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PROF C.R. SANE ORATION

Overview of Andrology in Animal Reproduction

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Increased efficiency of Animal Production could be achieved by improvement in husbandry, improvement of genetic potential of animals and by development of techniques to realise the full reproductive potential of both males and females. It is considered necessary to review the present status of Andrology - Physio Pathology of male reproduction - before adopting recent techniques and concepts to augment reproductive efficiency in male.

EFFECTIVE SCRENING OF THE MALES prior to use for breeding is obligatory to exploit the maximum genetic potential in any livestock enterprises. The characteristics of puberty and associated changes in the reproductive organs is the available tool for selection of a male for breeding. Lack of selection standards for the male in husbandry has limited the rate of genetic improvement in female.

A male's potential traits may be used to predict not only his reproductive potential but also the reproductive merit of his daughter as well. Among different external and internal morphometric changes considerable importance has been attached to weight and measurements of testis in the post natal male as a possible indication of puberty and ovalation rate in the daughter. Testicular size of males correlated positively to the ovulating rate in genetically related females and negatively to the onset of puberty. Measurement of tubular size may be used as a selection criteria in identifying superior males in the first year of post natal period. The size of the seminiferous tubule is an excellent index for assessing the reproductive efficiency. Diameter of tubule increased in a curvilinear manner when compared with age, body weight and testicular weight. Scrotal circumference which can be measured very early and accurately is an indirect assessment of tubular size. The pattern of testicular growth over a period of time may be more informative than any single measurement of testicular size.

Scrotal circumference is an indirect measurement of the testicular size and active spermatogenesis and it would be useful in evaluation for breeding soundness. Correlation of the scrotal circumference with the testicular weight was reported to be positive and highly significant. Measurement of the scrotal circumference may be a simple method of assessing puberty in bulls and will be a useful tool in the selection of early mating bulls. Scrotal circumference was positively and significantly correlated with body weight during 6 to 24 months of age.

The development of testis and other accessory reproductive organs and glands are closely dependent on the optimum level

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of testosterone secreted by the leydig cells under the influence of pituitory hormones. Several studies suggest that blood concentration of testosterone provide an indication of reproductive potential of the males. Estimation of testosterone during prepubertal stages would be an useful indication of male's reproductive merit and testicular function at later stage of development. In young and growing male during postnatal period nutritional deficiency especially low energy intake has been observed to retard sexual development and delay the onset of puberty.

PUBERTY in the male occure commonly at the time spermatozoa was released to make reproduction possible. Males are considered to have attained puberty when they developed the ability to produce viable spermatozoa, exhibited sexual aggressiveness and effected intromission and ejaculation. Presence of spermatozoa in the lumen of the majority of seminferous tubules as the sign of the puberty. Puberty was considered to have been achieved on production of an ejaculate containing 50 x 10^6 spermatozoa with a mass motility score of one and with not more than 30 per cent of abnormal spermatozoa. Presence of mature spermatozoa in the tail of epididymis was considered as the stage of physiological puberty.

In bulls puberty is defined as the age at which the first ejaculate contained a minimum of 50 x 10^6 total spermatozoa with atleast 10 per cent progressive motility. Marked difference in pubertal age and body weight were reported between various breeds of sheep. Genetic factors were considered to be responsible for the variation in pubertal age and body weight between breeds and within breeds. Average age at puberty was 10 to 16 months, in cattle and 15 to 24 months in buffalo. Body weight was considered to be a better guide to puberty than chronological age.

The importance of good nutriton for better body growth and reproductive soundness in males is not understood and appreciated by most of the farmers. Both quantity and quality of the feed could influence the testicular size and scrotal volume in the rams. Delay in the onset of androgenic activity was due to the lack of gonadotropins from the hypophysis (due to deficiency of endogenous GnRH release) and not due to an inability of testis to produce testosterone. The mechanism mediating the effect of nutrition on the sexual development of mammals were not well understood, especially in relation to hypothalamus and pituitory hormones.

TESTOSTERONE is the main androgen secreted in the testis, but it is converted peripherally in many of the androgen effector tissues, to the more active metabolic 5 alpha dihydro testosterone (DHT). This conversion takes place under the influence of 5 alpha reductase. This reaction is irreversible and this compound, unlike the other androgen cannot undergo peripheral aromatization to estrogen. DHT is the next active form of the hormone and it combines with the receptors in the cytoplasm of the cell. 5 alpha reductase enzymes appears to be present in most of its effector tissues. Embryonic differentiation of male reproductive organs, changes associated with puberty and growth is dependent on testosterone. The precise role of androgen in spermatogenesis is uncertain. The early embryonic differentiation of the ganocytes and final stages of the meiotic divisions that result in the formation of spermatides are considered to be androgen dependent.

In Eutherian mammals with the scrotal testis, thermal factor plays an equally important role in the maintenance of normal

spermatogenesis. Disturbance in the thermoregulatory mechanism of the scrotum has been shown to affect adversely the spermatogenesis resulting in maturation depletion of the germinal epithelium a characteristic factor of the tubular degeneration.

The THERMOREGULATORY MECHANISM of the scrotum becomes ineffective when whole body is exposed to high environmental temperature or due to high rise of body temperature in infectious diseases or due to localised heating of the scrotum by natural or artificial means. In all these conditions the testis is exposed to high temperature which is not conducive for the normal spermatogenesis. There is unequivocal evidence to show that the scrotal insulation causes tubular degeneration. The necessity of the testis temperature to be lower than that of the abdomen for proper spermatogenesis was first suggested by Crew (1922).

In India environmental temperature plays an important role in carrying testicular degeneration. High ambiant temperature during summer depresses fertility rate owing to the production of poor quality semen. Changes in the germinel epithelium is very specific. Pachytene spermatocyte and round spermatids are affected by heat stress resulting in maturatium arrest. In most of the cases the changes noticed is temporary and normalcy restored by 45-60 days period.

Lagerlof (1938) reported that testicular degeneration was the commonest type of bull infertility and 75% of bull with testicular pathology showed degeneration.

IN TESTICULAR DEGENERATION treatment with FSH increased the number of Type B spermatogonia and prevented the adverse effect on spermatogenesis produced by heat stress. Treatment with FSH-LH and glucocortecoid restored seminiferous tubular diameter, total spermatocyte and total spermatides to normal level. Treatment with GnRH reduced fertility and decreased androgen secretion by desensitization of pituitary and suppression of testicular function when given continuously. Extended treatment with Testosterone suppressed spermatogenesis, clomphine citrate treatment improved oligozoospermia and asthernozoospermia. PGF2 alpha increased sperm concentration but libido remained unaltered. Treatment with A, D, E had no effect. Oxytocin increased volume, mass activity, sperm concentration and live sperm and reduced reaction time.

The first comprehensive description of pathological sperm in the bull was published by Lagerlof (1934). Different diseases with fever, psychological exhaution could give an increased number of pathological sperm. These conditions produced atypical mitosis and other anomalies of the cell divisions in the testicles. It has also been shown that not all pathological sperm changes have a testicular origin. It has been shown that epididymal dysfunction can also result in pathological sperms. The majority of the pathological changes reflected a damaging influence of the heat on primary spermatocytes and spermatids but a small proportion of abnormal tails originated in the proximal epididymis. It has been shown that both in normal and in infertile bulls the composition of the sperm population leaving the testicle undergoes dramatic changes during the passage through epididymis (Rao, 1971). Independent of the degree of degeneration the numbers of pathological sperm decrease on the way to the ejaculate. The decrease is much more pronounced in bulls with testicular degeneration than in normal bulls. During the transport through epididymis the

frequency of abnormal tails increases very much and more in bulls with testicular degeneration than in normal ones.

The electron microscopical studies revealed that there are number of ultrastructural changes that could not be detected with light microscopy and that these changes had a higher frequency in bulls with low fertility than in normal bulls. It should also be stressed that the morphological changes are only one expression of abnormal conditions of the sperm. There are certainly other factors of biochemical and enzymatical nature and even other factors which would give a low fertility.

MALE FERTILITY: Evaluation of the male fertility should essentially involve determination of spermatozoal capacity to reach the site of fertilization in female genital tract, undergo capacitation, acrosome reaction, penetrate the oocyte and be incorporated by the oocyte, replicate their DNA, achieve syngamy and support normal embryonic development. Tests for sperm motility, sperm morphology, cervical mucus penetration test, sperm mitochondrial activity index, hypo-osmotic swelling test, acrosome integrity test, sperm chromatin structure assay and invitro acrosome reaction assay are some of the methods adopted to assess the fertilizing capacity of spermatozoa. However, Zona-free Hamster egg penetration assay is considered to be the best since it evaluates several attributed of fertilizing ability of spermatozoa such as capacitation, acrosome reaction, gamate membrane fusion, sperm chromatin decondensation. The correlation found between this test and fertility has resulted in its use in testing the fertilizing ability of spermatozoa of human and variuos livestock species. Sensitivity of this test has been further improved by using hetero spermic penetration. The major disadvantage of this test is that it cannot measure the ability of spermatozoa to bind to, or penetrate the zona pellucida which is largely a function of motility. A combination of motility assessment and zona-free Hamster oocyte penetration test might improve overall assesment of sperm quality.

The future thrust area in Andrology in animal reproduction should be to establish Accredited bull stations and AI centres as per O.I.E. code; Standardise clinical Andrological examination of breeding bulls used for AI; Studies on male infertility; post - ejaculation improvement of semen quality and application of biotechnological techniques such as sexing of sperm and sperm fertility assessment.

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Treatment of True Anoestrus in the Bovine with Progestagen and Oestrogen

2. Oestrous response and fertility in anoestrous buffaloes following natural service at the induced oestrus

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ABSTRACT

Sixty-five lactating and seven dry, parous, acyclic buffaloes were assigned to three treatment groups during their normal breeding season : (Group I) untreated control, 30 lactating animals; (Group II) 0.5 mg melengestrol acetate (MGA) per head daily orally for 14 days + 400 μg oestradiol benzoate (OB) injected 48 hr after the last day of MGA feeding, 35 lactating animals; (Group III) MGA+OB treatment as per Group II, 7 dry animals. Animals exhibiting standing oestrus were served by fertile bulls.

All treated animals exhibited oestrus but standing oestrus was observed in 60.0% and 85.7% of lactating and dry animals, respectively; with an overall incidence of 64.3%. In contrast, in the control group only 33.3% of animals exhibited standing oestrus and this difference was significant ($P<0.01$).

All the oestruses were not ovulatory. The overall incidence of ovulation at induced oestrus was 80.0% and 71.4% in lactating and dry animals, respectively and those did not differ from control (90.0%). The conception rate at the induced oestrus was 57.1% and 83.3% in lactating and dry animals, respectively and the overall conception rate was 62.9%. This did not differ from the control animals served at natural oestrus (50.0%). Following treatment ovarian cyclicity was established in 57.1% and 71.4% of lactating and dry animals, respectively with an overall incidence of 58.5%. This differed significantly ($P<0.05$) from untreated controlls (30.0%). These results indicate that a combined MGA+OB therapy is quite effective in inducing fertile oestrus in parous, anoestrous buffaloes during normal breeding season.

——x——x——x——

The objectives of this study were to examine : (1) the efficacy of a higher dose of oestrogen following progestagen treatment on the incidence of standing oestrus and (2) the fertility following natural service at the induced standing oestrus.

MATERIALS AND METHODS

The study included 65 multiparous, lactating and 7 dry anoestrus Murrah buffaloes; The critrion for considering them anoestrus, their housing & feeding schedule were similar to that described earlier (Shanker *et al.,* 1996).

After balancing for age, parity and post-partum interval, the lactating animals were allotted into two groups : (GroupI) untreated control, 30 lactating animals; (Group II) melengestrol acetate (MGA) 0.5 mg / animal / day given orally for 14 days plus Oestradiol benzoate (OB) 400 μg injected intramuscularly, 48 hr after the last day of MGA feeding, 35 lactating animals. MGA was mixed in the concentrate mixture and was fed at 0900 hr. Seven dry animals in Group III, were treated with MGA and OB, as described for Group II. The trial was conducted during November-February

which was normal breeding season for buffaloes.

For oestrus detection and natural service all treated animals were divided in groups of 5 each. A raddled fertile bull was kept with each group of 5 animals between 1100 and 1700 and 0300 and 0600 hr, and buffaloes were checked for acceptance at hourly interval. Oestrus was classified as standing and non-standing (Morrow *et al.,* 1976). The incidence of ovulation was ascertained by rectal palpation at 10 to 12 days after induced oestrus to monitor the presence of corpus luteum (CL). In the control group a raddled fertile bull was used with all 30 animals for oestrus detection and natural service. Pregnancy was confirmed by rectal palpation at 60 days after natural service.

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

RESULTS AND DISCUSSION

(a) Oestrous response

All treated animals exhibited oestrus (Table 1). However, standing oestrus was observed in 60.0% and 85.7% of lactating and dry animals, respectively and the overall incidence was 64.3%. Contrarily, in the control group only 33.3% exhibited natural standing oestrus and this incidence was lower than lactating, dry ($P<0.05$), and overall treated ($P<0.01$) animals. Furthermore, frequency distribution of post-partum oestrus interval revealed that while in the control group only 7/30 (23.3%) animals exhibited oestrus within 150 days after calving, in the lactating treated group higher ($P<0.01$) number (21/35, 60%) exhibited standing oestrus within this period.

In earlier study we observed 85.1% oestrous response in anoestrous buffaloes treated with MGA + 250μg OB with a non-standing oestrus incidence of 27.5% (Shankar *et al.,* 1996). In this study increasing the dose of OB from 250 to 400 ug did not reduce the incidence of non-standing oestrus although there was an improvement in overall oestrus response. Furthermore, while the incidence of standing oestrus was 25.7% more in dry animals, however, this increase was not significant. Nevertheless, these results indicate that physiological status of the animal may govern the oestrous response to treatment. Our results confirm earlier reports (Rajamahendran and Thamotharam, 1983; Rao and Sreemannarayan, 1983; Rao *et al.,* 1985; Shankar *et al.,* 1996).

(b) Ovulation

All the induced oestruses were not ovulatory (Table2) in lactating animals (Group II), 76.2% and 85.7% ovulated at standing and non-standing oestrus, respectively (Overall incidence 80.0%). In the dry animals (Group III), the respective values were 83.3%, 0.0% and 71.4%. In the control (Group I), 90.0% animals ovulated at the first natural oestrus & incidence of ovulation did not differ among groups indicating that a satisfactory incidence of ovulation can be achieved in anoestrous buffaloes following MGA and OB treatment.

(c) Conception rate

Table 2 reveals that the conception rate at the induced oestrus was 57.1% and 83.3% in lactating and dry animals, respectively with an overall conception rate of 62.9% which did not differ from the control (50.0%), However, it was much higher than 35.0% reported earlier following

A.I. at MGA+OB induced oestrus (Shankar *et al.,* 1996). Rao and Sreemannarayana (1983) also reported a conception rate of 36.0% in parous anoestrous buffaloes following A.I. at the oestrus induced with norgestomet and PMSG. Our results indicate that factors other than an altered endocrine milieu may account for a poor conception rate following A.I. at induced oestrus in the buffalo and these warrant investigation.

(d) Functional activity of induced CL and establishment of ovarian cyclicity

Since the conception rate at the induced oestrus was similar to the control, apparently in majority of animals the induced CL were functionally normal. Additionally, of 16 lactating animals exhibiting standing ovulatory oestrus (Table 1) 4 which did not conceive (Table 2) showed first natural oestrus with a mean cycle length of 20.7±0.89 days. Among 12 lactating animals exhibiting non-standing ovulatory oestrus (Table 1) 4(33.3%) exhibited first natural oestrus with a mean cycle length of 21.5±2.25 days. The remaining 8 animals became anoestrus. The overall mean cycle length of treated lactating animals was 21.0±1.98 days and was similar to control (20.9±0.92 days) (Table 2).

Our results further indicate that following treatment overian cyclicity was established in 57.1% and 71.4% of lactating and dry animals with an overall incidence of 59.5%. This differed significantly ($P<0.05$) from untreated controls in which it was established in 30.0% animals (Table 2)

It is concluded that MGA+OB therapy is quite effective in inducing fertile oestrus in parous anoestrous buffaloes during normal breeding season and this treatment should help reduce the intercalving interval in this species.

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Table 1. Oestrous response and ovulation in anoestrous buffaloes following oestrus induction with melengestrol acetate and oestradiol benzoate

Attributes	Lactating		Dry	Total treated
	Group I (Control)	(Group II) (MGA+OB)	Group III (MGA+OB)	(Group II+III)
No. of animals	30	35	7	42
No. in induced oestrus (%)	10 (33.3)[a]	35 (100.0[b]	7 (100.0)[c]	42 (100.0)[d]
Standing (%)	10/10 (100.0)	21/35 (60.0)	6/7 (85.7)	27/42 (64.3)
No.ovulated (%)	9/10 (90.0)	16/21 (76.2)	5/6 (83.3)	21/27 (77.8)
Non-standing (%)	—	14/35 (40.0)	1/7 (14.3)	15/42 (35.7)
No.ovulated (%)	—	12/14 (85.7)	0/1 (0.0)	12/15 (80.0)
Total ovulated / Total in induced oestrus(%)	—	28/33 (80.0)	5/7 (71.4)	33/42 (78.5)

* - In the control group all data pertain to natural oestrus.

a vs b = 5.54, ($P<0.05$) a va c = 6.33, ($P<0.05$) a vs c = 6.84, ($P<0.01$)

Table 2. Conception rate at induced oestrus and establishment of ovarian cyclicity in anoestrous buffaloes treated with melengestrol acetate and oestradiol benzoate

Attributes	Lactating		Dry	Total treated
	Group I (Control)	(Group II) (MGA+OB)	Group III (MGA+OB)	(Group II+III)
No. of animals	30	35	7	42
No. in oestrus	10	35	7	42
No.served by bull	10	21	6	27
No.conceived (%)	5/10 (50.0)	12/21 (57.1)	5/6 (83.3)	17/27 (62.9)
No. exhibiting 1st/2nd natural ovulatory oestrus	4	8	—	8
Cycle length following natural/induced oestrus (days, Mean±S.E.)	20.9 ±0.92	21.0 ±1.98	—	—
Establishment of cyclicity (%)	9/30 (30.0)[a]	20/35 (57.1)	5/7 (17.4)	25/42 (59.5[b])

X^2 a vs b = 6.12, ($P<0.05$)

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Progesterone Profile at pre, during and Post Superovulatory Treatment in Crossbred Cows

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ABSTRACT

Concentration of progesterone was studied in twelve crossbred cows treated either with pregnant mare serum gonadotropin (PMSG) or FSH and prostaglandin $F_2\alpha$($PGF^2\alpha$) 48 h later to induce superovulation. The mean pre-treatment serum progesterone concentration in PMSG and FSH treated group was 1.96±0.32 and 2.96±0.52 ng / ml, respectively. The progesterone concentration increased during gonadotropin treatment and decreased to a basal level of 0.29±0.06 and 0.04±0.20 ng / ml in PMSG and FSH treated animals, respectively at $PGF^2\alpha$ induced oesturs. The progesterone value increased steadily and reached value of 9.0±1.0 and 7.48±1.67 ng / ml in PMSG and FSH, respectively on day of flushing. It was observed that with an increase in the progesterone level during the first two days of treatment the quality of embryo recovered was decreased.

