



SUPEROVULATION AND EMBRYO TRANSFER IN CYCLIC LOCAL GOATS OF ASSAM

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ABSTRACT

A total of 30 cyclic Assam Local goats were selected for superovulation, embryo collection and their subsequent transfer to recipients. The experimental goats were divided into five groups each comprising 6 animals and treated with the hormonal combinations of PMSG750IU+PGF_{2α}5mg+HCG1000IU, PMSG750IU+PGF_{2α}10mg+HCG1000IU, PMSG750IU+PGF_{2α} 5mg+HCG750IU, MAP5mg+ PMSG750IU+PGF_{2α}5mg+HCG1000IU and Allylestrenol5mg+ PMSG 750IU+ PGF_{2α}5mg+HCG1000IU were administered in group I, II, III, IV and V respectively. The animals in group I, II and III were treated with allotted dose of PMSG after 48 hours prior to the single dose of PGF_{2α}. HCG was injected during induced oestrus just before or after mating. The goats of group IV and V were fed MAP and allylestrenol daily for 11 days starting within 6-15th days of the oestrous cycle and after 24 hours of last progesterone feeding PMSG was injected followed by PGF_{2α} and HCG as in above groups. The result indicated the mean time interval between PGF_{2α} and onset of oestrus and mean duration of superovulatory oestrus due to different hormonal treatment combinations were varied significantly (P<0.05) while oral administration of MAP or allylestrenol to the cyclic goats for a fixed period of 11 days before administration of PMSG was equally effective in suppressing occurrence of oestrus with significantly (P<0.05) shortest induced oestrus period. The superovulatory pattern due to

different treatment combinations also revealed that all the treatment combinations were equally effective although the mean ovulation rate were significantly ($P < 0.05$) different while progesterone primed groups showed higher ovulation rate in group IV and V respectively. The overall fallopian flushing resulted 81.69% and 76.47% with treatment A and B respectively while the uterine flushing resulted overall embryo recovery rate recorded as 54.74% and 57.52% with the same respective treatments. Out of twelve (4-8-cell) embryos transferred to the six recipient goats resulted three pregnancies (50%) followed by three kidding of full term kids upon fallopian transfer. Similarly, five pregnancies (41.67%) were occurred after uterine transfer of fifteen fresh embryos (>morula) to twelve recipient goats. It was found that 25% of fresh embryos were survived upon fallopian transfer while 33.33% of fresh embryos were survived upon uterine transfer with full term pregnancy. The gestation length of the recipients and body weight of the new born upon embryo transfer was almost similar to that of the normal pregnancy.

INTRODUCTION

Effective superovulation is the only reliable technique. For achieving maximum number of viable embryos for rapid genetic livestock improvement through multiple ovulation and embryo transfer technique (MOET). The Assam Local goats has some positive reproductive traits with better adaptability in sub-tropical humid zone. Although, superovulation in most of the livestock had been achieved successfully with the use of exogenous gonadotropins, steroids and prostaglandin information on different parameters like superovulatory response of different hormonal preparations in regards to embryo transfer technology in Assam Local goats in this North-East zone is meager. Keeping this in view, the present experiment was undertaken to study superovulatory response with administration of different hormonal combinations, embryo collection and their subsequent transfer in Assam Local goats.

MATERIALS AND METHODS

A total of twenty six healthy Local goats of Assam with the live body weight of 10-16Kg were used in the present investigation. These goats were maintained under semi-intensive system of management throughout the experimental period. The randomly selected goats were divided into five groups each comprising six animals. Though FSH and LH resulted better

recovery of viable embryos, pregnant mare serum gonadotropin(PMSG) was used as source of FSH which is very economic with single injection when FSH requires multiple injections with higher cost as compared to single injection of PMSG. So, five hormonal combinations were used for superovulation and embryo collection as shown in Table1. The hormones used for superovulation were PMSG(Folligon), Human Chorionic gonadotropin (HCG), PGF_{2α}(Dinofertin) and two progestational analogues viz. 6-Methyl-17-Acetoxy progesterone(MAP,Furlutal) and 17-hydroxy-17-Allyl-4estren(Allylestrenol, Gestanin) respectively.

