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Effects of different intravaginal progesterone releasing devices on estrous synchronization and LH surge in fat-tailed ewes during non-breeding season*)

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Summary

The aims of this study were to compare two methods of estrus synchronization and to evaluate the effectiveness of the PMSG treatment combined with P4 application. Fifty non-lactating seasonal anestrus fat-tailed ewes were randomly assigned into five groups. The controlled internal drug release devices (CIDR) were applied during day 14 in group I and in group II. Progesterone impregnated sponges were applied during day 14 in group III and in group IV. And then 500 IU PMSG was injected in group I and III i.m. intravaginal devices removed. Ewes in group V served as controls. There was no difference between the groups in the peak value of LH and LH surge. Although LH surge was seen in the control group's 5 sheep, none of the control ewes expressed estrus. Different progestagen treatments have no different results when they are evaluated in terms of the success of the estrus synchronization. PMSG application, after P4 treatment, increased the success of the synchronization.

Keywords: ewe, synchronization

Ewes are polyestric animals depending on seasons in terms of features of breeding. Applications of exogenous hormones for increased reproductive performance in domestic ewes are usually focused on estrous synchronization (14, 30). Thus, standing oestrus and breeding both occur within a short time period in synchronized ewes (12). Oestrus synchronization in goats and sheep is achieved by controlling the luteal phase of the estrus cycle, either by providing exogenous progesterone (P4) or by inducing premature luteolyzis (2, 29). Sponges that have been impregnated with either native P4 or an analogue and then inserted into the vagina for a given period of time have also been used. The controlled internal drug release (CIDR) devices consist of a nylon core surrounded by a silicone elastomer that is impregnated with P4, and were developed alternatively (14, 29).

Progesterone was known to suppress pulsatile LH release; frequency of LH pulses is thus low before regression of the corpus luteum. As P4 declines with luteal demise, this inhibition is removed and LH pulse frequency increases. The resulting rise in circulating LH provides a stimulus for the follicular phase increase in estradiol secretion from ovarian follicles. Estradiol, in turn, elicits the preovulatory LH surge that causes ovulation, and the formation of a corpus luteum (3). Therefore, P4 and LH measurements can be used as indicators of luteal activity and ovulation.

Pregnant mare's serum gonadotrophin (PMSG) is used for induced ovulation and oestrus in out of breeding season, and is used for more effective synchronization in breeding season (28). In addition, with an increased dose PMSG increases ovulation rate and twinning (25).

The aims of this study were to compare two methods of oestrus synchronization (CIDR devices and P4 sponges) for efficacy of synchronization and timing

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of endocrine events, and to evaluate the effectiveness of the PMSG treatment combined with P4 application.

Material and methods

This experiment was conducted at the University of Kafkas, Faculty of Veterinary Medicine, between April 2005 and May 2005. The Animal Handling and Ethical Board at the Faculty of Veterinary Medicine approved all experimental designs and applications. Fifty non-lactating Tushin breed (2-5-years-old and 39-46 kg body weight (BW)) ewes were housed in a flock barn accessed to a feeding lot and were fed with grass hay on an ad libitum basis. Grass hay (93.26% dry matter, 8.84% of CP, total fiber 31.1%, 2000 kcal/kg ME as fed basis) was served two times a day (09:00 and 15:00 h). Ewes were randomly assigned into five groups (average BW 41.5 kg in per group). All ewes were in the anoestrus season.

The controlled internal drug release devices (Eazi-Breed CIDR, 300 mg progesterone, Pharmacia Animal Health, Australia) were applied during fourteen days in group I (n = 10) and in Group II (n = 10) and then 500 IU PMSG (Choronogest PMSG, Intervet, Türkiye) was intramuscularly injected in group I after the removal of intravaginal devices. Progesterone impregnated sponges (Choronogest sponge, 40 mg Chronolone, Intervet, Türkiye) were applied during fourteen days in group III (n = 10) and in group IV (n = 10), and then 500 IU PMSG was injected intramuscularly in group III at sponge removal. Ewes in group V served as a control (n = 10) and no administration was applied.

