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Plasma Prolactin and Milk Production in Murrah *(Bos bubalis)* Buffaloes Fed with Elevated Energy Levels During Pre- and Postpartum Period

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ABSTRACT

Fifteen Murrah buffaloes of the Dairy Farm, Punjab Agricultural University, Ludhiana, were grouped (5 each) and provided with 100, 120 and 140 per cent energy of NRC rations during pre- and postpartum period. The animals on elevated rations performed better in terms of weight gain, milk yield, total fat and solid-not-fat than that of the control. The prolactin level ranged between 90 to 123.6 ng/ml during prepartum period which was significantly low ($P<0.01$) than that of postpartum (range 331.2±13 to 437±22 ng/ml). It was found to have positive correlation with milk yield. The results suggest that the buffaloes should be fed NRC Plus energy levels to achieve better milk production and general efficiency.

INTRODUCTION

Body conditions at parturition and postpartum body weight changes, play a significant role in the performance of dairy animals, which inturn is affected by pre- and postpartum feeding. It is customary to feed increasing amount of concentrates during last 6 to 8 weeks of pregnancy for development of mammary gland and build up of body reserves. Mostly we follow the feeding standards of NRC designed for cattle of temperate climate. A few reports are available on the level of energy rations required for milk production and prolactin levels (Seth *et al.,* 1978 and Singh and Barsaul, 1988). Therefore, the piece of work under report was undertaken to study the prolactin level, milk production and body weight changes as influenced by pre- and postpartum elevated energy supply.

MATERIALS AND METHODS

Dry and pregnant Murrah buffaloes (15) of the Dairy Farm, Punjab Agricultural University, Ludhiana were divided into 3 groups of 5 each on the basis of body weight, lactation number and expected date of calving and were kept on basal diet for 20 days. They were fed, during 56 days prepartum and 98 days postpartum, at either the normal (100% NRC) or the elevated (120% and 140% NRC) energy levels. The animals were kept individually under semi-loose housing system in a shed measuring 45.75 x 20.70 m including a sheltered area of 6.49 x 45.75 m. They were provided with weighed quantity of feed and fodder as per calculated requirement on the basis of body weight, level of nutrition and production after calving. Concentrate mixture (20% crude protein and 2.67 Mcal ME/kg), wheat straw and green fodder were fed throughout the experimental period. Body weights were recorded at day 45 prepartum and at day 4 postpartum till day 90 postpartum at 14 days interval. The milk yield was recorded daily throughout the experimental period. Milk fat was estimated by Gerber method (Aggarwal and Sharma, 1961) and solid-not-fat (SNF) was calculated according to the formula: $\frac{CLR}{4}$ + fat + 1.21 + 0.66 - fat where CLR is the corrected lactometer reading. Milk fat and solid-not-fat were determined by following the standard procedures.

Blood samples were collected from each experimental buffaloes at 14 days intervals by jugular venipuncture in prechilled dried glass test tubes containing EDTA and sodium fluoride. Plasma was separated by centrifugation (5°C) at 3000 rpm for 15 min. and stored at -20°C until further use.

Hormone assays: Plasma samples (in duplicate) were analysed in a single assay for prolactin concentration employing a prolactin RIA kit with I^{125} prolactin (Diagnostic Products Corporation, USA).

The data was statistically analysed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean body weight changes and prolactin concentration during pre- and postpartum periods,

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and the milk yield and its composition during 90 days postpartum are given in the table under the normal and elevated feeding regimens (Table 1).

During the prepartum period the animals on both normal and elevated feeding regimens showed substantial gain in body weight. The pooled mean body weight change per fortnight was found to be significantly (P<0.01) more in the elevated groups. The loss of weight was also more in these groups at parturition. It is interesting to note that during 90 days postpartum the absolute loss was markedly less in the elevated groups. The data further revealed that the animals on the elevated rations started gaining weight at higher rate as compared to animals on normal rations. These results showed that if the animals are fed on the high energy rations, body weight loss can significantly be reduced. Several workers have observed the beneficial effect of pre- and postpartum feeding in cattle (Anderson *et al.,* 1981; Ducker *et al.,* 1985) and buffaloes (Rutter and Randel, 1984; Kalsi and Takkar, 1985; Deshmukh and SenGupta, 1988). During prepartum the rate of gain was 0.73 kg/day in the normal while it was 0.88 to 1.09 kg/day in the animals on elevated rations. These observations correspond with earlier report in cow (Cowan *et al.,* 1981).

The changes in body weight during early lactation are said to effect the milk yield and postpartum reproductive efficiency (Youdan and King, 1977 and Bhalaru *et al.,* 1986). Usually, there is fall in weight during the first few weeks followed by a period of gain (McClure, 1965; Hickman *et al.,* 1971) and thus it may be economical to practice enhanced feeding of buffaloes to have better reproductive efficiency (Sharma *et al.,* 1990).

Prolactin hormone: The mean prolactin values during prepartum period was found to be 92.7±4.1, 98.5±8.9 and 102.2±7.8 ng/ml in normal, elevated 1 and elevated 2 groups, respectively (Table 1). It varied from 90 to 123.6 ng/ml. The prolactin levels were 361.2±5.8, 369.6±6.6 and 390.9±11.5 ng/ml during postpartum period which was significantly higher than that of prepartum levels. These findings are in agreement with those reported by Dindorkar *et al.* (1988) who observed that the prolactin level was minimum during advanced pregnancy. It could be due to high level of circulating steroids which render the mamary glands refractory to prolactin and glucocorticoids thereby inhibiting milk secretion (Meites, 1966).

The high level of prolactin corresponds with the increased milk production (Table 1). It was also found to be positively correlated with milk production (r = 0.30 to 0.55). Level of nutrition had been reported to increase the prolactin level (Sheth *et al.,* 1978).

Calf birth weight and sex: Plan of nutrition had non-significant effect on the birth weights of the calves and are in confirmity with those reported by Anthony *et al.* (1986) but differ with the findings of Bellow and Short (1978) who observed beneficial effect of elevated feeding on calf birth weight. The variation in calf birth weight was due to sex ratio in the different groups. Males were found to be heavier than the females.

Milk yield and composition: Level of nutrition was found to effect the milk yield and its composition significantly (P<0.01). Peak yield was achieved during the first fortnight in the animals on normal rations whereas it occurred in the second fortnight in the animals on elevated rations. It varied significantly among the groups and fortnights. Review of literature revealed conflicting reports on the effect of prepartum (Ceonon, 1981; and Deshmukh and Sengupta, 1988) and postpartum (Rao *et al.,* 1983; Poole, 1986; Deshmukh and Sengupta, 1988 and Singh and Barsaul, 1988) feed on milk production. Poole (1986) and Singh and Barsaul (1988) observed beneficial effect of additional feeding while Rao *et al.* (1983) and Deshmukh and Sengupta (1988) could not observe any significant increase in milk yield from elevated pre or/and postpartum feeding.

Milk yield and solid-not-fat were found to increase by enhancing the energy levels while there was a little decrease in the fat percentage (Table 1). This could be due to the simultaneous increase in the prolactin levels which was found to be positively correlated with milk production and solid-not-fat. The findings of the present study are in accordance with those of Sial and Shah (1968) but did not agree with Ducker *et al.* (1985).

Additional feeding might have helped in building up body reserves. The buffaloes who made greater weight gain before calving and lost their weight after parturition yielded more milk than that of control. Such observations were also reported by Kumar and Mudgal (1977) and Balasubramanya (1981).

Milk fat percentage was found to be slightly more in the control group. There was a negative correlation between the milk fat and milk yield. However, the total fat yield was found to be more in the elevated groups. The findings were in confirmity with those of Ducker *et al.* (1985).

Conflicting observations had been reported on the effected of nutrition on the milk fat and solid-not-fat. Deshmukh and Sengupta (1988) observed no difference in milk fat resulting from different levels of feeding. Likewise SNF content was also not effected (Ceonon, 1981; Deshmukh and Sengupta, 1988). The results on solid-not-fat were found to be in accordance with those of Kumar (1976) and Chaudhary *et al.* (1988). From the preceding discussion, it could be inferred that the buffaloes should be fed atleast 120 per cent rations to achieve better milk production and general efficiency.

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Table 1. Mean body weight changes (kg), prolactin (ng/ml), milk yield and its composition of Murrah buffaloes on normal and elevated energy rations.

Attributes	Feeding regimens		
	Normal	Elevated	
	100% (5)	120% (5)	140% (5)
1. Body Weight changes (kg/fortnight)			
Prepartum	10.7	15.3	12.3
Prepartum	-65.6	-71.2	-71.8
Postpartum	-3.2	+3.4	+15.0
2. Prolactin (ng/ml)			
Prepartum	92.7 ±4.1	98.5 ±8.9	102.2 ±7.8
Postpartum	361.2 ±5.9	369.6 ±6.6	390.9 ±11.5
3. Milk yield (kg/fortnight)**	111.7 ±9.9	128.3 ±6.1	133.6 ±5.8
Milk fat (%)*	6.85 ±0.2	6.6 ±0.1	6.5 ±0.2
Solid-not-fat (%)**	9.16 ±0.12	9.3 ±0.04	9.32 ±0.04
4. Calf birth weight (kg)	37.8	34.0	35.5
Sex ratio (male:female)	4 : 1	1 : 4	3 : 2

** Significant at P<0.01.
Figures in parentheses represent the number of animals.

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Circulating Level of Plasma Thyroidal and Adrenocortical Hormoes in Murrah Buffaloes During First Ten Months of Lactation

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ABSTRACT

Plasma triiodothyronine (T_3), Thyroxine (T_4) and cortisol were assayed in pluriparous Murrah buffaloes during Lactation. The average plasma (T_3) varied from 1.35 to 9.99 ng/ml. It attained the peak during third and fourth month of Lactation. Plasma cortisol level was low during early lactation phase, 0.98 $\pm$ 0.15 ng/ml as compared to mid and late lactation (1.16 $\pm$ 0.13 and 1.56 $\pm$ 0.19 ng/ml).

INTRODUCTION

Plasma thyroidal and adrenocortical hormones are of much significance during stress conditions, particularly the lactational stress. Present study was therefore undertaken to determine the circulating levels of plasma triiodothyronine, thyroxine and cortisol hormones during all the phases of lactation in Murrah buffaloes.

MATERIALS AND METHODS

The present study was carried out on nine apparently healthy pluriparous Murrah buffaloes, maintained at the University dairy farm under standard managemental conditions. All the animals were in their third lactation with average body weight 340 kg. Animals were kept on green fodder, wheat straw, concentrate and water was provided ad lib. Average ambient temperature during course of study was maximum of 19.5°C to 41°C and minimum of 6.0°C to 26.6°C. Buffalo calves were weaned immediately after parturition and all animals followed normal reproductive pattern. Blood samples were collected from jugular vein in heparinized glass vials under aseptic conditions at forthnightly intervals. Plasma was separated by refrigerated centrifugation at 2000 g for 30 minutes. Samples were stored at -20°C in different aliquots to avoid repeated thawing. Plasma triiodothyronine (T_3) was quantified by technique of Chopra *et al.* (1971) and thyroxine (T_4) was estimated by method of Abraham (1977) using RIA kits procured from BARC*. Plasma cortisol was assayed by method of Moore *et al.* (1985) using coat-a-count RIA kit procured from DPC** These kits were of high specificity. Results so obtained were analysed statistically as per Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

The average concentration of plasma T_3, T_4 and cortisol hormones has been presented in table I.

The average plasma triiodothyronine varied from 1.35 to 9.99 ng/ml during the study period. The T_3 level increased gradually during early phase of lactation attaining peak levels during third and fourth months of lactation. Thereafter the level followed a declining trend. Plasma thyroxine concentration also showed similar fluctuations during different months of lactation (Table I). Significantly ($P<0.05$) high thyroxine level was observed during the third and fourth months of lactation possibly due to dual stress of lactation and early gestation. Khurana and Madan (1986) reported low T_3 and T_4 concentration during late lactation, however their reported values were relatively lower as compared to one findings, possibly due to seasonal variation in study period. Kumar *et al.* (1990) also observed a change in thyroxine level in relation to stage of lactation and season. Lowest T_3 and T_4 values at the end of lactation were also reported by Malsh *et al.* (1980) thus confirming our findings.

Plasma cortisol level was low during early lactation phase, 0.98 $\pm$ 0.15 ng/ml as compared to mid and late lactation values of 1.16 $\pm$ 0.13 and 1.56 $\pm$ 0.19 ng/ml respectively. High cortisol concentration (Table I) during late lactation is due to physiological stress of gestation.

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Table I: Average concentration of plasma T_3, T_4 and cortisol hormones in lactating Murrah buffaloes.

Months of lactation	Milk yield/ day (kg)	Triiodo-thyronine (ng/ml)	Thyroxine (ng/ml)	Cortisol (ng/ml)	Tempera-ture (°C) Min.	Max.	Relative humidity (%) Min.	Max
I	11.10	2.24^{b} ±0.10	49.75^{b} ±3.34	0.84^{a} ±0.07	24.4	35.3	49	73
II	12.50	4.11^{c} ±0.53	53.42^{bc} ±2.11	0.73^{a} ±0.07	18.1	32.2	37	74
III	10.25	8.35^{d} ±0.48	60.16^{c} ±2.24	1.36^{a} ±0.27	12.5	28.0	40	76
IV	9.36	9.99^{d} ±0.54	65.33^{c} ±4.36	1.40^{a} ±0.33	6.0	21.6	45	96
V	8.25	1.67^{a} ±0.09	49.50^{b} ±2.35	0.86^{a} ±0.14	6.4	19.5	53	98
VI	7.78	1.50^{a} ±0.08	36.92^{a} ±1.85	1.23^{a} ±0.26	7.8	22.8	48	92
VII	7.24	1.35^{a} ±0.08	35.25^{a} ±2.05	0.94^{a} ±0.31	12.3	26.1	46	85
VIII	7.00	1.42^{a} ±0.09	36.67^{a} ±2.40	2.00^{a} ±0.72	17.8	36.5	29	54
IX	6.65	1.50^{a} ±0.07	30.00^{a} ±2.10	1.66^{a} ±0.52	23.2	41.0	21	68
X	5.80	1.48^{a} ±0.07	28.83^{a} ±2.12	1.63^{a} ±0.50	26.6	38.6	35	66

Each figure is a mean of 9 values
The figures having different superscripts within a column differ significantly ($P<0.05$) with each other.

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Blood Serum Cortisol and Total Cholesterol Levels Around Parturition in Surti Buffaloes

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ABSTRACT

An investigation was undertaken to estimate the circulating levels of blood serum cortisol and total cholesterol from 30 days antepartum to 7 days postpartum. The study revealed peak blood cortisol levels on the day of parturition (4.27 ± 7.34 ng/ml). The cortisol level declined precipitously at 12 hours postpartum and then declined to basal level in early postpartum period. The blood serum total cholesterol level declined significantly (P<0.05) from 173.94 ± 10.51 mg% to 148.29 ± 7.46 mg% between 30 days antepartum to 12 hrs antepartum respectively. The blood cholesterol level exhibited minor fluctuations in the early postpartum period.

INTRODUCTION

The recent literature on endocrine aspects of buffaloes around parturition is available for Murrah buffaloes (Lohan *et al.* 1987 and Kaker *et al.* 1990), but for other breeds of buffaloes such informations are lacking. Therefore, present investigation was undertaken to study the blood cortisol and total cholesterol profile around parturition stages in Surti buffaloes.

MATERIALS AND METHODS

Six pleuriperous Surti buffaloes of University farm kept under standard feeding and managemental conditions were considered for the present study. All the animals were clinically healthy and had normal time and act of parturition. The calves born were weaned right from birth. Buffaloes were milked within 30 min of foetal expulsion. Placenta got expelled out within 3 to 6.5 hrs of parturition. Blood samples were collected by jugular vein puncture from 30 days antepartum to 7 days postpartum (Table 1). The serum cortisol and total cholesterol were estimated as per the methods of Foster and Dunn (1974) and Schoenheimer and Sperry (1934) respectively.

RESULTS AND DISCUSSION

The serum cortisol levels differ non-significantly between different stages around parturition (Table 1). The peak level of cortisol (4.27 ± 1.34 ng/ml) was recorded on the day of parturition. Present observations are in agreement with the earlier report of Kaker *et al.* (1990). Elevated cortisol level obserbed around parturition indicated the involvement of this steroid in the events leading to parturition. In certain species it might be involved in initiating the metabolic changes in the placenta leading to increased estradiol synthesis required for increasing uterine tone before the onset of parturition (Kaker *et al.*, 1990). However, Jain and Madan (1985) are of the opinion that a precepitous rise in cortisol level on the day of parturition is because of parturition stress rather than its role in initiating the act of parturition. Surge in cortisol level during parturition may indicate adrenal cortical association with maternal milk synthesis and secretion (Convey, 1974) or increased in abdominal contractions (Echternkamp and Hansel, 1973). Injection of cortisol in antepartum sheep can induce the onset of parturition (Liggins, 1974). Similar are the observations for cattle (Fairclough *et al.* 1981) and buffaloes (Prakash and Madan, 1988). Therefore, the peak rise in cortisol level is attributed with the initiation of act of parturition in present study. This argument is further proved by declining value recorded after the act of parturition is over (Table 1).

Cholesterol being a precursor for steroid hormone synthesis, its concentration vary during different reproduction phases such as pregnancy, parturition and lactation (McDonald, 1980). Serum total cholesterol during present study revealed a significant (P<0.05) fall from 173.94 ± 10.51 mg% to 148.29 ± 7.46 mg% between day 30 antepartum to the day of parturition and then remain fluctuating

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around 140 mg% during 7 days postpartum (Table 1). The parallel findings about declining trend in total cholesterol in cattle and buffaloes approaching parturition have been reported by various workers Sharma and Luktuke (1981) and Pareek and Aminudeen (1985). Increasing level of triglycerides towards term might be the consequence for the reduction in cholesterol level in blood (Sato, 1978). The persistant lower level of cholesterol in blood serum during postpartum period indicate about the secretion of this important lipid in milk (Arave *et al.*, 1975 and Sato, 1978).

The present studies not only lay emphasis about the physiological requirement of cortisol and cholesterol around parturition but also may prove to be useful in detection of ensuing parturition in buffaloes.

Table 1: Blood serum cortisol and total cholesterol levels around parturition in Surti buffaloes.

