Effect of oestrus synchronization with different lengths of progesterone-impregnated sponges and equine chorionic gonadotropin on reproductive efficiency in Romanov ewes during the non-breeding season

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Abstract

This study aimed to determine the effects of different lengths of progesterone and equine chorionic gonadotropin (eCG) treatment on reproductive performance during the non-breeding season in ewes. Progesterone-impregnated sponges were inserted intravaginally for 7 d in Group 1 (G1, n = 24), 9 d in Group 2 (G2, n = 25), and 11 d in Group 3 (G3, n = 24). On the day of sponge removal, eCG (350 IU) was injected and 24 h later the ewes were exposed to rams. Pregnancy was diagnosed by transrectal ultrasonography 30 ± 3 d after mating. Oestrus symptoms of G2 and G3 started earlier than G1, but there was only a significant difference between G1 and G3 ($P = 0.013$). In G1, G2, and G3, the oestrus responses were 100%, 100%, and 95.8%, pregnancy rates were 79.2%, 72%, and 75%, lambing rates were 79.8%, 72%, and 75%, litter sizes were 2.5, 2.2, and 2.4, and multiple birth rates were 86.7%, 80%, and 85.7%, respectively ($P > 0.05$). In conclusion, the short-term progesterone-impregnated sponge and eCG treatment provided excellent oestrus induction. The effects of different days (7, 9, or 11) of progesterone treatment on reproductive indicators were similar; therefore, the stress caused by the inserted sponge can be reduced by short-term use.

Oestrus induction, pregnancy rate, sheep, short-term, sponge

Many methods have been developed to control reproductive activity in farm animals. The main purpose of these protocols is to increase reproductive efficiency by administering various hormones (Stevenson and Britt 2017; Kuru et al. 2018a). Sheep are seasonally polyoestrous and generally, there is no sexual activity during the non-breeding season; therefore, hormonal manipulation is needed to stimulate oestrus during this period (Abecia et al. 2012; Kuru et al. 2018a, 2020).

Progesterone and its analogues are used effectively in induction and synchronization of oestrus in ewes (Kuru et al. 2018a). The progesterone-impregnated sponge is inserted intravaginally for 10 to 14 d. Oestrus is detected approximately 30 to 37 h after removal of the progesterone-impregnated sponge (Kuru et al. 2020). Progesterone treatment should be combined with equine chorionic gonadotropin (eCG) (Abecia et al. 2012; Garoussi et al. 2020), a hormone with simultaneous follicle-stimulating-hormone- and luteinizing-hormone-like activities. Progesterone treatment is effective in the presence of sufficient concentrations of gonadotropins that initiate preovulatory events, and the gonadotropic effect is achieved by eCG injection (Powell et al. 1996).

Recently, oestrus synchronization protocols have been developed in small ruminants using short-term progesterone (Karaca et al. 2009). In these protocols, a progesterone-impregnated sponge is inserted into the vagina for 5 to 7 d. This period is shorter than the half-life of the corpus luteum. Therefore, a single dose of prostaglandin F2 alpha (PGF) treatment is added to the protocol as there may be a cyclic corpus luteum during

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the breeding season. The response to short-term or long-term progesterone treatments is generally similar (Cox et al. 2012; Martinez-Ros et al. 2019).

Romanov is a prolific sheep breed of a Russian origin. Romanov ewes have high adaptability and are successfully managed in different breeding systems, with high reproductive performance, lamb yield, and survival rates (Ricordeau et al. 1988, 1990; Đuričić et al. 2019). A large interval between the onset of oestrus and the luteinizing hormone peak is characteristic of Romanov ewes, and the oestradiol concentration in the follicular phase and the progesterone concentration in the luteal phase may be higher than in most sheep breeds. The high concentration of hormones may be due to the number of developing follicles and corpus luteum (Bindon et al. 1979; Cahill et al. 1981). Romanov ewes are characterized by early sexual maturity, a long mating season, and a short anoestrus period. Three successful lambing periods in two years are possible with oestrus synchronization (Ricordeau et al. 1990). While eCG is typically used in protocols during the non-breeding season, the classic dose of eCG may be reduced due to multi-follicle growth in this breed. Oestrus synchronization positively affects fertility and litter size during the non-breeding season (April-May) and the eCG dose should not exceed 300-350 IU (Cornu and Cognié 1985).

