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EDITOR

Dr. A. S. Kaikini
Ph.D., F.R.V.C.S. (Sweden)
Ex–Dean, Faculty of Veterinary Science, PKV

Correspondence Address :
Editor, The Indian Journal of Animal Reproduction
B-306, Ujwal Flats, Rahate Colony, Jail Road,
Nagpur-440022.

The Indian Society for the Study of Animal Reproduction

(Regd. No. Bom. 253/78)

Correspondence Address :

Hon. Secretary, Indian Society for the Study of Animal Reproduction
C/o Department of Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Marathwada Agricultural University,
PARBHANI-431402 (Maharashtra State)

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CONTENTS

NATIONAL SYMPOSIUM ON "RECENT TRENDS IN FERTILITY MANAGEMENT OF FARM ANIMALS" AND VII ANNUAL CONVENTION OF INDIAN SOCIETY FOR THE STUDY OF ANIMAL REPRODUCTION AT

Department of Animal Reproduction College of Veterinary and Animal Sciences, Mannuthy-680 651, Trichur, Kerala State.

AUGUST 22nd to 24th, 1988

Organised by :

Kerala Agricultural University, ISSAR and Indo–Swiss Project, Mattupetty

Persons interested to participate in the Seminar should send the Registration Fee by Bank Draft (DD) to the Organising Secretary before 15th July 1988 : Delegate Fee Rs. 150/–. Accompanying adult Rs. 100/– and Child Rs. 50/–.

For further details contact : The Organising Secretary, National Symposium on Fertility Management of Farm Animals, Department of Animal Reproduction, College of Veterinary and Animal Sciences, Trichur–680 651, Kerala State.

Dr. C. P. N. IYER

Professor and Head, Department of Animal Reproduction

Organising Secretary

Editorial....

We congratulate the Govt. of India for their recent kind gesture of reducing the customs duty on some of the essential inputs required for enhancing the genetic and production potential of Indian cattle by frozen semen technology, clinical therapy of hormonal infertility and embryo transfer technology. A copy of the Customs Notification No. 27/88 is published elsewhere in this issue for the benefit of our readers.

This notification restricts the exemption of customs duty in excess of 25% ad valorem for certain hormones and drugs, only to the Government Departments. The cattle owners and marginal farmers whose animals are attended to by private veterinary practitioners and service agencies are denied this facility with resultant prohibitive cost of Prostaglandin $F_2\alpha$ which is the obvious drug of choice.

In the First Asian Congress on Animal Reproduction held at Bombay in December 1985, it was estimated that there are over 8 million cases of hormonal infertility in cattle and buffaloes in India. Thus, besides other hormonal preparations, at least 5 million doses of Prostaglandin $F_2\alpha$ will be required per annum for treatment of such cases spread over Govt., Semi-Govt. and private sectors. The present prohibitive cost of $PGF_2\alpha$ seriously affects the 4·9 million cases of cows and buffaloes of breedable age, belonging to small and marginal farmers and private cattle breeders including the IRDP beneficiaries.

The only solution to this problem lies in the timely and ready availability of cheaper $PGF_2\alpha$ preparations for animal use. This is a peculiar veterinary gynaeco-clinical aspect which is very vitally linked to productivity of milch cattle and buffaloes. We therefore feel that the customs duty exemption be extended for import of raw material to the private sector Pharmaceutical industry involved in manufacture of $PGF_2\alpha$. The finished product could thus be available at a reasonable (40% less than the present) rate which will benefit all the consumers involved in milk production industry.

We sincerely request the twin doyens at the helm of Animal Sciences in the Country-the Deputy Director General (Animal Sciences), Indian Council of Agricultural Research and the Animal Husbandry

Commissioner with the Govt. of India, New Delhi, to initiate such a process early at the Govt. of India level.

Recently, the Technology Mission Adviser to the Prime Minister, Dr. Sam Pitroda announced that very shortly a National Dairy Commission is to be set up at the highest level, with the sole objective of intensifying milk production in the country. We therefore appeal to our dynamic young Prime Minister Shri Rajiv Gandhi to take a positive step as suggested above, in furtherance of the cause for an all round speedy socio-economic development of the Nation in its relentless onward march towards the 21 st Century.

EDITORIAL BOARD

Customs Notification

No. 27/88-Customs

GSR (E) – In exercise of the powers conferred by sub-section (1) of Section 25 of the Customs Act, 1962 (52 of 1962), the Central Government, being satisfied that it is necessary in the public interest so to do, hereby exempts the goods specified in the Table hereto annexed and falling within Chapters 29, 30, 84, 85 or 90 of the First Schedule to the Customs Tariff Act, 1975 (51 of 1975) when imported into India by a Government Department administratively concerned with the Animal Husbandry from–

(a) so much of that portion of the duty of customs leviable thereon which is specified in the said First Schedule, as is in excess of the amount calculated at the rate of 25 percent ad-valorem; and

(b) the whole of the additional duty leviable thereon under section 3 of the said Customs Tariff Act.

TABLE

(1) Automatic straw filling and sealing machine
(2) Automatic printing machine
(3) Programmed biological freezer
(4) Automatic semen analyser
(5) Photometer for semen analysis
(6) Overcheck Pregnancy detecting kit
(7) Freeze drier
(8) Tuberculin syringe
(9) Pregnant mare serum (PMSG)
(10) Prostaglandin PGF_{2} Alpha
(11) Human chorionic Gonadotropin hormone (HCG)
(12) Follicular stimulating hormone (FSH)
(13) Leutinizing hormone
(14) Lencomycin powder.

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Spermiograms Of Holstein Friesian Bulls And Their Relationship With Fertility

D. N. MOHANTY[1] and Y. G. DUGWEKAR

Department of Obstetrics and Gynaecology, College of Veterinary Science, Punjab Agricultural University, Ludhiana-141004

ABSTRACT

A total of 175 ejaculates from 17 healthy Holstein Friesian bulls of 13 to 65 months age were studied for their semen characteristics which were correlated with the fecundity rate of 8 bulls.

The mean values of different semen characteristics studied – volume, mass activity, initial motility, live sperm concentration and abnormalities of head, acrosome, mid-piece and tail obtained from a total number of 175 collections were : 4·89 ml, 2·74, 70·44%, 1323·41 millions/ml of semen, 5·46%, 5·16%, 2·40% and 4·88% respectively.

Individual motility registered correlation with sperm head abnormality ($r = -0·40$); percent live spermatozoa correlated with individual motility ($r = 0·87$) and head abnormality ($r = -0·45$) and sperm concentration in millions/ml of semen correlated with individual motility ($r = 0·93$), unstained spermatozoa ($r = 0·89$) and head abnormality ($r = -0·44$).

The mean incidence of total head, acrosome, mid-piece and tail defects studied in different age groups of experimental bulls revealed highest incidence of head (6·15%) and tail (7·80%) abnormality in group 1, acrosome defects (6·69%) in group 4 and mid-piece defects (3·75%) in group 2, respectively. Significant differences ($P < 0·05$) were observed among various groups in the occurrence of tail and acrosomal defects.

The average fecundity rate of 8 bulls was found to be 57·62 percent with significant correlation with mass activity ($r = 0·87$), individual motility ($r = 0·90$) and live sperm ($r = 0·81$).

* * *

Fertility of a bull is solely dependent on the qualitative and quantitative character of semen produced. Though certain environmental and managemental factors can influence the characteristics of semen, but the change in the character can only reflect the conditions of genital organs. The spermiogram of a bull is the key characteristic and it should be optimum in assessing the fertility of that bull. The present investigation was carried out in the Livestock breeding and Dairy farm, Patiala to correlate the sperm characteristics with fertility in Holstein-Friesian bulls.