Days	Cortisol (ng/ml) n=5 x ±SE	Total Cholesterol (mg%) n=6 x ±SE
30 days A.P.	1.55±0.32	173.94±10.51^{a}
25 days A.P.	1.29±0.16	177.03±7.21^{a}
20 days A.P.	1.06±0.31	171.64±5.49^{ab}
15 days A.P.	1.72±0.26	163.83±8.08^{abc}
13 days A.P.	2.15±0.70	155.04±4.83^{abc}
10 days A.P.	1.78±0.56	163.84±7.92^{1bc}
7 days A.P.	1.96±0.28	146.80±10.81^{abc}
5 days A.P.	2.17±0.42	174.21±24.03^{a}
3 days A.P.	1.82±0.58	160.81±15.01^{abc}
2 days A.P.	1.86±0.62	159.43±9.57^{abc}
1 day A.P.	1.39±0.16	155.23±6.79^{abc}
12 hrs A.P.	4.27±1.34	148.29±7.46^{abc}
12 hrs P.P.	2.65±0.66	149.03±8.88^{abc}
1 day P.P.	1.79±0.76	130.43±12.27^{c}
2 days P.P.	1.05±0.35	134.64±3.57^{c}
3 days P.P.	2.01±0.35	158.42±5.04^{abc}
5 days P.P.	1.55±0.33	136.67±8.93^{bc}
7 days P.P.	1.39±0.29	141.89±14.41^{abc}

A.P. = Antepartum
P.P. = Postpartum
abc = Means with different superscripts differ significantly with each other at 5% levels of significance.

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APPEAL TO CHAPTER SECRETARIES

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Exfoliative Vaginal Cytology and Serum Progesterone Levels in Normal and Abnormal Oestrous Cycle of Cow.

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ABSTRACT

Exfoliative Vaginal Cytology (EVC) studies along with levels of progesterone were carried out in 25 cows from a well established, "Cattle Breeding Farm, Kandivali, Bombay" in order to find if EVC could be employed as diagnosotic or prognostic tool. The normal cycling animals showed increased number of cornified cells (50.74 $\pm$ 2.23%) in oestrus. The higher level of progesterone in different groups of animals accompanied by more number of parabasal and intermediate cells. The higher count of leucocytes in repeat breeding animals indicated presence of infection. In anoestrus animals both EVC picture and progesterone levels showed variation in different animals indicating individual differences in ovarian abnormalities.

INTRODUCTION

With greater awareness for early and accurate diagnosis of reproductive disorders the need for clinico-pathological tests providing such an aid is acutely felt. The vaginal mucosa displays cyclic changes under the influence of changing levels of hormones - estrogen and progesterone. Although, considerable number of papers from foreign countries has been presented only a few reports, those of Hussain and Khan (1978 and 1979), Rao *et al.* (1979) and Subramanian and Pattabiraman (1988) on local and cross-bred animals in India are available. As these findings appeared some what conflicting, a detail work was undertaken specially with an interest to see how far cytological studies are helpful in assessing the status of bovine repeat breeding and anoestrus conditions.

MATERIAL AND METHODS

Present study was based on 47 Vaginal smears and serum samples collected from 25 cows from a well organized and maintained farm, "Cattle Breeding Farm, Kandivali, Bombay".

Based on the records, expression of oestrus signs and perrectal examination, material was collected from twelve normal cycling, ten anoestrus and three repeat breeding animals. On an average three samples per animal starting from oestrus, from the normal and repeat breeding animals were collected. Ten samples were collected from animals with postpartum anoestrus period of more than 90 days.

Vaginal smears were taken with tightly closed swab holder from the dorsolateral part of anterior vagina and fixed with ether - alcohol (1:1) mixture and preserved in it till they were subjected to Shorr's staining.

Size of 100 different types of exfoliated vaginal epithelial cells was measured from randomly selected fields using camera lucida. The exfoliated vaginal epithelial cells were classified on the basis of staining characteristics, mensuration and morphology of the cells.

The cell types were classified as follows:

1) Cornified cells - Large or small about 30 to 60 μ in size, round, flat or polygonal in shape with eosinophilic, pink, orange to red cytoplasm and dark gray or pyknotic nucleus.
2) Superficial squamous cells - Large about 30 to 60 μ, flat, usually polygonal cells, transparent and basophilic cytoplasm and dense, homogenous, pyknotic nucleus.
3) Intermediate cells - Smaller than superficial cells, 20 to 40 μ, polygonal in shape, basophilic, flat, spread out or folded cytoplasm and large vasicular nucleus with fine chromatin net-work.
4) Parabasal cells - Small, 15 to 25 μ, round or oval with opaque, usually basophilic, vacuolated cytoplasm and vesicular nucleus with coarse chromatin pattern.
5) Basal cells - Small, about 13 to 20 μ, round to oval with strongly basophilic cytoplasm and large nucleus occupying more than half of the cell. Epithelial cells were counted in addition to recording leucocytes per 100 epithelial cells. Differential leucocytes count was also recorded.

Part of M.V. Sc thesis submitted to KKV, Dapoli

Serum progesterone levels were estimated by Radioimmunoassays (RIA) performed by using coat-a-count RIA kits obtained from Diagnostic Product Corporation, U.S.A. The lower limit of sensitivity of assay system was found to be 0.1 ng of standard progesterone and it helped to measure progesterone in 100 ul serum samples.

RESULTS AND DISCUSSION

The measurements of cells noted here in general talliec with those reported by Miroud and Noakes (1990) in his work on bovines. *Stagewise EVC picture is presented in table 1.*

In Oestrus: The EVC Picture was characterised by significantly more number of cornified cells (50.74 ± 2.23%) and superficial cells (18.18 ± 4.38%) as compared to other stages and with significantly less number of leucocytes per 100 epithelial cells counted. Intermediate cells (17.2 ± 3.82%) and parabasal cells (13.9 ± 3.98%) were also noted. Similar cell picture in oestrus has been described by Rao *et al.* (1979) and Subramanian and Pattabiraman (1988) in bovines. They attributed the increased cornification noted during oestrus to the higher levels of estrogen present in the period. However, Hussain and Khan (1978) found more number of parabasal cells (92.97%) and absence of intermediate cells. They did not offer any comment on the findings.

In Mestestrus: Parabasal cells (35.36 ± 4.21%) and intermediate cells (22.56 ± 3.15%) were the predominant cells in metestrus period while basal cells and superficial cells were in minimum number.

Number of leucocytes per 100 epithelial cells counted was characteristically high (212.63 ± 59.55%). Most of the smears were characterised by the presence of R.B.Cs. The present findings could be well-correlated with the most recent work of Miroud and Noakes (1990). The presence of R.B.Cs was attributed by them to metoestral bleeding due to diapedesis or capillary bleeding from endometrium.

In Diestrus: This was characterised by presence of more intermediate cells (42.11%) and parabasal cells (41.86%) and significantly less number of cornified cells (12.04 ± 3.18) and superficial cells (4.0 ± 1.07%) compared to other stages. Similar findings in bovines have been recorded by Rao *et al.* (1979) and Subramanian and Pattabiraman (1988). However, Miround and Noakes (1990) found parabasal cells to be fewer in this stage. Increased number of parabasal and intermediate cells have been related to the higher level of progesterone in diestrus in humans (Raphael, 1976). The same situation appears to be true in bovines from the present study.

In Proestrus: Proestrus samples in sufficient number were not available.

Repeat Breeding Animals: The repeat breeder oestrus smear showed comparatively less number of cornified cells (26.1%) and more intermediate cells (32.8%) than that of normal oestrus smears. The leucocytes per 100 epithelial cells counted were extremely high (412%) as compared to the normal oestrus smears (12.54%). The higher count of leucocytes present in all the samples collected from repeat breeding animals was taken to mean presence of infection.

Anoestrus: The EVC picture was characterised by presence of more number of cornified cells (30.53%). In addition to this parabasal (29.12%) and intermediate (24.63%) cells and few leucocytes (49.17%) per 100 epithelial cells counted were also present. The EVC picture of individual animals showed wide variation probably because of varying ovarian abnormalities noted in anoestrus cows. Out of the 10 anoestrus animals six animals were with hard and inactive ovaries which showed completely different EVC picture from the cytological findings recorded by Hussain and Khan (1979) in similar condition.

Differential Leucocyte Count (DLC): The DLC carried out for the first time in such type of study indicated that PMN cells dominated the leucocytes in exfoliated vaginal cells irrespective of stages of cycle, normal and abnormal (Table 1). However, number of cells more than seen in normal cycling animals could be taken as an indication of infection in repeat breeder animals.

EVC and Progesterone Level:

Normal oestrous cycle: In the present study progesterone levels (Table 1) were minimum 0.31 ± 0.11 ng/ml during oestrus while levels were increasing from metestrus (0.73 ± 0.19 ng/ml) to diestrus (3.48 ± 0.43 ng/ml), then the values dropped down during proestrus (0.67 ± 0.11 ng/ml). This trend in serum progesterone profile was found to be similar to that reported by Deopurkar, (1990) for normal cycling animals. The predominance of cornified cells was noted when the progesterone levels were lower and with increase in progesterone level number of cornified cells decreased indicating inverse relationship with cornified cells. This supports the opinion of Roberts

(1971) who considered progesterone to be responsible for inhibition of keratinization of vaginal cells. In the present study parabasal and intermediate cells were more when progesterone concentration was maximum.

Repeat Breeding Animals: The progesterone level in one samples collected at repeat breeding oestrus was 0.12 ng/ml and appeared to increase to 0.89 ± 0.36 ng/ml during metestrus and 2.63 ± 0.03 ng/ml during diestrus. Further reduced levels (0.99 ± 0.59 ng/ml) were observed after 19th day postoestrus.

Although lower values were noted, they appeared to follow the trend of oestrus-metestrus-diestrus of normal cycling animals. However, these animals did not show reduction in the level after 19th day postoestrus exhibiting prolonged diestrus condition.

Anoestrus Animals: Progesterone levels of anoestrus animals were ranging from unmeasurable limit to 2.2 ng/ml. This suggested that the variation in EVC picture of individual animals could be due to presence of low grade hormonal activity in these animals.

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Table 1: Mean Exfoliated Vaginal Cells and Level of Serum Progesterone in the Stages of Oestrous Cycle

Sr. No.	Stage of Cycle	Cornified Cells% Total	Other Cells					Serum Progesterone ng/ml
			Superficial	Intermediate	Parabasal	Basal	Leucocytes	
1.	Proestrus	30.30 ±26.79	7.10 ±0.00	33.30 ±34.54	27.50 ±15.8	1.75 ±1.74	229.32 ±204.31	0.67 ±0.11
2.	Oestrus	50.74 ±2.23	18.18 ±4.38	17.20 ±3.82	13.90 ±3.98	0	12.54 ±1.62	0.31 ±0.11
3.	Metestrus	36.22 ±5.11	3.36 ±1.09	22.56 ±3.16	35.36 ±4.21	2.41 ±0.72	212.63 ±59.55	0.73 ±0.19
4.	Diestrus	12.04 ±3.18	4.00 ±1.07	42.11 ±3.72	41.86 ±2 69	0	109.76 ±25.92	3.48 ±0.43
In Repeat Breeding Animals								
5.	Oestrus	26.10	4.20	32.80	21.00	7.60	412.60	0.12
6.	Metestrus	21.25	1.32	34.79	42.65	-	506.51	0.89
7.	Diestrus	13.90	2.93	38.07	44.43	0.67	212.53	2.63
8.	Prolonged Diestrus	17.99	12.50	32.07	36.64	0.96	140.97	1.00
In Anoestrus Animals with								
9.	Smooth ovary	36 12	9.67	20 48	27.36	6.33	26.56	0.61
10.	Slight follicular development	28.53	15.18	22.82	30.27	3.18	88.23	0.03
11.	Cystic ovary	2.94	6.86	53.92	36.27	-	57.65	0.38
12.	Total	30.53	11.04	24.53	29.12	4.75	49.17	0.41

Mean ± S.E.

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Serum Progesterone Profile During Oestrous Cycle in Goat Treated with Different Doses of HCG

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ABSTRACT

The trend of serum progestrone level during oestrous cycle in goats treated with three different doses of HCG and in the control was observed to be similar. The mean serum progesterone level during oestrous cycle in treated (550 IU Vs 5.40 ± 0.25 ng/ml; 650 IU Vs 6.28 ± 0.18 ng/ml; 750 IU Vs 6.25 ± 0.15 ng/ml) as well as in the control gorup (5.62 ± 0.21 ng/ml) was found to be highest on day 12 of the cycle. The lowest value in all the groups was observed on the days of oestrus.

INTRODUCTION

The pattern of serum progesterone level during oestrous cycle in goat has been reported by many investigators (Heap and Linzell, 1966; Thorburn and Schneider, 1972; Jones and Knifton, 1972; Rahman, 1978; Mgongo *et al*, 1984; Baruah *et al*, 1987 and Wani, 1989). There is however, a paucity of comparative data on the levels of blood progesterone during oestrous cycle in goats treated with various doses of HCG. The present study reports the serum progesterone level during oestrous cycle in indigenous goats of Assam treated with three different doses of HCG.

MATERIALS AND METHOD

A total of 20 sexually matured healthy local goats of Assam, 2-3 year old, were included in this study. The animals were maintained under semi-intensive system of rearing.

The animals were divided into 4 groups, viz. A,B.C and D having 5 animals in each group. The animals of group A were served as control; while animals of group B,C and D received an intra-muscular injection of 550, 650 and 750 IU of HCG (Chorulon**) at 6 hours post onset of oestrus, respectively. The animals of control group received an intramuscular injection of distilled water at the same time schedule.

Collection and processing of blood samples: From animals of this experiment, about 5 ml of blood sample was collected by jugular venu-puncture at 2 days interval from day of oestrus (day 0) to day 18th of the cycle and the last collection was done on the day of next oestrus (day 0_1). After collection, the blood sample was allowed to clot and the serum was separated from clotted blood samples and stored at -20°C till processed futher.

Estimation of serum progesterone level: Serum progesterone level was estimated by RIA (Radio immuno assay) technique using kits procured from M/S Leeco Diagnostics Inc., Southfiled, Michigan.

RESULTS AND DISCUSSION

The serum progesterone profile in goats of all the four groups was lowest on the day of oestrus (Table-I). Then the serum progesterone level continued to incline from day to day 12 of the cycle and, thereafter, it continued to decline till the day of subsequent oestrus (day 0_1). The peak level of 5.62 ± 0.21, 5.40 ± 0.25, 6.28 ± 0.18 and 6.52 ± 0.15 ng/ml in group A,B,C and D, respectively, was observed on day 12 of oestrous cycle. This trend of progesterone level is in close agreement with that observed by Thorburn and Schneider (1972) in Saanan goats, Jones and Knifton (1972), Rahman (1978), Baruah *et al.* (1987) in goat and Wani (1989) in German dwarf goats of mixed inheritance. However, Thorburn and Schneider (1972) observed a maximum of 4 ng/ml progesterone concentration on about day 10 of the oestrous cycle in Saanan goats; on the other hand, Wani (1989) observed the progesterone level of 10.3 ng/ml on day 12 of the cycle. The findings of Thorburn and Schneider (1972) and Wani (1972) and Wani (1989) differ from the observation of the present experiment, which might be due to the differences of breed; as the present experiment was done on local goats of Assam; whereas

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** Chorulon- Chorionic gonadotropin B.Vet.C (Luteinizing hormone), Intervet, Holland

Thorburn and Schneider (1972) and Wani (1989) conducted their experiments of Saanan goats and German dwarf goats, respectively. Higher level of progesterone observed in the present investigation than that of Thorburn and Schneider (1972) also might be due to difference in assay procedure. The recovery rate of progesterone in their assay method ranged from 79-84 percent, whereas, the recovery rate of the present experiment was 94 percent for 5 ng of progesterone. Moreover, procedural losses were not included in the study of Thorburun and Schneider (1972).

There was apparently not much difference in the peak levels of progesterone profile in control group as well as the other three groups of goats treated with 550, 650 and utp IU of HCG. Peak level of progesterone was observed in all the groups of animals on day 12 of the cycle; however, slightly higher value of 6.52 ± 0.15 and 6.25 ± 0.18 ng/ml of progesterone was observed in 750 IU and 650 IU HCG treated group, respectively, on day 12 of the cycle. Asher and Smith (1987) stated that mid cycle progesterone concentrations were positively related to ovulation rate in does. In the present study, also, there was no significant difference of ovulation rate between the control and the treated groups. Hence, the non-significant difference of serum progesterone level between the control and treated groups could be due to similar ovulation rate.

Table 1: Serum progesterone profile (ng/ml) on various days of oestrous cycle in goat treated with three different doses of HCG (Mean ± S.E.).

Group	Dose	No.of animals	Day of oestrous cycle										
			0	2nd	4th	6th	8th	10th	12th	14th	16th	18th	0_1
A	Control	5	0.46 ± 0.07	0.73 ± 0.05	1.78 ± 0.16	3.28 ± 0.22	4.60 ± 0.15	5.16 ± 0.09	5.62 ± 0.21	4.24 ± 0.09	3.88 ± 0.22	1.64 ± 0.14	0.39 ± 0.03
B	550 IU HCG	5	0.41 ± 0.06	0.69 ± 0.06	1.86 ± 0.12	3.48 ± 0.23	4.12 ± 0.23	5.08 ± 0.18	5.40 ± 0.25	4.12 ± 0.26	3.22 ± 0.18	1.66 ± 0.19	0.71 ± 0.07
C	650 IU HCG	5	0.49 ± 0.08	0.84 ± 0.04	2.20 ± 0.09	3.56 ± 0.29	4.26 ± 0.29	5.30 ± 0.21	6.28 ± 0.18	5.08 ± 0.09	3.42 ± 0.21	1.78 ± 0.25	0.68 ± 0.08
D	750 IU HCG	5	0.42 ± 0.05	0.92 ± 0.03	2.14 ± 0.18	3.70 ± 0.18	4.42 ± 0.11	5.52 ± 0.14	6.52 ± 0.15	4.52 ± 0.16	2.72 ± 0.18	2.02 ± 0.16	0.81 ± 0.02

0 - Day of oestrus

0_1 - Day of subsequent oestrus.

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Efficacy of Receptal in Induction of Estrus in Buffaloes by Different Dose and Route of Adminsitration

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ABSTRACT

Twenty four anestrous murrah buffaloes were divided in to three groups of eight each. Group I received 2.5 ml Receptal Intrauterinely group II 1.5 ml Intramuscularly while group III Served as control. In both the treated groups 75 percent animals responded to treatment on an average interval of 33.33 ± 2.11 and 30.00 + 3.00 days respectively, as judged by presence of corpus luteum in one of the ovaries. The fertility rate was 75.50 and 12.5 percent for group I, II and III respectively.

INTRODUCTION

Anestrus is one of the major reproductive problems resulting in great loss to the dairy farmers. In anestrus animals, there is an inadequate hypothalamic stimuli for the release of gonadotropic hormones (Shokier, 1959). Receptal is a synthetic GnrH preparation which is reported to release endogenous gonadotropins and has been used in the treatment of anestrous condition (Nasr. *et al* 1983, Pottabiraman *et al* 1986). In the present experiment, Receptal has been used by different dose and route of administration to bring down the cost of treatment in anestrous buffaloes.

