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New Technique for Activating Reproductive System during Non-Breeding Season in Ghezel Ewes

Research Article

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ABSTRACT

Ghezel sheep is one of the main breeds in the north-west of Iran which is reared for meat, milk and wool production. Improving reproduction of this breed will have a significant impact on the economic development of local farmers. Therefore, in the present study the effect of simultaneous use of estrus synchronization (controlled internal drug releasing (CIDR) and equine chorionic gonadotropin (eCG)) and flushing diet on reproduction during non-breeding season in Ghezel ewes was determined. A total of 114 three-year-old Ghezel ewes were equally divided into three treatment groups. Group 1 was synchronizing with CIDR and eCG and was flushed for 40 days. Group 2 was flushed only for 40 days and group 3, the control did not receive any treatment at all. Rams also received a flushing diet concurrent with ewes. CIDR application was performed for 12 days, following CIDR withdrawal, each of the group 1 ewes received an intramuscularly injection with 400 IU eCG. Thereafter, all ewes were introduced to the rams. Pregnancy was determined by ultrasound at 50 days after introduction to the rams. Lambing rate in group 1 was 276.32% and the pregnancy rate was 100%. This result was significantly different from group 2 and 3. Although a minor improvement in pregnancy percentage was observed in group 2 in comparison with control group, the overall results suggested that flushing alone was not effective in activating the reproductive system in non-breeding season. This study showed that estrus synchronization using CIDR and eCG accompanied with flushing diet was a suitable technique to improve lamb production during non-breeding season in Ghezel ewes.

KEY WORDS CIDR, eCG, flushing diet, Ghezel ewes, non-breeding season, reproduction.

INTRODUCTION

To keep up with the increasing growth of human population, food production and supply must be increased, especially in developing countries. Meat and milk are high-value foods of animal origin and are crucial for human health. Therefore, optimization of animal production is necessary and new techniques must be researched to reach this goal. In many countries sheep are mostly reared for wool production, but the dual purpose option must be considered. The ovine industry in Iran mainly produces meat and wool is only a second priority product (Saadatnouri and Siahmansour, 2003; Khaldari, 2008). Ghezel sheep is an Iranian fat-tailed; medium-sized breed from the north-west provinces of Iran (including east Azerbaijan and west Azerbaijan provinces). The breed is multipurpose and meat, wool, skin as well as milk are valuable products of this sheep. Growth rate of this sheep is high and is about 200 g/day (Izadifard and Zamiri, 2007). The reproductive cycle is an annual cycle regulated by a photoperiod, but energy intake may also be a factor of interest (Rosa and Bryant, 2003). Environmental temperature, nutritional status, social inter-

actions, lambing date and lactation period modulate the reproduction of sheep (Rosa and Bryant, 2003). The technique of increasing in energy levels of diets during a short time interval known as flushing increases ovulation rate (Downing and Scaramuzzi, 1991) by increasing in FSH secretion rate in the luteal phase (Nottle *et al.* 1987).

Estrous synchronization is a technique which is used for coordinating estrus cycle and increasing in lambing rate (Khaldari, 2008). Estrous synchronization can be done using controlled intravaginal drug releasing devise (CIDR) or progesterone sponge alone or accompanied by gonadotropin (Rhodes and Nathanielsz, 1998). Equine chorionic gonadotropin (eCG) with a long half-life and no need for several injections is the main used gonadotropin for superovulation (Leyva *et al.* 1998; Ali, 2007; Wheaton *et al.* 1993).

Since the response of sheep to synchronization appears to be breed dependent, it is necessary to investigate whether flushing in combination with synchronization can successfully decrease the period of seasonal anestrus in Ghezel ewes or not. To the best knowledge of the authors, there are no reported studies regarding the use of flushing and synchronization with CIDR and eCG on the lambing rate of Ghezel ewes in the non-breeding season.

