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"Perspective and Prospective of the role of artificial insemination and gynaecology in enhancing livestock production*

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Country has made great advances in the fields of livestock production and dairy development. The production of milk, eggs and wool have increased many folds as compared to earlier years. However, the growth of human population has a limiting effect on the per capital availability of these vital livestock products. Further the constraints of overall poor productivity of our animals, insufficient availability of feeds, fodders and nutrients, inability to dispose of large number of unporductive animals, lack of adequate management, awareness in rural areas also have limiting effect on livestock production. These factors, alongwith agro - climatic and other environmental effects disturb reproductive cyclicity in our animals.

Reproductive efficiency of farm animals is directly related to their productiviey. Further optimum reproduction leads to augmentation of economic returns to livestock owners and rural poor. The improvemet in productivity of livestock and their raising in scientific manner by the weaker sections of the society in rural areas can become a major thrust area for effective social change.

Animal Husbandry and Dairy sectors contribute to about 31 per cent of the total agricultural output in the country. This includes apart from valuable livestock products contribution from draft animal power. Eighty per cent of the contribution of livestock comes from cattle and buffaloes.

The employment potential under Animal Husbandry sector alone is projected to be 48.85 million persons by 1995.

Success of dairy cattle and buffalo husbandry lies in ensuring proper and optimal reproductive rhythum. Enhancing cattle and buffalo productivity through cross - breeding with semen of superior exotic sires and genetic upgradation of buffaloes through artificial insemination have contributed greatly to the increased milk production in the country. However, the coverage through artificial insemination is hardly 15% and much more requires to be done in this field. As stated earlier the fertility of animals play a vital and important role in dairy economics. Any alterations or anomalies leading to reduced fertility in male and females affect overall returns in terms of milk yields and number of calves available for sale and replacement. Some of the problems of reproductive inefficiency infertility in cattle and buffaloes can be solved through proper and scientific application of artificial insemination technique and by employment of rigourously followed sexual health control measures.

Artificial Insemination technique is the main and basic tool in the hands of people interested in increased livestock production in the country. Rapid strides made in the field of Multiple Ovulation and embryo transfer (MOET) cannot replace the importance of artificial insemination in the field conditions. MOET will be useful for rapid multiplication of elite herds and availability of progeny tested superior sires through the use of Open Nucleus Breeding System. Government of India has sanctioned a National Science and Technology project on "Cattle Herd Improvement for Increased Productivity using Embryo Transfer Technology" through the Department of Biotechnology.

Under the leadership of National Dairy Development Board and Collaboration of N.D.R.I., I.V.R.I., N.I.I., and regional centres the infrastructure has been created for rapid multiplication of superior male and female. However, still the work done has to be further expanded and strengthened to make sure availability of Elite Sires for Artificial Insemination programmes in the country.

For rapid genetic improvement of the existing cattle and buffalo population the Artificial Insemination network will have to be strengthened. It is expected to provide Artificial Insemination services through 75,000 A.I. centres and perform 32.00 million inseminations by the end of 1994-95. It is also

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envisaged to have 48000 A.I. centres opened with a target of 22.00 million A.I. under Government whereas 27,000 A.I. centres with a target of 10.00 million A.I. will be functioning under Operation Flood in Co-operative Sector. This will help in gradual replacement of the existing non-descript animals by cross-bred and improved animals. It is necessary to mention here that A.I. infrastructure alone will not be sufficient to augment rapid production of cattle and buffaloes with higher productivity. In interior and remote areas and hilly tracts, natural service with bulls and buffalo bulls will still remain a major breeding programme. Government of India has rightly emphasised the need for strengthening the bull mother farms and sanctioning the national bull production programme. This will ensure supply of bulls and buffalo bulls of higher genetic constitution for natural service. However, final goal has to be 100% coverage through Artificial Insemination.

Artificial Insemination technique using frozen semen is becoming more and more popular over the years. The scientists working in the field of frozen semen production have standardised the techniques of semen dilution, cooling, equilibration and freezing of cattle and buffalo semen. Researches have shown that buffalo semen produced and frozen during cooler season when used during summer gives better conception rates, compared to the semen produced, frozen used during summer season. Addition of enzymes and additives like amylase, hylauronidase, acetylcholine etc., have found to give good results in buffalo frozen semen production. Rapid and slow thawing techniques have been compared. Importance of changes in acrosome during freeze-thaw procedures have been well documented. However, the problem of semen quality in cross-bred bulls and some of the buffalo bulls has to be investigated. It is found that 50% or more of cross-bred bulls are unfit for inclusion in breeding programme as their semen is poor in quality and freezability.

In the field application of artificial insemination technique, problems of availability and regular supply of liquid nitrogen have to be solved. The liquid nitrogen which was freely available from fertilizer plants is being diverted for production of other products. This leads to insufficiency and irregularities of liquid nitrogen supply to the field centres. This leads to loss of frozen semen doses, loss of faith of the farmer in the system which further retards the progress of cattle and buffalo improvement programmes. It is essential to ensure regular supply of liquid nitrogen through fertilizer plants and plants owned by Government and Co-operative sector. Infrastructure for rapid increase of A.I. progammes has to be ensured. There are problems of repairs and availability of spareparts for the existing liquid nitrogen plants. This needs to be sorted out at national level. Availability of sufficient number of sires particularly proven sires will go a long way in strengthening the network. The requirement of liquid nitrogen containers of different varieties and facilities for their repairs or replacement have to be streamlined. In the A.I. technique the role of the Scientist working in the Frozen Semen Production Station is most important. Production of good quality semen ensures better fertility. Regular, microbiological count of semen should be carried out by all the production centres. Sexual health of the bulls and buffalo-bulls should be properly monitored. The training, efficiency and attitude of inseminator plays a great role in the success of A.I. programme in the field conditions. Extension education of farmers and farm women in the techniques of heat detection and animal husbandry practices needs to be more emphasised. The extension activities in the animal husbandry and dairy sectors require much more attention as this is directly linked with livestock production programmes. The Officers and staff working in the field of frozen semen production and A.I. should be trained and retrained at well established training facilities or Institutes. As stated earlier, male and female infertility in cattle and buffaloes have detrimental influence on their productivity. To ensure optimum fertility regular monitoring of general and sexual health, analysis of reproductive records, study of the quantity and quality of feeds and fodder being given, examination of agro-climatic and management factors gives a good insight into the causes of reduced fertility. Clinical and laboratory examination of the semen, cervical mucus, blood serum etc., will help in arriving at the diagnosis. In certain cases bio-chemical examination of blood and study of the metabolic profile clarifies the picture. Radio immunoassays, Elisa technique and field testing kits have been employed to study the endocrinological status of infertile animals. However, such detailed examination is always not possible in field conditions. Popularity of A.I. and regular sexual health control activities have ensured availability

of diagnostic and therapeutic services at the door step of the farmers. The major problems in female infertility are delayed puberty, anoestrus, repeat breeding and reproductive infections. The field Veterinarian has to be well trained in clinical examination of infertile animals so that he can arrive at proper diagnosis. Insufficiency of quality feeds and fodder and parasitic infestations accompanied with managemental problems of detection of heat play a major role in reproductive disorders in female cattle and buffaloes. Agro-climatic factors especially during summer and extreme winter disturb reproductive rythem. The Cynaecologists working in the field conditions have a great responsibility of solving problems of infertility in females. Infertility in animals cause tremendous economic losses to the farmers and to the nation. Hence, the competance of the field Veterinarians as well as other staff has to be of high order. The system of monitoring of fertility of inseminated females have to be analysed with the fertility of the bulls and buffalo bulls whose semen is used as well as evaluation of the fertility results obtained by the inseminator. Similarly, training and refresher courses for field Veterinarians in Clinical and laboratory diagnosis of infertility problems are equally important.

Unless, artificial insemination network is properly strengthened and capability, efficiency and competance of field workers in A.I. and Gynaecology are increased the target of reaching milk production to the tune of 80 million tonnes by 2000 A.D. cannot be accomplished. Thus, a lot more emphasis will have to be given for providing sufficient funds and infrastructure, trained manpower, training and extension facilities, diagnostic laboratories, strengthening of Department of Gynaecology in the Veterinary Colleges. Presently, no special schemes operate for providing diagnostic and therapeutic services for reproductive disorders in the rural areas. A separate wing should be created in the State Directorates of Animal Husbandry to look after problems of infertility in cattle and buffaloes in the State with sufficient funds, infrastructure manpower and mobility so that these vital aspects of economic importance are given proper attention.

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Effect of Commercially Available Follicle Stimulating Hormone on in Vitro Maturation of Goat Oocytes

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ABSTRACT

The influence of follicle stimulating hormone (FSH, Folltropin) on *in Vitro* maturation (IVM) of goat oocytes was evaluated. Oocytes were isolated from the ovaries and cultured for 24 h in TCM 199 medium with different concentrations of 1, 10 and 20 ug / ml and differentiation of nuclear matrix to metaphase II stage of meiosis was taken as criteria of maturation. A higher maturation rate was observed when oocytes were incubated in the medium in the presence of FSH in comparison to incubation without FSH. The optimum concentration of FSH was found to be 10 ug / ml, which gave a maturation rate (91.8%). FSH at both low and high concentrations (1 ug and 20 ug / ml, respectively) was less effective. These studies demonstrate that commercially available crude FSH preparations have good biological activity and can be used successfully for *in vitro* maturation of goat oocytes.

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Most oocytes remain arrested in prophase of the first meiotic division for a very long period. The oocytes within the fully developed healthy graaffian follicles undergo final maturation when triggered by the lutenizing hormone surge. Early studies have revealed that mammalian oocytes resume meiosis spontaneously in culture medium containing serum, even in absence of exogeneous hormones (Pincus and Enzmann, 1935, Edwards, 1965). However, ova matured under such conditions fail to form normal pronuclei after sperm penetration and do not develop into blastocysts (Thibault *et al.,* 1975)

The maturation of bovine oocytes *in vitro* in presence of gonadotropins and estradiol, results in enhancement of the rates of maturation, fertilization and development to blastocyst stage (Sirard and First, 1988, Leibfried-Rutlege *et al.,* 1986). Various forms and origin of gonadotropins have been tried for *in vitro* maturation, such as oLH and oFSH (Moor and Trousnson, 1977), bLH (Brackett *et al.,* 1989), hMG (Galli and Moor, 1991), and commercially available FSH (Chauhan and Anand, 1991 and Saeki, *et al.,* 1990).

Information regarding the hormonal requirements and other conditions necessary for *in vitro* maturation (IVM) of goat oocytes are very limited (Chauhan and Anand, 1991). Since commercially available preparations of FSH which are the major source of the hormone for the large scale experimentations, are relatively impure and possess both FSH LH activities. Hence, it was pertinent to examine the effect of these preparations on *in vitro* maturation of goat oocytes.

MATERIALS AND METHODS

Isolation and culture of oocytes

Ovaries were collected from a local slaughter house and brought to the laboratory in normal saline within 2 h of slaughter, maintained at 25 to 30°C. Ovaries were washed 3 times in fresh normal saline and placed into a 60 x 15 mm plastic petri dish containing 5ml Tyrode - Lactate medium buffered with 4 - (2 hydroxyethyl) -piperazine ethane sulfonic acid (TL-HEPES, pH 7.4) supplemented with 4 mg / ml bovine serum albumin (BSA), 0.2 mM sodium pyruvate and 40 ug / ml gentamycin sulfate (Sigma Chemical

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Co., St. Louis, MO). Ovaries were carefully dissected with the help of scalpel and oocytes released in the TL Hepes medium. The medium containing released oocytes were collected in a conical tube and allowed to stand for 10 min at room temperature to allow sedimentation of the oocytes. The oocytes from the bottom of the tubes were aspirated and transferred to another petridish. The dishes were then searched under the stereomicroscope and only good quality oocytes enclosed with compact cumulus cells were used for maturation.

The collected oocytes were washed 3 times in TL Hepes medium and then transferred to the maturation medium TCM 199 supplemented with 20% Fetal Calf Serum (FCS) in the presence of 3 different concentrations of commercially available FSH (Folltropin, Vetrepharm Inc, Ontario, London Canada). In vitro maturation was carried out in five different treatment groups: group 1 - TCM 199 alone, group 2 - TCM 199 + 20% FCS, group 3 - TCM 199 + 20% FCS + 1 ug / ml FSH, group 4 - TCM 199 + 20% FCS + 10 ug / ml FSH and group 5 - TCM 199 + 20% FCS + 20 ug / ml FSH. 10 oocytes were placed in 50 ul maturation drops, covered under sterile paraffin oil at 38°C in 5% CO_2 in air and 95% relative humidity and incubated for 24 h.

After 24 h of culture, cumulus cells were removed, mechanically displaced with a small bore hand pulled glass pipette. The denuded oocytes were fixed in ethanol:acetic acid (3:1) for 24 h and stained with 1% aceto orecin stain and examined under a phase contrast microscope at 400x magnification. Based on change in their matrix, the oocytes were classified as germinal vesicle (GV), metaphase I (MI) and metaphase II (MII). The data were analysed by 2 x 2 Chi-Square analysis (Snedecor and Cochran 1967).

RESULTS

The oocytes were harvested from follicles having a diameter between 3-5 mm and those which were invested with compact cumulus mass were selected for maturation. About 495 good quality oocytes were used for this investigation in a total 4 replicates. The nuclear maturation of oocyte cumulus complexes was evaluated in different concentrations of folltropin.

Maturation rate of the goat oocytes in TCM - 199 alone was 47.9%. Addition of protein supplement in the form of 20% FCS significantly improved the maturation rate of goat oocytes (67.4%) ($P<0.05$). Addition of FSH improved the maturation rate. There was not any significant difference in maturation rate between medium containing 1 ug / ml FSH and medium containing no hormone, but 20% FCS. Maturation rate was significantly improved at the concentration of 10 ug and 20 ug FSH / ml as compared to 1 ug FSH ($P<0.05$). The highest maturation rate was observed (91.8%) when goat oocytes were cultured in the medium containing 10 ug FSH /ml. Further increase in the concentration of FSH to 20 ug /ml did not have any additive effect, in fact, the maturation rate was slightly lowered (82%) when the hormone was present at a concentration of 20 ug / ml (Table 1).

DISCUSSION

Three different concentrations of commercially available FSH were used for in vitro maturation of goat oocytes. In the present study, percent maturation rate was high in the hormone treated groups as compared to medium containing no hormones. A significantly higher maturation rate was observed at the concentration of 10 ug FSH / ml (91.8%). Overall maturation rate in the hormone treated groups was found higher in the our study when compared to the maturation rates observed by Chauhan & Anand, (1991). They observed a maximum maturation rate of 69.1% in Hams F-12 supplemented with BSA and FCS in the presence of FSH. Our results are in agreement with the results observed by Younis *et al.,* (1991). They observed 100% maturation rate of goat oocytes in the medium TCM 199 in the presence of 100 ug LH / ml and 90% in the presence of 5 ug FSH / ml. Sirard *et al.,* (1988) observed lower maturation rate in cattle when oocytes were cultured in the medium without hormone as compared to medium containing hormones. Saeki *et al.,* (1990) have also observed the effect of commercially available FSH on cattle *in vitro* maturation, fertilization and development. They did not observe any difference in frequencies of maturation, fertilization and development of embryos amongst the commercially available FSH and the standard hormone preparation LH and FSH generally available from National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), Maryland, USA. It has been reported

that the oocytes do not have gonadotropin receptors (Lawrence, *et al.,* 1980). The receptors are present only in the adjacent follicle cells. Therefore, signals induced by gonadotropins in follicle cells is transduced into the oocyte via gap junctions (Downs, *et al.,* 1988). Three different concentrations 1, 10 and 20 ug / ml of FSH were used in present study. The 10 ug / ml FSH concentration was found to be optimum to transduce signals to the oocytes and resulted in a higher maturation rate. Lower concentration of 1 FSH, 1 ug /ml does not seem to be sufficient to induce such signals while the 20 ug / ml concentration seems to cause a definite inhibitory effect on *in vitro* maturation of oocytes.

In conclusion, commercially available FSH (Folltropin) preparation can be used successfully for in vitro maturation of goat oocytes at the concentration of 10 ug / ml.

Table 1. Effect of follicle stimulating hormone (Folltropin) on *in vitro* maturation of goat oocytes.

Treatments	Conc FSH	No.of Trials	Total Oocytes	Cumulus expanded	Stages of maturation GV (%)	Met I (%)	Met II (%)
1. TC 199	—	4	96	No expan-tion	14 (14.58)	36 (37.50)	46 (47.91)[a]
2. TC 199+ 20% FCS	—	4	89	44 (49.43)	07 (7.86)	22 (24.71)	60 (67.41)[b]
3. TC 199+ 20% FCS	1ug	4	96	61 (63.54)	5 (5.20)	21 (21.87)	70 (72.91)[b]
4. TC 199+ 20% FCS	10 ug	4	86	52 (60.46)	1 (1.16)	6 (6.97)	79 (91.86)[bd]
5. TC 199+ 20% FCS	20 ug	4	128	96 (75.00)	2 (1.56)	21 (16.40)	105 (82.03)[bd]

a,b,c,d,e,f: Values in the same column with different superscript were significantly different at ($P<0.05$), 2 x 2 Chisquare.

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Circulatory Levels of Thyroid Hormones during Oestrous Cycle in Dairy Cows

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ABSTRACT

The serum triiodothyronine (T_3) and thyroxine (T_4) concentrations of 15 regularly cyclic Jersey cows were estimated by RIA. The mean levels of (T_3) and (T_4) showed the peak value of 1.86±0.06 ng / ml and 8.24±0.16 ug / 100 ml on day 'O' (day of oestrus). The average concentration of (T_3) and (T_4) on 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 16th, 18th and 20th day after oestrus were found to be 0.61±0.04, 0.62±0.06, 0.66±0.04, 0.66±0.06, 0.68±0.04, 0.70±0.06, 0.66±0.04, 0.70±0.06, 1.64±0.05 and 1.70±0.04 ng / ml and 5.20±0.12, 5.20±0.14, 5.64±0.12, 5.62±0.16, 5.64±0.18, 5.68±0.12, 5.62±0.14, 5.68±0.16, 7.64±0.16 and 7.94±0.12 µg / 100 ml respectively. The lowest values of (T_3) and (T_4) were observed on day 2nd of the cycle.

