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Effects of Breed and Progestin Source on Estrus Synchronization and Rates of Fertility and Fecundity in Iranian Sanjabi and Lori Ewes

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Abstract: A trial was conducted to evaluate the effects of FGA (Fluorogestone acetate) and CIDR (Controlled internal drug release) on the induction of estrus and pregnancy and fecundity rates of the Sanjabi and Lori sheep. A total of 360 Sanjabi and Lori sheep were randomly grouped into two treatments with intravaginal devices inserted for 13 days: Group FGA (40 mg FGA, n = 180) and Group CIDR (n = 180). All ewes received an i.m. injection of 400 IU eCG (equine chorionic gonadotrophin) at devices removal. Estrous was assessed by exposing all ewes to vasectomized rams at 12 h intervals. Cervical artificial insemination was performed 12 h after estrus onset. The overall estrus response was 72.5%. The source of progestin did not influence the efficiency of estrus response but a significant difference ($p<0.05$) was found between the breed groups (Lori: 88.6%, Sanjabi: 58.3%). Among the sheep that received either CIDR or FGA, estrus response was significantly ($p<0.05$) higher in the Lori (CIDR: 82.2%, FGA: 91.1%) than in the Sanjabi (CIDR: 64.4%, FGA: 52.2%) breed. The lambing and fecundity rates for all groups were 60.2% and 1.2±0.03, respectively. No significant differences in term of the lambing and fecundity rates were recorded between CIDR and FGA groups and among Lori and Sanjabi breed. The results of this study indicate the source of progestin or sheep breed did not influence the pregnancy and fecundity rates. The sheep breed influences the estrous response rate while the source of progestin did not affect the estrous response.

Key words: CIDR, FGA, estrus synchronization, fertility, fecundity

INTRODUCTION

Estrous synchronization is a valuable management tool that has been successfully employed to enhance reproductive efficiency, particularly in ruminants (Kusina *et al*., 2000). In small ruminants, estrus synchronization is achieved either by reducing the length of the luteal phase of the estrous cycle with prostaglandin F2α or by extending the cycle artificially with exogenous progesterone or more potent progestagens (Evans and Maxwell, 1986; Kusina *et al*., 2000; Jainudeen *et al*., 2000). As prostaglandin treatment is limited to the breeding season, different protocols of estrous synchronization using progestins have been introduced (Ainsworth *et al*., 1982; Hamra *et al*., 1989; Gourley and Riese, 1990; Haresign, 1992; Wheaton *et al*., 1993; Godfrey *et al*., 1997; Wildeus, 1999; Naqvi *et al*., 2001; Bretzlaff and Romano, 2001; Boscos *et al*., 2002; Ungerfeld and Rubianes, 2002; Timurkan and Yildiz, 2005; Luther *et al*., 2006). Treatment with intravaginal sponge impregnated with progestagen (Fluorogestone acetate, FGA) or intravaginal device containing progesterone (Controlled Intravaginal Drug Release, CIDR), for a period of 10-16 days and intramuscular injection of eCG at intravaginal device removal, have been successfully used to improve the reproductive performance in ewes (Haresign, 1992; Hill *et al*., 1998; Fukui *et al*., 1999; Kohno *et al*., 2005; Ucar *et al*., 2005; Gomez *et al*., 2006). The use of eCG in estrus synchronization protocols in sheep is well established. A single eCG (equine chorionic gonadotrophin) treatment, after progestin treatment, increases ovarian response, conception rate and percentage of multiple births from induced ovulations (Langford *et al*., 1982; Pearce and Robinson, 1985). However, ovarian response of sheep to estrous synchronization and subsequent fertility and fecundity rates varies with stress, environment, breed, season, the kind of progestagen employed, etc. (Doney *et al*., 1973; Das *et al*., 1999, Romano *et al*., 2000, 2001; Menegatos *et al*., 2003; Evans *et al*., 2004). Therefore, the recognition of the physiological state of females that influences estrus synchronization and subsequent fertility will increase our knowledge about their use.

