Received: 11 October 2022, Accepted: 07 November 2022

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 1, 88-93

Influence of Long and Short-term Progesterone Administration on Estrous Synchronization and Reproductive Performance in Ewes During May Season

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Abstract

Estrous synchronization is a promising management device to get better reproductive proficiency, particularly in ruminants. However, limited information is available about the influence of long and short-term progesterone administration on estrous synchronization and reproductive performance in ewes during May season, particularly in Egypt. In this study, a total of 144 ewes have been utilized for estrous synchronization and reproductive performance in ewes during May season. Ewes were separated into six groups with different treatments as follows: G1, control (natural mating), G2, ewes were treated with 20 mg Cronolone vaginal impregnated sponges (Flurgestone acetate, FGA). The sponge was remained intravaginal for 11 days. Each ewe was intramuscularly (i.m.) injected with 500 international unit (IU) Pregnant mare serum gonadotrophin (PMSG) on the day of sponge withdrawal (day 11), G3, ewes were treated with intra-vaginal sponges. The sponge was remained intra-vaginal for 11 days, on day 10, each ewe was injected with 25 mg ultra-short progesterone (P4) (1 ml Lutone). Each ewe was injected i.m. with PMSG (500 IU) on day 11. G4 ewes were treated with intra-vaginal sponges. The sponge was remained for 11 days. Teaser ram was introduced to treated ewes after sponge withdrawal. The 5th group (G5), ewes were treated with intra-vaginal sponges. The sponge was remained intravaginal for 11 days. On day 10, each ewe was injected with 25 mg P4 (1 ml Lutone). Teaser ram was introduced to treated ewes after sponge withdrawal. The 6th group (G6), the same treatment of G2, but the sponge was inserted intra-vaginal only for 6 days. Each ewe was i.m. injected with 500 IU pregnant mare's serum gonadotropin (PMSG) on day 6. Synchronization was better in G2, G3 and G6, respectively and all short-term group animals came into estrus. In addition, onset of estrus and its duration differs significantly among groups. Reproductive performance also showed a significant difference among groups and the short-term group has the highest fertility rate. Given the above information, our data concluded that progestagens synchronized estrus (long term or short term) and administration of PMSG (500 IU) at sponge withdrawal improves the reproductive efficiency of artificially inseminated crossbred ewes.

KEYWORDS
Estrous synchronization, Ewes, Non-breeding, May season, Short-term PMSG, Sponge

INTRODUCTION

Estrous synchronization is considered as a promising managemental tool which has been used effectively to increase reproductive performance, especially in ruminants (Kusina *et al.*, 2000; Safdarian and Hashemi, 2006). In sheep, conventional artificial insemination (AI) protocols necessitate regular estrus detection using teasers, which is extremely labor-intensive (Miranda *et al.*, 2017). Fixed timed artificial insemination (FTAI) saves time and effort in artificial insemination systems but requires accurate synchronization of ovulation (Vilariño *et al.*, 2017). Nutritional and/or hormonal therapies may promote sheep production processes, leading to increased estrus responses and pregnancy rates (Kridli *et al.*, 2003). Intravaginal instruments impregnated with progesterone or synthetic progestagen like fluorogestone acetate (FGA) or medroxy-progesterone acetate are used, which is considered the most common method of estrous synchronization in ewes (Fukui *et al.*, 1999; Karaca *et al.*, 2009) and used during breeding and non-breeding season (Romano, 2004). Estrus synchronization is effectively obtained if pregnant mare serum gonadotrophin (PMSG) and P4 impregnated intravaginal devices are used together (Romano, 2004). Treatment with PMSG shortens the period between the onset of estrus and ovulation (Dogan and Nur, 2006). In ewes, a single dose of PMSG can activate the follicular growth and increase the ovulation rate (Koyuncu and Ozis, 2010a). To obtain higher breeding rate during the non-breeding season, anestrum ewes should be isolated with male before the beginning of the regular breeding season. One of the most effective processes which stimulate ovulation is to introduce rams to ewes, that called the male effect or ram effect (Jordan, 2005), this will cause promotion of breeding and some cycle synchronization during anestrus between the flock's ewes (Chanvallon *et al.*, 2008). The complete isolation of the ewes from rams for a period before introduction is done as a familiar practice to obtain reproductive response (Jordan, 2005). LH releasing (short-term response) is induced when a ram is introduced into a flock, ac-

