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Factors Affecting Superovulatory Response And Embryo Yield In Jersey X Kankrej Cows

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ABSTRACT

Five Jersey X Kankrej (J X K) cows were superovulated 31 times either with FSH or PMSG preparations. Animal-to-animal variation ($P<0.01$) was one of the major fator contributing towards variable superovulatory response and yield of viable embryos. Mean ovulation rate, embryo recovery rate and transferable embryos were 6.69±0.73, 2.19±0.46 and 1.32±0.31 respectively. There was no significant influence of repeated superovulation, type / nature of gonadotropin used, day of initiation of superovulatory treatment during estrous cycle and season on superovulatory response and yield of embryos in J X K cows.

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A large number of factors contribute towards variability in superovulatory (SOV) response and yield of viable embryos (Monniaux *et al.*, 1983; Totey *et al.*, 1991 and Armstrong, 1993) in cattle. Unpredictable SOV response and low yield of viable embryos continue to be a major limiting factor in livestock improvement through embryo transfer. Therefore, the objective of this study was to determine (i) animal-to-animal variation (ii) the effect of repeated superovulation (iii) influence of type / nature of gonadotropin used (iv) effect of day of initiating superovulatory treatment and (v) effect of season on superovulatory response and yield of viable embryos.

MATERIALS AND METHODS

Five Jersey X Kankrej (J X K) crossbred cows 5 to 6 years of age, weighing 337.72±3.09 kgs were subjected to 31 times superovulation treatment over a period of 2 years. During the first year these cows were superovulated with preparations of Follicle Stimulating Hormone (FSH) and in the second year with Pregnant Mare's Serum Gonadotropin (PMSG). A total dose of each gonadotropin used for superovulation was as follows: (1) Folltropin (FSH-Vetrepharm) 35 mg i.m. in divided dosages for your days, morning and evening or (2) FSH, Indian Immunologicals (IIL) 44 mg i.m. in divided dosages for four days, morning and evening or (3) Folligon (PMSG-Intervet) 2000-3000 I.U. i.m., single dose or (4) Trophovet (PMSG–IIL) 2500 I.U. i.m., single dose. Initiation of SOV treatment was done either on day 9 or between 11 to 14 of the estrous cycle. In the earlier studies (Sarvaiya *et al.*, 1992) also no clear cut difference in response was noticed between (1) and (2) or (3) and (4) preparations of FSH and PMSG and hence the data were pooled for Folltropin and FSH (II L) treatment and Folligon and Trophovet treatments groups. A total dose of 35 mg Prostaglandin F_2 alpha was injected to induce estrus. Donors were inseminated with frozen semen during induced estrus. Embryos were recovered non-surgically and classified as transferable and non-transferable. The studies in two years period were divided into 3 seasons viz., winter (Nov-Feb), summer (March-June) and monsoon (July-Oct) to record the influence of season on superovulatory response and embryo recovery. Effect of above variables on superovulatory response and embryo yield was analysed by mixed model least squares analysis of variance (Harvey, 1960) using computor programme PC-2. Repeatability of the SOV response and embryo production during successive superovulatory attempts was calculated by standard procedures.

RESULTS AND DISCUSSION

The present study recorded a great animal-to-animal variation in mean ovulation rate and embryo yield among 5 cross-bred cows (Table 1). The difference was highly significant between cows ($P<0.01$). Similar observations were made by Hasler (1992), where embryo yield varied from 5.4 to 18.6 per cow. The ovarian

status of the donor at the time of hormone treatment appear to be a major determinant of the superovulatory response (Monniaux *et al.,* 1983 and Armstrong, 1993).

Effect of repeated superovulation on ovulation rate and embryo yield is presented in Table 2. Twenty nine flushing were made on 5 cows 2 to 7 times each at an interval of 94.88±10.15 days. Mean number of ovulations and embryo yield were quite variable from first to seventh treatment but there was no significant difference in response to superovulation and embryo yield from first to seventh treatment. Conflicting reports are available on the effect of repeated superovulation. Some authors recorded a decreased response and embryo recovery rate (Donaldson and Perry, 1983; Bastidas and Randel, 1987 and Totey *et al.,* 1992); others showed no effect or inconsistant relationship (Fielden and Hayman, 1982). The observations made in the present study corroborate with the latter study, where no definite trend in response was recorded. Decreased superovulatory response with repeated treatment has been attributed to refractoriness resulting in part from formation of antibodies against exogenous gonadotropins (Jainudeen *et al.,* 1966) or due to short term recovery time required after the previous superovulation (Lubbadeh *et al.,* 1980).

The repeatability estimates were medium (R=0.33) for ovulation rate and low for both embryo recovery rate (0.13) and transferable embryos (0.05). These low to medium levels of repeatability for superovulatory response do not encourage culling of donors strictly based upon ovarian response (Bastidas and Randel, 1987).

Effect of nature of gonadotropin (FSH or PMSG), day of estrous cycle and season on superovulatory response and embryo yield is presented in Table 3. Apparently higher ovulation rate, embryo recovery rate and transferable embryos were recorded with FSH treatment as compared to PMSG but there was no significant response difference between two types of gonadotropins. Although direct comparative trials on both the gonadotropin preparations are lacking on Indian crossbred cattle but available literature suggests that FSH preparations were superior (Madan, 1990; Sarvaiya *et al.,* 1992; Joshi *et al.,* 1993) over PMSG (Subramaniam *et al.,* 1990; Misra *et al.,* 1992). However, based on 39 selected references, Sienning and Wheeler (1989) concluded that PMSG work equally well.

Initiating superovulation treatment either on day 9 or between 11 to 14 had no influence on response or yield of embryos in the present study. Similar observation were made by Donaldson (1984) but others reported that day 10 or 11 of the estrous cycle was better for SOV response as compared to other days of the cycle (Agarwal *et al.,* 1991).

There was no significant effect of season on SOV response and yield of embryos but there was a tendency for better SOV response and recovery of embryos during winter as compared to summer and monsoon season (Table 3). Contrary to these findings, Totey *et al.,* (1991) reported that monsoon was better season compared to other seasons though no significant difference was reported between seasons.

It is concluded from the present studies that animals-to-animal variation was a major contributing factor towards variable SOV response and embryo yield as compared to other factors studied.

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Table 1: Animal-to-animal variations in superovulatory response and embryo yield in Jersey X Kankrej cows

Identification of cows		No.of times Superovulated	No.of ovulations	Embryo yield	
No.	Name		X±S.E. (Range)	Embryos recovered X±S.E. (Range)	Transferable embryos X±S.E. (Range)
1	Bagi	9	9.56±1.41 (6-18)	3.22±1.24 (0-10)	1.89±0.75 (0-7)
2	Valay	7	7.43±1.19 (3-12)	3.29±0.64 (1-5)	2.00±0.69 (0-5)
3	Suri	6	5.67±1.38 (0-8)	2.00±0.77 (0-5)	1.17±0.54 (0-3)
4	Gauri	6	2.50±1.14 (0-6)	0.50±0.34 (0-2)	0.50±0.34 (0-2)
5	Sarswati	3	6.67±0.88 (5-8)	0.33±0.33 (0-1)	0.00±0.00 (0-0)
Overall		31	6.69±0.73	2.19±0.46	1.32±0.31
Statistical difference			*	**	NS

** P<0.01: Means differ with each other at 1% level of significance
NS: Statistically non-significant difference between the means

Table 2: Effect of repeated superovulatory treatment in crossbred cows

Treatment attempts	No. of flushings	Interval between two treatments (Days)	No. of ovulations	Embryo yield	
		X±S.E.	X±S.E.	Embryo recovery rate X±S.E.	Transferable embryos X±S.E.
I	5	—	7.00±0.44	1.00±1.00	1.00±1.00
II	5	89.60±13.04	6.66±1.94	0.80±0.58	0.80±0.58
III	5	78.80±19.90	5.20±0.86	2.00±0.45	1.60±0.51
IV	4	110.00±20.66	6.75±3.90	3.25±2.29	2.50±1.55
V	4	82.25±23.92	4.50±1.85	1.75±0.85	1.75±0.85
VI	4	103.25±45.69	6.25±2.10	3.00±1.08	0.50±0.50
VII	2	131.50±32.50	12.50±2.50	3.50±1.50	0.50±0.50
Overall	29	94.88±10.15	6.52±0.77	2.00±0.43	1.28±0.33

Table 3: Effect of nature of gonadotropins, day of estrous cycle and seasons on superovulation and embryo yield in crossbred cows

Factors	No. of donors treated	No. of ovulation X±S.E.	Embryo yield	
			Embryo recovery rate X±S.E.	Transferable embryos X±S.E.
1. Nature of gonadotropins				
i) FSH	13	7.46±1.07	2.77±0.96	1.77±0.64
ii) PMSG	18	6.11±0.99	1.78±0.38	1.00±0.26
		NS	NS	NS
Day of estrous cycle				
i) Day 9	7	7.71±1.43	1.71±0.75	1.14±0.51
ii) Day 11-14	23	6.38±0.84	2.33±0.56	1.36±0.38
		NS	NS	NS
3. Effect of Seasons				
i) Winter (Nov-Feb)	10	7.40±1.16	3.20±0.83	2.00±0.60
ii) Summer (Mar-June)	9	7.78±1.85	2.20±1.13	1.11±0.75
iii) Monsoon (July-Oct)	12	5.25±0.81	1.50±0.45	0.92±0.26
		NS	NS	NS

NS: Statistical difference between the means is non-significant ($P<0.05$)

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Induction of Estrus in Non-cycling Surti Goats and their Endocrine Profils

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ABSTRACT

For induction of estrus in non-breeding season, multiparous Surti goats were injected with (1) Tonophosphan 15 ml in three doses on alternate days (2) Tonophosphan treatment followed by PMSG (500 IU) and (3) PMSG (1000 IU) alone, Estradiol-17B and progesterone were estimated at definite interval from samples of responded or non-responded goats of all the groups. The results revealed that 15.75, 56.75 and 77.0 percent goat responded by exhibiting estrus respectively with treatment 1 to 3. The goats conceived and kidded were 33, 100 and 100 percent respectively for three groups. Study on ovarian sterioids in responded and non-responded goats revealed that there was a definite drop in progesterone level and rise in estradiol-17B levels before the onset of the estrus. The levels of estradiol-17B levels before the onset of the estrus. The levels of estradiol-17B were significantly higher in goats treated with 1000 IU PMSG. The non-responded goats maintained higher progesterone level during treatment period and estradiol-17B levels did not show appreciable rise. This treatments, thus help to induce fertile estrus in non-cycling goats during non-breeding season.

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Most of the well defined Indian breeds of goats kidded only once in a year. Since average gestation period of the Surti goats is 144.0±1.5 days (Janakiraman and Mehta 1991) there is possibility of inducing second conception in a year. Various combinations of PMSG, FSH, Progesterone GnRH and $PgF_{2\alpha}$ have been used for induction of estrus in goat and sheep (Goswani *et al.,* 1990, Eiamvitayakorn *et al.,* 1988 and Mc Natty *et al.,* 1988). The present study therefore attempted to use three different treatment, viz. (i) Tonophosphan (Hoechst) (ii) Tonophosphan with PMSG (500 IU) (iii) PMSG (1000 IU) alone, towards induction of estrus in non-cycling goats.

MATERIALS AND METHODS

Twenty eight pluriparous non-cycling Surti goats were randomly selected for the study. The goats were maintained under the stallfed management. The study was carried out in monsoon (July & Aug.), a low breeding season. The treatment were as follows.

(i) 19 goats received 15 ml of tonophosphan in three doses at an interval of 48 hrs.

(ii) The goats of first treatment which did not respond, received 500 IU of PMSG after 72 hrs of the last Tonophosphan injection.

(iii) 9 goats received 1000 IU of PMSG alone.

All the goats were observed for estrous with the help of appronised buck at the interval of 6 hrs. The buck response and the behavioural symptoms of the female at estrus were recorded. Blood samples were collected at pre-treatment, during treatment and post-treatment. The responded goats were bred by natural service with the help of fertile buck.

The serum samples were analysed for estradiol-17B (Robertson et al. 1979) and progesterone (Kubasic *et al.,* 1984) by using standard RIA procedure. The statistical treatment for analysis of data was as per Snedecor and Cochran (1971).

RESULTS AND DISCUSSION

The number of goats responded and kidded thereafter in these treatments are shown in Table-1. In tonophosphan treatment the induction of estrus was only 15.75% but when it was followed by 500 IU of PMSG, the response was near to 4 times (Table 1). All the animals which responded in tonophosphan with PMSG treatment were conceived and kidded (100% conception). The percentage of induction of estrus and conception was highest in last treatment i.e. 1000

IU of PMSG dose (Table 1). The time taken for onset of estrus was 72, 80 and 43 hrs respectively for treatment 1 to 3.

The response to 1000 IU PMSG in Surti goats is closely comparable to earlier studies of Dutta *et al.,* (1990), Pandey *et al.,* (1991) and Sinha and Khan (1991) in goats and that of Fukai *et al.,* (1988) and Melbaum (1988) for sheep. As such no trials on induction of estrus with tonophosphan has been reported in literature.

Anestrus buffaloes maintained significantly lower phosphorus level (Sarvaiya, 1989) and Palmer *et al.,* (1941) and Morrow (1977) had shown possitive association between blood inorganic phosphate and hypothalamo-hypophyseal activity hence the trials with tonophosphan treatment for induction of estrus was conducted in goats. It was observed that tonophosphan alone can induce estrus in very small number of goats but can provide a very good priming effect to PMSG so that response can be induced even with half the dose of PMSG.

The time taken for induction of estrus after 1000 IU PMSG treatment is comparable with those of PMSG treatment (Naqvi and Kalra, 1990) and $PGF_2\alpha$ plus PMSG treatment (Melbaum, 1988) in sheep. However, time taken for induction of estrus after tonophosphan alone or followed by PMSG is significantly higher than reported for PMSG treatment.

The blood levels of progesterone and estradiol-17 B in responded and non-responded animals has been recorded and tabulated in Table 2. The persual of the data revealed the fact that all responded goat had exhibited a gradual decline in progesterone level towards onset of estrus and a steady rise in estradiol-17 B levels from pre-treatment to onset of estus. The blood levels of progesterone exhibited increasing tendancy right from 18 hrs after the onset of estrus. Totally opposite trend was shown by estradiol-17B to that of progesterone right from 18 hrs after the onset of estrus. The average levels of both progesterone and estradiol-17B were at par in all the responded goats of the three treatments. The non-responded animals exhibited significantly higher levels of progesterone throughout the period. The data revealed that animals responded to the treatment in view of fast dropping of progesterone levels. Non-responded animals maintained high progesterone level presumably due to retained corpus luteum. It is appreciable that till the corpus luteum is active, the tonophosphan and / or PMSG treatment can not induce follicular development and onset of estrus. In such cases treatment with $PGF_2\alpha$ followed by PMSG may help for positive response. The hormonal profile of responded animals during estrus phase are comparable to those reported for cycling goats by Baruah *et al.,* (1987) and Pathak *et al.,* (1990).

Overall 17 goats conceived and kidded, out of 28 goats under treatment where success rate was highest in treatment with 1000 IU PMSG. At present there is no comparable data available in literature for endocrine status and conception percentage after artificial estrus induction studies.

Table 1: Estrus Induction and Conception Rate in Non-Cycling Surti Goat After Tonophosphan and For PMSG Treatment

Treatment	n	In Estrus	Conceived and kidded	Non-responded
a) Tonophosphan	19	3(15.78%)	1(33.33%)	16(84.21%)
b) Tonophosphan 500 IU PMSG	16	9(56.25%)	9(100.0%)	7(43.75%)
c) PMSG 1000 IU	9	7(77.77%)	7(100.0%)	2(22.22%)
Total Treated	28	19(67.86%)	17(89.41%)	9(32.14%)

Table 2: Fluctuation in Blood Serum Estradiol-17 B and Progesterone Levels in Non Cycling Surti Goats Responded and Non-Responded to various Treatments of Estrus Induction

Hormone	Pre-treatment level	Tonophosphan Treatment TP			PMSG treament	Estrus onset	Hours after estrus onset				Average level
		TP-I	TP-II	TP-III			+6	+18	+24	+48	
I Tonophosphan treatment Responded Goats (n=3)											
Progesterone (ng/ml)	1.97 ±0.17	1.23 ±0.24	0.80 ±0.02	-	-	0.42 ±0.02	0.53 ±0.03	1.41 ±0.18	1.69 ±0.06	1.56 ±0.26	1.20 ±0.13
Estradiol-17 β (pg/ml)	20.26 ±2.71	22.93 ±2.45	34.17 ±2.95	-	-	50.27 ±7.78	36.07 ±2.57	6.00 ±0.23	7.33 ±1.85	8.30 ±1.54	23.22 ±2.76
II Tonophosphan followed by 500 Iu PMSG Responded Goats (n = 9)											
Progesterone (ng/ml)	2.37 ±0.27	1.83 ±0.11	1.66 ±0.13	1.60 ±0.12	1.47 ±0.23	0.47 ±0.05	0.51 ±0.15	1.15 ±0.12	1.57 ±0.11	1.59 ±0.20	1.42 ±0.37
Estradiol-17 β (pg/ml)	15.07 ±0.79	10.53 ±1.63	14.60 ±1.37	19.67 ±2.68	20.83 ±2.94	48.73 ±8.73	41.92 ±10.05	19.10 ±4.37	16.23 ±1.74	15.12 ±1.64	22.17 ±4.50
III PMSG (1000 IU) Responded Goats (n = 7)											
Progesterone (ng/ml)	1.97 ±0.17	-	-	-	1.23 ±0.24	0.48 ±0.02	0.53 ±0.03	1.41 ±0.18	1.69 ±0.06	1.56 ±0.26	1.23 ±0.13
Estradiol-17 β (Pg/ml)	20.26 ±2.71	-	-	-	22.93 ±2.45	50.77 ±7.78	36.07 ±2.57	6.00 ±0.23	7.33 ±1.85	8.30 ±1.54	23.22 ±2.76
IV Non Responded Goat (n = 7)						Hours Post PMSG					
Progesterone (ng/ml)	5.35 ±0.61	4.95 ±0.60	4.83 ±0.54	4.05 ±0.86	3.96 ±0.19	-	-	-	2.18 ±0.34	2.28 ±0.34	3.76 ±0.55
Estradiol-17 β (pg/ml)	21.95 ±12.89	21.25 ±9.76	26.95 ±11.25	25.39 ±10.10	21.88 ±7.88	-	-	-	21.88 ±8.02	21.88 ±8.02	22.36 ±9.37

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Induction of Estrus with Prostaglandin F_2 Alpha Treatment During Normal Estrous Cycle, Superovulation and After Embryo Recovery In Jersey x Kankrej (J x K) Crossbred Cows

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ABSTRACT

An investigation was undertaken to determine the interval from $PGF_2\alpha$ treatment to estrus in three categories of Jersey x Kankrej (J x K) crossbred cows viz., during normal estrous cycle (recipients), Superovulation and after embryo recovery. Single i.m. injection of 25 to 35 mg of $PGF_2\alpha$ synchronized the estrous cycle of 80.00% cows within 74.67±5.51 hr. of treatment. Estrus was induced earlier ($P<0.01$) during superovulation (48.78±2.45 hr., n = 23) as compared to normal cycling cows (74.67±5.51 hr., n=12) and after embryo recovery (24.31±3.21 days, n=22). Further it was observed, that return-to-estrus interval after embryo recovery was longer (30.60±4.15 days, n= 13) in high responding group of cows (≥ 7 C.L.) as compared to low responding (≤ 6 C.L.) group of cows (15.22±3.25 days, n=9, ($P<0.01$) to superovulation. While nature of gonadotropin used for superovulation (FSH or PMSG) or prostaglandin treatment on day of embryo recovery or day 6.5 to 8.5 of breeding had little influence on return-to-estrus interval after embryo recovery in J x K cows.

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Synchronization of donor-recipient estrus is the most venerable principle for successful embryo transfer programme (Sreenan *et al.*, 1975; Seidel, 1981). $PGF_2\alpha$ or its analogues increase the flexibility of timing superovulation in donors treated with PMSG or FSH (Betteridge, 1977). The onset of estrus generally occurs 12 to 24 hr. esrlier in superovulated than normal cycling cows and within 48 hr. after $PGF_2\alpha$ treatment during superovulatory treatment (Walten *et al.*, 1987). The use of $PGF_2\alpha$ or its analogue (PG) to synchronize estrous cycle or to induce estrus during superovulation or after embryo collection should be optimized (Desaulniers *et al.*, 1990).

Therefore, this is an attempt to document the interval between $PGF_2\alpha$ treatment and induction of estrus in three categories of J x K crossbred cows viz., during normal estrous cycle, superovulation and after embryo recovery.

MATERIALS AND METHODS

Six Jersey x Kankrej crossbred (J x K) cows kept under standard feeding and managemental practices at the R.B.R. Unit were used repeatedly for synchronization of estrous cycle, superovulation and embryo recovery. Estrus was synchronized in normal cycling cows (recipients) by single intra-muscular injection of 25 or 35 mg of prostaglandin F_2 alpha ($PGF_2\ \alpha$) during mid luteal phase of estrous cycle. Superovulation (SOV) was induced by using FSH (Indian Immunological Ltd. (IIL), (Hyderabad) or folltropin (Vetrepharm Inc., Canada) or PMSG (IIL) or Folligon (Intervet, Holland). A total of 44 mg of FSH (IIL) or 35 mg of folltropin was injected on day 11 to 14 of estrous cycle spread over 4 days in descending dose schedule. ($PGF_2\ \alpha$) (35 mg) was injected in two divided doses (20 + 15 mg) at 72 and 80 hr of initiation of SOV treatment. Superovulation with PMSG (2000-2500 I.U.) preparations was started on day 9 to 14 of estrous cycle. Luteolysis was induced using 25 or 35 mg of $PGF_2\ \alpha$ 48 hr after initiation of SOV treatment. A dose of prostaglandin (25 or 50 mg) was injected to a group of cows (n=16) on day of embryo recovery (day 6.5) or day 6.5 to 8.5 and another group of cows was kept as control (untreated group n=6). Depending upon the superovulatory response, cows were grouped into two categrories (≥ 7 C.L., high responding group; ≤ 6 C.L., low responding group) to study the influence of number of corpora lutea on return-to-estrus interval after embryo recovery.

