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Effect of fluorogestone acetate and eCG on reproductive parameters in lactating Pırlak ewes

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Abstract: It was aimed to determine the effect of intravaginal sponges impregnated with different doses of fluorogestone acetate (FGA) on some reproductive parameters in 360 Pırlak ewes at 30–60 days postpartum. The positive control group (n = 130) was treated with 30 mg of FGA for 12 days. On the sponge removal day, 600 IU of equine chorionic gonadotropin was injected intramuscularly and rams were joined into flocks 36 h later. Halved intravaginal sponges containing FGA were applied in the experimental group (n = 100) with the same protocol as in the positive control group. The estrous behavior of ewes in the negative control group (n = 130) was followed by joining rams into the flock without any treatment protocol. There was no significant difference in estrus, conception, lambing, single and twining rates, or single and twining rates per lambing between groups. The second estrus rate was 59.5% and 78.3% in the positive control and experimental group, respectively. Total lambing rate following first and second estrus was higher in the experimental group than in the positive control. The ewes in the negative control group did not show estrous behavior during the first or second estrus of other groups. It was concluded that the insertion of halved intravaginal sponges impregnated with 30 mg of FGA could cause more economical estrus synchronization.

Key words: Halved sponge, fluorogestone acetate, anestrus, estrus synchronization, Pırlak

1. Introduction

Ewes give birth once per year due to a long anestrus period after lambing. Various hormones are administered in order to obtain lambs twice in 1 year or three times in 2 years for the market (1). Therefore, several protocols have been developed to induce out-of-season estrus to raise and provide the lambs for the market year-round. Intravaginal sponges containing progesterone are commonly used for induction of estrus synchronization in ewes that are cyclic and in seasonal anestrus (2). Indeed, the most frequent route of application of progestagens is intravaginal sponges. Fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) or a controlled internal drug-releasing device (CIDR) containing 0.3 g of native progesterone is impregnated into the sponges (3).

It is known that the administration of progestagen-impregnated sponges decreases luteinizing hormone (LH) secretion (4) and increases follicle turnover (5). Maintenance of a high level of progestagens followed by rapid withdrawal is necessary for acceptable fertility in synchronized estrus (6). Moreover, it is postulated that lower doses of progestagens are sufficient to obtain optimal fertility following synchronization with intravaginal sponges (7–9). It has been reported that reducing the dose of FGA from 40 to 20 mg does not significantly affect ovarian follicular dynamics or other aspects of ovarian function (3).

To the best of our knowledge, there is no report on the application of exogenous progesterone at various dose levels for estrus induction and synchronization in Pırlak ewes during lactating anestrus. The present study was therefore designed to examine whether reducing the dose of FGA-impregnated intravaginal sponges would give satisfying results or not for the induction of estrus and subsequent fertility in lactating Pırlak ewes.

2. Materials and methods

The study was approved by the local animal ethics committee of Afyon Kocatepe University (AKÜHADYEK 169-12) in accordance with ethical principles that have their origins in European Union Directive 2010/63/EU. The experiment was conducted in Afyonkarahisar Province (38°44′N, 30°34′E; altitude 1034 m) during the out-of-breeding season. A total of 360 multiparous Pırlak

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ewes at 30–60 days in milk, aged between 2 and 5 years and weighing 50–60 kg, were used in the study. Animals bred in rural conditions were chosen from among ewes that did not show any clinical health problems in their previous parturition following pregnancy obtained by natural mating in the breeding season (July to August).

The animals were divided into experimental (n = 100), positive control (n = 130), and negative control (n = 130) groups. Animals in all groups were exposed to pasture feeding. The study was initiated in February for the positive and negative control groups, whereas data of the experimental group were obtained in March. The first day of treatments was accepted as day 0 (D 0) of the study. The positive control group (n = 130) was treated with 30 mg of FGA (intravaginal, Ova-Gest, Aydın İlaç, Turkey) for 12 days. On the day of sponge removal (D 12), equine chorionic gonadotropin (eCG) (IM, 600 IU, Pregnecol, Aydın İlaç) was injected and rams were joined into flocks 36 h later (D 14). The experimental group (n = 100) was treated for 12 days with halved intravaginal sponges (approximately 15 mg of FGA), which were cut with scissors from the middle point of the sponge (Figure). The remaining protocol was the same as that used in the positive control group. Accordingly, eCG was injected on the sponge removal day (D 12) and rams were joined to flocks 36 h later (D 14). At least one ram for ten ewes was allowed to stay for 36 h in flocks in both the positive control and experimental groups. During the joining of rams into the flocks, every ten ewes were naturally mated by one ram. When the ram mounted, it was accepted that standing heat was observed. After controlled mating, the ewes were then transferred into another paddock in which at least two rams were present. When the mounting of rams was not allowed by ewes, it was decided that standing heat had finished. The negative control group (n = 130) did not receive any treatment; only the estrous behavior was followed by the joining of rams into the flock and recorded throughout the study. Transrectal ultrasonography (USG) was performed (7.5 MHz linear array transducer, WED-3000 V, Mindray, China) for diagnosing the pregnancies 30 days after mating and the data were recorded. Ewes were observed until delivery and fertility parameters such as estrus rate (number of ewes showing standing heat/number of ewes in group); conception rate (number of pregnant ewes/number of ewes in group); lambing rate (number of ewes lambing/number of ewes in group); singleton, twinning, and triplet rates (number of ewes lambing singleton, twin, or triplet lamb(s)/number of ewes in groups); singleton, twinning, and triplet rates per pregnancy (number of ewes lambing singleton, twin, or triplet lamb(s)/number of ewes lambing in group); and fecundity rate (number of lambs born/number of ewes lambing in group) were calculated.