Romanov ewes are crossbred in many countries to improve the reproductive performance of local sheep breeds (Ricordeau et al. 1990; María and Ascaso 1999; Đuričić et al. 2019). They are preferred for crossbreeding due to their suitability to different breeding conditions, high reproductive efficiency, and the high survival rate of crossbreed lambs (Kutluca Korkmaz and Emsen 2016; Đuričić et al. 2019). Fat-tailed ewes have been crossed with Romanovs to increase reproductive performance in eastern Turkey (Kutluca Korkmaz and Emsen 2016).

This study aimed to determine the effect of different lengths (7, 9, or 11 d) of progesterone-impregnated sponge and eCG treatment on reproductive performance during the non-breeding season in Romanov ewes.

Materials and Methods

This study was carried out after obtaining approval from the Kafkas University Ethics Committee for Animal Experiments (KAÜ-HADYEK/2020-003), and permission from the Turkish Ministry of Agriculture and Forestry, Kars, Turkey.

Table 1. Composition of the concentrate mixture fed to ewes.

Ingredient	%
Barley	21.5
Wheat bran	16.6
Molasses	0.5
Corn	39.0
Vegetable oils	1.0
Cottonseed meal (38 CP)	5.0
Soybean meal (48 CP)	12.0
Marble powder	3.1
Salt	1.0
Vitamin and mineral premix*	0.3

CP - crude protein; * - contents of vitamin and mineral premix in 1 kg: 100 g of Na, 33 mg of I, 7 mg of Ca, 27 mg of Se, 3,000 mg of Fe, 2,660 mg of Mn, 167 mg of Cu, 10,000 mg of α-tocopherol, 300,000 IU of cholecalciferol, and 3,000,000 IU of retinol

Animals

A total of 73 Romanov ewes, ranging in age from 2 to 3 years, 50 to 60 d postpartum, without any clinical problems, were studied. The weight of the ewes was 40 to 50 kg and the body condition score (BCS) was 2.5 to 3.0 (1 = Extremely thin to 5 = Obese). Fertile Romanov rams (n = 12) were used for heat detection and mating.

Feed

The ewes were housed in intensive management conditions and during the study period alfalfa and hay straw were given as roughage. In addition, 450 g/ewe of concentrate feed (16% crude protein and 2,700 kcal/kg metabolisable energy) per day was provided. The content of the concentrate feed is presented in Table 1.

Oestrus synchronization protocols

The Romanov is a prolific breed and postpartum first oestrus can occur between 30 and 50 d. For this reason, ewes with at least 50 postpartum days were exposed to rams for 30 d and heat was followed at 12-h intervals (Plate VII, Fig. 1). At the end of this process, oestrus synchronization was performed on the ewes (n = 73) without heat. Sheep were divided into three

groups and synchronized with different lengths (7, 9, or 11 d) of progesterone-impregnated sponges and eCG hormone treatment during the non-breeding season. Age, body weight, and BCS of the groups were balanced.

In group 1 (G1, n = 24) a progesterone-impregnated sponge (60 mg of medroxyprogesterone acetate, MAP, Esponjavet®, Hipra, Turkey) was inserted into the vagina on d-0 and removed on d-7. In group 2 (G2, n = 25), a progesterone-impregnated sponge was inserted into the vagina on d-0 and removed on d-9. In group 3 (G3, n = 24), a progesterone-impregnated sponge was inserted into the vagina on d-0 and removed on d-11. In all three groups, 350 IU eCG (Oviser®, Hipra, Turkey) was injected intramuscularly when the sponge was removed (Plate VII, Fig. 1).

Oestrus detection and mating after synchronization

The rams were divided into three balanced groups according to their BCS and age. At 24 h after sponge removal, ewes were housed with rams for 4 d at a female:male ratio of 6:1. In a 4-d period, heat detection was made every 8 h. When the ewe mated, it was determined to be in heat and removed from the group (Kuru et al. 2020).

Diagnosis of pregnancy

Pregnancy was confirmed 30 ± 3 d after mating by transrectal ultrasonography with a 7.5 MHz linear probe (SonoSite Vet180® Plus, SonoSite, Inc., Bothell, Washington, USA). To improve imaging quality, the rectum was cleaned and ultrasonographic application was performed in the standing position. When the embryo was detected, the sheep was confirmed as pregnant (Kuru et al. 2018c).

