Bangl. J. Vet. Med. (2004). 2 (2): 147–150

EFFECTS OF ALFAPROSTOL AND LUPROSTIOL ON THE EMBRYO PRODUCTION WITHIN MOET TECHNIQUE IN BLACK BENGAL GOATS

M. I. Faruk, B. Z. Fatema[1], F. Y. Bari and M. G. S. Alam

Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT

The effects of Alfaprostol and Luprostiol on embryo production within multiple ovulation and embryo transfer (MOET) technique were studied on 16 Black Bengal goats during the period from January 2002 to June 2003. These 16 goats were randomly divided into two equal groups (A & B), each consisting of 8 goats. Each of the 16 goats was flushed in different times within MOET technique to determine the effects of alfaprostol and luprostiol on embryo production. Each group consisting of 8 donors was synchronized with alfaprostol (Gabbrostim®, VETEM, Italy) or luprostiol (Prosolvin®, Intervet International, Netherlands) @ 2 mg and 7.5 mg, equivalent to 1 ml / donor respectively. The donor goats were hand mated following the onset of oestrus, 1-2 times at 6 h interval depending on the duration of oestrus. The embryos were collected at Day 7 of mating using surgical recovery method. The mean number of ovulation in alfaprostol and luprostiol group was 8.50 ± 0.90 and 8.1 ± 0.76, respectively, where in both cases 900 iu PMSG was used for induction. There was no significant ($p > 0.05$) difference between this two groups on superovulation rate. The mean numbers of recovered, fertilized and transferable embryos were 5.4 ± 0.80 and 5.1 ± 0.61; 3.9 ± 0.52 and 2.6 ± 0.37 and 3.6 ± 1.6 and 2.4 ± 1.0, respectively, in alfaprostol and luprostiol treatment group. Like superovulation, there was no difference between the two treatment groups on recovered, fertilized and transferable embryos. The percentage of recovered, fertilized and transferable embryos were 63 ± 7.7 and 63 ± 3.74, 72 ± 4.55 and 51 ± 7.0 and 93.6 ± 1.6 and 90.48 ± 1.0 in alfaprostol and luprostiol treated groups, respectively. The significant difference was only existed in the percentages of fertilized embryos between the two treatment groups, where alfaprostol treated group had the significantly ($p < 0.01$) higher percentage of fertilized embryos.

Key words: Alfaprostol, luprostiol, effect, embryo transfer, ovulation, Black Bengal goats

INTRODUCTION

Multiple ovulation and embryo transfer (MOET) technique is mainly performed to speed up the process of genetic improvement of any economic traits of mother by increasing the selection intensity and accuracy of selection. Within the MOET technique synchronization of oestrus is required to induce oestrus in all donors and recipient at a time, facilitating AI and embryo collection and transfer process. The Black Bengal goat constitutes majority of goats' production in Bangladesh. But the problems of this breed are their small body sizes and high fat content in the meat. To increase the body size and to increase the lean meat it is necessary to breed the top quality she goats with the fertile buck. With natural breeding it will be a long time process to get this genetic improvement. MOET technique may be employed to speed up this process. Reports on the effects of synthetic prostaglandins on the success of MOET technique in goat are limited. This paper describes the effects of alfaprostol (Gabbrostim®, VETEM, Italy) and luprostiol (Prosolvin®, Intervet International, Netherlands) synchronization on the superovulatory response and embryo production.

MATERIALS AND METHODS

Synchronization

A total of 16 donor adult Black Bengal she-goats was purchased from the local market and this study was conducted during the period from January 2002 to June 2003. These 16 goats were randomly divided into two groups (A & B), each consisting of 8 goats. The oestrous cycles of 8 donor does were synchronized by two intramuscular injection of 1 ml (2 mg) alfaprostol (Gabbrostim®, VETEM, Italy) at 11 days interval. Luprostiol (Prosolvin®, Intervet International, The Netherlands) was injected in the same manner @ 1 ml (7.5 mg) in 8 donor does in another group.

Superovulation

PMSG, 900 iu (Folligon®, Intervet International, The Netherlands) was injected to induce superovulation in both types of synchronization treatment does. The superovulatory treatment was initiated at day 12 of the oestrus cycle (Day 0 = Day of oestrus). A luteolytic dose of synthetic $PGF_2\alpha$ (2 mg Alfaprostol, Gabbrostim®, Italy and 7.5mg Luprostiol, Prosolvin®, Intervet International, The Netherlands) was injected 48 h after injection of PMSG to induce oestrus in both types of induced does. The donor does were hand mated with fertile buck immediately after observing the symptoms of oestrus for 1-2 times, 6 h apart, depending on the duration of oestrus.

