RESPONSES OF THE BLACK BENGAL GOAT (*CAPRA HIRCUS*) TO PMSG AND SURGICAL EMBRYO RECOVERY WITHIN MOET TECHNIQUE

M. O. Faruk[1], F. Y. Bari, M. Shamsuddin, M. G. S. Alam and M. F. Islam[2]

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

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ABSTRACT

The aim of the present study was to determine the responses of Black Bengal Goat following synchronization with alfaprostol, superovulation with PMSG and embryo collection with surgical procedure. This research work was carried out at the Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU) during the period from January to December 2001. Three Flushing schedules have been carried out in one-year time consisting of 5 donor and 4 recipients in Flush I, 2 donors and 4 recipients in Flush II, and 4 donors and 3 recipients in Flush III. The donors as well as the recipients were synchronized with Gabbrostim® (Alfaprostol, VETEM, Italy) at the dose rate of 2-mg equivalent to 1ml/goat. Among 11 donors only 6 donors responded following first injection, but all donor responded with second and third injection of Gabbrostim®. The mean time of onset of oestrus and duration of oestrus in donor were 29.9 ± 9.20h, 50.0 ± 5.94h, 47.8 ± 4.05h and 20.9 ±5.10h, 27.9 ± 1.19h, 29.4 ± 1.36h respectively. Among 11 recipient does, only 6 responded following first injection of Gabbrostim, while that of second injection was 9. The mean time of onset of oestrus and duration of oestrus were 28.6 ± 9.69h, 40.1 ± 8.95h and 17.1 ± 5.05h, 22.5 ± 3.45h respectively. All 9 recipients responded in third injection and the mean time of onset of oestrus and duration of oestrus were 39.9 ± 5.06h and 27.4 ± 1.32h respectively. In all flushing schedules, superovulation was performed using PMSG (Folligon®, Intervet International B.V, Holland) at the dose rate of 900 IU, 800 IU and 700 IU respectively. Both natural service and AI was performed to fertilise the ovum after onset of oestrus. The superovulatory response was determined by counting the number of corpora lutea (CL) during collection of embryos. The percent of superovulatory donor goats were 100, 50 and 25% respectively. The mean number of ovulation was 13.6 ±3.6, 10.5 ± 8.5 and 3.5 ± 3.2 respectively. The embryos were collected surgically by using 50 ml Phosphate buffer saline (PBS). The embryos were counted and graded under stereomicroscope. The embryo collection rates varied from 0-100%. The recovered embryos were of 100% fertile. The total number of embryos recovered was 4, 8 and 8 with the percent of recovery 6, 38 and 61.5% respectively. In Flush I among 4 recovered embryos, 3 were Grade 1 morula and the rest embryo was Grade 2 late morula. In Flush II among 8 recovered embryos, 7 were Grade 2 early morula and rest embryo was Grade 2 late morula. In Flush III among 8 recovered embryos, 5 were Grade 2 morula and rests 3 were Grade 2 late morula.

Key words: Black Bengal goat, PMSG, embryo, MOET

INTRODUCTION

The Black Bengal goats are the predominant goat breed in Bangladesh and are very easy to rare. It is mainly used for meat, milk and leather production. But the problems of Black Bengal goat breed are their small body sizes (average body wt is 15 kg) and high fat content in the meat, both intra-muscular and subcutaneous. The people dislike the high fat content, mainly due to three-reason taste, wastefulness and health. Saturated fat content in the food has been found to be associated with the incidence of several increased chronic degenerative diseases involving the digestive system, circulatory system and heart (Keys *et al*., 1986).

To increase the body size and to improve the economic traits in goat it is necessary to bred the top quality buck with top quality she-goat. However, the mean ovulation rate of Black Bengal goat is two. Through natural breeding this will be a long-term process to get the increased number of goat with high economic traits. Superovulation within the MOET technique is mainly performed with the object of increased ovulation rate and hence increased supply of high quality embryos for transfer to increase the high quality offspring.