Reproductive indicators

Reproductive indicators such as onset of oestrus, oestrus response, conception rate, pregnancy rate, lambing rate, embryonic or foetal death, fecundity, litter size, birth type, and sex distribution were determined. Reproductive indicators were calculated according to the following formulas (Kuru et al. 2017):

Onset of oestrus = Time between sponge removal and the onset of heat
Oestrus response (%) = Ewes in heat/total ewes × 100
Conception rate (%) = Pregnant ewes/mating ewes × 100
Pregnancy rate (%) = Pregnant ewes/total ewes × 100
Lambing rate (%) = Ewes giving birth/pregnant ewes × 100
Embryonic or foetal mortality (%) = Ewes that were pregnant at diagnosis 30 d after mating but did not give birth/pregnant ewes × 100
Fecundity = Total lambs/ewes in heat
Litter size = Total lambs/ewes giving birth
Birth type (%) = Birth type (single, twins, triplets, quadruplets)/pregnant ewes × 100

Statistical analysis

Oestrus onset time (mean ± SEM) was analysed by one-way ANOVA, and differences between groups were determined with *post hoc* Tukey's honestly significant difference test. Differences between groups for other reproductive indicators were determined with the Chi-square test or Fisher's exact test. SPSS® (SPSS Version 26.0, Chicago, IL, USA) and GraphPad Prism® (Version 8.0.2, Graph Pad Software, San Diego, CA, USA) were used for analysis and $P < 0.05$ was considered significant.

Results

Progesterone-impregnated sponges never fell out of the vagina and no premature births were encountered during the study period. The onset of oestrus was significantly different between the groups ($P = 0.012$) and oestrus symptoms of G2 and G3 started earlier than G1, but there was only a significant difference between G1 and G3 ($P = 0.013$). Although the oestrus onset time was numerically different between G1 and G2, it was not significantly different, but we can say that there was a trend ($P = 0.07$). The onset of oestrus in G1, G2, and G3 was 40.6, 34.8, and 32.9 h, respectively (Fig. 2). There was no significant difference ($P > 0.05$) between the groups in other reproductive indicators (Fig. 2). Also, birth type and sex distribution (Table 2) between the groups were not significantly different ($P > 0.05$).

Discussion

Progesterone is frequently used for oestrus synchronization in small ruminants during the non-breeding season when a progesterone-containing device (sponge, etc.) is inserted into the vagina for 12 to 14 d. The duration of the sponge in the vagina is parallel to the