Materials and Methods

Semen from 17 healthy adult Holstein Friesian bulls was collected with an artificial vagina. Ten ejaculates were

[1] Present Address : Lecturer Grade I, Department of Gynaecology, Orissa Veterinary College, Bhubaneswar-751 003.

studied from each bull for macro and microscopical examination.

Semen volume was noted from the graduated semen collection tube. Colour, density, mass activity and individual motility was graded as per the norms laid down by Zemjanis (1970). Sperm concentration was estimated as per Herman and Madden (1953) with the improved Neubauer counting chamber. The percentage of live and dead sperm was estimated by means of differential staining technique (Blom, 1977). Morphological spermatozoan abnormalities were studied both in Giemsa and unstained semen samples preserved in buffered formal saline as per Saacke (1970). Fecundity rate of each bull from which semen was collected was calculated on the basis of data for fertility available at Indo-Swiss Project, Patiala.

Results and Discussion

The mean volume of ejaculated semen from Holstein Friesian bulls under study was 4·89 ml, with a range of 2·90 to 7·43 ml, which concurs with Foote *et al.* (1977). The mean mass activity of experimental bulls was found to be 2·74 (4 point scale).

The mean progressive individual motility observed (70·44%) differs from the findings of other workers which might be ascribed to differences in age, ambient temperature and season.

The mean live sperm count (79·07%) in the present investigation is in conformity with the results obtained by Bishop *et al.* (1954). Live sperm count was found to highly correlate ($r = 0·87$, $P < 0·01$) with initial motility of spermatozoa which denotes that increase in live sperm percent with rise in percent motile sperm, might indicate improvement in epididymal function with advancement of age.

The mean sperm concentration in ejaculates was found to be 1,323·41 millions/ml. It was observed to have high significant correlation ($r = 0·93$ and 0·89) with initial motility and live sperm count. This significant positive correlation between sperm concentration and live sperm count might imply increase in the production of live sperm along with increase in sperm output, accompanied with age.

The mean head abnormalities was 5·46% which has a low significant correlation ($r = -0·40$, $-0·45$ and $-0·44$) with percent motile sperm, percent live sperm and sperm concentration, which might indicate decrease in head abnormalities with attainment of mature age and increase in percent motile and live sperm count.

The mean percentage of acrosome abnormalities was found to be 5·16 percent. The discrepancy of the result as compared to other workers might be due to procedures adopted to study the abnormalities using giemsa stain (Saacke, 1970). Significant differences in acrosomal defects obtained between different age groups might relate to the inclusion of varying number of bulls of poor freezability in different age groups and wide variations in the incidence of acrosomal abnormalities between bulls (Table 1).

The mean percentage of middle-piece and tail abnormalities were found to be 2·40 percent and 4·88 percent. The significant differences obtained in the incidence of mid-piece and tail defects might be due to uneven number of bulls included in different groups studied.

The average fecundity rate of Holstein-Friesian bulls under study was recorded to be 57·62 percent. Highly significant ($P < 0.01$) correlations were obtained ($r = 0.87$, 0·90 and 0·81) in respect of mass activity, individual sperm motility and live sperm count, with the fecundity rate of bulls.

Sperm head and tail abnormalities were non-significantly correlated with fecundity rates. The lack of significance between fecundity rate and spermatozoan abnormality in the present investigation might be due to low incidence of abnormal spermatozoa.

The correlation among the parameters might signify better fecundity rate, using semen samples endowed with higher number of motile and viable spermatozoa.

The non-significant correlation of volume of semen and sperm concentration with fecundity rates might be explained from the utilisation of limited number of sperms in diluted semen, inseminated regardless of increased output of sperm with the advancement of age (Thibier and Colchen-Bourlaud, 1972).

Acknowledgements

The authors are thankful to the Director, Indo Swiss Project, Patiala and Dean, College of Veterinary Science, P.A.U., Ludhiana for providing the necessary facilities for conducting the research.

Table 1 : Variations in Seminal characteristics of Holstein-Friesian bulls.

Group	Age (months)	Sperm Conc. (millions/ml)	Live Sperm %	Individual motility (%)
		1	2	3
1	20–29	1074·00 ± 90·01	76·95 ± 1·55	68·00 ± 4·50
2	30–39	1155·29 ± 152·96	78·22 ± 2·01	68·96 ± 1·38
3	40–49	1385·83 ± 133·59	78·57 ± 2·18	70·08 ± 1·67
4	50 & above	1482·85 ± 115·88	81·20 ± 1·54	73·03 ± 0·56

Group	Age (months)	Total Head defects (%)	Total Acrosome defects (%)	Total M.P. defects (%)	Total Tail defects (%)
		4	5	6	7
1	20–29	6·15 ± 1·90	5·33 ± 1·07	3·23 ± 1·17	7·80 ± 1·45
2	30–39	5·15 ± 1·36	3·25 ± 0·52	3·75 ± 1·15	4·74 ± 1·45
3	40–49	5·76 ± 0·78	5·09 ± 0·69	2·20 ± 0·45	4·06 ± 0·55
4	50 & above	5·09 ± 0·48	6·69 ± 0·82	1·24 ± 0·34	4·77 ± 1·39

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Fructose And Citric Acid Concentration And Its Relation With Seminal Attributes In Bull Semen

A. K. Barik, B. N. Mohanty, S. K. H. Ray and D. N. Mohanty

Department of Gynaecology, Orissa Veterinary College, Bhubaneswar–751003

ABSTRACT

A total of seventy ejaculates from 4 pure bred and 5 cross-bred healthy bulls were examined to determine the relationship between physical and biochemical properties of semen.

The initial fructose and citric acid levels were 358·69 mg and 362·82 mg; 444·27 mg and 453·57 mg/100 ml in pure and cross-bred bulls, respectively. The semen of pure bred showed significantly higher ($P<0.01$) initial motility and live sperm, but no difference was found in the sperm concentration. The difference of citric acid concentration in the seminal plasma, between and within bulls was highly statistically significant ($P<0·01$).

* * *

Fructose and citric acid are two major biochemical components contributed by seminal vesicle in ruminants. The former being utilised by spermatozoa in vivo and vitro and the latter contributes the buffering mechanism of semen (Mann, 1964). Both substances are related to normal functioning of reproductive glands. Various authors have claimed a positive correlationship between initial fructose and metabolic activities of sperms (Redenz, 1933, Anderson, 1946, and Singh and Sadhu, 1978), whereas

Mann (1948) did not regard the same as a means to measure the metabolic activities of sperms. Rothschild and Barnes (1954) and Doicheva *et al.* (1978) found a positive correlation between citric acid and fructose content and also sperm concentration. The present investigation was aimed to assess the relationship of initial fructose and citric acid content of bull semen with the seminal attributes.

Materials and Methods

Collection of bull semen was made by standard method in the Department of Gynaecology. Routine evaluation of semen was conducted in respect of individual motility (Zemjanis, 1970), sperm concentration (Herman and Madden 1953) and live sperm concentration (Blom 1950). Initial fructose concentration and citric acid estimations were carried out according to Mann (1948) and Oser (1965) respectively. Fifty-one collections were made from pure bulls and 19 from cross-bred bulls. Data was analysed as per Snedecor and Cochran (1967).

