

FERTILITY RESPONSE TO PROLUTON DEPOT AND CIDR-B TREATMENT IN REPEAT BREEDER COWS

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ABSTRACT

A total of 30 repeat breeder cows were selected to assess the response of fertility to the treatment of Proluton Depot and CIDR-B. The animals were divided in five treatment groups. In group I the cows in oestrus were inseminated at 24 hours and 48 hours and then Inj. Proluton Depot 250 mg was given I/M. In group II & III CIDR-B and PGF₂, alpha was given in early and late diostrus, respectively. CIDR-B with "Cidrol" was given in early dioestrus in group IV and in late dioestrus in group V.

All the animals were observed for subsequent oestrus. The pregnancy diagnosis was confirmed by perrectal examination after 60 days. The percentage of conception was 33.33 in group I; 41.57 in groups II and III; 25.00 in group IV and V.

Repeat breeding is the most common reproductive disorder in crossbred cows both in organised farm and field animals. The exact cause of repeat breeding is still an enigma in many cows and hence termed as idiopathic or unexplained infertility. One of the causes of repeat breeding is hormonal imbalance and progesterone deficiency in particular. Many workers had used exogenous progesterone in repeat breeding condition and observed significant rise

in fertility. Wilt Bank et al (1956) injected 50 mg progesterone in 36 repeat breeder cows daily for 30 days after insemination and found that 16 cows (44.49%) had normal embryos. Lokhande et al (1983) treated 275 crossbred heifers with sialistic treatment, conception rate was significantly higher in sialistic treatment group heifers.

MATERIALS AND METHODS

A total of 30 healthy repeat breeder crossbrd cows comprising of 13 from organised farms and 17 from village adjacent to Akola town were selected for the present studies.

Cows for experimentation were identified after studying the breeding records both from the farms and the A.I. subcenters. These cow were subjected to gynaecoclinical examination for studying the status prior to start of treatment.

The Controlled Internal Drug Releasing (CIDR-B) is the new form of Progesterone Releasing Intravaginal Device. The cattle device is produced by coating a nylon spine silicon based elastomer (Dow corning 595) containing 1.9 gm (107 w/w) of progesterone. A nylon filament is attached to the device.

The treated cows were watched for subsequent oestrus and the cows which did not exhibit oestrus after insemination were examined for pregnancy diagnosis after 60 days to assess the fertility response.

The experimental animals were grouped at random into five groups of 6 cows in each group.

Group I: The cows in heat were inseminated twice (24 and 48 hours after the onset of oestrus) and then Inj. Proluton Depot (250 mg) was given I/m on second day of oestrus.

Group II: In this group the CIDR-B device was inserted during early dioestrus stage of oestrus cycle on day 10 Inj. Lutalyse (2.5 ml) was given I/m.

Group III: In this group the CIDR-B device was inserted during late dioestrus stage of oestrus cycle on day 10 Inj. Lutalyse (2.5 ml) was given I/m.

Group IV:- In this group the cows were treated with CIDR-B with "Cidrol" during early dioestrus stage of oestrus cycle.

Group V:- In this group the cows were treated with CIDR-B with "Cidrol" during late dioestrus stage of oestrus cycle.

In Gr. II, III, IV and V the device was removed on day 12 and then the cows were inseminated at 48 and 72 hr. after removal of device.

RESULTS AND DISCUSSION

In group I, 6 cows were treated

with Inj. Proluton Depot 250 mg. I/M on day 2 after insemination of which 2 cows (33.33%) conceived. Similar results were recorded by Wilt Bank et al (1956) in repeat breeder cows. The higher pregnancies with progesterone therapy were obtained by Jain (1985) in repeat breeder cows and also by Johnson et al. (1958) and Waltson et al (1990) So it could be concluded that conception rate in the repeat breeding syndrome can be improved by Inj. of Proluton depot.

In group II and III out of 12 cows treated with CIDR-B device and Inj. PGF2 alpha on day 10 of treatment, 5 cows (41.57%) conceived. The results were found similar to those of Kerr et al (1991) who treated the normal cows with CIDR-B and PGF2 alpha. Better results with this treatment were also reported by Lee and Chung (1989) and Cleeff et al (1991).

In the present study the cows treated at early dioestrus showed higher conception rate (50%) than the cows treated in late dioestrus (33.33%). During early dioestrus there exists progesterone deficient as per Kimura et al (1987) so that exogenous progesterone supplementation through CIDR-B device might have increased the progesterone level in blood.

In group IV and V treated with CIDR-B and estradiol 10 mg on day of insertion of device, out of 12, 3 cows (25%) were found to be pregnant. Present findings are in partial agreement with the findings of Dixit et al (1983) who treated anoestrus cows.

The present results differ from those of Lokhande et al (1983), Gyawu et al (1991) and Mac Millan and Peterson (1993), who reported higher pregnancies in normal cycling cows. The treatment of repeat breeder animals involves the correction of deficiency causing repeat breeding syndrome. In the present studies the conception rate was higher in the group treated with CIDR-B and PGF₂, alpha than in the cows treated with

CIDR-B and "Cidrol". These results differ with those of Lokhande et al (1988), Gyawu et al (1991) and Macmillan and Peterson (1993) who treated the normal cycling cows reported higher pregnancies rates in cows treated with PRID and O.B. than cows treated with PRID and PGF₂ alpha. It is concluded that CIDR-B with PGF₂ alpha treatment in early dioestrus might be reliable to correct the repeat breeder syndrome.

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PLASMA OESTROGEN CONCENTRATION IN NON-CYCLING MURRAH BUFFALO HEIFERS (BUBALUS BUBALIS)

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ABSTRACT

The present investigation was undertaken to estimate the basal circulating plasma oestrogen concentration in non-cycling Murrah buffalo heifers. Estrogen was estimated by radioimmuno assay in blood plasma. The plasma oestrogen concentration (pg/ml) ranged from 21.26 ± 0.54 to 23.25 ± 0.74 , 20.69 ± 1.04 to 22.34 ± 0.66 and 21.41 ± 1.07 to 24.49 ± 0.80 respectively in 12, 24 and 30 months old buffalo heifers. From the investigation it reveals that plasma oestrogen concentration in Murrah buffalo heifers was at similar level at different ages.

The basal circulatory concentration of gonadal hormone at different ages and reproductive stage in cattle have been extensively reviewed (Gonzalez-Padilla et al 1975a, b). The information available on buffalo reproductive endocrine profile during prepubertal and pubertal stages is not adequate. The present investigation was undertaken to estimate the basal plasma oestrogen concentration in Murrah buffalo heifers

of different age groups.

MATERIALS AND METHODS

Six anestrus Murrah buffalo heifers at 12 to 24 months and five heifers at 30 months age were selected from Institute's herd. A vasectomised bull was used at six hourly interval daily for 60 days to detect the estrus animals. Genitalia were palpated per rectum to observe the ovarian condition in respect of presence of follicle and corpus luteum. None of the heifers exhibited estrus symptoms and neither they were having follicle nor corpus luteum on their ovaries. Blood samples were collected for 19 days (0,2,4,6,8,10,14 and 19) through indwelling jugular cannulae in chilled heparine treated test tubes. Plasma was separated by centrifuging blood samples in refrigerated centrifuge and stored at -20°C . Oestrogen in blood plasma was estimated by radioimmuno assay (Singh and Madan 1998). The inter-assay and intra-assay coefficient of variation was 8.8% and 9.2% respectively. The results were statistically analysed. (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The basal plasma oestrogen

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concentration (Mean \pm SE) ranged from 21.26 ± 0.54 pg/ml to 23.25 ± 0.74 pg/ml, 20.69 ± 1.04 pg/ml to 22.34 ± 0.60 pg/ml, and 21.41 ± 1.04 pg/ml to 24.40 ± 0.80 pg/ml respectively in 12, 24 and 30 months aged heifers. Among the individual heifers the oestrogen concentration fluctuated from 19.18 pg/ml to 25.51 pg/ml, 19.48 pg/ml to 26.45 pg/ml and 18.00 pg/ml to 26.55 pg/ml respectively in 12, 24 and 30 months old buffalo heifers. Nearly same concentration of plasam oestrogen was recorded in three age groups of buffalo heifers.

The oestrogen concentration obtained in this study is higher than

the value recorded in cattle heifers (Hill et al 1972) but similar to the oestrogen value recorded in rural buffaloes (Madan et. al. 1983). The present investigation reveals that of plasma oestrogen levels in Murrah buffalo heifers remains the same from prepubertal to pubertal age where age of puberty in Murrah buffaloes is 29.8 to 34.2 months (Madan 1988).

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ESTRADIOL PROFILE OF RATHI CATTLE DURING ESTRUS CYCLE

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ABSTRACT

The estradiol profile of 4 Rathi cows was studied during the estrus cycle. On the day of estrus E_2 level was high and declined to low levels on the 5th day. Transient peaks of E_2 were seen on the 10th day.

Rathi cattle of the North West part of the Rajasthan are known to have week estrus expression, delayed post partum estrus and silent estrus (Purohit, 1995). Hormonal status of the breed has not been studied, hence as a part of preliminary investigation the estradiol hormone profile was studied during the estrus cycle.

MATERIALS AND METHODS

Estradiol hormone profiles were studied during the estrus cycle in four pure bred Rathi cows four months post partum belonging to the college dairy herd. Animals were closely observed prior to and during the anticipated estrus. Blood was collected in tubes containing EDTA prior to estrus (day 0), 8 hour after the first signs of estrus, at 24 hour intervals for 6 days and at 4 days intervals till the subsequent estrus. Plasma was separated

immediately and stored at -20 C till further assay. Plasma estradiol was estimated by RIA as previously described (Palta et al 1996). The results were analysed using standard statistical methods (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The overall mean estradiol before estrus, on the day of estrus and on the 5th, 10th, 14th, 18th and 22nd day of estrus was 8.00 ± 1.88 , 10.15 ± 2.18 , 4.97 ± 0.43 , 6.75 ± 1.15 , 5.93 ± 1.50 , 6.09 ± 1.04 and 9.53 ± 2.11 ng/ml respectively. A graphic representation of the hormone concentration during different days of the cycle in the 4 animals is presented in Fig. Peak E_2 concentration was seen on the day of estrus followed by a decline 24 hours later, which continued to reach low levels by day 5. In animal No 1 a transient rise was seen on day 3 followed by decline as also reported by Dobson et al (1973). Transient low peaks of E_2 were again seen on day 10 of the cycle as already reported (Bearden and Fuquay 1992).

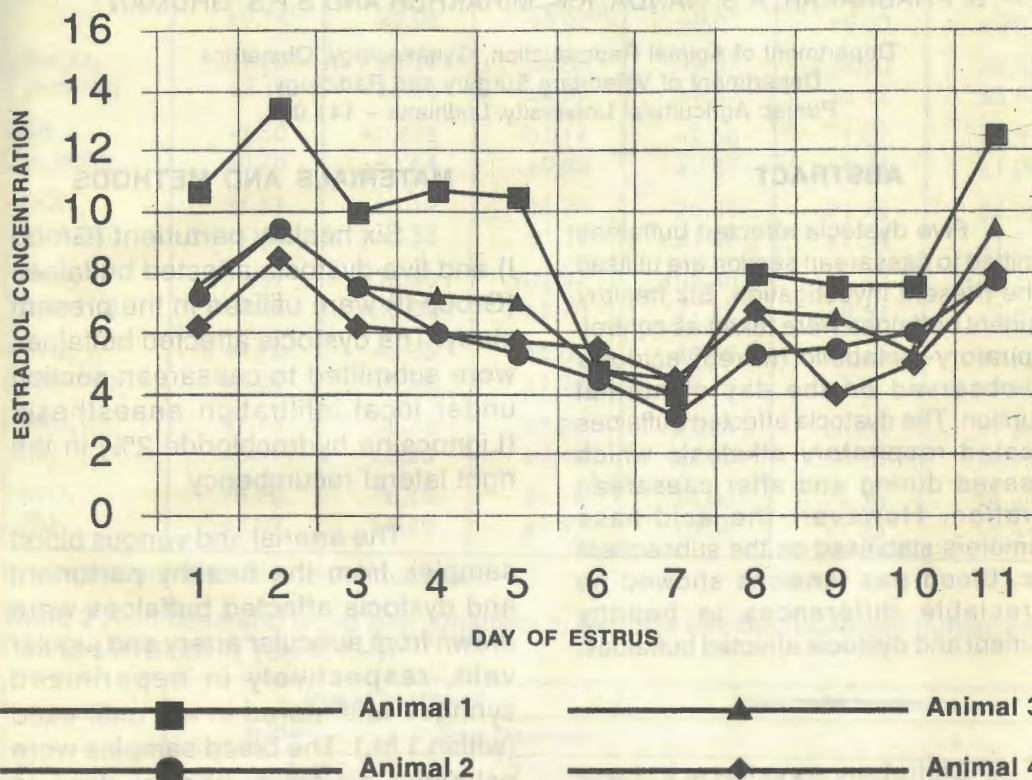
The E_2 concentration was again high on the day of subsequent cycle (day 22). In this study the peak E_2 values on the day of estrus are very low as compared to previous workers (Dobson et al 1973; Dobson 1978; Kesner and Convey 1982) probably due to the weak

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endocrine status of the cows of this breed. The animals of this breed have been known to be sluggish breeders with

silent estrus expression and delayed maturity, (Purohit, 1995).

PLASMA ESTRADIOL PROFILE OF RATHI CATTLE



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ACID BASE DISTURBANCES IN BUFFALOES WITH DYSTOCIA SUBMITTED TO CAESAREAN SECTION

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ABSTRACT

Five dystocia affected buffaloes submitted to caesarean section are utilised for the present investigation. Six healthy parturient buffaloes were taken as control. Respiratory-metabolic (mixed) acidosis was observed on the day of normal parturition. The dystocia affected buffaloes indicated respiratory alkalosis which increased during and after caesarean operation. However, the acid-base parameters stabilised on the subsequent days. Blood gas tensions showed no appreciable differences in healthy parturient and dystocia affected buffaloes.

Disturbances in the acid-base status of blood affects the body homeostasis and may have deleterious influence on body metabolism (Robertson, 1989). Evaluation of acid-base parameters of dystocia affected buffaloes might help to judge the actual status, so as to institute an appropriate fluid therapy. Therefore, the aim of present study was to monitor acid-base status and blood gases of caesarean operated buffaloes in comparison to healthy parturient dams.

MATERIALS AND METHODS

Six healthy parturient (Group I) and five dystocia affected buffaloes (Group II) were utilised in the present study. The dystocia affected buffaloes were submitted to caesarean section under local infiltration anaesthesia (Lignocaine hydrochloride 2%) in the right lateral recumbency.

The arterial and venous blood samples from the healthy parturient and dystocia affected buffaloes were drawn from auricular artery and jugular vein, respectively in heparinized syringes and stored in ice until used (within 1 hr.). The blood samples were collected on six periparturient days in group I buffaloes. In group II, blood collection was made immediately before and after caesarean operation and daily on two-post operative days.

The blood pH, PaCO₂ were determined with a blood gas analyser at 37°C. Actual bicarbonate of plasma (HCO₃) and base excess (BE) were calculated from Siggaard-Anderson alignment nomogram. Also, arterial (SaO₂) and venous (SvO₂) Oxygen saturations were derived from Severinghaus nomogram. Haemoglobin was estimated by Sahli's

Table 1. Acid-base and blood gas dynamics in healthy parturient buffaloes (Gp I, n=6).

Parameter	Days pre-partum			Day of calving	Days post-partum	
	3	2	1		1	2
PHa	7.452 ±0.04	7.422 ±0.02	7.420 ±0.02	7.410 ±0.01	7.429 ±0.03	7.450 ±0.03
PaCO ₂ (mm Hg)	31.80 ±5.11	38.12 ±0.69	36.88 ±3.99	39.57 ±0.60	33.90 ±3.77	35.88 ±3.67
BE (n mol/l)	-1.50 ±0.76	+0.875 ±1.64	-0.214 ±0.46	+2.50 ±0.87	-1.67 ±1.04	+1.33 ±1.26
HCO ₃ (m mol/l)	21.67 ±1.45	25.12 ±1.53	23.78 ±1.11	26.50 ±1.06	21.75 ±1.59	24.92 ±1.67
PaO ₂ (mm Hg)	128.07 ±29.03	118.90 ±11.09	109.97 ±9.70	108.40 ±7.32	115.37 ±8.59	108.70 ±9.82
PvO ₂ (mm Hg)	44.70 ±2.33	46.65 ±5.36	46.07 ±1.94	40.65 ±2.17	45.43 ±1.27	44.60 ±2.46
SaO ₂ (%)	97.73 ±0.97	98.12 ±0.29	97.52 ±0.49	97.74 ±0.30	98.88 ±0.35	97.86 ±0.25
SvO ₂ (%)	76.20 ±3.63	78.12 ±5.28	79.78 ±1.97	74.00 ±3.36	79.33 ±1.85	78.42 ±3.44

a, v represent arterial and venous blood.

