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Study of Reproductive Performance of Crossbred Ewes Treated with GnRH and PMSG during Breeding Season

Research Article

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ABSTRACT

Reproductive performance was evaluated in non-lactating crossbred ewes to which were administered the exogenous GnRH and PMSG. The ewes were randomly allocated in three treatment groups (n=40). After accurate detection of estrus and 2 hours prior to mating, 2.5 mL of distilled water and 2.5 mL of GnRH were injected intramuscular to each ewe of the first (control) and second group, respectively. In the third group, the ewes were pretreated with CIDR for 14 days and received 400 IU PMSG at the time of withdrawal of the CIDR. After injection of PMSG, fixed-time artificial insemination was performed with 0.5 mL of fresh diluted semen. No significant differences were observed in term of the pregnancy, lambing, and fecundity rates between ewes treated with GnRH and control group (87.5, 97.5, and 1.114% vs. 75, 82.5, and 1.1%, respectively). Twining rate was higher in the ewes treated with GnRH than synchrony or control groups (18.18, 4, and 6.5% respectively, $P<0.05$). In the artificially inseminated group, pregnancy, lambing and fecundity rates were 77.5, 62.5, and 0.81%, respectively. In conclusion, the results showed that treatment of ewes with GnRH at time of estrus and prior to mating, improved the conception and twining birth rates. Also injection of PMSG after CIDR removal caused an increase in efficiency of fertility rate and shorter breeding period.

KEY WORDS crossbred ewes, GnRH, lambing rate, PMSG.

INTRODUCTION

Reproduction efficiency plays a critical role in determining profit potential for livestock production systems. Hormonal treatment to control ovulation and reproduction is a prerequisite for successful breeding and increasing the number of pregnant females (Husein *et al*. 2005), resulting in short breeding period and more uniform newborn crop (Husein and Kridli, 2003). Pituitary gonadotropins are a key component for growth of ovulatory follicles in ewes. Growth of antral follicles beyond 2 mm in diameter cannot occur in the absence of gonadotropins (Driancourt *et al.* 1987). Gonadotropin releasing hormone GnRH (Akif Cam and Kuran, 2004), follicle stimulating hormone FSH (Boscos *et al*. 2002) and pregnant mare's serum gonadotropin PMSG (Dogan and Nur, 2006) increase the number of growing follicles, ovulation rate and litter size. The GnRH modulates the secretion of luteinizing hormone LH and FSH, and the endogenous surge of LH during estrus is needed for ovulation and luteinizing of granulosa and theca cells to form luteal cells for subsequent progesterone secretion (Henderson, 1979). Injection of GnRH at time of estrus, increases plasma progesterone concentrations and improves fertility (Zare Shahneh *et al*. 2008). A single injection of GnRH has also been widely used to manipulate the patterns of ovarian follicular development in cattle (Macmillan *et al*. 2003). The administration of the GnRH reduced the variation in the timing of the LH surge for goats, improving the

synchrony of ovulation (Pierson *et al*. 2003). Estrus in the ewe is a less obvious event than in the other ruminants (Ptaszynska, 2001). Hence, a detailed detection of estrus stages becomes crucial in this species, particularly in hand-mating or artificial insemination (AI). Synchronization of estrus has been used to increase reproductive efficiency in most domestic animals including ewes. Wheaton *et al*. (1993) reported that sponges and controled internal drug release CIDR were effective when used with PMSG in ewes. Pearce and Robinson (1985) reported that use of a single eCG injection in ewes, after progestin treatment, increased ovarian response, conception rate, and percentage of multiple births from the induced ovulations. Injection of PMSG is required to stimulate follicular growth, leading to a higher ovulation rate by an estrus animal out of the breeding season (Greyling and Van Niekerk, 1991). During the non-breeding season, Moeini *et al*. (2007) reported 72.5% of estrus and 60.2% of fecundity using CIDR-eCG in Iranian Sanjabi and Lori ewes. Moreover, high doses of PMSG has been shown a potential negative effect on pregnancy rates in cattle (Drion and Roover, 2001). Because of decreasing crossbred ewe's population in recent years (Jafari, 2008), the strategies which can improve population of this animal can be profitable for farmers and helps to conserve this animal. Therefore, the main objective of this study was to evaluate the effect of the hormonal treatment on lambing rate of crossbred ewes during breeding season.