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Apart from regulating basal metabolic rate, thyroid hormones play an important role in the maintainance of growth and reproduction. The thyroid gland contains large amounts of iodine. In humans hypothyroidim is beleived by many to be closely associated with sterility, infertility and abortion. Preliminary clinical studies in cattle suggest thyroprotein or triiodothyronine may be of value in certain type of female infertility such as "silent" estrus (Roberts, 1971) and thyroid activity is necessary for normal reproductive function. There is a correlation between thyroid functions and reproductive efficiency of the female animal and many workers have reported the hormonal profile of thyroid gland during different reproductive stages (Robertson and Falconer, 1961 and Vadodaria *et al.,* 1980). Baruah *et al.,* (1990) reported that the thyroid hormones level fluctuate during oestrous cycle in indigenous goats of Assam and the ovarian function could be determine by estimating the peripheral serum (T_3) and (T_4) levels. To establish the above findings, a similar type of work was taken on Jersey cattle and mereover, there is a paucity of information regarding the thyroid harmones level during oestrous cycle in cattle.

MATERIALS AND METHODS

Experimental animals: Fifteen regularly cyclic purebred Jersey cows were taken for the present investigation.

Nutrition and Management: The cows were subjected to the general management system applied on the farm and were fed hay, concentrate feed mixture and green fodder, whenever available, according to their requirements for maintenance and milk production. During the experimental period, the ambient temperature and relative humidity (RH) were 26.2° and 86.2% (Autumn season).

RIA technique: Blood samples were collected from each of the 15 experimental animals on day 'O' of the oestrus and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20th days post-oestrus. The serum was separated from blood samples and stored at - 20°C till the assay was carried out. All serum samples were assayed for (T_3) and (T_4) using RIA kit supplied by BARC, Bombay as per protocol supplied with the kit. All samples were assayed in duplicate and were critically evaluated.

Statistical analysis: The data were analysed statistically as per the standards and methods given by Snedecor and Cochran (1967). Critical difference (C.D.) test was done to see the significant difference of (T_3) and (T_4) values in different days of oestrous cycle.

RESULTS AND DISCUSSION

The different levels of triiodothyronine (T_3) and thyroxine (T_4) during oestrous cycle in Jersey cows are presented in the table 1. It would be evident from the table 1 that the mean concentrations of T_3 and T_4 in the blood of Jersey

cows varied significantly ($P<0.01$) between different days of the oestrous cycle. As revealed in the C.D. test (at 5% level), concentrations of T_3 and T_4 were found to be higher on the day of oestrus than other days, except 18 and 20th day of the oestrous cycle. The intra and interassay coefficients of variation for T_3 and T_4 assays were 5.6% and 10.6% and 5.2% and 11.2% respectively.

Higher values of T_3 and T_4 observed in this investigation during oestrus are similar to those reported by Sharma and Sharma (1976); Agarwal *et al.*, (1985) and Baruah *et al.*, (1990) in cyclic goats.

The higher values of T_3 and T_4 during oestrus, and day 18 and day 20 of postoestrus may possibly be associated with the increase levels of oestrogen hormone during those periods of the cycle. Concentrations of T_3 and T_4 are closely associated with increase in concentrations of circulating oestrogen (Ingbar and Woebar, 1974). Williams (1974) also reported that administration of oestrogen or androgen caused alteration in the binding of thyroid hormones in plasma and elevation of T_3 and T_4 concentration in blood might be due to the increased concentration of TBG as a result of high oestrogen level. The direct release of TSH from anterior pituitary coincident with release of FSH and LH in the ewe at ovulation (Robertson and Hutchinson, 1960), also contribute the explanation of higher levels of T_3 and T_4 at that time. The present study confirmed the earlier findings of the same author that ovarian function can be monitored by estimating the peripheral serum T_3 and T_4 levels.

Table 1. Serum Triiodothyronine (T_3) (ng / ml) and Thyroxine (T_3) (µg / 100 ml) levels in Jersey cows.

Days of oestrous Cycle	T_3	T_4
0 day	$1.86^{a}\pm0.06$	$8.24^{a}\pm0.16$
2nd day	$0.61^{b}\pm0.04$	$5.20^{b}\pm0.12$
4th day	$0.62^{b}\pm0.06$	$5.20^{b}\pm0.14$
6th day	$0.66^{b}\pm0.04$	$5.64^{b}\pm0.12$
8th day	$0.66^{b}\pm0.06$	$5.62^{b}\pm0.16$
10th day	$0.68^{b}\pm0.04$	$5.64^{b}\pm0.18$
12th day	$0.70^{b}\pm0.06$	$5.68^{b}\pm0.12$
14th day	$0.66^{b}\pm0.04$	$5.62^{b}\pm0.14$
16th day	$0.70^{b}\pm0.06$	$5.68^{b}\pm0.16$
18th day	$1.64^{a}\pm0.05$	$7.64^{a}\pm0.16$
20th day	$1.70^{a}\pm0.04$	$7.94^{a}\pm0.12$

Means bearing different superscript differed significantly.

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Some Blood Biochemical Studies in Rathi Cows and Heifers before and after Induction of Oestrous with Fertivet

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ABSTRACT

Fertivet was found to be more efficient in inducing fertile oestrous in true anoestrous Rathi Cows (83.3%) than in post partum anoestrous Rathi Cows(57.1%) and a shorter average span of 5.9 days was required in heifers as compared to average 8.75 days in cows for inducing oestrous. Analysis of blood glucose, serum calcium, serum phosphorous and total serum protein during fertivet induced oestrous showed higher values when compared with anoestrous but only serum calcium was significant statistically, however comparision of these values with those in normal oestrous cows showed no difference except for total serum protein which was significantly higher in normal oestrous cows.

——x——x——x——

The introduction of clomiphene citrate in the veterinary profession has been reported to be a good tool for treating anoestrous cows and buffaloes by various workers, Deshpande *et al* (1976), Kaikini *et al* (1977), Hukeri *et al* (1979), Dugwekar *et al* (1980) and Chandra Reddy, *et al* (1990) and in sheep and goat by Sinha *et al* (1980) and Sharma *et al* (1983). However no studies have been undertaken on the Rathi cows and heifers belonging to the Arid Tract and on the blood biochemistry of animals before and during estrus induced with clomiphene. The present study was taken up with a view to know the efficacy of the drug in Rathi cows and heifers and the impact of treatment if any on the blood serum constituents namely blood glucose, blood serum calcium, serum phosphorous and total serum proteins.

MATERIALS AND METHODS

Seven post partum anoestrous Rathi Cows and twelve anoestrous Rathi heifers free from any clinical genital infection and having smooth inactive ovaries without any C.L.or G.F. and in good bodily condition were selected for the study. the blood of the animals was collected prior to the start of therapy (Group I), for estimation of blood glucose, blood serum calcium, serum phosphorous and total serum protein.

One tablet of Fertivet (FVT 300, a product of Ar-Ex Labs, Bombay, containing cis-clomiphene citrate 180mg and trans-clomiphene citrate 120mg) was administered to all the animals as a drench dissolved in 250ml of water on the first day and on subsequent days the tablet was given orally mixed in jaggery for four days. The animals were closely examined for signs of heat after the administration of the 5th tablet. When the animals showed signs of heat blood was again collected for blood biochemistry (Group-II) and the animals were inseminated with frozen semen. Those returning to heat after 21 days and consecutively were again inseminated. Pregnancy diagnosis was done at 60 days post insemination. Blood was also collected from 6 Rathi cows which had come into heat without any treatment (control) for serum chemistry. The blood glucose, blood serum calcium, phosphorous and total serum proteins were estimated by standard methods given by Oser (1976).

RESULTS & DISCUSSION

The detailed results of Fertivet treatment in Rathi cows and heifers are presented in Table I. The most peculiar finding noted was that when the animals came into heat they had a marked oedema of the vulvular lips which was more marked in heifers.

It is clear from Table 1 that out of 7 cows treated with Fertivet only 4 cows (57.1%) came into heat whereas out of 12 heifers treated, 10 heifers (83.33%) did come into oestrous, further, heifer on an average required only 5.9 days (3-10) after completion of treatment to show signs of oestrous whereas cows required a longer average of 8.75 days (6-14).

The mean number of services required per conception was 2.0 in case of cows (50% conceived in 1st service and 50% in 3rd service), whereas heifers required a lower number of 1.28

Assistant Project Officer

services per conception (71.42% conceived in 1st service and 28.57% in 2nd service). The findings suggest that Fertivet is more effective in inducing oestrous in anoestrous Rathi heifers than anoestrous Rathi Cows.

The results of blood glucose, blood serum calcium, blood serum phosphorous and total serum protein in cows and heifers before and after Fertivet treatment and in normal estrus cows are presented in Table 2.

It is clear from the table that the values of blood glucose were higher during induced oestrous (Grp II) as well as during normal oestrous (control) when compared with anoestrous (Grp I) Group. However the difference between Grp I and Grp II and that between Grp II and control was non-significant indicating thereby that clomiphene has no effect on the blood glucose. The results are in accordance with Sharma *et al* (1984) who found higher values of blood glucose in normal cyclic cows in comparison with anoestrous cows.

Blood serum calcium values were significantly higher during Fertivet induced oestrous (Grp II) as well as during oestrous when compared with anoestrous group (Grp I) but, when values of induced oestrous (Grp II) were compared with normal oestrous Control group the difference was found to be non-significant indicating that the blood serum calcium is usually higher during a normal oestrous too, and that there is no relation of Fertivet treatment with the blood serum calcium. The results are in agreement with Dindorkar (1974) who found that values of serum calcium and phosphorous increase during oestrous and that anoestrous cows have lower values of serum calcium and phosphorous.

Blood serum phosphorous values were higher during induced oestrous (Grp II) as compared to anoestrous (Grp I) group but the difference was non-significant. Comparison of values of Grp II and Control revealed that the phosphorous values were slightly lower in the Control group, but the difference was non-significant.

Blood serum total protein values were non-significantly higher during induced oestrous (Grp II) when compared with anoestrous (Grp I) group, however comparison of values of induced oestrous (Grp II) and normal oestrous group (Control) showed significantly higher values in the control group. Larson and Kendall (1975) and Georgiev (1974) have reported similar findings of increase in total serum protein during oestrous.

It is revealed that clomiphene (Fertivet) tablet does induce fertile oestrous in post-partum anoestrous Rathi cows and true anoestrous Rathi cows and true anoestrous Rathi heifers, the efficacy of drug being more marked in heifers and that the drug has little or no relation with the blood glucose, blood serum calcium, blood serum phosphorous and total serum proteins.

Acknowledgement: The author is highly thankful to the Sales Manager, M/s Ar-Ex Labs, Bombay for the free supply of Fertivet tablets for the trial.

Table 1 Effect of Fertivet treatment in anoestrous Rathi Cows and heifers:

Group	No.of animals	Days req. induce estrus	No.of animals showing estrus	% of animals showing estrus	Services req. conception	Percentage of animals conceived Service		
						1st	2nd	3rd
Cows	7	8.75(6-14)	4	57.1%	2.0	50%		50%
Heifers	12	5.9(3-10)	10	83.33%	1.28	71.42%	28.57%	-
Total	19	6.5(3-14)	14	73.68%	1.44	66.6%	22.2%	11.11%

Table 2: Blood glucose, Serum Calcium, Serum phosphorous & total serum protein in different groups:

Group	No.of animals	Glucose mgs / 100ml	Calcium mgs / 100ml	Phosphorous mgs / 100ml	Total Protein gms / 100ml
Group - I	19 Mean	44.47	10.47	5.24	5.99
(Before Fertivet treatment-Anoestrous)	S.D.	3.26	0.904	0.89	0.79
Group - II	14 Mean	46.07	11.30	5.65	6.26
(During Fertivet induced oestrous)	S.D.	5.73	0.825	0.660	0.725
	't'	0.9828^{NS}	2.6265^{*}	1.406^{NS}	0.9693^{NS}
Control (Normal Oestrous)	6 Mean	47.16	10.95	5.49	7.52
	S.D.	4.66	0.6465	0.956	1.290
	't'	0.390^{NS}	0.8771^{NS}	0.306^{NS}	2.6964^{*}
		NS.....Non-Significant.		*.....Significant at P<0.05	

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Study of some Physical properties of Estrual Cervical Mucus in Synchronised Normal and Repeat Breeder Cross Bred Cows with reference to fertility

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The physical properties of estrual cervical mucus have a direct bearing on the fertility of an animal as it essentially undergoes certain changes during estrus phase for the passage of spermatozoa. Sperm penetration and survival were found to be correlated with flow elasticity and inversely with pH of cergvical mucus (CM)., The high conception rate reported with CM having low viscosity may be due to lower resistance of CM against spermatozoal motility., (Panchal 1988), typical fern pattern (Sharma *et al* 1987) and lowered pH.

MATERIALS AND METHODS

The experimental groups comprised of nine normal and repeat breeder cross-bred cows in each group. At least one regular estrus of all experimental animals was studied for physical attributes of CM. Later these animals were synchronised with single injection of Dinofertin 25 mg. I / m.

The CM samples were collected at mid stage of regular and induced estrus by syringe pipette teachnique as described by Reddy (1973). Physical properties of CM were studied as regards its colour, consistancy. Further arborisation fern pattern Hydrogen Ion concentration and motility arrestment score were recorded as per Sukhdeo and Roy (1971), and Panchal (1988). Thereafter, the animals were bred by A.I. Pregnancy was assessed on non-return basis and later confirmed by gynaecoclinical examination.

RESULTS AND DISCUSSION

a) Colour and Consistancy of CM

From each of normal and repeat breeder group, Out of six estruses with clear cervical mucus and thick consistancy four (66.67 per cent) and five (83.33 per cent) respectively were found to be fertile. Similarly in repeat breeder cows fertility was high in estruses with clear CM (80 per cent) and thick consistancy (60.00 per cent).

In combined experimental group (18 cows) out of 11 cows showing fertile estrus, maximum,eight (72.73 per cent) samples showed clear CM with thick consistancy., Fertility-rate was lower with CM of turbid colour and thin consistancy wherein only three (42.55 per cent) out of seven cases showed fertile oestrus. This is in aggreement with Basic (1962) and Mursepp (1967) who reported better conception rate with clear CM as compared to turbid CM. These results indicate that synchronisation treatment might help in improving quality of CM.

b) Arborisation Fern Pattern:

In normal cows after synchronisation the number of cows showing typical fern pattern reduced from seven to five (55.56 per cent) whereas in repeat breeders it increased from three to five (55.56 per cent). In combined group out of 11-fertile estrus maximum eight (72.37 percent) samples showed typical fern pattern while out of seven infertile estrus, three (27.28 percent) showed typical fern pattern. This is in agreement with Rao and Rao (1982) and Sharma *et al* (1987).

Out of six normal and five repeat breeder cows showing fertile estrus typical fern pattern was recorded in four (66.67 per cent) and four (80.00 per cent) cases of respective group at insemination.

c) Motility Arrestment Score

A higher fertility rate (63.64 percent) was observed in cases with Nil arrestment score O (V). Similar observations are reported by Panchal (1988). However, Reddy (1973) did not find any significant relation between fertility and *in Vitro* sperm motility in CM As stated earlier, this higher fertility rate in Nil arrestment group may be due to lowered resistance of CM against spermatozoal motility.

In normal and repeat breeder groups also, fertility rate was found to be more (66.67 and 40.00 percent) respectively in nil arrestment score group. This is in agreement with Panchal (1988). In synchronised oestrus there was no remarkable difference in motility arrestment scores of CM which is in agreement with Murlinath *et al* (1990).

d) Hydrogen Ion Concentration (pH):

The difference in pH may adversely affect the motility of spermatozoa (Gupta *et al*, 1981). The present investigation, however, revealed no significant difference in pH values of cows with fertile (8.00 + 0.078) and infertile estrus (7.98 + 0.084). Hartwig (1959) reported higher fertility rates above mean of 7.24 pH and Shehata *et al* (1978) reported maximum sperm penetration at 8.25 pH. Observations reported by these workers are thus in concurrence with the prsent findings. Although slightly higher (8.08 ±0.04) pH was reported in repeat breeder cows with fertile estrus, the difference was not significant.

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Serum Biochemical Profile in Repeat Breeders

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ABSTRACT

The serum biochemical estimations for copper, manganese, zinc, iron, calcium, phosphorus and vit. A were carried out in 10 repeat and 5 regular breeding cows at definite interval. The study revealed over all lower values of all the parameters except that of iron in repeat breeders. corrective therapy with Mineral-Vitamin Compound* @ 20 gms. per animal once daily for 15 days helped to raise the lower serum biochemical level of difference parameters under study in repeat breeders.

INTRODUCTION

Reproductive failure accounts for more than half of all losses resulting from disease of cattle. This picture is reflected in the increasing number of cases of infertility reported in Veterinary clinics and animal health camps (Kaikini, 1989). About 25 per cent dairy cows under the great stress for milk production are culled for reproductive reasons (Young et al 1983).

Inspite of optimum nutrition, there are still cases of irregular breeders and repeat breeders without any malformation or genital disorders detected on repeated gynaeco-clinical examinations.

It is possible that repeat breeding, in otherwise healthy cows may be due to mineral and / or trace elements imbalance or deficiency.

It was proposed to estimate calcium, inorganic phosphorus, zinc, copper, magenese, iron and vitamin A levels in blood serum of repeat breeding cows to see that the findings of this study could be useful as guidline for field Veterinarians.

MATERIAL AND METHODS

Ten Sahiwal cows at the college CBF which were chronic repeat breeders were considered for the study. These cows were apparently normal and healthy with well formed normal tubular genitalia and functional gonads confirmed on periodic gynaeco-clinical checkup. Five regular breeding Sahiwal cows were studied for determining the norms of regular breeding animals.

Serum examinations were conducted twice at an interval of 30 days before treatment and on 8th day post treatment serum calcium, in organic phosphorus, Vit.A, Zinc, Copper, Iron and Manganese levels were estimated by using standard procedures and use of atomic absorption spectrophotometer.

I. Examination for genital infection:

i) Sterilized swabs were used for collecting cervical mucous and were subjected to bacteriological examination for detecting any genital infection.

ii) Serum samples were subjected to standard tube agglutination test using Brucella abortus plain antigen.

Corrective supplemental therapy was resorted to by adding mineral vitamin mixture (ALVITE-M) at the rate of 20 gms. per animal per day once in the feed of each animal from repeat breeding group under study for a period of 15 days.

