

Full Length Research Paper

Effect of different oestrus synchronizations protocols on the reproductive efficiency of Dammar ewes in Yemen during winter

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This study compared the effect of oestrus synchronization using controlled-internal-drug-release-device (CIDR) and equine chorionic gonadotropin (eCG) on reproductive efficiency of Dammar ewes in winter. Data of forty-one ewes were divided into two groups; for long CIDR treatment (12 days, LT, n=25) and for short CIDR treatment (6 days, ST, n=16). The LT group was further divided into 3 subgroups to receive eCG either 48 h before CIDR removal (LT1), or at CIDR removal (LT2). Nevertheless, a third subgroup (LT3) received no eCG and served as the control variable. The ST group was also divided into two subgroups to receive eCG either at CIDR removal (ST1) or to receive no eCG (ST2). Five fertile rams naturally mated the ewes. Then in terms of oestrous responses, the pregnancy and lambing rates were 100 percent (100%) in ewes for all treatments. The onset and duration of oestrous was shorter in LTa1 than the remaining subgroups. Litter size in those of LT2 and LT1 subgroups was greater than those of LT3. Additionally, litter size in those of ST1 was greater than in those of ST2 subgroup. This indicated that eCG injection affected litter size. In conclusion, these results indicated that CIDR could successfully be used for either 6 or 12 days prior to ram introduction without having to administer eCG. However, eCG is important to induce multiple births.

Key words: Oestrus synchronization, sheep, mating, lambing, winter.

INTRODUCTIO

Progestogens were maintained during 6 to 14 days and were associated with or without pregnancy mare serum gonadotropin (PMSG) or PMSG and prostaglandin combinations (Ataman et al., 2005). Reduced fertility rates have been attributed to many factors including; heredity, breed, seasonality, age, environment, nutrition, diseases, semen quality, female reproductive status and hormonal treatment (Yavuzer, 2005). Recent study found out that short-term treatment (which is between 5 and 6 days) with different progestogen devices during the non-breeding season has been as effective as long-term treatment to induce oestrus and the subsequent fertility

(Ungerfeld and Rubianes, 1999). The effect of the length of a progestogen treatment for 12 days versus 6 days on synchronization efficiency using fluorogestone acetate (FGA) treatment with PMSG administration was applied at different times of sponge removal (Ustuner et al., 2007).

However, such efficiencies of oestrus synchronization in sheep or in others animals has not been studied in Yemen. This is the first experiment, which have been done and considerable reviewers can use it in the future regarding these findings in my country. The aim of this study was to evaluate the effect of long and short treatment of controlled internal drug release device (CIDR) with or without equine chorionic gonadotropin (eCG) on reproductive efficiency of Dammarian ewes in Yemen during winter regarding 12 versus 6 days. The outcome of this study should encourage farmers in

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Yemen to use oestrus synchronization techniques to improve management and increase lamb crop production rates.

MATERIALS AND METHODS

The study was carried out during winter at military of economic co-operative farm, located in Mabar area in Dammar province with latitude of 14°47'06"W, longitude of 44°16'35"E, and with altitude of 2327 m. Forty one (41) fat-tailed and healthy Dammar indigenous ewes were used in the study with their ages ranging from 2 to 4 year old, and weighing between 25 to 50 kg with a body condition score (BCS) of 2.5 to 3 on a scale from 0 (emaciated) to 5 (obese) (Romdhani et al., 2004). The ewes and five fertile rams for natural mating were treated with Evmectin for external and internal parasites while being grazed on natural pasture twice (morning and evening) on daily basis with mineral blocks as well as water in the pens being provided.

The ewes (n = 41) were divided into two groups; long term (LT, n = 25) and short term (ST, n = 16), and treated by CIDR device containing 0.3 g of progesterone (Eazi-breed CIDR; Pfizer New-Zealand) while being injected with eCG 300 IU (Folligon; Intervet, Boxmeer-Holland) at different time interval. The eCG was applied 48 h before the removal of CIDR, at the CIDR removal and control for those in the long term groups (LT1, LT2 and LT3) for 9 and 8 ewes and for those in the short term groups (ST1 and ST2) at CIDR removal and control for 8 and 9 number of ewes respectively Table 1. In additions, the rams were examined and confirmed to be free from any obvious abnormalities of the palpable reproductive organs and were treated with ivermectin for external and internal parasites. The five fertile rams which aged from 1.6 to 3 years old with bodyweight ranging from 40.7 to 50.5 kg were used for natural mating and heat detection in the ewes as the rams were introduced from 5 to 24 h after CIDR removal.

