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Laparoscope Assisted Embryo Transfer and Conception Rate in Farm Rambouillet Ewes

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ABSTRACT

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The present study was performed to assess the usefulness of laparoscope in the embryo transfer programmes in sheep and to evaluate conception rate after single, double and triple embryo transfer in farm bred rambouillet ewes. A total of 21 freshly collected embryos were transferred in 11 estrus synchronized ewes to yield nine lambs. The conception rate of single, twin and triplet embryo transfer was 66.67%, 50% and 100%, respectively with the lambing rate was 0.00%, 66.67% and 100%, in the respective groups. The embryo survival rate was 100% and 80% in twin and triplet embryo transfers. It was concluded that laparoscope is very useful tool in detection of ovulation and selection of suitable ewes for embryo transfer. Aid of laparoscope in embryo transfer programme reduces surgical risks and minimizes total time in embryo transfer.

Introduction

Sheep is a multi-facet utility animal reared mainly for the flesh and fibers, though it also produces some milk and has religious values too. Its contribution in the rural economy is important particularly in the mountainous areas of the country. Easy rearing under broad environmental conditions, short generation cycles and a high fecundity are the characters which need to be properly exploited to

enhance the contribution of sheep in the development of Indian economy. Embryo transfer is a technique by which elite female animals are superovulated to produce embryos which are collected from uterus prior to implantation and are transplanted in to the reproductive tract of synchronized recipient females to bear gestation and give birth of offspring (Gibbons and Cueto, 2011). It is a unique tool and has opened up many possibilities in enhancing production potential

in sheep by shortening the generational interval. Use of laparoscope minimizes surgical trauma and chances of post surgical adhesion (Gebrehiwot *et al.*, 2018). Various researchers had observed adverse effect of multiple pregnancies as lower birth weight (Alexander, 1974) and high mortality after birth (Huffman *et al.*, 1985) leading to retarded growth and poor performance in postnatal life (Brown and Radziewicz, 1998).

Ovine embryo transfer has not being extensively used in India, since CSWRI (ICAR) has made pioneering efforts on superovulation and surgical embryo transfer in the native Gargole sheep (Naqvi *et al.*, 2006). Transfer of multiple embryos in recipient reduces the cost during care of recipients, thus is economical, though various reports available on this are conflicting (Naqvi *et al.*, 2007) and finally requires to be tested for the specific sheep breed used. Sporadic trials have been made to superovulate ewes in the other parts of the country but this technology is yet to be adopted at sheep breeding farms. This work was performed to evaluate the usefulness of laparoscope in the embryo transfer programmes and to evaluate conception rate after single, double and triple embryo transfer in farm bred rambouillet ewes.

Materials and Methods

The present study was carried at Embryo Transfer Technology (ETT) laboratory at Govt. Sheep Breeding Farm (SBF), Sheep Husbandry Department of Jammu, Panthal, District Reasi, lying in the subtropical zone of Jammu and Kashmir, located at the latitude of 32°58'29"N and longitude of 74°58'35"E and 1671 meters above the mean sea level. The experiment was conducted during breeding season (August to November) months of the year 2015, 2016 and 2017, with environmental temperature fluctuating

between a minimum of 8°C and a maximum of 30°C. Eighty parous cyclic crossbred ewes aged between 6 and 8 years, in their 2nd to 4th parity, weighing between 40 and 50 kg and in good body condition (score > 3 out of 5) were selected for the experimentation.

All the ewes were kept under loose housing system in the animal sheds having concrete flooring, asbestos roofing and sufficient space for the free movement in a clean and hygienic condition. They were grazed daily from 07:00 AM to 06:00 PM on green pastures interspersed with seasonal shrubs with water ad libitum. Additionally each ewe was fed daily 6 kg green/dry fodder (Oat/maiz/barseem) and 500 gm concentrate mixture. All the ewes were examined using an ultrasound scanner to rule out pregnancy and any pathological defects of genitalia and ovarian abnormalities.

Estrus synchronization of donor and recipient ewes

All donor and recipient ewes were synchronized for estrus using polyurethane sponge impregnated intravaginal progesterone implant, kept *in situ* for 12 days. The recipient ewes received injection serum gonadotropin (eCG, Folligon, Intervet) @ 200 IU just after withdrawal of progesterone sponge while donor ewes received eCG at the time of first FSH injection. Estrus detection in donor and recipient ewes was carried out using an apronized ram an interval of 3 hrs after removal of the implant. Donor ewes were bred with quality ram while recipient ewes were strictly segregated to prevent breeding.

