The Indian Journal of Animal Reproduction; 25(2): 101-103; December 2004

Superovulatory responses of eCG and live births following surgical embryo transfer in Marwari ewes

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Received : March 18, 2002
Accepted : October 10, 2003

ABSTRACT

Multiple ovulation and embryo collection (n = 8) was done in Marwari ewes followed by their transfer to synchronised recipients (n = 7) Donor ewes were superovulated by injecting 1000 IU of eCG on day 12 of a progesterone implant under two groups. Group 1 (n = 4) received a commercially available progestagen implant CIDR-G whereas in Group II (n = 4) ewes received an indigenously prepared implant. Ewes were induced to estrus by a PG injection and mated to fertile rams at estrus. Embryos were collected surgically on day 6, their ovarian response recorded and embryos transferred surgically. Only 75% (3/4) ewes responded in both the groups. The number of CL/ewe, total ova recovery rate, fertilized eggs/ewe and number of morula/blastocysts/ewe were 7.0±2.51, 4.66±2.66, 3.66±2.18 and 3.0±1.52 in group 1 and 10.0±5.13, 9.34±4.37, 7.34±4.48 and 9.34±2.02 in Group II. Statistically all these were non significantly different except for the total ova recovery rate which was significantly higher ($P < 0.05$) in Group II indicating thereby that locally made sponges can replace commercial implants of progestagens. Three pregnancies and live births resulted from transfer of 12 embryos to 7 recipients.

Key words : eCG, embryo transfer, ewes, superovulation

The use of embryo transfer technology for promoting higher genetic gains in sheep has been slow (Cognie, 1999) however, significant numbers of sheep embryo transfers have been reported from Australia and South Africa (Thibier, 1996). A limited number of reports have also appeared in India (Naqvi *et al.*, 1996, 1998; Uphale *et al.*, 1998; Honnapagol *et al.*, 1999). A high degree of variability of the ovarian response to the superovulatory treatment is the most important factor limiting the use of embryo transfer on commercial scale in small ruminants (Cognie, 1999). One contributing aspect of this variability is the breed of the animal (Gulyani and Naqvi, 1994). In the present study we report the effects of superovulation in Marwari ewes employing eCG in combination with progestagen implants and the subsequent embryo recoveries hitherto not reported previously, in Marwari ewes.

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MATERIALS AND METHODS

Marwari ewes aged 1-2 years maintained under identical conditions at the department of Veterinary Obstetrics and Gynaecology were used during the months of August - October, 2001 for the study. Donor ewes were superovulated under two groups. In group (n=4) ewes received a commercial progestagen implant controlled internal drug release - Goats (CIDR-G, Inter Ag Hamilton NJ) followed by 1000 IU of eCG (Folligon, Intervet Holland) IM on day 12 of the implant. In group II (n = 4) ewes received a indigenously prepared progestagen sponge containing 0.350 g of progesterone (CDH, New Delhi) described elsewhere (Naqvi *et al.*, 1996) and 1000 IU of eCG IM on day 12 of the implant. Ewes in both groups were given in PG (Inj. Illiren, Hoechst Germany 1.5 ml IM) 48 h later followed by implant withdrawl. Ewes were detected in estrus by a teaser and mated to a fertile rain. Recipient ewes received a commercial progestagen implant CIDR-G that was withdrawn one day before withdrawl of implant from donor ewes.

Embryo collection and transfer : Embryos were recovered from donor ewes by midline laparotomy as close to the udder as possible. A finger was inserted into peritoneal cavity and

the genitalia were brought to the incision site. The number of CL were recorded on both the ovaries. A blunt incision was given towards the cervical end of one of the uterine horn by the scalpel or mosquito forceps and a 10 Fr foley Cathetor (Romsons Medicons, Agra) was introduced into the horn from this side. The bulb of the cathetor was inflated with 5-10 cc of air. The other end of the catheter was kept in a petri dish. 15-20 ml of warm DPBS (Himedia, Bombay) with 0.1% BSA was introduced from the fimrial end of the horn through a 18 gauze needle attached a syringe and collected in the dish. The bulb of the cathetor was then deflated and the cathetor was withdrawn. The same procedure was repeated in the other horn. Uterus was washed several times with heparinized saline and replaced back. The peritoneum and muscles were sutured with chronic catgut. The skin was sutured with simple interrupted sutures using silk thread. Animals were given 1 gm strepto penicillin IM (Hindustan Antibiotics, Pune) and 5 cc of diclofenac sodium (Inj Zobid, Sarabhai Chemicals, Mumbai) IM daily for 5 days post operative. Skin sutures were removed 7 days after operation.

Collected fluid was searched under sterozoom microscope. The embryos once located were given 2-3 washings in holding medium containing 0.4% BSA and loaded into 0.25 ml pre-sterilized mini semen straws (IMV, France) and transferred surgically the same day to synchronized recipients. Ewes were operated using the procedure used for donor ewes. A stab wound was made on the uterine horn ipsilateral to the CL bearing ovary and the straw having the embryo was introduced into it. Embryos were transferred to pushing through a AI gun plunger.

RESULTS AND DISCUSSION

The response of ewes to eCG in combination with the two progestagens is presented in Table 1. Seventy five percent (3/4) of the ewes responded to the progestagen treatment in both the groups. Ewes exhibited estrus in 28.5±2.0 and 26.9±2.15 hours in both the groups. Similar results on estrus induction time and superovulatory response have previously been documented by Naqvi *et al.* (1998) in Bharat Merino ewes and Uphale *et al.* (1998) in Malpura ewes. The latter workers however, recorded only 50 per cent ewes showing a ovulatory response. The number of CL recorded per ewe, total ova recovered, number of fertilized eggs recovered per ewe and the number of morula/blastocysts recovered per ewe were higher in ewes in group II but statistically these differences were non significant. The results are nearly similar to those recorded previously by Naqvi *et al.* (1998) and Updhale *et al.* (1998). Honnappagol *et al.* (1999) have however, recorded lower responses in crossbred sheep. A difference between these studies and the present one is that no progestagen implant has been used in these studies.

Gulyani and Naqvi (1994) have indicated that the age of the ewe, season of the year, breed, number of flushing and dose protocol for superovulation influenced the superovulatory responses in ewes.

Pregnancy rates and live births : Out of total 12 embryos transferred to 7 synchronized recipients three pregnancies and live births resulted giving a pregnancy rate of 25 per cent. Pregnancy rates in previous studies have shown to be 54% with transfer of *in vivo* embryos in Hungarian

Table 1. Effect of eCG on superovulation and embryo recovery in Marwari ewes

	Group I	Group II
No. of ewes treated	4	4
No. of ewes in estrus	3	3
No. of CL / ewe	7.0±2.51	10.0±5.13
No. of large unovulated follicles	1.67±0.66	1.34±0.66
No. of ewes flushed	3	3
No. of ewes yielding eggs	3	3
Total ova recovery rate	4.66±2.66	9.34±4.37*
Fertilized eggs/ewe	3.66±2.18	7.34±4.48
No. of morula/blastocysts/ewe	3.0±1.52	4.34±2.02

*Significant ($P < 0.05$)

Merino ewes (Cseh and Solti, 2001). Increased number of transferred emryos positively influence the pregnancy rate (Cseh and Solti, 2001). Lower number of transferred embryos in the present study could be one reason for the lower pregnancy rates.

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