Results and Discussion

The average motility of pure bred and cross bred bulls was 82·84 ± 0·77 and 75·26 ± 2·57 percent respectively, which was statistically significant. The percent of live sperms was 84·37 in pure bred and 75.63 in cross bred semen, the difference being statistically significant (Table 1). These findings were in close conformity with those of Kodagali (1963), Rao and Rao (1975) and Rao and Rao (1980). However, Saxena and Tripathy (1978) reported a low value for cross bred semen. It is therefore suggested that the higher values of motility and livability in pure breds might be due to genetic superiority of the qualitative characters, though there was statistical difference in pure bred and cross breds. The mean values obtained in this experiment were in normal range, with an individual variation of 50 to 90 percent in motility and 50 to 91 percent livability among cross breds. This difference was due to individual variations.

The sperm cell concentration (millions/ml) in the pure bred (1,003·92 ± 50·79) was lower than those of cross bred bulls (1,176·31 ± 16·13). It was higher than those reported by Saxena and Tripathy (1979) and Rao and Rao (1980), but is in close conformity with Kodagali (1963). The differences between the cross bred and pure breds are not statistically significant. The variation in the sperm concentration is due to sexual development, maturity of the bulls, feeding, reproductive health, size of the testes, season and geographic region (Salisbury, 1978). In the present study the sperm concentration might be less as the pure bred bulls were used extensively.

Initial Fructose Concentration

The mean initial fructose levels estimated in the present investigation were 358·69 and 362·82 mg/100 ml of semen (Table 1) in pure bred and cross bred bulls respectively. The differences between and within the bulls were statistically not significant. The values obtained in this experiment are in close agreement with Lopatko (1969) and Schons (1972). Higher initial fructose concentration has been reported by Singh and Sadhu (1978), Mohanty (1981) and Osman (1981). Lower value of fructose in the present study may be due to frequency of collection, breed and individual variation or due to some unobserved pathological condition of accessory glands

which have not been investigated (Mann, 1964 and Chaudhary and Sadhu, 1981).

Citric Acid Concentration

The mean citric acid content of the semen samples of pure bred and cross bred bulls investigated were 444·27 and 453·57 mg/100 ml. respectively (Table 1). The difference in citric acid content between and within bulls was statistically significant. These results were in conformity with the findings of Schons (1972) and Schons *et al.* (1974) but contradictory to Rothschild and Barnes (1954) and Doicheva (1979) who reported higher values. Factors such as breed (Schons, 1972); frequency of ejaculation (Boryczko, 1971); deficient in circulatory testosterone (Humphrey and Mann, 1949) and pathological condition of the testes and seminal vesicles (Schons *et al.* 1974) have been attributed to the variations in concentration of citric acid in seminal plasma in this investigation. The present findings indicate definite relationship between biochemical and physical characteristics in regulating their quality during processing and preservation.

TABLE 1 : Seminal attributes in bulls

Bulls	Initial motility %	Live sperm %	Sperm conc. millions/ml	Initial fructose conc. (mg/100ml)	Citric acid conc. mg/100ml
Pure bred N–51	82·84±0·77	84·37±0·81	1003·92±50·79	358·69±12·14	444·27±25·01
Cross bred N–19	75·26±2·57	73·63±2·51	1176·31±16·13	362·82±16·13	453.57±48·73
Over all N–70	80·78±0·97 t = 49·43*	81·72±1·03 t = 48·95*	1050·71±45·30 t = 0·696 NS	359·81± 9·81 F = 0·31 NS	452·22±22·97 F = 2·97*

Mean ± S. E. * Significant at p 0·01 level N. S. = Not Significant

N = Number of ejaculates studied

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Effect Of Extenders And Freezing On The Biometrics Of Goat Sperm Head*

B. C. DEKA[1] and A. R. RAO[2]

College of Veterinary Science, Tirupathi-517502, Andhra Pradesh, India

ABSTRACT

Fifteen ejaculates, 3 each from 5 indigenous goats, were extended without seminal plasma in egg yolk citrate fructose glycerol, Tris egg yolk citric acid fructose glycerol, skim milk egg yolk fructose glycerol and raffinose egg yolk glycerol extenders and frozen using liquid nitrogen vapour. Eosin-nigrosin stained semen smears were prepared from fresh semen, after cooling to 5° C, after equilibration and after freezing and thawing. Ten morphologically normal unstained

* Part of the Ph. D. Thesis submitted by the first author to the A. P. Agricultural University, Hyderabad.

Present Address : 1. Associate Professor, Dept. of Gynaecology, College of Vety. Science, Khanapara, Guwahati-781 022, Assam.
2. Dean, P.G. Studies, A. P. Agricultural University, Rajendranagar, Hyderabad-30.

sperms were selected at random in each smear and measurements of head length and head breadth were made at a magnification of 1000X using an ocular micrometer. The head length and head breadth of sperm varied from 8·122 ± 0·031 to 8·159 ± 0.031 micron and from 4·243 ± 0·011 to 4.286 ± 0·015 micron in different extenders at different stages of processing of semen. The head length and head breadth of sperm varied significantly ($P<0.01$) between bucks but not between extenders, between stages of processing of semen and due to interactions.

* * *

An ideal extender must maintain normal morphology of sperm during freezing in order to ensure better fertility. A few studies were made to record the effect of freezing on the biometrics of bovine sperm (Dui jn, 1974; Wells *et al.*, 1974 ; Bandopadhyay *et al.*, 1983). Perusal of available literature revealed no such study on goat sperm. The present work was undertaken to record the effect of four extenders on the biometrics of goat sperm head during freezing.

Materials and Methods

Fifteen ejaculates, 3 each from 5 indigenous goats, collected using artificial vagina were used for this study. If a buck donated less than 0·8 ml of semen, a second ejaculate was collected and pooled with the first ejaculate. After removing the seminal plasma as per Deka and Rao (1984), the sperms with little entrapped fluid were extended in egg yolk citrate fructose glycerol (Mathew, 1974). Tris egg yolk citric acid fructose glycerol (Hahn, 1972), skim milk egg yolk fructose glycerol (Rajkonwar *et al.*, 1977) and raffinose egg yolk glycerol (Paggi, 1971) by split sample technique. The dilution, cooling, equilibration, freezing and thawing were done as described by Deka and Rao (1985). The eosin-nigrosin stained semen smears were prepared as per Blom (1977) at different stages of processing of semen *viz.*, fresh semen, after primary dilution and cooling to 5° C, after equilibration and after freezing and thawing. Ten morphologically normal unstained sperms with clear outline of head were selected at random in each smear and measurements of head length and head breadth of sperm were made at a magnification of 1000X using an ocular micrometer. The head length and head breadth of sperm included only the maximum length and breadth of sperm head as described by Mukherjee and Bhattacharya (1949). The data were analysed using three-factor analysis of variance as per Snedecor and Cochran (1968).

Results and Discussion

The mean head length and head breadth of sperm in fresh semen were 8·129 ± 0·032 and 4·244 ± 0·010 microns respectively (Table 1). It was observed that during different stages of processing of semen in the four extenders, the mean head length and head breadth of sperm varied from 8·122 ± 0·031 to 8·159 ± 0·031 micron and from 4·243 ± 0·011 to 4·286 ± 0·015 micron, respectively. The head length and head breadth of sperm differed significantly ($P<0.01$) between bucks, but not between extenders, between stages of processing of semen and due to interactions. This is in agreement with the observations of Bandopadhyay *et al.* (1983) in buffalo sperm. On the contrary, Dui jn (1974) observed decrease in head length, head breadth and projected head area of bovine sperm after glycerolization and after freezing and thawing. On the other hand, Wells *et al.* (1974) observed

no effect of freezing on head length, but did record significant decrease in head width of bovine sperm.