Embryo production

Embryos were produced from superovulated donor ewes using purified porcine FSH given in six tapering dose protocol and eCG, injection given at the start of first FSH given

on day 10 after application of progesterone sponge. Donor ewes were treated in two FSH dose groups with/without hCG hormone given 24h after removal of implant, thus making four groups Group-I (80 mg FSH), Group-II (80 mg FSH+ 500 mg hCG), Group-III (100 mg FSH), Group-IV (100 mg FSH+ 500 mg hCG). On observation of estrus donor ewes were hand mated once and further same breeding ram was allowed to accompany over night with them.

Embryo collection was performed surgically after 5-6 days of breeding date in donor ewes. Retrograde flushing of the uterine horns were done using flushing media (Euroflush, IMV Technologies) with the help of IV cannula introduced near the bifurcation of uterine horns. The washings were collected through catheterization of fallopian tubes and collected in sterile beaker. Searching, isolation, evaluation and gradation of embryos were performed under stereo zoom microscope (Olympus, Japan) at 200X and were classified morphologically as per Gibbons and Cueto (2011).

Laparoscopy and embryo transfer

Suitable recipient ewes that have synchrony in onset of heat within 12 hrs with the donor were selected for embryo transfer. For evaluation of site of ovulation a 2.5 mm diameter and 30 cm length with 300 forward-oblique rigid laparoscope telescope (KARL STORZ) was used. Pneumoperitonium using 4-5 L CO₂ gas was achieved using by KARL STORZ- ENDOFLATOR device if required during the examination.

Ewes were restrained in dorsal recumbency on a special designed lap cradle with all the four legs firmly secured. Abdominal area anterior to udder was shaved and scrubbed with Savlon solution, dried with sterile gauze and sprayed with 70 % alcohol for

sterilization of clean skin. Sedation was achieved using Inj. xylazine @ 0.29 mg per kg body weight and local anesthesia by subcutaneous infiltration of Inj. lignocane 2% (~ 5ml) at the line of incision, 5-7 cm anterior to udder and 3-4 cm each side of the mid ventral line. Endoscope was sterilized by dipping in 70% alcohol solution followed by rinsing with double distilled water in between each examination.

Two small (2cm) incisions using angular blade were made in the skin, approximately 4-6 inches in front of the udder, on each side of midline avoiding blood vessels. Trocar and trocar sleeve for the laparoscope were inserted on the left side of midline by sliding it under the skin about one inch before inserting it into the peritoneal cavity, avoiding puncturing blood vessels or other structures. Trocar was removed as soon it entered in the peritoneal cavity. Insufflation of peritoneal cavity was done for better visualization if required using pneumoperitoneum apparatus.

Laparoscope was inserted through trocar sleeves to observe the reproductive tract. Manipulation probe was inserted through second incision and its entry viewed into peritoneum through the laparoscope. With the manipulating probe it was easier to bring the uterine horn under field of vision and observe surface structures on the ovaries (Fig. 2). Locally modified atraumatic Allis's forceps were used to hold one of the uterine horns for lifting it up to the outer surface opening for better visualization of the horn with naked eye for embryo transfer.

Embryos were loaded in tomcat catheter in between air spaces attached to 1 ml syringe. Ovaries were viewed through laparoscope with the aid of manipulating probe and uterine horn ipsilateral to the CL was gently lifted to the surface of skin and held between thumb and forefinger. An aperture was made in the

uterine horn, 1/3rd towards ovary with a small conical trocar and embryo loaded catheter was inserted into lumen of uterus towards uterotubal junction followed by embryo deposition (Fig. 3).

After suturing the incision site using catgut (no. 1) for muscles and nylon thread for skin, Betadine ointment was applied at the sutured site. Inj. Streptopenicillin (Dicrysticin, Sarabhai Zydus, India) @ 500 mg was given just prior to laparoscopy and continued for 5 days BID along with Inj. meloxicam (Melonex; Intas Pharma., India) @15 mg/ewe SID for 3 days. Ewes were placed in recovery pens and sutures removed after 8-10 days post surgery.

Recipient ewes post 30-40 days of embryo transfer were scanned with ultrasound machine (Prosound 2, Toshiba Aloka) using 5MHz transabdominal sector probe for presence of signs of pregnancy i.e. placentomes and fetus with fetal fluid.