MATERIALS AND METHODS

Buffalo cows, which were found true anestrous on two subsequent examination at an interval of 10 days and having 60 days post-partum period were included in this study. The animals were divided into three groups of eight (8) animals, each Group I received 2 ml Receptal (Hoechst, West Germany) by intra-uterine route, group II received 5 ml by intramuscular route while group III served as control. All the animals were examined per-rectally at an interval of 10 days for a period of 60 days to detect silent ovulation or changes in the ovaries. Estrus detection was done by the buffalo bull twice daily in addition to visual observations in the morning and evening. Those detected in estrus were served naturally & pregnancy diognosis was done 60 days post-service by rectal examination.

The results of the present experiment are presented in the table. In group I, the response of the experimental animals to GnRH is parallel to group II in terms of interval between injection/infusion to occurrance of corpus luteum. No animals in either group showed ovartsigns of estrus, but all responding animals ovulated quitly. The interval between injection to occurance of estrus treated with GnRH by intramuscular route observed in the present study (70.83 ± 6.32 days) is much higher than that reported in cows (Kodagali, 1981) or in buffaloes (Pattabiraman *et al* 1986). This disparity cannot be attributed to any apparent cause. It is quite likely that response to GnRH may vary with nutritional as well as reproductive status of the animals. However, the response obtained in the present study is close to earlier report in buffaloes (Nasr. *et al* 1983). In the present study GnRH caused quite ovulation/luitinization initially, followed by ovart estrus at on interval exceeding the normal cycle length. This can be attributed to further quite ovulations as was evident by changed location of initial corpus luteum. The reasons for these quite ovulations are not clear.

Intra-uterine dose of 2.5 ml Receptal was as effective 5 ml intramuscularly. Possible the dose of 2 ml intramuscularly should be as effective as 5 ml intramuscularly, as there is no local effect GnRH either on the tract or ovaries as observed in present study.

Table: Effects of Receptal by different dose and route of administration in anestrus buffaloes.

Group	No. of animals treated	No. of animals responding (%)	Interval between injection to occurance of corpus luteum (days)	No. of animals cycling	Interval between occurrance of C.L. to estrus (days)	No. of animals pregnant (%)
I (Intra-uterine)	8	6 (75)	33.33±2.11	6	70.83±6 32	6 (75)
II (I/M)	8	6 (75)	30.00±3.00	4*	63.00±4.62	4 (50)
III (Control)	8	1	40.00	-	-	1 (12.5)

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Super Ovulation and Embryo Recovery in Jamunapari Goats Using FSH of Equine Origin

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ABSTRACT

Seventeen pure bred parous Jamunapari goats aged 3½ - 4 Yrs of experimental herd of the institute were utilized as embryo donors in the present study. The goats were managed under semi-intensive husbandry conditions. Fifteen of seventeen goats (88.23%) exhibited oestrus within 96 h of sponge withdrawal. Oestrus in two goats was prolonged and split type. The mean duration of synchronized oestrus was 33.66±4.11h. Average ovulation rate, recovery rate of eggs/embryos and of transferrable embryos were 13.38±1.62 (4-26), 7.46±1.46 (2-20) and 2.07±0.78 (0-20) respectively.

INTRODUCTION

The embryo transfer technology is a valuable tool for fast exploitation of genetic potential of elite breeds of animals. The commonly used superovulatory agents are gonadotrophins either of pituitary origin (Armstrong *et al,* 1983[a]) or pregnant mare's serum (Armstrong *et al,* 1983[b]); agrawal *et al,* 1985). Source of follicle stimulating hormone has been found to affect the superovulatory response in different species of animals (Donaldson, 1989). The aim of the present study was to investigate the ovarian response in Jamunapari goats after treatment with FSH of equine origin.

MATERIALS AND METHODS

Seventeen purebred parous Jamunaprai goats of 3½ - 4 Yrs of age of the experimental herd of the institute were subjected to superovulatory treatment with the view to harvest embryos for embryo transfer work. The animals were managed under semi-intensive husbandry conditions. They were allowed for grazing 6-8 h daily supplemented with seasonal cultivated green fodder. They were also provided with 500 gms concentrate mixture with 16% digestible crude protein (DCP) and 80% total digestible nutrients (TDN). The experiment was conducted in June-July 1990. Veramix sponge (60 mg medroxy progesterone acetate, Upjohn, Ltd. UK) was placed intravaginally and kept insitu for 18 days to regulate the oestrous cycle. The superovulatory treatment using FSH of equine origin in descending divided dose schedule of 6, 4, 3 and 2 mg over 4 days in equally divided doses at 12 h interval was begun 48 h before the veramix vaginal sponge withdrawal. The does were observed for oestrus at 12 h interval using an aproned buck. In order to enhance ovulation of developed follicles, each doe received an intravenous injection of 500 I.U. of HCG (Chorulon, Intervet, Holland) at the onset of oestrus. Each doe was hand mated at 0.12 and 24 h of oestrus exhibition by buck of proven fertility.

Embryo collection: The embryo collection was made surgically as per the procedure adopted by Agarwal *et al,* (1982[a]). The genetalia was exteriorized through a mid ventral incision cranial to the mammory attachment. Oviducts were flushed with Dulbecco's phosphate buffered saline (D-PBS, GIBCO, USA) enriched with 5% goat serum in a retrograde fashion, 72-80 h after the onset of synchronized oestrus. Flushings were examined under sterio-zoom microscope under low magnification for morphological characteristics before further use.

RESULTS AND DISCUSSION

Fifteen does responded to treatment and exhibited typical standing oestrous behaviour within 96 h of progesterone impregnated vaginal sponge withdrawl. Majority (13) of the does exhibited oestrus within 36 h (12-36) following the sponge withdrawal. The mean sponge withdrawal oestrus interval observed in the present study is comparatively higher (34.13±5.17h) to the reported values of 27.60±3.50h (24-32) in Alpine goats (Baril and Vallet, 1990) and lower to that reported in Feral goats (1.7±0.4 days) by Armstrong *et al* (1983[a]). This difference seems to be due to difference in the agents used for oestrus synchronization and source of FSH. The overall oestrus duration was 33.60±4.11 h (28-84), which is comparable to the Feral goats (1.7±0.4 days) superovulated with the combination of FSH-P and Prostaglandin F2 alpha analogue i.e. cloprostenol (Armstrong *et al,* 1983[a]0. However the oestrus was prolonged and of split type in two does.

Agrawal (1986) in Barbari goats with PMSG and Goel and Agrawal (1990) in Jamunapari goats

with FSH-P (Schering Corp. USA) observed a mean ovulation of 7.83±1.01 and 8.80±1.73 respectively, which are much lower to the 13.38±1.62 (4-26) in the present study. The ovulation rate in the present study is in close resemblence to Alpine goats (12.70) as reported by Baril and Vallet (1990) but lower to 16.10±0.80 in Feral goats (Armstrong etal 1983). This variability in the superovulatory response is the main constraint of embryo transfer technology. There was no significant, difference in ovulation rate in left and right ovaries in the present study (90 VS 84; P>0.01). The egg/embryo recovery rate (7.46±1.46) in the present study is comparable to earlier reports in the same breed of goat (7.00±1.44), when FSH-P was used as superovulatory agent (Goel and Agrawal, 1990) but significantly lower to Angora and Barbari goats by FSH-P and PMSG as reported by Armstrong and Evans (1983) and Agrawal *et al,* (1982) respectively. If we compare the present egg/embryo recovery rate (55.74%) with the earlier report of Nuti *et al.* (1987) i.e. 63%, in slightly lower. The probable reason for the poor recovery becomes clear in the light of average ovulation rate of 13.38±1.62(4-26). Due to inconsistent response i.e. hypo to hyper stimulation of ovaries, egg/embryo pick up by fimbria was poor. Consequently a good number of egg/ embryos were dropped in abdominal cavity.

Inspite of moderate to optimum superovulatory response and embryo recovery rate a lower number of transferrable embryos could be recovered in flushings (2.07±0.78, range: 0-10). The fertilization depends upon a number of factors viz. timely breeding with proven sire, ovulation time and altered hormonal profile of the animal as a result of exogenous hormonal administration. The focal point for the poor results may lie in any of the following points. Keeping in view the range of ovulation timings, the mating was done at least twice and luteinizing hormone (Chorulon, Intervet, Holland) was administered exogenously to enhance the ovulation of developed follicles, the possibility of delayed ovulation, a reason for poor fertilization rate can not be ruled out. The LH contamination in FSH has deleterious effects an fertilization rate in cattle (Donaldson and Ward, 1986) and accordingly this may be another reason for poor fertilization rates in the present study.

It is concluded that although the superovulatory response was optimum by FSH of equine origin with acceptable degree of recovery rate, the recovery rate of transferrable embryos was not very satisfactory. Futher investigations are warrented before using this gonadotrophin as a reguar superovulatory agent for embryo transfer programme in caprine species.

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Effect of PMSG and HCG on Ovarian Follicular Growth in Local Goats of Assam

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ABSTRACT

The present experiment was an attempt to study ovarian follicular growh in local goats of Assam after administration of three different doses of PMSG and HCG. Laparotomy was conducted at 48 hours following onset of oestrus to record the number of different type of follicles on the ovarian surface. Follicles below 1 mm size were not counted. Number of different types of follicles i.e. small, medium and large, differed significantly ($P<0.01$) among the three different PMSG : HCG treated groups. Total follicular count was significantly higher (11.60 ± 0.31) in the group receiving 500 : 1000 I.U. of PMSG : HCG. The significantly lower number of total follicles in the group receiving 600 : 1000 I.U. of PMSG: HCG indicated a rapid growth and maturation of the follicles leading to eventual rupture and thereby increasing the ovulatory performance.

INTRODUCTION

For successful embryo transfer technology ovarian response must be altered by using gonadotropins or its analogues. Ovarian follicular growth can be induced by gonadotropin administration in domestic ruminants as reported by Mc Natty *et al.* (1982). Ovarian potentialities in local goats of Assam have not been exploited so far. The following work has been initiated to study the effect of different dose combination of PMSG : HCG on ovarian follicular growth in local goal of Assam.

MATERIALS AND METHODS

Forty sexually matured healthy local cyclic female goats of Assam, 2-3 years old, formed the subjects for the study. Two healthy vasectomized bucks were selected for detection of heat. The animals were maintained under semi-intensive system of rearing and were supplied with 250 g of concentrate mixture per doe per day and water *ad-libitum*. The animals were divided into four groups comprising of ten animals in each group. One group served as control, while the other three were used to be treated with 500, 600 and 700 I.U. of PMSG (Folligon**) respectively, on 15th day of the oestrous cycle and subsequently injected with 1000 I.U. of HCG (Chorulon***), at 6 hours post onset of oestrus. The control group received two injections of distilled water at the same time schedule. Heat was observed at 6 hours interval and detected with the help of a vasectomized buck. All the animals were laparotomised at 48 hours post onset of oestrus to record the number and size of unovulated and ovulated follicles in both the ovaries. Follicles were classified on the basis of sizes as small (1-3 mm), medium (3-6 mm) and large (> 6 mm). Follicles below 1 mm size were not counted.

Results were analysed statistically following the methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean number of small, medium, large and total of unovulated and ovulated follicles found in both the ovaries under three different PMSG : HCG treated and control groups are shown in Table 1.

Significantly ($P<0.01$) higer mean of small follicles was recorded in group II-B (3.80 ± 0.48). In group II-B_2 and II-B_3 the mean (0.60 ± 0.24 and 0.40 ± 0.24, respectively) number was very less even in comparison to the control (2.40 ± 0.24). This implies that use of 500 : 1000 I.U. of PMSG : HCG would contribute to the faster development of follicles keeping only few of them small at 48 hours post onset of oestrus. But further increase of PMSG might cause a very rapid conversion of the follicles, other than to the small type, resulting in presence of insignificant number of small typed follicles at 48 hours post onset of oestrus. However, the mean number of medium

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** Folligon - Inervet, Holland

*** Chorulon - Intervet, Holland

type follicles was significantly (P<0.01) higher (5.80 ± 0.37) in the control group than other PMSG : HCG treated groups. The cause might be attributed to the stimulatory activity of these superovulatory hormones either towards the formation of greater number of large preovulatory follicles or ovulatory follicles at 48 hours post onset of oestrus. Again the mean number of preovulatory or large follicles was significantly (P<0.01) higher in group II-B_1 and II-B_3 (4.40 ± 0.67 and 4.20 ± 0.37, respectively) than in group II-B_2 (1.20 ± 0.37) and control (0.40 ± 0.24). Presence of significantly greater number of preovulatory/large follicles in the treated groups could be explained by the fact that the PMSG : HCG contribute significantly to the formation of greater number of preovulatory/large follicles. These findings are in agreement with the report of Dhindsa *et al.* (1971) and Driancourt and Fry (1992). However, significantly lower number of follicles in the group that received 600 : 1000 I.U. of PMSG : HCG indicated a rapid growth and maturation of the follicles leading to rupture and thereby increasing the mean number of ruptured follicles (10.20 ± 1.28).

The mean number of ovulated follicles in all the treated groups were significantly (P<0.01) higher than control group. Significantly higher ovulation rate (10.20 ± 1.28) was recorded in group II-B_2 and lower (2.20 ±0.20) in conrol group. Although use of PMSG : HCG produced a significantly better ovulatory performance, a dose increase of PMSG from 600 to 700 I.U. with 1000 I.U. of HCG, instead of further improving the ovulation rate appeared to act negatively resulting into rather significantly decreased ovulation rate due to limitation of follicle receptor/threshold capacity of the follicles. This increased number of ovulated follicles due to PMSG : HCG administration was in conformity with the report of Rao *et al.* (1984) and Song and Iritani (1986).

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Table 1: Number of different type of unovulated follicles and ovulated follicles in three different PMSG : HCG treated and control groups

Groups	Dose PMSG: HCG I.U.	Type of unovulated follicles			Total of unovulated follicles Mean ± S.E.	Number of ovulated follicles Mean ± S.E.
		Small (1-3 mm) Mean ± S.E.	Medium (3-6 mm) Mean ± S.E.	Large (>6 mm) Mean ± S.E.		
II-A (Control)	Distilled water	2.40 ± 0.24^b	5.80 ± 0.37^a	0.40 ± 0.24^b	8.60 ± 0.62^b	2.20 ± 0.20^d
II-B_1	500 : 1000	3.80 ± 0.48^a	3.40 ±0.40^b	4.40 ± 0.67^a	11.60 ± 0.31^a	4.20 ± 0.20^c
II-B_2	600 : 1000	0.60 ± 0.24^c	2.40 ± 0.24^b	1.20 ± 0.37^b	4.20 ± 0.25^c	10.20 ± 1.28^a
II-B_3	700 : 1000	0.40 ± 0.24^c	3.60 ± 0.51^b	4.20 ± 0.37^a	8.20 ± 0.49^b	6.40± 0.24^b

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Response of Superovulation in Prepubertal Goats

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ABSTRACT

The experiment was conducted on a total of 16 prepubertal goats in four groups comprising of four goats in each group. Pregnant mare serum gonadotrophin and Human chorionic gonadotrophin, either alone or in combination was used to induce superovulation. Superovulatory response did not increase by increasing the dose of PMSG from 500 IU to 750 IU. Average number and percentage of ovulations increased significantly, ($P<0.01$) with the incorporation of HCG along with PMSG and the best results were obtained with 750 IU PMSG and 1000 IU HCG administered together. Highly significant effect ($P<0.01$) of treatments on the number of anovulatory follicles was observed, thus, anovulatory follicles were maximum in the group in which highest dose (750 IU) of PMSG alone was administered.

INTRODUCTION

The generation interval from birth to reproductive age can be reduced be obtaining embryos from prepubertal goats. The harvest of total number of embryos for transfer may be increased by including superovulation at prepubertal stage, thus exploiting the maximum reproductive potential of the donors which are generally of excellent pedigree. Superovulation in adult goats with hormonal treatments has been reported in literatures but such information in prepubertal goats is meagre (Shukla *et al.*, 1971; Soma and Sugie, 1971 and Song and Iritani, 1987). This experiment was therefore, undertaken to study the superovulatory response in prepubertal goats using PMSG and HCG either alone or in combination in varying doses.

MATERIALS AND METHODS

The experiment was conducted on a total of 16 prepubertal goats (4-5 months of age). The goats were managed under loose housing system and fed as per NRC standards. Study was conducted in four groups comprising of four goats in each group. Pregnant mare serum gonadotrophin (Folligon, Intervet) and Human chorionic gonadotrophin (Chorulon, Intervet) either alone or in combination were used to induce superovulation in different groups. Goats in group 1 were administered 500 IU PMSG; group II, 500 IU PMSG and 1000 IU HCG; group III, 750 IU PMSG and Group IV 750 IU PMSG and 1000 IU HCG through intramuscular route. Oestrus was detected after PMSG injection by visual inspecion, behavioural changes and with the help of a vigorous buck with good libido both morning and evening. Upon detection of oestrus HCG was administered. The oestrus goats were mated twice withbucks of known fertility at 24 hours interval. Response to superovulation was judged on the day of embryo collection by counting the number of corpora lutea, corpora haemorrhagica and anovulatory follicles obtained on both the ovaries. Statistical analysis was done according to the methods suggested by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

All the goats exhibited oestrus within 4 to 7 days of treatment with PMSG. Ovarian response to superovulatory treatment has been presented under table 1. Average response was identicalin group III (10.75 ± 2.14) and group IV (10.75 ± 2.72) whereas, it was low in group I (8.00 ± 1.22) and group II (7.25 ± 0.48). Analysis of variance did not indicate any significant difference between groups with different doses of gonadotrophins on ovarian response. Mc Donald (1980) opined that the prepubertal animals require superovulatory treatment with LH or LH like gonadotrophin after FSH treatment to induce superovulation. Song and Iritani (1981) also used PMSG and HGC in different combination for superovulation and embryo collection in immature (age 2 to 3 months) goats. It is evident from the table that average number of ovulation and ovulation percentage was the highest in group IV followed by groups II, III and I. Analysis of variance indicated a highly significant effect of different doses on the number of ovulations. It may be mentioned here that goats in groups II and IV received HGC in addition to PMSG whereas groups I and III received PMSG alone. This was in accordance with the finding of Rao and Ramakrishna (1987) who also oberved that ovulation were maximum in calves where HCG was administered along with FSH. Shukla *et al.*

(1971) noted that all the females in groups treated with pregnant mare serum and HCG had ovulated in comparison to none in the control group.