ISSN: 2090-6277/2090-6269/

companied by ovulation (long-term response). Within minutes, a pulse of LH can be noticed, while FSH or prolactin secretion does not alter as quickly (Poindron *et al*., 1980; Martin *et al*., 1983). If contact with males is maintained, ewes undergo a preovulatory LH influx that lasts about 36 hours (Oldham *et al*., 1978). At the same time, the production of FSH rises. (Martin *et al*., 1983). Ovulation occurs 48 hours after the male introduction and is referred to as "silent" since it is not accompanied by estrous activity (Oldham *et al*., 1978). Reviewing the available literature, little information is available about the potential influence of long and short-term progesterone administration on estrous synchronization and reproductive performance in ewes during May season. Clearly, the main purpose of this study was undertaken to acknowledge how certain hormonal treatments affected ewes' reproductive output during the non-breeding season (May).

MATERIALS AND METHODS

Ethical approval

The ethical approval of the present study was obtained from a guidance of the Research, Publication, and Ethics Committee of the Faculty of Agriculture, Mansoura University, Egypt, and the institutional Review approval Board Number is R/22.

Study area

The study was conducted at the Sakha Animal Production Research Station, Kafr Elshiekh, Egypt, during May season 2020, which is part of the Animal Production Research Institute, in collaboration with the Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt.

Animals and experimental design

The study was performed on 144 crossbred ewes breed (1/2 Rahmani x 1/2 Finish Landrace) with body weight (BW) of 45-50 kg and ages of 3-4 years. During treatment, ewes were placed in semi open sheds and were fed on concentrate feed and roughages in accordance with NRC guidelines (Council, 2000). The animals were divided into six treated groups according to age, BW and physiological status (24 ewes in each) as follows: The 1st group or control one (G1); ewes that were in estrus and able to mate naturally (No hormonal treatment). The 2nd group (G2); ewes were treated with 20 mg Cronolone vaginal impregnated sponges (Flurgestone acetate, FGA; Intervet International B.V. Boxmeer-Holland). This sponge was inserted intravaginally on day 0, and remained for 11 days. Each ewe was injected i.m. with PMSG (500 IU) on the day of sponge removal (day 11). The 3rd group (G3); ewes were treated with intravaginal sponges that was inserted on day 0, and remained intra-vaginal for 11 days. About 24 h before sponge withdrawal (day 10), each ewe was injected with 25 mg P4 (1 ml Lutone). Each ewe was injected i.m. with PMSG (500 IU) on day of sponge removal (day 11). The 4th group (G4); ewes were treated with intra-vaginal sponges, which was inserted on day 0 and remained intravaginal for 11 days. Teaser ram was introduced to treated ewes after sponge removal. The 5th group (G5); ewes were treated with intra-vaginal impregnated sponges. The sponge was inserted on day 0 and remained intravaginal for 11 days. About 24 h before sponge removal (day 10), each ewe was injected with 25 mg P4 (1 ml Lutone). Teaser ram was introduced to treated ewes after sponge withdrawal. The 6th group (G6); the same treatment of G2 in this experiment, but the sponge was inserted intra-vaginal only for 6 days. Each ewe was i.m. injected with PMSG (500 IU) on the day of sponge removal (day 6). The groups from 2 to 5 were subjected to AI at 48-56 h with fresh diluted semen after day 11, while group 6 was subjected to AI at 48-56 h with fresh diluted semen after day 6. The protocols for all groups were presented in Table 1.

