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The effect of hCG given on day 12 post-mating on ovarian function and embryo survival in Beetal goats in southern Punjab, Pakistan

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Abstract: The present study was conducted to determine the effects of human chorionic gonadotrophin on ovarian function and embryo development of Beetal goats reared under traditional conditions at the Sheep and Goat Research Station, Bahauddin Zakariya University, Multan. Fourteen Beetal goats at 4 years of age, weighing 40 ± 0.41 kg (mean ± SEM) were used. Their daily food was hay, green fodder, tree loppings, and 250 g of additional rations per goat. These goats were mated to buck at synchronized estrus by 2 i/m injections of dalmazin, given at 11-day intervals. These animals were divided into 2 equal groups (7/group) through random stratification by body weight. These were given either saline (Group I) or 300 IU human chorionic gonadotrophin (Group II) on day 12 post-mating. The blood samples were collected for progesterone. The animals were slaughtered on day 25 of pregnancy. Reproductive tracts were removed, corpora lutea, isolated, counted, and weighed. Embryos were also recovered, weighed, and measured for crown rump length, amniotic sac length, and the width and number of caruncles forming placentomes were counted as well. The results showed that human chorionic gonadotrophin increases the plasma progesterone concentration ($P < 0.05$). Treatment with hCG not only improves the conceptus growth but also increases the number of caruncles significantly ($P < 0.05$). In conclusion, the results of the present study revealed that treatment with human chorionic gonadotrophin may be luteotrophic and embryotrophic. The embryonic mortality was 27% in this breed. The reduction in embryo mortality was found after being given human chorionic gonadotrophin (17%), and thereby could improve embryo survival and increase litter size.

Key words: Embryo survival, ovarian function, hCG, progesterone, Beetal goat

Introduction

Pakistan, being an agricultural country, places a significant dependence on livestock for providing food of high nutritional value, and products like skin and wool used as raw materials for industries/export, and ensures the well-being of the rural populace. Livestock accounts for 52.2% of agricultural value added and about 11% of GDP (1). The role of livestock in a rural economy may be realized from the fact that 35 million of the rural population is engaged in raising livestock, with household holdings of 2-3 cattle/buffalo, and 5-6 sheep/goat per family. This helps them to derive 30%-40% of their income. Goats are an important national genetic source in Pakistan (2). There were an estimated 56.7 million goats during

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2007-08 in Pakistan, and the mutton production in the country at present was 0.740 million tons (1).

Substantial embryonic mortality has been reported in all mammals studied so far. It has been shown that this mortality may be the major cause of reproductive failure of the domestic species (3). A prenatal embryonic loss results in return to service that extend inter-birth intervals, and causes a reduction in litter size in prolific breeds. Therefore, it is essential to understand the physiological causes of these losses in productivity, and utilize this knowledge to enhance productivity (4).

Preimplantation embryonic loss is the major factor limiting optimum reproductive performance in farm animals. In sheep/goats, 30%-40% of fertilized eggs are lost during the first 3 weeks of pregnancy (4-6). Of this total loss, 70%-80% occur between day 8 and 16 after insemination (7). One of the likely major causes of embryonic loss is the inadequate luteal function (3,8). In attempts to reduce embryonic mortality, and to improve reproductive performance, progesterone supplementation during early pregnancy has been employed in sheep and cattle (7,9). There are studies showing that progesterone supplementation after breeding improves not only the pregnancy rate (10,11) but also stimulates subsequent fetal growth (12,13).

The administration of gonadotroph around the time of estrus onset has been shown to reduce the presence of large unovulated follicles at the time of embryo collection in goats that were superovulated with FSH (14,15). Armstrong et al. (16) administered either hCG or GnRH at the time of onset of estrus in goats superovulated with PMSG, and was unable to increase the number of ovulations, and reduce the number of follicles present in the ovaries during the subsequent luteal phase, thus suggesting that the problem was not an inadequate preovulatory LH surge, but rather a lack of response of follicles to the LH secreted at that time. Cam and Kuran (17) reported that both hCG and GnRH administration improved reproductive performance of ewes when administered on day 12 post-mating. It has also been reported that hCG administration can stimulate conceptus growth in utero, as determined on day 13 (18) and on day 25 (19).