The ewes displaying standing heat again following the joining of rams into the flocks in the positive control and experimental groups were mated and the data were recorded. Accordingly, the second estrus rate (number of ewes showing standing heat/number of ewes in group); overall lambing rate (number of ewes lambing after first and second mating/number of all ewes in group); overall singleton, twinning, and triplet rate (number of ewes lambing singleton, twin, or triplet lamb(s) after first and second mating/number of all ewes in groups); and overall fecundity rate (number of lambs born after first and second mating/number of all ewes lambing in group) were calculated. Standing heat detection in the negative control group was performed by the observation of mounting of rams on the ewes.

2.1. Statistical analysis

The chi-square test was used for analysis of estrus, conception, and singleton, twinning, triplet, and overall pregnancy rates, whereas fecundity rates were compared by independent sample t-test. SPSS 13.0 was used for all analyses. Fecundity rate was given as mean ± SEM and the data were considered to be significant at $P < 0.05$.

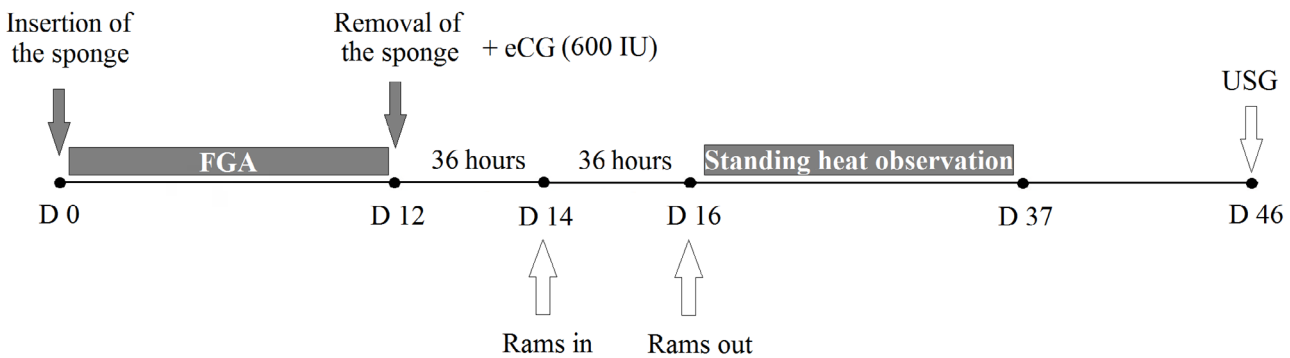

Figure. The days of estrus synchronization protocol, observation of second estrus, and ultrasonography in the positive control and experimental groups. Positive control group: 30 mg of fluorogestone acetate (FGA) (intravaginal), 600 IU of equine chorionic gonadotropin (eCG) (intramuscular); experimental group; 15 mg of FGA (intravaginal), 600 IU of eCG (intramuscular). D: day, USG: ultrasonography.

3. Results

It was observed that mucopurulent vaginal discharge was evident on the applicator on the first day of treatment in two ewes of the positive control group, whereas two sponges dropped on day 0 and day 1 of treatment. Moreover, sponges in the experimental group dropped on day 0 in six ewes and on day 1 in two ewes, while one ewe displayed continuous standing heat behavior following the joining of the ram into the flocks. It was seen that intravaginal sponges were retained at 98% (126/128) in the positive control group and at 92% (91/99) in the experimental group throughout 12 days. The ewes that dropped the sponges in the positive control and experimental groups did not show estrus following eCG injection and the joining of the ram. Therefore, the data obtained from four ewes in the positive control and nine ewes in the experimental group were discarded from the study and 126 ewes in the positive control and 91 ewes in the experimental group were evaluated.