Heat detection

Briefly, 6 h after the controlled internal drug release device (CIDR) removal; five fertile rams were introduced and subjected for heat detection and natural mating. The observation of the heat detection and mating were performed during all days, (day and night) in the pasture and pens. The ewes were considered in oestrus, when they allow the male to mount them. Oestrus in the ewes were observed during grazing in daylight and in the pens every 2 h during night, with some crucial assistance being offered to us by the shepherd and the technicians for observing the oestrus and mating. Following CIDR removal, some reproductive parameters were recorded with all the days of the experiments. This reproductive parameter includes the oestrus response, onset of oestrus, duration of oestrus and cessation of oestrus. The rams were used to have been mated with the ewes more than twice until the end of duration of oestrus. It has been shown that the occurrence of mounting is higher at night than during the day as cited by Hafez and Hafez, (2000) and Susan (2010).

Pregnancy diagnosis

Pregnancy diagnosis was done by traditional method before (CIDR) insertion and after three months following natural mating with normal examination of abdominal palpation by checking with hand in order to determine that the ewes were in the process of conception. The main reason for the use of traditional method is because there was neither scanning apparatus for veterinary usage in veterinary department nor was there in private clinics. However, Prof. Dr. Ghazi, A.J. in Yemen with an immense experience of

dealing with pregnancy diagnosis concerns traditionally in ewes suggested the use of traditional method for pregnancy diagnosis which was done by him.

Statistical analysis

In this study, the effects of CIDR based treatment with and without eCG injections was studied to investigate further statistical relationships of the parameters derived from ewes under study. The incidence of oestrus (onset of oestrus, oestrus response, cessation of oestrus and duration of oestrus cycle) and (pregnancy and lambing rates) were analyzed by using one-way analysis of variance (ANOVA). The effects of the treatments on the proportion of sheep showing oestrus and the proportion becoming pregnant were compared by using the Chi-square test based on the tool of statistical package for the social sciences (SPSS) (12) software.

RESULTS

The distribution of ewes exhibiting oestrus following synchronization treatment for long and short treatments during winter were 28 out of 41 which was 68.3% of ewes showing oestrus within 72 h whereas, 75% of ewes in those of ST1 and ST2 showed oestrus between 97 to 120 h. Similarly, 12.5% of ewes in ST1 showed oestrus during 121 to 144 h. The majority exhibiting oestrus in long time (LT) and short term (ST) groups were (68.29%) that showed oestrus between 0 to 97 h, compared with other range of time as shown in Figure 1. The onset of oestrus in those of LT1, was shorter than those in LT2, LT3 groups with significant difference at $P < 0.05$ in those of LT3 whereas, the onset of oestrus in ST1 was shorter than those in ST2, and with no significant difference in those of ST1 and ST2. These results indicate that the onset of oestrus occurred earlier in ewes with eCG-treated than in ewes with non-eCG treated with significant difference at $P < 0.05$; in LT3 and ST2 as shown in Table 2. Furthermore, the study shows that the oestrus response of ewes treated for long and short-term during with or without eCG at winter occurred in 41 ewes which were (100%) following CIDR withdrawal. Therefore, there was no significant difference in all treatments groups. These results indicate that the effectiveness of CIDR administered for 6 days or 12 days without eCG had an effect, thereby ensuring the acceptable oestrus response during the winter as shown in Table 2.

The duration and cessation of oestrus in LT1 group were shorter than that of LT2 and LT3, with significant difference within LT groups and those of ST1 were shorter than ST2, with significant difference at significant level of $P < 0.05$ within ST groups. Therefore, the duration of oestrus in ewes in ST group occurred earlier with non-eCG-treated than other ewes in eCG treated during winter as shown in Table 2. The pregnancy and lambing rates were 100% in both LT and ST groups during winter. However, the use of eCG-treated ewes had no obvious effect on the pregnancy and lambing rates in both LT and ST groups as shown in Table 3. The litter size during