Present address: [1]Department of Medicine and Surgery, Dinajpur Government Veterinary College, Basharhat, Dinajpur.

Embryo collection

The embryos were collected from donors using surgical embryo collection procedure at day 7 of natural service as described by Tervit *et al.* (1986) and Baril *et al.* (1989).

Preparation of Media

Commercially available phosphate buffered saline (PBS) with calcium and magnesium (PBS, Biochrom KG, Berlin, Germany) was supplemented with 2% bovine serum albumin (Albumin Concentrate®, 20% w/v, ICP, Aukland, Newzealand), penicillin and streptomycin (100 μg / ml, Streptopen®, 0.5 g vial, Renata Animal Health, Bangladesh) to complete the medium. Briefly, 9.55 g dry PBS was dissolved in 1 litter of deionized distilled water (Milli-Q® pluse, ultra pure water system, Milli Pore Corporation, USA). Calcium salt (132.46 mg $CaCl_2.2H_2O$) was added gradually and dissolved in PBS solution spinning the medium in a conical flask by hand movement. Magnesium salt (100 mg $MgCl_2.6H_2O$) was dissolved in the same way. Sixty (60) mg of penicillin and 100 mg of streptomycin were mixed in the PBS solution one after another. Prior to flushing 10 ml BSA was mixed slowly. Medium was prepared following standard aseptic measures.

Anesthesia and preparation of donors for collection

A sedative, chlorpromazine hydrochloride (Largactil®, Rhone-Poulenc, Bangladesh) was injected IM @ 0.1 mg / kg body weight approximately 15-30 minutes prior to surgery. The near half of the abdominal wall, anterior to the udder was clipped, shaved, scrubbed with an antiseptic solution (Hexisol®; 0.5% chlorhexidine gluconate, ACI, Bangladesh) and dried. Paravertebral nerves were blocked by injecting 12 ml of 2% lignocaine hydrochloride (Jasocaine®, Jason Pharmaceuticals Ltd., Bangladesh), 4 ml in last thoracic, 3 ml in each first and second lumbar and 2 ml in third lumbar vertebral spinal nerve. The skin and underline tissues were desensitized by subcutaneous infiltration of 5 ml of 2% lignocaine hydrochloride.

Procedure of flushing

The donor goat was placed in a wooden made cradle with the head suspended in downward position. The abdomen was covered with a sterile window towel cloth and secured with towel clips. A 1-2 inch para-midline incision was made cranially starting from very close to the udder. The reproductive tracts were exteriorized starting from the cervix to ovaries and anchored there to avoid repeated handling. Ovaries were examined carefully and CL was counted for number of ovulation. A bone pin was used to puncture the uterine horn just below the external bifurcation of horns. A 2-way 8 FG Foley catheter (FOLEYCATH™, WRP ASIA PACIFIC SDN, BHD, Selangor, Malaysia) with the stillete was inserted into the base of the uterine horn through the punctured wound. The stillete was taken out slowly and the balloon of the Foley catheter was infiltrated with 5 ml air. Another puncture wound was made in the tip of the uterine horn using a blunted 19-G needle attached with a 50 ml syringe containing 50 ml flushing medium. After flushing with the 50 ml medium, the uterine lumen was completely evacuated by blowing through 50 ml air using same needle and syringe. The flushing fluid containing the embryos was collected into a sterile collection pot. The Foley catheter was removed carefully after deflation of the balloon. The reproductive tract was washed with heparinized saline and return back into abdomen gently. All instruments were removed from the abdomen and the wound was closed surgically with sutures.

Post collection care and management

A single dose of $PGF_2\alpha$ (2 mg Alfaprostol) was injected intramuscularly to the donor does after flushing to induce luteal regression. An antibiotics, Streptopen® (0.5 g vial, Renata Animal Health, Dhaka, Bangladesh) was given intramuscularly as a protective measures against infection and 1.0 ml of Diclovet® (10 ml vial, Renata Animal Health, Dhaka, Bangladesh) was given intramuscularly to minimize the post operative pain and discomfort. Tetanus toxoid (1 ml, Tetavax®, Rhone-Poulenc) was also injected against tetanus in does.

Embryo searching

After collection, the fluid containing the embryos from each horn was divided into two parts in 2 petridishes. Each petridish was examined under stereomicroscope using 20x, to visualize the embryos. The embryos were counted.

Statistical analysis

Statistical analysis was done by using SPSS programme. The comparison between alfaprostol and luprostiol on superovulation, recovered, fertilized and transferable embryos were performed by using '*t*'-test for significance.