Examination of the ovaries for response invariably showed presence of certian number of anovulatory follicles which were maximum in Group III (9.50 ± 1.19) and Group I (7.25 ± 0.85) where PMSG were administered alone. Occurrence of anovulatory follicles was significantly (P<0.05) low in Group II and Group IV where HCG and PMSG combination was used. Analysis of variance showed hightly significant (P<0.01) effect of treatments on the number of anovulatory follicles. Rao and Ramakrishna (1987) observed a higher number of large anovulatory follicles with PMSG in comparison to FSH. They also observed that individual response to superovulation was variable comparison to FSH. They also observed that individual response to superovulation was variable from animal to animal and was not directly related to the age or body weight of the animals. Bhattacharya *et al.* (1989) also recorded a significant number of anovulatory follicles in cows superovulated with PMSG. The number of such follicles varied from animal to animal and also between treatments.

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Table 1: Average ovarian response to varying doses of gonadotrophins in prepubertal goats (n = 16)

Groups	CL	CH	AF	Total	Av. No. ovulations	Ovulation percentage
I	0.00	0.75 ± 0.48	7.25 ± 0.85	8.00 ± 1.22	0.75 ± 0.48	9.37
II	1.00 ± 0.71	2.50 ± 0.29	3.75 ± 0.48	7.25 ± 0.48	3.50 ± 0.65	48.27
III	0.25 ± 0.25	1.00 ± 0.71	9.50 ± 1.19	10.75 ± 2.14	1.25 ± 0.95	11.62
IV	5.00 ± 2.85	3.00 ± 1.58	2.75 ± 0.75	10.75 ± 2.72	8.00 ± 2.52	74.41

C.L. = Corpus luteum
C.H. = Corpus Haemorrhagicum
A.R. = Anovulatory follicles.

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Control of farrowing in Sows with Prostaglandin

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ABSTRACT

The study was conducted on 68 sows of Landrace, Tamworth and Large white York shire breeds. All the experimental sows were injected prostaglandin F_2 alpha intramuscularly on day 111 of gestation. The sows were divided into four groups and received 5 mg (group I), 10 mg (group 2) and 15 mg (group 3) of prostaglanding injection. The control (group 4) received 3 ml of normal saline. The interval between injection and farrowing, time of farrowing, litter size and piglet mortality within 24 hours were recorded. The average intervals between injection and farrowing were 27.87±2.23, 26.43±1.19 and 26.96±3.38 hr respectively for 5 mg, 10 mg and 15 mg prostaglandin F_2 alpha treated groups in comparison to 47.38±3.52 hr in the control group. There was no significant difference within the experimental groups of sows but revealed a significant (P<0.05) effect of administration of prostaglandin F_2 alpha on the interval between injection and farrowing. The average piglet mortality in experimental groups was 8.79 percent whereas it was 16.89 percent (P<0.05) in the control group.

INTRODUCTION

Controlling the farrowing time in sows offers better supervision of parturition process. This in turn helps in reducing piglet mortality as farrowing can be timed to take place during working hours of the day when an attendent is present to assist the delivery. The pre- and post partum losses of piglets due to unattendance has been estimated to be as high as 20 to 25 percent (Dziuk, 1977). For inducing parturition in sows several techniques have been employed, including administration of corticosteroids (First and Bost, 1979), oxytocin (Welk and First, 1979) and prostaglandin F_2 alpha (Diehl *et al.,* 1974; Stephens *et al.,* 1988 and Sinha *et al.,* 1990). Nevertheless, prostaglanding F_2 alpha has been reported to be most effective either alone or in combination with estradiol and Oxytocin (Gall and Day, 1987) and Xylazine (Ko *et al.,* 1989). Present investigation was conducted to study the different dose schedules of prostaglandin F_2 alpha tham salt (Lutalyse, Up John) for inducing desired time parturition in sows and to observe piglet mortality during first 24 hours of induced parturition.

MATERIAL AND METHOD

The study was carried out on 68 sows of Landrace, Tamworth and Large white Yorkshire breeds maintained at ICAR Pig breeding farm of college of Veterinary Sciences, Birsa Agricultural University, Ranchi during January, to December, 1990. The study was conducted in four groups and each sow was administered with an intramuscular injection of 5 mg (group 1, n = 15), 10 mg (group 2, n = 15) and 15 mg (group 2, n = 14) respectively of prostaglandin F_2 alpha (Lutalyse, Up John). The fourth group (control group, n = 24) received 3 ml of normal saline intramuscularly instead of prostaglandin F_2 alpha. Subsequent to injection all the sows were watched closely for farrowing symptoms. Advent of farrowing time was recorded when the first piglet was born. The interval between injection and farrowing, time of farrowing, litter size and piglet mortality within 24 hours were recorded.

RESULTS AND DISCUSSION

The average interval between injection of prostaglandin F_2 alpha and the number of sows farrowed during day and night in three experimental and control groups are presented in Table 1. It is evident from the table that the farrowing interval was shorter in experimental groups (27.87±2.23, 26.43±1.19 and 26.96±2.38 hours respectively for 5 mg, 10 mg and 15 mg prostaglanding F_2 alpha treated groups) in comparison to the control group (47.38±3.52 hours) of sows. Analysis of variance showed significant (P<0.01) effect of administration of prostaglandin F_2 alpha on the initation of farrowing from the time of treatment.

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However, the differences within the experimental groups were statistically not significant. This observation is very much similar to the findings of Stephens *et al.,* (1988) who reported that 91 to 100 percent of sows began to farrow within 30 hours after injection of prostaglandin F_2 alpha. However, Gall and Day (1987) observed slightly higher (31.3±1.40 hours) values than the present findings. Gall and Day (1987) further observed that by increasing the dose of prostaglandin F_2 alpha (10 and 20 mg) the sows were expected to farrow within a short interval of time. This could not be confirmed during the present study, however, the interval in group sows (5 mg PG F_2 alpha) was approximately one hour more than groups 2 and 3 (10 mg and 15 mg PG F_2 alpha), which was statistically not significant.

During the present study the average piglet mortality in experimental groups was lower (8.79%) in comparison to the control group (16.89%, Table 1). Chisquare test revealed a significantly (P<0.05) higher piglet mortality in the control group as compared to the experimental groups (Table 1). However, the differences in mortality among the three experimental groups were not significant. It may be inferred that since majority of farrowing in the experimental groups occurred during the day time, an attendent was present to assist the delivery process and hence the piglet mortality was reduced. Present finding corroborates the observation of Randall (1972) who also reported that since most of the still births that occurred during the later stages of parturition in swine, could be prevented by having an attendent at the time of farrowing.

Tabel 1: Farrowing time, interval between administration of PGF^2 alpha and farrowing and post natal mortality within 24 hours of birth in sows.

Group	5.00 AM 5.00 PM	5.00 PM 5.00 AM	% farrowed during day	Interval (hours)	Piglet born	No. & % Post natal mortality
1) 5mg	14	1	93.33	$27.87^a \pm 2.23$	145	11 (7.59%)
2) 10mg	13	2	86.67	$26.43^a \pm 1.19$	145	14 (9.79%)
3) 15mg	13	1	92.86	$26.96^a \pm 2.38$	133	12 (9.02%)
4) Control	14	10	58.33	$47.38^b \pm 3.52$	225	38 (16.89%)

Means bearing different superscripts in a column differ significantly (P<0.05).

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Superovulation And Embryo Recovery in Rabbits*

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ABSTRACT

Six Albino rabbits were supervulated by injecting 150 IU PMSG intramuscularly as a single dose to does followed 60 + 072 hrs later by natural mating with two fertile bucks. hcG 150 IU was injected intravenously post coitum to does as a single dose. Embroys were recovered from does 96 hrs post coitum by surgical method using DPBS. The number of CL and anovulatory follicles was 24.66±2.71 and 15.66±2.24 respectively. Mean percentage of eggs recovery and eggs fertilized was 37.04±10.09 and 91.41±5.77 respectively.

MATERIALS AND METHODS

Six Albino rabbit does weighing 1 kg to 1.5 kg and two fertile Albino rabbit bucks were used as experimental animals. All these selected rabbits were kept under identical feeding and managemental conditions. All the does and bucks were caged individually. Superovulation in does was achieved by injecting PMSG i.e., inj. Folligon (Intervet int., Boxmeer, Holland) 150 IU intramuscularly as a single dose. Natural mating with fertile bucks was arranged 60 to 72 hrs following PMSG treatment. hcG i.e., Inj. Chorulon (Intervet Int., Boxmeer, Holland) was injected intravenously 150 IU in does post coitum. By performing mid-ventral laparotomy of does, uterine cornuae and Fallopian ducts were flushed with Dulbeco's Phesphate Buffer saline (DPBS) with 2 per cent rabbit serum. Petridishes with flushed DPBS were examined under a stereoscopic microscope at 40 X magnification.

RESULTS AND DISCUSSION

Mean number of anovulated follicles was 15.66±2.24. These findings are in agreement with those of Illera *et al.,* (1988) but are lower than Kim *et al.,* (1988). Mean number of CL was 24.66±2.71. Present findings are in agreement with those reported by Illera *et al.,* (1988) but are superior than that (11.83 to 13.0) Taneja *et al.,* (1990). Mean percentage of ovulation to total ovarian activity in does was 61.48±3.05. These findings are in agreement with those of Illera *et al.* (1988). Mean percentage of eggs recovered from does was 37.04±10.09 and findings are in agreement with those reported by Taneja *et al.,* (1990) but lower than that (81.0 to 94.0%) Kim *et al.,* (1988). Mean percentage of eggs fertilized in does was 91.41+5.77 and findings are superior than those reported by Ross (1987). Mean number of abnormal embryos recovered was 2.83±0.43 and findings are in agreement with reported by Taneja *et al.,* (1990).

* Part of M.V.Sc., Thesis submitted by the Senior author to M.A.U., Parabhani-431402 (M.S.)

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Role of Some Minerals in Delayed Maturity of Cross-bred Cattle*

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ABSTRACT

A total of 592 F_1 cross-bred heifers examined with a history of anoestrus revealed that 30.90 per cent had developed genetalia but non functional ovaries. However, 42.06 per cent heifers clinically investigated were found to have hypoplastic genital organs and gonads. Serum samples collected from these heifers with developed but non functional ovaries revealed a high significant difference (P<0.01) with respect to inorganic phosphorous, Ca:P ratio, total proteins and zinc levels when compared with cycling heifers. The level of cholesterol in serum showed a significant difference (P<0.05) whereas the serum calcium levels did not differ significantly between the cycling and delayed matured heifers. The possible treatment of these animals found deficient were being discussed and corrective measures regarding the onset of oestrus in delayed matured heifers are advocated.

INTRODUCTION

The cross-bred heifers have shown the average age at maturity at about 15-18 months located at different agroclimatic conditions of the country. But in Orissa, the sexual maturity of F_1 crossbred Jersey heifers are round about 22 months (Choudhury, 1984). It is of common knowledge that nutritional deficiency can cause retarded growth and development of genital organs resulting in non functional ovaries in otherwise disease free animals. Therefore, the present study was undertaken to evaluate certain blood constituent levels in delayed matured croosbred heifers and to suggest some curative measures against local agroclimatic condition.

MATERIALS AND METHODS

A total of 592 F_1 crossbred heifers having passed 18 months of age but not exhibiting heat symptoms were examined and only a total of 62 animals with normal developed genitalia but smooth quiscent ovaries were taken up for investigation. A total of 20 normal cycling heifers with properly developed genital organs with functional ovaries were taken as control group. Blood was collected from non cycling animals on the day of examination and serum was separated to be kept in deep freeze (-20°C) for further analysis. Similarly blood was collected from cycling animals (control) for comparison. Blood serum collected from both these group of animals were analysed for cholesterol (Parekh and Jung, 1970), Calcium (Oser, 1965), Inorganic Phosphorous (Spectronic 20 manual), Total Protein (Spectronic 20 manual) and Zinc (Smith *et al.* 1979) concentration.

A total number of 62 animals were found to be deficient in phosphorous, total protein and zinc either singly or in combination when compared with the control ones. The normal range of Calcium, Phosphorous and Zinc as estimated by previous workers (Morrow, 1969 and Chandolia and Verma, 1987) were taken as the minimum standard for deficiencies. Animals found deficient in phosphorous were supplemented with Tonophosphan parenterally @ 5 ml twice a week for 3 weeks. Animals having low level of zinc in blood serum were supplemented with a special Trace-Min formula consisting of Zinc sulfate, Copper sulfate and Ferrous sulfate orally, @ 1 gram daily for 15 days. Similarly protein deficient animals were supplemented with a computed ration containing a minimum of 10 per cent for 1 month. Statistical analysis were done as per the methods described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean values of the blood constituents studied in both the delayed maturity and normal cycling heifers with the analysis of variance are presented in Table 1.

The significant difference (P<0.05) between the normal cycling and delayed matured heifers with respect to cholesterol might imply that concentration

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of cholesterol in blood and the synthesis of steroids are probably related to the energy status of the animals which finds the support of Velhankar (1973) who reported a positive correlation between blood cholesterol concentration and energy status of the animals. Therefore, the low cholesterol level during the present study might be a contributing factor in delaying the onset of maturity due to hypocholesterolemia.

Lower level of phosphorous in delayed matured heifers (P∠0.01) might be a contributing factor in inhibiting the anterior pituitary resulting in supression of both ovarian and genital activity. It can be further stressed that the imbalanced calcium phosphorous ratio (P∠0.01) might have resulted in pituitary gonadal disfunction and delay in the onset of maturity in cross-bred heifers (Morrow, 1977).

The highly significant difference (P∠0.01) with respect to total protein between both the group of animals finds support from Herrick (1977) and is further substantiated by Wiltbank (1965) that heifers maintained on low protein diet did not reach puberty earlier. As the studies were undertaken in field condition the discrepancy of protein content in the diet cannot be ruled out. Hence, the deficiencies of protein in diet and its disturbed metabolism and its utilisation might affect reproduction during the process of maturation amongst the heifers.

The significant difference (P∠0.05) between the zinc level is in agreement with Chandolia and Verma (1987) between cycling and delayed matured heifers, but Olivieri *et al.* (1977) found that zinc level was not related to fertility.

The discrepancy in the zinc level observed might be due to difference between individual animals and adoptability to different geo-climatic regions exerting a significant effect during which sampling was made.

The treatment done on the basis of the deficiencies and the levels of these constituents in animals exhibiting oestrus and not exhibiting oestrus at pre and post treatment period with the analysis of variance are presented in Table 1. A total of 44 animals (70.96 per cent) exhibited oestrus out of 62 animals treated. The regime of post treatment resumption of heat covered a period between 15 to 60 days. It was evident from the present investigation that in the group of animals exhibiting oestrus and not exhibiting oestrus, the post treatment values of serum constituents in all the blood parameters increased except in the calcium level that did not exhibited oestrus. High significant difference (P∠0.01) observed in group of animals those exhibited oestrus might be attributed to supplementation of required minerals and which might have enhanced the function of tubular genital tract and function of ovary. The animals that did not exhibit oestrus after treatment implies that these animals might be requiring more of a particular ingredient or might be due to some other causes which are not included in the present study.

Therefore thorough investigation keeping in view of various agro-climatic regions and deficiencies of blood constituents backed by diagnostic aids may be quite useful in tackling the serious problem of delay in maturity for subsequent fertility.

Table 1: Blood serum constituents (Mean ± S.E.) of normal cycling and delayed matured heifers and during pre and post treatment period with analysis of variance.

Sl. No.	Group	Total cholesterol (mg %)	Calcium (mg %)	Inorganic phos-phorous (mg %)	Calcium phos-phorous ratio	Total protein (gm %)	Serum zinc (mcg %)
(A)	**Non Treated animals**						
1.	Cycling heifers (20)	118.78±3.45	10.14±0.09	5.70±0.13	1.79±0.05	7.25±0.09	103.53±2.40
2.	Delayed matured heifers (62)	108.95±1.70	10.02±0.09	2.92±0.08	3.43±0.09	6.84±0.08	53.96±1.19
	F Value	6.77*	0.17^{NS}	6.81**	35.36**	6.88**	50.64**
(B)	**Treated animals**						
1.	Responded: 44						
	(a) Pre treatment	102.50±2.67	10.01±0.10	3.03±0.12	3.40±0.14	6.62±0.17	56.69±3.49
	(b) Post treatment	132.75±2.16	10.27±0.11	5.26±0.12	1.90±0.05	7.20±0.12	100.86±15.28
	F Value	25.97*	1.29^{NS}	30.30**	26.41**	6.51*	31.37**
2.	Not Responded: 18						
	(a) Pre treatment	107.50±2.77	10.60±0.17	2.90±0.14	3.81±0.21	6.69±0.17	59.16±3.80
	(b) Post treatment	116.66±1.98	10.40±0.16	3.26±4.24	3.25±0.09	7.05±0.11	66.16±3.26
	F Value	1.42^{NS}	1.23^{NS}	3.25^{NS}	4.89*	2.72^{NS}	1.75^{NS}

*P<0.05 = Significant at 5 per cent level. **P<0.01 = Significant at 1 per cent level. NS = Non significant

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Effect of Parity of some Productive and Reproductive Traits in Sahiwal Cattle *(Bos Indicus).*

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ABSTRACT

Breeding phenomenon in Sahiwal cattle was studied in order to elucidate the effect of parity on different reproductive and productive parameters. Parity was found to have significant effect on age at fertile service, age at calving, weight of male calves and lactation milk yield, whereas it had no significant effect on weight of female calves, gestation length, service period, intercalving period and lactation period. The study involved an analysis of 104-393 (number) observations for different economic characters.

INTRODUCTION

For formulating breeding programme for genetic improvement of dairy cattle on scientific lines, it is essential to have the basic information on productive and reproductive characters. Sahiwal, being one of the important breeds of dairy cattle of central and southern dry area of the Punjab State, the present study was undertaken.

MATERIALS AND METHODS

The study was based on the data collected from records of Sahiwal cattle maintained at college of agriculture, C.S.A. University of Agri. & Tech., Kanpur for the period 1963 to 1987. Age at fertile service, Age at calving, Weight of male and female calves at birth, Service period, Gestation length, Intercalving period and Lactation milk yield were studied for observation varying from 104-393 distributed over 8 lactations. Effect of parity was observed through analysis of various as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Age at fertile service increased from 945.65±33.15 days for the first lactation cows to 4121.22±109.42 days for the VIIIth lactation cows. Similarly age at calving increased from 1227.69±20.36 days to 4406.77±67.18 days for the same period. Weight of male calves ranged between 21.39±0.37 Kgs. (Ist lactation) to 23.73±0.72 Kgs. (Vth lactation). Similarly the weight of female calves ranged between 21.04±0.76 Kgs. (VIth lactation) to 21.75±1.70 Kgs. (VIIIth lactation).

