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Laparoscopic Transabdominal Transfer of Blastocysts in Korean Black Goats

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ABSTRACT

As a part of the effort to improve post-transfer survival rate of embryos in Korean black goats, a technique for laparoscopic uterine transfer of blastocysts was carried out. A total of 26 transferrable embryos (morula to expanded blastocysts) were transferred to 13 recipient goats via transabdominal laparoscopic method. In consequence of our hormone protocol, 65% of the recipients (13/20) were found to have synchronized estrus. After confirmation of corpus luteum in each recipient goat, a Babcock laparoscopic forceps was inserted into the lower abdominal cavity to hold a uterine horn and fasten it near the peritoneum without causing injury. Then 7.5cm long 16G IV catheter was inserted directly into the uterine lumen through the abdominal wall. After removal of the stylet of the IV catheter, the embryo transfer tube (identical in size to the stylet and loaded with blastocysts) was inserted into the uterine lumen through the catheter to unload the embryos. Of the 13 estrus synchronized recipients, 9 were transferred blastocysts and 4 were transferred molurae (2 embryos in each recipient) in uterine ipsilateral to the ovary with corpus luteum. Four of the 9 recipients which blastocysts were transferred using this method has been confirmed pregnant (44.4% pregnancy rate).

(Key words: Laparoscopic, Transabdominal transfer, Blastocyst, Korean black goat)

INTRODUCTION

The first successful embryo transfers in livestock species performed on sheep and goat by Warwick et al. (1935) transferred goat embryos washed from oviduct to opposite horn of same animal and transferred sheep or goat embryos to other animals in each species. The laparoscopic embryo transfer in sheep, successfully performed by Schiewe et al. (1984), transferred surgically recovered embryos to uterine horn by laparoscopy.

In general, the embryo transfer in goat has been performed by laparotomy methods (Shin et al., 2008). Repeated laparotomies and handling of the reproductive tract are stressful to the animal and can cause tissue damages and abdominal adhesions (Schiewe et al., 1984). Because of these disadvantages, such method requires relatively long intervals and reduces the number of times it can be applied to the same animal (Schiewe et al., 1984; Kraemer, 1989; Shin et al., 2008). In contrast, laparoscopy is a safe and less invasive surgical procedure with smaller incision sites, less surgical complications including bacterial infection and tissue dehydration, and also causing less adhesion formation than that incurred when using laparotomy (Schiewe et al., 1984).

So far, numerous studies regarding laparoscopic embryo transfer in goats have been published (Schiewe et al., 1984; Kraemer, 1989; Flores-Foxworth et al., 1992; Besenfelder et al., 1994; Kühholzer et al., 1998; Baldassarre et al., 2002), most of these were performed in large breed goats. In Korean black goats, the only laparoscopic embryo transfer was published by Shin et al. (2008), in which DNA microinjected early stage embryos were transferred laparoscopically into oviduct to produce transgenic goats. However, morula or blastocyst stage embryos needs to be transferred into the uterus, requiring a procedure that is substantially different from one used in oviductal transfers. Therefore, developing a laparoscopical uterine transfer technique is necessary for this species.

This study was aimed at developing an efficient and practicable method for a minimally invasive embryo transfer technique of Korean black goat blastocysts into the uterus directly with the aid of a laparoscope.

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MATERIALS and METHODS

1. Animals

This study was performed during the natural breeding season, from September to March, at the Experimental animal farm of Chungnam National University in Daejeon, Korea. A total of 49 female Korean black goats with body weight ranging from 15 to 30 kg were used as donors and recipients. Two male Korean black goats were used to breed the donor goats. All of the goats were fed TMR (Green-marble®; Geumgang TMR, Korea) with free access to water and trace-minerals.

