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Treatment of True Anoestrus in the bovine with Progestagen and Oestrogen

1. Initiation of ovarian cyclicity and fertility in anoestrous buffaloes following artificial insemination at the induced oestrus

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ABSTRACT

The efficacy of a 14 day progestogen (melengestrol acetate, MGA) treatment followed by a single I/M injection of oestradiol benzoate (OB) was evaluated for the initiation of ovarian cyclicity in parous, acyclic buffaloes. Treatment with MGA was less effective than additional treatment with OB in the induction of oestrus (56.0% vs 85.1%, $p<0.05$) and ovulation (42.8% vs 77.5%, $p<0.02$). Incidence of standing oestrus (42.8% vs 72.5%) as well as pregnancy rate at induced oestrus (7.1% vs 35.0%) were also lower ($p<0.001$ and <0.10, respectively) with MGA treatment. The ovarian cyclicity was initiated in 11.4%, 16.0% and 62.7% of control, MGA and MGA+OB treated animals, respectively; the incidence was higher ($p<0.001$)in MGA+OB treated animals in comparison to control and MGA treated animals. These results indicate that there are possibilities of inducing ovarian cyclicity in functionally anoestrous buffaloes with a combined progestagen + oestrogen therapy.

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True anoestrus is one of the major factors which affects reproductive efficiency of cattle and buffaloes in most tropical countries. Oestrus and ovulation can be induced in cyclic cows and buffaloes employing progestagens (Kordts *et al.*, 1974. Willemse *et al.*, 1982; Rao *et al.*, 1987) and progestagens and oestrogens (Ulberg and Lindlay, 1960; Foote and Hunter, 1964; Britt *et al.*, 1974). However, little systematic work on hormonal therapy for true anoestrus has been done in tropical regions of the world. This paper describes the results of a study which was done to evaluate the effectiveness of progestagen and oestrogen therapy in inducing ovarian cyclicity in anoestrous buffaloes.

MATERIALS AND METHODS

Animals and Treatment

Three trials were conducted in parous anoestrous Murrah-type buffaloes confirmed to be functionally anoestrus by the absence of a palpable corpus luteum (CL) associated with a toneless uterus on three consecutive examination of genital organs at 10 days interval.

The animals were housed in shaded half-wall sheds and were fed a ration of concentrate mixture (fortified with mineral mixture and salt), greens and roughages adequate to meet their mutrient reguirements for maintenance and/or production (Ranjhan, 1977). Water was available free choice.

In all trials, synthetic progestagen melengestrol acetate (MGA) (Upjohn) and oestradiol benzoate (OB) (Intervet, Holland) were employed. Melengestrol acetate was mixed with the concentrate mixture and was given orally at 0900 hr for 14 days. Forty

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eight hours after the last day of MGA feeding animals were given a single intramuscular injection of OB at the dose(s) schedule specified in each trial.

Trial 1 - Forty six, parous, acyclic, dry buffaloes were alloted randomly to five groups : (Group I) untreated control, 8 animals; (Group II) MGA 0.5 mg, 9 animals; (Groups III) MGA 1.0 mg, 9 animals; (Group IV) MGA 0.5 mg+OB 250 ug, 10 animals; (Group V) MGA 1.0 mg+OB 250 ug, 10 animals.

Trial 2 - Twenty one, parous, acyclic, dry buffaloes were alloted randomly into three groups of seven each: (Group I) untreated control; (Group II) MGA 0.5 mg; (Group III) MGA 0.5 mg+OB 250 ug.

Trial 3 - Forty, parous acyclic lactating buffaloes were employed between 3 to 5 months after calving. They were alloted randomly into 3 groups: (Group I) untreated control, 20 animals; (Group II) MGA 0.5 mg+OB 250 ug, 10 animals; (Group III) MGA 0.5 mg+OB 250 ug, 10 animals.

Trials 1 and 3 were conducted during December - February which was normal breeding season, while trial 2 was conducted during April-May which was a low breeding season for buffaloes.

OBSERVATIONS

Oestrus was detected with the help of vasectomized bulls (at 0600, 1200, 1800 and 2400 hr), oestrual turgidity of the uterus, the presence of mature Graafian follicle in the ovary on rectal palpation, the behavioural signs and cervical mucus fern pattern and was classified as standing and non-standing (Morrow *et al.*, 1976). Ovulation was monitored by recording the disappearance of a follicle of pre-ovulatory size, the appearance of ovulation depression and the subsequent development of CL in the same ovary.

In Trial 1 and 2 animals exhibiting induced oestrus were inseminated twice (a.m./p.m. schedule) with extended liquid semen and forzen-thawed semen of fertile bulls, respectively. None of the animals were inseminated in Trial 3. Pregnancy diagnosis was carried out within 45 to 60 days after insemination.

Establishment of ovarian cyclicity was calculated as follows:

$$\frac{\text{No. conceived at induced oestrus} + \text{No. returning to service after A.I. at induced oestrus and / or showing first natural oestrus}}{\text{Total number employed}} \times 100$$

Statistical significance of differences were determined by Chi-square or Student's 't' test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There was no effect of either the dose of MGA (0.5 mg vs 1.0 mg, Trial 1) or the dose of OB (200 ug.vs 250 ug Trial 3) on the incidence of induced oestrus and ovulation. Also various other response parameters did not differ among trials. Hence, the data for three trials have been pooled and summarized in Table 1.

(a) Oestrus response and ovulation

Animals did not exhibit oestrus during MGA feeding but palpation data revealed considerable follicular growth within 48 hr after MGA withdrawal, as it is in cattle (Zimbelman and Smith, 1968). Thus MGA may have a stimulatory effect on the

follicular growth (Pant and Sharma, 1979) as does progesterone in the ewe (Harned & Casida, 1971).

The MGA+OB treatment was highly effective ($p<0.005$) than MGA along in inducing oestrus (85.1% vs 56.0%). The incidence of standing oestrus was also significantly greater ($p<0.001$) in the former than in the latter group (72.5% vs 42.8%). During the period of trials which lasted for about ten weeks only 11.4% untreated animals exhibited natural oestrus and this differed significantly ($p<0.001$) from both treatment groups. These results confirm earliar reports in post-partum cows (Ulberg and Lindley, 1960; Foote and Hunter, 1964; Britt *et al.,* 1974) and further indicate that all the induced oestrus were no 'standing! further studies are required to ascertain if the interactions between dose and duration of MGA feeding with the dose of OB and interval between MGA withdrawal and OB injection govern the incidence of standing oestrus.

(b) Incidence of ovulation

All induced oestruses were not ovulatory. However, the incidence was significantly more ($p<0.02$) in animals exhibiting oestrus after MGA+OB treatment than in those given MGA alone (77.5 vs 42.8%). Of the total animals employed the incidence of ovulation was 11.4%, 24.0% and 65.9% in the control, MGA and MGA+OB groups, respectively. The difference between the first two groups was not significant, however, the differences between control and MGA+OB and between MGA and MGA+OB were significant ($p<0.001$).

Interestingly, MGA alone induced ovulatory oestrus in the anoestrous buffaloes and this confirms earlier reports in which MGA feeding reduced days open in post-partum cows (Kordts *et al.,* 1974. Britt *et al.,* 1974). We speculate that MGA may mimic the transient increase in circulating levels of progesterone that has been observed prior to first overt oestrus during resumption of ovarian cyclicity in post-partum cows (Manns *et al.,* 1983). Also withdrawal of MGA therapy should result in higher concentration of gonadotrophins in the plasma, leading to follicular development.

In our study oestrogen administration following progestagen priming also enhanced the ovulation rate, as it is in cattle (Foote and Hunter, 1964; Pant and Sharma, 1979). Presumably a greater magnitude and / or duration of LH surge following OB injection may account for this observation.

(c) Conception rate

The conception rate at induced oesturs was low in animals receiving MGA alone than in those given OB after MGA priming (7.1% vs 35.0%, $p<0.10$) and the latter is similar to the first service conception rate following AI in normal cyclic buffaloes (Pant and Roy, 1972; Cockrill, 1974). However, the near failure of conception in MGA treated buffaloes may be due to an aberration in the dynamics of follicular growth and this needs investigation.

(d) Establishment of ovarian cyclicity

Compared to control group MGA alone was ineffective in establishing ovarian cyclicity (11.4% vs 16.0%). However, oestrogen administration in MGA primed animals established ovarian cyclicity in 62.7% animals and this differed significantly ($p<0.001$) from control as well as MGA treated animals. These results indicate that it is possible to induce ovarian cyclicity in functionally anoestrous buffaloes with a combined MGA and OB therapy. Others have shown that although progesterone alone

was effective in inducing puberty in heifers (Gonzalez Padilla *et al.,* 1975 b), however, a combined progesterone and oestrogen therapy was more effective (Gonzalez Padilla *et al.,* 1975 a).

The mean values for oestrous cycle length in animals showing overt oestrus following the regression of induced CL were similar and they also did not differ from control animals indicating that the induced CL were functionally normal. This suggestion is supported from our observation that pregnancy was maintained in animals which conceived at induced oestrus.

In conclusion, our results indicate that there are possibilities of treating functional anoestrus in the buffaloes with a conbined MGA and OB therapy. However, plasma levels of gonadotrophins and ovarian steroids should be measured at and subsequent to induced oestrus for a complete understanding of the physiological mechanism(s) involved.

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Table 1. Effect of MGA and MGA+OB therapy on oestrous response, conception rate and establishment of ovarian cyclicity in functionally anoestrous buffaloes.

Attributes	Treatments		
	Group I (Control)*	Group II (MGA)	Group III (MGA+OB)
No. of animals	35	25	47
No. in induced (%) Oesturs	4 (11.4[a])	14 (56.0[b])	40 (85.1[c])
Standing (%)	3/4 (75.0)	6/14 (42.8[d])	29/40 (72.5[e])
Non-standing (%)	1/4 (25.0)	8/14 (57.2)	11/40 (27.5)
Interval (hr) from end of treatment to onset of oestrus (Mean± S.E.)	--	49.2±4.80	43.8±4.0
Duration (hr) of induced oestrus (Mean ± S.E.)	--	25.2±1.80	24.6±1.84
No. ovulated at induced oestrus (%)	4/35 (11.4)	6/14 (42.8[f])	31/40 (77.52)
No. ovulated out of total employed (%)	4/35 (11.4[h])	6/25 (24.0[i])	31/47 (65.9[j])
No. inseminated at induced oestrus (%)	--	14	20
No. conceived at induced oestrus (%)	--	1/14 (7.1[k])	7/20 (35.0[l])
No. exhibiting first natural ovulatory oestrus (%)	4/35 (11.4)	3/14 (21.4)	22/40 (55.0)
Cycle length following natural / induced oestrus (days) (Mean ± S.E.)	21.0±0.57	21.0±1.01	19.8±0.45
Establishment of cylcicity (%)	4/35 (11.4[m])	4/25 (16.0[n])	29/47 (62.7[o])

* - In control group all data pertain to first natural oestrus

X2 a vs b = 13.8, p<0.001
a vs c = 43.8, p<0.001
b vs c = 7.1, p<0.005
d vs e = 21.7, p<0.001
f vs g = 5.9, p<0.02
h vs j = 24.3,p<0.001
i vs j = 11.4, p<0.001
k vs l = 3.6, p<0.10
m vs 0 = 21.7, p<0.001
n vs 0 = 13.8, p<0.001

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Superovulatory Response and embryo recovery in Anoestrous Hariana Cows and Heifers*

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ABSTRACT

This experiment was conducted on Hariana cows and heifers (6 in each group) which were anoestrus, by using Syncro Mate-B (Norgestomet), FSH(E) and Fertagyl (GNRH) treatment regimen. Four cows and all the heifers responded to the treatment. The mean number of corpora lutea, unovulated follicles, embryos recovered, degenerated embryos and transferable embryos for the cows were 9.75±0.48, 1.00±0.41, 4.00±0.71, 1.25±0.25 and 2.75 ±0.85, while the corresponding values for heifers were 8.83 ±0.83, 0.67 ±0.33, 2.60 ±0.68, 1.40 ±0.25 and 1.20 ±0.49, respectively. These results show that anoestrous animals can also be superovulated.

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Generally superovulation is initiated by administration of gonadotropins in mid oestrous cycle (9-14 days), when there is a well developed corpus luteam on the ovary. But, in recent years inclusion of progestational compounds in the traditional superovulatory schedule, to induce superovulation inrrespective of stage of the oestroue cycle was reported. However, information on superovulating anoestrous animals is scanty. Hence, this experiment was conducted to study the superovulatory response and embryo recovery in anoestrous Hariana cows and heifers that were kept on artificial progestational phase by Syncro Mate-B implant.

MATERIALS AND METHODS

This experiment was conducted on 6 pubertal heifers beyond 3 years of age and 6 cows beyond 6 months post-partum that were under uniform managemental conditions, after ascertaining their non-cyclicity. Treatment regime for both cows and heifers was same. To synchronize oestrus, animals were implanted with Syncro Mate-B implant* (3mg.norgestomet) subcutaneously on external ear (midway between base and tip) and administered intramuscularly Syncro Mate-B injection* (3mg. norgestomet and 5mg. oestradiol valerate in 2 ml.). The implant was withdrawn after 9 days. Superovulatory treatment was started on 7th day of implantation by intramuscular administeration of 34mg. FSH-E (Follicle Stimulating Hormone-Equine) in a descending dose schedule for 4 days (7, 5, 3 end 2 mg.) at 12 hourly intervals. To synchronize ovulation Fertagyl (0.5 mg. of synthetic gonadotropin releasing hormone) was administered intramuscularly 24 hours after implant withdrawl. The animals were observed for oestrus at 6 hourly intervals starting from 12 hours, after implant withdrawl and the animals in standing oestrus were mated with fertile bull at 12 hourly intervals. On the day of flushing (7th day postoesturs) superovulatory response was ascertained per rectally by

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counting the number of C.L. (Corpora Lutea) and UOF (Unovulated Follicles). Embryos were recovered nonsurgically using Dulbacco's phosphate buffered saline supplemented with bovine serum albumin. The embryos recovered were evaluated based on the standard morphological examination.

RESULTS AND DISCUSSION

All the superovulated animals exhibited oestrus after removal of the Syncro Mate-B implant. The everage interval for the oestrous exhibition from the implant withdrawl was 25.00±4.75 hrs. in cows and 23.00±1.00 hrs. in heifers (Table) and similar results were reported by Prather *et al.,* (1984) who included PG and Syncro Mate-B for synchronization.

Animals with more than 2 ovulations were considered as responders and the details are presented in table. Four cows and all the heifers responed to the superovulation. In the responded animals, the number of CL and UOF were 9.75±0.48 and 1.00±0.41 in cows and 8.33±0.83 and 0.67±0.33 in heifers respectively, which did not differ significantly. The number of CL observed in the present study are comparable with the earlier obsrvations (Ramakrishna and Ramachandraiah, 1989) in crossbred cattle. Monmiaux *et al.,* (1983) reported inherent variability in ovarian follicular population, while Moor *et al.,* (1984) stated the variation in the population and stage of the follicles at gonodotropin treatment as potential causes of variation in the superovulatory response. The less number of UOF in the present study may be due to the beneficial affects of GNRH administered, eventhough comparative studies were not made.

The number of embryos recovered and transferable embryos were comparatively higher in cows (4.00±0.71 and 2.75±0.85) than in heifers (2.60±0.68 and 1.20±0.49), while the degenerated embryos were higher in heifers (1.40±0.25) than in cows (1.25±0.25). However, all these differences were statistically nonsignificant. The present findings are comparable to the observations of Singla and Madan (1990) in crossbred cattle, but Donaldson (1991) reported higher values for exotic breeds of cattle. Becker and Pinheiro (1986) reported lower embryo recovery rate and transferable embryos in Indian breeds of cattle with high ovulation rate and attributed this to the inability of the infundibulum in egg uptake in case of increased ovulations. One blastocyst was collected from each group, while the remaining were morulae / early blastocysts and all of them were fertilized. The recovery of mostly morulae / early blastocysts may be due to the synchronous ovulations as a result of GnRH inclusion in the treatment schedule and higher fertilization rate may be due to mating twice with a fertile bull at the time of synchronized oestrus. A positively significant (r=0.948) correlation was observed between number of embryos recovered and transferable embryos.

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Table 1. Comparison of superovulatory response, embryo recovery and their quality in superovulated (anoestrous) Hariana cows and heifers.

Parameter studied	Cows		Heifers	
	Total	Mean ±SE	Total	Mean±SE
Animals heated	6	—	6	—
Animals exhibited oestrus	6	—	6	—
SMB withdraw to oestrus interval, (hrs.)	—	25.00±4.75	—	23.00±1.00
Animals responded	4	—	6	— —
Animals flushed	4	—	5*	—
Number of CL	39	9.75±0.48	44	8.83±0.83
Number of UOF	4	1.00±0.41	4	0.67±0.33
Total embryos recovered	16	4.00±0.71	13	2.60±0.68
Degenarated embryos	5	1.25±0.25	7	1.40±0.25
Transferable embryos	11	2.75±0.85	6	1.20±0.49
Blastocysts	1	0.25±0.25	1	0.20±0.20
Morulae / early blastocysts	15	3.75±0.48	12	2.40±0.51

* All the embryos recovered were fertilized. Heifer No.1 was ot flushed due to catehteriztion problem.

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Studies on Superovulatory response in cows treated with PMSG or FSH during luteal stage of the oestrus cycle

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ABSTRACT

Lactating crossbred cows (Jersey x Red Sindhi) n=12) were superovulated either with PMSG (2000 IU) or FSH (18mg) beginning on day 11 of oestrus cycle and PGF_2 (25mg) was administered on third day of gonadotropin treatment in two doses at 12 hours interval. The embryo collection was carried out on day 7 after superovulatory oestrus. The number and quality of embryoes obtained were recorded. The mean number of corpus luteum, total freezable, transferable, degenerated and unfertilized embryoes did not differ significantly among PMSG (6.6±1.2, 4.84±1.60, 1.66±1.08, 2.00±0.85, 0.50±0.22 and 0.66±0.42) and FSH treatment (7.6±1.1, 5.3±0.55, 1.83±0.91, 1.83±0.87, 1.16±0.47 and 0.50±0.34) but PMSG treatment showed significantly higher number of unovulatory follicles ($p<0.01$)

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The response of the individual donors to the superovulatory drug is influenced by exogenous and endogenous factors. Information on the use of superovulatory drugs on indegenous cows and their crosses with exotic breeds in tropics is limited. Hence the present study was formulated to study the ovulatory response, fertilization rate, embryo quality and quantity in crossbred cattle using both PMSG and FSH.