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Sanjabi and Lori ewes are the most widespread breeds of Kermanshah and Lorestan provinces, Iran, respectively. There is no information regarding estrus synchronization efficiency and fertility, in these breeds, induced by hormonal treatment during the non-breeding season. Thus the current study was conducted to determine the effects of two different intravaginal devices (CIDR and FGA) and an injection of eCG on the efficiency of estrous synchronization and subsequent fertility and fecundity in these breeds during the non-breeding season.

MATERIALS AND METHODS

Animals: The present study was conducted at two local sheep farms located in Kermanshah (latitude 33°36′ N, Longitude 45°24′ E, altitude 1300 m) and Lorestan (latitude 35°15′ N, Longitude 48°30′ E, altitude 1400 m) provinces, Iran, during the non-breeding season from late May to July 2006.

A total of 180 Sanjabi (2-3 years old, 45.5±0.8 kg weight) and 180 Lori (2-3 years old, 42.5±0.5 kg weight) ewes were used in this study. The animals were kept indoors at night and outdoors most of the day. Indoors, the ewes were fed concentrated diets based on cottonseed meal, barley and wheat bran having 2400 kcal ME and 13% crude protein through the experimental period. During the day, the ewes had access to natural grazing. Salt and mineral blocks and water were available *ad libitum*.

Treatment: All ewes on farm A (n = 180 Snjabi Breed) and B (n = 180 Lori Breed) were randomly grouped into two treatments, based on body weight and age, with intravaginal devices inserted for 13 days: A) 40 mg FGA (Fluorogestone acetate, Chronogest®, Intervet, Netherland) (Groupl, n = 90 ewes/each farm) B) CIDR (Type G containing 0.3 g progesterone: Inter Ag, Hamilton, New Zealand) (Group 2, n = 90/each farm). All ewes received an intramuscular injection of 400 IU eCG (Folligon®, Intervet, Holland) at intravaginal devices removal.

In both farms A and B, estrous activity was assessed by exposing all ewes to vasectomized rams (1 ram per 10 ewes), fitted with a marking harness, at 12 h intervals, until artificial insemination. Semen from 10 Sanjabi (Farm A) and 10 Lori (Farm B) rams with probed fertility was collected using an artificial vagina. It was diluted with milk extender containing 1,000,000 IU penicillin G potassium. Cervical artificial insemination was performed 12 h after estrous onset. The ewes on both farms A and B were allowed to term to record and calculated the estrous response (ewes showing estrous/total ewes), pregnancy rate (ewes lambing/total ewes inseminated) and fecundity rate (lambs born/ewes lambing).

Parameters and statistical analysis: A randomized complete blocks design was applied, in which animals of two breeds represent blocks and factor treatment at 2 levels. The following model was used:

$$X_{ij} = \mu + \delta_i + \delta_j + \varepsilon_{ij}$$

Where:

X_{ij} = The amount of each observation,
μ = The mean of the population
δ_i = The effect of block,
δ_j = The effect of treatment and
ε_{ij} = The experimental error.

Effect of breed and treatment on estrous response rate and pregnancy rate were tested using Chi-square test. Effect of breed and treatment on fecundity rate was analyzed using t-test. Statistical analyses were performed using SAS (1999).

RESULTS

No intravaginal devices were lost during the trial and none of the ewes showed estrous while the intravaginal devices were in place.

The overall estrous response rate was 72.5% (261/360, Table 1). Overall the type or source of progestin did not influence the efficiency of estrous synchronization rate (CIDR: 73.3, FGA: 71.7%), but a significant difference ($p<0.05$) was found between the breed groups (Lori: 86.6%, Sanjabi: 58.3%, Table 1). Among the sheep that received either CIDR or FGA, estrous response rate was significantly ($p<0.05$) higher in the Lori (CIDR: 82.2%, FGA: 91.1%) than in the Sanjabi (CIDR: 64.4%, FGA: 52.2%) breed. In the Sanjabi ewes, there was a trend toward higher estrous detection rate in the group that treated with CIDR (64.4%) than in the group that received FGA (52.2%). On the contrary, in the Lori breed, there was a tendency toward reduced estrous detection rate estrous in the group that treated with CIDR (82.2%) that in the group that received FGA (91.1%).