Estrus was detected by external symptoms. Rectal palpations were carried out during preselection, prior to SOV treatment, around estrus, day of embryo recovery and at weekly interval upto subsequent estrus.

Statistical analysis of the data was made as per Snedecor and Cochran (1971).

RESULTS AND DISCUSSION

Of the total 15 estrous cycles synchronized 12 (80.0%) animals exhibited estrus within 74.67±5.51 hr. after (PGF_2 α) treatment. There was no significant influence of dose of prostaglanding F_2 alpha on the number of cows responded and interval between treatment and induction of estrus (Table 1). Interval between PGF_2 α treatment and induction of estrus in donor cows was shorter (48.78±2.45 hr.) as compared to normal estrous cycle / recipient cows (74.67±5.51 hr., P<0.01; Table 1,2). It was further observed that there was no influence of nature of gonadotropins (either FSH or PMSG) on interval between PGF_2 α treatment and estrus (Table 2).

Return to estrus interval after embryo recovery was observed to be 24.31±3.21 days (Table 2,3). Nature of gonadotropin used for SOV or treatment with 7 PGF_2 α on day 6.5 to 8.5 or just after embryo collection did not affect the return-to-estrus interval. However, return-to-estrus interval was significantly (P<0.01) longer for high responding group of cows (30.60±4.15 days) compared to low responding cows (15.22±3.25 days) to superovulatory treatment.

The results obtained in the present study in the control of normal estrous cycle of cows (recipients) and donors with prostaglandin are in confirmity with earlier findings (Desaulniers *et al.*, 1990; Manickam *et al.*, 1990; Kathiresan *et al.*, 1991) in crossbred and exotic cattle. The onset of estrus was earlier in donors (av. 48.78 hr) as compared to recipient cows which is comparable to exotic cattle (Mapletoft, 1986 and Walten *et al.*, 1987). Therefore the practice of treating the recipients with PGF_2 α 24 hr prior to the PG treatment of donor cows in exotic cows holds true in J x K crossbred cows for close synchrony between donor and recipients.

After embryo collection on an average 60 to 65% superovulated dairy cows came in to estrus within 10 days after PG treatment (Chupin *et al.*, 1984 and Ali-Dinar *et al.*, 1987). Similarly, Kathiresan *et al.*, (1991) observed that 50 mg of PGF_2 α treatment after embryo collection shortened the estrous cycle from 18 to 8.33 days in control (no treatment) and treated group respectively. However, in present study, unlike the previous ones, did not find any significant difference of PG treatment on return-to-estrous interval. Increased number of corporalutea significantly (P<0.01) delayed return-to-estrus interval (Table 3) after embryo recovery. This relationship may be suggest a function of high concentration of progesterone in controlling follicular growth, which is in agreement with the earlier works of Lucy *et al.*, (1990).

Table 1: Interval between PGF_2 α treatment and induction of estrus in normal cycling J x K cows.

Group	Dose of PGF_2 α treatment	Number of estrous cycles synchronized n	Number and percentage or response n (%)	Interval between treatment and induction of estrus (hr) X ± S.E.
I	25 mg	4	3(75.00)	64.00±8.01
II	35 mg	11	9(81.82)	78.22±6.64
	Overall	15	12(80.00)	74.67±5.51

Table 2: Interval between PGF_2 α treatment and superovulatory estrus and after embryo recovery in J x K cows.

Group	Gonadotropin used for SOV	Interval between PGF_2 α treatment and superestrus (hr.) X ± S.E.	Return to estrus interval after embryo collection (days) X ± S.E.
I	(a) FSH (IIL) 44 mg	48.00±5.85(7)	21.14±6.80(7)
	(b) Folltropin 35 mg	52.80±2.94(5)	28.40±3.37(5)
	Mean	50.00±3.57(12)	24.17±4.22(12)
II	PMSG	47.45±3.48(11)	24.48±5.19(10)
	Overall	48.78±2.45(23)	24.31±3.21(22)

Table 3: Influence of treatment and number of corporalutea on return-to-estrus interval after embryo recovery in J x K cows.

Group		Number of cows n	Interval between treatment to estrus (days) X±S.E.
I	Prostaglandin F_2 alpha treatment (25 to 50 mg)	16	24.00±3.97
II	Control (no treatment)	6	25.17±5 67
III	High responding cows to SOV (7-18 CL)	13	30.60±4.15**
IV	Low responding cows to SOV (0-6 CL)	9	15.22±3.25**
	overall	22	24.31±3.21

** P<0.01: Statistically means differ with each-other at 1% level of significance.

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Clinicopathological Studies in Female Infertile Buffaloes

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ABSTRACT

One hundred female buffaloes were classified according to the condition of the examined genital tracts into two groups. Hematological and serum biochemical examinations were carried out on control and diseased animals. Buffaloes of group I-suffering from cervicitis and endometritis revealed a significant leukocytosis manifested by neutrophilia, eosinophilia, monocytosis and lymphopenia, in addition, significant elevations in total lipids, α and γ globulins with significant increase in values of β-globulins. Buffaloes of group II.(suffering from ovarion inactivity) showed normocytic normochromic anemia with leucocytosis and neutrophilia, in addition, significant decrease in total protein, β-globulin, total lipids, cholesterol, iron and Vit. A and showed significant elevations in α and γ globulins. These results indicate that serum biochemical parameters are of great importance in diagnosis and differential diagnosis of infertility problems.

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Buffaloes are considered as one of the most important domestic animals in Egypt. They produce milk with a higher fat percentage than native cows.

Information about the changes in the blood constituents in relation to the different causes of infertility in buffaloes are lacking. Endometritis and ovarian inactivity are the main problems of buffaloes in Egypt (Shawki and Brinks, 1990).

The present work was carried out to provide more information about the variations in blood constituents associated with some forms of infertility (cervicitis-endometritis and inactive ovaries in Egyptian buffaloes). It is hoped that the hematological and biochemical changes would be helpful in the diagnosis and differential diagnosis of the etiology of these problems.

MATERIAL AND METHODS

This study was carried out on one hundred apparently normal female buffaloes at Oussime Village, Giza Governorate and El-Khanka farm, El-Kalubia Governorate.

According to the history of each animal and the condition of the examined gential tracts, animals were divided into two groups; Group I: buffaloes suffering from cercivicitis and endometritis (40 cases). Group II: buffaloes suffering from ovarian inactivity (35 cases). The rest of animals (25 cases) with normal ovarian function, were considered as control animals.

Blood samples were collected from the jugular vein of each animal. One sample was taken on EDTA for the hematological assay (erythrocytic count, hemoglobin content, packed cell volume, total and differential leukocytic count). Another sample was used for serum separation for biochemical analysis (total protein with its electrophoretic pattern, lipids, cholesterol, iron, copper and Vit. A). Statistical analysis of the data was done by the students "t" test according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The values of hemogram in group I were not significantly different from the control, while a normocytic normochromic anemia were observed in animals of group II (Table 1). These results agree with those of El-Sebaie *et al.,* (1988) and Emara *et al.,* (1989) who attributed these changes to low level of estrogen in cases of ovarian inactivity.

Evaluation of leucocytic patterns showed significant leucocytosis in group I manifested by neutrophilia, eosinophilia, monocytosis and lymphopenia. These findings confirm the previous reports by El-Sebaie *et al.,* (1988) who attributed such findings to defense of the body against infections and/or stress effects. Ahmed (1987) stated that eosinophila may be due to intiation of inflammatory responce and lysosomal activity while monocytosis may be due to removal of tissue debris and lymphopenia due to withdrawl

Table (1) Values of Hemogram in control, cervicities endometritis and inactive ovaries

Parameter / Groups of Buffaloes	RBS x $10^6\mu1$	Hb gm %	PCV %	Total leuco-cytic x$10^3\mu1$	Absolute Values / $\mu1$				
					Neutro-phils	Eosino-phils	Baso-phils	Mono-cytes	Lympho-cytes
Control	6.26 ± 0.27	12.00 ± 0.30	36.24 ± 0.71	6.700 ± 0.259	2247 ± 154	256 ± 23	18 ± 5	237 ± 20	3870 ± 159
Group I	5.83 ± 0.05	11.48 ± 0.25	34.93 ± 0.62	7.944** ± 0.193	3764** ± 144	383** ± 20	18 ± 5	375 ± 23	3333* ± 136
Group II	5.25* ± 0.20	9.93** ± 0.19	31.88** ± 0.70	7.325* ± 0.198	2726* ± 164	297 ± 18	15 ± 5	272 ± 17	4247 ± 138

+ M±S.E. * <0.05 ** <0.001

Table 2: Values of serum protein with its electrophoretic pattern in control, cervicitis - endometritis and inactive ovaries.

Parameter / Groups of Buffaloes	Total Protein gm%	Relative Percent					Albumin / Globulin ratio
			α-globulins	β-globulins	γ-globulins	Total globulins	
Control	9.10 ± 0.11	44.10 ± 0.77	13.50 ± 0.55	18.50 ± 0.44	24.50 ± 0.65	55.10 ± 1.10	0.80 ± 0.02
Group I	8.87 ± 0.16	41.60 ± 0.86	15.90* ± 0.63	10.50** ± 0.46	33.30** ± 0.97	58.30* ± 0.81	0.73* ± 0.02
Group II	7.66** ± 0.18	41.75 ± 1.48	15.89* ± 0.54	9.70** ± 0.85	33.46** ± 1.13	58.31 ± 1.44	0.72* ± 0.004

+ M ± S.E. * $P < 0.05$ ** $P < 0.001$

Table 3: Values of serum total lipids, cholesterol, iron, copper and vitamin A in Control, cervicitis - endometritis and inactive ovaries.

Parameter / Groups of Buffaloes	Total lipids mg %	Total cholesterol mg %	Iron mg %	Copper mg %	Vitamin A I.U. %
Control	441.70 ± 19.80	82.60 ± 3.71	226.47 ± 10.14	85.50 ± 4.42	55.14 ± 2.81
Group I	579.60** ± 20.80	88.70 ± 2.60	205.27 ± 4.07	82.17 ± 3.63	50.27 ± 2.07
Group II	387.20* ± 18.60	56.19** ± 1.90	198.57** ± 7.58	72.75* ± 3.23	43.20** ± 0.80

+ M ± S.E. * $P < 0.05$ ** $P < 0.001$

of the circulatory cells to be infilterated in affected tissue and got with inflammatory discharge.

Buffaloes of group II showed leucocytosis and neutrophilia. These findings agree with those of El-Baghdady (1979) who recorded the same picture in addition to eosinopenia while disagree with those of El-Shawaf (1984). The difference in leucocytic patterns may be attributed to the causative agents, degree of inflammation and immunological response of the animals.

Concerning the total serum protein of group I (Table 2), the obtained values showed no marked changes between normal control and diseased animals. The same results were reported by Said *et al.*, (1964) and Moustafa *et al.*, (1988). The protein fractions of the same group showed a highly significant decrease in β globulins and significant increase in α and γ globulins leading to a significant decrease in A/G ratio. The present findings agree with the picture of inflammatory diseases recorded by Reynolds and Freeman (1986) & Zilva *et al.*, (1988) while Enkhia *et al.*, (1982) contradicted the previous results as they did not observe any significant alterations.

On the other hand, there was a high significant decrease in the values of total serum proteins in group II. The same results were obtained by El-Baghdady (1979), El-Azab *et al.*, (1988) and Emara *et al.*, (1989). They attributed such changes to either environmental, seasonal variations or hormonal imbalances.

The studies done on the serum lipids (Table 3) showed a significant increase in its values in animals suffering from cervicitis endometrisis. These findings agree with those of El-Sabaie *et al.*, (1988) who attributed the obtained hyperlipaemia to faulty feeding management. While in case of group II, there were significant decrease in its values. The same findings were reported by Patel *et al.*, (1990) wherease nonsignificant decrease was recorded by Hassan *et al.*, (1991).

Values of serum cholestrol were decreased markedly in animals having ovarian inactivity. These findings are in accordance with those observed by Dutta *et al.*, (1988). It was generally accepted that cholesterol is an index towards steroid activity as recorded by Dhaliwal and Sharma (1990).

Regarding the values of serum iron and copper, there were nonsignificant decrease in animals of group I. These findings agree with Manickam *et al.*, (1977). On the other hand, these two minerals were significantly decreased in animals of group II. Similar results were reported by Sharma *et al.*, (1988). These results were confirmed by Desai *et al.*, (1982) who stated that both iron and copper behave in a regular way to be used as an indicator for FSH, LH and estrogen activity.

The values of serum vilamin A did not reveal any significant alteration in buffaloes suffering from cervicitis-endometritis, while a highly significant decrease was observed in buffaloes suffering from ovarian inactivity. These findings are supported by Emara (1987) and El-Garhi *et al.*, (1989) who explained the development of pituitary cysts which interfere with the production and release of gonadotrophins in Vit. A deficient animals.

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Enzymatic Attributes of Cervical Mucus in Synchronised Normal and Repeat Breeder Crossbred Cows.

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ABSTRACT

The respective levels of ACP, AKP, ALT, AST and LDH in normal and repeat breeder cows in fertile and infertile estrus on synchronisation were 16.10±2.99 Vs. 14.17±1.57 KA Units; 33.43±6.10 Vs. 28.03±4.18 and 22.70±2.21 Vs 22.73±3.31 KA Units; 26.36±4.70 Vs. 47.02±5.37 and 18.86±3.68Vs. 24.68±3.73 Units per ml.; 75.676±9.07 and 54.098±5.32 Vs. 75.91±6.16 Units per ml.; 133.855±15.96 Vs. 112.07±11.85 and 113.524±11.68 Vs. 148.375±10.89 I.U. per litre CM respectively with no significant difference in any group except in normal fertile and infertile estrus in regards of ALT activity.

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Cyclic changes in physico-biochemical attributes of cervical mucus are necessary for the passage of spermatozoa at the time of ovulation. Beyond these owing to the vital role of cervical mucus enzymes in fertility, some enzymatic changes are also expected. Acid phosphatase level is an useful index for fertility assesment with regards to estrogen level. Increasing ACP level might be beneficial for hydrolysing phosphomono-estres, and thus, may provide energy in the form of phosphatase, for normal reproductive functions, in normal cows (King, 1971). Decreasing tendency of AKP may favour folliculogenesis and further may enhance chance of conception in normal fertile cows while inverse is true in case of infertile cows. Increased trend of Lactate Dehydrogenase was reported by Sharma *et al.*, (1986)in serum, which might be responsible for production of more pyruvic acid which in turn, provides energy for normal reproductive functions.

The present studies envisage more consistent information regarding changes in Acid Phosphatase, Alkaline Phosphatase, Alkaline Transaminase, Aspertate Transaminase and Lactate de-hydrogenase enzymes in cervical mucus during synchronised oestrus as related to fertility.

MATERIALS AND METHODS

The experimental group consisted of nine normal and nine repeat breeder cross-bred cows belonging to Livestock Instructional Farm, P.K.V.,Akola. Later these animals were synchronised using single instramuscular injection of Dinofertin 5 ml (25 mg)I/m.

The CM (Cervical mucus) samples were collected aseptically during mid stage of regular and induced estrus using syring-pipette technique (Reddy,1973). The samples thus collected in clean, sterilised vials were kept at - 20°C till assayed. The CM samples were studied for enzymatic profile using standard methods, as regards Acid Phosphatase by King and King (1954), for Alkaline transaminase Aspartate Transaminase and Lactate dehydrogenase using 2-4 DNPH Method by Reitman and Frankel (1957).

The synchronised oestrus to which the cows conceived was classified as 'fertile' the other being 'infertile'.

The data thus obtained were statistically analysed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Out of all 18 (100%) cows exhibiting oestrus on synchronisation, 6 and 5 (out of 9 in each) were fertile in normal and repeat breeder group respectively. The CM analysis revealed following details. (A)Acid Phosphatase (ACP): In the present study the mean ACP level in synchronised fertile (14.24±2.212 KA Units) and infertile estrus (14.088±1.295 KA Units) did not differ significantly. Bugalia and Sharma (1988), however, reported significantly higher ACP activity in cervical mucus of fertile cows.

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The mean ACP activity in CM of normal cows showing fertile and infertile estrus was 14.07±1.57 and 12.02±2.99 KA Units respectively the difference being nonsignificant. In repeat breeder synchronised cows the ACP was 16.10±2.99 and 14.08±1.29 KA Units in respective groups. Thus cows with fertile estrus showed increasing trend of ACP in both normal and repeat breeder. These findings are in agreement with King *et al* (1945) and King (1971).

(B) Alkaline Phosphatase (AKP):

There was no significant difference in mean CM AKP activity of cows showing fertile (28.55±3.86 KA Units) and infertile estrus (25.007±2.78 KA Units). Similar trend was reported by Bugalia and Sharma (1988) who stated that the higher AKP activity in CM during fertile estrus may be associated with carbohydrate metabolism of cervix under the influence of estrogen which further enhance glycogen utilisation by spermatozoa.

The average AKP activity in normal cows showing fertile (33.43±6.18 KA Units) and infertile estrus (28.03±4.88 KA Units) and in repeat breeders showing fertile (22.70±2.21 KA Units) and infertile estrus (22.73±3.31 KA Units) did not differ significantly.

(C) Alanine Transaminase (ALT/GPT):

Slightly higher (Non-significant) level of ALT/GPT) was noted in cows showing infertile estrus (34.25±5.22 Units per ml) than that in fertile estrus (22.45±3.31 Units per ml) Similar observationa are reported by Sharma *et al.,*(1986).

Significantly higher ALT activity was noted in CM of normal cows showing infertile estrus (47.02±5.37 Units per ml) than those showing fertile estrus (26.36±4.70 Units per ml) Similarly on synchronisation repeat breeder cows showing fertile estrus had lesser (18.86±3.068 Units per ml) GPT (Non-significant) than cows with infertile estrus (24.68±3.73 Units per ml).

(D) Aspartate Transaminase (AST/GOT):

Present study, however did not reveal significant difference in fertile and infertile estrus (75.67±5.68 Vs 75.67±9.07 Unit per ml) in normal and (54.098±5.32 Vs 75.91±6.16 Units per ml) in repeat breeder cows respectively.

In pooled data, however, slightly higher non-significant values of AST activity was noted in infertile estrus (75.81±5.245 Units per ml) as compared to fertile estrus (65.86±5.095 Unit per ml).

(E) Lactate Dehydrogenase (LDH):

Present observations revealed no significant difference and specific trend in mean CM LDH activity of normal and repeat breeder synchronised cows. The LDH activity in fertile and infertile estrus was (133.855±15.96 Vs 113.524±11.68 I.U. per litre of CM) in normal cows and (112.07±11.85 Vs 148.375±10.89 I.U. per litre CM) in repeat breeder synchronised cows. Thus Higher LDH level was noted in fertile estrus of normal cows and infertile estrus of repeat breeder cows. These observations are similar to Sharma *et al.,* (1986). The synchronisation process may probably results in alteration in pyruvic acid production, which in turn provide energy in a better way for normal reproductive functions as suggested by Henry *et al.,* (1974) who found that LDH helps in conversion of lactic acid into pyruvic acid.

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Levels of Glucose, Calcium and Alkaline Phosphatase in blood with relation to retention of placenta in bovines.

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ABSTRACT

The levels of blood glucose, serum calcium and alkaline phosphatase was estimated on 260th to 270th day of gestation and on the day of parturition in a group of cows with and without retention placenta. The cows with retention of placenta had significantly ($P<0.01$) lower level of glucose and calcium, where as serum alkaline phosphatase had higher concentration. The role of calcium and glucose in the expulsion of placenta is discussed.

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The retention of placenta in cows are considered to be an important pathological condition among all obstetrical disorders, because of its world wide distribution and subsequent reproductive disorders with a long period of convalescence. Levels of glucose in blood, calcium and alkaline phosphatase in serum of pregnant cows and their effect on normal parturition has been reported by various workers (Pradhan 1978, Kulkarni *et al.*, 1984 and Pareek and Deen, 1985). The variations in the levels of these constituents in abnormal parturition and retention of placenta in cows has been reported with variable relationships (Dutta and Dugwekar, 1983, Agarwal *et al.*, 1985 and Rajpal and Vadnere, 1985). The present investigation has been carried out to find the levels of glulose, calcium and alkaline phosphatase at prepartum period and their relationship with retention of placenta in cows.

MATERIALS AND METHODS

Blood and serum was collected from 35 cross-bred Jersey cows of Livestock Farm, Khapuria, Cuttack on 260th-270th day of gestation and on the day of parturition. From those cows, 15 with retained placenta were taken as experimental animals and the other 20 which had normal expulsion of placenta were considered as control.

Levels of glucose in the blood samples from both the group of animals along with serum calcium was estimated by Nelson-Somogyi method (Oser, 1965) and Clark-Collip modification of Kramer-Tisdall method as described by Oser (1965). Serum alkaline phosphatase activity was quantitatively estimated by Ames Blood Analyser using a special kit.

