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Transcervical Embryo Recovery in Korean Black Goats: A Preliminary Experiment

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ABSTRACT

Four pluriparous Korean black goat does were superovulated with FSH and mated with fertile bucks. Anesthetized animals were placed in lateral recumbency, then size 8 Foley catheter was inserted into the uterus through the cervix under the vaginal speculum and the balloon was inflated to fix the catheter in the uterine body. The opposite end of the catheter was connected to a 3-way and a flushing medium was infused into the uterus. Modified Dubecco's PBS with 1% FBS was used as the flushing medium. Four goats were allocated in two groups depending on the type of medium infusion into uterus. Injection group; the flushing medium was injected into uterus and the infused medium was collected by to-and-fro method using a syringe. Gravity-flow group; the flushing medium was allowed to enter the uterus by gravity flow by lifting the medium bottle and drained out of the uterus into a collecting tube. All four goats had catheter inserted through the cervix and uteri flushed successfully. The volume (recovery rate) of recovered medium varied considerably from 87 ml/200 ml (43.5%) to 148 ml/160 ml (92.5%). Nine embryos/ova in total were recovered from Gravity-flow group goats. Although the embryo recovery rate was low, the possibility of a transcervical embryo recovery in Korean black goat had been proven in this preliminary experiment.

(Key words: transcervical, embryo, recovery, Korean black goat)

INTRODUCTION

Embryo collection in goats has been performed using a surgical approach (Armstrong *et al.*, 1983; Nowshari *et al.*, 1995; Shin *et al.*, 2008) especially in small breeds. As the surgical approach causes stress in the animal and may formulate adhesions, it is difficult to apply the procedure to the same animal repeatedly (Kraemer, 1989). Laparoscopic embryo collection results in fewer adhesions than surgical approaches (Flores-Foxworth *et al.*, 1992), but requires special equipments and highly trained personnel (Pereira *et al.*, 1998).

Transcervical embryo collection was avoided due to the difficulty of introducing a catheter through the cervix (Pereira *et al.*, 1998). Low economic value may also have been one of reasons that embryo transfer has not been frequently researched in goats (Fonseca *et al.*, 2013). Furthermore, since Korean black goats are smaller than other goat breeds, it is relatively more difficult to introduce catheter through the cervix nonsurgically.

Progress in biotechnology has resulted in transgenic animals capable of producing important biopharmaceuticals in their milk or other body fluids, and Korean black goat is an attractive animal to utilize in the field. A nonsurgical embryo transfer technique can be used avoid the disadvantages of surgical procedures such as adhesions and stress. This report is a preliminary experiment for a development in technique that collects embryo nonsurgically through the cervix in Korean black goats.

MATERIALS AND METHODS

1. Animals

Four Korean black goat does, with a body weight ranging from 31 to 34 kg (mean 32.25 kg), were used in this experiment. All of them were pluriparous and were subjected to the supreovulation treatment. The goats were fed Perfect 100® (Keumkang TMR Ltd, Korea) twice daily, with *ad libitum* access to water and mineral block. The animals were randomly

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divided into two groups according to flushing method (Injection and Gravity-flow).

2. Estrus Synchronization and Superovulation

All four goats were used in embryo recovery 22 days after intravaginal progesterone device (CIDR, progesterone, 0.3g; Pfizer, Auckland, New Zealand) insertion. The goats were inserted CIDRs on day 0, (11:00 a.m.) and 150 IU of eCG (Daesung P.M.S.G.®; Daesung microbiological labs, Korea) was administered I.M. on day 11 (05:00 p.m.). For superovulation, starting on day 11 (05:00 p.m.), FSH (Folltropin-V®; Bioniche, Belleville, Ontario, Canada) was given I.M. in equal eight doses (0.9 mg/1 dose/1 doe), twice daily (09:00 a.m. and 05:00 p.m.). The CIDR was removed on day 14 (09:00 a.m.). A single I.M. injection of 100 μg of GnRH (Gonadon; Dong- bang, Korea) was given for definite ovulation on day 14 (05:00 p.m.). The goats mated twice with a billy goat on day 15 (09:00 a.m. and 05:00 p.m.). The animals were starved from day 20 (09:00 a.m.) and the flushing was conducted on day 22 (01:00 p.m.).

3. Embryo Recovery

The equipment for the transcervical collection of embryos consisted of a duck-billed speculum, light source, Pozzi forceps, metal stylet with round tip, Foley catheter (8 French), 3-way catheter, petri dish, and microscope.