It is evident from the results of this study that neither freezing nor the extenders studied significantly affect the biometrics of buck sperm head.

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The authors are thankful to the Principal, College of Veterinary Science, Tirupati for providing the facilities for this study.

TABLE : 1 Head length and head breadth of buck sperm (Mean* ± SE) at different stages of processing of semen in egg yolk citrate fructose glycerol (EYCFG). Tris egg yolk citric acid fructose glycerol (TEYCAFG), skim milk egg yolk fructose glycerol (SMEYFG) and raffinose egg yolk glycerol (REYG) extenders.

Stages	Head length (μ)				Head breadth (μ)			
	EYCFG	TEYCAFG	SMEYFG	REYG	EYCFG	TEYCAFG	SMEYFG	REYG
Fresh semen	8·129 ± 0·032				4·244 ± 0·010			
After cooling to 5° C	8·142 ±0·031	8·122 ±0·031	8·148 ±0·031	8·151 ±0·033	4·243 ±0·011	4·261 ±0·011	4·258 ±0·013	4·278 ±0·015
After equilibration	8·154 ±0·031	8·151 ±0·032	8·152 ±0·029	8·143 ±0·030	4·255 ±0·011	4·258 ±0·012	4·257 ±0·011	4·281 ±0·015
After freezing and thawing	8·140 ±0·031	8·138 ±0·031	8·159 ±0·031	8·156 ±0·034	4·262 ±0·012	4·260 ±0·012	4·279 ±0·015	4·286 ±0·015

* 150 observations

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Bacteriological Studies On Pre-Frozen And Frozen Semen Of Bucks Of Different Breeds

S.N. SINHA[1], B.K. SINGH, B.K. TIWARY[2] and A.K. SINHA

Department of Gynaecology, Obstetrics and A. I. Ranchi Veterinary College, Ranchi–834 007.

The present investigation reports bacteriological studies of semen collected from 11 bucks representing Jamnapari, Barbari and Black Bengal breeds. Semen samples numbering 99, comprising 33 each of neat, diluted and frozen, were investigated. The average bacterial load per ml of neat semen from Jamnapari, Barbari and Black Bengal bucks were 4,477; 5,505 and 6,988 organisms respectively. Corresponding figures in diluted semen were 26,554; 15,832 and 37,851 organisms respectively, while in frozen semen the corresponding figures were 11,075; 6,293 and 6,981 organisms respectively. Among bacterial isolates, 40·72 percent were gram negative bacilli, 31·14 percent gram positive cocci, 22·75 percent gram positive *bacilli* with spores and 5·39 percent were gram positive bacilli without spores. *E. coli* was highest in predominance (8·38 %). *Staph epidermidis* (7·18 %) had the highest frequency among gram positive cocci, *Bacillus coagulans* (7·18 %) was most frequent among gram positive bacilli with spores, while *Corynebacterium pyogenes* (3·59 %) was found to be most frequent in occurrence among gram positive bacilli without spores.

Semen is quite vulnerable to microbial contamination and it is difficult to

Present Address :

1. Dept. of Gynaecology, Obstetrics & A. I., Bihar Veterinary College, Patna–800 014.
2. Dept. of Microbiology, Ranchi Veterinary College, Ranchi–834 007.

collect and process semen free from micro-organisms despite adoption of strict hygienic measures. Quantum of microbial load and type of micro-oganisms present in the semen have great impact on the success of artificial insemination programmes. There are few reports on bacteriological quality of bovine semen (Reddy *et al.*, 1971; Naidu *et al.*, 1982; Kher and Dholakia 1984). The present study was undertaken to investigate bacterial load and isolates in neat, diluted and frozen semen from bucks of Jamnapari, Barbari and Black Bengal breeds.

Materials and Methods

Semen was collected twice a week, with the help of sterilised artificial vagina from 4 Jamnapari, 4 Barbari and 3 Black Bengal bucks maintained under identical managemental conditions. Semen samples were diluted in Tris egg yolk fructose citric acid extender. After dilution, penicillin G Sodium 1000 i. u. & streptomycin 1000 microgram were added per ml of the diluted semen. Small quantities of neat and diluted samples were transferred to sterilised vials under strict sterile precautions, for bacteriological studies. Samples were frozen in Landshut straws as per Fougner (1976) and Bhandari *et al.*, (1982). Samples stored in liquid nitrogen for 7 days were investigated for bacteriological quality. The neat, diluted and frozen samples which were investigated were from the same ejaculate. For the study, 3 ejaculates from each buck, numbering 33 neat and their counterpart 33 each of diluted and frozen samples were investigated. Thus, a total of 99 samples were studied for bacterial load and isolates. The bacterial count of the samples was estimated by pour plate count method. Primary isolation of organisms was done on sheep blood agar plates. In furtherance of identification, the isolates of 4 broad groups of organisms viz. gram positive cocci, gram negative bacilli, gram positive bacilli with spores and gram positive bacilli without spores were put to various morphological biochemical and sugar fermentation tests. Results obtained were analysed as per criteria tables available in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbson, 1974).

Results and Discussion

The average bacterial load per ml of neat semen was 4477, 5505 and 6988 organisms in Jamnapari, Barbari and Black Bengal bucks respectively. In diluted semen the corresponding figures were 26554, 15832 and 37851 organisms, while in frozen semen the corresponding figures were 11075, 6293 and 6981 organisms respectively. The bacterial isolates were gram negative bacilli (40·72 %), gram positive cocci (31·14 %), gram positive bacilli with spores (22·75 %) and gram positive bacilli without spores (5·39 %). Among gram negative bacilli, *Escherichia Coli* had the highest frequency (8·38 %) whereas the frequency of others were *Pseudomonas aeruginosa* (7·18 %), *Proteus morgani* (3·59 %), *Aerobacter aerogenes* (3·59 %), *Paracolobacterium intermedium* (1·79 %), *Alcaligens faecalis* (1·79 %) *Citrobacter freundi* (3·59 %), *Klebseilla aerogenes* (1·79%), *Aeromonas liquefaciens* (1·79 %), and unidentified organism (1·79%) Among gram positive cocci, *Staphylococcus epidermidis* (7·18 %), *Staphylococcus aureus* (3·59%) *Micrococcus spp.*(2·99%), *Streptococcus fecalis* (3·59 %), *Streptococcus lactis* (5·39 %), *Streptococcus agalactiae* (1·79 %), *Streptococcus dysgalactiae* (1·79 %), *Streptococcus uberis* (2·99 %),

and *Enterococci* (1·79 %) were isolated. The gram positive bacilli with spores were *Bacillus coagulans* (7·18 %), *Bacillus polymixa* (4·79 %), *Bacillus cereus* (7·18 %) *Bacillus subtilis* (1·79 %) and *Bacillus brevis* (1·79 %). The gram positive bacilli without spores were *Corynebacterium pyogenes* (3 59 %) & *Corynebacterium Xeroxis* (1·79 %). This bacterial load is comparable to that in bull and buffalo bull semen (Naidu *et al.* 1982, Kher and Dholakia, 1984). The overall mean of bacterial count in the diluted semen was on higher side. It may be due to the fact that the extender containing egg yolk fructose and buffer was quite conducive to the growth of micro organisms. Penicillin G sodium and Streptomycin were added in the extender which indicated that some of the organisms which were present in the semen were insensitive to Penicillin and Streptomycin. Low bacterial count in frozen semen samples was obviously due to the effect of freezing. Bacterial species isolated from buck semen were by and large similar to those recovered from bull, buffalo bull and boar semen, with difference in frequency of occurrence of a particular species (Naidu *et al.* 1982, Kher and Dholakia 1984, Tamuli *et al.*, 1984). The study revealed that addition of antibiotics in the dilutor and storage of semen at ultra low temperature (–196° C) in liquid nitrogen were not of much help in rendering semen samples free of microbial contamination since they merely suppressed multiplication of bacteria, but failed to stop it.