Service period varied between 221.93±40.46 days (VIIth lactation) to 291.88±15.83 days (Ist lactation). Gestation period ranged between 284.14±1.20 days (Vth lactation) to 285.80±1.83 days (VIIth lactation). Intercalving period varied between 497.20±39.99 days (VIIth lactation) to 582.05±15.66 days (Ist lactation). Lactation period and milk yield were lowest in Ist lactation (291.37±13.36 days) and highest in VIIth lactation (1729.60±176.69 Kgs) respectively. The lactation period and milk yield were highest in VIIIth lactation (340.44±26.36 days) and in Vth lactation (1929.01±115.67 Kgs).

Parity significantly influenced the birth weight of male calves and lactation milk yield and had highly significant influence on age at fertile service and age at calving. No significant effect on weight of female calves, gestation length, service period, intercalving period and lactation period was observed.

Ponshke (1969), Kodagali (1980) and Velhankar (1973) reported the age at first fertile service as 1007.95±22.56 days; 34.90±5.63 months and 978.66±28.73 days respectively in GIR cattle. These values are higher than present findings, may be due to different locations, breed and animal husbandry practices followed. Aziz and Siddhu (1983) Tewari and Kushwaha (1987) reported lower age at first calving (38.98±1.48 months and 1095.09 days respectively). Juma and Kessair (1967) reported birth wt. of male calves in pure and crossbreds as 32.32 and 34.18 kg. respectively. Friesion calves had the highest birth weight 26.30±0.56 Kgs. and Sahiwal the lowest 21.11±0.45 Kgs. (Aziz and Sidhu, 1983) which is comparable to present findings. Higher service period (336.00 days) than present study (266.02±7.90 days was reported by Bhatnagar *et al.* (1983). While Agrawal *et al.* (1971) observed higher gestation period (294.1 days) as compared to present findings (285.27±0.35 days).

Basu *et al.* (1979) reported the calving interval to range between 350.22±56.39 to 410.46±34.62 days in Sahiwal, Tharparkar and Red Sindhi cows, lower than present findings 551.63±7.81 days, may be due to genotypic and environmental differences. An higher lactation milk yield reported by Singh and Desai (1967); Chopra *et al.* (1973) and Aziz and Sidhu (1983) were 2427 Kgs, 2312 Kgs. and 2754.56±125.80 Kgs. respectively, were comparable to the findings of present investigation.

It can be concluded from the findings that the productive and reproductive efficiency in Sahiwal cattle can be achieved at or after the second lactation. Best results can be obtained upto 3rd lactation. Parity did not significantly influence the birth weight of female calves, gestation length, service period, intercalving period, lactation period (Ist to VIIIth lactation).

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The Relationship Between Fertility and Milk Production in Jersey Cows

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ABSTRACT

The relationship between fertility as measured by the interval from calving to conception or service period (S.P.) and first lactation milk production in terms of total and 305-day yields, yields per day of lactation and per day of calving interval (C.I.) was investigated in a Jersey herd in Andhra Pradesh. The total and 305-day milk yields, lactation length (L.L) dry period (D.P.) and C.I. increased linearly with increase in S.P. The regression coefficients were significant and each additional day of S.P. resulted in increase of 2.49 Kg. of total lactation yield, 1.4 Kg. of 305-day yield, 0.7 days of D.P. and 1.1 days of C.I. The correlation between S.P. and lactation yield was positive and significant. The maximum milk production was recorded for the cows with S.P. of 151 to 180 days and a C.I. of 450 days. The yield per day of C.I. declined with increase in S.P.

INTRODUCTION

Fertility is important to keep cows in production and both these are determinants of economic returns of a Dairy. Since knowledge of the rationship between fertility and milk production is important for effective control of dairy production, the present study was undertaken in Jersey cows.

MATERIALS AND METHODS

A total of 277 first lactation records of the herd at the Jersey Cattle Farm, Banavasi, Andhra Pradesh covering a period of 14 years (1977 to 1990) were used in the study. The interval from calving to conception or service period (S.P.), lactation length (L.L.), milk production 305-days, total lactation, per day of L.L. and per day of calving interval (C.I.) dry period (D.P.) and C.I. were the traits considered. Records of cows with abortion, calving difficulties and short lactations were deleted.

The statistical analyses were carried out as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean service period (S.P.) was 161.8±8.25 days. The frequency distribution of S.P. at class intervals of 30 days showed that maximum number of cows (39%) conceived between 61 to 120 days postpartum (Table 1). About 11.2% and 9.4% of cows had intervals less than 60 and above 361 days respectively. The variation in S.P. was significant (P<0.01).

The mean total and 305-days first lactation milk yield were 2525.2±24.84 Kg. and 2310.4±21.50 Kg. and that of L.L. was 334.5±16.45 days. When those lactation measures were related to S.P., the lowest and highest values were observed for the cows with S.P. of loss than 60 days and above 361 days respectively and their relationship was linear. The regression coefficients indicated that each additional day of S.P. resulted in 2.49 Kg. more lactation milk, 1.4 Kg. of 305-day milk and 0.33 days of L.L. The correlation coefficients between S.P. and lactation yields were positive (0.4012 to 0.4158) and significant (P<0.01).

The different in 305-day milk yields between cows open for less than 60 (lowest production) and above 361 days (highest production) was 520 Kg., whereas the same for total lactation production was 1033 Kg., indicating greater persistency of milk production in the cows with higher S.P.

The mean milk yield per day of total lactation varied from 7.13 to 8.01 Kg. and that of 305-days, from 6.68 to 8.39 Kg. for the cows with different S.P. The highest means for both were recorded for the cows with S.P. of 151 to 180 days. However the different were not significant. The mean milk yield per day of C.I. varied from 4.28 to 6.45 Kg. The yields decreased with increase in S.P. Similar trend was observed for 305-day yields.

The mean first D.P. was 107.8±9.02 and that of first C.I. was 468.4±23.60 days. Both D.P. and

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C.I. increased linearly with increase in S.P. The regression analysis showed that each additional day of S.P. led to increase in 0.7 days of D.P. and 1.1 day of C.I.

The results of S.P. was higher than 104 days and milk yield lower than 3834 Kg. reported for Dairy cows in temperate climates (olds *et al.* 1979). But the results of effect of S.P. on milk production were consistent. According to Morrow *et al.* (1966), Spalding *et al.* (1975), the higher 305-day production was the cause of more days open, while smith and Legates (1962) opined that additional days open caused higher 305-day production. Delayed conception postpartum was not uniformly expensive for all breeds of animals and C.I. of 12 to 13 months was considered to be ideal (Leuca and Legates, 1969). In the present study the maximum milk production was recorded for the cows with a S.P. of 151 to 180 days with a mean C.I. of 450 days. The depressing effect of gestation on production in the cows conceiving early and increased persistency associated with additional days of S.P. may be accounted for higher production in these cows.

Significant effects of S.P. on L.L. persistency, D.P., and milk yield per day of C.I. were also observed by Olds *et al. (loc.cit).* Milk yield per day of C.I. decreases as S.P. increases because additional days of S.P. result in more days in milk which extend the low producing part of lactation and in more days dry.

Positive correlation between milk production and days open as observed in this study was also reported by Smith *et al.(loc.cit.)*

It is evident from this study that S.P., D.P. and C.I. were longer and milk production was lower in Jersey cows maintained under tropical climates compared to these in temperate climates. Cows with higher 305-day milk production tended to have longer S.P. The maximum milk production was recorded in cows with S.P. of 151 to 180 days and a C.I. of 450 days. Increase in S.P. was consistently associated with higher milk production, longer D.P. and C.I.

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Table 1. Relationship between fertility and first lactation milk production of Jersey Cows in Andhra Pradesh.

Service period (days)	n	First lactation Milk production (Kg.)		L.L. (days	D.P. (days)	C.I. (days)	M.Y. per day lact. (Kg)	M.Y. per day C.I. (Kg)
		305-days	Total lact.					
⩽60	31	2038.0	2065.6	274.2	51.9	326.1	7.53	6.33
61-90	55	2216.2	2290.8	301.5	53.7	355.2	7.60	6.45
91-120	53	2240.6	2338.9	318.4	64.9	383.3	7.35	6.10
121-150	39	2378.0	2550.9	333.7	80.8	414.5	7.64	6.15
151-180	24	2562.2	2854.3	356.3	93.5	449.8	8.01	6.35
181-270	25	2260.2	2598.4	364.5	136.3	500.8	7.13	5.19
271-360	24	2466.3	2998.2	397.9	196.5	594.4	7.54	5.04
361	26	2558.4	3099.5	402.4	321.0	723.4	7.70	4.28
Overall	277	2310.4	2525.2	334.5	107.8	468.4	7.55	5.42

n = No. of Observations;
L.L. = Lactation length
D.P. = Dry period;
C.I. = Calving interval;
M.Y. = Milk yield.

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I, Dr. S.R. Pattabi Raman, Editor of THE INDIA JOURNAL OF ANIMAL REPRODUCTION, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dr. S.R. Pattabiraman
Editor.

IJAR. 14(1), 1993; 35-36

Age at First Fertile Service in Gir Cattle*

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ABSTRACT

In a study of records of 23 years of age at first fertile service of 445 Gir cows, results indicated that this parameter was significantly affected by period of calving and not be season of calving. It was also observed that 74 per cent of total variability was due to genetic source.

MATERIALS AND METHODS

The records of 23 years on 445 Gir cows maintained at Cattle Breeding Farm, Junagadh were utilized. 23 years were grouped into 5 periods: P_1 (1966-70), P_2 (1971-75), P_3 (1976-80), P_4 (1981-85) and P_5 (1986-88) and the year was divided into 3 seasons: S_1 (June-September), S_2 (October-January) and S_3 (February-May). The records were analysed by fitting constants (Harvey, 1966). The linear model was used to find out the effect of period of calving and season of calving on age at first fertile service. The DMR Test (Kramer, 1957) was used to test the diferences among the means. Additive genetic variance was estimated by the paternal half-sib correlation method.

RESULTS AND DISICUSSION

Least square means and standard errors for age at first fertile service in each such class are presented in the Table. The overall average age at first fertile service in Gir Cattle was found to be 1386.1±26.6 days. This is almost similar to the estimate of 42.91±0.72 months obtained by Kodagali *et al.* (1986) but is higher than those reported by Ponkshe (1969) and Velhankar (1973) as 1007.95±22.56 and 978.66±28.73 days, respectively in Gir cattle. The highest age at first fertile service (1486.8±105.1) was recorded for P_1 period as compared to rest of the periods. The least squares analysis of variance indicated the highly significant ($P<0.01$) effect of period of calving and non-significant effect of season of calving on age at first fertile service. Presence of significant differences in the trait during different periods indicates that possibly managerial and feeding conditions did change over the years of which data were available.

High additive genetic variance for age at first fertile service (0.74±0.21) was observed in Gir cows. Taylor *et al.* (1978) obtained slightly lower value (0.59±0.66) in Malvi but it was accompanied by high standard error. The additive genetic variance for age at first fertile indicated that 74 per cent of the total variability was due to genetic source. Selection, therefore based on age at first fertile service might result in some improvement of the trait in Gir cattle.

Acknowledgements: We are grateful to Research Scientist, Cattle Breeding Farm, Junagadh and Director of Research & Dean, P.G., Gujarat Agril. University for the permission to work on the data. Thanks are also due to Indian Council of Agricultural Research, New Delhi, for the grant of Fellowship to the senior author.

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* Part of the Ph.D. thesis submitted to Gujarat Agril. University, Sardar Krushinagar by first author.

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Table: Least squares means and standard errors for the factors affecting age at first fertile service in Gir cattle.

Factor	Code	Number of observations	Least squares mean	Standard error
Overall		445	1386.1	26.6
Periods of calving				
1966-70	P_1	12	1486.8^{a}	105.1
1971-75	P_2	60	1248.4^{c}	47.2
1976-80	P_3	103	1395.5^{ab}	36.3
1981-85	P_4	148	1349.0^{bc}	30.3
1986-88	P_5	122	1451.0^{ab}	33.5
Seasons of calving				
June-September	S_1	80	1370.9	45.3
October-January	S_2	171	1398.6	32.6
February-May	S_3	194	1388.9	32.8

Means with the same superscripts do not differ significantly ($P<0.05$) within the effects.

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Estrus Induction and Fertility Following Intra-uterine Infusion of Prostaglandin F_2 Alpha and Lugol's Iodine in Subestrus Crossbred Cows.

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ABSTRACT

Thirty subestrus cows were randomly divided into three groups of ten animals each. Group I received intra-uterine infusion of 1 ml of Lutalyse, group II received 1 ml of Lutalyse alongwith 20 ml of dilute solution (1:25) of Lugol's iodine. While group III served as control receiving 1 ml of normal saline intra-uterinally. The interval between infusion to occurrence of estrus was 3.1±0.27, 3.9±0.09 and 11.5±3.19 days with a fertility rate of 50, 90 and 20 per cent, respectively. The number of animals detected in estrus in three groups were 4, 9 and 2, respectively.

INTRODUCTION

Subestrus is a major infertility problem affecting about forty per cent of cows in a herd, though they cycle normally. Today it is fairly believed that in bovine retention of the corpus luteum exists only in connection with pathological condition of the uterus (Chatterzee, 1988).

Prostaglandin F_2 alpha and its analogs have a luteolytic effect during the days 5 to 17 of bovine estrus cycle (Rowson, 1972). Subsequently, various workers have induced estrum and good fertility rate with intra-muscular injection (Dobeli *et al.* 1982, Kudlac and Vinkler, 1980) or intra uterine infusion (Nakahara *et al.* 1976 and Chouhan *et al.* 1982) of prostaglandin F_2 alpha.

Infusion of weak solution of iodine in subestrus cows induced estrus in 70 per cent of cows with 52 per cent fertility (Nakahan 1971).

Since administration of prostaglandin F_2 alpha or its analogs do not induce overt estrus in all the subestrus cows, the present experiment was undertaken to study the efficacy of intra-uterine infusion of prostaglandin F_2 alpha followed by dilute solution of Lugol's iodine in subestrus cows.

MATERIAL AND METHODS

Thirty subestrus crossbred cows belonging to dairy unit of Live Stock farm, Adhartal, were randomly divided into three groups of 10 animals each. Each animal was examined twice at an interval of 10 days to assure cyclicity of the experimental animals. Group I received intra-uterine infusion of 1 ml of Lutalyse (Unichem, Laboratories, Bombay) Group II received intra-uterine infusion of 1 ml of lutalyse followed by 20 ml of dilute solution of Lugol's iodine (DIS) 1:25). Group III served as control, infused with 1 ml of normal saline intra-uterinally.

Estrus detection was done by parading a bull daily in the morning and evening along with the visual detection. In addition, rectal palpation was performed on day 3rd and 4th post infusion to detect estrus. Animal exhibiting or detected in estrus were inseminated with fertile liquid semen. Pregnancy diagnosis was done by rectal pulpation 60 days post insemination.

RESULTS AND DISCUSSION

All the animals in both the experimental groups responded to intra-uterine infusion of 1 ml of prostaglandin F_2 alpha, however, the no. of animals detected in estrus in the group I was four as against ten in the group II. In control group III only two animals were detected in estrus.

The interval from infusion to occurrence of estrus was 3.1±0.3 in group I; 3.9±0.1 in group II and 11.5±3.2 in group III. These values agree with earlier reports (Moore, 1974; Nakahara *et al.* 1974 and Chouhan *et al.* 1982). However, the fertility of 50% and 90% in groups I & II in incontrant to 20% in group III achieved in this study is much higher than reported by other workers (chouhan loc. cit.). The high fertility rate agrees with other reports conducted at the same farm (Chatterzee, 1988).

In the group II, the detection of estrus as well as fertility was very high this may be attributed to intra-uterine infusion of dilute solution of Lugol's iodine followed by prostaglandin, which might have wiped off subclinical infection and possibly stimulated anterior pituitary to release more gonadotropins resulting in overt estrus and subsequently a high fertility. However, this remains speculative unless periestrus hormonal profile of these animals is studied.

Thus it can be concluded that prostaglandin F_2 alpha is effective in low doses when infused by intra-uterine alongwith dilute solution of Lugol's iodine in subestrus cows.

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ANNOUNCEMENT

National Symposium on "Role of Theriogenology for Augmenting Fertility in Domestic Animals" and XIth Annual Convention of ISSAR will be held at Yuba Bharati Krirangam, Salt Lake, Calcutta from 27 to 29th November 1993.

Scientists/Officers/Members who desire to attend the symposium and present papers are requested to contact for further details:-

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IJAR. 14(1), 1993; 39-40

Effect of Cortisol Incorporation on Preservability and Metabolism of Ram Spermatozoa Extended in EYMG Extender

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ABSTRACT

Pooled ram semen was extended in EYMG (Egg Yolk McIlIvaine Glucose) @ 1:1. Split samples were transfered to the tubes containing Cortison (Sigma) in the concentrations so as to give final levels as 0, 2 and 3 mcg per ml of extended semen. These samples were stored at refrigeration temperature for 24 hr.

Addition of cortison lowered accumulation of lactic acid although differences between 2 and 3 mcg levels were not significant in this respect. However, the values for 2 mcg were better than 3 mcg. A significant decrease in percent progressive motility was observed at 3 mcg level with a corresponding decrease in the number of live spermatozoa. The level of 2 mcg cortisol per ml better sustained most of the attributes observed during the storage.

INTRODUCTION

To improve the preservability and fertility of ram semen a number of additives have been used (Deoxy corticosterone acetate, White, 1953; Cortisol, Guyton, 1981; alphatoccopherol, Srivastava et al, 1987). High rate of lactic acid accumulation during the storage of ram semen at refrigeration temperature appears to be the main problem as it adversly effect the sperm plasma membrane and its permeability which ultimately lowers functional life span of spermatozoa. This study was designed to observe the effect of clucocorticoid 'Cortisol' on the metabolism and preservability of ram semen.

MATERIAL AND METHODS

Pooled ejaculates of adult Rambouillet rams collected in Artificial vagina in quick succession were evaluated and diluted in EYMG (Egg Yolk McIlIvaine Glucose) extender @ 1:1 (EYMG: Na_2HPO_4 1.78% aqueous w/v titrated with citric acid 1.68% aqueous w/v to bring pH to 7.0, 100 ml., Glucose 0.8g; Streptopencillin 300 mg; Egg Yolk 20.0 ml)

Three concentrations of cortison (SIGMA) viz 0, 2 and 3 mcg per ml of the extended semen were included in the experiment on the basis of the work done by Sonar, (1984). In this study only those semen samples were used which were having more than 70% motility and 3000 million sperms per ml. The extended semen was preserved at refrigeration temperature (3-7'C) for 24 hr. Semen quality parameters were observed by standard methods. Fructose was estimated by the method of Mann, (1948) and Lactate and Pyruvate using Lactate and Pyruvate kits No.826-UV & 726-UV (SIGMA), respectively. The observations were made at 0 hr post dilution and after 24 hr of storage.

student 't' test was used to compare the results as described by Croxton and Cowden (1966).