Therefore, if multiple ovulation and embryo transfer technique be applied within the breeding programme, it will take less time to increase the selection intensity through the mother line. Studies in sheep indicated that genetic gain per generation of selection could be doubled if each ewe supply 10 offspring per breeding year (Smith, 1986 and Bari *et al.,* 1999). Several researches have been conducted in Europeans countries in goats for better responses and yield of transferable embryos. For example, choice of better gonadotrophins (Armstrong and Evan 1983; Agrawal, 1986; Baril *et al.,* 1988 and Goel *et al.,* 1991), dose of gonadotrophins (Agrawal and Bhattacharyya, 1982), dose and device of synchronizing agent (Ishwar and Pandey, 1988; Nandy *et al.,* 1990; Martemucci, 1992), mating system (Tervit *et al.,* 1986; Baril *et al.,* 1989), transfer of embryos (Armstrong and Evan, 1983; Nagashima *et al.,* 1987; Baril *et al.,* 1989; Flores- Foxworth, 1997) and synchrony of oestruses between donor and recipients (Armstrong and Evan, 1983; Bondurant, 1986; Lewis, 1996). No published work has been reported on multiple ovulation and embryo transfer in Black Bengal goat in Bangladesh. Therefore the present work was designed to observe the responses of Black Bengal goat following superovulatory with PMSG and embryo recovery with surgical embryo recovery method.

MATERIALS AND METHODS

This study was conducted at the Department of Surgery and Obstetrics, BAU during the period from January to December 2001 under the BAURES project, Evaluation of Multiple Ovulation and Embryo-Transfer Technique in Black Bengal goat in Bangladesh. The eleven Black Bengal goat of 1 and 2 years old, weighing 10-15 kg were flushed as a donor in three times (Flush I, 5 Flush II 2, and Flush III 4) in one years time within the MOET treatment. The number of recipient does was also 11 (Flush I 4, Flush II, 4 and Flush III, 3). The goats were housed in well-ventilated, concrete floor; house with adequate light. The goats were fed sufficient green grass and a concentrate mixture containing equal amounts of wheat bran, crushed wheat, soybean bran, maize grain at the added with 2% iodinated salt and 1% vitamin and mineral premix at the rate of 0.5 kg/head. The goats were acclimatized for 3 months and checked for normal cycle before giving treatment.

Synchronization of oestrus

Synchronization of oestrus was done with two intramuscular injection of 1 ml equivalent to 2 mg Gabbrostim[®] (Alfaprostol, VETEM SPA, Porto Empedocle, Italy) at 11 days apart in both donor and recipient goats. The donor and recipient goats were observed for signs oestrus at 4h interval from 700h to 2000h. Onset of oestrus was detected on the basis of the behavioral and clinical signs.

Superovulatory response

Three doses of PMSG (Folligon[®], intervet International B.V., Boxmer, Netherlands) 900 IU (n = 5), 800 IU (n = 2) and 700 IU (n = 4) were used for superovulation. The superovulation treatment was initiated at Day 10 or Day 11 of the oestrous cycle (Day 0 = day of oestrus). A luteolytic dose of synthetic prostaglandin $F_2\alpha$ (alfaprostol, Gabbrostim[®], 2 mg, VETEM S.P.A, Porto Empedocle, Italy) was injected intramuscularly 48 hours after injection of PMSG to induce oestrus.

Natural service and artificial insemination

Both natural service and artificial insemination were performed to fertilize the superovulated ovum. The donor goats were inseminated at 6 hour after the onset of oestrus. Then the goats were allowed to serve by the buck at about 6 hour after insemination.