MATERIALS AND METHODS

Twelve lactating cows (Jersey x Sindhi) located in Farm owned by Tamilnadu Veterinary and Animal Sciences University were used. They were maintained under standard feeding and managemental conditions. Oestrus was synchronised using 25mg Dinoprost (PG, Lutalyse, Unichem Laboratory Ltd., Bombay). Observation for oestrus behaviour commencd, 48 hours after PG injections. The cows were then randomly assigned to receive either FSH (n=6) or PMSG (n=6) treatment, which was initiated on day 11 of the oestrus cycle. Cows received eight intramuscular injections of FSH (Folltropin, Vetrepharm, London, Canada) given twice daily for four days at doses of 3.6, 2.7, 1.8 and 0.9 mg (Total 18 mg equivalent to 400 NIH-FSH-P1) FSH per injection per day, respectively or a single intramuscular injection of 2000 IU of PMSG (intervet, Boxmeer, Holland). Luteolysis was induced by injecting, Dinoprost (25mg) at 48 and 60 hours after initiation of gonadotropin treatment. Observation for oestrus commenced 24 hours after the second PG injection. Flushing for embryoes was done on day 7 (day of onset of oestrus=day 0) non surgically after determining the numebr of corpora lutea, using standard flushing procedure. The collected embryoes were examined under stereo microscope, counted, graded on a scale of 1 to 5 as per the method described by Goulding *et al.*, (1991). Unfertilized eggs were not included in this grade 1 to 5 classification system.

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Data on number of corpus luteum, large follicles, embryoes (recovered, freezable, transferable and degenerated) and unfertilized eggs were analysed. Means were compared using t - tests.

RESULTS AND DISCUSSION

The number of ovulations (estimated) in PMSG treated cows ranged from 3 to 11 with an average of 6.6±1.2 per cow, whereas it ranged from 4 to 11 with an average of 7.6±1.1 per superovulation in FSH treated cow. The mean number of large follicles on day 7 for PMSG or FSH was 1.5±0.22 and 0.33±0.20, respectively ($p<0.01$). The statistical analysis of the frequency distribution for the number of large follicle showd that the Chi-square value of 7.2 is significant ($p<0.05$). The main contribution to the significant chi-square value is the high number (4/6) of FSH treated cows with no follicles compared with PMSG and nil number of FSH treated cows with two large follicles compared with PMSG treated cows (3/6).

Though the response to PMSG and FSH group did not show any statistical difference, the mean ovulation rate in donors responding to superovulation (3 CL) with FSH was higher than PMSG. Datta *et al.*, (1992), Agarwal *et al.*, (1993) and Kathiresan *et al.*, (1994) reported higher ovulation rate with FSH, whereas Saha Madan (1990) reported higher ovulation rate for PMSG administration. The unovulated large follicles on the day of flushing was significantly higher in PMSG than FSH treated group. These unovulated follicles may presumably be those which continue to grow due to longer half life (120 hours) of PMSG and failed to ovulate because of incomplete maturation at LH surge (Monniaux *et al.*, (1983).

The mean number of recovered embryoes was found to be 4.83 + 1.60 (ranged from 0 to 9) and 5.33 + 0.55 (ranged from 3 to 7) which was 72.50 per cent and 69.56 per cent of the total ovulations from the cows assigned to PMSG or FSH treatments. The mean number of freezable, transferable degenerated and unfertilized eggs in PMSG and FSH treated cows were 1.66±1.08, 2.00±0.85, 0.50±0.22 and 0.66±0.42 and 1.83±0.91, 1.83±0.87, 1.16±0.47 and 0.50±0.34, respectively. Five cows among PMSG treated and four cows in FSH group responded well and produced 5-9 embryoes. One animal did not respond to PMSG treatment. The number of transferable embryoes per donor in the two treatment was almost similar with per cent transferable embryoes being 75.77 and 65.66 per cent (including freezable embryoes) in PMSG and FSH group, respectively. Agarwal *et al.*, (1993) reported that recovery rate of transferable embryoes varies from 45 to 75 per cent.

The number of degenerated embryoes was higher following FSH treatment. Oliver *et al.*, (1984) observed that on administraiton of PMSG the number of ovulation and altered structures were smaller and hence less degenerated embryoes when compared with FSH.

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Synchronization of Oestrus of Tellicherry Goats Treated with Synthetic Progestagens and Fertility following Natural Services or A.I. with Frozen Semen*

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ABSTRACT

Twenty eight Tellicherry goats were divided into 2 groups (FGA and MAP groups). Each doe in FGA group was treated with 45 mg Fluorogestone acetate intravaginal sponge and MAP group does were treated with 60 mg Medroxy Progesterone acetate vaginal sponges for 18 days. At the time of withdrawal of sponges, each doe was given 600 I.U.PMSG. Oestrus was detected by using an apronised buck. Breeding at synchronized oestrum was carried out by using intact buck (N.S.) or artificial insemination (A.I). All does in FGA and MAP groups exhibited oestrus within 32-42 hours and 32-54 hours. Duration of oestrum in FGA and MAP groups was 29.28±1.62 and 45.57±2.27 hours. Duration of oestrum showed significant diffrence between 2 groups. The kidding rate through natural service was 71.43 and 57.14 per cent and the corresponding values through A.I. was 57.14 and 42.86 per cent in FGA and MAP groups respectively. Mean serum progesterone concentrations at the time of insertion of FGA and MAP sponges were 2.42±0.23 and 2.64±0.36 ng / ml and the corresponding values at the time of withdrawal were 2.64±0.18 and 2.88±0.36 ng / ml respectively. At oestrum, the mean progesterone level was 0.50±0.08 ng / ml to FGA group and 0.51±0.08 ng / ml to MAP group.

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Synchronization of oestrus has become a subject of major interest in recent years especially in goat industry. Progestagen impregnated intravaginal pessaries have been used to control the time of oestrus in different breeds of goats (Dhinsa *et al.*, (1971). However the effect of various progestagens for the control of time of oestrus and fertility rate have not been compared in Tellicherry goats in detail. Hence, the present study on Tellicherry goats was taken to assess the effect of Fluorogestone acetate and Medroxy progesterone acetate vaginal sponges on estrum, fertility and serum progesterone level.

MATERIALS AND METHODS

Twenty eight parous, healthy and cyclical Tellicherry goats weighing approximately 20-35 kgs were divided into 2 groups (FGA and MAP groups) comprising of 14 does in each group. Each doe in FGA group was treated with 45 mg Fluorogestone acetate vaginal sponge (FGA, Chronogest, Intervet International, B-V. Boxmeer, Holland) and MAP group does were treated with 60 mg Medroxy Progesterone Acetate (MAP, Veramix, Upjohn Limited, Sussex) sponge for 18 days. At the time of withdrawal of sponges each doe was given 600 I.U. Pregnant Mare Serum Gonadotrophin intramuscularly.

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Twenty four hours after the withdrawal of sponges, all the does were observed individually every 4 hours for signs of oestrus by using an apronised buck. Time from the removal of sponges to onset of oestrus was recorded in hours for both the treatment groups. Duration of oestrum was determined as the period between first and last mounting by the apronised buck (Doijode *et al.,* (1991). Does exhibited oestrus in FGA and MAP groups were divided into two sub groups as N.S. and A.I. groups. Natural service for N.S. group and Artificial Insemination for A.I. group was done at 48 and 60 hours of sponge removal. Kidding rate was calculated as number of does kidded to no. of does allowed for N.S. or A.I. in both the groups. Blood samples were collected at the time of insertion of sponges, withdrawal of sponges and at standing oestrum. Serum was separated and stored at -20°C until progesterone assay. Progesterone assay was done by RIA technique as described by Sharma *et al.,* (1987)

RESULTS AND DISCUSSION

All does in FGA group and MAP group exhibited oestrus within 32-42 and 32-52 hours, respectively. No significant difference was noticed between FGA and MAP groups in onset of oestrum (Table). Various authors recorded variable time of onset of oestrum with FGA (Pathiraja *et al.,* (1991) and Romano, 1993) and with MAP (Alacam *et al.,* (1985) sponges where they have not administered PMSG or administered lesser doses of PMSG. But in this study, it is concluded that administration of 600 I.U. of PMSG at sponge withdrawal was responsible for the earlier onset of oestrum and closer synchrony. This is in accordance with the observatins of Dhinsa *et al.,* (1971). Mean duration of oestrum in FGA and MAP group was 29.28±1.62 and 45.57±2.27 hours and the difference was statistically significant. Presence of large unruptured follicles on the ovaries of oestrus synchronized goats was reported by Dhinsa *et al.,* (1971). Chemineau *et al.,* (1982) observed a longer interval between the maximum oestrogen level and maximum gonadotrophin level at induced oestrus than at natural oestrus. In this study, it is concluded that any one or combination of the above observations might be the reason for the longer duration of oesrum in MAP treated group does.

Natural service resulted in 71.43 (5/7) per cent kidding rate in FGA group and 57.14 (4/7) per cent in MAP group. Similar findings were recorded by Romano (1993) with FGA sponge and Bowen (1988) with MAP sponge. A.I. had the kidding rate of 57.14 (4/7) per cent in FGA and 42.86 (3/7) per cent in MAP group. When compared to MAP group, FGA had higher kidding rate in natural service and A.I. The longer oestrus duration in MAP group might have caused asynchrony between the insemination and ovulation and reduced the kidding rate. In both the treatments N.S. had higher kidding rate than A.I. Baril *et al.,* (1993) opined that the synchronization of oestrus is not precise enough to allow for fertilization to occur in all the goats when A.I. is performed at a predetermined time after the end of progestagen treatment.

Mean serum Progesterone concentrations at the time of FGA and MAP sponges insertion were 2.42±0.23 and 2.64±0.36 ng/ml and at withdrawal they were 2.64±0.18 and 2.88±0.36 ng/ml respectively (Table). Similar trend in progesterone level was observed in goats by Chemineau *et al.,* (1982). No difference in the progesterone level at standing oestrus between 2 groups (0.05±0.08 vs

0.51±0.08 ng/ml) indicated complete luteolysis and therefore the difference in the fertility between two groups may not be due to the level of progesterone at standing oestrus. Similar level of P^4 at oestrus was recorded by Pathiraja *et al.,* (1991).

From this study it is concluded that though 100 per cent oestrus synchronization response was achieved with FGA and MAP sponges, FGA was found to be superior when compared to MAP in kidding rate.

Table 1. Onset and duration of oestrus, kidding rate and serum P^4 level in oestrus synchronized Tellicherry goats

Parameters		Treatment	
		FGA	MAP
Mean onset of oestrus (hours)		36.14±0.86	38.71±1.71
Mean duration of oestrus (hours)		29.28±1.62	45.57±2.27
Kidding rate (Percent)	N.S.	71.43	57.14
	A.I.	57.14	42.82
Serum P_4 level (ng/ml)	At sponge insertion	2.42±0.23	2.64±0.36
	At withdrawal	2.64±0.18	2.88±0.36
	At standing oestrum	0.50±0.08	0.51±0.08

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Estrus Synchronisation with Progesterone Impregnated Vaginal Sponges During Summer Season

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ABSTRACT

Estrus in sheep is synchronised using either progestins such as naturally occuring progesterone or synthetic progestagens (Robinson, 1979; Mauleon, 1979) or two injections of prostaglandin F_2 alpha (Cognie and Mauleon, 1983; Gordon, 1983) separated by an interval of 9-11 days. In the present study, locally made vaginal sponges impregnated with progesterone have been tested for their efficacy in synchronising estrus in native and crossbred ewes maintained in semi-arid tropical conditions during summer season.

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MATERIALS AND METHODS

A : Preparation of Vaginal sponges

Vaginal sponges were prepared in our laboratory using synthetic sponge foam and cotton thread. Flat sponge was cut into circular pieces of similar shape in two sizes Size 'A' : 35 mm diameter and 27 mm height, size 'B' : 42 mm diameter and 27 mm height, and were tied with 16-20 cms cotton thread. These sponges were sterilised by autoclaving. A solution of progesterone (0.175 g/ml) was prepared in ethanol and each sponge was loaded with 2 ml solution. The sponges were dried in hot air and kept in a sealed polythene bag.

B. Animals and Treatment

Vaginal sppnges (size A) impregnated with 0.35 g progesterone were put into vagina of 9 (group 1) and 10 (group 2) adult crossbred and native ewes during summer using glass speculum and plunger. The sponges were kept *in situ* for 12 days. Pregnant mare serum gonadotrophin (PMSG : 400 IU) was administered in group 2 ewes at the time of sponge removal. Ewes were detected in heat by parading aproned ram in the morning and evening. In another experiment, size A sponges were put into vagina of 19 and size B into 40 crossbred and Kheri ewes. During the trial observations were recorded on number of sponges lost and ease of removal.

RESULTS AND DISCUSSION

The use of exogenous progesterone has been demonstrated in controlling the cylcicity among ewes and mimic the function of corpus lueum (Harma *et. al.,* 1986; Wheaton et al, 1993) and also in initiating cyclicity effectively in temporarily non-cycling ewes (Jochle, 1993). PMSG is generally used with a progestagen to induce and synchronise estrus among anestrus / non-cycling ewes (Cunningham *et. al.,* 1980 and Hamra et al, 1989). All the ewes in group 2 treated with PMSG, exhibited heat within 48 h of sponge removal as compared to only half of the ewes in group 1. It indicated that only progesterone treatment could not induce the cyclicity in all the ewes but the addition of PMSG treatment initiated cyclicity effectively among all ewes during the temporary non-cycling stage. The mean time interval between sponge removal and onset of heat was more in group 1 than in group 2 but not statisticaly significant. Donor ewes treated with PMSG in a embryo transfer experiment, exhibited heat about 12 hrs earlier than the recipient ewes without PMSG treatment (Nagvi and Kalra, 1990). Progesterone is absorbed

effectively through vaginal wall and is sufficient to produce progesterone level present during luteal phase of cycle in ewes treated with 0.4 g progesterone loaded sponges (Hamra *et. al.,* 1986). In the present study, we used 0.35 g progesterone which appeared to be sufficient in bringing the animals in heat. Irrespective of size, no sponge was lost during 12 days period when kept *in situ*. General management and grazing did not influence the retention. All size A sponges could be removed easily but 20% size B sponges got retained and had to be removed with the aid of finger. It appeared that size 'B' was bigger for these animals. In conclusion, estrus in native and crossbred ewes could be induced and synchronised in temporarily non-cycling ewes using progesterone (0.35 g) impregnated sponges and PMSG (400 IU) treatment.

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Oestrus and Ovulation Response in Superovulated Goats in Relation to Age

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ABSTRACT

A total of forty five cyclic does belonging to three age group viz. A (1-2 years), B (2-3 years) and C (3-4 years) were treated with progesterone : PMSG and HCG. Overall oestrus response, duration and ovulation rate recorded in three groups were 91.11 per cent, 51.02±2.14 hours and 8.00±0.43 respectively. Oestrus duration was found to be significantly longer in the younger group (63.00±3.29 hrs) as compared to that of the older groups i.e. B (47.07±3.07 hrs) and C (42.00±1.65 hrs).

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The embryo transfer technology (ETT) has established itself as an important tool for livestock germ plasm improvement through rapid multiplication of superior donors. Selection of donors of right age so as to obtain optimum result is an important aspect of ETT. As such the present investigation was conducted to study the degree of oestrus and ovulatory response in superovulatory goats of different ages.

MATERIALS AND METHODS

A total of forty five normal, healthy, cyclic local goats of Assam, 1-4 years of age were selected for the present experiment. They were divided into three age groups of fifteen animals each viz., A (1-2 years), B (2-3 Years) and C (3-4 years). Animals were kept under semi-intensive system of rearing. To all animals, MAP (Forlutal) was administered orally at the dose level of 5 mg / animal / day for 14 days. On the day of withdrawal PMSG (Folligon, Intervet, Holland) at the dose level of 600 IU was injected intramuscularly. Animals showing oestrus was injected intramuscularly with HCG (Chorulon, Intervet, Holland) 6 hours after the onset of oestrus. Oestrus detection was done by vasectomised bucks every 6 hours interval throughout the study period. The very first acceptance of the buck by the female was considered as the onset of oestrus; and the duration of oestrus was the period between the first and last mounting by the vasectomised bucks.

Laparotomy was performed on day 5 post onset of oestrus for observation of ovulatory incidences. Laparotomy procedures were employed as per Hunter *et al.,* (1955) and Agrawal *et al.,* (1982) with slight modifications. The number of corpus haemorrhagicum or cherry red spot indicating ovulation was recorded in both the ovaries. Results were statistically analysed as per the methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The average oestrus duration, ovulation number and percentage of oestrus response following superovulatory hormonal treatment of different age groups hae been

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presented in Table 1. Age group differences were found to be significant (P/0.01) in respect of duration of oestrus.

All the animals of group B responded (100%) to the synchronised and superovulatory treatment, followed by group A (93.33%) and group C (80.00%). The average duration of oestrus was significantly longer ($p<0.01$) in group A (63.0±3.29 hrs) than group B (47.07±3.07 hrs) and group C (42.0±1.65 hrs) though there was no significant difference between group B and C. The overall values of oestrus duration (51.02±2.14 hrs) as observed in the present study was as compared to the reported values of Goswami (1989), Bonia (1992) and Chakravarty (1995). The longer duration of oestrus might be due to inclusion of younger animals (1-2 years) in the present study as compared to the animals used by the earlier workers.

The average ovulation number appeared to decrease with the advancement of age. The average ovulation numbers were recorded as 8.14±0.75, 8.07±0.75 and 7.75±0.76 in group A, B and C respectively. The group differences were however not significant. The overall average ovulation number combined for the three groups was 8.0±0.43, which is comparable with the findings of Ahmed and Maurya (1981), Doijode *et al.,* (1991) and Bonia (1992).

In order to study the association of age with the duration of oestrus and ovulation number, correlation coefficients were estimated. Age was found to be negatively correlated ($p<0.01$) with duration of oestrus (4=-0.6846±0.0851). However, age and ovulation number was not significantly correlated (r =-0.0208±0.1601). The estimated regression coefficient of oestrus duration on age (b = -0.9355±0.1595), which was significant ($p<0.01$) showed that for every one year increase in age of the doe, the oestrus duration reduced by nearly 1 hour. The regression coefficient of ovulation number on age (b = -0.0057±0.0435) was not significant.

Our results in the present experiment indicate that, when treated with progesterone : PMSG and HCG, younger (1-2 years) the animals longer is the duration of oestrus. Younger animals also tended to ovulate relatively more. This was perhaps due to prolonged and increased target cell response to the hormonal stimulations. Furthermore, the refrectoriness of ovarian sensitivity increases with the advancement of age, which might be a possible factor for decreased ovarian response to exogenous hormonal treatment in higher age group of animals. However, the breed, nutritional status, seasons, body weight, interactions between endogenous and exogenous hormones, individual variation and social factors may also influence oestrus duration and ovulation number (Hafez, 1987).

