

Production of Genetically Superior Goats through Embryo Transfer Technology in Sri Lanka

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ABSTRACT. *This study was conducted to establish techniques for embryo production and transfer in goats and also to examine the feasibility of application of such techniques in Sri Lanka to improve the reproductive efficiency of superior animals. Two groups of genetically superior does were inserted with progesterone releasing intravaginal pessaries (45 mg, Cronolone) on Day-1 of the program. The does in group one (n = 3) and group two (n = 4) were given injections of pure porcine Follicular Stimulating Hormone (pFSH, 20 mg/ml) and pure ovine Follicular Stimulating Hormone (oFSH, 0.88 mg/ml), respectively. On Day-8, all animals were injected with Pregnancy Mare Serum Gonadotropin (PMSG, 500 µg/ml). Subsequent injections of pFSH or oFSH were given in the morning and evening on Day-9 and Day-10. Does were injected with Prostaglandin F_{2α} (PGF_{2α}, 263 µg/ml) in the morning of Day-9 and vaginal pessaries were removed in the evening of Day-10. On the evening of Day-11, Gonadotropin Releasing Hormone (GnRH) injections were given and the does in estrus were kept for 48 hours with a Jamnapari buck for breeding. Seven days post-estrous, embryos were collected, surgically, by flushing the uterus with warm embryo flushing medium containing Lactated Ringers Solution with 1% bovine serum albumin (BSA). The quality of the embryos was assessed and the recovered embryos were transplanted surgically to estrus synchronized goat recipients (n = 8) at 7 days post-estrus.*

Following embryo transplantation, four does were found to be pregnant by ultra sound scanning at 35 days in pregnancy. One healthy female offspring (Peradeniya Kumari) was born at full term (150 days) to Group-1. Another four goat kids were born to Group-2, while one kid died. In the same group, one abortion was reported. During the first six weeks of birth, weight gain of first born kids to Group-1 and 2 were 152.3 g and 149.2 g /day, respectively. The results suggest that genetically superior goats can be produced using embryo transfer technology in Sri Lanka.

INTRODUCTION

Goats are highly prolific animals and breed throughout the year in tropics. They have the ability to thrive in diverse ecological and agro climatic zones in different agricultural systems. (Freitas *et al*, 2004).

Reproductive biotechnologies play a key role in improving animal reproduction. Multiple Ovulation and Embryo Transfer (MOET) is a technique which can be used to multiply genetically superior females by increasing reproductive efficiency. (Cognie *et al.*, 2003; Gonzalez-Bulnes *et al.*, 2004). In MOET technique embryos are collected from a genetically superior (donor) animal and are transplanted into another animal (recipient) for the rest of the development until term (Betteridge, 1981). This technique can be used to multiply genetically superior animals rapidly by increasing their reproductive efficiency; as an easy method to transport genetic material across the world at low cost and with minimum risk of spreading diseases, together with embryo sexing to get more female offspring from a genetically superior animal (Abeygunawardena, 2002a). It can also be used to conserve endangered species (Senger 1999). With the combination of embryo splitting, multiplication rate of the donor can be further accelerated and identical twins can be made according to the requirement of the research purpose. Embryos can be produced even from animals which have conception failures or inability to have normal pregnancies (Noakes, 1986). Several key steps including synchronization of estrous cycles of donor and recipient animals, superovulation of donor animals, artificial or natural insemination of embryo donor, recovery of embryo from donor animal, *in vitro* maintenance of quality embryos until transfer and transfer of embryo to recipient animals are involved in MOET process.

Usually Embryo Transfer (ET) is performed through a laparotomy under general anesthesia and two to four embryos are transferred to a recipient in one occasion in goat and sheep (Alexander, 2005). For a successful embryo transfer, uteri of both donor and recipient animals should be in the same stage of the estrous cycle (Senger, 1999). There have been no studies in Sri Lanka to investigate the feasibility of ET in goats. Therefore this study was carried out with the objectives of establishing techniques for embryo production and transfer in goats and evaluating the feasibility of application of this technique in Sri Lanka.

