Offsprings Produced by Transcervically Inseminating Frozen-thawed Semen into Uterus of a Estrus-induced Saanen Goat during Non-breeding Season

Hwan-Yul Yong*, Min-Ah Kim, Bok-Soo Bae, Seung-Dong Kim, Shin-Il Jo, Yang-Mook Lim, Mi-Hyun Yoo, Yong-Hee Ha, Chang-Shik Oh and Doo-Hee Kim

Seoul Zoo, Makgye-dong, Gwacheon 427-080, Korea

ABSTRACT

We report herein the successful results of estrus induction, sperm cryopreservation and kids born by transcervical insemination of frozen-thawed semen in a Saanen goat. Flugestone acetate (FGA: 60 mg) was inserted into vagina for 15 days. The goat was intramuscularly injected with 400 IU PMSG and 200 IU hCG (PG600®: Intervet, Korea) a day before withdrawal of the FGA sponge. Follicles and corpora lutea were identified on both ovaries by laparoscopy. Artificial insemination was performed 46 hours after removal of FGA sponge. The concentration of frozen-thawed semen was 3.975×10^8/ml and 0.5 ml of frozen-thawed semen was transcervically inseminated into uterine body under anesthesia. Three kids, all females, were born 144 days after artificial insemination. This is the first report producing kids by transcervical insemination of frozen-thawed semen in a Saanen goat of which the estrus was induced by FGA sponges, PMSG and hCG during non-breeding season in Korea.

(Key words : estrus, frozen-thawed, induction, insemination, Saanen)

INTRODUCTION

In a dairy industry, goats are one of animal resources to provide milk. Saanen goat is one of main animal species to produce milk in Korea. Goat milk is recently interested because its digestibility is better than cow milk as well as similar to human milk in Korea (Lim *et al.*, 2006; Sanz Ceballos *et al.*, 2009). Its breeding season is October to November, and kids are born next spring (Lim *et al.*, 2006).

Breeding dairy goats through a year is necessary to maximize milk productivity. To acquire this goal, it should be considered critical to develop artificial breeding techniques in this species such as estrus synchronization, sperm cryopreservation, and transcervical or laparoscopic insemination (East and Rowe, 1989; Baril *et al.*, 1993; Leboeuf *et al.*, 2000; Sohnrey and Holtz, 2005; Kausar *et al.*, 2009).

In order to artificially breed sheep and goats, various types of intravaginal progestagen implants have been extensively used to induce estrus and ovulation during breeding and non-breeding season (East and Rowe, 1989; Ritar *et al.*, 1990; Fonseca *et al.*, 2005; Bitaraf *et al.*, 2007; Kausar *et al.*, 2009).

The objectives of this study were to examine if FGA functions as a suppressant of ovarian cycle and elicits estrus induction when it is needed, and to establish the technique of semen cryopreservation.

MATERIALS AND METHODS

1. Animal

Cross-bred male and female Saanen goats were 2 to 3 years old, and had been raised at a petting section for children in Seoul Zoo. The female goat was multiparous and the male had been approved to be fertile.

2. Estrus Induction

The female Saanen goat was treated with two intravaginal sponges each of that contains 30 mg flugestone acetate (Syncrite-30®; Animal Health Supplies, Australia) on May 22, 2009. On the 14th day of the sponge insertion, pregnant mare serum gonadotropin (PMSG) 400 IU and human chorionic gonadotropin (hCG) 200 IU (PG600®; Intervet, Netherlands) was intramuscularly administered. Twenty four hours later, the sponges were removed.

3. Cryopreservation of Semen

Semen collection was conducted by electronic stimulator (ElectroJac IV, Neogen; USA) on April 14, 2009 (Mejia *et al.*, 2009). Briefly speaking, a conical rectal probe of the electro-

* Correspondence : E-mail : getzoopregnant@seoul.go.kr

nic stimulator (2.5 cm diameter) was inserted about 10 cm deep into the anus before applying multiple stimuli directly to anal inner walls and 1 ml of fresh semen was recovered, which motility was excellent. The semen was diluted with Triladyl (Minitüb, Germany)-based diluents (Triladyl: egg yolk: tri-distilled water, 1:1:3 (v/v), respectively). The diluted semen was cooled down to 5℃ for 2 hours and loaded into 0.5 ml straws before being exposed to liquid nitrogen vapor for 10 minutes. The frozen semen had 50~60% motility one month later when it was thawed in 38℃ water bath for 5 minutes. The final concentration of frozen-thawed semen was 3.975×10^8/ml.

