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Full Length Research Paper

Developmental competence, birth and survival of lambs following transfer of twin or triple embryos of dwarf size prolific donor into large size non-prolific recipient sheep

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The objective of the present study was to assess the embryo survival and development of progeny following transfer of either 2 or 3 embryos derived from dwarf size prolific Garole sheep into non-prolific large size Awassi x Malpura crossbred recipient ewes. Embryos were collected from donor ewes following induction of superovulation using FSH (5.4 mg Ovagen) and PMSG (200 IU) regimen. Estrus was synchronized in donor and recipient ewes by administering two injections of prostaglandin $F_2 \alpha$. The recipient ewes were divided into two groups and each recipient ewe received either 2 (Group 1) or 3 embryos (Group 2) of transferable quality in the uterine horn ipsilateral to corpus luteum. The recipient ewes of both the groups were examined for the presence of fetuses at 40 days of gestation by ultrasonography. The pregnancy and lambing percentages of ewes belonging to Group 2 were 57%, which was comparatively higher than Group 1 ewes where it was 42.9%. The survival of embryos was 38.1% in Group 2 and was higher compared to Group 1 (28.6%). The survival of lambs at weaning was higher in Group 1, compared to Group 2. The results indicate that survival of embryos and pregnancy rate was better following transfer of 3 than 2 embryos of prolific sheep to non-prolific sheep.

Key words: Microsheep, garole, embryo transfer, embryo survival, multiple births.

INTRODUCTION

The global spread of prolific sheep breeds from their country of origin has been possible through embryo transfer (Fahmy, 1996). Embryo transfer can play an important role for conservation, faster multiplication and propagation of prolific and endangered sheep breeds. Garole sheep is India's most valuable germplasm due to its high prolificacy and the ability to thrive well under harsh and adverse climatic conditions (Sharma et al., 1999). The DNA test has provided conclusive evidence that the origin of *FecB* gene in Booroola Merino strain was via the prolific dwarf Garole sheep (Davis et al., 2002). Garole sheep is found in the hot and humid Sunderban region of West Bengal weighing 10 - 14 kg at maturity. An average litter size of 2.27 lambs with 7.3% single births, 65.45% twins, 21.8% triplets and 5.45% quadruplets has been reported for garole ewes (Ghalsasi and Nimbkar, 1993). Garole rams produce good quality semen, which can be stored for short-term and long-term preservation (Joshi et al., 2001). There is an enormous potential for introgression of the *FecB* gene from its original carrier Garole sheep into large size non-prolific breeds. Success has been achieved in evolving new prolific germplasm by artificial insemination of Malpura ewes using diluted Garole semen (Naqvi et al., 2002) and subsequently multiplied by *inter se* mating among Garole x Malpura halfbreds (Sharma et al., 2004) . The segregation of *FecB* gene has been identified in Garole x Malpura crosses and their progeny (Kolte et al., 2005).

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Many homozygous carriers in the Garole breed have a maximum litter size of twins (Davis et al., 2002) and the effect varies according to the breed into which the gene is introgressed (Nimbkar et al., 2002). Our recent studies have shown evidence of production of large number of *in vivo* derived embryos in a single flush from a Garole ewe (Naqvi et al., 2006 a) and birth of heavier Garole lambs following transfer in large size recipients compared to contemporary lambs produced through hand mating of Garole ewes (Naqvi et al., 2006b). There is an economic incentive on transferring multiple embryos in recipient sheep to reduce the number of recipient ewes and additional burden on their upkeep. There are conflicting reports available on embryo survival in sheep as it remained unaffected (Armstrong and Evans, 1983), increased (Quirke and Hanrahan, 1977; Cseh and Seregi, 1993) or decreased (Mutiga, 1991). However, no report is available following transfer of multiple embryos derived from small size prolific donor sheep into large size non-prolific recipient sheep. It is also not known whe-ther the group survival of genetically distinct embryos of prolific sheep will be favourable in the maternal uterine environment of non-prolific large size recipient ewes in which the limitation associated with uterine size is compensated. The aim of the present investigation was therefore, to observe the developmental competence, birth and survival of Garole lambs following transfer of twin or triple embryos in proximity to its average litter size into large size non-prolific recipient ewes.

MATERIALS AND METHODS

The embryo transfer trial was carried out during late autumn of the year 2004. The experimental animals were maintained at the Institute's sheep farm at Avikanagar which is located at longitude of 75º - 28'E, latitude of 26º - 26'N and altitude of 320 m above mean sea level in the semi-arid region of the country. The climate of this region is essentially tropical. The highest temperature occurs from April to June when mean monthly temperature is about 42ºC and may reach up to 49°C. The rainfall is erratic and mainly concentrated during July to August. The precipitation ranges from 400 to 700 mm per annum.

