambing/lambing ewes).

Statistical analysis: The data for plasma P4 level, last application-estrus interval, fecundity and litter size were analyzed using ANOVA test. Differences in estrus, pregnancy, non-return, lambing and multiple birth rates among groups were assessed by Chi-square test.

Results

Mean plasma P4 concentrations on d -21, -11, 0 and on the day of mating in groups (ng/ml) (±S.E.M.) were summarized in *Table 1*. While there was no difference among groups on d -21 and -11 for plasma P4 levels, it was higher in groups PG and Pre-PG than that in Sponge and Pre-Sponge groups on d 0. Plasma P4 levels in all groups were low on the mating day and there was no difference among them.

As indicated in *Table 2*, the intervals from the last PG injection to estrus were statistically shorter in the PG group compared to Sponge group in the first estrus period. Last application-estrus intervals were similar in Pre-PG and Pre-Sponge groups in the subsequent/natural estrus.

The estrus, pregnancy, non-return, conception, lambing and multiple birth rates, fecundity and litter sizes in the PG, Pre-PG, Sponge and Pre-Sponge groups are given in *Table 3*. Estrus rate in Pre-Sponge group was significantly (P<0.05) higher than those in the other three groups. In Pre-Sponge group, while pregnancy and lambing rates were significantly (P<0.05) higher than those in the PG and Sponge groups, pregnancy rate was similar with that in the Pre-PG group. Lambing rate in Pre-Sponge group was significantly (P<0.05) higher than those in PG and Sponge groups but similar with that in Pre-PG group. Fecundity rate in Pre-Sponge group was higher than that in PG group. Non-return and multiple birth rates were similar in all groups, but multiple birth rate in Sponge group was numerically higher about 21-23% than those in the other three groups. Litter size in Sponge group was significantly (P<0.05) higher than those in the PG, Pre-PG and Pre-Sponge group groups.

Discussion

It is well known that one of the $PGF_{2\alpha}$ or P4-based synchronization protocols is mostly preferred for synchronization of the estrus cycle in sheep during the breeding season. However, when fertility obtained in first estrus mating following $PGF_{2\alpha}$ or P4-based synchronization applications are compared with that in mating at natural estrus, the results appear to be highly inconsistent and variable (Yu *et al*., 2018). In this study, considerable fertility parameters were obtained in Central Anatolian Merino sheep during the breeding season mated in the first hormonal or second natural estrus after the synchronization of the estrus cycle with a double dose $PGF_{2\alpha}$ injection 11 d apart or with $PGF_{2\alpha}$ injection at the end of the intravaginal P4-containing sponge application for 11 days.

First of all, in this study, high mean P4 levels determined in plasma obtained from blood samples taken on the 10th day before and on the day of initiation of estrus synchronization applications (on d -21 and just before $PGF_{2\alpha}$ or sponge applications on d -11) in all groups, which is an indicator of ovarian activity indicating that ovulation and functional corpus luteum(s) are formed in its follow-up (Bulbul *et al.*, 2014), and that the study was carried out during the breeding season.

Estrus synchronization protocols consisting of two injections of $PGF_{2\alpha}$ in sheep have been established regarding adequate injection interval for the development of the corpus luteum sensitive to the second injection of $PGF_{2\alpha}$ in sheep with luteolysis at the first injection or without the corpus luteum (Yu *et al*., 2018), as in cows. In this study, it was shown that estrus can be synchronized with two $PGF_{2\alpha}$ applications 11 d apart in Central Anatolian Merino sheep, both in the PG and Pre-PG groups, with the high P4 level on the second $PGF_{2\alpha}$ injection day and a high estrus rate. Although no studies have been conducted on the length and phases of the estrus cycle in Central Anatolian Merino sheep in previous extensive studies, the corpus luteum in sheep was found to be sensitive to $PGF_{2\alpha}$ at the age of 3 days, and it was stated that the interval between two $PGF_{2\alpha}$ injections could be 7-14 d (Fierro *et al*., 2011; Fierro *et al*., 2017).