Table 2 Acid-base and blood gas dynamics in dystocia affected buffaloes subjected To Caesarean Section (Gp II, n=5)

Parameter	Day of treatment		Days post- treatment	
	Initial	During Treatment	1	2
pHa	7.477 ±0.03	7.505 ±0.03	7.529 ±0.04	7.490 ±0.04
PaCO ₂ (mm Hg)	31.040 ±2.24	27.880 ±4.81	27.950 ±2.25	29.800 ±1.70
BE (m mol/l)	7.00 ±4.00	-1.10 ±2.70	1.50 ±3.50	0.25 ±3.25
HCO ₃ (m mol/l)	23.40 ±4.40	18.78 ±4.07	23.60 ±3.40	22.80 ±3.20
PaO ₂ (mm Hg)	103.44 ±3.35	105.78 ±13.61	118.40 ±53.60	89.35 ±3.65
PvO ₂ (mm Hg)	50.10 ±2.54	45.76 4.45	43.65 ±5.35	44.45 ±3.65
SaO ₂ (%)	97.90 ±0.13	97.96 ±0.26	97.15 ±1.65	97.05 ±1.05
SvO ₂ (%)	87.40 ±0.51	82.40 ±3.68	82.28 ±3.25	87.00 ±1.50

a, v represent arterial and venous blood.

haemoglobinometer as described by Schalm et al. (1975).

RESULTS AND DISCUSSION

In healthy parturient buffaloes (Group I : Table 1), fall in blood pH and PaO_2 on the day of parturition associated with non-significant increase in PaCO_2 indicated hypoventilation resulting in respiratory acidosis. However, a simultaneous increase in HCO_3 and BE suggested it to be a respiratory-metabolic (mixed) acidosis (Table 1). Elevated PaCO_2 at calving could result because of anoxia and exhaustion at calving (Szenci, 1985). Respiratory metabolic (mixed) acidosis in healthy parturient bovines was recorded by Szenci (1985) and Siddiquee (1988). Oxygen tensions in arterial blood in peripartal days did not show any appreciable change, however, a slight dip in oxygen tension that occurred at parturition could be due to pressure on diaphragm during labour pains. Also, no tissue hypoxia was indicated by arterial and venous oxygen saturations.

The initial blood pH and PaCO_2 in animals subjected to caesarean section (Group II; Table 2) indicated respiratory alkalosis. Since alkalosis was accompanied with a decline in the arterial PaCO_2 , it was assumed that the animals had been hyperventilating as a result of pain and discomfort of surgery. When compared with normally calving buffaloes, respiratory alkalosis increased during and after surgery. It appeared to being compensated partially as exhibited by a fall in the HCO_3 and BE levels, so, buffering mechanism of the body appeared to compensate the acid-base disorder to some extent as evident by subsequent acid-base changes. Ammann et al. (1974) found almost identical acid-base picture although, Szenci (1985) found acid-base values within physiological range in cows delivered through casesarean section. Oxygen tensions of arterial and venous blood were within safe limits and no tissue hypoxia was evident. Rather a decrease in PvO_2 was suggestive of a better tissue oxygen utilisation.

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EVALUATION OF LUPROSTIOL AND OXYTETRACYCLINE TREATMENT FOR RETENTION OF FETAL MEMBRANES IN COWS.*

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ABSTRACT

Cows with retained fetal membranes (RFM) were given single intramuscular injection of 15 mg luprostiol and/or two gram oxytetracycline per day for five days postpartum. Time taken for placental expulsion, physical nature of vaginal discharge and uterine involution were recorded. Neither luprostiol was effective in expelling RFM nor intrauterine oxytetracycline could prevent the onset of puerperal metritis secondary to RFM at the given dose. Involution of uterus was delayed in cows with puerperal metritis.

As an alternative to manual removal of RFM several ecobolics such as oxytocin, ergot derivatives variable β_2 adrenergic antagonists and calcium preparations were used with variable results (Roberts, 1971; Peters and Laven, 1996). In this study the efficacy of luprostiol, a $\text{PGF}_2\alpha$ analogue, to expel RFM and intrauterine oxytetracycline to prevent puerperal metritis in Frieswal (Friesian x Sahiwal) cows is evaluated and the results are presented.

MATERIALS AND METHODS

The study was conducted in the Military Dairy Farm, Barielly, U.P. Cows

that failed to expel the fetal membranes spontaneously with 24 hours after calving were considered as clinical cases of RFM. A total of 18 RFM cows were divided into three groups. viz.

Group I (n=8) : Each cow received single intramuscular injection of 15 mg luprostiol (Prosolvin)^a $\text{PGF}_2\alpha$ analogue between 24 and 30 hours postpartum and intrauterine oxytetracycline (Terramycin Bolus) two gram per day for five days postpartum.

Group II (n=6) : Treated intrauterine with oxytetracycline 2g/day for 5 days.

Group III (n=4) : Served as untreated control.

Time taken for the expulsion of RFM and changes in the odour of vaginal discharge during oxytetracycline therapy were recorded. Cows with purulent vaginal discharge were considered to have puerperal metritis. Rectal palpation at 4-5 days interval was done till involution of uterus as defined by Olson et al. (1986). Changes in vaginal discharge and uterine involution were also recorded on 25 non-RFM cows for comparison. The results were statistically analysed.

RESULTS AND DISCUSSION

The mean time taken for the expulsion of RFM after initiation of treatment was 102, 75.8 and 96 hours

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in group I, II & III respectively. The difference noticed among the three groups was not significant. The purulent uterine discharge was noticed in group I and II but the odour was mild similar to non RFM cows with metritis. On the contrary in group III the discharge was copious, purulent with repulsive odour. With regards to involution of uterus, in group I, the mean time taken for total involution was 27.25 days. The corresponding values in group II and group III were 28.6 and 31.75 days respectively. The difference was, however, not significant. A significant delay in involution was observed in non RFM cows with metritis (25.8 days) as against otherwise normal non-RFM cows (21.54 days).

The results of the present study indicate that luprostiol injection has not significantly helped in early expulsion of RFM. Similar observations with other PGF₂α analogues have been reported by earlier workers (Burton et al. 1987; Stevens et al. 1995). Considering the

complex process of placental maturation and separation and multifactorial aetiology of RFM (Roberts, 1971), it seems that the uterotonic effect of PGF₂α (Burton et al. 1987) alone is of little value in expelling RFM.

In this study, intrauterine oxytetracycline either alone or in combination with luprostiol could not prevent the onset of metritis but controlled the severity as evidenced by the favourable changes in the nature of uterine discharge (Paisley et al 1986 and Dutta and Dugwekar 1988 and Cairoli et al 1993). It has also been reported that intrauterine antibiotics destroyed the phagocytic capacity of uterine derived polymorphonuclear leukocytes (Paisley et al. 1986).

No significant difference observed in involution of uterus among RFM cows could be attributed to the uterine infection (Roberts, 1971) as evidenced by significant delay in uterine involution noticed even in non RFM cows with metritis.

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OESTRUS RESPONSE AND FERTILITY IN SUBOESTRUS BUFFALOES TREATED WITH INTRAUTERINE MEDICATION DURING LOW BREEDING SEASON.

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ABSTRACT

Fifty eight suboestrus pluriparous Murrah buffaloes with mild endometritis were subjected to intrauterine medication during low breeding season (May to August). Animal's of group I (n=26) received intrauterine 40 ml dilute (1:30) Lugol's iodine solution, whereas group II & III (n=16 each) were given intrauterine antibiotic therapy viz. 1000 mg Ampicillin (1g Roscillin) and 750mg Enrofloxacin (7.5ml Enrocin), respectively. Group IV (n=20) was kept as untreated control.

The oestrus response was more or less equal in all the treated Groups (I, II & III) i.e. 84.62, 81.25 and 75.00 percent within 6.5 ± 0.78 , 8.15 ± 1.56 and 10.67 ± 1.90 days with conception rate of 81.82, 69.23 and 83.33 per cent, respectively. In contrast, only 25.00 percent buffaloes of group IV responded to oestrus within 44.20 ± 13.73 days and 40.00 percent conceived.

Regular breeding and calving at appropriate time is the key for the success of dairy enterprise. Buffaloes are seasonal in oestrus expression. The sexual activity is highest between Sept. to March and lowest between May to August (Jain and Tailor, 1995.)

Lugol's iodine therapy has been tried by some workers in inducing oestrus (Chouhan and Singh, 1979) as well as intrauterine infusion of antibiotics is also useful in treating such cases (Agrawal and Pandit, 1991).

In the light of above facts that present study was planned to compare the efficacy of various intrauterine medicaments in suboestrus buffaloes during the low breeding season.

MATERIALS AND METHODS

The study was conducted on 58 pluriparous buffaloes between the age of 5 to 10 years from May to August 1996. After gynaeco-clinical examination, the animals with low grade endometritis and suppression of behavioural expression of heat were included in the study. These buffaloes were randomly divided into four groups. Twenty six animals in group I received intrauterine 40 ml dilute Lugol's iodine solution (1:30) where as 16 animals each in group II & III were given intrauterine 1000 mg Ampicillin (1g Roscillin) and 750 mg Enrofloxacin (7.5ml Enrocin) respectively after making 40 ml volume by dissolving in distilled water.

The oestrus was detected by parading the teaser buffalo bull after morning and evening milking and also by behavioural signs of oestrus. Confirmation of heat was done by gynaeco-clinical examination. For numerical expression of the intensity of oestrus and status of reproductive organs, score card was employed (Gautam, 1989). On that basis, the oestrus was classified as intense, intermediate and weak. The animals expressing standing heat were allowed natural service. The pregnancy diagnosis was done by per-rectal examination after 50 days post service.

RESULTS AND DISCUSSION

Amongst the buffaloes treated, 84.62 percent exhibited oestrus in group I, 81.25 percent in group II and 75.00 percent in group III with an average post-treatment interval of 6.5 ± 0.78 (2-13), 8.15 ± 1.56 (2-21) and 10.67 ± 1.90 (2-20) days respectively. Whereas, only 25.00 percent buffaloes of group IV expressed oestrus with an average post-treatment interval of 44.20 ± 13.73 (11-86) days. Maximum number of animals were observed in intense heat in group I (54.55 per cent) and group II (53.85 per cent) followed by group III (33.33 percent). In contrast, none of the animal expressed intense heat in control group IV. Highest conception rate was observed in group III (83.33 percent) followed by group I (81.82 per cent) and group II (69.23 per cent), whereas, it was only 40.00 percent in group IV.

The present study indicate the oestrus induction obtained within 2-13 days of treatment with lugols iodine in group I substantiate the findings of Nakahara et. al., (1971) and Gunther and Kunz (1975) in cows and Chouhan and Singh (1979) in buffaloes. Kendre and Bhosrekar (1996) also observed similar oestrus response (80.00 percent) in anoestrus buffaloes treated with intrauterine infusion of 10 ml povidine iodine solution. Besides its antiseptic activity, Glotra et. al., (1970) stated that Lugol's iodine casues local irritation in the endometrium and then release of PGF₂ alpha causing regression of corpus luteum and brought about reflex secretion of gonadotrophins from the anterior pituitary. More or less similar response in terms of oestrus induction and conception rate was obtained by Chouhan and Singh (1979) after intrauterine infusion of Lugol's iodine and antibiotics in suboestrus buffaloes having a low grade endometritis.

The study also indicate that the conception rate was lowest when the animals were bred in weak oestrus as compared to that in intense and intermediate oestrus. Maximum number of animals were observed with typical pattern of arborization in group I, II and III (59.09, 53.85 and 66.67 percent), with atypical pattern (36.66, 38.46 and 16.67) and least (4.54, 7.69, 16.66 per cent) with missing pattern, respectively. However, the animals in group IV showed higher percentage (60.00 percent) of missing pattern as compared to typical (20.00 percent)

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and atypical (20.00 percent) pattern. None of the animals conceived which had no arborization pattern of cervical mucus. Kumar (1989) also observed very poor conception when fern pattern was absent.

It is concluded that the use of antibiotics is beneficial for treating the cases of suboestrus associated with low grade endometritis. Enrofloxacin

is a new broad spectrum antibacterial compound encompassing gram negative and gram positive organisms including anaerobic pathogen population. The best conception rate obtained in the present study thus proves its efficient use in suboestrus cases. Lugol's iodine however, is the cheapest drug and its usefulness can not be overlooked.

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SERUM PHOSPHATASE AND TRANSFERASE ENZYMES IN NORMAL CYCLIC AND REPEAT BREEDER CATTLE

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ABSTRACT

The concentration of various enzymes and their interrelationship in normal cyclic and repeat breeder cattle was studied. The mean values of ACP, AKP, GPT and GOT were 0.91 ± 0.06 and 1.13 ± 0.05 ; 36.34 ± 0.52 and 56.14 ± 3.52 ; 12.30 ± 0.79 and 12.00 ± 0.60 ; 39.36 ± 2.28 and 40.13 ± 2.51 units/L in normal cyclic and repeat breeder cattle on the day of estrus (Day '0'), respectively. Significantly higher levels of AKP and ACP were recorded in repeat breeder cattle. From day '0' onwards, AKP, ACP and GPT showed decreasing trend while no changes were recorded with respect to GOT.

The nutritional deficiencies and deranged enzymatic activity affect the normal reproductive behaviour of animal and may lead to breeding problems. Therefore, an attempt has been made to investigate the levels of various serum enzymes, so as to ascertain their possible involvement and usefulness as a tool for clinical diagnosis of repeat breeders.

MATERIALS AND METHODS

The experiment was

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conducted at the Military Dairy Farm, Bareilly and A.I. Centre, IVRI, Izatnagar. The crossbred cattle were in the age group of 3 to 9 years and in between first and sixth lactation. Blood samples (10 ml) were collected from 8 normal cyclic and 8 repeat breeder cattle on day of estrus (Day '0') only. Remaining samples were obtained from the repeat breeder cattle at an interval of one day, i.e., Day '0' 10th and 20th following onset of behavioural estrus. The serum was separated with the help of Pasture pipette into a sterilized vials and stored at -20°C until assayed. Serum phosphatases were estimated according to Kind and Kings (1954). Transferases enzymes were estimated according to Reitman and Frankel (1957). The enzyme assay kits employed in the present study were procured from M/S Qualigens Fine Chemicals (Mumbai), India.

RESULTS AND DISCUSSION

A) Alkaline Phosphatase (AKP)

Alkaline phosphatase concentration on the day of estrus was significantly ($P < 0.001$) higher in repeat breeder cattle (56.14 ± 3.52 units/L) than normal cyclic cattle (36.34 ± 0.12 units/L). This was in agreement with

the findings of Sinha et. al., (1986) and Gandora et. al., (1993). In normal cyclic cattle decreased concentration of AKP might enhance the folliculogenesis and more conducive for fertility (Devarj, 1983; Sinha et. al., 1986). Nutritive material primarily glycogen is gradually stored in the uterine epithelium and musculature under the influence of estrogen (Old and Van Denmark, 1957) to be metabolised and utilised later by the implanting blastocyst and maintain the dynamic state of uterus (Hughes et.al., 1963). Maximum alkaline phosphatase activity in the endometrium has been reported to occur during progestational phase (Marinov and Lovell, 1968). In the light of present discussion it is clear that the low concentration of alkaline phosphatase help in accumulation of glycogen during estrogenic phase in normal fertile animal.

The study revealed that the higher alkaline phosphatase activity observed in repeat breeder cattle during estrogenic phase might indicate the possible early mobilization of glycogen thereby resulting in depletion of energy stock in the endometrium and death of the embryo due to starvation during progestational phase (Sinha et al., 1986).

The average values of alkaline phosphatase in repeat breeder cattle were 51.52 ± 4.61 , 48.92 ± 6.18 and 32.02 ± 3.66 units/L on day 0, 10th and 20th, respectively and they are in decreasing order. Significant ($P < 0.05$) differences were

noted between day 0 and 20 and 10 and 20 but not in day 0 and 10. Mehta et.al., (1989) has reported, increased activity of alkaline phosphatase from estrus to 1st trimester of pregnancy in normal cyclic cattle. It is apparent from the present finding that, there was a decreasing trend of alkaline phosphatase enzyme may lead to failure of implantation and early embryonic mortality in repeat breeder cattle.

B) Acid Phosphatase (ACP)

On the day of estrus the acid phosphatase level was significantly ($P < 0.05$) higher in repeater (1.13 ± 0.05 units/L) than normal cyclic cattle (0.91 ± 0.6 units/L). These observations are comparable with the findings of Sharma and Tripathi (1985) and Shukla (1989). The mean values of ACP in repeat breed cattle were 1.09 ± 0.22 , 0.97 ± 0.20 and 1.08 ± 0.14 units/L on day 0, 10th and 20th, respectively and the difference was not significant.

C) Alanine aminotransferase (GPT)

The study revealed a non-significant ($P < 0.05$) difference between normal (12.30 ± 0.79 units/L) and repeat breeder (12.00 ± 0.60 units/L of serum) during estrus. These observations are comparable with the findings of Gandotra et.al., (1993). GPT concentration in repeat breeder cattle were 9.90 ± 0.68 , 8.64 ± 0.84 and 6.24 ± 0.64 units/L on day 0, 10th and 20th, respectively and these values were in decreasing order. These values were highly significant ($P < 0.01$)

between day 0 & 20 and 10 & 20, where as value on day '0' and 10 were not - significant.

D) Aspartate aminotransferase (GOT)

Average level of GOT was slightly higher in repeat breeder cattle (40.13 ± 2.51 units/L) than normal cyclic cattle (39.26 ± 2.28 units/L) on the day of estrus. Similar observation have been reported by Gandotra et.al., (1993). In repeat breeder cattle mean concentration of GOT were $42.72 \pm$

3.10 , 44.64 ± 1.69 and 43.44 ± 4.35 units/L on day 0, 10th and 20th, respectively. However, Shukla (1989) reported a significantly higher trend from 1st day of estrus to 16th day of estrous cycle in repeat breeder cattle.