MATERIALS AND METHODS

Selection and preparation of goats for ET

Genetically superior embryo donor goats for the ET programme were selected on the basis of their production and reproduction performance and the pedigree records. Their health status was examined and anthelmintics and multivitamin injections were given. Four days after the injections 1 ml of Tetanus toxoid was given to all selected goats. The animals were provided with ad libitum forage, 650 g of concentrates and 60 g of mineral mixture daily for two months.

Superovulation of embryo donors

Selected genetically superior does were inserted with progesterone releasing intravaginal pessaries (45 mg, Cronolone, Intervet) in the morning of Day-1 of the superovulation schedule. Does were divided into two groups. Does belonging to the group one (n = 3) and group two (n = 4) were administered with 2.5 ml intramuscular (IM) injections of pure porcine Follicle Stimulating Hormone (pFSH); (Folltropin-V, 20 mg/ml NIH-FSH-P1, BIONICHE, Canada) and pure Ovine Follicle Stimulating Hormone (oFSH); (Ovagen™, 0.88 mg/ml NIADDK-oFSH-17-Standard, ICPbio Limited, New Zealand) respectively, on Day-8 of the program. In addition, 300 IU Pregnancy Mare Serum

Gonadotropin hormone (PMSG), (Folligon, Intervet International BV, Boxmeer-Holland) was given to all does on the evening of Day-8. Folliculogenesis and maturation were further supported with subsequent injections of 1.25 ml pFSH or oFSH in the morning and evening of Day-9 and Day-10 (Gonzalez-Bulnes *et al.*, 2004). Does were injected with 197 mg of Prostaglandin F_{2α} (PGF_{2α}, cloprostenol sodium; PGF Veyx fort, Veyx Pharma, Schwarzenborn) in the morning of Day-9 and vaginal pessaries were removed in the evening of Day-10. On Day-11, 1.25 ml of pFSH or oFSH and 1 ml of Luteinizing hormone releasing hormone (LHRH) (50 µl/ml, Depherelin Veyx Pharma, Schwarzenborn) injections were given in the morning and evening, respectively. Immediately after the LHRH injection does were kept for 48 hours with a genetically superior Jamnapari buck for natural breeding.

Synchronization of embryo recipients

In the morning of Day-1 intravaginal progestagen-impregnated pessaries (Chrono-gest, which contained 45 mg Flugestone acetate; Intervet), were inserted to all recipients, (eight crossbred does), and 125 µg of Prostaglandin F_{2α} (Veyx® fort, Veyx-Pharma GmbH, Schwarzenborn) was administered to each animal intramuscularly. On Day-17, vaginal pessaries were removed, and 400 IU of PMSG; (Folligon, Intervet) Intramuscular injections were given to each doe. The animals were observed for visible signs of estrus on the following day.

Surgical embryo collection

Seven days post mating, embryos were collected from three and four donors in groups one and two respectively, in the following manner. Intramuscular (IM) injections of xylazine 2% (0.2 mg/kg body weight) were given (Bishop, 2001) to sedate embryo donors. Ketamine Hydrochloride 10% (22 mg/kg body weight), was injected 20 minutes after the xylazine injection. Once the donors were anesthetized, they were kept on a surgical cradle in dorsal recumbent posture. After shaving of the ventral abdominal region of the animal the surgical site was scrubbed alternatively with 70% Isopropyl alcohol and Povidone Iodine solution three times each. The uterus and both ovaries were exteriorized through mid ventral laparotomy (7 cm). After measuring the length, width and thickness of each ovary, the number of corpora lutea in each ovary were counted. The uterotubal junction of the right uterine horn was pierced with a blunt ended 18G hypodermic needle and tip of the embryo flushing catheter (Tom cat catheter; 3 ½ FR, 14 cm; Sovereign™, Mexico) was inserted and pushed towards the uterine horn. Same uterine horn was pierced with a small artery forceps at the level of the bifurcation and two-way pediatric silicon elastomer coated Foley Catheter (8 Ch/Fr, 3/5 ml/cc; Unomedical, malayasia) was inserted. After inflating of the cuff, stylet of the foley catheter was removed. Fifty milliliters of Embryo flushing medium (Lactated Ringers Solution with 1% Bovine Serum Albumin) was passed through the flushing catheter while gently tapping on the uterine horn, fluid was collected into a 100 ml beaker. The same procedure was repeated for the left horn. After removal of both catheters, the incision on the uterus was sutured with 3/0 catgut (Ethicon). After application of hydrocortisone cream, the reproductive tract was repositioned in the abdominal cavity. Depending on the body weight, required doses of intra abdominal and intramuscular penicillin streptomycin were administered. Peritoneum and the muscle layers of the incision line were sutured using 1 USP chromic catgut using simple interrupted suture pattern. Skin incision was sutured with 0.45G nylon using simple interrupted suture pattern. Coumaphos, propoxur and