RESULTS AND DISCUSSION

Semen quality attributes observed at 0 and 24 hrs of storage did not show significant differences. However, progressive motility of samples containing 3 mcg cortisol showed significant deterioration ($P<.05$) during 24 hrs of storage (Table-1)

Although, the differences during the storage were insignificant within the three concentrations of cortisol, 2 mcg concentration was superior to other concentrations with respect of percentage of motile, live and abnormal spermatozoa. The number of progressively motile spermatozoa was comparable with the control. It appears that 2 mcg level of cortisol can better sustain the viability and motility of ram spermatozoa. Inspite of very efficiently sustaining the viability of ram spermatozoa at 3 mcg level, cortisol adversely effect the motility of ram spermatozoa during storage at refrigeration temperature. This observation is in agreement to those of White (1953) and Graves & Eiler (1979) who reported a depression in the motility of the semen samples containing corticosterone.

The observations of the metabolic parameters during storage (Table-II) showed a significantly high ($P<.05$) accumulation of lactate. This could be brought to lower level by addition of cortisol. None of the other values viz. pH, pyruvate and fructose showed significant difference during the storage.

The samples containing 2 mcg level of cortisol could sustain pH of the medium better whereas fructose utilization was poorer. Similar trends were observed by Guyton (1981) in the study of cells. However, there was negligible increase in the pyruvate level. Although the differences during the storage are significant in different concentration of cortisol, the values indicate that in samples containing 2 mcg cortisol there is greater flux of lactate to TCA cycle whereas at 3 mcg level pyruvate values were lowered to almost half of the initial value.

Our results are in agreement with those of Sonar (1984) who have shown 2 mcg level of cortisol to be insignificantly superior to the other concentrations with respect to percent motility, live spermatozoa, fructose utilization and lactic acid accumulation. It appears that cortisol may prove useful as an additive to chilled ram semen. A detailed study including fertility trial is required before drawing final conclusions.

Acknowledgements: Authors are thankful to the Director, CSWRI,Avikanagar for providing facilities and Sh. P.S. Rawat, for Statistical analysis.

Table-I: Effect of Cortisol on preservability of Ram Semen

S. No.	Cortisol level mcg/ml.	% Motility		% Progressive Motility		% Live Spermatozoa		% Abnormal Spermatozoa	
		0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
1.	0.0	70.00 ±3.87	62.50 ±3.09	57.50 ±4.03	50.00 ±4.28	76.80 ±2.39	70.20 ±4.98	11.10 ±1.91	8.30 ±2.13
2.	2.0	70.80 ±3.52	65.00 ±1.83	57.50 ±5.28	48.30 ±2.79	78.50 ±4.34	76.50 ±3.58	13.28 ±3.15	12.19 ±2.66
3.	3.0	71.70 ±3.33	56.70 ±8.72	62.50 ±4.23	46.70 ±4.01	74.70 ±3.26	74.00 ±2.27	14.14 ±1.46	11.82 ±2.84
				t = 2.71* (P<0.05)					

Table-II: Effect of Cortisol on metabolism of Ram Semen

S. No.	Cortisol level mcg/ml.	pH		Fructose mM/L		Lactate mM/L		Pyruvate mM/L	
		0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
1.	0.0	6.40 ±0.04	6.00 ±0.11	4.31 ±0.553	3.09 ±0.156	9.34 ±0.325	10.43 ±0.062	0.15 ±0.044	0.17 ±0.051
						t = 3.29* (P<0.05)			
2.	2.0	6.30 ±0.04	6.00 ±0.10	4.20 ±0.625	3.81 ±0.270	9.47 ±0.388	10.28 ±0.119	0.070 ±0.030	0.10 ±0.035
3.	3.0	6.30 ±0.05	6.10 ±0.13	3.96 ±0.669	3.40 ±0.213	9.47 ±0.505	10.32 ±0.081	0.24 ±0.027	0.12 ±0.039

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The Distribution of Carnitine and Sialic Acid in Different Parts of Epididymis of Black Bengal Goat (*Capra hirus*)**

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ABSTRACT

Twelve testicles of mature black Bengal Goat *(Capra hirus)* were collected and biochemical estimation of carnitine and sialic acid at different parts of epididymis viz. Caput, corpus & Cauda were carried out for observing the relationship of the two bio-chemical components with that of sparmatozoa. The results of the present investigation revealed that the free carnitine concentration at caput, corpus and cauda were 5 ± 0.28, 5.98 ± 0.25 & 17.96 ± 0.53/u gm/gm of wet tissues respectively and free sialic acid concentration of the corresponding parts were 112.92 ± 3.78, 154.42 ± 7.12 & 217.33 ± 5.06/u gm/gm of wet tissues respectively. The results were statistically analysed and discussed.

INTRODUCTION

The composition of fluids play role in maturation of sparmatozoa during epididymal transit (Bedford, 1966). For specifying and composition and basic understanding of factors responsible for sparm cell maturation during epididymal transit, several biochemical factors had been tested of which carnitine & sialic acid attracted the scientists very recently.

Carnitine present in exceedingly high concentration in the epididymis of rate was first made by Marquis & Frits (1965). Similar observations have been reported for rats and other mammals (Casillas, 1972; Casillas et.al., 1984; Jaulin et. al. 1987). Carnitine accumulates in the bovine spermatozoa as they mature in the epididymis and a close correlation between the Carnitine content of rabbit spididymal spermatozoa and their fertility was found (Casillas & Chaipayungpan, 1979). Several hypothesis have been proposed regarding the role of Carnitine in sperm cell maturation. Carnitine acts as a carrier of fatty acyl groups to reach the sites of/B-exidation (Casillas, 1972) and utilizes maximum energy producing pathways (Casillas et al. 1984). The sialic acid helps in capacitation (Hatree & Srivastava, 1965) in fertilization process (Warren, 1959) and in maturation (Gupta, 1974). It also acts as lubricant and reduce the friction of densely packed spermatozoa in the caudal epididymis (Hamilton, 1975).

An exiguous attempt has been made to study the pattern of distribution of total free carnitine & free sialic acid in the tissues of different parts of epididymis viz. caput, corpus & cauda of Black Bengal Goat *(Capra hircus)*. There is no available literature found in case of goat.

MATERIALS & METHODS

The whole of the epididymis from twelve (120 testicles of mature black Bengal Goat *(Capra hircus)* of approximately 2 years of age were collected and carefully trimmed of adipose and connective tissue along with superficial blood vessels. The organs were divided into caput, corpus and cauda sections. The three parts of epididymis were cut open and washed thoroughly with PBS. The tissue extracts were made by homogenization and repeated centrifugation.

The spectrophotometric assay of carnitine was carried out following the method suggested by Pearson et al. (1965). Sialic acid was quantitatively estimated by the method as suggested by Warran (1959). The results were analysed statistically by the method of Snedecor & Cochran (1967).

Materials: 5.5' - Dithiobis-nitrobenzene, Acetyl Co A, Carnitine acetyle transferase from pigeon breast muscle (1 mg/0.02 ml) and sialic acid (N-acetyl Neuraminic acid) were obtained from Sigma Chemical Inc. USA and the other reagents are of best grades available from Commercial sources.

RESULTS

The distribution of free carnitine and free sialic acid was examined in three parts of epididymis (viz. Caput, Corpus & Cauda). The one way classification of variance along with critical difference test have been performed. The results of which is shown in Table-1 & Table-2 respectively.

** Part of the M.V.Sc. Thesis of P.R. Ghosh

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The average concentration of free carnitine are 5.0 ± 0.2876, 5.9831 ± 0.2587, 17.9672 ± 0.5382/u Mol/gm of whole tissue (weight) in caput, corpus & Cauda respectively. The increase of carnitine content among the caput, corpus & cauda are statistically significant (P<0.05) (Table-2).

The average concentration of free sialic acid in caput, carpus and cauda are 112.92 ± 3.78, 154.42 ± 7.12 and 217.33 ± 5.06/u gm/gm of wet tissues respectively. The increase in sialic acid content among caput, corpus and cauda and statistically significant (P<0.05) (Table-2).

DISCUSSION

After analysis of results the present investigation reveals that the concentration of free carnitine is accumulated mostly in the cauda where the spermatozoa achieve maximum maturation during epididymal transit (Bedford, 1966). No available literature is found to support the present investigation. But qualitatively similar results were observed in at (Marquis and Fritz, 1965) : bull, bear and monkey Casillas, 1976). rabbit (casillas & Chaipayangpan, 1979), hamster (Casillas et al., 1984) and in bear (Jeulin et al., 1987). The excessive high concentration of carnitine in cauda reflects that the mature cell have an increased capacity of energy matabolism which is used to sustain highly motile cells after ejaculation.

The increased concentration of carnitine in epididymis maximizes the energy producing pathways of spermatozoa by helping in/B-oxidation with the formation of fatty acyl-carnitine which enables fatty acyl group to reach the site of/B-oxidtion which is inaccessible to extermitochondrial Co A and Co A derivatives (Casillas, 1972: Casillas et.al.1984). In this study measurement of concentration of carnitine in caput, corpus & cauda epididymis is carried out and the result of which reflects that the carnitine concentration is highest in cauda (Table-2) and probably helps in sperm maturation. Carnitine also can stimulate motility. Moderate concentration of carnitine as found in distal caput produce motility of spermatozoa from proximal caput. Thus the increased concentration at cauda may play a role in motility of the mature spermatozoa.

From the present investigation, it is seen that the sialic acid concentration in cauda in highest followed by corpus and caput respectively. It is known that high level of sialic acid has a possible role in sperm cell maturation in caudal region (Hamilton, 1975 and Gupta, 1974) and in stabilization of sperm membrane (Hatree & Srivastava, 1965; Hamilton, 1975). The highest concentration of sialic acid in caudal epididymis of black Bengal goat *(Capra hircus)* thus indicates that probably it has definitive role in sperm cell maturation.

Table 1: Table showing analysis of variance of concentration of free carnitine and free sialic acid in different parts of epididymis of black Bengal Goat *(Capra hircus).*

		Free Carnitine		Free Sialic Acid	
Source	d.f.	SS	MS	SS	MS
Between Segments	2	1250.81	625.405*	66334.4	33167.2*
Within Segments	33	5.02	0.1521	1004.5	30.4
Total	35				

* P<0.05

Table 2: Table showing results of critical difference test of carnitine and sialic acid in different parts of epididymis of Black Bengal Goat *(Capra hircus).*

PARAMETER	Parts of Epididymis		
	CAPUT (Mean ± S.E.)	CORPUS (Mean ± S.E.)	CAUDA (Mean ± S.E.)
Carnitine*	5.0 ± 0.2876^{a}	5.9831 ± 0.2587^{b}	17.9672 ± 0.5382^{c}
Sialic acid*	112.92 ± 3.78^{p}	154.42 ± 7.17^{q}	217.33 ± 5.06^{r}

P<0.05

* The Unit of carnitine is/μ Mol/gm of tissue (Wet weight)

** The Unit of sialic acid is/μ gm/gm of tissue (Wet weight)

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Revivability and Acrosomal Integrity of Buffalo Spermatozoa deep Frozen in straws using different equilibration and thaw rates.

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ABSTRACT

Semen of 4 Murrah bulls was tested in a factorial combination of 3 equilibration periods (EP:2h, 4h and 6h) and 2 thaw rates (TR: 37° for 30 S and 75°C for 9 S) to elucidate the optimal EP and TR procedures conducive for improved revivability and acrosomal integrity of sperm using Tris-EYG extender. The post-thaw motile sperm(MS) at 0 h averaged 57.9%, 58.7% and 65.0% for the three EP and 66.0% and 61.8% for the two TR respectively. EP and TR contributed significantly (P<0.01) to the variation of MS at 0 h and 1 to 6 h incubation at 37°C, duration of sperm survival(SS) at 37°C (EP only) and absolute index of sperm survial (AISS) at 37°C. Bulls differed in MS, SS and AISS. Interactions of TR X Bulls for SS (P<0.05) and EP X TR for AISS (P<0.01) were significant. Neither of the treatments had any effect on the percent of intact acrosomes (PIA), while EP affected the percent of damaged acrosomes (PDA) (P<0.01). The best rating of each of these measures: MS (72±1.27%), SS (5.25±0.38 h), AISS (180±10.9), PIA (83±5.2%) and PDA (41±2.2%) was observed for the semen equilibrated for 6 h and thawed at 37°C for 30 S.

INTRODUCTION

Information on deep freezing of buffalo semen using different extenders, glycerol levels, equilibration periods and thaw rates is available (Rathore, 1965; Flukiger *et al.* 1976; Tuli and Singh, 1981; Ahmed, 1984). However, the published results on the significance of equilibration periods on post-thaw motility are inconsistent, while those on the thaw rates are inadequate.

The present study was therefore undertaken to determine optimal rates of equilibration and thawing consistent with improved post-freezing revivability and acrosomal integrity of spermatozoa of Murrah bulls.

MATERIALS AND METHODS

Four Murrah bulls (6 to 9 years) at the Indo-swiss project, Visakhapatnam (18°N, 83°E) were used in the study. Semen was collected from each bull twice a week in A.V. of 8" size, after two false mounts. Four ejaculates from each bull with 70% motile sperm (MS) were extended in Tris-EYG extender to contain 60 x 10^6 sperm/ml, packed in 0.5 ml French medium straws at 37°C, equilibrated at 4°C, frozen by rapid horizontal vapour freezing method in static LN for 8 min. and then stored in LN for 24 hours.

Three equilibration period (EP) of 2 h, 4 h and 6 h and two thaw rates of (TR)-37°C for 30 S and 75° for 9 S were studied.

Four straws per treatment within each ejaculate were thawed and pooled in test tubes (12 x 75 cm) and incubated at 37°C for 6 h. Each sample was evaluated under a phase contrast microscope fitted with a biotherm at 37°C for MS immediately after thawing (0 h) and at hourly intervals for 6 h, sperm survival in hours at 37°C (SS) and the absolute index of sperm survival at 37°C (AISS). The absolute index, a measure of both post-thaw motility and survival of sperm at 37°C was the sum of products of time interval between motility estimates and average of every two consecutive estimates (Milovanof, 1962).

The percentage of total sperm with intact acrosomes (PIA) and those with damaged acrosomes (PDA) before and after thawing were estimated from Giemsa stained smears. Acrosomal damage included swollen, ruffled, fractured and separating acrosomes (Saacke *et al.* 1968).

A randomized block design was used and the means of each variable were analysed. (Snedecor and Cochran, 1980) with EP and TR as fixed variables and bull replicates as random variables in factorial arrangement. The EP, TR and bull means were compared by Duncan's multiple range test (Duncan, 1955). The MS data were analysed after are-sin transformation.

RESULTS

The averages of 16 ejaculates from 4 bulls were: Volume = 2.78±0.32 ml, motility = 78.1±2.1%, concentration = 1.31±0.42 (x 10^9) sperm/ml, PIA=91.2±4.8% and PDA-12.8±0.5%.

A summary of effects of EP and TR on MS after thawing and at hourly intervals for 6 h incubation at 37°C, post-thaw sperm survival at 37°C and absolute index of sperm survival is presented in Table-1 and on PIA and PDA in Table-2.

The pre-freezing MS at the end of 2, 4 or 6 h equilibration was similar. But the post-thaw MS at 0 and 1 to 6 h incubation was higher (P<0.01) for EP 6 as compared to EP 2 or EP 4. Likewise, the mean MS at different hours of incubation was higher (P<0.01) when thawed at 37°C for 30 S as compared to 75°C for 9 S. The treatment combination of EP 6 and TR 37°C for 30 S resulted in higher (P<0.01) SS and AISS, reflecting improved motility and survival of freeze-thawed buffalo spermatozoa.

The mean MS declined (P<0.01) at 0, 1 and 2 h incubation for EP 2 and EP 4 and was negligible after 4 h incubation as contrasted to EP 6. The mean SS and AISS also improved (P<0.01) with EP 6, as compared to EP 2 and EP 4, while the latter two did not differ.

The thaw rates produced contrasting effect on MS in that the reduction of motility was most evident at 0 and 1 h incubation when thawed at 75°C for 9 S. The mean AISS, but not the mean SS differed between TR which was higher (P<0.01) for 37°C for 30 S.

Bulls differed in MS at 0 h (P<0.05) and 3 h (P<0.01) in SS and AISS (P<0.01). Bull x TR interaction for SS (P<0.05) and EP x TR for AISS (P<0.01) were significant.

The mean post-thaw PIA was higher for EP 6 and 37°C for 30 s but the difference was not significant, while the incidence of injured acrosomes was significantly low (P<0.01) than in EP for 2 or 4 h. Thaw rates did not affect PDA.

DISCUSSION

Of the three EP and two TR tested, buffalo sperm equilibrated for 6/h and thawed at 37°C for 30 s was found to be the best both in respect of improved revivability and acrosomal maintenance. EP accounted for greater variation as compared to TR in all the measures studied. Maximal loss of motility was seen during the final step of deep-freezing and this loss was minimal when equilibrated and thawed for longer periods i.e., 6 h and 30 s. Significant motility loss of Murrah buffalo sperm during the final step of freezing was observed Matharoo and Singh (1980) and a minimum of 5 to 6 h EP was found to be essential to achieve optimal post-thaw survival of buffalo sperm by Vasanth (1979). Studies based on post-thaw motility and Sephadex filtration test also indicated that sperm of Nili - Ravi buffalo bulls extended in Tris-EYG and equilibrated for 7 h was superior than one of 5 h (Carbo et al, 1980). Ahmed (1984) obtained improved results following 4 h than 2 or 6 h EP, which however was based on post-thaw motility alone.

The present results revealed that equilibration for 6 h without regard to thawing procedure improved acrosomal maintenance with a lower incidence of damaged acrosomes. This shows that a minimum equilibration period of 6 hours is beneficial for the maintenance of intact and healthy acrosomes of buffalo sperm.

It is concluded that equilibration period of 6 h and thaw rate of 37° for 30 s seems to be beneficial to buffalo sperm revivability and maintenance of healthy acrosomes when deep frozen in medium French straws using Tris-egg yolk-glycerol extender.

