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RESEARCH ARTICLE

The Effect of GnRH (Dalmarelin) Given on Day 12 Post-Mating on Ovarian Function and Embryo Development in Lohi Sheep at Southern Punjab, Pakistan

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ABSTRACT

The present study was conducted at Sheep and Goat Research Station, Bahauddin Zakariya University, Multan. The objectives of this study were to investigate the effects of GnRH analogue (Dalmarelin) treatment on Day 12 of pregnancy on ovarian function, plasma hormone concentrations and embryo development to improve litter size. Sixteen Lohi sheep 3-4 years old, weighing 44±0.4 kg (Mean ± S.E.M) were selected. The daily feed/ration was hay, seasonal fodder, tree looping and 250g concentrate per sheep. Sheep were mated to ram at synchronized estrus by two i/m injections of 2ml PGF2α analogue (Dalmazin) given at 11 days apart. These animals were divided into two treatment groups (8/group) through random stratification by body weight. Animals were injected an either saline (group I) or 2ml GnRH (group II) on Day 12 post–mating. The blood samples were collected from jugular venipuncture (3ml) with a disposable syringe from 1 hour before and 0, 2, 4, 6, 8, 24, 48, and 72 hours after treatment. Plasma progesterone concentrations detected by ELISA were higher ($P<0.05$) in sheep as compared with saline treated controls. Oestradiol concentrations in GnRH treated animals were significantly ($P<0.05$) higher than those in the controls. The effects of GnRH on conceptus growth and litter size were determined by at slaughter on Day 25 of pregnancy. GnRH treatment increased ($P<0.05$) embryonic weight, amniotic sac width and length and crown-rump length as compared with controls. Results of present study demonstrated that treatment with GnRH may be luteotrophic and embryotrophic and thereby could improve litter size.

INTRODUCTION

The sheep is domesticated ruminant which plays vital role in the economy and culture of most peoples of the world by providing food and clothing. Pre-implantation embryonic loss is the major factor limiting optimum reproductive performance in farm animals. In sheep, 30–40% of fertilized eggs are lost during the first 3 weeks of pregnancy (Bolet, 1986; Michels et al., 1998). Of this total loss, 70–80% occurs between day 8 and 16 after insemination (Sreenan et al., 1996). One of the major causes of embryonic loss is likely to be the inadequate luteal function (Wilmut et al., 1986; Ashworth et al., 1989; Nancarrow, 1994).
Gonadotrophin supplementation during early pregnancy can reduce embryonic losses. A single injection of GnRH analogue, buserelin, given on Day 12 post-insemination can reduce embryo mortality in cattle and sheep (Peters et al., 1992; Beck et al., 1994; Khan et al., 2006). Buserelin injection on Day 12 can significantly improve fertility in cows by up to 12% (Macmillan et al., 1986; Peters et al., 1992) and in mature ewes by up to 10% (Beck et al., 1994; McMillan et al., 1986; Peters, 2005; Khan et al., 2007). GnRH treatment stimulates the release of pituitary gonadotrophins that improve luteal function and luteinize developing follicles (Beck et al., 1996a; Cam et al., 2002) may also help to establish accessory CL?. The net effect is a transitory increase in plasma progesterone and oestradiol concentrations, followed by a prolonged decrease in oestradiol concentrations (Beck et al., 1996a,b). It is assumed that this observed decrease in oestradiol concentrations as a result of buserelin (GnRH) treatment delays luteolysis, as higher

oestradiol concentrations are required to induce an increase in oxytocin receptor concentrations. Consequently, prostaglandin-$F_2\alpha$ ($PGF_2\alpha$) release is inhibited and that gives slow developing conceptuses more time to establish (Mann et al., 1995; Beck et al., 1996a). However, it is also possible that enhanced luteal function induced by LH release (as a result of GnRH treatment) may have a direct effect on conceptus growth and improve embryo viability by producing higher quantities of interferon-tau (IFN-ô) and blocking the luteolytic signal effectively. However, effect of GnRH on conceptus growth during early pregnancy is yet to be determined.

Enhanced luteal function and/or conceptus growth, hence improve the reproductive performance. GnRH administration post-mating has also been reported in cattle (Rettmer et al., 1992; Sheldon and Dobson, 1993; Drew and Peters, 1994). However some studies have reported that GnRH administration to have no effect on reproductive performance in cattle and sheep (Ryan et al., 1994; Tefera et al., 2001).

There are no reports on effects of GnRH treatment on Lohi sheep at Southern Punjab, Pakistan. The present study was initiated to determine the effects of GnRH treatment on day 12 of pregnancy, on plasma progesterone, ovarian function and embryo development.