Following superovulatory treatment and after detection of oestrus of donors breeding was done through natural service with healthy bucks. Then all donors were laparotomized for observation superovulation rate. Another twenty one nanny goats were taken and divided seven groups where each groups comprising three goats were superovulated with treatment combination PMSG750IU+PGF_{2α}5mg+HCG750IU(A) for embryo collection by fallopian flushing at 48, 72 and 96 hours and uterine flushing at 72, 96, 120 and 144 hours respectively after mating with good breeding bucks. Similarly another 21 goats were taken and divided into seven groups each comprising three goats and were treated with MAP5mg+PMSG750IU+PGF_{2α}5mg+ HCG750IU(B) for embryo collection by fallopian flushing at 48, 72 and 96 hours and uterine flushing at 72, 96, 120 and 144 hours respectively similarly after mating with good breeding bucks. Embryo recovery rate both from fallopian and uterine flushing was carried out by using TCM-199 media. Embryos of less than 8-cells were transferred surgically into the fallopian tube and embryos of more than 8-cells(morula) were transferred into the lumen of uterine horn 2-3cm from utero-tubal junction of the oestrus synchronized recipient goats while these goats were observed closely for non-return to oestrus till full term pregnancy and parturition.

RESULTS AND DISCUSSION

The result of the superovulatory response to the administration of different exogenous hormonal combinations in regards to oestrous behavior and ovulation and embryo recovery rate (Table 2, 3 and 4) indicated that all the treated animal groups (100%) of the experiment equally responded where all superovulated goats of all groups were exhibited oestrus(100%).

The mean interval between PGF_{2α} injection and onset of oestrous were recorded as 22.80±0.44, 20.00±0.39, 21.80±0.39, 25.40±1.20 and 24.40±1.41 hours and the mean duration of superovulatory oestrous due to different hormonal treatment were found to be 35.13±2.28, 42.20±4.98, 32.83±3.73, 25.52±0.74 and 24.4±1.33 hours for the experimental groups I, II, III, IV, and V respectively (Table-2) and suprovulatory induced man oestrus period were 35.00±0.28, 42.20±4.98, 38.80±4.98, 38.80±4.98 and 25.40±0.33 hours in respective treated groups and they were varied significantly(P<0.05) where critical difference test indicated that the progesterone fed animals shown significantly shorter oestrous period. The present findings were in good agreement with the result reported by Armstrong *et al.*(1983) while comparatively shorter interval was reported by some workers (Oh *et al.*, 1979, Armstrong and Evans, 1983) in contrary, a higher time interval was reported by others(Al-Wahab *et al.*,1982, Bondurant *et al.*,1984). Oral administration of MAP and Allylestrenol to the cyclic goats of the groups IV and V for a period of 11 days before administration of PMSG and PGF_{2α} was effective in suppressing occurrence of oestrus of the experimental goats till their withdrawal and the use of superovulatory doses of PMSG and PGF_{2α} injection. This findings are in agreement with findings of Ahmed and Maurya(1981).

The overall mean ovulation rate(Table 3) was recorded as 8.0±0.49, 7.60±0.91, 8.20±0.59, 10.60±0.62 and 12.40±1.80 with a range of 6-10, 5-10, 6-10, 8-13 and 5-19 numbers in group I, II, III, IV and V respectively where the mean values of ovulation rate were varied significantly (P<0.05) but critical difference test indicated that the treatment combinations given to the group IV and V significantly higher than that of group I, II and III while of IV and V; I, II and III did not differ significantly with highest ovulation number up to 19. The higher ovulation rate in progesterone primed goats of group IV and V might be due to higher serum progesterone concentration. Nearly similar ovulatory pattern in caprine was also reported (Pandey and Rathor, 1986). Higher ovulation rate in progesterone primed groups (IV and V) indicated that the use of progesterone for 11days appeared to enhance ovulation rate which was in agreement with the report of Bondioli and Wright Jr(1981) and Armstrong *et al.*, (1983). Injection of 750IU of PMSG followed by injection reduced dose rate of HCG 750IU and 5mg of PGF_{2α} might be considered to be economic superovulatory hormonal combination for cyclic goats of Assam.