4. Artificial Insemination

Forty four hours after withdrawal of the sponge, the Saanen goat, weighed 30 kg, was anesthetized by intravenously injecting 0.3 ml of xylazine (0.23 mg/kg; rompun®; Bayer Korea, Korea). The presence of follicles and corpora lutea on both ovaries was identified using laparoscopy (Stortz, Germany) and a vaginal speculum was positioned near to the cervical opening before 0.5 ml of frozen-thawed semen was inseminated into uterine body using goat AI gun. The goat was rapidly recovered from anesthesia by intramuscularly injecting 0.3 ml of atipamezol hydrochloride (50 ug/kg; Antisedan®; Pfizer Korea, Korea).

5. Analysis of Hormone Concentrations and Pregnancy Check

Blood samples were collected by jugular venipuncture. After centrifuging at 3,000 rpm for 20 minutes, the serum was transported to Neodin Lab (Neodin Inc., Korea) for analysis of estradiol and progesterone.

RESULTS

1. Changes of Hormone Levels

The decrease of estradiol level shown from FGA insertion (43.5 pg/ml) to PG 600 injection (36.7 pg/ml) means that ovarian activity had been well suppressed by FGA during vaginal insertion (Table 1). PG 600 accelerated follicular development and maturation, which was well indicated from the estradiol level rising up to 51.6 pg/ml when the sponges were with drawn (Table 1). When artificial insemination was performed, the estradiol level of 44.5 pg/ml was lower than that of sponge removal (51.6 pg/ml) (Table 1). Progesterone level was maintained between 2.74 and 4.49 ng/ml until around parturition (Table 1).

2. Parturition

Female kids weighed 2.65, 2.5 and 1.45 kg were born in October 30, 144 days after AI (Fig. 2).

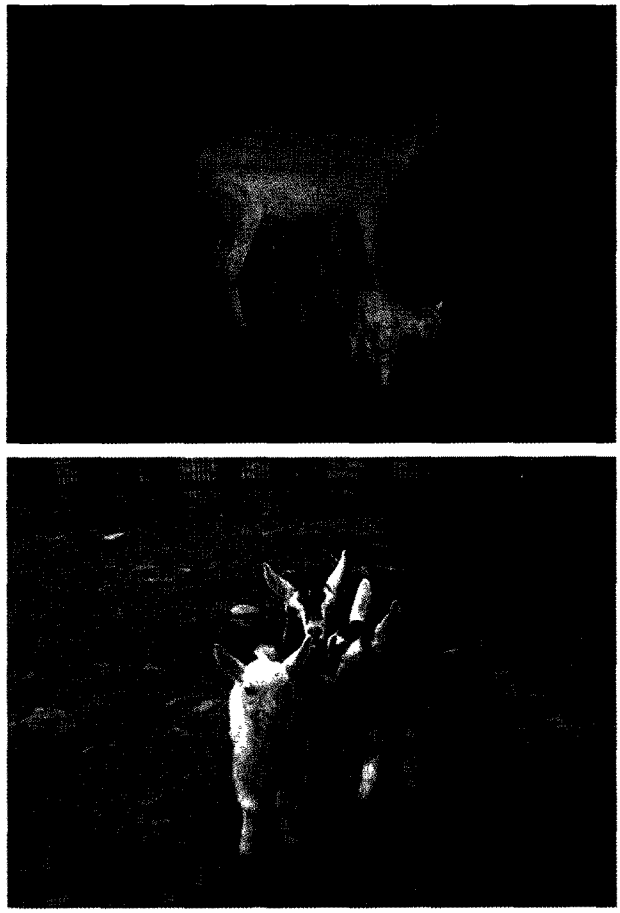

Fig. 1. Three 5-day-old kids born by transcervical insemination of frozen-thawed semen and their mother goat (A), and healthy kids 32 days after parturition (B).