Further study on the level of enzymes at different periods of the cycle in normal and repeat breeders with and without conception is required to assess their usefulness in clinical diagnosis of repeat breeders

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FACTORS AFFECTING SOME REPRODUCTIVE DISORDERS IN JERSEY AND JERSEY x RED SINDHI HALFBREDS

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ABSTRACT

In the present study the effect of genetic and non-genetic factors on some reproductive disorders viz; dystocia, still birth and abortion were studied utilizing records on 252 Jersey and Jersey x Red Sindhi half bred cows in Himachal Pradesh during the period (1987-94). The study revealed highly significant ($P < 0.01$) effect of sire on all the three reproductive disorders, while effect of the non-genetic factors like farms, season of birth, year of calving and parity were significant on occurrences of still birth and abortion but non significant on dystocia in both pure bred and half bred cows. The general heritability estimates were low to medium.

The genital disorders in dairy cows had significant influence on dairy economy directly as well as indirectly. It may have detrimental effect on individual cow's performance in future. Furthermore, it will cause sustainable problems if the disorder is highly inheritable. The present study was undertaken with the view to see the effect of genetic and non-genetic influences dystocia, still birth and abortion as well as Jersey X Red Sindhi half bred because the bulls of these breeds are frequently used for A.I. in field.

MATERIALS AND METHODS

The incidence of dystocia, still birth and abortion were recorded from the 252 Jersey and Jersey X Red Sindhi halfbred cows maintained at three organised dairy farms i.e. Govt. Jersey Breeding Farms, Palampur, Kamand and University dairy farm Palampur of Himachal Pradesh. The managerial practices followed at all these three farm were uniform. The data for the present study were classified according to genetic group (Jersey and Jersey X Red Sindhi halfbred), farm, year of birth (1987-1994) and season of birth (Winter, Spring, Rainy and Autumn). The data was first transformed for threshold characters and further analysed by using the least squares method (Harvey 1990) in order to account the effect of various genetic and non-genetic factors.

RESULTS AND DISCUSSION

The Least-squares analysis of variance for various genital disorders are presented in Table 1.

1. Dystocia :

The pooled mean for dystocia indicates that among the overall reproductive disorders under

consideration dystocia alone accounts for the 4.7%. This was much higher than those reported by Mukherjee et al (1993) in Karan Fries (2%) herds. The pooled heritability estimates for dystocia was 0.003 ± 0.076 while it was 0.004 ± 0.076 in purebred Jersey and 0.009 ± 0.077 in Jersey X Red Sindhi half bred cows. Study revealed significant effects of sire on dystocia while other factors like genetic group, farm, parity, season and year of birth had no effect on dystocia. Significant effects of parity were reported by Tomar et al (1975) and Mukherjee et al (1993), while non-significant effect of season and year of calving were observed by Mukherjee et al (1993) and Rawal and Tomar (1996). The non-influence of genetic group suggests that crossbreeding did not alter the incidence of dystocia. However effect of sires indicates that certain sires may be carrier of the genes influencing the trait.

2. Still Birth :

The least-squares mean for still birth was found to be 0.037 ± 0.101 (3.7%). The observed estimate is higher than that of reported by Mukherjee et al (1993) in Karan Fries herds (2%). The heritability for still birth was observed as 0.088 ± 0.081 . The effect of sire, genetic group, parity, year and season of birth were found significant on still birth. The present findings are corroborated with the observations of Mukherjee et al (1993) and Rawal and Tomar (1996); The latter also reported non-significant effect of parity and

season of calving on still birth in Sahiwal herds. The effect of sires further implies that some of the sires used in breeding are carrier of genes for genital disorders. Significant ($P < 0.05$) effect of age at first calving on this trait indicate that cows which calved at late stages faced with problems of these disorders.

3. Abortion :

The pooled least-squares mean for abortions was found 0.034 ± 0.108 (3.4%). The per cent of still birth was found 3.4% which is less than that reported by Mukherjee et al (1993) in Karan Fries (4.36%) herds. Result showed significant effect of sire, parity, farm and year of birth but non-significant effect of genetic group and season of birth on abortion. However significant effect of season and year of calving on abnormal calving were also reported by Mukherjee et al (1993). Rawal and Tomar (1996) reported significant effect of sire and period of birth but non-significant effect of parity and season of calving on abortion in Sahiwal herds.

The heritability estimates for dystocia, still birth and abortions were found to be 0.003, 0.13 and 0.03 respectively. As these are reproductive traits, the estimates are about to the expectations. The estimate for still birth is around 10% which indicates that some sires are heterozygous for the genes causing this disorder.

Table - 1 : Least-square analysis of variance (ANOVA) for reproduction disorders

S. No.	Source of variation	D.F.	Dystocia mean squares	F	Still birth Mean squares	F	Abortion Means Square	F
1.	Sire	25	0.512	1.895**	0.224	5.387**	0.239	4.894**
2.	Genetic group	1	0.0187	0.692	1.915	45.928**	0.093	1.902
3.	Farm	2	0.0475	1.757	0.579	13.890**	0.176	3.603*
4.	Parity	6	0.04512	1.668	0.406	9.742**	0.264	5.397**
5.	Season of Birth	3	0.05041	1.863	0.312	7.496**	0.0619	1.2666
6.	Year of Birth	7	0.0097	0.359	0.649	15.568**	0.534	10.922**
7.	Regression ;							
	i. AFC B Liner	1	0.05007	1.851	0.130	3.134	0.1846	3.77
	ii. AFC B Quad	1	0.022	0.843	0.209	5.020*	0.3162	6.457*
8.	Remainder	205	0.027	—	0.0147	—	0.0489	—

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SEASONAL VARIATIONS IN PHYSICAL ATTRIBUTES OF SEMEN IN MALABARI AND CROSS-BRED BUCKS OF KERALA*

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ABSTRACT

Seven adult bucks belonging to Malabari, and Alpine X Malabari breeds belonging to Kerala Agricultural University goat farm was used to study the seasonal variation in physical attribute of semen. Semen was collected on daily rotational basis, at weekly intervals from each bucks and was evaluated as a part of an year long semen evaluation study. Atmospheric temperature, relative humidity (RH) and day length (DL) were also recorded daily during the period. The semen parameters were analyzed for monthly, quarterly and half yearly variations and were correlated with major climate variables to determine the environmental regulatory factors.

Among the physical attributes of semen only PH and total sperms per ejaculate showed significant variation between months and quarters while none of the other variables such as volume, colour, density, mass activity, sperm, concentration and MBR time showed significant variation between these periods. Only PH of semen showed significant relationship with environmental variables and the correlation was negative with maximum temperature ($P < 0.01$) and

positive with relative humidity and day length ($P < 0.05$). The relationship of other attributes with environmental variables were not uniform and were non significant.

Even though Indigenous and cross-breed bucks in tropical zone maintains fertility through out the year (Cupps 1991, and Jainudeen and Hafez 1993), seasonal variation in semen quality is reported in many studies and various environmental variables are attributed to this variation. (Elwishy et al., 1970; Leidl et al. 1970 and Hoffman et al. 1972 Mittal 1986, 1987). Krishnakumar, (1992) has reported two seasonal peaks in reproductive activity of Nanny goats in Kerala and is attributed to variation in day length and feed availability in the region while there is no comparable studies for Bucks. Hence the present study was carried out to find out the seasonal variations in physical characteristics of buck semen and to investigate its environmental regulatory mechanisms.

MATERIALS AND METHODS

The study was carried out at Kerala Agricultural University Goat

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Farm. Mannuthy for a period of one year. Seven adult bucks of Malabari and their cross-breeds with Alpine maintained under intensive management were used for the study. Semen was collected regularly at 7 days intervals from each of the bucks on a daily rotation basis, using Danish type artificial vagina remodelled to suit the bucks. Immediately after collection, the sample was incubated in a water bath maintained at 37°C and the physical attributes were evaluated. Volume was measured from the calibrated 2 ml glass collection vial. Visual assessment of colour was done and was graded from 1-4 based on intensity of yellow colour. Density was assessed based on capacity and consistency of a small drop of semen taken on a glass slide and was also graded from 1-4. Assessment of mass activity was done by examining a small drop under the microscope (100 X) for eddies, currents and swirls and was expressed from 1 to 4 in terms of '+' signs.

For estimating pH, universal pH indicator solution (BDH) was used. Sperm concentration was determined using Haemocyto-meter counting method and the number of sperms per ejaculate was arrived by multiplying the volume of semen with sperm concentration. Metabolic rate of sperms was estimated at biweekly intervals using standard Methylene blue reduction (MBR) test.

During the period of the study daily recordings of maximum and minimum temperature and humidity using maximum-minimum and dry-wet bulb thermometer installed inside the animal shed was done and day length was recorded based on the sun set and sunrise given in calendar. The period of study was divided into quarters corresponding to four seasons of the region, and half years of long and short days for the purpose of analysis. The four seasons included Monsoon, post monsoon, spring and summer corresponding to the quarters June to August (JJA), September to November (SON), December to February (DJF) and March to May (MAM) respectively. The data were statistically analysed for seasonal variation and correlation with environmental variables.

RESULTS AND DISCUSSION

Seasonal average of environmental variables (Temperature, relative humidity and day length) and physical attribute of semen are presented in Table.

Average volume of semen collected during the entire period of study was 0.75 ml. Between the four seasons minimum volume was during the period from September to November and maximum volume was during the period from June to August which is in agreement with the report of Ibrahim and Yousri (1992) and Singh and Purbey (1994).

Colour of ejaculates varied from yellowish white to deep yellow. Among quarters intensity of yellow colour was more during DJF (2.72) and lesser during MAM (2.61). Of the 350 samples collected no sample was discarded due to occurrence of an unusual colour. Density of Semen was less during DJF and more during MAM (3.39 Vs 3.61) but the variation was non significant. Altogether density was better during long day period than short day (3.53 Vs 3.40).

The quarterly averages of sperm concentration varied from 4020 millions in MAM to 3339 Millions in SON. However the variation between none of these periods were statistically significant. The quarterly averages Sperms per ejaculate were highest in MAM (3133 millions) and the lowest in SON (2105 millions) Between half years the variation was from 2303 millions in short day period to 2998 millions in long day half year.

Even though volume and sperm concentrations did not vary significantly between season total number of sperms per ejaculate showed highly significant variations between seasons. Pattern of variation was similar to sperm concentration with maximum during MAM and lowest during SON.

Average mass activity of total samples were 3.31 Quarterly averages varied from 3.94 (SON) to 3.22 (JJA) and between half years variation was

from 3.69 in sort day period to 3.26 during long day period. However the variations were statistically non-significant.

Among the quarters pH was lowest during DJF (6.16) and the highest of 6.39 was during JJA. During the short day half year pH was lower compared to the long day half year. The variation was highly significant ($P<0.01$) between quarters and half years. Among quarters maximum time taken for MBR was during JJA and the minimum was during MAM and the values were 296 and 257 seconds respectively. During long day and short day periods average MBR time was almost the same. The variations were not significant between quarters and half years.

Highly significant variation ($P<0.01$) was observed in the pH of semen between seasons and half years and there was significant positive correlation with day length ($P<0.01$) and relative humidity ($P<0.05$) and negative correlation ($P<0.05$) with maximum temperature. While this finding concurred with the report of Reddy et al. (1989), it disagreed with Patil and Raja (1978).

Comparison of the variation in physical attributes of semen and the environmental variables showed that there was significant positive correlation of pH with relative humidity and day length ($P<0.05$) while negative correlation with maximum temperature

($P < 0.01$). Relative humidity was negatively related to other parameters such as volume, sperm concentration and sperm per ejaculate while positively related to MBR time though the correlation was non-significant.

The relationships of above parameters were positive with maximum temperature and day length in most cases though they were also non-significant.

TABLE

Seasonal environmental average of variables and seminal attributes in bucks

Environmental and seminal attributes	SEASONS			
	Post Monsoon (SON)	Spring (DJF)	Summer (MAM)	Monsoon (JJA)
Environment				
Max. Temp °C	31.20	33.38	35.73	29.26
Min. Temp °C	23.36	23.48	25.23	23.56
Rel. Humidity %	84.64	70.88	74.47	91.87
Daylength (h)	11.47	11.28	12.14	12.32
Semen				
Colour	2.71	2.72	2.61	2.65
Density	3.40	3.39	3.61	3.44
Mass activity	3.94	3.41	3.36	3.22
MBRT (Seconds)	266	257	296	289
Volume (ml)	0.65	0.75	0.79	0.82
Sperm Concentration				
Millions/ml	3339.0	3479.60	4020.0	3560.6
Sperms per ejaculate (Millions)	2105.0	2535.2	3131.0	2860.6
pH	6.22	6.16	6.26	6.39

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MORPHOLOGICAL CHANGES OF GOAT SPERMATOZOA DURING EPIDIDYMAL TRANSIT*

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ABSTRACT

The goat spermatozoa were obtained from Caput, Corpus and Cauda regions of epididymes and were studied for morphological changes. The migration of cytoplasmic droplets from proximal to distal regions of mid piece was observed during epididymal transit. The percentage of total abnormal sperms and head abnormalities decreased significantly from Caput to Cauda region; while the tail abnormalities significantly increased from Caput to Corpus with a marginal decrease from Corpus to Cauda region.

Morphological changes in spermatozoa such as location of cytoplasmic droplet and abnormalities during epididymal maturation were studied extensively by several investigators (Amann and Almquist, 1962 Gustafson and Crabo, 1971). These studies are scanty in goat species and hence the present study was taken up.

MATERIALS AND METHODS

Fifteen epididymes were obtained from adult healthy goats soon after slaughter from Ziaguda Municipal slaughter house. The epididymes were carried to the laboratory by preserving them in physiological saline. The epididymes were identified for three distinct regions viz., Caput, Corpus and Cauda regions. Isolation of epididymal spermatozoa was done according to Amann et al. (1982). Morphology of spermatozoa was studied by staining with Leishman's stain (Rao, 1953). The morphological studies were carried out using bifocal compound microscope (Spencers, U.S.A.) in high power (400X).

RESULTS AND DISCUSSION

The spermatozoa obtained from caput region showed high percent (77.27%) of proximal cytoplasmic droplets. The number of proximal droplets significantly decreased from caput to corpus (16.33%) and to cauda (6.07%) indicating maturational changes are associated with the transit of sperm in these parts of duct. Whereas the spermatozoa with distal cytoplasmic droplet increased from 5.73% in caput to 57.6% in corpus and to 75.2% in cauda epididymis. The present finding

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of the migration of cytoplasmic droplet from proximal to distal of midpiece of Spermatozoa during their transit from corpus to cauda epididymis is in agreement with findings of Bedford (1963) in rabbit, Rao (1971) in bull, Amann et al., (1982) in ram, and Sharma et al., (1989) in goats.

In our study the incidence of droplet free spermatozoa was somewhat higher in all the three regions than the observations of Sharma et al., (1989) in goats. Sharma et al., (1989) examined wet smears, while in the present study, the smears were stained. Such high incidence of droplet free spermatozoa in stained smears in comparison to wet film was reported by Bane and Olund (1966).

The incidence of abnormal spermatozoa in three regions of epididymis were also estimated. The abnormal sperm include the abnormalities of head and tail. The total abnormal sperm was more in the caput (29.47%) than in corpus (27.00%) and in cauda (21.40%). Thus there was a gradual decrease in the number of abnormal sperms during their transit in the epididymal duct. The present findings are similar to that of Gupta et al., (1996). However Amann and Almquist (1962) observed high incidence of abnormal spermatozoa in corpus (19.5%) than in caput (13%). The lower percentage of abnormal spermatozoa in cauda region (11.4%) of their observation was similar to the present findings. Contrarily, Sharma et al. (1989) observed increase in total abnormal sperm from caput to cauda region in

goats. This increase was however largely due to increase in sperm tail defects.

Among the total sperm abnormalities, the incidence of head abnormalities was higher in caput (14.93%) and showed a significant decrease in the corpus (7.33%) and cauda (3.53%). This indicates that the decrease in total sperm abnormalities were largely due to lowered incidence of head abnormalities as the sperm transit epididymal duct. Similar observations were made by Amann and Almquist (1962), Rao (1971) and Gustafson and Crabo (1971). Sharma et al., (1989) also observed a significant decrease in sperm head abnormalities of goat spermatozoa from caput (7.8%) to corpus (5.5%). However, they observed a marginal increase in these abnormalities in the cauda region (5.7%) when compared to corpus. The incidence of increased head abnormalities in the caput in the present observation is due to the fact that head abnormalities are mostly of testicular origin. The subsequent reduction in this incidence was most probably due to resorption or dissolution of abnormal sperm heads, since the epididymis is known to have greater resorptive capabilities (Rao, 1971).

The pattern of incidence of tail abnormalities along the epididymal duct was in marked contrast to that of abnormal heads. The incidence of tail abnormalities in corpus epididymis (19.67%) was higher than in caput (14.53%) and cauda (17.87%). This abnormality was mostly due to increased

presence of tailless sperms which were significantly higher in corpus region. The incidence of tail abnormalities was consistent while increasing significantly from caput to corpus with a nonsignificant decrease from corpus to cauda region. The present findings were in general agreement with the observations of Amann and Almquist (1962) and Rao (1971), who reported similar higher tail abnormalities in the corpus region.

