

THERIOGENOLOGY

LAPAROSCOPIC EMBRYO TRANSFER IN DOMESTIC SHEEP: A PRELIMINARY STUDY

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ABSTRACT

An effective, minor-invasive technique for embryo transfer in sheep was developed using a laparoscopic transabdominal approach. Twelve recipient ewes received embryos either by conventional laparotomy or by laparoscopy. The estrous cycle of recipient ewes was synchronized using a progestagen-impregnated vaginal pessary/pregnant mares' serum gonadotropin treatment regimen. Donor ewes were superovulated with follicle stimulating hormone or human menopausal gonadotropin, bred with a ram of one breed and laparoscopically inseminated *in utero* with semen from a different sheep breed. Five to six days after estrus, embryos were transferred laparoscopically into the terminal one-half of the recipient's uterine horn ipsilateral to the ovary with prominent corpus luteum development. Pregnancy was diagnosed by transrectal ultrasonic procedures, and by direct laparoscopic examination of the uterus. Of six laparoscopic transfers, three resulted in single births; one of six laparotomy transfers resulted in a live birth. Breed appearances of the four lambs born indicated that two of the offspring resulted from laparoscopic artificial insemination of the donor ewe. The results demonstrated that laparoscopic transfer of embryos was a rapid and safe procedure, easily applied to an ovine embryo transfer program and with potential for similar studies in other species.

Key Words: Embryo Transfer, Laparoscopy, Sheep

INTRODUCTION

Laparoscopy is a safe, minor-invasive surgical procedure serving a valuable role in biomedical research (1,2). Roberts (3) first reported an

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THERIOGENOLOGY

ovine laparoscopic technique using a refined fiber-optic system and trocar-cannula approach through the ventral abdominal wall. Laparoscopy has been effectively used in the ewe to study estrous behavior-pituitary-ovarian relationships (4,5), ovarian morphology (6,7) and ovulation rate (8,9). Laparoscopic pregnancy diagnosis (10), follicular aspiration (11) and more recently, artificial insemination (12) also have been shown to have practical application in sheep.

Direct access to the uterus of the ewe for embryo collection and transfer classically has been by surgical laparotomy (13-18). Repeated laparotomies and handling of the reproductive tract can traumatize tissues and increase the incidence of abdominal adhesions, thereby reducing the potential useful longevity of recipient ewes. Because of laparoscopy's minor intrusive nature, incision sites are smaller and the incidence of bacterial infection, tissue dehydration and adhesion formation is less than that incurred using laparotomy (7).

In our laboratory, the sheep serves a useful role in embryo research and as a model for similar developmental studies in rare or endangered hoofstock. Because wildlife species are stress-susceptible, a continuing effort is made to develop or modify manipulative procedures to be as atraumatic as possible. It was hypothesized that laparoscopy might be a safe, efficient method for transabdominal embryo transfer to a predesignated site within the uterine horn of the recipient. The objective of this study was to develop a laparoscopic technique for transferring sheep embryos that may have future application in non-domestic species. These preliminary results were collected as part of a more comprehensive study to evaluate endocrine function and ovarian activity in ewes receiving various gonadotropin treatments, the latter data being prepared for publication separately (19).

MATERIALS AND METHODS

Animals. Crossbred white-face ewes were housed in an outdoor, partially sheltered pen, fed a hay pelleted ration twice daily (0700 h, 1500 h) and provided free access to water.

Estrous synchronization, ovulation induction and embryo collection. The estrous synchronization/superovulation regimens used and estrus/ovulation/embryo results are the primary subject of another paper (19); however, general methods pertaining to these procedures are described here briefly. Sponge pessaries, each impregnated with a 60 mg medroxyprogesterone acetate suspension (MAP, Depo-Provera[®], Upjohn Co., Kalamazoo, MI) were inserted intravaginally on Day 0 in recipient ewes. On Day 11 of MAP treatment these ewes were injected with 400 IU pregnant mares' serum gonadotropin (PMSG, Gestyl[®], Diosynth, Chicago, IL; im) and the MAP-sponges removed 24 h later (Day 12).