——x——x——x——

A highly variable and unpredictable production of viable embryos in donor animals is a serious problem in bovine embryo transfer.

Several experiments have demonstrated that profiles of plasma progesterone in the superovulated cow differ from those in the untreated animals.

The objective of the present study is to estimate the progesterone level prior to, during and after the superovulatory treatment either with PMSG or FSH and to relate the hormone level with the quality of the embryo.

MATERIALS AND METHODS

Twelve lactating cows (Jersey X Sindhi) located in Tamilnadu Veterinary and Animal Sciences University Farms were used. They were on average 110 day post partum and were superovulated either with pregnant mare serum gonadotropin (PMSG) or Follicle-stimulating hormone (FSH). The gonadotropin treatment was initiated during mid luteal phase of the cycle, PMSG (2,000 IU, Folligon, Intervet International, Holland) was injected as single intramuscular injection. However, FSH (Folltropin) was given twice daily 12 hours apart 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). Luteolysis was induced by injecting Dinoprost (25 mg) at 48 and 60 hours after initiation of gonadotropin treatment. All cows were artificially inseminated with Frozen-thawed semen at 48, 56 and 72 hours after the first PG injection. Flushing was done on day 7 (day of onset of oestrus = day 0) nonsurgically, after determining the number of copora lutea, using flushing catheter (German Rusch 18 G). Embryo were examined, counted, graded on a scale of 1 to 5 as per the

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method described by Goulding et al (1991). Unfertilized eggs were not included in this grade 1 to 5 classification system. Blood samples were collected from the animals between 7.30 to 8.30 am by Jugular venipuncture into a centrifuge tube and allowed to clot at room temperature, on the day of gonodotropin treatment, on the day of Prostaglandin treatment, on the day of superovulatory oestrum, third day after superovulatory heat and on the day of embryo collection. After centrifugation at 3000 rpm for 10 minutes the serum was harvested and stored at -20°C until assayed for progesterone. Progesterone profile was studied by Radio-immunoassay as described by Sarma et al (1987). Interassay and intra assay variation were 11 per cent and 13 per cent, respectively.

RESULTS AND DISCUSSION

Mean plasma progesterone concentration in cows superovulated with PMSG and FSH are given in the table 1 and 2. The mean serum progesterone level during pre-treatment and superovulatory period in the PMSG treated animals was. 1.96±0.32 ng / ml (range 0.38 to 6.0 ng / ml) and in FSH treated animals was 2.96±0.52 ng / ml (range 1.6 to 4.5 ng / ml) and 2.91±0.25 ng / ml (range 2.0 to 3.8 ng / ml), respectively.

An increase in the level of progesterone immediately after superovulatory treatment was observed by Tamboura *et al.,* (1985) and Datta *et al.,* (1992) but no such increase was observed by Booth *et al.,* (1975), Yadav *et al.,* (1986). Increased progesterone levels during the first two days of gonadotropin treatment was the evidence of the luteotrophic effect of PMSG and FSH since they contain LH activity (Lindsell *et al.,* 1986).

The Progesterone value decreased to near basal levels of 0.29±0.96 ng / ml in PMSG treated animals and 0.40±0.02 ng / ml in FSH treated animals at superovulatory Oestrus. This was primarily due to luteolytic effect of PGF_2 to cause a decrease in progesterone secretion and thus initiate rapid follicular activity (Alcivar *et al.,* 1992, Agarwal *et al.,* 1993).

The progesterone level increased steadily after day 3 of suprovulatory oestrus. The mean values increased from 1.18±0.08 and 1.42±ng / ml on day 3 to mean high values of 9.0±1.0 ng / ml and 7.48±1.67 ng / ml on day 7 after superovulatory oestrus in PMSG and FSH treated animals, respectively. The increase in progesterone, in gonodotropin treated groups was due to formation of multiple corporalutea, however unovulated follicles were also reported to secrete appreciable amounts of progesterone (Booth *et al.,* 1975)

Abnormal progesterone profile was responsible for poor response to superovulation, more unfertilized ova and non-transferable embryos. In the present investigation two cows in FSH and one cow in PMSG treated group had a level of 0.4, 4.5 and 4.5 ml of progesterone. on the day of flushing. The reason attributed to such a low level of progesterone was the premature regression of the corpus luteum (Alcivar *et al.,* 1992). Both *et al.,* (1975) observed that 4 to 27 per cent of cattle suffered with premature regression of corpora lutea.

One cow in PMSG group had a low level of serum progesterone (0.38 ng / ml) on the day of PMSG administation and one cow in FSH group had low levels of progesterone concentration at the time of $PGF^2\alpha$ injection and both type of profile

resulted on and three viable embryo's, respectively. Another finding of this study was that with an increase in the progesterone concentration during first two days of treatment, the per centage of good embryos recovered were low. Tamboura *et al.,* (1985) postulated that the increase in progesterone concentration was due to premature luteinization of follicles. Such follicles could release oocytes which have not matured properly because of the hormonal micro environment and these oocytes will lead to abnormal embryos.

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Table 1. Mean (+SE) Serum Progesterone concentration in superovulated (PMSG) cows

Day of cycle	Range (ng / ml	Progesterone (ng / ml
11	0.38 - 2.5	1.96±0.32
13	0.32 - 6.0	2.95±0.95
0	0.06 - 0.55	0.29±0.06
3	0.9 - 1.4	1.18±0.08
7	4.0 - > 10.0	9.0±1.00

Table 2. Mean (+SE) Serum Progesterone concentration in superovulated (FSH) cows

Day of cycle	Range (ng / ml	Progesterone (ng / ml
11	1.6 - 4.5	2.96±0.52
13	2.0 - 3.8	2.91±0.25
0	0.34 - 0.5	0.40±0.02
3	1.2 - 1.8	1.42±0.10
7	0.4 - >1.10)	7.48±1.67

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Effect of PGF_2 Alpha Administration On Onset of Estrus, Subsequent Estrous Cycle Length and Fertility Following Embryo Collection in Superovulated Cattle

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ABSTRACT

Effect of administration of PGF_2 alpha on onset of estrus, subsequent estrous cycle length and fertility following embryo collection in superovulated crossbred cattle was studied. Onset of estrus in superovulated cattle following embryo collection with single injection of 25mg PGF_2 alpha was significantly delayed than the normal cyclic cows (16.36±8.36 Vs. 3.28±0.40 days, $P< 0.01$). The subsequent estrous cycle length following embryo collection was slightly shorter than that of spontaneous estrous cycle in control group (18.57±5.48 Vs. 20.16±1.43 days). The conception rate following superovulation and non-surgical embryo collection was lower than the normal cyclic control cows.

——x——x——x——

The major limitation of using superior dairy cows as donors in embryo transfer programme is prolonged open period following superovulation and embryo recovery. The delay in the onset of estrus following embryo collection interferes early rebreeding of donors and their availability for repeated superovulation. Administration of PGF^2 alpha following embryo recovery in cattle is an important advancement for bringing the donors in estrus early and reducing the open periods (Garcia *et al.*, 1983). The fertility has been reported lower following superovulation and embryo recovery (Greve and Lehn-Jensen, 1978; Rommel *et al.*, 1986 Bak *et al.*, 1987. Moyaert *et al.*, 1987). In the present paper effect of administration of PGF^2 alpha on onset of estrus, subsequent estrous cycle length and fertility following embryo collection in superovulated crossbred cattle is reported.

MATERIALS AND METHODS

The present study was conducted on 19 crossbred cows superovulated with PMSG. The donors were flushed by non-surgical method on day 6/7 post-estrus (day0 = day of estrus. Donors after flushing were treated with single intra-muscular injection of 25mg PGF^2 alpha (Dinofertin, Alved pharma and Food Ltd., India). A separate control group (n=6) was also maintained. The animals of this group were not superovulated and flushed but treated with 25mg PGF^2 alpha between day 10-12 of the estrous cycle (All animals had cyclic CL in their ovary). All animals were subjected to detection of estrus after PGF^2 alpha injection twice daily i.e. morning and evening with the help of a vasectomized teaser bull and visual observation of external estrus symptoms. Time taken for the onset of estrus and length of subsequent estrous cycle was calculated. Animals were inseminated with frozen semen at induced and subsequent estrus. All the animals were examined for pregnancy 45-60 days after insemination. Conception rate and number of insemination per conception were calculated.

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RESULTS AND DISCUSSION

Results pertaining to the effect of administration of PGF^2 alpha following embryo collection on return to estrus interval subsequent estrous cycle length and fertility is presented in Table 1. Onset of estrus after PGF^2 alpha injection following superovulation and embryo recovery (gr.I) was significantly delayed than the normal cycling control (gr. II) cows (16.36±8.36 Vs. 3.28±0.40 days; ($P<0.01$) indicating cows with higher ovulation rate required more time for the luteolysis than the non-superovulated normal cycling control cows. Superovulated animals following embryo recovery may require comparatively higher dose of PGF^2 alpha than the normal cyclic animals for the complete luteolysis and early return to estrus. Garcia *et al.,* 1983) has reported better results of inducing estrus in superovulated cattle with a higher dose of PGF^2 alpha (60 Vs. 30mg). In normal cyclic cattle the normal luteolytic dose of PGF^2 alpha or its synthetic analogue between day 5 to 16 of the cycle resulted decrease in serum progesterone, size of corpus luteum (Louis *et al.,* 1973) and return to estrus within 2-4 days (Agarwal *et al.,* 1987., Chauhan *et al.,* 1994) which is in agreement to the findings of the present study. Most of the workers have reported onset of estrus following embryo recovery with the use of normal luteolytic dose of PGF^2 alpha and its analogue within 8-16 days of time (Chupin *et al.,* 1984; Jones *et al.,* 1986; Yadav *et al.,* 1991) which is inagreement with the findings of the present study. However, others have reported slightly delayed onset of estrus following the administration of PGF^2 alpha (Holy *et al.,* 1991; Chauhan *et al.,* 1994). Comparatively longer return to estrus interval (as late as 30-38 days) following embryo recovery without PGF^2 alpha treatment has been reported (Greve-Lehn Jensen, 1978; Haupt, 1979; Ozil *et al.,* 1979).

The subsequent estrous cycle length in superovulated donors following embryo recovery was slightly shorter than that of spontaneous estrous cycle (18.57±5.48 Vs. 20.16±1.43 days), however, the difference was statistically non-significant. Greve and Lehn-Jensen (1978), Takahashi and Horita (1983), and Kharche (1989) also reported slightly shorter post superovulatory estrous cycle length which is inagreement with the findings of the present study.

A good number of animals (58.33%) conceived following superovulation and non-surgical embryo collection, however, they have taken comparatively more number of inseminations per conception than the control (4.85 Vs. 2.00). Most of the investigators (Greve and Lehn-Jensen, 1978; Haupt, 1979; Kruff and Lampeter, 1980; Cowen and Sosnik, 1987; Moyaert *et al.,* 1987; Prasad *et al.,* 1990) have reported lower conception rate and more number of inseminations per conception following superovulation and non-surgical embryo collection which is in agreement to the findings of the present study. However, others observed normal conception rate following superovulation and embryo collection in superovulated cattle (Takahashi and Horita, 1983; Chupin *et al.,* 1984, Bak *et al.,* 1989). The lower conception rate and more number of inseminations per conception in superovulated cattle following non-surgical embryo collection may be attributed to irregular estrous cyclicity, luteal deficiency and precoccious breeding after superovulation (Greve and Lehn-Jensen, 1978).

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Table 1. Effect of post flushing PGF_2 alpha treatment on return to estrus interval, subsequent estrous cycle length and fertility in cross bred cattle

Group / parameter studies	PGF_2 alpha injection following embryo recovery group I	PGF_2 alpha injection during mid luteal phase of normal estrous cycle (control) group II
Animals treated	19	6
Animals exhibited estrus (%)	18(94.73)	6(100)
PG to estrus interval (days)	16.36±8.36[A]	3.28±0.40
Subsequent estrous cycle length (days)	18.57±5.48	20.16±1.43
Cows inseminated	12	6
Cows pregnant	7	5
Conception rate (%)	58.33	83.33

Mean within a row with different superscripts are significantly different (P<0.01).

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Plasma Cholesterol Levels in Relation to Superovulatory Response and Embryo Recovery*

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ABSTRACT

Total cholesterol concentration was estimated in the blood plasma samples of superovulated animals collected at the time of Syncro Mate-B implantation, FSH start, Syncro Mate-B implant withdrawal, oestrus and flushing. Total cholesterol concentration was slightly higher in cows than in heifers at the time of Syncro Mate-B implantation and this trend continued throughout the period of study. The embryo recovery and transferable embryos were significantly higher (4.50±0.65 and 3.00±0.82) in the animals with cholesterol level of more than 160 mg./ 100 ml. than those of less than 160 mg./ 100 ml. (2.20±0.37 and 1.00±0.32). This shows that selection of donor cows basing on plasma total cholesterol levels may be benefecial.

——x——x——x——

There is a great variation in the superovulatory response and embryo recovery among the animals. Hence, there is a need to find the means to select the donor cows, using relatively simple techniques to have better superovulatory response. Estimation of total cholesterol level in plasma before superovulation was indicated as one such method (Kweon *et al.,* 1986 and Maruo *et al.,* 1987). Hence, this study was carried out to find out the relationship of the plasma total cholesterol level with superovulatory response and embryo recovery.

MATERIALS AND METHODS

Anoestrous Hariana cows and heifers (6 in each group) of Government Livestock Farm, Sector-1, Hisar were selected for the study. Animals were synchronized for oestrus using Syncro Mate-B* implant (3 mg. norgestomet) subcutaneously on external ear (mid way between base and tip) and injection (3 mg. n orgestoment and 5 ml. oestradiol valerate in 2 ml.) intramuscularly (-9 day). Superovulation was started with FSH-E (Follicle Stimulating Hormone-Equine) from the 7th day of implantation (-3 day) and the implant was withdrawn after 9 days (0 day). The animals were observed for oestrus starting from 12 hours of implant withdrawl and the animals were mated with a fertile bull during oestrus at 12 hourly intervals. Embryos were recovered on 7th day post oestrus. Total cholesterol concentration in blood plasma collected at Syncro Mate-B implantation, FSH start, Syncro Mate-B with drawl, oestrus and flushing was assayed by enzymatic method with Autopok kits ** using Auto Blood Analyser.

RESULTS AND DISCUSSION

Higher cholesterol concentration was observed in cows (157.50±5.12 mg./ 100 ml.) than in heifers (147.50±6.68 mg./ 100 ml / at the time of Syncro Mate-B implantation and this difference, though not significant continued throughout the remaining period of study. A small increase

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in plasma cholesterol concentrations was recorded both in cows and heifers up to oestrus followed by a similar decline at flushing (Table-1). Similar pattern was observed by Purohit and Kohli (1977) and Prasad *et al.,* (1984), eventhough the values reported by the above authors were slightly higher.

The animals were divided in to two groups, based on cholesterol concentration at the time of Syncro Mate-B Implantation (Table-2). Group-A, consisted of 4 animals (2 cows and 2 heifers) with cholesterol concentration of more than 160 mg./ml., while Group-B, consisted of 8 animals (4 cows and 4 heifers) with cholesterol concentration of less than 160 mg./100 ml. In Group-A, all the 4 animals responded and were flushed, while in Group-B, only 6 animals responded out of which 5 were flushed. The number of corpora lutea, embryos recovered, degenerated embryos and transferable embryos in high cholesterol and low cholesterol groups were 9.25±0.48 VS 9.20±1.07, 4.50±0.65 VS 2.20±0.37, 1.50±0.29 VS 1.20±0.20 and 3.00±0.82 VS 1.00±0.32, respectively. While the superovulatory response and degenerated embryos were similar in both the groups, significant differences were observed in embryo recovery ($P<0.01$) and transferable embryos ($P<0.05$). Contrary to the present findings, Maruo *et al.,* (1987) observed positive relationship between cholesterol level and ovulation rate. However, the present findings were in agreement with the positive correlations of plasma total cholesterol levels with embryo recovery observed by Maruo *et al.,* (1987) and with transferable embryos by Schaffer *et al.,* (1990). Kaneko (1980) reported that total cholesterol is one of the metabolites which represents the condition of lipid metabolism of cattle and suggested that the animals with total cholesterol concentration of 120-130 mg./100 ml. and more may be metabolically healthy and such animals will have better superovulatory response and embryo recovery. These results suggest that the donors with high cholesterol levels may be prefered for superovulation.

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Table 1. Comparison of plasma cholesterol levels (mg / 100 ml) in superovulated (anoestrus) Hariana cows and heifers.

Stage of superovulatory treatment	Cows (n=6)	Heifers (n=6)
SMB implantation	157.50±5.12	147.50±6.68
FSH start	160.17±5.45	148.50±5.39
SMB withdrawal	164.17±4.10	151.00±6.17
Estrus	169.00±5.90	157.83±9.27
Flushing	161.33±3.71	150.83±6.11

Values are Mean±SE

Table 2. Cholesterol concentration at SMB implantation in relation to superovulatory response and embryo recovery in (anoestrus) Hariana cows and heifers.

Parameter studied	Cholesterol concentration	
	>160 mg / 100 ml	<160 mg / 100 ml
Animals treated	4	8
Animals responded	4	6
Animals flushed	4	5a
Mean±SE of corpora lutea	9.25±0.48	9.20±1.07
Mean±SE of embryos recovered	4.50±0.65**	2.20±0.37**
Mean±SE of degenerated embryos	1.50±0.29	1.20±0.20
Mean±SE of transferable embryos	3.00±0.82*	1.00±0.32*

a - One heifer could not be flushed due to catheterization problem

* - Means within a row are significantly different (P<0.05)

** - Means within a row are significantly different (P<0.01) Value are Mean±SE

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Regulation of Oestrous Cycle in Crossbred Heifers

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ABSTRACT

Trials were carried out to study the efficacy of prostaglandin F_2 alpha in the management of oestrous cycle in crossbred heifers with fixed time insemination. Twelve heifers (75%) and 16 heifers (100%) responded to prostaglandin when administered as single and double injection schedule. The mean time taken for induction of oestrus was 56.66 h and the duration of oestrus was 21.86 h. Majority of experimental animals showed marginal increase in oestrul characters. The first insemination conception rate of experimental (25%) and control groups (6.25%) were significantly different. Experimental heifers required 1.88 as against 3.14 inseminations per conception.