The percentage of embryo recovery rate (Table 4) with the treatment combination of PMSG750IU+PGF_{2α}+HCG750IU (Treatment A) was highest with fallopian flushing at 48 hours(87.00%) followed by at 72 hours (81.82%) and 96hours(76.00) after mating while uterine flushing revealed lowest recovery rate at 72hours(34.78%) with highest recovery rate at 120hours(72.73%) followed by at 96hours (41.67%) and at 144hours(61.54%). The corresponding values with the treatment combination MAP5mg+PMSG750IU+PGF_{2α}+HCG750IU (Treatment B) were at 48hours (84.00%), at 72hours(71.43%) and at 96hours (72.73%) during fallopian flushing while uterine flushing revealed embryo recovery rate as lowest at 72hours(39.13%) with highest recovery at 120hours(72.73%) with at 96hours(59.09%) and at 144hours(54.29%) respectively. However, the overall fallopian flushing resulted 81.69% and 76.47% with treatment A and B respectively while the uterine flushing resulted overall embryo recovery rate recorded as 54.74% and 57.52% with the same respective treatments(A and B). The present findings were in agreement with result reported by other authors (Agrawal *et al.*, 1982, Indarjeet and Gupta, 1985) while no collection could be made at 96 hours which might be due to fast passing of falliopian embryo into the uterus due to serum oesrogenic activity. The least fallopian and uterine collection at 96 hours after mating could be due to critical conjuncture time of embryo transit from the fallopian tube to the uterine horns and it was lower than that of Mutiga and Baker(1985). Uterine embryo collection highest at 120 followed by at 144 hours than the report of other workers(Agrawal *et al.*, 1982, Tervit *et al.*, 1983, and Tervit *et al.*,1985). The variation might be attributed to the breed characteristics (Tervit *et al.*, 1985) and drugs for superovulation (Keiling *et al.*, 1986).

Out of twelve (4-8-cell) embryos transferred to the six recipient goats(Table 5) resulted three pregnancies(50%) followed by three kidding of full term kids upon fallopian transfer. Similarly, five pregnancies (41.67%) were occurred after uterine transfer of fifteen fresh embryos(>morula) to twelve recipient goats. It was found that 25% of fresh embryos were survived upon fallopian transfer while 33.33% of fresh embryos were survived upon uterine transfer. The gestation length of the recipients and body weight of the new born upon embryo transfer was almost similar to that of the normal pregnancy. Though, the number of observations in the present experiment was less nearly similar results reported by Agrawal *et al.*(1980) and Tervit *et al.*(1985). However, a higher percentage of pregnancy with better survivability of fresh embryos upon transfer in goats were reported by Armstrong *et al.*(1983). A positive pregnancy

upon transfer with extruded blastomeres during the present study too was in agreement with the report of Shea(1981) in bovine. Moreover, no information on superovulation, embryo collection and embryo transfer in Assam Local goats is available till date.

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Table 1. Treatment combinations used for for superovulation of cyclic Assam Local goats