RESULTS AND DISCUSSIONS

Bacteriological examination of the cervical mucous from the animals under study, revealed the presence of non-pathogenic staphylococci and contaminating bacilli. Animals also were confirmed free from brucellosis by standard tube agglutination test.

The biochemical estimations in repeat breeding cows at definate interval revealed overall lower serum levels of copper-mangenese, zinc, calcium, phosphorus and Vit. A as compared to that of five regular breeding cows as can be observed from Table-1.

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* 'ALVITE-M' (Alembic)

Table 1. Mean and standard error for different parameters pre-and post treatment in repeat and regular breeding cows.

Parameters (1)	Pre-treatment mean values for repeat breeder (2)	Post-treatment mean values for repeat breeders (3)	Mean values for regular breeders (4)
Copper	0.847 ±0.052	1.40 ±0.219	2.304 ±0.010
Manganese	0.170 ±0.009	0.21 ±0.032	0.464 ±0.007
Zinc	1.855 ±0.010	1.74 ±0.063	1.973 ±0.02
Iron	2.467 ±0.031	2.390 ±0.156	2.455 ±0.028
Calcium	6.60 ±2.56	7.32 ±0.331	9.84 ±0.659
Phosphorus	3.375 ±0.22	4.03 ±0.206	4.466 ±0.138
Vit.A	37.136 ±1.679	43.408 ±1.883	41.216 ±2.441

Table 2. 't' values of diff. parameters Pre / Post treatment in repeat and regular breeding cows.

Parameters (1)	Pre-treatment regular & repeat breeders (2)	Pre & Post treatment repeat (3)	Post treatment repeat and regular breeders (4)
Copper	32.44**	2.33*	2.722*
Manganese	21.00**	1.142 NS	5.183**
Zinc	5.756**	1.1641 NS	2.371*
Iron	0.244 NS	1.01 NS	0.693 NS
Calcium	5.595**	1.632 NS	3.559 NS
Phosphorus	3.296**	2.059 NS	1.306 NS
Vitamin 'A'	1.39 NS	2.359*	0.643 NS

* P<0.05 ** P<0.01 - N.S. - Non Significant

Copper: The levels of serum copper were found to be significantly lower in repeat breeders. Copper deficiency have effect on physiological functions in general and reproduction in particular (Hidiroglou, 1979).

Manganese: Serum mangenese levels found to be significantly lower. Impairment in reproduction such as delayed oestrus and conception in female animals is caused due to deficiency of mangenese in diet. (Rojas *et al.* 1965, Dyer *et al* 1965).

Zinc: Significantly lower values recorded for serum level in repeat breeders. Miller *et al* (1970) rightly stated that zinc is an essential nutrient, functioning largely on enzyme systems and its deficiency seriously impairs reproduction in females. The serum zinc level indicates gonadal status in animal (Kulkarni 1981).

Iron: The serum levels did not show any variation in our study cases.

Calcium and Phosphorus: Pretreatment serum calcium and phosphorus level were found to be significantly lower in repeat breeders than in regular breeders.

(a) Calcium plays an important role in sensitizing the tabular genitalia for the action of hormones thereby increasing the fertility rate in animals (Moddie 1965).

(b) Sukhija and Sengupta (1986) emphasized the need for certain levels of inorganic phosphorus for adequate secretion of estrogen.

Vitamin A: Vit. A level difference in regular and repeat breeders found to be non significant so, the role of Vit. A in present cases, could not be assesed.

The statistical analysis using 't' test revealed significant differences for copper, manganese, zinc, calcium and phosphorus level between these two groups (Table 2). However non-significant difference observed for serum, iron and Vitamin A level in them.

The overall effect of treatment resulted in considerable improvement in the biochemical values as seen in table 1.

However the levels of copper, manganese and zinc though improved after treatment could not reach near normalcy.

Cows with repeat breeding problem without any gynaecological abnormality or infection can be suspected for serum biochemical deficiencies. Significant difference existed between repeat breeding and regular breeding cows as regards serum copper, mangenese, zinc, calcium and phosphorus, levels improved after multi-mineral supplementation.

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Postpartum Oestrum in Relation to Plasma Levels of Copper and Zinc in Crossbred Cows

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ABSTRACT

Plasma concentrations of copper and zinc were estimated in postparturient crossbred cows in their first and second lactation in an effort to find whether any correlation existed between plasma levels of copper and zinc and the time of occurrence of postpartum oestrum. The results indicated that plasma levels of both copper and zinc were higher in cows returning to oestrum within 60 days postpartum than cows not returning to oestrum within 60 days postpartum in both the lactation I and II. The differences were found to be significant for copper ($P<0.01$) for cows of lactation I and for zinc ($P<0.01$) for cows of both lactation I and II.

——x——x——x——

Anoestrum has been reported as the single most frequent reproductive disorder which accounts for 68.6% of infertility problems in cows (Roine, 1973). The basic causes of anoestrum in dairy cows are not always apparent and many factors viz. nutrition, gross uterine pathology, chronic debilitating disease and management factors etc. are implicated in its causation (Chauhan and Kessy, 1984). Disturbances in hormonal and biochemical milieu including trace minerals may also cause anoestrous condition and their estimation might be a potential aid in characterizing this problem in postparturient cows. There are conflicting reports on the role of copper and zinc in causing postpartum anoestrum in cows. The present study was, therefore, undertaken to estimate plasma levels of copper and zinc in postpartum crossbred cattle returning to oestrum within 60 days postpartum and in those not returning to oestrum within 60 days postpartum.

MATERIALS AND METHODS

Twenty-four postparturient cows belonging to the Livestock Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar were used for the present study. Out of these cows 10 cows belonged to lactation I and of which 5 cows belonged to the genetic group 25% Sahiwal, 25% Jersey and 50% Red Dane (S x J x RD) and 5 cows belonged to the genetic group 25% Sahiwal, 25% Holstein Friesian and 50% Red Dane (S x HF x RD). All the cows of lactation II (n = 14) belonged to the genetic group (S x J x RD). Cows not exhibiting any behavioural sign of oestrum and changes in the reproductive organs as per rectal examination within 60 days postpartum were considered anoestrous. These experimental cows were grouped as follows -

Group I : Cows of lactation I and exhibiting oestrum within 60 days of calving (n = 4)

Group II : Cows of lactation I and not exhibiting oestrum within 60 days of calving (n = 6)

Group III : Cows of lactation II and exhibiting oestrum within 60 days of calving (n = 7)

Group IV : Cows of lactation II and not exhibiting oestrum within 60 days of calving (n = 7).

About 10 ml blood was collected by aseptic jugular vein puncture at parturition, on day 30 and day 60 postpartum in duplicate sets of clean and sterilized tubes containing 200 µl of 10% EDTA in normal saline. Plasma was separated and stored at -20° till estimation of copper and zinc levels. Both copper and zinc levels in plasma were estimated in diluted samples (dilution rate 1:25) using atomic absorption Spectrophotometer.

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RESULTS AND DISCUSSION

The mean levels of both plasma copper and zinc in different groups of cows are presented in Table 1. The results indicated that the mean plasma levels of both copper and zinc were higher in cows of group I and group III in comparison to the levels in cows of group II and group IV. The differences were found to be statistically significant ($P<0.01$) in all the cases except in case of copper in between group III and group IV cows. The higher levels of copper and zinc in plasma in cows returning to oestrum within 60 days postpartum (group I and III) in comparison to cows not returning to oestrum within 60 days Postpartum (group II and IV) indicate that plasma levels of copper and zinc in cows may be of importance in relation to the time of occurrence of postpartum oestrum. The present findings are in agreement with Dzenite (1965), Boiter *et al.,* (1988) and Manickam and Kathaperumal (1978) who indicated that copper and / or zinc levels may be related to the occurrence of postpartum oestrum. However, Larson *et al.,* (1980) did not find any relationship between serum copper and zinc levels and reproductive performance of cows and Alberio *et al.,* (1985) for copper and Olieveri *et al.,* (1979) for zinc with postpartum oestrum in cows.

Table 1: Mean levels of copper and zinc in different groups of cows

Group	Copper		Zinc	
	Mean Value (µg / dl)	t-value	Mean Value (µg / dl)	t-value
I	136.66 ±7.53 (n=12)		203.75 ±22.26 (n=12)	
		5.1228**		3.7622**
II	81.94 ±7.11 (n=18)		115.55 ±12.17 (n=18)	
III	121.19 ±5.40 (n=21)		187.28 ±16.72 (n=21)	
		1.9177		3.7144*
IV	107.61 ±4.56 (n=21)		110.14 ±12.31 (n=21)	

** ($P<0.01$)

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Studies on Certain Blood Constituents on the Day of First Oestrus in Jersey Heifers Fed with Different Levels of Dietary Protein

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ABSTRACT

Change of certain blood constituents were observed on the day of first oestrus in fifteen purebred Jersey heifers, fed three levels of dietary protein. The levels of haemoglobin and calcium did not differ significantly on the day of first oestrus than the preoestrus condition. However, the values of inorganic phosphorus, total protein, urea nitrogen, NPN, cholesterol and alkaline phosphates were significantly higher ($P<0.01$) on the day of oestrus than the average values of pre-oestrus condition. The dietary levels of protein also had a significant ($P<0.01$) effect on the serum total protein, urea nitrogen, NPN and cholesterol levels.

——x——x——x——

Optimum level of protein in the ration not only helps acceleration of growth rate, but also increase the reproductive efficiency, in growing animal. The plane of nutrition influences the blood picture to a great extent (Baruah *et al.*, 1988). In light of this, the study of blood composition has received great significance not only from nutrition but also from the point of reproduction. Moreover, certain blood constituents fluctuation during different reproductive stages (Devaraj *et al.*, 1985) and study of these blood constituents gives indication about the reproductive status of the animal but there is a paucity of information regarding the blood constituents of purebred Jersey heifers on the day of first oestrus. Therefore, keeping these views in mind, the present investigation was undertaken to study the different blood constituents on the day of first oestrus in purebred Jersey heifers, fed three different levels of dietary protein.

MATERIALS AND METHODS

Fifteen purebred Jersey heifers of 12-15 months of age were randomly distributed into three groups on the basis of their body weight and were fed with three levels of dietary protein along with measured quantities of paragrass and paddy stra for a period of 180 days. The three levels of protein received by the group viz. low protein (LP), medium protein (MP) and high protein (HP) were 301.5, 402.5 and 502.5g protein / 100 kg body weight respectively. The TDN and ME levels were 2.10 kg and 8.09 Mcal / 100 kg body weight as per NRC (1978) for growing heifers (Large breed). Rations were adjusted at regular intervals along with the change in body weight at fortnightly weighing. Blood samples were collected from each of the fifteen experimental animals just before the start of the experiment and which was used as initial (prefeeding) value. However, during the feeding trial, the blood samples from experimental animal of various groups were recollected at monthly interval till the day when animal showed signs of first oestrus. In each group about 30 samples for preoestrus condition and 5 samples for oestrus condition were examined. Haemoglobin was estimated from the blood by "Acid hematin method" as per method described by Baker *et al.* (1955). Serum Calcium, inorganic phosphorus, total protein, urea nitrogen, non-protein nitrogen, cholesterol and alkaline phosphates were estimated as per the method described by Webster, Jr. (1962), Kuttner and Liedenstein (1970), Kigsley (1970), Natelson-Scott and Beffa (1970), Folin-Wu (1970), Zlatkis *et al.*, (1953) and King and Armstrong (1970) respectively. All samples were estimated in duplicate and were critically evaluated. The data so obtained were subjected to statistical analysis as per the standard and methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

The levels of haemoglobin and calcium as observed in this study (Table) did not differ

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Table: Average values of certain blood constituents during preoestrus and oestrus condition in various experimental groups.

	Hb (g%)	Calcium (mg%)	Inorganic phosphorus (mg%)	Total protein (g%)	Urea nitrogen (mg%)	NPN (mg%)	Cholesterol (mg%)	Alkaline phosphatase K.A. (Unit/ 100 ml)
Low Protein group								
Preoestrus	$9.96^{a}\pm0.02$	$10.47^{a}\pm0.27$	$5.97^{a}\pm0.06$	$6.97^{a}\pm0.06$	13.18 ± 0.11	$29.94^{aa}\pm0.28$	$124.55^{a}\pm2.02$	$7.19^{a}\pm0.07$
Oestrus	9.94 ± 0.04	10.39 ± 0.14	6.88 ± 0.11	8.20 ± 0.12	14.12 ± 0.12	31.82 ± 0.14	197.88 ± 3.02	12.16 ± 0.13
't' value	0.32^{NS}	0.61^{NS}	7.58^{**}	4.90^{**}	7.34^{**}	5.95^{**}	20.20^{**}	33.81^{**}
Medium protein group								
Preoestrus	$10.09^{a}\pm0.03$	$10.47^{a}\pm0.13$	$5.86^{a}\pm0.05$	$7.34^{b}\pm0.08$	$15.07^{b}\pm0.17$	$32.15^{b}\pm0.20$	$116.74^{b}\pm0.69$	$7.24^{a}\pm0.12$
Oestrus	10.06 ± 0.05	10.41 ± 0.10	6.81 ± 0.10	9.99 ± 0.12	17.14 ± 0.17	35.03 ± 0.22	189.59 ± 1.37	12.19 ± 0.30
't' value	0.48^{NS}	0.41^{NS}	4.38^{**}	10.47^{**}	8.73^{**}	9.60^{**}	46.70^{**}	15.37^{**}
High Protein Group								
Preoestrus	$10.27^{a}\pm0.05$	$10.43^{a}\pm0.18$	$5.91^{a}\pm0.08$	$8.08c\pm0.05$	$16.76c\pm0.11$	$34.13c\pm0.25$	$110.49c\pm0.69$	7.20 ± 0.12
Oestrus	10.22 ± 0.03	10.35 ± 0.12	6.94 ± 0.07	10.18 ± 0.15	20.05 ± 0.10	37.58 ± 0.16	173.78 ± 1.67	12.18 ± 0.11
't' value	0.79^{NS}	0.42^{NS}	9.28^{*}	13.55^{*}	25.31^{*}	11.50^{*}	35.16^{*}	30.37^{*}

NS = Not significant ** = P<0.01

Means within the same column with different superscript are significantly different (P<0.01)

In each group preoestrus condition = Mean of 30 samples
oestrus condition = Mean of 5 samples

significantly on the day oestrus than the preoestrus condition. The values of inorganic phosphorus, total protein, urea nitrogen, non-protein nitrogen, cholesterol and alkaline phosphates were significantly higher ($P<0.01$) on the day of oestrus than the average values of preoestrus condition. All the experimental animal in this study came to oestrus within the period of feeding trial. The onset of first oestrus in heifers fed on high, medium and low levels of protein were 19.05 ± 0.26, 20.70 ± 0.50 and 21.88 ± 0.20 months respectively and it was found to be statistically significant ($P<0.01$).

Higher values of different blood constituents on the day of oestrus were also reported by various workers. Significant rise of serum inorganic phosphorus, total protein, urea nitrogen, NPN and alkaline phosphates might be associated with the high level of oestrogenic activity or due to increase metallic activity under the oestrogen phase of the cycle (Singh and Dutt, 1974). The increasing trend of total protein towards puberty and maturity showed its bearing on proper development and function of reproductive organs (Meecham *et al.*, 1963). The elevation of serum cholesterol level on the day of oestrus might be due to an increased endogenous biosynthesis of cholesterol rather than difference in the rate of catabolism (Block and Rittenberg, 1942).

Normal functioning of the reproductive system is dependent upon adequate dietary protein. The nutritional quality of protein fed as well as the protein proportion in the diet will not only influence the development and composition of gonad but the blood picture also. Hypophyseal gonadotrophin content is definately increase with high dietary protein along with increase levels of serum protein, urea nitrogen and NPN and which will lead to proper development of ovary and helps in attaining the puberty.

In this experiment, heifers fed on high protein showed earlier oestrus with significant changes of blood parameter. So considering this result heifers may be recommended to feed 502.5g / protein / 100 kg body weight.

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Accuracy of Prediction of LH Surge and Optimum Insemination time by Measurement of Vaginal Electrical Resistance in Cows

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ABSTRACT

Serum concentrations of LH, FSH and oestradiol and vaginal electrical resistance (RV) were determined in cows induced to oestrus with oestradiol - 17B and GnRH to elucidate their temporal relationships. Administration of GnRH resulted in an episodic release of LH and an a cute decline in RV which were closely synchronised. Although oestradiol failed to elicit LH release, RV did fall at a slower rate. Thus LH surge can be accurately predicted by measurement of RV and this will allow optimisation of insemination time, since the time of LH surge and ovulation are known to be fairly constant.

——x——x——x——

Accurate detection of oestrus is a key to successful insemination of cows and buffaloes. A technique that measures changes in electrical resistance of the vagina (RV) is suggested as a potential tool for improving the accuracy of oestrous detection since the RV changes throughout the cycle with the lowest values occuring around oestrus (Leidl and Stolla, 1976), mostly influenced by oestrogen and progesterone (Schams *et al* 1977) and the preovulatory surge of LH (Canfield and Butler, 1989). As the time from an LH Surge to ovulatin is fairly constant in the bovine, the ability to predict this particular parameter could be of practical use to determine optimum insemination time.

MATERIALS AND METHODS

A total of 30 post-partum cows, 5 cyclic and 25 acyclic were used in the study. For acyclic cows oestrus was induced by single instramuscular injections of 250 ug GnRH (Facterel, Ayerst Lab, Canada (n = 5) or 1 mg of oestradiol - 17B (n = 5). The cows were checked for oestrus and probed with a vaginal probe (Ovascan, Animark Inc. U.S.A.) twice daily and at 6th intervals for 24 h during natural or induced oestrous periods for determining the vaginal electrical resistance (RV).

Jugular blood samples were collected at 2h intervals before and at 0.5 h for 8 h, 1 h till 12 h and 2 h untill 24 h after administration of GnRH. The samples were allowed to clot at 4°C and the serum was removed and stored at - 20°C untill assayed.

The serum concentrations of LH, FSH were quantified by specific double-antibody RIA procedures described by Howland (1972) and Cheng (1978). Oestradiol - 17 B was determined by RIA method described by Yu *et al* (1974) from the samples collected at every second hour.

Statistical analysis was carried out as described in Snedecor and Cochran (1980).