Table 1. Average oestrus duration, ovulation number and oestrus response in three diffeent age groups of goat following superovulatory treatments.

Group of goats	Total number of goat treated	Oestrus response (%)	Oestrus duration (hours)	Ovulation number
A (1-2 years)	15	93.33	63.0^{a}±3.29 (19.56%)	8.14±0.75 (34.69%)
B (2-3 years)	15	100.00	47.07^{b}±3.07 (25.29%)	8.07±0.75 (35.83%)
C (3-4 years)	15	80.0	42.0^{b}1.65 (13.62%)	7.75±0.78 (34.85%)
Overall	45	91.11	51.02±2.14 (26.90%)	8.0±0.43 (34.35%)

Means within a column with at least one superscript in common do not differ significantly.

Within parenthesis are the coefficient of variation in percentage.

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Efficacy of Different Collection Methods for Oocyte Recovery in Sheep

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ABSTRACT

Methods of slicing, dissection, follicle puncture and aspiration were employed to recover oocytes from sheep ovaries collected at a local abattoir. Mean number of oocytes were 10.33, 11.13, 4.41 and 2.75 per ovary, respectively. The unit processing time taken was 1.32, 10.63, 1.32 and 1.77 min. This study revealed that the method of slicing is the best for oocyte retrieval in ovine.

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Ovaries of abattoir origin is a good and cheap source of primary oocytes for *in-vitro* culture (Agrawal, 1992). However, proper oocyte selection in the laboratory is crucial for successful embryo production *in-vitro* (Brackett and Zuelke, 1993). Several methods of oocyte collection have been tried with varying degree of success ingoat (Mogas, *et al.,* 1992; Pawshe *et al.,* 1994; Chakravarty *et al.,* 1994) and in sheep (Wahid *et al.,* 1992; Datta *et al.,* 1993). Hence the present study was an attempt to assess comparative efficiency of four different collection methods in terms of quality oocyte yield in a shortest time from ovine ovaries.

MATERIALS AND METHODS

Sheep ovaries (n=190) was collected at a local abattoir irrespective of seasons, and transported to the laboratory in sterile normal saline (30 to 35°C). Oocytes were retrieved following slicing (n=57), dissection (n=45), follicle puncture (n=44) and aspiration (n=44) and graded into good, fair and poor depending on the types of cumulus investments (number of cumulus layers), their compactness and ooplasm granulity as described by Das *et al.,* (1995). Oocytes were screened and classified under stereozoom microscope (Bausch & Lomb, USA) at low and high power magnification, respectively. The unit time required to process each ovary by different methods was recorded. The entire work was carried out at a room temperature ranging from 30-37°C.

Statistical analysis was done according to Snedecor and Cochran (1967). Student 't' test was employed and significant difference was evaluated at $p<0.05$

RESULTS AND DISCUSSION

The results obtained in the present study are presented in table-1. The mean number of oocytes recovered by follicle puncture and aspiration were significantly ($p<0.05$) less than by slicing and dissection methods in this study, However, it was nonsignificant between slicing and dissection. Slicing yielded relatively more oocytes than that reported in sheep (Datta *et al.,* (1993) and in goat (Singh *et al.,* 1992; Pawshe *et al.,* 1994). However, it was comparable to Agrawal, 1992 in goat. Wahid *et al.,* (1992) and Datta *et al.,* (1993) obtained

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6.4 and 5.4 oocytes per ovary, respectively, by follicle dissection method in sheep. On contrary to earlier observation, it was quite more in the present study. The number of oocytes retrieved following puncture was higher in comparison to goat (Pawshe *et al.,* 1994) and comparable (Datta *et al.,* 1993) in sheep and in goat (Mogas *et al.,* 1992; Pawshe *et al.,* 1994; Chakravarty *et al.,* 1994) with aspiration. On contrary, Wahid *et al.,* (1992) and Singh *et al.,* (1992) recovered comparatively more number of oocytes with the later technique in sheep and goat, respectively.

Number of good oocytes / ovary obtained here by slicing and dissection were significantly ($p<0.05$) higher than follicle puncture and aspiration (table 1) and was in agreement to Singh *et al.,* (1992) in goat. However, present study showed non significant difference between slicing and dissection as well as follicle puncture and aspiration. The per cent of good oocytes recovered by slicing, dissection and aspiration were much less as reported earlier (Datta *et al.,* (1993). But recovery rate of good oocytes was more with slicing than follicle puncture, dissection and aspiration. However, fair oocyte yield was relatively higher following dissection and slicing. Poor oocytes were significantly less by aspiration than the other three methods. Culturable quality oocytes (<2 layers of cumulus cells)/ovary obtained in the present study was in agreement to that observed in goat (Mogas *et al.,* (1992).

Mean processing time needed by dissection was significantly ($p<0.05$) higher than taken by slicing, puncture and aspiration methods. Similarly, aspiration took significantly ($p<0.05$) more time than slicing and puncture. But the later two were nonsignificant with respect to processing time (table-1). Slicing time in the present study was nearly comparable to that reported in goat (Pawshe *et al.,* 1994). It was, however, less than that recorded by Datta *et al.,* (1993) in sheep and Singh *et al.,* (1992) in goat. Dissection took maximum time in this study supporting the findings of Datta *et al.,* (1993) and Singh *et al.,* (1992). On contrary, Wahid *et al.,* (1992) took comparatively less time. Time required for follicle puncture method here was lower than reported by Pawshe *et al.,* (1994) Wahid *et al.,* (1992) and Datta *et al.,* (1993) observed mean processing time of 1.03 and 3.26 min. respectively for aspiration. However, our present finding were in agreement to Pawshe *et al.,* (1994) in this respect and differed from Singh *et al.,* (1992).

Although dissection method yielded a better oocyte recovery in this study but it was time consuming and laborious. Hence, slicing is considered to be simple, feasible and economic in terms of time taken and oocyte recovery. It can therefore, be used for maximum yield of culturable quality oocytes require for *in-vitro* maturation and fertilization (IVM-IVF) studies.

Table 1. Comparative efficiency of collection methods for oocyte retrieval in sheep.

Method(s) of collection	Number of ovaries	Classification of oocytes			Total oocytes	Mean processing time min.
		Good >5 layers (no.)	Fair 2-5 layers (no.)	Poor <2 layers (no.)		
Slicing	57	136	210	243	589	
i) Percent oocytes		23.09	35.65	41.26	100.00	
ii) Mean oocytes±SEM		2.39±0.40[a]	3.68±0.64[a]	4.26±0.94[a]	10.33±1.78[a]	1.32±0.10[a]
Dissection						
	45	81	217	203	501	
i) Percent oocytes		16.17	43.31	40.52	100.00	
ii) Mean oocytes±SEM		1 80±0.34[a]	4.82±0.69[a]	4.51±0.64[ac]	11.13±1.40[a]	10.63±0.81[b]
Folllicle puncture	44	38	60	96	194	
i) Percent oocytes		19.59	30.93	49.48	100.00	
ii) Mean oocytes±SEM		0.86±0.22[b]	1.36±0.29[b]	2.18±0.32[ad]	4.41±0.58[b]	1.32±0.09[2]
Aspiration						
	44	18	36	67	121	
i) Percent oocytes		14.88	29.75	55.37	100.00	
ii) Mean oocytes± SEM		0.41±0.10[b]	0.82±0.17[b]	1.52±0.28[b]	2.75±0.39[c]	1.77±0.11[c]

Values (Column wise) bearing different superscripts differ dignificantly ($p<0.05$ test)

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Compensatory Ovarian Hypertrophy Suppressing Activity In Buffalo Fetal Cotyledons and Its Absence in corpus Luteum, Amniotic and Allantoic Fluid

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ABSTRACT

Compensatory ovarian hypertrophy (COH) suppressing activity was examined in 20% ammonium sulphate precipitates from 0.9% saline extracts of fetal cotyledons and corpus luteum collected between 2-4 months of gestation. These extracts were injected into unilaterally ovariectomized mice (left side) on the morning of dioestrus, while control group of mice received bovine serum albumin. Mice were killed after 72 hours and the right ovaries were weighed. The degree of COH was expressed as percent increase in weight of right ovary to that of the left. COH suppressing activity could be detected in fetal cotyledons but not in corpus luteum. This inhibitory factor(s) is thermolabile and inactivated by trypsin digestion. No precipitation of proteins was found either in aminotic or allantoic fluid.

——x——x——x——

In addition to established role of progesterone in suppressing follicular development and ovulation during pregnancy (Rexroad and Casida, 1975) recent studies reveals that certain factor(s) are secreted from placenta and endometrium regulating follicular dynamics during pregnancy. A gonadal peptide inhibin, which selectively inhibits FSH secretion from pituitary has been detected from pig (Mason *et al.,* 1985) and cow follicular fluid (Robertson *et al.,* 1986). A substance from rat uterine epithelium inhibits prolactin secretion from pituitary (Goropse and Freeman, 1985). Similarly, an inhibin like factor, from buffalo fetal cotyledons has been detected (Maurya and Pant, 1990). The present experiment, therefore, was conducted to confirm the presence of Inhibin like activity in buffalo fetal cotyledons and to examine it in corpus luteum, amniotic fluid and allantoic fluid if any.

MATERIALS AND METHODS

Pregnant buffalo uteri (alongwith ovaries), betwen 2-4 months of gestation were obtained from abattoir, within one hour of slaughter. Ovaries from non pregnant animals containing corpus luteum were also collected. Materials were immediately transported to the laboratory in chilled (4°C) 0.9% saline. Gestation age were estimated by measuring crown-rump length of the fetuses (Singh *et al.,* 1963) after dissecting the uteri. Fetal cotyledons were carefully seperated from maternal caruncles without intermixing of the tissues. Corpora lutea were also dissected out from ovaries of pregnant as well as non pregnant animals. Fetal cotyledons and corpora lutea were processed in a similar manner seperately. Pools of tissues (80-100 g) were homogenised in three volume of chilled 0.9% saline. The homogenates were centrifuged at 4000 rpm for 30 min. at room temperature (about 20°C). The supernatants were treated with ammonium sulphate (20% W/V) and kept overnight in refregerator at 4°C to ensure complete preciptation of proteins. The precipitates

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were collected by centrifugation at 4000 rpm for 30 min. at room temperature, suspended in distilled water and dialyzed for 96 hrs against distilled water at 4°C. After dialysis, the material was used for bioassay. Allantoic fluid was collected in a beaker from outer allantoic sac with the help of a syringe and needle. Similarly amnoitic fluid was collected from inner amniotic sac. These fluids were centrifuged seperately at 4000 rpm for 30 min. at room temperature. Supernatant was collected and measured. For protein precipitation from these fluids same procedure as used with fetal cotyledons and carpora lutea tissues, was adopted. As there was no precipitation of proteins either in allantoic fluid or in aminotic fluid, accordingly, no further investigations were made.

Protein content of ammonium sulphate precipitate of fetal cotyledonary extract and corpora lutea extract was measured following Lowry *et al.,* (1951). Adult female mice of swiss albino strain 35-40 days old and weighing 20-35 g were used. They were kept under normal daylight schedule with free access to feed and water. Mice were unilaterally ovariectomized (left ovaries) on the day of dioestrus, under ether anesthesia between 10.00 and 12.00 h. The ovaries were weighed immediately. Ammonium sulphate fractions of fetal cotyledonary extract, trypsin digestd (50 ug trypsin/mg protein incubated for 2 h at 37°C in Tris-EDTA buffer, pH 7.4) extracts, heat treated extracts (67°C for 1 h and 100°C for 20 min.) were injected subcutaneously in the experimental group of mice while the control group received bovine serum albumin. Protein was injected @ 200 ug in 0.5 ml volume of 0.9% NaC1 to each mouse except in one group of recipients of ammonium sulphate precipitate of fetal cotyledonary extract, where dose rate was 100 ug in same volume. All the mice were sacrificed 75 h post injection and the right ovaries removed and weighed. The degree of COH for each mouse was calculated as the percentage increase in the weight of the right ovary (at sacrifice) as compared to left ovary (at hemispaying). In exactly similar manner the effect of ammonium sulphate precipitate of corpus luteum extract on COH in mice was observed against two control groups. Students it' test was used to analyse the data.

RESULTS AND DISCUSSION

Table 1 depicts COH of right ovary was significantly ($p<0.01$) supressed in the mice injected with 100 ug and 200 ug fetal cotyledonary extract in comparison to control (% 29.6±3.98 and 26.7±7.59 vs 64.9±8.32). Trypsin digestion of fetal cotyledonary extract and heat treatment of it at 100°C for 20 min. have completely abolished COH suppressing activity however heat treatment at 67°C for 1 h had no effect.

There was no significnt difference among groups in the mean COH following injections of bovine serum albumin, normal saline and corpus luteum extract (Table 2). This indicated an absence of COH suppressing activity in the ammonium sulphate precipitate of corpus luteum extract.

COH in mice was supressed on administration of protein from fetal cotyledonary extract at two dose levels of 100 ug and 200 ug, indicating the presence of inhibitory activity in fetal cotyledons, which was not dose dependent. Maurya and Pant (1990) also reported earlier the similar findings but at the 200 ug dose rate only. Interestingly, this activity was found absent in maternal caruncular tissue in an earlier study by Maurya and Pant (1990) and Mehrotra and Pant (1995).

Further the proteinaceous nature of the inhibitor was confirmed by subjecting it to heat treatment and by trypsin digestion. This study indicated that heat treatment of fetal cotyledonary extract at 100°C for 20 min. had completely abolished the activity while heating to 67°C for 1 h had no effect. This indicates that the inhibitor of fetal cotyledonary extract is more thermostable as compared to FSH suppressing activity in the bull seminal plasma (Peek and Watkins, 1979).

However, further studies are warented to determine the exact temperature (below 100°C) and duration of exposure at which COH suppressing activity of fetal cotyledonary extract may be completely abolished. Trypsin digestion also destroyed the COH suppressing activity confirming proteinaceous nature of material. We speculate that the inhibitor(s) isolated in this study may play an important role in the regulation of follicular dynamics during pregnancy.

In the present investigation we have also examined presence of this COH suppressing activity in corpus luteum. Results (table 2) clearly indicate an absence of COH suppressing activity in ammonium sulphate precipitates of corpus luteum. Rexroad and Casida (1975) suggested that the corpus luteum of gestation acted on follicles to increase atresia and alter their growth rates during gestation in cow. Present study revealed that mechanism by which corpus luteum may be concerned with increased folicular atresia during pregnancy is unlikely to be due to the secretion of inhibin like material. Further studies are required to ascertain the factor(s) secreted by the pregnancy corpus luteum that could alter the dynamics of follicular growth during pregnancy in cattle.

Table 1. Effect of ammonium sulphate proecipitate of fetal cotyledonary extract heat treated and trypsin digested extracts (mean±SE) on compensatory ovarian hypertrophy in mice.

Treatment	Dose ug/ .5 ml	No.of mice	Weight of mice (g)	Weight of left ovary (mg)	Weight of right ovary (mg)	% hypertrophy of right ovary
Bovine serum albumin	200. ug	6	26.8±1.35	1.98±0.19	3.29±0.42	64.9±8.32
Fetal cotyledons(F.C)	100 ug	6	26.3±0.49	2.48±0.28	3.21±0.37	29.6±3.98**
Fetal cotyledons	200 ug	6	25.8±1.19	2.49±0.24	3.1±0.30	26.7±7.59*
Heat treated F.C. 67°C (1 h)	200 ug	6	30.7±0.62	3.57±0.22	4.36±0.18	23.2±5.94**
100°C(20 min)	200 ug	6	29.3±0.99	3.75±0.47	5.8±0.52	59.5±8.49
Trypsin digested (F.C.)	200 ug	6	30.3±1.09	3.70±0.12	5.62±0.12	52.6±4.69

* Significantly different from control value ($p<0.01$)

** Significantly different from control value ($p<0.005$)

Table 2. Effect of ammonium sulphate precipitate of corpus luteum extract (mean ± SE) on COH in mice.

Treatment	Dose ug/ .5 ml	No.of mice	Weight of mice (g)	Weight of left ovary (mg)	Weight of right ovary (mg)	% hypertrophy of right ovary
Normal saline	0.5 ml	6	28.5±1.50	2.23±0.42	2.91±0.46	33.9±10.92
Bovine serum albumin	200 ug	6	24.0±1.15	2.3±0.81	3.06±0.44	34.5±6.26
Corpus luteum	200 ug	6	24.0±0.85	1.90±0.25	2.50±0.31	32.4±3.51

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Pattern of Oestrous Cycle and its abberrations in Repeat Breeding Crossbred Cattle

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ABSTRACT

Pattern of estrous cycle and its abberrations in repeat breeding crossbred cattle was studied. The mean length of normal estrous cycle in repeat breeding and normal cows were 20.6±0.11 and 20.45±0.25 days, respectively. Majority of normal cycles both in repeat breeding (79.33%) and normal (82.50%) cows were ranging between 19 to 23 days. The incidence of normal, long regular multiple irregular long and short cycles were 57.58, 13.24, 16.50 and 12.66% in repeat breeding and 51.61, 14.83, 19.33 and 14.19% in normal cows, respectively. Maximum proegnancy existed in both the groups (70.97% in RB and 50.63% in normal cows) when the length of estrous cycle was normal. Incidence of anovulation was higher in repeat breeding than the normal cows (26.53 vs 20.00%).

——x——x——x——

It is well documented that a large proportion of infertility problems in dairy cattle are centered around the disturbances in the estrous phenomena. Reproductive efficiency in cattle is affected adversely by the abberrations in the estrous cycle. Ovulation failure has been reported as one of the cause of repeat breeding in dairy cattle (Ayalon *et al.,* 1967; Mehta *et al.,* 1986). In the present paper pattern of estrous cycle, its abberration and incidence of failure of ovulation in repeat breeding crossbred cattle have been reported.

MATERIALS AND METHODS

A total of 521 estrous cycle from 89 repeat breeding and 155 cycles from 87 normal crossbred cows were analysed. The cycle of 17 to 25 days duration was considered as normal cycle (Diaz and Gonzalez, 1965). The inter estrual period falling out of the range of 17 to 25 days were considered as abberrations of estrous cycle. The cycle length below 17 days was considered as short cycles and more than 25 days duration were as longer cycles. The longer cycles were categorized into long regular multiples and irregular long cycles. The multiples of 21±4 days (21 x n±4 days) were considered as long regular cycle (double, triple etc). The longer cycle which did not fall within multiples regular cycle range considered as irregular long cycles. Existance of conception in each type of cycles was calculated. To study the ovulation failure 198 crossbred cows (100 - normal and 98 - Repeat breeding) were clinically investigated to find out occurring of ovulatory failure in them. All the cows were examined per rectum between day 9 to 12 following estrous for the presence of corpus luteum on the ovary. Animals with no palpable corpora lutea were classified under ovulation failure.