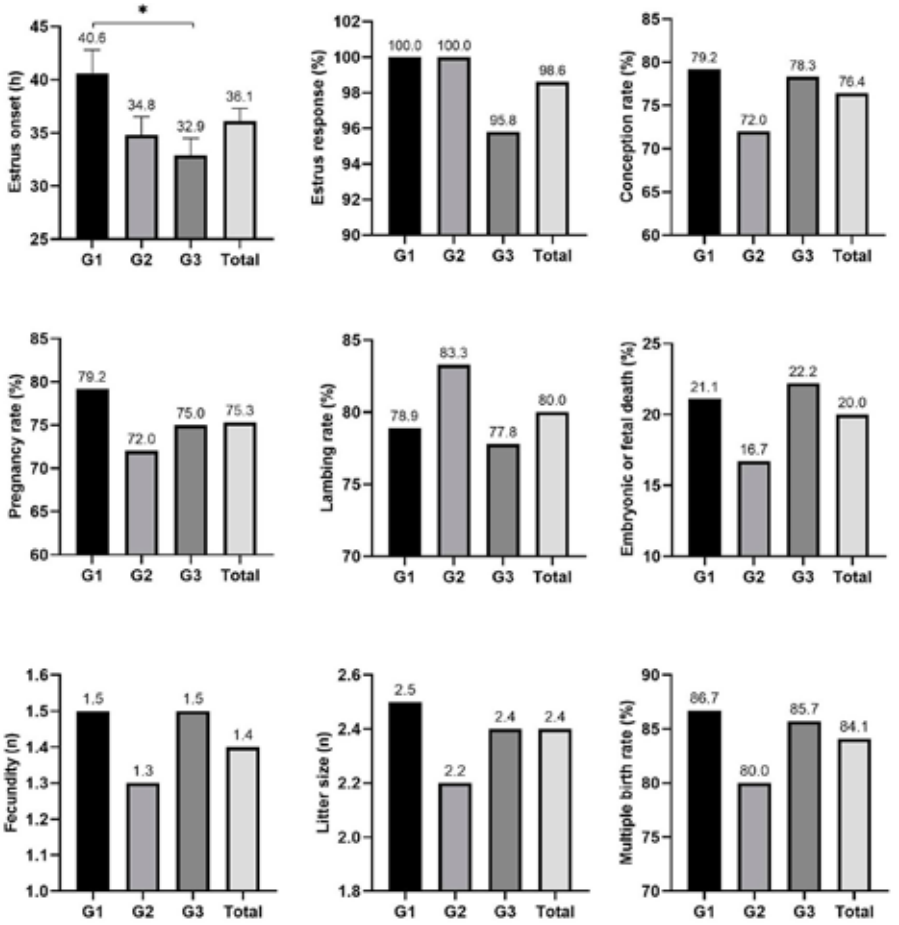

Fig 2. Onset of oestrus, oestrus response, conception rate, pregnancy rate, lambing rate, embryonic or foetal mortality, fecundity, litter size, multiple birth rate in groups. Oestrus symptoms started earlier in G3 than G1 and were statistically different in Tukey HSD test (P = 0.013). There was a significant trend at the onset of oestrus between G1 and G2 (P = 0.07). *: Shows the significant difference between G1 and G3. G1: 7-d progesterone-impregnated sponge + equine chorionic gonadotropin (n = 24). G2: 9-d progesterone-impregnated sponge + equine chorionic gonadotropin (n = 25). G3: 11-d progesterone-impregnated sponge + equine chorionic gonadotropin (n = 24)

Table 2. Type of birth and sex distributions in groups.

Indicator		G1 (n = 24)	G2 (n = 25)	G3 (n = 24)	Total (n = 73)
Birth type, %	Single	13.3 (2/15)	20.0 (3/15)	14.3 (2/14)	15.9 (7/44)
	Twins	40.0 (6/15)	46.7 (7/15)	35.7 (5/14)	40.9 (18/44)
	Triplets	33.3 (5/15)	26.7 (4/15)	42.9 (6/14)	34.1 (15/44)
	Quadruplets	13.3 (2/15)	6.7 (1/15)	7.1 (1/14)	9.1 (4/44)
Sex, %	Female	56.8 (21/37)	45.5 (15/33)	55.9 (19/34)	52.9 (55/104)
	Male	43.2 (16/37)	54.5 (18/33)	44.1 (15/34)	47.1 (49/104)

G1: 7-d progesterone-impregnated sponge + equine chorionic gonadotropin (n = 24); G2: 9-d progesterone-impregnated sponge + equine chorionic gonadotropin (n = 25); G3: 11-d progesterone-impregnated sponge + equine chorionic gonadotropin (n = 24)

dioestrus length. Synchronized oestrus occurs after the sponge is removed. In addition, eCG treatment increases reproductive efficiency in such protocols (Kuru et al. 2018a). In recent years, many studies have reported on the duration of the sponge in the vagina (Texeira et al. 2016; Kuru et al. 2017, 2018a, 2020). Shortening of the sponge treatment length had no negative effect on fertility (Kuru et al. 2018a). Short-term sponge treatment can provide better oestrus induction than long-term treatment (Sareminejad et al. 2014). In our study, we determined the effect of intravaginal progesterone-impregnated-sponge treatment (7, 9, or 11 d) shorter than the length of dioestrus (12 to 14 d) on reproductive efficiency in Romanov ewes during the non-breeding season. Literature searches did not reveal any similar comparative studies in this breed. Romanov ewes have high reproductive performance and multiple birth rates. The first ovulation time after birth is shorter in Romanov ewes than in many sheep breeds (Ricordeau et al. 1990). With this in mind, we initially used the ram effect, which is a non-hormonal method for oestrus synchronization. Oestrus synchronization was performed with progesterone-impregnated sponges and eCG in ewes that did not mate with 30 d of exposure to rams.