Nine days after a detected estrus, donor ewes received one of two 3.5 day gonadotropin regimens: 1) follicle stimulating hormone (FSH-P[®], Burns-Biotech, Omaha, NE, i.m. injections twice daily, total dosage 19 mg); 2) human menopausal gonadotropin (hMG, Pergonal[®], Serono Labs., Randolph, MA; i.m. injections twice daily, total dosage 1350 IU). At the subsequent estrus each donor was paired 24 h with either a Dorset or Suffolk ram for

natural mating. The ram not used for natural breeding was electroejaculated approximately 12 h after the onset of donor ewe estrus. Raw semen (0.1 to 0.2 ml) was laparoscopically deposited into each uterine horn using the technique described below for transabdominal embryo transfer. Surgical embryo collections were performed on Day 5 or 6 of the donor's estrous cycle (Day 0 = first day of estrus). Each uterine horn was flushed with PBI medium(20) + 20% heat-treated fetal calf serum, and recovered embryos were maintained at room temperature (21 to 25°C) until transferred. After evaluation, one or two randomly assigned late morula to blastocyst stage embryos were transferred into recipient ewes (within + 12 h of estrus synchrony of donors) by laparotomy (treatment I) or laparoscopy (treatment II). Using either technique, the embryos were transferred to the terminal one-half of the uterine horn ipsilateral to the ovary with the most prominent appearing corpus luteum or corpora lutea.

Anesthesia and general surgical procedures for recipients. Feed and water were withheld from ewes for 24 h. Atropine sulfate (Med-Tech, Elwood, KS; 0.22 mg/kg, i.m.) was given as a pre-anesthesia. Anesthesia was induced initially with xylazine (Rompun®, Bay Vet, Shawnee, KS; 0.22 mg/kg, i.m.) followed 5 min later with ketamine hydrochloride (Vetalar®, Parke-Davis, Detroit, MI; 11.0 mg/kg, i.m.). The xylazine-ketamine combination produced a surgical plane of anesthesia which was maintained with halothane via endotracheal intubation. After restraining the animal on a surgical table in dorsal recumbency, the ventral abdomen in clipped free of wool and surgically prepared. Standard laparotomy procedures, as initially described by Hunter and coworkers (13), were used for transferring embryos according to treatment I.

For laparoscopic transfer (treatment II), the surgical table was angled to 45° so that the ewe was in a head-down position. A pneumoperitoneum was produced by inserting a 120-mm long Verres needle intra-abdominally in the lower right abdominal quadrant midway between the flank and umbilicus. The needle was attached to an automatic insufflator (Richard Wolf Medical Instruments Corp., Rosemont, IL) by a flexible gas hose and approximately 2 l of 100% CO₂ were passed into the abdominal cavity. A small skin incision (1 cm) was made on the ventral-midline between the mammary gland and umbilicus and a 10-mm diameter trocar-cannula inserted through the peritoneum. The trocar was removed and replaced with a 10-mm, 180° laparoscope (Richard Wolf Instruments Corp.) attached to a high intensity fiber optic light source.

Using the Verres needle to manipulate, the uterine horns and the entire surface of each ovary were examined (Figure 1a). A 6-mm diameter accessory trocar-cannula was inserted into the caudal quadrant ipsilateral to the uterine horn selected for transfer. A modified Palmer forceps (Richard Wolf Instruments Corp.) was placed into the cannula and used to grasp and secure the terminal one-half of the uterine horn (Figure 1b). Embryos designated for transfer were drawn into a 3 1/2-FG tom-cat catheter (Soverign, 11.5 cm, Monoject, St. Louis, MO) attached to a 1-ml syringe. To avoid loss of embryos and identify their location within the catheter, one blank medium-air space buffer was arranged on each side of the medium aliquot containing the embryos (Figure 1e). For cannulation of the uterine lumen, a technique originally described for collecting uterine fluids from pigs (21) was modified and employed. A 16-ga 5.5-cm long Teflon catheter