MATERIALS AND METHODS

The present study was carried out on Lohi sheep at Sheep & Goat Research Station B. Z. University, Multan (Pakistan). Sixteen sheep of three to four years old, weighing 44±0.4 kg (Mean ± S.E.M) were used. The feeding of the animals was mainly through grazing of the available seasonal fodder, tree looping and also provided 250g addition ration per sheep. These sheep were exposed to fertile ram at synchronized estrus by two intramuscular injections of 2ml $PGF_{2\alpha}$ analogue (Dalmazin, Fatro Pharmaceutical Veterinary Industry, Italy) given at 11 days apart. These animals were divided on two treatment groups (8/group) through random stratification by body weight. Each animal was given either saline (group 1st) or 2ml GnRH analogue (Dalmaralin, Fatro Pharmaceutical Veterinary Industry, Italy) group 2nd on Day 12 post–mating. The blood samples were collected from jugular venipuncture (3ml) with a disposable syringe from 1 h before and 0, 2, 4, 6, 8, 24, 48, and 72 h after treatment for progesterone and oestradiol assays. It was transferred to centrifuge tubes and heparin was added for anticoagulation. Plasma was harvested after centrifugation of this blood for 15 minutes at 3000 rpm and stored at -20°C until the determination of hormones by ELISA. The animals were slaughtered on day 25 post-mating and their reproductive tracts were collected and transported back to the laboratory. Pregnancy was determined on the basis whether uterus of an animal contained a conceptus or not. Ovulation rate was determined for each pregnant animal by counting the number of corpora lutea on both ovaries. The conceptuses were carefully removed and embryo weight (g), amniotic sac width / length and crown-rump length (mm) determined. The viability of each embryo was assessed from its crown rump length, weight and stage of development. The number of caruncles was also counted for each animal.

Statistical analysis

The data for litter size and the number of viable embryos were compared among groups using Chi-square analysis, whereas the effects of treatment on embryo weight, amniotic sac width and length, crown-rump length and number of caruncles were compared using t test. $P<0.05$ was taken as statistical significance.

RESULTS

Plasma progesterone concentrations in control and GnRH on day 12 post-mating

Mean Plasma progesterone concentrations were significantly ($P<0.01$) higher in ewes treated with GnRH as compared to control group (Fig. 1). The progesterone concentrations in GnRH treated ewes were started to elevate at the start (0hour) of day 12 (control = 4.4±0.28ng/ml; GnRH = 4.9±0.28ng/ml) as compared to control group. At 2nd (control = 4.3±0.29ng/ml; GnRH = 6.3±0.35ng/ml), 4th (control = 4.2±0.29ng/ml; GnRH = 6.8±0.37ng/ml) and 6th h (control = 4.6±0.29ng/ml, GnRH = 7.2±0.38ng/ml), they were still high in GnRH treated group. At 8th h, it had reached at a maximum value (control = 4.5±0.28ng/ml; GnRH = 7.4±0.38ng/ml). It remained higher at 24th and 48th hour and then started to decline thereafter to reach basal values after 72 hours.

Plasma oestradiol concentrations in control and GnRH on day 12 post-mating

Mean plasma oestradiol concentrations were significantly higher ($P<0.05$) immediately after GnRH administration as compared to control group (Fig. 2). Oestradiol concentrations in both the groups (control and GnRH treated) overlapped at about 1st h after treatment. In GnRH treated animals oestradiol concentration started to increase from 2nd h (5.9±0.29pg/ml) as compared to control (4.6±0.35 pg/ml) and fluctuated more or less until 24 hours. At 48th hour there was maximum concentrations (control = 6.3±0.35pg/ml, GnRH = 7.9±0.36pg/ml,) in GnRH treated as well as control groups. However by 72 hours after treatment *i.e.*, at the end of sampling period, oestradiol concentrations were lower (control = 5.8±0.30pg/ml; GnRH = 6.0±0.30pg/ml) in both groups.