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Abnormalities In Landrace Boar Spermatozoa

M.K. TAMULI and C.K. RAJKONWAR

Department of Gynaecology, Obstetrics and Artificial Insemination, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781 022 Assam

The fertility of semen bears a high degree of relationship with the various abnormalities of spermatozoa. The report on the incidence of various abnormalities of spermatozoa in Landrace boars involving head, midpiece, tail and acrosome is scarce. The present study was undertaken to record the incidence of different types of sperm abnormalities in boar semen.

Materials and Methods

A total of 126 ejaculates were collected at three days interval from five Landrace boars of 13 to 15 months age, by 'simple fist' method using a dummy. Two semen smears were prepared from each ejaculate. One was stained with Carbol Fuchsin-Eosin (Lagerlof, 1934) to detect the various abnormalities of head and midpiece and the other was stained with Giemsa (Watson, 1975), to record the various acrosomal abnormalities at a magnification of 1000X. The incidence of proximal protoplasmic droplets, free heads and tail abnormalities were studied in neat semen smears (Hancock, 1959) of each ejaculate at a magnification of 450X under the phase contrast microscope. In each smear, percentage of abnormalities was counted out of 200 sperms. The sperm abnormalities were clasified as per Blom (1972) and Cerovsky (1977). Acrosomal abnormalities were classified as per Saacke (1970) and Blom (1973). Statistical analysis of the data was done as per Steel and Torrie (1980).

Results

The mean incidence of total head, midpiece and tail abnormalities was recorded as 2·01 ± 0 12%, 17·01 ± 0·88% and 1·20 ± 0·13%, respectively with 2·92 ± 0·15% total major sperm cell defects and each varied significantly ($P < 0.01$) between boars.

Under the major classification of head abnormalities, the mean incidence of different defects was : double forms, 0·007 ± 0·005%; pear shaped heads, 0·35 ± 0·04%; narrow at the base. 0·16 ± 0·03%; abnormal contours, 0·10 ± 0·02%; small abnormal heads, 0·12 ± 0·02% and free pathological heads, 0·007 ± 0·005%, whereas the mean incidence under the minor classification of head abnormalities was : narrow heads, 0·25 ± 0.05%; small normal heads, 0·22 ± 0·03%; elongated heads, 0·20±0·05%; giant heads-enlarged, globular or short broad, 0·40 ± 0·04% and free normal heads, 0·16±0·03%. The underdeveloped, decapitate and diadem heads could not be detected.

The total minor midpiece abnormalities, constituted by the abaxial implantation defect alone, ranged from 1·00 to 38·00% (mean 14·98 ± 0·85%), Under the major classification of midpiece abnormalities, the mean incidence of

different defects was : cork screw, 0·07 ±0·02%; other midpiece defects viz., tail stump-thickened, filliform or split, 1·05 ±0·10%; proximal protoplasmic droplets, 0·84±0·07%; pseudodroplets, 0 01±0·01% and 'Dag' defect, 0·15±0·2% The incidence of tail abnormalities recorded was : distal protoplasmic droplets 0·26±0.03%; simple bent tails, 0·86±0·12%; tails bent along the protoplasmic droplets 0 03±0·01% and terminally coiled tails, 0·03 ± 0·01%.

The mean incidence of total acrosomal abnormalities was 3·11±0·16% and varied significantly ($P < 0.01$) between boars. The mean incidence of different acrosomal abnormalities was : knobbed, 0·57 ± 0·06%; Swollen, 1·46 ± 0·12 %; ruffled, 0·4 ± 0·05%; separated completely, 0·45 ± 0·06%; separated incompletely, 0.07±0·01% and incomplete, 0·10±0·02%.

Discussion

The incidence of total head abnormalities was found to be lower than the findings of Murty (1974). The individual abnormalities of sperm heads under the major and minor classifications were found to be quite minimum and comparable with that reported by Cerovsky (1978). The total incidence of midpiece defects, excluding the abaxial implantation was lower than that reported by Murty (1974). The abaxial implantation of midpiece–the most noteworthy feature in boars–is not considered to be a sterilizing defect as in bull (Bierschwal and Hindrikse, 1959) and did not influence the non-return rate even when the incidence was as high as 20 to 80 per cent. The significant variation in head and midpiece abnormalities between the boars, recorded in this study supports the findings of Murty (1974). The incidence of total tail abnormalities was lower than that recorded by Murty (1974) and Sreekumaran (1974). The incidence of total acrosomal abnormalities was quite low. The knobbed spermatozoa, identical to persistent acroblast (Cerovsky, 1981) were found to be within the normal range.

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Studies On Some Blood Constituents In Normal Cycling, Fertile And Infertile Repeat Breeder Crossbred Cows

M.K. AWASTHI and K.G. KHARCHE

Department of Veterinary Obstetrics & Gynaecology, College of Veterinary Science & Animal Husbandry, Jabalpur–482 001 (M. P)

ABSTRACT

Investigation was conducted on 30 (J × Gir & H. F. × Gir) crossbred cows to study blood constituents in normal cycling, fertile and infertile repeat breeder cows. The blood glucose level averaged 54·21±2·55, 50·51±1·90 and 53·96±3·35 mg % in normal cycling, fertile and infertile repeat breeder cows, respectively, on day of estrus with statistically non-significant difference among three groups. The serum calcium level in corresponding groups was 7·86±0·12, 8·93±0·63 and 8·43±0·77 mg %, the difference among groups being non-significant. The infertile repeat breeders had significantly lower ($P<0.01$) inorganic phosphorus level than other two groups. The serum cholesterol level averaged 101·69±7·55, 115·91±8·28 and 118·84±0·05 mg %, respectively, in normal cycling, fertile and infertile repeat breeder cows with statistically non-significant difference. Among three groups in which haemoglobin concentration averaged 9·06 ± 0·29, 8·98 ± 0·29 and 8·85 ± 0·34 mg %, the difference was non-significant.

* * *

Considerable evidence exists in the literature on the role of specific nutrients influencing the fertilizability of ova and its survival in most species of domestic livestock (Lamond, 1979). Certain biochemical constituents in blood during estrus have been found to be associated with the fertility status of cows and their reproductive behaviour (Kumar *et al.* 1986). Hence the present investigation

was undertaken to ascertain the role of glucose, calcium, inorganic phosphorus, cholesterol and haemoglobin in normal cycling and repeat breeder cows.

Materials and Methods

The study was conducted on 30 (J × Gir & H. F. × Gir) crossbred cows maintained at the All India Co-ordinated Research Project on Cattle, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

Control group comprised of 10 normal cycling crossbred cows. Blood samples were collected from each cow during estrus phase prior to insemination. Treatment group consisted of 20 repeat breeder crossbred cows. Blood samples from all the cows of this group were collected during estrus phase after gynaeco-clinical examination, prior to insemination. Following insemination, all the animals were closely observed for return to estrus and later subjected to pregnancy diagnosis by rectal palpation on day 45 and were grouped in two subgroups as fertile estrous group consisting of cows diagnosed as pregnant on day 45 post insemination and non-fertile estrus group which included cows returning to estrus or diagnosed as non-pregnant on day 45.