Embryo collection

Embryos were collected from donor goats by surgical embryo collection method on Day 6 or 7 after natural service and AI. A sedative, chlorpromazine hydrochloride (Largactil[®], Rhone-Poulenc, Bangladesh), was injected IM at the dose rate of 0.1 mg/kg body weight approximately 15-30 minutes before surgery. The rear half of the abdominal wall, anterior to the udder, was clipped, shaved, scrubbed with antiseptic solution (Hexisol[®]; 0.5% chlorhexidine gluconate, ACI, pharmaceuticals, Ltd, Bangladesh) and dried. Paravertebral nerves were blocked by injecting 12 ml of 2% lignocaine hydrochloride, 4 ml in last thoracic, 3 ml in both first and second lumbar and 2 ml in third lumbar vertebral spinal nerve (Jasocaine[®,] Jayson pharmaceuticals, Ltd, Bangladesh). The skin and underline tissue was desensitized by infiltrating 5 ml of 2% lignocaine hydrochloride. The donor goat was suspended in head down position in the wooden made cradle. The abdomen was covered with a sterile towel cloth attached with towel clips. A 1-2 inch para-midline incision was made immediately cranial to the udder using regional paravertebral and local anaesthesia. Reproductive tracts were exteriorized

starting from the cervix to ovaries and anchored there to avoid repeated handling. Ovaries were examined and the number of ovulation was counted by counting the number of corpora lutea. After holding the uterus, a bone pin was used to puncture the uterine horn about 1-2 inch below the external bifurcation. An 8 french gauge foley catheter with the stilette was inserted into the base of uterine horn through the punctured wound. The stilette was taken out and the balloon of the foley catheter was inflated with 5-ml air. A puncture wound was made in the tip of the horn using a blunted 19-G needle attached to a 50 ml syringe containing 50 ml flushing medium (PBS solution containing a balanced salt solution, supplemented with a protein source, BSA and antimicrobials). After flushing through the 50-ml medium the uterine lumen was completely evacuated by blowing through 50-ml air using same needle and syringe. A total of 100-150 ml of medium was used in two to three flushes of individual horn. The flushing medium containing the embryos was collected into a pre-warmed sterile pot. The flushing procedure was then repeated for the other horn. The tip of the uterine horn was placed back into the abdominal cavity and the foley catheter removed carefully after deflation of the balloon. All the instruments were removed from the abdomen and the wound was sutured aseptically.

Post collection care and management

The skin wounds of all donors goats were covered with benzoin seal. The donor goats were immediately injected with 2mg alfaprostol and 0.5 gm streptomycin with 5 lac penicillin (Streptopen®, 0.5gm, Renata Animal Health, Dhaka, Bangladesh) to induce oestrus and protect from microbial infection.

Selection and grading of collected embryos

The flushed out concentrated medium was divided in to two portion and placed in a searching petridish (100-mm diameter, CORNING® New York, USA) and examined under a stereomicroscope (20X, Binocular Stereomicroscope®, XTS-110, China) to visualize the embryos. The identified embryos were individually picked up with a micropipette controller (Assistent Micro Classic® No. 558, Kart Hecht GmbH & Co. KG, Germany) and placed in a small sized petri dish (35 mm diameter, FALCON®, Betcon Dickinson Company, Japan) containing fresh, filtere-sterilized (0.22μm pore size, Minisart®, Minitub GmbH, Tiefenbach b. Landshut, Germany), medium. The embryos were washed 4 times in the same way and evaluated subsequently. The embryos were classified in four groups according to the method described by Flores- Foxworth (1997) and Bari *et al.*(1999). Grade 1- the embryo was nearly perfect with more than 98% of the cell mass active and healthy, Grade 2- about 70 to 98% of the cell mass was active and healthy and some extruded blastomeres were found, Grade 3- a poor quality embryo with less than 70% of the cell mass active and healthy and several extruded blastomeres were present, Grade 4- degenerated embryos with no active cell mass and distinct plasma membrane.

The data were analyzed statistically by the paired t-test.

RESULTS AND DISCUSSION

Synchronization

The time of onset of oestrus and the duration of induced oestrus after injection of Alfaprostol within three Flushing schedules are shown in Table 1 and 2.