RESULTS AND DISCUSSION

Results revealed no difference in the mean length (20.67±0.11 vs 20.45±0.25 days) and incidence (57.58 vs 51.61%) of normal estrous cycle between repeat breeding and normal crossbred cows. The frequency distribution of normal cycles (20-21 Days) was more or less similar in repeat breeding and normal cows. Incidence of short (12.66 vs 14.19%), multiple long

(13.2 vs 14.83%) and irregular long (16.50 vs 19.35%) cycle did not differ much between repeat breeding and normal cows. Most of the authors have reported average length of normal estrous cycle in between 20 to 21 days and its incidence 40 to 60 per cent (Badawy *et al.,* 1973; Luktuke *et al.,* 1973; Coetzer *et al.,* 1975) which is in agreement to the findings of the present study. However, others observed higher incidence of normal cycle (Diaz and Gonzalez 1965; Johnson and Oni 1986). On the other hand few authors have reported lower incidence of normal cycle (Martinez *et al.,* 1984; Soto and Gonzalez, 1992). The difference in the incidence of normal cycle might be due to diffeence in breed, plane of nutrition, managemental and agroclimatic conditions.

Most of the authors have reported almost similar incidence of these types of cycle in cattle (Eswaraiah, 1976; Sharma *et al.,* 1984. Martinez *et al.,* 1984). Occurrence of short cycle might be due to follicular cyst in the ovary or split estrus. Multiple long cycle might be due to weak/silent heat and shorter duration of estrus which remained unnoticed. However, occurrance of irregular long cycles might be due to early embryonic mortality (Sreenan and Diskin, 1983).

Pregnancy results revealed that higher proportion of cows both repeat breeding (70.97%) and normal (50.63%) became pregnant when the estrous cycle was normal indicating conception had tendency to occur when the estrous cycle was normal. Lower conception rate however, was obtained in both repeat breeding and normal groups experiencing short (3.23 vs 12.66), multiple long (12.90 vs 18.99), and irregular long (12.90 vs 17.72) cycles, As observed in the present study lower conception rate has also been recorded in cows experiencing short or irregular long cycle (Vandemark and Moller, 1950; Olds and Seath, 1951).

Incidence of anovulation was higher in repeat breeding (25.55%) than the normal cows (20.00%) which offers a possible explanation of one of the causes of repeat breeding. Higher incidence of anovulation in repeat breeding cows has also been reported by Ayalon *et al.,* (1967), Mehta *et al.,* (1980) and Khanna and Sharma (1992) which is in agreement with the results of the present study. The incidence of anovulatory estrus in normal cows is almost similar to Glod (1961) however, it was lower than Choudhary *et al.,* (1965) and Sampat Kumar and Iya (1966). On the other hand it was higher than Graden *et al.,* (1968) and Narsimha Rao and Kesavamurthy (1972). The differences in the incidence of anovulation might be due to difference in breed, endocrinological constitution, agroclimatic and managemental conditions.

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Microbial and Biochemical Profile in Repeat Breeder Cows.

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ABSTRACT

Of sixty Cervico VAginal discharges cultured from repeat breeder cross bred Jersey cows forty six samples were found to be positive and were subjected to antibiotic sensitivity test. The incidence of bacteria in descending order were E.Coli, Staphylococci, Streptococci, Cornyebacterium, Proteus and Psudomonas. Out of thirteen selected antibiotics Gentamycine and Chlromphenicol are found to be most effective. Fifteen blood samples each at random from repeat breeding cows with uterine infection and without uterine infection were compared with healthy cycling cows for certain biochemical constituents. Significantly low levels of glucose, inorganic Phosphorus, heamaglobin and total protein were recorded in both the groups. Repeat breeding may be due to the combination of nutritional and infectious causes and deficiencies unassociated with infection alone can cause the condition.

——x——x——x——

Increasing number of Infertility and repeat breeding among dairy cows are reported from animal health camps and veterinary clinics. Under field conditions where the level of management is not always upto recommended practices, conditions like infections of reproductive tract and nutrient deficiencies can precipitate into failure of conception or early embroyonic death.

MATERIALS AND METHODS

Sixty Jersey Cross bred repeat breeder cows attending to various infertility camps were selected. Cervico Vaginel discharges were aseptically collected during estrus period and were inoculated on blood agar, Mecconkey agar and nutrient agar. 46 isolates thus obtained were bacteriologically identified as per Cruickshank *et al.,* (1975) and subjected to antibiotic sensitivity testing using 13 antibiotic discs supplied by M/s. Span diagnostic, India as per the method of Ellner (1978).

Fifteen blood samples from these animals collected from Jugular vein at random as two separate aliquots using sodium flouride and heparin as anticoagulents (Group.I). Similar number of blood samples at random were collected from cross bred repeat breeder cows that showed no uterine infection (Group.II). Fifteen blood samples were collected from healthy cross bred cows that conceived with 1-3 inseminations and were considered as controls (Group.III). Blood with sodium flourids was used for estimation of blood glucose (Varlay, 1967). Plasma was separated from heparinised blood and utilised for estimation of total protein, albumin, Inoragnic phosphorus, calcium and cholesterol as per Varlay (1967). Haemaglobin was estimated by Cyanmeth-Haemaglobin method using the reagent kits supplied by M/s. Span Diagnostic, India, Statistical analysis was carried out using 't' test as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Of 60 samples processed organisms could be isolated from 46 (76.5%) while the remaining 12 (20.6%) were bacteriologically sterile indicating that this condition is not entirely due to bacterial infection of reproductive tract. Among the isolates E.coli predominated (18) followed by Streptococcus sp(6) Stephylococcus

sp(7) Corynebacterium sp(5) Salmonella sp(4) Proteus sp(3) and Psudomonas sp(3). A considerable variation was observed in the suspectability pattern of different isolates against antibiotics used. The organism are highly suseptable to Gentamycine (89.1%) Chlromphenicol (73.9%) and Nalidaxic Acid (73.9%) followed by Kenamycine (69.5%) Nitrofurazone (65%), Tetracyclin (58.6%), Co-trimethaxazole (58-6%), Strephomycine (34.7%), Sulfamethaxazole (14.5%) and Pencillin (13%). Sharda *et al.,* (1991), and Krishnan *et al.,* (1994) observed highest sensitivity to gentamycine and Chlromphenicol to the organisms isolated from cervico Vaginal swabs of repeat breeder cows. Lower than 50% of sensitivity to some antibiotics may be due to resistant strains, requiring proper evaluation before their actual use. Nalidaxic Acid is 100% sensitive to E.Coli and can be used as an alternative to Gentamycine to avoid excess use of the later and to prevent resistant strains.

Both the groups of repeat breeder cows showed significantly lowered glucose, total protein and inorganic phosphorus agreeing with the observations of Dutta *et al.,* (1991). Hypoglycemia at estrus and shortly after service can cause lowering of glucose of mucosa of genital tract causing lack of energy for spermatzoa to fertilise ova (Mcclure, 1968).

Rupde *et al.,* (1993) Khan and Iyer (1993) recorded low inorganic phosphorus in repeat breeder cows. Blood *et al.,* (1980) observed that Inorganic phosphorus tends to fall following insufficient dietary intake. Phosphorus is essential for transfer of biological energy particularly through ATP and its deficiency may interfear with fertilisation and may cause early embryonic death. Calcium levels however are unaffected in contrary to the findings of Rupde *et al.,* (1993) in both the groups.

Lowered protein levels in repeat breeding cows were recorded by Dutta *et al.,* (1991). Dietary insufficiency or inanition can reduce blood protein level where Vit.A and phosphorus deficiency are complicating factors (Robert 1971). Lower levels of albumin though statistically insignificant and significantly low levels of haemaglobin can indicate long standing low protein status and concentration of albumin is directly related to number of services required for conception (Blood *et al.,* 1989). Wagner (1962) related low haemaglobin levels to anoestrus, delayed postpartum breeding and possibily repeat breeding. Lowered tissue oxygenation may be the contributing cause. However the levels of haemaglobin in the blood that actually effects the reproductive function should be worked out.

Cholesterol levels of repeat breeder cows are statistically insignificant but lower than controls which reflect low saturated fat diet (Benjamin 1985) and can contribute to low energy status due to which follicles fail to develop and early embryos are affected (Robert, 1971).

Undernutrition and inanition can reduce the secreation of Gonadotrophins and administration of harmones were unable to sustain complete reproductive response (Mustgaard 1969).

The present study recommends a comprehensive approach towards the tackling the cases of repeat breeding cows giving due emphasis to infectious and nutritional status, more to the later under marginal managemental conditions.

Acknowledgement: The author is extremely thankful to Director (AH) Andhra Pradesh for providing the facilities.

Table 1. Biochemical Profile of Repeat Breeder Cows.

Parameters	Repeat Breeder with Uterine infection	Repeat Breeder without Uterine infection	Control
Glucose mg%	45.72±3.81*	45.6±1.52**	62.20±5.74
Total Protein g%	5.98±0.09*	6.00±0.14*	6.05±0.18
Albumin g%	2.58±0.67NS	2.83±0.20NS	3.38±0.07
Inorganic Phosphorus mg%	4.33±0.14**	4.51±0.18**	5.96±0.18
Calcium mg%	9.79±0.52NS	9.85±0.21NS	9.95±0.25
Serum cholestrol mg%	73.4±8.34**	77.8±6.45**	83.0±6.05
Haemoglobin g%	9.78±0.23NS	9.71±0.2NS	10.92±0.22

Significant at 5% (*) at 1% (**)
Not significant (NS).

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Treatment of Uterine Torsion in Buffaloes - Modification Of Schaffer's Method

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ABSTRACT

Detorsion of uterus was tried by either Schaffer's method or other three modifications viz., Sharma method, peg method and double plank method in 52 buffaloes and cows with uterine torsion of 180° - 360° at end term.

It is concluded that Sharma's modification of Schaffer's detorsion method was most effective in 18 out of 20 buffaloes (90%) and suitable for removal of uterine torsion in pregnant buffaloes.

——x——x——x——

Uterine torsion is the single largest cause of maternal dystocia in buffaloes with incidence as high as 67% (Singh *et. al.,* 1978). This obstetrical malady could be successfully resolved in cows by Schaffer's method (Schaffer, 1946 Roberts and Hillman, 1973) The decrease detorsion success in buffaloes compared to cows using of Schaffer's method remains unexplained. Modification to this method or invention of some suitable alternative detorsion method is, therefore, warranted. The present study explains the success of various alternative detorsion methods devised to relieve the uterine torsion in buffaloes.

MATERIALS AND METHODS

Fifty two buffaloes and cows suffering from uterine torsion at the end of gestation were included. Each case of less than 24-hour duration and was apparently free from palpable utero-mental adhesions. The animals were randomly subjected to rolling to remove the torsion emloying either of the four detorsion methods used in this study. The rolls were repeated until complete detorsion was achieved or upto a maximum of four rolls. The Schaffer's method was tried on 10 buffaloes and 10 cows with uterine torsion. The modifications named as (a) Sharma's modified method, (b) Peg method and (c) Double plank method, were tried on 20, 6 and 6 buffaloes with uterine torsion, respectively. The Sharma's modification involved applying pressure on abdomen of the buffaloes casted laterally on the side of torsion with a 12" long 8" wide wooden plank kept in position by 2 persons at the lower end. In addition, one man controlled the pressure during rolling by holding the upper end of the plank while standing on the other side of the animal. The fore and hind legs of the buffalo were tied separately and were pulled to roll the animal.

In peg method, lower end of the plank pressing the abdomen of buffalo was temporarily fixed in the ground using two 15" long angled iron pegs while one person controlled the pressure at the free end of the plank. If more than one roll was required, the animal was rolled back to its original position without distrurbing the position of the pegs and the operation was repeated.

In the double plank method two 12' long wooden planks joined with hinges at one end were spread on the ground. The buffalo was casted with the side of torsion on one plank, while the other plank was used to press the abdomen of animal. One

person controlled the pressure by holding the free end of upper plank while the animal was being rolled.

RESULTS AND DISCUSSION

Using Schaffer's method, the detorsion was successfully achieved in all the 10 cows (100%) but in only four out of ten buffaloes (40%). Discouraging detorsion results using Schaffer's method in buffaloes were reported by other workers also (Singh *et. al.,* 1978; Ahmed *et. al.,* 1980). Pressure applied on buffalo abdomen by the person standing on the ground end of the plank appeared to be either insufficient or inappropriate probably because of capacious abdomen (Singh *et. al.,* 1978) and / or thicker and heavy abdominal musculature in buffaloes than in cows.

Complete detorsion was achieved in 18 out of 20 buffaloes (90 %) treated by Sharma's modified technique. The advantage of this technique appeared to be the better fixation of the pregnant horn through the pressure being modulated by a person at the free end of the plank. This obviates that the buffaloes need more pressure than cows for successful detorsion. This modification resulted into a success rate already reported by Schaffer's method in cows (Roberts and Hillman, 1973; Arthur, 1966). Through Peg method, detorsion could be achieved only in one out of six buffaloes (16%). Although the use of pegs resulted in reducing the number of persons involved in the detorsion operation, fixing the pegs in ground, recasting the buffaloes for repeated rolling precisely at a place near to the pegs and slipping of plank out of pegs were the problems which made this operation combursome. Using double plank method, detorsion was achieved in only three out of six buffaloes (50%). Because of problems encountered during recasting the buffaloes on the plank and the fear of damage to the animal's spinal cord by the pressure of plank underneath the animal, this method had to be abandoned.

The uterus in each animal after various operations was intact. Live calf was delivered only in three buffaloes each subjected to Schaffer's method and to Sharma's modified technique while it was dead in all the rest included in this study. The calf survivability in three cases subjected to Sharma's modified technique suggested this technique to be harmless to the live fetus.

In the present study the detorsion success of Sharma's modified technique in buffaloes was similar to that of Schaffer's method in cows. No harmful effects of the extra pressure exerted in this modification were evident. The other modifications viz., Peg and Double Plank methods, though eliminated the need of help from extra persons, the practical difficulties encounterd left them unsuitable.

Acknowledgement: The help in evolving the Modified Sharma's technique by Dr. R.D. Sharma, Professor Emeritus, is acknowledged with reverence.

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Congenital Anomalies in Calves and Kids

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ABSTRACT

An analysis of congenital malformations among calves and kids showed a higher incidence in calves. Females were affected more. Involvement of digestive system was found maximum followed by muscular, urogenital and ocular system. Integumentary and nervous systems were least affected. Common anomalies were atresia ani and associated defects, prolapse of viscera through umbilicus, dermoid cysts, umbilical hernia and schistosomus reflexus.

——x——x——x——

Congenital anomalies may be caused by arrest, suppression or distortion of organ analogs at different stages of embryological development, genetic or environmental factors or by interaction of both. Eventhough neonatal losses due to congenital malformations form a very small percentage, a knowledge of local pattern of appearance of such malformation will be most helpful for the purpose of elimination in future. Various congenital anomalies of calves, lambs and kids had been reviewed by Singh (1989) and Angus (1992). The objective of the present paper is to document the observations made on clinical cases of congenital anomalies among calves and kids for a period of 10 years.

MATERIALS AND METHODS

Ninty cases of congenital anomalies in calves and kids presented at the Department of Surgery, college of Veterinary and Animal Sciences, Mannuthy formed the subject for this study. The defects were diagnosed and analysed according the species, sex and the body system involved and were presented in table 1.

RESULTS AND DISCUSSION

Occurance of anomalies was observed more in calves (68.8%) than in kids (31.1%). Female animals were affected more (53.3%) than the males (44.4%). Singh (1989) observed more number of incidence in males. Even though no defenite cause for this sex difference could be found from this analysis, the ignorance of the farmers in treating the male infants born with anomalies might have been a reason for this.

Even though congenital malformations often involve multiple body systems, particularly where environmental influences have operated at some specific time during foetal development (Angus, 1992). Involvement of digestive system is found to be more (46.6%) followed by muscular system (23.2%) and urogenital system (10%) skin defects and affections of nervous system were the least in occurance. Singh (1989) also observed almost a similar trend in the systems involved.

Digestive system: The most common defect observed in this study was atresia ani (12.2%). Atresia ani alone was observed in 11 animals (4 calves and 7 kids), but in 22 animals (52.4%), it was found to be associated with abnormalities of other body systems mainly urogenital system. Atresia ani is reported to be the most common congenital defect of lower gastrointestinal tract in animals (Jubb *et al.*, (1993). Singh (1989) also noticed atresia

ani as the most common defect of digestive system with a higher incidence in lambs than calves and kids. Atresia Ani et recti conditions stands next to atresia ani (8.9%) followed by Atresia ani with rectovaginal fistula (6.7%) which was observed in calves alone.

Muscular system: Prolapse of viscera through the umbilicus (6.7%) was found to be the most common anomaly of the muscular system. It was seen more in calves than kids. Incidence of hernias and schistosomus reflexus (4.4% each) were followed by incidence of contracted tendon at knee and fetlock joints.

Urogenital system: Incidence of urethral diverticulum in male kids formed the major part of anomalies of urogenital system (5.6%). In 16 animals (5 kids and 11 calves), defects of urogenital system were found to be associated with digestive system. Hypospadias and urethral diverticulum were seen only in kids.

Ocular system: Unilateral dermoid cyst (5.6%) and bilateral dermoid Cysts (3.3%) in calves were the only anomaly observed in ocular system. Similar observations were reported by Singh (1989) in calves.

Skeletal system: Bilateral dislocation of patella (3.3%) abscence of pubis and amelia (calves), Scoliosis and imperfect fusion of Sacrum (kids) were the major abnormalities observed in skeletal system.

Acknowledgement: The authors are thankful to the Dean, College of Veterinary and Animaol Sciences, Mannuthy for according sanctions to publish this paper.

Table 1. Distribution of Congenital anomalies among calves & kids.