Jugular blood samples were collected for P4 determination every day into anticoagulant tubes (4 mL), starting from the day before vaginal devices/sponges application until 30 days after devices/sponges removal. A more intensive sampling was conducted immediately after PMSG treatment and up to 96 h thereafter, at 2 h intervals. Blood samples were centrifuged (Hettich, Universal 3R, 3000 per/min., 10 min.); plasmas were then separated and stored at –20°C until the analysis. Plasma samples were analyzed using a double-antibody EIA technique for determination of P_4 (18) and LH (17). The range of standards for P_4 and LH was from 0.25 to 16.0 ng/mL and 0.8 to 50 ng/mL, respectively. Intra- and interassay coefficients of variations were 8% and 9% for P_4, 11% and 13% for LH, respectively.

Oestrus observations with the aid of teaser rams were carried out concurrently with the blood sampling at 2 h intervals. Oestrus behavior toward rams was recorded until the rams were refused.

Mean and standard error of mean (SEM) were calculated by using the Minitab statistical package (Version 11.2, Minitab Inc., State College, PA, USA). Parameters in groups were compared with sample t test.

Results and discussion

Following P_4 treatment, all PMSG applied ewes showed oestrus. These animals expressed oestrus earlier and longer than those not applied PMSG (fig. 1). Simultaneously, there is a statistical difference of the onset of LH surge, time of maximum level of LH between the PMSG applied and non-applied groups. While there was a difference among CIDR applied groups, PMSG used and non-used groups, in the manner of duration of LH surge. However, there was no difference in sponge applied groups. Moreover, there was no difference between the control group and the other groups for duration of LH surge (tab. 1).

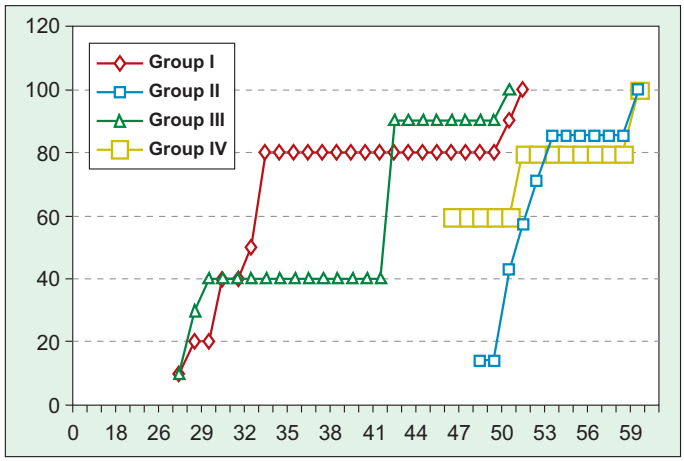

Fig. 1. Distribution of ewes express estrus depicted cumulative percent among treatment groups

Tab. 1. Results acquired in the research

Parameters	Group I (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	Group V (n = 10)
Number of ewes express estrus	10^{a} (100%)	7^{ad} (70%)	10^{a} (100%)	5^{bd} (50%)	0^{c} (0%)
Interval from Sponge/CIDR removal to estrus (h)	34.5 ± 2.9^{a}	51.9 ± 1.3^{b}	37.2 ± 2.0^{a}	49.6 ± 2.0^{b}	0
Duration of estrus (h)	42.2 ± 5.5^{a}	24.0 ± 1.5^{b}	32.5 ± 2.3^{a}	23.8 ± 1.5^{b}	0
Onset of LH surge (h)	36.2 ± 3.8^{a}	47.7 ± 1.4^{b}	33.0 ± 1.4^{a}	48.0 ± 3.5^{b}	65.5 ± 10.4^{ab}
Time of maximum level of LH	40.0 ± 4.0^{a}	52.3 ± 1.7^{b}	36.6 ± 1.5^{a}	52.0 ± 3.5^{b}	70.0 ± 10.7^{ab}
Duration of LH surge	9.2 ± 0.6^{a}	11.3 ± 0.4^{b}	9.6 ± 0.4^{ac}	10.5 ± 0.5^{abc}	11.0 ± 0.6^{abc}
Peak value of LH (ng/mL)	20.4 ± 3.3	17.7 ± 4.1	21.0 ± 2.1	23.6 ± 3.1	23.6 ± 4.0
Area under curve (AUC) (ng/mL*h)	83.6 ± 9.7^{a}	100.8 ± 21.0^{ad}	89.4 ± 8.8^{ac}	93.0 ± 14.0^{ad}	126.2 ± 10.7^{bd}
P4 levels (ng/mL), prior to devices/sponge application	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.04	0.5 ± 0.1
P4 levels (ng/mL) (during devices/sponge application)	4.1 ± 0.2^{a}	4.7 ± 0.2^{b}	0.7 ± 0.04^{c}	0.6 ± 0.04^{c}	0.8 ± 0.05^{d}

