

FERTILITY MANAGEMENT FOR OPTIMISING PRODUCTION IN FARM ANIMALS

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The livestock industry depends on proper reproduction resulting into optimum production. Livestock reproduction and production should be viewed not only from the economic point of view but from sociocultural and ecological point of view also. Two major problems confronting us today are population explosion and malnutrition. Limiting the growth of human population and increasing the quality and quantity livestock production are the main solutions to the problems of the developing Nations like ours. This can be achieved only if the animals have a long useful life with maximum production span and minimum offspring losses. More offspring per animal would allow judicious selection for replacement stock without which it may be difficult to improve the genetic merit of the herd from generation to generation. Consequently good fertility management of farm animals providing valuable protein viz. milk, meat and other by products becomes inevitable.

Reproductive science relates to the productive science and the

developments over the past few decades have supported the very cause of reproductive health programmes. The animals with reproductive disorders should be given ample opportunity to achieve an acceptable calving interval within time limits necessary for profitability. Reproductive disorders should be detected as soon as possible and timely therapy instituted. But therapeutic heroics for chronically infertile livestock constitutes an uneconomical practice. Need of the hour is to conduct work on such maladies and to pass on the fruitful findings for execution to the field to minimise their frequency if it is not possible to abolish them completely^{1, 5, 13, 16}.

Management techniques should provide the normal livestock with the greatest possible opportunity to achieve a short delivery period. By employing proper managerial measures on natural reproductive phenomena the animals are allowed to perform efficiently to full potential. The farm animals are usually normal and with efficient management

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controlling the human errors responsible for the prolonged calving period better returns can be obtained. Fertile semen must be deposited aseptically to a healthy reproductive tract at a right time and at right place with optimum number of sperms. The owner or manager and labour force must be systematically trained in the techniques necessary to perform their jobs. This performance should be regularly and objectively evaluated.

It must be ensured that along with lactating females adequate nutrition is made available for growers, cycling, pregnant, and more importantly to the breeding males. Adequate housing facilities should be provided and it must be seen that the animals are free from infectious diseases. Facilities must exist to restrain number of large animals in a short time with little manpower. Bovine herd should be looked everyday for application of heat detection aids, genitalia examinations and inseminations.

Animals identification by branding, notching, ear tag and records on individual basis using cards are the systems which are most compatible. Records have no value unless they are evaluated for improving the herd performance with sincerity. Cows and buffaloes should be segregated as per their reproductive status for most efficient heat detection. For management purposes, the important parameters are calving interval, average days in milk at first service, first service

conception rate and culling percentage for reproductive efficiency. These days electronically processed records are very convenient to handle, effective and quick to evaluate.

After each herd check, the Animal Reproduction Specialist must compute the 24-day heat detection percentage and number of cows/buffaloes presented for pregnancy diagnosis and found to be open. It should be ensured that the entries are properly made, particularly if there is absentee ownership. The monthly conception rate obtained by each inseminator should be reviewed. Fertility management incorporates male and female. With the advent and boost to the A.I. technology it becomes extremely important to evaluate and select our half of the herd judiciously. Grading and scoring of males for sexual behaviour, seminal characteristics including cryopreservability and sperm biometry can be employed effectively¹⁵. Major hurdle in the spread of A.I. technology and its advantages initially was the inferior keeping quality of the semen of the farm animals. Successful attempts have been made by various workers to improve the efficacy of semen diluters¹⁴.

Seminal characteristics of the farm animals are indicative of assessing fertilising power of the semen to be used in the field for artificial insemination. With the advent of this technology semen was imported and crossbreeding came into practice. It has been concluded that

crossbreeding has a direct effect on the age at first calving, breeding efficiency and milk production⁶. Studies have clearly shown that inbreeding causes lowered reproductive efficiency in most of the species of the animals including farm animals. The effect is more noticeable as the percentage of inbreeding increases. High level of inbreeding reduces fertility rate and increases the incidence of expression of recessive lethal genes. Therefore, judicious crossbreeding programme coupled with preservation of our own Country's precious livestock germ plasm very carefully is wanted earnestly.

Application of A.I. calls for timely detection of estrus, pregnancy and treatment of reproductive disorders. Today our farmers have a better choice to induce or synchronise the estrus. Confirmation of estrus, early pregnancy and differentiation of ovarian cysts can be done using enzyme linked immunosorbant assay kits. Fertility in farm animals can be monitored this way very easily and conveniently¹¹. Linear array scanner is playing a major role in overcoming the limitations of various diagnostic techniques used earlier and is helping in visualising the unseen mysteries at click of a button. It has helped to locate and understand the internal environment and observe the changes on daily basis. Pregnancy as early as 22 days in bovine, porcine and canine can be diagnosed. The pathological conditions like pseudopregnancy and pyometra can be differentiated and

progressive foetal development can be monitored adequately using the technique.

In animal reproduction artificial insemination has greatly contributed to the upgrading of livestock. Similarly there is innovative embryo transfer technique². Now it is possible to augment the production of animal protein at a faster rate than utilising the traditional methods of breeding. This innovative technique had added a new dimension to animal reproduction and breeding. It calls for proper estrus synchronisation and superovulation⁹.

With all the diagnostic and potential technologies available, gestational care of the animal plays a pivotal role in optimising the production in livestock. A number of factors are said to influence the gestation period. Similarly body weight and pelvic conformation change proportionately during pregnancy and puerperium. Any deviation from the norms during pregnancy may result in calving problems and eventually result in greater losses^{7, 8}.

Control of the severe problems of contagious genital infections has focused attention on other causes of abortion and infertility¹². The judicious use of hormones coupled with PGF2 α as an aid for cure in treating certain sterility problems has been recognised. With more and more number of Animal Reproduction Specialists taking active interest in this field many livestock

farmers are realising the value of the routine preventive health care or practices for pregnancy and infertility examinations in maintaining good reproductive and productive levels in a herd. A well managed dairy herd should have 65 to 70 per cent of the cows conceive on first service with an

average of 1.3 to 1.7 services per conception. Thus proper reproductive health care during adverse climatic conditions, pregnancy, parturition and post-partum period can minimise losses in farm animals followed by optimum fertility and production^{3, 4, 10}.

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THE EFFECT OF SYNCRO-MATE-B ON FOLLICULAR DYNAMICS DURING SUPEROVULATION IN CROSSBRED COWS*

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ABSTRACT

The study was designed to find out the influence of synchro-mate-B on follicular dynamics during superovulation in crossbred cows. Twelve cows were selected and their oestrus was synchronized with synchro-mate-B. The implants were kept in situ for 9 days. Superovulation was initiated on day 7 of the implant period. Progesterone, oestradiol, luteinizing hormone and follicle stimulating hormone were assayed. Based on the hormonal profile, a hypothetical model of follicular dynamics during superovulation in SMB treated cows is proposed that Synchro-mate-B demised the dominant follicle and initiated the emergence of new follicular wave between days 4 and 5 of the implant period. The newly developed follicle attained its dominant stage on day 7 of the implant period i.e. on the day of initiation of superovulatory treatment, and it became persistent dominant follicle during superovulatory treatment i.e. late phase of the implant period.

Several authors incorporated synchro-mate-B into superovulation regimen to initiate superovulation at any stage of oestrous cycle (Parther et al., 1984; Almedia, 1987; Bo et al., 1996) but the results in terms of embryo recovery and quality were inconsistent. Several studies illustrated the influence of synchro-mate-B on follicular dynamics in unstimulated cows that synchro-mate-B regressed the dominant follicle and initiated new follicular wave at any stage of oestrous cycle (Bo et al. 1994) and, in contrast, maintained the dominant follicle throughout the implant period (Kojima et al. 1992), whereas, such information in superovulated cows was scarce and hence the present study.

MATERIALS AND METHODS

Twelve nonpregnant, nonlactating, healthy, parous, cyclical crossbred cows, aged between 3 and 6 years and weighing 300 to 400 kgs, having an average oestrous cycle length 18 to 21 days were utilised for this study. All the cows were maintained under uniform managerial

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conditions and fed with adequate quantity of feed and fodder. The oestrous cycle was synchronized with Synchro-Mate-B system (SMB - Animals Health Inc., USA). All the cows, in the luteal phase of oestrous cycle, received 6 mg norgestomet ear implants (17- α acetoxy-11- β methyl-19-nor preg-4-3, 20 dione) plus an intramuscular injection of 3 mg norgestomet and 5 mg oestradiol valerate on the day of implant insertion (day - 0). The implants were removed on day. 9. On day 7 of the implant period, the superovulatory treatment was initiated with 400 mg NIH-FSH-P₁ (Folltropin - V, Veterphram, Canada) in 8 divided descending intramuscular doses of 80/80, 60/60, 40/40 and 20/20 mg for 4 days at 12h interval. An intramuscular injection of 25 mg PGF2 α was administered 12h before the implant removal. Embryos were collected nonsurgically on day 7 post oestrus.

Blood samples (10 ml) were collected from jugular vein in 10mg EDTA treated tubes from the day of implant insertion to implant removal at 24 h interval and stored at - 20°C until hormonal assay. Plasma concentration of progesterone and oestradiol was measured with solidphase radioimmunoassay kit (Coat-A-Count, Diagnostic products corporation, Los Angles, USA). Plasma concentrations of LH and FSH were measured by double antibody

RIA method (WHO; Sufi et al 1986). Purified bovine LH (USDA-bLH-B-6, AFP-1174-B) and LH antiserum (NIDDK-anti-oLH-1, AFP-192279) were used. The sensitivity of this assay was 0.14 ng/ml, and intra and interassay coefficient variations were 4.90 to 8.48 and 5.4 to 9.9 per cent, respectively. Purified bovine FSH (USDA-bFSH-I-2, AFP-5318c) and FSH antiserum (NIDDK-anti-oFSH-1, AFP - 5288113) were used. The sensitivity of this assay was 0.2 ng/ml, and intra-and interassay coefficient variations were 5.70 to 8.90 and 5.4 to 12 per cent, respectively. The radioactivity was counted by gamma counter. All collected data were analysed statistically (Snedcor and Cochran, 1989).

RESULTS AND DISCUSSIONS

Progesterone, oestradiol, luteinizing hormone (LH) and follicle stimulating hormone (FSH) profiles during superovulation in syncro-mate-B treated cows are presented in fig 1. The hypothetical model of follicular dynamics during superovulation in SMB treated cows is depicted in fig 2.

During the SMB implant period, with in 24-48 hrs of initiation of SMB treatment, the progesterone level suddenly declined from 2.8 ng/ml to 0.5 ng / ml and the same level was maintained throughout the implant period. It is in accordance with the findings of Kogima et al. (1992). Odde (1990) suggested that the

dramatic fall in progesterone was due to the luteolytic effect of oestradiol valerate, administered on the day of SMB implant insertion, over the existing corpus luteum. Peters (1984) reported that norgestomet concentration in the circulation was nondetectable by radioimmunoassay since the synthetic progestin did not cross react with labelled progesterone antibody. Hence, it is inferred that the progesterone recorded might be the endogenous progesterone.

The luteinizing hormone concentration gradually increased from the day of implant insertion (0.58 ng / ml) to implant removal (2.2 ng/ml). This increase in LH concentration indirectly reflected the increased LH pulse frequency. Sanchez et al. (1993) reported that norgestomet mimicked the LH secretory pattern of the follicular phase and not of the luteal phase of the oestrous cycle during the implant period. Sanchez et al. (1995) stated that in norgestomet treatment, the progesterone released from the implant was depleted slowly during the later phase of the treatment period, and the progesterone released from the implant was not sufficient to suppress the LH pulse frequency, and in turn increased the LH pulse frequency and maintained the dominant follicle.

The fluctuations in follicle stimulating hormone and oestradiol profile were inversely related with each other. The oestradiol concentration dramatically increased four fold from 70.70 pg/ml to 265.58

pg/ml, whereas the FSH concentration decreased from 58.53 ng/ml to 40.53 ng/ml within 24 hr of SMB treatment. Kazmer et al. (1987) stated that in SMB treated cows, the sudden increase in oestradiol was due to the administration of oestradiol valerate. Then, the oestradiol declined to basal level (65 ng/ml) on day 4 of implant period and again started to increase and reach the concentration of 105.60 pg/ml and 142.76 pg/ml on day 7 of implant period and on the day of implant removal, whereas, FSH showed a small surge (81ng/ml) on day 4 of the implant period and then started to decrease towards the day of implant removal. These fluctuations in oestradiol and FSH concentrations (fig. 1) indicated that regression of the existed follicle and emergence of new follicular wave between days 4 and 5 of the implant period. Our findings are in accordance with that of Bo et al. (1995) that in SMB treated cows the administration of oestradiol valerate caused regression of dominant follicle and initiated the emergence of new follicular wave within 4.3 day of initiation of SMB treatment and Adams et al. (1992) that each emergence of follicular wave preceded by small FSH surge. After day 5 of implant period the oestradiol concentration showed increasing trend, it indicated the growth of the newly developed follicles, it was further favoured by the findings of Revah and Butler (1996) that follicular growth and oestradiol concentration had positive relation. The high concentration of oestradiol (105ng/ml) recorded on the day 7 of

FIG-1. HORMONAL PROFILE DURING SUPEROVULATION IN SYNCRO-MATE-B TREATED COWS

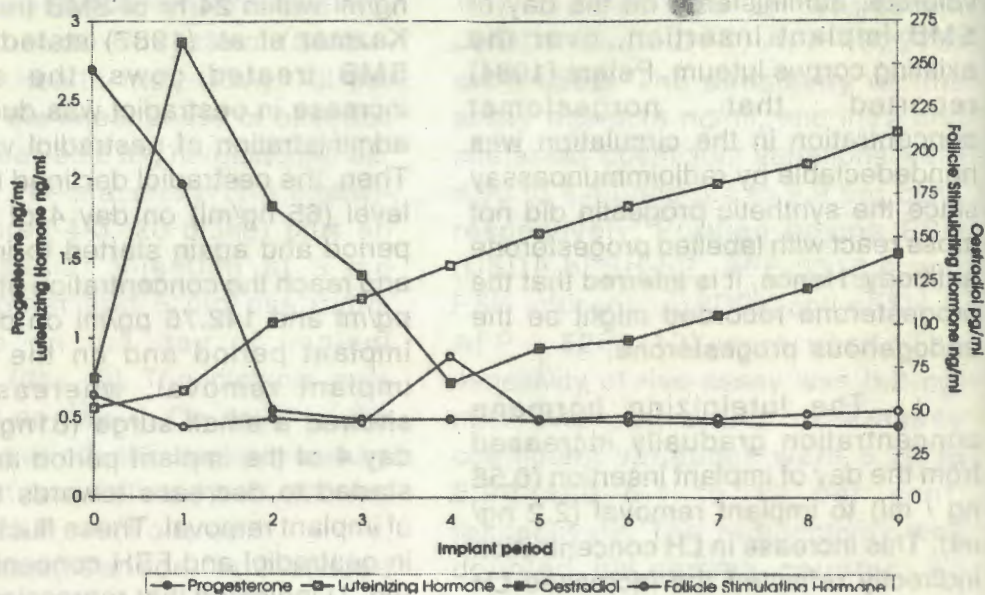
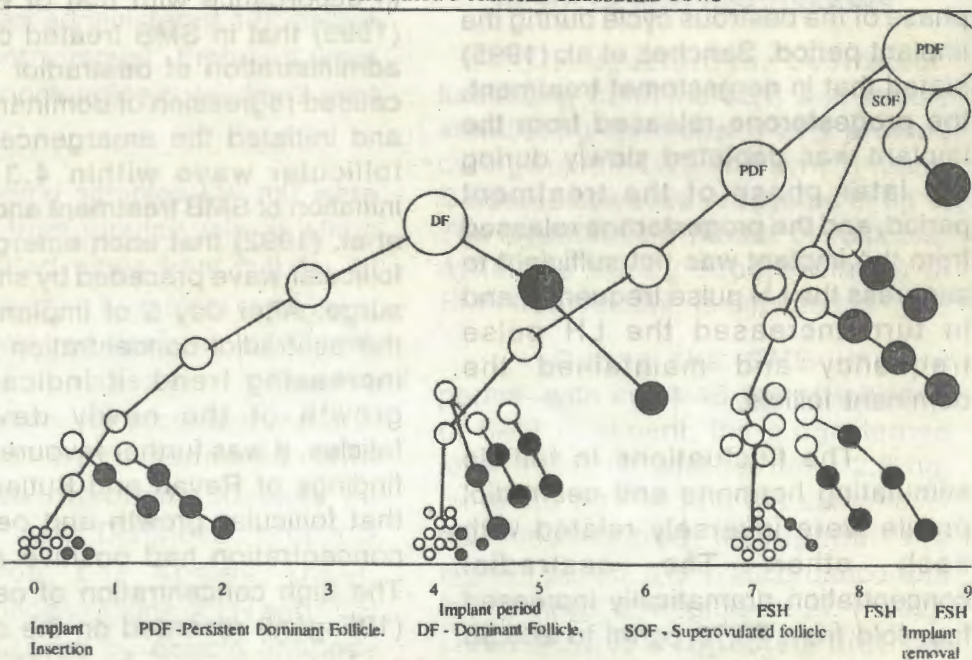


FIG-2. A HYPOTHETICAL MODEL OF FOLLICULAR DYNAMICS DURING SUPEROVULATION IN SYNCRO-MATE-B TREATED CROSSBRED COWS



implant period. i.e. on the day of initiation of superovulatory treatment, indicative of presence of dominant follicle. Our results support the findings of Bondenstein et al. (1996) that the newly developed follicle attained its dominant stage within 2 days of emergence. Sanchez et al. (1995) reported that dominant follicle secreted more amount of oestradiol, and Thatcher et al. (1996) stated that dominant follicle secreted more amount of inhibin that suppressed the FSH synthesis. In the present study the high concentration of oestradiol (142.76 pg/ml) and low level (49.74 ng/ml) of FSH on the day of implant removal clearly indicated the presence of dominant follicle i.e. progestogen induced persistent follicle.

In the present study, based on the hormonal profile, a hypothetical model (fig 2) of follicular dynamics during superovulation in SMB treated cows is proposed that Synchro-mate-B demised the dominant follicle and initiated the emergence of new follicular wave between days 4 and 5 of the implant period. The newly developed follicle attained its dominant stage on day 7 of the implant period i.e. on the day of initiation of superovulatory treatment

and it became persistent dominant follicle during the later phase of the implant period. Several studies reported that embryo recovery and transferable embryos were poor in syncro-mate-B treated cows. In present practice, in syncro-mate-B regimen, super-ovulation was initiated on day 7 of the implant period i.e. during the presence of dominant follicle, it might be the reason for the poor embryo recovery in syncro-mate-B treated cows since Guilbault et al. (1991) reported that presence of dominant follicle at the initiation of superovulatory treatment decreased the superovulatory response. Hence, further research with the aid of ultrasonography is needed in this area to manipulate the duration of implant period and day of initiation of superovulatory treatment for better utilization of syncromate-B-system in multiple ovulation and embryo transfer programme in cows.

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COMPARISON OF SURGICAL AND LAPAROSCOPIC EMBRYO RECOVERY IN BARBARI GOATS*

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ABSTRACT

Twelve non-pregnant, healthy, parous, cyclical Barbari breed of goats were randomly divided into two equal groups namely Group I, utilized for surgical embryo recovery and Group II utilized for Laparoscopic embryo recovery. All the 12 goats were synchronized with Norgestomet ear implant (3 mg) plus PGF₂ α and superovulated with PMSG - 1000 IU. Estrus was detected with the help of an apronized buck. The goats were bred with a fertile buck during estrus. The embryos were recovered on day 7 after the first service in both the groups. The overall mean embryo recovery rates were 61.36 per cent and 18.92 per cent for Group I and Group II respectively.

Recent studies of ET in small ruminants have developed laparoscopic and non-surgical procedures for both embryo collection

and transfer. The relatively atraumatic method for embryo collection produce results that are competitive with surgical methods for uterine stage embryos (Kraemer, 1989). With this in view the present study was conducted to compare the surgical and laparoscopic embryo recovery in Barbari goats.

MATERIALS AND METHODS

Twelve non-pregnant, healthy, parous, cyclical Barbari breed of goats weighing approximately 15-25 kg obtained from University Research Farm of Tamil Nadu Veterinary and Animal Sciences University were randomly divided into two equal groups namely Group I, utilized for surgical embryo recovery and Group II, utilized for laparoscopic embryo recovery.

Superovulation and breeding : All the 12 goats of both groups were synchronized with Norgestomet ear implant (3 mg) (Syncromate-B, SMB, Animals Health Inc., USA) left 11 days insitu and PGF₂ α - 0.225 mg (Iliren, Hoechst, Wiesbaden, Germany) was given on day 10 intramuscularly. Superovulation was carried out by single injection of PMSG - 1000 IU (Folligon, Intervet International, B-V-Boxmer, Holland) intramuscularly on

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day 9, Oestrus was detected with the help of an apronized buck. At oestrus the goats were bred with a fertile buck and hCG-750 IU (Chorulon, Internet International, B-V-Boxmer, Holland) was given to each goat. The embryo recovery was carried out in both the groups on day 7 following the first service.

Surgical Procedure : The goats (Group I) were starved by withholding feed for 24 hours and water for 12 hours prior to surgery. Anaesthesia was induced using a combination of xylazine - 0.22 mg and ketamine HCl- 15 mg per kg body weight intramuscularly. They were laparotomised by midventral incision and the reproductive organs were exposed. The uterine horns were flushed using Dulbecco's Phosphate Buffered Saline (DPBS; Sigma Chemicals, St. Louis, USA) with 10 percent Foetal calf serum (FCS: Sigma, St. Louis, USA) as outlined by Buckrell and Pollard (1997) with slight modification of insertion of Foley - catheter just cranial to the bifurcation. After completion of uterine horn flushing, the genital organs were repositioned and the muscle and skin sutures were applied as per the standard technique.