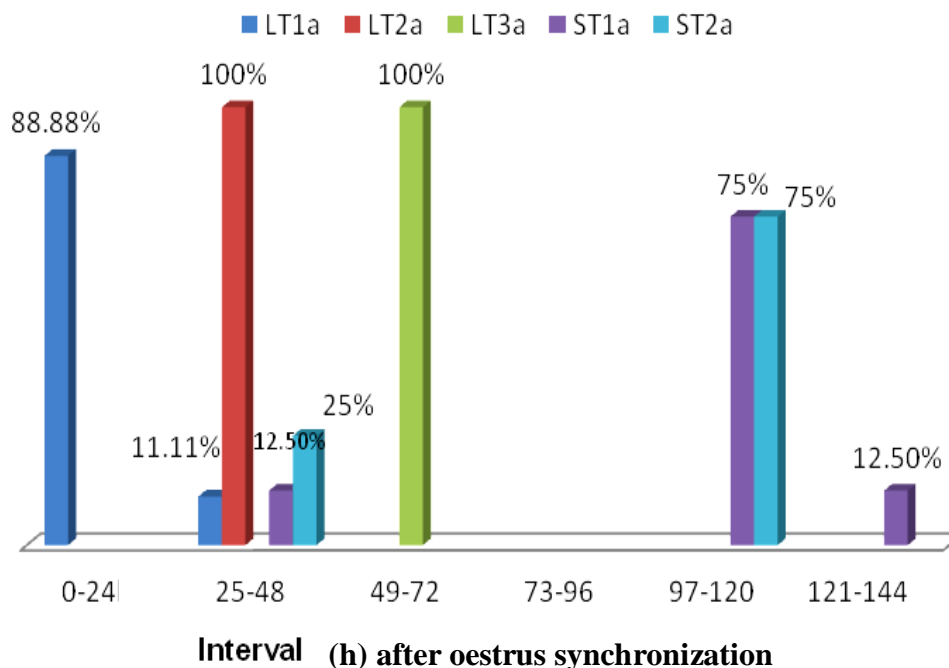


Figure 1. Percentage distribution of ewes exhibiting oestrus for both long and short-term treatments in winter.

Table 1. The experimental design during winter.

Experiment 2	Number of ewe	Subgroup	Time of eCG injection 300 IU
CIDR for 12 days.G.2.1	9	LT1	CIDR + eCG before 48h CIDR removal
	8	LT2	CIDR + eCG at CIDR removal
	8	LT3	CIDR control without eCG
CIDR for 6 days.G.2.2	8	ST1	CIDR + eCG at CIDR removal
	8	ST2	CIDR control without eCG
Total	41		

winter in those of LT2 and LT1 were higher than those of LT3, with significant differences at significant level of $P < 0.05$ among them.

It was also noticed that the litter size in those of ST1 was higher than in those of ST2 with no significant difference in ST groups. Such results indicate that eCG injection had an effect on the litter size in LT and ST groups. Therefore, we conclude that there were significant differences between LT and ST groups as shown in Table 3. The twinning and fecundity rates in LT2 were higher than that of LT1, LT3, as were similarly in those of ST1 groups, and were higher than that of ST2 with significant differences observed within and between both LT and ST groups. These results indicate that the time of eCG injection had an obvious effect on the twinning

and fecundity rates in LT and ST as shown in Table 3. Figure 1 shows the percentage distribution of ewes exhibiting oestrus following long and short-term treatments in winter.

DISCUSSION

In our study, there is no CIDR lost during the experiments period in winter. Eighty ewes (100%) in ST1 group exhibited oestrus within 24 to 144 h following progesterone withdrawal. This result is in consistence with the results obtained by Ungerfeld and Rubianes (1999), Das et al. (2000), Simonetti et al. (2000) and Vinales et al. (2001) that showed that interval onsets of

Table 2. The onset, duration, cessation and oestrus response in Dammar ewes using oestrus synchronization protocols for 6 and 12 days with CIDR and different time injection of eCG during the winter.