——x——x——x——

Management of oestrous cycle as an alternative to routine oestrus detection has gained momentum in recent times. The purpose is to control the time of oestrus and therefore the time of ovulation. Favourable results stimulated several workers to undertake detailed trials to evaluate the efficacy of PGF_2 alpha with various dose regimens and under different routes of administration and insemination at detected oestrus and at fixed time. The objective of the present investigation was to evaluate the efficacy of administration of PGF_2 alpha by single or double injection regimen in the management of oestrous cycle in crossbred heifers and fertility of fixed time insemination at induced oestrus.

MATERIALS AND METHODS

Materials for the present study consisted of 48 crossbred heifers of breedable age maintained under identical condtions of feed and management at Kerala Agricultural University Livestock Farms. They were subjected to detailed clinico-gynaecological examination and those found to be cycling were selected and randomly allotted to the following three treatment groups.

Group I

Sixteen animals were subjected to intramuscular administration of 25 mg of PGF_2 alpha (***Lutalyse 5 ml) when they had a functional corpus luteum as determined by rectal palpation. Out of 12 heifers responded to treatment six heifers were inseminated 72 h and the remaining 96 h after the administration of Lutalyse.

Group II

Sixteen cycling heifers with apparently normal reproductive health were administered intramuscularly two injections of Lutalyse, 25 mg each 13 d apart. Among them eight were inseminated 72 h and the remaining 96 h after the administration of the second dose of Lutalyse.

Group III

Sixteen heifers were watched for natural oestrus and inseminated (control).

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*** Lutalyse (Inj.): 5 ml. (Upjohn)
Each ml. contains Dinoprost Tromethamine equivalent to Dinoprost 5 mg.

Observations in respect of oestrus response and time taken from the administration of PGF_2 alpha to the onset of oestrus in groups I and II and duration of oestrus, intensity of oestrus, conception rate and number of inseminations per conception in all the groups were studied and analysed.

RESULTS AND DISCUSSION

Perusal of data in table 1 revealed that out of 16 heifers in group I which were treated with Lutalyse, 12 evinced oestrus while all the heifers in group II responded to treatment. Statistical analysis revealed significant difference in oestrus response between group I and II. The present study indicated that single as well as double injection schedule of PGF_2 alpha were effective in inducing oestrus in crossbred heifers. However, the response after single prostaglandin administration was found to be lower in heifers, which might be attributed to the unresponsiveness of corpora lutea to PGF_2 alpha at the time of administration (Singh *et al.,* 1979). Arthur *et al.,* 1989) suggested that double injection schedule gave better response in heifers. A marginal decrease in oestrus response was noticed during summer probably due to high ambient temperature which affected the normal hormonal profile of crossbred heifers in the tropics. The time taken for induction of oestrus in group I and II was 53.50 and 59 h respectively. Similar observations were made by Pant *et al.,* (1992). Slight variations in the time taken for the induction of oestrus in different studies could be due to variation in the stage of corpora lutea at the time of administration of prostaglandin. Seasonal influence on the time taken for induction of oestrus after the administration of prostaglandin was observed, least during rainy season and maximum during winter. This could be attributed to the better response of animals to prostaglandin during the favourable months of the year.

The duration of oestrus in heifers ranged from 12 to 30 h (mean 20.00 h), 18 to 36 h (mean 23.25 h) and 12 to 30 h (mean 20.75 h) in group I, II and III respectively without any significant difference. In the induced oestrus significant variation in the duration of oestrus was observed between winter and rainy season, during winter the duration of induced oestrus was significantly longer.

All experimental animals which responded to PGF_2 alpha showed marginal increase in vulval oedema, hyperaemia of vaginal mucosa, oestrual discharge and tonicity of uterine horns compared to control animals. The present study revealed that luteolysis and subsequent changes in the reproductive tract brought about by exogenous PGF_2 alpha were similar or even better than that caused by endogenous PGF_2 alpha. Majority of experimental animals in both the groups showed medium to high intensity of oestrus compared to natural oestrus. It may also be noted that the percentage of weak oestrus were more in natural oestrus compared to induced oestrus indicating beneficial effect of PGF_2 alpha in the detection of oestrus by better and pronounced oestrus signs.

Perusal of data also revealed that in group I, the first insemination conception rate and overall conception rate were 33.33 and 66.67 per cent when inseminated 72 h post-treatment, while the respective values were 33.33 and 50 per cent when inseminated 96 h post-treatment. A marginal increase in the overall conception rate was observed in heifers inseminated 72 h after the administration of the second dose of PGF_2 alpha, 12.50 per cent of animals conceived at first insemination while the overall conception rate was 62.50 per cent.

The corresponding values at 96 h insemination were 25 and 50 per cent. Thus it could be seen that when double dose regimen was practised, better first insemination conception rate was observed in heifers when inseminated 96 h post-treatment. Among control animals the first insemination conception rate and overall conception rate were 6.25 and 43.75 per cent. The first insemination conception rate of experimental heifers was significantly higher than that of control animals. The number of inseminations required per conception was 1.50 and 1.33 and 2.20 and 2.25 at 72 h and 96 h inseminations in group I and II respectively as against 3.14 in the control heifers. From the present study it could be stated that PGF_2 alpha could be successfully used for induction of oestrus in crossbred heifers.

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Table 1. Effect of PGF_2 alpha in the induction of oestrus in crossbred heifers

		Groups		
		I	II	III
1. No. of animals treated		16	16	—
2. No. of animals evinced oestrus		12	16	—
3. Time taken for induction of oestrus(h)		53.50	59.00	—
4. Duration of oestrus (h)		20.00	23.25	20.75
5. Intensity of oestrus (%)				
a. High		25	38	13
b. Medium		67	56	43.5
c. Low		8	6	43.5
6. First insemination conception rate (%)				
	AI at 72 h	33.33	12.50	6.25
	AI at 96 h	33.33	25.00	
7. Overall conception rate (%)				
	AI at 72 h	66.67	62.50	
	AI at 96 h	50.00	50.00	43.75
8. No. of AI per conception				
	AI at 72 h	1.50	2.20	
	AI at 96 h	1.33	2.25	3.14

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Pregnancy Diagnosis in cows by Radioimmunoassay And Milk Ejection Induced By Luteal Oxytocin

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ABSTRACT

Progesterone hormone was analysed by RIA in the milk samples of 50 cross-bred cows, 17-20 days after artificial insemination for pregnancy diagnosis. The cows having progesterone level 5 ng / ml or above were classified as pregnant. These animals were found pregnant by rectal palpation on day 45. Other 50 cows of various stages of lactation and pregnancy (2 to 6 months) were administered intravenously non-luteolytic doses of prostaglandin after removal of milk from cestern by placing teat syphon induced milk ejection within few seconds in the cows with functional corpus luteum and were confirmed pregnant by rectal palpation. The effective dose of prostaglandin was 600 micg / cow. This method of pregnancy diagnosis and presence of corpus luteum in lactating cross bred cows can replace the expensive radioisotopic immunoassay not available under field conditions.

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The per-rectal examination of pregnancy is laborious and fails to diagnose early pregnancy, thus losing valuable time. Radio immunoassay (RIA) has been used for quantitative estimation of progesterone hormone (Laing and Heap, 1971; Bloomfield *et al.,* 1986 Gao *et al.,* 1988), which has high diagnostic value for pregnancy status. The RIA demand handling of radioisotopes and the use of costly scientillation counters and have not reached to common farmers, Veterinarians and livestock production people. The evaluation of rapid milk ejection on administration of non-luteolytic doses of PGF_2 alpha seems to work well on lactating cows for early pregnancy diagnosis (Watches *et al.,* 1984). Wathes *et al.,* (1983) first time reported that luteal oxytocin secreted from corpus luteum caused the increased intra-mammary pressure in rats. The corpus luteum of cows also had oxytocin like activity (Fields *et al.,* 1983).

The method of rapid milk flow test on lactating cows may replace the costly methods like RIA and enzyme link immunoassay. With this objective the present study was undertaken to compare the doses, reliability and cost between RIA and prostaglandin induced milk flow test (PG-IMFT) to confirm the pregnancy status of dairy cows.

MATERIALS AND METHODS

In the present study 10 ml milk samples from each of 50 cross bred cows of Livestock Research Center, 17-20 days post A.I. were collected for pregnancy diagnosis by RIA. Each sample comprised of pooled milk from all the 4 quarters. The samples were preserved with sodium azide and stored at - 20°C pending estimation of progesterone. The samples were defatted by centrifugation at 2000 g for 45 minutes at 4°C before estimation of hormone. The RIA technique used was according to Kamonpatona *et al.,* 1983). Pregnancy status of the cows confirmed by RMFT and by rectal palpation on day 45.

In the second part of the experiment, 50 cows of various stages of lactation and pregnancy (2 to 6 months) were selected from livestock Research Centre of the university and were administered non-luteolytic doses of prostaglandin (Lutalyse). Animals were divided into five groups of 10 each and each group were administered with 125, 250, 600, and 1250, 2500 mic g of prostaglandin intravenously after draining the milk from each quarter with the help of teat syphon. Prostaglandin caused release of oxytocin from corpus luteum and thereby contraction of the milk alveolous resulted in rapid milk flow in few seconds. If the corpus luteum was not present then the flow of milk was not observed. The presence of corpus luteum / pregnancy further confirmed by per-rectal palpation.

RESULTS AND DISCUSSION

The cows having progesterone level 5 ng / ml and above were considered pregnant. On rectal palpation, these cows were found pregnant and the RMFT also showed positive results. Twenty five cows having progesterone value in the range of 5.6 to 16 ng / ml milk were considered pregnant, whereas 21 cows having progesterone level in the range of 0.44 to 0.96 ng / ml with an average of 0.68 ng / ml milk were considered non-pregnant. Four cows having progesterone level 4.4 ng / ml were considered doubtful by RIA but these cows were found pregnant on rectal palpation on day 45 post A.I. and with RMFT. The progesterone values for pregnant cows monitored by RIA are in agreement with the findings of Nut *et al.,* (1974); Heap *et al.,* (1979) and Karagianidis (1990).

The RMFT was taken up on cows of all stages of pregnancy with different doses of prostaglandin. The dose of 600 mic g / cows seems to be quite reasonable. The lower doses not work and higher doses are costly and there is a risk of abortion with application of higher dose of prostaglandin. However, in our study no abortion occured with higher doses as 1250 and 2500 mic g / cows. The results of RMFT are in agreement with Abele (1987); and Labussiere *et al.,* (1992).

The amount of milk ejected after prostaglandin administration and milk yield per day of the cow were not correlated. However, this depends on stage of lactation, and the month of pregnancy of the cows. The cost of 600 mic g prostaglandin is Rs. 2.88 which is within the reach of a farmer and test can be done at farmer's door. The non-luteolytic cheap dose of prostalgandin can confirm the presence of corpus luteum. The RMFT positive test is also indication of pregnancy at early stage (17 days), helpful in the treatment of persistent corpus luteum, pyometra, sub-clinical causes of infertility and controlled breeding in dairy cattle to avoid losses due to decreased reproductive efficiency.

The 'PG - IMFT (RMFT) thus, offer a simple, easy to interpret, rapid, and cost effective method for pregnancy diagnosis as well as for the detection of luteal activity, as a substitute of RIA in lactating cows.

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A Comparative study on the number and type of Pre-Puberal Goat Oocytes Recovered by three different methods

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ABSTRACT

Pre-puberal goat overies were collected from the local abattoir and transported to the laboratory in normal saline supplemented with 50 μg / ml gentamycin. After thorough washing in Phosphate Buffer Salin (PBS), oocytes recovery were made following three different methods. The recovery of grade I oocytes (0.75±0.48 and 0.50±0.26 respectively) were significantly ($P< 0.05$) higher in slicing and dissection than aspiration (0.25±0.25) methods. On overall basis, although, a higher number of oocytes recovered per ovary were observed in slicing (2.17±1.28) and dissection (1.50±0.54) but no significance difference was found with that of aspiration (1.21±0.74).

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Recent developments in embryo biotechnology including in-vitro maturation and fertilization of oocytes are expected to offer new dimensions in research and development for further application in the improvement of farm animals. The availability of good quality and large number of oocytes recovered per ovary has been an important consideration in the in-vitro production of embryos. Mammalian ovaries contain thousands of oocytes, but till now its potential could not be utilized fully. Therefore, a comparative study on the number and type of oocytes recovered by three different methods were made.

MATERIALS AND METHODS

Ovaries (115) of pre-puberal goats were collected from a local abattoir and transported to the laboratory in normal saline supplemented with 50 μg / ml gentamycine. Ovaries were washed thoroughly in PBS. Follicles measuring 2 to 6 mm in diameter were counted for individual ovary. For recovery of oocytes from the folicles, the ovaries of the individual animal were assigned to three techniques i.e. Aspiration, Dissection and Slicing alternatively.

Aspiration: Follicles measuring 2-6 mm in diameter were aspirated by a 2 ml disposable syringe attached to a 20-guage needle with PBS. The content of the follicles were aspirated out and placed in an embryo collection dish containing PBS for evaluation under stereozoom microscope.

Dissection: The follicles measuring 2-6 mm in diameter were dissected out from the ovaries. The stromal tissues were then removed from the follicles as far as possible. The follicles were then taken in a petridish containing PBS and ruptured by using two pairs of pointed dissection forceps under stereozoom microscope. The oocytes recovered were transferred to an embryo collection dish containing PBS using pasteur pipette for evaluation.

Slicing: Ovaries were placed in a petridish containing 2-4 ml of PBS and held with a pair of forceps. The visible surface of the follicles were carefully dissected, and finally the whole ovary was sliced with the help of scalpel blade into thin pieces. Large pieces of ovarian tissues were removed by thorough washing. The petridish was

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observed under stereozoom microscope at 25 X and the oocytes were transferred to an embryo collection dish containing PBS for evaluation.

The isolated ocytes were washed three times in TCM-199 supplemented with 10% heat inactivated goat serum (HIGS) and classified into three categories according to the cumulus cell characteristics i.e. Grade I, II and III.

Grade I — Oocytes with one or more complete layers of cumulus cells adhering to the zona pellocida.

Grade II — Oocytes having scattered envelope of cumulus cells.

Grade III — Oocytes devoid of any cumulus cells.

The students t-test was emlployed to assess the effect of the recovery method on the number and type of oocytes.

RESULTS AND DISCUSSION

Significantly higher ($P< 0.05$) Grade I oocytes were recovered per ovary by slicing and dissection method (0.75±0.48 and 0.50±0.26 respectively) than that of aspiration (0.25±-.20) methods. For grade II oocytes also slicing and dissection recorded higher value (0.76±0.47 and 0.46±0.23 respectively), however, no significant difference were observed between dissection and aspiration (0.331±0.26). There were significant difference among three recovery methods for grade III oocytes. The total number of oocytes recovered per ovary by slicing (2.17±1:28) and dissection (1.50±0.54) method were more than that of aspiration (1.21±0.74) though, the results were not differ significantly. Our results in the present observation are comparable to the reports of Mogas *et al.,* (1992) and Wahid *et al.,* (1992). However, the number of oocytes recovered per ovary was less which may be due to the small structural size of the prepuberal goat ovary. The slicing and dissection methods yield better result interms of grade I, II and total number of oocytes recovered per ovary, as small and medium sized follicles embedded deeply within the cortex of the ovary can be easily cut out or sliced, which might be missed out during aspiration. The present finding supported the reports of Ball *et al.,* (1983) andn Pawshe *et al.,* (1994) that oocytes remain firmly attached to the small and medium sized follicles before cumulus expansion and can not be aspirated, but can be easily recovered from small follicles when the slicing method is employed. Slicing of ovaries is a simple and efficient tool for recovering good quality oocytes but the aspiration technique was found to be very laborious and time consuming becasue of the small structural size of the goat ovary.

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Induction of Oestrus in Buffaloes Using a Herbal Medicine*

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ABSTRACT

A clinical trial was conducted in 110 anoestrus Surti buffaloes using single dose (Janova* capsules - 3 per day for two days orally): Group-I (n=76) and double dose (Janova* capsules - 4 per day for three days orally) Group-II (n=34) schedules. Among the buffaloes treated, 60.53 (n=46) and 52.94 n=18) per cent buffaloes could be followed in group I and II, respectively, with oestrus induction rates of 75.55 (n=34) and 83,33 (n=15) per cents, respectively. Among buffaloes inseminated at induced oestrus, only 8.57 (n=3) per cent and none conceived in group I and II, respectively.

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Among the various aetiological factors of bovine female infertility the functional forms of infertility constitute a major share. Since the hormonal therapy for anoestrus is very costly, a non-hormonal herbal preparation (Janova) was tested in the present study for its efficacy for induction of oestrus in Surti buffaloes.

MATERIALS AND METHODS

The clinical trial was conducted in 110 Surti anoestrus buffaloes at A.I. Centre, Veterinary College, Anand during November, '94 to March, '95. The buffaloes were selected at random after thorough gynaeco-clinical examination per-rectally along with a history of prolonged anoestrus. The buffaloes having smooth and inactive ovaries with no functional structures as well as uterine pathology were selected for the trial.

Among the buffaloes selected, 76 were treated with single dose of Janova capsules (3 per day orally for two days): Group-I and 34 were given double dose (4 capsules per day orally for three days):Group-II. The animals were kept under observation by the farmers for exhibitory signs of oestrus. The farmers were advised to wait and watch for 10 days post treatment and to present the animal for AI on noticing the oestrus signs earlier or on day 10 post treatment and also for followup on observing no oestrus for 10 days.

On followup examination the animals were examined per-rectum for the genital changes, characteristics to oestrus, viz., developing or matured Graafian follicle, uterine tonicity, cervical relaxation as well as congestion of vaginal mucosa and the findings were recorded. The animals induced in oestrus were inseminated and followed for return to oestrus and/or pregnancy 6 to 8 weeks post insemination. The observations made were tabulated and analysed.