RESULTS

R.V. PATTERN

The RV of acyclic cows displayed a more or less constant pattern throughout the recording period. In cyclic cows it gradually declined to some low value around the time of standing oestrus. When oestrus was induced with single GnRH injection, the RV showed a sharp decline reaching the nadir within 6 h (40%) and 12 h (60%), while that for cows pretreated with oestradiol 6 h prior to GnRH, 60 and 40% of cows had lowest RV at 6 and 12 h of GnRH injection respectively. The cows that received oestradiol alone also had altered RV pattern characterised by gradual decline to lowest values at 18 (50%) and 24 h (50%) of injection. Subsequent readings slowly increased to preinjection levels. Individual cow differences in RV were high. The average rate of decline of RV was 36, 26, 33 and 34% respectively in natural, GnRH, E_2 + GnRH and E_2 treated cows respectively.

Harmone levels: Administration of GnRH resulted in a surge release of both LH and FSH within 1.5 to 2 h elevating serum concentrations from mean basal levels of less than 1 ng / ml

to mean peak heights (LH 8.82 ng / ml, FSH-1.65 ng / ml) while in E_2 pre-treated cows the levels rose to mean peak heights (LH 21.4 ng / ml, FSH 2.59 ng / ml) before declining to basal levels within 5.5. to 7 h. Although ovulation occurred and RV declined in all the GnRH treated cows, behavioural signs were exhibited by only a few cows. The serum oestradiol (E_2) levels increased within 2 h following E_2 injection from a mean basal level of 11.3 pg / ml to 92.9 pg / ml. However, single E_2 treatment was not accompanied by ovulation although RV declined. In acyclic cows, no alteration in any of the hormones studied was seen.

DISCUSSION

The RV patterns in induced and normal oestrous cycles were similar with lowest values recorded on the day of oestrus. The RV declined to the lowest values within 4 to 10 h of GnRH-induced peak release of LH both in E_2primed and non primed cows and the lowest RV and the LH peak were very closely synchronised. Administration of single E_2 also produced similar change in RV but at a belated interval of 18 h to 24 h indicating that both the hormones act synergestically at the time of oestrus, the effect of LH being acute as compared to that of E_2. Since the interval from the LH surge to ovulation is about 25 h in the cow (Schams *et al,* 1977) prediction of LH surge by measuring RV alone would allow for determination of optimal insemination time to enhance fertility irrespective whether the ovulation is accompanied by behavioural signs or not. However, each individual animal has its own baseline resistance level and associated decline during oestrus.

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Histomorphological study of uterus from non-ovulated side in Surti buffaloes during various phases of estrous cycle

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The reproductive organ shows many significant histomorphological changes during various phases of estrous cycle. Considerable work has been reported on histomorphological picture of gonad and tubular genitalia of the buffaloes. Less information is available on the histomorphological changes in the uterus of Surti buffaloes, especially from the non-ovulatory side. Hence, an attempt has been made to study the histomorphological structure of uterus during various phases of estrous cycle in Surti buffaloes.

The histomorphological study was carried out on the uterus, taken from Cranial 1/3rd of uterine horn of non ovulated side and were preserved in 10 per cent neutral buffered formalin. Sections of 7-10 µm thickness were cut on cryostat microtome and stained with Harris' haematoxylin and eosin (Humason, 1966).

The results are presented in Table 1 and 2.

Endometrium: The uterine epithelium was simple columnar type with patches of pseudostratified columnar epithelium. Proestrus and estrus showed tall columnar cells, while matestrus and diestrus showed low columnar cells and the results are in agreement with Cole and Cupps (1969) and Sundravadanan and Venkataswamy (1973), but differ with the findings of Hafez (1974) in farm animals.

As the epithelium was tall columnar type during proestrus and estrus phases, the thickness of epithelium was more during proestrus while, it was less during metestrus phase, due to low columnar epithelium. El-Sheikh and Abdelhadi (1968) reported that the thickness of epithelium was 14.24 µm in Murrah buffaloes, which is in close agreement with 14.44±1.68 µm thickness of epithelium of all the four phases in Surti buffaloes.

The thickness of endometrium was found to be minimum in estrus and maximum in diestrus phase of estrous cycle in Surti buffaloes. Similar findings were reported by Sundravadanan and Venkataswamy (1973) in cattle. Endometrium was found to be thinner during the follicular phase but increased in thickness conspicuously during luteal phase in Surti buffaloes, which is similar to the findings of Nalbandov (1964) and Hafez (1974) in farm animals, while Cole and Cupps (1969) opined that in domestic animals the thickness of endometrium was lowest during proestrus and greatest during estrus and it became thinner during metestrus and diestrus phases of estrous cycle.

Endometrial gland: The height of glandular epithelium was less during proestrus and more during diestrus phase of the estrous cycle and the research are in agreement with the findings of Janakiraman *et al.,* (1976). Whereas, Singh and Sharma (1985) reported minimum height in late luteal phase and maximum in mid luteal phase of estrous cycle of buffaloes.

The total number of uterine glands per unit area was minimum in proestrus and maximum in estrus phase of the cycle. The uterine glands gradually decrease in number from estrus to diestrus phase. Similar results were reported by Janakiraman *et al.,* (1976). The differences between the various phases were significant.

The lumen diameter of uterine gland was found to be smaller in estrus and larger in metestrus phase. Similar findings were reported by Singh and Sharma (1985). However, Janakiraman *et al.,* (1976) reported maximum diameter of uterine gland at estrus phase and

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Table 1: Mean, SE and CV% of thickness of epithelium of mucosa, lumen diameter of uterine glands, height of glandular epithelium, number of uterine gland and thickness of endometrium in uterine horn of non-ovulated side of Surti buffalo (6) during various phases of estrous cycle.

Character	Thickness of epithelium of mucosa (μm)		Thickness of the endometrium (μm)		Lumen diameter of uterine gland (μm)		Height of glandular epithelium (μm)		No. of uterine gland per mm^2	
Phases	Mean ±SE	CV%	Mean ±SE	CV%	Mean ±SE	CV%	Mean ±SE	CV%	Mean ±SE	CV% CV%
Proestrus	15.75 ±2.64	41.00	2121.39 ±225.50	26.04	25.38 ±3.28	31.69	9.01 ±0.30	8.14	31.54[a] ±3.29	25.52
Estrus	14.70 ±1.65	27.44	1777.09 ±137.30	18.93	19.60 ±2.37	29.60	10.79 ±0.84	18.96	90.77[b] ±8.31	22.42
Metestrus	13.07 ±1.19	22.39	2118.48 ±77.05	8.91	62.83 ±7.48	29.16	10.91 ±1.25	28.08	50.26[a] ±3.99	19.44
Diestrus	14.23 ±1.68	28.96	2777.11 ±424.44	37.44	43.75 ±5.12	28.66	12.31 ±1.36	27.00	39.15[a] ±9.29	58.12
Overall average	14.44 ±0.56	7.70	2198.51 ±209.12	19.02	37.89 ±9.78	51.61	10.75 ±0.68	12.57	52.93 ±13.19	49.83

Means showing the different superscripts differ significantly ($P<0.05$)

Table 2. Mean, SE and CV% of thickness of entire myometrium, thickness of circular muscle layer, longitudinal muscle layer, stratum vascular and perimetrium in uterine horn of non-ovulated side of Surti buffalo (6) during various phases of estrous cycle.

Character	Thickness of entire Myometrium (μm)		Thickness of circular muscle layer (μm)		Thickness of stratum vascular (μm)		Thickness of longitudinal muscle layer(μm)		Thickness of perimetrium (μm)	
Phases	Mean ±SE	CV%	Mean ±SE	CV%	Mean ±SE	CV%	Mean ±SE	CV%	Mean ±SE	CV%
Proestrus	2122.36^{a} ±213.21	24.61	757.57 ±75.64	24.46	864.27 ±150.54	42.67	458.81^{b} ±74.22	39.62	363.85^{a} ±26.89	18.10
Estrus	1648.03^{a} ±358.35	53.26	570.18 ±105.51	45.33	700.34 ±211.76	74.07	361.81^{a} ±58.54	39.63	267.99^{a} ±84.72	77.43
Mestestrus	2136.03^{ab} ±279.23	32.02	828.38 ±59.00	17.45	596.55 ±144.86	59.4	420.01^{ab} ±58.65	34.21	541.26^{b} ±72.55	32.83
Diestrus	2910.97^{b} ±313.24	26.36	909.86 ±143.60	38.66	812.86 ±115.37	34.77	968.06^{c} ±84.83	21.46	770.18^{c} ±84.38	26.84
Overall average	2204.35 ±261.44	23.72	766.50 ±72.46	18.91	743.51 ±59.76	16.07	552.17 ±140.05	50.73	485.82 ±110.40	45.45

Means showing the different superscripts differ significantly (P<0.05).

minimum at late luteal phase in Surti buffaloes. They have reported lowest diameter at late follicular phase and highest at mid luteal phase.

Thickness of Myometrium and Perimetrium: The thickness of entire myometrium was found to be maximum in diestrus phase and minimum in estrus phase and the findings are in step with the report of Shukla *et al.,* (1973). However, Sundravadanan and Venkatswamy (1973) reported more thickness of myometrium in follicular phase and less in luteal phase of estrous cycle in cattle.

The thickness of circular muscle layer was less in proestrus phase and more in diestrus phase. While Shukla *et al.,* (1973) reported lowest thickness during estrus and highest during early follicular phase in Surti buffaloes. The present study also showed gradual increase in the thickness of circular muscle layer from proestrus to diestrus phase.

The thickness of stratum vascular was maximum in proestrus and minimum in metestrus phase. The longitudinal muscle layer showed similar trend to that of entire myometrium, which was more thick in diestrus phase and less in estrus phase, while reverse changes has been reported by Shukla *et al.,* (1973) in Surti buffaloes.

The change in thickness of perimetrium was also revealed more thickness in diestrus and less in estrus phase and difference was found to be significant.

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Histology and Histochemical Studies on Corpus Luteum of Superovulated and Control Crossbred Cows

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ABSTRACT

Histology and Histochemical studies were undertaken on the corpus luteum (CL) of four superovulated and four control cows at day 7 of the cycle. The histological observations revealed that granulosa lutein cell population was significantly higher in control cows than that of superovulated cows. The granulosa lutein cell population also significantly varies between the CL of each superovulated cows. 5-3 β-hydroxysteroid dehydrogenase (-3 βHSD) and glucose- 6-phosphate dehydrogenase (G-6-pD) activities were found to be mainly localized in the granulosa lutein cells in superovulated CL. A marked decrease in these enzyme activities were noted in the superovulated CL than the control CL. Between the CL of the same superovulated cows, the enzyme activities varied markedly.

——x——x——x——

Plasma steroids concentration (oestrogen, progesterone) increases during various phases of oestrous cycle. During mid luteal phase, blood progesterone level of normal cycling cow increases to a peak level (Wetteman, *et al.,* 1972; Kotwica and Williams; 1982). The blood progesterone level increased in superovulated animals during luteal phase but not enough to correlate with the number of corpora lutea formed during superovulation (Solti, *et al.,* 1978; Saumande, 1980). Several workers studied the normal cyclic corpus luteum (Rubin, *et al.,* 1963; Savard, *et al.,* 1963; Donaldson and Hansel, 1965; Keyes and Wiltbank, 1988). However, no report so far is available on histology and histoenzymology of superovulated CL. The present study was undertaken to study the steroidogenesis in CL of superovulated as well as control cows to know the reason, why the progesterone concentration in superovulated cow does not increase proportionately with the number of corpora lutea formed as compared to the control CL.

MATERIALS AND METHODS

Day 7 corpora lutea were collected from four superovulated and four control cyclic crossbred cows. The superovulation treatment was induced with 40 mg of FSH-P (Burns Biotec, U.S.A.) in decreasing dose level (7, 6, 5, 2 mg twice daily) for 4 days. A single prostaglandin F-2 alpha-analogue (Carboprost-tromethamine, Upjohn, U.K) injection of 500 µg was given on day 3rd of the treatment. The control cows were also given a prostaglandin (PG) injection on the same day. When these cows came to standing oestrus, they were injected with 150 µg of gonadotropin releasing hormone (Cystorelin, Ceva Laboratory Inc., U.S.A.). The corpora lutea were enucleated on day 7 of the superovulatory oestrus, after performing laparotomy and washed in cold saline. Each CL was divided into two equal portions. One portion was quickly freezed at -20°C in a cryostat while other portion was fixed with Bouin's fluid.

For histochemical study, 10 µ frozen sections were cut by cryostat and were taken on cover slips. The lipid was extracted by chilled acetone (-4°C) for 10 minutes. They were then washed with Tris buffer and incubated for 1 hr at 37°C in a media made for -3 βHSD according to Deane and Rubin (1965). Control sections were processed side by side with a similar media except the substrate, pregnanolone. After incubation, the sections were washed with 15% phosphate buffer saline and fixed in phosphate formol solution. They were then examined under microscope after glycerine mounting.

For G-6-PD activity, similar cryostatic sections were made as in case of - 3βHSD and taken on cover slips. The sections were then covered with a media meant for G-6-PD as described by Brandau, *et al* (1968). A control

group of sections were also run side by side without the substrate, glucose-6-phosphate disodium salt. The sections were kept at 37°C for 30 minutes for proper colour development and formazan deposition. The enzyme reaction was then blocked by phosphate formol buffer. They were then dehydrated in alcohol and cleared in xylene. The permanant slides were made by DPX mounting. Deposition of purple colour formazan granules and intensity of colour development in the localized area or cells indicated the presence of enzyme.

For histology, the tissues fixed in Bouin's fluid were dehydrated in graded alcohol and cleared in xylene. The paraffin blocks of these tissues were made and 4 μ section of the tissues were cut by a rotary spencer microtome and stained with hematoxyline and cell count from periphery of the CL in between trabeculae was done under microscope from ten areas (each area of 3.14 square mm) of each section in four of such sections from each CL.

RESULTS AND DISCUSSION

Studies on histology and histochemistry of properly lutenized CL of control cows showed that the - 3βHSD enzyme was mainly localized in the theca lutein cells than that of granulosa lutein cells. Similar observation has been made by other workers in case of mammalian ovaries (Hansel, *et al* 1973; Lobel and Levy, 1986; Mazumdar and Nandy, 1987). The G-6-PD, known as a supportive enzyme to steroidogenesis has also been found to be higher in the theca lutein cells, as has been reported by other workers (Savard, *et al* 1963; Mazumdar and Nandy, 1987).

In case of ovarian CL of superovulated cow, the - 3βHSD and G-6-PD activities were mainly localized in the granulosa lutein cells. There was little or no activity recorded in the theca lutein cells of superovulated CL. When the CLs of the same cow were studied variations in the enzyme activities, it was noted that there existed variations in the enzyme activities among the CLs of the same superovulated cow. Body tries to maintain the therapeutic hormone levels within it's normal range by quickly catobolizing the hormones or by decreasing the synthesis. Thus the autoregulation mechanism gets activated in such cases to control the hormone level within the physiological range. It is quite natural if the progesterone concentration increases proportionately with the increased number of CL, then the hormone level should increase by 10 to 20 fold, as theca lutein cells known to synthesize the maximum progesterone in CL (Donaldson and Hansel, 1965; Lobel and Levy, 1986; Mazumdar and Nandy, 1987). High level of progesterone has got adverse effect on the cow causing several disturbances in body metabolism. Thus in the CL of superovulated cow, probably decrease the progesterone synthesis as has been observed in this experiment.

The biometry of granulosa lutein cell number from specific site of each CL in control and superovulated crossbred cows showed that the average granulosa lutein cell number in normal CL is three times more than that of superovulated CL (Table-1). Even the granulosa lutein cell number varied widely among the CL of each superovulated cow (Table-2). The - βHSD activity was mainly localized in the granulosa lutein cells of a superovulated CL. Therefore, it is presumed that the cow tries to regulate the steroidogenesis by decreasing the granulosa lutein cell number too. On the other hand it has also been reported that the mitotic index of granulosa lutein cell is very poor and limited to 3 to 4 days after ovulation and further division in granulosa lutein cell was not observed. Furthermore, the mean weight of the seven day old corpora lutea (1.95 gm) on superovulated ovaries (Donaldson, 1985) were less than half of the normal weight 4.6 gm (Donaldson, *et al* 1965) irrsepective of the number of corpora lutea on the ovary. The above finding also supports our hypothesis of regulation of hormone synthesis of the corporalutea to its physiological limit.

The level of oestradiol 17-β in superovulated cows also supports the above hypothesis as oestradiol level at oestrus did not increase proportionately with the number of developing follicles (Henricks, *et al* 1973; Kharche, 1989). So it is presumed that the autoregulation mechanism to control the hormone level is much more active in superovulated cows than the control cows.

Table 1. Mean Number of Granulosa Cell / Area of Corpus Luteum of Control and Supervulated Cows

S.No.	Control Cows	Superovulated Cows
1	2085.75	417.09
2	2127.00	743.55
3	1949.00	1300.81
4	1945.63	543.89
x	2026.84[a]	751.33[b]
S.E.	±46.68	±195.09

Note: Mean values with different small letters are significantly different at 5% probability level (P<0.05).

Table 2. Mean Number of Granulosa Cell / Area of Each Corpus Luteum Among Superovulated Cows

S.No.	Cow No.I	Cow No.II	Cow No.III	Cow No.IV
1	282.50	586.25	1405.50	867.00
2	326.00	674.00	1503.00	734.50
3	314.25	803.00	2445.00	402.50
4	396.00	773.50	1453.75	512.25
5	201.00	1038.00	1270.75	549.75
6	435.50	706.75	1207.00	435.00
7	414.50	686.75	1440.75	325.50
8	721.25	757.50	1010.00	328.00
9	456.75	333.75	992.50	559.00
10	448.25	833.50	1020.25	615.25
11	479.50	1018.50	990.50	602.50
12	529.50	775.00	879.75	579.00
13		660.25		715.00
14		771.25		389.25
15		787.00		
16		594.25		
17		507.50		
18		1077.25		
x	417.09	743.55	1300.81	543.89
S.e.	±38.54	±43.42	±121.64	±42.65

Significantly different at 1% probability level (P<0.01).