RESULTS AND DISCUSSION

The levels of glucose, calcium and alkaline phosphatase activity in both the group of cows having retention of placenta and without retention are presented in Table 1. The variations in the values of different blood constituents were compared by Fisher's 't' with the values being presented in Table 2.

In the groups of cows without retention of foetal membrances, the level of blood glucose on 260th-270th days of gestation increased on the day of parturition. Similarly cows with retention of placenta had also increased level of glucose on the day of parturition than on 260th-270th day. On comparision the level of glucose on the of parturition in both the groups a higher difference ($P<0.05$) was marked. However, when the levels of glucose on 260th-270th day of gestation and day of parturition was compared in both the groups of cow (Table 2) a highly significant difference ($P<0.01$) was marked, which was in accordance with the observation of Dutta and Dugwekar (1983) and Agarwal *et al.*, (1985). The significantly higher glocose level on the day of parturition in normal cows might be an indication of hyperglycemia caused by physiological stress. The significantly lower level of glocose on the day of parturition in cows with retention of foetal membrane is attributed for atony of uterine tissue and less contraction causing delay in parturition.

The level of calcium (Table 1) in cows with retention of foetal membrane was lower on the 260th-270th day of gestation (9.68±0.37 mg%)

Table 1: Blood constituents (Mean ± S.E.) of cows with and without retention of foetal membranes on 260th-279th day of gestation and on the day of parturition.

Constituents	Without Retention of foetal membranes (20)		With Retention of foetal membranes (15)	
	260th - 270th days of guestation	On the day of parturition	260th - 270th days of guestation	On the day of parturition
Blood Glucose (mg %)	54.55 ± 2.21	62.68 ± 3.06	43.12 ± 1.99	51.07 ± 1.89
Serum Calcium (mg %)	11.63 ± 0.54	9.17 ± 0.20	9.68 ± 0.37	8.32 ± 0.25
Serum Alkaline Phosphatase (unit / litre)	250.67 ± 15.00	326.00 ± 22.92	305.00 ± 48.81	586.50 ± 65.46

Figures in the parentheses indicate the number of animals under investigation

Table 2: Test of significane ('t' test) of blood constituents of cows with and without retention of foetal membranes on 260th - 270th day of gestation and on the day of parturition.

Groups	Blood Glucose	Serum Calcium	Serum Alkaline Phosphatase activity
WRFM X RFM on 260th - 270th day of gestation	3.52**	2.68*	1.25^{NS}
WRFM X RFM on the day of parturition	2.85**	2.69*	2.61*
WRFM on 260th - 270th day of gestation X WRFM on the day of parturition	2.16*	7.50**	2.75*
RFM on 260th - 270th day of gestation X RFM on the day of parturition	2.78*	3.01**	2.24*

WRFM — Without retention of foetal membrane
RFM — Retention of foetal membrane
* — Significant at 5% level (P < 0.05)
** — Significant at 1% level (P < 0.01)
N.S. — Not significant

and day of parturition (8.32±0.35 mg%) than that in cows without retention of feetal membranes. On statistical comparision (Table 2) significantly ($P<0.05$) higher calcium was observed on 260th-270th day in normal cows in comparision to the cows with retention of placenta. A highly significant difference was observed ($P<0.01$) in the calcium level of cows with retention of placenta on 260th-270th day of gestation and day of parturition. This finding is in close confirmity with the observation made by Boiter *et al.*,(1972) and Shukla *et al.*,(1983), but contradicts the findings of Tsolov (1962). The disturbance in the calcium metabolism and its utilization by the tissue results in the atony of the internal organs. During pregnancy specially as the late stage, there is excessive mobilisation of calcium. It is therefore suggested that probably due to less availability of calcium to the uterine tissue has resulted in atony of uterus, with decrease contraction and retention of footal membrances.

The level of alkaline phosphatase in serum in both group of cows was significantly higher ($P<0.01$) on the day of parturition than the 260th-270th days of gestion. The present observation is in confirmity with Dutta and Dugwekar (1983) who observed sharp increase on the day of parturition in cows having retention of placenta, but contradicts the findings of Sahukar *et al.*,(1985) and Rajpal and Vadnere (1985). A significantly higher level of serum alkaline phosphatase in cows with retention of placenta might be due to the leakage of more amount of this enzyme from the inflammed and necrotic tissue in the retention of placenta.

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Induction of Lactation in Crossbred Heifer and Subsequent Fertility

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ABSTRACT

Induction of lactation and subsequent fertility were studied in six crossbred infertile heifers (average weight 260 kg) or more than three years of age. On day of treatment, heifers were injected with estradiol valerate (0.1 mg/kg body weight) followed by subsequent daily injections of estradiol valerate (0.1 mg/kg body weight) and 17 alpha hydroxyprogesterone (0.25 mg/kg body weight) for seven days.

All heifers showed estrus within 48-72 hrs of PMSG treatment (1000 IU intramuscular) on day 17th. They were given 2.5 ml GnRH (Receptal, Hoechst) on standing estrus and showed regular estrus cycle of 19 to 21 days. All heifers became pregnant after four months of treatment.

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It has been demostrated that precosious puberty can be induced in heifers with hormonal treatments such as progesterone and estradiol 17 B injection (Gonzalez-Padilla *et al.,* 1975), estrogen and progesterone (Gonzalez-Padilla *et al.,* 1975; and Short *et al.,* 1976), gonadotrophins (Glencross, 1980) or repetative injections of GnRH (McLeod *et al.,* 1984).

Under village condition the level of infertility goes upto 40% or even higher (Pandey, 1986). Artificial induction of lactation in infertile heifers and cows hes been experimented with varied success (Fleming and Head, 1986 and Purohit *et al.,* 1991). Hence, hormonal treatment to overcome delayed puberty, infertility and agalactia in crossbred heifers would be of great advantage, particularly intropical countries where husbandry and nutrition often only marginally adequate.

MATERIALS AND METHODS

Six crossbred infertile heifers of three to four years of age with average weight of 260 kg, having average genitalia with small and smooth ovaries were included in this study. The heifers were dewormed (Penacure - Hoechst) one week before the commencement of hormonal treatment and fed with balanced ratio of feed and fodder along with 25 gm of vitamin and mineral supplement for 7 days.

On the first day of hormonal treatment, heifers were given intramuscular injection of estradiol valerate (Progynon depot, German Remedies Ltd) at the dose rate of 0.1 mg/kg body weight, followed by injections of estradiol valerate (0.1 mg/kg body weight) and 17-alpha hydroxyprogesterone (Duraprogen Unichem Lab., Bombay) at the dose rate of 0.25 mg/kg body weight for subsequent 7 days. The teats of heifers were given first stimulus from fifth day onwards for five to ten minutes in the morning and evening. From eleventh to fifteenth day, deep intramuscular injection of 40 mg Dexamethasone (Dexona-Cadilla, Ahmedabad) were given. During the same period, intramuscular injection of Terramycin (Sarabhai) were also given to avoid chances of infection after Dexamethasone treatment. The heifers were fed with 40 mg of Suplivite-M (Sarabhai, Baroda) to meet mineral requirement for milk production and 40 gm of Galog (Indian Herbs, Saharanpur) for increased milk production. On sixteenth day, intravenous injection of 450 ml Mifex (M&B, Bombay) alongwith 8 mg of Dexamethasone and intramuscular injection of 15ml tonophosphon (Hoechst, Bombay) were also given. The milk produced during twenty first day of treatment was fed to the heifers under treatment for 28 days.

On day 17th heifers were injected with 1000 IU of PMSG (Folligon, Intervet, Holland) through intramuscular route. The heifers were observed for normal estrous cycle and injected with 2.5 ml of GnRH (Receptal-Hoechst) at the time of artificial insemination.

RESULTS AND DISCUSSION

The size of udder grows faster from 8th day of treatment upto 15th day. Initially, small quantity of water milk start coming which later on turn milky and yellowish milky appearance. Initially, milk was curdled on boiling due to steroid secretion.

The initial milk yield was nearly 1/2 to 1 litre per milking but it gradually increased to average peak yield of 5 litres/day and maintained for approximate 5 months after that milk yield was gradually reduced to 1/2 litre per milking.

All heifers were detected in estrus on an average 72 hrs post PMSG treatment. They resumed cyclic activity and conceived after artificial insemination. However, Glencross (1980) reported failure of ovulation in prepubertal heifer treated with PMSG and HCG.

A proper proportion of estrogen and progesterone is essential for initial follicular development. The effectiveness of treatment would be very high after giving PMSG. The PMSG further stimulate the follicular development initiated by estrogen and progesterone and also increased luteinising hormone production. Ovarian inactivity in heifers is associated with a low frequency of LH episode due to hypothelamic inhibition of GnRH release (McLeod *et al.,* 1984). However, the use of GnRH at the standing heat helps in development and maturation of follicle and increase LH surge which insure complete ovulation.

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Effect of 'Chorulon' Administration to Improve the Conception Rate in Rural Bovines

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ABSTRACT

Ovulatory disturbances on account of hormonal imbalance, especially of LH are major cause for failure of fertilization and subsequent poor conception. Attempts have been made to treat such cases using hormones with variable results (Singh *et al.*, 1985; Sankaralingam and Duraisamy, 1986). An attempt was made to observe the role of 'Chorulon' in the improvement of conception in rural cows and buffaloes, which failed to conceive after two inseminations.

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MATERIALS AND METHODS

Ninety six randomly selected animals (39 cows and 57 buffaloes) presented at clinics of field extension programme of the institute for A.I. were, used for the study. These animals were regularly cyclic and had normal genitalia. The animals were divided into two groups. Sixty animals (26 cows and 34 buffaloes) in Group I (Control) did not receive any treatment. Thirty six animals (13 cows and 23 buffaloes) in Group II (treated) were injected (i/m route) 1500 i.u. of chorulon (made in Holland by Intervet Co.) just before insemination with good quality (above 40% PTM) semen. These animals were inseminated only once and followed up 60 days after treatment to record pregnancy status.

RESULTS AND DISCUSSION

The overall conception (CR) in control group was 31.66% (19 conceived out of 60 animals). The CR in control cows was 30.76% (8 out of 26) and in buffaloes 32.35% (11 out of 34). In the treatment group, however, 14 animals (6 cows and 8 buffaloes) conceived with an overall conception rate of 38.88%; the CR in treated cows was 46.15% and in buffaloes 34.78%. The study indicate that chorulon can be effectively used to improve the CR in bovines. It is also supported by the views of other workers who found a better CR with Chorulon thereby. Singh *et al.*, (1985) claimed 100% results with chorulon in treating repeaters while Sankaralingam and Duraisamy (1986) observed 72% CR with 1500 i.u. chorulon in bovines. The better effect after chorulon administration is conceivable as this consists of HCG or LH like activity. LH is responsible for ovulation and in the deficieny of LH possibly the ovulation may not take place; but after administration of LH like substances probably the ovulation is ensured and subsequent conception takes place. When 1500 i.u. chorulon was given 6 hrs before A.I. Sandhu and Singh (1992) found an overall CR of 67.2% in repeater cows.

A definite improvement in cows but not in buffaloes after chorulon administration might be due to species difference and low hormonal profile which needs further investigation.

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Trichomoniasis in Cattle in North West Rajasthan

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ABSTRACT

Trichomoniasis was diagnosed in 29 cows out of 103 (28.16%) abortions in sporadic cases reported in the clinics. Microscopic examination of fresh uterine or cervico-vaginal exudate of abortion under the phase contrast microscope is recommended which revealed the presence of highly motile protozoa in abundance with a characteristic movement of the flagellae, axestyle and the undulating membrane.

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Trichomoniasis a venereal disease of cattle caused by *Trichomonas foetus*, is characterised by infertility, early abortion and post coital pyometra. The first incidence of Trichomoniasis in India was recorded in Calcutta in a 5 year old Tharparkar cow with servere vaginitis and in a 2 months pregnant cow in a dairy farm of Bengal Veterinary College by Das *et al.*, (1956). They also recovered the caustive organism from the placental fluid. Later on the incidence of Trichomonas infection was reported in Bihar by Ishque and Kuppuswamy (1957) in the vagina of a cow, subsequent attempts in this direction were made by Prasad and Narayanan (1962) and Settergren and Soderlind (1966).

MATERIALS AND METHODS

The uterine or cervico-vaginal discharge and wherever possible, the foetal membrances, foetal fluids and the foetus of 103 abortion cases of cows presented in obstetrics and gynaecology clinic from August,1988 to December,1991 were tube and petri dishes. Fresh smear from foetal membrances, foetal fluids, stomach contents of the foetus and cervico-vaginol discharge were examined microscopically under phase contrast microscope under high power (1x1000) for Trichomonades. Fixed smears were stained with Giemsa's stain and subjected to further examination and identification.

RESULTS AND DISCUSSION

From a total of 5831 clinical cases of various reproductive disorders recorded in the obstetrics and gynaecology clinic there were 173 cases of abortion. Species wise number of abortions recorded in cattle, buffalo and goats was 103,22 and 448, respectively. Subsequent investigation revealed that out of 103 abortions in cows, 29 were due to trichomoniasis amounting to 28.16%. This clearly indicates the prevalence of trichomoniasis and its transmissian was from the community bulls spreding be disease in the local cows. Abilt and Ball (1978) also reported that out of 7 cows which aborted following service by infected bulls only 3 were positive for T. foetus while the rest were negative.

In the positive cases, the fresh wet smear examination of the cervico-vaginal discharge and foetal fluid of the cows with early abortion under the phase contrast microscope, revealed characteristic movements of the axestyle and undulation membrane very clearly. The organisms were found in abundance. These were also noticed in the wet smears from foetal stomach contents and placenta. The examination of foetal membranes revealed the presence of whitish surface of the cotyledons with placentitis and degenerative changes. The organism was identified as *Trichomonas foetus* on staining with Giemsa's stain and culture. 81% of the cases of abortions were from 6 to 18 weeks of gestation. In general after abortion the involution of the uterus was fast (8 to 15 days) and 73% of the animals come in heat in 3 to 25 days after abortion. However the owner of the animals was advised not to bread on 3 subsequent heats. A sexual rest of 60 days was given before the animals were bred again by artificial insemination.

On subsequent days Post-abortion sampling of vaginal mucous revealed that the concentration

of T.foetus organism decreased and vanished completely after 4 days. Jubb and Kennedy (1970) also reported similar findings. This was due to the confinement of organism on the surface of the uterine mucosa (Parson *et al.,* 1976). Following abortion the organisms seem to get diluted and washed out as such reduced in numbers and decreased intensity.

This smears gave the best results in revealing trichomonads, where as in thick smears these organisms could easily be mistaken for typical dysplasea of uterine mucosa.

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Concentration of Gentamicin in Endometrial Tissue Subsequent to Muscular and Uterine Routes of Administration in Cows.

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ABSTRACT

Disposition of gentamicin in crossbred cows was investigated in the endometrial tissues subsequent to intramuscular and intrauterine routes of administration. It is observed that the drug was not able to reach endometrial tissue in sufficient quantities after intrauterine administration (10mg/kg). However, upon intrauterine administration (5mg/kg) the drug was detectable in endometrial tissue upto six hours.

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Gentamicin, a broad spectrum bactericidal aminoglycoside antibiotic, has gained considerable clinical significance in Veterinary Medicine (Conzelman, 1980). Pharmacokinetic parameters for gentamicin have been defined after intravenous administration in cows, buffaloes, horses, sheep, dogs, cats, rabbits, ponies and several avian species (Garg *et al.,* 1989 a, Garg *et al.,* 1989 b, Pedersoli *et al.,* 1980, Wilson, *et al.,* 1981, Riviere and Coppoc, 1981, Jernigan *et al.,* 1988) and after intramuscular administration in sheep and cats by several authors (Wilson *et al.,* 1981, Jernigon *et al.,* 1988). However, the reports on disposition of gentamicin in genital tissues subsequent to intramuscular or intrauterine routes of administration are scanty. The present investigation was therefore, conducted to study the disposition of the antibiotic in the endometrial tissue subsequent to intramuscular and intrauterine routes of administration in estrus cows.

MATERIALS AND METHODS

Twelve crossbred cows of Ranchi Veterinary College dairy farm were selected for determining endometrial tissue levels of gentamicin. Their body weight ranged between 155-410 kg. The animals were maintained on standard ration and partial grazing. Drinking water was supplied *ad lib*. Vaccination and other measures were done as per routine. The experimental animals were divided into two groups, each group consisting of six animals. In both the groups of cows, estrus was induced with the help of PGF_2 alpha (Lutalyse, Unichem Laboratories). In group I gentamicin sulphate was administered intramuscularly at the rate of 10 mg/kg body weight at induced estrus. In group II gentamicin sulphate was administered through intrauterine route at the rate of 5 mg/kg body weight. The endometrial samples were collected at 0 (just before drug administration), and 15, 30, 60, 120, 360, 480, and 720 minutes after drug administration in sterilized petridishes. Immediately after collection the biopsy samples were kept in refrigerator at 4°C and subjected to analytical drug determination within 24 hours of collection. The endometrial biopsy was obtained using aseptic precautions with the help of a hiopsy catheter of trocar and canula type. To determine the concentration of gentamicin Microbiological Assay Technique (Cruick-shank *et al.,* 1975) was adopted.

RESULTS AND DISCUSSION

Subsequent to intramuscular administration, gentamicin was not detected in majority of endometrial tissue samples (Table 1). It was not detected in any of samples obtained from cow no. 1, 12 and 13 whereas, it was detected in samples from cow no. 2, 9 and 10. These findings do not agree with those of Al-Guedawy *et al.,* (1983) who observed that approximately 183.7 /ug and 39.4 /ug gentamicin accumulated in the uterine lumen of cows six hours after intramuscular administration at the rate of 4 mg/kg body weight and 2 mg/kg body weight, respectively. Although, Bhatt (1988) was able to detect gentamicin in healthy and diseased uterine tissue after administration at a dose level

of 4 mg/kg body weight; the drug was not detectable when administered at a dose level of 2 mg/kg body weight in diseased uterine tissue and concentration otained was not significant in healthy uterine tissue. Further-more, the concentration in uterine tissue was above MIC levels only upto two hours and declined to traces at four hours. This can be explained on the findings that protein binding of gentamicin is of very small degree (Ziv, 1980). Amount of its free portion generally governs the level of a drug in the tissues. The variations in the drug concentration have been attributed to PH, protein concentration and presence of other agents during determination (Ziv, 1980).

Subsequent to intrauterine administration, the mean endometrial tissue concentration of gentamicin in cows was 5.16±0.19 ug/kg at 15 minutes (Table 2). The peak mean drug concentration of 64.54±0.32 ug/g attained at 240 minutes which declined rapidly to 7.00±0.19 ug/g at 360 minutes. The drug was not detected in endometrial tissue samples collected at 480 and 720 minutes. At-Gurdawy *et al.,* (1983) reported that following intrauterine infusion of gentamicin in the cows the majority intrauterine infusion of gentamicin in the cows the majority of the dose (70.6%) remained in the uterine lumen throughout the six hours period. Transport of gentamicin through uterine membrane appears to be affected by the osmolarity of the vehicle, since 17% and 82% of infused gentamicin remained in the uterine lumen at six hours post infusion using water and saline vehicle, respectively (Al-Guedawy *et al.,* 1983). Pathology of the uterus has also been reported to affect the transport of gentamicin and Bhatt (1988) recorded that the drug could be detected at 18 and 24 hours after infusion in diseased and clinically normal uterine tissue, respectively.

Table 1: Gentamicin Concentration (ug / g in bovine endometrial tissue following a single intramuscular dose of 10 mg / kg body weight.

CASE NO.	TIME IN MINUTES							
	15	30	60	120	240	360	480	720
1	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	6.00	ND	ND
9	ND	ND	ND	ND	ND	8.25	ND	ND
10	ND	ND	ND	ND	8.50	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND	ND
13	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not detected.

Table 2: Gentamicin Concentration (ug / g in bovine endometrial tissue following a single intrauterine dose of 5mg / kg body weight.

CASE NO.	TIME IN MINUTES							
	15	30	60	120	240	360	480	720
3	4.00	11.25	17.25	39.50	67.00	8.50	ND	ND
4	5.25	10.75	18.50	38.75	63.25	6.00	ND	ND
5	4.00	11.50	15.75	50.00	65.75	7.25	ND	ND
7	5.75	14.75	19.00	37.00	64.50	7.00	ND	ND
8	6.00	11.50	19.25	34.00	64.00	7.25	ND	ND
11	6.00	11.00	19.00	32.75	62.75	6.00	ND	ND
Mean	5.16	11.79	18.12	38.66	64.54	7.00		
± S.E.	0.19	0.30	0.27	1.23	0.32	0.19		

ND = Not detected.

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Breeding Behaviour of Indigenous Goats in Semi-Arid Zone

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ABSTRACT

Two hundred twelve Jamunapari, 205 Barbari and 58 Jakhrana does (adult cyclic) maintained at the institute livestock farm under semi-intensive management system comprising of 4-6 hrs daily grazing in forest area and supplementation with 500 gms concentration mixture and bhoosa/green fodder *ad-libitum* in the sheds were used. Incidence of oestrus was studied round the year at 12 hrs interval in 1986 and 1987. Monthly incidence of oestrus (tupping percentage) was calculated for each breed. A difference in oestrus pattern was observed between months and among breeds. The highest incidence of oestrus was observed in May to July and September to October in Jamunapari and Barbari. However, the incidence of oestrus from December to April was almost absent in Jamunapari and poor in Barbari goats. Jakhrana did not show a uniform pattern and exhibited oestrus round the year though there was monthly difference in tupping percentage. The data indicate a trend of seasonality in oestrus exhibition in all the three breeds. The trends was most distinct in Jamunapari and least in Jakhrana, Barbhari was intermediate.