RESULTS AND DISCUSSION

Among the buffaloes treated and followed in Groups I and II, 75.55 (n=34) and 83.33 (n=15) per cent buffaloes responded to treatment of single and double dose of Janova capsules, respectively, with follicular development on either of the ovaries. The observations made for mean

* Janova Capsules, Dabur Ayurvet Limited, New Delhi-110 001.

are presented in Table 1. Buffaloes conceived during the induced oestrus were 8.57 (n=3) per cent in Group-I whereas none of the animals conceived in Group-II.

The oestrus induction rate was observed to be comparatively higher with the Group-II than the Group-I, the difference being non-significant, suggested that considerably good proportion of animals can be induced for oestrus using single dose treatment instead of using, double dose and thereby curtailing the cost incurred for the treatment. Secondly, the mean oestrus induction interval has been practically double (16.54 days) in Group-I as compared to that of Group-II (8.53 days). As far as the uterine tonicity, cervical relaxation and oestrus nature are concernced, the response observed in Group-I has been better than that of Group-II. The per cent conception rate obtained in the single dose group has been superior to the double dose group.

Acknowledgement: Authors are thankful to Dr. K.N. Vyas, Principal, Veterinary College, Anand to provide necessary facilities for the trial and to M/s Dabur Ayurvet Ltd., New Delhi-110 001 for free supply of drug.

Table 1. Oestrus induction and pregnancy rates in Surti buffaloes treated with herbal medicine - Janova*.

Sr. No.	Treatment Response		Group - I		Group - II	
			n	Per Cent	n	Per Cent
1.	Buffaloes Treated		76	100.00	34	100.00
2.	Buffaloes Followed		46	60.53	18	52.94
3.	Oestrus Induced		34	75.55	15	83.33
4.	Mean Oestrus Induction Interval (D)		16.54		8.53	
5.	Genital Response:					
	i. Uterine Tonicity	— Good	10	28.57	00	00.00
		— Moderate	21	60.00	11	73.33
		— Mild	4	11.43	4	26.67
	ii. Cervical Relaxation	— Full	22	62.86	1	6.67
		— Partial	12	34.29	12	80.00
		— No	1	2.86	2	13.33
6.	Nature of Oestrus	— Good	9	25.71	1	6.67
		— Medium	22	62.86	10	66.66
		— Poor	4	11.43	4	26.67
7.	Buffaloes Conceived		3	8.57	0	00.00

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Efficacy of Exapar in Post-Parturient Disorders with Retained Placenta in Bovines

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ABSTRACT

Oral administration of EXAPAR - a herbal preparation for treatment of post-partum disorders associated with retention of placenta in 31 cows and buffaloes, resulted in expulsion of membranes, restoration of lochial discharge and involution of uterus. It proved to be an effective and safe uterine cleansing agent for post-partum reproductive health in bovines.

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Post-parturient retention of foetal membranes causes economic loss due to loss of milk and delay in involution of uterus and subsequent conception, early embryonic mortality and the problems of repeat breeding or even permanent infertility (Narasimhan and Deopurkar, 1994). The present study was conducted to evaluate the efficacy of EXAPAR (Dabur Ayurvet Ltd.) in breedable cows and buffaloes.

MATERIALS AND METHODS

The study was carried out on 31 animals (18 Hariana cows and 13 Murrah buffaloes) with retained placenta, in four villages of Gurgaon district. The animals included three cases of abortion. Complications like foetid discharge were present in several animals, post-parturient metritis in four and pyometra in one animal. History regarding calving number, breeding and managemental practices was recorded from the owners.

All the animals were administered Exapar orally, 250 ml divided in daily dosage of 100 ml on the first two days and 50 ml on the third day. Supportive antibiotic therapy, intrauterine or parenteral was given to check infection. The animals were observed for shedding of placenta and nature of discharge. They were rectally examined for seven weeks to note the involution of uterus.

RESULTS AND DISCUSSION

In 15 of the animals, placenta was removed manually on owner insistence, while in others including those having partially retained placenta, Exapar medication resulted in expulsion in 4 to 8 (average 5) hours. Uterine discharge ceased within five days indicating that the drug increased the uterine tone and potentiated the action of antibiotic therapy administered in complicated cases. In five of the 11 retained placenta cases without manual removal and an equal number of manual removal cases, exapar was the only therapy. There was no adverse effect of Exapar medication except slight straining in one or two buffaloes. This was however, not much as to produce prolapse of uterus or vagina.

Involution of the uterus ensured in all the 31 animals (100.0%), 17 of them achieving it in 26 to 35 days, whereas another 14 (45.6) showed complete involution in 36 to 45 days. Timely involution of uterus has a bearing not only on cyclicity but also on milk yield.

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Postparturient oestrus was confirmed within 90 days in all cases where follow-up could be possible.

One of the major preventive measures to check postparturient reproductive failure is the care and follow-up of animals which had retention of placenta, calving abnormalities and abnormal discharge. Various treatments tried including ergot, oxytocin and estrogen, are not satisfactory (Arthur, 1979). Oral administration of herbs with proven ecbolic as well as uterine cleansing and restorative effects therefore, appears to be a safe and effective option both therapeutically and prophylactically. Exapar is a combination of such herbs with documented action profile e.g. *Aloe Barbadensis* (Gupta, 1972), *Aristolochia indica* (Chopra *et al.,* 1982), *Gloriosa superba* (Tewari *et al.,* 1967), *Lepidium sativum* (Nadkarni, 1954), *Leptadenia reticulata* (Satyavati *et al.,* 1976), *Peganum harmala* (Kapoor, 1990), *Plumbago zeylanica* (Kapoor, 1990), *Rubia cordifolia* (Nadkarni, 1954), to name a few.

In the present trial, Exapar gave desired level of activity in different puerperal disorders, and resulted in expulsion of foetal membranes, restoration of lochial discharge and involution of uterus. The shedding of foetal membranes in an average of 5 hours achieved in the present trial compares favourably with the expulsion time observed in a study on another herbal therapy (Nehra, 1987). The time for complete involution of uterus observed herein was not much in excess of the normal involution period recorded for Hariana cows (average 33.5±1.0 days) and Murrah buffaloes (average 38.3±1.2 days). Additionally, Exapar helped in the treatment of metritis through expulsion of the foetid uterine contents and regeneration of the endometrium. Thus Exapar appears to be an effective uterine cleansing agent to ensure postparturient reproductive health of breedable animals.

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Biometrics of Non-Gravid Genitalia of Local Non-Descript Goats of Andhra Pradesh*

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ABSTRACT

Biometrical studies were made on one hundred non-gravid genital tracts of local non-descript goats of similar age and weight. In general, the reproductive tract of these animals resembles that of miniature form of bovine female genitalia. The average weight of entire genitalia was 78.20±2.92gm. The number of cervical folds were 4. The number of caruncles in right and left and both cornua together were 38.30±1.24, 38.20±1.10 and 78.50±1.95 respectively.

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This study was done to establish the norms of female reproductive tract of local non-descript goats of Andhra Pradesh.

MATERIALS AND METHODS

One hundred non-gravid genital organs of local non-descript female goats were collected from an abattoir of Hyderabad, Andhra Pradesh. The genital organs were freed from all extraneous tissues and weighed. The different parts of the reproductive tract were measured by the technique followed by Hadi (1965) and Sharma (1978). The data was statistically analysed as per Snedecor and Cochram (1967).

RESULTS AND DISCUSSION

The weight of genitalia in local does was 78.20±2.92gm. Similar weight of genitalia was reported by Chinchkar *et al.,* (1990). However, Srivastava *et al.,* (1984) reported an average weight of 26.24 gm in Angora crossbred goats. The difference possibly may be due to variation in the size of the animals studied.

The average length of vulva and vagina of local does recorded in this study was 3.00±0.08 and 7.20±0.15cm, respectively. The present values are almost similar to those reported by Basu *et al.,* (1961) and Chinchkar *et al.,* (1990).

The cervix in local she-goats measured 3.90±0.14 cm in length, 2.15±0.03cm in width and 0.92±0.02 cm in thickness. These values are almost similar to the observations of Singh *et al.,* (1974) and Chinchkar *et al.,* (1990). The number of cervical annular folds (3.28±0.70) compare favourably with the reports of the above mentioned workers.

The mean greater curvature of the right and left uterine cornua was 12.07±0.48 and 12.60±0.47 cm respectively. The greater curvature of the left cornua was found to be slightly longer than the right one. The width of the right and left cornua was 6.81±0.29 and 10.80±0.28cm respectively. The width of the left cornua was found to be significantly ($P<0.01$) higher than the right one. Almost similar values were reported in does by Chinchkar *et al.,* (1990).

* Form part of the M.V.Sc., thesis submitted by the Senior author to the Andhra Pradesh Agricultural University.

The number of coruncles present in right and left uterine cornua were 38.30±1.24 and 38.20±1.10 (Total 78.50±1.95). The total number of caruncles observed in this study was found to be far lower than the reported values of Chinchkar *et al.,* (1990), who noticed higher number of caruncles (116.20±5.67) in does. Whereas, Hadi (1965) reported a much lower value of 64.84±9.90 in his study on goat genitalia. The higher values obtained by Chinchkar *et al.,* (1990) might be due to inadequate number of animals taken for his study. The lower values reported by Hadi (1965) might be due to the variation in the size of the animals taken for his investigation.

The present study revealed some differences in length measurements of Fallopian tubes, left oviduct being somewhat longer (15.60±0.46cm) than the right one (15.00±0.4cm). Similar observations were reported by Hadi (1965) and Singh *et al.,* (1974). The values found in this study are in contrast to the observations made by Chinchkar *et al.,* (1990) who recorded the measurement of right Fallopian tubes to be somewhat longar than the left tube. However, the measurements of width at three different places did not differ much between the right and left Fallopian tubes. Similar trend of observations were also reported in their studies by Hadi (1965) and Singh *et al.,* (1974).

The right and left ovaries weighed 0.95±0.04 and 0.90±0.05 gm respectively. The average length, width and thickness of right ovary are 1.80±0.04, 1.10±0.03 and 0.63±0.02 cm respectively. Whereas, the measurements of length, width and thickness of left ovary were 1.92±0.03, 1.24±0.03 and 0.60±0.02cm respectively. The ovarian measurements of this study compare favourably with those reports in does by Singh *et al.,* (1974). Basu *et al.,* (1961) and Das *et al.,* (1982) have reported somewhat lower values of ovarian measurements in does belonging to states of Rajasthan and Assam, respectively.

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Study of Farrowing Pattern and Suckling Behaviour in Desi Pigs

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ABSTRACT

Sixteen, randomly selected, healthy, desi sows, kept in isomanagerial managemental condition were the source for study of farrowing pattern. The usual farrowing took place between 2.00 P.M. to 8.00 A.M. in lateral recombancy except few which were either in ventral or in standing posture. About 95.2 per cent piglets were born showing anterior presentation. Mean parturition time was two hours and nine minutes and the interval between two consective birth of piglets ranged from 4 to 26 minutes. The order of suckling establishes within 20 to 40 hours after farrowing and there is a definite priority for selecting the anterior teats. The interval between two sucklings varied from 30 to 40 minutes and in night hours it was even more.

——x——x——x——

The knowledge of farrowing pattern and suckling behaviour are essential in economic rearing of pigs. Such knowledge helps in intensification of management during farrowing time and rearing of piglets during preweaning period resulting in saving of large number of piglets up to weaning. Proper suckling not only enables the piglets in faster growth but also leads to desirable weaning weights which in turn is correlated with postweaning body weights. Thus the present study was undertaken.

MATERIALS AND METHODS

Sixteen, randomly selected, healthy desi sows kept in isomanagerial confinement system were the source for the study of farrowing pattern. 120 piglets resulting from sixteen farrowings, were the source for study of suckling behaviour. The following parameters were considered for the present study - Posture of female, presentation of foetus, farrowing time (Total duration of parturition including placental expulsion), time interval between two consecutive birth of piglets, establishment of teat order, milk intake during suckling period, diurnal pattern and incidence of distocia, if any.

RESULTS AND DISCUSSION

Normal farrowing time of desi sows varied in between afternoon (2.00 P.M.) to morning hours (8.00 A.M.). The presence of an observor was not tolerated by the farrowing sows and they became upset and disturbed by it, hence hidden observation was done. For comfortable expulsion of foetus most of the desi sows preferred lateral recombant position. During interval between subsequent expulsion of foetuses the sows change their position by gently standing up and later relaxing in lateral recombancy. Left recombancy was more common than right recombancy however the sows rarely change their sides from one recombancy to another until and unless they are disturbed. In the present study 95.2 per cent farrowing occurred in lateral recombancy, 3 per cent in ventral and 1.8 per cent in standing position. Standing position is usually opted by primipara sows for the expulsion of first foetus. Sometimes due to external disturbances, the sows opt standing position. The present findings are in close agreement with the reports of Gotz (1991) and Raychoudhary *et al.,* (1995) in Hampshire sows. Most piglets were born partly covered with fetal membranes and the piglets escaped from these coverings by themselves. Similarly the umbilical cord

in all most all the cases broke down by the piglet movement.

Out of 120 piglets born, 95.2 per cent piglets were born with anterior presentation while 4.8 per cent with posterior presentation. The total average time required for complete farrowing which included placental expulsion also was two hours and nine minutes. The present report is lesser than the reports of Davidson (1953) and Raychoudhary *et al.,* 1995) and is in close confirmation with Hafez (1993). However, the range of duration of farrowing varied from half an hour to seven hours, which is in confirmation with Hafez (1993). This difference of time may be attributed to difference of breed and the managemental situations to which the animals are exposed.

Interval between two consecutive births varied from 4 to 26 minutes and the average time period required was 12 minutes. The variation in interval between two consecutive births may be due to health of piglets, physical status of dams, age of dams and the amount of physical exercise during pregnancy. No case of distocia was recorded which might be due to proper feeding, management, health cover during gestation period and probably due to less number of farrowings studied.

Establishment of teat order was formed within 20 to 40 hours after farrowing. It was observed that stronger piglets tend to suck first and they prefer anterior teats and they maintain their choice throughout the suckling period. The option for anterior teats may be because of the easy approach to catch hold the teats. Usually the pelvic teats are more oedematus for first few days of parturition; hence they are difficult to catch hold and suckle and also due to presence of hind limbs the approach to pelvic teats is not easy. The flow of milk to pelvic teats is always expected to be more because of directly supply of mammary vein. It is suggested that weaker piglets should be assisted to catch hold of pelvic teats. Stronger piglets after consuming the milk of their selected choice tend to interfere with other piglets for want of more milk. The present findings regarding selection of teat is in conformation with the reports of Hafez (1993) and Roychoudhary *et al.,* (1995).

By taking the body weights both before and after suckling (urine and faces were taken care of) it was estimated that piglets suckle about 30, 34.8 and 40.1 gram of milk respectively in first, second and third week of age, per day per suckling. These findings are in confirmation with the report of Gotz (1991) and is in contrast to the reports of Hafez (1993).

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Half life of different motility characteristics of Jersey-Haryana cross bull spermatozoa

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ABSTRACT

Quantification of continuance of sperm motility and different velocity characteristics in definite term of respective half life period has been attempted. The half life period of continuance of number of progressively motile sperm, mean gross velocity and mean velocity of only motile sperm in ejaculated semen samples of first generation Jersey-Haryana bulls at 38±1°C are 3 hr 12 min, 4 hr 54 min and 3 hr 37 min respectively. Relation of continuance of number of progressively motile sperm with gross mean velocity, mean velocity of only motile cells, initial gross mean velocity and initial mean velocity of only motile sperms have been estimated.

——x——x——x——

Assessment of motility is important in identifying ejaculates that appear to be normal / subnormal (Sullivan and Elliot-1968, Shanon and Curson - 1972). Current methods for assessing sperm motility are primarily visual, and the results are expressed in comparative scale than in absolute terms. Most investigators, especially in India, attached to Artificial Insemination units depend entirely on graded estimates of vigour of swirls of wave formation in undiluted sample and percentage of progressively motile sperms in diluted semen sample.

While objective assessment of individual sperm movement has received greater attention during post fifties (Rothschild 1953; Cummings 1954; Enhorning 1968; Elliot *et al.,* 1973) only scattered reports on the actual velocity of spermatozoa of farm animals are available. Philips and Andrews (1937) reported on velocity of ram spermatozoa. Moeller (1950), Baker *et al.,* 1957), Rickmenspoel (1957), Van Demark *et al.,* 1958, 1959) and Van Dam (1958) reported on bull sperm velocity. Velocity and progressive motility with particular reference to its fertility has been reported by Gechwend (1986) with computerised method of videomicrography. Chakrabarti (1993) asserted that the rate of velocity (retardation) of spermatozoa is an objective index predicting livability and quality of sperm. The present article is an attempt to quantify continuance of sperm motility and velocity characteristics in definite term of its half life.

MATERIALS AND METHODS

Sperm velocity was estimated by simple method of Baker *et al.,* (1957) as modified and adopted by Chakrabarti (1993). Studies were undertaken on 38 ejaculates from 3 first generation Jersey-Haryana cross bulls regularly used for breeding purpose. The nonreturn rate of cows inseminated with diluted semen of the 38 ejaculates under studies were 58%. Velocity and motility measurements were conducted at 38°±°C. Measurements were taken every 30 minutes.

Calculation: The sperm velocity in a sample has been found to decrease exponentially with time (Van Duijn 1962;

Chakrabarti 1993). In terms of exponential decay the velocity would become zero only at an infinite time. This obviously is impossible in instant case. The last sperm in reality would become immotile (zero velocity) at a very finite time indeed. It would, however, hold over 2 half periods. Total period of sperm motility continuance is accepted as having 2 half life periods.

For exponentially decaying relation the full life period of the matter is dependent on the time constant (1 / T) of decay, which in fact is the rate of change with unit time. The reciprocal of time constant in an exponential decay relation yields the total life period. In a variable where rate of decay is exponential throughout last bit 'T' is infinity. Half life period in such case is obtained by multiplication of 'T' by 0.69. But in case of sperm life, period of its motility or velocity is very much finite and time limited. The half life period in this case is obtained by multiplying total life period by 0.5 (Van Duijn 1962).

Mean gross velocity in mm / min ($\bar{V}$) was determined following Baker *et al.*, (1957). $\bar{V}$ gives mean velocity of all cells in suspension including those of zero velocity (non-motile). The mean velocity of only motile cells in mm / min ($\bar{U}$) were found by dividing $\bar{V}$ by fraction of motile cells present at the time (Chakrabarti, 1990).