Explanations: a, b, c, d – supercripts within the same row indicates significance ($P < 0.05$)

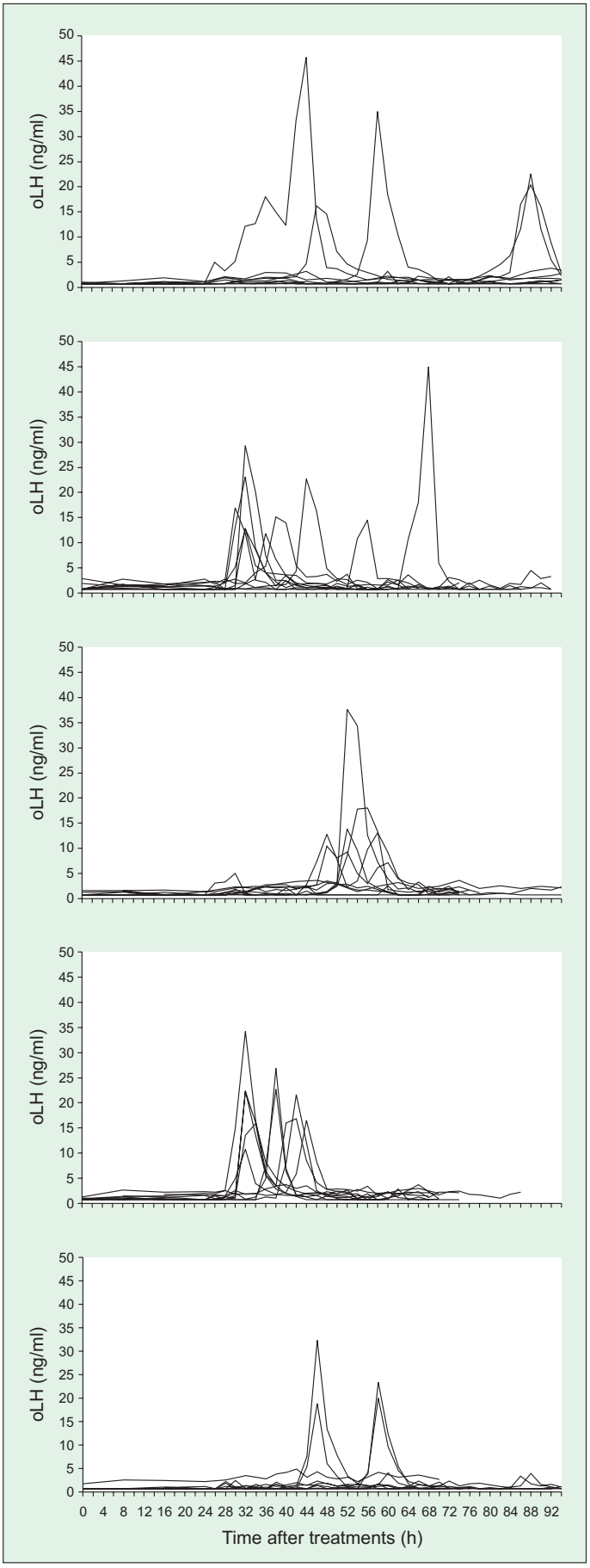

Fig. 2. The LH concentrations of individual fat-tailed ewes after estrus synchronization (CIDR+PMSG, CIDR, Sponge+PMSG, sponge and control group; respectively)

There was no difference between the groups in the peak value of LH (fig. 2). The difference was determined between the PMSG used groups and control group in the point of AUC. Although LH surge was observed within the control group in 5 sheep, none of the control ewes expressed oestrus. P_4 level was similar in all groups prior to intravaginal sponge and silicon application; however, P_4 levels were higher in CIDR applied groups during the treatment.