Human chorionic gonadotrophin has been reported to stimulate blastocyst expansion, and larger blastocysts secrete more IFN-τ (18), which by down-regulation of oestradiol and oxytocin receptors suppresses $PGF_2\alpha$ release more effectively. Consequently, the luteolytic mechanism is either blocked or delayed, and this gives blastocysts more time to establish. However, it is yet to be investigated whether the increased blastocyst expansion following hCG treatment leads to increased conceptus growth and improved placentation.

Concerned with the economic viability of the goat enterprise, it is necessary to evaluate the reproductive and productive potential of this breed, and gain a thorough knowledge of its reproduction physiology. Limited and incomplete studies exist on the reproductive parameters to help improve herd management (20,21). In particular, there is, as of yet, no information on the gonadotroph supplementation of this breed. It is expected that the information generated will be used to develop a package for profitable goat farming.

Materials and methods

The present study was carried out on the Beetal goat at Sheep and Goats Research Station, Bahauddin Zakariya University, Multan (Pakistan). The Beetal is one of the most famous breed of goats in Pakistan with a small litter size. The objective of this study was to determine the effect of hCG on ovarian function and embryo development to improve litter size. Fourteen goats of 4 years of age, weighing 40 ± 0.4 kg (mean ± S.E.M.) were used. Different fodders such as various types of grasses, *Medicago sativa*, and *Trifolium alexandrium*, were available at the station in different seasons for free grazing. Animals were also provided 250 g of additional rations (equal mixture of cotton seed cake + wheat husk) per goat. These goats were exposed to fertile buck at synchronized oestrus by 2 intramuscular (i/m) injections of 2 mL $PGF_2\alpha$ analogue (Dalmazin, Fatro Pharmaceutical Veterinary Industry, Italy) given 11 days apart. The animals were divided into 2 treatment groups (7/group) through random stratification by body weight. They were given either saline (Group I) or 300 IU hCG (N. V. Organon Oss Holland) (Group II) on day 12 post-

mating. The blood samples were collected from the jugular vein with the help of disposable syringes 3 days before and after treatments for hormonal assays from day 12 to day 15 and were slaughtered on day 25 of pregnancy. Heparinized blood samples were centrifuged for 10-15 min at 3000 rpm. The serum was harvested with the help of a micropipette, and was stored at -20 °C until the determination of progesterone by ELISA. Corpus luteum (CL) and embryos were isolated, counted and weighed (g). The amniotic sacs' width and length were measured (mm). The number of caruncles forming placentome was counted as well.

Statistical analysis

The results are expressed in percentages and mean ± SEM. The values between various groups were compared with chi-square (χ^2) and paired t-test. $P < 0.05$ was taken as statistical significance.

Results

The Figure shows that plasma progesterone concentrations were significantly ($P < 0.01$) higher in goats treated with human chorionic gonadotrophin (hCG) as compared to the control group. The progesterone concentration in hCG treated goats was elevated at the start (0 h) of day 12 (control = 4.2 ± 0.15 ng/mL; hCG = 4.8 ± 0.9 ng/mL) as compared to the control group. At hour 2 (control = 4.4 ± 0.25 ng/mL; hCG = 6.4 ± 0.32 ng/mL), hour 4 (control = 4.2 ± 0.26 ng/mL; hCG = 6.8 ± 0.37 ng/mL) and hour 6 (control = 4.6 ± 0.29 ng/mL, hCG = 7.2 ± 0.38 ng/mL), progesterone concentration was still high in the hCG treated group. At hour 8, it had reached a maximum value (control = 4.5 ± 0.28 ng/mL; hCG = 7.4 ± 0.38 ng/mL). It would remained high at the 24th and 48th h, and then started to decline thereafter to reach basal values after the 72nd h.

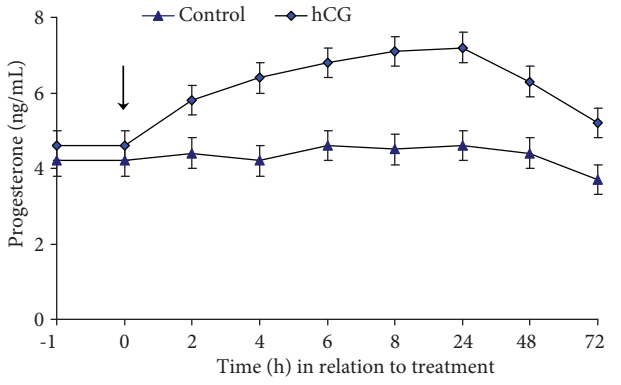

Figure. Mean ± SEM plasma progesterone concentrations on day 12 post-mating in Beetal goats

The results relating to the ovulation rate, embryo viability, and placentation of the Beetal goats treated with hCG or saline (control) on day 12 post-mating are given in Table 1. The number of corpora lutea, and number of embryos recovered were significantly ($P < 0.05$) different in hCG treated group as compared to the saline group.

