EFFECTS OF ALFA PROSTOL AND LUPROSTOL ON THE EMBRYO PRODUCTION WITHIN MOET TECHNIQUE IN BLACK BENGAL GOATS

N. I. Faruk, B. Z. Patnaik, 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 Luprostol 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 alternate times within MOET technique to determine the effects of alfaprostol and luprostol on embryo production. Each group consisted of 8 donors was synchronized with alfaprostol (Gebrostenol, VETEM, Italy) or luprostol (Proseostrol, Intervet International, Netherlands) at 2 mg and 7.5 mg, equivalent to 1 ml/100 kg, respectively. The donor goats were bano bano followed the onset of estrus, 1-2 times at 6 hours interval preceding the duration of estrus. The embryos were collected at Day 7 of mating using surgically removed cervix method. The mean number of ovulation in alfaprostol and luprostol group was 5.0 ± 0.0 and 8.1 ± 0.76 respectively, where in both cases 900 IU PMSG was used for induction. The mean fetal number of recovered, fertilized and transferable embryos were 5.4 ± 0.8 and 5.2 ± 0.2, 3.9 ± 0.52 and 2.6 ± 0.37 and 3.6 ± 1.6 and 2.4 ± 1.0 respectively, in alfaprostol and luprostol treated group. Similar procedure was followed in仿 each group throughout the experiment. The percentage of recovered, fertilized and transferable embryos were 36 ± 7.7% (63 ± 3.14), 72 ± 4.55 and 31 ± 7.7 and 93 ± 6 and 90 ± 5 and 1.0 in alfaprostol and luprostol treated groups respectively. The significant difference was only existed in the percentage of fertilized embryos between the two treatment groups, where alfaprostol treated group had significantly higher percentage of fertilized embryos.

Key words: Alfaprostol, luprostol, 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 the 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 goats with the fertile back. 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 effect of synthetic prostaglandins on the success of MOET technique in goats are limited. This paper describes the effects of alfaprostol (Gebrostenol, VETEM, Italy) and luprostol (Proseostrol, Intervet International, Netherlands) synchronization on the superovulatory response and embryo production.

MATERIALS AND METHODS

Synchronization

A total of 16 donor adult Black Bengal doe-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 oestrus cycles of 8 donor does were synchronized by two intramuscular injection of 1 ml (2 mg) of alfaprostol (Gebrostenol, VETEM, Italy) at 11 days interval. Luprostol (Proseostrol, Intervet International, Netherlands) was injected in the same manner 48 hr (7.5 mg) in 8 donor does in another group. Superovulation

PMOS, 900 IU (Soligon, 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 luteolysis dose of synthetic PGF2a (2 mg alfaprostol, Gebrostenol, Italy) and 7.5mg Luprostol, Proseostrol, Intervet International, The Netherlands) was injected 48 hr after injection of PMOS to induce oestrus in both types of induced does. The donor does were mated with fertile buck immediately after observing the symptoms of oestrus for 1-2 days, 6 hr apart, depending on the duration of oestrus.
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 Bazzi 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 (Albunin Concentrate, 20% w/v, JCP, Austria, Norwadler), penicillin and streptomycin (100 μg/ml Streptomycin, 0.5 μg/ml, Renana Animal Health, Bangalore) to complete the medium. Briefly, 9.55 g dry PBS was dissolved in 1 liter of distilled water (Milli-Q® water, ultra pure water system, Millipore Corporation, USA). Calcium chloride (1.132.66 mg CaCl22H2O) was added gradually and dissolved in PBS solution spinning the medium in a conical flask by hand movement. Magnesium salt (100 mg MgCl2 6H2O) was dissolved in the same way. Sixty (60) mg of penicillin and 100 mg of streptomycin were mixed in the PBS solution and another. Prior to flushing 10 ml BSA was mixed slowly. Medium was filtered following standard aseptic measures.

Anesthesia and preparation of donors for collection

A sedative, chloropentone hydrochloride (Lazac®, Pharma-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, attention to the udder was clipped, shaved, scrubbed with an antiseptic solution (Hexacide®, 0.5% chlorhexidine gluconate, Aci, Bangladesh) and dried. Paravertebral nerves were blocked by injecting 12 ml of 2% lignocaine hydrochloride (Lascaine®, Javon 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 underlie tissues were desensitized by subcutaneous infiltration of 5 ml of 2% lignocaine hydrochloride.