2. Estrus Synchronization and Ovulation Induction

The estrus cycles of the donors were synchronized with the intravaginal insertion of a controlled internal drug-releasing devices (CIDR, progesterone, 0.3 g; Pfizer, Auckland, New Zealand) for 10 to 12 days, and the CIDRs were removed 24 hours before mating with male goats (Shin et al., 2008). Superovulation was induced by a combined treatment of FSH (Folltropin-V®; Bioniche, Belleville, Ontario, Canada), eCG (Daesung P.M.S.G.®; Daesung microbiological labs, Korea) and GnRH (Gonadon®; Dongbang, Korea). FSH (0.9 mg/goat, 12 hours interval, IM) was administrated to the goats 8 times, starting at 60 hours before CIDR removal and 1 day after CIDR removal. The eCG (150 IU, IM) was administrated at the time of the first FSH administration, $PGF_2α$ (Lutalyse™; Pfizer, Belgium, 5 mg, IM) was administrated at 48 hours before CIDR removal and GnRH (100 ㎍, IM) was administrated at 12 hours after CIDR removal to induce ovulation. The donors that showed estrus behavior were mated with male goats twice at 24 and 36 hours after CIDR removal.

The estrus synchronization of recipient goats was performed in a similar procedure to the donors: CIDR of recipient was inserted at the time of donor insertion and removed at 24 hours earlier than donor. A single dose of 100 ㎍ of GnRH was administrated at 60 hours before CIDR removal.

3. Embryo Recovery

The embryos were surgically recovered 7 days after the first mating with male goat. The donor and recipient goats were fasted 72 hours prior to surgery. Lower abdominal and inguinal area were clipped for the surgical procedure. Atropine sulfate (Atropine sulfate inj; Dai Han Pharm, Korea, 0.22 mg/kg) was subcutaneously injected as a preanesthetic agent 15 minutes before the induction of anesthesia. Anesthesia was induced by intravenous tiletamine-zolazepam (Zoletil 50®; Virbac, Carros, France, 2.0 mg/kg) and maintained with isoflurane (Ifran®; Hana Pharm, Korea) under pure oxygen. The goats were positioned in head-down Trendelenburg about 30°. Lower abdomen of the goats was aseptically prepared with povidone iodine (Povidone; SF. INC, Korea) and 70% alcohol.

Before laparotomy, ovaries were examined by laparoscopy to determine degree of ovarian response to the superovulation treatment. To produce pneumoperitoneum a Veress needle was inserted into abdominal cavity at the left lower abdominal quadrant cranial to udder. Intraabdominal pressure was set at 12 mmHg by insufflating CO_2. A 5 mm skin incision was made on the ventral-midline between mammary gland and umbilicus and primary trocar (5.5 mm) was inserted. A 5.5 mm secondary trocar was inserted at 1~2 cm cranial to the primary trocar. A rigid telescope (5mm, 30°; Schölly® FIBEROPTIC GMBH, Germany) and laparoscopic Babcock forceps were inserted through the cannulas. Both ovaries were examined laparoscopically, and confirmed the presence of corpora lutea and evaluated for the number of ovulation spot by counting the number of corpora lutea.

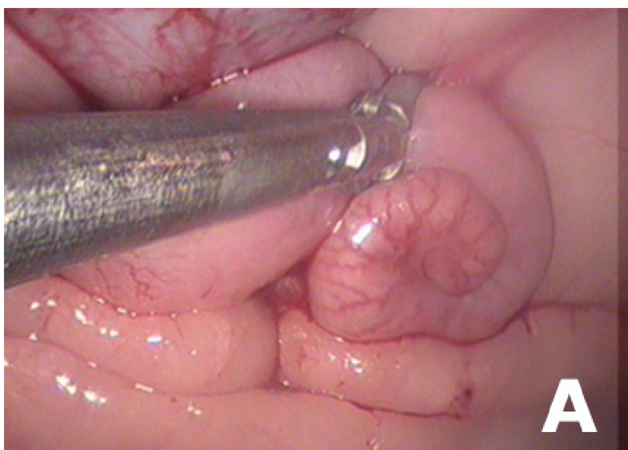
After examination of ovaries, cannulas were removed and a ventral midline incision for laparotomy was made by connecting and elongating the two 5 mm small skin incisions for primary and secondary trocars. Reproductive tract of superovulated donor was exteriorized, and both ovaries were examined again to evaluate ovarian response exactly. For collecting the embryos, a stab-incision was made at the 1 cm proximal to the bifurcation of uterine horns. An 8 Fr Foley catheter (Foley balloon catheter; Sewoon medical, Korea) was inserted into each uterine horn through this stab-incision, and the catheter bulb was inflated with air. A 3.5 Fr×12 cm catheter (Catheter, artificial fertilization; Dukwoo medical, Korea) was inserted into the fimbriated end of the oviduct. The oviduct were flushed with 50 ml of warm (37.5℃) PBS (Gibco, Invitrogen, USA), containing 4% FBS (Gibco, Invitrogen, USA), direction of oviduct to uterine horn. After the removal of the indwelt oviductal catheter, the uterus was flushed to-and-fro about 15 to 20 times with total volume of 100 ml PBS via the 8 Fr Foley catheter. After flushing of the oviduct and uterine horn was completed, the other oviduct and uterus