The results regarding conceptus growth (mean ± SEM) in goats given saline or hCG on day 12 post-mating, and slaughtered at day 25 post-mating are presented in Table 2. The mean weight of the embryos was significantly higher ($P < 0.05$) in hCG treated group as compared to the saline group.

Table 1. The ovulation rate, embryo viability, and placentation of the Beetal goat injected with hCG or saline (control) on day 12 post-mating.

	Control	hCG
No. of goats treated	7	7
No. pregnant at slaughter (%)	5 (71)	6 (86)
No. of CLs	11[a]	18[b]
No. of embryos recovered	8[a]	15[b]
Embryo loss	27%[a]	17%[b]
No. of placentomes		
Left horn	35.33 ± 6.69[a]	75 ± 12.28[b]
Right horn	40.66 ± 0.33[a]	79 ± 12.52[b]
Total	57 ± 6.50	154 ± 12.40

The values in a row with different superscripts are significantly different ($P < 0.05$).

Table 2. Conceptus growth (mean ± SEM) in goat given saline or hCG on day 12 post-mating and slaughtered at day 25 post-mating.

	Control	hCG
Mean weight of the embryo (g)	0.20 ± 0.05[a]	0.34 ± 0.11[b]
Luteal weight (g)	0.95 ± 0.23[a]	0.94 ± 0.14[a]
Mean amniotic sac width (mm)	8.0 ± 0	9.4 ± 0.11
Mean amniotic sac length (mm)	10 ± 0	12.5 ± 0.11

The values in a row with different superscripts are significantly different ($P < 0.05$).

Discussion

The results of the present study support the hypothesis that gonadotrophin supplementation on day 12 of pregnancy in the goats 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-τ (18), which plays an important role in preventing or weakening the luteolytic signal.

Khan et al. (22) studied GnRH/hCG effect on day 12 of post-mating in ewes and ewe lambs and reported the same results that hCG treatment significantly ($P < 0.05$) increases plasma progesterone concentrations in ewes. The progesterone concentrations remained higher for 2-8 h, and then started to decline thereafter to reach basal values by the 72nd h after treatment. Beck et al. (23) also reported that hCG or GnRH treatment increases plasma progesterone concentrations on day 12 post-mating in ewes. This effect of hCG on plasma progesterone concentration during the present study is in agreement with the previous findings in cattle (24). The administration of hCG agonist on day 12 post-mating (before the time of maternal recognition of pregnancy) improved embryo survival, pregnancy rate, and litter size. Placentation was improved in hCG treated animals, as the number of placentomes was increased. The greater number of placentomes resulting in a larger overall surface area might improve attachment of the embryos, and therefore reduce embryo losses by improving placentation (22,24).

The hormonal treatments used in the present study prevented the mortality of the embryo by stimulating the fetal growth, which resulted in an increased weight. The beneficial effects of hCG administration on embryo survival may be through the stimulatory effect of hCG-induced progesterone on fetal growth, because it has been reported that progesterone supplementation increases subsequent fetal growth. Findings of the present study are in agreement with Macmillan et al. (10) and Khan et al. (22). HCG administration on day 11.5 post-mating increases pregnancy rate in sheep, as reported previously by Khan et al. (19). GnRH administration on day 12 post-mating improved the kidding rate, as reported by Cam and Kuran (17). The effect of the hCG on the pregnancy rate and fetal weights could be attributed to its effects on progesterone production and uterine secretions, which were embryotrophic. It is possible that the supplementation of progesterone, or administration of GnRH or hCG may be more effective in reducing embryonic mortality in these climatic conditions.

In conclusion, the results of the present study showed that hCG administration improved embryo growth and placentation of goats effectively when administered on day 12 post-mating. The embryonic mortality was 27% in this breed. The reduction in embryonic mortality was found after hCG (17%), thus suggesting that such a treatment has a potential to improve the fertility of goats. This could be used to increase litter size.

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