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The Effect of GnRH Given on Day of Mating on Ovarian Function and Reproductive Performance in Lohi Sheep

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ABSTRACT

The present study was conducted to investigate the effects of GnRH (Dalmeralin) treatment given on the day of mating on reproductive performance in Lohi sheep. Seventy six ewes 3-5 year old, weighing 44 ± 0.4 Kg were put to rams at synchronized estrus using two intramuscular injections of 2 ml $PGF_{2\alpha}$ analogue (Dalmazin) 11 days apart. These animals were divided into two equal groups through random stratification by body weight and were given either saline (group 1) or 2 ml GnRH (group 11) on day of mating. A total of 16 ewes were slaughtered on day 25 of pregnancy (eight ewes from each treatment group). GnRH administration on the day of mating increased ($P<0.01$) plasma progesterone concentration compared to control. There was a tendency for a higher non-return rate and a lower number of ewes returned to estrus in the GnRH treated ewes, but the difference between the two groups was non significant ($P>0.05$). Pregnancy rate was higher in GnRH treated group (83.3%) than control (60%) group ($P<0.05$). The ewes in GnRH administered group had more twins ($P<0.05$) than those in control group (28 v 10). In conclusion, GnRH administration improved reproductive performance of ewes when administered on the day of mating, probably through its beneficial effect on embryo survival by enhancing luteal function.

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INTRODUCTION

Small ruminants play an important role in the economy of Pakistan by providing annually 578 tones of meat, 32.1 million skins for expanding leather industry, 41.0 tones of wool for carpet industry earning Rs. 4.5 billion in foreign exchange plus providing jobs to thousands of the families. This is the major source of livelihood of over 1.2 million livestock farmers, especially in the arid regions (Anonymous, 2007-08).

The efficiency of production in sheep depends heavily on the reproductive performance of females. The incidence of twin births in Lohi breed of sheep is low and considerable economic advantages would accrue from the availability of effective methods of increasing the reproductive rate in this breed.

Embryonic mortality during early pregnancy causes a significant reduction in the reproductive performance of farm animals. During the first 3 weeks of pregnancy, 30-40% of fertilized eggs are lost in sheep and goats (Nancarrow, 1994; Michels *et al*., 1998). Of this total loss, 70-80% occurs between days 8 and 16 after insemination (Sreenan *et al*., 1996), the majority of this is the result of embryo mortality (Beck *et al*., 1996). Fertilization failure accounts for only 5-10% of losses (Wilmut *et al*., 1986). One of the major causes of embryonic loss is thought to be the inadequate luteal function (Ashworth and Bazer, 1989; Nancarrow, 1994).

There are conflicting reports on the effect of GnRH on pregnancy rate in cattle. GnRH treatment post-mating in some studies has been shown to increase pregnancy rate (Macmillan *et al*., 1986; Sheldon and Dobson, 1993; Drew and Peters, 1994; Anjum *et al*., 2009), while some studies have failed to demonstrate any such beneficial effect (Jubb *et al*., 1990; Ryan *et al*., 1994).

GnRH agonist treatment at the time of mating results in a surge release of LH, causing improved luteal function and luteinisation of developing follicles (Beck *et al*., 1996), which may stimulate conceptus growth (Khan *et al*., 2007) and larger conceptuses may block luteolytic signal more effectively by secreting higher levels of interferon-tau (IFNtau) (Nephew *et al*., 1994), thereby reducing embryo mortality. This involves a single injection of GnRH or hCG at the time of mating, thus inducing ovulation and luteinisation at the appropriate time relative to insemination. Although the evidence for

the efficacy of this approach is not conclusive, GnRH treatment on the day of insemination can increase pregnancy rate in cattle by 6 to 7% (Peters *et al*., 1992).

The present study was conducted to investigate whether a GnRH injection on the day of mating has any effect on luteal function, embryo viability, placentation and overall reproductive performance in Lohi sheep.

MATERIALS AND METHODS

Experimental animals

The present study was conducted at Sheep and Goat Experimental Station, Bahauddin Zakariya University, Multan and Allahdad Livestock Farm Jehanian, District Khanewal, Pakistan. A total of 76 Lohi sheep 3-5 years old and weighing 44 ± 0.4 Kg were used. All ewes had free access to water, shade and trace mineral salt blocks and were fed seasonal fodder and tree loopings alongwith 250g of concentrate mixture per head per day.

Treatments and post treatment monitoring

Estrus was synchronized in all the animals using two intramuscular injections of 2 ml $PGF_{2\alpha}$ analogue (Dalmazin, Fatro Pharmaceutical Veterinary Industry, Italy) given at 11 days apart. Ewes in estrus were mated naturally. The animals were divided into two treatment groups through random stratification by body weight. On day of mating, ewes of group 1 were treated with single injection of 2 ml GnRH analogue (Dalmeralin, Fatro Pharmaceutical Veterinary Industry, Itlay) and animals of group 11 acted as control receiving only saline. Ewes returning to service were monitored to calculate non-return rates. At lambing, number and weight of each lamb were recorded.