RESULTS AND DISCUSSION

Effects of alfaprostol and luprostiol on superovulatory response and embryo production in Black Bengal goats are shown in Table 1.

Based on ≥3 superovulation rate, 100% donor does superovulated following induction with 900 iu PMSG in both alfaprostol and luprostiol synchronized groups. The number of superovulation varied from 4-11 and 4-10, respectively. The mean numbers of superovulation were 8.5 ± 0.90 and 8.1 ± 0.76 in alfaprostol and luprostiol groups respectively. There was no significant difference ($p > 0.05$) between these two types of synchronizing agent on superovulation rate.

Table 1. Effects of alfaprostol and luprostiol on superovulation and embryo production in Black Bengal goats by 900 iu of PMSG (Mean ± SE)

S/N	Parameters	Alfaprostol[1] (n = 8)	Luprostiol[2] (n = 8)	Level of significance
1.	Incidence of superovulation ≥3	8/8 (100%)	8/8 (100%)	NS
2.	Number of superovulation	8.5 ± 0.90	8.1 ± 0.76	NS
3.	Number of embryos recovered	5.4 ± 0.80	5.1 ± 0.61	NS
4.	Percentage of embryos recovered	63 ± 7.7	63 ± 3.74	NS
5.	Number of fertilized embryos	3.9 ± 0.52	2.6 ± 0.37	NS
6.	Percentage of fertilized embryos	72 ± 4.55	51 ± 7.00	S
7.	Number of transferable embryos	3.6 ± 1.60	2.4 ± 1.0	NS
8.	Percentage of transferable embryos	93.6 ± 1.6	90.48 ± 1.0	NS

[1]Gabbrostim®, VETEM, Italy, [2]Prosolvin®, Intervet International, The Netherlands, S = Significant ($p < 0.01$), NS = Not significant ($p > 0.05$).

The mean numbers of recovered, fertilized and transferable embryos were 5.4 ± 0.8 and 5.1 ± 0.61; 3.9 ± 0.52 and 2.6 ± 0.37; 3.6 ± 1.60 and 2.4 ± 1.0, respectively following alfaprostol and luprostiol injection. There was no significant difference between the two treatment groups on these parameters. The mean percentage of recovered, fertilized and transferable embryos were 63 ± 7.70 and 63 ± 3.74; 72 ± 4.55 and 51 ± 7.00; 93.6 ± 1.6 and 90.48 ± 1.0, respectively in alfaprostol and luprostiol treated groups. The significant difference ($p < 0.01$) was only existed on percent of fertilized embryos where alfaprostol group had the significantly higher number of fertilized embryos.

In this experiment, the ovulation rate was 8.5 ± 0.90 and 8.1 ± 0.76, in both the synchronized groups, respectively, where the both groups were induced by 900 iu PMSG. Although the mean ovulation rate in the present study following synchronization with alfaprostol was similar to results observed by Agrawal (1986), however, it was little bit higher to results observed by Biswas *et al.* (2000), involving cloprostenol in both cases. As stated above this discrepancy could be due to difference in types of synthetic prostaglandins, individual response of animals and accuracy in the observation of oestrus.

Regarding superovulatory hormones, the type and dose of PMSG used in this experiment is consistent with the other reports (Armstrong and Even, 1983; Agrawal and Goel, 1998, and Pargonkar *et al.*, 1994), although, there are some disagreement (Cameron *et al.*, 1988; Maracek *et al.*, 1989; Doijode *et al.*, 1992; Riesenberg *et al.*, 2001, and Gogai *et al.*, 2001). The difference of breed, batches and lots of PMSG, age, parity and nutrition condition in different experiment can be responsible for this disagreement. In this experiment there was no difference between alfaprostol and luprostiol on superovulation rate.

Success of MOET technique not only depends on superovulation rate but also on the embryo yield. In the present study the percent of recovered embryos in both treatment groups were 63%, while the percent of fertilized and transferable embryos were 72% and 51% and 93.6% and 90.5%, respectively. In this experiment the percent of recovered embryos were relatively low compare to other studies (Tervit *et al.*, 1986 and Baril *et al.*, 1989). Surgical embryo recovery procedure was used to recover the embryos. The Black Bengal goat is said to be a dwarf breed and there is a lot of subcutaneous fat which interfered to exteriorize the uterus through small incision and repeated attempts to grasp the uterus, resulting in damage of some of the embryos. Moreover, PMSG is used for superovulation, which could be responsible for lower embryo recovery rate. In most of the cases premature luteal regression was observed on

the ovaries, which seems to have an effect on embryo recovery rate in the goat induced with PMSG (Stubbings *et al.*, 1986; Schiewe *et al.*, 1990). This percentage of recovered and transferable embryos were similar with Mahmood *et al.* (1991), however, embryo recovery percentage was higher in other published works in goats (Cox *et al.*, 1987; Ocampo *et al.*, 1988, and Doijode *et al.*, 1992) using lutalyse. This difference could also be due to difference in recovery method, skillness of operator and embryologist. In the present study, there was no difference between the two treatment groups in either percent of embryos recovered or transferable embryos. However, significant difference was observed on percent of fertilized embryos, where alfaprostol group had significantly ($p < 0.01$) higher percent of fertilized embryos. Here, there was no difference between them on number of embryos recovered.