THERIOGENOLOGY

placement unit (Cathlon IV[®], Jelco Lab., Rariton, NJ) was inserted through the abdominal wall at the site directly ventral to the secured and elevated uterine horn. The catheter was gently inserted into the uterine horn (Figure 1c) and upon penetrating the lumen, the stylette needle was withdrawn slightly within the catheter cannula and the latter further advanced (Figure 1d). Generally, the catheter was considered intraluminal when it was passed approximately 2 cm and could be manipulated back and forth freely. The tom-cat catheter containing the embryos was guided through the cannula (Figure 1e), and after it could be freely maneuvered within the lumen the embryos were slowly expelled (Figure 1f). The catheters were withdrawn from the uterine horn, the laparoscope and ancillary instruments removed and the incision sites sutured. Antibiotic (Dual-Pen[®], Med-Tech, Elwood, KS; 20,000 units/day) was injected (i.m.) for three consecutive days post-surgery.

Each recipient ewe was subjected to transrectal probe ultrasound twice (Sheepreg[®], Animark, Aurora, CO) 4 to 5 wk later. Any ewe with an unclear diagnosis was subjected to a laparoscopic examination at 9 to 10 wk after transfer to confirm pregnancy. All ewes were permitted to carry pregnancies to term.

RESULTS

After surgical preparation of the recipient female the laparoscopic transfer procedure required approximately 15 min including the time needed for suturing the two incision sites. Penetration of the uterine horn with the Teflon catheter caused no apparent tissue trauma and withdrawal of the catheter after embryo deposition resulted in negligible hemorrhage from the puncture site.

Data indicated that laparoscopic embryo transfer resulted in pregnancy rates comparable to or greater than those performed by laparotomy (Table 1).

TABLE 1. COMPARATIVE EMBRYO TRANSFER RESULTS

Embryo transfer treatment	Gonadotropin treatment of donors	No. of recipient ewes	No. of pregnant ewes	Overall pregnancy rate(%)
I Laparotomy	hMG	4 (n=6)	1	16.6
	FSH-P	2	0 ^a	
II Laparoscopy	hMG	2 (n=6)	1 ^a	50.0
	FSH-P	4	2	

^a Fetal resorption was diagnosed in an additional ewe.

Pregnancy occurred independent of the two donor gonadotropin treatments. Three ewes were confirmed pregnant by ultrasonic detection of a fetal heart