Anomaly observed	Calves		Kids		Total	Percentage
	Male	Female	Male	Female		
DIGESTIVE SYSTEM						
1. Abnormal pharynx with Double tongue	1	—			1	1.1
2. Agenesis of anus and valva	—	1	—	1	2	2.2
3. Atresia ani	2	2	3	4	11	12.2
4. Atresia ani with prolapse of colon	—	1	—	—	1	1.1
5. Atresia ani with rectovaginal fistula	—	6	—	—	6	6.7
6. Atresia ani with rectovesical fistula	—	—	1	0	1	—1.1
7. Atresia ani with rectourethral fistula	—	—	1	0	1	1.1
8. Atresia ani with urethral diverticulum	—	—	1	—	1	1.1
9. Atresia ani with Hypospadia and rectovesical fistula	—	—	1	—	1	1.1
10. Atresia ani et recti	4	1	1	2	8	8.9

11.	Atresia ani et recti with Taillessness	1	—	—	—	1	1.1
12.	Atresia ani et recti with rectovaginal fistula	—	1	—	—	1	1.1
13.	Atresia ani et recti with rectovesical fistula	1	—	—	—	1	1.1
14.	Aplasia of rectum	1	1	—	—	2	2.2
15.	Recto vaginal fistula	—	2	—	—	2	2.2
16.	Stenosis of anus with rectovaginal fistula	—	1	—	—	1	1.1
17.	Agenesis of anus and vaiva with rectovaginal fistula	—	—	—	1	1	1.1
		10	**16**	**8**	**8**	**42**	**46.6**
MUSCULAR SYSTEM							
18.	Prolapse of viscera through umbilicus	1	3	—	2	6	6.7
19.	Umbilical hernia	3	1	—	—	4	4.4
20.	Schistosomus reflexus	2	2	—	—	4	4.4
21.	Extended joints	2	0	—	—	2	2.2
22.	Unilateral contracted tendon (Knee joint)	—	—	1	0	1	1.1
23.	Bilateral contracted tendon (Knee joint)	1	1	—	—	2	2.2
24.	Bilateral contracted tendon (Fetlock joint)	0	2	—	—	2	2.2
		9	**9**	**1**	**2**	**21**	**23.2**
UROGENITAL SYSTEM							
25.	Urethral diverticulum	—	—	5	0	5	5.6
26.	Hermaphrodite	—	—	—	—	2	2.2
27.	Pervious urachus	2	0	—	—	2	2.2
		2	**0**	**5**	**0**	**9**	**10.0**
OCULAR SYSTEM							
28.	Unilateral dermoid Cyst	2	3	—	—	5	5.6
29.	Bilateral dermoid Cyst	0	3	—	—	3	3.3
		2	**6**	**—**	**—**	**8**	**8.9**
SKELETAL SYSTEM							
30.	Absence of Pubis	0	1	—	—	1	1.1

31.	Bilateral dislocation of Patella	1	2	—	—	3	3.3
32.	Amelia	0	1	—	—	1	1.1
33.	Scoliosis	—	—	0	1	1	1.1
34.	Imperfect fusion of Sacrum	—	—	0	1	1	1.1
		1	4	0	2	7	7.7
SKIN DEFECTS							
35.	Foetal anasaraca	1	1	—	—	1	1.1
		1	1	—	—	1	1.1
NERVOUS SYSTEM							
36.	Hydrocephalus	1	0	—	—	1	1.1
		1	0	—	—	1	1.1
		26 (28.8%)	**36 (40%)**	**14 (15.6%)**	**12 (13.3%)**	**90**	**100**

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Karyological Evaluation of AI Bulls and Potential Young Sires*

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ABSTRACT

A cytogenetic screening of 86 breeding bulls and the potential young male animals belonging to eight genetic groups was conducted in different farms of Madhya Pradesh. The animals were randomly analysed for detection of chromosomal abnormalities. Only five bulls had abnormal percentage of polyploids and breaks/gaps. Out of these, except for one Jersey bull which was azoospermic, all the other bulls were normal without any reproductive disorders. The semen collection of the azoospermic bulls revealed watery semen devoid of spermatozoa. The karyological analysis of this bull revealed the breaks/gaps in 4.63 percent and the polyploid cells in 2.77 percent of the metaphase plates screened.

——x——x——x——

Bulls used in artificial insemination are potentially capable of producing thousands of offsprings and should be routinely examined for chromosomal abnormalities. The association of transmissible chromosomal aberrations with fertility may cause great economic losses to the farmers.

MATERIALS AND METHODS

The number of young and mature bulls from 8 genetic groups included for the cytogenetic studies are indicated in table 1. Apart from these bulls, 2 males of identical twin pair and 3 male partners of heterosexual twin pairs of unknown genetic groups from field conditions were also studied cytogenetically. Out of 86 males studied, 62 were mature and 24 were young bulls between 6 to 15 months of age.

The blood samples from all the animals were cultured for obtaining the chromosomes using simplified bovine lymphocyte culture method (Eldridge, 1982). About 100 metaphase plates from each animal was screened for the presence of chromosomal abnormalities.

The semen samples from 8 bulls were also studied for evaluation of semen attributes like volume, concentration, initial motility and percent live and dead count. The cytogenetic screening of these males was conducted simultaneously in order to correlate the semen characteristics with chromosomal anomalies.

RESULTS AND DISCUSSION

The details about the number of animals studied from different genetic groups, number of animals with abnormal metaphases and percent abnormal metaphase observed are indicated in table 1. It is apparent from the table that excluding the twin males, out of 81 bulls screened, only 5 males were found to have chromosomal abnormalities. One Gir bull has 2.94 percent of breaks/gaps, however, the semen of the bull was normal having no report of fertility problem. One Jersey bull had 4.63 percent of polyploid cells and 2.77 percent of breaks/gaps. One bull from HG group had fairly high percent (4.16%) of polyploid cells, but the animal was normal in all respect. One bull each from HJG and

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BHG group showed abnormal percentage of breaks/gaps, however no reproductive problem were observed.

Although the bulls of the identical twin pair was normal cytogenetically, all the three male partners of the heterosexual twinpairs showed different percentage of chromosomal chimerism. The details regarding chromosomal chimerism of these bulls has been discussed in a separate article (Sarkhel and Katpatal, 1994).

Seven out of eight bulls had normal semen picture (Table 2). Although J 399 had 3 percent polyploid cells, it had normal semen characteristics and fertility.

It was interesting to note that the semen of the J 421 was watery, devoid of spermatozoa. The testicles of the bull were normal and the libido was satisfactory. The bull was declared as a case of azoospermia and was subsequently culled from the herd. The cytogenetic screening of the bull revealed normal chromosome number of 60, XY. The bull, however, had abnormal frequency of breaks/gaps (4.63%) and polyploid cells (2.77%). Figure 1 shows the metaphases and karyotypes of this bull indicating the breaks/gaps. Dunn *et al.,* (1980) reported a Hereford bull with testicular hypoplasia and azoospermia but with good libido, however cytogenetically bull was 61, XXY. Stanik and Izarikova (1984) reported the abnormally high percentage of autosomal breaks in bull with impaired spermatogenesis, azoospermia, poor semen quality and testicular hypoplasia.

The polyploids and breaks/gaps in low frequency (<2%) are considered as a natural event of mitosis without much genetic significance. However, their high frequency has been reported to be associated with some reproductive disorders in male (Stanik *et al.,* 1980, Stanik and Izarikova, 1984). Yadav (1981) reported large number of breaks/gaps in one Sahiwal cryptorchid bull.

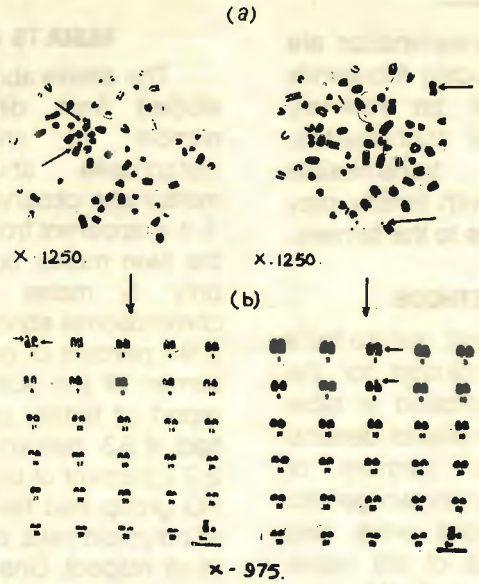

Fig.1 Metaphases and their karyotypes of an azoospermic bull. Arrows indicate the breaks/gaps.

Table 1. Summary of chromosomal abnormalities observed in bulls of different genetic groups.

Genetic groups	No. of A.I. bulls/young male	No. of animals with abnormal metaphase	No. of abnormal metaphases Total No. of cells scored Polyploidy	Gap/Break
Hostein Friesian (HF)	5	0	0	0
Jersey (J)	10	1	5/108 (4.63)	3/108 (2.77)
Gir (G)	5	1	0	3/102 (2.94)
1/2J x 1/2G (JG)	5	0	0	0
1/2HF x 1/2G (HG)	18	1	5/120 (4.16)	0
1/2J x 1/4HF x 1/4G (JHG)	14	0	0	0
1/2HF x 1/4J x 1/4G (HJG)	16	1	0	4/122 (3.28)
1/2BS* 1/4HFx 1/4G (BHG)	8	1	0	4/120 (3.3)
Identical twins	2	0	0	0
Males of heterosexual twin	3	Chimerism		

Figures within parenthesis indicate the percent abnormal metaphases. * Brown Swiss

Table 2. Semen analysis and the chromosome aberrations of the eight bulls of different genetic groups.

Genetic group	Bull No.	Volume (ml)	Conc. of sperms. (Million/mm^3)	Initial motility (percent)	Percent Live	Chromosome abnormalities Breaks/ gaps	Polyploid cells
HG	HI-6	5.75	1015	72.75	82.00	0	0
HG	HI-2	5.75	1041	71.25	80.00	0	0
BHG	C-140	6.90	1240	74.85	85.00	0	0
BHG	C-145	6.30	1090	73.25	83.00	0.05%	0
BHG	C-299	5.25	1061	76.25	86.00	0	0
BHG	C-411	5.40	1102	75.50	84.00	0	0.5%
Jersey	J-399	4.80	1160	75.25	85.00	1.5%	3.0%
Jersey	J-421	1.50	0	0	0	4.63%	2.77%

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Assessment of Sperm Kinematics of Frozen-Thawed Ram Spermatozoa

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ABSTRACT

Ram spermatozoa is susceptible to freezing. In an effort to develop a freezing protocol for cryopreservation of ram semen, a need was felt to quantify changes which occur in the spermatozoal motion due to freeze-thaw process. The aim of the present study was to apply computer-assisted sperm analysis technique for objective assessment of spermatozoal track in pre and post-thaw conditions. Prior to freezing there was not much difference in sperm kinematics of samples assessed during post-dilution and post-equilibration stages. However thawing affects sperm velocity, motility and amplitude of lateral head displacement of ram spermatozoa. The application of this technique offers a high degree of accuracy and a large range of capabilities which are useful for gaining deeper understanding on the motion characteristics of frozen-thawed ram spermatozoa.

——x——x——x——

Cryopreservation of ram semen is dependent upon number of interrelated factors (Fiser and Fairfull, 1986; Pontbriand *et al.,* 1989, Holt *et al.,* 1992). In order to optimize various variables for achieving good post-thaw survival of ram spermatozoa consistantly there is a need to specify and quantify sperm motion characteristic during various stages of cryopreservation. The aim of the present study was to apply computer-assisted sperm analysis (CASA) technique for distinguishing spermatozoal motion of ram spermatozoa in pre and post-thaw conditions.

MATERIALS AND METHODS

Adult Malpura rams were used as semen donars. Ejaculates of good quality semen were pooled and extended @ $1x10^9$ spermatozoa per ml (Mathur *et al.,* (1991) in Egg yolk tris glycerol extender. The extended samples were filled in 0.25 ml size straws, equilibrated and then frozen @ -5°C per minute in a programmable cell freezer. Straws were stored in liquid nitrogen until required. Thawing was done at 50°C for 10 seconds in a water bath. Fifty observations of post-thaw samples from three freezings were analysed by CASA by using a HTM-S (Version 7.2Y) Hamilton-Thorn Motility Analyser (Hamilton-Thorn Research, Inc., USA). The analyser set up was as follows : Temperature : 37°C; Chamber: Makler (10 um); Image type: Phase contrast; Acquisition rate: 25 frames per second; Acquisition time : 0.8 seconds; Minimum contrast: 8; Minimum size: 6; LO/HI size gates: 0.6 to 1.8; LO/HI intensity gates: 0.6 to 1.8; Magnification : 2.17.

Freshly diluted, equilibrated and thawed samples were suitably diluted (10 to 50 folds) in isotonic sodium chloride or sodium citrate solution for taking observations. The HTM-S parameters included in the analysis were a) percent motility, b) mean track speed or curvilinear velocity (CLV), average path velocity (APV) and mean progressive velocity or straight line velocity (SLV), c) percent rapid (VAP>25 um/second), medium (10<VAP<25) um/second) and

slow (0<VAP<10 um/second) moving spermatozoa, d) percent mean linearity and straightness, e) mean amplitude of lateral head displacement (ALH) and f) mean beat frequency for all observations.

RESULTS AND DISCUSSION

Visual estimates of sperm motility during cryopreservation of ram semen provide a simple but subjective assessment of sperm motion parameters. These estimates cannot specify changes which occur in the motion characteristics due to freeze-thaw process. The application of CASA technique enables rapid, precise and objective evaluation of sperm motion (Budsworth *et al.,* 1988, Amann, 1989, Suittiyotin and Thwaites, 1992, Yeung *et al.,* (1992). Apart from identifying motile and immotile spermatozoa it can also compute the sperm velocity and track dimensions of the motile spermatozoa. Sperm velocity and motility are known to be correlated with fertility (Holt *et al.,* 1985, Aitken 1990). Within broad limits, it is not the proportion of motile sperm that is important, the velocity of moving sperm and the way they move rather than quantity (Moore, 1989).

There was not much difference in sperm Kinematics assessed immediately after dilution at 30°C as compared to values obtained after equilibration at 5°C (% motility 94 vs 95; % rapid 92.5 vs 92; CLV 144 vs 139 um/second, APV 112.5 vs 108.5 um/second; SLV 87.5 vs 85 um/second and amplitude of lateral head displacement of spermatozoa 7.5 vs 7.2 um, respectively). It indicates that slow cooling of extended semen samples prior to freezing is not detrimental to the velocity and motility of ram spermatozoa. After thawing there was a decrease in the values of spermatozoal (% motility 69.8; % rapid 47.1; CLV [illegible] um/second; APV 80.6 pm/second; SLV 69.2 um/second and ALH 6.0 um). There was a higher relative distribution of motile cells towards medium and slow categories which was not prominent in samples assessed prior to freezing. Observations with experimental cryomicroscopy has indicated that freeze-induced membrane damage in ram spermatozoa in manifested after thawing (Holt *et al.,* (1992). Although cryomicroscopic studies were not undertaken in this experiment but it appears that the higher coefficient of variation in the post-thaw values obtained with CLV APV SLV and percent rapid, medium and slow categories of spermatozoa as compared to percent post-thaw motility was the manifestation of freeze-induced membrane damage which was more clearly evident on the behaviour of sperm velocity as compared to motility. However, there was not much difference in percent linearity, percent straightness and mean beat frequency of spermatozoa at various stages of cryopreservation (Tables I).

Application of CASA has been very useful in conjunction with programmable freezing during our efforts to develop improved freezing protocols for ram semen as it can help in differentiating and quantifying the relative distribution of motile spermatozoa.

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Table 1. Sperm kinematics of ram spermatozoa during cryopreservation.

S.No.	Semen Characters	Dilution (Mean±S.E.)	Equilibration (Mean±S.E.)	Thawing (Mean±S.E.)	(Range)	(% CV)
1.	**Motility Estimates (%)**					
	Motility	94.0±2.00	95.0±0.00	69.8±1.61	50-88	16.3
	Rapid	92.5±1.50	92.0±1.00	47.1±1.50	19-68	22.5
	Medium	1.0±0.00	3.0±0.00	15.3±0.73	8-26	33.6
	Slow	0.5±0.49	0.0±0.00	7.1±0.43	3-17	42.8
2.	**Sperm Velocities (μm/second)**					
	Curvilinear	144.0±3.99	139.0±4.99	99.2±2.44	58-165	17.4
	Average path	112.5±5.49	108.5±3.5	80.6±2.37	43-145	20.8
	Straight line	87.5±4.49	85.0±3.99	69.2±2.35	32-131	24.0
3.	**Track Dmensions and Frequency**					
	Linearity (%)	60.5±0.49	60.5±0.49	63.1±0.87	48-76	9.8
	Straightness (%)	75.5±1.50	75.0±1.00	77.9±0.73	56-86	9.6
	Amplitude of Lateral head displacement (μm/Sec)	7.5±0.04	7.2±0.85	6.0±0.13	3.9-8.1	16.2
	Beat frequency (Hz)	10.4±1.00	10.9±1.70	10.4±0.99	8.1-12.4	9.5

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Effect of Ultra low Temperature on Acrosomal Integrity of Buck Spermatozoa in Tris and Citrate Dilutors.

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ABSTRACT

Fifty ejaculates obtained from five non-discript adult deshi bucks were used to study the effect of deep freezing on acrosomal changes in buck spermatozoa. The semen samples were diluted in TEYFCAG (Tris-Egg yolk-Fructose-Citric Acid Glycerol) and EYCFG (Egg yolk-Citrate-Fructose-Glycerol) dilutors and frozen in liquid nitrogen and preserved for 24 hrs. The percent intact acrosome (PIA) declined from 88.76±0.43 in prefreeze to 68.90±0.82 in post thaw semen when diluted in TRIS and from 85.98±0.47 to 56.70±1.15 when diluted in EYC dilutors. In post-thaw semen the percent of swollen, ruffled, fractured, separating and entirely lost acrosomes were estimated as 14.68±0.21; 7.94±0.35; 1.82±0.12; 3.12±0.16 and 3.98±0.20 in TRIS and 19.30±0.69; 10.84±0.46; 2.78±0.14; 4.20±0.24 and 5.22±0.29 in EYC dilutor respectively. The total acrosomal damage increased from 11.28% to 24.04% in TRIS and from 13.98 to 42.34% in EYC dilutor due to freezing.

——x——x——x——

Acrosome contain several hydrolytic enzymes which are involved in the fertilization process (Mann and Lutwak-Mann 1981). The equitorial segment of the acrosome is very much important because it is this part of the spermatozoa along with the anterior portion of the post acrosomal region., which initially fuses with the oocyte membrane during fertilization. (Hafez, 1987). Any alteration or loss to acrosomal structure and content might affect the fertilizing capacity of spermatozoa. Studies on the acrosomal integrity has been conducted extensively in bull and ram semen, but report in buck semen is scanty. As such, present investigation, reports the effect of freezing on changes in acrosomal structure in buck spermatozoa.