sulfanilamide containing powder (Negasunt, Bayer Polychem, India) and povidone iodine solution were applied on the site after suturing.

Evaluation of embryos

Embryos were isolated from flushing medium using a wire trawl just prior to the evaluation process. The quality of the embryos was assessed and the collected embryos were graded as excellent, good and poor (IETS, 2008).

Surgical embryo transfer

Eight crossbred recipient does were divided into two groups and were subjected to 12 hours withholding period of feed and water. Blastocyst or morulae stage embryos were transferred on the same day as described below, to four recipients in each group.

Recipient does were placed on a surgical cradle in dorsal recumbency. The lower abdominal area of the doe was shaved and disinfected using povidone iodine and 70% isopropyl alcohol. The cradle was tilted 60° with the head facing down. An incision of approximately 1.5 cm was made in the skin about 3 cm to the left of the mid line and about 5 cm from the udder, using a no. 23 scalpel blade. A trochar and cannula were inserted into the abdominal cavity through the incision made on the skin. The trochar was removed and a laparoscope (6.5 mm diameter) was carefully inserted through the cannula into the abdomen. Another incision was made on the right side in the same manner and a Babcock forcep was introduced. The uterine horns were visualized using the laparoscope and the ovaries were carefully inspected to find the ovary with large corpora lutea (CLs). The tip of the respective horn was grasped and 3-4 cm of the horn exteriorized using the Babcock forcep. At the same time the embryos were loaded into the tip of a 3.5 Fr tom cat catheter with the help of an insulin syringe. The embryos were loaded to the tip of the catheter in the following manner: First, a small amount of embryo holding medium (Vigro holding plus, AB technology) was aspirated into the tip of catheter, then an air bubble, then the embryos with medium and finally another air bubble. The uterine horn was punctured (very close to the utero-tubular junction) using a blunt 18 G needle and the tip of the catheter was inserted into the lumen of the uterine horn and the embryos were expelled. Before releasing the horn into the abdomen, the tom cat catheter was examined to confirm that all the embryos were expelled successfully. This was carried out by washing and pipetting the catheter with small amount of embryo holding medium in a 35 mm dish, under the microscope. The abdominal incisions were sutured using 1-USP-chromic catgut with far-near-near-far suture pattern. A long acting Penicillin Streptomycin injection was administered. The does were monitored post-operatively for few hours and released to the shed.

Pregnancy diagnosis

Pregnancy confirmation was carried out on 35 days post ET, using an ultra sound scanner attached to a 7.5 MHz linier type per rectal probe.

Data analysis

Mean values of the parameters measured during the superovulation and embryo transfer process were compared between groups using Student's 't' Test. Significant difference was determined at $p < 0.05$ level.

RESULTS

The parameters measured during superovulation and ET process are given in Table 1. The number and the quality of the embryos recovered from each group of does are shown in Table 2. Following embryo transplantation, four does were found to be pregnant. One healthy female goat kid (Peradeniya Kumari, Figure 1) was born with 3.6 kg birth weight at full term (150 days) to Group-1. Another four kids with birth weight of 3.2 kg (♀), 1.8 kg (♀), 1.6 kg (♂) and 1.2 kg (♂) were born to Group-2. The third kid died shortly after birth. In Group-2 one abortion was noticed.