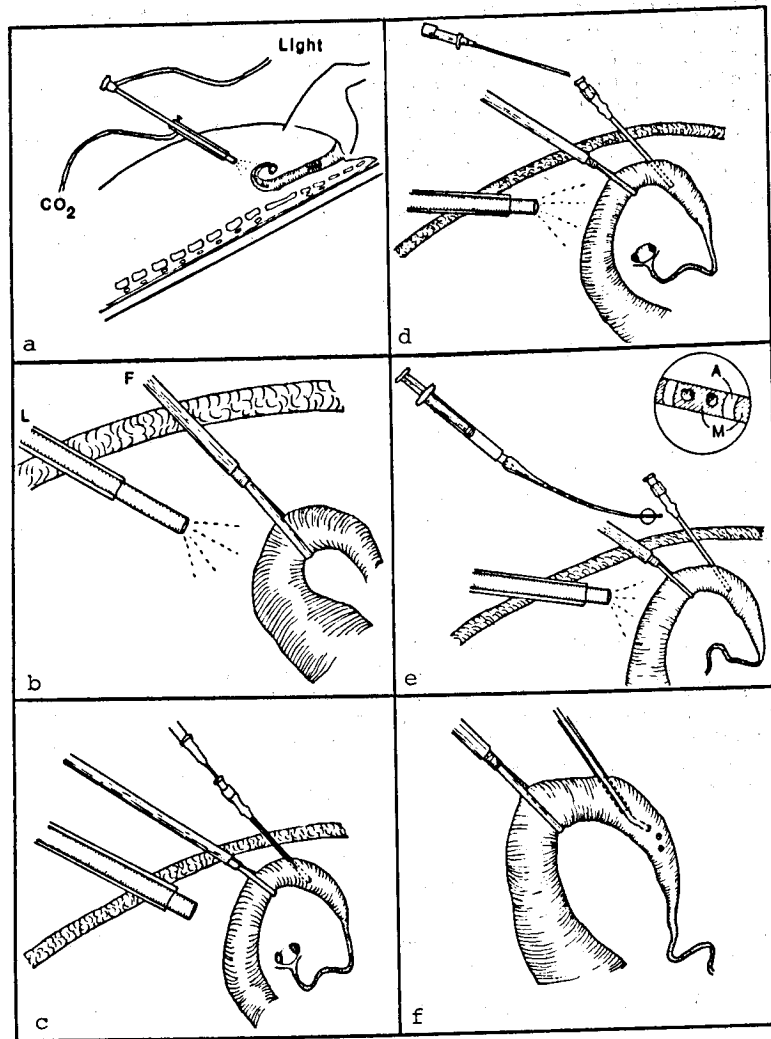


Figure 1. Laparoscopic embryo transfer in the ewe. The lapro-
scope initially is used to identify the reproductive tract(a) and
the ancillary forceps are used to grasp the uterine horn(b). The
horn is then elevated and a catheter placement unit punctured
through the abdominal wall and inserted into the uterine lumen(c).
The 16-ga stylette needle is withdrawn from the cannula(d). Em-
bryos are aspirated into a tom-cat catheter(e) with an air and
medium interface, and the latter guided through the cannula and
extended into the uterine lumen to deposit the embryos(f).
L = laparoscope; F = forceps; A = air space; M = medium.

THERIOGENOLOGY

beat. A strong uterine blood flow was detected in the fourth ewe on two occasions, with final diagnosis of pregnancy confirmed by laparoscopy. Laparoscopic examinations were performed on two other ewes with equivocal ultrasound diagnosis (one ewe from each treatment group). In each ewe the uterine horn receiving the embryo was distinctly enlarged; however, neither female aborted or produced a live birth. Based on the 12 recipients used, four ewes produced lambs (33%): one by laparotomy and three by laparoscopic transfer. Examination of the Dorset or Suffolk breed characteristics of the offspring indicated that two of the four lambs resulted from the laparoscopic artificial insemination rather than the natural mating.

DISCUSSION

These results demonstrate the feasibility and efficiency of using laparoscopic techniques for embryo transfer of sheep. Transabdominal uterine horn cannulation under direct laparoscopic viewing was a rapid, minor-invasive procedure which had distinct advantages over the major surgical approach of laparotomy. Although the pregnancy rate of laparotomized ewes was less than that described in some other ovine embryo transfer studies (17,22), the overall pregnancy rate (33%) was comparable to results obtained by Armstrong and Evans (18). The laparoscopic examinations conducted 9 to 10 wk after transfer in two ewes indicated uterine enlargement. The failure of the latter two ewes to lamb suggest the possibility of fetal resorption.

The study also demonstrated the usefulness of laparoscopic techniques for direct pregnancy diagnosis and intrauterine deposition of semen, confirming earlier reports (10,12). It would appear that these ancillary laparoscopic procedures are extremely valuable in sheep embryo transfer and related reproductive research. In addition, these techniques may have important application in similar studies involving rare, non-domestic species in which less intrusive, atraumatic surgical procedures are highly desirable.

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TherIOGEnology

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THERIOGENOLOGY

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