Results and Discussion

The results obtained from the four recipient groups after embryo transfer, are presented in the table 1, the success rate after embryo transfer shows the effectiveness of laparoscopy use in this study. It is evident from the results that conception rate of triplet embryo transfer was highest (100%) with high lambing rate (100%). The conception rate of single embryo (group-I) and twin embryo (group-II) transfers were also satisfactory (66.6% and 50%, respectively). Loss in conception occurred due to abortion occurring at day 45 and 140 days of gestation period under the group I and no lambing occurred in single embryo transfer, while one of the recipients in the group II bearing twin fetuses died unfortunately due to wild animal attack (Fig. 5). These results are quite satisfactory and are better than the previous reports of 29% to 65% pregnancies and 46% -

80 % lambing after embryo transfer in sheep (Ishwar and Memon, 1996; Gibbons *et al.*, 2019; Folchet *et al.*, 2000; Dattena *et al.*,2000). Brown and Radziewicz (1998) reported higher pregnancy rate after transfer of twin (64%) and single (39%) *in vitro* derived embryos. Variations in the conception and lambing rate depends on various factors such as the developmental stage of embryo at the time of transfer, synchronization between donor and recipient, age of embryo donor, embryo storage, and embryo production method (*in vivo* or *in vitro*) (Thompson *et al.*, 1995; Dattena *et al.*, 2000).

The present study transferred only excellent and good quality embryos (Fig. 1) which has resulted high conception rate. The high conception rate obtained in this experiment shows precision in quality and selection of transferable grade (Grade-I and Grade-II) embryos (Angela *et al.*, 2006). These results are comparable with the reports of Rizzo *et al.*, (2012), who observed lambing rate of 83.6% and survival rate of 63% after embryo transfer. They also reported that conception rate in multiparous recipient ewes were better than nulliparous and for best results two embryos should be transferred to recipient ewes having more than two corpora lutea. The high gestational loss after single embryo transfer in our experiment is mainly due to lower number of animals used in this group. Gibbons *et al.*, (2011) suggested single embryo transfer as the viability of fetus is equal to the twin embryo transfer, around 41% and 50% for transfer of morulae and blastocysts, respectively. Higher perinatal mortality occurs even under the extensive conditions and ultimately penalize the final yields of the embryo transfer though twin and triplet embryo transfer reduces maintenance cost on recipients (Naqvi *et al.*, 2007). Fernandez Abella (2015) observed 75% and 90% of lamb survival for twin and single lambs, respectively. The lamb survival observed in our research was 100 % in twin

embryo transfer while it was 80 % in the triplet transfer group (Table 1, Fig. 4). In previous reports greater lamb survival for single lamb (80%) than for twin lambs (43%)

was observed by Brown and Radziewicz (1998) from IVF derived embryos, while Naqvi *et al.*, (2007) suggested transfer of triplets for economic concerns.

Table.1 Embryo transfer and lambing in different recipient groups

Recipient groups (number of recipient used)	Number of embryo transferred in each recipient	Ewes conceived (%)	Lambing /conceived ewes (%)	Gestational loss (%)	Number of embryo transferred	Lambs born	Efficacy of embryo transfer	Lamb survival after birth (%)
Group-I (3)	1	2(66.67%)	0*	2 (100%)*	3	0	0.00%	-
Group-II (6)	2	3(50.00%)	2/3(66.67%)#	1(33.33%)#	12	4	33.33%	4(100.00%)
Group-III (2)	3	2(100%)	2/2(100%)	0(0%)	6	5	83.33%	4(80.00%)
Total (11)	-	7(63.64%)	4/7(57.14%)	3(42.86%)	21	9	42.86%	8(88.89%)

*Both conceived ewes aborted at 45 day and 140 days of gestation.

#One animal unfortunately killed in wild animal attack bearing twin fetuses



Fig.1 Transferable grade sheep embryo (morula stage)



Fig.2 Laparoscopic evaluation of ovulation in recipient ewe



Fig.3 Laparoscope assisted embryo transfer (loaded in tomcat catheter)



Fig.4 Lambs born after embryo transfer



Fig.5 Fetuses recovered after postmortem of recipient ewe died in wild animal attack

There are a great number of environmental factors that influence reproduction (Bronson, 1985) and many of them influence prenatal survival, besides this various maternal and embryonic factors accrues to increased embryonic death after embryo transfer (Wilmut *et al.*, 1986). The embryo transfer efficacy calculated on the basis of lamb born out of total embryos transferred was 42.86% in this experiment is comparable with results of Naqvi *et al.*, (2007), though it is lower than global standard (Gebrehiwot *et al.*, 2018) and leaves scope for further investigations in refining this technique.

We can conclude that the pregnancy rate following fresh embryo transfer in recipient ewes in the sheep farm is 63.64%. The lambing rates of conceived ewes are 57.14% with 42.86% gestational loss. The efficacy of embryo transfer was high (83.33%) in triplet embryo transfer, whereas lamb survival after birth was highest (100%) in the twin embryo transfer. Laparoscope was effectively used in embryo transfer programme at farm level. High gestational loss after embryo transfer requires much attention and needs special care of pregnant ewes to make the programme efficient and viable. The embryo transfer efficiency obtained after twin and triplet embryo transfer in this experiment is as par global embryo transfer efficiency and recommends its use for the sheep farm.

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