RESULTS AND DISCUSSION

The sperm velocity holds exponential relationship with time upto about 200 min post ejaculation at 38°C after which the retardation rate increases many times. Similar phenomenon has been noted by Rickmenspoel (1960). The sudden acceleration of decay rate and denial of exponential relationship after a certain period of time remains unexplained. Accordingly velocity recorded at different time intervals upto 200 min post ejaculation has been considered for statistical analysis. From regression analysis values of different motility characteristics at zero hour, just on ejaculation, is estimated and called initial values. For sake of brevity abbreviations of the characteristics as also their initial values are used hereinafter viz. % individual motility (IM, IM_o, % progressively motile sperm (N_{mot}, $N_{o\ mot}$, mean gross velocity ($\bar{V}$, $\bar{V}_o$), mean velocity of motile cells ($\bar{U}$, $\bar{U}_o$).

Initial values of sperm motility characteristics of Jersey-Haryana bulls are : IM_o - 99.8%, N_{omot} - 94.23%, $\bar{V}_o$ - 4.74 mm / min and $\bar{U}_o$ - 6.7 mm / min. Van Duijn (1962) reported $\bar{V}_o$ as 6.36 mm / min. Data with regard to IM_o and N_{omot} are not available for comparison.

The half life period of $\bar{V}$ is noted to be 4 hours 54 min or grossly 5 hrs; for $\bar{U}$ it is 3 hr 37 min or grossly 3.5 hrs. The half life of $\bar{V}$ is thus 1.35 times that of $\bar{U}$. Half life of continuance of N_{mot} is 3hr 12 min and that of IM is 3hr 45 min. Beek and Salisburry (1943) reported that the period of continuance of motility of bull spermatozoa in dilute semen at 46.5°C was over an hour. Survival time for bull sperm has been reported by Krolinski (1979) to be 95.5 min. at 46.5°C. Half life period of number of normally moving sperm was reported to be 60.0±44 (S.D). hr. when stored at 1.9°C (Rickmenspoel, 1960).

Half life period of continuance of progressive motility bears a positive correlation, significant at $P<0.05$ with $\bar{V}_o$, and a negative correlation, significant at $P<0.001$ with $\bar{U}_o$. 22.5% and 61.94% of the variation in $\bar{V}_o$ and $\bar{U}_o$ respetively are dependent on the variation of half life period of progressive motility of spermatozoa in the sample. It seems that $\bar{U}$ is important in reflecting the viability as also the

continuance of sperm motility of the sample. Further from the regression analysis of number of progressively motile cells on velocity characteristics it is noted that number of progressively motile sperm is significantly positively correlated with $\bar{V}$ and $\bar{U}$, but negatively with $\bar{V}_o$ and $\bar{U}_o$. Individual motility also bears significantly positive correlation with number of progressively motile sperms. The relations are significant at $P<0.001$. 82.43%, 50.47%, 51.998 % 47.817% and 66.635% of variation in total number of progresively motile sperm is dependent on IM, $\bar{V}$, $\bar{U}$, $\bar{V}_o$ and $\bar{U}_o$ respectively of the sperms in the sample. Van Duijn (1962) observed negative correlation between mean velocity and concentration of normally moving spermatozoa. Further half life of continuance of N_{mot} in a sample bears significant negative correlation with decay rate of $\bar{U}$ ($-d\bar{u}/dt$). About 69% of variation in $-d\bar{u}/dt$ is due to variation in the half life of N_{mot}. The higher half life of motile sperms indicates lower decay rate, greater liveability and very likely fertilisability of the sample. Velocity decrease may be a better index of fertility (Rickmenspoel 1960). Rice *et al.,* 1957) recorded that semen capable of maintaning good motility during storage also showed superior fertilising capacity.

Above observations lead that mere statement of percentile value of progressive motility is vague and possibly mismomer with regard to prediction of semen quality. Half life of N_{mot} sperms describes the sum aggregate health of sperm in the sample more objectively and statistically valid form. It is accordingly suggested that the practice of declaring semen quality through subjective percentile value of progressive motility should be radically revised in favour of half life of N_{mot} in the sample.

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Critical note on Haemocytometric Estimation of Sperm concentration

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ABSTRACT

Red blood cell (R.B.C.) and White blood cell (W.B.C.) counting chambers of haemocytometer have been used seperately in determining sperm cell concentration by direct method. Sperm cell concentration obtained through use of the two chambers have been compared. Determination of sperm cell concentration through use of 4 corner and 1 central medium squares of R.B.C. chamber is much advantageous and preferred as it is supported by valid statistical logics.

——x——x——x——

One of the most important characteristics of ejaculated semen is its sperm content. It contributes significantly towards the fertility status of semen as well as fecundity of the male. The ideal method for determining sperm concentration in a sample must be simple, rapid and accurate. Classically sperm concentration in a sample is determined by direct cell count using Neubauer counting chamber. This method is in regular use in the field laboratories of our country. The use of Red blood cell (R.B.C.) chamber has been traditionally in vogue and referred to by Salisbury *et al.*, (1943), Coffin (1953), Rice (1957), Roberts (1971), Hafez (1980). Sone *et al.*, (1982), however, suggested counting in White blood cell (W.B.C.) chamber. Present note attempts to assess merits of each method, R.B.C. and W.B.C. chamber, in determining sperm concentration in a sample. Such assessment becomes further imperative in view of its inclusion in undergraduate curricula of Agricultural and Veterinary Universities.

MATERIALS AND METHODS

Semen samples were collected from 3 different bulls of central semen collection station at Bengal Veterinary College, Calcutta. Sperms were immobilised by adding 5 mg of sodium fluoride to each 1 ml of semen sample, and kept at 30°C. 0.1 ml of this semen sample was mixed with 9.9 ml of 2.9% sodium citrate dihydrate solution to make the dilution 1 : 100. Thoroughly mixed 1 ml of this solution was then mixed with 9 ml of 2.9% sodium citrate dihydrate solution to make the final dilution of 1 : 1000.

Haemocytometer chamber was next charged with 1 : 1000 diluted sample carefully avoiding any overflow. After charging, the haemocytometer was allowed to stay undisturbed for 1 minute for settlement of the cells. Using a medium power (x10) the ruled area was surveyed to check that the cells were evenly distributed. The high power (X 45) objective was then swung into position and with illunination suitably reduced the cells in 80 tiny (tertiary) squares were counted, i.e. the central and 4 corner medium (secondary) squares of the centrally located R.B.C. chamber. Total number of sperm in 5 medium (80 tiny) squares is then multiplied by 5 to obtain sperm in 25 medium (400 tiny) squares of diluted sample. The value is then multiplied by 10×10^6 to obtain sperm concentration per ml of neat semen (Raphel 1976). Seperately total number of sperms in all 25 medium squares are also counted directly and the number is multiplied

by 10 x 10^6 to get count per ml of neat semen. Number of sperm in all 64 medium (secondary) squares of all the 4 W.B.C. chambers at 4 corners of the same charged haemocytometer are also counted and overall average for 16 squares (1 sq mm) are taken. This is then multiplied by 10 x 10^6 to obtain sperm concentration in neat semen (Sane *et al.,* 1982)

RESULTS AND DISCUSSION

With placement of coverslip over the counting chambers a capillary space of 0.1 mm depth is created. In this capillary space, 4 W.B.C. chambers are placed peripherally at 4 corners of the centrally located R.B.C. chamber. On charging the capillary space with the cell suspension the cells are randomly dispersed in the fluid filled capillary space subject to the wall effect of the capillary. The fluid in the capillary space is influenced by the air current at the four sides of the chamber. At the outer slit like openings of the external edge of the haemocytometer the atmosperic air current is most effective and creates a drift in capillary fluid. Because of the drift there is lateral gliding / shifting of cell particles as also drying of the capillary fluid, initiated at extreme periphery and gradually advancing centrally with time. The head of sperm cell being small, much smaller than W.B.C. are more affected by this fluid drift. Thus counting of cells in W.B.C. chamber is greatly affected unless counting is made in moist / wet chamber. The centrally placed R.B.C. chamber is protected from this adverse effect for some time during which counting can be completed comfortably.

The sperm head size is much smaller than that of W.B.C. They compare with those of human R.B.C. with regard to size. Sperm cells are, therefore, likely to be concentrated centrally in a wet film as on neubauer counting chamber under the cover slip. Counting of sperm cells in R.B.C. chamber, therefore, fulfils condition of random sampling much satisfactorily. Further even after 1 : 1000 dilution of normal neat semen the number of sperm per c mm is much higher than that of W.B.C. in a normal blood sample after 1 : 20 dilution. Advantage of R.B.C. chamber over W.B.C. chamber for counting large number of cell particle under microscopic field is implied. Raphel (1976) suggested that when white blood cell count is very high it might be necessary to use the red cell counting technique instead of W.B.C. counting chamber for estimating W.B.C. concentration in the sample.

Counting of sperm cells in all 64 secondary squares of 4 W.B.C. chamber under low power objective (Sane *et al.,* (1982) is much time consuming, tedious putting stress on eye. This increases accumulated errors in counting. Method of Sane *et al.,* (1982) involves counting of cells in 4 sq mm area. Counting of 5 medium (secondary) squares of R.B.C. chamber counts cell content in 0.2 sq mm area only and is, therefore, less tedious and much less time consuming. Use of high power (x 45) objective makes counting under R.B.C. chamber further easier and comfortable with much less stress on eye. This also minimises accumulated error in counting.

Counting of sperm cells in all 4 W.B.C. chambers considers sperm cells at 4 corners of 1 sq mm each of a large 9 sq mm area. It fails to include representative sample from centrally dispersed cells. It thus fails to fulfil the primary condition of random sampling. The design of counting cells in 4 corner and 1 central medium squares of R.B.C. chamber avoids this deficiency as well. Studies on analysis of variance of

means of counts in 4 W.B.C. chambers vary significantly.

Correlation coefficient (r_1) between total number of cells obtained from count in 5 and 25 secondary squares of R.B.C. chamber is 0.9749, probable error of r_1 being 0.00789. Correlation coefficient (r_2) between sperm count obtained through 16 medium squares of W.B.C. chamber and 25 medium squares of R.B.C. chamber is 0.897, probable error of r_2 being 0.0288. Probable error of r_2 is much higher than that of r_1. Correlation coefficient (r_3) between sperm count through 16 medium squares of W.B.C. chamber and that through the 5 medium squares of R.B.C. chamber is 0.8993, probable error of r_3 being 0.0304, close to that of r_2.

Because of the sampling error in counting through W.B.C. chamber it becomes imperative to note that r_2 differs significantly from r_1. This is tested following through 'z' transformation of 'r' values. r_1 and r_2 differ significantly ($p = 0.0278$). This significant difference further affirms sampling error in counting through W.B.C. chamber.

From the correlational relationship between different methods of counting it is noted that 80.87% of variation in sperm count through medium squares of R.B.C. chamber is dependent on variation in sperm count through 16 medium squares of W.B.C. chamber. 80.46% of variation in sperm count through 25 medium squares of R.B.C. chamber is dependent on variation in sperm count through 16 medium squares of W.B.C. chamber. But 96.04% of variation in sperm count through 25 medium squares of R.B.C. chamber is dependent on variation in sperm count through 5 medium squares of R.B.C. chamber. Thus counting of sperm content through 5 medium squares of R.B.C. chamber is statistically more advantagious.

Finally it is to be noted that repeatability of haemocytometer count is only 0.64 (Hickman 1958). Counting of sperm concentration through centrally located R.B.C. chamber (400 tertiary squares) have been preferred by most workers as it stands on valid statistical logics. Counting through peripherally located W.B.C. chambers induces wider departure from that of R.B.C. chamber and would reduce the repeatability further.

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A Simplified Nigrosine-Eosin-Geimsa Staining Technique to Distinguish Acrosome Damaged Live and Dead Sperms

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ABSTRACT

A simple and rapid procedure for nigrosine-eosin-geimsa staining to distinguish acrosomal damage of live and dead sperm population and to study the acrosomal morphology is described. Twenty semen samples from two bucks were subjected to nigrosine-eosin-geimsa staining at 0, 24 and 48 h storage under refreigeration, revealed that eventhough a high proportion of sperms developed acrosomal damage with storage time, count of live sperms with damaged acrosome was negligible, indicating that acrosomal damage may be developing only during or after death of the spermatozoa.

——x——x——x——

Nigrosine-eosin is the most common staining technique for distingushing live and dead sperms in a dried smear (Campbell *et al.,* 1956) and Geimsa staining sppears to be the most proven method for evaluating acrosomal status (Watson, 1975 and Saacke *et al.,* 1968). Even though incidence of acrosomal damage can be studied, categorisation of acrosomal damage between live and dead sperms was difficult by above staining methods. Recemtly Tamuli and Watson (1994) have reported a staining technique combining nigrosine-eosin with geimsa to distinguish changes in live sperm sub-population. A more simplified procedure of nigrosine-eosin-geimsa (NEG) staining is described in a study to find out the propotion of acrosome damaged live and dead spermatozoa in buck semen at different hours of preservation.

MATERIALS AND METHODS

The study was conducted at Kerala Agricultural University Goat Farm, Mannuthy. Twenty semen samples collected from two bucks at 3 days intervals were used for the study. Nigrosine-eosin stained smears were prepared (1 drop of 1% aquous eosin and 3 drops of 10% aquous nigrosine were taken and a small drop of semen was mixed with it and smears were prepared after 10-15 seconds) immediately after collection and also at 24 and 48 h of storage under refrigeration in Tris egg Yolk diluent (Tris hydroxy methyl methylamine 2.42%, Citric acid - 1.36%, Fructose 1%, Egg yolk 10% and antibiotics). Dried smears were examined for dead and live sperm counts and then the same smear was stained with Geimsa as follows.

The smear was washed in slow running tapwater till the nigrosine-eosin stain was completely washed off and was dried in air. (Before and after washing, few slides at random were counted and compared for the average number of sperms per microscopic field to ensure that sperms were not washed away from the smear). The smear was then covered with Geimsa stain (BDH) for 3 min, washed in tap water, dried in air and examined under oil immersion objective of the microscope. From each smear a total of 333 sperms were counted for the staining characterestics. The same procedure was used for fresh semen and extended semen at 24 and 48 h of storage.

RESULTS AND DISCUSSION

Average number of sperms counted per microscopic field did not show much variation before and after washing (100V/s 99.67%). Based on the staining pattern four categories of sperms were distinguished and were seperately counted.

1. Live with intact acrosome (L.I). characterised by unstained acrosome and post acrosomal region, eventhough slight demarcation between these two regions were present.

2. Live with damaged acrosome (L.D). Here post acrosomal region is unstained with violet decolouration of acrosome. Count of such sperms were negligibly small in all the smears examined.

3. Dead with intact acrosome (DI). Post acrosomal region is stained violet or bright pink and acrosome unstained, such sperms required careful differentiation from Dead sperms with detached acrosome.

4. Dead with damaged acrosome (ED) Here the whole sperm head is stained violet or pink and in a good propotion of such sperms the acrosome showed partial or complete detachment.

The present findings concurs the report of Tamuli and Watson (1994). Counts of acrosome damage in live and dead sperms included swollen, ruffled, detached, partly separated and degenerated acrosomes. The size, shape and other morphological characters of intact acrosome also could be clearly visualized. Integrity of memberane is indicated by unstained acrosomes and post acrosomal regions since damage to membrane allows eosin stain to enter inside, small quantity of eosin is retained inside after washing and counterstaining with Geimsa may be imparting bright pink or violet colour to these previously eosin stained areas.

Average counts of LI, LD, DI and DD sperms in the semen samples studied 81.45, nil, 12.00, 6.55 and 39.57, 0.18, 49.45, 10.80 and 22.74, 0.36, 50.55, 26.35 at 0, 24 and 48 h respectively. Live sperms with damaged acrosome was negligible (0.18%) eventhough total damaged acrosome count was very high at 24 and 48 h storage which implies that acrosomal damage might be developing only during or after death of the sperms. On comparison, dead sperm count in nigrosine-eosin smears was lower than that in nigrosine-eosin-geimsa smears (13.6 V/s 18.55, 48.65 V/s 60.25, 63.28 V/s 76.90 respectively at 0,24 and 48 h of storage) which may be due to counting of some dead sperms with intact acrosome as live sperms in the former technique.

This staining technique is very simple and much more useful, to distinguish acrosome damage in live and dead sperm population. The technique requires very less time and no fixative is needed in comparison to the procedure described by Tamuli and Watson (1994). The acrosomal integrity of live sperms after Freezing using the technique requires further study.

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Biochemical Study of Epididymis of Surti Buffalo male calves during Developmental stages.

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ABSTRACT

Study was performed to know the biochemical characters of caput, corpus and cauda epididymis of Surti male calves during developmental stages and to correlate with the physiological functioning of the gland. Most of the biochemical characters showed positive correlation with development except for the corpus epididymis. Some biochemical characters show decrease in pre puberal animal (Stage II) and some in puberal stage while all the characters rose to a significant levels in post puberal animals (Stage IV). This reflects their utilization and synthesis, under different physiological conditions.

——x——x——x——

As such no work has been reported on the structural, functional and biochemical aspects of sex accessary glands of Surti male. This is an attempt to provide some biochemical aspects of epididymis in relation to developmental stages.

MATERIALS AND METHODS

Epididymis (left and right) were removed surgically and seperated in caput, corpus and cauda from normal healthy male calves of Surti buffalo at four different developmental stages : Stage I (impuberal) - one month age; stage II (pre puberal) - 10 months age; stage III (puberal) - 18 months age and stage IV (post puberal) - 24 months age. The parts were cleaned, weighed and homogenized in cold buffer for biochemical analysis. The estimates analysed were, nucleic acids (Schneider 1957), phosphatase enzymes (King and Armstrong 1934), total protein (Lowry *et al.,* 1951), cholesterol (Schoenhemer and Sperry 1934) and inorganic phosphorus (Fiske and Subbarao 1925). Statistical analysis was carried out by the help of computer (HCL) to know the variation between and within the groups.

RESULTS AND DISCUSSION

Average concentration of different biochemical characters have been represented in Table - 1.

Nucleic acids: Both, deoxy ribose nucleic acid (DNA) and ribose nucleic acid (RNA) showed a statistical difference between the stages ($P<0.01$).

The trend of DNA and RNA was increasing as the age advanced. (Table-1). This increase in nucleic acids can be atributed to the proliferative changes due to secretary activity for different types of protein and enzymes required for maturation process and their contribution towards seminal plasma. Remarkable increase in RNA content of caput epididymis of animals of stage IV, may be attributed to the synthesis of specific RNA during sexual maturity under influence of sex hormone which has positive correlation ($r = 0.608$). Same type of observations have been reported by Ghyselink *et al.,* (1989) for rat epididymis.