Results and Discussion

The mean blood glucose level in the present study was found comparatively higher in normal cycling cows (54·21± 2·55 mg %) as compared to fertile repeat breeder (50.5±1·90 mg %) and infertile repeat breeder (53·96±3·35 mg %) with non-significant difference among three groups. These findings approximate with the findings of Lamothe and Guay (1970). Sharma *et al.* (1984) and Kumar *et al.* (1986). Hafez (1969) reported that since FSH is a glycoprotein, carbohydrate portion is essential for the biochemical activity of hormones. McClure (1965) opined that blood glucose level may be used to assess the reproductive function in dairy herd. In the present study none of the cows showed weak estrus and non-significant difference in blood glucose level was recorded. Hence, blood glucose level probably does not affect their fertility status.

The average serum calcium level (8·93±0·63 mg %) was higher in fertile repeat breeders, while normal cycling cows recorded lowest (7·86±0·42 mg %) serum calcium level with non-significant difference among three groups.

These findings are in agreement with the findings of Lamothe and Guay (1970) and Sharma *et al.* (1984). Morrow (1969) reported that calcium deficiency did not affect reproductive performance of the cows. The role of phosphorus in regulation of estrus system and fertility is well established. Bhaskaran and Abdullakhan (1981) reported that marginal deficiency of phosphorus is sufficient to cause disturbance in pituitary ovarian axis without manifestation of specific deficiency symptoms. Infertile repeat breeders had significantly lower (3·73±0·29 mg %) inorganic phosphorus level than normal cycling (5·06±0·19 mg %) and fertile repeat breeder cows (4·99±0 25 mg %). Similar observations were reported by Kumar *et al.* (1986).

There exists a correlation between the gonadal steroids and cholesterol metabolism (Robinson, 1957). In the present study, the infertile repeat breeder cows had higher serum cholesterol level (118·84±7·05 mg %) than other two groups

with non-significant difference among three groups. Similar findings were reportted by Sharma *et al.* (1984). The level of serum cholesterol is influenced by pituitary FSH which suppresses the production of cholesterol (Lynn *et al.* 1965).

In the present study, the normal cycling cows recorded higher (9·06±0·29 mg %) haemoglobin level, while infertile repeat breeder cows had (8·85±0 34 mg %) lowest value with non-significant difference among three groups, thus suggesting that haemoglobin probably does not play significant role in their fertility status. These findings are in agreement with the findings of Kumar *et al.* (1986).

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Serum Concentration Of Certain Minerals In Anestrus Cows And Buffaloes

Y.P.S. DABAS[1], S.P. SINGH[2] and O.P. SAXENA[3]

College of Veterinary Science, G. B. Pant University of Agriculture & Technology, Pantnagar-263145 (Nainital) U.P.

ABSTRACT

Serum concentrations of calcium, inorganic phosphorus, magnesium, sodium, potassium, copper and zinc were estimated in normal and anestrus crossbred cows and Murrah buffaloes. The anestrus cows and buffaloes were found to have significanty lower concentration of inorganic phosphorus and copper in the serum compared to the normal cycling animals. Supplementation of anestrus animals with Nuvimin Forte (Sarabhai Chemicals) restored serum inorganic phosphorus, copper and other mineral concentrations and 45 out of 60 anestrus cows and 27 out 46 anestrus buffaloes returned to normal heat within one month.

* * *

Dietary mineral elements are known to affect the physiological functions in general and reproduction in particular (Hidiroglou, 1979). Besides working as co-factor or activators of enzyme systems, the elements like calcium, have been found to sensitize the female tubular genitalia for the action of hormones (Moddie, 1965). Deficiency or excess of mineral elements like sodium, phosphorus, copper and zinc have been found to be associated with subnormal fertility and anestrus conditions (Ahlswede, 1972).

In the present study, serum concentration of certain minerals in normal and anestrus cows and buffaloes were monitored to investigate the inter-relationship between the mineral levels and the prevailing anestrus condition in the dairy animals.

Materials and Methods

A total of 77 crossbred cows and 61 adult Murrah buffaloes aged 3–8 years, reporting at college clinics and Government Veterinary Hospital, Rudrapur constituted the experimental animals. Based on the rectal examination and past history, these animals were divided into 2 groups.

Gr. I. Control : Seventeen crossbred cows and 15 Murrah buffaloes served as controls. These animals were showing normal estrous cycle and were free from any abnormalities of the genital organs.

Group II. Anestrus animals : Sixty crossbred cows and 46 Murrah buffaloes which did not exhibit estrus for more than one year following parturition,

1. Asstt. Professor, Department of Vety. Clinics.

2. Prof. and Head, Vety. Physiology, Himachal Pradesh Agriculture University, Palampur-176 061.

3. I/c Vety. Clinics.

constituted this group. Their ovaries were found to be soft and small without any developing follicles. All the animals were being maintained by the owners under traditional husbandry practices.

Serum samples were obtained from the control as well as anestrus animals and analysed for calcium (Spandrio, 1964), inorganic phosphorus (Goldenberg and Fernandez, 1966), magnesium (Basinski, 1965), sodium and potassium (Flame photometrically), copper (Gubler *et al*, 1959) and zinc (Devies, 1968).

After collecting the initial blood samples, each anestrus animal was offered 60 g Nuvimin Forte (Sarabhai Chemicals) and ad lib licking salt brick daily for 15 days. Ovaries of these animals were also massaged per rectum twice a week and each animal was observed for signs of heat for one month. Serum samples of these animals were again collected at the end of one month period and analysed for aforesaid mineral elements. The results were statistically analysed as per Snedecor and Cochran (1967).

Results and Discussion

The serum concentration of calcium, magnesium, potassium and zinc were within normal range and did not differ significantly between cycling and anestrus animals (Table 1). However, the level of serum inorganic phosphorus was found to be significantly lower ($P < 0.05$) in anestrus cows and buffaloes, compared to the normal cycling animals. The significance of optimum dietary phosphorus supply and its effect on reproductive processes has been studied by several investigators. Snook (1958) reported depressed or irregular estrus in lambs and cows which were grazing on phosphorus deficient pastures. There was slightly subnormal inorganic phosphorus content in the blood of these animals. In the experiments of Scharp (1979), the fertility of dairy cows with subnormal serum phosphate was found improved when defluorinated super phosphate was added to their drinking water.

In the present study, while the concentration of serum sodium was within normal range in buffaloes, its level in anestrus cows was found to be significantly lower than the cycling cows. Correlations have been made between the sodium status and infertility, using the sodium concentration in the saliva as the criterion. Ahlswede (1972) found that with concentration of sodium lower than 144m Eq/l, irregular estrus occurred in cows. Although salivary sodium was not monitored in our experiments, it is likely that lower serum sodium in anestrus cows might have contributed to this condition.

The most striking change among the mineral elements was observed in the concentration of copper. The serum copper concentration in anestrus cows and buffaloes was very low compared to the level in normal cycling animals. Copper has been directly associated with delayed and depressed estrus (Howell and Hall, 1970). Arthur (1975) reported that deficiency of copper and phosphorus was the most likely dietary cause of anestrus in cows under British farming conditions. Munro (1957) also reported low plasma copper level in anestrus cows. Interestingly, estrogen hormone has been found to increase the copper level (Sato and Henkins, 1973). The low level of serum copper in the anestrus cows and buffaloes

in the present experiment could have been due to low circulating estrogen in these animals, as their ovaries were underdeveloped and inactive.