The incidence of bent and detached tails were significantly higher in the cauda region (12.0%) when compared to caput (6.2%) and corpus (7.0%). Lower percentage of coiled tails

and bent midpiece were observed in different regions of the epididymis during the present investigation. The increase in tailless sperms in corpus may be due to the acquisition of the moderate motility by spermatozoa in this region (Amann et al., or due to changes in ductal fluid environment (Salisbury, 1961). When certain spermatozoa, whose vitality and resistance is low, are exposed to these changes may develop tail deformities in all the regions of epididymis (Rao, 1971). The reduced incidence of tailless sperms in cauda than corpus in our study may be due to active phagocytosis in the cauda region (Roussel et al., 1967), since the spermatozoa usually spend more period of time in this region.

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BIOCHEMICAL AND MOTILITY CHARACTERISTICS OF GOAT EPIDIDYMAL SPERMATOZOA

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ABSTRACT

The total lipids and phospholipids of sperm plasma membrane significantly decreased while cholesterol, cholesterol/phospholipid ratio increased significantly from caput to corpus and corpus to cauda. The protein composition of sperm plasma membrane showed a loss of high molecular weight membrane proteins (77 kd, 70 kd, 50kd, 47kd, and 37kd), while an increase in a low molecular weight protein (31 kd), during epididymal transit. The computer aided motility analysis done on spermatozoa isolated from different regions of epididymis indicated flagellar movements in majority of caput sperm with less than 1% motile sperm, while corpus sperm showed 5% progressively motile sperm with majority of sperm showing circling movements. However majority of the cauda sperm (approximately 60%) showed progressive

motility. There was a gradual increase in different motility characteristics as the sperm moved from caput to cauda region.

Changes in sperm plasma membrane composition and organization during their transit in epididymal duct have been attributed to the maturational process, Lipid changes in goat sperm plasma membrane during epididymal maturation were reported by Rana et al (1991) and changes in protein composition was observed by Chatterji and Majumder (1989)

In the present study attempts were made to study the lipid profiles and protein composition in goat sperm plasma membranes from three different regions of epididymis and to correlate these changes to the pattern of their motility.

MATERIALS AND METHODS

Epididymes were obtained from six adult healthy goats soon after slaughter from Ziaguda municipal slaughter house. The epididymes were wrapped in aluminium foil and carried to the laboratory by keeping in ice. However, for motility studies epididymes were carried by placing them in native medium A (Rana et al, 1987).

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Isolation of epididymal spermatozoa was done according to Amann et al (1982). Isolation of plasma membrane of epididymal spermatozoa was essentially carried out by aqueous two-phase polymer method as described by Rana et al (1987) with slight modifications.

Protein content of the membrane preparation was estimated according to the method of Markwell et al (1978). Lipid extraction from sperm plasma membrane was carried out by the methods of Folch et al (1957).

Phospholipid estimation was carried out by the procedure described by Bartlett (1959) where as total cholesterol estimation was carried out as per Zlatkis et al., (1953).

SDS-PAGE analysis of sperm plasma membrane proteins was done according to Laemmli (1970). Silver staining of the gels was carried out as described by Blum et al (1987). Relative amounts of proteins were qualified by densitometry using UVG GDS 2000 Gel documentation system. (Mitsubishi Co.)

Computerised analysis of Motility Characteristics of spermatozoa was assessed using HTM-S motility analyser version 7.2 (Hamilton Thorn Research Inc., Danvers. M.A., USA.)

RESULTS AND DISCUSSION

Initially the protein content of sperm plasma membrane preparations was estimated and the results of lipid profiles was expressed on the basis of the protein content of isolated plasma membrane rather than on cell number, because the yields of membrane are

likely to differ in different lots of same cell population as well as in cells of varying physiological states.

The total lipids, phospholipids and cholesterol content of plasma membrane of spermatozoa obtained from caput, corpus and cauda are presented in Table 1. The total lipids significantly decreased as the sperm moved from caput (890 ± 10.16) into corpus (730 ± 8.90) and to cauda (650 ± 12.60 ug/mg) regions. Similar to the total lipids, the phospholipid levels also showed a significant decrease from caput to corpus and to cauda region. However, cholesterol content of sperm plasma membrane increased significantly from caput to corpus and to cauda. The cholesterol to phospholipid ratio increased significantly during sperm transit from caput (0.13 ± 2.14) to corpus (0.18 ± 1.36) and corpus to cauda (0.31 ± 2.05). The results on the lipids of epididymal sperm plasma membrane based on protein content was in agreement with the values of Rana et al (1991) in goals. The present observation of decrease in total lipid from caput to cauda was largely due to decrease in phospholipid content which contributed to 75% of membrane lipids. High concentration of phospholipids in caput spermatozoa and its subsequent decrease during epididymal transit may be due to their utilization as a source of energy for spermatozoa (Arora et al 1975). Decrease in phospholipid during maturation may further explain the greater susceptibility of cauda epididymal spermatozoa to cold shock than caput epididymal spermatozoa. (Hammerstedt et al., 1979; Scott et al., 1967). Increased cholesterol content and cholesterol/

Phospholipid ratio during epididymal transit may be responsible for less stable or more fluid membranes. (Rana et al, 1991).

The Protein composition of goat sperm plasma membrane was studied using SDS-PAGE and densitometric scanning. The results indicated that there was loss of certain membrane proteins with molecular weights of about 77 kd, 70 kd, 50kd, 47kd, and 37kd during epididymal transit. Whereas a low molecular weight polypeptides such as 55kd, 31kd increased as sperms travel to cauda region. Relative amount of a protein with molecular weight of about 42kd was more in corpus than caput and cauda.

Similar decrease in proteins with high molecular weight in protein composition of spermatozoa was reported earlier in goat spermatozoa (Chatterjee and Majumder, 1989). The loss of large molecular weight proteins during epididymal maturation may be due to proteolytic activity of epididymal fluid, which may have resulted in increased concentration of low molecular weight polypeptide in the cauda sperm.

Path velocity (VAP), progressive velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and Beat-Cross frequently (BCF) of motile spermatozoa are presented in Table 2. Goat caput epididymal spermatozoa exhibited only flagellar movement, but no progressive motility. Less than 1% of caput spermatozoa were motile. In the corpus, majority of spermatozoa exhibited

circling movements. Approximately 5% of corpus spermatozoa exhibited progressive motility. However, majority cauda epididymal spermatozoa exhibited greater progressive motility. VSL, VAP and VCL of motile spermatozoa gradually increased from caput to corpus and corpus to cauda epididymis as reported by earlier investigators (Gaddum, 1968; Hinton et al., 1979; Amann et al., 1982; Yeung et al., 1992; Girijadevi and Shivaji, 1994). Motility status of spermatozoa largely depends on the availability of energy sources, and it's metabolites such as cAMP (Cornwall et al., 1986;) Increased efficiency of glucose utilisation was another factor responsible for increased sperm motility as sperm traverse through epididymis. Motor apparatus in the spermatozoa matures during epididymal transit, which is responsible for increased sperm motility. (Mohri and Yanagimachi, 1980).

During epididymal transit there might be a decline in fresh weight of spermatozoa as there was decrease in lipid and protein content. Due to this decrease in lipid content and dehydration during epididymal transit, increased specific gravity (Lindahl and Kihlstrom, 1952) and decreased volume i.e., decrease in surface area of spermatozoa were noticed. As the surface area decreases the resistance offered by the medium also decrease. Decline in fresh weight of spermatozoa and the decrease in resistance offered by the medium result in increased sperm motility.

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Table 1: Plasma membrane lipid profile of spermatozoa obtained from different regions of goat epididymis

Lipids	Caput	Corpus	Cauda
Total Lipids*	890 ^a ± 10.16	730 ^b ± 8.90	650 ^c ± 12.60
Phospholipids*	668 ^a ± 12.44	560 ^b ± 12.59	406.7 ^c ± 10.94
Cholesterol*	84.2 ^a ± 3.04	98.2 ^b ± 2.84	125.2 ^c ± 4.35
Cholesterol/ Phospholipid**	0.13 ^a ± 2.14	0.18 ^b ± 1.36	0.31 ^c ± 2.05

Note: * Expressed as ug/mg of membrane protein

** Expressed as weight : weight

The data shown were mean of three experimental values

Figures bearing different superscript within rows differ significantly.

Table: 2 Motility characteristics of goat epididymal spermatozoa

Motility	Caput	Proximal Cauda	Distal Cauda
Path Velocity (VAP $\mu\text{m}/\text{sec}$)	88.5 ^a ± 1.98	113.0 ^b ± 2.47	120.4 ^b ± 3.04
Progressive Velocity (VSL $\mu\text{m}/\text{sec}$)	69.67 ^a ± 1.85	82.8 ^b ± 0.76	94.6 ^c ± 1.68
Curvilinear Velocity (VCL $\mu\text{m}/\text{sec}$)	126.46 ^a ± 5.12	216.66 ^b ± 1.78	216.6 ^b ± 4.14
Amplitude of lateral head Displacement (ALH μm)	8.9 ^a ± 0.61	9.3 ^b ± 0.44	9.2 ^{ab} ± 0.31
Beat Cross frequency (BCF Hz)	18.2 ^a ± 0.7	57.5 ^b ± 1.46	55.2 ^b ± 1.67

Note: Means with different superscripts, within a row, differ significantly ($P < 0.05$) The values are averages of 3 experiments.

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LEVELS OF CERTAIN IONS AND TRACE MINERALS IN PATANWADI AND CROSSBRED RAM SEMEN.

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ABSTRACT

The seminal plasma of 210 semen ejaculates obtained from Patanwadi and crossbred (Patanwadi x Russian Merino) rams was studied for the levels of ions and trace minerals. The mean values of sodium and potassium were significantly lower (110.48 ± 2.48 and 61.03 ± 1.65 mg. %) in Patanwadi rams in comparison to cross breeds (118.31 ± 2.35 and 66.69 ± 1.73 mg %). The average concentration of Zn was also recorded higher in Patanwadi rams whereas, Fe and Cu levels remained elevated in crossbred rams. Pottasium was found to be correlated negatively with initial motility (-0.163). Iron and copper exhibited positive relationship with sperm concentration. A positive correlation of copper with pH was also recorded.

The ionic composition of semen is of vital importance in maintenance of viability and metabolism of spermatozoa. It has

been reported to be associated with seminal characters and fertility (Kaludin, et al., 1983). Further, any alteration in concentration of ions may cause irreversible loss of sperm motility (Mann and Mann, 1984). The information on ionic composition of ram semen is scanty. Therefore, this study was undertaken to determine certain ion and trace minerals in the seminal plasma of Patanwadi and crossbred rams, and also to find out their correlation, if any, with other physical attributes of semen.

MATERIALS AND METHODS

Six healthy mature Patanwadi and crossbred rams (Patanwadi x Russian Merino), 3 rams of each breed, were included in the present study. Total 210 semen ejaculates, 35 from each ram, were obtained twice weekly by artificial vagina and assessed immediately for physical characters viz., volume, pH, mass activity, initial motility, sperm concentration, live and dead spermatozoan percentages. Subsequently, semen samples were centrifuged at 5000 rpm to separate the seminal

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plasma which was stored at -20°C until analysed for sodium and potassium (Oser, 1979). The levels of zinc, copper, iron and manganese were determined by Atomic Absorption Spectrophotometer technique. The data was analyzed and correlations between ionic constituents and physical characters of semen were worked out (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Mean concentration of various ions and trace minerals in the seminal plasma of Patanwadi and crossbred rams is given in Table-1. The mean values of each attribute in both the breeds of ram are comparable with the earlier reports (Mann and Mann, 1981 and Klaudin et al., 1983).

Sodium and potassium in the seminal plasma of crossbred rams were significantly higher (118.31 ± 2.35 and 66.69 ± 1.73 mg %) in comparison to Patanwadi rams (110.48 ± 2.48 and 61.03 ± 1.65 mg %). However, the ratio of Na and K (1.92 ± 0.07) was found non-significant between the two breeds of rams. The present findings are in accordance with the reports of Rai et al. (1976) and Profirov et al. (1986). Comparatively higher and lower values of these two constituents than the present values have also been documented (Thakar and Pandey, 1976 and Gonzalez et al., 1984). Such

fluctuations are obvious due to a wide range of variation in ionic composition of seminal plasma (Mann and Mann, 1981). Potassium was found to have significant negative correlation with initial motility of spermatozoa (-0.163), indicating its lower concentration, associated with cell movement. Thakar and Pandey (1976) also reported similar findings.

The concentration of Zn was recorded to be higher in seminal plasma of Patanwadi rams while, Fe, Cu and Mn levels remained elevated in crossbreds. The differences in the mean values of these elements, except manganese were significant in the two breeds of rams. Influence of breed character on concentration of Zn has been reported (Karaglannidis et al., 1985). A Significant negative correlation of Zn, and positive correlations of both Cu and Fe were discernible with the concentration of spermatozoa. Copper also showed significant positive relationship with the pH of semen. A close relationship of Cu and Fe elements with the concentration of spermatozoa in cattle and buffalo bulls. (Mishra et al., 1989 and Zaghloul et al., 1991) and of Zn with sperm motility in rams Kaludin et al., 1983) has been reported. The present findings suggest the involvement of these elements/minerals in maintaining the number of spermatozoa in a semen ejaculate, and also in providing congenial milieu for their survival.

Table 1: Ions and trace elements in seminal plasma of Patanwadi and crossbred rams.

Element / Bread	Patanwadi (n = 105)	Crossbred (n = 105)
Ions (mg %)		
Sodium	110.48 ± 2.48 ^a	118.31 ± 2.35 ^b
Potassium	61.03 ± 1.65 ^a	66.69 ± 1.73 ^b
Na : K ratio	1.96 ± 0.07	1.92 ± 0.07
Trace element (µg / 100 ml.)		
Zinc	40.60 ± 2.94 ^b	27.33 ± 2.49 ^b
Iron	42.21 ± 2.36 ^a	55.25 ± 2.42 ^b
Copper	2.34 ± 0.25 ^a	4.22 ± 0.45 ^b
Manganese	16.28 ± 0.73 ^a	17.96 ± 1.18 ^b

Means with different superscripts in row differ significantly at 5 percent for ions, and at 1 percent for trace elements.

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**THE EFFECT OF EXERCISE ON SEMINAL ATTRIBUTES OF MEHSANA
BUFFALO BULLS IN DIFFERENT SEASONS***

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ABSTRACT

Mehsana buffalo bulls were subjected to forced exercise for one hour (2.62 km) on alternate days. Semen quality measures improved significantly due to reduction in sperm abnormalities in all three seasons. However, the better results on semen quality improvement were noticed in summer and monsoon seasons.

Exercise to bulls keep them in good physical health. Beneficial effect of exercise on ejaculate volume, mass activity, sperm concentration, motility and sperm-ejaculate were observed by number of workers (Bhosrekar and Nagpaul, 1972; Poroshin and Oboskalov 1976; Morinets and Vernigor 1979; Tizol et al., 1987; and Adwani et al., 1995) in dairy cattle bulls. However, reports on effect of exercise on buffalo bull semen characteristics were very

scanty (Matharoo et al., 1985). The present study was therefore taken on Mehasana buffalo bulls to know the effect of exercise on semen characteristics.

MATERIALS AND METHODS

Present investigation was carried out at Pashu Samvardhan Kendra, Jagudan of Mehsana District Co-operative Milk Producers Union Ltd., Mehsana, Gujarat having semi-arid tropical climate. Twelve Mehsana buffalo bulls (2.5 to 5 yrs. old) were divided equally into control and exercise groups. In exercise group bulls were subjected to forced exercise for one hour (2.62 km) in the morning on alternate days. Other management and feeding practices were remained same for both the groups.

Semen collection and semen quality of bulls were examined once a week. Semen quality measures were done as per the methods suggested by Bhosrekar (1990). Acrosomal integrity of sperms measured as per the method suggested by Hancock (1952). The experiment was carried out in three seasons i.e., winter (November, February), summer

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(March-June) and monsoon (July-October) according to climatological variation Data were subjected to least squares of variance (Harvey, 1987).

RESULTS AND DISCUSSION

Initial Seminal Attributes:

The per cent co-efficient of variation were very high for most of the initial seminal attributes except pH. The overall least-squares means of initial seminal attributes differed significantly ($P \leq 0.01$) among experimental groups except motility and total sperm/ejaculate. The bulls under exercise group had significantly ($P \leq 0.01$) higher mass activity (2.12 ± 0.06 vs 1.87 ± 0.06) sperm concentration 1471.03 ± 29.30 vs $1023.79 \pm 30.00 \times 10^6/\text{ml}$ and lower ejaculate volume 2.09 ± 0.12 vs 3.47 ± 0.13 ml and pH 6.86 ± 0.01 vs 6.89 ± 0.01 than control group. The result was in contrast to Matharoo et al. (1985) in Murrah bull due to difference in breed, location and climatological factors. The same trend as per the overall observation was noticed for initial seminal attributes in different seasons in both experimental group, but the differences were non-significant.

Semen-quality measures :

The overall percentage of livesperm, spermhead abnormalities, mid-piece abnormalities, total sperm abnormalities acrosomal integrity and

post-thaw motility were respectively 83.58 ± 0.52 , 2.20 ± 0.09 , 2.98 ± 0.10 , 2.34 ± 0.12 , 7.52 ± 0.16 , 93.39 ± 0.19 and 52.83 ± 0.46 in exercised bulls. The corresponding values in control bulls were 81.42 ± 0.53 , 2.57 ± 0.09 , 3.77 ± 0.11 , 3.33 ± 0.12 , 9.68 ± 0.16 , 91.89 ± 0.19 and 51.71 ± 0.45 .