Semen dilution and artificial insemination (AI)

The Ewes in all treated groups responded to estrus after AI application with fresh extended semen. Tris-yolk extender was used for the extension of fresh semen at a rate 1:4 to obtain sperm concentration about $300x10^6$ sperm/ml. Before semen dilution, the diluent was slowly shacked and heated to 37 °C in a water bath. The volume of raw semen was determined, and a liquor of raw semen was taken for sperm motility assessment (Bane, 1952). For AI application, a fine inseminating pipette with simple blunt bent end with using of a vaginal speculum. Semen was deposited into the cervix of ewe as far as possible (about 1 cm).

Blood sampling

In G1: blood samples were taken twice weekly within the start up to the end day of the experiment. However, in G2, G3, G4, G5 and G6: samples were taken on days 0 (insert sponge), 6, 9, 10, 11, 13 (AI), 14 and 43 days. Blood samples were collected into tube containing heparin. The blood samples were centrifuged to separate plasma at 2500 rpm for 15 min., plasma was kept in plastic tubes and stored at -20°C until estimation of progesterone (P4) level.

Table 1. Summary of the treatment designs and groups.

Treat. Group	Day 0	Day 6	Day 10	Day 11	AI
G1			Control, natural mating		-
G2	Sponge insertion (SI)	-	-	Sponge withdrawal +500 IU PMSG*	48-56 h
G3		-	1ml lutone** (25 mg P4)	Sponge withdrawal +500 IU PMSG	
G4		-	-	Sponge withdrawal + male effect	
G5		-	1ml lutone (25 mg P4)	Sponge withdrawal + male effect	
G6		Sponge withdrawal +500 IU PMSG	-	-	

*: Folligon, Intervet International B.V. Boxmeer-Holland; S: Sponge; **: Lutone (each 1 ml lutone contains 25 mg progesterone in oily solution).

Progesterone hormone assay (P4)

The P4 level were detected by Quantitative methods in blood plasma using P4 radioimmunoassay kit (Catalog No. 1188 manufactured by Immunotech, France) as described by Bojanic *et al.* (1991).

Statistical analysis

The presented data were analyzed using SAS software program (SAS., 2000), GLM analysis of variance (ANOVA). Duncan Multiple Range test (Dancan, 1955) was used to get the mean separations among the experimental groups at P<0.05.

RESULTS

Estrous rate

As shown in Table 2, data related to estrous rate was significantly higher (P<0.05) in G2, G3 and G6 than in G1, whereas the highest rate was recorded among ewes of G6 (100%), followed by G2 (87.5%) and the lowest in G3 (50%) as compared to 25% in G1. This indicated that ewes treated with short term sponge (6 days) and PMSG at sponge withdrawal showed estrous response with 100% estrous synchronization.

Estrous characteristics

The results showed that time of estrous onset from sponge withdrawal was significantly (P<0.05) the latest (58.75 h) with moderate duration (29.25 h) in G6. While the earliest estrous onset and the shortest duration were significantly (P<0.05) obtained in G2 and G3. However, ewes in G4 showed significantly (P<0.05) moderate time of estrous onset (54.75 h) with the longest duration (31.50 h) (Table 3). Regarding comparing long with short term treatment (G2 vs. G6), long term treatment (12 d) experienced delayed estrous activity, but it did not affect estrous duration as compared to short term treatment. However, the superiority was in estrous rate, being higher in short than long term treatment.

Reproductive performance

Pregnancy, lambing and fertility rates

The present study showed that pregnancy and lambing rates were significantly (P<0.05) higher in control ewes (G1) than all treated ewes (G2-G6). Meanwhile, among treatment groups,

Table 2. Effect of treatment on estrous rate of ewes in experimental groups during May season.

Experimental group	Number of treated ewes	Estrous occurrence	
		n*	%
G1 (control)	24	6	25.0^{c}
G2 (S_{d11}+ $PMSG_{d11}$)	24	21	87.5^{a}
G3 (Sd11+ P4 d10+PMSG d11)	24	12	50.0^{b}
G4 (S11d+M d11)	24	6	25.0^{c}
G5 (Sd11+P4 d10+Md11)	24	9	37.5^{bc}
G6 (S_{d6}+$PMSG_{d6}$)	24	24	100^{a}

Columns containing different superscripts letters have a significant difference at P <0.05.
*Based on number of lambed ewes. S: Sponge; P4: Progesterone; PMSG: Pregnant mare serum gonadotrophin; M: Male effect.