Before the procedure, abdominal and perineal region were washed and shaved. Point one mg/kg of xylazine (Rompun®; Bayer Korea, Korea) for pre-medication and 2 IU of oxytocin (YN-oxytocin; Yoonee Chemical, Korea) per a goat were given I.V to dilate the cervix (Sohnrey and Holtz, 2000.; Flohr SF *et al.*, 1999; Khalifa RM *et al.*, 1992), and antibiotics (DS PPS®, benzathine penicillin G 300,000 IU, procaine penicillin G 450,000 IU and dihydrostreptomycin sulfate 600 mg per doe; Dae Sung Microbiological Labs, Korea) given I.M.. Anesthetized animals were placed in lateral recumbency on a surgical table for flushing, followed by maintenance with isoflurane (Ifran®; Hana Pharm, Korea). Gel was used to lubricate the speculum, which was introduced into vagina (Fig. 1). A light source aided in finding the cervical opening. External os of the cervix was clipped using a Pozzi forceps and retracted gently into vagina. The metal stylet was introduced through the cervix to confirm cervical dilation. Thin stylet was inserted into foley catheter to allow it to be introduced through the cervix. The catheter was passed through the cervix and the balloon was inflated with 5 ml of flushing medium. The catheter was then drawn back against the internal opening of the cervix to ensure a tight seal, and maintained a gentle traction in order to prevent leakage of the flushing medium. The opposite end of catheter was connected to a 3-way and the flushing medium was infused into the uterus. Modified Dubecco's PBS with 1% FBS was used as the flushing medium.

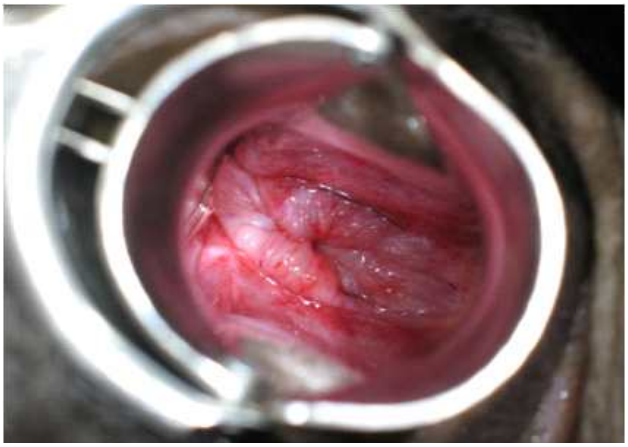

Fig. 1. External os of the cervix was observed through the vaginal speculum.

Four goats were allocated in two treated groups, with two goats in each group, depending upon the type of medium infusion into uterus.

1) Injection Group (Group 1)

Ten to 20 ml of flushing medium was injected slowly into uterus using syringe until resistance was felt. Then the infused medium was collected by pulling back the syringe plunger slowly using 3-way catheter (Fig. 2). Injection and retrieval of the medium was repeated 10 to 20 times.

2) Gravity-Flow Group (Group 2)

The flushing medium was allowed to enter the uterus by gravity flow by lifting the medium bottle about 1 m above the goat's uterus until the flow was stopped. Close the inlet valve and then open the outlet valve to drain out of the uterus into a 50 ml Falcon tube. Infusion and retrieval of the medium was repeated 20 times.

After the majority of fluid was recovered, the balloon on the foley catheter was deflated and the catheter withdrawn. The collecting tube containing the flushing medium from the uterus were left in a 37℃ waterbath for 15 minutes to settle down

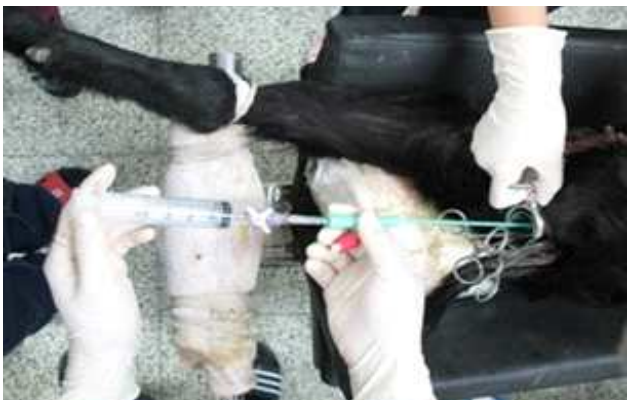

Fig. 2. Flushing the uterine horns to-and-fro using 3-way catheter and syringe connected to the Foley catheter introduced into the uterine body through the cervix.

the embryos, then the upper portion of the fluid was gently siphoned off. The remaining medium were poured into petri dishes and were examined under ×10 magnification stereomicroscope for presence of embyos.

4. Laparoscopic Identification

At the end of flushing, animals were placed in Trendelenburg position for laparoscopy. The abdomen was insufflated with CO_2 using a Veress needle. Two 1 cm incisions was made in the skin and two stab wounds were made in the abdominal wall with trocar, each 2 to 3 cm from the midline and approximately 5 cm anterior to the udder. Two 5 mm laparoscopic cannulas were equipped into wounds for a laparoscope and a laparoscopic grasping forceps. The whole genital tract, especially ovarian response such as follicle or corpus luteum, was examined.

