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Original article

Different estrous induction protocols during non-breeding season in Assaf ewes

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ABSTRACT

This experiment was conducted to investigate the effect of pregnant mare serum gonadotrophin (PMSG) levels and the progestagen method on estrus response, onset and duration of estrus, lambing rate and litter size. A total of 20 Assaf ewes were used in the experiment, which was conducted during April, a month that is considered as non-breeding period in Palestine. Ewes were treated with intravaginal sponges containing 60 mg medroxyprogesterone acetate (MAP). Seven days later, sponges were removed and 10 new sponges were inserted to 10 of the experimental ewes. Following withdrawal of sponges at day 14, 5 ewes from each treatment groups were injected intramuscularly with a pregnant mare serum gonadotrophin (PMSG) at level of 300 or 600 IU. The results showed that level of PMSG and progesterone application methods had no significant effects on the tested parameters. This finding indicated that low level of PMSG can be applied for estrus synchronization. Results showed that using one sponge followed by a 300 IU dose of PMSG could induce estrus successfully with low cost comparing to application of two sponges and high doses of PMSG.

1. Introduction

Sheep production is one of the most important agricultural sectors in Palestine. Sheep population was estimated to be about 744 thousand heads (PCBS, 2008). This sheep population is composed of the Awassi breed

which comes in the first place followed by the Assaf breeds (Goot, 1986). Palestine is located along the line 14-34 and 40-35 east and between latitudes 30-29 and 15-33 in the north. The peak of ewes breeding activity occurs in the period from September to November and lambing activity in months of February to April. Because sheep are seasonally polyestrous, an attempt to mate at a frequency greater than once a year will require one breeding season during or near anestrous. Without intervening treatments during anestrous, little ovarian and estrous activity occurs, and pregnancy and conception rates are low, especially if out-of-season breeding occurs during the early postpartum period. The major problems that faces the sheep farmers in Palestine is reproduction management (estrus synchronization), this is necessitates looking for reproductive techniques, which could help the farmer and decrease the cost of production.

Different methods of estrus synchronization were investigated worldwide, such as introduction of rams (Romano et al., 2001; Rosa and Bryant, 2002; Chanvallon et al., 2010), progesterone (Fukui et al., 1999; Boscos et al., 2002; Husein and Kridli, 2002; Karaca et al., 2009), prostaglandins (Ataman et al., 2006; Ataman and Aköz, 2006; Sözbilir et al., 2006) and melatonin (Stellflug et al., 1988; O'callaghan et al., 1991; Carlson, 2000). Several researchers aimed to investigate the efficiency of synchronization using different progesterone treatments outside the natural breeding season. Greyling and Van der Nest (2000) used different progestone levels in natural season followed by injection of PMSG. There was no attribution in synchronization efficiency related to different doses of progesterone levels, but the interval of estrus after removal of sponge until induce of the estrus were shorter at low levels of MAP than with high levels of it. In addition, others reported that the duration of estrus are shorter in MAP/PGF2α treatment compared to MAP alone treatment (Ahmed Muna et al., 1998; Dogan and Nur, 2006).

Ovulation rate in ewes is lower when induced during anestrus than during the breeding season. Ovulation rates were increased by injection of gonadotropins such as equine chorionic gonadotropin (eCG), follicle stimulating hormone (FSH), human chorionic gonadotropin (hCG) andmixed gonadotropin preparations, such as PG 600 which contains 400 IU PMSG and 200 IU hCG (Knights et al., 2003). Some researchers studied the effect of different PMSG doses on pregnancy and lambing rate in different sheep breeds. Rad and Farzaneh (2007) used CIDR-G and different levels of PMSG (i.e. 300, 400, 500 and 600 IU) in Balouchi ewes, and reported that twining rates were higher when using 600 IU of PMSG compared to 400 or 300 IU. Other researchers used different levels of PMSG (500, 600, 750 IU) in breeding season, and reported that pregnant rate and litter size were increased when 600 and 750 IU PMSG were used (Timurkan and Yildiz, 2005). Akoz et al. (2006) used 30 mg, 40 mg FGA with different doses PMSG (300, 500, 700 IU), they reported that injection of 700 IU of PMSG was more effective in pregnant rate and lambing rate regardless of the dose of FGA. The average interval from sponge removal to onset of estrus was studied by Fonseca et al. (2005) who reported that the interval was 49.7 ± 15.7 hr after injection of 200 IU.

Intravaginal sponges have some side effects such as vaginal contamination and infections when used for long periods. The hormone contained in the sponge may be diminished with time. The objectives of this study were to investigate the effect of refreshment intravaginal sponges and different PMSG levels on estrus response, onset and duration of estrus, lambing rate and litter size in Assaf ewes in north West Bank of Palestine.