Supplementation of anestrus cows and buffaloes with Nuvimin Forte (Sarabhai Chemicals) partially restored the serum levels of most of the minerals, but especially those of inorganic phosphorus and copper. Strikingly, the serum copper level in anestrus cows and buffaloes rose from pre-treatment level of 130–141 μg/dl to 169–170 μg/dl after mineral supplementation. Consequently, 45 out of 60 anestrus cows and 27 out of 46 anestrus buffaloes came into normal heat within one month. However, the remaining animals could not come into estrus dne to one or the other reasons.

TABLE 1 : Serum Concentration of minerals in normal cycling and in anestrus cows and buffaloes before and after mineral supplementation (Mean ± S.E.)

Minerals	Cyclic		Anestrus		After mineral supplementation	
	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
Calcium (mg/dl)	11·50±0·30	11·20±0·15	9·8±0·50	9·5±0·2	10·7±0·61	10·95±0·5
Inorganic phosphorus (mg/dl)	6·50±0·20	6·2±0·25	4·30±0·31	4·0±0·3	5·8±0·52	5·75±0·4
Magnesium (mg/dl)	2·83±0·15	4·0±0·20	2·10±0·08	3·85±0·17	2·75±0·25	3·8±0·2
Sodium (mEq/l)	151·5±8·0	149±7	145±6·8	147±5	146±7·6	145±4·5
Potassium (mEq/l)	4·65±0·33	5·5±0·35	4·45±0·45	5·6±0·2	4·5±0·5	5·4±0·4
Copper (μg/dl)	185±11	181±10	130±8	141±7	170±10	169±5
Zinc (μg/dl)	310±13	298±10	305±9	303±9	307±11	298±11

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Total Cholesterol Concentration In Uterine Secretions Of Buffaloes During Certain Phases Of Reproduction

S.K. SHASHIKUMAR[1] and B. MUNILAL DUBEY[2]

Department of Gynaecology and Obstetrics, Veterinary College, Bangalore-560 024

ABSTRACT

The total cholesterol concentration in uterine secretions of baffaloes is reported during different phases of reproduction. Its concentration was higher during dioestrus, than in oestrus and pro-oestrus. In early pregnancy, its concentration was lower than dioestrus. It is hypothesised that progesterone level influences cholesterol concentration in oestrus cycle. During pregnancy, the lower concentration of cholesterol is attributed to the level of progesterone and utilisation of cholesterol by the developing conceptus.

In pro-oestrus, oestrus, dioestrus and early pregnancy, the total cholesterol concentration was 125·80 ± 4·96, 54·40 ± 6·15, 207·03 ± 10·04 and 156·10 ± 11·71 mg/100 ml, respectively.

* * *

During various phases of reproduction, uterine secretion serves as a vehicle, nutrient medium both for the spermatozoa and embryo. The chemical nature and physical properties are undoubtedly of great importance to successful reproduction. Embryo transfer experiments in the cow have demonstrated the critical importance of the stage of uterine environment for the viability and development of the embryo (Rowson *et al.* 1972; Sreenan and Beehan 1974). This knowledge has led to explore the biochemical constituents of uterine secretions. However, fat which is a source of energy has not been studied much. Hence, in this study, cholesterol as an index of fat has been studied quantitatively in the uterine secretions of buffaloes during certain phases of reproduction.

Materials and Methods

A total of 45 nonpregnant and 15 early pregnant uteri (25-30 days) of healthy buffalo cows were collected from the abattoir and transported to the laboratory in an ice packed thermocool box. The non-pregnant genitalia were categorized into pro-oestrus, oestrus and dioestrus phases by studying the ovarian morphology, as per Choudary *et al.* (1968) and Abul Fadle *et al.* (1974) for follicular and corpus luteum size. Based on the biometry of the conceptus (Arthur, 1968), the pregnant uteri of 25 to 30 days were grouped as early pregnancy.

The uterine secretions from non-pregnant uteri were collected by adopting the method of Olds and Van Demark (1957) with slight modifications-a wooden

1. S. K. Shashikumar, Instructor, Department of Anatomy, Veterinary College, University of Agricultural Sciences, Bangalore-24.
2. B. Munilal Dubey, Professor, Department of Gyanaecology and Obstetrics, Veterinary College, University of Agricultural Sciences, Bangalore-24.

roller pin was used in the collection technique. In early pregnant uteri, a dorsal transverse incision was made anterior to the os internus, the uterine horns were lifted from the tubal end and observed for escape of conceptus with the fetal sac. Wherever the conceptus did not escape freely from the uterus, such uteri were considered as uteri with implanted embryos and those uteri were excluded. A ligature was then applied anterior to the incision and uterine secretions were collected in the same manner as that of non-pregnant uterus. The samples collected were stored at–4°C and were analyzed for total cholesterol as per Henley (1957). Statistical analysis of the data was done as per Steel and Torrie (1960).

Results

There was a significant ($P \leqslant 0.01$) variation in the concentration of total cholesterol among the phases of oestrus cycles studied. The concentration was highest during dioestrus and lowest during oestrus. In early pregnancy, its concentration was significantly lower ($P \leqslant 0.01$) than dioestrus and higher ($P \leqslant 0.01$) than pro-oestrus and oestrus (Table 1).

Discussion

There are no reports in the available literature to state that uterine secretions of buffalo contain cholesterol. However, the demonstration of cholesterol sulfatase enzyme in the uterine secretions of hamster (Legault *et al.*, 1980) and its possible role in capacitation of spermatozoa, indicates the presence of cholesterol in the uterine secretions of the other species too. The uterine endometrium contains cholesterol as cell ingredient, and its synthesis increases under the influence of oestrogen (Aizwa and Mueller, 1961). The release of the cell ingredients into the uterine secretions is progesterone dependent and the type of secretion is apocrine (Hafez *et al.*, 1975). Probably due to such changes in the present study, there was a higher concentration of cholesterol in dioestrus than in pro-oestrus and oestrus.

It is known that the release of cholesterol and other cell ingredients is progesterone dependent. In early pregnancy upto 40 days, Thorburn and Schneider (1972), observed lower concentration of blood progesterone than during dioestrus. For the establishment of corpus luteum to produce optimum level of progesterone, the period required is shorter in pig and about 50 days in sheep (Moor, 1968). During this period sufficient luteotrophic hormone (LTH) is not present to maintain corpus luteum (Heap *et al.* 1973). In cows, it is suggested that Luteinizing hormone is the major component of LTH complex which stimulate progesterone synthesis from the corpus luteum after conception (Heap *et al.* 1973). These findings indicate lower concentration of cholesterol in early pregnacy than dioestrus in the present study, perhaps due to lower concentration of progesterone during early pregnancy.

In rats, lipids present at the implantation site are utilized by the blastocyst (Boshier, 1976). The cholesterol ester and cholesterol free in rabbit placenta & foetus increased as pregnancy advanced (Boyd, 1935),which perhaps means that cholesterol is essential for the conceptus and its source for an unimplanted embryo is uterine secretions. Constant utilization of cholesterol by the conceptus may result in its depletion. Probably due to this process,

besides the lower concentration of progesterone in early pregnancy, the total cholesterol concentration in uterine secretions was lower than in dioestrus.

TABLE 1 : Total cholesterol concentration (mg/100 ml) in uterine secretions of buffaloes during certain phases of reproduction.