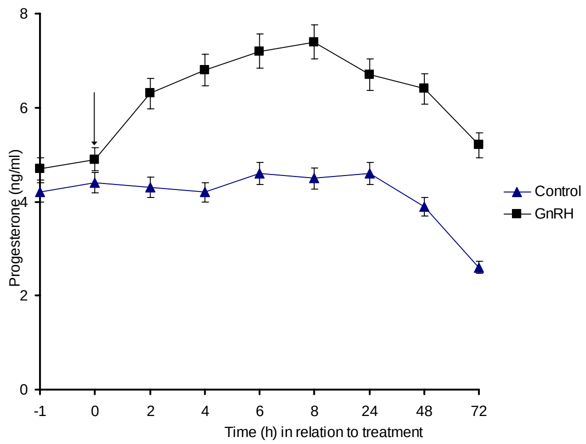

Fig. 1: Mean plasma progesterone concentrations in Lohi sheep given saline or GnRH on day 12 post-mating

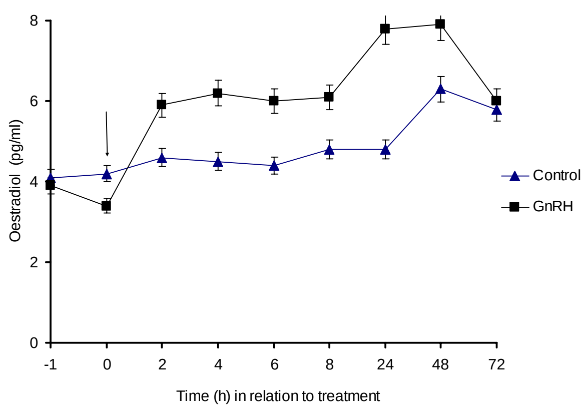

Fig. 2: Mean plasma oestradiol concentrations in Lohi sheep given saline or GnRH on day 12 post-mating

Litter size and ovarian characteristics of sheep slaughter on day 25 of pregnancy

The number of viable fetuses and litter size distribution in sheep slaughtered on day 25 post- mating from control and GnRH administered groups are presented in Table 1. Sixteen slaughtered sheep eight from each treatment group were all pregnant. More sheep had twins in the group GnRH (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.

The results of embryonic development in sheep slaughtered on day 25 post-mating are given in Table 2. The embryo weight and number of caruncles forming placentomes were greater (P<0.05) in the GnRH treated sheep as compared with control group. Moreover amniotic sac width, crown-rump length and amniotic sac length were significantly (P<0.05) greater in GnRH treated sheep as compared with control group.

Table 1: Numbers of viable embryos and litter size, of Lohi sheep in saline or GnRH on day 12 post-mating and slaughtered on day 25 of the pregnancy.

	Control	GnRH
Total number of sheep treated	8	8
No. of ewes pregnant	7	8
No. of viable fetuses		
Single	5	3
Twin	4	10
Total no. of fetuses	9	13
Litter size	1.12	1.62

Table 2: Means ± S.E.M embryo development, number of caruncles, crown rump length and amniotic sac width/length of Lohi sheep in saline or GnRH on day 12 post-mating and slaughtered on day 25 of the pregnancy (n=16).

	Control	GnRH
Embryo weight (g)		
Single	0.193±0.001[a]	0.207±0.004[b]
Twin	0.170±0.004 [a]	0.180±0.00[b]
No. of caruncles	88.14±1.1[a]	97.12±1.7[b]
Amniotic sac length (mm)		
Single	14.2±0.3[a]	15.6±0.3[b]
Twin	12.0±0.4[a]	13.2±0.2[b]
Amniotic sac width (mm)		
Single	11.0±0.3[a]	13.3±0. 3[b]
Twin	10.4±0.4[a]	12.1±0.2[b]
Crown rump length (mm)		
Singles	11.6±0.5[a]	13.6±0.3[b]
Twins	11.0±0.4[a]	12.1±0.3[b]

Values within the same rows that do not have a common superscript are significantly different (P<0.05)

DISCUSSION

The results of the present study showed that the increase in plasma progesterone concentrations in sheep as a result of GnRH treatment is in agreement with the previous work of Beck et al. (1996a,b); Nephew et al. (1994) and Khan et al. (2006) in sheep and Macmillan et al. (1986) in cattle. Beck et al. (1996a) showed an increase in plasma concentrations of progesterone after GnRH (buserelin) injection on Day 12 post-mating, Nephew et al. (1994) reported an increase in plasma progesterone concentrations as a result of hCG administration on Day 11.5 post-mating, in ewes. Whereas Khan et al. (2007) proved an increase in plasma progesterone concentration as a result of GnRH or hCG treatment on Day 12 post-mating in ewe and ewe lambs.

The increase in plasma progesterone concentrations after GnRH treatment on Day 12 of pregnancy suggests that GnRH through LH release may provide luteotrophic stimulation to corpus luteum. 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 of pregnancy seems to be beneficial for improving fertility by decreasing embryo mortality.