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Comparative Assessment of Certain Biochemical And Mineral Constituents of Seminal Plasma And Their Interrelationships in Ox And Buffalo Bulls*

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ABSTRACT

The profile of various biochemical and mineral constituents were studied in the seminal plasma of 36 ejaculates each from 3 HF and 3 Murrah bulls. The values were initial fructose 515.20±35.42 & 604.44±27.96 mg%; total protein 7.88±0.33 & 4.30±0.12 g%; total cholesterol 79.25±3.15 & 73.13±1.86 mg%; acid soluble phosphate 65.57±3.47 & 91.00±91.00±3.32 mg%; ester phosphate 55.37±3.21 & 79.79±3.07 mg%; inorganic phosphate 10.31±0.48 & 12.28±0.37 mg%; sodium 337.37±3.82 & 295.11±4.64 mg%; potassium 96.22±4.86 & 103.56±3.15 mg% and chloride 334.59±15.56 & 269.13±7.85 mg%, respectively. The levels of all traits, except cholesterol and potassium, differed significantly ($P<0.01$) between them. Fructose content had significant ($P<0.05$) positive correlations with protein & cholesterol (0.71; 0.38) and negative correlations with phosphate, potassium and chloride (-0.46; -0.83 & -0.34,resp) in Friesians and with protein and potassium (-0.37 each) in Murrahs. Protein was negatively correlated with phosphate, potassium and chloride (-0.46;-60 & -0.59) in Friesians. Cholesterol had negative correlation with phosphate (-0.33) in Friesians and positive correlations with protein and phosphate (0.35 each) in Murrahs. Sodium and potassium had significant negative and positive correlations only with phosphate (-0.45;+0.60) in Friesians and with chloride (-0.37; +0.46) in Murrahs.

The literature about the absolute concentration and the specific role played by various biochemical, enzymatic and mineral constituents of bull and buffalo semen in sperm motility, metabolism, keeping quality and freezability / fertility is rather inconsistent with regards to the levels and correlations within and between species. Buffalo semen has been reported to possess considerably low levels of most of these constituents (Singh *et al.,* 1969; Banerjee and Ganguli, 1973; Dhami *et al.,* 1990). The object of this study was to know and compare the seminal biochemical and mineral profiles and their interrelationships between the ox and buffalo bulls.

MATERIALS AND METHODS

Seventy two weekly collected semen ejaculates from 3 Holstein Friesian and 3 Murrah buffalo bulls were utilized in this study during the winter months. The bulls were maintained under identical nutritional and managerial practices at the Germ Plasm Centre of IVRI, Izatnagar (UP). Following collection and evaluation, the samples were centrifuged at 2500 rpm for 30 minutes. The seminal plasma siphoned out was stored at - 15°C till assayed. The levels of various biochemical and mineral constituents viz., initial fructose, total protein, total cholesterol, acid soluble, ester and inorganic phosphates, chloride, sodium and potassium were estimated in the seminal plasma using standard analytical procedures described elsewhere (Dhami, 1992). The data on various traits were analysed statistically for cattle and buffalo semen separately using 'F' test and their correlation coefficients were worked out. The species difference in the pooled means of each trait was compared by 't' test.

RESULTS AND DISCUSSION

The pooled means of various constituents studied and their interrelationships observed in cattle and buffalo semen plasma are shown in Table 1.

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Fructose, Protein and Cholesterol:

The seminal plasma of Friesian bulls contained significantly (P<0.0) lower initial fructose and higher total proteins than the Murrahs, but the cholesterol content did not differ between them (Table 1). The bull effect was significant for all three traits only in Friesians. Similar observations were also made by Abdou *et al.* (1976) for fructose content of HF and Murrah bulls. Banerjee and Ganguli (1973) and Dhami *et al.* (1990) however reported significantly higher values for both fructose and protein, and Prabhu *et al.* (1973) and Kumar *et al.* (1988) observed 2 to 5 times higher values for cholesterol in cattle than the buffalo semen. Further, the cholesterol content of 49 and 62 mg% recorded in buffalo semen plasma by Prabhu *et al.* (1973) and Varshney *et al.* (1978) was quite lower than the present findings. The fructose content had significant (P<0.01) positive correlations with protein and cholesterol and negative correlations with phosphates, potassium and chloride in Friesians. Whereas in Murrahs, it revealed significant negative correlations only with protein and potassium. Further, the protein content showed significant negative correlations with phosphates, potassium and chloride in Friesians, but had positive correlation only with cholesterol in Murrahs. The association of cholesterol with phosphates was significantly negative in Friesian and positive in Murrahs (Table 1). The trend of protein content also revealed positive associations with freezability and fertility of semen in different bulls studied.

In semen, fructose is an important metabolite for bovine sperm and its level reflect the sperm quality, its metabolic activity and the secretory function of the vesicular glands. While protein provides an amphoteric property to seminal plasma. The correlation findings of seminal fructose and protein observed with other constituents in our study were in close proximity to those reported by Patel *et al.*, (1989) in crossbred bulls. Kaker and Arora (1976) however found significant negative correlation of fructose with protein. While Dabas *et al.*, (1984) and Kumar *et al.*, (1988) failed to observe any correlation between protein, cholesterol, lipids, anions and cations in bull and buffalo semen. Like phospholipids, cholesterol also acts as cryoprotective and an insulating agent for sperm membrane, thereby causing a decrease in its permeability and increase in sperm survival (Dabas *et al.*, 1984).

Inorganic, acid soluble and ester phosphates:

The means of inorganic, acid soluble and ester phosphates content in the seminal plasma of Friesian bulls were significantly (P<0.01) lower than those of Murrahs. The variation between bulls was also significant for all three traits among both the species, except inorganic phosphate in Murrahs (Table 1). These findings were in agreement with Singh *et al.*, (1969) and Ibrahim *et al.*, (1985). Further the values of acid soluble and inorganic phosphate in semen of both the species were little lower than those reported by Flerchinger and Erb (1955) and Patel *et al.*, (1989), but were higher than those of Banerjee and Ganguli (1973), Dabas *et al.*, (1984) and Kumar *et al.*, (1988).

In Friesians, the contents of acid soluble, ester and inorganic phosphates had significant (P<0.01) negative correlations with fructose, protein and sodium, and positive correlations with potassium. Whereas in Murrahs, inorganic phosphate was negatively correlated with sodium and positively with acid soluble and ester phosphates. Remaining all correlations were not significant (Table 1). There was a positive association between phosphate content of seminal plasma and fertility status of bulls under study. Dabas *et al.*, (1984), Kumar *et al.*, (1988) and Belorkar *et al.*, (1991) however, did not find any association between inorganic phosphate and acid soluble / phospholipid phosphorus, cholesterol or mineral elements in cattle and buffalo semen. The presence of high amount of phosphate ions in seminal plasma of Murrah bulls observed might be due to accumulation of metabolic product of spermatozoa and indicated that AKP-ACP and ATPase enzymes were more active so as to liberate more phosphate ions in buffalo than the cattle semen (Dhami, 1992). These findings tend to show that higher phosphate in semen was not compatible for better quality and preservability of bovine semen.

Sodium, Potassium and Chloride:

The overall means of sodium and chloride contents were significantly (P<0.01) greater in Friesians than the Murrahs, but the potassium content did not vary between them (Table 1). Singh *et al.* (1969) however, reported lower

values for all three elements in cattle than the buffalo semen. Our findings in respect of potassium level in Murrahs compared well with those of Dhami *et al.* (1990), although the sodium content reported by them was very low. Further, the contents of all three elements varied significantly only among Friesian bulls. The levels of chloride in semen influence the sperm membrane potential and motility in association with anions. They also are directly related with the maintenance of excitability of sperm, optimum semen pH and constant osmotic pressure within and without the sperm cells.

In our study, the sodium and potassium contents of seminal plasma had significant but negative and positive correlations, respectively, only with phosphates in Friesians and with chloride in Murrahs. Further, sodicum was positively associated with potassium in Murrahs, in contrast to negative association in Friesians (Table 1). The high levels of potassium are known to inhibit fructose utilization, oxygen uptake and sperm motility (Dhami *et al.,* 1990), and hence its negative association observed with fructose and positive association with chloride and phosphate ions are conceivable. Cragle *et al.* (1958) however reported significant negative correlations of chloride with both sodium and potassium in bull semen. Patel *et al.* (1989) found positive association between sodium and potassium in bovine semen. While Belorkar *et al.* (1991) reported most correlations of chloride to be negative and nonsignificant in crossbred bulls. Thus, the excess chloride was not conduceive to better sperm quality in bovines. The present findings showed that the cattle and buffalo semen varied widely in respect of the levels of most biochemical constituents and their interrelation-ships in inverse manner. This may be responsible for the differences noted in the keeping quality, freezability and fertility of their semen at large.

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A Comparative Study on Quality of Chilled Bull Semen Preserved in French Medium Straw And Plastic Vial

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It is a common practive to use 1 ml plastic vial and 0.5 ml straw for storage of chilled and frozen bull semen, respectively. In view of better hygiene, safety and ease during handling and artificial insemination using straws, some studies have been initiated to compare the efficacy of straws and ampoules or vials in preserving bull semen (Rajamannan, 1970; Jondet, 1972; Pickett *et al.*, 1976; Pace and Sullivan, 1978). The present study has been undertaken to study the quality of bull semen in respect of motility, livability, transaminase release and bacterial load when preserved at 5°C in plastic vials and French medium straws.

A total of 20 ejaculates collected with the help of standard artificial vavina at weekly interval from 4 bulls maintained at Indo-Australian Cattle breeding project, Khanapara were taken for the study. All ejaculates had atleast a mass activity of ++(+) and volume 3 ml. Immediately after collection the semen samples were extended (1.20) in egg-yolk citrate extender (Salisbury *et al*,1941) containing 800 I.U. penicillin and 1000 µg streptomycin per ml. The extended semen was then divided into two fractions and packaged one fraction in French medium straws and other fraction in one ml plastic vial and prserved under refrigeration (5°C upto 72 hours. Samples of semen just before packaging (O hour) and at 24, 48 and 72 hours of preservation in straws and plastic vials separately were examined for progressive motility of spermatozoa, live sperm count, intact acrosome, bacterial load, GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase) activities. Progressive motility and live sperm count were estimated using conventional methods.

Intact acrosomes were counted in semen smears stained with giemsa stain as per Watson (1975). GOT and GPT activities were estimated using the method of Reitman and Frankel (1957). The bacterial load of semen was determined by pour plate method of Cruickshank *et al*, (1975).

The date were subjected to statistical analysis as per Snedecor and Cochran (1967) and compared between the two methods of packaging-straw and vial.

Average values for sperm motility, live sperm count, intact acrosome, bacterial load and transaminase activity of semen preserved for different hours in French medium straws and plastic vials have been prsented in Table.1.

There was no significant difference in quality between French medium straws and plastic vials preserved semen upto 72 hours as indicated by the values of 't' (Table 1), however percentage of progressively motile sperm, live sperm and spermatozoa with intact acrosome remained higher throughout the entire period of preservation in French medium straws. The bacterial load of the semen was also found to be lower though nonsignificantly in French medium straws. GOT and GPT release which was indicative of sperm cell injury (Graham and Pace, 1967) was also found to be similar in both methods of preservation. The apparently superior quality of chilled semen preserved in French medium straws as observed in the present study might be attributed to availability of greater surface area to volume ratio (Jondet, 1972), lesser agitation of spermatozoa (Sane *et al*, 1954) and lesser chance of leakage (Van Demark, 1949) in French medium straws as compared to in plastic vials. Marvin and Sullivan (1978) also reported superior quality of semen in French medium straws as compared to glass ampoule and attributed it to hygienic handling of semen in straws. From the point of hygiene and ease of handling, packaging of chilled semen in French medium straws appeared more suitable than that in plastic vial. However further study is suggested with larger samples for confirmation of the fertilityof french medium straws preserved semen.

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Table 1. Average (Mean ± SE) values for characteristics of chilled semen preserved in French Straw and plastic vial at different hours of preservation

Semen Characteristics	Method of Packaging	0 hour	24 hours	48 hours	72 hours
Motility (%)	Straw	74.41±0.40	65.00±1.95 (1.83)	55.83±1.42 (0.74)	48.33±1.23 (1.51)
	Vial		60.42±1.38	53.75±1.20	45.42±1.38
Live sperm count	Straw	89.50 ±70	83.83 ±76 (1.08)	78.02 ±0.58 (1.05)	73.83 ±0.74 (1.20)
	Vial		82.42 ±1.00	77.25 ±0.90	72.50 ±0.76
Intact acrosome (%)	Straw	93.92 ±0.43	89.42 ±0.85 (1.02)	84.08 ±1.24 (0.84)	79.42 ±1.34 (1.44)
	Vial		88.25 ±0.69	82.50 ±1.30	76.58 ±1.33
Bacterial load	Straw	186.67 ±98.68	372.50 ±155.77	417.08 ±161.12	490.33 ±200.24
	Vial		408.33 ±159.89	434.16 ±167.86	566.67 ±228.12
GOT activities (unit / ml / million)	Straw	0.64 ±0.03	0.94 ±0.06 (1.35)	1.02 ±0.06 (1.94)	1.48 ±0.09 (0.50)
	Vial		0.96 ±0.05	1.20 ±0.07	1.54 ±0.09
GPT activities (unit / ml / million)	Straw	0.31 ±0.01	0.36 ±0.02 (0.40)	0.42 ±0.04 (0.51)	0.57 ±0.04 (0.01)
	Vial		0.37 ±0.02	0.46 ±0.04	0.57 ±0.03

Figures in the parentheses indicate 't' value signifying the difference between straw and vial.

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Effect of Spermatozoan concentration on freezability of bull semen[1].

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ABSTRACT

The semen from pure bred(9) and cross-bred(8) bulls were diluted at a concentration of 15 x 10^6, 20 x 10^6 and 30 x 10^6 spermatozoa per 0.25 ml of frozen semen straw. The effect of dilution rate on the freezability was determined. The post thaw motility and livability in samples with 15 x 10^6 spermatozoan concentration differed highly significantly ($P<0.01$), and proved to be the best in comparison to 20 x 10^6 and 30 x 10^6 concentration.

The widespread use of artificial insemination technique of cattle has demanded the increased utilization of bulls with superior genetic ability. To meet this demand researchers have been made to determine the minimum number of spermatozoa per dose of insemination without sacrificing the quality and fertility of semen samples. A spermatozoan concentration of as low as 3 x 10^6 per dose of semen was reported to have normal conception rate (Jondet, 1969 and Jondet, 1972). But, Nair (1975) and Jani *et al.* (1984) opined that a minimum 10 million viable spermatozoa was necessary to have an overall conception rate of 50.00 per cent. Keeping this in view an investigation was made to find out the minimum concentration of spermatozoa required in frozen bovine semen and find out their motility and livability.

MATERIALS AND METHODS

Seventeen semen samples were collected from pure bred (9) and cross-bred(8) bulls of Frozen semen bank, Cuttack and processed for freezing. An initial dilution, with Tris egg yolk glycerol diluent (TEYG), was made to the neat semen to obtain 30 x 10^6 spermatozoa per 0.25 ml dose of insemination and further diluted to have 20 x 10^6 and 15 x 10^6 spermatozoa per 0.25 ml straws. The dilutions were made in such a way to get equal number of 0.25 ml straws of 30 x 10^6, 20 x 10^6 and 15 x 10^6 spermatozoan concentration, and these samples were frozen, equillibrating semen for 5 hours. The post thaw motility and live sperm percentage was estimated immediately after freezing.

RESULTS AND DISCUSSION

The prefreezing motility percentage in three dilution rate and the corresponding values at post thawing immediately after freezing is presented in Table 1. It was obvious from the study that the ejaculates from the pure bred and cross-bred bulls did not differ much with regard to quantitative and qualitative characters are concerned. The decrease in the post thaw motility as compared to the pre-freeze motility when the concentration of spermatozoa increase finds the support of Wettwer (1969). However, Graham *et al.* (1957) did not find positive correlation between the equillibration time and motility. Analysis of variance showed a significant difference in pre-freeze and post thaw motility at various concentration of semen ($P<0.01$) of both pure bred and cross-breds. The least significant range test revealed that in both type of semen samples the difference between 15 x 10^6 and 20 x 10^6 and 15 x 10^6 and 30 x 10^6 were statistically significant. Hence, the results indicate that 15 million spermatozoa was having the highest freezability which is in close confirmation with Miczkovic *et al.* (1973) who observed 5 to 10 per cent higher post thaw motility in higher dilutions of semen.

The overall post thawed live spermatozoa percentage in different sperm concentrations varied between 61.46 per cent to 64.54 per cent (Table 1) with a highly significant ($P<0.01$)

1. Part of the M.V.Sc. thesis submitted by the first author to O.U.A.T., Bhubaneswar.

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difference between the various concentration of semen samples. The post thaw live percentage between 15 x 10^6 and 20 x 10^6, and 15 x 10^6 and 30 x 10^6 in different semen samples were statistically significant ($P<0.01$). The better post thaw sperm survival in the concentration of 15 x 10^6 can be concluded that in a higher dilution the spermatozoa are exposed to a wider environment of dilutor components in comparision to the spermatozoa in higher concentration thus acquiring comparitively better protection.

The percentage of conception rate for inseminations at 5 hours equillibration time varies from 53.84±0.71 per cent for 20 million sperm concentration to 67.64±3.51 per cent in 30 million with 15 million sperm concentration showing the conception rate of 56.82±6.82 per cent for a total of 210 number of inseminations carried out. No statistically significant difference was noticed between the fertility rate and different concentrations, which is in agreement with Graham (1959) and Philipsen (1983). For economic point of view it can be advocated that a low concentration of 15 x 10^6 spermatozoa per insemination dose of 0.25 ml French straws and 5 hours equillibration time before freezing can be adopted without much detoriation in overall conception rate.

Table 1: Pre-Freeze motility and Post thaw motility and livability in different concentrations of spermatozoa (percentage) at 5 hour equillibration.

Spermatozcan 0.25 ml.	Concentration/	Pure Bred (9)	Cross-Bred (8)	Overall (17)
15 x 10^6	Pre Freeze motility	74.85±2.54	74.03±2.27	74.48±1.65
	Post Thaw motility	68.50±2.45	64.37±2.22	66.59±1.70
	Post Thaw livability	66.95±2.42	61.72±2.24	64.54±1.76
20 x 10^6	Pre Freeze motility	73.34±2.42	72.13±1.85	72.78±1.51
	Post Thaw motility	65.22±2.28	62.83±0.49	64.03±1.26
	Post Thaw livability	64.19±1.98	60.22±0.68	62.35±1.21
30 x 10^6	Pre Freeze motility	74.24±2.39	73.00±1.47	74.21±1.43
	Post Thaw motility	66.15±2.45	60.65±1.44	63.61±1.62
	Post Thaw livability	63.95±2.09	58.56±1.44	61.46±1.48

Mean±S.E.
Figures in parentheses indicates the number of ejaculates under study.