In the Sponge and Pre-Sponge groups, the mean plasma P4 concentration in both groups was approximately 1 ng/ml on the day the 11-d application was terminated, indicating that the sponge application duration constitutes the luteal phase length required for regression of the cyclic corpus luteum in sheep (Hameed *et al*., 2021). With the applications of FGA or medroxyprogesterone acetate (MPA), which is placed into the vagina as a sponge, for 9-19 d, it is aimed to create an artificial luteal phase effect that mimics the corpus luteum while suppressing new estrus and ovulation until the application is terminated (Wildeus, 2000; Hameed *et al*., 2021). Similar to this study, it has been reported that similar P4 profiles occur in various studies in which FGA or MPA were administered at

the same or close durations (Menegatos *et al*., 2003; Naderipour *et al*., 2012). The compatibility of the results among the studies can be explained by the high rate of endogenous luteolysis in sheep due to the very low variation in the length of the estrus cycle, which lasts for an average of 17 d in sheep, the rapid luteolysis of CL and the sudden decrease in peripheral P4 level (Bartlewski *et al*., 2011), and the suppression of estrus and ovulation by exogenous P4 administration during the application (Yu *et al*., 2018).

Table 1 Mean plasma P4 concentrations (ng/ml) on d -21, -11 (first $PGF_{2\alpha}$ in PG groups or sponge application in Sponge groups), 0 (second $PGF_{2\alpha}$ in PG groups or $PGF_{2\alpha}$ application in Sponge groups) and on the day of mating in groups (±S.E.M.)

Groups	d -21	d -11	d 0	Mating day
Pre-PG	2.36±0.31	3.38±0.31	5.29±0.36 [a]	1.10±0.08
PG	3.05±0.28	3.28±0.37	5.37±0.40 [a]	1.16±0.09
Pre-Sponge	3.08±0.42	3.74±0.34	1.23±0.17 [b]	1.05±0.05
Sponge	2.63±0.31	4.12±0.57	1.13±0.12 [b]	1.16±0.10

[a,b] Values within a column with different superscripts differ significantly at $P<0.05$

Table 2 Mean last application-estrus intervals (h) in groups (±S.E.M.)

	First period	Second period
Pre-PG	54.63±1.40[ab]	440.20±3.52
PG	52.50±1.36[b]	-
Pre-Sponge	55.97±1.38[ab]	446.50±3.31
Sponge	58.30±1.36[a]	-

[a,b] Values within a column with different superscripts differ significantly at $P<0.05$

Table 3 Estrus and fertility rates at mating period in groups

	Pre-PG	PG	Pre-Sponge	Sponge
n	50	50	51	51
Estrus rate (%)	90.00[b]	80.00[b]	100.00[a]	78.43[b]
Pregnancy rate (%)	80.00[ab]	66.00[b]	90.20[a]	64.71[b]
NRR (%)	95.56	90.00	94.12	87.50
Lambing rate (%)	76.00[ab]	62.00[b]	88.24[a]	62.75[b]
Fecundity	1.02[ab]	0.80[b]	1.11[a]	0.96[ab]
Multiple birth rates (%)	27.50	29.03	26.67	50.00
Litter size	1.28[b]	1.29[b]	1.24[b]	1.53[a]

[a,b] Values within a column with different superscripts differ significantly at $P<0.05$

One of the most important data showing the effectiveness of these protocols is the degree of synchronization of estrus of the sheep in the herd. Although this rate is affected by many intrinsic and extrinsic factors, the rate of ewes showing estrus in 3-5 d period should be as high as possible (Kose *et al*., 2022). In this study, a 5 d period was defined for all groups as the estrus detection and mating period. Following the synchronization protocols, the estrus rate in the Sponge and PG groups during the first estrus detection period when mating was to be performed was very close to each other and was approximately 80%. These rates were close to the values in some previous studies in which $PGF_{2\alpha}$ and FGA were tested in the same study (Gonzalez-Bulnes *et al*., 2005; Naderipour *et al*., 2012; Hasem *et al*., 2015). It is thought that the main factors of this result were carrying out the present study during the breeding season when $PGF_{2\alpha}$ is effective, performing the $PGF_{2\alpha}$-based synchronization protocol with a double dose application, and injecting the $PGF_{2\alpha}$ at the end of the P4-based synchronization protocol. In this study, estrus rate after hormonal administration in the PG and Sponge groups may be lower than the rates reported in several studies (Moakhar *et al*., 2012; Hasani *et al*., 2018), but it should be taken into account that estrus rate is affected by many factors such as treatment schedule, eCG application breed, diet, season and active ingredient differences (Wildeus, 2000; Fierro *et al*., 2013).