Four clinically healthy and cycling ewes of Garole and 14 Awassi x Malpura crossbred of 4 - 7 years of age were used as embryo donor and recipient, respectively for this study. The body weight of Garole and Awassi crossbred ewes ranged between 12.5 to 16 and 31 to 48 kg with the mean ± SEM of 14.4 ± 0.95 and 38.2 ± 1.18 kg, respectively. All the animals were grazed 8 - 10 h daily on natural vegetation interspersed with seasonal shrubs and herbs. In addition to grazing, garole ewes were provided concentrate mixture of 150 g/ewe/day while it was 300 g/ewe/day for Awassi crossbred ewes. The concentrated mixture contained 18% crude protein (CP), 65% total digestible nutrient (TDN), 1% mineral mixture, 1% common salt and vitamins (A and D_3).

Estrus in embryo donor ewes was synchronized using two injections of prostaglandin $F_2 \alpha$ (PGF) (Lutalyse; Pharmacia N.V./S.A. Puurs- Belgium) @ 7.5 mg each, administered intramuscularly at 10 days interval. Superovulation in donor ewes was induced with the use of pregnant mare serum gonadotrophin (PMSG) and follicle-stimulating hormone (FSH) of ovine origin (Ovagen, ICP, New Zealand). Superovulatory treatment was commenced three days prior to second PGF injection. Each of the donor ewes received a total dose of 5.4 mg FSH (NIADDK-O FSH-17) twice a day (morning and evening) at a constant dose over a period of four days. The ewes were treated with 200 IU PMSG (Folligon, Intervet-Netherlands) intramuscularly at the commencement of the superovulatory treatment.

Estrus in ewes was detected by parading aproned ram of high sexual vigour at 6 hourly intervals up to 4 days from the day of second PGF dose.

Donor ewes exhibiting behavioural estrus were mated twice a day (morning and evening) with a ram of proven fertility. Donor ewes were subjected to ovarian examination with the aid of laparoscope (Karl Storz, Germany) followed by laparotomy for embryo recovery (Naqvi et al., 2001, 2006a, b) between 3 to 4 days after mating. In brief, the animals were fasted for at least 24 h prior to laparoscopy and/or laparotomy. The abdominal area anterior to udder was shaved and sprayed with 70% alcohol and the ewes were sedated with Xylazine hydrochloride (Xylazine, Indian Immunologicals, India) and locally anaesthetized by infiltration of lignocaine hydrochloride (Xylocaine, Astra Zeneca, India). All the ewes treated for superovulation were examined by laparoscopy and found worth attempting for surgical embryo collection.

For embryo collection the reproductive tract was visualized through the laparotomy and the uterine horn was carefully exposed to at least the bifurcation of the two horns.

The flushing media (20 ml Dulbecco's phosphate buffered saline supplemented with 2% Bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) was introduced into the base of the uterine horn using the blunt Jelco needle with syringe. The medium was flushed towards the tip where it was collected through a polythene catheter (OD 3 mm) introduced into the fallopian tube through fimbrae. The flushed media was collected in a graduated glass tube and the process was repeated for the other uterine horn.

The recovered fluid was transferred into a sterile petri dish for searching and examining the embryos under a Stereozoom microscope (Nikon, Japan) equipped with a warm stage set at 37°C and 50X magnification. The fertilization of ova was verified by cleavage.

The embryos were evaluated for quality under Inverted microscope (Olympus, Japan) having warm (37°C) stage platform at 400X magnification. The quality of transferable embryos was assessed according to the morphological criteria based on symmetry of the cells, shrinkage, vacuolization or lysis (Robertson and Nelson 1998). The good quality embryos were utilized for transfer into recipients.

Estrus in recipient ewes was also synchronised using PGF as in donor ewes. The recipient ewes in close synchrony with donor ewes were divided into two groups of 7 each. Each recipient received either 2 (Group 1) or 3 embryos (Group 2) of transferable quality in uterine horn ipsilateral to the right or left ovary possessing corpus luteum. Embryo transfer in ewes was performed by a modified laparoscope aided procedure (Naqvi et al., 2001) as described by McMillan and Hall (1994).

At 40 days of gestation, all the recipient ewes were examined for the presence of foetuses by using a real time Desktop Veterinary Ultrasound Scanner system equipped with convex array 5.0 MHz / 40R / 60D transducer (Model SA-600V, MEDISON Company, Limited. Korea). The recipients were monitored routinely throughout the pregnancy till lambing.

The data on number of ewes diagnosed pregnant by ultrasonography, lambs born per recipient; birth weight and weaning weight of lambs at 3 months of age were recorded. The mean values were analyzed by student t-test and the proportions by using chi-square test (Snedecor and Cocharan, 1980).

Table 1. Pregnancy and lambing rates of non-prolific recipients following transfer of twin or triple embryos derived from prolific Garole sheep.