In sheep and goats, the progesterone-containing devices can perfectly synchronize oestrus during the non-breeding season; however, progesterone treatment should be combined with eCG injected at the end of the progesterone treatment or 1 to 2 d before. In our previous studies on different sheep breeds, we detected oestrus symptoms on average 31 to 45 h after sponge removal (Kuru et al. 2017, 2020). Oestrus onset time is typically later with short-term progesterone-based protocols compared to long-term (Özyurtlu et al. 2011; Sareminejad et al. 2014; Texeira et al. 2016). However, in some studies, no significant difference was found between the onset of oestrus with short-term or long-term progesterone treatments (Ataman et al. 2006; Husein et al. 2007; Amer and Hazzaa 2009). After 12-d flugestone acetate (FGA) and eCG treatment, oestrus started mostly between 24 and 36 h and the mean onset of oestrus was 30.3 h in Romanov x Pelibuey ewes (Macías-Cruz et al. 2013). In our study, the onset of oestrus was between 32.9 and 40.6 h, and oestrus started later with 7-d progesterone treatment compared to 9- or 11-d treatment. However, the oestrus onset time was significantly different in G1 and G3 ($P = 0.013$), and this result was consistent with our previous studies (Kuru et al. 2017, 2020). In the non-breeding season, the oestrus rate was determined as 95.5% to 100% in Romanov ewes after 14-d FGA and 250 to 300 IU eCG treatment (Cornu and Cognié 1985), and 100% after 14-d FGA and 140 to 280 IU eCG treatment in Romanov x Pelibuey ewes (Macías-Cruz et al. 2013). After 6-, 9-, or 12-d MAP + 300 IU eCG treatment, the oestrus rate in Santa Inês ewes was 72.7%, 72.7%, and 80%, respectively (Texeira et al. 2016). In our study, the rate of oestrus was 100%, 100%, and 95.8% in G1, G2, and G3, respectively. In Romanov ewes, 7-, 9-, or 11-d MAP + 350 IU eCG treatment provided excellent oestrus induction. Short-term (7-d) progesterone-impregnated sponge treatment may be preferred for oestrus induction in ewes that are not in heat after the ram effect. In our previous study, we determined that intravaginal progesterone-containing devices cause vaginitis and increase serum oxidative stress markers (Oral et al. 2015; Kuru et al. 2018b). Therefore, the stress created by the sponge can be reduced with a short-term use compared to a long-term use.

Almost all Romanov ewes mate during the year and are pregnant and have high reproductive performance. The mean conception rate was 93.8% in Romanov ewes (n = 5379) followed for 5 years (Đuričić et al. 2019). The conception rate was 89.5% and 90.7% in Romanov ewes after 14-d FGA and 300–350 IU eCG treatment, respectively during the non-breeding season. The conception rate was 90.9% after 14-d FGA and eCG treatment in Romanov ewes with a 90-d birth-mating interval (Cornu and Cognié 1985). In this study, the conception rate in G1, G2, and G3 was 79.2%, 72.0%, and 78.3%, respectively, and the short-term protocol had an effect similar to 9-d or 11-d progesterone

treatments. Similarly, the pregnancy rate was not significantly different between the groups ($P > 0.05$). Considering the pregnancy rate, we determined the short-term protocol (7-d) to be preferable in the non-breeding season to increase fertility in Romanov ewes. Supporting this view, the conception rate after 7- or 12-d MAP + eCG treatment during the non-breeding season in Akkaraman crossbreed ewes was 85.7% and 76.9%, respectively (Ataman et al. 2006).

In different studies, the lambing rate was 83% (14-d FGA without eCG) (Ortiz 1988), 80.7% to 82.6% (14-d FGA and 300 to 350 IU eCG), and 86.4% (14-d FGA and 400 to 450 IU eCG) (Cornu and Cognié 1985) in Romanov ewes. The lambing rate was 65% after 12-d FGA and 140 to 280 IU eCG treatment during the non-breeding season in Romanov x Pelibuey ewes (Macías-Cruz et al. 2013). In addition, embryonic deaths reportedly vary between 17.4% to 19.3% (Ricordeau et al. 1988) or 5% to 28% (Cornu and Cognié 1985) in Romanov ewes. In our study, the overall lambing rate was 80% and embryonic death was 20%. In Romanov ewes, the risk of embryonic death is higher in synchronization with progesterone and eCG during the non-breeding season. Embryonic deaths increase especially in oestrus induction during the early postpartum period and are higher in young ewes than mature ones. There is also a positive correlation between the ovulation rate and embryonic deaths (Cornu and Cognié 1985; Ricordeau et al. 1988). In our study, embryonic deaths directly decreased lambing rates. An injection of 350 IU eCG may have increased the ovulation rate. Factors such as the young age of the ewes and the induction of oestrus during the non-breeding season may have affected the embryonic/foetal death rate. Therefore, the dose of eCG can be reduced and ewes with a longer postpartum period can be selected for oestrus induction.

Fecundity was 1.95 in Romanov ewes in the breeding season (Đuričić et al. 2019) and 1.4 in Romanov x Pelibuey ewes after 12-d FGA and eCG treatment during the non-breeding season (Macías-Cruz et al. 2013). In our groups, G1, G2, and G3, fecundity was 1.5, 1.3, and 1.5, respectively. Numerically, the highest fecundity was at G1 and G3, but there was no significant difference between the groups. Fecundity may be slightly low due to oestrus induction during the non-breeding season. In our previous study, we determined that progesterone-containing devices increase inflammation markers due to vaginitis (Kuru et al. 2015). Therefore, if the short-term protocol (7-d progesterone) is preferred for oestrus induction in ewes, they are exposed to the sponge for less time, thus the severity of vaginitis may be reduced.