Procedure of flushing

The donor groin was placed in a wooden made crate with the head supported 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 ovaries 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 PG Foley catheter (FOLEYCATH®, WRAP ANA PACIFIC SDN, BHD, Selangor, Malaysia) with the stilette was inserted into the base of the uterine horn through the punctured wound. The stilette was taken out slowly and the ballon of the Foley catheter was inflated with 5 ml air. Another puncture wound was made in the tip of the utero-vaginal 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 horn 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 PGE2 (2 mg Alfragide®) was injected intramuscularly to the donor does after flushing to induce luteal regression. As antibiotics, Streptomycin (0.5 μg/ml, Renana Animal Health, Dhaka, Bangladesh) was given intramuscularly as a protective measures against infection and 1 ml of Dilucove® (10 ml vial, Renana Animal Health, Dhaka, Bangladesh) was given intramuscularly to maintain the post operative pain and discomfort. Tetanus toxoid (1 ml, Tetavis®, 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 perifluids. Each perifluid was examined under stereoscope using 20x, to visualize the embryos. The embryos were counted.

Statistical analysis

Statistical analysis was done by using SPSS programme. The comparison between alfaprost and liposar on superovulation, recovered, fertilized and transferable embryos were performed by using ‘t’-test for significance.

148
RESULTS AND DISCUSSION

Effects of alfalfa and lupin on superovulatory response and embryo production in Black Bengal goats are shown in Table 1. Based on 23 superovulation rate, 100% donor does superovulated following induction with 900 IU PMSG in both alfalfa and lupin treated synchronised groups. The number of superovulation varied from 4.1-4.0, respectively. The mean numbers of superovulation were 8.5 ± 0.90 and 8.1 ± 0.76 in alfalfa and lupin treated groups respectively. There was no significant difference (p > 0.05) between these two groups of synchronizing agent on superovulation rate.

Table 1. Effects of alfalfa and lupin on superovulation and embryo production in Black Bengal goat by 900 IU of PMSG (Mean ± SD)

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Alfalfa 1 (n = 8)</th>
<th>Lupin 2 (n = 8)</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Incidence of superovulation 23</td>
<td>8/8 (100%)</td>
<td>8/8 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>Number of superovulation</td>
<td>8.5 ± 0.90</td>
<td>8.1 ± 0.76</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>Number of embryos recovered</td>
<td>5.4 ± 0.00</td>
<td>5.1 ± 0.61</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>Percentage of embryos recovered</td>
<td>63 ± 7.7</td>
<td>65 ± 7.9</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>Number of fertilized embryos</td>
<td>3.9 ± 0.52</td>
<td>2.4 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>Percentage of fertilized embryos</td>
<td>72 ± 4.35</td>
<td>71 ± 7.00</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>Number of transferable embryos</td>
<td>3.6 ± 1.80</td>
<td>2.4 ± 1.00</td>
<td>NS</td>
</tr>
<tr>
<td>8</td>
<td>Percentage of transferable embryos</td>
<td>93.6 ± 1.6</td>
<td>90.48 ± 1.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Gabbhunjan, YETEM, Italy, "Pulsolvin", Interter 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 ± 1.00 and 2.4 ± 1.0, respectively following alfalfa and lupin injection. There was no significant difference between the two treatment groups in these parameters. The mean percentage of recovered, fertilized and transferable embryos were 63 ± 7.70 and 63 ± 3.54; 72 ± 4.35 and 71 ± 7.00; 93.6 ± 1.6 and 90.48 ± 1.0, respectively in alfalfa and lupin treated groups. The significant difference (p < 0.01) was only existed on percent of fertilized embryos where alfalfa 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 of PMSG. Although the mean ovulation rate in the present study following synchronization with alfalfa was similar to results observed by Agrawal (1986), however, it was higher than results observed by Beas et al. (2000), involving clomiphene in both cases. As stated above this discrepancy could be due to difference in types of synthetic progesterogens, individual response of animals and accuracy in the observation of estrus.

Regarding superovulatory hormones, the type and dose of PMSG used in this experiment is consistent with the other reports (Armstrong and Egan, 1983; Agrawal and Cost, 1998, and Purgoslin et al., 1994), although, there were some disagreement (Cameron et al., 1988; Mesecar et al., 1988; Dookdeo et al., 1992; Rottiers et al., 2001, and Gogii et al., 2001). The difference of breed, batches and lot of PMSG, age, parity and nutrition condition in different experiment can be responsible for this disagreement. This experiment there was no difference between alfalfa and lupin 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 percentage of fertilized and transferable embryos were 72% and 51% and 75.6% and 90.5%, respectively. In this experiment the percent of recovered embryos were relatively low compared to other studies, Terki et al., 1986 and Barli et al., 1989. Surgical embryo recovery procedure was used in this experiment. The Black Bengal goat in this study was 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.
REFERENCES


14. Rosenberg S, Mkerizecke-Cilmmann S and Monette B (2001). Ultrasonic survey of follicular development following superovulation with a single application of gP2, meS or meG in goats. SMALL Ruminant Research 40: 83-93.