After the P4-based synchronization protocols, it was determined that the onset times of the first estrus were delayed compared to the onset of estrus in the $PGF_{2\alpha}$-based synchronization groups, and the delay in the Sponge group was statistically significant compared to the PG group. It was determined that this effect continued on the onset of second estrus, but it was not statistically significant. It was also reported that sheep synchronized with synthetic P4, similar to our study, showed estrus later than those synchronized with natural P4, and they also determined that LH release was delayed in these sheep

(Menegatos *et al*., 2003). However, it is well known that, the onset of estrus in sheep is associated with the secretion of estradiol secreted from antral follicles in the pre-ovulatory period, it is caused by a continued increase in estradiol secretion after a decrease in P4 concentration by luteolysis and accompanied by an increase in the frequency of LH release from the anterior pituitary, and it continues with the production of estradiol, which continues until the increase in P4 at the follicle level with the effect of the LH peak (Nan *et al*., 2021). It has been reported that this mechanism, which indicates the onset of estrus, changes with exogenous applications, and the estradiol-producing abilities (Gonzalez-Bulnes *et al*., 2005) and growth rates (Letelier *et al*., 2011) of pre-ovulatory follicles decrease in sheep synchronized with FGA compared to ewes synchronized with $PGF_{2\alpha}$. Another study reported that estradiol levels were significantly lower in ewes synchronized with MPA on the 2nd day after cessation of administration, very close to the onset of estrus, compared to sheep synchronized with natural P4-containing controlled internal drug release (CIDR) and $PGF_{2\alpha}$ (Naderipour *et al*., 2012). In this study, the elimination duration of P4 in peripheral blood following sponge withdrawal may have a slight effect on the delay of the onset of the first estrus in the P4 treated groups, as Menegatos *et al.* (2003) put forward.

Another important result of this study is that when the estrus of the sheep is synchronized with the $PGF_{2\alpha}$ or P4-based synchronization protocols, the following second estruses are synchronized in a short time interval in the new estrus cycle, that is, the synchronized state of the estruses continues. In the study, the synchronization effect of hormonal applications on the duration of the estrus cycle continued in the next estrus cycle. Previously, it was stated the second estrus of that not conceiving to the first service in synchronized ewes will be within about 16-17 d after the first mating (Knights *et al*., 2006). Similarly, Pabuçcuoğlu *et al.* (1996) reported the first natural estrus occurs on the 18th day after the induced estrus after estrus synchronization with the P4-based synchronization protocol in Kıvırcık ewes.

The other remarkable result of this study is that the estrus rate during the second estrus detection period (5 d period starting on day 16 after the last application) when mating was performed in both the Pre-Sponge and Pre-PG groups is higher than the estrus rate of ewes that were mated in both the Sponge and PG groups during the first estrus detection period (the first 5 d period after the last application). But, Pabuçcuoğlu *et al.* (1996) stated no differences between the first natural estrus rate or induced-estrus rate after the P4-based synchronization protocol. Mainly, we suggest that follicular wave and luteal development were synchronized simultaneously effectively after the pre-synchronization protocols, consisting of two $PGF_{2\alpha}$ applications 11 d apart or intravaginal treatment of P4 as a sponge for 11 d and an intramuscular injection of $PGF_{2\alpha}$ at the time of sponge removal. Moreover, in these results, there may be the therapeutic effects of the hormones used in the synchronization protocol on various reproductive disorders (luteal cyst, follicular cyst, inactive ovary, subestrus, subclinical endometritis, etc.) in sheep. Reproductive disorders, for example, follicular cysts, endometritis, etc. in ewes are ignored mostly in the breeding season, yet it was stated that these disorders may be high rate and cause the reduction of the reproductive efficiency of ewes (Silva *et al*., 2020). In addition, the fact that some of the negative effects that may occur in the first estrus following the estrus synchronization with hormonal manipulation did not occur during the second natural estrus may have been effective in the higher estrus rate in the second period (Pinna *et al*., 2012). These findings show that in Central Anatolian Merino sheep, estrus can be synchronized with both $PGF_{2\alpha}$ and P4-based methods, and the synchronization of estrus will continue in subsequent estrus and the advantages of hormonal estrus synchronization will not be lost with cycling. The continuation of the synchronization of estrus within the second estrus cycle is important in several ways. First, if the pregnancy rate is low at the mating/AI in the first estrus for various reasons, the subsequent estrus detection of non-pregnant ewes becomes easier, and it enables the second mating/AI to be done more easily and at a low cost. Secondly, the longer the mating season and the related lambing season in the flock and the risk of negativities in the implementation of other practices (vaccination, weaning, fattening, etc.) in herd management are reduced. Third, when the effects of exogenous hormones are not desired to occur in the results of the experiments to be carried out by the researchers, it is possible to conduct the experiments easily (Kiyma *et al*., 2016; Kose *et al*., 2022).