Table 3. Onset and duration of estrus from sponge withdrawal in ewes of treatment groups during May season.

Treatment Group	Ewes observed in heat	Estrous characteristics	
		Onset (h)	Duration (h)
G2 (S_{d11}+ $PMSG_{d11}$)	21	53.50±1.88^{b}	27.50±0.82^{b}
G3 (Sd11+ P4 d10+PMSG d11)	12	52.50±0.73^{b}	28.50±1.29^{b}
G4 (S11d+M d11)	6	54.75±1.77^{ab}	31.50±1.50^{a}
G5 (Sd11+P4 d10+Md11)	9	57.25±1.68^{ab}	30.00±1.60^{ab}
G6 (S_{d6}+$PMSG_{d6}$)	24	58.75±2.10^{a}	29.25±0.52^{ab}

Columns containing different superscripts letters are significantly different at P<0.05.
S: Sponge; P4: Progesterone; PMSG: Pregnant mare serum gonadotrophin; M: Male effect.

Table 4. Effect of treatments on pregnancy, lambing and fertility rates of ewes in experimental groups during May season.

Treatment	Treated ewes (n)	Ewes in heat (n)	Pregnant ewes		Lambed ewes		Fertility rate
			n	%	n	%	%
G1 (control)	(24)*	6	6	100^{a}	6	100a	25.0^{b}
G2 (S_{d11}+ $PMSG_{d11}$)	24	21	18	85.7^{b}	18	85.7^{a}	75.0^{a}
G3 (Sd11+ P4 d10+PMSG d11)	24	12	6	50.0^{c}	6	50.0^{b}	25.0^{b}
G4 (S_{11d}+M_{d11})	24	6	3	50.0^{c}	3	50.0^{b}	12.5^{b}
G5 (S11+P4 $_{d10}$+M_{d11})	24	9	3	33.3^{d}	3	33.3^{c}	12.5^{b}
G6 (S_{d6}+$PMSG_{d6}$)	24	24	18	75.0^{b}	18	75.0^{a}	75.0^{a}

Different superscript letters across columns indicate significant differences among groups at P<0.05. S: Sponge, P4: Progesterone, PMSG: Pregnant mare serum gonadotrophin; M: Male effect.

the pregnancy and lambing rates were statistically significant (P<0.05), whereas the parameters were the highest in G2 and G6, moderate in G3 and G4, and the lowest in G5. In term of fertility rate, ewes in G2 and G6 showed significantly (P<0.05) higher fertility rate (75% in each), while those in other groups showed poor fertility rate, ranging from 12.5 to 25% (Table 4).

Litter size and fecundity

During long term treatment, ewes in G2 also yielded the highest lambs' number (27/group), indicating significantly (P<0.05) higher litter size and the highest fecundity (1.5 lambs/ewe and 112.5%) due to greater twins number as compared with other treatment and control groups. In addition, ewes in G6 (short term treatment) showed higher pregnancy, lambing and fertility rates with higher litter size (1.33/ewe) and fecundity (100%), being insignificantly lower than those in G2. Although ewes in G5 recorded significantly (P<0.05) the highest litter size but only one ewe in this group lambed twin lambs. On the other hand, litter size and fecundity of ewes in G3, G4 and G5 did not significantly differ from that in control ewe (Table 5). These results indicated that sponge (long for 11 d or short term for 6 d) plus PMSG at sponge withdrawal resulted in the best results of litter size, fertility and fecundity during May season.