2. Materials and methods

Twenty Assaf ewes were used in this study, the ewes were 3-5 years old, 65-70 kg average body weight and four healthy Assaf rams aged 3-4 years weighing 90-100 kg. This study was carried out in special farm owned by Gazal family at Sabastia village near Nablus city, Palestine. All ewes had previously lambed and weaned their last lamb. Estrous induction and synchronization was conducted during out of the natural breeding season. The animals were kept indoor at night and had access to natural grazing area for most of the day. Indoor ewes were offered diets of concentrated feed with wheat straw. Water and mineral licks were available *ad libitum*. Ewes were distributed randomly in a factorial design. The ewes portioned into 4 groups with 5 ewes in each, as the following:

Group I: Received hormonal treatments comprised of intravaginal sponges impregnated with 60 mg medroxyprogesterone acetate (MAP) (Syncro-Breed, The Advanced Veterinary Manufacturing Co. Ltd/Palestine) sponges were inserted for 14 days plus an intramuscular injection of 300 IU of PMSG (AVCP Manufacturing Co. - Ramallah - Palestine) at time of sponge removal.

Group II: Received the same progesterone treatment as in group I, but instead of 300 IU of PMSG, ewes were injected with 600 IU at sponge removal.

Group III: Received the same type of sponges used in all groups, but sponges were replaced by a new one at the 7th day of treatment. The new inserted sponges were removed after 7 days and the ewes were injected with 300 IU PMSG at sponge removal.

Group IV: Received the same progesterone treatment as group III but the PMSG dosage was as in the group II (i.e. 600 IU). The four groups are summarized in Table 1.

At the day of sponge removal and PMSG injection, 4 teaser rams were allowed to run with the treated ewes. Estrus was monitored every 6 hours for 3 days. Ewes that detected by teaser rams have been introduced to the rams to be bred. Following sponge removal some reproductive parameters were recorded and evaluated for each animal of the four groups. These parameters were: Incidence of estrus, onset of estrus, duration of estrus, pregnancy rates, lamb ingrates and litter size.

Table 1
Treatment groups and experimental design of Assaf ewes.

Treatment/Groups	Progesterone treatment	PMSG treatment
Group I	60 mg MAP for 14 days	300 IU PMSG
Group II	60 mg MAP for 14 days	600 IU PMSG
Group III	1st 60 mg MAP sponge for 7 days 2nd 60 mg MAP sponge for another 7 days	300 IU PMSG
Group IV	1st 60 mg MAP sponge for 7 days 2nd 60 mg MAP sponge for another 7 days	600 IU PMSG

2.1. Statistical analysis

All data were analyzed using the SPSS Inc (2008) using one way ANOVA (SPSS Inc: SPSS 16 for windows. Statistical package for the social sciences, Chicago).

3. Results and discussion

3.1. Estrus response

Out of the 20 Assaf ewes used in the trial, 16 ewes exhibited signs of estrus during the 120 hr observation period. However estrus responses were 80% for both first and second groups and 60 and 100% for the third and fourth groups, respectively (Table 2). The percentage of ewes exhibiting estrus in this trial was comparable to values reported by (Simonetti et al., 2000; Dogan and Nur, 2006). However Dogan and Nur (2006) reported 88,9% estrus response by using 60 mg of MAP and 500 IU of PMSG during non- breeding season. Using MAP at level of 60 mg resulted in 80.9% estrus response (Simonetti et al., 2000). Higher estrus responses (i.e. 100%) were observed in Karakul ewes when dosed with 60 mg MAP along with 500 IU of PMSG (Hashemi et al., 2006). The slight differences between the results obtained in the current study and those reported by other research might be due to breed of sheep and the season in which the studies were executed.

3.2. Onset of estrus

The time from sponge withdrawal to estrous and the duration of the induced estrus period following the two methods of sponge treatment for the two different PMSG doses are shown in Table 2. A summary of the distribution of animals showing estrus is set out in Table 3. The time from sponge withdrawal to the onset of estrus was not significantly different between the two progesterone application methods or the two PMSG doses. The time to estrus was slightly longer (60.7 hr) in the groups treated with one sponge for 14 days and 300 IU PMSG compared to the other groups (51.3, 52.6 and 46.8 hr).