The semen quality in bulls under exercise group showed significant ($P < 0.01$) improvement that motility except post thaw motility compared to control. Tizol et al. (1987) reported better semen quality in exercise groups of Holstein bulls than non-exercise group. Sperm head, midpiece and total sperm abnormalities significantly reduced ($P < 0.01$) in exercise group in all the three seasons except sperm head abnormalities in winter season. The sperm head (1.97 ± 0.16 vs 2.82 ± 0.16) mid-piece (3.04 ± 0.18 vs 4.04 ± 0.18) and total abnormalities (7.46 ± 0.28 vs 10.37 ± 0.27) reduced significantly in summer and monsoon season in exercised group compared to control group. In winter the reduction noticed was not significant. Improvement was also observed in live-sperm count, Sperm tail abnormality, post-thaw motility and acrosomal integrity in exercise group compared to control group in all seasons, however, the differences were non-significant.

According to Singh et al. (1980) potassium along with water is

eliminated from tissue during exercise. This in turn stimulate the aldosterone secretion. The aldosterone had extra-gonadal effect especially on epididymal sperm reserve. This would be the reason for significant improvement in semen quality in bulls subjected to exercise.

It was inferred that Mehsana buffalo bulls exercising in morning hours could be able to improve the semen quality during summer and monsoon season due to reduction in sperm-abnormalities.

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FREEZABILITY OF BUFFALO BULL SEMEN USING DIFFERENT EXTENDERS

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ABSTRACT

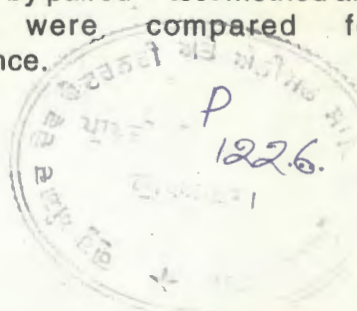
The objective of the present investigation was to study the effect of various extenders on the progressive motility in frozen semen from Murrah buffalo bulls. Fourteen split ejaculates were frozen in LN_2 vapours to compare Tris buffer with Laiciphos (IMV) and Biociphos (IMV) with regards to their comparative efficacy on semen freezability. The before freezing and after freezing post thaw motility was found to be significantly different between Laiciphos and Tris extenders ($P < 0.05$). However did not differ significantly between Laiciphos and Biociphos. It is therefore inferred that tris-based extender is better for buffalo semen freeze preservation than the Laiciphos and Biociphos extenders.

The frozen buffalo semen is important for any buffalo genetic improvement programme. The use of frozen semen has considerably improved the availability of superior germplasm. The problems and prospects of buffalo semen freeze preservation has been recently reviewed (Jindal and Chopra, 1993, Jindal, 1998). Although a satisfactory post thaw motility is obtained by the method developed at CIRB (Jindal,

1998) it was considered appropriate to see that how the milk based extenders (Biochiphos and laiciphos) compare with the tris based extenders being currently used.

MATERIALS AND METHODS

Fourteen split ejaculates of six Murrah buffalo bulls were frozen in LN_2 vapours to evaluate the effect of different extenders on freezability of the semen. Every ejaculate was divided into three equal parts and diluted in Tris buffer, IMV Laiciphos and Biociphos. Final glycerol concentration was kept at six per cent. The freeze preservation of buffalo bull semen was done as per procedure described by Jindal (1994). The sperm motility was assessed before filling the semen into straws and after thawing at $37^\circ C$ in a water bath. The sperm motility was assessed at low magnification under phase contrast microscope (Nikon). The sperm motility was assessed by at least two technicians and a mean of the two values was considered as the sperm motility for that sample. The data was analyzed by paired 't' test method and means were compared for significance.



RESULTS AND DISCUSSIONS

The mean values of the sperm motility before freezing was 60.35 ± 1.61 , 57.85 ± 3.98 and 63.21 ± 7.73 percent in Biociphos, Laiciphos and Tris extender, respectively. The before freezing motility was found to be significantly different between Laiciphos and Tris extenders ($P < 0.05$). However, the motility did not differ significantly between Laiciphos and Biociphos. When means were compared the highest pre-freezing sperm motility was found in Tris followed by Biociphos and Laiciphos. The Tris buffer had highest (43.92 ± 1.75 per cent) post-thaw sperm motility followed by Laiciphos (37.85 ± 3.73) and Biociphos ($37.50 \pm$

2.90). The overall Tris buffer-based extender was considered better than other two IMV skimmed milk based-Laiciphos and Biociphos extenders for better semen freezability. Laiciphos gave better post thaw sperm motility in comparison to biociphos. Contrary to our observations, Galli et al (1991) found Laiciphos to be better than Tris for buffalo semen freeze preservation. Aleandri et al (1996), who conducted trial with IMV Laiciphos and Biociphos on preservation of buffalo semen and found that the total sperm motility was better in Biociphos than Laiciphos. It is therefore inferred that Tris buffer extender gave better sperm motility than Laiciphos and Biociphos extenders for buffalo semen freeze preservation.

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EFFECT OF FREEZING ON CYTOMORPHOLOGICAL CHANGES AND INTRA-CELLULAR ENZYME RELEASE IN BUCK SPERMATOZOA*

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ABSTRACT

Using artificial vagina, 70 ejaculates were collected from five Tellicherry bucks aged between two and five years. These ejaculates were frozen in Tris-based extender containing 20 per cent egg yolk and 7 percent glycerol. The diluted semen was equilibrated for 4 h before freezing. The mean percentages of swollen, separating, entirely lost acrosomes and total acrosomal damages were 4.25, 0.46, 2.32 and 7.03 at pre-freeze and 9.09, 0.87, 3.50 and 13.47 at post-thaw stages respectively. The mean GOT and GPT enzyme levels were 151.70 and 15.66 and 216.07 and 21.29 units per ml of extra cellular medium at pre-freeze and post-thaw stages respectively. The differences in various types of acrosomal damages and enzyme levels between bucks and between processing stages were highly significant. ($P \leq 0.01$).

During the process of freezing, acrosomal damage and leakage of intra-cellular enzymes from

spermatozoa are inevitable in spite of addition of egg yolk and cryoprotectant. Although, there is voluminous literature available on the freezing of bovine and bubaline semen, the reports on freezing of goat semen is meagre. Hence, the present study was undertaken to investigate the extent of cryoinjury caused to buck spermatozoa due to freezing based on intracellular enzymes (GOT and GPT) and to study the cytomorphological changes of buck spermatozoa on freezing.

MATERIALS AND METHODS

Seventy ejaculates were collected from the five Tellicherry bucks aged between two and five years using artificial vagina. Semen samples were collected from the bucks with an interval of two or three days between collections. The semen samples with mass activity scores of 5 or 4 were used for the present study. The semen samples were diluted 1+5 at 37°C with Tris diluent containing 20 percent egg yolk and 7 percent glycerol. The diluted semen samples were equilibrated for 4h at 5°C, packaged in French medium straws (0.5ml) and frozen in liquid nitrogen vapour. The frozen straws were then stored in liquid nitrogen container. After 24h, the straws were

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thawed in water at 37°C for 30 seconds. The post-thaw motility of spermatozoa was assessed in a phase contrast microscope.

The cryoinjury to buck spermatozoa was studied by assessing the extent of damage to the acrosomes and by estimating the concentration of GOT and GPT enzyme levels in the extra-cellular medium just before freezing (pre-freeze) and after freezing and thawing (post-thaw). The acrosomes were studied using Giemsa staining technique (Watson, 1975) and the damaged acrosomes were classified according to Watson and Martin (1972). The GOT and GPT enzymes were estimated by colorimetric method as per Reitman and Frankel (1957). The data were analysed statistically (Snedecor and Cochran, 1967) for interpretation.

RESULTS AND DISCUSSION

The mean pre-freeze and post-thaw motility of goat semen recorded in the present study were 76.80 and 54.50 percent respectively. The mean acrosomal damage during pre-freeze and post-thaw stages of processing of buck semen are given in Table. The mean percentages of various types of acrosomal damage showed highly significant differences between stages of processing. All types of acrosomal damage increased significantly ($P < 0.01$) between pre-freeze and post-thaw stages of processing of goat semen. The proportion of swollen acrosomes was found to increase more rapidly than

separating or entirely lost acrosomes. On the whole, there was a drastic increase of swollen, separating and entirely lost acrosomes in the post-thaw semen samples. The total acrosomal damage increased from 7.03 to 13.47 percent in the post-thaw semen samples. Earlier reports revealed that the total acrosomal damage increased from 4.43 to 11.70 per cent (Deka and Rao, 1986) and 11.28 to 24.04 per cent (Singh and Purbey, 1992) in goat semen.

The mean levels of intracellular enzymes, GOT and GPT at pre-freeze and post-thaw stages are presented in Table. The mean values of GOT and GPT differed highly significantly ($P < 0.01$) between the two stages of semen processing. It could be seen that there was an overall leakage of 42.43 percent GOT and 40.23 per cent GTP in frozen-thawed goat semen. This increase might be due to the cryoinjury to spermatozoa during freezing and thawing in spite of the cryoprotection offered by the egg yolk and glycerol. Even though, the leakage of both the enzymes is around 40 per cent, the corresponding reduction in sperm motility is only about 29 percent (from 76.80 per cent at pre-freeze to 54.50 per cent at post-thaw). Similar significant enzymes leakage between bucks and between pre-freeze and post-thaw stages were reported by earlier workers (Tiwari et al., 1985; Singh et al., 1992). The leakage of the intracellular enzymes is due to the result of cryoinjury caused to the acrosome of buck spermatozoa during the process of freezing and thawing.

TABLE

Means (\pm SE) of acrosomal damage (percent) and levels of intra-cellular enzymes (units per ml of extra-cellular medium) at pre-freeze and post-thaw stages of processing of buck semen.

Acrosomal Damage							
Swollen acrosome		Separating acrosome		Entirely lost acrosome		Total acrosomal damage	
PF	PT	PF	PT	PF	PT	PF	PT
4.25 ^b \pm 0.10	9.09 ^a \pm 0.10	0.46 ^b \pm 0.14	0.87 ^a \pm 0.12	2.32 ^b \pm 0.06	3.50 ^a \pm 0.11	7.03 ^b \pm 0.15	13.47 ^a \pm 0.12

Intra-Cellular enzymes			
GOT		GPT	
PF	PT	PF	PT
151.70 ^b \pm 1.62	216.07 ^a \pm 2.68	15.66 ^b \pm 0.015	21.96 ^a \pm 0.32

PF = Pre-freeze

PT = Post-thaw

Means bearing different superscript differ significantly ($P < 0.01$)

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SIMULTANEOUS DETERMINATION OF VIABILITY AND ACROSOMAL STATUS OF BOVINE SPERMATOZOA BY DUAL STAINING TECHNIQUE

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ABSTRACT

With the aim of simultaneous determination of viability and acrosomal status of bovine spermatozoa, the two common staining techniques Nigrosin-Eosin (NE) staining technique and the Giemsa staining technique were combined. The proportions of live bovine spermatozoa as determined by the NE technique and the Nigrosin-Eosin-Giemsa (NEG) technique were similar. Acrosomal status determined by the NEG technique did not differ significantly from that of giemsa staining technique. This staining technique for identifying live and dead bovine spermatozoa and acrosomal status in the same smear is capable of determining four categories of spermatozoa, live acrosome intact, live acrosome-reacted or damaged, dead acrosome intact and dead acrosome-reacted or damaged.

technique (Hancock, 1952) is the most common method for evaluating the acrosomal status in a dried smear. Presently available techniques for evaluating the acrosomal status of live and dead spermatozoa in the same smear are either tedious (triple stain technique by Vazquez et al., 1992) or requires costly equipments like fluorescence microscopy and phase contrast microscopy (DeLeeuw et al., 1991). Tamuli et al., (1994) developed a simple staining technique for simultaneous determination of viability and acrosomal status in boar spermatozoa. Present study indicate use of combined nigrosin-eosin and giemsa staining techniques using BALL's fluid fixation for simultaneous determination of viability and acrosomal status.

MATERIALS AND METHODS

Fresh semen samples were collected from six bulls (5 ejaculates each) maintained at the Southern Regional Station of National Dairy Research, Adugodi, Bangalore.

Nigrosin-eosin (NE) staining method :

The nigrosin-eosin stain was prepared by modifying the method of Tamuli et al., (1994). Nigrosin (10 g) was dissolved in double distilled water (50 ml) by boiling

Assessment of both the viability and the acrosomal status of spermatozoa is essential for the assessment of semen quality for predicting the fertility of a particular bull or in the evaluation of semen preservation methods. The nigrosin-eosin staining is the most commonly used technique for distinguishing live and dead spermatozoa in a dried smear (Hancock, 1951). Giemsa staining

The nigrosin was filtered through a glass filter paper into a measuring cylinder containing water soluble eosin (1.25 g). To this, 7.5 ml of 50 mM glucose and 7.5 ml of tartrate phosphate buffer (TPB : di sodium hydrogen orthophosphate anhydrous, 50mM, potassium dihydrogen orthophosphate, 25mM ; potassium sodium tartrate, 77 mM) were added by repeated rinsing of the beaker containing nigrosin. Then the final volume was made to 100 ml and kept at 4°C for use. The semen smears were prepared by mixing the semen and stain in the proportions 1:3 for 30 seconds before preparing smear and drying quickly on a warm plate.

Nigrosin-Eosin-Giemsa (NEG) Stain

Method : For NEG staining NE stained smears were fixed for 30 min. in BALL's fluid (saturated picric acid: formaldehyde (40%): and glacial acetic acid in the ratio of 15:5:1 respectively).

The slides were rinsed in slow running water for 10 min. Then the slides were rinsed once in distilled water. Later the slides were stained with 6% Giemsa working solution for 3 hrs. at 37°C (Fresh working solution was prepared by mixing 90 ml of carbondioxide free water, 6 ml of geimsa stain and 4 ml of phosphate buffer pH 7.4 on the day of use). After 3 hours the slides were dipped in flowing tap water and rinsed once with distilled water and air dried. The smears were examined under oil immersion at a magnification of 1000x and 200 sperm cells were scored.

Thirty semen samples from 6 bulls were stained first with NE examined

and then stained with Giemsa and re-examined under 1000X. The percentage of live cells was compared in the slide from counts made by NE and after NEG staining.

The data were analysed by student's test as described by Snedekar and Cochram (1967).

RESULTS AND DISCUSSION

NE stained smears when examined under bright field microscopy dead sperms were dull pink with an ill-defined margin where as live sperms remained unstained and glittered. In NEG stained smears, the live spermatozoa appeared pink and the dead ones appeared purple in the post acrosomal region and acrosomal status was easily evaluated from the deeply stained acrosomes. The percentage of live spermatozoa on the same slide on the NE smear (74 ± 13.6) and then again from the NEG smears (72.6 ± 18.2) were similar ($P < 0.05$). The mean percentage of acrosomal damage observed in the NEG smear was similar to that recorded for the same animal using giemsa stain (13.88 ± 3.66) alone. P-182450

The results indicated that the NEG staining technique can be successfully utilised to identify live and dead spermatozoa and acrosomal status of bovine spermatozoa in the same smear. Though slightly higher percentage of live cells were observed in NE smears as compared to NEG smears in the same slide the difference was insignificant. The percentage of acrosomal damage observed in the NEG smears were similar to that recorded for

the same animal using Giemsa staining technique.

Our present findings of similar percentage of live cells in NE and NEG staining in bovines is in agreement with the findings of Tamuli et al., (1994) in pigs. However, the same authors have reported the higher percentage of live spermatozoa in the NE smears than in the NEG smears in rams. The variation in the results could be due to species variation. In NEG staining technique, NE stained slides can be stored and stained later with giemsa stain for screening.

Spermatozoa which have lost their plasma membrane integrity produces a purple coloration of the post acrosomal region by NEG staining and

purple coloration is also observed in the acrosome as soon as deterioration begins. The NEG staining procedure is a reliable technique, and more simple than that described by Tarbot and Chacon (1981) to distinguish four sub groups of spermatozoa, live acrosome intact, live acrosome reacted (damaged) dead acrosome-intact and dead acrosome-damaged (reacted). The visual distinction of different sub groups of spermatozoa requires little experience. Assessment of the percentage of live acrosome-intact spermatozoa is an essential parameter in determining quality of semen and will assist in the assessment of potential fertility in bulls before they are used for artificial breeding programmes.

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TESTOSTERONE LEVELS IN MADRAS RED RAM LAMBS MAINTAINED UNDER GRAZING AND FEEDLOT SYSTEMS*

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ABSTRACT

The mean plasma testosterone levels in Madras Red ram lambs were 0.09 ± 0.01 , 0.18 ± 0.02 , 0.27 ± 0.04 , 0.27 ± 0.03 and 0.43 ± 0.07 ng/ml at 4, 5, 6, 7 and 8 months of age respectively. Significant differences were noticed in the plasma testosterone levels between age groups. The overall mean plasma testosterone level of Madras Red ram lambs under grazing system was significantly lower than ram lambs under feedlot system.

Study carried out by Schanbacher et al., (1974) on peripheral levels of testosterone in developing cross bred rams suggested that the steroidogenic function of the ram testis commences at approximately the same time as spermatogenesis. Under conditions of inadequate or poor nutrition, the androgenic function of the testes was

found to be retarded more markedly than spermatogenesis (Skinner and Rowson, 1968). The object of this report is to find out the effect of two types of feeding systems on the plasma testosterone levels.