——x——x——x——

Oestrus / oestrous cycle profile is an important trait which affects the overall reproductive efficiency of an animal. The knowledge of oestrus / oestrous cycle profile is equally important for undertaking embryo transfer programme. In general tropical breeds are considered, non-seasonal (Rajkonwar and Borgohain, 1978; Kaura; 1952; Devendra and Burns, 1970; Mazumdar and Mazumdar, 1983), while temperate breeds are seasonally polyoestrus (Devendra and Burns, 1983). There are certain goat breeds which kid twice a year (Masud, 1964). The restricted seasonally in oestrous activity in the tropical goats is an adaptive effort by different breeds in relation to changes in ambient temperature, onset of rain and availability of herbage (Roy *et al.*, 1962). Tropical goats start exhibiting oestrus shortly after during dry season. Diffirent agroclimatic factors, managemental practices, feeding pattern and photo-periodicity are some important factors, which are known to influence the oestrous activity. Meagre information on oestrous activity in Jamunapari goat is available (Singh and Singh, 1974; Khan *et al.*, 1978; Wani, *et al.*, 1980), but in Barbari and Jakhrana such information is scanly. An attempt has been made to study the oestruos pattern in Jamunapari, Barbari and Jakhrana goats in different months of the year.

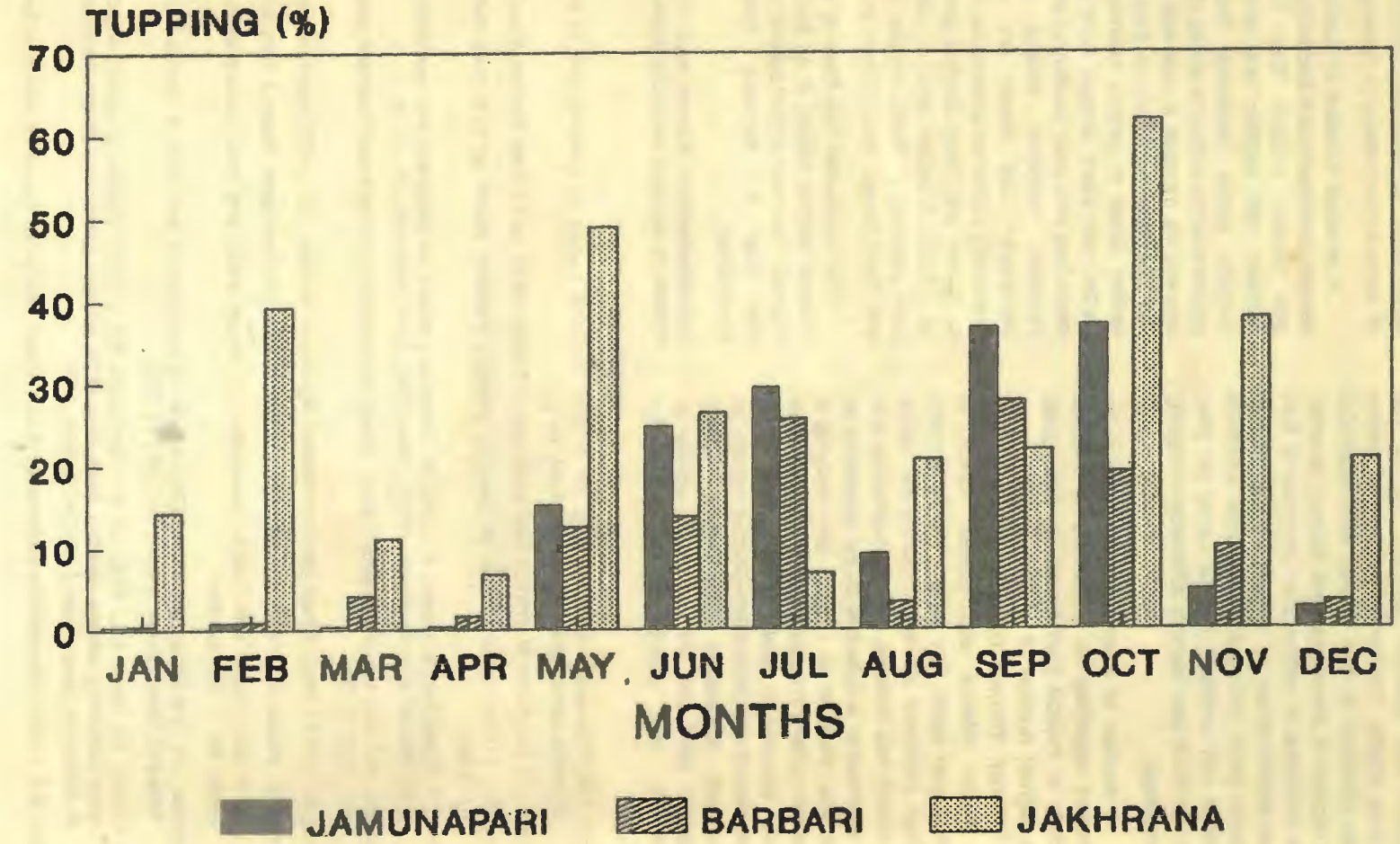
MATERIALS AND METHODS

A total of 475 adult cyclic goats (Jamunapari: 212, Barbari: 205, Jakhrana: 58) were taken for present investigation. Goats were maintained under semi-intensive management system comprising of 4-6 hrs daily grazing in forest area and supplementation with 500 gms conc. mixture and bhoosa/green fodder *adlibitum* in sheds. Aproned bucks were used for oestrus detection. Incidence of oestrus was studied at 12 h interval round the year in 1986 and 1987. Frequency of oestrus was calculated monthwise for different breeds studied.

RESULTS AND DISCUSSION

Frequency of oestrus and percent incidence in Jamunapari Barbari and Jakhrana goats are presented in fig 1. The salient signs of oestrus were frequent bleating, wagging of tail, frequent micturition, homosexual behaviour, tendency to seek the buck. Vulvar discharges were thin and watery during early oestrus, mucinous during mid oestrus and thick and cheesy in late oestrus. They teased other goats either in oestrus or non-oestrus. The oestrus symptoms were more pronounced in Barbari breed as compared to the other two breeds. A difference in oestrus

FIGURE 1. SEASONALITY IN BREEDING BEHAVIOUR IN GOATS



pattern was observed between months and among breeds. The highest incidence of oestrus was observed in May to July and September to October in Jamunapari and Barbari. However, the incidence of oestrus from December to April was almost absent in Jamunapari and poor in Barbari goats. Jakhrana did not show a Uniform pattern and exhibited oestrus round the year, though there was monthly difference in tupping percentage.

A number of reports are available in the literature regarding the seasonality of oestrous activity in tropical goat breeds. In local goats the highest number of oestrous cycles occur in October, followed by June and the lowest in March (Mishra and Biswas, 1966. In Jamunapari goats, Roy *et al.,* (1962) reported a high incidence of oestrus during the period of rainfall and herbage growth, is possibly due to oestrogenic substances in the vegetation at that time. Wani *et al.,* (1980) also reported a seasonal pattern in oestrus activity in Jamunapari goats. Majority of goats exhibited oestrus in June-August and September-October months. In study with Jamunapari goats Sharma, (1985) recorded 69% and 68% oestrous activity in June-July and October-November months respectively. All these data confirm the findings of present observations in Jamunapari goats.

A seasonal variation in oestrus occurrence has also been reported in Barbari goats by Prasad and Pandey, (1981). They indicated that probably mild weather and moderately high humidity during September had stimulating influence on the endocrine glands of female goats, whereas the increase in cold with less humidity had a diminishing effect on their reproductive system. Goats in general (Jamunapari, Beetal, Barbari and Black Bengal) have two breeding peaks May and June, September and November. However, restricted kidding seasons in Jamunapari and Beetal are indicative of seasonality in oestrus appearance in comparison to Barbari and Black Bengal goats. (Singh *et al.,* 1982). A similar trend in seasonality has been reported in Beetal (Amble *et al.,* 1964) and Black Bengal goats (Singh *et al.,* 1985).

It is concluded that there is a seasonality towards oestrus display in all the three breeds of goats. The trend is most distinct in Jamunapari and least in Jakhrana. Barbari stands intermediate.

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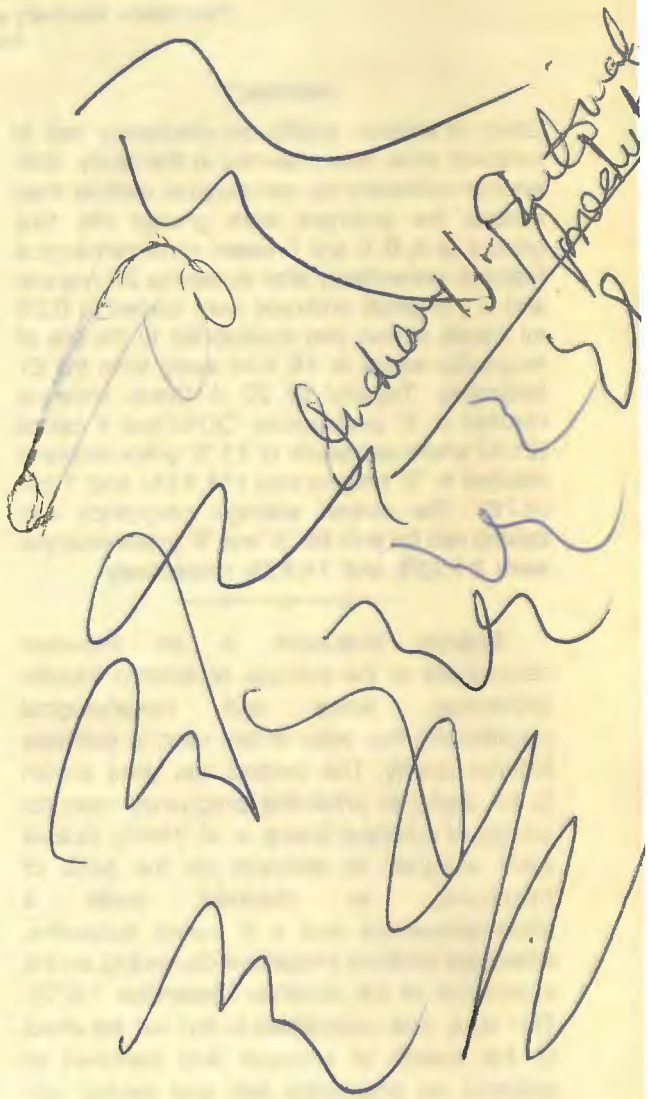

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Effect of Transport of Embryos on Pregnancy and Calving Rate in Crossbred Cows

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ABSTRACT

Effect of embryo quality on pregnancy rate in recipient cows was observed in this study. After embryo collection by non-surgical method from donors, the embryos were graded into four groups as A, B, C and D based on morphological features. Immediately after screening 20 A grade and 21 B grade embryos were loaded in 0.25 ml french straws and transported to the site of recipients, which is 45 Kms away from the ET laboratory. Transfer of 20 A Grade embryos resulted in '6' pregnancies (30%) and 5 calves (25%) where as transfer of 21 'B' grade embryos resulted in '2' pregnancies (14.33%) and 1 calf (4.76). The overall average pregnancy and calving rate for both the 'A' and 'B' grade embryos were 21.95% and 14.63% respectively.

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Embryo evaluation is an important determinant of the success of embryo transfer technology. Gross and morphological classification has been widely used to delineate embryo quality. This method has been shown to be useful in predicting pregnancy rates for groups of embryos (Laing *et al.*, 1988). Grades were assigned to embryos on the basis of morphology as observed under a stereomicroscope and it is purely subjective, somewhat arbitrary procedure depending on the experience of the observer (Betteridge 1977). This study was undertaken to find out the effect of the quality of embryos and transport of embryos on pregnancy rate and calving rate under field conditions in crossbred recipient cows.

MATERIALS AND METHODS

Embryos were collected from five crossbred donor cows located at embryo transfer unit, Animal Biotechnology Department of Madras Veterinary College. Embryos were collected non-surgically by ebb and flow two way technique on 7th day after the first insemination according to the method described by Newcomb *et al.*, (1978) and Elsden *et al.*, (1976). PBS containing 4G / L of BSA was used for collection. Immediately after locating the embryos, they were transferred to culture discs containing PBS with FCS for evaluation. The embryo quality was assessed under stereomicroscope at 90 x and graded into grade A, B, C and D according to the recommendation of embryo transfer training programme, National Biotechnology Centre, Indian Veterinary Research Institute, Izat Nagar, India. Immediately after grading, 'A' grade and 'B' grade embryos were loaded individually in 0.25 ml french straws (IWV France) as described by Elsden and Seidel Jr., (1984). The ready to transfer 'embryo straws' were transported to the Livestock Research Station, Kattupakkam, which is 45 kms away from the embryo transfer unit. The straws were transported in thermosflask containing water at 37°C. All transfers to the recipients in this study were carried out non-surgically within 8 hours after collection and the embryos were deposited in the middle of the uterine horn ipsilateral to the corpusluteum by using Cassou AI pipette. Only the recipient cows synchronised for estrum within ± 12 hours of the donors were used in this study. Pregnancy diagonosis was based on rectal examination at 35 days or more and all pregnancies were followed until parturition. The results were analysed by Chi square test as per Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

In all 61 embryos were collected by 11 flushings of which 20, 21, 11 and 9 were graded as A, B, C and D respectively. Only 'A' and 'B' grade embryos were transferred to the recipients.

The transfer of 20 'A' grade embryos resulted in 6 pregnancies (30%) and 5 calves (25%) and the transfer of 21 'B' grade embryos resulted in '3' pregnancies (14.3%) and 1 calf (4.76%). The overall pregnancy and calves born were '9' (21.95%) and 6 (14.63%). One cow received 'A' grade embryo aborted at 6 months of gestation and two cows received 'B' grade embryos aborted at 2 months and 6 months respectively.

Subramaniam *et al.,* (1990) recorded 29.16% pregnancy rate and 20.08 calving rate in their 'on farm embryo transfer' in cross bred cows in India. In their work the donors and recipients were maintained at a particular farm. In this study, although the difference in pregnancy rate between 'A' and 'B' grade embryos was not statistically significant, the 'A' grade embryos seemed to result in a higher pregnancy rate. Regarding calving rate it was significantly higher in 'A' grade embryos (25%) when compared to 'B' grade embryos (4.76%). Christie (1982) opined that the quality of embryos can be related to pregnancy rate on transfer. The results of this study indicate that in crossbred cows quality of embryos has got important effect on pregnancy rate and calving rate and when the embryos are going to be transported for a long distance in "ready to transfer staws" for transfer to recipient cows under field conditions, 'A' grade embryos should be selected.

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Arterial Pattern of Uterus in Buffaloes

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ABSTRACT

Arterial pattern of uterus of buffaloes was studied using Radiographic technique. Lead oxide solution was used as radio opaque material. Normal nongravid and gravid genitalia of she buffalces were utilised. Ovarian artery gave one prominent anastomosing branch to uterine artery. The arteries on either side of the uterus were interconnected with prominent anastomosing arches at the level of the body of the uterus. Arcuate arteries which extend from the mesometrial to antimesometrial border of uterus were less coiled in gravid genitalia.

——x——x——x——

Uterine, uterine branches of ovarian and vaginal arteries which supply the uterus undergo structural changes during different physiological reproductive status. Such changes in the vascular architecture are reported to occur under the influence of ovarian hormones (Dobrowolski and Hafez, 1970). Changes during pregnancy was more dramatic and dynamio (Gillet, 1972. Vascular architecture of uterus has been studied in detail in sheep, cow, swine, dogs and cats (Delcampo and Ginther, 1973 & 1974). Except for the reports of Mobarak (1969) and Panchamukhi and Mudholkar (1971) there is paucity of information on uterine arteries in Buffaloes

MATERIALS AND METHODS

Genitalia of She-buffaloes with intact uterine and ovarian arteries were collected from slaughter house. Twenty normal sized genitalia and 15 gravid genitalia were used. The uterine artery on one side was cannulated with 18 G blunt hypodermic needle and fixed with an artery forceps. All other cut arteries were ligated and the other side uterine artery was clamped with an artery forceps. Lead oxide suspension (20%) in liquid soap was mixed well and injected into the cannulated artery with 20 ml glass syringe under steady digital pressure. About 60 ml of the suspension was injected into one uterine artery. The injection procedure was repeated on the other uterine artery. After thorough washing the genitalia were radiographed. The skingrams were studied for the arterial pattern.

RESULTS AND DISCUSSION

The uterus was mainly supplied by uterine artery which divides into primary, secondary and tertiary branches in the broad ligament. The pattern of branching on one side differed from that of the other side. At the mesometrial border the artery divided into several branches some of which course cranially along the border upto to the ovarian end of the uterine horn before entering into the uterine wall (Fig 1). The arteries after entry into the uterus divide into number of coiled arteries and proceed towards the antimesometrial margin as arcuate arteries. These arcuate arteries were highly tortuos and encircle the horn to meet at the antimesometrial border (Fig 3) Hansel and Asdell (1951) and Yamanchi and Sasaki (1968) also reported similar observation in cow.

Ovarian artery divide into a coiled branch which reached the ovary and almost a straight branch to the uterus. The uterine branch traversed in the broad ligament and reached the cranial part of the uterine horn. It gave off a prominant anastomosing branch to one of the branches of the uterine artery. In some of the genitalia two separate uterine branches of the ovarian artery were noticed. Ginther and Delcampo (1974) described arterial connections between the uterus and the uterine branch of ovarian artery. Panchamukhi and Mudholkar (1971) recorded in buffaloes only one uterine branch of ovarian artery which divided into a fallopian and a cornual branch. They did not record the anastomosing branch between the ovarian and uterine arteries as observed in the present investigation.

The uterine branch of the vaginal artery was observed to be much coiled unlike in cow (Ginther

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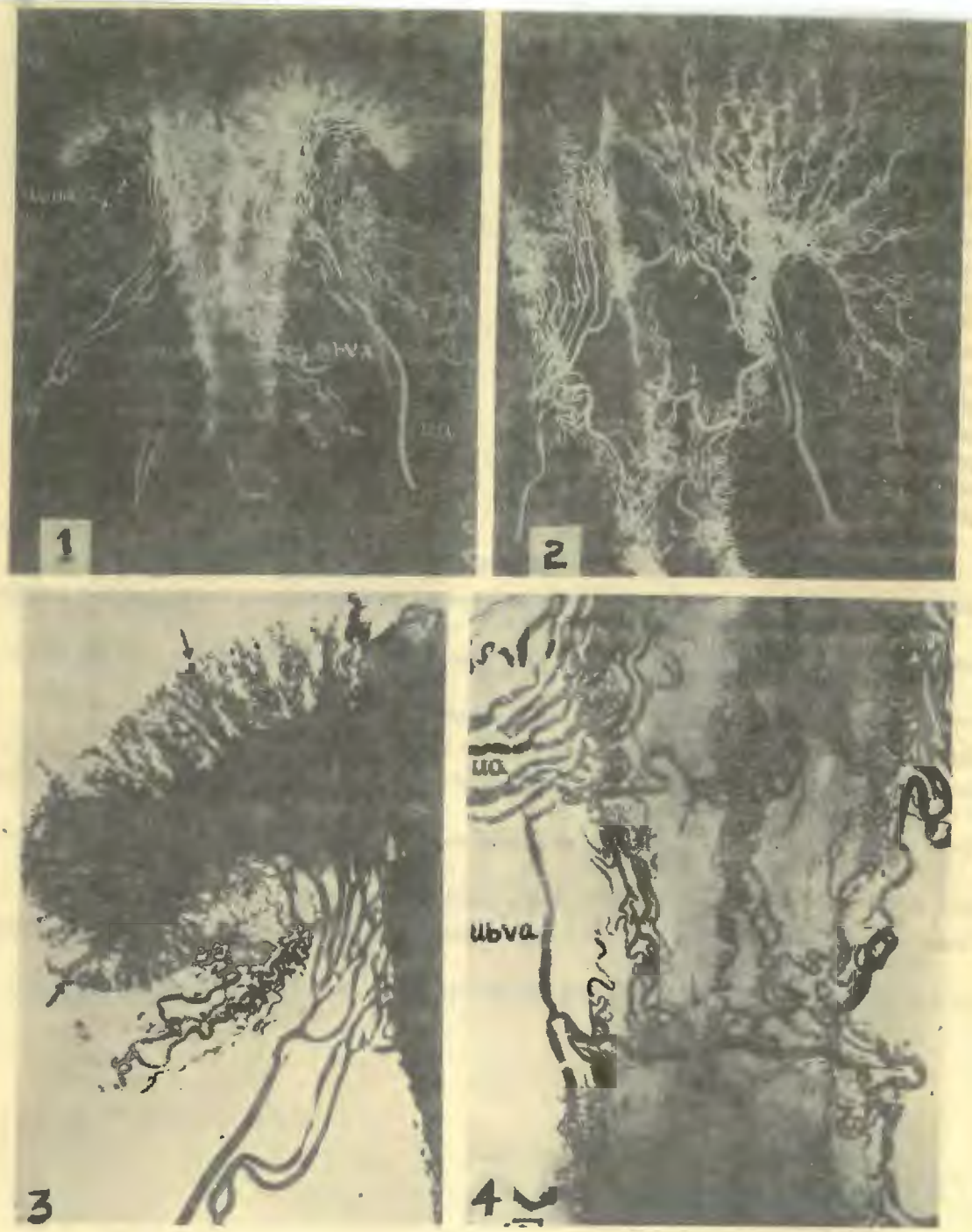

Fig. 1. Arterial pattern of non gravid genitalia showing ovarian, uterine and vaginal arteries

Fig. 2. Arterial pattern of Gravid genitalia showing arcuate arteries and arterial anastomosis

Fig. 3. Uterine horn showing distribution of arcuate arteries

Fig. 4. Distinct arterial anastomosis between left and right side uterine arteries

and Delcampo, 1974) and joined with a branch of uterine artery on either side. It provided fine arterial branches to the body of the uterus and cervix.