The results of this study lead to the conclusions that both alfaprostol and luprostiol may be suitable for synchronization of oestrus within MOET technique in Black Bengal goats in respect of superovulation and embryo production.

REFERENCES

1. Agrawal KP (1986). Hormonal control of ovulation and induction of superovulation in Barbari goats used as donors in embryo transplantation studies. *Indian Journal of Animal Reproduction* 7: 81-83.
2. Agrawal KP and Goel AK (1998). Efficacy of different gonadotrophins on superovulation and embryo recovery in Jamunapari goats. *Indian Journal of Animal Reproduction* 19: 102-105.
3. Armstrong DT and Even G (1983). Factors influencing embryo transfer in sheep and goats. *Theriogenology* 19: 31-42.
4. Baril G, Casamitjana P, Perrin J (1989). Embryo production, freezing and transfer in Angora, Alpine and Saanen goats. *Zuchthug* 24: 101
5. Biswas S, Ghosh BB, Bandyopadhyay SK, Roy MM and Senapati PK (2000). Response of Buserelin (GnRH) on synchronization of oestrus and multiovulation in Black Bengal goats treated with PMSG and $PGF_{2\alpha}$. *Journal International* 4: 290-293.
6. Cameron AWN, Battye KM and Trounson AO (1988). Time of ovulation in goats (*Capra hircus*) induced to superovulate with PMSG. *Journal of Reproduction and Fertility* 83: 747-752.
7. Cox JE, Maria AS, Mora G and Olguin L (1987). Effects of hCG administered late during the oestrous cycle on the ovulatory response of PMSG/PGF–superovulated goats. *Agro-Ciencia* 3: 129-134.
8. Doijode SV, Bakshi SA, Pargaonkar DR and Markandeya NM (1992). Studies on surgical method of embryo recovery in Osmanabadi and crossbred goats. *Indian Journal of Dairy Science* 45: 570-571.
9. Gogai AK, Borgohain BN, Deka BC and Chakravarthy P (2001). A study on the superovulatory response and embryo recovery rate in Assam local goats. *Indian Journal of Animal Reproduction* 22: 26-29.
10. Mahmood S, Koul GL and Biswas JC (1991). Comparative efficacy of pFSH and PMSG on superovulation in Pashmina goats. *Theriogenology* 35: 1191-1196.
11. Maracek I, Elecko J, Hendrichovsky V, Lazar L, Krajnicakova M, Schvarc F, Seregi J, Treuer A and Gabris G (1989). Hormonal treatment of goats during a successful embryo transfer at a large farm. *Veterinarstvi* 39: 199-202.
12. Ocampo MB, Uenishi RS, Valdez CA, Murralla Z, Hishinuma M and Kanagawa H (1988). Superovulation response of upgraded indigenous Philippine goat. *Japanese Journal of Veterinary Research* 36: 249-255.
13. Pargonkar MD, Bakshi SA, Pargonkar DR, Tandle MK and Doijode SV (1994). Studies on superovulation response of goats treated with PMSG. *Indian Journal of Dairy Science* 47: 149-150.
14. Riesenberg S, Meinecke-Tillmann S and Meinecke B (2001). Ultrasonic survey of follicular development following superovulation with a single application of pFSH, eCG or hMG in goats. *Small Ruminant Research* 40: 83-93.
15. Schiewe MC, Howard JG, Goodrowe KL, Stuart LD and Wildt DE (1990). Human menopausal induces ovulation in sheep but embryo recovery after prostaglandin $F_{2\alpha}$ synchronization is compromised by premature luteal regression. *Theriogenology* 34: 469-486.
16. Stubbings RB, Bosu WTK, Barker CAV and King GJ (1986). Serum progesterone concentrations associated with superovulation and premature corpus luteum failure in dairy goats. *Canadian Journal of Veterinary Research* 50: 369-373.
17. Tervit HR, Goold PG and McKensie RD (1986). Development of an effective goat embryo transfer regime. *Proceeding of New Zealand Society of Animal Production* 46: 233.