MATERIALS AND METHODS

Fifty ejaculates from five adult non-discript Indian bucks of 2-3 years age were used in this study. The bucks were maintained under semi-inensive management regime and were housed away from the does in the animal shed of the Institute. The bucks were allowed for grazing for 4-6 hours daily and were fed 200gm of concentrate mixture each. The semen was collected twice a week in artificial vagina using anoestrus doe. After the initial evaluation of semen for its physical characteristic, the samples having high motility (mass motility 4 and progressive motility above 80%) were split into two. The first part was diluted in TEYFCAG (Tris-3.028%, Egg yolk-20%, Fructose 1.250%, Citric acid 1.675%, Glycerol-7%), and the second part was diluted in EYCFG (Egg yolk-20%, Sodium citrate-2.98%, Fructose-1.250% and Glycerol7%). The semen samples were diluted at the rate of 1:20 with freshly prepared dilutors in one step. The samples were filled in medium french straws and frozen in liquid nitrogen after providing a combined cooling and equilibration time of two hours. After preservation for 24 hours, the straws were thawed dipping in water at 37°C for 30

seconds. The acrosomal morphology was studied in freshly collected semen, pre-freeze semen and post-thaw semen using Giemsa stain as per method described by waston (1975). A total of 200 spermatozoa per slide were examined under oil immersion objective (100 x) of microscope. The acrosomal abnormallities were clasified as swollen, ruffled, fractured, separating and entirily lost (Fig 1-5) (Saacke *et al.,* 1968, Watson, 1975). The statistical analysis was done as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The results of the present study indicated significant variation between bucks in the values of intact acrosomes ($p<0.01$), swollen acrosomes ($p<0.01$), ruffled acrosomes ($p<0.05$) and fractured acrosomes ($p<0.05$) (Table 1). The mean of all the types of acrosomes differed significantly ($p<0.01$) between dilutors. Loss to acrosomal structure was observed less in TEYFCAG dilutor, whereas it was more pronounced in the samples diluted in EYOFG. The intact acrosomes which were 94.16% in fresh seman (pooled samples) declined to 88.76% and 68.90% in pre-freeze semen diluted in Tris based diluter whereas it declined drastically to 85.98% and 56.70% in pre-freeze and post-thaw semen respectively in citrate based dilutor. This indicated that Tris dilutor provided better protection to acrosomal structure than citrate dilutor in ultra low temperature. Probably the buffering ability of Tris was better than that of citrate based dilutor. This fact is also supported by Edwin *et al.,* (1975) who offered the view that Tris dilutor readily penetrates the sperm cells and acts as an intracellular buffer, which reduces the damage caused to spermatozoa due to cold shock at ultra low temperature.

The alteration acrosomal structures increased progressively and significantly ($p<0.01$) between stages of freezing process. The total acrosomal abnormalities increased from 5.85% in fresh semen (pooled) to 11.28% and 31.04% in pre-freeze and post-thaw semen respectively in Tris and to 13.98% and 42.34% in pre-freeze and post-thaw semen respectively in citrated dilutor. Amongst the different types of acrosomal abnormalities recorded, swollen acrosomes constituted highest percentage and was significant ($p<0.01$) between bucks, between dilutors and between stages of freezing process. Similarly ruffled, fractured and separating acrosomes were also significantly ($p<0.01$) influenced by the three factors. However enterily lost acrosomes was influenced significantly ($p<0.01$) only by dilutors.

The report on acrosomal changes in goat semen is scanty. However the values in the present investigation are nearer to those reported by Memon *et al.,* (1985) and slightly higher than Deka and Rao (1985, 1987) who also observed better results in Tris than EYC dilutor. Lower percentage of acrosomal abnormalities than the present investigation was reported by Chaudhary *et al.,* (1988). Das and Raj Konwar (1994, 1994[a]) during freezing of buck semen.

The acrosome sustain damage during processing of semen and freezing or preservation of spermatozoa. The damage may occur during dilution and cooling; and according to Tasseron *et al.,* (1977), 50% of the damage takes place at this stage. In the present study too, damage of acrosomal structure during processing and freezing was significant. According to Jones and Martin (1973) and Jones and Stewart (1979), the alteration takes place during their exposure to cold, which causs a

Table 1. Mean ± S.E. and 'F' value of Normal and abnormal acrosomes (%) obtained during freezing of buck spermazoa (n=50)

Acrosomes (%)	Fresh semen	Diluted semen				F value		
		TEYFCAG		EYCFG				
		Pre-freeze	Post-thaw	Pre-freeze	Post-thaw	Between bucks (d.f.=4)	Between dillutors (d.f.=1)	Between stages of freezing (d.f.=2)
Intact acrosomes	94.16 ±0.25	88.76 ±0.43	68.90 ±0.82	85.98 ±0.47	56.70 ±1.15	9.27^{xx}	162.76^{xx}	731.38^{xx}
Swollen acrosomes	2.30 ±0.13	4.32 ±0.25	14.18 ±0.57	5.14 ±0.26	19.30 ±0.69	4.10^{xx}	52.40^{xx}	456.43^{xx}
Ruffled acrosomes	1.91 ±0.08	3.86 ±0.21	7.94 ±0.35	4.52 ±0.21	10.84 ±0.46	2.67^{x}	4.72^{xx}	157.25^{xx}
Fractured acrosmes	NIL	0.44 ±0.10	1.82 ±0.12	0.84 ±0.09	2.78 ±0.14	2.74^{x}	33.39^{xx}	97.82^{xx}
Separating acrosomes	1.04 ±0.06	1.54 ±0.13	3.12 ±0.16	1.96 ±0.12	4.20 ±0.24	3.17^{x}	32.55^{xx}	68.39^{xx}
Entirely lost acrosomes	0.60 ±0.06	1.12 ±0.13	3.98 ±0.20	1.52 ±0.11	5.22 ±0.29	1.96	41.78^{xx}	204.82^{xx}

n= Number of observations, S.E.= Standard error, d.f.= degree of freedom
TEYFCAG= Tris Egg yolk Fructose Citric Acid Glycerol; EYCFG= Egg yolk Citrate Fructose Glycerol.
x= significant at 5% level. xx= significant at 1% level.

considerable ultrastructural changes of acrosome leading to the disruption of the plasma and the outer acrosome membrane resulting in the loss of acrosomal content. However, the cellular integrity and permeability may alter during storage and aging (Saacke and White 1972; Varanavaskii, 1978).

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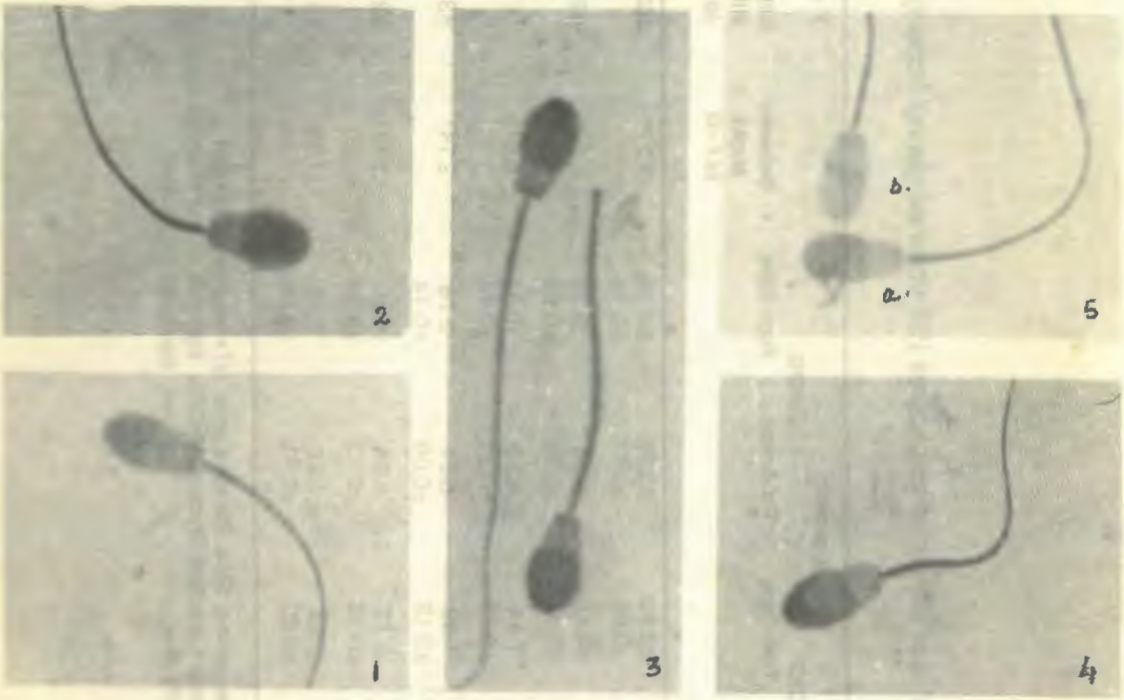

Fig. Different acrosomal changes:
1. Normal 2. Swollen 3. Ruffled
4. Fractured 5. Separting and lost

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Studies on the occurrence and freezability of static ejaculates in buffalo bulls.

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ABSTRACT

In the present investigation involving 178 ejaculates collected from 9 Murrah buffalo bulls, a frequency of 46.0 per cent of static ejaculates was observed. The freezability (per cent of ejaculates successfully frozen) of static ejaculates vis-a-vis normal ejaculates was 87.8 vs 95.8 percent. The average post thaw motility of frozen semen was 40.1±0.84 in case of static ejaculates whereas it was 44.3±0.75 per cent in case of normal ejaculates. The discard rate of semen after freezing was 12.2 and 4.2 per cent in case of static and normal ejaculates respectively. The results indicate that although static ejaculates result in a little higher percentage of discard rate after freezing, with a little lower average post thaw motility majority of these can be perfectly freezable.

——x——x——x——

Buffalo semen is generally characterised by two types of mass activity viz ejaculates having wave motion similar to those observed in cattle and ejaculates found to be of cell mass (static) without sperm activity. Sahni (1990) reported that the percentage of ejaculates showing normal wave activity in buffalo semen averaged 30-50 compared to 80-100 in cattle. Sahni and Mohan (1990) also reported that the occurrence of static ejaculates was fairly high (40-100%) in all the buffalo bulls used during winter season at IVRI Izatnagar. Abhi *et al.*, (1968) also reported an average of 38% incidence of static ejaculates in buffalo bulls. Freezability of static ejaculates collected in buffalo bulls is studied and reported in this paper.

MATERIALS AND METHODS

Semen samples were collected from 9 bulls under progeny test programme of AICRP using artificial vagina. About 178 ejaculates were collected during Nov.-March of the year. All the bulls were kept under a standard feeding and management conditions at the institute and were offered liberal quantities of green fodder and wheat straw in addition to concentrate mixture.

The semen samples were evaluated for volume, colour, consistency and mass activity on a warm stage (37°C) using a Nikkon Phase contrast Microscope attached with a video monitor. All the glasswares used were sterilized in oven prior to use and kept at 37°C.

The semen without any wave motion on microscopic examination were classified as static. Such samples were diluted with tris diluter in the ratio of 1:2 and again examined. A majority of such samples showed good motility after dilution and were processed for freezing as described earlier (Jindal 1994). Normal ejaculates having +++ or more mass activity on a scale of four were also similarly procesed for freezing. A total of 178 ejaculates (82 static and 96 normal) were evaluated. The straws were thawed for 30 sec. in a water bath at 40°C and examined on a warm stage for post thaw motility.

RESULTS AND DISCUSSIONS

A frequency of occurrence in static ejaculates was found to be 46 per cent

(Table) and was less than reported earlier by Sahni and Mohan (1990) and more than that reported by Abhi *et al.,* (1968). The variation in the percentage of static ejaculates could be due to different in management, season etc.

The freezability of static ejaculates vis a vis normal was 97.8 vs 95.8 precent. The average post thaw motility of frozen semen was 40.1±0.84 in case of normal ejaculates. The discard rate of semen after freezing was 12.2 and 4.2 percent in case of static and normal ejaculates respectively. The study indicate that although, static ejaculates result in a little higher percentage of discard rate after freezing and a little lower average post thaw motility, there seems to be no reason to reject them from further processing for freezing at the initial mass activity evaluation.

Table 1 Frequency of occurrence and freezability of static ejaculates in buffalo bulls.

No. of bulls used	9
Total ejaculates collected	178
Static ejaculates observed	82 (46.0)
Normal ejaculates observed	96 (54.0)
Frozen successfully:	
i) Static	72 (87.8)
ii) Normal	92 (95.8)
Post thaw motility observed:	
i) Static	40.14±0.84
ii) Normal	44.13±0.79
Discard rate:	
i) Static	(12.2)
ii) Normal	(4.2)

Figures in parentheses are in percent.

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Effect of Caffeine on Buffalo Semen Cryopreservation

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ABSTRACT

Ten ejaculates, each from five fertile buffalo bulls exhibiting ≥70 per cent initial motility were extended in Tris-egg yolk (T_1) and Tris-egg yolk caffeine (T_2 with glycerol (7%) and subsequently frozen at - 196°C. The sperm motility, livability and morphology during prefreezing (fresh, extended and after equilibration) and 24 h after freezing was examined in both the extenders. Caffeine puriss used as additive in the concentration of 7 mM to Tris-egg yolk glycerol showed harmful effect on spermatozoan motility, livability and morphology during cryopreservation.

——x——x——x——

Caffeine, a cyclic AMP phosphodiesterase inhibitor increases the extracellular cAMP level by thirty five per cent (Schoff and Lardy, 1987). The role of caffeine in better preservation of buffalo semen has been demonstrated by several workers (Miyamoto and Nishikawa, 1979, Singh *et al.,* (1986), Gehlaut and Srivastava, 1987 and Hukiri and Shinde, 1988). In the present study caffeine puriss in concentration of 7 mM was added in Tris-egg yolk glycerol extended semen and its effect was seen at various stages of freezing.

MATERIALS AND METHODS

A total of fifty ejaculates (ten from each) from young healthy buffalo bulls were collected in artificial vagina (42-45°C) at weekly intervals. After initial evaluation of semen (spermatozoan motility ≥ 70 per cent), it was diluted in Tris-egg yolk glycerol (T_1). and its modified form containing caffeine puriss (T_2). Composition of extenders is given as:-

Tris-egg yolk glycerol (T_1)

Tris (Hydroxymethyl amino methane)	3.280 g
Citric Acid	1.675 g
Fructose	1.250 g
Distt. Water	100.00 ml
Egg yolk : Buffer	1:4
Glycerol	7%
Volume taken	20 ml

Tris-egg yolk caffeine-glycerol (T_2)

Stock solution was prepared by dissolving 136 mg of caffeine puriss in 5 ml of Tris buffer (7 mM). I ml of stock solution was mixed with 19 ml T_1 extender to prepare 20 ml T_2 extender.

The extended semen was then frozen and preserved at -196°C. The spermatozoan motility, live sperm count, sperm head and tail abnormalities and per cent intact acrosomes were examined in fresh semen (Stage I), after complete extension (Stage II), after equilibration (Stage III) and 24 h after freezing (Stage IV) in each ejaculate, Tris-egg yolk extended semen (T_1) and its modified form (T_2)

RESULTS AND DISCUSSION

The mean percentage of motile spermatozoa and live sperm count at various

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stages of freeze preservation of buffalo semen extended in T_1 and T_2 are compared. The average spermatozoan motility of fresh semen was recorded as 73.86±0.29 per cent. The average per cent spermatozoan motility after complete extension(Stage II), after equilibration (Stage III) and post freeze thaw (Stage IV) in the two extenders was 71.06±0.33, 69.38±0.23, 43.36±0.55 in T_1 and 70.22±0.28, 68.92±0.27, 39.92±0.64 in T_2 respectively. Statistical analysis revealed that the treatment T_2 showed significantly ($p<0.05$) lower motility than T_1 at post freeze thaw stage. The percentage of live spermatozoa in fresh buffalo semen was recorded as 83.43±0.45. The values at stage II, III and IV were as 78.90±0.71, 76.69±0.69, 52.33±0.69 in T_1 and 78.69±0.63, 76.14±0.63, 47.24±0.45 per cent live sperm in T_2. Treatment T_2showed significantly ($p<0.05$) reduced live sperm count as compared to T_1 at post freeze thaw stage. There was non-significant difference in terms of motility and live sperm per cent between T_2 and T_1 upto equilibration stage.

The sperm head tail abnormalities and intact acrosomes in fresh semen were found to be 2.17±0.04, 8.15±0.33 and 94.42±0.07 per cent, respectively. Statistical analysis revealed that head anormalities (3.05±0.05; 3.72±0.04 and 4.88±0.04) and tail abnormalities (14.69±0.66, 18.05±0.77 and 23.39±0.62) were significantly ($p<0.05$) high and intact acrosome percentage significantly ($p<0.05$) low (87.57±0.44; 82.69±0.61 and 63.02±0.62) in T_2 instages II, III and IV respectively when compared to T_1 extended semen.

It has been reported that the stimulatory effect of caffeine is more prounounced in semen having initially poor motility (Schoefeld *et al.,* 1973, 1975, Schill, 1975 and Schill *et al.,* 1979). Lately, Mouss (1983) observed that in dose >6 mM caffeine exhibited a slight insignificant stimulation that reversed later into an inhibitory effect on all parameters of sperm motility in a dose of 60 mM/ml. Results of the present study also support these views. All the semen samples taken for the study were of good quality (with initial motilit of at least 70 per cent) and the caffeine concentration was 7 mM which resulted in non-significant change in sperm motility and live sperm count in comparison to T_1 upto post equilibration stage.

Contractile proteins, spermosin and flactin, present in sperm tail are similar in behaviour and molecular structure to myocin and actin of vertibrate skeletal muscle. It is observed that when skeletal muscle is exposed to caffeine, large quantity of lactic acid produced, oxygen consumption is much increased and there is strong muscle twitch, with higher concentration of caffeine contracture results. Harrison *et al.,* (1980) observed that longer incubation of spermatozoa with caffeine had a deleterious effect on spermatozoal uitrastructure and elemental composition. The semen samples were frozen approximately 6 h of incubation with caffeine resulted in poor post-thaw motility and livability in comparison to Tris egg yolk glycerol.

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A Study on the Physical Characters of Semen of Hybrid (Broiler and Layer) and Deshi Cross Fowl

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ABSTRACT

Semen characteristics in terms of volume, motility, sperm concentration, percentage of live spermatozoa and different types of abnormalities of head, midpiece and tail were evaluated for three genetically different groups of fowl namely Hybrid Broiler, Deshi Cross and Hybrid Layer. The mean values of the above characteristics were respectively 0.26±0.02, 0.17±0.01 and 0.15±0.001 ml; 75.0±1.27, 73.33±1.38 and 77.63±0.87%; 1.80±0.1 x 10^9, 2.06±0.08 x 10^9 and 1.63±0.09 x 10^9 per ml; 85.57±1.72. 87.79±0.92 and 87.76±0.99%; 8.60±0.63, 8.53±0.86 and 6.41±0.6%; 1.54±0.21, 1.94±0.25 and 1.57±0.16%; 3.46±0.32, 3.49±0.21 and 3.91±0.28% in the three groups and semen quality of Hybrid Broiler was superior followed by Deshi Cross and Hybrid Layers.