Table 1. Effect of alfaprostol on the onset of oestrus and duration of oestrus in donors and recipients in Flush I, II and III. (mean ± s.e.) h

Flush I + Flush II + Flush III	No. of donor injected	No. of donor responded	Time of onset of oestrus	Duration of oestrus
1st injection	11	6 (54.5%)	29.9 ± 9.20	20.9 ±5.10
2nd injection	11	11 (100%)	50 ± 5.94	27.9 ±1.19
Flush I + Flush II + Flush III	No. of recipient injected	No. of recipient responded	Time of onset of oestrus	Duration of oestrus
1st injection	11	6 (54.5%)	28.6 ± 9.69	17.1 ±5.05
2nd injection	11	9 (81.8%)	40.1 ±8.95	22.5 ± 3.45

Table 2. Comparison of onset of oestrus and duration of oestrus between donor and recipient does following 3rd injection of prostaglandin (mean ± s.e.) h

Donor/Recipient	No. of doe injected	No. of doe responded	Time of onset of oestrus	Duration of oestrus	Significance of difference
Donor	11	11 (100%)	47.8 ± 4.05	29.4 ± 1.36	NS
Recipient	9	9(100%)	39.9 ± 5.06	27.4 ± 1.32	

*NS= Not significant.

In donor goats, the percentage of goats showed oestrus after 1st, 2nd and 3rd injections were 54.5, 100 and 100% respectively. The mean time of onset of oestrus was 29.9 ± 9.20 (s.e.), 50 ± 5.94 (s.e.), 47.8 ± 4.05h (s.e.) respectively. The mean times of duration of oestrus were 20.9 ±5.10 (s.e.), 27.9 ± 1.19 (s.e.) and 29.4 ± 1.36h (s.e.) respectively. In recipient goat, the percentage of goats showed oestrus and the mean time of onset of oestrus were 54.5, 81.8, and 100%, respectively following first, second and third injection of alfaprostol. The mean time of onset of oestrus and duration of oestrus was 28.6 ± 9.69 (s.e), 40.1 ± 8.95(s.e) and 39.9 ± 5.06h (s.e) and 17.1 ± 5.05 (s.e), 22.5 ± 3.45 (s.e) and 27.4 ± 1.32h (s.e) respectively.

In paired t-test, there was no significant difference between the donor and recipient goats on the onset of oestrus and duration of oestrus after third injection of prostaglandin, where the donors only had PMSG injection (Table 2). The plasma progesterone concentrations plummeted to below basal levels after the alfaprostol injection (Figure 1 & 2).
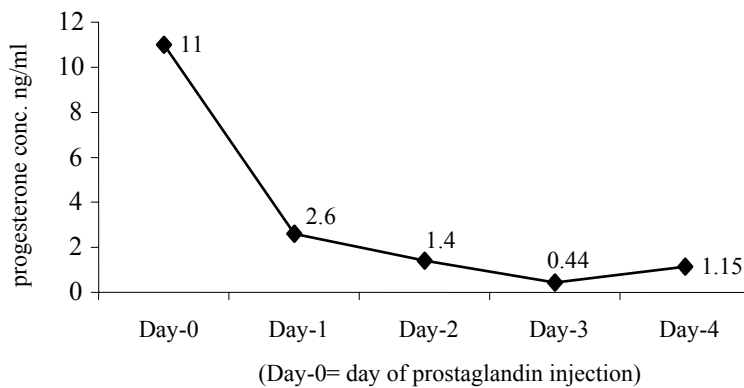