MATERIALS AND METHODS

Twenty healthy Madras Red ram lambs born at Livestock Research station, Kattupakkam,, Tamil Nadu were utilized for this study. The ram lambs were weaned at three months (90 days) of age. At the time of weaning the ram lambs were equally and randomly divided into two groups of different feeding systems namely grazing and feedlot systems. Ram lambs under grazing system were sent for grazing daily. Concentrate feed at the rate of 100g per lamb from 3 to 5 months of age and 200 g per lamb from 5 to 8 months of age was fed in bulk for all ram lambs as a supplement to grazing. Under feedlot system each ram lamb was kept in separate wooden partitioned pens. They were fed individually ad libitum with pellet feed comprising of 50 percent concentrate and 50 percent lucerne meal from 3 to 8 months of age.

Every month, two ram lambs from grazing and two ram lambs from feedlot systems were utilized for

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estimation of testosterone. The blood was collected from each ram lamb 8 times at hourly interval starting from 8.00 A.M. to 3.00 P.M. by jugular vein puncture. Immediately after blood collection, the plasma was harvested into separate sterile vials, properly labelled and stored at -20°C in deep freeze. A total of 180 blood samples were collected from 20 ram lambs at monthly interval from 4 months to 8 months of age. The testosterone in the plasma was estimated by Radio Immuno Assay as per the method described by Catling and Sutherland (1980). The mean testosterone value of 8 samples collected from each ram lamb was utilized for statistical analysis. (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

In the present study, the mean plasma testosterone estimated in Madras Red ram lambs was 0.09 ± 0.01 , 0.18 ± 0.02 , 0.27 ± 0.04 , 0.27 ± 0.03 and 0.43 ± 0.07 ng/ml at 4,5,6,7 and 8 months of age respectively (Table 1). These values were comparable to the findings of Schanbacher et al., (1974) and Yarney and Sandford (1989) in cross bred ram lambs. In contrast, Sandford et al., (1982) reported higher values in exotic ram lambs. The low plasma testosterone level in the present study might be due to the influence of indigenous breed and climate. Adeyeno et al., (1990) also stated that low values of plasma testosterone was characteristic of the indigenous rams reared in the tropical and equatorial climate.

Significant differences were noticed in the plasma testosterone levels of Madras Red ram lambs between age groups. The plasma level was low at four months of age and then it increased two-fold by five months of age. By eighth month, the testosterone level has increased by five fold. Venugopal Naidu (1991) observed spermatogonia at fifth month, primary spermatocytes at sixth month and complete spermatogenesis by eighth month of age in rams. The changes in testosterone level observed in this study coincide with changes in seminiferous epithelium and confirm the earlier report that the testosterone plays an important role in the final maturation of spermatozoa and also in attainment of the sexual maturity in male goat (Chakraborty et al., 1989). Schanbacher et al., (1974) suggested that the steroidogenic function of the ram testes commenced at approximately the same time as spermatogenesis. The presence of mature type of Leydig cells at five months of age coincided with the increase in the testosterone level and thereafter there was increase in the number of mature Leydig cells and testosterone concentration (Venugopal Naidu, 1991). Reviers et al., (1985) reported that the plasma testosterone concentration was correlated with the cross-sectional area of Leydig cells and total volume of Leydig cells per testis.

The overall mean plasma testosterone level in the grazing system (0.19 ± 0.02 ng/ml) was significantly lower than feedlot system

(0.30 ± 0.50 ng/ml) in Madras Red ram lambs during postnatal period (Table). There was no difference in testosterone level between grazing and feedlot systems at four months of age, but the difference was noticed at five months of age which increased to the maximum by eight months of age. Martin et al., (1987) also observed an increase in the testosterone level after nutritional supplementation in rams. The androgenic function of the testes was found to be retarded by inadequate or poor nutrition (Skinner and Rowson, 1968). The delay which under feeding causes in the onset of androgenic activity was due to the lack of gonadotropins from the hypophysis (Mann, 1964).

The individual testosterone value of plasma samples of lambs observed to fluctuate at lower level without attaining peaks at 5 months of

age in ram lambs kept under both grazing and feedlot systems. At 6 months, the peaks were observed in lambs under feedlot system. At 7 months of age clear cut peaks were observed in lambs under both systems. At 8 months of age, the amplitude of peaks were observed in lambs under both systems. However, the amplitude of peaks were high in feedlot system than in grazing system.

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Mean Plasma testosterone level (ng/ml) in Madras Red ram lambs

Age (Months)	Grazing System	Feedlot System	Mean
4	0.09 ± 0.02	0.10 ± 0.02	$0.09^a \pm 0.01$
5	0.15 ± 0.02	0.21 ± 0.02	$0.18^{ab} \pm 0.02$
6	0.23 ± 0.03	0.31 ± 0.04	$0.27^b \pm 0.04$
7	0.22 ± 0.06	0.32 ± 0.06	0.27 ± 0.03
8	0.28 ± 0.06	0.58 ± 0.13	0.43 ± 0.07
Overall Mean	$0.19^a \pm 0.02$	$0.30^b \pm 0.05$	—

Means bearing different superscripts differ significantly ($P < 0.01$).

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**INDUCTION OF ESTRUS AND OVULATION IN POST-PARTUM ANESTRUS
CROSSBRED COWS WITH SHORT-TERM
STERIOD TREATMENT.**

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The short term therapy for induction of ovulatory estrus and fertility in lactating true anestrus cows with considerable success has been reported by several workers (Fotte and Hunter, 1964; Britt et. al., 1974; Gonzalez et. al., 1980 and Shanker, et. al., 1996). This paper describes the results of a study done to evaluate the effectiveness of progestogen and estrogen therapy in anestrus crossbred cows, maintained at Military Dairy Farm, Bareilly (U.P.)

MATERIALS AND METHODS

Fourteen post-partum anestrus (100-150 days) crossbred cows were examined on two occasions at an interval of 10 days to exclude possibility of cyclicity and were randomly dividend into two groups, Group A (n=8) received i.m. injections of 50 mg progesterone (Proluton depot. German Remedies Pvt. Ltd., Bombay) daily for a period of 5 days and 5 mg estradiol valerate (Progynon depot) on 7th day Group B (n=6) served as control.

All the animals were closely observed for the signs of estrus. Cows detected in induced estrus were

inseminated twice (am/pm schedule) with frozen-thawed semen and pregnancy diagnosis was done 45 days after insemination. Ovulation was confirmed by identification of corpus luteum 5 to 7 days after estrus per rectal examination.

RESULTS AND DISCUSSION

Out of eight hormone treated cows, 2 showed pronounced estrus symptoms on an average interval of 2.5 ± 0.5 days with cent percent fertility which indicate that induced estrum was ovulatory and corpus luteum were functionally normal. Four (67%) of six animals established overian cyclicity within the mean interval of 24.66 ± 4.66 days of treatment and were conceived by subsequent inseminations. The average number of service per conception was 1.5 ± 0.5 in treated cows. Similar estrus interval in response to short term steroid treatment has been reported by Thakur (1989) in buffaloes. However, the response to the treatment is better than earlier report in cows with same drug schedule (Gonzalez and Ruiz, 1978; Gonzalz et. al., 1980). This may be attributed to size of experiment

herd, season, plan of nutrition and difference in post partum period.

In the control group, out of six animals only 2(33%) animals showed estrus with conception following in seminations during the period of study. Thus, it clearly shows that the short-

term steroid treatment used in this study is effective for treating functional anestrus in cross bred cows. However, to understand the complete physiological mechanism involved in, the plasma levels of pituitary gonadotrophins and ovarian steroids should be estimated.

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FACTORS INFLUENCING PREGNANCY RATE THROUGH EMBRYO TRANSFER IN CATTLE

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A number of factors have been reported affecting the success rate through embryo transfer in cattle (Critser et al., 1979; Wilmut et al., 1985). Quality of embryo has been considered as a good predictor of pregnancy. Embryos of excellent / good quality produced higher pregnancy rate than the fair or poor quality embryos (Schneider et al., 1980). Transfer of blastocyst resulted in better survival rate than the morula in cattle (Riha et al., 1987). Similarly, surgical transfer produced higher pregnancies than the nonsurgical transfers (Hahn et al., 1980). In the present paper influence of quality of embryos, embryonic development and type of transfer (surgical vs. nonsurgical) on pregnancy rate through embryo transfer in cattle have been reported.

MATERIALS AND METHODS

A total of 105 cattle embryos collected from superovulated donors on day 7/8 post - oestrus were transferred into the synchronised recipients. Healthy reproductively sound, well fed and managed Cows only were used as recipient's. Standard hormonal protocol was adopted both for superovulation of donors and synchronisation of recipients. Prior to transfer, quality and

stage of embryonic development was determined morphologically (Morula and Blastocyst) under stereozoom microscope. The embryos were graded as excellent / good, fair, poor, degenerated and unfertilized as per Shea (1981). Ninety five embryos were transferred nonsurgically using Cassou embryo transfer gun under epidural anaesthesia. Ten embryos were transferred surgically through laparotomy performed under local anaesthesia as per Newcomb (1979). The horn was exposed and embryos were transferred at the tip of the uterine horn. In both the methods, embryo was transferred into the uterine horn ipsilateral to the ovary bearing corpus luteum. All the recipients were subjected to pregnancy diagnosis per rectum 45-60 days following transfer of embryos.

RESULTS AND DISCUSSION

Results pertaining to the effect of embryo quality, indicate transfer of excellent / good embryos ($n = 48$) produced higher pregnancy rate than the fair ($n = 52$) quality embryos (39.50 vs 7.11%). Poor quality embryos ($n = 5$) however, did not produce any pregnancy. With regards to embryonic development out of 105 embryos 46 were morula and 59 were blastocyst. Transfer of

blastocyst resulted in higher pregnancy rate than the morula (23.72 vs. 17.79%) indicating better survival rate of embryos in vivo with the advancement of stage of embryonic development. Chung et al. (1983) and Riha et al. (1987) have also reported similar observations. On the other hand, Butler and Biggers (1989) did not observe any correlation between the quality of embryos and conception.

Irrespective of stage of development and quality of embryo the surgical transfer resulted higher pregnancy rate than the nonsurgical transfer (50.0 vs. 17.89%). The overall pregnancy rate was 20.95%. Better embryo survival by surgical transfer as observed in the present study has also been reported by other workers (Hahn et al., 1980; Takahashi, 1980).

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RELATIONSHIP OF CERTAIN BIOCHEMICAL ATTRIBUTES IN CERVICAL MUCUS WITH CONCEPTION RATE IN COWS

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Normal biochemical constitution of cervical mucus (CM) is prerequisite to fertility of animals. Deficiency or excess of certain biochemical constituents can be lethal to sperms (Stegmayr and Ronquist, 1982) and affect conception rate of buffaloes Vadodaria and Prabhu (1990). The present study was undertaken to estimate the levels of calcium, inorganic phosphorous, glucose and total protein in CM during fertile and non-fertile estrus of cows in Himachal Pradesh to assess their relationship with fertility.

MATERIALS AND METHODS

A total of 55 adult, healthy and cycling cows (Jersey, Jersey x Red Sindhi) belonging to H.P.K.V. Dairy Farm Palampur were selected for the present study. The animals were kept under standard conditions of feeding and management. At estrus all the animals had good uterine tone clear, copious, stringy cervical mucus (CM) with typical fern pattern was collected aseptically before insemination and stored at -20°C till analysis. Calcium, inorganic phosphorus, glucose and total protein were estimated with the help of AMES CH 100 SEAC semi-auto analyser (Miles India Ltd., Baroda). Animals were examined per rectum, 3 months post-

insemination, for pregnancy diagnosis and grouped as conceived (Gr I: n=24) and non-conceived (Gr II: n=31). The statistical significance was determined by Student's 't' - test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Calcium :

The mean level of calcium in CM of group I (7.59 ± 0.39 mg/dl) was significantly higher ($P < 0.05$) than that of group II (5.64 ± 0.43 mg/dl). Similar level of calcium in the CM of fertile cows have been reported by Sharma and Tripathi, (1989). However, Shanker et. al. (1984) have reported higher values, of calcium in the CM of cows that failed to conceive. Calcium is one of the major cation in CM which stimulates glycolysis thereby sustaining the viability, motility and metabolism of the sperm (Sidhu and Guraya, 1985), whereas excessive levels can inhibit sperm motility (Vadodaria and Prabhu, 1990).

Inorganic Phosphorus :

There was no significant difference in the mean values of inorganic phosphorous in the CM of group I: 1.08 ± 0.11 and group II: 1.06 ± 0.15 mg/dl. These values stimulate the levels in the CM of infertile cows,

whereas higher values have been recorded in cows during fertile estrus (Krishnaswami and Uthappa, 1984). Inorganic phosphorus is essential for energy transformation at cellular level and is associated with maintenance of sperm glycolysis and respiration (Lardy and Phillips, 1943).

Glucose :

The mean value of glucose in the CM of group I (1.20 ± 0.31 mg/dl) was not significantly different than in group II (0.96 ± 0.31 mg/dl). Sidhu and Guraya (1985) have reported very little sugar in the cervico-vaginal mucus. The glucose could not be detected in 12 animals of group I and 9 animals of group II. Glucose benefits conception as it can be utilised as a source of energy (Wani et. al., 1979).

Total Protein :

Total protein content in CM of group I (0.20 ± 0.03 mg/dl) higher ($P < 0.05$) than in CM of group II (0.12 ± 0.01 mg/dl). Comparatively higher values of total protein in CM of fertile and infertile cows have been reported earlier (Wani et.al., 1979). Protein in CM improve sperm transport and regulate its osmolarity; consistency, threadability and buffering capacity (Goel et. al., 1974).

Cervical mucus of normally conceiving cows had significantly higher ($P < 0.05$) levels of calcium and total protein, and nonsignificantly higher inorganic phosphorus and glucose than the cows which failed to conceive.

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EFFECT OF MEDICINAL PLANTS ALOE BARBADENSIS AND ARISTOLOHIA BRACTEATA ON ONSET OF PUBERTY IN RATS*

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Several herbal medicines (medicinal plants) are identified as potent agents for regulation of fertility in females. Plants like Aloe barbadensis and Aristolohia bracteata are used as emmenagogue for the treatment of amnorrhoea and various uterine disorders (Anon, 1948) A Preliminary study was made to observe the effect of the above medicinal plant extract on puberty in immature female rats and the results are reported in this paper.

MATERIALS AND METHODS

Mature fresh green leaves of Aloe barbadensis and whole plant of Aristolohia bracteata were collected, shadow dried, powdered and stored at room temperature. The powdered plant material was exhaustively extracted with 70 percent ethanol, concentrated under reduced pressure to a semisolid mass and was made free from solvent. The extract material was stored at 4°C in closed container until use. Extracts of both the plants Aloe barbadensis (ALE) and

Aristolohia bracteata (ARE) were suspended in vehicle Polysorbate 80 (SRL) @ 0.1ml/g with distilled water and solutions were prepared freshly before administration. Equal volume of Polysorbate 80 was taken with distilled water and this served as vehicle for the control animals.

A total number of 24 immature female albino rats (IVRI 2 CQ strain) of 22 days old were procured from Laboratory Animal Resource section, IVRI and acclimatized to laboratory conditions. The animals were kept in polypropylene cage and maintained on balanced ration, provided by Feed Technology Unit, IVRI. The feed @ 15-20gm/rat/day in addition to milk @ 10ml/rat/day was given and all rats had free access to clean drinking water.

The rats were divided into three groups of 8 each. Group I served as control and received only the vehicle. Groups II and III received ALE and ARE respectively at the dose rate of 500mg/kg b.wt. daily from 26 to 35 days of age. The animals were observed for onset of puberty (day of vaginal opening) twice daily from day 36 onwards till all the animals attained puberty. Weight of each animal was

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observed once in five days and on the day of vaginal opening, data recorded and analysed statistically.

RESULTS AND DISCUSSION

In the rat, vagina opens between 35 and 90 days of age (Bivin, 1980) and varies greatly with the strain and rate of growth (Bennett and Vickery, 1970) and environmental factors (Bronson and Rissman, 1986). In the present study the onset of puberty observed at 63.25 ± 2.0 days in the ALE followed by 64.125 ± 3.80 days in ARE treated groups and 73.125 ± 2.48 days in the control

group. Both the treated groups attained puberty significantly earlier than the control ($P < 0.05$) and there was no significant difference between the treated groups. The body weight at the time of vaginal opening was higher in treated groups (GII 90.25 ± 4.23 , GIII 101.25 ± 5.95) than in control group GI (85.75 ± 8.31). However the difference observed between treated and control groups was not significant.

The results of the above study indicate that both ALE and ARE have effect on the early onset of puberty and on the increase in body weight in rats.

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IN VITRO SENSITIVITY PATTERN OF BACTERIA ISOLATED FROM UTERINE SECRETIONS OF REPEAT BREEDING COWS.

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The beneficial effect of antibiotic therapy after in-vitro sensitivity tests in repeat breeding cows has been reported by various workers (Kharade et al., 1983; Rajeev Krishnan et al., 1984; Das et al., 1996 and Gupta et al., 1997). This therapy gains more significance in field where the level of management is not generally optimum and therefore have higher incidence of reproductive tract infections leading to failure of conception or early embryonic death. Hence the present work was designed to study in vitro antibiotic sensitivity pattern of the various bacterial isolates obtained from uterine secretions of repeat breeding cows.