Large litter size and multiple births in Romanov ewes are generally due to a high ovulation rate. The ovulation rate is 2.74 at the age of 10 months, 4.25 in the adult, and does not much exceed 6. The mating period, age, and ovulation rate may affect the litter size but the eCG dose does not (Ricordeau et al. 1990). In various studies, the litter size in Romanov ewes varied between 1.61 and 3.11 (Ricordeau et al. 1988; Đuričić et al. 2019). The litter size varied between 2.00 and 2.74 in Romanov ewes after treatment with progesterone and eCG during the non-breeding season (in May) (Cornu and Cognié 1985; Ricordeau et al. 1990; Đuričić et al. 2019). Litter sizes in prolific ewe breeds after short-term or long-term progesterone treatment during the non-breeding season were 2.2 to 2.5 or 2.4, and there was no significant difference (Martinez-Ros et al. 2019). In our study, there was no significant difference between the groups (2.5, 2.2, and 2.4, respectively) and short-term progesterone treatment did not have a negative effect on litter size. Some breeders believe that short-term progesterone treatment cannot increase lamb yield during the non-breeding season. The lamb yield rate in G1 should eliminate such concerns.

The high ovulation rate directly affects the multiple birth rate in Romanov ewes. Some problems (high embryonic death, foetal losses, and lamb death) frequently occur in multiple births (especially triplets, quadruplets, and quintuplets). Despite this, Romanov ewes are more productive than many local breeds and provide more economic gain (Ricordeau

et al. 1990). The multiple birth rate was 87.3% in Segureña x Romanov ewes (Martinez-Ros et al. 2019) and 92.3% in Romanov x Pelibuey ewes (Macías-Cruz et al. 2013) after FGA and eCG treatment during the non-breeding season. After short-term (first group: PGF + 7-d FGA + eCG, second group: 7-d FGA + eCG + PGF) or long-term (14-d FGA + eCG) progesterone treatment during the non-breeding season, the rate of multiple births in prolific ewe breeds was 69.2% to 78.0% and 75.0%, respectively (Martinez-Ros et al. 2019). In our study, the rate of multiple births in G1, G2, and G3 was 86.7%, 80.0%, and 85.7%, respectively. Age, feeding, breeding season, and eCG injection are directly related to multiple births which may explain the difference in results between studies.

In conclusion, progesterone-impregnated sponge treatment for a shorter time than the length of dioestrus provided excellent oestrus induction in Romanov ewes without heat after ram effect during the non-breeding season. Even a short-term (7-d) progesterone treatment was similarly effective on reproductive indicators compared to other treatments (9- or 11-d). Thus, the severity of vaginitis caused by the progesterone-containing devices and the stress caused by the inserted sponge can be reduced with short-term use without affecting birth rates.

Conflict of Interest

The authors declare that there is no conflict of interest.

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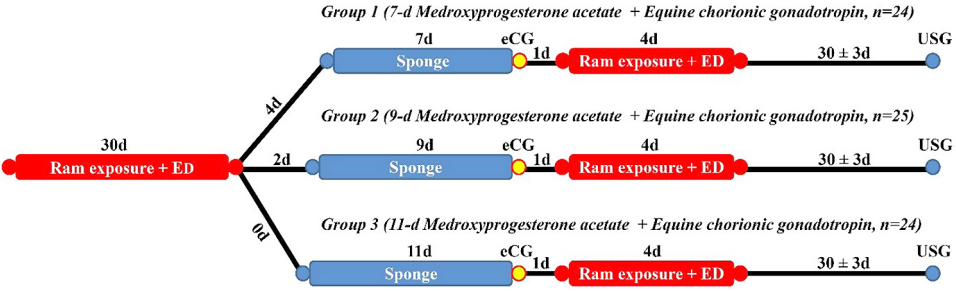

Fig 1. Schematic view of the treatments.

d – day; USG - transrectal ultrasonography; ED - oestrus detection. The ewes were exposed to the ram for 30 d and oestrus was followed, and the ewes without heat were divided into 3 groups and synchronization protocol treatment was performed. When ewes were exposed to the ram for 30 d, oestrus was followed every 12-h. Oestrus detection, which started a day after the sponge was removed, was performed for 4 d at 8-h intervals