Groups	Drug combination	Doses	Mode of Administration
I	PMSG	750IU	i/m, Single injection within 6-16 th day of the cycle
	PGF _{2α}	5mg	i/m, Single injection 48 hours after PMSG injection
	HCG	1000IU	i/m Single injection during oestrus just before or after mating
II	PMSG	750IU	i/m, Single injection within 6-16 th day of the cycle
	PGF _{2α}	10mg	i/m, Single injection 48 hours after PMSG injection
	HCG	750IU	i/m Single injection during oestrus just before or after mating
III	PMSG	750IU	i/m, Single injection within 6-16 th day of the cycle
	PGF _{2α}	5mg	i/m, Single injection 48 hours after PMSG injection
	HCG	750IU	i/m Single injection during oestrus just before or after mating
IV	MAP	5mg	Fed orally for 11 days daily starting between days 6-16 th day of the cycle
	PMSG	750IU	i/m, Single injection after 24 hours of last MAP withdrawal
	PGF _{2α}	10mg	i/m, Single injection 48 hours after PMSG injection
	HCG	750IU	i/m Single injection during oestrus just before or after mating
V	Allylesrenol	5mg	Fed orally for 11 days daily starting between days 6-16 th day of the cycle
	PMSG	750IU	i/m, Single injection after 24 hours of last Allylestrenol withdrawal
	PGF _{2α}	10mg	i/m, Single injection 48 hours after PMSG injection
	HCG	750IU	i/m Single injection during oestrus just before or after mating

Table 2. Oestrus pattern during superovulation of cyclic goats with different treatment combination

Groups	Number of treated goats	Day of treatment started oestrous cycle	Number of goats exhibited oestrus	Time taken for onset of oestrus following last injection Mean±SE(hours)	Duration of oestrus of donors
I	6	6-16 th day	6	22.80±0.44 (21-24)	35.00±0.28 ^a (34-36)
II	6	6-16 th day	6	20.00±0.39 (19-21)	42.20±4.98 ^a (34-63)
III	6	6-16 th day	6	21.80±0.39 (21-23)	38.80±4.98 ^a (24-56)
IV	6	6-16 th day	6	25.40±1.20 (21-30)	25.52±0.34 ^b (24-26)
V	6	6-16 th day	6	24.40±1.41 (21-30)	25.40±0.33 ^b (24-26)

Mean values with comm. Superscripts are not significantly different with the column and figure in parentheses indicate range of variation of individual value

Table 3. Effect of different treatment combinations on superovulation pattern of cyclic goats

Groups	Number of treated goats	Mean ovulation rate		
		Right ovary	Left ovary	Overall (Right + Left ovary)
I	6	4.40±0.36 (3-5)	4.60 ±0.22 (3-7)	8.20±0.49 ^b (6-12)
II	6	4.40±0.46 (3-6)	3.20± 0.52 (2-6)	7.60±0.91 ^b (5-12)
III	6	4.60±0.61 (3-7)	3.60±0.57 (2-6)	8.20±0.59 ^b (5-13)
IV	6	5.60±0.80 (4-6)	5.20 0.33 (4-7)	10.60±0.62 ^a (8-16)
V	6	6.45± 0.97 (4-9)	6.20 ±0.34 (1-8)	12.40 1.30 ^a (5-19)

Table 4. Embryo recovery from superovulated donor goats with two treatment combinations at different hours after breeding

Collection of embryos after breeding at	Superovulatory treatment combination PMSG750IU+PGF _{2α} +HCG750IU		Superovulatory treatment combination MAP5mg+ PMSG750IU+PGF _{2α} +HCG750IU	
	Fallopian tube collection	Uterine collection	Fallopian tube collection	Uterine collection
	Total No.E/No. CL	Total No.E/No. CL	Total No.E/No. CL	Total No.E/No. CL
48hours(3)	21/24=87.00%		21/25=84.00%	
72hours(3)	18/22=81.82%	8/23=34.78%	16/22=72.73%	9/23=39.13%
96hours(3)	19/25=76.00%	10/24=41.67%	15/21=71.43%	19/35=54.29%
120hours(3)		24/33=72.73%		24/33=72.73%
144hours(3)		16/26=61.54%		13/22=59.09%
Overall recovery rate	58/71=81.69%	58/106=54.72%	52/68=76.47%	65/113=57.52%

Within parentheses indicated number of goats, No.E=Number of embryos collected, No.CL=Number of corpus luteum present in both the ovaries of superovulated goats