MATERIALS AND METHODS

This trial was performed at the animal research station of the College of Agriculture in University of Tabriz, Iran. It has an altitude of 1567 m above sea level and latitude of 38˚ 07′ N and 46˚ 29′ E, with an average rainfall and temperature of 4 mm and 25 ˚C, respectively, for July (Institute National de Meteorological). Non-lactating crossbred ewes (n=120, 2 to 5 year old, average body weight ±SEM= 47.9±4.6 kg; body condition score ±SEM= 2.6±0.2, in 1-5 scale) were used for this study. Estrus was detected by 6 teaser rams.

The ewes were kept indoors at night and outdoors most of the day. Indoors, the ewes were fed concentrated diet based on cottonseed meal, barley and wheat bran having 2450 kcal ME and 14% crude protein through the experimental period. Water and a mineral supplement were available *ad libitum*.

Animals (Baluchi×Moghani, Ghezel×Baluchi, Arkhar-Merino×Ghezel and Arkhar-Merino×Moghani) were randomly allocated in three treatment groups (n=40). Each genetic group classified to three treated groups randomly. A fter accurate detection of estrus and 2 hours prior mating, 2 mL distilled water and 2.5 mL GnRH (CinnaRelin; CinnaGen Biopharma Co, Tehran, Iran) were injected intramuscular to first (control) and second group, respectively. In these two groups ewes were mated with the proven rams. In the third group, the ewes were pretreated with CIDR (EAZI-BREED; Pfizer New Zealand Ltd, Auckland, New Zealand) for 14 days and received 400 IU PMSG (folligon; Intervet International B.V., Boxmeer, Holland) at the time of withdrawal of the CIDR. After injection of PMSG, fixed-time AI with 0.5 mL fresh diluted semen (approximately 2×10^8 spermatozoa/mL) was performed in these animals.

Statistical analysis

The experiment was laid out in a completely randomized design. The mean values for estrus response, duration of pregnancy, Lamb birth weight, pregnancy, lambing, and fecundity rates were analyzed by ANOVA (SAS, 2003); post hoc comparisons were made using Turkey's test. Statistical significance was defined as $P<0.05$. Pregnancy, lambing, and fecundity rates were calculated as follows (Zeleke *et al*. 2005):

Pregnancy rate= (ewes lambing/ewes inseminated) × 100
Lambing rate= (lambs born/ewes inseminated) × 100
Fecundity rate= (lambs born/ewes lambing) × 100

RESULTS AND DISCUSSION

The highest pregnancy, lambing, and fecundity rates were recorded in ewes received GnRH 2 hours prior mating compare to ewes given PMSG at the time of withdrawal of CIDR and hence AI at fixed time or control group ($P<0.05$, Table 1). The results showed that the group receiving GnRH on 2 hours prior to mating had higher lambing and twining rates than the other groups (Table 1 and 2). All the ewes used in the third group, exhibited overt sings of estrus during the 24-48 hours observation period. However, the differences among any of the groups in terms of the duration of pregnancy and lambs birth weight rate were statistically insignificant ($P>0.05$, Table 2). Increasing sheep productivity by increasing lambing frequency and fecundity is considered important for crossbred sheep production in northwest Iran. On the other hand, increasing rate of fecundity in sheep offers the best opportunity to increase the efficiency of lamb meat production. Hence, in this study to increase fertility rate and induce follicular growth, an administration of GnRH and PMSG was used. The results showed that injection of GnRH at the time of estrus and prior mating increased lambing and twin rates during breeding season. These results are in line with previous reports in ewes (Eppleston *et al*. 1991; Safranski *et al*. 1992; Turk *et al*. 2008; Zare Shahneh *et al*. 2008). In contrast, Naohisa *et al*. (1999) reported that a single injection of GnRH or hCG at day 12 after CIDR removal did not improved fertility in Suffolk ewes.

Table 1 Effect of administration of GnRH and PMSG on pregnancy, lambing and fecundity rates in crossbred ewes

Treatment	n	Pregnancy rate (%)	Lambing rate (%)	Fecundity (%)
Control	40	75 (30/40)	82.5 (33/40)[a]	1.1 (33/30)[a]
GnRH	40	87.5 (35/40)	97.5 (39/40)[a]	1.114 (39/35)[a]
AI-PMSG	40	77.5 (31/40)	62.5 (25/40)[b]	0.81 (25/31)[b]

Means within a column with different superscript are significantly different (P<0.05).