Laparoscopic Procedure : Standard laparoscopic equipments and instruments (M/S. Karl Storz GmbH and Co. Tuttlingen, Germany) were used for this study at the Laparoscopy Unit of the Department of Clinics, Madras Veterinary College.

Goats were starved and anaesthetised similar to surgical procedure. Anaesthetised goats were placed in dorsal recumbency and suspended head down on a hydraulic operation table at an angle of 45 degrees.

Pneumoperitoneum was created using the Verres needle connected to the electronic carbon dioxide endoflator. Following insufflation of the abdomen, three stab incisions were made on the ventral abdominal wall anterior to the udder as described by Nellenschulte and Niemann (1992) with slight modification.

The uterine horn ipsilateral to the ovulated ovary was grasped near the bifurcation using a grasping forceps. A small puncture was made on the uterine wall just cranial to the forceps by using a Steinman pin (size 3 mm) and the two-way Foley-catheter (size 10 FG) with a metal stylet (K-wire 1mm) was gently introduced. The balloon was inflated with 3-5 ml of air and the metal stylet withdrawn. With 5-10 ml of the flushing medium, the embryos were recovered by retrograde manner in a slow and continuous process. Both uterine horns were repeatedly flushed for 3-4 times. The recovered medium was screened under zoom stereo microscope. After laparoscopy, the intraabdominal CO₂ gas was eliminated by manual pressing of the abdominal surface. The skin stab incisions were closed surgically. The goats subjected to both surgical and laparoscopic embryo

collection were given antibiotic Benzathine penicillin 40,000 IU per Kg body weight i/m. The sutures were removed on day 7 in laparoscopic group and day 9 in surgical group.

During embryo collection in both the techniques, ovarian response and embryo recovered were assessed and recorded. Based on ovulation spots on the ovary and number of embryos recovered percentage of embryo recovery was worked out. Both left and right ovary put together the overall recovery rate was derived. After completion of the embryo collection, all the goats (Group I and Group II) were maintained for two weeks and then slaughtered for necropsy findings, and histological study. The collected data were analysed statistically as per the procedures described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Surgical Technique : The over all embryos recovered from the uterine horns by surgical technique (without clamping the utero-tubal junction) was 0, 62.5, 80, 50 and 66.67 per cent for the goats # 1,2,3,4,5 and 6 respectively. The overall mean embryo recovery rate was 61.36 per cent. However, the mean embryo recovery rates of 57.9 per cent and 67 per cent were reported by Lima et al. (1996) and Nuti et al. (1987) respectively, who adopted orthograde flushing of uterine horn. The variation in the embryo recovery rate in the present study may be due to the

flushing technique (Retrograde) adopted. Moreover, clamping of utero-tubal junction was not done while flushing. Generally, the embryos recovered from uterine horn is less as compared to the tubal flushing, since, the caprine uterus has distinct cotyledons that may hinder the free flow of embryos along with the medium.

Laparoscopic Technique : In the present study, the overall embryos recovered from the uterine horns by laparoscopic technique (without clamping the utero-tubal junction) was 0,33.33,25,40,0 and 12.5 per cent for the goats # 1,2,3,4,5 and 6 respectively and the overall mean embryo recovery rate was 18.92 per cent. Nellenschulte and Niemann (1992) who reported the mean embryo recovery rate of 6 per cent, when embryos were collected from uterine horn without clamping the utero-tubal junction by laparoscopic technique. However, Foxworth (1992) reported the embryo recovery rate in laparoscopic technique ranged from 0 - 100 per cent. Moreover, McKelvey et al. (1986) reported a low embryo recovery rate of 35 per cent in first collection but a high recovery rate of 76 per cent and 66 percent in subsequent second and third collections. The lower embryo recovery rate obtained in this study may be due to the embryo collection carried out without clamping the utero-tubal junction, retrograde method of flushing adopted and also individual variation among the goats.

In the present investigation, all the goats subjected to surgical embryo collection showed extensive adhesions involving abdominal wall, greater omentum, uterus, uterine horns, oviduct and ovaries at the time of slaughter. This might be due to trauma and extensive manipulation. This prevents further embryo collection procedure. In contrast, the goats subjected to laparoscopic embryo collection had no adhesions but at the site of puncture the endometrium was prolapsed as reported by McKelvey et al. (1986). The prolapsed mass would have occurred due to the intra abdominal CO₂ pressure created during laparoscopic procedure, which is similar to the findings of

Neuhaus et al. (1998). Histological study of the endometrial prolapsed mass revealed the presence of normal endometrial glands with few inflammatory cells. These inflammatory cells might be due to incomplete healing of the punctured site.

It is concluded that laparoscopic technique is superior to surgical technique, since, it is less traumatic, promotes faster healing, requires less post-operative care and hospitalization. Repeatability is high as postoperative complications like adhesion is very low. Moreover, with experience and skill, this laparoscopic technique of embryo collection can be performed with speed and in a short time.

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EFFICIENCY OF NORGESTOMET AND PGF2 α WITH PMSG, GnRH AND FSH ON INDUCTION OF OESTRUS IN ANOESTRUS BUFFALOES*

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ABSTRACT

Efficiency of a short-term progestagen, Norgestomet, and PGF2 α with PMSG, GnRH or FSH on induction of oestrus was assessed on eighteen anoestrus buffaloes. Oestrus induction and ovulation was achieved in all buffaloes (100%). Higher conception rate of 83.33% was obtained in buffaloes treated with Norgestomet + PGF2 α + PMSG combination, or Norgestomet + PGF2 α + GnRH combination, whereas the conception rate in Norgestomet + PGF2 α + FSH treated group was 66.66%. However, the difference was non-significant.

Anoestrus in cattle and buffaloes has been treated with progestagens alone (Roche, 1976) or in combinations with gonadotrophic hormones (Rao and Sreemannarayana, 1983) or prostaglandins (Joshi et al., 1990) with variable results. Further, the administration of PMSG and PGF2 α in conjunction with a short-term

progestagen treatment resulted in better control of ovulation and high calving rate in cows (Chupin and Pelot, 1980). The present study was undertaken to determine the efficiency of Norgestomet and PGF2 α with PMSG, GnRH and FSH to induce cyclicity in acyclic buffaloes.

MATERIALS AND METHODS

The present study was conducted at Artificial Insemination centre, IVRI and private dairy farms in and around Bareilly. Twenty four acyclic murrah buffaloes were selected based on both the absence of corpus luteum for two successive examination at 11 days interval and plasma progesterone analysis. Buffaloes were randomly divided into four groups, each consisted of 6 animals. Group I served as control. Group II, III and IV were given treatment as follows.

On day 1 of the experiment, all the buffaloes in group II, III and IV received 3 mg. norgestomet ear implant (Crestor, Intervet) along with intramuscular injection of 3 mg. norgestomet and 5 mg. oestradiol valerate. On day 7 all buffaloes were administered with 15 mg of PGF2 α (Prosolvin, Intervet). On day 9, ear implants were removed and the

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following hormones were administered.

Group II: 600 IU PMSG (Folligon, Intervet) - Intra muscular injection

Group III: 250 ug GnRH (Fertagyl, Intervet) - Intra muscular injection

Group IV: 15 units FSH (Super-OV-Ausa international, USA) subcutaneously in divided doses, 2.5 units each in morning and evening on 7th, 8th and 9th days.

Oestrus detection was carried out using teaser twice daily, morning and evening, and buffaloes that showed oestrus were inseminated with frozen semen. All the buffaloes were examined on day 10 of induced oestrous cycle per rectally to assess whether ovulation has taken place or not and blood was collected for the estimation of progesterone. Pregnancy was confirmed by rectal palpation 45-60 days post-insemination. Standard statistical methods of Snedecor and Cochran (1989) were used to analyse the data.

RESULTS AND DISCUSSION

All the buffaloes in group II exhibited oestrus, of which four buffaloes showed typical fern pattern of cervical mucus and all conceived at first insemination. The rate of ovulation was 100%. The conception rate to 1st and 3rd inseminations were 66.66 and 83.33%, respectively. These findings are in agreement with those of Narasimha Rao et al. (1985) and Joshi et al. (1990), who also reported 100% ovulation rate in

anoestrus buffaloes treated with Norgestomet, PGF2 α and PMSG. However, the conception rate to 3rd insemination obtained in our study was higher than that reported by them. Chupin and Pelot (1980) achieved a high calving rate of 65.4% after two predetermined AI in dairy cows following progestagen implant, PMSG and PGF2 α treatments and they attributed this to precise control of oestrus and ovulation and to similarity in the sequences of events in treated animals to those observed during normal oestrus.

In group III also all the 6 buffaloes showed oestrus, of which three had typical fern pattern of cervical mucus and all conceived at 1st insemination. The rate of ovulation in this group was 100%. The conception rate for 1st and 3rd inseminations were 50 and 83.33%, respectively. The percentage of induced oestrus obtained in this study is higher than those reported by Rao and Rao (1983) and Saini et al. (1986) who reported 50% and 53% induced oestrus in treated anoestrus buffaloes. It may be due to the fact that progesterone treatment allow better synthesis and storage of pituitary gonadotrophins and consequently higher gonadotrophic surge on removal of the implants. Further, the administration of GnRH on the day of removal of progesterone implant may help in the release of stored gonadotrophins. This may account for the higher ovulation rate and conception rate obtained in this group.

All the six buffaloes in group IV showed oestrus and the ovulation rate in this group was also 100%. The cervical mucus of two buffaloes showed typical fern pattern, however, three buffaloes conceived to 1st insemination. The conception rate for 1st and 3rd inseminations were 50 and 66.66%, respectively. The overall conception rate obtained in this group is less compared to other groups. These findings are comparable with those of Sheffel (1982) who also reported an ovulation rate of 94.17% in post-partum anoestrus beef cows with 10 mg FSH (in four divided dose) before implant removal.

The precise role of PGF2 α in induction of oestrus in true anoestrus is poorly understood. However, it is suggested that PGF2 α may be important in folliculogenesis and ovulation following the LH surge induced by progestagen withdrawal in anoestrus buffaloes.

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COMPARATIVE EFFICACY OF DIFFERENT HORMONAL METHODS ON SUPEROVULATION AND EMBRYO RECOVERY IN BLACK BENGAL GOATS*

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ABSTRACT

Eighteen parous, cyclical pure Black Bengal goats (approx two years of age) were taken, they were divided randomly into three equal groups (Groups A, B and C). All three groups were treated with double doses of Prostaglandin F₂ alpha at an interval of 11 days to induce estrus, on day 9th of first PG application 1000 IU PMSG was injected to induce superovulation. Group B was treated with 750 IU HCG six hrs after onset of estrus. Group - C received 8 µg GnRH analogue, Buserelin, 24 hrs after last PG injection. Group - A neither received HCG nor GnRH. Does with superovulatory estrus were provided natural service 12 hourly till the end of estrus. Laparotomy was performed on day - 6 of superovulatory estrus.

A non - significant increased ovulation, embryo recovery, transferable embryo and decreased unovulated large follicle were recorded in group - C (PMSG + GnRH) and group - B (PMSG + HCG) than group - A treated only by PMSG.

It has been reported that better superovulatory response was obtained by FSH compared to PMSG (Armstrong et. al. 1983 a, b; Pendleton et. al. 1992) but PMSG is a suitable gonadotropin due to it's easy availability, single does schedule and lower cost. When GnRH or HCG used with PMSG, it gave better superovulatory response (Jabbour and Evans, 1991; Akinlasotu and Wilder, 1993; Krisher et. al. 1994; Sharma et. al. 1994 Cox et. al. 1987 and Goel and Agarwal 1990) possibly by promoting LH surge of higher intensity which may augment the ovulatory process.

This study has been conducted to investigate wheather GnRH or HCG can improve the superovulatory response, embryo recovery and embryo quality when used with PMSG in Blank Bengal goat.

MATERIALS AND METHODS

A total eighteen parous cyclical pure Black Bengal goats were taken for this study. Animals were reared under semi intensive management system.

Superovulation : All the does were devided randomly into three equal groups (n = 6). All groups were synchronized by double doses of PGF₂ alpha analogue (Tiaprost trometamol 0.294 mg, Illiren - Hoechst Roussel Vet). Animals were

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superovulated by 1000 IU PMSG (Folligon - Intervet International - Holland) on day 9 of first PG injection

Breeding : Goats were allowed natural mating at 12 hourly till the end of estrus under strict supervision.

Embryo Collection : Laparotomy was performed on day - 6 of superovulatory estrus. Embryo collection was done by flushing the oviduct using the media composed of D-PBS containing 0.2% BSA (Fraction - V, Sigma, USA). During egg collection both right and left ovaries were closely examined for number of ovulation, unovulated large follicle, presence of premature regressing corpora lutea. Harvested embryos and unfertilized eggs were examined under zoom stereo microscope for grading based on their morphological characteristics as excellent, good, fair and poor.

The data thus generated has been statistically analysed as per Snedecor and Cochran (1980).

RESULTS AND DISCUSSION.

The results are presented in the table. Non significant higher number of ovulation (10.17 ± 0.31) was observed in Group - C followed by Group - B and A ($P > 0.05$). Unovulated large follicles were found to be less in the Group - C (2.17 ± 0.13) than others but the different between three groups were not significant ($P > 0.05$). Prematurely regressing corpora lutea was noted in

three groups. The mean number of ovulation in group - A was in agreement with the finding of Rosnina et. al. (1992). From reports of Cox et. al. (1987), Goel and agarwal (1990), Sharma et. al. (1994), it was observed that goats treated with PMSG and HCG gave significant higher number of ovulation than the goats which did not receive HCG treatment. In the present investigation animal receiving HCG did not show any significant increase in number of ovulation. It is also reported that use of GnRH or its analogue with superovulatory drugs, ovulation was significantly higher and also decreased number of unovulated large follicle (Jabbour and Evans 1991, Akinlasotu and Wilder 1993; Krisher et. al. 1994). In the present investigation similar result was observed but the changes observed were non significant ($P > 0.05$).

Seventeen out eighteen treated does with three or more corpora lutea were used for recovery of eggs. The mean number of eggs recovered was 4.80 ± 0.28 , 5.50 ± 0.27 , 7.50 ± 0.40 respectively for group - A, B and C with non significant difference ($P > 0.05$). Higher number of embryos were recovered from group - C (6.00 ± 0.30) than others but the different were not significant statistically ($P > 0.05$). Fertilization percentage was near about same in three groups.

A total 81 embryos were recovered from three groups. Most of these were in morula stages (53.08%), early blastocyst stage (28.39%), rest

were 2-8 cell stages (12.39%) and few degenerative (6.19%). This was in agreement with the finding of Rosnina et. al. (1992). A non - significant ($P > 0.05$) higher number of excellent, good and fair embryos were obtained from group - C than other but more number of poor embryos were found to be in group - A. Similar finding has been reported previously by Armstrong et. al. (1983a) and Tervit et. al. (1983). The reason for premature regressing corpora lutea in goats are unknown but may be related to excessive recruitment of new follicle that produces high level of estrogen (Armstrong et. al. 1983a). It seems

likely that the failure to recover higher number of embryos and also transferable embryos under this condition is a reflection of abnormalities in embryo transport, as a result of endocrine abnormalities associated with the luteal failure.

In this study additional ovulatory drugs HCG or GnRH analogue, Buserelin with PMSG gave better result in respect of ovarian response and embryo recovery than treated only by PMSG. But GnRH or HCG failed to improve ovulation rate, embryo recovery and transferable embryos significantly.

TABLE 1: Ovarian response of superovulated goats (Mean \pm SE)

	Group - A PMSG	Group - B PMSG + HCG	Group - C PMSG + GnRH
Number of does treated	6	6	6
Number of does in estrus	6	6	6
Ovulation / does *	7.17 \pm 0.28	8.50 \pm 0.19	10.17 \pm 0.31
Number of unovulated large follicle (> 5 mm)	4.17 \pm 0.28	3.33 \pm 0.22	2.17 \pm 0.13
Number of does flushed (does with > 3 CL)	5	6	6
Eggs recovered / doe *	4.80 \pm 0.28	5.50 \pm 0.27	7.50 \pm 0.40
Recovery (%)	49.62	59.83	64.10
Embryo / doe*	3.40 \pm 0.24	4.67 \pm 0.25	6.00 \pm 0.30
Fertilization %	76.56	86.80	87.97
Morula / doe*	1.80 \pm 0.19	2.33 \pm 0.18	3.33 \pm 0.33
Blastocyst / doe*	1.00 \pm 0.19	1.33 \pm 0.19	1.67 \pm 0.17
Excellent embryo / doe*	0.40 \pm 0.12	1.67 \pm 0.13	1.83 \pm 0.23
Good embryo / doe*	0.40 \pm 0.09	1.17 \pm 0.11	1.50 \pm 0.20
Fair embryo / doe*	0.80 \pm 0.13	1.67 \pm 0.10	2.00 \pm 0.15
Poor embryo / doe*	1.60 \pm 0.17	1.17 \pm 0.19	0.67 \pm 0.15

* The values are not significant at 5% level.

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OXYTOCIN CONCENTRATION IN THE CORPUS LUTEUM OF THE BUFFALO (BUBALUS BUBALIS) *

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ABSTRACT

The corpora lutea (CL) from different stages of oestrous cycle and pregnancy were collected from buffaloes (non - descript breed, of varying age and parity) slaughtered between 3 - 4 A.M. following Arabic method. Extracted oxytocin was bioassayed on oestrogenized virgin rat uterus. The mean luteal oxytocin concentration was low (30.61 ± 4.41 ng/g) in early (Days 4- 6). The concentration significantly increased (104.69 ± 12.73 ng / g) in mid (Days 10 - 12) and declined significantly in late (Days 16 - 18) cycle CL (49.87 ± 5.95 ng /g). Concentration in CL of previous cycle (CA) was lower (12.20 ± 1.86 ng /g) compared to dioestrus CL. Additionally, the mean luteal oxytocin concentrations in early and mid pregnancy were 4.79 ± 0.90 ng / g and 2.98 ± 1.03 ng / g respectively and was significantly less compared to non-pregnant corpus luteum. Biological activity of luteal extract as well as standard oxytocin was inhibited by boiling, oxytocin specific antagonist, sodium thioglycollate and alpha - thioglycerol.

Variety of peptides viz., oxytocin, vasopressin, oxytocin related neurophysin and relaxin are known to be synthesized by the corpus luteum (Wathes, 1984; Schams, 1987). Of those peptides, oxytocin appears to play an important role in the regulation of luteal function. Oxytocin in the corpus luteum was demonstrated in the goats (Ott and Scott, 1910). Subsequently, its presence was confirmed in sheep (Wathes and Swann, 1982). However, Fields et al. (1987) reported immuno - histochemically localized neurophysin in large luteal cells of the non - pregnant buffaloes. Thus, the present study was designed to quantitate the concentration of bioactive oxytocin in the buffalo (*Bubalus bubalis*) corpus luteum.

MATERIALS AND METHODS

The corpora lutea (CL) from different stages of oestrous cycle and pregnancy were collected from buffaloes (non-descript breed, of varying ages and parity) slaughtered between 3- 4 a.m. at local Abattoir, Mathura. The ovaries were collected within 30-60 minutes after slaughter.

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Ovaries with CL were separated and placed in polyethylene packets containing chilled normal saline (0.9% w/v) and transported to laboratory in a thermos flask containing ice mixed with freezing mixture (Potassium nitrate and sodium chloride, 1 : 4) for further processing. The stage of pregnancy was ascertained by measuring curved crown and rump length (CRL) of the foetus (Soliman, 1975). Corpora lutea of oestrous cycle were categorized into four groups viz., of early (Days 4 - 6), mid (Days 10 - 12), late (Days 16 - 18) dioestrus and of previous cycle (corpus albicans) based on their colour, size, consistency, nature of connective tissue pleats, vasculature and presence or absence of mid cycle follicles in the contralateral ovary of the same animal.

Luteal tissue oxytocin was extracted following the method described in Indian Pharmacopoeia (1955) for posterior pituitary with certain modification. The extracts were stored at -20°C pending bioassay. The pH of the extract was adjusted to 6.5 - 7.0 using 0.1N - NaOH solution at the time of assay. Oxytocin was bio-assayed on isolated oestrogenized virgin female albino rat uterus (Perry, 1970). Concentration of oxytocin in test samples were calculated from a graph plotted with ordinate representing the magnitude of contractions and abscissa showing the varying concentration of standard

oxytocin was expressed in ng/g weight of tissue (1 IU = 2 / ug, Wathes, 1984).

Specificity of standard oxytocin (OT) and unknown tissue extracts was tested using various agents namely : boiling in boiling water bath for 90 - 120 minutes, sodium thioglycollate (20 μl , 0.04M/3 ng OT for 90 minute at 30°C) causing dissociation of disulphide linkage (Ames et al 1950). Alpha thioglycerol (0.1 ml, 1 : 10 in Dejalon solution per 10 ml bath) a specific in - vitro oxytocin receptor blocker (Martin and Schild, 1965) and oxytocin specific antagonist (40 ng antagonist / 10 ml bath / 2 ng oxytocin). (Manning and Sawyer, 1989) gifted by Maurice Manning.

Analysis of variance and Duncan's multiple range test was used to compare the means as per the method of Steel and Torrie (1984). Intra and Inter-assay coefficient of variation was compared using Snedecor, (1952).