The reproductive parameters obtained from the positive control and experimental groups are shown in Table 1. Estrus, conception, and lambing rates did not show any significant difference between groups. Estrus rate observed 36 h after removal of vaginal sponges and treatment with eCG in the positive control and experimental groups was 96.8% and 97.8%, respectively. It was seen that both singleton and twinning rates and those rates per pregnancy did not differ between groups. Triplet lambing was not seen in the positive control group, whereas three ewes in the experimental group had triplet lambing and this difference was significant ($P < 0.05$). However, triplet lambing rate per pregnancy did not show a significant difference statistically. Fecundity rates in the positive control and experimental groups were 1.36 ± 0.06 and 1.57 ± 0.09, respectively, but there was no significant difference between groups.

The reproductive parameters obtained from the positive control and experimental groups after the second standing heat following the joining of the ram and mating are given in Table 2. In the present study, 44 ewes in the positive control group and 36 ewes in the experimental group displayed standing heat behavior and mated 14–21 days after removal of the intravaginal sponge. The estrus rate was higher ($P < 0.05$) in the experimental group (78.3%) than in the positive control group (59.5%). Additionally, two of 30 ewes showing no second standing heat in the positive control group (6.7%) and 10 (100%) ewes in the experimental group displayed estrus 14–21 days later following second estrus mating ($P < 0.001$). The lambing rates were evaluated based on the data obtained after all standing heat behavior and mating. It was determined that the overall lambing rate was higher ($P < 0.05$) in the experimental group (89%) than the positive control group (76.2%). Overall singleton and twinning rates did not differ between groups, whereas overall triplet rate was higher in the experimental group ($P < 0.05$). Moreover, the overall

Table 1. Reproductive parameters obtained following first standing heat after treatment with 30 mg and 15 mg of fluorogestone acetate (FGA) in the positive control and experimental groups.

Parameters	Groups	
	Positive control (n = 126)	Experimental (n = 91)
Estrus rate (%)	96.8 (122/126)	97.8 (89/91)
Conception rate (USG) (%)	41.3 (52/126)	49.5 (45/91)
Lambing rate (%)	41.3 (52/126)	49.5 (45/91)
Singleton rate (%)	26.2 (33/126)	24.2 (22/91)
Singleton rate per pregnancy (%)	63.5 (33/52)	48.9 (22/45)
Twinning rate (%)	15.1 (19/126)	22 (20/91)
Twinning rate per pregnancy (%)	36.5 (19/52)	44.4 (20/45)
Triplet rate (%) *	0 (0/126)	3.3 (3/91)
Triplet rate per pregnancy (%)	0 (0/52)	6.7 (3/45)
Fecundity rate	1.36 ± 0.06	1.57 ± 0.09

* There is a significant difference between groups ($P < 0.05$).

Table 2. Second and third estrus rates and reproductive parameters obtained following all standing heat behavior after treatment with 30 mg and 15 mg of fluorogestone acetate (FGA) in the positive control and experimental groups.

Parameters	Groups	
	Positive control (n = 91)	Experimental (n = 126)
Second estrus rate (%) *	59.5 (44/74)	78.3 (36/46)
Third estrus rate (%) *	6.7 (2/30)	100 (10/10)
Overall lambing rate (%) *	76.2 (96/126)	89 (81/91)
Overall singleton rate (%)	61.1 (77/126)	63.7 (58/91)
Overall twinning rate (%)	15.1 (19/126)	22 (20/91)
Overall triplet rate (%) *	0 (0/126)	3.3 (3/91)
Overall fecundity rate	1.19 ± 0.04	1.32 ± 0.06

* There is significant difference between groups ($P < 0.05$).

fecundity rate in the positive control and experimental group was 1.19 ± 0.04 and 1.32 ± 0.06, respectively, but the difference was not significant. It was determined that the ewes in the negative control group (n = 130) started to allow the mounting of rams in April and the estrus rate was 10.7% (14/130) at the end of April.

4. Discussion

It has been stated that the efficiency of intravaginal sponges is related to the retention time of sponges in the vagina; hence, commercially available intravaginal sponges have high (>90%) retention rates (2). In the present study, it was found that the retention rate of intravaginal sponges applied for 12 days in the positive control and experimental group was 98% (126/128) and 92% (91/99), respectively. Therefore, it is concluded that it is possible to use halved sponges successfully, since they have retention rates similar to those of whole sponges.

The observation of no standing heat in ewes in the positive control and experimental groups after the dropping of the sponges on the first and second days of treatment suggested that normal luteal function and follicular development were related to the duration time of the intravaginal sponge, in accordance with other reports (10,11).