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Studies on Some Biochemical Constituents of Patanwadi Ram Semen*

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ABSTRACT

Estimation of some biochemical constituents of Patanwadi ram semen showed seasonal influences. Significant seasonal variations were observed in initial fructose and total cholesterol while total protein content was not affected by the season.

——x——x——x——

The study of biochemical properties of semen has assumed a considerable importance not only for evolving suitable semen extenders and preservation techniques but also as an aid for andrological assessment of rams. However, such studies on ram semen have not been carried out in detail. Hence the present study is an attempt to make available the information on some biochemical constituents of Patanwadi ram semen.

MATERIALS AND METHODS

Studies were undertaken on six adult healthy breeding rams of Patanwadi breed, belonging to A.I.C.R.P. (fine wool), Livestock Research Station, Gujarat Agricultural University, Sardar Krushinagar. The semen was collected at weekly intervals with the help of artificial vagina during pre-breeding (February-March), breeding (April to 2nd week of June) and post-breeding (Third week of June to end of July) season as determined by coinciding with the heat period in ewes. In all a total of 150 (25 samples from each rams) ejaculates were collected. For initial fructose biochemical procedure described by Mann (1964) was followed from the protein free filtrate, prepared from 0.1 ml of neat semen. For total protein and total cholesterol the weekly semen samples of all the six rams were pooled and seminal plasma was separated by centrifugation. In all a total of 25 pooled semen samples (25 samples from each ram) were analysed biochemically. Total protein was estimated by biuret method and total cholesterol was estimated as per Henly (1957). The data were statistically analysed as per Snedecor and Cochran (1967).

RESULT AND DISCUSSION

Initial fructose:

The initial fructose content (mg%) averaged 434.35±7.25 in Patanwadi rams. The present findings corroborate with the findings of Knight (1973) and Abdulla *et al.* (1974) in Ossimi and Rahmani rams and are also in close approximation with the findings of Markandya and Paragoankar (1990) in Osmanbadi buck semen. Fructose content in semen originates in the seminal vesicle under hormonal influences and the activity of sex glands from time to time (Mann 1964).

The observed highly significant ($P<0.01$) differences between seasons recorded highest value during breeding season which differed significantly from the rest of the seasons (Table-1). However, the differences in fructose content during pre-breeding and post-breeding seasons were not of real nature. Similar significant seasonal variations were recorded by Cupps *et al.* (1960) in Suffolk Hampshire and Rambouillet rams and Mittal (1980) in Magra and Marwari rams.

Total Protein:

The total protein content (g%) averaged 4.79±0.17 in seminal plasma of Patanwadi rams. These findings are comparable with the findings with the findings of Markandya and Paragoankar (1990). However, Chand *et al.* (1985) reported slightly lower values (3.25 and 3.41 g%) in Nali and Lohi breeds. This might be due to the differences in breed group. The protein contents during pre-breeding, breeding and post-breeding seasons are given in the Table-1. The observed differences between the seasons were not of real nature.

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Total Cholesterol:

The total cholesterol (mg%) averaged 24.84±2.74 in seminal plasma of Patanwadi rams with a range of 9.55-66.64 mg per 100ml. The total cholesterol content obtained during pre-breeding, breeding and post-breeding seasons is given in Table-1. The observed differences between the seasons were significant (P<0.01). The cholesterol content was highest during breeding season and lowest during post-breeding season. This could be due to well known differences in the levels of androgens between breeding and non-breeding seasons. But the cholesterol content during pre-breeding season differed significantly neither from breeding season nor from post-breeding seasons.

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Table 1. Average (Mean±S.E.) values of biochemical estimates of seminal plasma in Patanwadi rams during different seasons.

Sr. No.	Initial fructose (mg%) (neat semen)	Total Protein (g%)	Total Cholesterol (mg%)
Seasons: Pre-breeding (S_1)	419.78±14.60	4.61±0.37	23.47±6.18
Breeding (S_2)	454.96±9.35	5.01±0.06	26.98±3.39
Post-breeding	416.54±10.48	4.64±0.27	12.85±1.21
Average	434.35±7.25	4.79±0.17	24.84±2.74

Table 2. Mean square values of biochemical constituents for different seasons.

Sr. No.	Constituents	d.f.	M.S.S. Value	'F' Value
1	Initial Fructose	2	22743	5.0526**
2	Total Protein	2	2.40	2.49
3	Total Cholesterol	2	862.68	6.2459**

** Highly significant (P<0.01).

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Microbial flora of bovine semen and their antibiotic sensitivity pattern

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Many venereal diseases of bovines like trichomoniasis, Campylobacteriosis, Brucellosis, Listeriosis, Leptospirosis and possibily bovine tuberculosis have been transmitted extensively by AI. To inhibit the micro-organism in the semen before using for AI, some antibiotics like Penicillin, Streptomycin or Sulpha drugs are used in semen.

In the present study attempt has been made to find out the microbial quality of bovine semen as well as their antibiotic sensivtivity pattern.

A total of 12 breeding bulls maintained at bull centre, College of Veterinary Sciences, Pantnagar (Nainital) were used in the present study. About 2 ml, of semen was collected from each bull, asceptically in sterilized test tube and brought to the laboratory in an ice box. Seven semen samples were collected from each bull. Isolation and identification of bacteria were done as per method described by Cruickshank *et al* (1975). Blood agar plates (10% bovine blood) were used for the inoculation of semen samples. Inoculated samples were incubated aerobically at 37°C for 24-48 hours.

All the isolated strains were tested for *in vitro* sensitivity to 24 antibiotics by single disc diffusion method (Ellner, 1978) with commercially available biodiscs (Hi-Media Laboratories, Bombay, India).

A total of 88 semen samples were tested, of which 59 samples were found positive for one or more than one type of bacteria. Out of 59 positive samples, 48 and 11 yielded one and more than one type of bacteria respectively. Twenty nine samples were found negative for any type of bacteria. The different type of bacteria isolated frm bovine semen were *Staphylococcus aureus* (6), *Staphylococcus epidermidis* (13), *Streptococcus* spp (3), *E. coli* (11), *Citrobacter spp* (7), *Proteus spp* (8)Pseudomonus aeruginosa(7), *Bacillus spp*(3) and *Corynebacterium* spp (1). Brucella organisms could not be isolated from any sample of bovine semen. However, the isolation of *Pseudomonas aeruginosa* in the present study is significant as these orgnaisms are considered to be abnormal microflora of semen and may reduce the survivability of spermatozoa and fertility of cows. The organisms thus isolated presently are also distributed widely in nature and have been reported to be associated with wide variety of reproductive disorders in cattle (Roberts, 1971; Barth *et al*, 1981; Rahman *et al*, 1983; Saikia *et al*, 1987). A considerable variation was observed in the susceptibility patterns of different isolates against the various antibiotics tested during the study. The organisms were highly susceptible to Ampicillin (86.4%), Gentamycin (77.9%) and Chloramphenicol (76.27%) followed by sulphamathizole and Polymyxin B (33.8% each), Cephaloridine and Streptomycin (22.0% each), Nitrofurantoin, Triple sulpha and Methicillin (37.2% each), Vancomycin (20.3%), Oxytetracycline (45.7%), Erythromycin and Chlortetracycline (40.6% each), Cotrimaxazole (55.9%), Amoxycillin (44.0%), Neomycin (18.6%), Novobiocin (64.4%), Cloxacillin (32.2%), Tetracycline (54.2%), Nalidixic acid (28.8%) and Trimethoprium (59.3%), Kanamycin, Penicillin and Furazolidone (0.0%). This finding is in agreement with the findings of Saikia *et al* (1987). The combination of Penicillin and Streptomycin are the oldest and widely used preparation in semen dilutors and to reduce bacterial population. However, the present study revealed that these two drugs have little antibacterial effect on the bacterial flora of bovine semen. On the other hand drugs like Ampicillin, Gentamycin and Chloramphenicol have been found to be most effective. It is recommonded that one of these drugs should be used in semen dilutor in order to reduce bacterial load of semen.

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Effect of Glycerolisation Procedure on the Quality of Frozen Buffalo Semen

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In an attempt to standardise the glycerolisation procedure for buffalo semen a two step dilution was found to be significantly better to single step dilution at room temperature. Further to ascertain, the effect of addition of half of the portion of diluent having no glycerol and having 3% glycerol at room temp (20°C) and other half having 14% and 11% glycerol respectively after cooling to 5°C in cold handling cabinet, on quality of frozen semen in terms of forward motility, live count, acrosomal maintenance, percent sperm filtered through sephadex G-15 and release of Aspartate Amino transferase in extra cellular medium, the present studywas undertaken.

Ten adult Murrah buffalo bulls between the age group of 4 to 5 years and weighing 400 to 600 kg were selected. The semen collection was done once a week. The procedures for semen collection, evaluation, diluent (Tris - egg yolk - citric acid - fructose), dilution (two step dilution technique) and equilibration were as per Bhosrekar el al (1991).

Two treatments of glycerol addition were followed. In one 'A' portion of diluent contained 'No glycerol' and other 3% glycerol while 'B' portion of diluent was having 14% and 11% glycerol respectively, so that on final dilution the concentration of glycerol will be 7%. The quality control tests for frozen semen followed were post thaw motility (PTM) live count, acrosome maintenance, per cent sperm filtered through sefadex G-15 and aspartate amino transferase release (Bhosrekar et el 1991), to asses the effect of two glycerolization procedurs. From the figures of drop in PTM, live count, acrosome maintenance and AAT release the procedure of adding 3% glycerol to portion 'A' seems to be slightly better though statisticaly non significant (Table). This may be due to sensitization effect of glycerol on spermatozoa thereby reducing the risk of osmotic shock by exposing the spermatozoa to sudden higher concentration of glycerol. Bower *et al*(1973) also observed no obvious effect on the release of AAT when up to 2% glycerol containing diluent was added at 37°C. They further recorded that dilution with increased glycerol concentration at 37°C caused increased release of enzyme in Boar semen.

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Table. Effect of different glycerolization procedures

Sl. No.	Parameters	Values before freezing		Values after freezing and thawing	
		3% glycerol Mean + SE	No glycerol Mean + SE	3% glycerol Mean + SE	No glycerol Mean + SE
1.	% drop in forward motility	8.00 ±0.39	7.63 ±0.40	11.13 ±0.49	11.75 ±0.68
2.	% drop in live count	8.99 ±0.84	9.76 ±0.83	12.98 ±0.92	13.14 ±0.90
3.	% drop in acrosome maintenance	2.39 ±0.28	2.74 ±0.23	2.56 ±0.23	3.04 ±0.23
4.	% sperm filtered through sephadex G—15	—	—	48.69 ±1.25	48.69 ±1.25
5.	AAT Release	757.50 ±113.57	734.20 ±103.77	734.20 ±58.96	658.13 ±80.04

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Preculture Staining of Buffalo Follicular Oocytes

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A study was undertaken to determine the preculture meiotic (dictyate) stage of buffalo follicular oocytes required for *In-vitro* maturation. In all 20 ovaries from slaughtered buffaloes were aspirated with the help of 10 ml syringe fitted with 18 guage needle. Follicular fluid viewed under steriomicroscope and 21 oocytes were recovered. These oocytes were incubated in 3% sodium citrate solution (Kinis, 1988) for about two minutes and cumulus cells sorrounding oocytes were removed by repeated pipetting with fine glass pasteur pipette. These nude oocytes were then placed on a clean glass slide and compressed by coverslip and were sealed with rubber cement. These slides were fixed for 24 hours in acid-alcohol 1:3 (1 part glacial acetic acid and 3 parts ethanol). After 24 hours, the slides were stained with 1% aceto-orcein stain (1 g synthetic orcein powder + 45 ml glacial acetic acid + 55 ml distilled water, boiled and filtered, (Sanderson and Stewart, 1961) and observed under compound microscope to evaluate the meiotic stage of preculture oocytes.

Out of 21 oocytes examined 61.90% (13/21) possessed a germinal vesicle. Hunter *et al* (1972) showed 93% of preculture cattle oocytes possessed an intact germinal vesicle. Leibfried and First (1979) assessed nuclear configuration of pre-culture oocytes and found 84.40% (878/801) of all oocytes examined possessed germinal vesicle. Akufo *et al* (1987) found all except one (98.15%) out of 54 oocytes remained in the dictyate stage. All these scientists have worked on cattle ovaries. Our observations are lower than those reported by these investigators which may be due to species variation.

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Summer breeding in Surti buffaloes

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This study was planned to investigate the possible tools to improve breeding and fertility of buffaloes during summer months.

The experiment was conducted at All India Coordinated Research Project on Buffaloes, Livestock Research station, Vallabhnagar during May and June, 1989. The heat detection was done by parading vasactomized twice a day. Buffaloes in oestrus were artificially inseminated twelve hours after onset of oestrus and pregnancy diagnosis was done 60-90 days of last insemination. Buffaloes were exposed to showering 4 times between 11.30 A.M. to 3.00 P.M. during May and June.

Under non-showering group out of 12 buffaloes 10 (83.33%) exhibited heat and 3 (25.9%) become pregnant, while out of 64 heifers 37 (57.81%) exhibited heat and of which 25 (39.96%) were pregnant. The conception rate was 30 and 67.57 per cent respectively for buffalo and heifer. The conception rate was higher in heifers as compared to buffaloes although per cent animals exhibiting heat was significantly higher in buffaloes indicating that May and June months are more favourable for breeding of heifers as compared to buffaloes.

The results indicate that is is possible to bread buffaloes and heifers during off - seasons (May and June). The fertility level of buffaloes and heifers can be considered as fairly satisfactory although the fertility (81.13%) was less than that observed by Ray *et al* (1968) in buffaloes.

For improving conception rate in buffaloes 46 buffaloes were exposed to showering out of these 42 (91.31%) exhibited heat of which 18 buffaloes (39.13%) conceived with a conception rate of 42.86%. This is considerably higher than non-showering buffaloes. Out of above 46 lactating buffaloes, which was exposed to showering 35 were complete anoestrus and remaining 11 were normal cycling buffaloes. About 91 percent buffaloes exhibited heat in both the groups. However, the conception rate was lower by 9 per cent in anoestrus group.

The percent animals exhibiting heat was higher in showering group as compared to non-showering group. It therefore, appears that greater facility for thermolysis by frequent showering of water on the body of the buffaloes and heifers would have improved the conception rate by decreasing the parental loss in the same way as observed by Ray *et al* (1968) in buffaloes, where the conception rate was improved by 15 per cent.

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Reproduction Traits of Red Sindhi Halfbreds

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

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

FRS	JRS
Period 1 (1976 to 1981)	period 1 (1974 to 1975)
Period 2 (1982 to 1988)	Period 2(1976 to 1981)

Seasons

1. S_1 June to September
2. S_2 October to January
3. S_3 February to May

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

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

Where,

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

Collected data were used for calculation of genetic group differences.

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

Period of Calving:

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

Season of calving

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

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Table: Least - squares means of age at first calving and 1st calving interval (days) of Red Sindhi halfbreds

Effect	Age at first calving			Calving interval		
	n	Mean	SE	n	Mean	SE
			Friesian x Red Sindhi halfbreds			
Over all mean	28	1059.05	37.32	23	380.27	51.11
Period of calving						
P1 (1976 to 81)	14	1052.81	58.80	12	402.22	80.52
P2 (1982 to 88)	14	1065.27	43.69	11	358.32	59.83
Season of calving						
S1 (June to Sep)	11	1029.24	52.26	10	378.74	71.58
S2 (Oct to Jan)	14	1090.79	41.59	10	316.78	56.96
S3 (Feb to May)	3	1057.10	95.26	3	445.28	130.46
			Jersey x Red Sindhi halfbreds			
Over all mean	30	1007.99	67.86	27	384.51	30.45
Period of calving						
P1 (1974 to 75)	17	809.53[b]	103.72	16	415.30	46.54
P2 (1976 to 81)	13	1206.46[a]	100k.88	11	353.71	45.27
Season of calving						
S1 (June to Sep)	12	1116.33	114.02	12	436.87	51.17
S2 (Oct to Jan)	12	921.00	100.50	10	358.42	45.10
S3 (Feb to May)	6	986.66	143.75	5	358.24	64.50

Similar superscript did not differ significantly from each other.

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Studies on age at First Lambing in Sheep

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ABSTRACT

Age at first lambing of 488 ewe - lambs of Muzaffarnagri breed (M) and crossbreds with Dorset (D) and Suffolk (S) were studied under tropical conditions. The effects of genetic group, season and year of birth were determined on age at first lambing. Least square analysis of variance revealed significant effect of genetic group, season and year of birth on age at first lambing. Dorset x Muzaffarnagri (F1) had significantly the lowest age (589.46±36.98 days) at first lambing in comparision to other breeds. The lambs born in season-I (March - April) had significantly higher age at first lambing (785.59±20.08 days) than those born in season - II (September - October). Age at first lambing was highest in the ewe lambs born in 1978 (919.29±29.86 days) and it was lowest for the year 1984 (623.33±44.98 days).

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Age at first lambing is one of the most important trait of reproduction which has a great bearing on the flock productivity in sheep. Considerable variations have been observed among various Indian breeds of sheep on this trait. It is essential to study the various factors which affect the age at first lambing and altimately theoverall productive life of sheep. Accordingly the prsent study was under taken in Muzaffarnagri and its croses with Dorset and Suffolk and effect of genetic group, season and year of birth on age at first lambing was investigated.

MATERIALS AND METHODS

Ewes (native and crossbreds) after attaining the age of nine months were introduced for heat detection twice daily by parading vasectomised / approned rams in the flock, maintained under semi-intensive system of management. Age at first lambing refers to the age of an ewe from the date of birth to the date of first lambing and recorded in days. The data was recorded for a period of 8 years i.e. from 1977 to 1984. Only normal lambings were considered in the prsent study. Least square analysis of variance and least square means alongwith their standard errors were obtained for all the main effects such as genetic group, season and year of birth on age at first lambing as suggested by Harvey (1975).