RESULTS AND DISCUSSION

The mean (\pm s.e.) oxytocin concentration in the CL of cyclic and pregnant buffaloes and in the CL of previous cycle (CA) as measured by bioassay (minimum assay sensitivity ranged between 0.5 - 6.0 ng / 10 - 15 ml bath) are listed in Table. Intra and inter-assay coefficient of variation were 9.9 and 8.5% respectively. Also, biological activity of luteal extract as well as standard oxytocin was

Table : Oxytocin concentration (ng/g wet weight) of Buffalo corpus luteum as affected by the stage of the oestrous cycle and pregnancy.

Stage of the oestrous cycle / pregnancy	No. of Observation	Meant s.e.m.	Range
Stage of the cycle			
Early	15	30.61 ± 4.41 ^{bc}	9.6 – 73.0
Mid	15	104.69 ± 12.73 ^a	38.6 – 216.0
Late	15	49.87 ± 5.95 ^b	20.4 – 82.7
Overall	45	61.72 ± 7.70	9.6 – 216.0
CL of previous Cycle (CA)	10	12.20 ± 1.86 ^c	* 0.0 – 18.0
Stage of Pregnancy			
Early	15	4.79 ± 0.90 ^c	* 0.0 – 12.0
Mid	10	2.98 ± 1.03 ^c	* 0.0 – 8.9
Overall	25	3.74 ± 0.97	* 0.0 – 12.0

* Not detectable. Means within a column with different superscripts differ significantly, Duncan's multiple range test was performed at 1% level.

inhibited by boiling, oxytocin specific antagonist, sodium thioglycollate and alpha thioglycerol. Perusal of the table reveals that the concentration of oxytocin in buffalo luteal tissue was significantly low ($P < 0.01$) in early cycle and increased significantly ($P < 0.01$) nearly three-and-half - times in mid cycle CL. The oxytocin concentration significantly declined ($P < 0.01$) by nearly 50% in late cycle corpus luteum. In the CL of previous cycle (CA) the oxytocin concentration was lower than recorded during all the three stages of the oestrous cycle. (Table).

The present investigation indicates that the corpus luteum of cyclic buffaloes is a rich source of oxytocin and also, the pattern of change of luteal oxytocin

concentration of different stages of the oestrous cycle was similar to those reported in cow and sheep (Schams, 1987), however, the yield was much lower compared to cow (Fields et al., 1983), sheep (Wathes and Swann, 1982). This variability in concentration could be attributed to a species differences besides extraction and assay procedures.

Compared to cyclic CL there was a significantly low oxytocin concentration in the corpus luteum of early ($P < 0.01$) (Days 35 - 115) and mid (Days 130 - 220) pregnant buffaloes. This finding is in agreement with earlier reports in sheep and cattle (Wathes and Swann, 1982., Sheldrick and Flint, 1983, Schams, 1987). In buffalo, Fields et al., (1987) also observed similar results by immuno-

histochemical localization. In pregnant sheep this decline in oxytocin may be either due to a uterine factor or prolongation of luteal life span (Sheldrick and Flint, 1983) possibly favouring nidation.

These results based primarily on the bioactivity indicates that the preptide from the Buffalo luteal tissue is oxytocin - like material. However, further studies are required to purify, sequence and to substantiate the physiological role of oxytocin in the buffalo as reported for cow and sheep.

ACKNOWLEDGEMENTS

Thanks are due to Dean, College of Veterinary Science, Mathura and Director IVRI Mukteswar, for providing facility and leave respectively for carrying out this study and Dr. G.S. Bisht for statistical analysis. Grateful thanks are also due to Prof. Maurice Manning, Department of Biochemistry and Mol. Biology, Medical College of Ohio, Toledo for generous gift of oxytocin antagonist.

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EFFECTS OF BETA-ADRENERGIC AGONIST (ISOXSUPRINE) ON CORTISOL CONCENTRATIONS IN UTERINE TORSION IN BUFFALOES

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ABSTRACT

Effects of isoxsuprine hydrochloride on plasma cortisol concentrations were studied in buffaloes that got caesarean section for uterine torsion. For this 60 mg of isoxsuprine hydrochloride was administered, intravenously, 15 minutes prior to caesarean section in seven buffaloes. Seven other buffaloes having uterine torsion also got operated in the same manner and served as operated control, while five other normal parturient buffaloes were used for normal controls. Plasma cortisol was measured in plasma at 0, 2, 4, 6, 24, 48 and 72 hours postpartum / post caesarean section. Buffaloes suffering from uterine torsion had higher concentrations of plasma cortisol than normal parturient buffaloes that remained elevated till 48 hr. Administration of isoxsuprine hydrochloride prior to surgery had no significant effect on plasma cortisol concentrations. It is concluded that isoxsuprine hydrochloride is not useful in eliminating stress of uterine torsion.

Dystocia due to uterine torsion is very common in buffaloes particularly in and around Haryana

State (Gupta et al. 1981; Manju et al. 1985; Khatri et al. 1986).

In cases of uterine torsion that are not resolved through rolling, caesarean section is the only alternative method that help in delivery of foetus, but add surgical stress to the animal. Monitoring and management of such stress might be helpful in recovery of animals. There are many approaches to manage such stress, but application of B-adrenergic agonist, has been advocated by Ahlers and Anderson (1967). The present study was designed to evaluate effect of Isoxsuprine which is a B-adrenergic agonist on cortisol concentrations in uterine torsion in buffaloes.

MATERIALS AND METHODS

Study was conducted on 14 Clinical cases of uterine torsion in buffaloes brought to the veterinary clinics, Haryana Agricultural university Hisar for treatment. Five pregnant buffaloes maintained by the Buffalo Research Center at the University farm, under identical conditions of feeding and management were also included in the study to serve as the normal parturient controls. These

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animals were kept under observations for the symptoms of impending parturition and the calving process completed under vigilance.

The diagnosis of torsion of uterus in the clinical cases was confirmed by rectal and vaginal examination. The cases of uterine torsion were randomly assigned to two groups namely, isoxsuprine group ($n=7$) and operated control ($n=7$). Isoxsuprine group received 60 mg of isoxsuprine hydrochloride (Duvadilan), intravenously, 15 minutes prior to surgery. The delivery of the foetus was achieved by lapro - hysterotomy in all the cases and uterus detorted through the laprotomy - wound after the withdrawal of fetus. All the animal received supportive therapy (4 litres Ringer's lactate I/V during operation and next 2 days, 50 I.U. Oxytocin. I/V during operation, 50 ml Chromostat I/V during operation, Metranidazole 2 gm I/V for 3 days, Streptopencillin 5 gm I/M for 3 days and dressing of suture line with Betadine).

Blood samples were collected in heparinized vials from all the animals at 0, 2, 4, 6, 24, 48 and 72 hours of caesarean section / postpartum. Cortisol concentrations were determined by solid phase radioimmunoassay using coat - a - count kits supplied by Diagnostic Products Corporation, Los Angeles (USA). The intra-assay variation was 3.0 to 5.1%, while inter - assay variation was 4.0 to 6.4%.

RESULTS AND DISCUSSION

Plasma cortisol concentrations have been shown in table. The plasma cortisol concentrations were significantly ($P<0.05$) elevated in buffaloes affected with uterine torsion than normal parturient ones and remained significantly ($P<0.05$) higher up to 48 hr. The plasma cortisol levels in the torsion affected buffaloes ranged between 10.62 to 63.61 ng/ml prior to surgery where as the concentrations in the normal buffaloes on the day of calving ranged 0.66 to 1.68 ng/ml. The higher cortisol concentrations in uterine torsion could be due to stress of torsion as suggested (Manju et al., 1986 and Prabhakar, 1995). The wide variation in cortisol concentrations among buffaloes could be due to individual sensitivity to stress (Hudson et al. 1976; Heuwiesser et al., 1987). Continuation of higher cortisol concentrations had been observed for various periods in uterine torsion cases. Siddiquee (1988) observed higher cortisol concentrations upto 24 hr, while Manju et al. (1985) observed this up to 5 days. This variation could be due to variation in tissue damage (Schmidt and Booker, 1982) due to caesarean section as decline in cortisol concentrations was faster after rolling than caesarean section (Ghuman, 1995).

The plasma cortisol concentrations in buffaloes receiving isoxsuprine were not significantly ($P > 0.05$) different from buffaloes not receiving this drug (operated control),

however, the plasma cortisol concentrations declined significantly by 48 hr post caesarean in operated control buffaloes than isoxsuprine treated buffaloes. There was no significant change in cortisol concentrations upto observation period of 72 hr in animals treated with

isoxsuprine. This difference might suggest that interference in animals physiology through such drugs is not good as similar observation were made with the use of tranquilizers (Ghuman, 1995). It is concluded that isoxsuprine is not useful in eliminating stress of uterine torsion.

TABLE : Plasma cortisol concentrations (ng / ml) in buffaloes receiving isoxsuprine, operated control and normal parturient buffaloes.

	0 hr.	2 hr.	4 hr.	6 hr.			
Operated control group (n = 7)	35.64 ^{ax} ± 8.20	31.21 ^{ax} ± 8.6	31.23 ^{ax} ± 13.01	29.81 ^{ax} ± 0.25	20.38 ^{abx} ± 5.99	9.71 ^{bxy} ± 6.31	8.22 ^{bxy} ± 7.11
Isoxsuprine group (n=7)	25.93 ^{ax} ± 8.42	31.91 ^{ax} ± 7.96	26.97 ^{ax} ± 5.80	33.17 ^{ax} ± 6.41	16.44 ^{abx} ± 9.53	18.26 ^{abx} ± 8.51	13.10 ^{abx} ± 3.10
Normal Parturient buffaloes (n = 5)	1.04 ^{ay} ± 0.22	0.98 ^{ay} ± 0.13	1.28 ^{ay} ± 0.17	0.98 ^{ay} ± 0.13	0.86 ^{ay} ± 0.19	1.61 ^{ay} ± 0.93	1.74 ^{ay} ± 1.16

Different superscripts of a, b show significant difference ($P < 0.05$) between periods within group (horizontal comparison), while x, y show significant difference ($P < 0.05$) between groups, within period (vertical comparison).

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DISTRIBUTION PATTERN, HISTOLOGY AND HISTOMETRY OF THE UMBILICAL AND PLACENTAL VESSELS IN BUFFALOES*

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ABSTRACT

The umbilical cord was composed to two thick arteries and two thin veins. A pair of artery and vein diverged at right angles to traverse as placental vessels in the allanto chorion. Anastomosis of the umbilical arteries at the level of divergence and inter connecting branches of the placental vessels supplying the cotyledons were commonly noticed. The umbilical vessel had exceptional vascular structure. They were characterized by inner longitudinal and outer circular muscle fibres with uniformly distributed elastic fibres. The veins at later stages of gestation did not differ from the arteries in their histological feature. The diameter and thickness of the umbilical artery was more than the vein upto 95 days of gestation but subsequently there was no difference between their sizes. The rate of increase in the size of the umbilical artery was maximum upto 95 days of gestation whereas in the vein it was maximum upto 130 days of gestation.

Histological features of the placental and umbilical vessels vary markedly from that of the other arteries and veins of the body (Carleton and Short, 1956). Further, they undergo extensive structural changes along with the growing placenta and fetus (Brosens et al., 1967). There is paucity of such information on the umbilical and placental vessels of buffaloes.

MATERIALS AND METHODS

Gravid genitalia of non descript buffaloes of different parity were collected from Madras corportion slaughter house and brought to the laboratory for detailed investigation. The gravid genitalia was washed and cut opened along the dorsal curvature without damaging the foetal membranes. The membrane was cut opened along the antimesometrial border and both gravid and non-gravid sides of the placenta were spread out on an illumination box to make direct observation.

Curved crown rump (CVR) length of the fetus was measured

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and the stage of gestation was calculated based on the formula described by Soliman (1975). In all 32 genitalia were utilised for the study. Based on CVR they were divided into 5 groups. Group I : 65 days; Group II : 95 days; Group III : 130 days, Group IV : 155 days, Group V : 195 days.

With the diffuse bright light provided by the illumination box and with stereo zoom microscope the distribution of placental vessels were studied and photographed whenever necessary. Histometric analysis of the umbilical vessels was carried out with calibrated ocular micrometer.

RESULTS AND DISCUSSION

In the present study the umbilical cord of buffaloes was observed to be composed of four blood vessels and one allantoic duct surrounded by mucous connective tissue. These structures and the amniotic membrane covering the umbilical cord showed distinct changes as the foetus grew and the stage of gestation progressed from 56 to 220 days (Fig 1).

Umbilical Vessel : The umbilical vessels revealed a pair of thick and a pair of thin vessels. By dissecting the foetus and tracing the vessels it was confirmed that the thick vessels with the allantoic duct were umbilical arteries and thinner ones were veins. The arteries and veins remained separate throughout their course in the umbilical cord.

A pair of umbilical artery and vein diverge at right angles and pass along the mesometrial border of the allantochorion. The vessels traversing through the gravid side, were observed to be thicker than those of the non-gravid side. However, in both sides the artery remained thicker than the vein (Fig 2).

The umbilical arteries at the level of divergence were united by a distinct anastomosing arch. The anastomosing arch was noticed at all stages of gestation (Fig. 3). At early stages it was slender and indistinct but became more prominent subsequently.

Placental Vessels : The placental artery and vein as they traverse along the allantochorion did not shown any anastomosis. They gave off branches at regular intervals at right angles to supply the cotyledons. At very early stage of pregnancy (CVR - 7.0 cm.) even before the formation of cotyledons branches from the placental vessels could be identified diverging and terminating at different levels in the allantochorion. On the gravid side group of vessels arise at the same place to supply about 3 to 5 cotyledons. In certain other places inter connecting branches between the smaller vessels supplying the cotyledns were noticed. Frequency of such interconnections increased as the gestation advanced.

Compared to the angiographic technique after injecting radio-opaque material adopted by

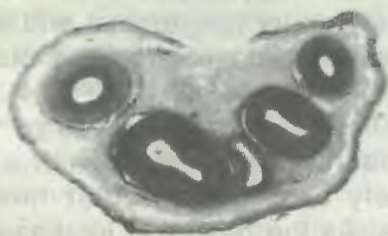


Fig. 1



Fig. 2



Fig. 3



Fig. 4

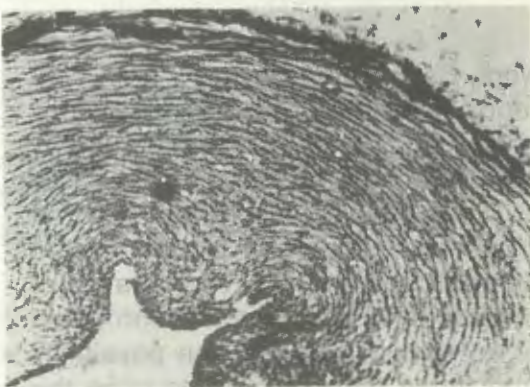


Fig. 5

Fig. 1 C.S. of umbilical cord - arteries (a); veins (v) and allantoic duct (ad).

Fig. 2 Thick placental artery and thin placental vein.

Fig. 3 Anastomosis between the two umbilical arteries.

Fig. 4 Umbilical artery (56 days) inner longitudinal and outer circular muscle fibers.

Fig. 5 Umbilical artery (109 days) - Inner and outer circular muscle fibers. Collagen fibers in tunica adventitia.

earlier workers (Steven, 1964) use of illumination box for examining the distribution of umbilical and placental vessels as adopted in this work was found to be easier, economical and gave good results.

Two umbilical arteries and two umbilical veins were observed in the all genitalia examined in the study. This agrees with the observation of Steven (1968) in sheep and Roberts (1971) in bovine. Sloss and Duffy (1980) reported two umbilical arteries and a single umbilical vein or sometime two veins in cattle.

As observed in the present work, Barcoft and Barron (1946) also reported that paired branches were given off from the placental vessels at right angles opposite to each row of cotyledons. Fine branches of the placental vessels which terminate at different levels at very early stages of pregnancy even before the formation of cotyledons suggest the possibility that these vessels predetermine the site of formation of cotyledons.

The anastomosis noticed between the umbilical arteries and between the branches of the placental vessels recorded in the present study has no comparable information in the available literature. Steven (1964 and 1968) reported anastomosis between umbilical vein at the bifurcation in sheep uterus. He noted that large anastomosis between umbilical arteries were relatively uncommon.

Histological Changes of Umbilical Vessels : At very early stages of gestation (CVR - 6.0 cm; 56 days) the umbilical artery showed two distinct layers of inner longitudinal and outer circular muscle fibres. The elastic fibres were almost absent (Fig. 4). The umbilical vein at this stage was comparatively very small with a few loosely arranged circular muscle fibres. As the gestation increased to about 77 days (CVR - 10.7 cm) the umbilical arteries showed increased amount of outer circular and inner longitudinal muscle fibres. The amount of elastic fibres was however, remained negligible. In the umbilical vein, at this stage of gestation, the circular fibres were more than the previous stage but longitudinal muscle fibres remained undifferentiated.

As the gestation advanced to 109 days (CVR - 17.9 cm) the umbilical artery showed distinct area of inner longitudinal and outer circular muscle fibres (Carleton and Short, 1956). The elastic fibres were seen distributed uniformly in the outer circular muscle fibres (Fig 5). The outer most adventitia layer was less prominent but rich in collagen fibres (Shrivastava et al., 1980). The umbilical vein at this stage showed well-demarcated inner longitudinal and outer circular muscle fibres. Uniformly distributed elastic fibres could be identified in between the muscular fibers (Tsumara et al., 1981). As the stage of gestation advanced there was progressive increase in the distribution of muscle and elastic fibres.

Histometric Changes of Umbilical Vessels : The diameter and thickness of the umbilical vein was less than that of the umbilical artery upto 95 days of gestation. Subsequently the umbilical vein measured almost similar to the artery. The diameter of umbilical artery increased from 681μ at 65 days to 3266μ at 195 days of gestation. The corresponding increase in thickness was 233 to 1581μ . The diameter of umbilical vein increased from 334μ at 65 days to 3435μ at 195 days of gestation. The corresponding increase in thickness was 101 to 1362μ .

The differential rate of increase in the diameter and thickness of the umbilical artery and vein noticed between 65 to 130 days of gestation may be to accommodate the increased blood flow required by the growing foetus and foetal membrane (Schmidt et al., 1964). This was achieved by decrease in umbilical vascular resistance during early stages of pregnancy and by increased blood pressure during latter part of gestation (Hafez, 1980).

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GROSS, HISTOLOGICAL AND HISTOCHEMICAL CHANGES OF AMNIOTIC PLAQUES IN BUFFALOES DURING DIFFERENT STAGES OF GESTATION*

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ABSTRACT

Amniotic plaques were noticed from 79 to 220 days of gestation. Their size and shape varied distinctly with the stages of gestation. They were made of polygonal or oval cells with granular cytoplasm, rich in glycogen and free of mucin and lipid. At later stages of gestation they showed superficial and deeper regions which were composed of flattened scale like cells and clear polygonal cells respectively.

Amniotic plaques are peculiar structures seen on the inner side of the amnion. Their size, distribution, histology and histochemistry have been reported in bovine (Santoni, 1936; Germain et al., 1974 and Arthur, 1975) and in Camel (Morton, 1961). Most of the reports were confined to a particular stage of pregnancy. In the present investigation gross histological and histochemical changes of amniotic plaques during different stages of gestation in buffaloes are studied.

MATERIALS AND METHODS

Gravid genitalia of non descript buffaloes of different parity were collected from Madras corporation slaughter house and brought to the laboratory for detailed investigation. The gravid genitalia was washed and cut opened along the dorsal curvature without damaging the foetal membranes. The membrane was cut opened along the antimesometrial border and both gravid and non gravid sides of the placenta were spread out on an illumination box to make direct observation on the distribution of amniotic plaques. Pieces of amniotic plaque were collected for histological and histochemical examination.

Curved crown rump (CVR) length of the foetus was measured and the stage of gestation was calculated based on the formula described by Soliman (1975). In all 32 genitalia were utilized for the study. Based on CVR they were divided into 5 groups. Group I: 65 days; Group II: 95 days; Group III: 130 days; Group IV: 155 days; Group V: 195 days of gestation.

Gross observation of amniotic plaques : By the illumination box and with stereo zoom microscope the

* Part of the MVSC thesis submitted by the first author to the Tamil Nadu Agricultural University, Coimbatore.

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distribution of amniotic plaques were studied and photographed.

Histological and Histochemical examination : As per the standard histological technique (Druy and Wellington, 1967) the fixed tissues were processed. Thin paraffin section of amniotic plaques were subjected to the following histochemical techniques. (Mcmanus and Mowry, 1960).

1. Periodic acid Schiff and haematoxylin stain, 2. Periodic acid Schiff and Haematoxylin stain with diastase pre-treatment for glycogen, 3. Alcian blue for identifying mucin. 4. Sudan black stain for lipids.

Histological and histochemical features were studied in detail and micro photographs were taken with Carl Zeiss microscope.

RESULTS AND DISCUSSION

Macroscopical changes:

Group I: The amniotic membrane did not show any evidence of development of amniotic plaques.

Group II: The amniotic plaques appeared as small white dot like elevations (CVR-15 cm; 96 days). They were closely placed near the umbilical cord and spread out uniformly all around to a diameter of about 6-8 cm.

Group III: Distinct changes were noticed in this group. The plaques were raised structures of uniform size and were yellow in colour. The

periphery of the plaques were more dense than the central portion. They were radially distributed on the amniotic membrane to a diameter of about 15-20 cm. At many places, they coalesce to form a continuous sheet of thickened area (Fig. 1).

Group IV: During this period, there was no marked variation in the gross appearance of the amniotic plaques.