MATERIALS AND METHODS

In the present study, 114 three-year-old Ghezel ewes with average body weight of 66 kg were used. Animals were selected from a commercial flock in Sofyan area, east Azerbaijan province, Iran. All procedures used in this experiment was warranted based on University of Tabriz Animal Care and Ethics Committee. Ewes were randomly allotted to one of 3 groups. Group 1 was flushed for 40 days and was synchronized with a CIDR and eCG, group 2 was only flushed for 40 days and Group 3 was the untreated control group. The ewes in group 1 and group 2 were flushed from 2 March till 11 April 2014 (Table 1). Rams also received the flushing diet during the period they spend with the ewes. A progesterone-releasing device (CIDR), (Pfizer, New Zealand) was placed intravaginally in group 1 ewes 7 days after flushing and was removed after 12 days. During 12 days of CIDR placement, seven ewes from group 1 showed mild vaginitis. After CIDR removal, group 1 ewes were injected intramuscularly with 400 IU eCG (Pregnecol, Bioniche, Australia) and were introduced to the rams, as sameas the ewes of group 2 and 3 with 1/10 ratio of males/females. Twenty four hours after CIDR removal and introduction to the rams of the flock, ewes in group 1 started showing estrus signs. Maximum estrus was at 48 h after CIDR removal. All matings were recorded during 5 days after CIDR removal. Fifty days after ram introduction, pregnancy was determined by ultrasound using a sector 5.0 MHz probe. Ewes in the group 1 received adequate supplementary diet during three weeks before delivery in order to prevent pregnancy toxemia. Ewes' delivery started at the end of August 2014 and continued to the first few days of September.

Table 1 Ingredient and nutritional composition (dry matter basis) of ewes' rations

Ingredients	Dietary treatments	
	Flushing diet[1]	Basal diet[2]
Alfalfa hay (%)	28	28
Barley straw (%)	29	29
Pelleted concentrate[3]	25	25
Barley (%)	15	15
Wheat bran (%)	3	3
Barley grain-for flushing diet (g)	400	0
Alfalfa hay-for flushing diet (g)	15	0
Chemical component		
Digestible energy (Mcal/kg)	4.29	2.8
Total digestible nutrients (%)	98.6	64
Crude protein (%)	16.4	11.4
Calcium (%)	3.62	3.2
Phosphorus (%)	3.77	2.05

[1] Ewes in treat1 (synchronization with CIDR and eCG+flushing) and treat 2 (flushing) groups fed with the flushing diet.
[2] Ewes in control group fed with the basal diet.
[3] Pelleted concentrate which contained 14% crude protein composed of the following ingredients including: barley grain 25%, corn grain 32%, soybean meal 15%, cottonseed meal 15%, beet sugar pulp 5%, molasses 5%, vitamin (A and D) and mineral supplements 3%.

Almost all ewes delivered without any help, except three. Finally, all the data had been recorded and were analyzed using a chi-square test. Effects were also considered to be significant at levels of probability less than 5%.

RESULTS AND DISCUSSION

All ewes in the group 1 were judged pregnant by ultra sound diagnosis, while only four ewes in group 2 were found pregnant. All ewes in the control group were non-pregnant. Lambing rate and pregnancy percentage both affected significantly ($P<0.0001$) by treatments. Pregnancy percentage was 100% in the group 1, but only 11% in the group 2, while none of the control group ewes were recognized pregnant (Table 2). Lambing rate was 276.32% in the group 1, while it was 15.79% in group 2 and 0 in group 3 (Table 3). Thirty eight ewes in the group 1 delivered 105 lambs, included 1 singleton, 9 twins, 26 triplets and two quadruplets (Figure 1). Ewes in the group 2 delivered only six lambs, included 2 singletons and 2 twins (Figure 1).

In this study, it was shown that the estrus cycle of Ghazal sheep in the non-breeding season and successive lamb production could be attained by flushing and estrous synchronization techniques. Finally, the technique resulted in high lambing rate. Under our experimental conditions, flushing alone could not shorten seasonal anestrus effectively, though some weak responses were observed. In a similar study on Sanjabi ewe breed in Iran, it was shown that CIDR synchronization in the non-breeding season increased the

estrus prevalence (Mamoui *et al.* 2009). In Jordan, Husein *et al.* (2007) reported that 12 days intravaginally application of fluorogestrone acetate accompanied with eCG injection in Avasi ewes also improved reproductive performance in non-breeding season.

Martinez *et al.* (2015) using a special protocol [7-day treatment with an intravaginal progesterone-releasing device (IPRD), administration of GnRH (50 or 100 µg) at IPRD insertion on day 0 and equine chorionic gonadotropin (eCG) and prostaglandin F2α at IPRD removal on day 7], reported a significant increase in oestrus, ovulation, pregnancy and lambing rates out of the breeding season in adult Romney-cross and Dorset-cross ewes.