RESULTS AND DISCUSSION

Least squares analysis of variance revealed that the genetic group and year of birth has highly significant ($P<0.01$) effect on the age at first lambing while the effect of season of birth was significant ($P<0.05$).

The overall age at first lambing averaged 733.558±17.079 days. The least square means for age at first lambing in different genetic groups were 702. 188±12. 846, 589.458±36.981, 918.643±63.490, 783.047±35.220, 664.799±55.792 and 743. 211±35.813 days for Muzaffarnagri, Derset x Muzaffarnagri (F1), Suffolk Muzaffarnagri (F1), Dorset x Muzaffarnagri (F2), Suffolk x Muzaffarnagri (F2) and 1/4S 1/4D 1/2M breeds respectively. It was observed that Dorset x Muzaffarnagri (F1) crosses had the lowest age at frist lambing (589.458±36.981 days) While Suffolk x Muzaffarnagri (F1) had the highest (918.643±63.490 days.). Among first generation, Dorset crosses lambed significantly at an early age where as second generation, Suffolk crossbreds were found to lamb at an early age. However, the native Muzaffarnagri ewes were observed to have the age at first lambing in between the two corssbreds.

Duncan's multiple range test revealed significant differences between Muzaffarnagri and the two crossbreds of first generation, where as the difference was a non significant among Muzaffarnagri and crossbreds of second generation and 1/4S 1/4D 1/2M Present finding is in close agreement with the reports of Amble and Malhotra (1968), Acharya (1972), Sinha *et al* (1979), Tomar (1985) and Reddy *et al* (1986) in various native breeds and crossbreds. However, Kishore *et al* (1982) observed a non-significant effect of genetic group on age at first lambing.

Season of birth influenced the age at first lambing significantly indicating that the ewe lambs born in season I had significantly higher age at first lambing (758.59±20.05 days) than those born in season II 708.52±20.73 days). Similar results have been reported by Malik *et al* (1978) in Rambouillet crosses with Chokla, Malpura and Jaisalmeri. Sinha *et al* (1979), Kishore *et al* (1982) and Reddy *et al* (1986) also reported significant effect of season on age at frist lambing in different sheep breed and breed crosses. However, the present results do not agree with the findings of Koul (1979) and Tomar (1985) who reported non-significant effect of season of birth on age at first lambing in first generation of Coimbatore, Corriedale and their crosses.

Year of birth was found to have highly significant effect on the age at first lambing. Ewe lambs born in the year 1977 and 1978 differed significantly than those born in year 1979 to 1984, however, the difference between 1977 and 1978 was non-significant. Age at first lambing was highest in the ewe lambs born in 1978 (919.28±29.86 days) while it was lowest for the year 1984 (623.33±29.86 days) while it was lowest for the year 1984 (623.33±44.98 days). The effects of year of birth on age at first lambing was observed to be significant in various Indian breeds and their exotic crossbreds as reported by Narayanaswamy *et al* (1976), Sinha *et al* (1979), Reddy *et al* (1984) and Tomar (1985) where as Reddy *et al* (1986) did not observe any significant effect of year.

Age at first lambing decreased from 919.29 days in the year 1978 to 623.33 days in 1984 with a difference of 296 days. Such an improvement over the 7 years period has a great bearing on the overall productivity of an animal. The drastic improvement study may be mainly due to genetic manipulation (Selection response) coupled with better management practices.

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Antibiotic Sensitivity Pattern of Microorganisms Causing Endometritis in Cattle

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ABSTRACT

Clinical gynaecological investigations revealed 34.67% of 2760 cows suffering from endometritis. Cervicoveginal mucus samples collected from 89 cows and 127 bacterial isolates were identified as *E.Coli* (31.49%) Bacillus (22.83%), Streptococcus (16.53%) Staph. aurius (12.59%), Staphylococcus (6.29%) Corynebacterium (6.29%) Pseudomonas (2.36%), Pasteurella and Klebsiella (078%) each. Invitro antibiotics sensitivity. revealed nitrofurazone the most effective antibiotics followed by chloramephenicol, kanamycin and tetracycline while trimethoprim was least sensitive.

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The genital tract infection infemales usually occurs during oestrus, coitus, artificial insemination and post-partum period. The most common reproductive tract infection has been observed by the authors through coitus using communal / local bulls for breeding purposes. Many workers reported the isolation of microorganisms and usefulness of antibiotics in the treatment of genital tract infection (Dholakia *et al* 1987, Sharma, 1988 Venketeshwaran *et al* 1991, Sharda *et a*/1991).

MATERIALS AND METHODS

A total of 89 cervico-vaginal mucus samples were collected aseptically from endometritis cases with sterile cotton wool swab and uterline glass catheters. The incidence of genital tract infection and invitro antibiotics sensitivity pattern of microorganisms prevelant in North West part of Rajasthan in cattle are presented in Table I and II. Each sample was incubated on blood agar and Maeconkey's media and the isolates thus obtained were subjected to various bacteriological tests and identified as per Cruickshan *et al* (1973). These isolates were further subjected to invitro antibiotic sensitivity testing using the disc supplied by Banner *et al* 1966. Seven antibiotics discs were used namely Gentamycin, Trimethoprim, Streptomycin Chloramphenical, Tetracyclin, Kanamycine and Nitrofurazone.

RESULTS & DISCUSSION

During 18 months period 2760 cow were presented in the clinics of Obstetrics and Gynaecology. Clinical gynaecological investigation revealed that 957 cows (34.67%) were suffering from endometritis. The incidence of endometritis cases during winter summer and rainy season was 34.36, 34.62 and 35.08 per cent, respectively and there was no significant seasonal variation.

The organisms were isolated from 69 samples (77.52%) out of 89 samples, while remaining 20 samples (22.48%) were found bacteriologically sterile. In all 127 isolates were investigated for the presence of microorganisms which were identified as E coli, Bascillus, Streptococcus, Staph aurius, Staphylococcus, Corynebacterium pyogenes, Pseudomonas, Pasturella and Klebsiella.

The percentage of E.coli was maximum (31.49) followed by Bascillus (22.83). The lowest percentage of Pseudomonas, Pasteurella and Klebsiella infection was recorded. In the most of the cases E.coli infection was recorded, and these findings are in close agreement with Sharda *et al* (1971). The antibiogram of isolates were sensitive to more than one antibiotic. The percentage sensitivity of isolates towards different antibiotics was Gentamycine 31.88%, Trimethoprim 23.18%, Streptomycin 31.88%, Chloramphenical 47.82%, Terramycin 31.88%, Kenamycin 31.88% Nitrofurazone 68.11%. The Nitrofurazone is highly sensitive in 68.11% followed by chloramphenical 47.82%

The finding in relation to sensitivity with chloramphanical is in agreement with that of Venketeshwaran and Rajeshwar (1991). Trimethoprim is found to be the lowest sensitive to the microorganism with 23.18%. However the results of Sharda *et al* (1991) are contradictory with the present findings. They suggested that combination of Gentamycin and Streptomycin had better results.

Table: I Incidence percentage of Microorganisms isolates in endometritis.

Microorganisms	Isolates	Incidence percentage
E.Coli	40	31.49
Bascillus	29	22.83
Streptococcus	21	16.53
Staph aurius	16	12.59
Staphylococcus	08	6.29
Corynebacterium pyogenes	8	6.29
Pseudomonas	03	2.36
Pasteurella	01	0.76
Klebsiella	01	0.78
Total	127	

Table-II Sensitivity percentage of antibiotics in endometritis.

Antibiotics sesitivity	Sensitivity	%Sensitivity
Gentamycine	22	31.88
Trimethoprim	16	23.18
Streptomycin	22	31.88
Chloramphenicol	33	47.82
Tetracycline	22	31.88
Kenamycine	22	31.88
Nitrofurance	47	68.11

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Microbiological Study of Gynaecological Infections in Cattle

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ABSTRACT

Studies on gynaecological infections in cows at "Cattle Breeding Farms" located at Kandivli, was undertaken. Thirteen isolates were obtained from the samples collected in the form of uterine tampoons. From five cows showing gynaecological problems aetiological agents were identified as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella* sp. Most of the infections were of mixed type; *E. coli* and. *S. aureus* were detected in all the cases. In one case Pseudomonas was found to be involved in the infection.

Antibiotic sensitivity patterns of the isolates were examined by disc sensitivity method. Maximum number of isolates viz., eight out off thirteen were sensitive to chloramphenicol, seven were sensitive to streptomycin. Interestingly twelve of these isolates were resistant to penicillin.

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Gynaecological infections in cattle are of serious concern for two major reasons; some of them can be the cause of transient or permanent infertility in cows (Deopurkar, 1990). Secondly these infections can be the source of zoonoses, (Koshi and Meyers, 1967; Lampert 1947).

The indiscriminate and prologned use of antibiotics in the absence of drug sensitivity results had contributed to the emergence of resistant strains of bacteria. Thus isolation, preliminary identification and determination of drug sensitivity of the causative organisms have become very important for effective therapy of gynaecological infections and to limit the development of drug resistant strains.

MATERIAL AND METHODS

The clinical samples used in the present study were from cows showing gynaecological infections. The clinical signs of infection were repeat breeding. Cervicovaginal samples were collected from by tampoons on the day of oestrous in Stuart's medium. They were put in semisolid nutrient agar in tubes 6" x 1" which were then wax sealed and transported to laboratory.

0.85% sterilised saline was added to above tubes and the contents were macerated well. The samples from five different cows were analysed.

The macerate obtained as stated above was used for enrichment cultures. Viz., Mannitol salt agar, Dettol agar, MacConkey's agar. Colonies obtained on above enrichment media were subjected to preliminary characterisation and pure cultures were then preserved on nutrient agar slant for future studies.

Different antibiotics used were ampicillin (10 µg), carbenicillin (50 µg) cefazolin (30 µg,) cephaloridine (30 µg,) cefotaxime (30 µg) chloramphenicol (30 µg), erythromycin (15 µg), neomycin (30 µg), penicillin-G (10 units), rifampicin (5 µg), streptomycin (10 µg).

The thirteen different pure cultures obtained were identified by biochemical testing.

RESULTS AND DISCUSSION

Thirteen different isolates were obtained and identified as *E. coli, S. aureus, S. typhi, P. aerugenosa.* The occurence of *E coli* was frequent.

The antibiograms of various isolates have shown that *S. aureus* strains found in most of the cases were highly sensitive to chloramphenicol, streptomycin followed by neomycin. Most of the strains of *E. Coli* isolated were sensitive to chloramphenicol, neomycin and streptomycin arranged in the order of sensitivity. *P. aerugenosa* was isolated from only one case and was resistant to all the antibiotics tested in our study. *S. typhi* found in one case was found to be sensitive to chloramphenicol, neomycin, cephaloridine and streptomycin while resistant to cefazolin, cefotaxime, penicillin and ampicillin.

Out of thirteen isolates maximum number of isolates viz., eight were found to be sensitive to chloramphenicol, seven to streptomycin, six to neomycin, four to cephaloridine and two to erythromycin.

The frequent occurence of *E. coli* strains in these infections reflect the possibility of infection caused by the opportunistic pathogen which must be otherwise the normal flora of the ulerine tract of the cow.

The drug sensitivity pattern revealed the sensitivity to chloramphenicol, streptomycin, neomycin, cephaloridine, erythromycin as 61.53%, 53.84%, 46.15%, 30.76%, 15.38%, respectively.

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Leptospiral Abortions in Sheep

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Seroprevalence of ovine leptospirosis has been reported in various parts of India (Paragoankar and Ramakrishnan, 1963; Sawhney and Saxena, 1967; Rajasekhar and Nanjiah, 1971). However studies on clinical ovine leptospirosis with abortion are limited. The present report deals with the serological study undertaken in aborting ewes.

MATERIALS AND METHODS

Sera samples from 118 sheep with the history of pyrexia, jaundice and abortion were collected in Tirunelveli district, Tamilnadu. Samples were tested by microscopic agglutination test (MAT) using four-day-old live cultures of the leptospiral serovars - *autumnalis, ballum, bataviae, canicola, grippotyphosa, hebdomadis, icterohaemorrhagiae, pomona* and *pyrogenes,* as suggested by Faine (1982). Sera which gave a titre of 160 and above were considered positive (WHO, 1967). Sera samples were also tested for brucellosis by rapid plate agglutination test.

RESULTS AND DISCUSSION

Of the 118 sera samples tested, 45 (38.14%) had leptospiral antibody titres ranging from 320 to 1280. Serovar *pomona* predominated (71.11%) followed by serovars *icterohaemorrhagiae* (20,00%) and *grippotyphosa* (8.89%) The sera samples were negative to *Brucella melitensis* or *Brucella abortus* antigens. Abortions in pregnant ewes occurred through out the stage of gestation. Sheep are considered to be relatively resistant to leptospirosis (Rajasekhar and Nanjiah, 1971). However, Nillar *et al.* (1977) and Ellis *et al* (1983) reported the occurrence of abortions, stillbirths and weak lambs in leptospiral infections in sheep, as observed in the present study. Further, high titres of 1280 were recorded in the present study indicative of recent infection in sheep. The study also proved the involvement of serovar *pomona* as the major serovar causing abortions and infertility in sheep in Tirunelveli district, Tamilnadu.

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Clinical Management of Antepartum Prolapse And Subsquent Induction of Parturition in A Murrah Buffalo.

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Antepartum cervico-vaginal prolapse of a Murrah buffalo was succesfully treated. The pregnancy was followed upto term and prolongned gestation of the buffalo was terminated with induced parturition. Response to the treatment for the cervico-vaginal prolapse and induced parturition in the case is discussed.

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Case Report A ten years old multiparious seven months pregnant Murrah buffalo from buffalo unit, Veterinary College, Udgir was presented to the clinic with a history of prolapse. The animal was also reported to have noticable vaginal and perineal relaxation. It was observed that a foot ball sized mass of vagina with closed cervix was hanging through vulval opening. The animal was straining intermittantly with concurrent rectal prolapse.

TREATMENT AND DISCUSSION

Antepartum prolapse : Injection xylocaine HCL 2% 5 ml was administered epidurally. Urinary bladder was releived by lifting the prolapsed mass. Thorough cleaning of prolapsed mass was carried out with lukewarm water and mild anteseptic solution. Anteseptic jelly was applied on prolapsed mass. Reposition of prolapsed cervico-vaginal mass was carried out. Vulval sutures were taken with Gartner's needle and umbilical tape. Injection Duvadilan containing isoxsuprine HCL (Duphar-Interphran Ltd, Bombay - 18) 30 mg were given daily intramuscularly for five days. Injection Proluton Depot containing progesterone (German Remedies ltd., Bombay-93) 250 mg. were given intramuscularly twice in a week for a month. Vulval sutures were removed on fifth day of prolapse.

Clinical management of the antepartum prolapse was successful and the pregnancy was continued smoothly upto term.

Prolonged Gestation : The animal failed to calve on expected date of calving. The gestation period was prolonged by 20 days without any signs of approach of parturition. Viability of foetus was monitored regularly by per rectal examinations.

Prolonged gestation observed in this case appears to have been caused possibly due to continuous progestrone administration. Though progestrone administration has successfully retained the prolapse till completion of gestation, it has further prolonged the gestation by about 20 days.

Induction of Parturition : Injection Dexona containing Dexamethasone (Cadila Vet,Ahmedabad-8) 20 mg and Injection Epidosin containing Valethamate Bromide (TTK Pharma Ltd.,Madras - 43) 40 mg. were given seperately along with 5% injection Dextrose saline 450 ml intravenously. The animal was kept under constant observation to record behavioural changes and signs of parturition. After 15 hours of treatment the animal became restless with frequent movements and looking at the haunches. The first water bag reptured 20 hours after treatment. The per vaginal examination revealed that the cervix was fully dilated and foetus was present in birth canal with normal parturient position. Slight traction was applied on the forelimbs securing the head of the foetus and a live male calf weighing 34 kg was delivered.

Vashista *et al* (1991) reported observations on Dexamethasone induced parturition in buffaloes with gestational problems. Although the dose of Dexamethasone used for induction of parturition was same in the present case with that of above, but injection Epidosin was also employed additionally for cervical dilatation. Time required for induction of parturition in the present case was less than 24 hours after the treatment as against the range of 24 to 30 hours in the above report. Mannual assistance during the parturition was not required essentially in the present case whereas this is almost mandatory in all cases as reported earlier. The overweight of the foetus could be attributed to prolonged gestation in this case.

Postpartum involution : Injection oxytocin 50 iu, was given intramuscularly immidiately after

calving for rapid uterine involution and placenta was expelled 6 hours after the parturition. Uterine involution of the animal completed after 25 days of parturition.

Retention of placenta invariably encountered in induction of parturition was not recorded in the present case. Involution of uterus was rapid in the present case possible due to use of injection oxytocin as against normal time of 30 days as reported by Arthur *et al.* (1989). The involution of the uterus was monitored regularly by clinicogynaecological examination and was completed by 25 days with no purperal complications. It appears, therefore, that the antepartum prolapse in a buffalo can be managed with progestrone therapy. The dexamethasone induced parturition along with use of epidosin and oxytocin may be beneficial to avoid possible purperal complications.

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Effect of Feeding Time on the onset of Oestrus in Friesian X Tharparkar Heifers

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ABSTRACT

From present study it is difficult to conclude about the effect of the time of feeding of concentrate on the onset of oestrus in Friesian x Tharparkar heifers. Although there was an increase in the number of oestrus heifers in the morning, when the concentrate was fed in the evening and Vice-cersa.

——x——x——x——

Certain managemental practices influence oestrus behaviour and its intensity in cows, specially in heifers. Some changes in managemental practice could help in increasing breeding efficiency of cows (Pandey, 1981). The present study was designed to observe the effect of change in the feeding time on the time of onset of oestrus in Friesian x Tharparkar heifers.

MATERIALS AND METHODS

The experiment was conducted on the 18 crossbred heifers (1/2 Holstein Friesian x 1/2 Tharparkar) maintained at Livestock Farm, Department of Animal Production & Management, Veterinary College, Jabalpur. The heifers, selected for the study, were of body weights between 180-300 kg. and age of 23-36 months, having normal reproductive organs. The heifers were randomly distributed into three groups considering their body weight and age. Prior to start of experiment, these heifers were maintained under uniform management and feeding practice prevailing on the livestock farm. Vasectomised bull was paraded among the heifers in morning and evening for heat detection and oestrus behaviour of heifers were recorded. Each group, consisting of six animals, was distributed as follows:-

Group I served as control, in which concentrate mixture was fed twice daily i.e. at 9.00 A.M. and at 4.00 P.M. Heifers of group II were offered concentrate mixture only once at 9.00 A.M. everyday, whereas, animals of group III were allowed concentrate mixtuure once daily at 4.00 P.M. Various visual signs exhibited by heifers during course of heat detection were recorded in the morning and evening.