was identically flushed. The flushing medium was collected in sterile conical tubes (Centrifugal Tube, 50ml; LMS, Japan). These collection tubes were allowed to stand in a 37.5℃ water bath for approximately 10 minutes, then the supernatant was removed and aliquots from the bottom of each tube were transferred to sterile Petri dishes. Recovered embryos were evaluated morphologically under a stereomicroscope (Wild M5A; Wild Heerbrugg, Switzerland).

After recovering embryos, $PGF_{2}α$ (5mg, IM) was administrated to prevent pregnancy by uncollected embryos.

4. Laparoscopic Embryo Transfer

The recipient goats were aseptically prepared and positioned on the surgery table identically to the donor goats. Pneumoperitoneum was obtained by CO_2 insufflation through a Veress needle. Intraabdominal pressure was set at 12 mmHg during laparoscopic procedure. For the trocar placement, two 5 mm skin incisions were made at left and right lower abdominal quadrant, 5 cm cranial to the mammary gland and 2~3 cm apart from midline. Two cannulas were placed at abdominal wall, and a 5 mm, 30°, rigid telescope and laparoscopic Babcock forceps were inserted through the cannulas. Peritoneoscopy was performed to examine both ovaries. The uterine horn ipsilateral to the ovary with luteal body was grasped with laparoscopic Babcock forceps at middle part, about 5 mm diameter of uterine horn, and elevated close to abdominal wall (Fig. 1 A). A 16 G, 80 mm long IV catheter (3S-cath+, IV catheter; Dukwoo Medical, Korea) was inserted through the abdominal wall between two cannulas at the ventral-midline (Fig. 1 B). The catheter was gently inserted into



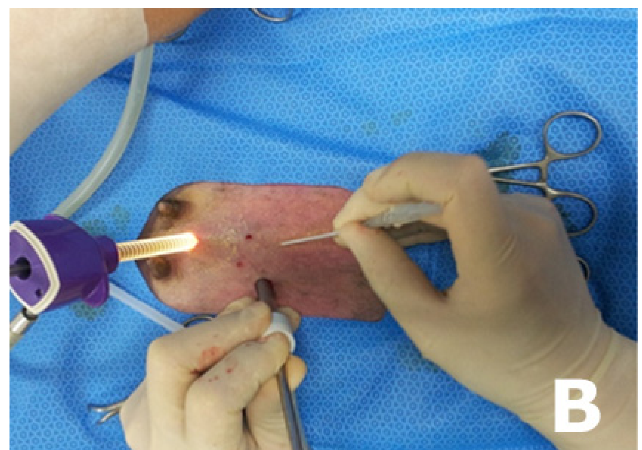
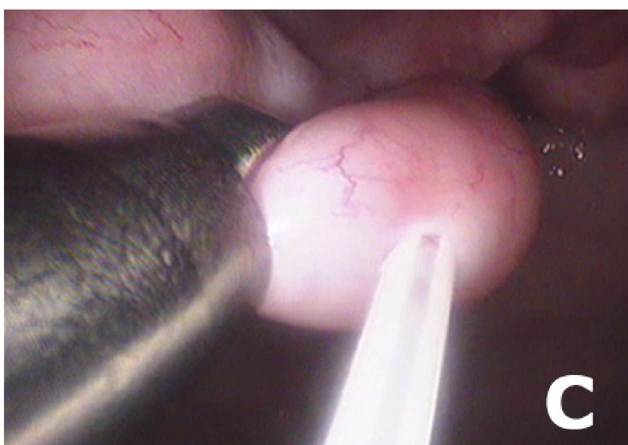


Fig. 1. Grasp of a uterine horn to transfer the embryos laparoscopically (A) and insertion of a 16G IV catheter through the abdominal wall (B).