Parameters	Group 1 (2 embryos/ewe)	Group 2 (3 embryos/ewe)	Overall
No. of recipients	7	7	14
No. of embryos transferred	14	21	35
No. (%) ewes pregnant*	3 (42.9)	4 (57.1)	7 (50)
Number (%) of embryo survived*	4 (28.6)	8 (38.1)	12 (34.3)
No. (%) of ewes lambing	3 (42.9)	4 (57.1)	7 (50)
Type of birth			
Single	2	1	3
Twin	1	2	3
Triplet	-	1**	1**
Total lambs	4	8	12
Lamb survival (%)at weaning	3 (75)	5 (62.5)	8 (66.6)
Number (%) of peri and postnatal losses	11 (78.5)	16 (76.1)	27(77.1)

* At 40 days of gestation.**Includes one still birth.

RESULTS

Table 1 summarizes the data on pregnancy and lambing rates of non-prolific embryo recipient ewes following transfer of 2 or 3 embryos per ewe, which were derived from dwarf size prolific Garole sheep. The ovarian examination through the aid of laparoscope at the time of embryo transfer revealed the monotocus character of recipients since all had single ovulation point. Ultrasonography of ewes after 40 days of gestation confirmed pregnancy in 57% of ewes in which 3 embryos/ewe was transferred. The pregnancy rate in this group was relatively higher, compared to 42.9% in ewes, which received 2 embryos/ewe. The fetuses counted were identical with the number of lambs born in each group but the survival of embryos and their development to term was relatively higher in ewes that received 3 embryos, compared to ewes that received 2 embryos. The values for pregnancy and lambing rates, however, did not approach to statistical significance due to the relatively low number of recipients. The mean gestational length of ewes carrying single, twin or triplet fetuses was 148.3, 146.7 and 146 days, respectively. Likewise, the mean birth weight of lambs produced as singleton, twin and triplet lambs was recorded as 1.60, 1.57 and 0.68 kg, respectively (Not given in the Table 1). The percentage of peri and postnatal losses were similar on transferring 2 or 3 embryos per ewe.

DISCUSSION

The results of this study indicate that small size prolific Garole ewes are suitable as an embryo donor and provide an ample scope for transfer of *in vivo* derived embryos into large size recipient ewes. Apart from possessing the fecundity gene responsible for increasing the ovulation rate, the high incidences of multiple births in garole sheep (Ghalsasi and Nimbkar, 1993) is possible because its small maternal size environment can also sustain the survival and development of multiple embryos up to parturition. It was worth investigating to know if same number of embryos derived from prolific sheep can also develop to term in the uterine environment of approximately 2.65 times higher body size non-prolific sheep, which provided more space for embryo development than small size garole ewes. The gestational length did not significantly vary in the ewes carrying single, twin or triplet foetuses indicating that the uterine capacity of large size non-prolific recipient is not a limiting factor on the development of embryos up to term.

The present study gave an indication that incidence of embryonic mortality up to 40 day of gestation was reduced when the number of transferred embryos was increased. Higher pregnancy rate of 55.2% has also been reported in Hungarian Merino ewes following transfer of two embryos per recipient, compared to 45.6% in case of single embryo transfer (Cseh and Seregi, 1993).

Nancarrow (1994) has reported that there is a possibility of an interaction between embryos that lead to each other's survival. In this study the embryo survival up to 40 days of gestation and also up to term was 38.1% when 3 embryos were transferred per ewe and was relatively higher as compared to transfer of two embryos per ewe where it was 28.6%. In the present study, all the embryos were transferred to the ipsilateral uterine horn. It has also been reported that the transfer of two embryos to the ipsilateral or both uterine horns does not influence the survival of embryos (Torres and Sovellec, 1987). Transuterine migration of embryos occurs when two or more ova are shed from a single ovary (Scanlon, 1972) or multiple embryo transfer in single uterine horn (Rowson et al., 1971) . Migration of embryo to contra-lateral horn may occur even when that horn is not asso-ciated with a corpus luteum and the chances are greater for the survival of non-migrant embryos (Doney et al., 1973). Since embryos in this study were transferred unilateral, therefore, there was greater possibility of an interaction between embryos. Breed difference was observed between control Merino and prolific Booroola Merino with respect to embryo/ fetal losses when the ovulation rate exceeded to 2 in control or 3 in Booroola Merino ewes (Bindon and Piper 1986). In this study, all the recipient ewes had a single ovulation point, which confirmed their monotocous breed character. Our results were also in accordance with the findings of Mutiga (1991) that multiple transfer of embryos in tropical sheep increased the number of lambs born per pregnant ewes.

In this study the postnatal losses increased with the number of embryos transferred and also with the litter size because transfer of 14 embryos at the rate of 2 per ewe and 21 embryos at the rate of 3 per ewe in equal number of recipients delivered 3 and 5 weaned lambs, respectively. Although the percent survival of Garole lambs up to weaning, produced on transfer of 2 embryos per ewe, was relatively higher but the percent of total peri and postnatal losses was comparable to the lambs produced on transfer of 3 embryos per ewe.

This study indicates that transfer of 3 embryos of prolific garole lambs per non-prolific ewe will be favourable for the overall production of lambs and it can also minimize the number and the maintenance cost on the upkeep of recipient ewes. Further research is required to intensify this work on a large scale and also study the postnatal growth of prolific garole lambs produced from large size non-prolific recipients up to puberty.

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