Accuracy of synchrony of the ewes in the present study was similar to that reported by others. Stenbak et al. (23) observed that hormonally induced ewes usually exhibit oestrus at 24-48 h after the removal of progestagen implants or pessaries. When the P_4 treatment finished, Greyling and Brink (10) determined oestrus about 31 h later, Van Cleff et al. (27) reported oestrus 36 h later, and Godfrey et al. (9), reported oestrus 34-40 h later. In the present study, oestrus was determined 34,5 ± 2,9 – 37,2 ± 2 h later in ewes treated with PMSG, and it was determined 49,6 ± 2 – 51,9 ± 1,3 h later in ewes not treated with PMSG. The average intervals between withdrawal of exogenous P_4, the onset of oestrus, the duration of oestrus, for ewes treated with P4 and progestagens are within the range reported in previous studies (10, 15, 27).

Although progesteron pessaries reduced the number of growing follicles; during sponge insertion the ovulation rate is significantly reduced. However PMSG treatment restores the ovulation rate by reducing the atresia rate of pre-ovulatory follicles. The PMSG treatment supported the growth of small follicles; i.e number of small follicles is increased and a significant increase is observed in oestrous and ovulation rate (4). In the current study, all the PMSG applied and some of those not applied PMSG sheep expressed oestrus. This result reveals that PMSG administration causes oestrus behavior. Furthermore, earlier onset of oestrus and longer oestrus duration in PMSG treated ewes leads to the assumption that PMSG trial. PMSG treatment regulates follicular development and increases oestrus rate. Romano et al. (20) reported that when injected immediately after the removal of the progestagen sponge, PMSG reduces the interval from sponge removal to oestrus.

Plasma P_4 values reach peak values of 2.1 ng/mL within 24 h following CIDR insertion, and stay at relatively stable levels between d 1 and 13 (1.9 ng/mL) (28). In the current study, plasma P_4 level was 0.3-0.6 ng/mL prior to CIDR insertion. In group I and II, the ewes that had the CIDR device applied, P_4 level was 4.1 ± 0.2 ng/mL and 4.7 ± 0.2 ng/mL, respectively. There was no important difference in P_4 level in the sponge applied groups. The lower P_4 level in these groups could be due to the method that cannot measure the P_4 inside the sponge.

In ewes, the „ram effect" has been extensively used to induce oestrus out of season (26). This effect is mediated through changes in pulsatile GnRH release

from the hypothalamus selectively increasing tonic LH (29). The introduced rams induce a rapid increase in LH pulse frequency. This is followed by an LH surge similar to that observed during the follicular phase of the oestrous cycle (26). Although ewes in control groups did not express oestrus symptoms, LH surge was noted. Godfrey et al. (8) stated that when the ewes in anestrus season kept together with rams oestrus may be induced. Nevertheless oestrus symptoms become indefinite probably due to premature lysis of corpora lutea.

In this study, the interval between time of pessaries removal and both the time of onset and reaching to maximum level of LH surge were shorter in Sponge/CIDR+PMSG groups than sponge and CIDR groups. These reductions, however, have been shown in earlier studies by other authors (1, 19). These observations may be the result of an increasing estrogen level caused by PMSG action on the follicles, since it had been reported that PMSG might increase not only the number of follicles, but also the growth rate of the large follicles (21). It has also been noted that oestradiol is derived almost entirely from developing ovulatory follicles (5), and levels of circulating oestradiol increase follicles size dependant manner (6, 7, 22). Results of studies by Moenter et al. (16) have clearly demonstrated that regardless of the season, a rise in estradiol concentration during the late follicular phase levels acts centrally upon the GnRH neurosecretory system to initiate a large and abrupt release of GnRH coincident with the onset of the preovulatory LH surge.

There was no difference ($P > 0.05$) among treatment groups in the peak value of LH surge in the current study; our values, however, were lower than that reported by Kaya et al. (13), whose study was conducted during the breeding season with the same breed. This difference may be a due to seasonal effects, as it has been reported by Thimonier (24) that LH concentration in the pituitary is reduced to 50% during the non-breeding season. There were significant differences between PMSG administrated ewes and the control group for AUC. Peak value of LH was similar in all groups, but durations of LH surges were shorter in PMSG administrated ewes compare to others. This difference for AUC may be due to its shortness.

In conclusion, no differences were found following treatments for the success of oestrous synchronization. However, PMSG application, after P4 treatment, increased the success of the synchronization rate. Moreover, keeping ewes together with rams could increase LH level even in the anestrus season.

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