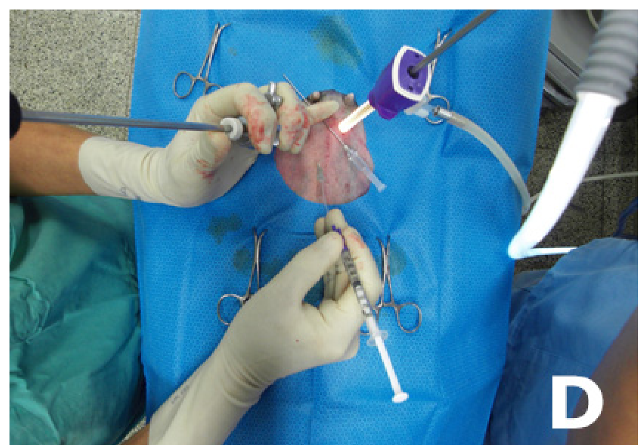


Fig. 2. A 16 gauge (G) IV catheter was inserted in a uterine horn, the stylet needle was removed (C) and insertion of a ET catheter through a 16 G IV catheter and release of embryos into the uterine lumen (D).

the uterine horn. After penetrating the lumen, the stylet needle was removed and the catheter further advanced to direction of the oviduct (Fig. 2 C). Embryos were loaded into a 3.5 Fr, 12 cm embryo transfer (ET) catheter attached to a 1 ml syringe, and two blastocysts per one recipient goat were transferred. 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 containing the embryos. The ET catheter containing blastocysts was inserted into uterine horn through IV catheter, and the embryos were slowly released into the lumen of uterine horn (Fig. 2 D). After transfer, the ET catheter was washed with medium, then examined for any remaining embryos.

The pregnancy was diagnosed by transrectal ultrasound scanning (Sonovet 2000; Medison, Korea) using a transrectal 5-MHz linear probe (LV4-7AD; Medison, Korea) more than 30 days after embryo transfer. Re-examination of pregnancy was made about 60 days and 120 days after transfer using abdominal ultrasonography.

RESULTS

The total number of different embryo stages and the recovery rate of embryo from superovulated donor goats are shown in Table 1. Eighteen out of total 29 superovuation-treated donor goats were confirmed the ovulation points. The total ovulated points by counting the number of corpora lutea were 167, the number of recovered embryos and oocytes were 96, and the recovery rate was 57.5%. Fifty out of total 96 recovered structures were confirmed to have normal morphologies and developmental stages (morula to expanded blastocyst).

In recipient goats, 13 out of 20 synchronization-treated goats were confirmed to have ovulated, and 26 embryos which had developed beyond morula stage were transferred to the 13 recipients; 8 morulae, 12 blastocysts and 6 expended blastocysts (Table 2). Two embryos in each recipient were transferred in the uterine horn ipsilateral to the ovary with luteal body(ies). The pregnancy diagnosis was performed using transrectal ultrasound after more than 30 days since embryo transfer and re-examined by abdominal ultrasound at around 60 and 120 days. Four recipient goats were diagnosed pregnant (Table 3

Table 1. Responses of ovarian stimulation after superovulation and the stages of recovered embryos.

No. of donors		Ovulation	Recovered structures				
Treated	Responding (%)		1~16 cell	M	BL	ExB	Total (%)
29	18 (62.1)	167	46	18	19	13	96 (57.5)

[*] M : morula, BL : blastocyst, ExB : expanded blastocyst

Table 2. The number of recipients estrus synchronized and the number of embryos transferred.

No. of recipients		No. of embryos transferred			
Treated	Responding(%)	M	BL	ExB	Total
20	13 (65)	8	12	6	26

[*] M : morula, BL : blastocyst, ExB : expanded blastocyst

Table 3. The results of pregnancy diagnosis according to the stage of transferred embryos.