Table 2 Treatment groups by pregnancy

Treatments	Pregnancy		Total
	0 (non-pregnant)	1 (pregnant)	
Group 1	0	38	38
	0.00	33.33	33.33
	0.00	100.00	
	0.00	90.48	
Group 2	34	4	38
	29.82	3.51	33.33
	89.47	10.53	
	47.22	9.52	
Group 3	38	0	38
	33.33	0.00	33.33
	100.00	0.00	
	52.78	0.00	
Total	72	42	114
	63.16	36.84	100.00

Frequency missing= 1.

Almadaly *et al.* (2015) has been reported that several synchronization protocols [including: intramuscular injection of 20 mg progesterone (P4) for 12 days and eCG on day 12; two doses of prostaglandin (PG) F2α with 9 days interval and eCG on day 9; whole (20 mg), or halved (10 mg) progesterone releasing intravaginal device (PRID) for 6, 8 and 14 days, PGF2α a day prior to PRID removal and eCG at PRID removal] all had an increasing effect on ewes' fertility (estrus, pregnancy and lambing rates) during the non-breeding season in Rahmani ewes. Flushing and synchronization (intravaginal fluorogestrone acetate+injection of 600 IU pregnant mare serum gonadotropin (PMSG)) in Ak-karaman sheep breed (turkey) was associated with increased multiparity and weaning rate in comparison to non-treated animals (Esen and Bozkurt, 2001). Gardón *et al.* (2015) using 60 mg medroxyprogesterone acetate (MAP) sponge for 12 days with or without 450 IU of eCG (equine chorionic gonadotropin) at the time of sponge showed the efficacy of the synchronization method with MAP and the reduction in the time to estrus induced by eCG in Spanish Merino ewes during the breeding season.

Sabra and Hassan (2008) reported that using a flushing diet including 58% corn meal, 41% soybean meal, 0.01% mineral supplement and 1% salt during one month before mating increased estrus prevalence, lambing percentage, and birth weight in comparison with control receiving no flushing diet. Emsen and Yaprak (2006), in another study in turkey, reported that estrus synchronization (intravaginal fluorogestrone acetate and intramuscular injection of 500 IU eCG) in treatment group accompanied with flushing in both treatment and control groups during one month before mating, resulted in sooner return of estrus cycles in the treatment group rather than the control group in Avasi and Red Karaman sheep breeds.

It was also reported that lambing rate and multiparity were higher in ewes of both breeds in the treatment group rather than the control group. On the other hand, lambing rate was increased in control group with flushing in comparison with ewes with no flushing and synchronization (Emsen and Yaprak, 2006). Although ewes' flushing in breeding season showed some improvement in reproduction of control group in the study of Emsen and Yaprak (2006), results of the present study showed that using flushing without estrus synchronization in non-breeding season had almost no effect on reproduction.

Most progesterone compounds including CIDR, fluorogestrone acetate and medroxy progesterone induce estrus in anestrus ewes (Ungerfeld and Rubianes, 2002). The results of the present study proved the ability of CIDR in initiating of estrus cycles in Ghezel ewes during non-breeding season.

Some previous studies in Iran showed the beneficial effects of melatonin in combination with CIDR and PMSG during non-breeding season on the initiation of estrus cycle and improvement of reproductive performance in Lori and Taleshi ewes (Mousavi and Sokhtehzari, 2011; Rasolikhah *et al.* 2013). Our results suggested that even without melatonin, using this protocol in group 1 in Ghezel ewes activated their reproductive system in non-breeding season.

An interesting result of the present study was about the high lambing rate in the group 1, which was 276.32%.

Almadaly *et al.* (2015) reported a range of 0 to 100% in lambing rate using several synchronization protocols during the non-breeding season in Rahmani ewes in which two protocols [two doses of prostaglandin (PG) F2α with 9 days interval and eCG on day 9; halved (10 mg) PRID for 8 days, PGF2α a day prior to PRID removal and eCG at PRID removal] had the best lambing rates (100%). Mohajer *et al.* (2012) used synchronization with CIDR for 14 days and injection of 300 or 500 IU PMSG at the time of CIDR removal accompanied with flushing during breeding season and reported no more than 113% lambing rate (ranged from 87-113%) in Zel ewes.