Progesterone profile

During transit time prior to May season, P4 level of the control ewes was above 0.1 ng/ml, then declined nearly mid-May. This finding indicated the higher incidence of ovarian activity of some ewes in the control group during May season. Also, this finding might suggest the activity of estrous during mid-May. In G2, P4 level showed a marked increase by advancing time of sponge insertion, but after sponge withdrawal and PMSG injection, P4 level decreased indicating response of ewes to be in heat at the time of AI (day 13) and this trend was observed in pregnant ewes. In G3, P4 level increased during sponge insertion in both pregnant and non-pregnant ewes. Also, P4 level showed additional increase by P4 treatment (day 10) in both pregnant and non-pregnant, then decreased at sponge withdrawal and PMSG injection in pregnant and non-pregnant. In G4, pregnant ewes responded to sponge insertion by increasing P4 level, but slight increase in P4 level occurred during sponge insertion in non-pregnant ewes. The same trend was observed in pregnant and non–pregnant ewes

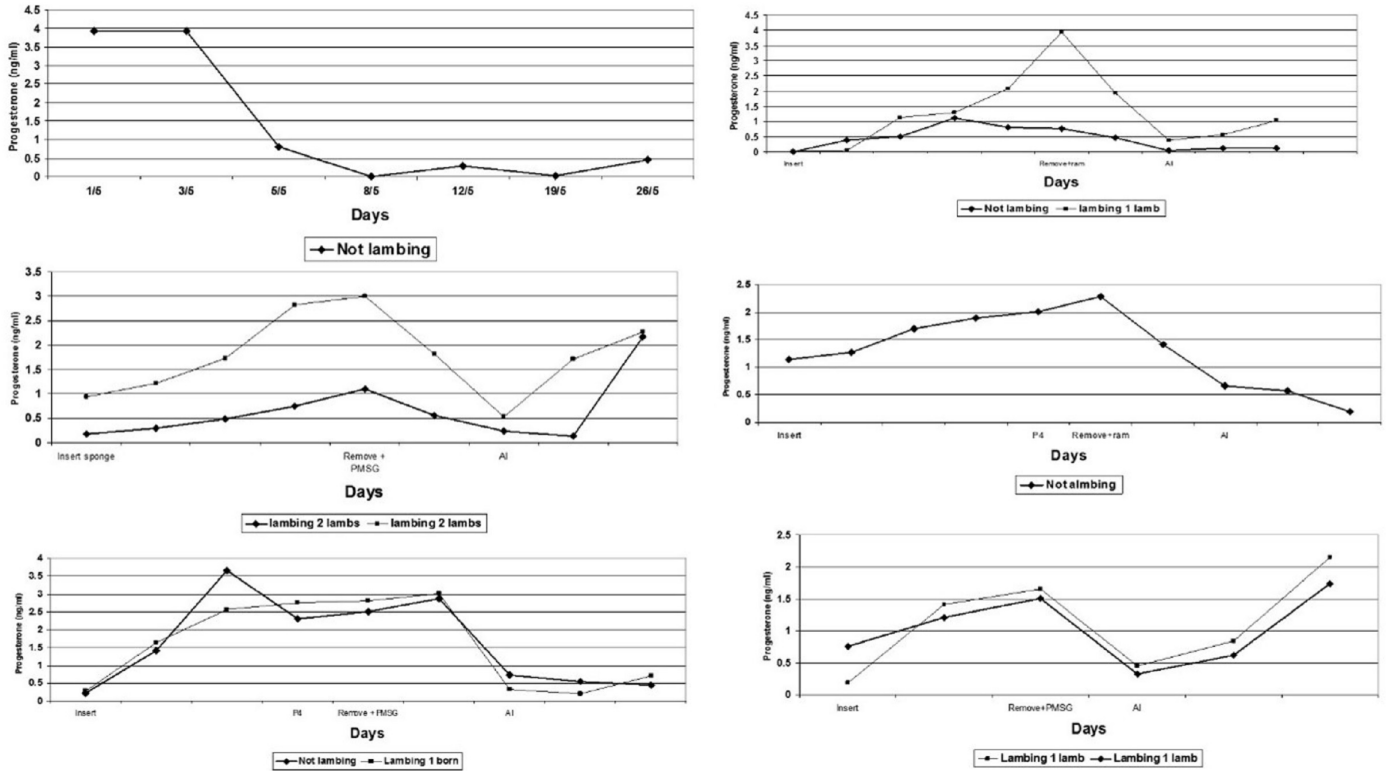

Fig. 1. Changes in P4 concentration among all groups.