In the present study, two distinct pattern of arterial anastomosis between left and right side were noticed. The uterine branch of vaginal artery showed a prominent anastomosing arch at the level of the body of uterus. From this arch net work of fine arteries ramify along the mid line of the two uterine horns (Fig 4). In the second pattern three anastomosing arches between the uterine branch of vaginal artery and fourth arch between the uterine arteries. Dobrowolski and Hafez (1970) noticed vascular anastomosis between the two horns in sheep. Ginther and Delcampo (1974) reported in cow a branch of the uterine artery of one side to cross the midline as a prominent arch and anastomose with the uterine branch of the vaginal artery of opposite side. The pattern of vascular anastomosis observed in buffalo differed from the reports on cow and other species. Panchamukhi and Mudholkar (1971) who studied the vascular supply in buffalo did not mention about the anastomosing arches.

In the gravid genitalia (Fig 2) the ovarian artery did not show much variation from that of the nongravid normal genitation. The arcuate arteries tends to be straight and thicker than that of the nongravid genitation. The arcuate arteries were mostly confined to the uterine horns while a portion of the body of the uterus appeared to be devoid of arcuate arteries. The anastomosing arterial arches were similar to that of the nongravid uterus.

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Effect of Age on Body Growth and Testicular Development In Crossbred Bulls

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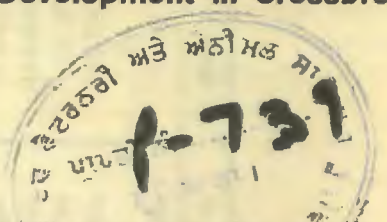

ABSTRACT

The body and testicular measurements were taken from 40 Crossbred bulls of 12 to 105 months of age having 75% exotic inheritance over a period of 12 months, to determine the influence of age of the bull on body, scrotal and testicular growth. The body weight and hear girth increased linearly with advancing of age while the scrotal and testicular growth showed a curvilinear relationship with age. The growth of testicules reached its maximum size by 60 months of age.

—•—x—x—x—

The pattern of prepubertal growth and development of testis in mammalian species in general is sigmoid in nature. The rate of growth of the bovine testis is slow during the initial period of postnatal life and rapid after onset of puberty (Lagerlof 1934). Extensive information is available on testicular development in European breeds of cattle but such information is very limited in Indian breeds, more so in crossbred bulls. Hence, the present work was undertaken to know the effect of age on body growth and the testicular development in 75% crossbred bulls.

MATERIALS AND METHODS

Four hundred and twenty body, scrotal and testicular measurement were collected over a period of 12 months from 40 crossbred bulls, aged between 12 to 105 months with 75% exotic inheritance, stationed at AICRP on Cattle, Lam Farm, Guntur (A.P.). To know the affect of age of the bull on body, scrotal and testicular development the bulls were divided into 5 age groups. The measurements were carried out as described by Hahn *et al.*, (1969). The data was subjected to statistical analysis as per Snedecor & Cocharan (1967).

RESULTS AND DISCUSSION

The mean values of body, scrotal and testicular measurements of different age groups were presented in table-1.

The statistical analysis of the data revealed a significant positive correlation ($P<0.01$) between age of the bull and body weight ($r = 0.81$) and herat girth ($r = 0.83$). Similar observations were made by earlier investigators (Coulter and Foote 1976 and Calo *et al.*, 1973) in European breeds.

The Scrotal circumference increased significantly ($P<0.01$) with advancing of age however, this increase was more rapid upto 36 months of age. A curvilinear relationship was observed between age and scrotal circumference. The scrotal circumference was significantly correlated ($P<0.01$) with body weight ($r = 0.79$) and heart girth ($r = 0.77$). The scrotal skin thickness increased with increase of age and differed significantly ($P<0.01$) among age groups.

The length of right and left testicles increased significantly ($P<0.01$) with advancing of age upto 60 months. The width and thickness of both testicles also followed similar pattern as that of length of testicles. The present observations indicate that the growth of testicles was more rapid in young age especially after puberty and reaches their maximum size by about 60 months, which was in agreement with the earlier observation of Largerlof (1934) this might be responsible for rapid increase in scrotal circumference upto 36 months of age. After 60 months of age, the increase in testicular size was not significant with age. Further it was noted from the present measurements that the size of the right testicle was consistently larger than the left testicle in all groups of bulls similar to the findings of Abdel Raouf, (1960) and Sakala (1964).

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Table 1: Scrotal and testicular measurements in 75% exotic crossbred bulls of different age groups (Mean ± Se)

Parameters	Group I	Group II	Group III	Group IV	Group V
1. Age of bulls (months)	< 24	24 - 36	36 - 48	48 - 60	> 60
2. Number of bulls	8	6	7	5	14
3. Body weight (kgs)	282.70[a]	355.80[b]	426.30[c]	533.97[d]	609.53[e]
	± 44.10	± 72.49	± 75.99	± 64.78	± 70.19
4. Heart girth (cm)	133.14[a]	164.96[b]	180.33[c]	188.62[d]	199.83[e]
	± 10.77	± 10.71	± 9.91	± 8.85	± 7.97
5. Scrotal circumference (cm)	27.18[a]	30.14[b]	30.52[c]	35.97[d]	34.86[e]
	± 2.57	± 2.14	± 2.31	± 4.08	± 1.90
6. Scrotal skin thickness (cm)	0.53[a]	0.67[b]	0.74[c]	0.87[d]	0.88[d]
	±0.09	± 0.09	± 0.10	± 0.10	± 0.14
7. Length of testis (cm)	9.29[a]	10.04[b]	10.41[b]	12.85[c]	12.13[c]
	± 1.27	±1.09	± 1.21	± 1.47	± 1.06
Right	9.15[a]	9.86[ab]	10.05[b]	12.49[c]	11.90[c]
Left	± 1.23	± 1.15	± 1.27	± 1.74	± 1.09
8 Width of testis (cm)	4.85[a]	5.60[b]	5.78[b]	7.01[c]	6.91[c]
Right	± 0.42	± 0.64	± 0.58	± 0.89	± 0.79
Left	± 4.79[a]	± 5.60[b]	± 5.82[b]	± 7.16[c]	± 6.92[c]
9. Thickness of testis (cm)					
Right	4.81[a]	5.73[b]	5.82[b]	6.84[c]	6.79[c]
	± 0.55	± 0.67	± 0.62	± 0.83	± 0.72
Left	4.84[a]	5.57[b]	5.70[b]	7.09[c]	6.90[c]
	± 0.49	± 0.72	± 0.75	± 0.86	± 0.75

abc mean values in rows bearing with atleast one common type superscript are not significantly different at $P < 0.01$, $P < 0.05$.

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Biometrical Aspects of Gonads and Accessory Glands with Hormonal Profile of Surti Buffalo Calves at Different Age Groups

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ABSTRACT

This study was conducted to understand the development of gonad and accessory glands in male buffaloes from early age to maturity. Totally 24 male calves of different age groups (Group I - 15 to 30 days, Group II - 8 to 10 months, Group III - 14 to 16 months, Group IV - 20 to 24 months) were studied to estimate and record the progressive changes in this aspects. The biometrical characteristics studied were weight, width, length and circumferance of testis, epididymis (caput, corpus and cauda) and seminal vesicles of all groups. Serum testosterone and LH were also estimated.

The results indicated that there was progressive and proportionate increase in all the biometrical observation of testis and accessory glands as the age advances indicating proper growth of glands for reaching puberty by 16 months of age. This has been supported by serum testosterone and LH levels. Both the estimates had shown physiological levels in the animals of Group II (14 to 16 months). The biometrical and harmonal estimates revealed that sensitization for puberty begins around 7 to 9 motnhs and puberty is attained by 16 months is this breed of buffalo males.

——x——x——x——

Buffalo males proved themselves as good breeding sires, work animals and meat sources. Hence greater attention is needed for rearing male calves to attain better body weight and early maturity. Studies on biometry and hormonal aspects of Surti male calves from birth to maturity is presented in this paper.

MATERIALS AND METHODS

Buffalo male calves born in the farm of Reproductive Biology Research Unit, Faculty of Veterinary Science and Ani. Hus., Gujarat Agriculatural University, Anand were utilized for this investigation. All the calves were weaned at birth. The calves were put in 4 age groups and each group had 6 calves. Group I 15 to 30 days, Group II 8 to 10 months, Group III 14 to 16 months, Group IV 20 to 24 months.

All these calves were managed in the farm with standard management practices as per Janakiraman (1985). Right and left testes were collected alongwith complete epididymis and seminal vesicle. Weight width, length and circumference were recorded immediately with help of thread and scale. Small portion of epididymis with testis were squeezed with drop of glass distilled water on a slide to observe spermatozoa. Both testosterone and LH were estimated by radio immuno assay as per the method described by Patel *et al.*,(1992).

Statistical analysis of the results was carried out by a computer employing randomized factorial analysis and Duncon significance test. Correlation and regression analysis was also carried out.

RESULTS

The average values for biometrical characteristic of testis, epididymis (caput, carpus and cauda) and seminal vesicle and hormonal levels have been tabulated in Table - 1.

It was clear that differences in all the characteristics due to age group were highly significant for all the glands (except circumferance for three parts of the epididymis). All the characteristics showed linear increase as the age group advanced.

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Hormonal study revealed that serum testosterone also showed linear increment in the estimate as the age advanced with some fluctuation. It ranged between 1.14±0.2 and 2.21±0.17 ng/ml. The difference between the age group was significant. Serum LH levels fluctuated in the range of 3.53±0.43 to 6.32±0.54 ng/ml with an overall mean of 4.91±0.58 ng/ml (Table 1). The differences between the age group were statistically significant (P<0.5).

Presence of spermatozoa were recorded in testes, cauda and caput epididymis of all the calves of third and fourth groups. Two animals showed spermatozoa in cauda epididymis of Group II.

DISCUSSION

Biometrics of testis: The overall results have suggested that even though there are visual difference in weight, length, width and circumference for left and right side testes, the difference was statistically non significant. Report of Ansari *et al.,* (1972) is also in agreement with the present results. However, the work carried out by Joshi and Karche (1990) on crossbred bulls has shown statistical difference in biometrical observation of left and right testis. Higher testical weight in right side of cattle bull has been reported by Ahmed *et al.,* (1985), Marka *et al.,* (1989) and Babu Rao and Ram Mohan Rao (1990). This may be explained as species difference.

The results of present study on Surti buffalo calves, clearly showed that difference in the biometrical observations were negligeble for animals of lower age and as the age advances the differences become more but not significant. This shows positive association between age and biometrical characters of testis. Similar associations have been recorded by Ahmed *et al.,* (1985) in Nili Ravi buffalo bulls and Joshi and Kharche (1990) in crossbred bulls.

Biometrics of Epididymis: The biometrical observations were taken separately for caput, corpus and cauda epididymis. Overall weight of complete epididymis in the present study observed as 4.15 gm, which is lower than reported values for Nili Ravi buffaloes (Mansoor Ali *et al.,* 1988), whereas Chandrapal and Bharadwaj (1988) reported considerably low values for corpus epididymis compared to present results. This may be explained as breed variation.

The results indicated that most of the biometrical characteristics of all the parts of epididymis showed increase as the age advances. Same types of observations have been reported by Goyal and Dhingra (1973) in Murrah buffaloes.

Biometrics of seminal vesicle: The results of Chandrapal and Bharadwaj (1984) on seminal vesicle of Murrah buffalo and of Osman and Zaki (1971) on bovine showed significantly higher values for thickness, length and weight compared to present results. However, the results of Joshi *et al.,* (1967) on non descript buffaloes seminal vesicle are in agreement with the present results.

Testosterone: The average testosterone was 1.74±0.23 ng/ml. irrespective of age groups. The variation between the age groups was statistically significant (P<0.01) (Table 1 and 2).

The concentration of testosterone increased significantly as the age advances reaching peak in the last group. Similar levels have been reported by Devaraj (1982) for Surti male calves. Whereas the levels were remarkably low in Egyption buffalo calves (Hemeida, 1985).

Present results showed significant increase in serum testosterone level in Group III (14 to 16 months of age). This is the pubertal age in this breed (Devaraj, 1982) where the first ejaculate is also possible.

The correlation study between testosterone and weight of testis (r = 0.476) weight of cauda epididymis (r = 0.335) and width and length of caput (r = 0.350 and r = 0.40) showed positive and significant relationship. Peak testosterone was recorded in animals of Group IV (20 to 24 months) which can be explained as peak activity of interestitial cells at sexual maturity.

Lueteinizing Hormone: The level was highest in animals of Group I which decreased upto Group III whereas in Group IV it again increased. Similar

results have been reported in the ICAR report on buffalo (ICAR, 1990). Such high level at early age is also documented by Rawlings *et al.,* (1972) and Manord *et al.,* (1979). The explanation given is lack of sensitivity at hypothalamo - hypophysial - gonadal axis which gets established as age advances (Agarwal *et al.,* 1983).

Table 1: Average levels of biometrical and hormonal observations of four groups of Surti male calves.

Organ	Character	Group			
		I	II	III	IV
Testis	Wt(gm)	1.21±0.04	6.22±0.61	16.50±1.81	28.40±2.27
	W(cm)	1.81±0.21	2.17±0.15	3.07±0.21	4.16±0.36
	L(cm)	2.54±0.10	3.56±0.24	5.60±0.30	5.76±0.38
	C(cm)	4.95±0.16	6.61±0.47	9.87±0.83	10.88±0.82
Caput	Wt(gm	0.30±0.03	0.94±0.08	1.87±0.24	4.62±0.72
epididymis	W(cm)	1.35±0.07	2.07±0.16	2.51±0.26	3.18±0.20
		2.20±0.05	2.50±0.27	3.30±0.47	4.25±0.29
		3.50±0.20	4.45±0.43	4.44±0.04	5.77±1.29
Corpus	Wt(gm)	0.74±0.12	0.87±0.18	0.94±0.16	1.80±0.82
epidymis	W(cm)	1.26±0.67	1.37±0.39	1.20±0.20	1.36±0.42
	L(cm)	4.19±0.51	4.05±0.62	4.52±0.73	5.19±0.93
	C(cm)	2.24±0.45	2.49±0.87	1.92±0.37	1.57±0.38
Cauda	Wt(gm)	0.22±0.02	0.95±0.12	1.14±0.19	2.13±0.18
epididymis	W(cm)	1.11±0.10	1.65±0.18	1.82±0.12	2.41±0.04
	L(cm)	1.45±0.11	2.00±0.16	2.37±0.16	2.93±0.24
	C(cm)	2.31±0.13	3.79±0.40	4.36±0.54	3.71±0.99
Seminal	Wt(gm)	0.25±0.01	0.98±0.18	1.29±0.10	4.87±0.47
vesicle	W(cm)	0.86±0.08	1.60±0.20	2.39±0.27	3.63±0.33
	L(cm)	1.52±0.15	3.51±0.28	3.82±0.29	5.67±0.43
	C(cm)	2.54±0.22	3.58±0.46	5.07±0.64	9.85±0.88
Testosterone (ng/ml)		1.41±0.20	1.71±0.29	1.91±0.28	2.21±0.17
LH(ng/ml)		5.18±0.75	4.61±0.53	3.53±0.43	6.32±0.54

Table 2 Analysis of Variance

Characters Source	df	Testosterone MSS	Leutisizing Hormone MSS
Age	3	38.41 **	155.84 **
Time	1	4x99 *	85.33 **
Breed	2	13x70 **	31.16 **
Age x Time	6	0x10 NS	4.10 NS
Age x Breed	6	4x45 *	66x373 **
Time x Breed	2	1.225 NS	0.068 NS
Age x Time x Breed	6	2.363 **	1.436 NS
Error	120		
Total	143		

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Type of Bacteria and its Load in Fresh Semen of Cross-Bred Bulls

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ABSTRACT

A study was undertaken to investigate the type of bacteria, its load and antibiogram pattern in the first and second ejaculates of fresh semen of cross bred bulls collected using the same artificial vagina. The average bacterial load per ml in the first ejaculate was 1100 as against 4860 in the second ejaculate. Seven genera of bacteria were isolated from fresh semen and 10 genera from artificial vaginal washings recovered after semen collection. All the isolates from semen were found to be sensitive to ciprofloxacin while only 67 per cent of the isolates showed sensitivity to streptomycin, chloramphenicol and gentamicin. Though tetracycline recorded 50 per cent sensitivity, all the isolates were resistant to penicillin.

——x——x——x——

Microorganisms may be present in bull semen and transmitted to cows at natural or artificial breeding causing genital diseases. The organisms may be bacterial, chlamydial, mycotic or viral and classed as pathogenic, potentially pathogenic or nonpathogenic. These agents may be present or incorporated later and survive in semen during collection, processing, freezing and storage. The principal part of the contamination is saprophytic or opportunistic pathogens from the prepuce and in some cases, from more highly situated parts of the male genital organs (Gangadhar *et al.*, 1986). The present study was taken up to assess the type of bacteria and its load in the first and second ejaculates and the antibiogram.

MATERIALS AND METHODS

A total of 20 semen samples, 10 each from first and second ejaculates of four crossbred bulls were taken with sterile precautions. The same artificial vagina was used for both collections. Standard Plate Count (SPC) method (Benson, 1990) was used to estimate the bacterial load in the semen samples. In order to limit the number of colonies per plate between 30 and 300, semen samples were diluted with sterile peptone water. One half millilitre of whole semen was diluted with 9.5 ml of sterile peptone water. From this 0.5 ml was taken and transferred immediately into 15 ml of melted Meuller Hinton (MH) agar kept in a waterbath at 50°C. The agar was then poured into sterile petridish. After the agar has solidified, the plate was incubated at 37oC. for 24-48 h and examined. The colonies were counted using Quebec colony counter.

Artificial vaginal washings were taken using sterile peptone water immediately after semen collection. The washings were cultured on MH agar. The colonies obtained from semen and artificial vaginal washings were subcultured on MH agar and were subjected to identification upto genus level as described by Cowan (1974). Antibiotic sensitivity of bacterial organisms isolated from semen was carried out by the standard disc diffusion technique employing chloramphenicol, ciprofloxacin, gentamicin, penicillin, streptomycin and tetracycline.

RESULTS AND DISCUSSION

Mean bacterial load in the first ejaculate was 1100 organisms per ml as against 4860 organisms per ml in the second ejaculate. More than a four fold increase in the bacterial load of the second ejaculate can be attributed to a greater contamination of the artificial vagina with the preputial discharge. Hence it is advisable not to use the same artificial vagina more than once even for the same bull.

Bacterial isolates obtained from the semen and artificial vagina were identified and are presented in Table I. Both saprophytic and pathogenic organisms could be isolated from semen and artificial vaginal washings with a predominance of saprophytes in the latter. The results of the present study are well in confirmity with the published report of Kher and Dholokia (1986).

All the isolates were found to be sensitive to ciprofloxacin, while only 67 per cent of the isolates showed sensitivity to streptomycin, chloramphenicol and gentamicin. Tetracycline recorded 50 per cent sensitivity. All the isolates were resistant to penicillin. From this study it could be said that penicillin and streptomycin as semen additives would not fully serve the purpose of reducing the bacterial contamination.

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Table 1: Bacterial isolates from fresh semen and artificial vaginal washings of crossbred bulls

Genus	Fresh Semen		Artificial Vaginal washing	
	Number	%	Number	%
Corynebacterium	2	18.2	1	7.7
Alcaligenes	4	36.3	3	23.7
Kurthia	1	9.1	1	7.7
Streptococcus	1	9.1	2	15.3
Micrococcus	1	9.1	1	7.7
Enterobacteriaceae	1	9.1	1	7.7
Pseudomonas	1	9.1	1	7.7
Staphylococcus	..	..	1	7.7
Eubacterium	..	..	1	7.7
Haemophilus	..	..	1	7.7
Total	11	100	13	100

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Prevalence of Microbes in Frozen Cattle Semen and Their Antibiotic Spectra

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ABSTRACT

Frozen semen samples of cattle were examined by standard methods for microbial contamination. For the bacterial isolates *in vitro* antibiogram studies were carried out with 17 antimicrobial agents. No sensitivity was noticed to nearly all the antimicrobials tested except to amikacin, tobramycin, gentamicin and kanamycin. The results suggest that these could be added to semen diluents instead of conventional antimicrobials.

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Semen gets contaminated with microorganisms either from within the animal or extraneously during collection, processing and preservation. Microbial sterility is compulsory for the quality of both neat as well as frozen semen since successful cattle improvement programme depends on it. Studies on microbial flora of frozen bovine semen (Mohanty *et al.,* 1988) and buffaloe semen (Gangadhar *et al.,* 1986) have been done in India by a few authors. In most frozen semen centres in India Penicillin and Streptomycin are added to control some specific and nonspecific bacteria. There are reports indicating that these antibacterials are no longer effective (Rahman *et al.,* 1983)

The present study was aimed to asses the nonspecific bacterial load of cattle frozen semen and the drug sensitivity of isolated bacteria to antimicrobials so that alternatives could be found in place of penicillin and streptomycin.