Although varied results have been obtained from estrus synchronization studies (12–14) during different breeding seasons, it is obvious that success rates are generally satisfying. Uçar et al. (12) reported that the estrus rate in Akkaraman, Dağlıç, İvesi, and Sakız ewes during the breeding season was 100% following FGA (40 mg) treatment for 14 days and eCG (500–600 IU). Treatment of ewes during the transition period or out of season had similar success rates. Accordingly, İvesi ewes had an estrus rate of 90% following treatment with 30 mg of FGA during the transition period (13), whereas it was reported that 12 days of treatment with 40 mg of FGA and 500 IU of eCG caused 100% estrus rate during anestrus (14). In the present study, the estrus rate observed 36 h after removal of vaginal sponges and treatment with eCG in the positive control and experimental groups was 96.8% and 97.8%, respectively. This result was consistent with the above-mentioned studies. Moreover, this finding also supports other reports (7–9), which mentioned that halved sponges had similar estrus rates as compared to whole sponges.

In the protocols that have been used for the combination of varied dosages of FGA and eCG during the breeding season, the interval between the observation of standing heat and eCG administration varies between 34 and 49 h (15–17), while the interval is between 33 and 43 h out of season (14). The interval between the observation of standing heat and removal of the vaginal sponge (MAP, 60 mg) or eCG (400 IU) treatment with a vaginal sponge (MAP, 60 mg) in ewes in the southern hemisphere during breeding and out of season varies between 30 and 37 h (8). The standing heat behavior takes at least 19 h (18) and at most 34 h (17). The present study revealed that standing heat ended 24 h after the very first joining of the ram and halved sponges had similar effects as whole sponge.

In the present study, conception and lambing rates were 41.3% in the positive control group, whereas those rates were 49.5% in the experimental group and the difference was not significant. However, these findings were higher at 67.4%–91.7% (14,19) or lower at 35% (13)

in some other reports. It is suggested that the weaning age (4.16 months on average) of Pırlak ewes housed in rural areas (20) may be the main factor causing discrepancies with other reports. It has been reported that increasing prolactin concentration due to stimulations during the lactation period has a suppressive effect on gonadotropins and therefore breeding activity is inhibited by the negative effect of pulsatile LH release and the first LH peak in suckling or lactating ewes (21). Another study (22) revealed that lactating ewes that were not suckling their lambs and were at least 90 days in milk had high lambing rates (85.5%). It is suggested that the lower lambing rates obtained in this study may be due to the lactation status or the continuation of suckling. Moreover, it was seen that halved sponge treatment had similar pregnancy rates as compared to whole sponges and it may be applied successfully in the field.

In the present study, singleton and twinning lambing rates and those rates per pregnancy as well as fecundity rates in the positive and experimental groups did not show any significant difference. It has been reported that fertility parameters are lower in ewes treated with only FGA than those treated with FGA and eCG (23,24). Moreover, the dosage (25) or treatment procedure (subcutaneous or intramuscular) (23) of eCG increases the fertility parameters. No differences in lambing and fecundity rates obtained in this study may be due to the usage of eCG in the same treatment model (600 IU, IM) in both groups. However, the ewes showed triplet lambing in the experimental group ($P < 0.05$), but that is probably due to the individual discrepancies of ewes.

In the present study, the second estrus rate was higher in the experimental group (78.3%) than the positive control group (59.5%) ($P < 0.05$). Therefore, the overall lambing rate was also higher ($P < 0.05$) in the experimental group (89% vs. 76.2%). The breeding season is around the end of summer or beginning of autumn in Turkey (23). Ataman et al. (10) reported that the end of the nonbreeding season or the beginning of the transition period varies between 1 and 31 May. However, it is suggested that estrous activity of ewes in Afyonkarahisar may be observed around the last week of April, since some ewes in the negative control group (10.7% (14/130)) displayed estrous activity. Moreover, higher second estrus and overall lambing rates in the experimental group may be due to ewes having a short anestrus period and the beginning of the transition period at April.

It has been reported that lambing, singleton lambing, twin lambing, multiple lambing, and fecundity rates are 79.25%, 73.16%, 24.62%, 26.84%, and 1.26 in Pırlak ewes during the breeding season without any synchronization protocol (20). It is suggested that the fertility parameters obtained in this study during the nonbreeding season are satisfying results as compared to the breeding season.

The fertility parameters of Pırlak ewes housed in a rural area in Afyonkarahisar, which were first reported by this study, provide practical information that would be helpful before using synchronization protocols. It is concluded that treatment of ewes with halved intravaginal sponges containing 30 mg of FGA for 12 days and injection of 600 IU of eCG can be successfully used in the field for estrus synchronization. Besides the ram effect, spring pastures initiate estrous activity and this season may be the transition period for Pırlak ewes. Therefore, it is suggested that treatment of ewes by intravaginal sponge and eCG out of season in rural areas in the pasture period and the separation of lambs from the flocks should be taken into account to obtain high estrus synchronization and lambing rates.

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