MATERIALS AND METHODS

Seventyfive uterine secretion samples of repeat breeding cows were collected aseptically with the help of sterilised glass pipettes during oestrus period. All these samples were

inoculated on Nutrient agar, MacConkey's agar and Blood agar plates for cultural examination Crickshank et al. 1974. In all 89 isolates of bacterial nature were obtained (Seh et al., 1999 a, b) out of which sixty were subjected to antibiotic sensitivity testing using disc diffusion method as per Bauer et al. (1966). Seven antimicrobial discs were used namely gentamicin (10 mcg) ciprofloxacin (5 mcg), norfloxacin (10 mcg), chloramphenicol (30 mcg), nitrofurantoin (300 mcg), chlortetracycline (30 mcg), erythromycin (15 mcg).

RESULTS AND DISCUSSION

The organisms were isolated from 73 (97.7%) out of 75 samples, while the remaining 2 (3%) samples were bacteriologically sterile. The various bacterial isolates have been described (Seh et al., 1999 a, b). All the isolates were found sensitive to various antimicrobials. The sensitivity pattern of the isolates revealed that 98.48% organisms were sensitive to gentamycin, 92.42% to ciprofloxacin and chloramphenicol, 71.21% to norfloxacin, 59.09% and 56.06% to chlortetracycline and nitrofurantoin respectively. Erythromycin was the only antibiotic which showed least effectiveness (3.03%).

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Very few reports are available in the literature regarding maximum sensitivity of uterine cultures to ciprofloxacin (Das et al., 1996 and Baishya et al., 1998). One isolate of proteus spp. showed simultaneous resistance to all antimicrobials except ciprofloxacin, whereas two strains of staphylococcus showed total resistance to gentamicin. A total of 13 isolates showed simultaneous resistance to 2 or more antimicrobials.

The high sensitivity pattern of

the isolates to gentamycin observed in the present study was in accordance with those reported by earlier workers (Sirhoi et al., 1989; Singh et al., 1995 and Gupta et al., 1997).

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HISTOMETRY OF ENDOMETRIAL GLANDS IN HEALTHY AND ENDOMETRITIS COWS*

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The uterine biopsy examination was found to be useful to study the cyclic changes in the uterus and to confirm the clinical cases of endometritis (Dawson 1977). Knowledge on the status of endometrial glands during gynaecological disorder is essential for diagnosis of pathological alterations.

The present study was undertaken to record morphometric studies of endometrium in cows affected with endometritis and healthy cows.

MATERIALS AND METHODS

One hundred and fifty two endometrial biopsies were collected from cows affected with endometritis by using Albuchin's Uterine biopsy catheter (Griffin et al., 1974).

Endometrial biopsies were fixed in Bouin's fluid and processed to obtain 5 to 6 microns thick paraffin sections. Sections stained with Ehrlich's haematoxylin and Eosin were

subjected to histometrical observation. The diameter of the gland and height of glandular epithelium were measured and expressed in microns. The data was subjected to statistical analysis as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The mean values of glandular cell height in healthy cows were 11.14 ± 0.21 microns where as they were 8.42 ± 0.53 microns in endometritis cows. The mean diameter of endometrial glands was 53.42 ± 0.63 microns in healthy cows and 44.46 ± 1.34 microns in cows affected with endometritis. Statistical analysis revealed that glandular cell height and diameter of endometrial glands in healthy cows differed significantly ($P < 0.01$) from that of cows affected with endometritis.

The mean glandular cell height and diameter of endometrial glands were low in endometritis cows when compared to that of healthy cows. Gonzalez et. al. (1985) reported that diameter of endometrial glands to be 52.4 ± 17.2 microns in category 4 endometrium. The finding of the present study are in accordance with the reports of Gonzalez et. al. (1985). Singh et al. (1987) reported the

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diameter of endometrial glands to be 76.40 ± 3.16 microns in repeat breeding cows. The results revealed that there was a significant decrease in size of glandular cell height and diameter of endometrial glands in cows affected with endometritis.

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EFFECT OF PMSG ON INDUCTION OF ESTRUS IN ANESTROUS EWES

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The sexual cycles in the ewes depend more upon the season than upon the pubertal age of the young female. The conception generally occurs in the first or second year of life. In this investigation effect of PMSG has been evaluated in anoestrus ewes.

MATERIALS AND METHODS

Twenty Rahmini sheep in a farm near a village, Gize province, Egypt were used in this investigation. They were 3 years old and suffered from delayed estrus and anovulation. A fertile ram of good quality semen was left with them for natural mating purposes for the whole breeding season. None of these ewes came in estrus or became pregnant.

Single injection of 500. I.U. PMSG (Gestyl : 80% FSH and 20% LH; Organon, Egypt) was given Intramuscularly to 15 ewes during non-breeding season. Five ewes were

given normal saline solution, served as control group.

RESULTS AND DISCUSSION

The administration of PMSG during non-breeding season resulted in appearance of estrus in all experimental ewes - within observation period of one month. While the other five control ewes failed to exhibit any estrus manifestations. The natural mating was done by a fertile ram. The conception rate was 86.7% and lambing was 79.9%

The anoestrus is uncommon in ewes except in unthrifty animals (Arthur et al 1986). However physiological anoestrus is observed in non-breeding season. The PMSG has been successfully used for synchronization of estrus in ewes (Hafez, 1987). This investigation adds the use of PMSG for successful conception and lambing in anoestrus ewes.

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A NOTE ON EFFECT OF SEASON ON OESTRUS AND CONCEPTION IN ONGOLE COWS

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Effect of season on fertility was reported in Cattle, Higher fertility rates were reported in Zebu Cattle during summer in the countries near to equator (Ian Gordon 1996). The present study was undertaken to know the effect of season on incidence of oestrus and conception rate in Ongole Cows under village conditions.

MATERIALS AND METHODS

A total of 517 Ongole cows and heifers maintained by small and marginal farmers under traditional system in the villages were artificially inseminated with frozen semen under field associate herd scheme during 1994 - 98 constitutes the material for this study. The pregnancy was confirmed by rectal palpation on 60th day post - insemination. The year was divided into three seasons viz., summer (March - June), Rainy (July - October). Winter (Nov. - Feb.). The data was analysed as per Snedecor and Cochran (1967), to know the effect of season on incidence of oestrus and conception rates (%).

RESULTS AND DISCUSSION

From the present study it was observed that the incidence of oestrus in Ongole cows was higher in summer (48%) as compared to rainy (23%) and winter (28%) seasons. The seasonal difference with regard to incidence of oestrus was found to be highly significant $P < 0.001$ but the same was nonsignificant between years. The higher incidence of oestrus in summer might be due to increased day light, temperature (Hafez, 1980) and existence of seasonal mechanism for ovulation in cattle (Ian Gordon, 1996).

The effect of season on conception rate in summer, rainy and winter seasons were 58.4%, 52.4% and 53.1% respectively. Analysis of data revealed that, the conception rate did not significantly vary from season to season. This is in agreement with the observations of Lakshmi Narayana and Rao (1983) in non-descript cattle.

It is concluded that season has got highly significant effect on the incidence of oestrus and had no effect on conception rates in Ongole cows.

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SERUM PROGESTERONE LEVEL IN REPEAT BREEDING COWS.

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Luteal disfunction leading to inadequate progesterone production during early luteal phase of the cycle could be a cause of early embryonic mortality. In the present study serum progesterone estimation and ovarian changes as determined by per rectal examination at different intervals were done to assess the cause for repeat breeding.

MATERIALS AND METHODS

The experiment was conducted at the military dairy farm, Bareilly, and A.I. Centre, IVRI Izatnagar. The cross-bred cows were in the age group of 3 to 9 years and in between first and sixth lactation. Blood samples (10ml) were collected and simultaneously rectal examination was carried out in 8 repeat breeder cattle on the day of estrum (day '0') and on 10th, 20th and 30th day post estrum. The serum was separated and stored in sterilized vials at 20°C until assayed for progesterone by ELISA

(Joyce et al., 1978 and Dhoble et al., 1986).

RESULTS AND DISCUSSION

Mean progesterone concentration on 0, 10th, 20th and 30th days were 1.98 ± 0.47 ; 2.43 ± 0.75 ; 1.97 ± 0.59 and 1.85 ± 0.78 ng/ml of serum respectively, in repeat breeding cows.

Critical rectal examination of ovarian changes in 8 repeat breeding cows during estrous cycle revealed 2(25%) cows with ovulatory heat and normal cycle length, 3(37.5%) cows with anovulatory heat and normal cycle length and the remaining 3(37.5%) cows with ovulatory heat and prolonged estrous cycle of 32-37 days in interval. Kimura et al., (1987) reported that only 38% repeat breeding cows had normal progesterone profile and 62% had an abnormal profile during luteal phase of the cycle indicating luteal phase disfunction in repeat breeding cows.

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A 61, XXY CHROMOSOME COMPLEMENT IN SUBFERTILE JERSEY CROSSBRED BULL

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Cytogenetic studies in animals as well as humans have identified chromosomal abnormalities as one of the reasons contributing to reduced fertility and infertility. The commonly found X-chromosome anomalies in domestic species are aneuploidy, trisomy (XXX) and monosomy (XO), mosaicism and chimerism. Non disjunction of the x-chromosome during meiosis can result in XO, XXX or XXY zygotes. However, in cattle, the incidence of trisomy - X is very rare (Pinheiro et al. 1987), this paper describes the occurrence of XXY, which appears to be first case in a crossbred bull.

MATERIALS AND METHODS

Heparinised blood was collected from a 22 months old Jersey and Sindhi crossbred bull and chromosomal smears were prepared using short term lymphocyte culture as described earlier (Patel et al. 1997). Giemsa stained 50 metaphase fields were screened under light microscope to detect possible chromosomal abnormalities.

RESULTS AND DISCUSSION

The normal somatic chromosome number of the cattle is 60 ($2n = 60$). All autosomes are

acrocentric and sex chromosomes (X and Y) are submetacentric in exotic cattle (*Bos taurus*). However Y chromosome is acrocentric in indigenous cattle (*Bos indicus*). The sex chromosomes were submetacentric in the crossbred bull (*Bos taurus* x *Bos indicus*). It was possible to identify x chromosomes even without G-banding because of their submetacentric appearance. All scored metaphase cells of the bull, exhibited 61, XXY because of an additional X-chromosome. The bull had normal phenotypes and normal testis size and scrotal circumference was 28 cms. The bull was giving semen of poor quality with 90% dead sperms.

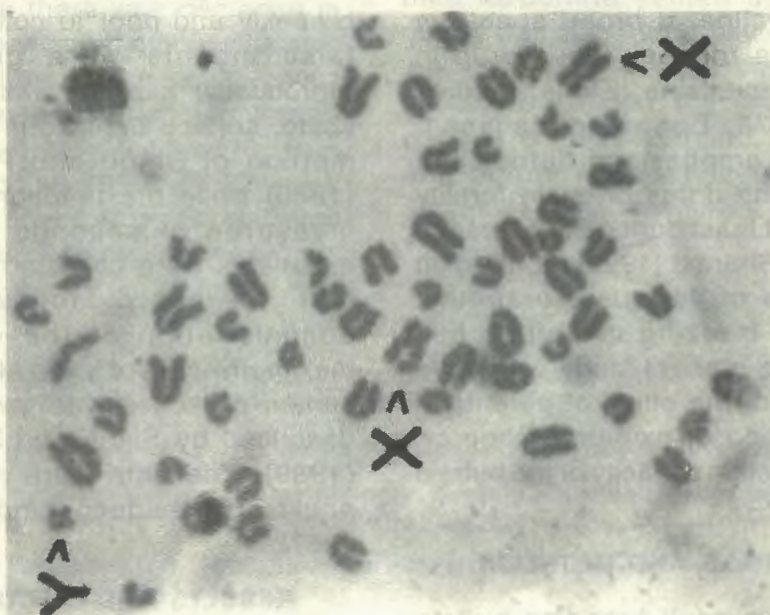
One case of trisomy - X (51, XXX) in a 10 year old river buffalo which calved twice but failed to conceive subsequently, was reported by Yadav and Balakrishna (1982). Similar case was also reported by Rieck et al (1970) in German simmental cattle (61, XXX) which gave birth to a male calf with a normal Karyotype (60, XY). An examination of these cases indicates that the phenotypes and reproductive status of trisomy - X cases are variable. Most of the trisomy - X (61, XXX) cases were reported in females except one

(XXY) reported by Dunn et al (1977) in a bull with testicular hypoplasia. However this case of XXY had no testicular hypoplasia but had poor quality of semen because of more dead sperms. Human cytogenetic surveys have revealed that the frequency of trisomy - X is considerably higher among the mentally retarded and among psychiatric patients than in the general population (Kidd et al 1963). Considering the various types of

chromosomal abnormalities reported in human studies, it is difficult to believe that livestock population are comparatively free of these abnormalities.

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EFFECT OF OXYTETRACYCLINE ON THE REPRODUCTIVE CAPACITY OF BUFFALO BULLS

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Antibiotics are regularly and effectively employed for the treatment of various bacterial infections and inflammatory conditions of the breeding bulls (Abbitt et al., 1984). Oxytetracycline, a broad spectrum antibiotic is routinely used drug in veterinary medicine. Earlier studies (Taylor 1975, Egan and Hammad, 1976) has emphasized detrimental effects of the drugs on male fertility potential, at least during the course of treatment. Sharma (1992) reported on the course of various therapeutic agents on freezing of buffalo bull semen. The present study was done to find out of the effect of parenteral administration of oxytetracycline on the reproduction capacity of the buffalo bulls.

MATERIALS AND METHODS

Four adult breeding buffalo bulls stationed at Punjab Agricultural University's Dairy Farm, were used for this study. Andrological examination of the bulls was conducted before the start of experiment and the bulls having the similar physical traits and reproductive capacity and spermograms were selected for this study. Before giving any treatment three pretreatment ejaculates were taken. Oxytetracycline (Wockhardt

Ltd. Mumbai) was administered @ 5 mg./kg body weight intramuscularly on both sides of the neck for 7 days consecutively. Semen was collected by artificial vagina method from each bull biweekly and prior to collection, 2-3 false mounts were given. The reproductive capacity was studied by libido, service behaviour as per the method of Singh and Pangawkar (1989) while the reaction time was measured in seconds. Eighteen ejaculates were collected from each bull biweekly following the administration of the drug. The maximum score of libido at the time of semen collection was given four as described by Singh and Pangawkar (1989). The data were statistically analysed (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The mean libido score was expressed in percentage and was 83.3 ± 3.55 percent in the bulls in the pretreatment samples, which was markedly decreased to 56.3 ± 6.25 in the first ejaculate after oxytetracycline treatment. The libido score was significantly reduced in 1st and 11nd ejaculate (7 days) and then the value returned towards pretreatment side.

Similar to the present results, Abbitt et al (1984) have also reported a significant decrease in the libido after the parenteral administration of dihydrostreptomycin and oxytetracycline in bulls. The mean score of service behaviour before administration of oxytetracycline was 55.8 ± 1.92 percent. The value decreased significantly ($P < 0.1$) and were 42.3 ± 2.23 percent after drug administration and there was significant decrease upto 4th (14 days) ejaculate. As there was decrease in libido, the service behaviour was also affected and there was simultaneous decrease in its value. The mean reaction time before drug administration was 59.8 ± 1.62 which increased to 86 ± 1.44 on 1st ejaculate and there was significant increase upto

7th ejaculate (24 days). This indicates that the bulls have taken more time to donate semen. The reason for increase in reaction time may be due to adverse effects of oxytetracycline and decrease in the level of blood testosterone (Vijaya et al, 1995).

Abbitt et al (1984) have reported that after dihydrostreptomycin and oxytetracycline administration, the penile erection was inhibited and bulls have taken more time to donate semen and semen quality was poor. Since oxytetracycline inhibited penile erection, this antibiotic should be avoided, if possible during periods of peak demand for semen and before conducting a breeding soundness examination.

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DISPOSAL PATTERN OF BREEDING BULLS IN KERALA.

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The present study was undertaken to assess the disposal pattern of breeding bulls maintained in the frozen semen production stations of Kerala Livestock Development Board.

MATERIALS AND METHODS

The data pertaining to the study was collected from the three bull stations - Matupatti, Dhoni and Kulathupuzha over a period of five years from 1993 to 1997. The individual bull's production particulars were scrutinised and the major reasons for the disposal of the breeding sires were analysed.

RESULTS AND DISCUSSION

Altogether 469 bulls were disposed from the three frozen semen stations during the five years; Matupatti 122 bulls (26.01%), Dhoni 188 bulls (40.09%) and Kulathupuzha 159 bulls (33.90%).

It was observed that many of the bulls were culled for more than one reasons. In Matupatti station 40.16 per cent of the bulls were disposed due to poor libido, 34.43 per cent on account of poor semen quality, 17.21 percent for having attained the stipulated production target and 14.75 per cent due to poor semen freezability; as against 35.64 per cent, 50.0 percent, 10.11 per cent and 17.02 percent respectively at the Dhoni

station. At Kulathupuzha station 48.43 per cent bulls were disposed due to poor semen quality, 34.59 on account of poor libido and 16.35 per cent for having attained the production target.

Thus the four major attributes for the disposal of bulls were poor semen quality (45.42%), poor libido (36.46%), attainment of production target (14.07%) and poor semen freezability (10.87%). In addition to poor semen quality and poor libido Kotayya and Narasimha (1981), Narasimha Rao (1984) and Rao et. al., (1993) reported that sizeable number of bulls are being disposed off on account of disease too. In the present study disposal due to diseases was only 4.26 per cent, probably because of the sound management practises being followed in the semen production stations.