For progesterone assay, blood samples (5 ml) were collected from jugular vein with disposable syringe 1 hour before and 2, 4, 6, 8, 10, 12 and 14 days after mating. Blood samples were centrifuged at 3000 rpm for 15 min to separate the plasma that was stored at -20^{0}C until it was assayed for progesterone by ELISA.

On day 25 post-mating, 16 animals (8/group) were slaughtered. Their reproductive organs were brought to laboratory. The numbers of corpora lutea on ovaries, embryos in the gravid uteri and caruncles forming placentomes for each group were recorded.

Progesterone assay

Plasma progesterone concentrations were determined by ELISA, using a commercial diagnostic kit (Biocheck, Inc. Foster City, USA) with method described by Mutayoba *et al*. (1990). The samples and reagents were allowed to reach room temperature (25^{o}C) and mixed thoroughly by gentle inversion before use. The standards, controls and unknowns were assayed in duplicate. The limit of the sensitivity was 0.3 ng/ml and inter and intra-assay coefficients of variation were 12.6 and 7.1%, respectively.

Statistical analysis

Comparisons between treatment groups were analyzed by student's t-test for placentome characteristics, gestation length and lamb birth weights, while chi-square was used to compare ovulation rate, non return rate, pregnancy rate and litter size. Data were analyzed by using computer software Minitab.

RESULTS

Plasma progesterone concentrations

Plasma progesterone concentrations of Lohi sheep in saline-treated control and GnRH treated groups are presented in Fig. 1. Mean plasma progesterone concentrations increased significantly with advancing pregnancy in both groups from day 2 onwards. Mean plasma progesterone concentration of GnRH treated group tended to be significantly higher (P<0.05) as compared to control group.

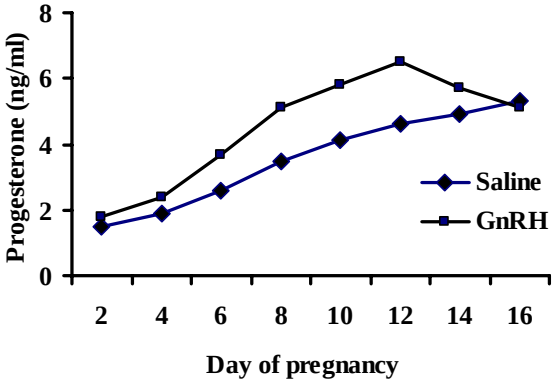

Fig. 1: Mean plasma progesterone concentrations at different days of pregnancy in Lohi sheep given saline or GnRH on the day of mating.

Lambing performance

The number of ewes returning to estrus, non-return rate and number of lambing in saline and GnRH treatment groups are presented in Table 1. There was a tendency for a higher non-return rate and a lower number of ewes returned to estrus in the GnRH treated ewes, but the difference between the two groups was non significant (P>0.05). GnRH treatment on the day of mating increased lambing rate when compared to the control group (83.3 v 60.0%, P<0.05).

The ewes in GnRH treated group had more twins (P<0.05) and consequently they had a higher total number of lambs born and a larger litter size than those in control group (Table 2). There was no difference (P>0.05) in gestation length between control and GnRH administered ewes (Table 2). However, administration of GnRH on the day of mating affected birth weight of single and twin lambs (P<0.05). Lambs from GnRH treated ewes were heavier than those from control ewes. The birth weight of single lambs was higher than those of twins (P<0.05).

Ovulation rate, luteal function, embryo viability and placentation

The data on ovulation rate, number of placentomes and number of embryos recovered from the Lohi sheep that were slaughtered at day 25 post-mating are presented in Table 3. There was significant (P<0.05) difference between the two groups in number of caruncles forming placentomes in both uterine horns and there was a higher ovulation rate in GnRH treated group compared to control

Table 1: Reproductive performance of Lohi sheep given saline (control) or GnRH on the day of mating

Groups	No. of sheep treated/mated	Return to estrus	Non return rate	No. of lambing (%)	No. of lambs			Litter size
					Single	Twin	Total	
GnRH	30	4	0.87	25 (83.3)[a]	11	28[a]	39	1.6
Control	30	7	0.77	18 (60.0)[b]	13	10[b]	23	1.21

Values within a column with different superscripts differ significantly (P<0.05).

Table 2: Gestation length and birth weight of Lohi sheep given saline (control) or GnRH on the day of mating

Groups	Gestation length (days ± SEM)		Birth weight (Kg ± SEM)	
	Singles	Twins	Singles	Twins
GnRH	147.4 ± 0.3	146.0 ± 0.3	4.53 ± 0.11[a]	3.51 ± 0.08[a]
Control	146.7 ± 0.5	146.4 ± 0.1	3.83 ± 0.10[b]	3.08 ± 0.07[b]

Values within a column with different superscripts differ significantly (P<0.05).