Table 2 Mean of twining rate, duration of pregnancy and lamb birth weight among treatment groups

Traits	Control	GnRH	AI-PMSG
Twining birth	2 (6.5%)[b]	6 (18.18%)[a]	1 (4%)[b]
Duration of pregnancy (d)	149-157	148-155	149-153
Lamb birth weight (kg) (mean±SEM)	5.1±0.4	4.3±0.4	4.6±0.4

Means within a row with different superscript are significantly different (P<0.05).

Injection of GnRH at time of estrus, increased plasma progesterone concentration and improved fertility rate (Zare Shahneh *et al.* 2008). A dose of GnRH can increase the number of gonadotropin dependent follicles, which grow up until pre-ovulatory phase in response to FSH (Lopez-Alonso *et al*. 2005). The GnRH surge making simultaneous control of FSH and LH surge due to ovulation, but the second FSH surge was not affected (Bowen *et al*. 1998). In ewes, pre-ovulation induction of LH/GnRH surge more likely involves the sequential action of estrogen on the arcuate nucleus + preoptic area (ARC+POA) cell populations (Sirjani *et al*. 2011). Reyna *et al.* (2007) reported that GnRH application had positive effect in synchronizing the time of ovulation but had no effect on the growth or atresia of the ovulatory or subordinate follicles. The response to GnRH depends on the moment of the cycle at which the hormone is administered (Geary *et al*. 2000). It was also showed that a GnRH injection, 24 hours after CIDR removal could enhance the number of embryos in multiple ovulation and embryo transfer (MOET) programs (Menchaca *et al.* 2009).

The effect of GnRH on embryo survival may occur through GnRH-stimulated LH surge stimulating production of progesterone by CL and/or causing ovulation and the formation of accessory CLs (Khan *et al.* 2003). In another study, because of ovulation rate and prolificacy are the lowest during the spring / summer period gonadotropin treatment at the end of progesterone treatment has been used to increase prolificacy of anestrous ewes (Safranski *et al.* 1992). When GnRH was used in the non-breeding season, and withoutprogestogen priming, it resulted in the formation of short-lived corpus luteum (CL) (Hunter *et al.* 1986) and fewer CL than ovulated follicles (Bartlewski *et al.* 2001). This problem may be overcome by progestogen treatment before the administration of GnRH (McLeod and Haresign, 1984).

Administration of GnRH eliminates the large follicles by ovulation of atresia and induces the emergence of a new follicular wave within 3 to 4 day at any stage of the estrus cycle in cattle (Twagiramungu *et al.* 1992).

Zeleke *et al*. (2005) reported that the administration of PMSG, 24 hours prior to or at progestogen sponge withdrawal was essential to obtain better fertility rates in ewes with induced estrus following AI. Hence, to obtain better results, the present study used injection of PMSG, at the time of withdrawal of the CIDR as an aid to synchronization of ovulation. Kim *et al*. (2005) indicated that PMSG has both FSH-like activities and LH-like activities which stimulate the growth of ovarian follicles. Mohd Azam *et al*. (2009) reported that the administration of PMSG lead to a decrease in the progesterone levels, thus stimulating estrus and ovulation in the treated animals and also reported that the groups of crossbred ewes that received PMSG injections came into estrus (onset of estrus) earlier, as compared to the group which did not receive PMSG injections. Simonetti *et al*. (2008) concluded that 500 IU of eCG given after 12 days of progestogen treatment increased serum concentration of estradiol during the periovulatory period, particularly in anestrous ewes; this probably resulted in the synchronous estrus and ovulation in anestrous ewes. There are several possible mechanisms by which eCG increases the number of large follicles. It may enhance the entry rate of small and medium follicles into larger sized follicles and it may also prevent the occurrence of natural follicular atresia (Mandiki *et al.* 2000). Greyling and van Der Nest (2000) indicated that the administration of 300 IU PMSG at sponge withdrawal showed no significant effect on the number of goats exhibiting estrus. Results of the present work indicated that estrus was induced in 100% of the ewes, 24 to 48 hours after CIDR removal. This technique (CIDR+PMSG) has an estrus response similar to the 100% obtained by Hashemi *et al*. (2006) in Karakul ewes, but higher than the 66.0% obtained with Romney Marsh ewes (Gatica and Correa, 1993).