Table 3 Treatment groups by lambing rate

Treatments	Lambing number					Total
	0	1	2	3	4	
Group 1	0	1	9	26	2	38
	0.00	0.88	7.89	22.81	1.75	33.33
	0.00	2.63	23.68	68.42	5.26	-
	0.00	33.33	81.82	100.00	100.00	-
Group 2	34	2	2	0	0	38
	29.82	1.75	1.75	0.00	0.00	33.33
	89.47	5.26	5.26	0.00	0.00	-
	47.22	66.67	18.18	0.00	0.00	-
Group 3	38	0	0	0	0	38
	33.33	0.00	0.00	0.00	0.00	33.33
	100.00	0.00	0.00	0.00	0.00	-
	52.78	0.00	0.00	0.00	0.00	-
Total	72	3	11	26	2	114
	63.16	2.63	9.65	22.81	1.75	100.00

Frequency missing= 1.

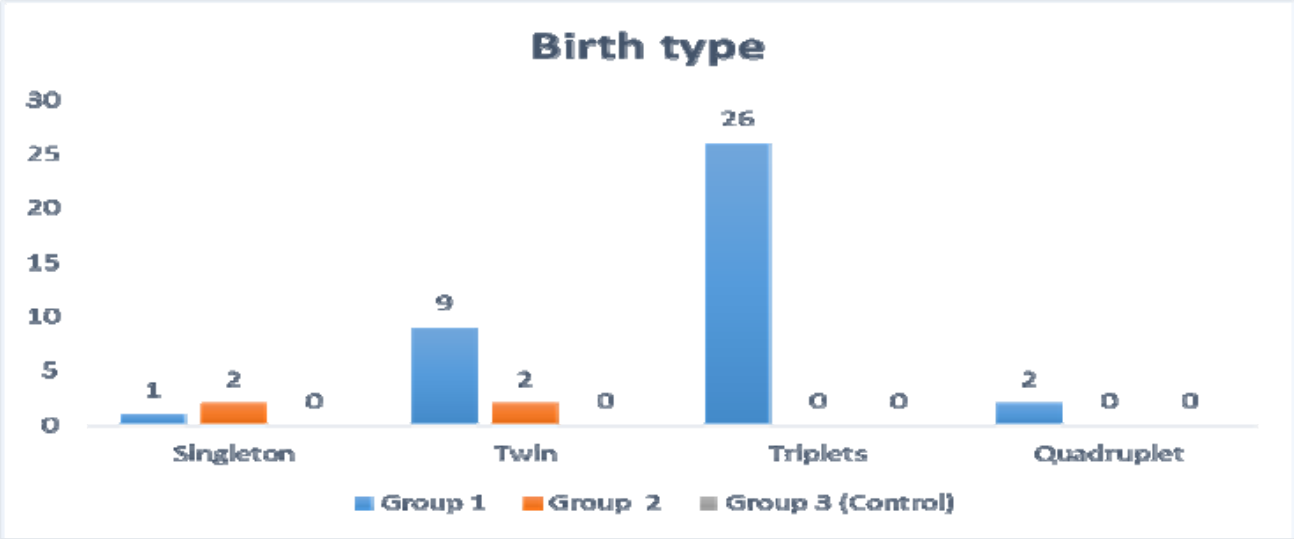

Figure 1 The effects of synchronization with CIDR and eCG and flushing (group 1), flushing (group 2) and no treatment (group 3) on singleton, twin, triplet and quadruplet births in Ghezel ewes in the non-breeding season in Ghezel ewes
Each group included 38 ewes

Also, the highest lambing rate in the experiment of Gardón *et al.* (2015) using estrus synchronization during the breeding season in Spanish Merino sheep was 1.85 lamb per each ewe which was lower than what was observed in the present experiment. Then, higher lambing rate in the present experiment is a breed dependent response. It seems that the genetic potential of Ghezel ewes has not been exploited to its maximum potential.

As in the present study there is no report regarding hormonal profile during flushing and synchronization, therefore, for better understanding the mechanisms, we suggest future complimentary studies consider doing the hormonal profile evaluation.

CONCLUSION

The anestrous period of Ghezel sheep can successfully be shorted by using a treatment protocol with CIDR and eCG in combination with flushing which could be a suitable technique to improve lamb production during non-breeding season in Ghezel ewes.

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