Table 5. Effect of treatments on litter size and fecundity of ewes in experimental groups during May season.

Experimental group	N	Lambed ewes (n)	Number of lambs	Litter size Lamb/ewe	Fecundity (%)
G1 (control)	24	6	6	1.00^{c}	25.0^{b}
G2 (S_{d11}+ $PMSG_{d11}$)	24	18	27	1.50^{b}	112.5^{a}
G3 (Sd11+ P4 d10+PMSG d11)	24	6	6	1.00^{c}	25.0^{b}
G4 (S_{11d}+M_{d11})	24	3	3	1.00^{c}	12.5^{b}
G5 (S11+$P4_{d10}$+M_{d11})	24	3	6	2.00^{a}	25.0^{b}
G6 (S_{d6}+$PMSG_{d6}$)	24	18	24	1.33^{bc}	100^{a}

Different superscript letters across columns indicate significant differences among groups at P<0.05. N: Number of treated ewes; S: Sponge; P4: Progesterone; PMSG; Pregnant mare serum gonadotrophin; M; Male effect.

by introducing ram. In G5, further increase in P4 level was noticed post P4 injection, but P4 level did not reach the minimum level of estrous. This may indicate delaying ovulation with timing AI in this group. In G6, the response of ewes during sponge insertion appeared by increasing P4 level during short term treatment for 6 days, then P4 level reduced at sponge withdrawal to be at minimum values at AI. This trend indicated higher P4 level (P4 peak) before AI and pregnancy of most ewes in this group. All changes in P4 among groups was shown in Figure 1.

DISCUSSION

Estrus synchronization is an important strategy to improve the reproductive proficiency of the herd, particularly in ruminants. Among others, estrus synchronization with progestogens when used as sponges or intravaginal devices are the most used in these protocols, which associated with FTAI, seemed to be a useful tool in improving the productive and reproductive efficiency of herds. The present study provided interesting information about the potential influence of long and short-term progesterone administration on estrous synchronization and reproductive performance in ewes during May season. In the present work, the rate of estrous was markedly larger when progesterone + PMSG were provided for a short period of time at sponge withdrawal time than other treatments. These results are similar to the results stated in a previous study (Nasroallah *et al*., 2012), who reported that all Dammar ewes were given controlled internal drug release (CIDR) 6 days + equine chorionic gonadotrophin (eCG) responded to estrous. However, Ustuner *et al*. (2007) showed that estrous response of Awassi ewes treated with sponge (short term 6 days) + PMSG 24 h before withdrawal, sponge (short term 6 days) + PMSG at withdrawal and sponge (short term 6 days) + PMSG after 24 h from withdrawal were 75, 83.3 and 72.7%, respectively, versus 100% for ewes treated with long term sponge (12 days) for the same protocols. In harmony with our findings, Ustuner *et al*. (2007) revealed that the beginning of estrus from sponge withdrawal of Awassi ewes treated with sponge (long term, 12 d) and PMSG administration was applied 1 day before and 1 day after sponge removal were 30.0, 38.18 and 31.8 h versus 62.0, 70.8 and 89.25 h for sponge short term (6 days) treatment of the same protocols, respectively. They also found that estrous duration was longer in long term than short term treatment. This difference may be related to the levels of P4, within two days of pessary administration, a peak was noticed, followed by a gradual decrease over the remaining period (Husein and Kridli, 2002; Yavuzer, 2005), However, the final phases of follicular development usually take 4-5 days (Wright *et al*., 1983). The decline in P4 might become attributed to the sponge being washed out by vaginal fluid (Wheaton *et al*., 1993). In sheep, lower P4 levels were often linked to the development of persistent follicles, extended luteal function, and decreased fertility (Johnson *et al*., 1996).