Table 3: Number of viable embryos, number of placentomes, ovulation rate and litter size in Lohi sheep given saline or GnRH at time of mating and slaughtered on day 25 post-mating

Groups	No. of sheep slaughtered	No. (%) of sheep pregnant	No. of CL's	Ovulation rate	No. of placentomes		No. of embryos recovered			Litter size
					Left horn	Right horn	Singles	Twins	Total	
GnRH	8	7 (87.0)	15	2.1	115.6 ± 4.2[a]	112.3 ± 3.2[a]	2	10[a]	12	1.7
Control	8	5 (62.5)	8	1.6	94.2 ± 3.7[b]	96.3 ± 2.7[b]	4	2[b]	6	1.2

Values within a column with different superscripts differ significantly (P<0.05).

but the difference was non significant. Out of 16 slaughtered ewes (8/group), 12 were pregnant. More ewes had twins in the GnRH group (P<0.05) and consequently they had a higher total number of viable fetuses and a larger litter size than those in the control group, but the difference was non significant.

DISCUSSION

In the present study, changes in the plasma progesterone concentrations resulted from GnRH treatment of Lohi sheep on day of mating are similar to those reported in previous studies both in cattle and ewes (Rettmer *et al*., 1992; Mann and Lamming, 1999; Beck *et al*., 1996; Khan *et al*., 2007; Lashari and Tasawar, 2007). This elevated level of progesterone was considered to be responsible for the rapid blastocyst growth, resulting in more IFN-tau secretion and consequently, improved embryo survival. The enhanced conceptus growth and placentation after GnRH treatment in the present study supported the findings of Nephew *et al*. (1994).

The increase in plasma progesterone concentrations after GnRH treatment on day of mating suggests that GnRH through LH release may provide luteotrophic stimulation to CL, which could explain the increase in CL weight observed in the ewes given this treatment (Farin *et al*., 1988). This luteotrophic stimulation may either be in the form of conversion of small luteal cells to large luteal cells which then secrete higher concentrations of progesterone (Farin *et al*., 1988) or may even be due to an increase in the size of large luteal cells (Fitz *et al*., 1982). Whatever the mechanism of higher progesterone secretion from the CL after GnRH treatment, this additional luteotrophic support at this stage seems to be beneficial for improving fertility. When GnRH analogue was given on day 12 after artificial insemination in cows, serum progesterone concentration was not affected, while serum LH levels increased significantly compared to controls (Yildiz *et al*., 2009).

The results of the present study showed that GnRH administration on day of mating improved rate and number of lambs born. This is in agreement with the previous findings of McMillan *et al*. (1986); Cam *et al*. (2002); Khan *et al*. (2007); Peters *et al*. (1992) and Lashari and Tasawar (2007), who observed positive effect of GnRH administration on the day of mating on embryo survival in sheep and cows. Lambing rates were 17% higher in GnRH administered ewes which resulted in an improved reproductive performance in ewes given GnRH on the day of mating compared to control group.

The results of the present study provide evidence that GnRH causes ovulation and the formation of accessory CLs, since higher number of CL was observed in GnRH given ewes at slaughter on day 25 of pregnancy. This is in agreement with the findings of Mann and Picton (1995), Beck *et al*. (1996) and Cam *et al*. (2002) that GnRH administration results in an increased number of CL.

The beneficial effect of GnRH supplementation prior to insemination increases conceptus growth (Garrett *et al*., 1988; Kleemann *et al*., 1994; Lashari and Tasawar, 2007). This would have helped improve embryo survival as larger conceptuses produce more IFN-tau, thereby more effectively suppressing the luteolytic mechanism and allowing more time for the establishment of pregnancy (Nephew*et al*., 1994). However, the present study is in agreement with Cam *et al*. (2002), who reported no difference in gestation length of both treatment groups.

Placentation in the present study also improved in GnRH treated animals, as the number of placentomes also increased in GnRH treated ewes compared to control. It could be hypothesized that a greater number of placentomes resulting in a larger overall surface area might improve attachment of the embryo(s) and therefore reduce embryo losses by improving placentation. Moreover, although pregnancy rate was similar for both groups on day 25, there was a tendency for more GnRH treated animals to lamb, suggesting that more pregnancies were maintained in this group.

There are a number of possible explanations regarding the effect of GnRH on conceptus growth. It may have been through a direct effect on the maturing oocyte that is carried over into embryogenesis and implantation or alternatively, GnRH treatment might have advanced ovulation, thereby allowing more time for embryo growth and development (Kleemann *et al*., 1994). The present results are consistent with an earlier study in cattle in which hCG treatment on day of insemination induced the formation of accessory corpora lutea but had no effect on plasma progesterone concentrations during early pregnancy (Rajamahendran and Sianangama, 1992).

In conclusion, the results of the present study showed that GnRH administration on the day of mating improved the reproductive performance in Lohi sheep. GnRH may be luteotrophic or embryotrophic and thereby could have improved the embryo survival.

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