Stage of embryos transferred[*]	No. of recipients	No. of pregnant recipients	No. of offsprings
M	4	0	0
BL	6	3	3
ExB	3	1	2 (twins)
Total	13	4	5

[*] M : morula, BL : blastocyst, ExB : expanded blastocyst

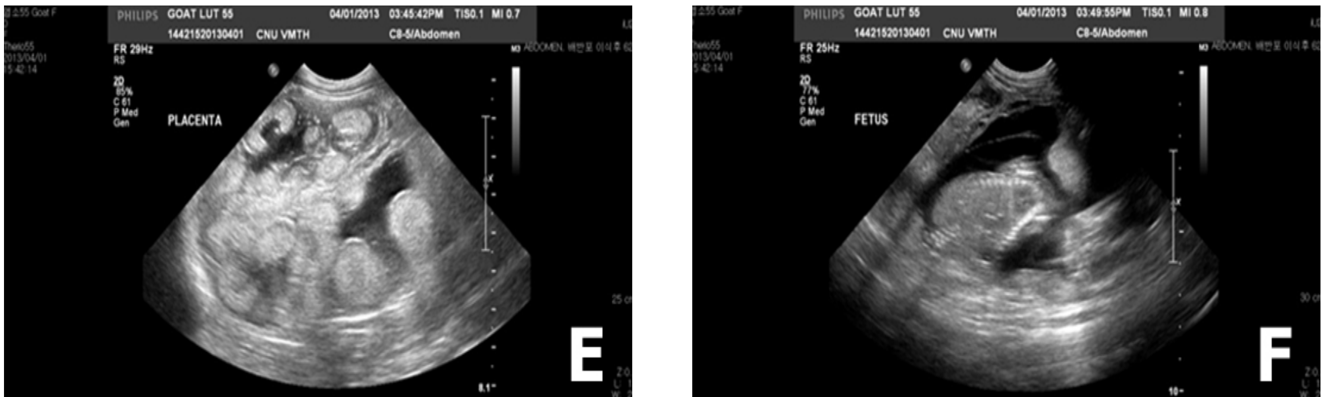


Fig. 3. Ultrasonogram of placentomes (E) showing round or doughnut shapes and fetus (F) at 62 days after transfer of blastocysts in a Korean black goat.

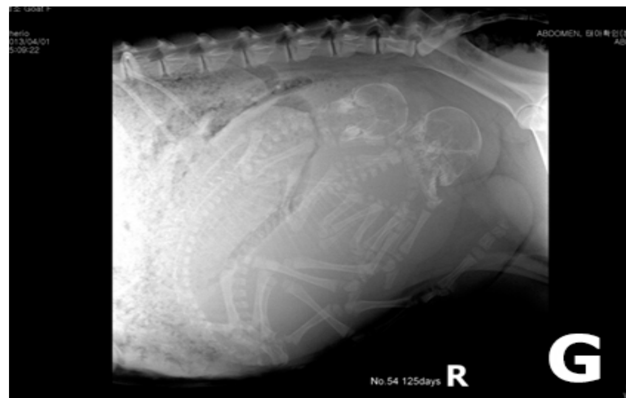


Fig. 4. Two feti were showing on radiograph at 125 days after transfer of blastocysts in a Korean black goat (G).

and Fig. 3). Three of the four pregnant recipients had single fetus and one had twins (Table 3 and Fig. 4). All fetuses were born normal and healthy.

DISCUSSION

Korean black goats still retain much of their wild nature in comparision to other domesticated goat species. Because wildlife species are stress-susceptible, a continuing effort is made to developmental studies in rare or endangered hoof-stock (Schiewe et al., 1984). Surgical procedure like laparotomy is a major limiting factor in small ruminant embryo transfer (Ishwar and Memon, 1996). Laparotomy also has many disadvantages such as tissue damages and abdominal adhesions (Schiewe et al., 1984; Kraemer, 1989; Shin et al., 2008). In comparison, laparoscopic embryo transfer can alleviate such problems (Flores-Foxworth et al., 1992; Kühholzer et al., 1998). Furthermore, the use of laparoscopy for the confirmation of ovulation could prevent needless surgery in unovulated goats in an embryo transfer program.

Data from Schiewe et al. (1984) and Shin et al. (2008) indicated that laparoscopic embryo transfer resulted in pregancy rates comparable to or greater than those performed by laparotomy. Transabdominal uterine horn cannulation under direct lapasoscopic viewing was rapid, minor-invasive procedure which had distinct advantages over the major surgical approach of laparotomy (Schiewe et al., 1984). The result of this study confirmed that the laparoscopic transfer of blastocysts into uterine lumen through direct loading the embryos via inserted 16 G IV catheter is a simple yet very effective method in Korean black goats. In this experiment, all pregnancy was not from transfer of morulae but blastocysts, it meant that the uterine environment at day 7 after estrus was more suitable for transferred blastocyst(s) than morula(e).

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