Phosphatase enzymes: The differences in these enzymes (both AKP and ACP) due to developmental stages was statistically significant ($P<0.01$). Chauhan and Sharma (1988) also reported same trend for both these enzymes in goat. However, the values

reported are quite high, which may be due to the species and the methodological variation. They used Bodansky method.

Both the enzymes had higher activity in caput and cauda epididymis compared to corpus epididymis (Table-1) indicating physiological importance of these two parts. The activities of both the enzymes were elevated to a significant level in animals of stage IV. This is explained as the functioning of these parts in mature animals. Glover and Nicander (1971) explained same importance with same type of results in mammlian epididymis. Alkaline phosphatase had a positive association with sex hormone (r = 0.409). Same type of relationship has been recorded by Mehta *et al.,* (1985) in rats.

Total protein: Differences in the protein concentration of the parts of the epididymis due to developmental stages were statistically significant at 1 per cent level. Higher levels of protein ($P<0.01$) of caput and cauda epididymis (Table-1) are attributed to their secretary property (Ghose *et al.,* (1989).

There was an increasing trend for protein concentration of the epididymal parts in relation to the age of the animals. Same type of trend has been reported for testicular protein by Champawat *et al.,* (1995). Protein concentration showed positive correlation with weight (r=0.588 for caput and r = 0.633 for cauda) and with sex hormone (r = 0.382 for caput and r = 0.412 for cauda).

As the age advances towards the sexual maturity, protein concentration of caput and cauda increases significantly explaining its association with functional status (secretary) of epididymis.

Cholesterol: Both the total of free cholesterol showed significant variation due to stage of animal for cauda epididymis only ($P<0.01$).

Decrease in cholesterol (total and free) in cauda epididymis of animals in stage II (Table-1) can be attributed to either utilization in the initial phase of pre pubertal stage. Towards the maturity (stage III and IV), these estimate showed slight elevation which can be explained as physiological functioning of these parts as well as influence of sex hormones also.

Inorganic Phosphorus: There was a significant increasing trend for phosphorus concentration ($P<0.01$ Table -1).

Decrease in phosphorus concentration of caput and cauda (group II) can be explained as utilization in the early phase of physiological activity. This was supported by correlation study with weight caput (r = 0.357) and cauda (r = 0.548). Same type of observation were recorded for testicular tissue of these animals (Champawat *et al.,* 1995).

Fall in most of the biochemical characterastic of epididymis, (caput and cauda) in the animals of stage II or III (pre pubertal) reflects the triggering of the physiological functioning of organ as secretary gland.

Table 1. Average concentration (±SE) of biochemical characterastics of epididymis of male calves of Surti buffalo.

Characters	STAGE I			STAGE II			STAGE III			STAGE IV		
	CAP.	CORP.	CAUD.	CAP.	CORP.	CAUD	CAP.	CORP.	CAUD.	CAP.	CORP.	CAUD.
DNA (mg / gm)	5.25	3.61	5.38	6.27	4.27	6.75	3.84	3.21	5.51	6.28	5.56	6.51
	0.19	0.26	0.23	0.45	0.39	0.51	0.22	0.34	0.69	0.39	0.40	0.69.
RNA (mg / gm)	5.65	3.53	5.61	4.01	2.32	4.44	5.77	3.57	5.34	8.22	5.24	6.76
	0.29	0.34	0.32	0.37	0.36	0.36	0.31	0.35	0.38	0.40	0.75	0.28
AKP (KAU / gm)	3.66	1.44	2.79	3.79	0.94	3.07	3.32	1.52	3.41	6.07	2.15	6.54
	0.60	0.15	0.41	0.53	0.11	0.45	0.52	0.17	0.56	0.62	0.17	0.35
ACP (KAU / gm)	6.73	2.42	6.19	4.55	2.72	4.56	6.10	3.28	5.01	7.52	2.01	7.59
	0.48	0.22	0.32	0.53	0.25	0.56	0.46	0.48	0.69	0.50	0.30	0.36
Protein	59.58	18.45	54.25	69.70	19.80	55.59	54.76	12.80	47.37	100.94	25.69	108.95
(mg / gm)	0.99	1.45	3.25	1.21	0.91	3.58	1.23	0.88	1.60	0.73	1.25	4.86
Cholesterol	1.93	1.30	1.20	2.13	1.32	0.68	1.65	1.35	1.50	1.79	1.39	1.28
(free) (mg / gm)	0.34	0.19	0.23	0.19	0.16	0.08	0.20	0.19	0.25	0.18	0.14	0.16
Cholesterol	5.57	4.22	5.31	5.20	4.73	4.11	5.97	5.56	5.67	5.69	5.30	6.65
total (mg / gm)	0.45	0.33	0.54	0.32	0.37	0.27	0.17	0.45	0.39	0.17	0.37	0.32
Phosporus	2.06	0.85	2.34	1.91	1.53	1.67	2.91	1.98	3.42	4.37	2.78	3.81
(mg / gm)	0.23	0.09	0.19	0.21	0.31	0.22	0.21	0.23	0.31	0.51	0.30	0.40

CAP = Caput, CORP = Corpus, CAUD = Cauda
AKP = Alkaline Phosphatase
ACP = Acid Phosphatase.

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Studies on Extracellular Transaminase Activity of Preserved Boar Semen with BTS, Kiev and BL-1 Dilutors

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ABSTRACT

One hundred twenty five (125) semen samples from 5 boars, diluted with BTS, Kiev and BL-1 diluters and preserved for 0.24,48, & 72 hours were utelised to assess extracellular activity of Aspartate amino transferase (AST) and Alanine amino transferase (ALT). Significantly higher extracellular activity of AST (560.46±24.284 IU/ 10^9sperms) and ALT (131.96±6.548 I.U./ 10^9 sperms) were recorded with BL-1 dilutor at 72 hours in comparison of other stages of preservation. Activity of both AST and ALT showed increasing trend from 0 to 72 hours with all the three diluters. Significant effect of diluters ($P<0.01$) on the activity of AST and ALT was recorded at 24, 48 and 72 hours of preservation but no effect of diluters in freshly diluted semen.

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The sperm acrosome and its enzymes play essential role in penetration and fertilization of mammalian ova (Stambough and Buckley, 1969). Leaks of enzymes from spermatozoa, subjected to preservation are accompanied by lowering of their biological value. Pace and Graham (1970) found a positive correlation between AST activity and fertility of bulls. GPT activity was also found positively correlated with mass-activity, sperm concentration and fertility (Khokhar,*et al.,* 1987). However, limited studies have been carried out on the activity of transaminases during liquid preservation of boar semen. Therefore, the present study was carried out to determine the variation in activity of transaminases during preservation of boar semen, diluted with BTS, Kiev and BL-1 dilutors.

MATERIALS AND METHODS

Five (5) semen samples from each of 5 boars, maintained at pig breeding Farm, Kankey, Ranchi were utelised for this study The level of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) was estimated in seminal plasma of freshly diluted and preserved semen samples of 24, 48 and 72 hours of duration following the method of Reitman and Frankel (1957) using the diagnostic reagent kit of span Diagnostics private limited, Surat.

RESULTS AND DISCUSSION

The average values of AST in preserved semen samples with BTS, Kiev and BL-1 indicated significant variation in its activity at different hours of preservation with the three dilutors which corroborates with the finding of Strzezek, *et al.,* (1979) and Azwi, *et al.,* (1990). Higher value of this enzyme was recorded with BL-1 dilutor in comparision of BTS, and kiev dilutor. The effect of dilutors on AST (Table-5) was highly significant at 24, 48, and 72 hours of preservation, which is in accordance with the finding of Bower,*et al.,* (1973). However, the extracellular activity of AST in semen, diluted with BST, and kiev dilutors did not differ between them during the course of preservation.

The lowest value of extracellulor ALT was recorded at 0 hour (45.56±0.828, 41.72±0.0857 and 42.2±0.743 i.v./ 10^9 sperms) with BTS, Kiev and BL-1 dilutor respectively and their values increased with

enhancement of preservation time. Analysis of variance revealed significant effect ($P<0.01$) of preservation time on extracellular level of this enzyme was significant at 24, 48, and 72 hours of preservation only which corroborates with the finding of Strzezek *et al.,* (1979) and Azawi *et al.,* (1990). Zahariev, *et al.,* (1974), however, recorded lower activity of ALT in bull seminal plasma after freezing in comparison of neat semen. Mann and Mann (1981) reported that the activity of transaminases is distinctly lower in seminal plasma than spermatozoa and after centrifugation, the activity in supernatant is positively correlated with sperm density. Variation in extracellular level of enzyme, probably occurs due to presence of decaying spermatozoa. Lower activity of extracellular transaminases in preserved semen with BTS and kier dilluter reflected the importance of protective properties of ingredients, particularly EDTA added in the diluter. Zanarier *et al.,* (1974) and Khokhar *et al.,* (1987) had found a significant correlation between ALT level and percentage of metile spermatozoa, sperm concentration and fertility enhancement in activity of extracellular transaminases of boar semen during preservation with BST, Kiev and BL-1 diluter probably indicated the extent of membrane damage.

Table 1. Mean extracellular AST activity during preservation with different dilutors (Units / ^{9} sperms)

Dilutor	Hours of preservation				
	0 hr.	24 hrs	48 hrs	72 hrs	Overall
BTS	188.64^{Ad}	362.64^{Ac}	646.00^{Ab}	765.36^{Aa}	490.66
	±2.591	±4.034	±4.527	±4.527	±22.953
Kiev	188.72^{Ad}	367.88^{Ac}	654.36^{Ab}	776.40^{Ba}	496.84
	±1.885	±4.701	±5.440	±3.386	±23.362
BL-1	190.56^{Ad}	520.56^{Bc}	693.08^{Bb}	837.64^{Ca}	560.46
	±2.32	±2.928	±2.209	±3.216	±24.284

Values bearing same small superscripts in a row did not differ significantly.
Values bearing different capital superscripts in a column differed significantly.

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Semen Quality and Release of Certain Enzymes During the Course of Freezing Bull Spermatozoa

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ABSTRACT

Freezing caused significant reduction in sperm motility and live sperm count and elevation of acrosomal abnormality, GOT, GPT and hyaluronidase activity of bull spermatozoa. Acrosomal abnormality, GPT and hyaluronidase activity of the spermatozoa increased significantly at the begining of the freezing process.

——x——x——x——

Leakage of transaminase and hyaluronidase from the spermatozoa into the surrounding medium indicates sperm cell damage (Foulkes and Watson, 1975; Mann and Mann, 1981) and occurs well before physical characteristics of the spermatozoa are affected. A study was conducted to record motility, live sperm count, acrosomal abnormality, GOT, GPT and hyaluronidase activity of bull spermatozoa at different stages of freezing.

MATERIALS AND METHODS

Twelve ejaculates from 3 Jersey bulls were frozen in tris-egg yolk-fructose-glycerol extender following 4 hour equilibration at 5°c. Sperm samples were examined for motility, live sperm count, acrosomal abnormality, GOT, GPT and hyaluronidase activity immediately after collection (fresh), during equilibration (0,2 and 4 hours) and immediately after freezing. Sperm motility and live sperm count were estimated by conventional methods, acrosomal abnormality by the method of Watson (1975), GOT and GPT activity by the method of Reitman and Frankel (1957) and hyaluronidase activity by the method of Rogers and Morton (1973).

RESULTS AND DISCUSSION

Variations in physico-biochemical characteristics of bull spermatozoa at different stages of freezing have been shown in Table 1.

Sperm motility and live sperm count were unaffected till the end of equilibration but decreased significantly after freezing. Acrosomal abnormality increased significantly at the end of equilibration and further after freezing. Freezing caused significant elevation of transaminase (GOT and GPT) and hyaluronidase activity of the spermatozoa, which indicated sperm cell damage due to freezing (Mancini *et al.*, 1964; Crabo *et al.*, 1971). Elevation of GPT and hyaluronidase activity cf the spermatozoa, however, was significant from the begining of equilibration and was indicative of bulll sperm damage occurring well before actual freezing.

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Table 1. Physico-biochemical characteristics of bull spermatozoa at different stages of freezing (n = 12).

Sperm	Fresh	During equilibration			After freezing
		0 hour	2 hour	4 hour	
Motility (%)	72.52^{a}± 2.03	70.83^{a}± 1.61	70.83^{a}± 1.61	69.58^{a}± 1.79	50.42^{b}± 1.30
Live sperm (%)	82.83^{a}± 1.17	82.20^{a}± 1.28	81.38^{a}± 1.71	80.75^{a}± 1.77	66.50^{b}± 2.25
Acrosomal abnormality (%)	8.75^{a}± 0.67	11.95^{a}± 0.78	12.98^{ab}± 0.84	17.30^{b}± 1.54	37.25^{c}± 3.01
GOT activity (unit / 10^{8} sperm)	30.60^{a}± 4.18	31.15^{a}± 0.93	32.78^{a}± 2.54	34.60^{a}± 2.73	52.62^{b}± 2.87
GPT activity (unit / 10^{8} sperm)	0.37^{a}± 0.06	12.75^{b}± 1.51	13.50^{bc}± 0.86	13.60^{bc}± 0.84	15.27^{c}± 0.96
Hyaluronidase activity (unit)	2.16^{a}± 0.13	5.98^{b}± 0.14	7.08^{b}± 0.49	7.17^{b}± 0.57	14.87^{c}± 1.33

Means bearing similar superscript in a row do not differ significantly.

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Studies on Age at First Collection and Semen Quality in Young Jersey x Kankrej crossbred Bulls

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Age at first semen donation in artificial vagina was used as a criterion to signify puberty as per Kotayya *et al.,* (1972) and Bhavsar *et al.,* (1974) and an attempt was made to compare the age, body weight, scrotal circumference and semen characteristics of JKF_1 and JK **Inter-se** bulls at first semen donation.

Under standard feeding and management conditions the average age, body weight, scrotal circumference at first semen donation of JKF_1 did not differ much from that of JK **Inter-se** young bulls. In the JKF_1, the first semen donation could be obtained at the average age of 62.00±1.29 weeks when the average body weight was 267.00±8.63 kg and scrotal circumference was 26.55±0.82 cm. In JK **Inter-se,** the average age at first semen donation was 64.00±1.91 weeks and scrotal circumference was 25.18±0.87 cm. However, the average body weight was quite lower (242.50±7.77 kg) than that of JKF_1, bulls. The earliest semen donation was observed at the age of 59 weeks in JKF_1 bulls. The earliest semen donation was observed at the age of 59 weeks in JKF_1 in comparison to 61 weeks in JK **Inter-se.** The latest semen donation was observed as late at 69 weeks in JK **Inter-se** in comparison to 65 weeks in JKF_1 bulls. The results of the present study thus indicate that JKF_1 and JK inter-se bulls do not differ in their age at first semen donation. But, the most conspicuous point is that JK **Inter-se** bulls donated semen at a lower body weight compared to JKF_1 bulls.

The findings of the present study are in accordance with that observed in **Bos taurus** x Ongole crossbred bulls (*Rao et al.,* 1979). Various other workers too, reported that crossbred bulls on average matured around 12 to 26 months of age (Koul *et al.,* 1979; Bhosrekar, 1990). A comparable scrotal circumference of 27.9 cm at pubety in other crossbred bulls was also observed.

In order to ascertain the interval between puberty and maturity the semen quality obtained after 5 to 6 weeks after first collection was compared with the quality of semen obtained at first collection.

The observations of different seminal attributes of consecutive four collections taken on alternate day after 5 to 6 weeks, indicate that the ejaculate volume, mass activity, sperm count and per cent live sperm count improved much over that of first collection. The ejaculate volume, sperm concentration and per cent live sperm count in JKF_1 improved from 2.05 to 2.45 ml, 1.25 to 1.88, 150.00 x 10^6 to 405.63 x 10^6 per ml, 57.00 to 73.00 per cent respectively. The corresponding vlaues observed in JK **Inter-se** bulls were 1.75 to 2.50 ml, 1.25 to 2.50, 112.50 x 10^6 to 565.00 x 10^6 per ml and 30.00 to 68.50 per cent. However, the overall seminal picture indicate that the normal norms are not established till 67 to 68 weeks in the experimental Jersey x Kankrej crosses as evident from the values reported for adult Jersey x Kankrej crossbred bulls

(Patel *et al.,* 1986). The ejaculate volume, mass activity, sperm count, per cent live sperm count reported were 7.09 ml, 3.68, 1235.48 x 10^6 per ml, and 89.53 per cent respectively. The present results thus suggested that Jersey x Kankrej crossbred bulls should be used for semen collection only after 18 to 20 months of age.

Similar observations of gradual improvement in the quality of the semen with advancement of age in crossbred bulls were reported (Rao and Rao, 1978; Raja and Rao, 1983).

In the adult Jersey x Kankrej crossbred bulls the scrotal circumference has been reported to be 36.93±0.72 cm (Patel *et al.,* 1986) and during the present investigation it was observed to be 34.50 cm in the adult Jersey x Kankrej crossbred bulls maintained at Livestock Research Station, Anand. The scrotal circumference recorded at 67 to 68 weeks in the experimental Jersey x Kankrej bulls (28.50 cm) indicated that at the stage of puberty and maturity the gonadal size was about 75 to 80 per cent of that of adult size.

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Preputial Detachment and Penile Release in Growing Jersey x Kankrej Bull Calves

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In the newborn calf, the glans penis is firmly adhered within the preputial sheath by a connective tissue band known as frenulum. The penis, therefore, can not be extended and erected. On approaching puberty, partial protrusion of penis occurs during mounting following separation from preputial sheath. But, complete erection of penis, eventual mating and ejaculation of spermatozoa occurs at puberty as reported by Jainudeen and Hafez (1987). An attempt was made in the experimental Jersey x Kankrej crossbred bull calves to study the age of preputial detachment and penile release. The bull calves were allowed to run along with healthy heifers / cows in heat on that day. This was done regularly at weekly interval.

The average age, body weight, body measurements and scrotal circumference of the experimental Jersey x Kankrej bull calves at complete penile release are presented in Table-1.

The results indicate that under standard feeding and management conditions the average age, body weight, body measurements, and scrotal circumference at complete release of penis from the prepuce of JKF_1 did not differ significantly from that of JK **inter-se** bull calves. The complete release of penis from prepuce occured in JKF_1 at an average age of 46.50±2.22 weeks when average body weight was 217.13±8.12 kg. In JK **inter-se,** the average age of penile release was almost the same. (46.50±3.77 weeks), but the average body weight was quite lower (170.75±19.40 kg) than that of JKF_1. The scrotal circmference at the age of complete penile release was found to be 20.83±0.55 and 20.30±1.61 cm respectively in JKF_1 and JK **inter-se** bull calves. The earliest complete release of penis from the preputial sheath was observed at an age of 37 weeks in JK **inter-se** in comparison to 41 weeks in JKF_1 bull calves; whereas the latest release of 51 weeks in JKF_1. The results of the present study suggested that complete release of penis occurs in JKF_1 and JK **inter-se** corsses at the same age (47 weeks) when scrotal circumference is around 20 cm.