In addition, short-term progestogen sponge treatment (5-7 days) has also been shown to be effective in inducing and synchronizing estrous in sheep during breeding and non-breeding seasons (Vinoles *et al*., 2001; Ataman *et al*., 2006). Reduced sponge insertion time can help to maintain increased P4 levels after pessaries being removed, as well as decrease the risk of vaginal contamination. Regarding the reproductive performance, Ustuner *et al*. (2007) found that fertility and lambing rates with long term + PMSG were lower than short term protocols. In this aspect, Husein *et al*. (1998) mentioned that breed, heredity, environmental conditions, management, and ewes' reproductive soundness are all factors responsible for low fertility rate. Embryonic mortality has long been thought to be the most significant constraint to the reproductive performance in mammalian species. Luteal inadequacy caused by environmental factors such as heat stress or poor nutrition has been identified as a major cause of sheep embryonic loss (Wilmut *et al*., 1986). Nearly similar results of fecundity were obtained by Safdarian *et al*. (2006) on Karakul ewes using CIDR + eCG. The results were obtained by Koyuncu and Ozis (2010b) on Kivricik ewes treated with sponge + PMSG at sponge withdrawal and Nasroallah *et al*. (2012) on Kermani ewes treated with CIDR +eCG. On the other hand, higher fecundity rate was obtained by Nasroallah *et al*. (2012) on Dammar ewes treated with CIDR (12 d) + eCG (144%) or CIDR (6 d) + eCG (137%). The pregnancy rate found in this study is similar to that found in a previous study (Simonetti, 2000). High LH pulse frequency and the growth of "persistent follicles" containing aged oocytes are linked to different types of estrous synchronization. P4 is also useful in synchronizing estrus for precisely designed artificial insemination, particularly when combined with PMSG. Furthermore, gonadotrophins may be combined with injectable progesterone to ameliorate pregnancy rates (Simonetti, 2000).

Similarly, to our hypothesis, Graylings *et al*. (1997) reported that the halved sponge group has slightly elevated mean LH concentrations with higher conception and lambing rates ($P < 0.01$) as compared to the whole sponge group. In a previous experiments, the subluteal levels of P4 (1-2 ng/mL) were effective in suppressing the occurrence of preovulatory surges of LH (Stock and Fortune, 1993). Administration of PMSG caused the progesterone level to decrease, thus stimulating estrus and ovulation in treated animals. The PMSG has both FSH-like activities and LH-like activities which stimulate the growth of ovarian follicles (Kim *et al*., 2005). This is consistent with a previous study by Stubbings *et al*. (1986), who reported that PMSG administration was used to induce superovulation in does. However, Walsh *et al*. (2007) concluded that hCG treatment did not improve the reproductive performance in estrous-induced ewes. Exogenous progesterone, administered by an intravaginal system to non-pregnant cows who had not shown estrous, increased the likelihood of pregnancy following artificial insemination (AI) at a set time. Progesterone therapy plus GnRH injections during proestrous might result in decreased fertility rates, as this treatment brought about the formation of recurrent Ewes carrying single or twin fetuses have blood P4 concentrations that are comparable to those recorded by researchers (Ezzo and Shalaby, 1990; Amiridis *et al*., 2002).

CONCLUSION

The study concluded that progestagens-synchronized estrus sponge (long for 11 d or short term for 6 d) plus PMSG during May season at sponge withdrawal improves the reproductive activity of artificially inseminated crossbred ewes, including litter size, fertility, and fecundity. Moreover, ewes treated with a short-term sponge (6 days) and PMSG at sponge withdrawal have an estrous response and 100% estrous synchronization with highest fertility rate and a higher P4 level (P4 peak) before AI and pregnancy of most ewes. The present study provides interesting findings which pay the attention about the potential future use of progesterone sponge with other drugs like nitric oxide donors to improve subsequent fertility of sheep flock.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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