Group V: In this group the plaques were flat and shrivelled with angular edges and irregular borders (Fig.2). However, they continued to be yellow in colour. They were sparsely distributed and most of them sloughed off easily. The macroscopical features of the amniotic plaques observed in this study agree with the earlier reports of Santoni (1936), Amoroso (1952) and Naaktgeboren and Zwillenberg (1961). In the present study, the plaques could be identified as early as 79 days of gestation (CVR - 11.1 cm). They become shrivelled and withered off when the gestation was 220 days (CVR - 65.0 cm). Roberts (1971) also observed the plaques during third to seventh month of gestation. However, Arthur (1975) observed the plaques to disappear even by sixth month of gestation whereas Germain et al., (1974) and Hagez (1980) observed the plaques to persist till late gestation.

Histological Changes:

Group II: The plaques were well-demarcated raised areas of cellular proliferation. The basal layer was made up of single row of cuboidal cells with large vesicular nuclei. The

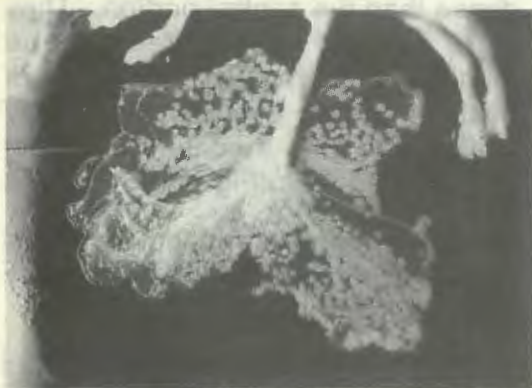


Fig. 1



Fig. 2

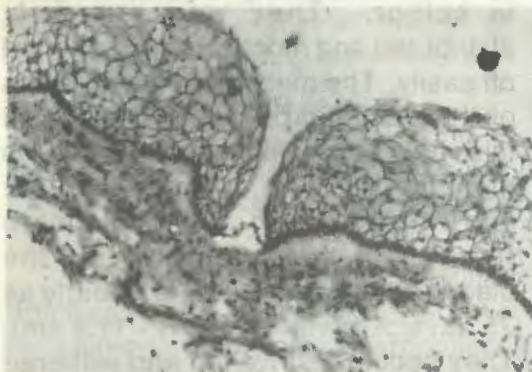


Fig. 3

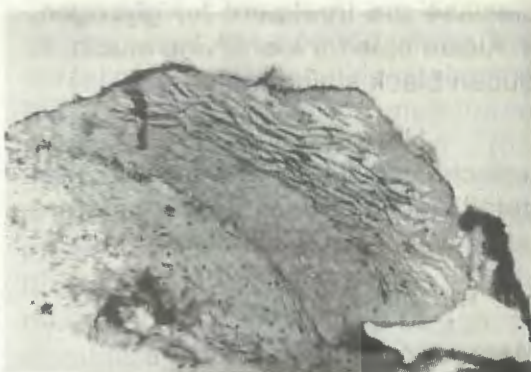


Fig. 4

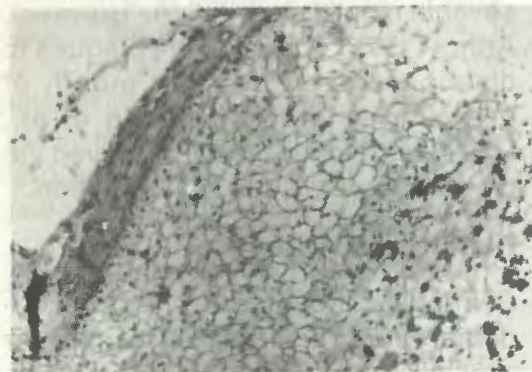


Fig. 5

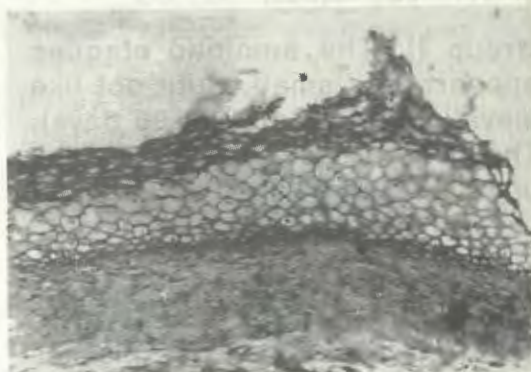


Fig. 6

Legend for figures in Page 136 / 42

proliferated superficial cells were large polygonal in shape. The lightly stained cytoplasm of these cells showed fine granules and vacuoles. They had distinct centrally placed nucleus. The free surface of the plaque was limited with a layer of flattened cells with elongated nucleus (Fig.3. CVR - 12.9 cm: 90 days).

Group III: The polygonal cells were more in number and larger in size. The cytoplasmic granules were prominent and larger and the nuclei were placed in an eccentric position. At this stage of gestation (CVR - 17.8 cm: 109 days) the plaques showed two regions. The superficial region was composed of irregular polygonal cells with deeply stained granular cytoplasm. The deeper region towards the basal layer was composed of polygonal or oval shaped cells with fine cytoplasmic granules and vacuoles.

Group IV: The superficial layer of cells became more flattened. The oval and polygonal cells in the deeper region were smaller in size and the cytoplasm was free of granules. The nucleus in most of them were either absent or pyknotic. As the gestation advanced to 162 days; CVR 39.5 cm, the superficial cells appeared almost like scales while the cells closer to the basal layer continued to be polygonal in shape with clear cytoplasm and indistinct nucleus.

Group V: The plaque appeared smaller and irregular in shape. The superficial cells were like flattened scales, the deeper portion very close to the basal

cells was composed of a few layers of polygonal cells with clear cytoplasm and often with out nucleus (Fig.4)

Morton (1961) noted that the large pustules were made of columnar type of cells. Initially the cells were observed to be oval or polygonal in shape but as the gestation advanced they became flattened and scale like. Roberts (1971) also described the plaques as keratinized epithelial cells.

Histochemical Changes:

Periodic acid schiff reaction (PAS): The fine cytoplasmic granules noticed at the early period of gestation (CVR - 12.4 cm) were faintly PAS positive. However, after diastase pre-treatment they were negative for PAS reaction (Fig. 5) indicating that the granules were made of glycogen. The distinct cytoplasmic granules were not stained with Alcian blue indicating that the cytoplasmic granules were not made of mucin. Fresh amniotic plaques stained with sudan black revealed no reaction, suggesting that the plaques were free of lipids. Subsequent to this group, only the superficial flattened cells continued to show PAS positive granules, while the deeper cells closer to the basal layer did not show PAS positive reaction (Fig. 6).

Regarding histochemistry presence of glycogen observed in this study confirms the earlier reports of Arthur (1975) and Hafez (1980). Further, it was shown that the plaques do not contain mucin or lipid. The glycogen appeared initially as fine

cytoplasmic granules and subsequently as prominent deeply stained granules when the gestation reached 109 days (CVR - 17.0 cm). In flattened scale like plaques the glycogen was confined more to the

superficial region. The yellow colour noticed may be due to the presence of glycogen. There are no comparable information in the literature on the distribution of glycogen in the amniotic plaque at different stages of gestation.

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Legend of figures in page 134/40

- Fig. 1** Gross photograph of distinct raised amniotic plaques - 130 days gestation.
- Fig. 2** Gross photograph of flat and shrivelled amniotic plaques - 90 days gestation.
- Fig. 3** Micro photograph of amniotic plaques - Note polygonal cells with granular cytoplasm - 90 days - PAS & H - 125 X
- Fig. 4** Micro photograph of amniotic plaques - flattened scale like superficial cells and polygonal cells with clear cytoplasm - 190 days - PAS & H, 125 X
- Fig. 5** Micro photograph of amniotic plaques - after diastase treatment. Note unstained cytoplasm of superficial cells - 110 days - PAS & H, 125 X
- Fig. 6** Microphotograph of amniotic plaques - Note PAS positive superficial cells and clear, basal polygonal cells - 160 days - PAS & H - 125 X

CIRCULATING LEVELS OF STEROID HORMONES IN FEMALE CAMELS DURING PERI-PARTURIENT PERIOD

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ABSTRACT

Blood samples from eight pregnant camels were analysed for Estradiol-17 B, Progesterone and Cortisol on different days falling one month before and two months after parturition using RIA technique. The mean estradiol levels were many folds higher during the last month of gestation (440 to 550 pg/ml) than those recorded after parturition (10 to 40 pg/ml). The mean progesterone concentration (2.2 ± 0.17 ng/ml) declined from last month of gestation through parturition to barely detectable values in post-partum samples. The mean cortisol concentration elevated to a peak level of 21.5 ng/ml on day prior to parturition and then sharply declined to basal levels of about 9.0 ng/ml by day six post-partum which were maintained upto two months.

Under normal physiological conditions, the pregnancy of a female is terminated at parturition which is a complicated process controlled by a

series of neuroendocrine signals. The present study was undertaken to investigate the changes in steroid hormone levels during a period one month before and two months after parturition in camels.

MATERIALS AND METHODS

Single humped pregnant camels (*Camelus dromedarius*) in their last month of gestation maintained under standard conditions of feeding and management at National Research Centre on Camel, Bikaner were used for this study. Blood samples from eight camels were collected on days 28, 21, 14, 7, 5, 3 and 1 pre-partum and 1, 3, 6, 9, 13, 20, 27, 34, 41, 48, 55 and 62 post partum through jugular venipuncture. Sera samples were analysed for Estradiol-17 β , progesterone and cortisol using Cost-A count specific RIA kits supplied by Diagnostic Products Corporation, USA. The protocol was followed as supplied with the kit with minor modification.

RESULTS AND DISCUSSION

The Estradiol-17 β concentration was 25 to 50 folds higher during the last month of gestation than the values recorded after parturition; the

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range being from 440.0 ± 97.95 to 552.0 ± 89.35 pg/ml during pre-partum and from 40.25 ± 2.03 to 38.75 ± 5.04 pg/ml during post-partum period. Following parturition, there in estradiol concentration from a value of 440.0 pg/ml on day-1 pre-partum to a level of 38.75 pg/ml on day-1 and 21.13 pg/ml on day-3 post-partum. The concentration declined gradually through post-partum period to a lowest value of 10 pg/ml by two months. The pre-partum values were significantly higher ($p < 0.01$) than those during post-partum period. However, the differences between post-partum stages were not significant. Similar trend was recorded in other species of animals such as cattle (Kaker et al., 1984; Sawada et al., 1988), buffalo (Arora and Pandey, 1982) and sheep (Robertson and Smeaton, 1973). The camel allantoic fluid has been shown to contain high level of estrogens on day of parturition (Elias et al., 1984) indicating that placenta may be the probable source of this hormone. Moreover, foetal goands might also be contributing to the pool as reported for the mare (Pashen and Allen, 1979). The rapid decline in Estradiol-17 β after parturition could be due to detachment of placenta and loss of circulation between mother and the foetus.

The mean progesterone level was high (2.2 ± 0.17 ng/ml) during last month of gestation and started falling from day-5 pre-partum to a level of 1.32 ng/ml on day-1 before parturition. On following delivery of calf, the progesterone concentration sharply

declined to a lowest value (0.11 ng/ml) by day-3 and then fluctuated within narrow limits of 0.06 and 0.16 ng/ml upto 2 months postpartum. The analysis of variance revealed that the stage to stage variations were significant ($p < 0.01$) during pre-partum period only. The trend appeared to be similar as reported for other species (Kaker et al., 1984; Sawada et al., 1988; Arora and Pandey, 1982; Prakash and Madan, 1985; Tsang, 1978). The fall in progesterone level at the time of parturition could be attributed to the regression of CL. Foetal glucocorticoids also act as a stimulus for the synthesis of PGF 2α by the uterus (Baetz and Leiting, 1980) which in turn causes luteolysis and checks the production of progesterone (Rawlings and Ward, 1978).

The mean concentration of cortisol in the serum of pre-partum camels fluctuated between 9 and 13 ng/ml from day-28 to day-3 pre-partum but increased upto a level of 21.5 ng/ml on day prior to parturition. The cortisol levels declined soon after parturition to attain a basal value of about 9.5 ng/ml by day-6 post-partum which were then maintained upto two months. Rise and fall in cortisol levels around the day of parturition was statistically significant ($p < 0.01$). Elevated cortisol levels in camel observed around parturition indicated the involvement of this steroid in parturition in this species as well. The high cortisol levels, during a short period after the delivery of calf, may

be a residual effect of the stress of parturition (Smith et al., 1973) and may have a physiological role in providing extra energy by virtue of its glycogenolytic and glycogenic

property. Normal levels after about a week after parturition coincide with the result of other workers Agarwal et al. (1992) and represent to cater day to day need of the animal.

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SERUM ENZYME CHANGES AROUND PERIPARTURIENT PERIOD IN FEMALE CAMEL

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ABSTRACT

Circulating levels of enzymes AST, ALT and ALP were estimated in serum samples of eight pregnant camels (*Camelus dromedarius*) belonging to National Research Centre on Camel, Bikaner during one month before and two months after parturition. The enzymes were estimated by autoanalyzer immediately after separation of serum. The study revealed that the ALP was significantly higher around parturition whereas AST and ALT elevated during later stages of parturition period.

these animals were collected by jugular venipuncture on days 28, 21, 14, 7, 5, 3 and 1 pre-partum and 1, 3, 6, 13, 20, 27, 34, 41, 48, 55 and 62 post-partum in glass tubes for separation of serum. Three enzymes viz., Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were estimated immediately after separation of serum, in Beckman DU Clinical System, using Lupin Reagents Kits. The data were statistically analysed in ECIL Micro-32 Computer.

MATERIALS AND METHODS

Eight single humped healthy pregnant she-camels (*Camelus dromedarius*) in the last month of gestation were used for this study. The animals belonged to National Research Centre on Camel, Bikaner and were kept under standard conditions of feeding and management. Blood samples from

RESULTS AND DISCUSSION

The mean concentration of AST, ALT and ALP in the serum of dam during prepartum stages varied from 51.97 ± 3.15 to 60.57 ± 2.82 IU/L; 4.93 ± 0.54 to 7.71 ± 0.66 IU/L and 89.52 ± 7.00 to 117.73 ± 11.77 IU/L, respectively, whereas, during post-partum stages the values varied from 50.77 ± 2.83 to 66.64 ± 4.2 IU/L; 6.08 ± 0.52 to 11.10 ± 1.11 IU/L and 98.84 ± 8.19 to 150.37 ± 16.48 IU/L, respectively. The enzyme AST exhibited wide fluctuations without any particular trend during last month of gestation. However, the levels elevated just after parturition (62.69 ± 3.58 IU/L) followed by an initial fall upto day - 13, (50.77 ± 2.83 IU/L) and then

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elevation upto 64.30 ± 3.89 IU/L by one and a half months postpartum. The minor fluctuations at various stages of prepartum and post-partum stages were not significant statistically. Lower AST activity in late gestation of cows (Murtuza et al., 1980) and higher values at parturition as compared to pre-partum and post-partum levels (Trende and Mulling, 1982) partially support the present findings. Macros (1980) also recorded lower AST values during later stages of pregnancy in cows which rose sharply just before calving and remained high until three weeks post-partum. Contrary to our results, Boots and Ludwick (1970) reported highest SGOT activity in first stage of lactation which decreased with advancing stages of lactation. Beaunoyer (1992) advocated that the AST is mostly bound to the mitochondria and therefore, marked elevation in the blood indicates serious cell damage if the cause of elevation in the cell lysis. The ALT levels exhibited a gradual ascending trend from one month pre-partum (4.93 ± 0.54 IU/L) to 6.08 ± 0.52 IU/L just after parturition and then increased to 11.10 ± 1.11 IU/L by two months post partum. The analysis of variance revealed that the post-partum values during later half were highly significant ($P < 0.01$) statistically. A little lower value of ALT around parturition and higher values during early lactation was encountered in cows (Murtuza et al., 1980). On the other hand, Hafez et al. (1983) found rising ALT levels in buffaloes during a period of four weeks before parturition indicating species variation. The

values of ALP activity obtained in this study ($91-151$ IU/L) are higher than those reported for post-partum camel (31.5 to 37.1 IU/L) by Elias and Yagil (1984). The variation may be due to difference in storage duration of serum and the technique used for determination of enzyme activity. However, the trend observed in camel is consistent with the findings of Kendal and Harshbarger (1962), who found higher phosphates activity in the serum of cows after parturition as compared to 1-4 days before the expected date of parturition. The ALP levels increased from day-14 pre-partum (89.52 ± 7.0 IU/L) to day-1 pre-partum (117.73 ± 11.77 IU/L) with a mild drop after delivery of the calf (108.36 ± 12.49 IU/L). Thereafter, the values further elevated to attain the highest level (150.37 ± 14.48 IU/L) on day-13 post-partum followed by a declining trend until two months post-partum (98.84 ± 11.19 IU/L). The variations between stages were highly significant ($P < 0.05$). Similarly, higher ALP activity in non-pregnant camels than those in early and late pregnancy has been reported (Eltohamy et al., 1986). Murtuza et al. (1980), however, reported significantly lower ALP activity in early lactating than late pregnant cows. Denielli (1951) postulated that many of the phosphates found in the animal cells are the integral part of the contractile proteins. The fraction of ALP on the cellular surface is to provide focal points for release of the energy required for protein concentration and extension. According to him, this

enzyme acts mainly as a phosphokinase (and not as a phosphates), transferring phosphate group from a high energy bond to a low energy, the difference in energy between the two bindings being made available for protein concentration. On the contrary, Deshpande and Janakiraman (1988) suggested the ALP are associated with non-muscular tissues are therefore, may not be influenced by physical stress. The

drastic changes in the levels of ALP seem to be associated with the stress of foetal enlargement, wear and tear at the time of parturition and onset of lactation.

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COMPARATIVE EFFICACY OF HERBAL PREPARATIONS IN THE MANAGEMENT OF ANOESTRUS IN NON-DESCRIPT RURAL BUFFALOES*

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ABSTRACT

The comparative efficacy of *Abroma augusta* (Ulatkambal), *Nigella sativa* (Kalonji) alone or in combination in inducing oestrus in non-descript acyclic buffaloes was evaluated. The results indicated that a combination of *Abroma augusta* and *Nigella sativa* in 2:1 ratio at 15 g does level was superior as it could induce ovulatory heat in 50% acyclic buffaloes (3/6) in 12 ± 1.73 days and two of these buffaloes (3/6) conceived. No activity was found with *Abroma augusta* and *Nigella sativa* at 5 g dose levels.

Post-partum anoestrus is one of the most common reproductive disorder responsible for production losses in rural buffaloes. Despite multiple etiology, use of hormonal preparations in the management of anoestrus syndrome is wide spread. These drugs have their own fall out such as hyperstimulation of ovaries, high cost and nonavailability in rural areas. During recent years, much interest is being exhibited in the use

of herbal drugs which are reputed for this cheapness, effectiveness, without harmful side effect and local availability in rural areas. For oestrus induction, herbal preparations such as Aloes compound (Deshpande, 1983), Estrona (Shah and Derashri, 1985), Prajana (Kodagali et al., 1991), Hitrit (Reddy et al., 1994) and Janova (Singal, 1995) have been tried in animals with variable results. Therefore, the present investigation was undertaken to evaluate the therapeutic efficacy of *Abroma augusta* (Ulatkambal) and *Nigella stiva* (Kalonji) either alone or in combination in the management of post-partum anoestrus in non-descript buffaloes.

MATERIALS AND METHODS

A total of 62 non-descript acyclic buffaloes formed the material for the present study. These acyclic buffaloes were randomly divided into three groups (I, II and III) of 18 acyclic buffaloes each and remaining 8 acyclic buffaloes served as untreated control. The buffaloes of group I, II and III were further subdivided into three subgroups consisting six acyclic buffaloes as Ia, Ib, Ic; IIa, IIb, IIc and IIIa, IIIb, IIIc. These acyclic buffaloes of three sub groups of I were treated with *Abroma augusta* root powder, at

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5, 10 or 15 g twice daily p.o. for 15 days respectively. Similarly 2.5, 5, or 7.5 g of *Nigella Sativa* seed powder twice daily p.o for 15 days to three subgroups of II respectively. A combination of *Abroma augusta* and *Nigella Sativa* at 2 : 1 ratio was given at 5, 10 or 15 g, twice daily p.o. for 15 days to three sub groups of III respectively. The treated buffaloes were watched for onset of oestrus upto one month. Buffaloes which responded to the herbal drugs with manifestation of ovulatory oestrus were bred artificially and pregnancy was confirmed 60 days post AI.

RESULTS AND DISCUSSION

Acyclic buffaloes were characterized by smooth and inactive ovaries on rectal examination done on two successive occasion at an interval of 11 day and the absence of heat for last three months after calving. Since no literature was available on the dose schedule of these herbs in animals, present doses were extrapolated from the doses mentioned for human by Nadkarni (1954) and one lower and one higher dose was used.

Abroma augusta remained totally ineffective @ 5 g twice daily orally. However, the herb could induce oestrus in the three of the six buffaloes (50%) @ 10 g dose level in 13 to 20 days (16.67 ± 2.03 days). Of these three, two buffaloes (66.67%) showed ovulatory heat and one buffalo conceived. Increasing the dose to 15 g level appears to afford no extra advantage as only two of six buffaloes (33.33%) could show signs of oestrus

in 12 to 16 days (14.00 ± 2.00 days). Of these, 50% (1/2) buffaloes showed ovulatory heat and conceived. Sawale and Dhoble (1999) used a herbal mixture consisting of Ulatkambal (*Abroma augusta*) @ 50 g orally as single dose in acyclic cows and found 60% oestrus induction with 66.6% conception rate in the mean time of 19.99 days. Induction of oestrus by *Abroma augusta* root powder in acyclic buffaloes might be due to its uterotonic activity (Mishra et al., 1966) and estrogenic activity (Tewari et al., 1976).