Table 1: Effect of equilibration periods and thaw rates on revivability of buffalo spermatozoa frozen in Straws

	Equilibration 2 h		Equilibration 4 h		Equilibration 6 h	
	TR1 (37°C for 30 S)	TR2 (75°C for 9 S)	TR1	TR2	TR1	TR2
Prefreezing % MS	71.8		72.5		72.9	
Post-thaw % motile sperm at 37° incubation for						
0 h	63.5	59.4	62.5	61.4	72.1**	64.6
1 h	38.8	34.4	42.0	34.0	59.4**	49.1**
2 h	9.0	20.6	20.6	22.8	39.6**	35.9**
3 h	4.4	5.2	8.1	7.4	25.5**	19.1**
4 h	2.5	0.5	5.6	1.9	12.7**	7.6
5 h	0.4	0.6	1.3	0.6	4.1**	2.0
6 h	0	0	0.6	0	1.6**	1.4
Overall mean	16.9	17.2	20.1	18.3	30.7	25.7
Post-thaw sperm Survival at 37°C (SS)	2.6±0.37	2.7±0.23	3.0±0.47	3.0±0.28	5.3±0.38**	4.3±0.35**
Absolute index of sperm survival (AISS)	83.0±8.36	87.5±8.80	105.9±11.76	94.1±10.31	180.8±10.85**	144.4±10.69**

** Significant at 1% level (∠P0.01)

Table 2: Effect of equilibration periods and thaw rates on acrosomal integrity of buffalo spermatozoa deep frozen in medium straws:

	THAW RATES		
	TR1 (37°C for 30 S)	TR2 (75°C for 9 S)	Overall
Percentage of intact acrosomes			
EP (2 h)	75.0	65.0	73.3
EP (4 h)	74.0	83.0	75.0
EP (6 h)	83.0	78.0	80.0
Percentage of damaged acrosomes			
EP (2 h)	69.0	75.0	67.3
EP (4 h)	58.0	59.0	60.3
EP (6 h)	40.9**	42.0**	40.7**

**Significant at 1% level (P∠0.01)

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IJAR. 14(1), 1993; 48

Reduction of Fertility in Male Chokla Rams Isoimmunised with Semen and Testicular Homogenate*

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Fertility rate in mammals can be controlled by autoimmunization with semen and homologous testicular homogenate. (Voisin and Toullet, 1969, Jain and Vyas, 1984). But limited work is reported on the production of infertility consequent upon to auto and isoimmunization with semen in rams. Therefore the present investigation was planned to study the possibility of producing immunological control of fertility in rams after immunising them with their semen and testicular homogenate.

Semen was collected as reported earlier (Jain and Vyas, 1984) in the morning hours from 10 chokla rams by using transistorised electroejaculator. The collected semen was examined for volume, colour, mass activity, pH, sperm concentration and live and dead count. The isoimmune response in rams was produced by giving a suspension of 1 ml pooled semen, 2 ml of 10% testicular homogenate, 1 ml of incomplete Fruends adjuvant and 1 ml of a mixture of antibiotics (20,000IU of procaine penicillin and 20,000ug of streptomycin in distilled water) for a period of 28 days. Auto immunization with the semen and testicular homogenate resulted in varied response on the testes of rams. The damage was studied by staining the tissues collected after 45 days of last injection from different regions of the testes by routine method of Haemotoxilin and eosin (H.E.), Masson's trichrome (Singh, 1969); PAS and Giems as stain (Pearse., 1960).

A remarkable difference in the density of semen, sperm concentration and abnormal sperm percentage was observed in the isoimmunized rams after three weeks of immunizations. Extensive degeneration of the spermatogenic epithelium vacoulization, hypospermatogenesis, oedema and fibrosis of interstitial tissues, thickening of the basement membrane, and partial obstruction of seminiferous tubules were demonstrated after 45 days of immunization. These results were in agreement to the work done in bulls (Menge and Christian, 1971), in guineapigs (Johnson, 1970). However, Busy (1965) reported no such changes in rams. Moreover, large number of leucocytic infitration and orchitis type reactions in the testes were reported. (Menge and Christian 197.1) in bulls.

The present investigation indicates that the fertility can be impaired by producing hypospermatogenesis by autoimmunization with semen. However, complete aspermatogenic condition may be created after subjecting rams for a longer period of auto-immunization with highly purified and more potent semen antigen.

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* This work was done at the College of Veterinary and Animal Science, Bikaner.

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Effect of Washing on The Quality of Goat Semen During Preservation At +5°C

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The study on the effect of washing on the preservation of goat semen is meagre (Deka and Rao, 1986). The present study was undertaken to record the effect of washing on sperm motility and acrosomal integrity during preservation of buck semen upto 72 hours at +5°C.

Twenty eight ejaculates, 7 from each of 4 Assam local bucks aged 3 to 4 years were collected using artifical vagina. Immediately after collection, each ejaculate was split into two halves. One half was extended (1:10) with tris extender (Foote, 1970). The pH of the extender was adjusted to 6.8. The other half was diluted (1:10) with tris buffer and centrifuged at 3000 RPM for 5 minutes. The clear supernatant was aspirated out. Tris buffer was added again to the sediment and the washing was repeated. The sediment, after double washing, was extended (1:10) with tris extender, considering the original volume prior to washing for extension. The extended semen was preserved at +5°C in a refrigerator for 72 hours. The preserved semen was evaluated for sperm motility and acrosomal changes at 0, 24, 48 and 72 hours of preservation. The sperm motility was estimated by using conventional method. The morphological changes of acrosomes were studied in Giemsa Stained Smears (Watson, 1975) and were classified into swollen, separating and entirely lost acrosomes (Watson and Martin, 1972). Two hundred sperm in each stained smear were studied at a magnification of 1000X for recording different morphological changes of acrosomes. The statistical analysis of the data was made as per Snedecor and Cochran (1967) after angular transformation of the percentages.

The mean sperm motility and various acrosomal changes in unwashed and washed semen at different hours of preservation at +5°C are presented in Table 1. The sperm motility was significantly ($P<0.01$) higher in washed semen than in unwashed semen during preservation at +5°C. This supports the findings of earlier workers (Iritani *et al.*, 1961; Deka and Rao, 1986). The poor sperm motility observed in the unwashed semen during preservation in tris extender might be due to egg yolk coagulating enzyme present in the seminal plasma that causes hydrolysis of lecithin into fatty acids and lysolecithin which is toxic to sperm (Aamdal *et al.*, 1965). Out of 28 split semen samples preserved without washing, 0 per cent sperm motility was recorded in 3 samples (10.71%) at 48 hours and in 4 samples (14.29%) at 72 hours of preservation. On the other hand, no washed semen sample showed 0 per cent sperm motility during preservation. Similar observation was also reported by Deka and Rao (1986). The incidences of various acrosomal changes were significantly ($P<0.01$) higher in unwashed semen than in washed semen during preservation. This might be due to increased number of dead sperm in unwashed semen resulting in increased incidence of acrosomal changes. The sperm motility decreased significantly ($P<0.01$) while the various acrosomal changes increased significantly ($P<0.01$) during preservation of unwashed and washed semen at +5°C.

It is evident from the present study that washing of goat semen improved its quality during preservation at +5°C.

Table 1: Sperm motility and various acrosomal changes (Mean ± SE) in unwashed and washed buck semen at different hours of preservation in Tris extender at +5°C.

Preservation period (hours)	Sperm motility (%)		Swollen acrosome (%)		Separating acrosome (%)		Entirely lost acrosome (%)		Total acrosomal changes (%)	
	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
0	87.79 ±0.67	87.85 ±0.62	1.09 ±0.13	1.77 ±0.16	0.39 ±0.11	0.60 ±0.08	0.84 ±0.16	1.22 ±0.18	2.66 ±0.20	3.99 ±0.22
24	78.54 ±0.81	81.90 ±0.63	3.62 ±0.14	2.65 ±0.11	1.18 ±0.07	0.91 ±0.14	2.63 ±0.15	1.68 ±0.08	7.62 ±0.26	5.41 ±0.20
48	56.03 ±5.69	74.15 ±0.86	6.64 ±0.17	4.43 ±0.14	2.46 ±0.90	1.72 ±0.16	3.83 ±0.16	2.66 ±0.09	13.00 ±0.28	8.90 ±0.20
72	41.51 ±5.50	67.17 ±0.59	9.17 ±0.17	6.76 ±0.16	7.43 ±0.27	4.44 ±0.18	10.07 ±0.34	5.99 ±0.21	26.85 ±0.59	17.29 ±0.47

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IJAR. 14(1), 1993; 51

Age at First Fertile Service in Jersey Cattle

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Age at first fertile service plays vital role in reducing the unproductive life of animal. Thus, the cost of raising dairy animal is minimum (Taylor et. al.; 1978). Ulmek (1991) reported that selection based on age at first fertile service in Gir cattle might help in bringing improvement in the trait. Therefore, studies on age at first fertile service in Jersey cattle were undertaken.

The date recorded on age at first fertile service pertaining to 424 Jersey cows maintained at Exotic cattle Breeding Farm, Tathawade, Pune (Indika) were considered for the present study. The periods of birth were formed assuming that year to year difference within a period due to different environmental conditions was small. Therefore, the date of 18 years were grouped into 3 periods of birth:P1 (1972-77), P2(1978-83) and P3(1984-90). Each year was sub-divided into 3 seasons viz, rainy (June-September), Winter (October-January) and Summer (February-May). The data on age at first fertile service in Jersey cattle were analysed by least-squares method (Harvey, 1966). The period and season, in which the animals were born, considered as sources of variation. The linear model was used to findout the effect of period and season of birth on age at first fertile service. The heritability of the trait was estimated by patternal half-sib correlation method (Becker, 1975).

The least-squares means and standard errors for age of first fertile service in each sub-class are presented in Table '1'. The Overall average age at first fertile service in Jersey cattle was 639.80 $\pm$ 7.24 days. The results are in agreement with those of Pyne *et.al.* (1988) in Holstein-Friesian X Hariana. D'souza *et.al.* (1979) observed the lower age at first fertile service in Red Sindhi X H.F. (592.0 $\pm$ 17.30) and Red Sindhi X Red Dane (574.4 $\pm$35.9 days).

Analysis of variance indicated that the age at first fertile service was significantly ($P<0.01$) influenced by the period of birth. Variations in the age at first fertile service might be attributed to the difference in feeding from P1 to P3. However, season of birth did not influence the trait. Similar results were reported by Ulmek (1991) in Gir cattle.

The heriatability estimated for age at first fertile service was 0.61 $\pm$ 0.23. Ulmek (1991) reported similar heritability estimate (0.74 $\pm$0.21) in Gir cattle. The additive genetic variance for age at first fertile service indicated that 61 per cent of the total variability was due to genetic source, this magnitude was encouraging. The results revealed that reduction in age at first fertile service might be brought about by selecting the animals based on their age at first fertile service. Thereby, reducing the unproductive life and minimising cost on rearing.

Acknowledgements: The authors are greatful to the Director of Animal Husbandry, Maharashtra State for permiting to use the date and Dr. B.S. Joshi, Superintendent, Exotic cattle Breeding Farm, Tathawade (Pune) for providing facilities.

Table 1: Least-square means for age at first fertile service (days).

Factors	Code	Average ± S.E.
Over all mean		639.80 ± 7.24 (424)
Period of birth		
1972-77	P1	603.91 ± 16.59 (108)
1978-83	P2	622.61 ± 11.92 (151)
1984-90	P3	692.89 ± 11.46 (165)
Season of birth		
Rainy (June-September)	S1	629.16 ±14.50 (137)
Winter (October-January)	S2	646.04 ± 12.12 (147)
Summer (February-May)	S3	644.20 ± 12.64 (140)

Figures in parantheses indicate the number of observations made.

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IJAR. 14(1), 1993; 53

Studies on Some Blood Constituents in Normal Cycling and Anoestrus Mutton Synthetic Ewes

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A study was conducted on 30 apparently healthy adult mutton synthetic (Dorset and Suffolk x Sonadi and Malpura) corss bred ewes to determine the blood constituents in normal cycling and anoestrous ewes during autumn breeding season. Haemoglobin, total serum protein and mineral levels in cycling and anoestrus ewes did not differ significantly and were within normal range.

Investigation was carried out on 19 apparently healthy mutton synthetic cross bred ewes (age 2.5 - 5 years) which did not conceive since last 3 consecutive breeding seasons. Eleven adult normal cycling ewes were kept as control. All the animals were maintained under standard management and nutritional practices at the institute farm. At the onset of autumn breeding season blood was collected from jugular vein and haemoglobin was estimated. The serum was separated by centrifugation for estimating total protein, sodium, potassium, calcium, magnesium and inorganic phosphorus by following the standard methods described by Oser (1965). The estimated values were compared by using student 't' test (Croxton and Cowden, 1966).

The average body weight of the anoestrus ewes was 29.86 ± 3.6 kg (22.5 to 37.0 kg) which were slightly lower as compared to the control group animals. The mean haemoglobin value in the experimental group was slightly lower whereas total protein values were marginally higher in experimental group ewes as compared to control (Table-1) but the differences were non-significant. However these values were within the normal range (Greenwood, 1977; Caballero et al., 1992). The mean serum sodium, potassium, calcium, magnesium and inorganic phosphorus levels also showed non-significant differences between the two groups (Table-1). These values were within normal range (Kaneko, 1980; Hooda and Kalra, 1985). It appears from this study that there was no correlation between these blood constituents and anoestrous condition.

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Table 1: Blood constituents of normal cycling and anoestrus mutton synthetic ewes.

Parameter	Normal cycling ewes (n=11)	Anoestrus ewes (n=19)
Haemoglobin (g/100ml)	10.20±0.28	9.62±0.20
Total protein (g/100ml)	6.31±0.10	6.41±0.13
Albumin (g/100ml)	2.26±0.03	2.25±0.03
Globulin (g/100ml)	4.05±0.09	4.16±0.12
A/G ratio	0.56±0.01	0.55±0.02
Sodium (meq/l)	175.10±5.83	186.61±3.17
Potassium (meq/l)	3.99±0.24	3.87±0.15
Calcium (mg/100ml)	7.85±0.79	8.15±0.60
Magnesium (mg/100ml)	2.75±0.09	2.80±0.06
Inorganic Phosphorus (mg/100ml)	5.19±0.28	5.43±0.18

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Retained Afterbirth Due to Leptospiral Infection in Cattle

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Bovine Leptospirosis is known to cause enormous economic loss to the cattle industry. A number of aspects of reproductive wastage in cattle associated with leptospiral infection have been highlighted by studies of naturally infected cattle, which include abortion, premature birth of live calves, still birth, birth of weak term calves which may not survive, decreased milk production and infertility. Ellis and Michna (1977) demonstrated leptospires in placenta from animals which produced live healthy calves as long as 60 days after infection. Many aspects of Leptospirosis in cattle are still inadequately defined. This present study was undertaken with a view to determine association of Leptospiral infection in retained afterbirth.

Serum samples were obtained from fifty one cows which retained the afterbirth beyond twelve hours of delivery. These cows attended Madras Veterinary College hospital for treatment of retained afterbirth.

The serum samples were screened for Leptospiral infection using Microagglutination test (Wolff, 1954) against six Leptospiral antigens representing six serogroups viz. 1) Autumnalis 2) Canicola 3) Grippotyphosa 4) Hebdomadis 5) Icterohaemorrhagiae and 6) Pomona.

Seven to ten day old cultures of Leptospires grown in EMJH medium were inactivated by addition of formaldehyde solution to a final concentration of 0.2 per cent v/v (Turner, 1968) and these were utilized as antigen for the test. Titre value of 1:80 and above was considered positive for leptospiral infection based on the work of Ratnam *et al* (1980). Foetal cotyledons from placenta were collected for histopathological study.

Eight (15.7 per cent) out of the fifty one cows with retained afterbirth showed positive titres of 1:80 and above. Positive titres were observed against Canicola, Grippotyphosa, Hebdomadis, Autumnalis and Pomona. Predominance of serogroup varied in different places, Autumnalis in Tamil Nadu (Ratnam *et al* 1980). In this study Autumnalis showed positive titres in maximum number of samples screened.

Michna (1967) revealed serological evidence of Leptospirosis in retained afterbirth. Ellis *et al* (1985) reported retained afterbirth in natural Leptospiral infection whereas Ayacardi *et al* (1982) observed retained placenta in experimental Leptosprial infection. In the present study positive titres observed indicated a possible association of leptospiral infection in ratained afterbirth.

Histopathology of Feotal Cotyledons revealed oedema, necrosis, haemorrhage and mild cellular infiltration. Severe oedema of the allanto-chorion (Murphy and Jensen, 1969); Focal necrosis and hydropic degeneration of the Villi (Elliz and Michna, 1977) were reported in experimental Leptospiral infection. The results of histopathology and serology observed suggested leptospirosis as a cause for retained afterbirth. However further investigations are necessary in this area.

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IJAR. 14(1), 1993; 56

Age at First Calving in Two and Three Breed Gir Crosses

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Age at first calving is an important reproductive and economic trait. It gives valuable information about the reproductive efficiency. Cross breeding of dairy cow with proven sires of exotic breeds is now a routine practice. To assess the effect of exotic inheritance of AFC in two and three breed Gir crosses this investigation was undertaken.

Data on 879, two and three Gir crosses comprising of 230 FG (50% Friesian and 50% Gir); 110 JG (50% Jersey + 50% Gir); 241 FJG (50% Friesian + 25% Jersey + 25% Gir); 149 JFG (50% Jersey + 25% Friesian + 25% Gir), 149 BFG (50% Brown Swiss + 25% Friesian + 25% Gir) maintained at AICRP on Cattle, Mahatma Phule Krishi Vidyapeeth, Rahuri spread over a period of 18 years from 1971 to 1988 were used for present study. Corrected data for non-genetic effect on year and season of calving was analysed to study the effect of exotic inheritance on their age at first calving by applying least-squares technique (Harvey, 1966).

The overall least-squares means of age at first calving was estimated to 901.80 ± 21.48, 770.39 ± 31.06, 873.28 ± 20.98, 945.68 ± 26.69 and 921.53 ± 26.69 days for FG, JG, FJG, JFG and BFG respectively. Wagh et al. (1988) reported slightly higher AFC in same genotypes than those in the present study. Genetic group had significant effect on AFC. Duncan's multiple range test revealed that JG halfbred differed significantly lower AFC over all genotypes in the present study. Prabhukumar et al. (1991) also reported higher AFC ($P<0.01$) in all crosses over Jersey halfbreds of Ongole. It is also found that FJG showed significantly lower AFC over JFG. Conribution of genetic group (R^2 value) was 5.37 per cent to the total variability.

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Reproductive Traits of Red Sindhi Halfbreds

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Age at first calving (AFC) plays an important role in reproduction. Non-genetic factors affect the AFC. Such information of Red Sindhi halfbreds under Maharashtra condition is very scanty.