Table 1: Response of Sanjabi and Lori ewes (%, No./total) to estrus synchronization

	Ewes in clinical estrus		
Treatments	Sanjaabi	Lori	Average of groups*
Group 1(CIDR)	64.4 (58/90)^a	82.2 (74/90)^b	73.3 (132/180)
Group 2(FGA)	52.2 (47/90)^a	91.1 (82/90)^b	71.7 (129/180)
Average of breeds	58.3 (105/180)^a	86.6 (156/180)^b	72.5 (261/360)

Means in the same row with different letter (a, b) differ significantly ($p<0.05$), *: Means in the same column were compared ($p>0.05$)

Table 2: Lambing rate (%, No./total) in Sanjabi and Lori ewes after estrus synchronization

Treatments	Sanjaabi	Lori	Average of groups*
Group 1 (CIDR)	62.1 (36/58)	66.2 (49/74)	64.4 (85/132)
Group 2 (FGA)	48.9 (23/47)	59.8 (49/82)	55.8 (72/129)
Average of breeds	56.2 (59/105)	62.8 (98/156)	60.2 (157/261)

Means in the same row with different letter (a, b) differ significantly ($p<0.05$), *: Means in the same column were compared ($p>0.05$)

Table 3: Mean number of lambs born per ewe lambing after estrus synchronization

	Lambs born per ewes lambing		
Treatments	Sanjaabi	Lori	Average of groups*
Group1 (CIDR)	1.3±0.1	1.2±0.10	1.2±0.04
Group 2 (FGA)	1.2±0.1	1.3±0.10	1.3±0.10
Average of breeds	1.2±0.1	1.3±0.04	1.2±0.03

Means in the same row with different letter (a, b) differ significantly ($p<0.05$), *Means in the same column were compared ($p>0.05$)

The lambing rate for all groups was 60.2% (157/261, Table 2). No significant differences in term of the lambing rate were recorded between CIDR and FGA (64.4% versus 55.8%) groups and between Lori and Sanjabi (62.8 versus 56.2, Table 2) groups. Among the sheep that received either CIDR or FGA, the fertility rate tend to be high in the Lori (CIDR: 66.2%, FGA: 59.8%) group, compared to the Sanjabi (CIDR: 62.1%, FGA: 48.9%, Table 2) group. In both Sanjabi and Lori breeds, lambing rate was higher in the group that received CIDR (Sanjabi: 62.1%, Lori: 66.2%) than in the group that treated with FGA (Sanjabi: 48.9%, Lori: 59.8%, Table 2), although this difference was not significant ($p>0.05$).

The overall mean number of lambs born per ewes lambing was 1.2±0.03 (Table 3).

No significant differences were observed either between treatments (CIDR: 1.2±0.04, FGA: 1.3±0.1) or between breeds (Sanjabi: 1.2±0.1, Lori: 1.2±0.04, Table 3).

DISCUSSION

In the current study, no ewes showed estrous while the intravaginal pessaries were in place. Therefore, it can be accepted that the dose of progestagen in the FGA sponges and CIDR device absorbed from the vagina during treatment was sufficient to suppress the preovulatory discharge of pituitary gonadotropins (Baired *et al*., 1975). No CIDRs or intravaginal sponge were lost throughout the experiment period, in agreement with the observation of Romano (1996). Other authors have reported a high number of CIDRs lost in ewes (Welch *et al*., 1984; Ainsworth and Downey, 1986; Maxwell and Barnes, 1986; Rhodes and Nathanielsz, 1988). Previous experience with the use of CIDRs in ewes, techniques employed in inserting the sponge (Romano, 1998) and factors such as intravaginal sponge texture and consistency could influence sponge retention in the vagina (Alifakiotist *et al*., 1982). In addition, other disadvantages described by Greyling and Brink (1987) while using CIDRs dispensers, such as difficult insertion was not observed in the present study.

In this study, estrous was induced in 72.5% of the Sanjabi and Lori ewes treated with CIDR and FGA, 24-60 h after intravaginal devices withdrawal. No significant difference in overall percentage of ewes exhibiting estrous was recorded between ewes synchronized with CIDR or FGA (73.3% versus 71.7%, Table 1). In several experiments, during the breeding and non-breeding season (Crosby *et al*., 1991; Fukui *et al*., 1999; Ungerfeld and Rubianes, 2002; Kohno *et al*., 2005; Luther *et al*., 2006; Hashemi *et al*., 2006), similar results were observed. But Knight and Hall (1988) cited the estrous response following CIDR removal to be significantly lower than following sponge treatment (87% versus 94%). This was, however, related to higher loss of CIDRs, compared to sponges (63% versus 0.8%).