Nigella sativa induced oestrus in one of the six buffaloes (16.67%) @ 2.5 g dose level in 20 days, though the heat was ovulatory (100%), but animal failed to conceive. At 5 g dose level, none of the six buffaloes evinced heat. However, at 7.5 g dose level, two of the six buffaloes (33.33%) showed oestrus sign in 11 to 19 days (15.00 ± 4.00 days) and one conceived. El-Shama et al. (1996) used "Reproton", a herbal mixture containing *N. sativa* in acyclic Egyptian buffaloes with persistent corpora lutea and found 85.7% ovulatory oestrus with 83.3% conception rate. The efficacy of *Nigella sativa* in inducing oestrus appears to be due to its estrogenic activity (Siddiqui et al., 1988 and Bhatnagar, 1996).

Combination of *Abroma* and *Nigella* (2:1) induced oestrus in two buffaloes (33.33%) out of the six buffaloes at the rate of 5 g in 13 to 21 days (17.00 ± 4.00 days). Of these, only one buffalo (50%) showed

ovulatory oestrus but remained unconceived. Whereas, at 10 g dose level, it could induce ovulatory oestrus in only one (16.67%) of the six buffaloes in 17 days and the animal conceived. However, at highest dose level (15 g), ovulatory oestrus was observed in three of the six buffaloes (50%) in 9 to 15 days with conception in two animals only. On comparison of all the treatments, it is evident that combination of *Abroma* and *Nigella* in 2:1 ratio at 15 g dose level was superior in inducing ovulatory oestrus in comparatively shorter time (12 ± 1.73 days) with 66.67% conception.

The higher efficacy of the combination appear to be due to synergistic action of these herbs owing to their estrogenic activity (Tewari et al., 1976 and Siddiqui et al., 1988). Because of limited number of observation, a large scale trial is indicated for definite conclusion.

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EFFICACY OF JANOVA AND EXAPAR COMBINATIONS IN TREATING ANOESTRUS IN RURAL CATTLE

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ABSTRACT

One hundred forty nine anoestrus cattle (104 cows and 45 heifers) were treated with three treatment regimens (A, B and C) comprising of Janova (J), Exapar (E) and Mineral (M) combinations. In true anoestrus animals oestrus was induced in maximum cases (58.18%) with treatment A (J + E + M) followed by (52.00%) with treatment B (J + E) and treatment C (J) (47.52%). It was better (63.23 and 55.55%) with treatment A and B in winter and in cows (60.00, 56.00 and 58.00%) than in heifers. In anoestrus cows associated with persistent corpus luteum, treatment A could induce higher response (58.18%) than treatment B (46.15%) and treatment C (20.00%). The post treatment duration for oestrus was shorter with treatment A (19.40 days) and showed an increase with B and C. The least squares analysis revealed highly significant effect of ovarian status on Post Treatment Oestrus (PTE) and Post Treatment Conception (PTC). Thus Exapar and Janova combinations showed better induction of oestrus as compared to Janova or Exapar alone.

suboestrus continue to be the most common cause of infertility. It is increasingly evident that herbal medicines with no side effects are of specific value in treating anoestrus in cattle. Therefore, systematic trials were undertaken to assess the efficacy of herbal formulation of Janova and Exapar from Dabur Ayurved in treating anoestrus in rural cattle.

MATERIALS AND METHODS

A total of 149 anoestrus cases including 104 cows with history of post partum anoestrus for more than 3 months and 45 pubertal heifers above 3 years of age were selected after examining per rectally for ovarian status. The animals with ovaries without palpable features (true anoestrus) and having persistent corpus luteum (functional) with a history of uterine disorders were grouped separately and assigned randomly to the A, B and C treatment regimen. Treatment A comprise of Exapar (E) 100 ml for 5 days followed by Janova (J) 3 capsules for 2 days (day 6 - 7 and 17 - 18) and mineral tablets for day 1 to 20. Treatment B consisted of similar doses of E and J only and treatment C with only J. Treatment D included only E to the cows with persistent corpus luteum. The trials were carried out in summer (March to June) and winter (November

In indigenous cattle late maturity, prolonged post partum anoestrus and high incidence of

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to February). The data were analysed with least squares technique according to Harvey (1975).

RESULTS AND DISCUSSION

I) True Anoestrus Cows (PTE): The response with only Janova (C) was 47.82% which is lesser than what reported by Signal (1995). The results suggest that with an addition on Exapar (E + J) the response could be improved to 52.00% (B) and an additional supplementation of minerals to 58.00% (A). The efficacy of treatment A and B was comparatively better (63.23 and 55.55%) in winter but not with treatment C (40.00%). The favourable season with nutritional adequacy might have helped activation. However, no such seasonal influence could be observed in heifers. The response with A, B and C regimens was higher (60.00, 56.25 and 58.33%) in cows that (55.02, 44.04 and 36.34%) in heifers, respectively.

II) Functional Anoestrus (PCL): In this group treatment A, B and D induced 58.33, 46.50 and 20.00% cows to oestrus. With treatment A and B better response (67.50 and 50.00%) was observed in summer as compared to (50.00 and 33.30%) winter. Thus the response on cows with functional anoestrus showed a different trend as compared to those in true anoestrus cows. The

results indicated that in addition to Exapar the ovarian stimulation with Janova and minerals help faster induction of oestrus.

Duration for Oestrus Induction and Conception The oestrus induction (PTE) duration in cows was least (19.40 days) with treatment A which increased (23.40 days) with treatment B and maximum (30.85 days) with treatment C. In heifers, however, it was minimum with treatment B (21.58 days) and maximum with treatment C (45.56 days). Thus the intervals were longer in heifers and with treatment C in particular indicating that only Janova treatment is not adequate for induction of oestrus.

The post treatment conception (PTC) was almost similar with treatment A and B (31.92 and 31.00 days) in cows and (34.72 and 30.00 days) in heifers. The interval was longer and almost similar with treatment C in cows and heifers (46.00 and 46.25 days) in cows but longer with treatment D (34.11 days). Similar trend was observed in PTC interval also.

The least squares analysis of PTE and PTC did not revealed significant effect of season, health, treatment and history. However, highly significant effect ($p < 0.01$) of ovarian status was observed on both PTE and PTC.

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INCIDENCE OF PARTURIENT DISORDERS IN FRIESWAL CATTLED.K. MANDAL¹, A.K. MATHUR², S. MUKHERJEE¹ AND S.P. SINGH³

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ABSTRACT

Two thousand three hundred and sixty eight calving records of Frieswal cows (Holstein - Friesian x Sahiwal having 5/8 HF inheritance), maintained at Military Farm, Meerut, distributed over a period of 5 years (1994 - 1999) were studied to find out the incidence of parturient disorder and its seasonal variation. The overall incidence of abortion, still birth, premature birth, retention of placenta, dystocia and total calving disorders were 2.50 ± 0.02 , 3.52 ± 0.02 , 0.42 ± 0.02 , 11.83 ± 0.03 , 0.42 ± 0.01 and 21.32 ± 1.36 per cent, respectively. Significant seasonal variation in occurrence of abortion, still birth and total parturient disorders were observed. The incidence of overall parturient disorders were highest during rainy (25.88 ± 0.05 per cent) followed by summer (21.29 ± 0.07 per cent) and winter (17.12 ± 0.04 percent) season. It was concluded that extreme summer and rainy season (June - October) are the most vulnerable period for incidence of parturient disorder in Frieswal cattle

Parturient disorders are the important factors affecting the reproductive efficiency of dairy animals, which in-turn causes a heavy

economic loss in dairy farming. It has been reported that about 18-40% of cattle are disposed off annually due to infertility and sterility (Iyre 1978) of which parturient disorders have a major contribution. The current article reports about the incidence of parturient disorders in Frieswal cows for which the available information is very scanty.

MATERIALS AND METHODS

The incidence of parturient disorders viz. abortion, still birth, premature birth, retention of placenta and dystocia of 2368 calving records were studied in Frieswal cows, a cross between Holstein - Friesian and Sahiwal (exotic inheritance 5/8 Holstein - Friesian), maintained at Military Farm, Meerut during the period from April 1994 to March 1999. All the cows were maintained at standardized management conditions as per Military Farm's Schedule. The incidence of a particular disorder was coded as 1 and non - occurrence as zero. The recorded data were classified into five years (periods) and each year was subdivided into three seasons viz. Summer (April - June), Rainy (July - October) and Winter (November - March) according to the climatological conditions.

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The frequencies of occurrence of parturient disorders were first converted into percentiles of total number of calving in a month of a year. Then the percentile data were transformed by Arc Sine transformation technique (Snedecor and Cochran, 1967). The transformed data were analyzed to study the effect of season and period through Least - Square analysis with the help of Mixed Model Least - Square and Maximum Likelihood Computer Programme, PC-2 version (Harvey 1990).

RESULTS AND DISCUSSION

The results are presented in Table 1 and Table 2. The overall least square for abortion, still birth and premature birth were 2.50 ± 0.02 , 3.52 ± 0.02 and 0.42 ± 0.02 per cent, respectively. Amble and Jain (1967) reported a total of 11.0% abortion, still birth and premature birth in Holstein - Friesian x Sahiwal crosses, which was much higher than the values obtained in the present findings. Significant seasonal variation was observed in abortion and still birth rate in Frieswal cows, with highest incidence during rainy season. Similar findings were also reported by Katoch et al (1999) in Jersey x Red Sindhi crosses. However, in the present investigation, no significant variation in still birth and abortion rate due to period of birth has been observed. The seasons or periods were not found to significantly affect the premature birth rates of Frieswal cattle.

The overall least square mean of occurrence of retention of placenta in Frieswal cows was 11.84

± 0.03 per cent, which was similar to the findings of Pandey et al (1981) in Gir crossbred. However, Holstein - Friesian crosses with native breed like Tharparkar and Hariana were found to have less incidence (6.90 - 8.00 and 1.40 percent, respectively) of retention of placenta (Sinha et al, 1978 and Pandey et al, 1981) than the Sahiwal crosses as obtained in the present investigation.

No significant seasonal and periodical variation in retained placenta was observed in the present findings. The highest incidence of retention of placenta was observed during rainy season (13.34 ± 0.08), similar to the findings of Agarwal et al (1984) in crossbred cows. However, Pandit et al (1981) and Shukla et al (1980) reported its highest occurrence during summer season. Significant monthly variation was observed in the present study with lower values obtained during March, April & May and higher values during June, July and August. From February onwards the significantly lower incidence of retained placenta might be due to availability and feeding of green Berseem which contain Ca, P and Vitamin A in sufficient amount.

The incidence of dystocia in Frieswal cows was 0.42 ± 0.01 per cent, much lower than the reports, Kaikini et al (1981), Pandey et al (1981) & Verma and Mishra (1984) and higher than the reports of Katoch et al (1999) in different crossbreds. Incidentally in the present study significant periodical variation was observed in occurrence of dystocia with unexpectedly sudden rise during

TABLE 1: Season-wise Least - Square means of various parturient disorders in Frieswal cattle.

Particulars	Seasons			
	Summer	Rainy	Winter	Overall
Abortion **	3.35 ± 0.07 ^a	5.68 ± 0.05 ^a	0.99 ± 0.04 ^b	2.50 ± 0.02
Still Birth *	2.19 ± 0.10 ^a	6.15 ± 0.07 ^b	2.79 ± 0.06 ^a	3.52 ± 0.02
Premature Birth	0.63 ± 0.07	0.58 ± 0.05	0.17 ± 0.04	0.42 ± 0.02
Retention of Placenta	11.36 ± 0.11	13.34 ± 0.08	10.90 ± 0.06	11.84 ± 0.03
Dystocia	0.13 ± 0.04	0.41 ± 0.03	1.07 ± 0.02	0.42 ± 0.01
Total				
Parturient disorder	21.29 ± 0.07 ^{ab}	25.88 ± 0.05 ^b	17.12 ± 0.04 ^a	0.42 ± 1.36

Means with similar or no superscript do not vary significantly; ** (P < 0.01); * (P < 0.05)

TABLE 2: Year-wise Least - Square means of various reproductive disorders in Frieswal Cows.

Particulars	Periods				
	1st	2nd	3rd	4th	5th
Abortion	3.67 ± 0.10	1.90 ± 0.10	1.85 ± 0.10	3.65 ± 0.10	1.79 ± 0.10
Still Birth	3.55 ± 0.13	2.30 ± 0.13	3.30 ± 0.13	6.09 ± 0.13	2.88 ± 0.13
Premature Birth	0.70 ± 0.09	0.58 ± 0.09	0.54 ± 0.09	0.11 ± 0.09	0.34 ± 0.09
Retention of Placenta	10.37 ± 0.13	9.52 ± 0.13	13.63 ± 0.13	16.83 ± 0.13	9.58 ± 0.13
Dystocia **	0.16 ± 0.05	0.01 ± 0.05	0.04 ± 0.05	2.52 ± 0.05	0.95 ± 0.05
Total Disorder	21.71 ± 0.01	19.17 ± 0.01	21.54 ± 0.01	25.85 ± 0.01	18.60 ± 0.01

** (P < 0.01)

the year 1997 - 1998 which might be due to some changes in managerial factors or any other paternal, maternal or foetal cause.

The overall incidence of total parturient disorder was 21.32 ± 1.36 per cent in Frieswal cows. Deshmuk and Kaikini (1999) reported almost similar rate of incidence in Jersey x Sahiwal crosses. The occurrence of total parturient disorders showed significant seasonal variation with the highest values observed during rainy season (25.88 ± 0.05 per cent) and lowest during winter season (17.12 ± 1.36) The month-wise analysis of

variance showed that from May onwards there was a significant and gradual increase in parturient disorders, which might be due to the heat and humidity stress during summer and rainy season. The gradual reduction in its incidence from December onwards might be attributed to the favourable climatic conditions during winter season. Hence, it was concluded that extreme summer and rainy season (June - October) are the most vulnerable period for the incidence of parturient disorders in Frieswal cows under the existing farm condition.

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TOTAL SERUM PROTEIN, ALBUMIN AND GLOBULIN IN PREGNANT ALSATIAN BITCHES*

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ABSTRACT

Seventeen apparently normal healthy Alsatian bitches with known history of mating were selected at random. Blood samples were collected on 21-25 days, 30-35 days and 45 to 50 days of mating and total serum protein, albumin and globulin were estimated. Values of non-pregnant bitches were compared with those confirmed as pregnant. The mean total serum protein and globulin were significantly higher in pregnant than that in non-pregnant bitches, however the mean serum albumin content did not differ.

University Veterinary Hospital Kozhikode with the known history of mating. The interval from mating to pregnancy diagnosis was calculated by considering the date of first mating as day 0. At 21 to 25 days, 30-35 days and 45 to 50 days of mating, blood samples were collected and serum was separated for the estimation of total serum protein and albumin kit. Values obtained albumin and globulin by standard Biuret and BCG Dye binding method using Stangen total protein from non-pregnant bitches were compared with those confirmed as pregnant.

Stress of pregnancy has been reported to bring about significant changes in the haemogram of bitches. This study was undertaken to investigate the changes if any in total serum protein, albumin and globulin during various stages of pregnancy in Alsatian bitches.

MATERIALS AND METHODS

Materials for the study consisted of 17 apparently normal healthy Alsatian bitches aged 1 1/2 and 6 years selected at random from those presented at A.I. centre Mannuthy and

RESULTS AND DISCUSSION

The serum protein of pregnant (6.9 to 8.6 g/dl) and non-pregnant (6.3 to 7.0 g/dl) bitches was within the range reported by Schalm et al. (1975). The mean values in pregnant and non-pregnant bitches (Table) revealed that the values were higher in pregnant bitches than those in non-pregnant ones which agrees with the findings of Lackhoff and Walden (1984) and Prabhakaran et al. (1996). This might be attributed to increased levels of globulin during gestation.

The range of serum albumin in pregnant bitches (4.0 to 4.9 g/dl) and in non-pregnant bitches (4.0 to 4.5 g/dl) is

Part of M.V.Sc. Thesis of first author, approved and submitted to the Kerala Agricultural University.

Table : Mean serum protein, albumin and globulin in pregnant and non-pregnant bitches

Sl. No.	Parameter	21-25 days mean \pm SE		30-35 days mean \pm SE		45-50 days mean \pm SE	
		Pregnant	Non-Pregnant	Pregnant	Non-Pregnant	Pregnant	Non-Pregnant
1.	Total Serum** protein (g/dl)	7.03 \pm 0.06	6.68 \pm 0.08	7.59 \pm 0.10	6.80 \pm 0.06	8.01 \pm 0.13	6.79 \pm 0.06
2.	Serum albumin (g/dl)	4.27 \pm 0.05	4.18 \pm 0.05	4.46 \pm 0.08	4.30 \pm 0.07	4.48 \pm 0.09	4.26 \pm 0.04
3.	Serum** globulin (g/dl)	2.80 \pm 0.03	2.50 \pm 0.04	3.30 \pm 0.04	2.50 \pm 0.04	3.53 \pm 0.05	2.52 \pm 0.05

Inference : ** Significant at 1 per cent level ($P < 0.01$) in pregnant bitches.

almost within the range noted by Singh et al. (1987). The mean serum albumin obtained (Table) does not significantly differ between pregnant and non-pregnant bitches.

The serum globulin in pregnant bitches (2.7 to 3.7 g/dl) and in non-pregnant bitches (2.3 to 2.7 g/dl) was within the normal range reported by Schalm et al. (1975). The mean values were found to be significantly ($P > 0.01$) higher in pregnant bitches than that in

non-pregnant bitches and agrees with the observations of Gentry and Liptrap (1981), Concannon et al. (1996) and Prabhakaran et al. (1996). This might be due to inflammatory reaction exerted by implantation in pregnant bitches.

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POSTNATAL DEVELOPMENT OF SEMINAL VESICLE IN MADRAS RED RAM LAMBS MAINTAINED UNDER DIFFERENT FEEDING SYSTEMS*

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ABSTRACT

The histology, histometry and histochemistry of seminal vesicles of Madras Red ram lambs during postnatal period from 1 to 8 months of age maintained under grazing and feed lot system were studied. Increase in size of the alveoli, presence of PAS - positive granules in the cytoplasm, accumulation of granular material in the lumen and decrease in the epithelial height indicated the establishment of secretory activity of the seminal vesicle by seven to eight months of age in Madras Red Ram lambs.

The scientific reports on seminal vesicle development in ram lambs were scanty except few reports on seminal vesicles weight (Louda and Stolc, 1981; Dady 1984 and Yarney and Sandford, 1989). Hence, the study on postnatal development of seminal vesicle was under taken in Madras Red ram lambs maintained under grazing and feed lot systems.

MATERIALS AND METHODS

Twenty six healthy Madras Red ram lambs born at Live-stock Research Station, Kattupakam, Tamil Nadu were utilized for this study. All the ram lambs from birth to weaning were allowed to suckle the milk from their respective ewes. The lambs also had access to *ad libitum* concentrate feed and good quality hay or green grass.

Pre-weaning group: Six ram lambs, i.e., two ram lambs at birth, one month and three months of age were slaughtered and collected the seminal vesicles for the present study.

Post-weaning group: The remaining twenty ram lambs were weaned at 3 months (90 days) of age. At the time of weaning, the ram lambs were equally and randomly divided into two groups of different feeding system were sent for grazing daily. Concentrate feed at the rate of 100 gm. per lamb from 3 to 5 months of age and 200 gm. per lamb from 5 to 8 months of age was fed in bulk for all the ram lambs as a supplement to grazing. Under feed lot system, each ram was kept in separate wooden partitioned pens. They were fed individually *ad libitum* with pellet feed

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comprising of 50 per cent concentrate and 50 per cent lucerne meal from 3 to 8 months of age. Every month, two ram lambs from grazing and two ram lambs from feed lot systems were slaughtered from 4 to 8 months of age and seminal vesicles were collected for the present study.

Immediately after slaughter small tissue pieces from all seminal vesicles were cut and fixed in Bouin's solution for 24 hours. Adopting the standard methods described by Luna (1968), the tissues were dehydrated in ascending grades of alcohol, cleaned in xylol and embedded in paraffin, sections of about 5-6 mm thickness were cut with microtome. The sections were mounted on clean slides and stained with Hematoxylin and Eosin (Humason, 1972) and periodic acid-shiff reagent (Drury et. al., 1967) for histological and histochemical studies.

RESULTS AND DISCUSSION

Pre-weaning group: The seminal vesicles in ram lambs from birth to three months of age were enclosed in a capsule of a loose, irregularly arranged connective tissue and smooth muscle fibres. The interlobular septa of variable thickness, was given off from the capsule, divide the gland into numerous lobules. The seminal vesicles presented a stroma and a paranchyma of alveoli. The alveoli were round or oval in shape and varied in sizes. The interalveolar space was filled with fibromuscular connective tissues.

Post-weaning group: At four months of age, the alveoli were small and lined by pseudostratified columnar epithelium. The cytoplasm of the epithelial cells showed few small vacuoles and eosinophilic granular material. The nucleus was round to oval in shape. The interalveolar connective tissue was more abundant. Majority of the alveoli were luminized. The lumen of the alveoli was empty. The alveoli were smaller in ram lambs of both grazing and feed lot systems (Fig. 1).

At five months of age, the histological features of the seminal vesicles appeared same as in four months aged ram lambs. These changes coincided with appearance of mature Leydig cells, spermatogonia and slight rise in testosterone concentration in blood (Venugopal Naidu, 1991).