Lack of significant differences in terms of time to the onset between ewes treated with the two different intravaginal sponges demonstrate a similar efficiency of the two synchronization methods in inducing estrus during the non breeding season in the Assaf ewes. Use of one sponge as in the group I and II is cheaper than the other two methods used in other groups. The time of estrus onset results (46.8 - 60.7 hr) of the current trial were in agreement with the previous findings of Greyling and Van der Nest (2000) (50.7 ± 26.3 hr) and with Dogan and Nur

(2006) 30 and 60 hr. To the contrary, Ahmed Amer and Maher Hazzaa (2009) noted that the time from sponge withdrawal to the onset of estrus to be later in ewes treated with FGA for 12 d. Simonetti et al. (2000) recorded estrus to occur 55.94, 56.74 and 57.7 hr after using sponges impregnated with 40, 50, 60 mg progesterone respectively. Comparing the results of this study to those obtained by Akoz et al. (2006) who used different concentrations of progesterone in sponges, it is cleared that there was no significant differences between using of two sponges or different progesterone levels in the sponge.

As mentioned in Table 3, using two sponges may accelerate the estrus induction after sponge withdrawal. Two of the ewes treated with one sponge showed estrus signs after 73 hr of sponge withdrawal.

Table 2
Mean estrus responses following estrous synchronization with the aid of different progesterone protocols and different PMSG doses.

Treatment	Groups		n	Estrus response (%)	Onset of estrus (hr)	Duration of estrus (hr)
One sponge method (14 days)	Group I	300 IU PMSG	5	4 (80)	60.7 ±20.3	26.4±4
	Group II	600 IU PMSG	5	4 (80)	51.3 ±25.2	12.1 ±7.3
Two sponge method each (7 days)	Group III	300 IU PMSG	5	3 (60)	52.6 ±10.9	12.8 ±8.3
	Group IV	600 IU PMSG	5	5 (100)	46.8 ±14.3	13.7 ±11.3

Table 3
Distribution of the estrus response at different time intervals among treatment groups of Assaf ewes.

Treatment		n	Time intervals (hr)				
			0-24	25-48	49-72	73-96	97-120
One sponge	300 IU PMSG	5	0	1	2	1	0
	600 IU PMSG	5	0	2	1	1	0
	Total	10	0	3	3	2	0
Two sponge	300 IU PMSG	5	0	1	2	0	0
	600 IU PMSG	5	0	2	3	0	0
	Total	10	0	3	5	0	0

3.3. Duration of estrus

Duration of estrus are shown in Table 2. This parameter was not significantly different in all treatment groups ($P>0.05$). However, the duration of estrus was slightly longer (i.e. 26.4 hr) in ewes who had sponges for 14 d and 300 IU PMSG dose compared to other treatment groups. In the group one (sponge for 14 days and 300 IU PMSG) compared to the results of our study which showed a shorter duration of estrus compared to other research with an average of 36 hr (Dogan and Nur, 2006; Hashemi et al., 2006). The reason of short estrus duration period in this study and the variation in two groups (300 IU PMSG and 600 IU PMSG) may be due to lower estrogen in blood during the non-breeding season and to breed differences, age and geographical location (Evans, 1986; Gordon, 1996; Hashemi et al., 2006).

3.4. Litter size

The estimated litter size at different for the different levels of PMSG treatment are shown Table 4. Out of 16 ewes that observed in estrus, 22 lambs were obtained. The number of lambs obtained from ewes (i.e. 7 ewes)

treated with 300 IU PMSG was 10, however, 12 lambs were obtained from ewes (i.e. 9 ewes) receiving 600 IU PMSG.

The mean litter size in the PMSG groups was estimated to be 1.43 for the ewes injected with 300 IU PMSG compared to 1.33 for those injected with 600 IU PMSG. Our results were in agreement with Pollot and Gootwine (2004) who reported that the mean litter size in Assaf breed to be 1.57. Results of this research showed that increasing the PMSG dose from 30 to 600 IU had no advantage in increasing twinning rate and litter size in spite of the fact that increasing PMSG dose has positive effects on improving these parameters. However, in the present study there was noticeably increase in the litter size in 300 IU MAP ewes.

Opposing the phenomenon of gonadotropin effects on ovulation rate, gonadotropin hormones function to increase litter size and twining rate, it was reported that the using of 400 IU PMSG increased the litter size when compared to ewes injected with 200 or 300 IU PMSG (de la Cruz et al., 1990; Toteda et al., 1990).

Table 4
The litter size estimation for the different levels of PMSG treatment in Assaf ewes.

Treatment/Groups	Number of lambing ewes	Number of lambs (litter size)
300 IU PMSG	7	10 (1.43)
600 IU PMSG	9	12 (1.33)

4. Conclusion

It can be concluded that changing sponges or increasing the dose of PMSG was of no advantage since the half dose of PMSG has the same effects on number of ovulations and liter size. Similarly changing the sponge had no effect on the rate of synchronized ewes. Significant savings could be achieved by applying 300 IU PMSG with one sponge. However, more research on the tested parameters is required, especially when dealing with larger herds of ewes.

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