MATERIALS AND METHODS

Frozen semen samples from 78 animals of different ages maintained at Cattle Breeding Farm, Eachenkottai, Thanjavur district, Tamil Nadu were studied for the prevalence of microorganisms. The samples were mostly from Jersy bulls but also included eight Murrah bulls and 10 Jersy cross bred bulls. All the animals were in good general and reproductive health and free from any known specific diseases. The samples in straws were brought to the laboratory in liquid nitrogen containers thawed at 37°C and examined. Bacterial count was determined by the method of Miles and Misra, (Miles *et al.,* 1938). Samples were then seeded on common and special media so as to facilitate the growth of common and fastidious microbes. After counting the colonies, representative ones were subcultured for study of the isolates by staining characters. No further attempt was made to identify the isolated organisms to generic or species level. *In vitro* antibiogram test was carried out with 17 antibacterial agents using biodiscs (Span Diagnostics or HI Media, India) by paper disc diffusion technique of Bauer *et al.,* (1966).

RESULTS

The bacterial load of frozen semen samples examined varied from 1.3×10^3 to 2.0×10^4 per ml for 40 positive samples. From 55 out of 78 samples tested, 68 microorganisms comprising 39 gram positive and 36 gram negative and 3 yeasts were isolated. Of the bacterial isolates, 34 Gram positive and 22 Gram negative were taken up for antibiogram studies. More than 60% resistance was noticed to all but kanamycin, gentamicin, tobramycin and amikacin with respect to both groups of bacteria. Gram negative organisms were found to be more resistant to antimicrobials than the other group.

The Gram positive isolates were sensitive to amikacin, tobramycin, gentamicin and kanamycin to the tune of 94.12%, 91.18%, 88.24% and 67.65% respectively. Whereas with the other group maximum sensitivity was observed with tobramycin (81.82%) followed by

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amikacin (77.27%), gentamicin (68.18%) and kanamycin (59.09%), (Table-I).

DISCUSSION

In the present study the bacterial load ranged from 1300 to 20,000 organisms per ml of frozen semen. This load was low when compared with that of Ibrahim *et al.* (1983) but closely agrees with the report of Mohanty *et al.*, (1988) whose range was 120 to 16,800 in frozen bovine semen.

Resistance noticed in this study to penicillin and streptomycin was 100% and above 68.17% respectively though resistance to most others are on the high side. Studies in India (Rahman *et al.*, 1983, Singh *et al.*, 1990) and abroad (Wayda, 1991) have established that most frozen isolates were resistant to these two antimicrobials especially to penicillin which corraborates with the present findings. It was observed that aminoglycoside antimicrobials like gentamicin, tobramycin and kanamycin were most inhibitory to both gram positive and Gram negative isolates. Similar results were recorded by Singh *et al.*, (1990), with frozen semen of crossbred bulls but tobramycin was not included in their study. In another study earlier addition of gentamicin rather than streptopenicillin was found to lower the bacterial load of frozen bull semen (Ahamed *et al.*, 1989), which also agrees with the result of the present report.

Gangadhar *et al.*, (1986) observed that combination of chloramphenicol and kanamycin was superior in reducing the bacterial load of buffloe frozen semen. But in the present study chloramphenicol was found to be ineffective though kanamycin susceptibility was 63.57% which thus partly agrees with the previous report. In general Gram negative group was more resistant to antimicrobials than the other group which might be because of the presence of drug resistant plasmids in most of them.

It may be concluded that tobramycin, gentamicin, amikacin and kanamycin might well be added to diluents either alone or in combination to counteract microbial contamination. But before that, studies are needed to find out the nonspermicidal levels of amikacin and tobramycin as safe levels of other two antimicrobials are already known (Ahamed *et al.*, 1989; Salisbury *et al.*, 1978)

Table 1: Resistance of bacterial isolates from semen to antimicrobials

Name	Gram Positive %	Gram Negative %
Colistin (10 g)	100.00	100.00
Methicillin (5 g)	100.00	100.00
Sulphamethizole (300 g)	91.18	91.00
Nitrofurantoin (300 g)	88.24	72.73
Penicillin-G (10 U)	88.24	100.00
Polymyxin-B (300 U)	85.29	72.73
Clindamycin (2 g)	85.29	100.00
Cephaloridine (30 g)	82.35	95.45
Tetracycline (30 g)	82.35	91.00
Erythromycin (15 g)	75.53	81.82
Streptomycin (10 g)	64.71	72.73
Ampicillin (10 g)	64.71	91.00
Chloramphenicol (30 g)	61.76	77.27
Kanamycin (30 g)	32,35	40.91
Gentamicin (10 g)	11.76	31.82
Tobramycin (10 g)	8.82	18.18
Amikacin (30 g)	5.88	22.73

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Effect of Caffeine On Motility and Fertility Frozen Goat Semen

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ABSTRACT

Addition of caffeine during deep preservation of goat semen in Tris medium was found to have a stimulatory effect on sperm motility. The sperm showed the highest motility in extender with 2 mM caffeine (56.750%) in comparison to the extender with 5 mM (49.388%) or Tris control (47.805%). There was no significant effect on live sperm and acrosomal damage % due to addition of caffein in the extender. Fertility rate with Tris +2 mM caffeine extended semen (59.183%) was higher than that with the Tris diluted semen (50.0%).

——x——x——x——

Freezing and thawing inflict considerable damage to the sperm with loss of motility and concommitant loss of fertility. In the past, attempts have been made to improve the motility of spermatozoa as well as its fertility by incorporating various motility enhancing agents including the methyl Xanthines (Caffeine) as additive in fresh, chilled and frozen semen of livestock and human beings (Amelar *et al.*, 1980). Reports on the effect of caffeine on goat sperm motility and fertility is lacking. Therefore, in the present investigation an attempt was made to study the effect of different levels of caffeine on post thaw motility live sperm % and acrosomal abnormality % and fertility of frozen goat sperm of three different breeds.

MATERIALS AND METHODS

A total of 12 bucks of Beetal and Black Bengal breeds (3 each) and crossbreds (6) were used for this study. One hundred and eight collections (9 from each buck) were take twice weekly. Immediately after collection and motility evaluation, the semen samples having the mass activity of + 4 or above (Sinha, 1986) were extended (1:15 dilution rate) in Tris extender (Raju and Rao, 1983). Extended semen samples were divided into three equal fractions, one of which served as control. In other two fractions Caffeine was added in a concentration of 2 and 5 mM, respectively. All the samples were packaged in Landshut minitube, equilibrated at 4° to 5°C for 5 hours and frozen and preserved in liquid nitrogen.

All the caffeine treated and control semen samples were evaluated 24 hours after freezing. Progressive motility percentage of the spermatozoa was estimated by modified haemocytometer method while the live sperm and acrosome damage was evaluated using eosin-nigrosin stain (Hancock, 1951) and giemsa stain (Watson, 1975) respectively. Estrus goats were inseminated using 2 mM and 5 mM caffeine treated and control frozen semen (49, 20 & 32 goats respectively). Conception rate was calculated on the basis of pregnancy diagnosis / actual kidding. Results were statistically analysed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean post thaw percentage of progressive motility, live sperm and acrosomal damage in different caffeine treated and control samples were presented in Table 1. It is evident that spermatozoa showed the highest progressive motility percentage in Tris extender containing 2 mM caffeine in comparison to Tris wth 5 mM caffeine and also the Tris control extender in all the three breeds. Irrespective of breeds as well, the post - thaw motility was significantly ($P<0.05$) higher in 2 mM caffeine extender than the 5 mM caffeine or control Tris extender. The reports regarding the effect of caffeine on post-thaw goat sperm motility are scarce. However, the results of present study is in agreement with the observations of earlier workers on ovine (Dominguez *et al.*, 1985), bovine (Thakur, 1992) and bubaline (Hukeri, 1989) frozen semen. During the present study

Table 1: Mean percentages of progressive motility, live sperm and acrosome abnormality of thawed sperm frozen with and without caffeine in different breeds in goats

Seminal atteibutes	Semen dilutors	Breeds of goats			Overall (108)
		Beetal (27)	Black Bengal (27)	Crossbred (54)	
Progressive motility % caffeine	Tris	50.220^{b} ±0.836	45.220^{b} ±0.710	47.888^{c} ±0.510	47.805^{c} ±0.408
	Tris + 2mM caffeine	55.230^{a} ±0.839	56.750^{a} ±0.958	56.750^{a} ±0.579	56.750^{a} ±0.407
	Tris + 5mM caffeine	51.222^{b} ±0.569	46.166^{b} ±0.768	50.080^{b}49.388^{b} ±0.429	±0.389
Live Sperm %	Tris	68.44 ±0.742	65.925 ±0.667	67.074 ±0.474	67.129 ±0.352
	Tris +2mM caffeine	68.777 ±0.634	66.055 ±0.991	67.470 ±0.535	67.444 ±0.443
	Tris +5 mM caffeine	68.222 ±0.711	66.440 ±0.922	67.694 ±0.922	8.351 ±0.172
Acrosome abnormality %	Tris	7.722 ±0.463	9.185 ±0.564	8.370 ±0.393	8.351 ±0.172
	Tris +2 mM caffeine	7.050 ±0.691	7.720 ±0.761	7.910 ±0.456	7.763 ±0.330
	Tris +5mM caffeine	8.380 ±0.626	9.270 ±0.748	7.972 ±0.458	8.402 ±0.336

Figures having different superscript within a column differ significantly (P<0.05)
Figures in the parenthesis represent the number of observations.

2 mM caffeine was found superior to 5 mM caffeine. Dominguez *et al.,* (1985) reported that caffeine had a dose dependent effect on the motility of frozen ovine spermatozoa and 2 mM caffeine was preferable. The mean live sperm and acrosomal abnormality percentage in different dilutors were not significantly affected by addition of caffeine in any breed of goats (Table 1).

The overall conception rate was 59, 55 and 50% with the 2 mM caffeine, 5 mM caffeine and control extender, respectively which did not differ significantly. Nevertheless, the conception rate of semen preserved with 2 mM caffeine was apparently higher than the control sample. This finding is in agreement with the observations of Hukeri (1989), Thakur (1992) and Bhosrekar *et al.,* (1992), where in higher conception rate with caffeine treated frozen semen was recorded in cattle and buffalo.

Motility stimulation of spermatozoa by caffeine has been postulated to be due to inhibition of phosphodiesterase activity and the subsequent rise in the intrasperm cAMP level (Bhatnagar *et al.,*1979). Elevated intrasperm cAMP inturn increases energy production by accelerating the glycolysis and TCA cycle which is utilized by the motile apparatus of the spermatozoa (Haesungcharern and Chulavatnatol, 1973). Intracelluar cAMP also acts as a regulator of Ca ion fluxes in the spermatozoa (Peterson *et al.,* 1979 and Aitken *et al.,* 1983). It appears reasonable to infer, therefore, that due to aforesaid reasons caffeine was able to alleviate the effects of freezing / thawing to a great extent and the sperm showed good motility during the present study.

The fertility trial was conducted on a smaller population of goats and results indicated an apparent increase in conception with caffeine treated semen samples. It has been reported that there is an increased concentration of intracellular cAMP during sperm capacitation (Morton and Albagli, 1973). Since caffeine increased cAMP, it was presumed that it might play an important role in capacitation and fertilization. However, experimentation on a larger population is needed for substantiation of the later findings.

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Preservation of Osmanabadi And Crossbred Buck Semen At Refrigerant Temperature

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ABSTRACT

Tris-Fructose-Citric acid (Mathews), Tris-Fructose-Citric acid (Hahns) and Laiciphos extenders were used for preservation of buck semen at refrigerant temperature. Tris-Fructose-Citric acid (Hahns) was found superior over other two extenders giving 52 per cent motile sperms at 72 hours of preservation of both Osmanabadi and crossbred buck semen. Out of six additives used in Tris-Fructose-Citric acid (Mathews), namely EDTA-potassium, EDTA-Calcium, Prostaglandin, Seminal plasma, Caffeine and Chlorpromaxine hydrochloride, the EDTA-Potassium and prostaglandin were more effective than other additives. EDTA-potassium as additive gave 60 per cent motile sperms for both Osmanabadi and crossbred buck semen while prostaglandin gave 53 and 58 per cent motile sperms for Osmanabadi and crossbred buck semen at 72 hours of preservation respectively.

——x——x——x——

Artifical insemination in goats has not gained momentum as in cattle. Studies on preservation of buck semen were carried out by Nimkar (1977), Deka and Rao (1985). Osmanabadi is a dual purpose bread from Marathwada region. Studies on preservation of crossbred buck semen are not reported in literature. Present studies were undertaken to determine the effect of dilutors on preservation of Osmanabadi and crossbred buck semen at refrigerant temperature and also to know the effect of various additives on semen preservation.

MATERIALS AND METHODS

Four Osmanabadi and four crossbred bucks (Alpine x Osmanabadi) of 2 to 5 years age with good servicing ability and sex libido were selected for present study. Semen from each buck was collected twice a week over a period of two months using artifical vagina method. Ejaculates with optimum mass activity (+++) and initial motility (70-80) were extended and preserved at refrigerant temperature. Extenders used were Tris-Fructose-Citric acid (Mathews etal 1984), Tris-Fructose-Citric acid (Hahns, 1972) and Laiciphos.

Tris-Fructose-Citric acid (Mathews) extender was used with six different additives Viz. EDTA-Potassium, EDTA-Calcium, Prostaglandin, Seminal plasma of bull, Caffiene puris and Chlorpromaxine hydrochloride.

One per cent solution of EDTA potassium and calcium salts were prepared separately. These solutions were added to extender at 5 per cent level. Prostaglandin F_2 was added to the extender at 0.03 mg per ml of extended semen. Seminal plasma from Red Kandhari bull was added to the extender at equal quantity to that of semen volume. Caffiene puris solution 2.72 per cent was prepared and from this solution 0.05 ml was added to 9.5 ml of extended semen.

The motility of extended semen samples was recorded after 0,24,48 and 72 hours of preservation at 5°C.

RESULTS AND DISCUSSION

Percentage of motile spermatozoa after 72 hours of preservation at 5°C for Osmanabadi buck semen was observed as 52, 49 and 39 per cent in Tris (Hahns), Tris (Mathews) and Laiciphos extenders respectively. Motile spermatozoa after 72 hours of preservation at 5°C. for crossbred buck semen were observed as 52, 47 and 30 per cent in Tris (Hahns), Tris (Mathews) and Laiciphos extenders respectively (Table). Significant difference was observed in between the extenders, the breeds and also within the individual bucks ($P<0.01$), at 72 hours of preservation.

Present findings are in agreement with Mathew (1983) who reported better preservation

of buck semen in Tris at 6°C. Parandekar (1987) observed similar motility pattern after preservation of Osmanabadi buck semen at 5°C. Motility of semen after preservation at 5°C with Laiciphos extender is found lower as compared to the findings of Gonzales and Stagnaroc (1976) who recorded it as 68 per cent. Motility of 39 per cent on preservation of semen with Laiciphos extender is found lower as compared to the findings of Gonzales and Stagnaroc (1976) who recorded it as 68 per cent. Motility of 39 per cent on preservation of semen with Laiciphos extender is higher than the observations of Baviskar (1985) who reported it as 30 per cent in Osmanabadi buck semen.

Percentage of motile spermatozoa in buck semen of Osmanabadi breed extended with Tris (Mathews) after 72 hours of preservation at refrigeration temperature were respectively 60, 53, 51, 50, 49, 23 and 49 per cent on addition of EDTA-Potassium, Prostaglandin, EDTA-Calcium, Seminal plasma, Caffiene, Chlorpromazine hydrochloride and tris (control), whereas for Osmanabadi crossbred semen the values were 60, 58,55, 55, 50, 20 and 47 per cent respectively (Table).

Addition of EDTA-Potassium, Prostaglandin, EDTA-Calcium and Seminal plasma as additives was observed to favour the semen preservation of both Osmanabadi and crossbred bucks at 5°C. Malik *et al.*, (1985) and Parandekar (1987) also reported that addition of seminal plasma favours semen preservation. Significant increase in survival rate of spermatozoa was reported by El-Gaffary (1986) on addition of prostaglandin in diluted semen.

The effect of dilutors and additives on semen preservation at refrigerent temperature.

	Motility Percentage on preservation of semen at 5°C. (Mean of 16 observations)							
	Osmanabadi buck semen				Cross-bred buck semen			
	0 hr	24 hrs	48 hrs	72 hrs	0 hr	24 hrs	48 hrs	72 hrs
Dilutors:-								
Tris (Hans)	67	59	55	52	68	59	54	52
Tris (Mathews)	68	57	52	49	68	57	51	47
Laiciphos	67	55	46	39	66	57	47	39
Additives:-								
EDTA-Potassium	70	65	62	60	71	66	62	60
Prostaglandin	70	63	56	53	70	66	62	58
EDTA-Calcium	69	61	55	51	71	65	60	55
Seminal Plasma	68	61	54	50	72	64	60	55
Caffiene	68	59	52	49	68	59	53	50
Chlorpromazine hydrochloride	63	40	29	23	61	40	28	20
Tris (Mathews) control	67	57	52	49	68	57	51	47

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Physico-Biochemical Characteristics of Semen In Relation To Libido And Service Behaviour of Buffalo Bulls*

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ABSTRACT

Seminal ejaculates from six healthy, sexually mature buffalo bulls were evaluated for physico-biochemical characteristics and their correlations were recorded with the libido and service behaviour. The overall mean per cent libido score, reaction time and service behaviour score were 72.22±0.07, 59.13±2.98 sec and 56.6±0.20, whereas the mean volume, mass activity, sperm concentration, progressive sperm motility, fructose and citric acid content were rated as 3.34±0.13 ml, 3.08±0.05, 1273.05±43.26 millions per ml, 72.08±0.42 per cent, 511.80±19.83 mg % and 404.17±175.24 mg respectively. A significant ($P<0.05$) correlation of libido score was found with progressive sperm motility ($r = 0.80$), seminal fructose ($r = 0.94$) and citric acid ($r = 0.84$). Reaction time was correlated with sperm concentration ($r = -0.89$), sperm motility ($r = -0.73$), fructose ($r = -0.91$) and citric acid ($r = -0.86$) whereas service behaviour was correlated significantly with sperm motility ($r = 0.65$).

——x——x——x——

Good libido and mating ability are important components of sexual behaviour in bulls. Proper understanding of sexual behaviour may help to develop improved practices and procedures of collecting and processing semen. Saxena and Singh (1974) reported a good relationship between libido and total number of sperm ejaculated and the semen quality. The present paper communicates the relationship of physical and biochemical characteristics of semen with libido and service behaviour of buffalo bulls.

MATERIALS AND METHODS

Six sexually mature, healthy buffalo bulls stationed at Dairy Farm, Punjab Agricultural University, Ludhiana were included in the present study. The bulls were maintained under identical conditions with regard to nutrition and management. The animals were given exercise for 30 min. on alternate days in the morning by manual exercise.

The libido and service behaviour of bulls was recorded at the time of semen collection as described by Singh and Pangawkar (1989) and was expressed in percentage. The reaction time was recorded as the interval between the moment bull approaches the dummy and the first mount with or without protrusion of penis outside the sheath and recorded in seconds.

The semen was collected regularly from bulls with the help of artificial vagina (42-45°C) with minimum six ejaculates from each bull. Each ejaculate was evaluated for physical characteristics *viz.* volume, mass activity, individual motility, sperm concentration by standard procedure of Herman and Madden (1953). The seminal fructose was estimated by the method of Mann (1948) while citric acid was measured according to Saffron and Denstedt (1948). The data was analysed statistically according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The overall mean per cent libido score was recorded to be 72.72±0.07. Singh and Pangawkar (1989) has reported a higher value of per cent libido score in ox bulls which indicates that buffalo bulls have relatively poor libido. The difference in libido score might be attributed to species variation as it has been reported to be stongly influenced by genetic factors (Chenoweth, 1983). The reaction time for buffalo bulls was 59.13±2.90 seconds. Bhosrekar *et al.,* (1988)

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have reported an average reaction time of 3.09 minutes. The shorter reaction time reported in the present study might be due to climatic conditions, fodder availability, time of semen collection and training of bulls at early age (Bhosrekar *et al.,* 1988).

The service behaviour of buffalo bulls were recorded by noting a series of events taking place at the time of semen collection. A maximum score of 13 was given for each collection and the average score value for a particular bull after collecting six ejaculates (78 score for each bull) was expressed in percentage. The overall mean score value for buffalo bulls was recorded to be 56.62±0.20 per cent. Similar findings were reported by Bhosrekar *et al.,* (1988) who opined that proper training of bulls with uniform management practices like least changes in attendants, bull pens, collection procedures and collector helps in setting up uniform behavioural pattern for getting optimum results.

The overall mean semen volume, mass activity, individual motility, sperm concentration were 3.34±0.14 ml, 3.08±0.05, 72.08±0.42 per cent and 1273.05±43.26 millions per ml respectively. The seminal fructose and citric acid content were 511.80±19.83 mg per cent and 404.17±5.24 mg per cent, respectively. The seminal values reported in the present study are within the normal range of fertile semen and presented to calculate correlation with other characteristics.

Correlation studies (Table) revealed significant ($P<0.05$) correlations of libido score with spermatozoan motility ($r = 0.80$), seminal fructose ($r = 0.94$) and citric acid content ($r = 0.84$). Saxena and Singh (1974) also reported a good relationship between libido and semen quality. Significant ($P<0.05$) negative correlations were obtained between reaction time and sperm concentration ($r = -0.89$), sperm motility ($r = -0.73$), seminal fructose ($r = -0.91$) and citric acid content ($r = -0.86$). These correlations suggest that bulls who require shorter period for sexual excitement donate semen of superior quality. Service behaviour score was positively ($r = 0.65$) correlated with spermatozoan motility. The present study indicates that buffalo bulls showing good libido and service behaviour with less reaction time are more likely to donate semen of better quality.

Table 1: The correlation coefficients between testicular measurements and physico-biochemical characteristics of buffalo bull semen.