At six months of age, the interalveolar connective tissue was reduced. The cytoplasm of the epithelial cells were granular and eosinophilic. The nucleus was round. Majority of the alveoli were wide and open. The lumen was empty except in some tubules of the feed lot system ram lambs which contained eosinophilic granules. The alveolar size of the seminal vesicle had increased in ram lambs of both systems but it was smaller in ram lambs of grazing system than in ram lambs of feed lot system (Fig. 2). These changes are correlated with appearance of spermatocytes in the

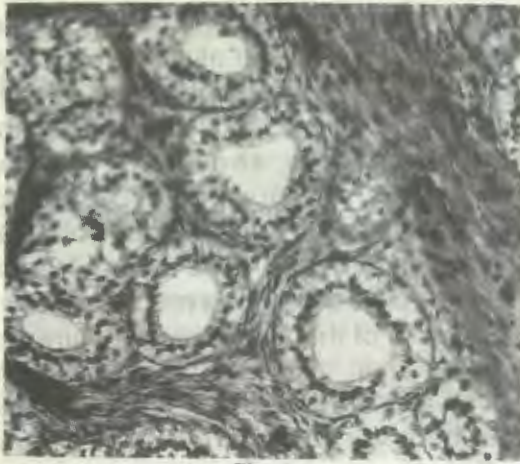


Fig. 1

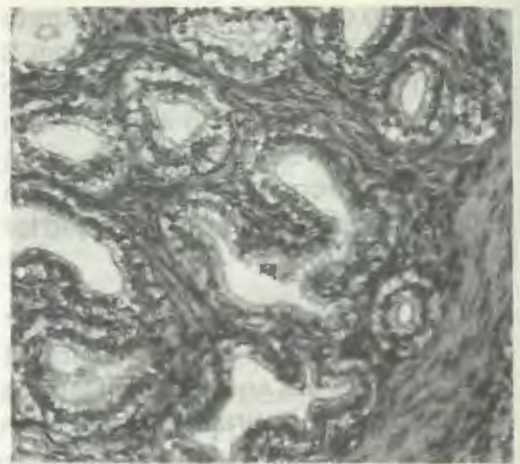


Fig. 2



Fig. 3

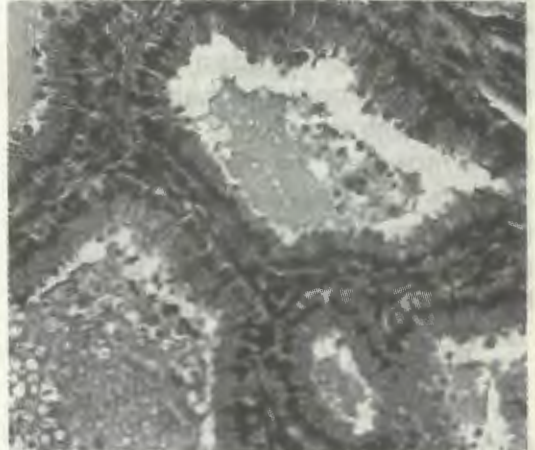


Fig. 4



Fig. 5



Fig. 6

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testis and increased testosterone concentration in blood (Venugopal Naidu, 1991).

At seven months of age, the interalveolar connective tissue was markedly reduced. The cytoplasm was granular and deeply stained. The nuclei was round and placed almost horizontal to the basement membrane. The lumen of the alveoli was empty in ram lambs under grazing system (Fig. 3). In feed lot system, the lumen of alveoli contained amorphous material of eosin coloured coagulum (Fig. 4). These changes were coincided with active spermatogenesis in testis and increased testosterone concentration in blood (Venugopal Naidu, 1991).

The histological features of the seminal vesicles at eight month aged ram lambs showed similar observations as in seven months. In grazing system, most of the alveoli were empty except few tubules which contained secretory material (Fig. 5). In feed lot system, majority of the alveoli were filled with eosinophilic secretory material (Fig. 6). The free luminal surface of the cells in some alveoli was fringed with secretory streamers and appeared as brush border. Similar histological observations were reported by Aitken (1955) in rams and Gupta and Yashwant Singh (1988) in goats.

The dense PAS - Positive granules were attached to the luminal border of epithelial cells indicated the release of secretion into the lumen of

alveoli of the seminal vesicles from seventh month onwards. The cytoplasm of the epithelial cells both at luminal border and basal surface showed PAS positive reaction. The interalveolar connective tissue also showed PAS activity. Similar PAS positive reaction was described in adult seminal vesicles of rams by Aitken (1959).

The epithelial height of seminal vesicles was 31.83 ± 0.40 , 28.98 ± 0.12 , 24.15 ± 0.22 , 17.94 ± 0.61 and 16.47 ± 0.50 μm in ram lambs at 4, 5, 6, 7 and 8 months. Significant differences in epithelial height of seminal vesicles were noticed between age groups, where as significant differences were not observed between ram lambs of grazing (24.15 ± 1.69 μm) and feed lot systems (23.60 ± 2.10 μm). In the present study, the epithelial height of the seminal vesicles decreased from four months to eight months of age. This was in agreement with John (1985) who reported that epithelial height of seminal vesicles gradually declined from one month to 24 months of age in Murrah buffalo calves.

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Legend of Figures in page 156 / 62

- Fig. 1** : Microphotograph of seminal vesicle - 4 months - Grazing system. Note the small alveoli lined by pseudostratified columnar epithelium. (H & E x 160)
- Fig. 2** : Microphotograph of seminal vesicle - 6 months - Feed lot system. (H & E x 160)
- Fig. 3** : Microphotograph of seminal vesicle - 7 months - Grazing system. Note the small and empty alveolar lumen. (H & E x 160)
- Fig. 4** : Microphotograph of seminal vesicle - 7 months - Feed lot system. Note the large alveolar lumen filled with amorphous granular material. (H & E x 160)
- Fig. 5** : Microphotograph of seminal vesicle - 8 months - Grazing system. Note the distended but empty alveoli. (H & E x 160)
- Fig. 6** : Microphotograph of seminal vesicle - 8 months - Feed lot system. Note the distended alveoli densely filled with secretory material.

HISTO-PATHOLOGICAL CONDITIONS OF MALE GENITALIA OF BUFFALOES.**KALI CHARAN AND R.P. VERMA***

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ABSTRACT

Sixty one male gonads alongwith copulatory organs were collected from slaughtered adult river buffaloes and subjected to histo-pathological assessment. Different types of abnormalities and their percentage of incidence in testis, epididymis and urethra were reported.

Obscure changes detected histopathologically in the male genitalia is likely to affect breeding efficiency of bulls adversely. Keeping this in views, the present study on histopathological investigations of male gonads and urethra were undertaken to explore the changes of the apparently normal organs.

MATERIALS AND METHODS

A total of 61 pairs of male gonads alongwith its copulatory organs were collected from mature buffaloes, slaughtered at Bareilly abattoir.

The genital organs were externally appeared normal. The specimen of testis, epididymis and urethra were further incised for gross

abnormalities and then fixed in 10% formal - saline solution for appropriate period. The pieces of 4-5 mm size thickness of aforesaid specimen were processed adopting standard procedure for histo-pathological study.

RESULTS AND DISCUSSION.

In the present study, out of 61 morbid genitalia 38 organs (62. 29%) were having histopathological abnormalities, while remaining 23 organs (37.70%) were not having any grossly detectable abnormal change.

Out of 61 organs examined, the incidence of abnormalities in testis, epididymis and urethra were 32.79%, 18.03% and 11.47% respectively. The incidence based on 38 affected organs were 52.63%, 28.95% and 18.42% in testis, epididymis and urethra respectively.

The incidence on basis of 61 male genitalia examined, depletion of germinal lining (Fig 1) coupled with fibrosis and hyperplasia and sloughing of epithelial cells admixed with spermatozoa in epididymis were found to be the highest (13.11%). It was followed by lymphoid aggregation and diffuse infiltration in the subcutaneous tissues in urethra (11.47%) (Fig 2). Degeneration of

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seminiferous tubules with calcification (9.84%), Degeneration and sloughing of germinal layer (4.92%), Depletion of germinal lining (3.28%), Discontinuity of pseudostratified tall columnar epithelium of ductal epididymis (3.28%), Testicular cyst (1.64%) and Calcification in epididymis (1.64%) were found in descending order.

The incidence on basis of 38 male affected organs, were maximum (21.05%) in Depletion of germinal lining coupled with fibrosis. Hyperplasia and sloughing of epithelial cells admixed with spermatozoa in epididymis (21.05%), followed by Lymphoid aggregation and diffuse infiltration in the

subcutaneous tissues in urethra (18.42%), Degeneration of seminiferous tubules with calcification (15.79%), Degeneration and sloughing of germinal layer (7.89%), Depletion of germinal lining (5.26%), Discontinuity of pseudostratified tall columnar epithelium of ductal epididymis (5.26%), Testicular cyst (2.63%), Calcification in epididymis (2.63%) in descending order.

Histopathological findings agree with the reports of (Rawal, 1972, Chaudhuri, 1977 and Maurya et al, 1968). It is evident that histopathological abnormalities in apparently normal tests and epididymis are responsible for affecting reproductive efficiency of breeding Bulls.

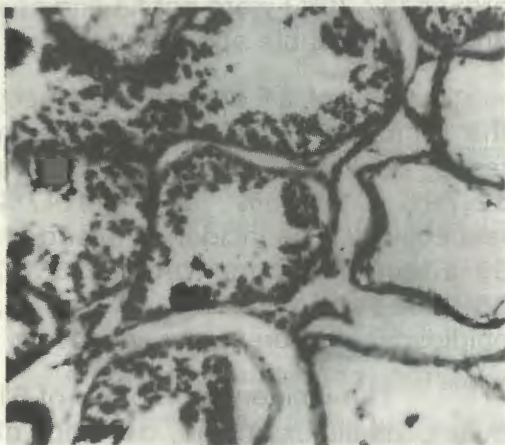


Fig 1. Depletion and sloughing of epithelial cells in testis.

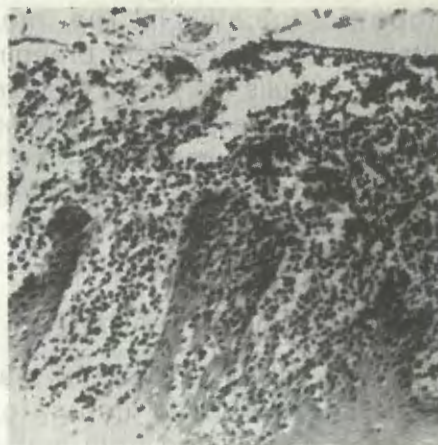


Fig 2. Lymphoid aggregation in urethra

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THE EFFECT OF SEASON ON SEMINAL CHARACTERISTICS, FREEZABILITY AND STRAW PRODUCTION IN HOLSTEIN FRIESIAN BULLS*

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ABSTRACT

The present study was conducted to assess the seasonal variation on seminal parameters and frozen semen straw production in Holstein Friesian bulls. The seasonal variation on all the seminal characteristics studied and number of straws obtained per collection was significant. The mass activity, initial motility and post thaw motility was normal during all the seasons. The results obtained on ejaculate volume, sperm concentration and number of straws produced per collection indicated that the semen production potentiality of Holstein Friesian bulls was better during summer and north - east monsoon, mediocre, during winter and below average during south-west monsoon.

Both the quality and quantity of bovine semen has been reported to vary remarkably during the extremes of summer and winter seasons (Dhami et al, 1987). This is particularly so in the northern states

of our country (Bhattacharya et al., 1975). The present study was aimed to ascertain seasonal variation on the seminal characteristics, freezability and straw production in Holstein Friesian bulls under agroclimatic conditions, Bangalore, India.

MATERIALS AND METHODS

The data on semen production generated over a period of 9 years (January 1989 to December 1997) from 15 German Holstein Friesian bulls was utilized for the study. On each collection normally two ejaculates and rarely one ejaculate was obtained from each bull. The total number of collections made during the period from all the bulls were 7,960. The total number of ejaculates available from these collections was 15, 727. The information available for each ejaculate were volume, mass activity and sperm concentration. Further, data was also available on initial motility, post thaw motility and the number of frozen semen straws (German) produced per collection. As per the Department of Meteriology, Government of India the year was divided into four seasons namely, winter (January - February), Summer (March - May), South-west monsoon (June - September) and north - east

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monsoon (October - December). The number of ejaculates available during winter, summer, south-west monsoon and north-east monsoon were 2660, 3676, 5577 and 3814 respectively. The data was analyzed by mutivariate analysis of variance (ANOVA) and by Fischer's protracted least significant test (Stell and Torrie, 1980) to know the effect of seasons on different semen production characteristics mentioned above.

RESULTS AND DISCUSSION

Semen volume : The overall mean volume of semen based on 15,727 ejaculates was 5.35 ± 0.02 ml (Table) which is in conformity with the findings of Nazir et al. (1987) and Pangawkar and Sharma (1989). The seasonal variation in mean ejaculate volume was significant ($P \leq 0.05$; Table). The superiority of summer in mean ejaculate volume produced (5.49 ± 0.03 ml) is in agreement with the observations made by Stalhammer et al. (1989). The lowest mean ejaculate volume (5.18 ± 0.03 ml) recorded during south - west monsoon contradicts the reports of Bhosrekar et al. (1989) and Sagdeo et al. (1991) who have reported highest volume of semen during rainy season. Further, the highest mean ejaculate volume recorded during summer differed significantly from that of south - west monsoon and winter but not from that of north - east monsoon.

Mass activity : In the present study the mass activity was recorded in 0.5 scale and corresponding

percentage was recorded directly. The overall mean percent mass activity was 81.00 ± 0.05 (Table) which works out to be 4.05 in 0-5 scale. The mass activity recorded in summer ($81.57 \pm 0.10\%$) was significantly high ($P \leq 0.05$) and confirms the findings of Sagdeo et al. (1991) and Dhami et al. (1998). The lowest mass activity recorded during north-east monsoon was statistically similar to that of winter but lower than that of south - west monsoon. In spite of significant seasonal variation it appears that the mass activity was normal for further processing of semen in all the seasons.

Sperm Concentration : The overall mean sperm concentration recorded was 0.93 ± 0.01 billions per ml (Table) which is in conformity with Sharma et al. (1991) and Dhami et al. (1998). The seasonal variation in sperm concentration was significant ($P < 0.05$) with highest and similar values during winter and summer (0.94 ± 0.01 billions per ml.) which further did not differ from the mean for north - east monsoon. The mean sperm concentration recorded during south-west monsoon was significantly lower than the values for all other seasons. The present findings confirms the observation of Amir et al (1982) who recorded no difference in sperm concentration during summer and winter and partly agrees with Tomar et al. (1985) and Dhami et al. (1998) who have recorded highest sperm concentration during winter and summer respectively.

TABLE : Seminal traits in Holstein Friesian bulls during different seasons.

Seminal traits / Season	Volume (ml)	Mass activity (%)	Sperm concentration (10^9 / ml)	Initial motility (%)	Post thaw motility (%)	Semen straws per collection
Winter	5.33 $\pm 0.04^b$	80.86 $\pm 0.15^{bc}$	0.94 $\pm 0.01^a$	80.53 $\pm 0.18^b$	43.18 $\pm 0.13^c$	281.62 $\pm 4.15^b$
Summer	5.49 $\pm 0.03^a$	81.57 $\pm 0.10^a$	0.94 $\pm 0.01^a$	80.03 $\pm 0.26^c$	42.80 $\pm 0.17^c$	291.00 $\pm 3.48^a$
South-west monsoon	5.18 $\pm 0.03^c$	80.96 $\pm 0.10^b$	0.91 $\pm 0.01^b$	80.75 $\pm 0.19^b$	43.92 $\pm 0.16^a$	265.80 $\pm 2.58^c$
North - East monsoon	5.46 $\pm 0.03^a$	80.63 $\pm 0.13^c$	0.93 $\pm 0.01^a$	81.17 $\pm 0.22^a$	43.51 $\pm 0.22^{bc}$	289.54 $\pm 3.51^a$
Overall mean	5.35 ± 0.02	81.00 ± 0.05	0.93 ± 0.01	80.64 ± 0.08	43.43 ± 0.09	280.19 ± 1.65

Note : Means bearing a common superscript in columns do not differ significantly with each other ($P \leq 0.05$)

Initial motility : The season wise variation in mean percent initial motility was significant ($P \leq 0.05$) with highest (81.17 ± 0.22) and lowest (80.03 ± 0.26) during north - east monsoon and summer respectively. Bhosrekar et al. (1989) also recorded highest percent initial motility during rainy season. In spite of significant variation, the percent motility recorded in the initial present study during all the seasons was normal for further processing of semen.

Post thaw motility : The overall mean post thaw motility was 43.43 ± 0.09 per cent (Table) which confirms with reports of Dharni et al. (1991). The season wise mean per cent post thaw motility differed significantly ($P \leq 0.05$) with highest value during south - west monsoon (43.92 ± 0.16). The mean post thaw motility recorded in the remaining three seasons did not vary significantly. Similarly, Sagdeo et al.

(1991) have also reported highest post thaw motility during monsoon that during winter and summer. Further, similar to the present findings Amir et al. (1982) found no variation in post thaw motility during summer and winter. Though there was significant variation, considering the prefreeze sperm concentration which was 30 million, the mean post thaw motility recorded during all the seasons was normal for utilization.

Semen Doses (Straws) Produced : The overall average number of frozen semen straws harvested per collection was 280.19 ± 1.65 and ranged between 80 and 1050. The highest mean number of straws per collection was during summer (291.00 ± 3.48) which differed significantly from those of winter and south west monsoon but not from that of north - east monsoon. The mean number of straws obtained per collection during south-west monsoon

was significantly lower than all other seasons. Stalhammer et al. (1989) recorded highest number of straws per collection during summer in Sweedish Friesian bulls. When compared with the overall mean (280.19 ± 1.65), the number of straws obtained per collection during summer and north - east monsoon, winter and south-west monsoon was above

average, average and below average respectively.

In conclusion, the performance of Holstein Friesian bulls was above average, average and below average during summer and north - east monsoon, winter and south - west monsoon respectively.

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EFFECT OF TWO DIFFERENT SEMEN EXTENSION METHODS ON THE FERTILITY OF FROZEN BULL SEMEN**S. SUDHEER**Deputy Manager, Frozen Semen Bank, K.L.D. Board (Indo-Swiss Project),
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The technique of deep freezing of bovine semen is continuously being improved to enhance post-thaw revival and fertility among inseminated females. At the Mattupatti Frozen semen production station, semen is being extended by the conventional double extension method described by Polge and Rowson (1952). Now many semen production stations have simplified this technique and are employing a single phase extension procedure for processing semen. Field studies comparing the two extension methods are scanty. Therefore it is envisaged to take up a comparative study of single and double extension methods based on fertility estimates, which are the real means of evaluating the effectiveness of various semen processing protocols.

MATERIALS AND METHODS

Five purebred and five crossbred bulls of the Mattupatti semen production station were selected for the study. Semen was collected from the bulls using the artificial vagina. The semen thus harvested was split into two fractions and evaluated separately. Using a phase contrast microscope provided with a warm stage facility the initial

motility of the split ejaculates was evaluated. Sperm concentration was estimated using a photometer. One half of the ejaculate was extended by the single extension method (glycerol was added initially in the extender) and the other half by the double extension method (glycerol was slowly added to the semen cooled to 5°C in 1.5 to 2 hours) using Tris extender. The semen was extended such that each French mini paillette contained 24 million sperms. The extended semen was filled into precooled paillettes of two different colours (clear for single extension and yellow for double extension). After giving an equilibration period of 4-5 hours, freezing was carried out by rapid horizontal vapour freezing in wide mouthed refrigerators of 330 litres capacity. From each bull 300 doses of semen was prepared i.e., 150 doses by each of the extension methods. Thus 3000 doses of semen were prepared from the bulls in two different coloured palliattes. The semen was supplied to 5 AI centers such that each center was allotted 60 doses from each bull (30 doses produced by each of the extension method). As the palliattes were thoroughly mixed before supplying to the AI center, the inseminators got the opportunity to use

the semen doses with out any bias. The conception rate was assessed separately for the two treatments based on pregnancy diagnosis conducted two months after insemination. Statistical analysis of the data was carried out as described by Snedecor and Cochran (1988).

RESULTS AND DISCUSSION

The post-thaw revival rates for the first and second treatments were 53.33 ± 0.86 and 55.00 ± 0.93 per cent respectively. Even though the revival rates were higher for the second treatment, the difference was not statistically significant. The result of the study are in accordance with the findings of Foote (1970) in related studies.

The conception rate for the single extension method ranged from 38.4 to 51.62 per cent with a mean conception rate of 44.0 ± 1.27 , where as for the second treatment conception rate ranged from 40.44 to 46.92 per cent with a mean of 43.35 ± 0.73 . However, the statistical analysis of the data revealed no significant difference in fertility rates between the two treatments. Foote (1970) also

reported that there was no difference in fertility rates when glycerol was included initially in Tris-fructose-egg yolk extender or when it was added slowly after cooling the extender to 5°C

The present study has revealed that both the extension methods are equally efficient in the processing of bovine semen in relation to fertility. However, the single extension method helps in totally avoiding the preparation of the second extender and the laborious process of glycerolisation. Thus the on-line production activities in the laboratory becomes simpler and time and labour saving.

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IMMUNOGLOBULIN CONCENTRATION IN SERUM AND CERVICAL MUCUS DURING OESTROUS CYCLE IN REPEATER CROSSBRED COWS

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Role of immune system in the control of reproduction in farm animals is attracting increased attention of researchers (Grossman, 1985, Widders et al., 1985; Parr and Parr, 1994 and Brenner et al., 1995). The immune system is regulated directly or indirectly by gonadal steroids in both pregnant and non-pregnant state. In many species the uterus is more susceptible to uterine infection in the progesterone dominated luteal phase than in the oestrogen dominated follicular phase of the oestrous cycle (Watson et al., 1987). Humoral immunity has a pivotal role in maintaining aseptic uterine environment (Parr and Parr, 1994). Thus, in the present study attempt has been made to estimate immunoglobulin concentration during different stages of the oestrous cycle of repeater crossbred cows.