It is clearly seen that pregnancy rates, birth rates and fecundity parameters were significantly higher in Pre-PG and especially in Pre-Sponge groups than those in other groups. Pre-PG and Pre-Sponge groups may have better pregnancy rates, birth rates and fecundity parameters than the other two groups because of the higher estrus rates in these groups and the negative effects of P4 and $PGF_{2\alpha}$-based hormonal estrus synchronization on fertility in the first estrus after hormonal applications. As a matter of fact, in some studies, it has been reported that fertility is negatively affected by P4-based synchronization applications because of vaginitis, breakdown of LH release, ovulation mechanism, sperm viability and transport in the female genital tract, etc (Gonzalez-Bulnes *et al*., 2005; Letelier *et al*., 2011; Manes *et al*., 2014; Manes *et al*., 2016). In addition, in $PGF_{2\alpha}$-based synchronization protocols, fertility may be adversely affected by effects such as changes in the LH release from the pituitary gland, the development of the pre-ovulatory follicle and corpus luteum (Letelier *et al*., 2011) and as a result, accelerated pre-ovulatory follicle development accompanied by low P4 concentration and insufficient P4 production from the corpus luteum developing after insemination (Fierro *et al*., 2011).

In this study, the litter size in the Sponge group was higher than that in all other groups. It is well known that in sheep, the number of lambs per birth is closely related to the number of ovulations. On the other hand, it has been shown before that ovulatory follicles in sheep originate from large follicles in the ovaries during luteolysis (Souza *et al*., 1997), the dominant follicle formed in cows does not have a suppressive effect on other follicles and small follicles can continue

to develop (Driancourt, 1994), and although ovulation is suppressed by exogenous P4 administration, the fertility ability of large follicles does not decrease due to aging (Evans *et al*., 2001). Along with these reports, high of the number of large follicles in the follicular stage (Gonzalez-Bulnes *et al*., 2005) and the number of ovulations (Letelier *et al*., 2011) in sheep synchronized with P4-containing sponge compared to sheep synchronized with $PGF_{2\alpha}$ provide strong support for the explanation that the number of lambs per birth is higher in the ewes that became pregnant when mated in the first estrus following the synchronization of ewes with P4 in our study. In addition to these, in sheep synchronized with $PGF_{2\alpha}$, it has been reported that the embryonic mortality rate in twin pregnancies (Fierro *et al*., 2011) and fetal loss rate (Dursun, 2019) are higher.

It was concluded in this study that; a) in Central Anatolian Merino sheep, P4 and $PGF_{2\alpha}$-based estrus synchronization may have a negative effect on fertility in the first estrus following the synchronization protocol, b) after P4-based and $PGF_{2\alpha}$-based synchronization applications, second estruses are synchronized in a narrow time interval in the new estrus cycle, that is, the synchronized state of estrus continues, c) fertility loss that may occur in estrus induced by exogenous hormone application can be prevented by detecting the estrus of the following cycle and mating, d) in studies where hormone use in sheep is not appropriate or in cases where hormonal residue is not desired for the consumer but synchronization is desired, second synchronized estrus resulting from presynchronization applications can be used.

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