Parameter	Sperm concentration (m / ml)	Individual motility	Fructose (mg %)	Citric acid (mg%)
Libido score (%)	0.59	0.80**	0.94**	0.84**
Reaction time(s)	-0.89**	-0.73**	-0.91**	-0.86**
Service behaviour score (%)	0.14	0.65*	0.48	0.59

* Significant at 1% probability level.

** Significant at 5% probability level.

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Interference of Protein in the Cholesterol Estimation of Seminal Plasma in the Bovines*

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ABSTRACT

The total cholesterol content in the seminal plasma of 3 Murrah, 3 Friesian and 3 Crossbred bulls was estimated before and after deproteinization of samples with acetone-alcohol mixture. Total protein was also determined simultaneously in whole samples. Highly significant ($P<0.01$) positive correlations of 0.85, 0.67 and 0.74 were observed for the three breeds between total protein and cholesterol estimates of whole seminal plasma as against negligible ones (0.33, 0.18 and 0.27) with the actual cholesterol contents of deproteinized samples. The total protein contents in the seminal plasma of Murrah, Friesian and crossbred bulls averaged 4.45 ± 0.16, 8.30 ± 0.28 and 8.97 ± 0.49 g%, respectively ($P<0.01$). The cholesterol contents of whole samples were 134.03 ± 4.27, 461.21 ± 18.65 and 550.64 ± 21.16 mg% ($P<0.01$) and of deproteinized samples as 71.02 ± 1.91, 78.49 ± 2.33 and 73.20 ± 3.88 mg%, respectively. These findings suggested very high interference of total protein contents in the cholesterol estimates of whole seminal plasma. Hence the total cholesterol should only be determined in the protein-free filtrates of seminal plasma to obtain reaslistic and accurate values while comparing different breeds / species of animals.

——x——x——x——

The protein and cholesterol contents are known to provide amphoteric property of seminal plasma and protective mentle of sperm plasma membrane. However, the mean values of total protein and cholesterol as determined by using different assay techniques with or without deproteinization of seminal plasma have been reported to vary greatly from 1.99 to 12.48 g% and 45 to 752 mg%, respectively, in ox and buffalo bulls (Singh *et al.*, 1969; Prabhu *et al.*, 1973; Saxena and Tripathi, 1979; Nema *et al.*, 1983; Kumar *et al.*, 1988; Dhami *et al.*, 1990). These undue variations in the profiles really indulged the authors to look into the realistic findings of cholesterol contents of seminal plasma by using two different assay techniques simultanesouly in relation to the protein contents of bull and buffalo semen.

MATERIALS AND METHODS

This comparative study was conducted during the period from October to February 1990-91 on semen of 3 Murrah, 3 Friesian and 3 triple crossbred bulls, aged 3 to 5 years. The bulls were maintained under uniform feeding and managerial regimens throughout the study at the Germ Plasma Centre of IVRI, Izatnagar. Semen was collected at weekly interval from each bull and was evaluated for its quality by routine tests. All the initially nonmotile / static ejaculates and another randomly selected 7 to 15 motile ejaculates (total 18) obtained from each bull were centrifuged at 2500 rpm for 30 minutes. The seminal plasma siphoned out was stored in sterile vials at -15°C till assayed. The total protein content of seminal plasma was determined by using Biuret reagent (Henry *et al.*, 1957) and the total cholesterol was estimated in whole samples without deproteinization or in the protein-free filtrates of alcohol-acetone treated / deproteinized samples as per King and Wotten (1956). The data on protein and cholesterol contents of each breed were analyzed separately using simple and / or factorial CRD and the correlation coefficients between them were worked out as per the standard statistical procedures.

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RESULTS AND DISCUSSION

The overall mean total protein content of seminal plasma found in Murrah bulls (4.45±0.16 g%) was significantly (P<0.01) lower than in the Friesian or crossbred bulls (8.30±0.28 and 8.97±0.49 g%). These findings supported the earlier observations of Singh *et al.*, (1969), Dabas *et al.*, (1982) and Dhami *et al.*, (1990). Also the initially static ejaculates had significantly (P<0.01) lower total protein content than in the motile ejaculates in both Murrah and Friesian bulls (Table 1). These observations compared favourably with the reports of Nema *et al.*, (1983) and Dhami and Kodagali (1989). The bull to bull variation was also significant for total protein contents in all three breeds / species (Table 1, 2) and agreed with most of the above reports. The seminal proteins are said to be more like milk-glycoproteins and influence the salt-mineral balance of semen (Crabo and Jayendran, 1979). It consists of nonprotein nitrogen, amino acids and peptides which contributes towards the amphoteric property of seminal plasma (Dabas *et al.*, 1982; Dhami *et al.*, 1990).

The total cholesterol content of whole seminal plasma, when estimated without deproteinization, revealed highly significant (P<0.01) differences between breeds studied, the values being higher in ox-bulls than buffalo-bulls. But the same when estimated in the protein-free filtrates of deproteinized samples did not vary significantly (Table 1, 2). These findings on the cholesterol estimates of whole samples compared well with the observations of Saxena and Tripathi (1979), Nandroo *et al.*, (1987) and Kumar *et al.*, (1988) in their comparitive studies on cattle and buffalo semen / seminal plasma without deproteinization. These estimates were indeed highly erroneous in all above reports, since they are greatly influenced by the protein contents of seminal plasma especially in ox-bulls due to its higher protein content which interfers with colour complex. Further, the real cholesterol contents of deproteinized samples of Murrah, Friesian and crossbred bulls obtained in the present study were slightly higher than the values of 49 to 62 mg% reported in either cattle or buffalo breeds by Varsheney *et al.*, (1978), Nema *et al.*, (1983) and Verma *et al.*, (1985), but were lower than those reported by Prabhu *et al.*, (1973). The findings of significantly higher total cholesterol content even in deproteinized samples of cattle than the buffalo semen reported by Prabhu *et al.*, (1973) were, however, not obsered in our study with protein-free filtrates.

There were very close parallelisms (P<0.01) between the total protein and the cholesterol estimates of whole samples with the correlation coefficients of 0.85, 0.67 and 0.74 in Murrah, Friesain and crossbred bulls, respectively. However, no such relationships were observed for protein and the actual cholesterol contents of deproteinized samples in any of the breeds, and their correlation coefficients in fact were very negligible (r = 0.33, 0.18 and 0.27, respectively). Dabas *et al.*, (1982) and Nandroo *et al.*, (1987) also did not find significant correlations of total protein with cholesterol in bull and buffalo seminal plasma. The cholesterol contents estimated with either of the methods were significantly (P<0.01) lower in static than in motile ejaculates of Murrah bulls. But it did not vary significantly in Friesian bulls. Nema *et al.*, (1983) and Verma *et al.*, (1985), however, failed to observe significant difference in the cholesterol content of static vs motile and good vs poor quality ejaculates in Surti and crossbred bulls, respectively. The bull to bull variation was significant (P<0.01) for cholesterol contents of whole seminal plasma only in ox-bulls.

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Table 1: Cholesterol content of seminal plasma as influenced by its protein content and type of ejaculates in Murrah, Friesian and crossbred bulls.

Breed/ species	Bull No./ Semen type	No.of Sample	Total Protein (g%)	Total cholesterol (mg%)	
				without deproteinization of samples	following deproteinization of samples
Murrah	M-1248	18	4.06±0.15^{a}	130.28±05.17	68.69±2.91
	M-1281	18	4.72±0.25^{b}	143.06±09.14	72.01±3.55
	M-1296	18	4.58±0.29^{b}	128.75±7.29	72.41±3.45
	Static	17	3.84±0.20^{x}	119.22±06.84^{a}	63.46±3.87^{x}
	Motile	37	4.73±0.28^{y}	140.83±05.18^{y}	74.45±1.98^{y}
	Overall	54	4.45±0.16**	134.03±04.27**	71.02±1.91
Friesian	F-594	18	7.19±0.46^{a}	405.78±23.88^{a}	80.22±4.36
	F-625	18	8.14±0.25^{b}	586.38±20.64^{b}	80.68±4.14
	F-678	18	9.60±0.29^{c}	391.47±12.20^{a}	77.57±3.79
	Static	9	7.14±0.63^{x}	438.45±26.59	76.01±6.11
	Motile	45	8.53±0.36^{y}	474.87±22.18	84.40±3.22
	Overall	54	8.30±0.28	461.21±18.56	78.57±2.33
Crossbreds	CB-201	7	9.76±0.85^{b}	510.36±30.30^{a}	73.25±7.98
	CB-1290	7	9.88±0.88^{b}	638.96±26.15^{b}	74.86±7.01
	CB-1296	7	7.26±0.40^{a}	502.60±30.33^{a}	71.49±6.05
	Overall (motile)	21	8.95±0.49	550.64±21.16	73.20±3.88

** P<0.01 between breeds: Column means bearing uncommon superscripts differed significantly between bulls or semen types within the breed.

Table 2: ANOVA (MSS) showing the effect of bull, semen type and its interaction on total protein and cholesterol content of seminal plasma of Murrah Friesian and crossbred bulls.

Source of variation	d f	Total protein	Cholesterol without de-proteinization	cholesterol after de-proteinization
Murrah bulls (MB)	2	2.212*	1111.17ns	73.681ns
Semen types (ST)	1	9.330**	5477.48**	1419.331**
MB x ST	2	0.369ns	951.35ns	58.326ns
Remainders	48	0.899	899.22	185.914
Friesian bulls (FB)	2	26.816**	141618.02**	50.749ns
Semen types (ST)	1	14.644**	5954.95ns	770.636ns
FB x ST	2	2.767ns	6189.69ns	771.572*
Remainders	48	2.250	4559.99	273.835
Crossbred bulls	2	15.368*	41056.31**	19.804ns
Remainders	18	3.853	5884.04	348.360

* P<0.01 ** (P<0.01); ns = nonsignificant.

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Morphological changes of acrosome at different stages of processing of buck semen during freezing with fructose egg yolk glycerol extender*.

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ABSTRACT

Fifteen pooled ejaculates from 6 Beetal bucks were used to study the different morphological changes of acrosome during different stages of freezing in fructose egg yolk glycerol extender with 5, 6 and 7 percent glycerol levels and 1,3 and 6 hours of equilibration periods.

The overall mean percentage of swollen, separating and entirely lost acrosome did not differ significantly between glycerol levels but differ significantly between equilibration periods.

The lowest percentage of swollen, separating and entirely lost accrosome were recorded after 1 hour of equilibration. After freezing, the lowest percentage of swollen, separating and entirely lost acrosome was recorded at 3 hours equilibration period with 7 percent glycerol level.

——x——x——x——

Though several studies were made onr am and bovine semen to study the acrosomal integrity with different extenders, reports on buck semen are scanty. Hence, the present study was undertaken to record the effect of fructose egg yolk glycerol extender on acrosomal integrity during freezing of buck semen.

MATERIALS AND METHODS

Fifteen pooled ejaculates from 6 Beetal Bucks kept in the Goat Research Station, Burnihat, Assam Agricultural University were used for the study. The semen was collected once in a week with artificial vagina. Three different percentage of glycerol viz. 5,6 and 7 and three different equilibration periods viz. 1,3 and 6 hours were used during the processing of buck semen for freezing in fructose egg yolk glycerol extender.

The morphological changes of acrosome were studied using Giemsa stain as per the method of Watson (1975) and they were classified as swollen, separating and entirely lost acrosome (Watson and Martin, 1972).

The statistical analysis of the datas were made as per the methods of Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

The percentage of swollen, separating and entirely lost acrosome at different stages of processing the semen for freezing are presented in Table I. and Fig. I.

The incidence of acrosomal defects did not differ significantly between bucks in the study. The values in fresh semen were found to be lower than the findings of Deka (1984). The increase in percentage of damaged acrosome alongwith the decrease in glycerol concentration, observed in the present study was in accordance with the finding of Jainudeen and Dass (1982). A higher percentage of damaged acrosome could be recorded with 5 percent glycerol level than 7 percent. But, Deka (1984) recorded higher percentage of damaged acrosome with higher percentage glycerol in buck semen. Bucker *et al.*, (1977) reported that the percentage of glycerol did not affect the percentage intact acrosome whereas Curiel and Mendez (1981) reported 31.5 percent abnormal acrosome with 5 percent glycerol level.

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Table 1: Percentage of swollen, separating and entirely lost acrosome (Mean* ± SE) in FEYG** extender at 5, 6 and 7 percent glycerol level at 1, 3 and 6 hours of equilibration during different stages of freezing.

	5 Percent glycerol			6 percent glycerol			7 percent glycerol		
Stages	Swollen	Separating	Entirely lost	Swollen	Separating	Entirely lost	Swollen	Separating	Entirely lost
Fresh semen	2.50 ± 0.20	0.08 ± 0.90	0.06 ± 0.03	2.50 ± 0.24	0.08 ± 0.09	0.06 ± 0.03	2.50 ± 0.24	0.08 ± 0.09	0.06 ± 0.03
	Overall mean - 0.0880±0.12			Overall mean - 0.88±0.12			Overall mean 0.88±0.12		
After glycerolization	2.87 ± 0.45	0.31 ± 0.20	0.09 ± 0.08	4.33 ± 0.59	0.11 ± 0.17	0.09 ± 0.08	4.83 ± 0.68	0.39 ± 0.19	0.09 ± 0.08
After equilibration									
1 hour	9.23 ± 1.46	1.28 ± 0.25	0.45 ± 0.18	8.90 ± 1.10	0.19 ± 0.83	0.29 ± 0.14	7.98 ± 0.91	0.47 ± 0.17	0.27 ± 0.18
3 hours	13.29 ± 0.19	0.43 ± 0.22	0.62 ± 0.26	11.47 ± 0.88	0.95 ± 0.34	0.47 ± 0.17	11.97 ± 0.82	0.45 ± 0.18	0.29 ± 0.20
6 hours	15.22 ± 1.91	1.06 ± 0.20	0.82 ± 0.30	14.70 ± 1.22	0.84 ± 0.36	0.27 ± 0.23	15.59 ± 0.84	1.26 ± 0.23	0.81 ± 0.42
After Freezing									
1 hour	17.05 ± 0.66	1.59 ± 0.32	1.28 ± 0.23	16.55 ± 0.69	1.08 ± 0.21	0.48 ± 0.17	16.63 ± 0.64	1.40 ± 0.44	0.52 ± 0.27
3 hours	18.45 ± 0.72	2.56 ± 0.40	0.86 ± 0.28	17.71 ± 0.90	1.24 ± 0.47	0.81 ± 0.42	15.78 ± 0.42	1.06 ± 0.29	0.29 ± 0.17
6 hours	21.96 ± 0.69	1.73 ± 0.32	1.77 ± 0.39	21.77 ± 0.64	1.89 ± 0.31	1.77 ± 0.34	21.33 ± 0.95	1.89 ± 0.62	1.77 ± 0.40

*, 15 observations.
**, Fructose egg yolk glycerol extender.

It was recorded that the increase exposure with glycerol was detrimental to the integrity of acrosome during freezing. This was in agreement with the findings of Deka (1984) in buck semen. Similarly, a lower percentage of damaged acrosome was recorded by Aguirre *et al.*, (1978) When the semen was equilibrated for 2 and 4 hours than 5 and 22 hours.

Gonzalez (1976) obtained higher percentage of conception rate at 1 hour of equilibration period in ram semen. Patt and Nath (1969) reported that 6 hours of equilibration was more detrimental than 1 hour equilibration in freezing of ram semen. The same observation could be recorded in the present study.

The mean percentage of swollen, separating and entirely lost acrosome after freezing was much lower than the findings of Chaudhury (1985) and was in accordance with the findings of Deka (1984). The overall mean percentage of total acrosomal changes was found to increase significantly ($P<0.01$) due to freezing. The observation was similar to the findings of Deka (1984) in buck semen.

Gokcen and Asti (1980) recorded 20.70 percent damaged acrosome at equilibration stage in ram semen. Milovanov *et al.*, (1970) recorded damaged acrosome upto 90 percent and Neves (1983) recorded 40 percent deduction of intact acrosome during freezing in buck semen.

These variations of damaged acrosome could be due to the different in composition of extenders (Wilmut and Polge, 1972), species of animal used (Watson and Martin, 1972) and on the rates of freezing and thawing (Coulter and Foote, 1974).

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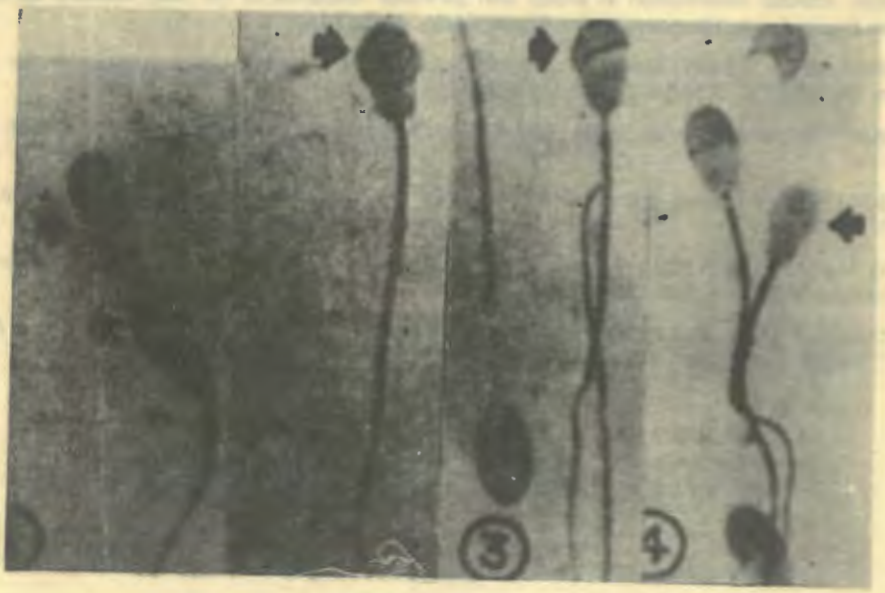

Fig 1.Different Morphological changes of acrosome
Normal acrosome
1.2 Swollen acrosome
1.3 Separating acrosome
1.4 Entirely lost acrosome

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Study on Freezing of Boar Semen in Three Extenders*

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ABSTRACT

The sperm rich fractions of 30 ejaculates collected using gloved hand technique from 5 Hampshire X Indigenous boars were used to study the relative efficacy of Lactose egg yolk glycerol (LEYG), TES Tris glucose egg yolk glycerol (TESTGEYG) and Tris egg yolk dextrose EDTA citric acid glycerol (TEYDEDCAG) extenders for freezing of semen. Prior to processing, the semen was held at 24°C for 3 hours. After 3 hours of equilibration at 5°C, the semen was frozen in 0.5 ml French straws using liquid nitrogen. The sperm motility and acrosomal changes were studied after equilibration and after freezing. In LEYG, TESTGEYG and TEYDEDCAG extenders, the sperm motility was 74.44±0.74. 67.88±0.62 and 68.25±0.81 per cent respectively after equilibration and 50.51±1.33, 37.96±0.74 and 33.94±0.79 per cent respectively after freezing and the intact acrosome was 80.42±0.68, 76.71±0.74 and 77.02±0.81 per cent respectively after equilibration and 58.26±0.81, 48.96±0.91 and 45.79±0.87 per cent respectively after freezing. The sperm motility and intact acrosome were significanty ($P<0.05$) higher in LEYG extender than in TESTGEYG and TEYDEDCAG extenders.

It is evident from the study that LEYG extender is superior to TESTGEYG and TEYDEDCAG extenders for freezing of boar semen.

——x——x——x——

The quality of frozen semen depends largely on the extender used for freezing. Although a few studies were reported from other countries to evolve a suitable extender for freezing of boar semen, there was diversity of opinion about the best extender (Schorner, 1974; Rillo *et al.*, 1980; Paquignon, 1985). The present study was undertaken to record the relative efficacy of 3 extenders for freezing of boar semen.

MATERIALS AND METHODS

Semen was collected using gloved hand technique from 5 Hampshire X Indigenous boars (2 boars of 50% and 3 boars of 75% exotic inheritance). The comparative efficacy of 3 extenders *viz.*, Lactose egg yolk glycerol (LEYG) (Park and Pursel, 1985), TES Tris glucose egg yolk glycerol (TESTGEYG) (Moore and Hibbitte, 1977 and Tris egg yolk dextrose EDTA citric acid glycerol (TEYDEDCAG) (Pursel *et al.*, 1978) was studied using sperm rich fractions of 30 ejaculates comprising 6 from each boar showing initial sperm motility of 70 per cent or more by split sample technique. The extenders were prepared in 2 fractions (non-glycerolated and glycerolated fractions). Antibiotics @ 60 mg penicillin and 100 mg streptomycin per 100 ml of the extenders were added to all the extenders. The pH of TESTGEYG and TEYDEDCAG extenders was adjusted at 7.2 Prior to use, LEYG extender was made clear by centrifugation at 3000 r.p.m. for 15 minuted.

Immediately after collection the sperm rich fraction of the ejaculate was held in a BOD incubator at 24°C for 3 hours. after that it was split into 3 parts and centrifuged at 1000 r.p.m. for 10 minutes at 24°C. The supernatant fluid was discarded and the centrifugate was resuspended (1:1) in non-glycerolated fraction of the extender. It was then cooled to 5°C in 1.5 hours. The glycerolated fraction of the extender (amount equal to that of non-glycerolated fraction) was added to the initially extended semen at 5°C in 3 steps at 15 minutes intervals. After 3 hours of equilibration at 5°C, the semen packed in French straws (0.5 ml) was frozen by exposing it to liquid nitrogen vapour for 10 minutes inside a freezing container, 5 cm above the liquid nitrogen level. The frozen semen after 12-16 hours of storage in liquid

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nitrogen was thawed in water at 40°C for 12-15 seconds. The semen was evaluated after equilibration and freezing for its sperm motility and acrosomal changes. The acrosomal changes were studied using Giemsa staining technique of Watson (1975). The statistical analysis of the data was done as per Snedecor and Cochran (1967) after angular transformation of the percentages.