Parameter	Experimental group				
	12 day			6 day	
	LT1a	LT2a	LT3a	ST1a	ST2a
No of ewes	9	8	8	8	8
Onset of oestrus (h)	1.3±2.1 ^b	7.0±0.0 ^b	6.0±0.0 ^{a,b}	1.8±11.8 ^a	03.6±9.5 ^a
Oestrus response (%)	(100%) ^a	(100%) ^a	(100%) ^a	(100%) ^a	(100%) ^a
Duration of oestrus (h)	5.6±1.7 ^b	8.0±0.0 ^b	8.0±0.0 ^a	8.5±0.3 ^{a,b}	4.5±0.5 ^a
Cession of oestrus (h)	7.0±3.4 ^b	5.0±0.0 ^b	04.0±0.0 ^{a,b}	17.1±17.9 ^{a,b}	45.6±8.8 ^a
Time of mating (h)	0.5±1.8 ^b	0.0±0.0 ^{ab}	6.0±0.0 ^b	13.5±12.1 ^a	22.1±8.8 ^a

^{a,b,ab}Values with different superscripts in the same row and experiment differ significantly at P<0.05

Table 3. The pregnancy, lambing and the litter size in Dammar ewes using oestrus synchronizations protocols for 6 and 12 days with CIDR and different time injection of eCG during the winter.

Parameter	Experimental group				
	12 day			6 day	
	LT1a	LT2a	LT3a	ST1a	ST2a
No. of ewes	9	8	8	8	8
Pregnancy rate (%)	9 (100) ^a	8 (100) ^a	(100) ^a	(100) ^a	(100) ^a
Lambing rate (%)	9 (100) ^a	8 (100) ^a	8 (100) ^a	(100) ^a	(100) ^a
Mean days of pregnancy period	149.8±0.20 ^b	150.7±0.52 ^b	151.2±0.67 ^{a,b}	50.7±0.79 ^b	52.5±0.42 ^a
Twining rate (%)	4(44.4%) ^a	4(50%) ^a	0(0%) ^b	(37.5%) ^{a,b}	(25%) ^{a,b}
Single rate (%)	5(55.51%)	4(50%)	(100%)	(62.5%)	(75%)
Fecundity rate (%)	13(144%) ^{a,b}	12 (150%) ^a	8 (100%) ^b	1(137.5%) ^{a,b}	0 (125%) ^{a,b}
Liter size	(1.4±0.17) ^{a,b}	(1.5±0.18) ^a	(1.0±0.00) ^b	1.37±0.18) ^{a,b}	.25±0.16 ^{a,b}
Male	8 (61.5) ^a	4 (33.3) ^a	(50.0) ^a	(45.5) ^a	(60.0) ^a
Female	5 (38.5) ^a	8 (66.7) ^a	(50.0) ^a	(54.6) ^a	(40.0) ^a

^{a,b,ab}Values with different superscripts in the same row and experiment differ significantly at P<0.05.

oestrus occurred between 24 to 144 h following progesterone withdrawal. The interval onsets of oestrus in this study were earlier in the use of CIDR+ eCG for 12 days instead of used CIDR without eCG for 12 to 6 days during winter. The result of this study is in consistence with the results obtained in the study of Husein et al. (1998), Amer and Hazza (2009), Dogan and Nur (2006) and Mustafa et al. (2007). Their results showed that the time of oestrus of ewes were earlier in the use of progestogen and gonadotropin for 12 days rather than the use of progestogen without gonadotropin for 12 to 6 days during the non-breeding season.

The interval onset of oestrus in those of LT groups with eCG treatment for 12 days happened earlier than in those of ST groups with eCG treatment for 6 days. The results of this study are in accordance with the findings of the study conducted by Mustafa et al. (2007) and Amer and Hazza (2009) during non breeding season for (4 to 12 days) and (12 to 6 days) respectively. The reason for such differences of the interval onset of oestrus in this study in LT group with eCG treatment for 12 days being

earlier than in those ST with eCG treatment for 6 days may be due to the use of CIDR after day 0 as the progesterone concentration increased gradually. Thereafter, from day 5 until day 20 thereby reaching the maximum between day 11 and 20 in the ewes treated for 6 days (Moeini et al., 2007; Mustafa et al., 2007; Amer and Hazza, 2009). Maybe the higher level of progesterone (P4) in eCG-treated of ewes for 12 day may be largely attributed to high ovulation rates and formation of many extra corpora lutea due to the gonadotropic activity of eCG (Greyling et al., 1988; Robinson and Scaramuzzi, 1994).