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In practice, hybrids are not used for further breeding as it is expected that the heterotic effect shown in the hybrids would revert back in the next generation. Accordingly there is very little information regarding the male reproductive efficiency of the hybrid breeds as well as of their Deshi Cross maintained in India. The present investigation was, therefore, aimed at the evaluation of the semen qualities of the cocks of these varieties in order to assess their reproductive efficiency.

MATERIALS AND METHODS

The present study was based on three varieties of cocks namely Hybrid Broiler, Deshi Cross and Hybrid Layer having four cocks in each variety. All the cocks were 40-45 weeks of age at the time of collection and average body weight of cocks were between 2.75 kg to 4 kg and reared under uniform feeding and management in deep litter system. From each of the cocks ten collections were made at an interval of seven days, by massage method and the ejaculates were measured with the help of tuberculin syringe. The motility and concentration were assessed by examination under the microscope.

The percentage of live spermatozoa and abnormal spermatozoa were calculated over a total of 200 sperms, after preparing semen smears and staining them with Eosin and Nigrosin. Statistical parameters of mean were worked out by the procedures outlined by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Volume: The mean semen volume were 0.26±0.02, 0.17±0.01 and 0.15±0.001 ml respectively in Hybrid Broiler, Deshi Cross and Hybrid Layer. Renden and Pierson (1982) recorded semen volume of 0.14 ml in broiler breed. Cheng *et al.*, (1987) reported semen volume between 0.26 to 0.28 ml in Layers. Available literatures did not idnicate any

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record about the volume of semen in Deshi Cross cock. The semen volume varied highly significantly between the genetic groups and volume of semen in Hybrid Broiler was significantly higher than those of the other two groups. Similar observation was made by Hafez (1968).

Motility: The sperm motility was 75.0±1.27 per cent in Hybrid Broiler, 73.33±1.38 per cent in Hybrid Deshi Cross and 77.63±0.87 per cent in Hybrid Layer breed. However, Kamar and Badreldin (1959) reported a motility of 4.7 for white Leghorn and 4 for New Hampshire. The variations between three groups were significant. It was also observed that the motility of Hybrid Deshi Cross breed was significantly less than Hybrid Layer breed. However, no significant difference was observed between Hybrid Broiler and Hybrid Deshi Cross breed and between Hybrid Broiler and Hybrid Layer breeds.

Concentration of spermatozoa: The mean values of sperm concentration were $1.80\pm0.10\times10^9$, $2.06\pm0.08\times10^9$ and $1.68\pm0.09\times10^9$ per ml in Hybrid Broiler, Deshi Cross and Hybrid Layers respectively. Seid and Al-Soudi (1975) found the concentration (x 10^9 per ml) of sperm of broiler, layer and indigenous cock to be 3.29, 3.19 and 3.01 for respective breeds. These values were higher than the sperm concentration recorded in this study. Present study revealed significantly higher sperm concentration between the genetic groups. The sperm concentration in Hybrid Deshi Cross breed was significantly higher from other two breeds.

The percentage of live spermatozoa: The Hybrid Broiler, Hybrid Deshi Cross and Hybrid Layer breeds showed a mean percent of 85.57±1.72, 87.79±0.92 and 87.76±0.99 respectively. The live spermatozoa for three breeds did not vary significantly and it was also observed that all the three breeds were statistically similar as regard to live spermatozoa percentage. Banerjee and Katpatal (1972) obtained live sperm percentage of 82.86 in White Leghorn and 72.63 in Deshi cocks.

The percentage of abnormal spermatozoa: As regard to spermatozoan abnormality percentage in Hybrid Broiler, Deshi Cross and Hybrid Layer there was no significant variations due to genetic group could be observed in abnormalit, percentage of head, midpiece and tail of spermatozoa. Sevine *et al.,* (1983) found abnormal sperm head ranging from 60.2 to 67.7 percent, abnormal midpiece 24.12 to 26.28 percent and abnormal tails 6.01 to 6.76 percent; where total abnormality ranged from 5.44 to 6.76 and these records varied widely from those made in the present study.

Since the percent abnormal spermatozoa in the three groups were uniformly distributed, the semen quality of Hybrid Broiler could be considered as the superior followed by Deshi Cross and Hybrid Layer with a little discrepancy of motility percent.

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Studies on Biochemical Composition of Buck Semen*

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ABSTRACT

Biochemical analysis of buck semen revealed the initial fructose, total reducing substance, total protein and total cholesterol concentration as 667.72±16.11 mg per cent and 125.65±3.70 mg per cent, respectively. No significant variation was observed between bucks for above constituents. The mean values for electrolytes viz. sodium, potassium, magnesium and inorganic phosphorus concentration were 42.56±1.97 mEq per litre, 59.57±2.25 mEq. per litre, 4.23±0.21 mg per cent and 14.67±0.74 mg per cent, respectively.

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On scanning the available literature, it is observed that only few reports are available on biochemical composition of buck semen. The present study therefore was undertaken to evaluate certain biochemical constituents in buck semen.

MATERIALS AND METHODS

Six healthy bucks procured locally and belonging to all India Co-ordinated Research Project on Goat for Meat Production, located at College of Veterinary Sc. & A.H., MHOW, were included in study. They were kept under uniform managerial conditions. The semen was collected using artificial vagina, twice weekly during morning hours. Thirty six semen samples (six from each buck) were collected and centrifuged and seminal plasma, thus obtained was used for estimation of biochemical constituents.

Biochemical constituents viz. initial fructose, total reducing substances, total protein, total cholesterol, sodium potassium, magnesium and inorganic phosphorus, were estimated using standard methods. The statistical analysis was done according to Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

The overall range and mean and standard error of the biochemical constituents in buck seminal plasma is given in table 1.

Initial fructose concentration in the present investigation is in accordance with the earlier report (Patil, 1970; Igboeli, 1974; Dunder *et al.,* 1983 and Markandeya & Pargaonkar, 1989). However, it is slightly higher than the findings of Roy *et al.,* (1950) and Sane *et al.,* (1982), as they found 465.00 and 250.00 mg per 100 ml of semen, while it is lower than the findings of Barakat *et al.,* (1972), Varshney *et al.,* (1977) and El-Sayed *et al.,* (1983) as they reported 820.00, 1294.50 and 806.11 mg per 100 ml of semen.

The value of total reducing substance in the proesent finding are in accordance with the earlier reports in buck. Varshney *et al.,* (1977) who found 548.00 mg per 100 ml of semen. The total protein concentration found in the present study is slightly higher than the findings of Dunder *et al.,* (1983), who found 2.99 gm protein per 100 ml of semen and it is lower than the findings of Patil (1970) who found 11.60 gm per 100 ml of semen. Protein is essential for buffer action, ionic

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equilibration and protective action. The value of total cholesterol in the present study is in agreement with the findings of Varshney *et al.,* (1977). Cholesterol esters are thought to be a storage form of free cholesterol for the biosynthesis of steroid hormones.

Sodium level in the seminal plasma is slightly lower than the findings of Igboeli (1974), Varshney *et al.,* (1977) & Markandeya and Paragoankar (1990, b). Because of the proesence of sodium and chloride ions an arbaraceous crystal pattern is frequently seen in air dried specimen of seminal plasma (Mann & Mann, 1981). The potassium level is in agreement with the findings of Nair *et al.,* (1982) and Dunder *et al.,* (1983). The values are slightly higher than the findings of Igboeli (1974), Varshney *et al.,* (1977) and Sane *et al.,* (1982), and lower than the findings of Markandeya and Paragoankar (1990, a).

The level of magnesium in the present study is in agreement with the findings of Varshney *et al.,* (1977) and Dunder *et al.,* (1983). However, it is lower than the findings of Pandey *et al.,* (1982), as they found 11.43 and 11.03 mg per cent in Saanen and Barbari buck semen. Magnesium is required for the proper action of adenylate cyclase enzyme which form cyclic AMP from ATP (Mann and Mann 1981).

Inorganic phosphorus level is in accordance with the findings of Pandey *et al.,* (1982) and Markandeya and Paragoankar (1990, a & b). However, these value are slightly higher than the reports of Varshney *et al.,* (1977) as they found 10.59 mg per cent in Barbari buck semen. Inorganic phosphorus is required in various phosphorylation reaction for the formation of phospholipids, phospho-proteins etc, (Mann & Mann. 1981).

Variation in the concentration of biochemical constituents studied was found to be statistically non significant within bucks.

Table 1. Overall Range and Mean±SE of The Biochemical Constituents In Buck Seminal Plasma

Biochemical constituents	Range	Mean±SE
Initial fructose (mg per cent)	457.95-830.57	667.72±16.11
Total reducing substance (mg per cent)	1018.50-2110.50	1463.86±41.26
Total protein (gm per cent)	3.69-5.72	4.69±0.07
Total cholesterol (mg per cent)	79.34-171.90	125.65±3.70
Sodium (m Eq per litre)	28.75-72.50	42.56±1.97
Potassium (Meq per litre)	40-90	59.57±2.25
Magnesium (mg per cent)	2.14-6.76	4.23±0.21
Inorganic phosphorus (mg per cent)	3.56-24.14	14.67±0.74

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Follicle Dissection Vis-A-Vis Oocyte Retrieval From Goat Ovaries

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The large scale embryo production through *in vitro* fertilization of *in vitro* matured oocytes of abattoir origin has gained promineance in recent years. The method of folicle dissection has been tried to maximize the number and quality oocyte retrieval in domestic ruminant (Katska, 1984; Lonergen *et al.,* 1991; 1992; Selvaraj *et al.,* 1992, Mogas *et al.,* 1992; Datta *et al.,* 1993 and Das *et al.,* 1995). The present study was designed to see the availability of different sized (in dia.) follicles and oocyte quality recovered from those follicles obtained from goat ovaries.

Ovaries were collected at a local abattoir. Folicle dissection was carried out using two fine watchmaker forceps under stereozoom microscope at low power magnification. Dissected folicles were then measured approximately with the help of a geometrical divider and scale and grouped in small (upto 2.0 mm.), medium (2.0 to 5.0 mm) and large (above 5.0 mm). On rupture of individual follicle, oocyte was recovered and assessed as good, fair and poor depending on their cumulus investment, compactness and ooplasm granulity.

The results obtained in the present study are presented in the Table 1. The small sized follicles were recovered maximally (68.67%) as compared to medium (27.11%) and large size (4.22%) in this study. The number of oocytes recovered from such follicles was just double than from medium and large follicles as a whole (Table 1). This was well comparable to that reported in goat (Martino *et al.,* 1994). Large sized follicles were more prone for rupture might be a reason of recovering less number of such follicles in this study besides their low availability (Table 1). However, small and medium sized follicles were good in number yielding an average recovery rate of 89.86 per cent oocytes was in agreement to Selvaraj *et al.,* (1992) and Lonergan *et al.,* (1992) but lower than those reported by Katska (1984) and Mogas *et al.,* (1992)

The per cent of culturable quality oocytes were retrieved more from medium sized follicles (Table 1). It may be possible that these follicles exposed to better hormonal stimuli and action growth factors (Lonergan *et al.,* 1992) than small sized follicles in this study. However, the present observation differed from that recorded by Selvaraj *et al.,* (1992). Small follicles yielded high per cent of poor quality oocytes (Table 1). Poor growth and dvelopment of such follicles which usually houses immature oocytes might be the possible reason.

It may be concluded that a good number of antral follicles could be isolated from abattoir recovered ovaries. Follicle size 2.0 to 5.0 mm in dia. could yield better quality oocytes. Availability of follicles upto 2.0 mm dia. may be a good source of oocytes for basic studies and may be tried for IVM-IVF providing adequate hormonal support *in vitro* culture.

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Table 1. Oocyte retrieval from different sized (dia.) follicles recovered from abattoir goat ovaries.

Follicle size (mm)	No.of follicles isolated	No.of oocytes recovered				oocyte recovery rate
		Good >5 layers	Fair 2-5 layers	Poor <2 layers	Total	
1. Small (upto 2.0)	114 (68.67)	11 (10.89)	22 (21.78)	68 (67.33)	101 (100.00)	101/114 (88.60)
2. Medium (>2.0 to 5.0)	45 (27.11)	9 (21.95)	15 (36.59(	17 (41.46)	41 (100.00)	41/45 (91.11)
3. Large (above 5.0)	7	Nil (4.22)	1 *16.67)	5 (83.33)	6 (100.00)	6/7 (85.71)
Total	166 (100.00)	20 (13.51)	38 25.68)	90 (60.81)	148 (100.00)	148/166 (89.16)

The figures in the parentheses are in percent.

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Evaluation of sire and heritability estimates on mortality in triple crossbreds.

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The data on 445 calves (221 males and 224 females) of JFG crosses (50% Jersey + 25% Holstein - Friesian + 25% Gir), spread over from (1980 to 1990), were collected from the records maintained at Cattle breeding farm of this University. Twelve sires were used for breeding purpose. The analysis of variance was done by using the following model (Singh and Parekh, 1982).

Yijl = U + bi + aj + cl + eijl

Where, Yijl - no. of calves died in ith season, jth year and lth sex. U-is the mean number of calves died per cell (bi-is the season effect, aj-is the year effect, cl-is the sex effect and eijl-is the random error). The season, year and sex effects from non-genetic origin were neutralised and corrected data were used for correct evaluation of sire. The heritability was estimated by intra-sire correlation method. The model used for sire analysis was-Yij=M + Si + eij.

These studies revealed that overall 21.34% mortality were recorded 16.74% in males and 25.89% in females indicating higher mortality in females. Highly significant effect of sex on mortality was observed ($p<0.05$), which were contradict.. y with the findings recorded by Verma *et al.,* (1980). Highest mortality was recorded during rainy season (30.07%), then comes winter (18.91%) and lastly summer (16.46%). The highly significant effect of season on mortality was observed in these studies which are in agreement with Verma *et al.,* (1980) and Tajane *et al.,* (1983). The mortality ranged between 11.11% to 27.27% (yearwise). There was non significant effect of year on mortality, while significant year effect was recorded by Tajane *et al.,* (1983) and Patil *et al.,* (1991). Sirewise mortality combined for both the sexes ranged from 13-40% to 33.33% indicatd that B-119 sire proved to be the best and B-157 had worst performance. Non-significant sire effect on mortality was observed in these studies which were in accordance to Tomar and Verma (1988) and Tomar and Tripathi (1992). The heritabiit estimated was (h^2 = 0.0155), indicating that sire did not influence the mortality and desirable effect of least mortality in offsprings could not be achieved through sire selection because most variations are non-transmissable.

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Age, Body Weight and Trans-Scrotal Circumference (TSC) in Mehsana Buffale Bulls.

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The objective was to study at different ages their body weight and TSC and the relationship between age, body weight and Trans-scrotal circumferance (TSC) in the Mehsana buffale bulls.

The material for study consisted of the body weights of 34 bulls with 444 observations from 5 to 28 months and 40 to 47 months. Two hundred thirty five TSC measurements were made from 21 bulls of 6 to 26 months age. TSC was measured using flexible cloth tape at the broadest portion of the testes and scretum (in cms) according to Foote (1969). The body weight in kg. was calculated according to Shastry and Thomas (1976). Monthly measurements and calculations were made for a period of two years. The correlation between age, body weight & TSC in the bulls studied was calculated on regression analysis according to Snedecor and Cochran (1971).

Age (months)	Body weight (kg)	TSC (cms)
6	75.00±4.29	8.81±0.59
12	121.59±4.10	11.22±0.44
18	230.70±11.00	18.75±0.93
24	323.07±16.10	23.92±0.65

The regression analysis showed that there was positive highly significant correlation between body weight and TSC. (cc r : 0.741**; ($p<0.01$).

Foote (1984) reviewed general evaluation of male reproductive capacity. The size of the testis (TSC) proved superior to other parameters. Careful palpation technique will help to correctly assess the TSC. Nema and Kodagali (1994) found that in Surti breed buffalo bulls testis size was highly related to many of the satisfactory semen characters and high TSC value bulls produced better semen quality and good sperm morphology. Differences amongst buffalo bulls existed for the trait TSC and selection for high TSC value bulls deserve application in the field.

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Effect of Storage of Bovine Cervical Mucus on Sperm - Migration *IN-VITRO*

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Cervical mucus from cows is used as a test medium for sperm migration *in vitro* (Berrang 1951). The spermatozoa of bulls migrate a different distance depending upon the quality of the mucus (Murase and Braun 1990). Mucus stored for a longer period without any change in its properties could be used for evaluation of sperm migration in number of samples. The purpose of the present study is to see how long the mucus can be stored and can be used for sperm migration test *in vitro*

Cervical mucus was collected from estrous cows using plastic insemination sheath as per Dabbas and Maurya (1988). The mucus samples which had tertiary branches in their fern pattern and with spinnbarkeit of more than 5cms were alliquoted into 2ml. Storage vials. Half of such sample vials were stored at refrigeration temperature (5°C) and the remaining vials were stored at -20°C.

In Vitro Cervical Mucus penetration Test was performed as per the method described by Anil Kumar and Devanathan, (1995). The distance the vanguard or foremost spermatozoa travelled in a fixed time (30 minutes) was measured in millimeters (SPD).

Frozen semen samples from 6 Jersey bulls processed in 0.5ml. French Medium straws were tested against the mucus samples (fresh & stored). Cervical mucus stored at 5°C and -20°C were tested on day 0, 1, 3, 7, 10 and 15 of storage. Difference in SPD between the days of storage of mucus was tested by factorial experiment in Randomised Block design.

Results of sperm migration tests in cervical mucus stored at 5°C and -20°C are proesented in the table. In the present study storage time had a significant effect on sperm penetration ($p<0.01$). There was a gradual decline in sperm migration from 35.56±1.373 mm on the day of mucus collection to 23.86±0.983 on 10 days of storage, thereafter a sudden decline to 19.94±0.727 mm on 15 days of storage at 5°C. But there was a gradual decline in SPD from 35.56±1.373 to 23.61±1.041 upto 15 days of storage at -20°C. This was in agreement with the findings of Kummerfeld *et al.,* (1981) who found a significant decrease in SPD when mucus samples were stored only for few hours at 4°C and 21°C.

The mucus starts liquefying or loosing its rheologic properties after 15 days of storage at 5°C and after 30 days of storage at -20°C. Hence further testing of the sperm penetration distance in cervical mucus become increasingly difficult. The lose or decay in the rheologic properties of cervical mucus was explained by a model suggested by Lee *et al.,* (1977).

Result presented here provide evidence that the quantitative sperm migration with bovine cervical mucus were greatly affected or influenced by the storage of mucus at 5°C or -20°C, in addition to the difference in SPD due to individual mucus donors.