RESULTS AND DISCUSSION

The mean sperm motility and acrosomal changes after equilibration and freezing in different extenders are presented in Table 1.

The sperm motility after freezing in all the 3 extenders was markedly lower than that after equilibration. This was in agreement with the observations of Moore and Hibbitt (1977) and Vengust *et al.,* (1983). The sperm motility recorded after freezing in the 3 extenders was higher than that recorded by Paquignon (1985) in semen extended in Lactose egg yolk-OEP and Glucose egg yolk extenders and frozen in pellets, 0.5 ml and 5 ml straws. The sperm motility after freezing in LEYG extender was observed to be higher than that of Park and Pursel (1985) in semen frozen in 5 ml straws using same extender. In TESTGEYG extender, the sperm motility after freezing was higher than that recorded in pellet frozen semen by Moore and Hibbitt (1977), Schorner (1974) and Vengust *et al.,* (1983) recorded higher post-thaw sperm motility in Tris extender than that obtained in this study. The higher sperm motility after freezing recorded in the present study than that in the studies of Park and Purse (1985) and Moore and Hibbitt (1977) might be due to holding of undilute semen at 24°C for 3 hours and freezing semen in 0.5 ml straws.

The mean sperm motility after equilibration and freezing differed significantly ($P<0.01$) between extenders but not between genetic groups of boars (50% and 75% exotic inheritance) and due to extender X genetic group interaction. Critical difference test showed that sperm motility after equilibration and after freezing, irrespective of genetic group of boars was significantly ($P<0.05$) higher in LEYG extender than in TESTGEYG and TEYDEDCAG extenders (Table 1). This could be due to the presence of sugar lactose that might have better cryoprotective action than other sugars or it could be that LEYG extender might be best suited for the set of conditions of processing and freezing followed in the study.

The percentages of intact acrosomes recorded after freezing in the 3 extenders were comparable to the values of normal apical ridge in semen frozen in pellets and straws (Aalbers *et al.,* 1985). The percentage of intact acrosome was significantly ($P<0.05$) higher in LEYG extender than in TESTGEYG and TEYDEDCAG extenders (Table 1). Similarly, Paquignon (1985) observed that the percentage of sperm with normal acrosome was higher in Lactose egg yolk-OEP extender than in Glucose egg yolk extender and was still higher with straws than with pellets. Rillo *et al.,* (1980) reported that lactose containing extender reduced acrosomal damage during freezing than did glucose containing extender. Lactose was reported to provide best protective action on acrosome during freezing (Wilmut and Polge, 1977). This sugar might have nutralised the deleterious effect of glycerol on acrosome of boar sperm. The incidences of swollen acrosome were much higher than that of separating and entirely lost acrosomes. The incidences of swollen, separating and entirely lost acrosomes were lowest in LEYG extender (Table 1).

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Table 1: Sperm motility and various acrosomal changes (Mean* ± SE) after equilibration and freezing of boar semen in different extenders.

Extenders	Sperm motility (%)		Intact acrosome (%)		Swollen acrosome (%)		Separating acrosome (%)		Entirely lost acrosome (%)	
	E	F	E	F	E	F	E	F	E	F
LEYG	74.44^{a} ±0.74	50.51^{a} ±1.33	80.42^{a} ±0.68	58.26^{a} ±0.81	14.68^{a} ±0.41	35.11^{a} ±0.67	3.50^{a} ±0.25	4.63^{a} ±0.26	1.58 ±0.14	1.82^{a} ±0.13
TESTGEYG	67.88^{b} ±0.62	37.96^{b} ±0.74	76.71^{b} ±0.74	48.96^{b} ±0.91	16.75^{b} ±0.46	40.78^{b} ±0.62	4.51^{b} ±0.24	7.51^{b} ±0.29	1.96 ±0.16	2.56^{b} ±0.17
TEYDEDCAG	68.25^{b} ±0.81	33.94^{c} ±0.79	77.02^{b} ±0.81	45.79^{b} ±0.87	17.23^{b} ±0.65	43.53^{c} ±0.65	4.03^{ab} ±0.25	7.57^{b} ±0.23	1.61 ±0.11	3.01^{b} ±0.15

* 30 Observations

E: After equilibration, F: After freezing

Means bearing different superscripts within column differ significantly ($P<0.05$)

LEYG: Lactose egg yolk glycerol

TESTGEYG: TES Tris glucose egg yolk glycerol

TEYDEDCAG: Tris egg yolk dextrose EDTA citric acid glycerol

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Effect of Preputial Washings on Semen Quality of Buffalo Bulls*

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The initial quality of semen can be improved hygienically by adopting preputial washing before semen collection as reported by Kher and Dholakia (1984) and Gangadhar *et al.,* (1986). In the present communication a detailed study has been conducted to see the effect of preputial washing on the improvement of semen quality and further preservation at +5°C and -196°C.

A total of 36 ejaculates, six from each buffalo bullmaintained at Livestock Farm, PAU, Ludhiana were taken at weekly interval in sterilised artificial vagina at 42°C -45°C. Three collections were taken with and three without preputial washings from each bull. Preputial washing was done by infusing 100 ml of sterile, warm (37°C) normal saline solution, just one hour prior to actual semen collection. Treatment To (without preputial washing), T_1 (Ist washing), T_2 (2nd wasing) and T_3 (3rd washing) was given. The semen samples were evaluated and then preserved at +5°C for 72 hours and frozen at -196°C. The spermatozoan motility and live sperm count was estimated in fresh preserved (+5°C for 72 hours) and post-thawed (24 hours after freezing) semen.

The average spermatozoan motility was recorded as 73.05±0.83, 76.16±0.76, 9.00±2.12 and 83.83±2.11 per cent in the treatments T_1, T_2, T_3. The motility was recorded highest in T_3 and lowest in To treatment. The spermatozoan motility in chilled semen (+5°C) at 0, 24, 48 and 72 hours of preservation was recorded as 75.40±1.31, 53.98±1.93, 35.53±1.77 and 18.02±1.92 per cent respectively and showed a significant (<0.05) increase in sperm motility after each treatment (T_0 to T_3) at all stages of semen preservation. The spermatozoan motility observed in post freeze thaw semen was 43.22±1.25, 49.00±2.30, 51.33±2.26 and 55.16±2.56 per cent in the treatments of T_0 to T_3 respectively. These findings are in conformity with the observations of Bhavsar *et al.,* (1986) and Arora (1990).

Mean live sperm count 80.50±0.82, 84.33±0.04, 86.16±1.47 and 88.66±1.26 in the fresh semen was recorded in treatments T_0 to T_3 respectively. These observations are comparable with the reports of Bhavsar (1986), Tripathi and Saxena (1988) and Vyawanare *et al.,* (1989). Significantly ($P<0.05$) higher live sperm count was recorded in the treatments T_1, T_2 and T_3 compared to T_0. This indicated that preputial washing had got beneficial effect on the live sperm count due to more hygienic measures taken while collecting semen from bulls.

Live sperm count in the chilled semen (+5°C) from zero to 72 hours of preservation were recorded as 81.22±0.91, 64.73±1.88, 43.23±2.37 and 25.35±2.08 per cent respectively. The percentage of livesperm increased significantly ($P<0.05$) from treatments T_1 to T_3 which is obviously due to the beneficial effect of the preputial washing as explained earlier i.e. due to bacterial count changes. There was a significant ($P<0.05$) decrease in the per cent live spermatozoan from zero to 24, 24 to 48 and 48 to 72 hours of preservation in all treatments. These above variations might be due to the effect of storage time of semen.

The live sperm count in post freeze thaw semen was recorded as 51.11±1.38, 57.33±2.69, 62.16±2.60 and 65.83±2.70 per cent in treatments T_0, T_1 and T_3 respectively. The differences were found to be significant among the treatments. The better post thaw live sperm count in T_3 treatment was due to minimum bacterial contamination and improvement in hygienic quality of semen before freezing. However, under practical conditions, it is not possible to produce semen free from micro-organisms, as contamination with few non-pathogenic organisms is unavoidable. Hence, it is better to provide preputial washing, once a week for getting good quality of semen from buffalo bulls.

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Effect of Hydrogen Ion Concentration on The Quality of Frozen Buffalo Semen*

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Hydrogen ion concentration (pH) of extender is one of the important factors which affect the quality of frozen semen. Perusal of available literature revealed limited study on the effect of pH of extender on freezability of buffalo semen (Chockalingam *et al.,* 1979). The present study has been planned to record the effect of pH of extender on the sperm motility, live sperm count and acrosomal morphology of frozen buffalo semen.

A total of 40 ejaculates comprising 8 ejaculates each from 5 Murrah buffalo bulls were used to study the effect of pH 6.5, 6.75 and 7.0 of Tris extender on quality of frozen semen by split sample technique. Tris extender (Foote, 1970) having pH 6.5, 6.75 and 7.0 was prepared in two fractions (non-glycerolated and glycerolated). Immediately after collection, the semen was split into 3 parts and extended (1:5) using non-glycerolated fraction of the extender having three different pH. The extended semen was then cooled gradually from 35°C to 5°C in 1.5 hours. The precooled (5°C) glycerolated fraction of the extender (amount equal to that of non-glycerolated fraction) was added to the respective primary extended semen at 5°C in 3 equal parts at an interval of 15 minutes. After 5 hours of equilibration at 4°C-5°C the semen was vapour frozen in medium sized (0.5 ml) French straws and stored in liquid nitrogen. After 14 hours of storage, the frozen semen was thawed in warm water (37°C) for 12-15 seconds for evaluation. The sperm motility, live sperm count (Blom, 1977) and acrosomal changes (Watson, 1975) were studied at two stages i.e. after equilibration and freezing. The sperm motility was estimated at a magnification of 400 x using phase contrast microscope. A total of 200 spermatozoa per slide were studied at a magnification of 1000 x for estimation of percentages of live sperm and acrosomal changes. The statistical analysis of the data was made after angular transformation of the percentages (Snedecor and Cochran, 1967).

The mean sperm motility after equilibration and freezing in Tris extender having pH 6.5,6.75 and 7.00 was 69.83±0.42 and 45.33±0.67, 74.38±0.30and 51.83±0.77, and 72.35±0.49 and 47.68±0.75 per cent respectively. The corresponding values for live sperm count were 77.04±0.42 and 54.40±0.41, 79.93±0.26 and 56.76±0.36 and 78.34±0.26 and 55.35±0.37 per cent, and for total acrosomal changes were 16.90±0.36 and 33.23±0.24, 13.01±0.24 and 23.55±0.70, and 15.13±0.25 and 29.44±0.48 per cent. The sperm motility and percentage of live sperm decreased significantly ($P<0.01$) while the incidence of total acrosomal changes increased significantly ($P<0.01$) due to freezing. The mean sperm motility and percentage of live sperm were significantly ($P<0.01$) affected by pH of the extender. This supports the observations of Chockalingam *et al.,* (1979). On the contrary, Sall *et al.,* (1980) reported that adjustment of pH to 6.8 or without adjustment of pH (pH 6.61 to 6.75, mean 6.70) did not significantly affect the post thawing motility of buffalo semen frozen in Tris extender. In the present study, out of the three pH of Tris extender, the pH 6.75 yielded the highest sperm motility and percentage of livesperm. The percentage of total acrosomal changes was not significantly affected by pH of the extender. The interaction between stage (after equilibration and feezing) and pH of the extender was found to be non-significant for sperm motility, percentage of live sperm and incidence of total acrosomal changes which showed that the main effects were independant. From the present study it may be concluded that pH 6.75 of Tris extender resulted better quality of frozen buffalo semen.

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Study on the Morphological Features of Oocytes in Goat

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Studies on ovaries obtained from abbatoirs have suggested that follicular oocytes could be the source of embryos for embryo transfer in livestock (Pineda and Bowen, 1980). The present study was undertaken to assess the rate of recovery of oocytes with different layers of cumulous cells harvested by aspiration from ovaries and by flushing of reproductive tracts of goat obtained from abbatoir.

A total of 46 numbers of reproductive organs of goat were collected in normal saline within 2 hours of slaughter from a local abbatoir. The number of visible follicles were counted on surfaces of both the ovaries. The ovulatory incidences, if any, were recorded. The oocytes from ovarian follicles were aspirated out using a glass syringe (2 ml) and disposable needle, and then placed in a watch glass containing normal saline. Uterotubal flushing was made, if ovulation was evident. For this, a plastic tube was inserted through the infundibulum, and the other end of the tube was held over a watch glass. Either through the uterotubal junction or through the base of the horn, depending on the stage of the CL, 2-10 ml of normal saline was pushed towards the infundibulum with the help of a hypodermic needle and a glass syringe and the flushing was collected over a watch glass. After collection, examination was made under stereo zoom microscope for studying the number and morphology of oocytes for each ovary / tract. Oocytes were categorized as 'A' having 2-3 layers of cumulous cells, 'B' - having atleast one layer of cumulous cells, 'C' - having only scattered envelope of cumulous cells and 'naked' - without any cumulous cells adhering to the oocytes (Singh and Sarma, 1991).

The results obtained in the present study were presented in Table 1. The recovery rate of oocytes from the ovarian follicles was in corroboration with that reported by Mogas *et al.*, (1992). However, it was found to be much lower than that of Singh and Sarma (1991). In the present study, number of visible follicles and percentage of oocyte recovery were higher in the right overy. This supports the observation of Balasubramanian *et al.*, (1991). The percentage of different categories of oocytes recovered in the present study were comparable with those recorded by Balasubramanian *et al.*, (1991) and Singh and Sarma (1991). On the contrary, Mogas *et al.*, (1992) found lowest incidence (11.2%) of grade I oocytes with 3 layers of cumulous cells and higher incidence of grade II (42.9%) oocytes with 1-2 layers of cumulous cells. The incidence of ovulation in the present study was very low. The rate of recovery of oocytes from the tract was 80 per cent and the oocytes were found naked and unfertilized.

The results of the present study suggest that oocytes can be harvested by aspiration technique from the ovaries obtained from abbatoir which may be used for *in-vitro* maturation and fertilization.

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Table 1: Recovery rate of various categories of oocytes from ovaries and reproductive tracts of goat obtained from abbatoir.

Attribute	Right		Left		Total	
	No.	%	No.	%	No.	%
Ovary examined	46	-	46	-	92	-
Visible follicle	196	-	162	-	358	-
Mean visible follicle / ovary	4.26	-	3.52	-	3.89	-
Oocytes recovered from follicle						
Grade A	28	25.00	18	22.50	46	23.50
Grade B	24	21.42	16	20.00	40	20.00
Grade C	16	14.28	12	15.00	28	14.54
Naked	44	39.28	34	42.50	78	40.62
Total	112	57.14	80	49.38	192	54.18
Mean oocyte recovered / ovary	2.43	-	1.74	-	2.08	-
Ovulation	16	-	4	-	20	-
Egg recovered from tract	12	75.00	4	100.00	16	80.00

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Treatment of Infertility in Bitch due to Osclitoris

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Early in the foetal period external undifferentiated genitalia becomes modified so the genital tubercle forms the clitoris which lies in a fossa and does not hinders with normal functioning in bitches. The present report puts on record successful treatment of infertility in a Doberman bitch due to rare occurrence of Osclitoris.

A 9 months old 18 Kg, pedigreed Doberman black bitch was presented to the clinic with the complaint of presence of some protruding mass through the vulvar lips. When in heat, she allowed the male to mount but copulation was not taking place. She was straining while urinating.

Gynaeco-clinical examination revealed that the protruding mass (Fig. 1) was actually enlarged clitoris with a bony feeling within it. It measured 4 cm in length with maximum diameter 1.3 cm. On catheterisation the urethral orifice was normal and provided normal flow of urine.

The animal was premedicated with diazepom @ 3 mg / kg body weight and epidural anaesthesia was induced by 7 ml of 2% lignocaine hydrochloride. The soft tissues encircling the bony mass were incised and a bone measuring 2.7 cm in length and 0.7 cm in diameter at maximum width was removed after separating it from the surrounding mass. The remaining stump was transfixed with No. 3 / 0 chromic catgut at the base to avoid haemorrhage. Post-operative administration of 1 g streptopenicilline and 4 mg betamethasone intra-muscularly for 5 days and local application of nitrofurazone ointment provided uneventful recovery.

Osclitoris in a bitch.

About 10 days after the operation the animal started urinating normally. It expressed normal oestrus after 3 months and mating occurred normally followed by confirmation of pregnancy.

Roberts (1971) stated that clitoris in dog measures about 0.6 cm in length and 0.2 cm in diameter. In the present case the clitoris was much enlarged due to presence of bony mass within it which might have caused mechanical obstruction at copulation. Once the obstruction was surgically removed the animal got cured and conceived. Hare (1976) also reported presence of osclitoris in dog amongst the cases of non-drug induced intersexes studied. Peggy and Patricia (1985) described that osclitoris is a rare consequence of faulty sexual differentiation during embryonic stage or due to hyperadrenocorticism. It is characteristic of clitoral hypertrophy and contains bone in it. The clitoris protrude through the vulvar lips and ressembles like a penis.

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A Case of Dystokia due to Dilopagus Monster (Craniopagus twin) in a Goat

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Dystokia due to duplication of cranial end of the foetal body is uncommonly found in bovines and still rarely in goats. Successful treatment of dystokia due to Craniopagus (Siamese) twin monster in goat is presented.

A non descript goat was presented for the treatment of dystokia. The water bags had ruptured long back and the foetus was jammed in the birth canal. Since vaginal delivery through traction was difficult, it was decided to extract it through the caesarean section. The animal was premedicated with triflupromazine hydrochloride intra-muscularly @ 4 mg / kg bodyweight and epidural anaesthesia was induced by injecting 10 ml of 2% lignocaine hydrochloride at lumbosacral space. Caesarean section was done at the mid line region between umbilicus and pubis (Pandey *et al., 1987).* Post-operative care included intra-muscular injection of 2.5g of streptopenicilline for 5 days and topical dressing of wound with nitrofurazone ointment. The skinsutures were removed on 10th day. Animal showed an uneventful recovery.

The extracted foetus was a fully grown female monster with two heads. Autopsy findings revealed that both the heads were joined to the thorax by two separate necks. The caudal end of the body below the conjoined cranial portion was single. The organs of thorasic, abdominal and urogenital systems also appeared grossly normal.

Craniopagus goat menster

Roberts (1971) described that conjoined twins in which the component parts are symmetrical are called Dilopagus monsters or Siamese twins which arise from a single ovum and are monozygotic. It occurs one in 100,000 in bovine births and still rarer in other species. Arthur (1956) reported that amongst conjoined twings Craniopagus class are united at the heads, which may be facing in the same or in the opposite direction, as found in the present case also (Fig. 1). Jones and Hunt (1983) stated that many congenital anomalies are essentially unknown, however, the important known causes are prenatal infection with a virus, poisons ingested by mother, vitamin deficiency (A and folic acid), genetic factors etc. Embryonic duplications are malformations due to abnormal duplication of the germinal area giving rise to foetuses whose body structures are partially but not completely duplicated.

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Double Os Uteri Externum in a Cross-Bred Cow

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In all the domestic animals there is a great variety of anomalous developments of the vagina, cervix ad uterus which arise singly or together from the Mullerian ducts (Laing, 1979). Most of the developmental defects in the tubular portions of the bovine reproductive tract are probably hereditary in origin, although, some cases may be congenital. Genetic or congenital anomalies of the cervix of cattle are not uncommon. Double external os of the cervix in cattle is occasionally observed and is believed to be an instance of failure of Mullerian ducts to fuse. Affected cattle usually conceive and calve normally. Rarely the foetal limbs may pass through either openings and result in dystocia (Roberts, 1971). The expulsion of the after birth may be impeded by the structural aberration (Arthur, 1989). A case of double external os of cervix in a cross-bred cow is reported.

A cross-bred cow aged seven years belonging to Livestock Research Station, Thiruvizhamkunnu was culled on account of failure to conceive even after repeated inseminations. Perusal of the breeding history revealed that it had a normal first calving. The genitalia was collected after slaughter for detailed examination. The external os of cervix was found to have a septum dividing it into two which did not extend into the cervical canal (Fig.I). All the other segments of the reproductive tract were found to be normal. The condition was diagnosed as double os uteri externum.

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Genitalia with double as uteri exterum

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Perivaginal Cyst In A Non Descript Cow

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A non descript cow aged six years was presented to the clinics with history of intermittant vaginal prolapse. Per rectal examination of the animal indicated cyclic ovaries. Per vaginal examination of the animal revealed a distended cyst obliterating vaginal passage. The cyst was lemon sized, filled with fluid, soft inconsistancy and attached to ventro-lateral surface of vagina. Exsteriorisation of the mass by gental traction on the vaginal wall did not exposed the mass completely for detailed check up.

The cyst was punctured with sterile needle and contents of the cyst were collected for microbiological and histological examination. Culture sensitivity test of the cystic fluid showed no pathogenic organisms. Microscopic examination revealed clear fluid without cellular contents. Lugol's iodine two per cent 15 ml was infused in the cystic sac followed by perentral antibiotics. Animal was examined per vaginally after eight days and no recurance was obsurved.

Cysts of gartner's duct and bartholin's glands are occasionally encountered in cattle on the floor of vagina and they are usually associated with cystic overies (Jones and Hunt, 1983), but in present case there was no evidence of cystic overies. The dilatation of gartner's canal as vaginal cyst is associated with highly chlorinated napthalene poisoning (Sastry, 1983). However, in the present case no clinical manifestations were noticed in relation to poisoning.

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