In this study, the onsets of oestrus in those of LT groups were shorter than those of ST groups. These results are in consistency with the results of those obtained by Ustuner et al. (2007). In his study, Ustuner et al. (2007) concluded that the onset of oestrus in those of LT were shorter than in those of ST groups in ewes synchronized for 12 day versus 6 day with different significant level at (P < 0.05) in those of ST and LT groups during the breeding season. In the present study,

the onset of oestrus in those of LT1 and LT2 were shorter than in those of LT1 in ewes synchronized for 14 days during breeding season which is in consistency with the results of those obtained by Karaca et al. (2010). Whereas, the onset of oestrus in those of ST1 and ST2 were longer than in those of ST and ST1 as well as in ST2 in ewes synchronized for 6 and 8 days during breeding season which is in consistency with the study obtained by Ali (2006) and Karaca et al. (2010) respectively. This study also showed that at the onsets of oestrus in LT1 and LT2, the durations were shorter than in those of LT (MAP + 500 IU PMSG) for 12 days in ewes during non-breeding season consistent with that obtained by Dogan and Nur (2006). Although such differences on the results on the onset of oestrus may be indefinite or attributed to differences in breed, season, nutrition, the use of gonadotrophins and the presence of the male after progesterone removal, all factors were known to influence the parameter in onset of oestrus in ewes (Zelege et al., 2005).

The oestrus responses in the present study in LT groups are similar to the result of LT after sponge removal, which is consistent with that obtained by Ustuner et al. (2007). It is comparable with the results of LT2 and LT3 at 93 and 100% for 12 days CIDR-eCG at CIDR removal and for 12 days MAP-500 IU eCG at sponge removal, respectively during non breeding season also consistent with the study of (Hashemi et al., 2006; Safdarian et al., 2006). The oestrus response in the present study in LT and ST were higher than that of ST1, LT1 and ST2 (7-day-GnRH-FGA-eCG-PGF_{2α}), (12-day-FGA-eCG-PGF_{2α}) and (7-day-FGA-eCG-PGF_{2α}) as indicated by Karaca et al. (2008). The reason of the higher oestrus response in this study may be due to no lost in any CIDR during the experiments. Other factors such as season, breed of sheep, type of progestogen, type of gonadotropin may also contribute (Evans et al., 2004). There may be other reason due to the differences in nutritional factors and the location (Koyuncu and Ozis, 2010). Alternatively, geographical location, particularly degree of latitude has a significant impact of oestrus (Riera, 1982). The duration of oestrus in this study in ST1 and LT2 groups were longer than those of ST and LT (34.4 and 36.7 h) groups respectively during breeding season which is consistent with the study of (Ustuner et al., 2007).

The duration of oestrus in those of LT1 in this study was shorter than in those LT using medroxyprogesterone acetate (MAP) obtained by Evans et al. (2004). The reason of the short duration of oestrus result in this study may be attributed to lower oestrogen level in the blood, breed differences, age and geographical location as cited by Hashemi et al. (2006). Maybe longer duration result due to the high oestrogen level in the blood produced following the induced luteolysis and the stimulation of follicular growth in the ovary by follicle-stimulating hormone (FSH) or exogenous eCG and high levels of

serum oestrogen concentrations which could be responsible for a prolonged duration of the oestrus period (Ahmed et al., 1998). The cessations of oestrus in this study showed significantly difference at ($P < 0.05$) during eCG-treatment in those of LT and ST groups than in non-eCG in those of LT and ST groups. The results in cessations of oestrus in this study are consistent with the results obtained by Ustuner et al. (2007). Furthermore, the cessations of oestrus in the present study in those of ST1 and ST2 were longer than those of ST at 102 h of ewes with FGA or MAP during non breeding season similar to that obtained by Ustuner et al. (2007) were longer than in those of ST at 64 h that was obtained by Evans et al. (2004).

The reason for longer cessations of oestrus in this study than others in the literature may be due to the difference of gonadotropins, device of progesterone, the dosage of eCG, time of injection of gonadotropins, progestogen treatment protocols (Amer and Hazzaa, 2009). In this study, 41 ewes became pregnant from natural service; representing 100% lambing of ewes in those of ST and LT groups. These results of the pregnancy and lambing rates in this study are higher than those of LT obtained by Mustafa et al. (2007) and Amer and Hazzaa (2009). The pregnancy rate in the present study in those of ST was higher than in those of ST1 and ST2 in ewes synchronized for 8 days during breeding season which is similar to that of the results obtained by Karaca et al. (2010) but was higher than in those of ST result with that obtained by Karaca et al. (2009). Therefore, the reason for the higher pregnancy rate of ST, in present study may be due to the ovulation of newly recruited growing follicles for those of ST for 6 days as reported by Vinales et al. (2001). Another possibility is because it might be due to the difference of the dosage of gonadotrophins, the breeding season, progestogen treatment protocols, and the ewe breed (Dogan and Nur, 2006). At the same time reproduction in sheep is influenced by numerous factors, which include genetic potential, nutritional status, environmental factors, day length or photoperiod effects, health status and other factor (Silva et al., 1998).