MATERIALS AND METHODS

A total of 28 Holstein - Sahiwal crossbred cows (Control n =

9, Repeaters n = 19) with 62-80% exotic blood, 3-9 years of age and 1-6 lactations maintained under uniform housing, feeding and managerial conditions at Military Dairy Farm, Bareilly were included in the present study. Blood from these cows were collected by regular venipuncture during oestrus (Day 0), early (Days 3-5) and Mid (Days 10 - 11) luteal phases of the oestrous cycle in a single cycle only. Separated serum and oestral cervical mucus (collected just before insemination) were stored at -20°C. Immunoglobulin (Ig) concentration was estimated in serum (McEwan et al., 1970) and in oestral cervical mucus also was estimated with the method of McEwan et al. (1970) with slight modification. 5ml of zinc sulphate solution (250 mg/l, w/v) was taken in each stoppered culture tube (Borosil, India). To this 0.5 ml of diluted (1 : 1) cervical mucus mixed with 0.6 ml of double distilled water and finally vortexed was added. The mixture was allowed to incubate for 60 minutes at $25 \pm 1^\circ \text{C}$ for turbidity reaction to take place and reading was taken at 580 nm. in a spectro photometer (106 systronics). Data were analysed using standard statistical methods (Snedecor and Cochran, 1967).

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Table 1 : Mean \pm Se of serum immunoglobulin concentration (mg/ml) during different stages of oestrous cycle in control and repeater crossbred cows.

Groups	Stages of oestrous cycle		
	Oestrus	Luteal Phase	
		Early	Mid
Control	12.13 \pm 1.23 ^{NS} (7.91 - 19.80) (n = 9)	13.21 \pm 1.37 ^{NS} (8.08 - 19.00) (n = 7)	12.54 \pm 0.88 ^{NS} (8.62 - 17.22) (n = 9)
Repeaters	(12.73 \pm 0.95) ^{NS} (7.24 - 21.02) (n = 19)	13.52 \pm 0.82 ^{NS} (8.18 - 18.88) (n = 15)	11.47 \pm 0.51 ^{NS} (9.05 - 14.45) (n = 10)

NS = Non significant; n = number of observation; Figures in parenthesis indicates range.

RESULTS AND DISCUSSION

Serum immunoglobulin Intra and Inter - assay coefficient of variations were 7.13 and 13.13 per cent respectively while in oestral cervical mucus variations were 23.29 and 15.24 per cent respectively. Serum immunoglobulin concentrations are contained in the Table.

Perusal of the table shows that on the day of oestrus and early luteal phase the immunoglobulin concentration was non-significantly higher in repeater compared to control crossbred cows. However, during mid-luteal phase the Ig level was non-significantly lower in repeater crossbred cows. Also, the immunoglobulin concentration both in control and repeater crossbred cows increased from oestrus to early - luteal phase and then declined during mid-

luteal phase of the oestrous cycle.

In oestral cervical mucus also the immunoglobulin concentration was non-significantly higher in repeater (0.34 ± 0.05 mg/ml) compared to control (0.27 ± 0.03 mg/ml) crossbred cows. In cows, Brenner et al. (1995) sub-typed immunoglobulin and reported a significant change in IgG concentration during oestrus and luteal phases of the oestrous cycle. In mare, Widders et al. (1985) did not find any cyclical variation in serum IgG and IgA level but the changes were significant for IgM. In the present study the immunoglobulin concentration showed non-significant variation during different stages of oestrous cycle.

It is concluded, therefore, that sub-class quantification of immunoglobulins may show significant change. Thus, further study in this regard and its clinical implication may

help in modulating reproductive performance of repeat - breeder.

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between 97 to 144 hr. A total five buffaloes (25%) failed to exhibited estrus within 10 days of treatment. These results are in agreement with the earlier reports (Rao and Rao, 1979; Singh et al. 1979; Pant and Singh, 1991). The incidence of estrus (75%) observed in this experiment after treatment is similar to the earlier report of Singh et al. (1979).

The first service conception rate with frozen semen in treated and control group were 40% and 46.66%

respectively and the difference was nonsignificant ($P > 0.05$). The results suggested that fertility of PGF2 α treated animals are comparable with that of control. However, Pant and Singh, (1991) have reported higher conception rates following natural service at PGF2 α induced estrus.

The results indicated that PGF2 α could be employed for the treatment of subestrus and improvement of breeding efficiency in buffaloes.

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TREATMENT OF REPEAT BREEDING CROSS - BRED CATTLER.K. RAJANGAM¹, N. JEYARAM² AND J. JOHNSON RAJESWAR³Veterinary University Training and Research Centre
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Intrauterine infusions in repeat breeders, has been a subject of considerable controversy, with reports of success (Roberts, 1971; Oxender and Seguin, 1976) as well as failures (Ulberg et al 1952; Roberts, 1956 and Gibbons 1959).

This study presents an attempt to treat the repeat breeders intravaginally, a much easier method, with a drug based on antibiogram studies.

MATERIALS AND METHODS

Twenty one repeater cows and four heifers, that were brought to the veterinary dispensary, Kurunthancode and to the infertility camps, were taken up for studies.

The vaginal discharge from each animal was collected by sterile swabs under aseptic conditions and were subjected to in-vitro antibiotic sensitivity tests. Bio-discs of Span Diagnostics (India) Ltd. and Mueller - Hinton agar of Hi-Media Laboratories (India) Ltd. were used for sensitivity

tests and standard procedure described by Bauer et al. (1966) was followed.

The antibiotic found most effective in-vitro, was used for in-vivo treatment. In this study Nitrofurantoin was found to be highly effective (72 per cent) in-vitro and the preparation "METREA" bolus (Nitrofurazone 60mg and urea 6.0 gm) of Western Remedies (India) Ltd., was used for in-vivo treatment, post-insemination, intra-vaginally, at two boluses a day for three consecutive days.

Six cows and three heifers, from which vaginal mucus was tested for antibiotic sensitivity, were available for the preliminary treatment trail. All the nine animals were followed up after treatment, pregnancy confirmed by per rectum examination sixty days post insemination and the calves were verified post-partum.

RESULTS AND DISCUSSION

The results of in vitro drug sensitivity tests performed on vaginal discharge samples collected from twenty five repeater cows and heifers revealed bacterial growth of all vaginal discharge samples, were sensitive to more than one antibiotic drug with

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varying degrees of susceptibility in - vitro. It was observed that all the twentyfive discharge samples tested were completely resistant to Penicillin. This observation concurs with earlier reports of Sinha et. al., 1977; Murthy and Rao, 1979; Pandit et. al., 1982; Ambrose and Pattabiraman, 1993.

Sensitivity to Nitrofurantoin was found to be the highest (72 per cent), followed by Chloramphenicol (68 percent) and Ampicillin (36 per cent). This result agrees with the earlier findings of Ambrose and Pattabiraman (1993). In their sensitivity studies performed on uterine discharges highest sensitivity was observed for Nitrofurantoin (62.34 percent) and Chloramphenicol (45.46 percent). It suggests that the infections occurring in the uterus and the vagina, are of similar type and the sensitivity pattern of organisms from vaginal discharge should give an indication of the treatment to be given for sub-clinical and clinical infections of the uterus also.

All the nine cows and heifers conceived after specific post-insemination intra-vaginal treatment with "Metrea" bolus. Pattabiraman et. al., (1983) also found "Orpisol" (Nitrofurazone 60 mg. and Urea 6.0g) to be effective, in 84 per cent of cows treated intra-uterine for puerperal infections.

The powerful antibiotic activity of the nitrofurazone, combined with the proteolytic effect of urea in "Metrea", may be the reason for complete control of infection in the reproductive tract, enabling one hundred per cent conception. This preliminary treatment trial suggest that intravaginal approach, if not better, is as good as intra-uterine route. the quick solubility of "Metrea" bolus, is an added advantage. Intravaginal approach is effective and much easier than intra-uterine route under field conditions, however, the results should be confirmed with larger number of animals in a controlled study.

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HISTOLOGY AND HISTOCHEMISTRY OF THE CERVIX UTERI OF GADDI SHEEP

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Restall (1966), Ramachandraiah et al. (1980) and More (1984) have described the structure of the cervix uteri of sheep in varying details. Present paper records the histological and histochemical alterations in the cervix during different phases of sexual (ovarian) cycle and senility in the Gaddi (Kashmere) sheep which is the means of livelihood of a large population of North Western Himalayan region of the country.

MATERIALS AND METHODS

Fourteen genitalia from healthy, adult, non-pregnant Gaddi sheep classified as five genitalia each of luteal and follicular phases and four of senile (7-8 years of age) phase were used in the study. Thin pieces of tissues from the internal os, middle and external os of the cervix uteri were fixed and processed adopting standard procedure (Luna, 1968 and Cock, 1974). Thin paraffin sections were cut and stained for routine histomorphology, connective tissue fibres and elastic fibres. The sections were also stained for histochemical demonstration of neutral and acidic muco-polysaccharides glycogen (Luna, 1968 and Chayen et al., 1969)

and lipids. In order to show the concentration of histochemical components in the tissues the grading of staining intensity (no reaction to intense reaction) was done based on microscopic examination.

RESULTS AND DISCUSSION

Histomorphology : The lining epithelium was simple columnar to stratified columnar with goblet cells in the cyclic animals and low columnar in senile phase. Ramachandraiah et al., (1980) observed only simple tall columnar epithelium. The cell height was reduced in the luteal phase as compared to follicular phase whereas minimum cell height occurred in senile animals. Ramachandraiah et al. (1980) observed reduced cell height in luteal diestrual phase. Lot of streamers on the cells revealed merocrine mode of secretion as also reported in goats by Joshi et al. (1976). The epithelial height was more at the base of the crypts than at the apices of the folds during different phases of cycle as also reported by Sundaravandanan (1974) in buffalo and Sato et al. (1982) in cattle. The cervical lumen showed a lot of cells with eosinophilic mucoid materials in the follicular phase. Joshi et al. (1976)

observed maximum secretion in the estrus phase in goats cervix. Simple coiled tubular cervical glands arose due to invagination of the surface epithelium as also observed by Joshi et al. (1976) and Ramachandraiah et al. (1980). Ramachandraiah et al. (1980) found more cervical glands in the cranial portion of the cervix than in the caudal portion. The glands were diminished in the senile phase.

Histochemistry : Moderates to strong PAS reactivity was observed in the epithelial cells of the cervix uteri and cervical glands. Distal border of the epithelial cells and secretory border of the glandular cells showed intense alcianophilia. Secretions also showed intense reaction with and

Alcian blue. Strong reaction for Best's carmine was also observed in the supranuclear zone of the epithelial cells. Epithelium showed diffused or fine granular reaction for neutral lipids (triglycerides) in the supranuclear zone of the epithelium. The endothelium of the blood vessels showed strong reaction for bound lipids. Lamina propria and tunica muscularis showed much less intensity of carbohydrates and fats. Particularly the glycogen was absent.

Concentration of Carbohydrates was marked during follicular phase, diminished in luteal phase and completely absent in the senile phase.

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STUDIES ON THE PLASMA PROGESTERONE LEVELS IN CROSSBRED HEIFERS FED WITH POULTRY LITTER DIETS

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The effects of individual feed stuff, especially non-conventional feed like poultry litter in the production and functioning of reproductive hormones is not available in the literature. In view of this, plasma progesterone profile was studied using crossbred heifers fed with concentrate mixture having poultry litter as a source of non-protein nitrogen.

MATERIALS AND METHODS

Twenty crossbred heifers aged about 3-6 months were grouped randomly into four groups of five each and fed with basal diet as green sorghum or green oats. Sun dried poultry litter was taken as an ingredient of concentrate mixture at the levels of 0.0, 25.0, 37.5, and 50.0 per cent (Table 1) and offered to the heifers in group I, II, III, IV, respectively to meet the requirements of nutrients as per NRC (NRC, 1988). Fresh clean drinking water was provided sufficiently.

The heat detection in heifers was carried out throughout the experimental period by observing the

discharge of cervicovaginal mucus, behavioural and physiological changes. Blood samples were collected from the jugular vein on the day of estrus and on the 8th day of estrus cycle for 8-9 consecutive estrus cycles, using vials containing EDTA 1 ng/ml. Plasma was separated from the blood by centrifugation at 5000 rpm at 4°C for 20 minutes and stored at -20°C, until assayed.

The cycles in the heifers were synchronized by injecting prosolvin @ 1.00 ml intramuscularly twice at the interval of 11 days so that the animals come in heat almost at the same time. Thereafter, artificial insemination was done. The blood samples were also collected on 18th day after A.I. for plasma progesterone estimation to diagnose the pregnancy. Confirmation of pregnancy was done on the 45th after A.I. by rectal palpation method.

Concentration of progesterone in the blood was determined by standard RIA technique. The data were analysed by two factorial completely randomized design.

Table 1. Composition of experimental diets (kg/100 kg)

S. No	Feed ingredients	Treatments			
		I	II	III	IV
1.	Mustard cake	30.0	22.0	15.0	15.0
2.	Ground wheat	33.0	25.0	22.0	16.0
3.	Deoiled rice bran	34.0	25.0	22.5	16.0
4.	Poultry litter	0.0	25.0	37.5	50.0
5.	Mineral mixture	2.0	2.0	2.0	2.0
6.	Common salt	1.0	1.0	1.0	1.0
	Total	100.0	100.0	100.0	100.0

RESULTS AND DISCUSSION

The average plasma progesterone values of the heifers as measured by RIA at different reproductive stages have been shown in Tables 2. The average values of progesterone on the day of estrus were found as 0.51 ± 0.10 , 0.51 ± 0.04 , 0.39 ± 0.05 and 0.53 ± 0.09 ng/ml of plasma in groups I, II, III and IV, respectively. Earlier workers also reported similar results

(Chandrashekar and Rao, 1990; Rastogi and Agarwal, 1988; Kurde et al., 1993). The plasma progesterone values on the 8th day of estrus cycle were 3.11 ± 0.28 , 2.83 ± 0.48 , 3.39 ± 0.39 , 3.36 ± 0.42 in groups I, II, III and IV, respectively. The values of progesterone varied significantly ($P < 0.01$) between 1st day and 8th day of estrus cycle in all the groups. The work done by earlier workers was in agreement with these results (Dutta et al., 1989; Pope et al., 1976).

Table 2: Average progesterone levels in blood plasma of heifer at estrus and at 8th day of estrus

Sl. No	Treatments	Progesterone At estrus	Levels (ng/ml) At 8th day of estrus
1.	Group I (Control)	0.51 ± 0.10	3.11 ± 0.28
2.	Group II (25.0 per cent PL)	0.51 ± 0.04	2.83 ± 0.48
3.	Group III (37.5 per cent PL)	0.39 ± 0.05	3.39 ± 0.39
4.	Group IV (50.0 per cent PL)	0.53 ± 0.09	3.36 ± 0.42

The progesterone values in the blood samples of eleven animals out of 20 showed decreased trend after 18th day of A.I. compared to the values on 8th day. This made clear that the corpus luteum (CL) was reducing in size. All these animals showed signs of heat within 18th to 22nd day of A.I. The mean plasma progesterone concentrations of heifers not conceived was 0.67 ng/ml (range 0.10 + 3.10). This agrees with the report of Shemesh et al., (1983) Inseminated heifers having higher values of progesterone on 18th day

post insemination (mean 5.73 ng/ml - range 3.80 to 7.20) were found to be pregnant when confirmed by rectal palpation method on 45th day of A.I.

The accuracy of RIA in the present study for pregnancy was 87.5 per cent while for non-pregnancy it was 100 per cent.

Therefore, it was concluded that poultry litter feeding upto 50 per cent level of concentrate mixture did not affect the secretion of progesterone at various stages of reproduction.

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SERUM THYROID HORMONES LEVELS IN CAMEL NEONATES (CAMELUS DROMEDARIUS)

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Thyroxine (T4) and Triiodothyronine (T3) levels in neonate calves in some way help the calf to adapt itself to extra-uterine life, the environmental condition of which are very different from that of intra-uterine life. Under the influence of higher thyroid status the newborn mobilizes its resources to derive more energy through elevation of BMR and blood glucose (Roy et al, 1957; Lagerscrantz and Slotkin, 1986). This study reports the levels of neonate camel thyroid hormones from birth to 62 days.

MATERIALS AND METHODS

Sera samples from eight neonate (seven female and one male) calves born out of eight randomly selected she-camels, were collected on day of birth followed by day 3, 6, 13, 20, 27, 34, 41, 48, 55 and 62 post birth. These samples were analysed for T4 and T3 using specific RIA kits procured from Bhaba Atomic Research Centre (BARC), Bombay, following protocol supplied with kit with

minor modifications. The assays were critically evaluated for their validity. The results were analysed statistically using completely randomised design and student "t" test in ECIL Micro 32 computer.

RESULTS AND DISCUSSION

The Thyroxine (T4) concentration in the camel calves at birth was 650.0 ng/ml which declined sharply to 462.0 ng/ml by day-3 and 301.25 ng/ml by 20th day post-birth and stabilized at this level by day 62. The concentration during week after birth was significantly higher than those at later stages ($p < 0.01$).

The triiodothyronine (T3) level in newly born calves was also several fold higher than the adult camels. Like T4 the highest values (14.4 to 16.8 ng/ml) were recorded during first week of life which subsequently declined to 8.90 ng/ml, almost half of its initial value by day-20 and then gradually attained a low level (4.0 ng/ml) after two months of birth. The analysis of variance revealed that the initial levels were significantly higher than those at later stages ($p < 0.01$).

The values of T4 and T3 in neonatal camel calves reported earlier by Agarwal et al., (1992) were slightly

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lower than those encountered in this study but the declining trend and sustained higher levels than adults were consistent with the findings of this study. Likewise, higher levels of thyroid hormones at the time of birth followed by decreasing trend during the first two to three months of age have also been reported in other domestic species (Khurana and Madan, 1984; Agarwal, et al., 1989; Nathanielsz, 1969 and Baruah et al,

1987). Although the exact physiological significance of this hyper thyroid status in camel calves is not known, it has been suggested that the higher levels, somehow help the calves to adapt to extra uterine life, Roy et al (1957) have opined that under the influence of higher thyroid status, the newborn mobilizes its resources to derive more energy which is possible due to high blood glucose levels and peak BMR value at birth.

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FACTORS INFLUENCING INCIDENCE OF DYSTOCIA IN BITCH**SHAMBU BENNUR, A. KRISHNASWAMY AND T.G. HONNAPPA**

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In a study by Linde - Forsberg and Forsberg (1983) the frequency of puppy deaths upto 3 weeks following normal parturition was 11.9%. In comparison to 22.3% of pups born dead or dying during abnormal parturition (Darvelid and Forsberg 1994). In view of considerable economical loss the kennel owners may suffer as a consequence of abnormal parturition, it is most surprising to note that the studies with regard to the factors influencing frequency of dystocia, various etiological factors and guidelines on selection of treatment procedures are limited. (Gaudet, 1985, Darvelid and Forsberg 1994). The present paper reports the influence of some factors on the incidence of dystocia in canines.

MATERIALS AND METHODS

The data relating to the present investigation were obtained from the clinical records of canine dystocia cases presented to the department of Gynaecology and Obstetrics between January 1989 to December 1996 as well as the clinical cases of dystocia handled during the

course of this investigation, that is between January 1997 to August 1998. The detailed proforma regarding different parameters considered for the present investigation was specifically prepared and the data was transferred to the proforma for the purpose of analysis. The clinical findings in dystocia both retrospective and prospective were pooled and analysed to study influence of breed, age, size, parity and duration of gestation on the incidence of canine dystocia.

RESULTS AND DISCUSSION

A total of 84 cases, 44 of which were retrospective and 40 prospective cases were available in 75 patients comprising of 13 different breeds. the highest frequency of dystocia was recorded in German Shepherd (29.3%) followed by Pomeranian (17.3%), Boxers (13.3%) and Dachshunds (10.7%). Chi square analysis (Snedecor and Cochran, 1967) revealed a significant effect of breed ($p \leq 0.01$) on the frequency distribution of dystocia in canines (Table) Although several studies have documented significant effect of breed of dam on the incidence of dystocia

Table 1 : Frequency of dystocia in different breeds

Breed	No. of Cases	Percentage
Dachshund	08	10.7
Pomeranian	13	17.3
Cocker Spanial	02	2.7
English Terrier	01	1.3
Golden Retriver	01	1.3
Labrador Retriver	05	6.7
German Shepherd	22	29.3
Irish Shutter	01	1.3
Doberman	04	5.3
Swis mountain	01	1.3
Great Dane	03	4.0
Boxer	10	13.3
Non Discriptive	04	5.3
TOTAL	75	

χ^2 df = 79.48**

12

in canines (Freak 1962; Smith, 1974; Arthur et al., 1989). German Shepherd do not find a mention in the available literature as breed being prone for dystocia. The higher frequency of dystocia in German Shepherd observed in the present study is perhaps due to their high population in Bangalore City as compared to other breeds. A relatively higher frequency of dystocia as observed in the present study has also been previously reported (Smith, 1974 Arthur et al., 1989). Factors suggested to be responsible for the predisposition of Boxer to dystocia include peculiar shape of foetal head making it difficult to enter the pelvic inlet (Arthur et al., 1989). Dachshunds are reported to exhibit an anatomically abnormal pelvis and found to be prone for particular type of inertia (Freak 1948).

Small sized breeds had a

frequency occurrence of 32% dystocia compared to 68% in medium and large sized breeds. Although Christiansen (1984) claimed that dystocia mainly occur in miniature breeds, the study of Darvelid and Forsberg (1994) was inconclusive. The present result suggested that the greater in the body size higher is the incidence of dystocia.