During the first six weeks weight gain of the first kid born to Group-1 and group-2 were 152.3 g and 149.2 g /day, respectively.

Table 1. Size of the ovaries, number of corpora lutea and number of embryo produced in two groups of recipients

Parameters	Group 1	Group 2
Mean size of the ovary (cm) \pm SEM		
Length	2.4 \pm 0.1	2.5 \pm 0.2
Width	1.2 \pm 0.1	1.7 \pm 0.1
Thickness	1.1 \pm 0.1	1.5 \pm 0.2
Number of corpora lutea (rang)	6-10	11-17
Mean number of corpora lutea	7.6 \pm 1.2	14.25 \pm 1.2
Mean number of embryo per animal	4.3 \pm 2	4.25 \pm 2

Table 2. The number and quality of embryos recovered

Animal group	Hormone used	Quality of embryos produced		
		Excellent	Good	Poor
Group 1	pFSH	02	08	03
Group 2	oFSH	04	11	02
Total		06	19	05

DISCUSSION

The experiments presented in this article resulted in healthy live offspring claiming the first born kid through embryo transfer technology in Sri Lanka.

The donors and recipient animals showed estrus after 24-36 hours of removal of spongers. The common signs of estrus to be noticed were swollen hyperemic vagina, clear color less vaginal mucus discharge, frequent wagging of the tail and restlessness (Abeygunawardena, 2002), but the animals did not exhibit all the signs at a given time. Signs of estrus are

reported to depend on the number of factors, such as health status of the animal, nutrition, environment and breed etc (Stephen, 1971a).



Figure 1. The first ET born kid ('Peradeniya Kumari')

The flushed embryos were in different developmental stages such as compacted morulae, blastocyst stage and expanded blastocyst stages. This could be due to the asynchrony of ovulation and fertilization of oocytes (Cognie *et al.*, 2004). A few unfertilized and degenerating embryos were also found.

Responses of embryo donors for the superovulatory treatment were different. Because the animals' response to exogenous hormones depends on several factors such as the level of nutrition, age, breed and reproductive status of the embryo donors (Gonzalez-Bulnes *et al.*, 2004). In the present experiment the range of total and transferable embryos recovered were 0-9 and 0-7 from a donor respectively. Usage of recently improved gonadotrophin preparations and programmed insemination protocols used in this experiment could not avoid this variability.

Low estrogen level produced by the granulosa cells of developing follicles exerts negative feedback on the secretion of gonadotropin. Similarly inhibin secreted by the developing follicles also exerts selection inhibitory action on the secretion of FSH (Greenwald and Terranova, 1988). The number of ovulations and transferable embryos following administration of commercial FSH preparations depends on the number of small antral follicles (2-3 mm) present in the ovaries. Similarly the presence of large follicles (>6 mm) at

the onset of the superovulatory treatment, exerts inhibitory effect on the final number of transferable embryos recovered (Cognie *et al.*, 2003).

One abortion was reported in this study. Viability of the fetus or the embryo depends on the functional corpus luteum, alterations of the preovulatory follicles and level of the ovarian abnormalities (follicular cysts, lutenised cysts etc) (Gonzalez-Bulnes *et al.*, 2004). Out of five kids resulted from the experiments one died shortly (within 20 minutes) after parturition. Kidding was delayed one hour from the first kidding and it was found cyanosed. With the artificial respiration it survived for twenty minutes. Cause of the death could be due to hypoxia, presence of placental detachment without being expelled out of the fetus. Delayed kidding may be due to the fetal malpositioning, calcium deficiency, uterine inertia, abdominal muscle fatigueness and nutritional deficiencies etc (Stephen, 1971b).

The last triplet of kids had relatively low birth weights. The reason could be due to the high nutritional demand for all three fetuses from the same maternal tissues throughout their gestation period.

CONCLUSION

It is feasible to produce genetically superior goats through the use of embryo transfer technique in Sri Lanka. Further studies are needed to optimize the technique.

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