After laparoscopic examination was completed, 5 mg of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Lutalyse^{TM}; Pfizer, Belgium) was administered I.M. to prevent pregnancy by remaining embryos.

RESULTS

Although it was quite difficult to pass the foley catheter through the cervix, all four goats had catheter introduced through the cervix and uteri flushed successfully. The volume of the recovered medium were varied significantly. As shown in Table 1. recovered volume (recovery rates) of the flushing medium from four goats were 87 ml/200 ml (43.5%), 102 ml/200 ml (51.0%), 148 ml/160 ml (92.5%) and 173 ml/220 ml (78.6%), respectively.

The total number of embryos/ova recovered were 9 (recovery rate; 19.0%) which consisted of 7 morulae , one 2-cell and one degenerated from group 2 (Gravity-flow method) goats. Most of them were from goat #3 (Fig. 3). No embryos were found in group 1 (Injection method, goat #1 and #2). These figures are somewhat disappointing considering the number of corpora lutea (total 42) counted by laparoscopy.

DISCUSSION

The major limiting factor in small ruminant embryo transfer

Table 1. Results from two different flushing method of transcervical embryo recovery in four Korean black goats

Group (method)	Goat #	Flushing medium*		No. of corpus luteum**	No. of embryos recovered
		Infused	Recovered (%)		
Injection†	1	20 ml×10 times	87 ml (43.5)	9	0
	2	10 ml×20 times	102 ml (51.0)	14	0
Gravity-flow‡	3	8 ml×20 times	148 ml (92.5)	11	8 (Morula: 7, 2-cell: 1)
	4	11 ml×20 times	173 ml (78.6)	8	1 (Deg; 1)

* Modified Dubecco's PBS with 1% fetal bovine serum.

** Confirmed by laparoscopic examination after flushing.

† Flushing medium was injected slowly into the uterus using syringe until resistance was felt. Then the infused medium was collected by pulling back the syringe plunger slowly using 3-way catheter.

‡ Flushing medium was allowed to enter the uterus by gravity flow by lifting the medium bottle about 1 m above the goat's uterus untill the flow was stopped and drained out of the uterus into a 50 ml Falcon tube.

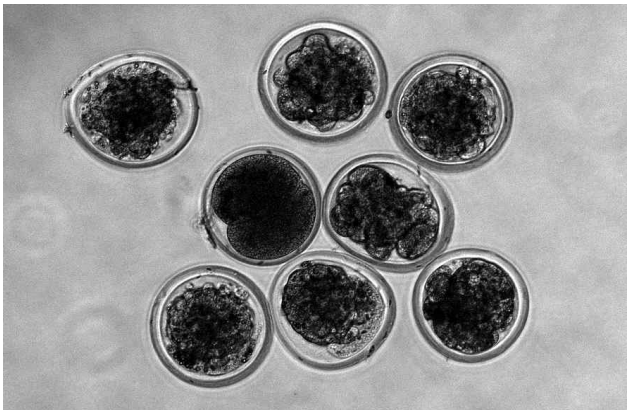

Fig. 3. Seven morulae and one 2-cell embryos from transcervical collection of the Korean black goat #3.

is the need for surgical procedures for both embryo recovery and transfer. While surgical procedures cause stress and adhesions in the animal, transcervical recovery technique can circumvent those disadvantages.

There are several reports of using $PGF_{2\alpha}$ before the flushing (Pereira *et al.*, 1998; Sohnrey and Holtz, 2000; Fonseca *et al.*, 2013). Pereira *et al.* (1998) suggested that the critical contribution to success was the induction of luteal regression by injection $PGF_{2\alpha}$ 8 or 16 hours before flushing. However, the goats in this experiment showed that even without an administration of $PGF_{2\alpha}$ prior to flushing, there was no significant difficulty in inserting the foley catheter through the uterine cervix. However, as many studies reported that $PGF_{2\alpha}$ leads to higher embryo recovery rate (Bretzlaff *et al.*, 1983; Jarrell and Dziuk, 1991; Pereira *et al.* 1998), injecting $PGF_{2\alpha}$ before the procedure should be considered for future experiments.

The recovery rate of flushing medium in goat #1 was very low compared to the results of other subjects (Nagashima *et al.*, 1987; Pereira *et al.* 1998). It is supposed that there was a significant leakage due to high pressure when injecting the medium into the uterus of goat #1.

Since a goat's uterus has a tortuous structure, nonsurgical embryo collection is very difficult, and is evidently shown in the low recovery rates of medium and embryo in this experiment. This should be addressed in further studies.

In conclusion, nonsurgical transcervical embryo recovery is possible in Korean black goat and may provide new prospects for embryo transfer in the species.

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