Data of 28 Friesian x Red Sindhi and 30 Jersey x Red Sindhi cows on age at first calving (AFC) and calving interval (CI) was collected for 15 years (1974 to 1981) from history and pedigree sheets maintained at Agriculture college Dairy Farm, Pune. Data were grouped in different periods and season of calving on the basis of meteorological basis as follows:

FRS
Period 1 (1976 to 1981)
Period 2 (1982 to 1988)

JRS
Period 1 (1974 to 1975)
Period 2 (1976 to 1981)

Seasons
1. S_1 June to September
2. S_1 October to January
3. S_1 February to May

These traits were analysed for least square analysis (Harvey, 1966) as follows:

$$Y_{ijk} = u + A_i + B_j + e_{ijk}$$

Where,
Y_{ijk} is the k^{th} observation of j^{th} season of calving and i^{th} period of calving.

Collected data were used for calculation of genetic group differences.

The overall least-squares means for AFC and CI were 1059.05 and 380.27 days, respectively for FRS. Corresponding figures were 1007.99 and 384.51 days respectively for JRS. It was revealed that AFC for Jersey inheritance was lesser than Friesian inheritance. This might be due to its breed characteristics. Least-squares analysis was carried out separately for each genetic group due to non-similarity of data in different periods. Both genotypes did not differ significantly from each other for both traits. Non-significant differences due to genotypes on AFC and CI were reported by Kale (1984) in triple Gir crosses. The contribution of genetic group to the total variation in AFC and CI were 1.67 and 0.66 per cent, respectively.

Period of calving: Differences due to period of calving were significant for AFC in JRS only. This trait was non-significant for period of calving in FRS. The contribution of period of calving to the total variation in AFC and CI were 0.15 and 1.26 per cent, respectively in FRS. While these figures were 25.23 and 4.34 per cent in JRS. DMR test revealed that cows calved during period 2 (1976 to 1981) showed significantly higher in AFC traits than cows calved during period 1 (1974 to 1975) in JRS only. This indicate favourable environmental condition during period (1974 to 1975) for AFC. These findings of significant influence of period of calving on AFC were corroborated with results of Katoch (1990) in local exotic and various crossbreds.

Season of calving: Season of calving and non-significant effect on both reproductive traits in FRS and JRS. Contribution of season of calving on AFC and CI was only 3.83 and 5.37 per cent for FRS and 5.14 and 7.00 per cent in JRS, respectively. Non-significant influence of season of calving on both traits were reported by Gosavi (1987) in various crossbreds.

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Clinical Trials with Fostim* (Serum Gonadotrophin) to Induce Ovulatory Heat

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The incidence and management of true anestrum were recognised as age old problems in cattle breeding programmes. There is a wealth of documentation on various therapies to induce estrum in cows and buffaloes. (Sadasiva Rao and Rao (1985) Shaw and Derashri (1985) Rao & Naidu (1987) Reddy *et al* (1990) Iyer & Sreekumaran (1992).

20 animals of Dairy Experimental Station, Rajendranagar, comprising of 10 crossbred cows and 10 buffalo-heifers with similar number of controls were taken for this study. The cross-bred cows with parity between 2-4 and weighing about 320 kgs were given 1500-2000 I.U. of serum gonadoprophin (Fostim) intramuscularly. Similarly the Buffalo-Heifers weighing about 300 kgs were given 1500 I.U. serum gonadotrophins (Fostim). All the above animals had smooth inactive ovaries and were clinically healthy.

Of the 10 cross-bred cows, 7 animals evinced heat within 10 days and 2 in about 20 days time and out of which 5 animals conceived by natural service, while the remaining one animal failed to come to heat. In the control groups, 2 animals came to heat, of which one conceived by natural service and one had anovulatory heat.

Where as in the graded murrah heifers 8 animals came to heat within 10 days period and the remaining two showed heat within 15 days. Perrectal palpation confirmed seven ovulatory heats, of which 6 animals conceived to natural service. In the control group, 3 came to heat and one conceived to natural service.

From the above results it could be concluded that serum gonadotrophic hormone is very effective in inducing fertile heat in both the cross-bred cows and buffalo heifers.

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IJAR. 14(1), 1993: 59

Uterine Torsion alongwith Haemoperitoneum in a Buffalo

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Causes of Uterine torsion are unknown. Physical injuries and violent actions of animals may lead to torsion of a gravid uterus. A pregnant nondescript buffalo of 8 years age was presented to the polyclinic with a history of horn butting by other buffalo 5 days before. The animal was reluctant to move. The animal adopted a typical posture of extended neek, half closed eyes, protrusion of tongue and expressing severe abdominal pain.

Clinical observations : Temperature was 103°F with dyspnea. Abdominal percussion revealed accumulated gases in the peritoneal cavity but ruminal tympany was absent. Per rectal examination of the animal revealed uterine torsion of 360° and absence of fremitus.

Treatment :The animal was rehydrated by infusing isotonic saline 4 litres and dextrose solutions 3 litres intravenously. Rolling of the animal to relieve uterine torsion was not undertaken due to poor condition of the animal. Hence, cesarean section under local infiltration anaesthesia was conducted by modified young's approach over left abdominal wall in right lateral recumbency of the animal. As soon as the peritoneum was opened gasses with putrified smell escaped with a great force followed by oozing 6-8 litres of blackish, tarry red, partly coagulated blood, bloodclots and fluid. It resulted into partial relief to the animal by resumming normal respiration. No fresh internal bleeding was noticed. However, a diffused peritonitis was noticed. A full grown, dead, putrified foetus was removed from the uterus. The flacid, thin walled uterus was sutured with continuous Lembert pattern using chromic catgut No. 1. The peritoneal cavity was washed with isotonic saline solution mixed with antibiotics. About 3 litres of isotonic saline solution was left in the peritoneal cavity and the laparotomy wound was closed as usual. Post-operatively antibiotic and supportive therapy was followed. The animal recovered completely within a fortnight.

In the present case torsion of uterus might have been caused due to violent actions and horn butting over the abdomen by other animal. Sane *et al*(1982) have reported external injury by horn butting as an exciting cause of uterine torsion. The haemoperitoneum might had occurred due to rupture of some blood vessels with subsequent internal haemorrhage but the exact location of rupture of vessels could not be detected during the operation.

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A Rare Case of Foetal Mummification in Goat

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A non descript multiparous goat aged 4 years was presented to the Veterinary Dispensary on date 19/5/92 (OPD No.197/92) with the history that, it has completed the gestation period, there is constant straining, had previous normal kiddings and was bred by natural service.

Per vaginal examination of the goat revealed the presence of kid at the cervix so gentle traction was applied and a viable, completely developed male kid was removed. It is followed by gentle removal of two more kids, in which the first kid was completely mummified and the appearance was like that of a dried chicken, without hair development. The other kid was completely developed viable female. All the three kids were in anterior presentation.

History given by the owner revealed that no such incidence occurred during earlier pregnancies and the other goats mated to the same buck delivered normal viable kids.

One or more mummified foetuses present in the uterus with one or more normal viable foetus is observed uncommonly in goats. Foetal mummification associated with a persistant corpus luteum is rarely observed in goats (Roberts, 1971). On screening the available literature, Markandeya *et al*(1991) reported a case of foetal mummification in goat, where, out of three foetuses two were mummified.

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IJAR. 14(1), 1993; 61

Dystocia Due to Pelvic Deformity In A Goat

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Dystocia due to pelvic abnormalities or injuries to pelvic bones is rare. Fractures of pelvis with secondary deformity and exostoses is seen most commonly in small animals met with accidents by motor vehicles. These may result in a stenosis of birth passage, resulting in a severe dystocia at Parturition (Roberts, 1971). Deshmukh (1975) recorded an incidence of 1.20% cases of dystocia due to pelvic deformity in buffaloes. Kunthawar and Kaikini (1968) recorded a rare case of maternal dystocia due to pelvic constriction in a horned goat.

A non-descript full term pregnant pluriparous she goat aged about $3_1/_2$ years was brought to the Veterinary Hospital, College of Agriculture, Pune (Case No.3266/186, dt. 28.2.92) with intermittent straining and signs of parturition since last night. Vaginal examination revealed that cervix was dilated completely and fingers could be passed through the os externa with little difficulty. Further exploration of the birth passae revealed that there is bony prominance protruding in the birth canal causing hindrance to pelvic inlet. The entry of the foetus into pelvic inlet caused resistance and hence strong traction was applied and one dead female kid was removed. The afterbirth was shed soon after the dystocia was relieved.

On further inquiries, owner told that the goat delivered two kids normally at previous kidding. Then she met with a rickshaw accident and there was fracture of pelvic bone which healed subsequently. On examination of pelvic bone, it was found that the pelvic was constricted and false joint was formed which caused stenosis of birth canal.

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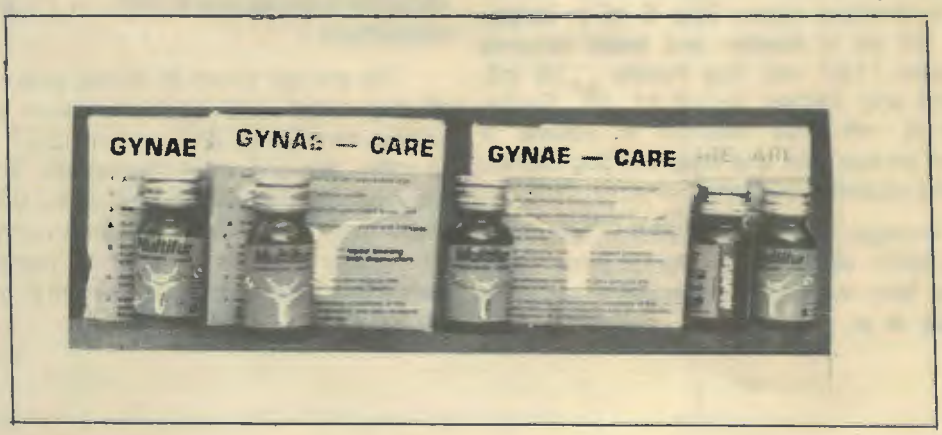

IJAR. 14(1), 1993; 62

A Note on Evaluation of Dog Semen

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The literature available on seminal attributes of dog viz. volume, motility, sperm concentration, live percentage and abnormal percentage of spermatozoa is meagre and based on small number of observations (Hancock and Rowland, 1949; Nooder, 1950; Harrop, 1955; Perez Garcia, 1957; Power, 1962; Heywood and Shortwell, 1971; Deshpande *et al.*, 1970; Dubiel, 1972; Chatterjee *et.al.*, 1976; Hendriske and Antonisee, 1984; Wong and Dhaliwal, 1985; Gunzel, 1986). In the present study, 48 ejaculates were collected from 4 mongrel dogs at 3 days interval by digital manipulation method (Macpherson and Penner, 1967) under all aseptic and hygienic precautions. Neat semen evaluation was done by standard techniques and presented in table 1.

In the present study the average volume, colour and consistency and pH were 2.57 ml, cloudy and milky thin and 6.57 respectively. These observations resemble with observations made by Harrop (1955) on 100 ejaculates from several breeds of dog as 2.01 ml; Deshpande *et al.*, (1970) as 2.06 ml in I neranian breed of dog. However in breeds like ey Hound the average volume as 7.5 ml has been reported by Power (1962); similarly Deshpande *et. al.*, (1970) reported average volume of 4 ml in Shetland Sheep Dog, 5 ml in Golden retriever, 5.9 ml in Alsatian and lesser volumes in Pekinaese (1.87 ml), Toy Poodle (1.35 ml), Dachshund and Afghan Hound (1 ml), Cocker Spaniel(1.25 ml). This variation in volume is dependant on age, size and weight of dog (Dubiel, 1972) and probably on breed.

The average mass activity (2.05) and initial motility (3.60) observed in the present study compared fairly well with observations made by Deshpande *et. al.*, (1970).

In the present study the average sperm concentration of 48 ejaculates in 264 millions/ml as compared to 275 millions/ml(19 ejaculates) reported by Hancock and Rowland (1949) and 85-588 millions/ml by Nooder (1950); while Harrop (1955) reported less average concentration of spermatozoa (125 millions/ml) on 100 ejaculates belonging to several breeds of dogs.

The average percentage of live spermatozoa (82.81%) obtained in 48 ejaculates in the present study compares fairly well (80% to 82%) with Hancock and Rowland (1949) and Perez Garcia (1957). While Gunzel (1986) in his observations of 74 ejaculates in various breeds of dogs reported average percentage of live spermatozoa as 88.80%.

In the present study of 48 ejaculates the average percentage of morphologically abnormal spermatozoa is 10.08% which compares fairly well with the observations of Wong and Dhaliwal (1985) as 9% based on 8 ejaculates. While Hancock and Rowland (1949) reported less percentage (average 4.8%). Perez Garcia (1975), Chatterji *et.al.* (1976), and Hendrikse and Antonisse (1984) reported higher average percentage of morphologically abnormal spermatozoa as 16%, 14.7% and 14.07% respectively.

The average values of normal seminal attributes of dog semen (48 ejaculates) such as volume, colour, consistency and pH were 2.57 ml, cloudy to milky, thin and 6.57 respectively. The average mass activity and initial motility was 2.05 and 3.60 respectively. Whereas total sperm concentration, live percentage and percentage of morphologically abnormal spermatozoa were 264 millions/ml, 82.81% and 10.08% respectively.

Table 1. Observations on Seminal Attributes of Dog

Sl. No.	Seminal attributes	No.of ejacu-lates	Average (Means)	S.E.	S.D.	C.V.%
1.	Volume (ml)	48	2.57	0.14	0.65	25.50
2.	Colour & Consistency	48	CLOUDY AND MILKY THIN			
3	pH	48	6.57	0.04	0.18	2.65
4.	Mass activity	48	2.05	0.10	0.50	24.30
5.	Initial motility (+1 to +5)	48	3.60	0.09	0.47	13.09
6.	Sperm concentration (Millions/ml)	48	264	15.00	72.63	27.50
7.	Live sperm %	48	82.81	0.93	5.00	5.48
8.	Morphologically abnormal Sperm %	48	10.08	0.95	5.00	45.25

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Biochemical Studies on Post-partum Anoestrus in Mehsani Buffaloes

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The study was conducted in thirteen Mehsani buffaloes. The groups were of true post partum anoestrus animals diagnosed 60 days post partum (n=6) and normal cycling (n=7) buffaloes maintained under uniform managemental conditions at the Livestock Research Station of the university. The blood samples were collected once only directly from the jugular vein and the condition was confirmed again after 30 days of blood sampling for the presence of C.L. or follicle. The serum was separated and stored at -20° till analysed.

Total proteins, albumin, globulin and A/G ratio were estimated by modified Biuret and Dumas methods adopted by span diagnostics Pvt. Ltd. (India). Total cholesterol (Henley, 1957), Magnesium (Anderson, 1957), Transaminases (Wootton, 1964), LDH (Wootton and Freeman, 1982) and Cholinesterase (Fishman and Green, 1961) were also estimated. The data were subjected to student's 't' test to confirm the significance of the mean values between the groups (Snedecor and Cochran, 1980).

The anoestrus buffaloes had non-significantly higher levels of total proteins (9.41 ± 0.67 g%), globulin (5.94 ± 0.74 g%) and albumin (3.47 ± 0.09 g%) as compared to the corresponding values in normal cycling buffaloes although, the A/G ratio was found to be slightly higher in normal cycling buffaloes (0.74 ± 0.14) than the anoestrus buffaloes (0.62 ± 0.09). The values of magnesium in anoestrus and normal cycling buffaloes were 4.48 ± 0.38 and 3.99 ± 0.16 meq/1, respectively. Nearly equal levels of total cholesterol were observed in both anoestrus (162.00 ± 3.53 mg%) and normal cycling (163.43 ± 22.14 mg%) buffaloes. The levels of GPT were 128.57 ± 33.43 and 150.58 ± 34.99 μ mol/mint/1 in anoestrus and normal cycling buffaloes, respectively. The estimates of GOT were 21.40 ± 5.57 μ mol/mint/1 in anoestrus and 33.34 ± 4.34 μ mol/mint/1 in normal cycling buffaloes. The difference between two groups of buffaloes were not significant. The concentration of lactic dehydrogenase (LDH) in anoestrus and normal cycling buffaloes was 686.14 ± 33.30 and 717.58 ± 23.42 IU/mint/1, respectively. The difference between these two groups was non-significant. While all the above estimates were non-significant for the group differences, the difference turned out to be highly significant in the case of Cholinesterase ($P \leq 0.01$). The levels of this enzyme were 4.35 ± 1.93 and 13.95 ± 1.91 μ mol acetylcholine hydrolysed/ml/h in anoestrus and normal cycling buffaloes, respectively. This indicates that ChS might be responsible for post-partum anoestrus in Mehsani buffaloes.

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IJAR. 14(1), 1993; 65

Observations on Dystocias due to Emphysematous Foetuses in Buffaloes

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Foetal emphysema in dystocias following foetal death and its invasion by micro-organisms from vagina is very common in buffaloes. So present report has compared the efficacy of two methods in 21 dystocias due to foetal emphysema in buffaloes and observations has been recorded.

21 buffaloes suffering from dystocias who started calving 15-30 hrs back and grossly mishandled and extensively tried in field to relieve the dystocias were presented to the Clinics. Vaginal examination revealed a dry, swollen and congested birth canal with emphysematous foetuses tightly packed in it. General condition of the animals was poor but were in a position to stand up and walk. In 17 cases abdominal contractions were going on while in 4 buffaloes contractions had completely ceased.

In this study, foetuses were removed manually and by caesarean section. In 16 buffaloes, following epidural anaesthesia and cleaning hind quarter and birth canal with antiseptic solution, foetuses were removed manually. After thorough lubrication of birth canal, with the help of foetotomy knife, approchable portion of the foetus was given multiple stab wounds to relive the absorbed gases and thereby sufficient space was created to correct the postural abnormalities of the foetuses. Following adequate lubrication, traction was applied over the foetal head and neck and simultaneously incisions were continued on chest and abdominal area to reduce the foetal size which paved the way for foetal delivery. In the remaining 5 buffaloes caesarean section was performed as per standard method by giving incision parallel and lateral to the milk vein. In both the groups same suitable therapy was given to check haemorrage, shock, infection and other post treatment complications.

In the cases of manual delivery, out of 16 buffaloes, 14 animals survived and only 2 died within two hours of foetal delivery. All buffaloes experienced metritis even after suitable therapy. In one buffalo during forced traction cervical rupture took place which on involution resulted in fibrosis of the cervix. In second group, out of 5, three animals succumbed within 3 days of caesarean. Two buffaloes which survived suffered from metritis.

In these types of dystocias, animals are already under stress due to toximea and are unable to tolerate the further stress of surgery. Further, there is risk of peritonitis and doubtful fertility after caesarean section. Manual delivery excludes the chances of peritonitis due to contaminated uterine fluid. However, manual delivery is laborious and involves the risk of tearing of birth canal and shock due to over exhaution in prolonged cases. It can be concluded that manual delivery should be attempted in early dystocias due to emphysematous foetuses. However, in long standing cases caesarean section should be performed promptly.

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