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Table 1. Sperm Penetration distance (mm/30mts) of frozen thawed bull spermatozoa in cervical mucus stored at 5°C and-20°C

Days of storage	Sperm Penetration Distance (mm)	
	5°C	-20°C
0	35.56±1.37	35.56±1.37
1	32.42±1.17	32.14±1.25
3	29.64±1.16	30.17±1.15
7	26.47±1.06	28.28±1.13
10	23.86±0.98	26.44±1.14
15	19.94±0.73	23.61±1.04

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Cesarean section in a sow - A case report

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Cesarean sections of native black pig is uncommon. A non - descript black sow aged about 18 months was presented to the Casulaty section of Veterinary College hospital. Namakkal. The anamnesis revealed that the animal had delivered one piglet 48 hours before and subsequently there was straining with no progression in labour.

On clinical examination, the general condition of the animal was satisfactory with elevated temperature, pulse and respiration. The vulval lips showed severe oedema and there was a foetid discharge. Vaginal examination and the delivery of the foetus per vaginum was not possible because of severe oedema. A cesarean section was performed after confirming the presence of the fetus in the uterus radiographically.

The animal was restrained on laternal recumbency using ketamine hydrochloride at the dose rate of 15 mg/kg body weight which resulted in good analgesia and muscular relaxation. A horizontal incision was made on the right lower flank. The gravid horns were exteriorized and three emphysematous fetuses along with their placenta were removed. One fetus from the right horn and two from the left horn were removed by separate incisions over each horn. The uterine incisions were repaired with inversion sutures, using catgut. The abdominal muscles and skin were repaired by interrupted sutures using chromic catgut and silk thread respectively. The animal was administered with fluids, antibiotics, and antihistaminics post operatively and the recovery was uneventful.

An important indication for cesarean section in sows is uterine inertia (Renard *et al.,* 1980) which was suspected to be the cause of dystokia in the present case. This delay in expulsion of the fetuses due to uterine inertia resulted in emphysema of the fetus as suggested by Arthur *et al.,* (1989). The low horizontal right flank incision 6 cm above and parallel to mammal was found ideal for approaching the uterus in this case as indicated by Mather (1966).

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Dystokia due to perosomus elumbis monster in a She buffalo (Bubalus Bubalis)

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In cattle, the defects of the Vertebral column are not uncommon. Perosomus elumbis which is primarily a defect in the development of axial and appendicular skeleton occurs in ruminants and swine.

A graded Murrah buffalo aged about 5 1/2 years attended the Veterinary College hospital, Namakkal with the history of dystokia. Vaginal examination revealed fully relaxed cervix and the fetus was in posterior presentation. The tail and ankylosed hind limbs were present in the vaginal cavity. A still born monster was delivered per vaginum by forced traction.

The monster fetus showed congenital abnormality of the skeletal system. The spinal cord was developed upto the thoracic region. The lumbar and sacral regions showed only a flat bone. The fetus had deformed pelvis and arthrogryposis of the fore and rear limbs (Fig). The fetus weighed 20.4 kg. The monster was a typical perosomus elumbis as per the classification of Roberts (1971) and Arthur *et al.,* (1989).

Fig. Perosomus Elumbis monster

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IJAR. 17(1), 1996; 69-70

Recurrent Prolapse due to Vaginal Lipoma and its Management in a Buffalo

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Prolapse of vagina due to vaginal fibroma has recently been reported (Kochhar *et al.,* 1995). The tumours and pedunculated growths in the vagina and cervix often tend to be a potent cause of irritation causing chronic prolapse (Anderson and Davis, 1958). The present communication describes a case of vaginal lipoma in a buffalo which caused recurrent prolapse of vagina.

An eight year old Nili Ravi buffalo was brought to PAU Veterinary Clinics with the complaint that a whitish tennis ball shaped structure was seen along with vaginal prolapse. Also, there was history of repeat breeding. On vaginal exploration, a round mass with greasy feeling was observed to be attached to the ventral part of the distal one-third of vagina. It was greyish white, moderately firm in consistency and movable (Fig.).

The animal was administered epidural anaesthesia and the perenium was scrubbed with antiseptic solution. The vaginal growth was explored after separating the vulvar lips and was ligated and transfixed at base using chromic catgut no.3. The mass was dissected and the bleeding vessels were cauterised. The site was painted with oint. Soframycin. In addition, Antibiotics (Streptopenicillin 2.5 gm i.m.) and Analgesics (Algesin, 15 ml i.m.) were administered for 5 days. The animal made uneventful recovery and subsequently came into oestrus after 3½ months and was successfully inseminated.

The histopathlogical examination of the tissue revealed it to be a lipoma. Lipomas of the vagina and vulva have predominantly been reported in canines (McEntee, 1990). Its occurrence in the genital tract of bovines and as such to cause prolapse of vagina has not been reported, although some workers (Nair and Sastry, 1954; Nanda *et al.,* (1987) have attributed the prolapse of vagina to tumours of the genital tract which may induce continuous irritation and straining. Excessive deposition of fat in the perivaginal tissues may also enhance the mobility of the vagina (Arthur, 1975) aiding its prolapse.

The treatment of benign tumours involved its surgical excision following which there was no straining and the recurrent vaginal prolapse was retained (Kochhar *et al.,* 1995). The vaginal lipoma in the present case might have hindered with the natural / artificial insemination and thus caused repeat breeding. The surgical excision of the tumourous mass lead to the reduction of the vaginal prolapse as well as initiation of the normal reproductive cycles.

It thus appears that the vaginal tumours, if benign should be surgically excised for successful management of the reproductive problems.

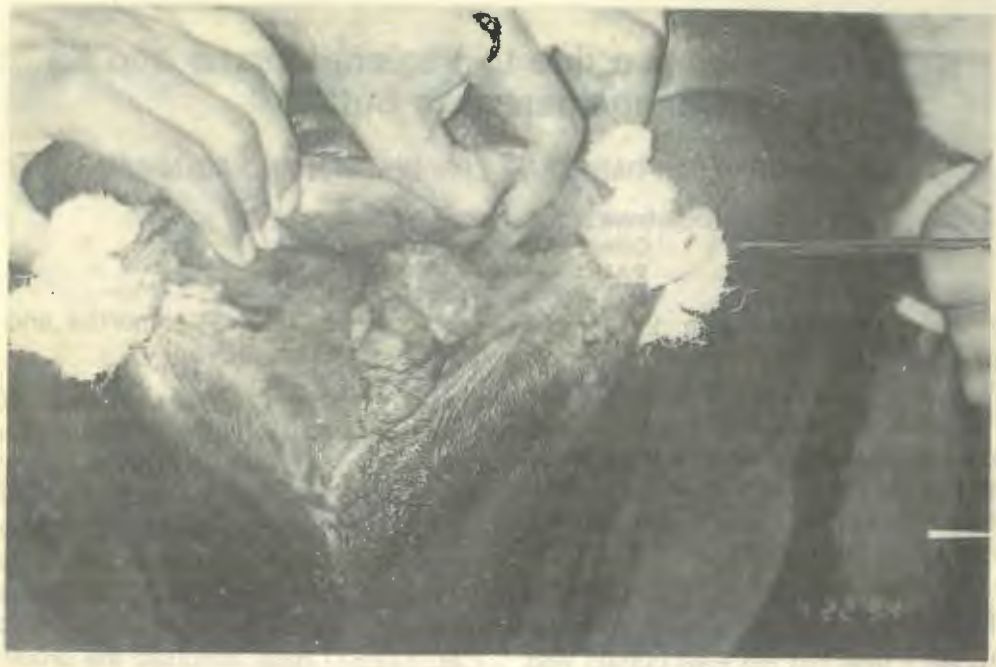

Fig. Vaginal Lipoma in a Buffalo

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Dystokia Due to Fetal Ascites in Murrah Buffaloe - A Case Report

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The incidence of fetal dystokia due to ascites or hydroperitoneum has been reported in Ayrshire, Friesian and Swedish low land cattle. However, dystokia due to fetal ascites has been reported in indigenous cow (Pandit and Singh, 1990) and in non - descript buffaloes (Iyengar, 1943; Sastri *et al.*, 1975 and Devanathan *et al.*, (1990).

A pleuriparous Murrah buffaloe aged 7 years was brought to the Veterinary College clinic for the treatment of dystokia. The animal had completed the gestation period and expressed symptoms of initiation of parturition 48 hours back. Strong straining resulted into expulsion of two limbs of fetus into birth canal. Then traction was applied by owner and local veterinarian without any success.

On general examination animal was found dull, depressed, recumbent and exhausted. the pulse rate, rectal temperature and respiration rate were within normal limits. The hooves of fetal limbs were dried and vulva appeared swollen and cyanotic. Pervaginal examination revealed dried and lacerated birth canal. The dead fetus was in posterior presentation and dorso - sacral position. Fetus could not be palpated beyond hind quarter. On per rectal examination, the abdomen of fetus was found exceptionally very large in size. As delivery of fetus through traction or fetotomy was not possible, it was decided to perform caesarean section.

Treatment- Before operation, animal was treated with enough intravanous fluid, cortisone and antibiotics. The caesarean section was performed under local infiltration analgesia at left paramedian site, following all aseptic precautions. The head and ferelimbs of the fetus were removed without any difficulty but distended abdomen could not be removed. Therefore, the abdomen was punctured at umbilicus in situ and about 10 litres of fluid was drained off and fetus was removed by applying mild traction. The placenta was found necrotic, fragmented, and removed mannually. The surgical wound was closed in routine manner & animal was kept indoor for next 3 days for observation & further necessary treatment.

Fetal Examination - The fetus was fully grown and the abdomen was still greatly distended (Fig.). The total weight of fetus before dissection was 70 kg. During dissection 36 litres of clear watery fluid was removed from the abdomen of fetus. The detailed post - mortem examination of fetus revealed no abnormalities except ascites.

Fetal ascites may occur due to diminished urinary excretion (Jubb and Kennedy, 1970). Occasionally, ascites is associated with a dropsical condition of uterus, mesotheliomas of fetal abdomen and Brucellosis (Roberts, 1971). According to Sloss and Dufty (1980), the obstruction of lymphatics prevent the circulation of peritoneal fluid which may cause fetal ascites. In the present case, dropsy of fetal membranes was not noticed as observed by Sastry *et al.*, (1975).

Fig. Note the greatly distended abdomen of fetus.

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A note on Abortions consequent to Fetal Ascites / Anasarca in Cattle

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The causes of abortion in animals are numerous but if a similar defect appears quite frequently in related individuals, a genetic cause should be suspected. Amongst the various agents causing abortion, genetic or chromosomal causes have been considered to be less serious. Abortion of congenital dropsical fetuses have been reported in Aryshire and Swedish Lowland cattle, attributed to autosomal recessive genes (Donald *et al.,* 1952; Herrick and Eldridge, 1955).

Five abortions in Jersey cows with varying degree of fetal ascites and anasarca were reported in HPKV Dairy Farm between August, 1992 and February, 1993. Earlier Artificial insemination using semen from different bulls was carried out. One of the cows aborted at 9 months, whereas the remaining aborted at around five months of gestation (Fig.). The abortions were spontaneous in all the cases expect one, in which fetus was extracted applying traction. On tracing back the ancestor lines, it was observed that Bull No.J-96 was the sire of all the cows that had aborted.

In many diseases inherited as autosomal recessive defect, neither the sire nor the dam is usually affected, but the disease comes from both. The genetic defects are more common after inbreeding resulting in natural mutation of the recessive characters which become dominant (Roberts, 1971). The placental membranes (Ayyappan *et al.,* 1993) or fetus or both are affected irrespective of the cause of abortion (Roberts, 1971). Congenital dropsical fetuses have also been reported to result from electrolyte imbalance (Faber and Anderson, 1990).

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Fig. Foetal ascites

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Dystocia Due to a Fetal Monster in a Buffalo - A case Report

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Monsters are mostly encountered in cattle with an overall incidence of one in 100,000 bovine births. They are rare in other species (Roberts, 1971). Though dystocia due to duplication of cranial end of foetal body is uncommon, a diplopagus monster in a goat (Pandit *et al.,* 1994), a dicephalus dipus hexabrachius (Urankar *et al.,* 1994) and a dicephalus distorms tetrapus dibrachius (Chauhan and Verma, 1995) monsters in buffaloes have been reported. A rare case of "Siamese" twins with symmetrical heads was recorded in a buffalo.

An 8 years old buffalo in 3rd parity with history of dystocia was presented for treatment. Both the previous calvings were reported to be normal. The gestation period was over and the water bags had reptured 4-6 h before. Pervaginal examination, after epidural analgesia and proper lubrication, revealed the fetus in anterior longitudinal presentation. Fetus appeared to be double headed with both the fore limbs and one head presented in the birth canal. The second head caused postural problem and revealed to be obstructed at the pelvic brim. Traction was applied after correction and a dead female fetus was extracted.

The fetal monster was a conjoined twin monster. It was fully developed and revealed duplication of the head. Both the head components were equal and of subnormal size which were fused at the neck region. All the appendages and caudal portion of the body were normal. It was a rare fetal monster classified as "Dicephalus dipus dibrachius" arising from a single ovum (Roberts, 1971).

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Fig. Dicephalus dibrachius Monster

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Mummification of Male Bovine Fetus Co-Twin to a Female

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Mummification of single fetus in case of bovine twin pregnancies is a rare occurrence. The present communication describes the maintenance of a twin pregnancy carrying one live and one dead fetus in bovine where placental anastomosis is a regular phenomenon during twin pregnancies.

CASE REPORT

A seven years old pregnant imported Holstein-Friesian cow died due to chronic hoof problem. The postmortem examination revealed that the right uterine horn was enlarged while the left horn was very hard and firm. On incising the uterine horns a female growing fetus of approximately seven months age and a male mummified fetus having 35 cm Croup Rump (C-R) length were recovered from right and left horns, respectively. Examination of ovaries revealed the presence of a single yellow-orange coloured well developed corpus luteum (CL) on the right ovary and a small but distinct brown coloured regressing CL on the left ovary. History indicated that the cow was inseminated 204 days back and the service period was 130 days. In her four previous normal calvings neither twinning nor mummification was recorded. The case report clearly revealed the presence of bicornual pregnancy with dizygotic twinning. However, one or more mummified fetuses with viable fetus are rarely observed in cattle.

When fetal mummification affects twin pregnancies in bovine, both fetuses are usually diseased. This is due to anastamosis of their allantoic blood vessels and consequent exposure of both fetuses to the same deleterious influences (Arthur *et al.*, (1989). In the present case the estimated age of mummified fetus was 142 days as per Arthur *et al.*, (1989). Roberts (1971) observed that mummification occurs mostly between fourth to sixth months of gestation and Williams (1940) believed that fetal death, at this stage, occurs due to interplacental hemorrhage and a resultant failure of placental function. However, possibility of fetal hypoxia due to umbilical constriction or torsion can not be ruled out.

In the present case pregnancy was probably maintained by the progesterone secreted from the CL of right ovary and placenta of the viable fetus as CL of the left ovary was found regressed. The cause of fetal death does not appear to be due to lysis of CL. As at this stage pregnancy could be maintained by the progesterone secreted by the CL of the contralateral ovary and placenta of both fetuses.

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Fetal Maceration Causing Utero-enteric Fistula in a Buffalo

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Occurrence of fetal maceration has been recorded in several domestic species (Roberts, 1971). The macerated fetus either remains inert in the uterus or leads to pregnancy toxaemia due to invasion of saprophytic bacteria (Roychowdhary, 1982). Occasionally the fetal bones even pierce into myometrium, when the uterus contracts further (Gahlot, 1983).

A seven month pregnant buffalo had symptoms of imminent abortion, which however subsided due to administration of progesterone by a local vet. Three months later it had been diagnosed to be a case of fetal maceration. Treatment with injections of 80 mg of Diethyl stilbestrol and 40 mg of Dexamethasone had failed to evacuate the uterus. Instead about a moth later, faeces were noted with utero-vaginal discharge; hence, the animal was presented to the University Veterinary Clinics for treatment.

The buffalo was frequently straining and appeared exhausted. The temperature, respiration and heart rate were however, within normal limits. The cervix was only one finger open. The vaginal walls were soiled with faecal material. The closer vaginal examination with the help of speculum failed to establish any recto-vaginal fistula. On per-rectal palpation, the uterus was found on the pelvic brim and displaced towards the right side. The fetal fluids were absent and the uterus was tightly contracted on the crumpled bony fetal mass inside. A part of uterus was adhered to the surrounding viscera on the right lateral and ventral aspects. Lapro-hysterotomy was undertaken to evacate the uterus of its contents.

On laparotomy the uterus was found to be firmly adhered to the intestines and the mesentry. Incision was made on the right horn of the uterus. A large amount of faecal material collected in the uterus was siphoned out. The fetal bones along with some shreds of decomposed musculature were removed. The faecal material however continued pouring into the uterus. The suspected tract of entry of faeces from the gut into the uterus could not be located due to extensive adhesions. The animal was thus euthenised after the closure of the incision.

The necropsy revealed a fistulous tract from the uterus to the intestines. The fistulation is suspected to have occurred due to piercing of naked fetal bones into the intestine through the contracting uterine wall. The use of estrogens might have further accelerated the piercing of the fetal bones through its contractile effects on the myometrium. The injuries thus caused lead to extensive adhesions between the uterus and intestines. The utero-enteric fistula so formed was the source of faecal material being voided through vagina.

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Dicephalus Ischiopagus Monster in a cross bred Cow

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Ischiopagus featuses are joined at the lower pelvic region and the bodies extend in a straight line and the heads in the opposite directions (Roberts (1971). A case of Dicephalus Ischiopagus monster calf in a cross bred cow is recorded.

CASE REPORT

A cross bred (Deshi x HF) cow aged about 7 years at full term pregnancy and in acute labour causing dystokia was presented to State Animal Health Centre, Bethuadahari, Nadia, W.B. The cow had already completed three lactation with normal calving. The pervaginal examination of the cow revealed, fully dilated cervix followed by anterior longitudinal presentation of fetus with two fore limbs and one hind limb. While correcting the position of hind limb it was noticed that the hind limb originated lateraly just caudal to the base of a fore limb with two tails. The delivery was effected with simple forced extraction. When the fetus came out of the birth canal it was noticed that two seperate heads were situated in the opposite direction and the bodies were extended in a straight line, joined at lower pelvic region. The fetus was alive for a very short time. The fetus had two pair of fore limbs, one pair of hind limb (total six limbs), two tails, two anal opening and two female external genitalia. According to Roberts, this type of congenital defect can be classified as "Dicephalus Ischiopagus dipus tetrabrachius monster calf" (Fig).

This special type of conjoined twins arise fromsingle ovum and are monozygetic (Arthur, 1956; Bowen, 1966 and Roberts, 1971). Dystokia is a most common sequelae of monstrosities in bovines (Parkinson, 1974).

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Fig. Dicephalus Ischio pagus dispus tetrabrachius monster calf