The difference in age or breed probably account for the slightly better results in our experiments. The lamb birth weight was not affected by treatment, that agree with results obtained by Titi *et al*. (2010) in Awassi ewes, while disagrees with results obtained in the goats (Titi *et al.* 2010).

Average weight of lambs born from ewes that received 2.5 mL GnRH (4.3±0.4 kg) was lower than the lambs from females that did not receive GnRH (5.1±0.4 kg). It can be because of the increase of lambing rate in the ewes received GnRH and this attributes to production of more lambs with lower weight mean (Alifakiotis, 1986). The cause of different findings reported by different researchers on lamb birth weight can be explained by the differences in management systems, age of dam, body condition, and breed of the experimental animals.

Similarly, duration of pregnancy also did not affected by treatment, that agrees with results obtained by Khaldari *et al*. (2003) in Zandi ewes during breeding season. Ewes, those 35 days (after second estrus period) after insemination did not display estrus behavior, are considered as pregnant ewes. L ambing rate in the inseminated ewes was lower than 15% of pregnant ewes (Figure 1), the reasons for these differences may be outlined as follows: some of ewes showed pseudo estrus, fetus abortion in early pregnancy because environmental factors and genetics detects.

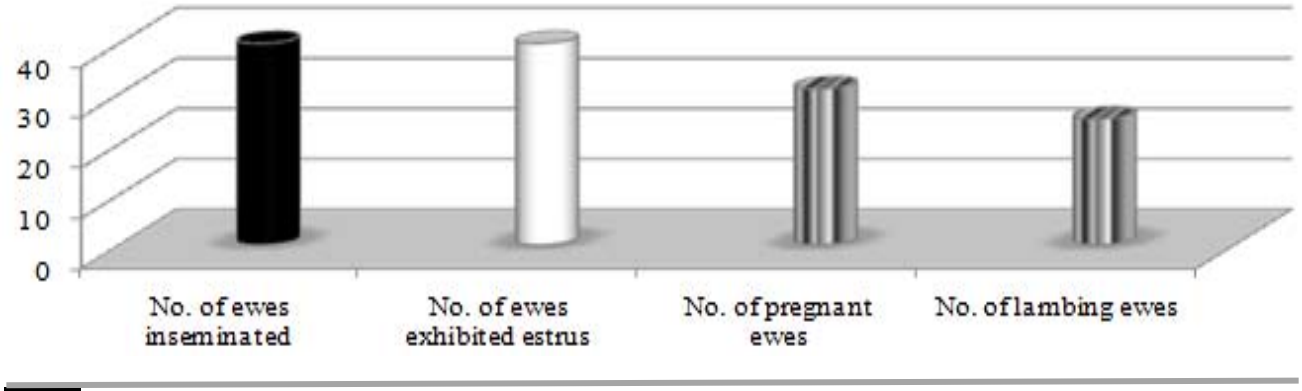

Figure 1 Reproductive performance of ewes with diluted semen in skim milk in AI-PMSG group

Cooper (1982) reported that mean abortion in early pregnancy was about 25%. Another reason for difference between pregnancy and lambing rates may be that long half-life of eCG leads to the formation of large anovulatory follicles which negatively affect early embryonic development and oviductal transport (Husein and Ababneh, 2008). Results of the present work about artificially inseminated ewes are in line with results obtained by Jafariahangari *et al.* (1997) in Moghani ewes, reported that mean pregnancy and lambing rates are equal to 75 and 55% during the breeding season, respectively. Dogan and Nur (2006) obtained 76.5% pregnancy rate with AI at a fixed time after the use of the MAP-PMSG protocol during nonbreeding season in Kivircik ewes. In the present study, a single 2.5 mL GnRH or 400 IU PMSG was employed. Therefore, administration methods of GnRH or PMSG (dose and injection number) as well as the injection timing at the induced luteal phase should be involved in a future study.

CONCLUSION

Results of the present study indicated that the administration of GnRH at the moment of estrus detection and prior mating improved their conception rate and twining birth rate in crossbred ewes during the breeding seasons, and also a single injection of PMSG after CIDR removal increases efficiency of fertility rate and may shorten the breeding period, which in turn may result in increased profit through better animal productivity.

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