The average age at first protrusion of penis during mounting observed in the present study occured at a later age than those reported in dairy bulls (Almquist and Amann, 1976). This might be due to the differences in breeds, feeding and management practices involved.

Table 1. Mean age, body weight, body measurements and scrotal circumferences of Jersey x Kankrej bulls at complete penile release.

Attributes	JKF_1 Mean±S.E.	JK Inter-se Mean±S.E.	Overall Mean±S.E.
No. of observations(n)	4	4	8
Age (weeks)	46.50±2.22	46.50±3.77	46.50±2.03
Body weight (kg)	217.13±8.12	170.75±19.40	193.94±13.10
Body measurements:			
Body length (cm)	116.63±1.83	114.00±4.45	115.31±2.28
Height at withers (cm)	113.75±1.03	107.75±4.38	110.75±2.37
Heart girth (cm)	137.75±1.11	128.50±5.30	133.13±3.06
Scrotal circumference (cm)	20.83±0.55	20.30±1.61	20.56±0.79

ANOVA showing differences of genetic groups for age, body weight, body measurements and scrotal circumference at complete penile release in Jersey x Kankrej bulls.

Sources	d.f.	M.S.S. of					
		Age (weeks)	Body weight (kg)	Body length (cm)	Height at withers (cm)	Heart girth (cm)	Scrotal circum-ference (cm)
		NS	NS	NS	NS	NS	NS
Genetic group	1	0.00	4301.2825	13.78250	72.00	171.120	0.55120
Error	6	38.33	884.9882	46.364375	40.58333	58.62583	5.75792
Total	7						

NS = Not significant

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Bovine Spermatozoan Motility Behaviour in Cervical Mucus and its Relationship to Fertility

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Sperm simulatory techniques like sperm penetration tests and swim up techniques were evolved (Suttiyotin *et al.,* 1992) using cervical mucus for objective measurement of semen quality. Computerized semen analysis allows sperm behaviour evaluation in a more detailed manner in luminal fluids of female genitalia. Such information would be of considerable interest for clinical evaluation of bovine fertility. In the present study an attempt has been made to relate spermatozoan concentration and sperm motility pattern when frozen thawed semen samples from Holstein bulls with known fertility were subjected to penetrate and migrate through cerivcal mucus, using a Hamilton - Thron International visual Optical System Motility Analyser (HTM - IVOS).

Cervical mucus collected from oestrus cows and bulls with known fertility (as judged by NR rates) whose semen cryopreserved were used in the above study. 10 µl of cervical mucus were placed on a prewarmed glass slide and covered with a cover slip. At one edge of the cover slip, one drop of the semen sample under test was placed and the whole set up was loaded on to the stage of HTM - IVOS where samples were evaluated for sperm cell concentration and motion behaviour with software supplied along with instrument. The data was analysed for correlation coefficient as per Snedecor and Cochron (1965).

The results have been presented in Table. It was observed that spermatozoa took ten minutes to reach the centre of the fluid from the site of contact by migration and penetration through cervical mucus which is in agreement with Gaddum - Rose *et al.,* (1980). The concentration of spermatozoa in semen samples obtained from different bulls with different fertility, had no relation with fertility status of the bull, since the bull with fertility of 41% had a higher spermatozoan concentration than in bull with 50% fertility, which is in agreement with the observations of Mortiner *et al.,* (1986). A look at the table reveals a significant ($P<0.05$) positive relation ($r = 0.88$) between sperm motility % of semen samples and fertility. Higher the motile sperm cells in the semen, the fertility of the bull is high and the present findings are in agreement with the observations made by Murase *et al.,* (1990). Further, higher the number of progressively motile sperm cells in the semen, higher will be the fertility rate. Galli *et al.,* (1994) also observed the similar pattern which is useful in andrological studies. An increase in the number of spermatozoa possessing linear motion, high path velocity and progressive velocity had a significant positive correlation with fertility as observed in our results. Earlier studies by Murase and Brown (1987) also indicated that conception rate in cows increases with increasing values for permeability of cervical mucus to spermatozoa, in particular with rate of forward motion of spermatozoa. Thus the pattern of bovine cervical mucus penetration and migration by spermatozoa can provide a useful information in andrological studies with respect to sperm behaviour and sperm interaction on female genitalia for selecting samples of semen with optimal charecteristics for higher fertility.

Table 1. Sperm concentration, motility & fertility parametres during sperm migration in cervical mucus. (Mean±S.E.)

Parametre:	HF1	HF2	HF3	HF4	HF5	HF6	HF7
Concentration	26.5	47.5	38.4	23.4	55.0	24.6	41.1
(X 16^6 / ml)	±0.54	±0.19	±0.19	±1.17	±1.45	±2.20	±1.20
Motility (%).	48.5	53.0	34.5	47.5	52.0	68.5	69.0
	±5.51	±1.00	±1.50	±0.49	±8.02	±4.43	±1.43
Progressive Motility (%)							
a. Rapid	01.5	03.5	00.0	04.0	06.5	08.5	18.0
	±1.50	±2.50	±0.00	±0.00	±1.50	±0.49	±0.23
b. Medium	14.0	34.5	23.5	18.5	03.7	05.5	57.0
	±3.00	±0.49	±0.49	±0.49	±0.19	±1.50	±0.23
c. Slow	15.5	13.5	04.0	00.5	15.5	00.5	03.0
	±4.30	±2.50	±2.00	±0.49	±1.50	±0.49	±0.12
d. Static	29.5	46.0	52.0	25.5	55.0	03.5	07.0
	±5.43	±1.00	±1.00	±1.50	±3.93	±2.50	±0.33
Path Velocity	46.8	44.9	44.9	68.5	48.8	46.5	64.5
(µm / sc)	±4.41	±2.85	±0.07	±0.04	±1.65	±5.01	±0.49
Progressive	35.8	35.8	37.2	58.4	66.5	42.2	57.5
Velocity (µm / sc)	±1.34	±1.14	±1.29	±2.45	±4.31	±3.15	±±0.23
Linearity (%)	46.0	±52.5	±71.5	±56.5	±52.0	±68.5	±80.0
	±3.00	±1.50	±6.51	±2.50	±2.00	±2.50	±0.12
Fertility (%)	41.0	48.3	—	50.2	50.4	55.4	58.8

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Induction of Estrous in Anestrous Murrah Buffaloes with Low Doses of Receptal and Lutalyse

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To combat anestrous in buffaloes, different remedial measures have been proposed, but recently gonodatropin releasing hormone has shown potential in the treatment of anestrous in buffaloes (Pattabiraman *et al.,* 1986; Dhoble and Gupta, 1986). In this experiment, low dose of Receptal has been tried to reduce the cost of treatment in anestrous murrah buffaloes, maintained at L.S.F., Dairy Unit, J.N.K.V.V., Adhartal, Jabalpur during the period from August to October, 1995.

Ten post-partum anestrous (90 days) murrah buffaloes were examined on two occasions at an interval of 15 days to exclude possibility of cyclicity and were injected 2 ml of Receptal (Hoechst, India). In other group seven animals similarly examined were kept as control. Rectal palpation was done every 15 days post-treatment for presence of corpus luteum. Animals having corpus luteum were treated with PGF_2 alpha (Lutalyse - upjohn). A murrah buffalo bull was paraded twice daily in the morning and evening for detection of estrous and natural service. Pregnancy diagnosis was done 45 days after natural service.

Out of ten animals injected with Receptal 6 animals showed pronounced estrus on an average interval of 21.16 days with cent-percent fertility. Three of four animals showed presence of corpus luteum on day 30 and one on day 45, post-treatment, and showed estrus on an average interval of 9.25 days after infusion of 5 mg of lutalyse with fertility. Similar interval in response to GnRH has been reported by Sankhyan (1992). However the conception rate in the present study is higher than reported by Kodagali *et al.,* (1981) in cows and Pattabiraman *et al.,* (1986) in buffaloes. This may be attributed to size of experimental herd, season and plan of nutrition.

In the control group, out of seven animals, 3 animals showed estrus with conception following natural service. Thus it clearly shows that Receptal even in low dose of 2 ml has a clear-cut effect in initiating estrus and/or forming corpus luteum, which was fully sensitive to luteolytic effect of lutalyse. However, the response to reduced dose of Receptal (2 ml I/m) is not so precise as with 5 ml as reported by Sankhyan (1992). Thus, it can be concluded that Receptal even in reduced dose is able to induse estrus / corpus luteum in treated animals with very good conception rate during the specified period under study.

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Hormotone Treatment for puerperal uterine soundness in Murrah buffaloes

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Expulsion of the placenta within the stipulated period is more important for subsequent reproductive efficacy as it helps in initiating involution of the uterus and appearance of post-partum heat. The establishment of regular oestrous cycle after parturition in cows and buffaloes is delayed for a variable period of time (Hukeri, 1995). Any invasive procedures of the reproductive tract is possibly contra indicated to attain the normal reproductive efficiency. Hence, the use of these procedures should be the definite exception rather than the rule (Wheeler, 1994). Looking to the above facts the efficacy of Hormotone* liquid was tried on the puerperal uterine health in Murrah Buffaloes.

The clinical trial was conducted on 50 healthy advanced pregnant Murrah buffaloes aging between 6-12 years. These animals were randomly divided equally into Hormotone treated and untreated control groups. To the buffaloes of Hormotone treated group 200 ml of Hormotone liquid was administered orally within one hour of parturition. Subsequently, the animal dropping placenta within 12 hours post-partum was administered 150 ml of the drug daily for 4 days. The animal failing to drop the same within the period was also medicated similarly with the only difference of a 150 ml additional dose at 12 hours also. The duration of expulsion of placenta, a cessation of lochia, involution of uterus and interval from calving to appearance of first post-partum oestrus was recorded as described by Salisbury *et al.*, 1985). In treated and control groups when the placenta was not expelled upto 48 hours of parturition it was removed manually followed by 2 doses of intrauterine administration of 4 Furea boluses on alternate days.

A significantly earlier expulsion of foetal membrane ($P<0.01$) was recorded in 88 per cent buffaloes of Hormotone treated group (5.45±0.45h) as compared to 80 per cent buffaloes of untreated control group (7.38±0.64h). The present findings are in agreement with the reports of Dindorker *et al.*, (1982), who also reported less duration (26.35±12.47 minutes) for expulsion of foetal membranes in Hormotone treated buffaloes than the untreated buffaloes (304.17±35.25 minutes).

Earlier cessation of lochia was recorded in Hormotone treated (10.36±0.71 days) than the untreated (12.16±0.89 days) buffaloes.

The uterine involution was significantly earlier ($P<0.05$) in Hormotone treated (22.32±0.82 days) than the untreated control 25.60±0.99 days) buffaloes confirms the findings of Dindorker *et al.*, (1982) who also observed significantly

M/s Charak Pharmaceuticals, Umergaon, Gujrat, containing Ashok, Dashmool, Jamboobeej, Satawari, Ashwagandha, Dodhar, Bhangra, Harde, Baheda, Amla, Anantmool, Jaswand, Karini, Bale, Garbha, Deodar, Kamal, Laveng, Pipali, Jeera, Nagarmoth, etc.

earlier involution of gravid cornua in Hormotone treated than untreated cows. They also observed earlier uterine involution in Hormotone treated than untreated buffaloes.

Eighty eight per cent buffaloes of Hormotone treated group expressed the first post partum oestrus much earlier with a mean duration of 74.78±4.66 days as compared to 80 per cent buffaloes in control group with a mean durationof 87.00±4.97 days, suggested that ingredients present in this drug have uterine stimulatory effect and are capable of stimulating ovarian functions by correcting hormonal balance (Sagdeo, 1982) and increasing ovulatary functions (Dindorker *et al.,* 1982).

It can be concluded that post-partum administration of Hormotone results into early expulsion of placenta, disappearance of lochia, involution of uterus and appearance of first post partum oestrus. Furthermore, if the placenta was not expelled within 12 hours post-partum than manual interference was mandatory, as none of the animals dropped the foetal membranes by itself if the retention was more than 12 hours.

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Oestrus Synchronization By Intravulvo Submucosal Injection of PGF_2 alpha In Goats

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Induction of luteolysis using PGF_2alpha or its analogues is the most widely used method for synchronization and is administered through different routes either as a single dose or as double blind trial (Narayana and Honnappa, 1986; Shivkumar, 1993 and Chauhan *et al.,* 1994). Luteolytic dose of PGF_2 alpha can be considerably reduced by intravulvo submucosal route of administration (Horta *et al.,* 1986 and Chauhan *et al.,* 1986) and the proportion of animals synchronized by a single injection can be increased if the animals are treated during the luteal phase of the cycle (Schama and Karg 1982).

The present investigation was to study the effectiveness of a single injection of PGF_2 alpha by intravulvosubmucosal route after palpation of the tubular reproductive tract by a method evolved by Kutty and Sudarsanan (1996) to assess the phase of the cycle.

MATERIALS AND METHODS

Twenty does with history of repeated cycles belonging to KAU Goat farm, Mannuthy formed the materials, which were selected based on palpation of tubular reproductive tract for lack of tonicity. PGF_2 alpha (Lutalyse - UpJohn) was given intravulvosubmucosally at 3 dose levels of 3.5, 2.5 and 2.0 mg to six does each and 2 animals were given a dose 1.5 mg each. All the animals were observed for signs of oestrus at 48, 72 and 96 h using a vasectomized buck and also by speculum examination, for internal changes. Animals showed oestrus signs were inseminated using fresh semen extended in goat milk antibiotic extender. Pregnancy diagnosis was done by palpating the tubular genital tract (Kutty and Sudarsanan, 1996) at one and two months and compared with date of kidding for confirmation.

RESULTS AND DISCUSSION

All the animals showed oestrus changes such as cervical dilatation and mucous discharge irrespective of the dose levels tried and is in agreement with the report of Chauhan *et al.,* (1994) that no significant influence for dose of PGF_2 alpha on the proportion of animals responded to the treatment. Behavioural oestrum of a lesser intensity was observed only in five animals at 72 to 96 h which included wagging of tail and standing to be mounted while rest of the does failed to show any behavioural signs and failed to be detected by the buck. Of the 18 does, 16 showed cervical dilation and mucous discharge at 48 h, while all the does had these changes at 72 h and in 17, this changes continued upto 96 h and is similar to the report of Pant *et al.,* (1992); Shivkumar, (1993). Only three animals (from different dose levels) were diagnosed pregnant and conception percentage was 15, while the average

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conception of untreated does during the month was 20.8%. The present finding supports the report of Pant *et al.,* (1992) that fertility at the synchronized oestrus is generally lower in lactating dairy animals and there is considerable year to year and season to season variations between and within farms. Contrary to the present finding Shivkumar (1993) reported 85% conception in does synchronized using PGF_2 alpha by intramuscular route.

It is concluded that even though a very low dose level of PGF_2 alpha 1.5 - 2 mg administered intravulvosubmucosally after luteal phase assessment ensures synchronization in 100% of goats, the conception rate was not encouraging.

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Successful Pregnancy, Superovulation and Embryo Fertility of A Cyclic Non-Breeder Cow

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The present communication puts on record a case of repeat breeder which failed to conceive by conventional therapy and ultimately conceived by a frozen embryo of another cow.

CASE HISTORY AND REPRODUCTIVE MANAGEMENT

An eight years old elite Holstein-Friesian cow with a standard lactation yield of 7283 Kg failed to conceive after several inseminations in her fourth lactation. It was then decided to superovulate this cow with a hope to get a few viable embryos. Superovulation was induced on day 10 of estrous cycle with multiple injections of FSH (Folltropin-V, Vetrepharma Inc. Canada) in descending dose schedule, twice daily for 4 days (2x100mg, 2x80mg, 2x40mg). 50 mg Lutalyse (Upjohn, USA) was administered in two equal divided doses at 48 and 60 hrs after initiation of superovulatory treatment. Donor was inseminated with high pedigree imported frozen semen at 48, 60 and 72 hrs after first prostaglandin injection. Embryo collection (non-surgical), evaluation, freezing (1.0m Glycerol), thawing and transfer were made as reported by Mishra *et al.,* (1990) on day 7 after first insemination. Immediately after each flushing 2.5 gm streptopenicillin (Dicristicine-S, Sarabhai) was infused intrauterine and 25 mg Lutalyse was injected i.m. to cause lysis of multiple corpa lutea. First flushing of donor was done 11 month post calving and subsequently on 52 and 30 days of first and second flush respectively, using same protocol. Again, donor failed to conceive when inseminated in second estrus after third flush using 100 ug Gn-RH (Fertagyl, Intervet, Holland) i.m. at the time of AI. A frozen embryo of another cow after thawing was transferred non-surgically in this cow.

RESULTS AND DISCUSSION

The donor cow responded well to the superovulatory treatment. Both the ovaries got enlarged greatly in each treatment. The number of CL counted in both ovaries during first, second and third flushing was 10, 17 and 12 respectively. Total eggs recovered in respective flushing were 9, 16 and 12. Total recovered viable embryos, degenerated embryos and unfertilized ova were 2, 4 & 3 infirst, 15, 0 & 1 in second and 11, 1 & 0 in third flush. Among recovered eggs, 89.18% were fertilized and 81.08% were viable indicating that repeat breeding in the cow might be due to developmental abnormalities of eggs rather than failure of fertilization. Out of total recovered viable embryos, 25 embryos were frozen and 5 were transferred fresh in the recipients. Later on, 12 frozen embryos were thawed and transferred into the recipients. On Gynaecological examination 60 days post transfer 2 recipients with fresh embryo (pregnancy rate 41.7%) were found pregnant. These findings indicate that embryos from the cyclic non-breeder cow were capable of full development in recipient. However, interaction between the mother (repeat beeding cow) and its embryo might not suitable for further development/

implantation. Interestingly enough, the donor cow was conformed pregnant 60 days post transfer. There appears to be no reason to believe that any abnormality existed in ovary, bursa, salpinx and FSH / LH releasing system. Kharche *et al.,* (1995) also reported that infertile cow may be superovulated for viable embryos and later on may be inseminated to get pregnancy.

It is inferred from the above findings that some non-specific factors may be responsible for repeat breeding. Further investigations on this line is, therefore, needed in order to understand proper explanation of interaction between embryo and uterine milieu.