RESULTS AND DISCUSSION

Time of Oestrus: Groupwise 75, 45 and 58 per cent of heifers exhibited oestrus in the morning, while 25,55 and 40 per cent came into heat in the evening in group I, II and III respectively but on overall, 60 per cent of heifers showed oestrous in the morning and 40 per cent in the evening. Awadelkariem and Mc Caughey (1984) with the help of television camera noticed very high percentage of heifers showing oestrus at 9.00 A.M. to noon with evening feed at 8.30 P.M. and heifers which were fed at 8.30 A.M., exhibited higher level of oestrus from 12.00k noon to 12.00 night.

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Factors Affecting Secondary Sex Ratio In Friesian - Sahiwal Crossbreds

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More female births are to be needed for running of a successful dairy enterprise, thus studies of sex ratio has its significance. The conception, birth and maturity are three vital points in the life of an animal, the sex ratio at these points are known as primary, secondary and tertiary sex ratio respectively. Amongst these, secondary sex ratio is most important and has been taken for the present study.

Data on 9375 calves born at four Militaryfarms during the period of fiveyears from April 1986 to March 1991 were taken. The data were divided into five periods on yearly basis and each year was, further, subdivided in to four seasons, i.e. Summer (May to July), Winter (Nov. to January), North-East Monsoon (August to October) and South-West Monsoon (Feb. to April). The two genetic groups of Higher cross (above 50%) and lower cross (50% and below) of Friesian bloods were made. The Chisquare test was applied to know the difference between the number of female and male births.

The overall sex ratio of male to female births was observed as 51.40 : 48.60 in the present study. Chisquare values were indicative of slightly higher percentage of male births but statistically insignificant, than those of females. Similar trends have been reorted by different workers indifferent breeds (Tomar and Singh, 1973 in zebu cattle; Slob, 1968 in Bos taurus; Tomar *et al*, 1976 and Patel *et al*., 1988 in Crossbred cattle and Chaurasia *et al*1985 in buffaloes). This tendency of higher male births was noticed in all the dairy farms, which ranged from 50.20 to 54.70 per cent. Chi-square values showed significantly higher male births in lower genetic herd but this difference was not noticed significant in higher crosses. Allthough seasonal variation had no significant influence on sex ratio, still increasing tendency of female births was observed from summer to winter months. Hussain and kumar (1984) also mentioned higher percentage of female births in winter season. Thus present study, in general, indicates that selection of sex-ratio of progeny may not be helpful in breeding programme.

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Treatment of Anoestrum In Cross-Bred Cattle With CoCu-H*

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Anoestrum in heifers and cows is the most common cause of infertility in cattle. Considering the importance of this condition and mineral deficiency as a possible etiological factor especially under field conditions, trials were carried out to study the efficacy of CoCu-H tablets to induce heat in anoestrous cows and heifers to restore fertility.

One hundred and fifty animals (40 heifers and 110 cows) with true anoestrum during routine field visits were included in the study. Ten cows and eight heifers showing true anoestrous condition were treated as control. The experimental animals were administered CoCu-H 2 tablets daily for 21 days.

Twentynine out of 40 heifers (72.5 per cent) and 90 out of 110 cows (81.2 per cent) showed oestrus at an average interval of 23.5 days after starting the treatment. The time interval from the beginning of treatment to the onset of oestrus averaged 28 days in heifers and 19 days incows. Among the control animals only two cows and one heifer evinced oestrus within a period of 62 days and 93 days respectively. All the animals showing oestrus were inseminated and 15 heifers (51.7 per cent) and 46 cows (51 per cent) conceived with an overall conception rate of 50.4 per cent. Among control animals also one cow conceived. Successful treatment of anoestrous animals by administration of minerals has also been reported earlier (Deshpande and Sane, 1977; and Pillai, 1980).

* CoCu-H by Respel Pharma containing Cobalt, Copper, Manganese, Magnesium, Zinc, Iron, Iodine, Selenium and Strontium

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Maternal Dystocia Followed by Uterine Prolapse in a Buffalo - A Case Report

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A buffalo with the history of straining and slight vaginal prolapse was presented to Veterinary Polyclinic, College of Veterinary and Animal Sciences, MAU, Parbhani. The buffalo was showing all the signs of approaching parturition. Per rectal examination revealed buffalo as advanced pregnant, foetus was live in anterior presentation and foetal parts were palpated intrapelvic. Per vaginal examination revealed that the external os uterus was partially dilated, cervical seal liquified and no further progress in first stage of parturition.

The buffalo was given Inj.Epidosin (TTK Pharma Ltd.) 15 ml and Inj. Oxytocin (Electrosol Pharma) 6 ml both intramuscularly and she was kept under observations for 6 hours. But there was no response to treatemnt. Thereafter Inj. Dexona (Cadila Veterinary) 8 ml was given intramuscularly. Per vaginal examination revealed dilatation of cervix. Foetus was removed manually by applying gentle traction. Following the withdrawal of normal live foetus, there was complete uterine prolapse. Epidural anesthesia was given with Lignocaine HC1 (BAIF Laboratories). The prolapsed portion was cleaned with potassium permagnate solution and repositioned manually and Furea boli (Eskayef Ltd.) were inserted intrauterine. Vulval matress sutures were placed. Buffalo was given Inj.Vetopen (Hindustan Antibiotics Ltd) 2.5 gm intramuscularly, Inj. Borocalcinate (BAIF Laboratories) 450ml intravenously and Liquid Furacin (Eskayef Ltd) 30 ml intrauterine for 4 consequative days. Vulval sutures were removed after 10 days. The buffalo showed uneventful recovery.

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Uterine Prolapse in Caprines

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Prolapse of the uterus is a common complication of the third stage of labour in the cow and the ewe and less frequently in the mare and bitch (Roberts 1971). The incidence of uterine prolapse recorded during 1985 - 1990 at the Madras Veterinary Hospital constitutes 4.6 per cent of the post - partum complications

Case History:

Case No.1 A local non-descript goat approximately of 8 years of age was brought to the Obstetrics unit of Madras Veterinary College (LAC-OP-5196) with the history that it gave birth to two live kids about 22 hours ago. Though kidding was normal shedding of placenta normal, it subsequently, developed complete eversion of the uterus.

Case No.2 A non-descript she goat aged 4 years was presented to the Obstetrics Unit (No. 4618) with the history that it had kidded 2 dead kids one fully grown and the other one premature 6 hours before. Complete eversion of the uterus with foetal membranes followed. The general condition of the animal was good.

Treatment:

The animal was put in dorsal recumbency with elevation of hindquarters as suggested by Arthur *et al* (1989) and adopting standard technique. Lubrication of uterine mass was done and reduction was performed with foetal membranes. Following successful reduction, no vulvar retention sutures were applied. Oxytocin 15 Iu. intravenously, the antibiotic therapy with Oxytetracycline bolus I gm intrauterine, antihistamines and 5% Dextrose saline as intravenous injection was given for 5 days post reduction. The recovery was uneventful in both the cases.

Discussion:

Prolapse of the uterus is considered to be an emergency and needs immediate intervention in order to save the life of the dam. In the present case, the part of the foetal membranes retained were not removed before reduction as they were fastly adherent. Arthur *et al* (1989) also advocated non-removal of the placenta. It is preferrable to leave them attached and return them with the uterus, failure to detach them at this stage will not significantly affect the prognosis when the animal is under antibiotic cover and sterile precautions during treatment. In the present case, supportive therapy with Oxytocin and antibiotic led to an uneventful recovery.

Acknowledgement: The author express their thanks to Dr. M.S. Dewan Muthu Mohamed, (since deceased), Director of Clinics, Tamil Nadu Veterinary and Animal Sciences University for taking interest in the Obstetrical and Gynaccological work and also for providing the necessary facilities.

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Hysterectomy of Everted Uterus In a Goat

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Eversion of the uterus is observed most commonly in the cow and ewe, occasionally in sows and rarely in dogs, cats and mares. Hysterectomy or amputation of everted uterus is undertaken only when replacement of a badly torn, lacerated, necrotic, infected uterus would result in death due to toxaemia (Roberts, 1971). The operation is also adopted as a last resort in those cases in which it is physically impossible to replace the uterus, beause of the unfavourable texture of the organ (Arthur, 1989). The prognosis is always guarded to poor because of the complexity of the surgical procedure and the deteriorating condition of the animal preceding operation. Although the prognosis is poor there are many recorded cases of successful amputation or hysterectomy. Hysterectomy of the everted uterus in a complicated post-partum eversion of the uterus in a goat is reported.

A female goat, aged about 5 years was brought to Veterinary Dispensary, Mukkulam, Kerala with postpartum eversion of uterus.The everted uterus was found hanging upto the hock. The endometrium was found to be severely lacerated with oedema and necrosis of mucosa. The everted mass was also found smeared with faeces, dirt and blood clot. Temperature and pulse of the animal were normal. Since reduction of the mass was impossible without rupture of the uterus, it was decided to carry out amputation of the everted uterus.

Epidural anesthesia was effected by administering 3 ml of xylocaine 2 per cent at lumbo-sacral site. A longitudinal incision about 7 cms long was made in the everted uterus on the dorsocaudal surface between the rows of caruncles. The uterine blood vessels in the mesometrium were carefully ligated in two places using No. 1chromic Catgut and the broad ligament was severed from the uterus by incising between the ligatures. The cranial portion of the vagina anterior to the everted cervix was drawn out of the vulva. A stout transfixing and encircling ligature using twine was applied on the vagina anterior to the everted cervix. Then the everted mass was excised at the body of the uterus caudal to the everted cervix. The stump was closed by putting continuous sutures using No.1 Chromic Catgut and replaced into the pelvic cavity through the vulval opening.

The animal was given Penidure LA-24** and Tetanus toxoid***. The goat had an uneventful recovery in a week's time.

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* Xylocaine - Astra
** Penidure LA-24 - Wyeth
*** Tetanus toxoid - Serum institute of India

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A Monostomus Dicephalus Male Buffalo Calf Monster

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It is wellknown that conjoined twins arise from a single ovum and are monozygotic. Further, it is reported that duplications may lead to doubling of the cranial end of the body, while the caudal end remains single and the duplication in the cranial region - Dicephalus or two heads with distomus or monostomus is occasionally seen (Roberts, 1971).

A similar case was reported in a male buffalo calf born to a 7 years old buffalo cow. The buffalo cow had completed 3 lactations and was presented with a history of dystocia. The pervaginal examination revealed anterior longitudinal presentation with lateral deviation of head. While correcting the lateral deviation of head it was noticed that another head was attached to the fetus and later that was also corrected and the delivery was effected with forced extraction. When the fetus came out of the birth canal, it was noticed that two seperate heads were attached to a single body and the calf was alive for about an hour subsequent to which it died. According to Roberts this type of congenital defect can be classified as a Monostomus Dicephalus buffalo calf monster with two fore limbs, two hind limbs with a single spine and a tail.

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A Dorsoventral Postcervical Band In a Parous Cross Bred Cow

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Developmental defects of the Mullerian duct at the region of the cervix produce conditions such as Uterus didelphys, double external os of the cervix or a dorsoventral postcervical band. Invariably all these conditions would lead to dystokia and occasionally to retention of the placenta in the first calving itself (Arthur *et al*, 1989). An unusual case of the persistence of a dorsoventral postcervical band in a parous cow is reported.

A crossbred cow aged about 6 years belonging to the University Livestock farm, Mannuthy, which had one calving was slaughtered being sterile and its reproductive organs were harvested for detailed study. The cow had calved only once nearly 2 years back and had retained placenta which was manually removed. The left ovary was 2.9 x 1.9 x 1.6 cm in size with 2 medium sized follicles measuring about 1 cm and a few regressed corpora lutea. The right ovary was 3 x 2x 1.5 cm with fewsmall sized follicles. Neither of the ovaries reveal any functional or regressing corpus luteum. There was lesion suggestive of extensive perimetritis. The external os of the cervix revealed a dorsoventral postcervical band about 4 cm in width, which gave the appearance of a true double external os of the cervix. There was no connection between the external os of the cervix and the band. The fact that the cow did not suffer from dystokia in the first calving inspite of this band is quite baffling. It is possible that the foetus could have negotiated through one side of the band without any impediment and hence an uneventful calving. The retention of the placenta could be on account of the placenta getting entangled in the band.

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Torsion of Pregnant Horn in a Cow - A case Report

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Torsion of uterus is commonly a complication of late first stage or early second stage of parturition (Arthur *et al*, 1982) and occasinally occur during advance pregnancy in bovines. In multitocous animals, specifically the bitches, the rotation of pregnant horn (Jones and Joshua, 1982) as well as non-pregnant horn (Hower *et al*, 1980) has been reported. The present report puts on record an unusual case of torsion of pregnant horn in front of the intercornual ligment in cattle.

Case History, Diagnosis and Treatment:

A crossbred cow of 3 years age and in full term was presented to the Veterinary Clinics of the Punjab Agricultural University, Ludhiana (SU-541,1990) with the history that the animal did not show any signs of parturition at the due date. The animal had shown signs of abdominal pain and impending parturition i.e. relaxation of pelvic ligaments and letdown of milk about two months ago which subsided subsequently. Feeding and water intake by the cow was normal at the time of presentation

The vulva and vagina was narrow and hand could not enter it. On per-rectal examination cervix, body of uterus and left non-pregnant horn along with the ovary could be palpated in the pelvic cavity while the pregnant right horn had about 180° left side torsion. The fluid thrill in the pregnant horn was present. Cotyledons, fetal parts and fremitus could not be appreciated.

Laprohysterotomy was performed from right paralumbar fossa and about 15 litre brownish fluid was evacuated. Detorsion was achieved following extraction of seven month fetus along with loosely attached dry and leathery placenta. Animal recovered uneventfully thereafter following antibiotic, analgesic and sterioid therapy.

Torsion of uterus affects individual animals, the etiology of which is still obscure. The various maternal predisposing factors put forward for its occurrence include instability of the pregnant uterus in the pendulous abdomen of the animal due to slipping or falling. The high incidence of uterine torsion in cows could also be due to sub illial attachment of mesometrium (Arthur *et al*, 1982), however, in the present case, the involvement of broad ligament was not evident where only the pregnant horn revolved along its longitudinal axis without the involvement of either body of uterus or the non-pregnant horn. Similarly, involvement of single pregnant horn in torsion had been reported earlier in bitches and cats (Jones and Joshua, 1982).

Asymetry and imbalanced suspension of the pregnant horn created by non-gravid horn is responsible for the torsion of uterus Sloss and Dufty, 1980) which proved to be contrary in the present case. Thus, the assumption that uterus rolls towards and over the non-gravid horn during torsion (Cuq and Agba, 1975 and Frerking *et al*, 1975) is doubtful. This contradiction gains support from the earlier findings that the uterine torsion can occur in a didelphic uterus also (Dhaliwal *et al*, 1988).

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Treatment of Mucometra in Cross - Bred Cattle

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Mucometra or the accumulation of mucus secretions in the bovines uterus has been reported to be associated with various anomalies of the reproductive tract or secondary to cystic ovarian diseases and hyperestrogenism (Mc Entee, 1990; Rameshkumar *et al.*, 1987; Roberts, 1986). The present report is based on four clinical cases of post-service closed mucometra (0.02% of the total 4565 infertility cases) attended at the Gynaecology Clinic of the College of Veterinary Science and Animal Husbandry, Anand during the year 1991-92.

HISTORY AND CLINICAL EXAMINATION

The animals were brought to the hospital with the history of prolonged anoestrous (5 to 11 months) after natural or artificial breeding. All the four cows were apparently healthy and in their first to third lactation. Clinical examination of these cows revealed enlarged, fluid filled uterus, located deep in the abdominal cavity and the cervix was stretched beyond the pelvic brim resembling 4-8 months of gestation in different animals. However, no characteristic definite sign of pregnancy could be palpated. Corpora lutea were present on the ovaries of two animals. Per-vaginal examination revealed closed cervix. No abnormal secretions were observed in the vagina or fornix.

THERAPY

All the four cows were treated with a single injection of Dinoprost tromethamine (Lutalyse, Unichem Lab., Bombay) 25 mg i.m. and Dexamethasone sodium (Dexona - Vet, TTK Pharma., Bombay) 20 mg i.m. Three of the four cows responded to the treatment with relaxation of cervix within 72 hours. Approximately 12 to 20 litres of clean mucoid discharge was voided by the animals after relaxation of cervix. The clinical examination on 7thday post-treatment revealed involuted uterus and resumption of cyclic ovarian activity as indicated by the presence of growing follicles or corpora lutea. At this stage 2.5 g Strepto-Penicillin (Dicrysticin-S, Sarabhai Chem., Baroda) was administered intrauterine in 20 ml of sterile distilled water to check the uterine infection. Sexual rest was adviced for two cycles. One of the three cows conceived to second insemination following sexual rest, while the other two did not report for follow-up.

The fourth cow which did not respond to above treatment was subjected to laparo-hysterotomy. Approximately 20 litres of mucoid fluid was evacuated from the uterus. Dicrysticin-S 2.5 g was infused i.ut. for next three days. Clinical examination on 10th day post-operative revealed complete involution of uterus. The animal was, however, not brought for the follow-up afterword.

DISCUSSION

The clinical cases reported in the present studywere with the history of post-service anoestrum. Mc Entee (1990) reported 14 cows with mucometra secondary to cystic follicular degeneration and some of the cows showing signs of nymphomania. However, in the present study, none of the cows had shown nymphomania. Congenital malformations of cervix and / or uterus were reported as possible causes of mucometra in bovines by McEntee (1990) and Roberts (1986). However, the clinical examination of the pleuriparous cows reported in our study did not reveal any such lesion. It is possible that the mucometra might have occurred due to anovulation and persistant graafian follicle for a prolonged period or a follicular cyst resulting in hyperestrogenism followed by resorption / degeneration of the cyst in the due course of time. Roberts (1986) reported that the fertility in cows having mucometra is questionable. In the present study, only one confirmed pregnancy was obtained out of four cows treated

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