The results of the present study show that, there was a tendency toward reduced estrous detection rate in the group that treated with CIDR (82.2%) than in the group that received FGA (91.1%) in the Lori ewes. On the contrary, in the Sanjabi ewes, there was a trend toward higher estrous detection rate in the group that treated with CIDR (64.4%) than in the group that received FGA (52.2%). This finding is in agreement with and contrary to the observation of Knight and Hall (1988) who cited the estrous response following CIDR removal to be significantly lower than following sponge treatment (87% versus 94%). This was, however, related to highest loss of CIDRs, compared to sponges (6.3% versus 0.8%). In this study, no CIDRs or intravaginal sponge were lost throughout the experiment period so the differences observed between CIDR and FGA in term of estrus detection, in both Sanjabi (CIDR: 64.4%, FGA: 52.2%) and Lori (CIDR: 82.2%; FGA: 91.1%) ewes, show that sheep breed may influence the estrous response. It has been reported that (Naqvi *et al*., 1999a, b; Das *et al*., 1999; Romano *et al*., 2000; Romano *et al*., 2001; Menegatos *et al*., 2003; Evans *et al*., 2004) some factors such as season, breed of sheep, type of progestagen, use of eCG and ram introduction might influence the estrous onset. Overall, Absence of significant difference in term of estrous response between ewes treated with CIDR or FGA (Table 1) demonstrates a similar efficiency of the two intravaginal devices in inducing estrous during the non-breeding season. These two types of devices can be used interchangeably, based on the availability and cost effective.

The results of this study demonstrate that the estrous response of Lori ewes to CIDR and FGA (Overall: 86.6%; CIDR: 82.2%; FGA: 91.1%) was significantly higher ($p<0.05$) than that for the Sanjabi ewes (overall: 58.3%, CIDR: 64.4%; FGA: 52.2%). This is most likely due to the fact that the Lori is a more prolific breed than the Sanjabi breed. Jainudeen and Hafez (1987) also reported prolific sheep respond better to hormonal treatment for the purpose of estrus synchronization than less prolific breeds. This finding is in agreement with the finding of Emsen *et al.* (2006) who reported higher estrous response in Red Karaman (92.5%) compared to Awassi (73.3%) ewes, when using FGA and 500 IU eCG during the breeding season. To the contrary, Boscos *et al.* (2002) recorded similar estrous response to MAP and 10 IU FSH or MAP and 400 IU eCG during the mid-breeding season in Chios and Berrichon ewes. Also Ungerfeld and Rubianes (2002) found no significant difference between Polwarth and Polwarth x Ile de France ewes regarding estrous response after short term priming with MAP, FGA or CIDR in combination with eCG during the non-breeding season.

In the present study, the overall estrous response using CIDR device and eCG was 73.3%, which is lower than the 93.3% obtained by Hashemi *et al.* (2006) in Karakul ewes. Estrus synchronization response ranging between 90 and 100% has been recorded in other experiments carried out during the non-breeding season and involving treatment with CIDR and eCG (Crosby *et al.*, 1991; Van Cleef *et al.*, 1998; Godfery *et al.*, 1999; Ungerfeld *et al.*, 2002; Kohno *et al.*, 2005; Luther *et al.*, 2006; Hashemi *et al.*, 2006). In the current study, the overall estrus response using FGA sponge and eCG was 71.7%, which is similar to 73 and 77% reported by Mutiga and Mukasa-Mugerwa (1992) and Rajamahendran *et al.* (1993) in Menze and Dorset ewes, respectively, but lower than the 100% reported in Hampshire and Montadale ewes (Luther *et al.*, 2006). Estrous synchronization success ranging between 75 and 100% has been obtained in other experiments carried out during the non- breeding season and involving FGA with eCG in Suffolk ewes (Crosby *et al.*, 1991), in Menze ewes (Mutiga and Mukasa-Mugerwa, 1992), in Dorset ewes (Rajamahendran *et al.*, 1993), in Polwarth and Polwarth x Ile de France ewes (Ungerfeld and Rubianes, 2002) and in Awassi and Red Karaman ewes (Emsen and Yaprak, 2006). The differences reported by different researchers on estrous response rate can be explained by the differences in body condition, breed and management.