Acknowledgement: The authors are most grateful to Dr. R.S.Gill, General Manager (Farms), NDDB, New Delhi and Dr. A.K.Singh, General Manager, Animal Breeding Centre, Salon, Rae Bareli, for their valued guidance.

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The Use of Tocolytics in Veterinary Obstetrics

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The word tocolytic is a Greek derivative (tokos = birth or labour; lysis = dissolution). Tocolytic drugs have been used in bovine obstetrics for the treatment of uterine spasm, incomplete dilation of the cervix, uterine torsion and prolapse,during foetotomy and to aid exteriorisation of the uterus during caesarean operation (Jonker *et al.,* 1991). Clenbuterol is a recently developed tocolytic drug. It produces myometrial relaxation by stimulating only the beta receptors in the myometrial cells (Menard and Segundo, 1987 and Plant and Bowler, 1988). Among all the drugs tested, beta adrenergics are considered as best and effective, besides they have lowest rate of side effects (Brockway *et al.,* 1987).

Eighteen cross bred pregnant cows in the first and second stage of labour were randomly selected from Military Dairy Farm, Hebbal, Bangalore. Clinical evaluation of these animals revealed no evidence of pregnancy abnormality. These animals were divided into three groups of six animals each. The work was planned for parturation postponement due at night and for reduction of dystocia.

Group I : Control group no treatment.

Group II : Intramuscular administration of clenbuterol 300 ug in the first stage of labour.

Group III : Intramuscular administration of clenbuterol 300 ug in the second stage of labour.

Spontaneous contractions were successfully abolished within 14 to 20 minutes after injection. In the six cows treated with clenbuterol in the first stage of labour; parturation was delayed for 7 to 10 hours. In cows treated with clenbuterol in the second stage of labour parturation was delayed for 2 and 3 hours. Clenbuterol is widely used in veterinary practice as a tocolytic in farm animals, to provide therapeutical interruption of parturation. Present data show that duration of delaying calving in the first stage of labour are much more compare with the second stage of labour, but in both of them result were satisfactory.

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Treatment of Cystic Ovarian Degeneration in Yak

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Cystic Ovarian Degeneration in dairy cattle is one of the major condition causing infertility in cattle. Cystic condition arises due to failure of the hypophysis to release sufficient amount of Lutenising Hormone (L.H.) to produce ovulation and proper development of corpus luteum (Roberts, 1971). There is no report of Cystic Ovarian Degeneration in yak. A second calver yak cow exhibited first postpartum heat after twelve months, in March, the beginning of breeding season in high altitude. It came to heat with normal discharge and oestrus length i.e. about 18 to 20 hrs, even though it was served 21 days ago. On palpation no evidence of ovulation or formation of corpus luteum in the ovary was noticed. It came to heat for the 3rd time in sequence exactly after 21 days with prolong oestrus duration accompanied with peculiar posture of raised tail for three to four days (Fig-1). During that period there was mild rectal prolapse due to relaxation of pelvic muscles, which was corrected by manual replacement (Mondal *et al.,* 1995).

Further examination revealed the presence of a fluctuating cystic structure of 2.5 to 3 cm size in the left ovary. On repeated examination as suggested by Arthur *et al.,* (1989) it was confirmed to be a case of Cystic Ovarian Degeneration.

Initially the yak cow was treated at first with Lutalase 5 ml. intramuscularly (Upjhon, Bombay) and the animal with out any response. On examination the structure was found in the ovary and the animal came to heat again with a long oestrous duration and scanty discharge. It was then treated with single dose of Chorulon (i/m 1500 I.U. Intercare) to lutenise the cyst followed by 5 ml. of Lutalase (i/m) after 10 days of Chorulone treatment to induce oestrus. Animal came to heat after 72 hrs and served by a yak bull. Interestingly, it again came to heat though it was pregnant which was confirmed after 60 days. It calved a normal male calf after 262 days of pregnancy.

This is a first case report of follicular cyst in yak.

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Ventral Hernia of the Gravid Uterus in a Cow - A case Report

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Extensive ventral hernia of the gravid uterus is a rare, but a potentially serious accident of advanced pregnancy in dairy cattle. The condition may occur either as a result of severe traumatic blow or due to a weak abdominal musculature (Arthur, 1964). In view of the rarity of the condition, the present paper places on record, a case of extensive ventral hernia observed in a dairy cow.

CASE HISTORY

A seven year old crossbred Holstein Friesian cow was referred to our clinic with the history of extensive swelling of the ventral abdomen. The animal had been purchased by the present owner when it was around four months pregnant. The owner noticed a swelling about 2 months back just in front of the udder on the right abdominal floor. Initially the swelling was small, about the size of a foot ball, and later proceeded gradualy so that in about a months time, the distention was so extensive that it extended from infront of the udder to sternum anteriorly and well below the hock ventrally.

The swelling was most prominent on the right side and the udder was deflected to one side. The animal had extreme difficulty in getting up and had a slow laboured gait. The appetite had considerably reduced. A hard movable mass could be palpated in the distended area on the right side of the ventral abdomen. A detailed rectal examination revealed that the cervix was well beyond the pelvic brim and the fetal parts could barely be palpated. However, uterine thrill was clearly evident indicating the presence of a live foetus.

Vaginal examination revealed soft pliable cervix which admitted two fingers, but the vagina appeared to have been pulled anteriorly. There was also a considerable accumulation of urine in the anterior vaginal floor. The sacrosciatic ligaments and vulva had also relaxed considerably. The history and observations made on the animal clearly suggested it to be a case of extensive ventral hernia of the abdomen.

LINE OF TREATMENT

Although the exact duration of pregnancy was not available with the owner, the mammary gland changes odema and relaxation of vulva and sacrosciatic ligaments suggested of an imminent parturition. Since the ventral hernia was extensive and dystocia was anticipated an elective caesarean section with the object of saving the life of the calf and the repair the hernia if possible was decided. Caesarean section was carried out through ventral paramedian incision using local and paravertebral anaesthesia. A live healthy female calf was delivered through the hysterotomy incision. The placenta was left inside and the uterus was sutured using standard procedure. Further exploration revealed an extensive ventral hernia and it was impossible to bring the hernial edge together, therefore, ventral hernia was not

repaired and the rest of the incision wound was surgically repaired. By day 10 post-surgery, the animal was active and moving about freely although the ventral distention still persisted. By two months post-surgery, the veterinarian who had referred the case informed that the ventral distension had considerably reduced and the abdomen had almost returned to its normal contour. Spontaneous reduction of the ventral hernia following delivery has been previously reported by Arthur (1964) and Robert (1986). Furthermore, ventral hernia may reoccur during subsequent pregnancies and hence rebreeding of such animals is questionable.

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Atypical Adenomatous Endometrial Hyperplasia in a Bitch

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The adenomatous endometrial hyperplasia is reported in a pomeranian bitch.

A 3½ years old bitch was presented to the clinic with the complaint of recurrent vulvar bleeding which was severe during oestrus. The oestrous cycle was erratic and matting never occurred. The growth of the animal was stunted as compared to its litter mates. Hormonal therapy was never used in the animal.

Clinical and haematological studies viz. RBC (3.69 x 10^6 µl), WBC (15.09 x 10^3 µl), haemoglobin (8.00 g/dl), PCV (27.00%), neutrophil (80.00%), lymphocytes (17.00%), eosinophil (3.00%) and total protein (4.4 g/dl) lead to the tentative diagnosis of chronic uterine pathological condition. Hence, ovariohysterectomy operation was performed under Diazepam pre-medication and epidural anaesthesia. Theuterus was tentimes larger than the normal size and it's lumen was not palpable as it appeared to be filled with some mass. The left ovary was normal, whereas, the right ovary was four times larger than the normal size and was cystic.

The longitudinally incised uterus was completely filled with the bulging cysts of various sizes. Fluid oozed from the ovarian and uterine cysts on puncturing. Histopathological examination revealed regularly arranged columnar epithelial cells lining the dilated endometrial glands and hyperplasia of the stroma.

Jones and Munt (1983) described similar observations for adenomatous endometrial hyperplasia in bitch as reported in the present case. Some workers (Sherman and Brown, 1979; Robbins and Kumar, 1987) considered this malady as the precursor of endometrial carcinoma in women. Morrow (1984) stated that the hyperplasia is caused by a relative or absolute hyperestrinism, as seen with polycytic ovaries or by the prolonged use of exogenous estrogens. In the present case also the occurrence of cysts in the right ovary might have elevated blood oestrogens, causing endometrial hyperplasia in the bitch.

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A Case of Uterine Prolapse in Goat

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The incidence of uterine prolapse was recorded by various workers (Rasbech *et al.,* 1967; Odegaard, 1977; Deka and Sarma, 1978). Prolapse of uterus occurs occasionally in sows and rarely in bitch, cats and mares. There is only one available literature regarding prolapse of uterus in goat (Deka and Borgohain, 1979) in North-eastern region. Therefore, the present study deals with a rare case of uterine prolapse in goat.

CASE REPORT

A non descript local goat of first lactation was brought by a private farmer to the clinics of the Department of Gynaecology, Obstetrics and Artificial Insemination, College of Veterinary Science, Khanapara, with the following history.

The she goat delivered one female kid in the morning at 5-00 a.m. After few minutes of delivery she developed abdominal straining at frequent interval. At 6-00 a.m. the uterus of the goat got porlapsed. The fetal membrane was connected with the prolapsed uterus.

CLINICAL FINDINGS

The she goat was found standing and drowsy. The whole uterus was completely everted out as an irregular big mass. On palpation the organ was found tense with reddish discolouration. A part of fetal membrane was found hanging down from a prolapsed uterine horn reaching the operation table (Fig. 1). The mucosa was inflammed. The prolapsed organ was soiled with blood clots and dust. During examination the animal was straining vigorously.

TREATMENT

After restraining the animal in recumbent position 3 ml of Lignocaine (BAIF) was injected epideurally. The prolapsed organ and the surrounding area were washed with weak potassium permanganate (1:10,000) solution. The parts of placenta attached to the prolapsed organ were separated out from the maternal caruncles. The urinary bladder was evacuated. Small blocks of ice were placed over the prolapsed organ to reduce the size of the prolapsed organ. Strepto-penicillin powder (Dicrysticin-S : Sarabhai Chemicals) and Tegeron (M & B) were applied on the prolapsed part. After that, the prolapsed organ was replaced with gentle pressure using palms of both hands. One lubricated hand was introduced gently to ensure complee replacement of the uterus. Two pairs of loop suture with black braided silk were given on either side of the vulva at the hair line. Loops were bound with cotton gauge in the form of figure '8' to close the vulvar lips to prevent recurrence.

One course of Oxytetracycline (Otim-Indian Drugs and Pharmaceuticals Ltd.,) at the dose rate of 2 ml continued daily for 5 days. One hundred ml of calcium borogluconate (Calborol : M & B) injected i/vly. Suture was removed next day. The goat was discharged as cured on 5th day of treatment.

The cause of uterine prolapse in the present case might be retention of placenta in the gravid horn after delivery of kid and uterine inertia. Treatment of uterine prolapse may be made as early as possible to avoid much injury to the organ and to have a good prognosis to the life of the suffering animal.

Acknowledgement: The authors are thankful to Dr. B.K. Bhattacharyya, Associate Professor and Dr. T.C. Bora, Professor, Department of Gynaecology, Obstetrics and Artificial Insemination, College of Veterinary Science, Khanapara for their co-operation during the treatment of the case.

Fig.
Prolapsed Uterine horn in a goat

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Effect of Heat stress and Hormone Treatment on the Histology of the Corpus Luteum

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Heat stress was observed to significantly affect folliculogenesis (Antoine and Pattabiraman, 1994). It was also recorded that heat stress increased the incidence of atretic follicle. However gonadotropic hormone treatment to heat stressed rats counteract the adverse effect of heat stress (Antoine and Pattabiraman, 1995). The present investigation is on the effect of heat stress and Gonadotropic hormone treatment on corpus luteum.

MATERIALS AND METHODS

Utilising BOD incubator, healthy adult albino female rats aged 120±5 days were heat stressed at 40.0±0.5 C and RH of 70 per cent for two hours daily for five days. Serum gonadotropin FSH (Biochem) 10 IU was administered intraperitoneally on day one and luteinizing hormone (Intervet) 10 IU was given subcutaneously on day 3 of heat stress period.

Fifty seven rats were divided into Heat stress (n = 16), Hormone treatment (n = 16), Heat stress and hormone treated group (n = 16) and untreated control group (n = 9). Four rats in treatment group were sacrified on 5, 10, 15 and 25 days after the beginning of heat stress. The untreated control rats were sacrificed separately in batches of three. The ovaries were collected and processed for histological examinations (Humason, 1979).

Serial thin sections were made to study the number of corpora lutea, number of luteal cells per microscopic field at 675 x magnification and the characteristic histological variation in the treated and control group. The data were statistically analysed adopting standard procedures (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The luteal cells of control rats were polygonal in shape with vesicular nucleus and prominent nucleolus. The cytoplasm was granular. In heat stressed rats on day five and ten there were well defined large vacuoles with infiltration of deeply stained polygonal cells in the corpus luteum. The cytoplasm of these infiltrated cells were eosinophilic and the nucleus were pleomorphic with condensed chromatin. The number of corpora lutea decreased from 5.0±2.0 to 3.0±1.6 by day 25 post heat stress. This could be due to the after effects of increased number of atretic follicles with corresponding reduced number of normal pre-ovulatory follicle. The number of luteal cells, cellular and nuclear diameter were reduced from 58.0±21 to 46.0±1.3 (day ten), 17.1 to 14.3 micron (day ten), and 7.5 to 5.7 micron (day 5) respectively from control group. The Histometric observation though statistically not significant observed to be less than that of the control. Homma *et al.,* 1972) observed decreased number and size of corpora lutea in rats to continuous illunination.

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In hormone treated rats on day five, the corpus luteum with distinct large cavities limited with a membrane containing amorphous granular material were noticed. Howe *et al.,* 1964) also reported such partially luteinized corpus luteum in hormone treated heifer calves. The number of corpora lutea increased to 7.0±3.6 on day five, but remained normal (5.0±2.0) subsequently. This could be possible by reversing the process of atresia which subsequently ovulate to form corpora lutea. The number of luteal cells declined from 58.0±2.1 to 50.0±1.6, but the cellular and nuclear diameter increased from 17.1 to 19.5 micron and 7.5 to 9.1 micron on day 5 post treatment. The increased size of the luteal cells could be the reason for the decline in the number of luteal cells per unit area. These changes could be due to the effects of luteinizing hormone.

In heat stressed and hormone treated rats there was no change in the number of corpora lutea during post treatment period. There was no significant change in the diameter of the luteal cell but the diameter of the nucleus continued to be 8.5 microns during post treatment period in contrast to 7.5 of control.

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Improvised artificial vagina for rabbit semen collection

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In livestock production plans artificial insemination plays an important role. It has already been proven in cattle development programmes that artificial insemination is an improved technique for rapid multiplication of superior germplasm. In view of this the technique of artificial insemination is being adopted in other species of livestock including meat producing animals involving rabbits. However the technique needs to be modified with regard to particular species for successful operation.

For artificial insemination, collection and evaluation of semen through A. V. (Artificial vagina) is the most important aspect. In the present study a specific attempt was made to improvise an artificial vagina for collection of semen from rabbit. The model improvised is cheaper and easy to prepare and also gives good results.

A hard P.V.C. pipe of 2.5 cm internal diameter and 12 cm length was used as the main body of A.V. the edges of this pipe were smoothened. A bicycle rubber tube of similar diameter and length was fixed internally to the pipe and was everted over the edges so as to avoid injury to the penis of the breeding buck and also to hold glycerine. A bicycle valve was fixed over the pipe 3 cm from one end to pour glycerine in the A.V. Glycerine helps to maintain the internal temperature of the A.V., (Paufler 1986). Human condoms was used as latex liner and fixed in A.V., which gave softer feel and lubrication to the A.V. The A.V. before use was kept in hot air oven or suspended in hot water at 60° wrapped in plastic bag, so as to get 50° C internal A.V. temperature. Method of semen collection was different than the cattle. In cattle, breeding bull is taken to the teaser cow whereas in rabbits teaser doe was taken to breeding buck (male rabbit).

By using this A.V. method 0.5 to 1.0 ml semen was collected twice in a week in morning hours. Volume collected was same as reported by Sinkovics *et al.,* (1983) and Paufler (1985). Motility of sperms was 60-80% which is in agreement with the report of Sinkovics (1983). Average total sperm count was 32 x 10^7 i.e. at par with the report of Paufler (1985). Semen collected from rabbits was diluted 10 times with egg yolk citrate dilutor and was used for artificial insemination.

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Quail egg yolk in semen extenders

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Egg yolk containing diluents are commonly used for preservation of Buck semen under refrigeration and deep freezing (Sinha *et al.,* 1987; Sinha *et al.,* 1991; Pintado and Perez, 1992, Singh *et al.,* 1992 and Kakadiya and Kavani, 1995) and yolk is usually collected from chicken egg. The present investigation was carried out on the efficacy of quail egg yolk in buck semen extenders in comparison to that from chicken for the advantages of (1) low cost (2) larger proportion of yolk and (3) Minimum wastage of nutrigious albumen. One quail egg is sufficient for extension of semen from 2-3 bucks for routine use in a farm.

The study was carried out at Kerala Agricultural University Goat Farm, Mannuthy. Twenty semen samples were collected from 2 bucks of one year of age at 2 days interval. After preliminary evaluation, semen was subjected to split extension in two extenders. (Extender-I containing Tris - 2.42%, Citric acid - 1.36% Fructose - 1% and quail egg yolk 5-10%. Extender-II chicken egg yolk instead of quail egg yolk). Semen samples diluted at 1:5 ratio and packaged in 2 ml vials were stored under refrigeration

Semen stored in extender I showed sedimentation leaving clear supernatent and microscopic examination revealed only immotile sperms in this supernatent. All the motile sperms were in the sediment. Motility assessed after mixing of both layers revealed better grading in extender I, than in extender II. The modility was 82.30, 58.75, 52.60 and 35.10 per cent in extender I and 82.30, 51.25, 39.10, 22.60 inextender II at 0, 24, 48 and 72 hours of storage. In extender II no sedimentation was noticed as in extender I.

Sedimentation of motile and immotile sperms along with yolk particles restricting the motility of sperms may be a contributory factor for better preservability in quail egg yolk diluent. Fertilizability and freezability of buck semen in this extender needs further study.

The present trial indicates that quail egg yolk can advantageously be replaced for chicken egg yolk in buck semen extenders.

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