Table 1. Changes of estradiol and progesterone from FGA insertion to parturition in a Saanen goat

Date	May 13	Jun 5	Jun 6	Jun 8	Jul 22	Aug 8	Aug 21	Sep 8	Sep 22	Oct 6	Oct 21	Nov 2
Estradiol (pg/ml)	43.5	36.7	51.6	44.5	27.4	34.4	39.4	46.7	46.8	49.6	50.5	31.8
Progesterone (ng/ml)	0.01	0.01	0.01	0.01	4.49	4.43	3.99	4.01	3.42	2.74	3.25	0.05

DISCUSSION

As shown in the present study, fifteen days as the period of FGA sponge insertion seemed to be proper in a Saanen goat. The dose of FGA, the period of sponge insertion, the time of gonadotropin injection were different depending on subspecies of goats (Loubser *et al.*, 1983; Ritar *et al.*, 1984; Ritar *et al.*, 1990; Romano, 2004; Bitaraf *et al.*, 2007; Khanum *et al.*, 2008). Like medroxyprogesterone acetate (MAP) and Controlled Internal Drug Release devices (CIDR), FGA used in this study was enough to suppress the fluctuation of pituitary gonadotropins (Table 1) (East and Rowe, 1989; Bitaraf *et al.*, 2007; Kausar *et al.*, 2009). As a preliminary experiment, oral administration of MGA (melengestrol acetate) was not available to induce estrus (not published).

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and PMSG are considered as critical factors that affect onset of estrus and determination of optimal AI timing (Romano, 2004). But, in the present study, $PGF_2\alpha$ was not used for luteolysis at the removal of intravaginal progestagen devices (Moore *et al.*, 1988; Rita *et al.*, 1990). The follicles seemed well matured and the oocytes were successfully fertilized by frozen-thawed spermatozoa after PG 600 injection and sponge removal, which were identified by laparoscopy observing corpora lutea (not shown). At first, artificial insemination was supposed to be performed by laparoscopy that was failed by mild hemorrhage occurred in the process of CO_2 gas infusion. Therefore, against our intention, the goat was transcervically inseminated under anesthesia. We think the use of anesthesia would not be necessary in the transcervical insemination of domestic goats if a suitable restraint device is prepared.

As the intravaginal sponges were inserted, it would be beneficial to cut the long string connected to sponges after insertion because goats have a tendency to pull or chew strange objects seen at the vulvar regions of the other goats even though the strings were originally designed to make operators pull out easily at the time of sponge removal. Moreover, the use of sponges caused mild vaginitis in this study but not as serious as the animal needed to be treated (Romano, 2004).

Pregnancy test was mostly accomplished by serum test of progesterone levels (Currie and Thorburn, 1977; Fleming *et al.*, 1990). The progesterone level was maintained between 2.7 and 4.5 ng/ml during pregnancy (Table 1). We do not know when estrous behavior started and how long it continued. It was not difficult to penetrate through cervical folds when artificial insemination was performed 46 hours after sponge removal. However, the time of artificial insemination may be beneficial if it was conducted less than 46 hours after sponge removal. The commencement and duration of estrus after device withdrawal could be different according to the types of intravaginal pessary and the use of gonadotropins or $PGF_2\alpha$ (Romano, 2004; Bitaraf *et al.*, 2007).

Kidding rate by transcervical insemination is considered to be better than by laparoscopy and litter size was low without preceding hormone treatment (Sohnrey and Holtz, 2005). In this study, we did not use $PGF_2\alpha$. The use of $PGF_2\alpha$ may not be important as much as the use of gonadotropins. The average of litter size is two in goat species. Three kids could be the result of well-developed follicles in the ovaries of which the function had been temporarily suppressed by FGA and initiated again by gonadotropins (Fig. 1). We did not know exactly the reasons that all the kids were female. The reason we could assume is that the artificial insemination was just once conducted and the environment of female reproductive track might be "amicable" only to X-bearing spermatozoa.

Artificial breeding techniques in various kinds of domestic goats can be applied and contributed to conservation of endangered wild goats.

In conclusion, the use of FGA and PG 600 is efficient to induce estrus and frozen-thawed spermatozoa collected from electrical shock is competent to fertilize oocytes ovulated by exogenous hormones during non-breeding season in a dairy Saanen goat.

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(접수: 2010. 4. 20 / 심사: 2010. 4. 23 / 채택: 2010. 5. 3)