Disposals due to poor body confirmation, poor dam's yield, old age, vicious temperament of bull, rupture of nasal septa, accidents, sperm abnormalities, hypoplasia of testicles, death and miscellaneous reasons constitute 7.03, 2.98, 4.26, 1.92, 1.92, 0.64, 1.07, 1.71, 0.85 and 1.91 percent respectively. Similar observations were also made by Kotayya and Narasimha Rao (1981) and Narasimha Rao (1984) in related studies.

Agewar distribution of disposed bulls showed that 48.19 per cent of the culled bulls were below 30 months, 25.16 percent between 30 to 60 months and 26.65 per cent above 60 months of age. The mean age at disposal was 36.85 ± 19.30 , 40.66 ± 23.77 and 51.11 ± 31.64 months for Matupatti, Dhoni and Kulathupuzha stations respectively.

Poor semen quality (54.87%) and poor libido (50.0%) were the major reasons for disposal in animals below 30 months. The results of the study agreed with the findings of Bhosrekar (1988), Mathews (1988) and Sasikumar (1993). Poor semen quality, poor libido, poor semen freezability and attainment of production target constitute 43.39, 35.60, 10.77 and 2.54 per cent respectively of the disposal for bulls aging between 30 to 60 months. Maximum number of bulls (40.0%) above 60 months of age were disposed off for having attained the production target.

A decline in disposal rate due to poor semen quality and libido was observed in the study as age advanced (54.87% at < 30 months; 43.39% at 30-60 months; and 16.00% at > 60 months). Similar observations of improvement in the quality of semen with advancement of age in bulls were reported (Rao and Rao, 1978; Raja and Rao, 1983 and Singh Laishram and Patel, 1996). However an increase in reaction time was generally experienced in breeding sires owing to the multiplicity of collection frequencies.

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BRUCELLA ABORTUS IN FROZEN SEMEN FROM SERONEGATIVE BULLS

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Modern trends in reproduction like artificial Insemination have created potential problems in the mode of transmission of disease like brucellosis. Earlier reports suggested that the bull played a less significant role in the spread of brucellosis. But A.I. has brought renewed interest that there is a possible chance of transmission by one infected bull semen to many animals. (Bendixen and Blom, 1947). Intrauterine insemination with semen containing virulent *Brucella abortus* organisms produced a high percentage of infection, but midcervical insemination did not produce infection (Lambert et al., 1963). There are also reports that some bulls give negative results in seroagglutination test but actually sheds virulent *Br. abortus* in their semen (Christensen, 1948). An attempt was made to isolate the organism in frozen semen from seronegative bulls.

MATERIALS AND METHODS

A total of 146 frozen semen samples were collected from three frozen semen production centres in Tamil Nadu and transported in liquid nitrogen containers. All these samples were collected from bulls which showed negative seroagglutination

results in periodical testing for brucellosis. These frozen semen samples were inoculated in Tryptose broth (Himedia) and incubated overnight in anaerobic jar at 37°C. Subsequently they were streaked onto tryptose agar plates (Himedia) and incubated in anaerobic condition with 5% CO_2 at 37°C for 7 days. Smears were made from individual colonies and subjected to Grams staining. Biochemical tests were carried out as per the manual of determinative bacteriology by (Buchanan and Gibbons 1974). Animal pathogenecity test was conducted in guinea pigs by subcutaneous inoculation of 0.5 ml of overnight broth culture.

Slide agglutination test was also carried out with *Brucella abortus* antiserum obtained from IVP, (Ranipet), by mixing equal quantities of saline suspension of the organism and serum.

RESULTS AND DISCUSSION

Five isolates of *Brucella* species was isolated from 146 frozen semen samples. Typical granular colonies were noticed in Tryptose agar. Individual colonies on Gram's staining of the smears revealed Gram negative coccobacilli. Based on the

biochemical tests it was identified as *Br. abortus*.

Slide agglutination tests of two isolates showed coarse granular agglutination and the remaining three showed fine granular agglutination within 2 minutes.

In animal pathogenicity test typical orchitis developed within 14 days which was first observed on the right testis followed on the left testis later on. In the sacrificed guinea pigs *Br. abortus* was recovered from the testis. The serum from guinea pigs was tested by slide and tube agglutination tests which proved to be positive for *Brucella*. The antibody titre (IU/ml) varied from 80-160.

The occurrence of *Br. abortus* in bull semen has been reported earlier by Bendixen and Blom (1947a) and Deka et al., (1982). Transmission of *Brucella* by artificial insemination was documented by Bendixen and Blom (1947a). This result concurs with the finding of Christensen (1948) that some bulls give negative results in sero agglutination test but actually shed virulent *Br. abortus* in semen.

The presence of *Br. abortus* in genital organs seems to have an undesirable effect on semen quality and A.I. of such semen samples constitute a health hazard in the spread of brucellosis

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CAESAREAN SECTION IN A DROMEDARY CAMEL A CASE REPORT

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The incidence of dystocia in dromedary camels is known to be as low as 4.6% (Tibary and Anoussai 1997). Under Indian conditions the incidence, although not reported is considered to be still lower in camels reared under their natural habitat. The various reasons of dystocia for which caesarean section is indicated in the camel include narrow maternal pelvis (Sharma and Pareek 1970), uterine torsion (Elias 1991, Nigam et al 1977, Petris 1956), fetal maceration (Gahlot et al 1983) and fetal postnatal abnormalities (Gera and Datt 1981). A case of dystocia due to lateral deviation of the head with fetal emphysema is reported.

Case History :- A female camel in its second parity was presented to the clinic one day after the onset of dystocia. The animal was in good general condition. The fore limbs of the fetus were projecting out of the birth canal. On examination it was found that there was a lateral deviation of the head with fetal emphysema. After infusion of 5 litres of sterile liquid paraffin into the birth canal the head deviation was corrected by manipulation, but the fetus could not be removed by traction. Attempts to reduce the fetal size by partial fetotomy of the head and limbs followed by traction also failed to expell

the fetus, due to light contracture of uterine muscles. Caesarean section was hence performed using the left paralumbar fossa approach.

Surgical Procedure:- The animal was secured in a sitting position and sedated with 8cc of Inj. Xylazine (Xylocad, Cadila) given intravenously. Preoperatively 4 litres of dextrose saline, multivitamins and dexamethasone (80 mg) was administered I/V. The fluid therapy with dextrose saline was continued till 2 hours postoperatively. The surgical site was prepared by shaving and scrubbing a 50 cm wide triangular area on the left paralumber fossa. The incision site was infiltrated with 60cc of 2% local anaesthetic. A 35-40 cm skin incision was made on the left paralumbar fossa about 4 cm below the second lumbar transversè process and parallel to the last rib extending through the skin and muscle. The first muscular layer (external abdominal oblique muscle) was incised followed by separation of the second and third abdominal muscles (internal abdominal oblique muscle and the transverse abdominal muscle). The retroperitoneal adipose tissue was grasped with tissue forceps and pulled towards the surgical site. A small incision was made with blunt dissecting scissors to uncover the

peritoneum. Blunt dissection of the peritoneum was done carefully making sure not to cut the underlying spleen. The uterus was grasped and brought to the surgical site and a 30 cm incision was made on the larger curvature. The fetus was expelled and the uterus was lavaged with 5 litres of sterile normal saline. 4 gms of Strepto-penicillin was sprinkled in the uterine horn and the uterus was sutured with continuous lembert suture using double layers of chromic catgut No. 2. The peritoneum, muscular layers and subcutaneous tissues were closed separately with simple continuous sutures using double layers of chromic catgut No. 2. The skin incision was closed by interrupted mattress sutures using silk No. 4. A clean cloth was tied around the belly at the incision site.

Post Operative care:-

The incision site was cleaned daily. Fluid therapy with 6-8 litres of Dextrose normal saline was continued for another 3 days. Four gms of Ampiclox 8 hourly daily was administered for 5 days. 30 ml of Inj. Diclofenac sodium was given at 12 hourly intervals for 5 days. Inj. Belamyl 20 ml daily for 3 days was also administered. The animal was given laxative diet for 3 days. The animal started eating normally from the second day post operative and was discharged on the 5th day. A slight swelling developed on the 5th day just below the incision site, but there was uneventful recovery and the skin sutures were removed 20 days post operative. The left paralumbar fossa was a good site for caesarean operation in the camel but due care should be exercised while cutting the peritoneum and avoid injuries to the spleen.

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**MULTIPLE CERVICAL TUMOURS AS THE CAUSE FOR RECURRENT VAGINO-CERVICAL PROLAPSE IN A CROSS-BRED COW
A CASE REPORT**

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Cervical tumours in cattle are very rare (Kohli and Bishnoi, 1980). Robers (1971) has described fibroma, fibrosarcoma, leiomyoma and carcinoma of cervix. A multiple pedunculated tumours of cervix in a cross-bred cow, which was treated successfully is reported here.

Case History and Treatment : A Jersey-Sindhi cross-bred cow aged about 8 years, in third parity, having a previous history of recurrent vagino-cervical prolapse occurring mostly during oestrus, was presented to the polyclinic with the prolapse of vagina and cervix. Gynaeco-clinical examination of the animal revealed multiple tumorous growths attached with three separate fleshy stalks (measuring 8-12 cm in length) to the ventral and lateral aspects of cervix. Epidural anaesthesia was induced by injecting 10 ml of 2% lignocaine hydrochloride. After cleaning properly the prolapsed organs, all the tumorous growths were excised from the cervix, and the haemorrhage was controlled by ligating the blood vessels. The cut edges of the cervix were approximated by simple interrupted sutures using chromic cat gut of no. 1

size. The prolapsed organs were replaced, and 5% povidone solution was sprayed on the cervix and the vagina. Injection oxytetracycline dihydrate /L.A. (Pfizer Ltd.) 30 ml was given intramuscularly and it was repeated after 3 days. The animal made an uneventful recovery.

The animal was reported in oestrus but eversion of genitalia was not observed. Insemination was deferred at this heat and luprostiol, a PGF₂ analogue (Prosolvlin, Intervet International B.V. Boxmeer, Holland) 2 ml was injected intramuscularly 10 days after estrus. Fixed time inseminations at 72 and 96 hours after Prosolvin injection were carried out which resulted in conception as confirmed by rectal palpation after 60 days of insemination.

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A CASE REPORT OF NEBOTHIAN CYST IN A CROSSBRED COW

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Nebothian cyst or Cervical Cyst were rare in occurrence in cattle. These cyst were observed usually on the external os and they were of thin walled and covered only by mucus membrane. (Roberts 1971; Laing et al, 1988). In this article the management of a case of Nebothian cyst has been reported.

Case History and Treatment : A well maintained cross bred cow was brought to the infertility clinic with a history that not settling for pregnancy in spite of repeated insemination and the last insemination was done five months back. The animal was subjected to routine gynaecological examination to ascertain the soundness of the reproductive organ.

The rectal examination revealed a doughy mass about four inches in diameter over the left lateral wall of the cervix. An attempt was made to retract the mass towards the vulval lips after administering epidural anesthetic of one ml. of Xylazine to restrain the animal. Since the animal

was a parous one with pendulous genitalia the mass was easily brought to the level of vulval lips. A 16G needle was inserted through the vaginal wall into the mass. A thick vicid fluid flowed through the needle. Then a small nick was made at the punctured site to clear the vicid fluid material. The vicid fluid material was collected in a sterilized tube to ascertain the nature of the fluid. It was confirmed that the material was not pus since it did not revealed any pus cells. Hence mass was considered to be retention cyst that developed due to the occlusion of the duct of cervical glands as observed by Laing et al, 1988.

Povidone iodine was infused through the surgical wound. The animal was further administered with Ampiciline 2.0 gm. per day for three consecutive days. The animal was checked after fifteen days and there was no recurrence of the condition and hence was advised to breed the animal in the next oestrus period.

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UTERINE PROLAPSE IN SHEEP AND ITS MANAGEMENT

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In small ruminants uterine prolapse following normal delivery or dystocia is not frequently encountered (Dhaliwal et al. 1989). The present case of bicornual prolapse after manipulative delivery is documented.

History : An ewe was presented to the department clinic with the history of assisted delivery of a lamb and bicornual uterine prolapse there after. On examination the prolapsed uterine mass was soiled, inflamed and lacerated with edematous maternal caruncles (Fig).

Treatment : The animal was given epidural anaesthesia using 4 ml of 2% lignocaine hydrochloride. The prolapsed uterus was cleaned with 2% Potassium permanganate and the lacerations were sutured with cat gut no. 1.0. The prolapsed mass was then lubricated and repositioned properly (Roberts, 1986). Retention of prolapsed part was achieved by applying purse string suturing to the vulvular lips which was removed after seven days. The animal was given Oxytetracycline 500 mg i/m for five

days. The other drugs given were Novalgine 5ml i/m for three days, 60 mg Dexona i/m, Normal Saline 1 litre and Calcium borogluconate 250 ml intravenously on the first day. The ewe had an uneventful recovery and conceived normally and delivered a live lamb in the next breeding season.

The relaxed ligament along with tenesmus and uterine atonicity seemed to be the predisposing factors for the occurrence bicornual of uterine prolapse in the present case.



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DISCEPHALUS - BIATLANTICUS MONSTER IN A BUFFALO.

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Congenital defects are defined as abnormalities of structure or function that are present at birth. They may affect a single anatomical structure or function, an entire system, parts of several systems or both a structure and a function (Morrow 1980). Reporting of such incidence will provide information on the frequency of various defects. The present paper is to keep it on record an incidence of Dicephalus monster in a non descript buffalo - cow.

This monster was encountered from 3 months gravid uterus of a non descript buffalo cow, during the routine survey of an abattoir to study the incidence of various reproductive disorders in buffalo cows.

On examination, the monster found to have two normal heads with two atlases and were free from attachment. The caudal portions of the two atlases were fused and continued with single second cervical vertebrae (Axis). The remaining portion of the body was apparently normal. All the thoracic, pelvic and abdominal organs were of a single fetus (Fig).

Adsul et al (1992) and Saleem et al (1996) reported duplication of both head and neck in Nagpuri and non descript buffaloes, respectively. But, similar type of monster described in this study was reported in a Murrah buffalo at birth by Bishnoi et al (1992).



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UTERUS UNICORNIS - A CASE REPORT

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Most of the developmental defects in the tubular portions of the bovine reproductive tract are probably hereditary in origin although some cases may be congenital. Literature emphasises the hereditary nature of these defects and the danger of their spread by bulls used for artificial insemination (Roberts 1971).

Segemental aplasia involving the uterine horns is not uncommon and if only one horn is involved, the condition is called uterus unicornis. These animals are usually infertile with prolonged

intervals between oestrous periods and repeated services per conception

A cross-bred Holstein Friesian heifer aged three years was presented in an infertility camp with the history of failure of conception even after repeated inseminations. On detailed clinic - gynaecological examination, the right uterine horn was found to be missing. All other segments of the reproductive tract were found to be normal. Later the animal was sacrificed, the reproductive tract was subjected to detailed examination and the condition was confirmed as uterus unicornis. (Fig.)

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CONJOINED TWIN MONSTROSITY IN A COW - A CASE REPORT

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Conjoined twin monster arises from a single ovum and is monozygotic. The occurrence of such monstrosity is (Roberts 1971). A case of a "Dicephalus dipus tetrabrachius," conjoined twin monster is reported.

Case History :- A non-descript cow aged 6 years belonging to village Mehtipur, Dt. Bareilly with full term second pregnancy was presented at the Polyclinic of the Institute. The cow was straining for six hours after the rupture of waterbag but no part of the fetus came out. On examination the case at that stage was diagnosed as dystocia due to breech presentation of fetus.



Correction :- The animal was restrained properly and epidural anaesthesia was induced with 6 ml of 2% xylocaine hydrochloride. After proper lubrication the fetus was taken out successfully applying mutational operation and judicious traction.

Description of the Monster:- The fetus was a female conjoined twin monster having thin hair, without signs of putrefaction. The monster was fully developed having duplication in the cranial region which resulted into two heads (Dicephalus) (Fig.) Eyes and ears were normal. The two bodies joined with each other in a face to face position starting from sternal region (Thoracopagus) and had four forelimbs (tetrabrachius). The monster had two hind limbs and two tails (dicaudatus). This was a rare case of Dicephalus dipus tetrabrachius conjoined twin monster as per the classification of Roberts (1971).

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MULTIPLE CONGENITAL ANOMALIES IN A KID

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A non descript 2 years old goat was presented to clinic with a history of dystocia from last 12 hours. The history revealed that the goat had earlier two normal kiddings. Gynaecological examination revealed the presence of a dead fetus which was in anterior presentation. The head and forelimbs were lying in the vaginal passage. Following epidural anesthesia with 2% Lignocaine hydrochloride and lubrication of the birth canal a dead and malformed kid was taken out. On careful observation it was found that the kid was showing multiple malformations like

brachygnathia, arthrogryposis and Kyphosis (Fig.)

Arthrogryposis is a deformity of the limb characterised by curvature or retention of a joint in a flexed or extended position (Roberts, 1982). These multiple anomalies could be due to inheritance/ in utero intoxications or in utero infections of the embryo and manganese deficiency. The vertebral malformations occasionally occur without any grossly apparent deviation in the vertebral column or compromise of the vertebral canal (Drew and Hexander, 1985).



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