Although many factors (type of intravaginal device, dose and timing of eCG injection, semen type, time and number of AI, breed and age of ewes, season and others) affect the fertility of ewes with synchronized estrus and ovulation, the fertility results at the first estrus after hormonal treatment are contradictory (Hamra *et al.*, 1986; Greyling and Brink, 1987; Crosby *et al.*, 1988; Wheaton *et al.*, 1993). The present study found no significant differences in the pregnancy and fecundity rates among CIDR and FGA groups as well as Sanjabi and Lori groups (Table 2 and 3). This agrees with other reports (Gordon, 1983; Fukui *et al.*, 1993, 1999; Romano *et al.*, 1996; Boscos *et al.*, 2002; Ungerfeld *et al.*, 2002; Kohno *et al.*, 2005; Emsen and Yaprak, 2006; Luther *et al.*, 2006). On the other hands, there are reports showing that one intravaginal device is superior to or inferior to other. Crosby *et al.* (1988) found that the lambing rate with natural mating was significantly lower in progesterone treated ewes than in MAP or FGA treated ewes. Wilson and Maxwell (1989) compared CIDR and FGA (30 mg) sponges in, 1,058 Merino ewes inseminated with frozen semen and found that significantly more ewes treated with FGA sponges became pregnant than those treated with CIDR. On the other hand, the effectiveness of CIDR on synchronizing estrus and a lambing rate similar to that with the MAP or FGA sponge in cyclic ewes has also been reported (Hamra *et al.*, 1986; Greyling and Brink, 1987; Wheaton *et al.*, 1993). Recently, Hill *et al.* (1998) reported that significantly fewer pregnancies after AI with frozen semen were obtained in ewes treated with MAP sponges (64.4%) than with FGA (40 mg: 72.1%) and CIDR (71.1%) treatment. But, no significant difference in the fertility of ewes treated with FGA and CIDR was found in the present study, although CIDR appears to be superior to FGA. These results indicate that the source of progestin and sheep breed does not affect the pregnancy rates following AI. Similar effects for CIDR and FGA spoges on pregnancy rates are likely due to comparable rates regarding the release of progestin from these sources. In fact, it has been demonstrated that the insertion of CIDR's resulted in a sharp increase in serum progesterone concentration in sheep for 3 or 4 days-followed by a decline 6 days after treatment (Wheaton *et al.*, 1993; Rubianes *et al.*, 1998). Similar pharmacokinetic results have been reported using intravaginal sponges containing MAP (Greyling *et al.*, 1994) and FGA (Gaston-Parry *et al.*, 1988) in ewes. However despite these similarities between progestin types, Wilson and Maxwell (1989) did report higher pregnancy rates in ewes treated with FGA sponges, when compared to CIDR devices during the breeding season. Therefore, the source of progestin used for estrous synchronization and AI in sheep may still warrant further investigation. Progestogen plus eCG treatment permits a wide degree of variation in conception rate among breeds,

flocks and years and in some cases high incidence of complete embryo loss and infertility (Haresign, 1992). So this information should be taken into account as different sheep breeds were used.

CONCLUSIONS

In conclusion, the results of present study indicate that:

- The dose of progestagen in the FGA sponges and CIDR device absorbed from the vagina during treatment was sufficient to suppress the preovulatory discharge of pituitary gonadotropins.
- The sheep breed influences the estrous response rate while the source of progestin did not affect the estrous response.
- Similar efficiency of the two intravaginal devices in inducing estrous during the non-breeding season show that these two types of devices can be used interchangeably, based on the availability and cost effective.
- There were no significant differences in the pregnancy and fecundity rates among CIDR and FGA groups as well as Sanjabi and Lori groups.
- Administration of progestin using intravaginal devices and eCG to Sanjabi and Lori ewes in the non breeding season appear to be effective in the synchronization of estrous and parturition and in increasing mean litter size and rate of pregnancy.

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