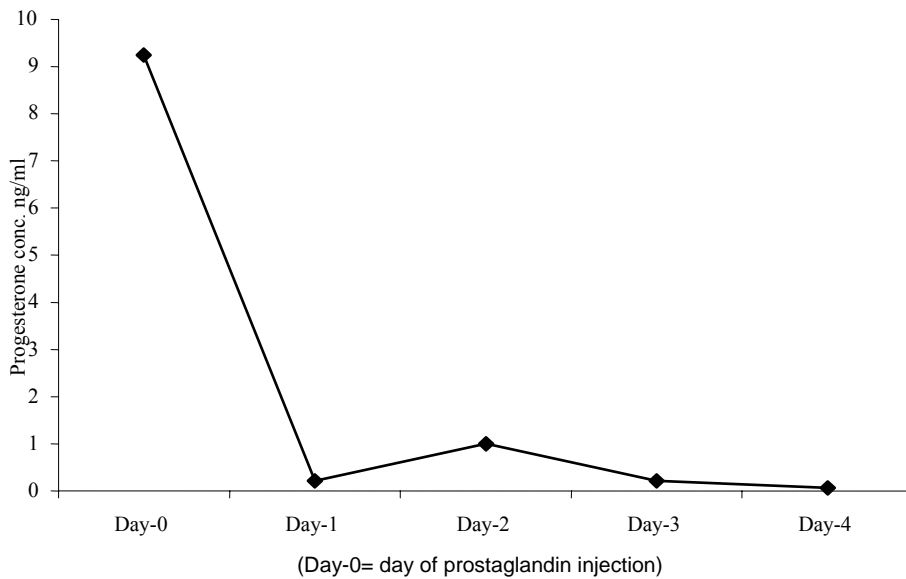Fig. 1. Plasma progesterone concentration in does treated with alfaprostol

Fig.2. Plasma progesterone concentration in does treated with alfaprostol

Superovulation
Effects of PMSG on superovulatory response and embryo yield of Black Bengal goats is shown in Table 3.

Table 3. Effects of PMSG and surgical embryo recovery procedures in the Black Bengal Goat (mean ± s.e.)

Parameters	Flush I (900 IU PMSG)	Flush II (800 IU PMSG)	Flush III (700 IU PMSG)
Incidence of superovulation ≥ 3	5/5 (100%)	1/2 (50%)	1/4 (25%)
superovulation/donor goat (mean ± s.e.)	13.6 ± 3.6 (4 – 25)	10.5 ± 8.5 (2 – 19)	3.5 ± 3.2 (5-8)
Embryo recovery percentage	6 (4/64) (0-100%)	38(8/21) (0-50%)	61.5 (8/13) 100%
Percentage of fertilized embryos	100	100	100
Quality of embryos	Grade 1 morula-3 Grade 2 late morula- 1	Grade 2 early morula-7 Grade 2 late morula-1	Grade 2 morula-5 Grade 2 late morula-3

Superovulatory response

Based on ≥3 superovulation rate, the percent of superovulatory donor goats were, 100, 50 and 25% following stimulation with 900, 800 and 700 I.U. PMSG, respectively. The ovulation rate varied from 2-25. The mean number of superovulation in 900, 800 and 700IU PMSG were 13.6±3.6, 10.5 ± 8.5 and 3.5 ± 3.2 respectively.The superovulatory response was higher in Flush I with 900 IU Folligon® (PMSG), (13.6 ± 3.6) compared to 800 and 700IU PMSG.

Embryo recovery

The embryo recovery rates varied from 0-100%. The total number of embryo recovered were 4, 8 and 8 with the percent of recovery 6, 38 and 61.5% in Flush I, II and III, respectively. The recovered embryos were of 100% fertile.

Embryo quality

In Flush I among 4 recovered embryos, 3 were Grade 1 quality morula and the rest embryo was Grade 2 quality late morula. In Flush II among 8 recovered embryos, 7 were Grade 2 quality early morula and rest embryo was Grade 2 quality late morula. In Flush III among 8 recovered embryos, 5 were Grade 2 quality morula and rest 3 was Grade 2 quality late morula. In most of the cases no embryos were transferred to the recipients due to unavailability of recipients correspond to the donors oestrus or unavailability of embryos from the donor corresponds to the recipient in oestrus. In Flush II among 8, a total of 3 embryos were transferred, 2 in right horn and 1 in left horn. However, the recipient showed heat within 3 weeks of transfer.