The increase in oestradiol concentrations after GnRH treatment, in sheep confirm the earlier work of Nephew et al. (1994) and Khan et al. (2006) they reported an increase in plasma concentrations of oestradiol in pregnant ewes given GnRH treatment on Day 12 post-mating. However, the results of this study do not support the hypothesis that GnRH treatment may act to improve embryo survival by lowering oestradiol levels and disrupting the luteolytic mechanism because of lower oestradiol concentrations (Mann et al., 1995; Beck et al., 1996a) as post-treatment oestradiol concentrations remained elevated.

The increased plasma concentrations of oestradiol obtained after GnRH treatment may play a role in the establishment of pregnancy and therefore, should not be ignored. Synthesis and release of oestradiol by swine blastocysts has been reported to be one of the important factors that contribute to the uterine environment favourable for embryonic growth (Geisert et al., 1982). It has been shown that the ovine blastocyst also synthesises oestradiol coincident with blastocyst elongation, perhaps in a manner similar to that of swine, and may therefore make the uterine environment conducive to embryo survival (Nephew et al., 1989). There is a possibility that both progesterone and oestradiol function cooperatively to induce blastocyst growth and prevent luteolysis (Nephew et al., 1994). The origin of the increase in oestradiol concentrations is not clear from the results of present study. Its source may either be healthy growing follicle(s) that respond to GnRH treatment to secrete more oestradiol or alternatively, growing blastocysts may produce more oestradiol.

The results of the present study showed that administration of GnRH agonist on day 12 post-mating before the time of maternal recognition of pregnancy improved pregnancy rate and litter size. Various studies have reported that GnRH administration improves reproductive performance in sheep during the breeding season (Macmillan et al., 1986; Beck et al., 1994, Khan et al., 1999; Cam et al., 2002). Cam and Kuran (2004) studied that hCG and GnRH administration on day 12 post-mating improved the lambing rate (22%) and twining rate (31%). The results of the present study are in agreement with these findings. The low pregnancy rate in the control group may have reflected the well-known genetic effect of breed on embryonic mortality (Nancarrow, 1994).

Heat stress can reduce the embryonic survival by its direct effect on embryo and possibly also by its indirect effect on plasma progesterone concentration. It has been reported in cattle that plasma progesterone concentration (Jonsson et al., 1997; Wolfeson et al., 2000) and in vitro progesterone production by luteal cells (Wolfenson et al., 2000) from heat stressed animals during summer months are lower than those during winter months. Delayed effect of heat stress on cattle fertility has also been reported during autumn (Roth et al., 2000). There is no information on such effects of heat stress on sheep fertility.

The results of present study supported the hypothesis that gonadotrophin supplementation on Day 12 post-mating in the sheep improves fertility by enhancing conceptus growth and placentation. It is assumed that conceptuses with a significantly greater mass would tend to be more viable than smaller conceptuses of the same age as larger conceptuses secrete higher quantities of IFN-*ô* (Nephew et al., 1994; Khan et al. 2007) that plays an important role in preventing or weakening the luteolytic signal.

The increase in the number of caruncles after GnRH treatment was probably the result of the larger conceptuses in this treatment group being able to form more attachments with uterine. This suggests that this treatment promotes implantation, which may help improve long term embryo growth and survival.

The reasons for the stimulatory effects of GnRH treatment on conceptus growth and placentation are unclear. However, it seems unlikely that GnRH can stimulate conceptus growth directly, because there is no evidence to suggest that the ovine conceptus has binding sites for GnRH. This assumption gains support from the work of Wilmut et al. (1985) who showed that uterine environment could be advanced by exogenous progesterone treatment so that a Day 6 uterine environment could be made suitable for the development of a Day 10 embryo (Lawson and Cahill, 1983). The treatment increased plasma progesterone concentrations, which may stimulate secretion of embryotrophic substances, for example, endometrial proteins (Ashworth and Bazer, 1989; Nephew et al., 1994; Khan et al., 2006), which may be associated with accelerated conceptus growth.

Some studies report improvements while some others report no effect of such hormonal treatments in reproductive performance of sheep and cattle (Jubb et al., 1990; Ryan et al., 1994; Tefera et al., 2001). It is possible that the supplementation of progesterone or administration of GnRH may be more effective in reducing embryonic mortality in these climatic conditions.

In conclusion, the results of the present study showed that GnRH (Dalmarelin) administration on Day 12 post-mating effects ovarian function in sheep. This treatment also improved conceptus growth. Moreover, GnRH may improve implantation by increasing the number of placentomes forming the placenta. In addition the results indicated that GnRH has luteotrophic and embryotrophic effect. This treatment also improved the reproductive performance of Lohi sheep by increasing pregnancy rate and number of lambs born.

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