The results obtained in the study by Moeini et al. (2007) showed that the lambing rates during the non-breeding season for 13 days in ewes was 60.2%, which was lower than the results in this study. In addition the study done by Dogan and Nur (2006) showed that the pregnancy rate was 54.5% during the non-breeding season by using MAP + 500 IU PMSG for 12 days including artificial insemination (AI) with diluted fresh semen which was lower than the pregnancy rate in this present study with natural service for 12 days. The reason for the difference in the result from the present study and the result from other researches on pregnancy and lambing rate may be due to the differences between natural service and artificial insemination, breed, nutrition, device of progesterone (CIDR or sponge), the dosage of eCG, time of

injection of gonadotropins and progestogen treatment protocols (Amer and Hazzaa, 2009). It seems that the use of natural service is better than the artificial insemination (AI) in ewes. Therefore, the result of the pregnancy and lambing rate showed higher percentage as achieved using natural mating in this study than in other experiments during the use of artificial insemination.

The litter size in those of LT1 in this study have similar results in those of ST1 and LT1 groups for 7 to 12 days obtained by Karaca et al. (2008) also for 12 days that was obtained by Ataman et al. (2006). In addition to the litter size, those of ST2 were comparable to those of ST for 4 days FGA during non-breeding season as indicated by Mustafa et al. (2007). The litter sizes in ewes during oestrus by Ataman et al. (2006) and Ali (2007) showed that the litter size was higher in those of the 12 days CIDR-eCG than in those of 6 days. Their result is consistent with the result of this study which showed that the litter size was higher in those of the 12-day CIDR-eCG than in those of 6 day. The litter size in this study in those of LT1 was lower than the litter size in those of ST2 1.8 (FGA for 7 day + GnRH at FGA insertion and PGF_{2α} and eCG 400 IU 24 h before FGA removal) in ewes during breeding season that was obtained by Karaca et al. (2008). The reason for the lower result of the litter size in this study when compared with the result obtained from the study by Karaca et al. (2010) may be due to the use of GnRH in the short-term progestogen program may likely improve multiple lambing rates and litter size at the synchronized oestrus (Husein and Kridli, 2003; Karaca et al., 2009).

The twinning rate in the present study in those of LT was comparable with LT 51.6% in ewes' synchronization for 14 days and PMSG during breeding season obtained by Koyuncu and Ozis, (2010). Whereas in those of LT2 and LT1 were higher than in those LT 36.7% in ewes synchronized for 12 day using FGA + 300 IU PMSG obtained by Anilkumar et al. (2010) and higher than in ewes synchronized for 12 days versus 7 days during breeding season in those of ST1, LT1 and ST2 obtained by Karaca et al. (2008). The differences in the twinning rate may be due to the differences of gonadotropins, device of progesterone (CIDR or sponge), the dosage of gonadotropin given, the time of injection of gonadotropins and progestogen treatment protocols (Dogan and Nur, 2006; Amer and Hazzaa, 2009).

Conclusion

The administration of eCG in this study improved the fecundity in the Dammar ewes during the short or long CIDR treatment. These results in the present study are consistent with the results obtained by Ali (2007) which showed that the administration of eCG supported and improved the fecundity but did not support the fertility in the Ossimi ewes. The fecundity rate in those of LT2 was

higher than those for 12 days in MAP-500 IU eCG at sponge removal during breeding season (Safdarian et al., 2006). A similar study showed result higher than those of LT in ewes treated for 14 days with AI as obtained by Zeleke et al. (2005); also, higher than in those of LT in ewes treated for 12-day- FGA-eCG obtained by Mustafa et al. (2007). The reason for the difference in the results on the fecundity rate may be due to the difference breed, seasons, difference of gonadotropins, used device of progesterone (CIDR or sponge), the dosage of gonadotropin, time of injection of gonadotropins, progestogen treatment protocols (Dogan and Nur, 2006; Amer and Hazzaa, 2009). In conclusion, these results indicate that CIDR could successfully be used for either 6 or 12 days prior to the ram introduction without having to administer eCG. However, eCG is important to induce multiple births.

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