Synchronization of oestrus is usually performed to have the entire animal in oestrus and offspring at the same time. Within a MOET programme, synchronization of oestrus gives the opportunity to have the donor and recipient animals at the same stage of cycle at the time of embryo transfer which is essential to achieve maximum survival of embryos (Moor and Rowson, 1966), since the degree of synchrony is one of the factors influencing the success of embryo transfer (Torres *et al.*, 1987). Synchronization of oestrus with synthetic prostaglandins resulted in 100 percent donor were in oestrus and 41.66 percent conception/pregnancy rate (Pandiya and Rathor, 1986) within MOET programme. The injection of cloprostenol resulted in 87.5% donor goat in heat. The mean time of onset of oestrus was 38.4 ± 0.3h (s.e.) and duration of oestrus was 40.8 ± 0.2h (s.e.) respectively (Armstrong *et al.*, 1983b).

In the present study, Donor and recipients goats were synchronized with Alfaprostol. The percent of donor goats showed heat were 54.5, 100 and 100% following first time, second time and third time injection of Alfaprostol. The mean time of onset of oestrus in donor and recipient varied from 29.9 ± 9.20 (s.e.) to 50 ± 5.94h (s.e.) and 28.6 ± 9.69 (s.e.) to 40.1 ± 8.95h (s.e.) respectively. This mean time of onset of oestrus in donor after Alfaprostol injection is similar to some published works (Armstrong *et al.*, 1983b; Bretzlaff *et al.,* 1983; Alam *et al.*, 1989; Biswas *et al.*, 2000). However, this is lowered than those reported by Pandey and Ishwar (1990) and higher than those reported by Holtz and Sohnrey (1992) and Perera *et al.* (1978). The discrepancy could be due to the fact that although synthetic prostaglandins were used in all works to synchronize the oestrus in goats, however, they were the products of different laboratories of having different qualities and doses. Like the mean time of onset of oestrus in donor and recipients goats, the mean time of duration of oestrus in donor goat were comparable to some of the published work (Holtz and Sohnrey, 1992; Martemucci, 1992) and dissimilar to some of the works (Pandey and Ishwar, 1990; Armstrong *et al.*, 1983b; Biswas *et al.*, 2000). The explanation of this dissimilarity of duration of oestrus is similar to mean time of onset of oestrus, however there were also some limitations at the beginning of the work about the carefulness of observation on the animals. In recipient goats both mean time of onset of oestrus and mean time of duration of oestrus was lower compared to donor. It is difficult to explain why in recipients the mean time of onset of oestrus was lower. The recipient's chosen were comparatively weaker than donor. Also there could be possibility of error in counting the hour. Further work is going on. These will elucidate the explanation.

In paired t-test, the absence of difference between the donor and recipient on the onset of oestrus and duration of oestrus following third injection of prostaglandin indicates that PMSG did not have any influence on the onset of those parameters.

MOET has the potential to speed up the process of genetic improvement by increasing selection intensity through the female line, but for this potential to be realized it requires a high superovulatory response. Among a number of factors, choice of gonadotrophin for superovulation of donor animal is the first important one, which

can affect the responses. It is desirable to have preparation, which results in consistently high superovulatory responses with minimum variation between animal. Furthermore the development of persistent follicles is highly undesirable because it compromises fertilisation, leads to premature activation of the oocyte and suboptimal conditions in the oviduct / uterine environments all of which leads to premature embryo recovery rate, embryo quality and subsequent embryo survival.

The ovulation rate within the MOET Technique can be said as superovulation if the donor produced ≥ 3 ovulation following treatment with gonadotrophin. In the present study the percent of animal showed superovulation following first induction was 100%. However, the incidence of superovulatory animal was gradually decreased following succeeding times. The interval between successive stimulation with PMSG was about a month within this study. There is also evidence of poorer responses at subsequent treatments with PMSG (Saumande and Chupin, 1977; McKelvey and Robinson, 1986) probably as a result of production of antibodies against PMSG (Jainudeen *et al*., 1966). There were incidences of cyst formation in the donor goats. Moreover, each time the embryos were flushed with surgical procedure. There is evidence that surgical flushing result in lower ovulation rate (Fukui *et al*., 1985; Torres and Sevellee, 1987). The lower ovulation rate could be due to difficulty in seeing the whole surface of the ovary due to adhesion following laparotomy or could also due to tissue damage or infection.