The incidence of dystocia was highest in bitches aged 2-4 years (50.7%) and gradually declined with advancing age. Bitches less than 4 years of age accounted for nearly 62% of dystocia cases. These results are similar to those of Gaudet (1985) and Darvelid and Forsberg (1994) who reported highest incidence of dystocia in bitches aged 2-4 years and 2-3½ year respectively. The incidence of dystocia was lowest in animals aged 6 years and above and may probably due to the fact that the most canine

owners may prefer not to breed animals after 5 years of age.

The present study also showed that the incidence of dystocia was higher in pluripara (60.7%). These animals had delivered 1 to 5 times. The frequency was similar to those reported by Dervelid and Forsberg (1994).

The duration of pregnancy in bitch is said to be 63 ± 5 days when calculated from the first day of mating. (Concannon et al. 1985). The mean duration of gestation in the present study was 65 ± 1.08 days and ranged between 59 to 71 days. Thus the gestation period of all the animals examined during the present study were well within the normal range of gestation period.

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STUDIES ON CAUSES AND TREATMENT OF DYSTOCIA IN CANINESSHAMBU BENNUR¹, A. KRISHNASWAMY AND T.G. HONNAPPADepartment of Gynaecology and Obstetrics, Veterinary College,
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In a retrospective study of 128 cases of dystocia in bitch Gaudet (1985) reported that 60% of the cases were deemed to be maternal anatomical or physiological abnormality. In a similar study involving 182 cases of dystocia Darvelid and Frosberg (1994) found that 75.3% of cases were of maternal origin. The treatment of dystocia, foetal or maternal have involved a use of ecbolics oxytocin and calcium to augment uterine contraction, digital manipulation, forceps delivery and laparohysterotomy (Roberts, 1986, Arthur et al., 1989). The present paper is an attempt to critically analyse the various treatment procedures adopted to relieve dystocia in canines.

MATERIALS AND METHODS

The data relating to the present investigation was obtained from clinical records of canine dystocia presented to the department of Gynaecology and obstetrics between January 1989 to December 1998. Every clinical case was carefully examined to evaluate the cause of dystocia and the causes were grouped as either foetal or maternal. Based on the cause the patient was subjected to any of the following treatment procedures.

a) Oxytocin only b) Oxytocin and digital manipulation c) oxytocin, calcium and dextrose d) oxytocin, calcium, dextrose and manipulation e) caesarean section only f) oxytocin, calcium, dextrose and cesarean section.

RESULTS AND DISCUSSION

The results are summarised in the table. Out of dystocias considered for the present investigation 86% were of maternal origin and the rest were of foetal origin. Gaudet (1985) and Darvelid and Forsberg (1994) have also reported that maternal causes of dystocia outnumber the foetal causes in bitch. The present study also showed that primary complete uterine inertia accounted for nearly 70% of maternal causes of dystocia. This observation has also been reported earlier by Darvelid and Forsberg (1994). The condition is said to have a hereditary disposition in some breeds (Freak, 1962) and stated to be more frequent in dams of 5 years and more, which could not be ascertained in the present study as it was more common in animal aged 2-4 years. A low litter size has also been reported to be associated with primary complete uterine inertia (Darvelid and Forsberg,

1994). In the present study however the litter size in animals with primary complete uterine inertia ranged between 2 and 9 with only 6 animals having litter size of 3 or lower in number (4.3%). Primary complete uterine inertia was also encountered in 2 other animals with litter size of 9. Freak (1962) considers complete uterine inertia in animals with high litter size to be due to excessive uterine distension.

Primary partial uterine inertia was the second most common cause of maternal dystocia, the condition being encountered in 27.8% of cases. This incidence is almost similar to 30.7% which reported by Darvelid and Forsberg (1994). Only two other cases of maternal dystocia were encountered in the present study namely a case each of vaginal septum and uterine torsion. Vaginal septum as a rare cause of maternal dystocia has been reported by Freak (1962). Uterine torsion has also been reported to be comparatively rare in canines (Bennett, 1980). In the present study both primary complete and partial uterine inertia were encountered more frequently in medium and large sized breeds.

Foetal cause of dystocia encountered in the present study were relatively few ($n=12$) and consisted of over sized foetus ($n=6$) breech presentation ($n=3$) and lateral deviation of head ($n=3$). In other reports foetal malpresentation, position and posture as a cause of dystocia were reported to range from

5.4% (Freak, 1962) to 24.7% (Darvelid and Forsberg, 1994).

Medical treatment involving oxytocin calcium or dextrose was used in an attempt to augment uterine contraction only in cases of primary complete and partial uterine inertia. The use of oxytocin alone or oxytocin followed by digital manipulation relieved dystocia in 10% of cases of uterine inertia. This success rate compares favourable with those obtained by Gaudet (1985). When oxytocin was supplemented with calcium dextrose, dystocia could be effectively relieved in 47.1% of patients with uterine inertia. It is possible that substantial number of inertia cases are caused by subclinical hypocalcemia and hypoglycemia (Bennett, 1974, Gaudet, 1985) and therefore cases of uterine inertia are preferably approached by medical treatment comprising of oxytocin calcium and dextrose rather than oxytocin alone.

In the present study uterine inertia could not be overcome by medical treatment in 21.4% of cases and had to be subjected for caesarean section. In other reports caesarean section followed by pre-treatment with drug was employed in 14.7% (Gaudet, 1985) to 47% (Darvelid and Forsberg, 1994).

Caesarean was the only line of treatment employed to relieve dystocia due to uterine inertia in 21.4% of cases. Further 25% of maternal dystocias also required caesarean section. Therefore irrespective of

causes of dystocia medical treatment with or without digital manipulation was successfully employed in 59.5% of

cases. In other studies caesarean section was employed much more frequently (Gaudet, 1985).

Table : Material and Foetal causes of Dystocia in bitches

Type of causes	Number of cases	Percentage
Material	72	(86)
Primary complete Uterine inertia	50	69.4
Primary partial Uterine inertia	20	27.8
Uterine torsion	01	1.4
Vaginal septum	01	1.4
Foesal	12	(14)
Oversized foetus	6	50
Breech presentation	3	25
Laternal deviation of head	3	25

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LIFE TIME CALVINGS FROM JERSEY AND RED SINDHI X JERSEY COWS IN HIMACHAL PRADESH

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The present study deals with the comparison of lifetime performance of exotic and their crossbred cattle maintained at Livestock Farm, Himachal Pradesh Krishi Vishwavidyalaya, Palampur.

MATERIALS AND METHODS

Records on 230 cows comprising 60 New Zealand imported Jerseys, 125 farm bred Jerseys and 45 Red Sindhi x Jersey halfbreds for 18 years were used to estimate the lifetime calf crop. These animals were maintained under loose housing system and standard managerial conditions. The present study deals the average herd life (yrs), life time calves born, calves given alive, female calves born, male calves born, female calves reaching to Age at first calving and sex ratio.

The mean, standard error and analysis of variance were calculated as per Snedecor and Cochran (1967). The sex - ratio was calculated and the Chi-Square test was used to see the significance of deviation from the expected ratio.

RESULTS AND DISCUSSION

The results are summarised in the Table. The imported Jersey had significantly higher herd life (10.15 ± 0.23 years), life time calves born

(6.466 ± 0.164), calves born alive (6.216 ± 0.155), female calves born (3.155 ± 0.177) and male calves born (3.220 ± 0.171); however, the differences between the genetic groups for average number of female calves reaching to age at first calving were statistically non significant. The differences between the farmbred Jerseys and Red Sindhi x Jersey half breds were statistically non significant for all these traits.

The sex ratio in imported Jersey in male and female were 55 and 45 % respectively, whereas in farm bred Jersey and Red Sindhi x Jersey half breds were 53 and 52 in male and 47 and 48 female respectively. Chi square test, however, reveal statistically non significant departure from the expected ratio.

The percentage of calves born alive out of the life time calves born in the 3 groups was 96.13, 95.85 and 97.92% respectively indicating no significant difference in the three groups with respect to prenatal mortality.

The percentage of the female calves that could reach to the age at first calving out of the total female calves born alive was found to be 48.81, 71.68 and 79.33% respectively in IJ, FBJ and RS X J respectively

Table 1 : Life time calf crop from Jersey and RS x Jersey cows in an organised Herd.

Genetic Group	Average herd life (years)	Life Time Calves born	Calves given alive	Female calves born	Male calves born	Female calves reaching to AFC		
Imported Jersey	10.15a \pm 0.23	6.466 ^a \pm 0.164	6.216a \pm 0.155	3.155a \pm 0.177	3.220 ^a \pm 0.171	1.54 \pm 0.121	45	55
Jersey Farm bred	7.62b \pm 0.16	3.624 ^b \pm 0.154	3.467 ^b \pm 0.154	1.932 ^b \pm 0.115	2.081 ^b \pm 0.108	1.385 \pm 0.08	47	53
RedSindhi x Jersey	6.37b \pm 0.28	2.888b \pm 0.268	2.818b \pm 0.265	2.103b \pm 0.214	1.852b \pm 0.172	1.666 \pm 0.173	48	52
Overall	7.562 \pm 0.16	4.221 \pm 0.14	4.065 \pm 0.137	2.331 \pm 0.097	2.372 \pm 0.090	1.489 \pm 0.064	50	50

P (< 0.01) Means with atleast one common superscript do not differ at 1% level of significance.

indicating clear cut superiority of the crossbreds over the pure exotics in their survival and adaptability. The farm bred Jersey were superior to the imported Jersey in this respect.

It may be concluded that Jersey crossbreds are better in survival of the calves upto the breeding age than pure Jersey. Further the farm bred Jersey show better adaptability than the imported ones on the basis of female calves reaching to the age at first calving.

Singh and Tomar (1989) and Dalal et al (1991) have reported lower value for herd life whereas Roy et al (1990), Gandhi and Gurnani (1990) and Jadhav et al (1993) have reported higher values in Sahiwal and Holstein Friesian x Sahiwal. Sahota and Gill (1990) reported life time calf born values towards lower side to the present findings whereas Roy and Tripathi (1990) reported higher values in Holstein x Zebu crosses.

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A HERMAPHRODITE KID WITH GENITAL AGENESIS AND ATRESIA ANI

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Congenital malformations such as atresia ani and hermaphrodite have been reported in new born animals (Laxmipathi, 1989). The present communication deals with a case of kid having hermaphrodite with genital agenesis and atresia ani.

CASE HISTORY AND CLINICAL EXAMINATION

A 2 days old kid was presented with the history of atresia ani and absence of defecation. Close examination revealed presence of thin, small sized penis devoid of external orifice. Further there was evidence of female organs like teats and vulva which was closed. There was also complete closure of anal opening. Hence the case was diagnosed as hermaphrodite with attresia ani.

TREATMENT AND DISCUSSION

The animal was operated for Aresia ani. First surgical site was prepared aseptically and local infiltration anaesthesia with 2% lignocaine hydrochlorid was given. A circular

incision was given on skin over closed anal part. After removing circular piece of skin anal mucosa was exteriorized and opening established. This mucosa was sutured with skin incision by simple interrupted sutures using nylon. After operation animal started passing faeces but it did not pass urine and subsequently died.

Hermaphroditism is a condition in which sexuality of an individual is confused by presence of anatomical structures of both sexes. (Birade, 1997). Hermaphrodites are of two types true and pseudo-hermaphrodites. True hermaphrodites are animals having gonads of both sexes where as pseudo hermaphrodites are animals having gonads of one sex but external genitalia of opposite sex (Bhikane and Kawitkar, 1997). Roberts (1982) reported occurrence of hermaphroditism and atresia ani in Sanen. Asdell and Eaton goats. Further he mentioned that the condition appears to be inherited as a simple recessive character associated with hornlessness.

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CONJOINED MONOZYGOTIC TWINS IN A BUFFALO - A CASE STUDY

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Conjoined twins due to embryonic duplication was reported to be common in cattle compared to other species with an occurrence of one in one lakh births (Roberts, 1971). This communication describes a rare case of conjoined twins in a buffalo.

A seven year old, second lactation, Murrah buffalo with a history of prolonged labour pains was examined per vaginum after giving epidural anaesthesia. It revealed the presence of two dead fetuses simultaneously engaged at the pelvic inlet in posterior longitudinal presentation. Since, the birth canal was roomy and the fetuses were relatively small in size, they were delivered by forced traction using foot snares after proper lubrication. Both the fetuses were males and hence were presumably of monozygotic origin. They were joined only superficially by a band of skin from region of umbilicus upto pubis along the length of linea alba (fig). Probably both the fetuses have shared common placenta. If embryonic duplication occur at the stage of

bilaminar germ disc just before appearance of primitive streak, then the fetuses will have common placenta. (Noden and Delhunta 1985). Since the attachment is in the region of abdomen it can be considered as Gastrophagus Diplopagus twins.



Fig. Gastrophagus diplopagus buffalo twins.

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DICEPHALUS BUFFALO NEONATE - A CASE REPORT

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Malformations due to abnormal duplication of germinal area will give rise to fetuses with partial duplication of body structures. Duplication of cranial part of the foetus is more common than that of caudal portion (Roberts 1971). The incidence of foetal monsters, though rare, was reported by Pandit et al., (1994) in goats and Chauhan and Verma (1995) in buffalo.

A 10 year old, pleuriparous buffalo with normal gestation was observed to be showing prolonged labour pains with both fore limbs projecting out of vulva. Per vaginal examination following epidural anaesthesia revealed a double headed dead foetus in anterior longitudinal presentation with both heads butting against maternal pelvic brim. Since, the dams vaginal passage was roomy and the fetus was relatively small, it was delivered by forced traction after minor correction of posture following retropulsion and proper lubrication. The foetus was fully developed with

both heads of equal size and fused at the neck region (Fig.). The remaining part of the body is normal. The present case can be classified as "Dicephalus, distomus, tetraophthalmus, tetraotus, dipus, dibrachis monster", as per Roberts (1971).



Fig. Dicephalus Manster

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A CASE OF DOUBLE EXTERNAL OS OF THE CERVIX IN A HARYANA COW

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Double cervix and other congenital anomalies of the tubular genitalia are occasionally reported in exotic (Roberts 1971) but rarely reported in Indian cattle. Depending upon the extent and severity of the defect, the animal may either be sterile (Kumar and Prasad 1999), may have impaired or normal fertility (Roberts 1971). This communication reports a case of true double external os of the cervix in a Haryana cow detected during gynaecological examination of more than 2500 cows over a period of 5 years.

A farm bred Haryana heifer aged 3 1/2 yrs. that had not shown any signs of oestrus was subjected to routine gynaecological examination per rectum. It was found that the external os protruding into fornix vagina was bifurcated into two parts separated at an angle of 30°. The cervix appeared to be flatter and wider than the normal cervix. The other parts of genitalia including uterine body, horns and ovaries did not reveal any abnormality. Per vaginal examination revealed that the external os of cervix

was divided into two parts each with a small but separate opening. Metal catheter was passed through both openings. It did not go beyond 1 cm into both as the canals appeared to be too narrow. Ultrasonographic examination was conducted using a Vet Scan 200 machine and a linear 5 MHz probe to confirm diagnosis and check patency of canal in both parts. The animal was diagnosed to be having a true double external os of the cervix. The animal exhibited first oestrus after about two months and was artificially inseminated. The insemination catheter could be passed through easily from both openings. The animal conceived at the first service and had an eventless gestation and subsequent parturition. Similarly, it had no problems with conception or calving for the second time and is presently pregnant for the third time.

It has been reported that this condition is hereditary and may be transmitted as a recessive gene (Roberts 1971). In the present case, 11 daughters sired by the same bull (half sisters) did not have any developmental defect.

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PARASOMUS ELUMBIS WITH ATRESIA ANI IN A NEW BORN COW CALF.**DEBASIS JANA AND MOUSUMI GHOSH**

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Atresia of anus has been reported frequently as congenital defect in calves (Lenghans and White 1973, Venugopalan 1982, Nayak et. al 1994) but the incidence of parasomus elumbis with atresia ani as congenital defect is very rare occurrence. Parasomus elumbis is occasionally seen in cattle and swine and is characterised by a lack of vertebrae and spinal cord caudal to the thoracic region (Roberts, 1972). The present communication reports such a rare incidence of parasomus elumbis with atresia ani in a new born calf.

A seven years old full term pluriparous deshi cow at fourth parity was brought to the clinic with the complaint of dystocia. Straining started since last late night and water bag ruptured at about 9 a.m. in the morning. The case was handled unsuccessfully by a Live stock Development Assistant. The general condition of the animal was good and all the physiological parameters were within the normal range. Pervaginal examination revealed a fully dilated cervix with a live deformed foetus in posterior dorso sacral presentation with both the hind limbs flexed and fixed cranially into the pelvic floor and

dystocia resulted due to such abnormal positioning and lack of flexibility.

After inducing epidural analgesia, correction of both the hind limbs was made and by forced traction as per standard obstetrical technique, a live foetus was relieved. The foetus was very much weak, physically handicapped with marked skeletal deformity and was with poor vigour. Organogenesis was complete except atresia ani. Both the fore limbs were morphologically and functionally normal but both the hind limbs were deformed, and curved elliptically, the foetus had a very short pelvis and varying degrees of ankylosed joints of the rear legs. The bony pelvis was short and deformed and the pelvic symphysis that developed by the union of the hip bones were found defective and morphologically the left one was deviated from the right one. This condition was diagnosed to be parasomus elumbis as per Roberts (1972). The sex of the foetus was male and survived only one and half hours due to its inherent weak condition.

Atresia ani that occurred in this case could be associated with abnormal chromosomes.

Developmental anomalies of the foetal life, failure of anal membranes to become perforated, failure of bowel to become canalised and interruption

of the foetal blood supply to the anus, may lead to atresia and and or atresia ani et recti (Singh et. al 1993).



Fig. *Parasomus elumbis* in a calf

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UROGENITAL ANOMALY IN MALE KIDS**C. JAYAKUMAR AND A.M. VAHIDA,**

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Congenital anomalies of urogenital system of male goats although uncommon, occurrence of pseudohermaphroditism, gonadal hypoplasia, urethral diverticulum and hypospadias have been reported (Shivaprakash et al., 1995). To keep on record, detection of two cases of urethral diverticulum together with cryptorchidism in the offsprings born to different sires in two successive freshenings of a goat is the present report.

Two male goats of 14 and 5 months of age with history of difficult micturition and dribbling of urine were brought to the Veterinary clinic at Livestock Research Station, Thiruvazhamkunnu under K.A.U. On examination, both had fluid filled bulge on the ventral aspect of ischium and pubis (Fig.) On application of pressure on the bulge, there was dribbling to continuous flow of urine. There was recurrence of the bulge indicating that it was a urethral diverticulum. Further examination revealed that the older one had bilateral cryptorchidism while the other had unilateral cryptorchidism.

Detailed investigation could not be carried out because the goats were intended for atonements.

Less commonly occurring cryptorchidism in goats was reported to be due to autosomal sex-linked recessive gene (Roberts, 1971). Urethral anomalies due to failure of fusion of urethral sinus resulting in urethral diverticuli have been reported (Cohrs, 1967). The urethral diverticulum and cryptorchidism in the cases under report having been seen in the offsprings of two successive freshenings of the same goat may presumably be due to genetic factors inherited from the mother.

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DICEPHALUS MONSTER IN AN INDIAN WATER BUFFALO (*BUBALUS BUBALIS*)

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Bovine fetuses with congenital defects are usually born dead and anomalies of musculo-skeletal tissues are common in monstrosities (Roberts, 1986). Dystocia is a most common sequelae of monstrosities in bovines (Bugalia et al., 1985). Fetotomy and Caesarean section are indicated in bovine dystocia due to monstrosities (Parkinson, 1974; Herr, 1979).

HISTORY AND CLINICAL EXAMINATION

A multigravida murrah buffalo in second stage of labour was brought to the veterinary clinics for treatment. Obstetrical examination revealed a dead fetus in posterior presentation. Mutation and forced extraction with adequate lubrication of birth passage were unsuccessful to deliver the foetus. Therefore, it has decided to relieve dystocia by fetotomy.

TREATMENT

Hind quarters and perineum of the buffalo were thoroughly cleaned with soap and water and disinfected with savlon. Posterior epidural anaesthesia was induced in the buffalo using 5 ml of 2% procaine hydrochloride (Novocaine, Pfizer Limited, Bombay, India). The birth passage was adequately lubricated with liquid paraffin for fetotomy.

The wire loop of the Thygessons fetotome was placed over one of the hind foot of the fetus and passed up to lie anterior to the external angle of the ilium where the skin incision was given to retain the loop keeping the head of the fetotome lateral to the anus including the tail, to prevent slipping of the wire loop down the limb during sawing. Similarly, the second limb was severed and removed by fetotomy. Subsequently forced extraction was attempted but was unsuccessful. Obstetrical examination at this stage revealed double heads of the fetus engaged in the pelvic inlet. Thus the monster was repelled caudally to continue fetotomy. Trunk was bisected and amputation of both the forelimbs was performed using schriever's

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wiresaw introducer to place the wiresaw loop between the neck and forelimb and placing the head of the Thygeson's fetotome over the severed end of the spinal column. The other forelimb was amputated in a similar manner and the remaining two joint heads were removed by manual traction.

RESULTS AND DISCUSSION

Dystocia due to dicephalus monster in a buffalo was treated by fetotomy. Complete skeletal

duplication in bovine twins with sternal fusion has been reported in a buffalo (Bugalia et al. 1985). Congenital duplication of cranial and caudal regions and fetal extremities in bovines warrant either fetotomy or caesarean section (Parkinson, 1974; Herr, 1979). Fetotomy was preferred over caesarean section in the present case as the buffalo was in second stage of labour with dead fetus and without toxæmia. Parkinson (1974) indicated caesarean section in pleuriparous cows with prolonged dystocia.

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