In the present study, the mean ovulation rate was 13.6±3.6 (s.e.), 10.5 ± 8.5 (s.e.) and 3.5 ± 3.2 (s.e.) respectively following 1st, 2nd and 3rd stimulation. The ovulation rate is satisfactory. This ovulation rate is comparable to that of other researchers within the same breed following induction with PMSG (Biswas *et al*., 2000) and in other breed (Armstrong *et al.,* 1983a, Agrawal 1986, Cameron *et al*., 1988), although they used relatively higher doses for superovulation. Here the breed difference could be override by the hormone stimulation. The ovulation rate in the present study is higher in 1st and 2nd flush compare to some of the published works (Riesenberg *et al*., 2001, Eiamvita Yakorn *et al*., 1988, Doijode *et al*., 1992). Although the breed difference could be a possible cause, however there could be also variation in FSH and LH ratio within the PMSG in different batches and lots of PMSG produced in different laboratory. Increase in LH ratio compare to FSH could interfere with the ovulation rate in superovulatory process. Moreover, Riesenberg *et al*. (2001) used comparatively high dose compare to this experiment. Black Bengal breed is prolific, the mean ovulation rate is two. It has been observed that prolific breed response more compare to non-prolific breed (Fernie *et al*., 1993). Even then care should be taken for explanation of high ovulation rate in this experiment, as the numbers of animals were small.

Three dose units 900 IU, 800 IU and 700 IU were used to induce the superovulation. The response was better with 900 IU (13.6±3.6). However, it was not possible to compare the results with different doses using significant test. Since the number in successive induction, which responded was very lower. Therefore, more works need to standardize the dose effects.

Success of multiple ovulation and embryo transfer process depend not only the superovulation rate but also on the embryo recovery rate and quality of embryo. Surgical procedure was used to flush the embryos in this experiment. The embryo recovery rate was very lower in this experiment and lowered to some of the published works, which were flushed with surgical procedure (Tervit, 1983, 1986, Patil, 1984 and Baril, 1989). The embryo recovery rate varied from 0-100% in individual cases. The explanation for lower recovery rate could many. Although there were available literatures about the surgical flushing of embryos in other species, however the literature for such flush in goat was not able to find out. The Black Bengal goat is a dwarf breed and there is a lot of subcutaneous fat, which interfered to exteriorize the uterus through the small incision, resulting in disruption of some of the embryos. Moreover during first time flush there were limitations with the embryologist. PMSG was used to induce superovulation, which could result in premature embryo production and lower embryo recovery rate. In this experiment it seems that problem with the flushing and limitation of the embryologist could be the possible cause for lower response because in the subsequent flush with the advance of time (during 3rd flushing) the embryo recovery percentage was increased compare to first and second flush.

Percent of embryos fertilized in this experiment was 100%. Both natural service and artificial insemination were used to fertilize the embryos. This could the possible explanation for 100 percent fertilization rate of the number of embryos recovered.

In this study the embryos were recovered in Day 7 in flush I and flush III and Day 6 in flush II. In flush II there were two early morula. During flush I due to heavy subcutaneous fat it was difficult to take out the entire

reproductive tract through the incision and it interfered the whole flush from tip to bifurcation of the horn. Therefore in second time the embryos were flushed on Day 6, thinking that embryos will be near the tip or middle of horn so that entire embryos will be able to flush out. Although it increased the embryo recovery rate compare to first time flush, however, 2 embryos were in early stage morula.

Regarding the embryo transfer, in flush I and flush III it was not possible to transfer the embryo due to unavailability of recipients correspond to donor or the recipient were there, but it was not possible to recover any embryo from the correspond donor. In flush two 3 embryos were transferred in one recipient, 2 in right horn and 1 in left horn. However, during exteriorization of the horn of recipient- the CL was destroyed. Therefore the recipient showed heat within 3 weeks.

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