Reproductive phase n = 15	Mean±S.E.	Range	Statistical comparison	Difference value	LSDT value	Inference
Pro-oestrus	125·80±4·96	97·00–170·00	Pro-oestrus to Oestrus	71·40	16·93	**
Oestrus	54·40±6·15	15·00–100·00	Pro-oestrus to Dioestrus	81·23	16.93	**
Dioestrus	207·03±10·04	150·00–280·00	Oestrus to Dioestrus	152·63	16·93	**
Early pregnancy	156·10±11·71	82·50–255·00	Pro-oestrus to early pregnancy	30·30	16·93	**
			Oestrus to early pregnancy	101·70	16·93	**
			Dioestrus to early pregnancy	150·93	16·93	**

'F' Observed (d.f. 56) 54·21 **, n – number of samples, S.E. – standard error, d.f. – degree of freedom, LSDT – Least square difference test, ** – Significant at $P \leqslant 0{\cdot}01$.

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Incidence of Anoestrum Among Crossbred Cattle (Holstein X Local) of Chotanagpur Region

JASWANT SINGH, B.K. SINGH and M.P. SINHA

Department of Gynaecology and Obstetrics, Ranchi Veterinary College, Kanke, Ranchi-834 007

ABSTRACT

The study was conducted on anoestrous crossbred cattle. The overall incidence of anestrum was recorded to be 45·05 %. The incidence was higher (59·82 %) in heifers than in cows (36·47 %). The maximum incident of anoestrum was observed during May (67·50 %) and minimum during February (32·0 %). Seasonwise incidence was 46·71 43·15, 48·38 and 42·79 % during winter, spring, summer and autumn respectively. Age/parity had significant influence on the incidence of anestrum, which was higher in younger animals and in second calvers.

* * *

Anoestrum is one of the most common condition causing infertility in dairy cattle. Information on the incidence of anoestrum in crossbred cattle is scanty. In view of the escalating importance of crossbreds and the economic losses

resulting from anoestrum, the present study was undertaken to record the incidence of this condition in crossbred heifers and cows of this area.

Materials and Methods

637 crossbred cattle (234 heifers and 403 cows) owned by the local farmers formed the material for the study. The animals were maintained under traditional husbandry practices. The heifers of two or more years of age and the cows which had not shown signs of oestrum 60 days or more post partum, were included in this study. The incidence of anoestrum was calculated and the influence of months seasons, age and parity was also recorded.

Results and Discussion

The incidence of anoestrum in crossbred cows was recorded to be 36 47 %, which is comparable (34 62 %) with the findings of Rao and Kotayya (1976). A low incidence of anestrum (14 %) was recorded in cattle by Naidu and Rao (1980) which might be due to variations in breed, feeding levels, agro-climatic conditions and age of animals. The incidence of anestrum in crossbred heifers was recorded to be 59·82 %, which was much higher than the reports of Luktuke and Sharma (1978) and Naidu and Rao (1980). The overall high incidence (45 05%) during this study might mainly be due to poor nutrition, since the farmers in and around Ranchi are generally poor. Similar values (47·76 %) were recorded by Rao and Murthy (1972). The incidence of anoestrum in heifers was found to be significantly higher than in cows. Monthwise incidence of anestrum recorded was highest (67 50 %) during May and lowest (32·00 %) during February. This is in agreement with the findings of Dessouky *et al.*, (1969).

The seasonwise incidence of anoestrum was 46·71, 43·15, 48·38 and 42·79 % during winter (November to January) Spring (February to April), Summer, (May to July) and Autumn (August to October) respectively. This is similar to the findings of Dessouky and Juma (1973) who reported the highest incidence of anestrum during Summer and the lowest during Autumn season. Roine (1973) reported significant influence o seasons on the incidence of anestrus in cattle whereas in the present study the influence of season was nonsignificant. This might be due to breed differences or high altitude location of Ranchi (625 metres above sea level), where aeasonal variation in temperature is not sharp.

The influence of sequence of calving on incidence of anestrum was noted (Table–1). The maximum incidence (16·2 %) was recorded during second calving and minimum (6·8 %) during first calving. The maximum occurence of anestrum during second calving might be due to lactationnal stress caused by high milk yield during this period.

The incidence of anoestrum was higher (41·8 %) in heifer of younger age (< 3 years) and lower (25·6 %) in heifers (> 3 years). Similar findings were recorded by Fielden *et al.*, (1973).

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TABLE 1 : Influence of age/parity on the incidence of anoestrum in crossbred cattle.

Age/parity	Number of anoestrum cases	Percentage of incidence
Heifer up to 3 years	31	41·8
Heifer above 3 years		
1st calving	19	25·6
2nd calving	5	6·8
3rd calving & above	7	7·6

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Multiple Births In Bubalus bubalis (Murrah Buffaloes) : Cytogenetical Investigations

B.R. YADAV, C.R. BALAKRISHNAN[1] and O.S. TOMER[1]

National Institute of Animal Genetics, NDRI Campus, Karnal–132 001, India

ABSTRACT

Sex chromosome chimaerism has been studied in six female and three male calves of *Bubalus bubalis* (Murrah buffaloes) belonging to two sets of triplets and four sets of twin heterosexual births. A close parallelism of XX/XY within the members of multiple births has been recorded. These six cases of multiple births were encountered from a large population of Murrah buffaloes with private owners. However, the incidence of multiple births is relatively low in organized herds. Only two sets of twin births, one set of still-birth males and another set of normal females were encountered from 2,757 calvings in

[1] National Dairy Research Institute, Karnal–132 001, India.

NDRI herd over a period of 16 years (1971–1986).

* * *

Buffalo being a uniparous species, multiple births are very rare. Recently, Tiwana *et al.* (1985) have reported two such cases among 2,325 calving of Murrah and Nili-Ravi buffaloes. Chromosome examination of calves born as co-twins or co-sibs in multiple births in Murrah buffaloes with private owners were carried out during the course of present investigations.

Materials and Methods

Bubalus bubalis (Murrah buffaloes) constituted the material for the present investigations. Blood samples from two sets of heterosexual triplets (one female and one male in one set and only one female in other set) and four sets of heterosexual twins (both female and male co-twins in two sets and female members in other two sets) were taken and short-term lymphocyte cultures were set up as per modifications of usual culture method described by Yadav and Balakrishnan (1985, a). Chromosome preparations were made by routine procedure. More than 50 complete metaphase plates were examined for each calf. Breeding data of NDRI herd from 1971 to 1986 was examined for multiple births.

Results and Discussion

Diploid number of chromosomes in all the calves was 50, the characteristic of river buffaloes (Fischer and Ulbrich, 1968; Yadav, 1981). An admixure of cells with 50 XX and 50 XY chromosome complements including the usual 5 pairs of submetecentrics was observed in all cases (Fig. 1–4). The XX bearing cells ranged in female co-sibs from 13.00 to 73·55% while in males the variation was from 41·05 to 61·30%. The corresponding range of XY cells was 26·45 to 87·00% in females and 38·70 to 58·95% in males (Table 1). Parallelism of XX/XY chromosomal chimaerism was observed in three sets where both male and female co-twins/co-sibs were available for investigations (Table 2).

Incidence of twinning in the herd maintained by the National Dairy Research Institute was one still birth male twin set and another normal female twin set among 2,757 calvings over a period of 16 years. However, Tiwana *et al.* (1985) have reported two twin births in 2,325 calvings in buffaloes from the herd maintained at PAU, Ludhiana. Twinning and multiple births in cattle are reported to vary in frequency between 0·2 to 3 per cent (Marcum, 1974). In buffaloes they are even rarer, ranging between 0·015 and 0·63 per cent (Tantawy and Ahmed, 1957; Ferrara, 1960; Fadzil, 1969; Goswami and Nair, 1968).

Sex chromosome chimaerism is a better method for diagnosis of freemartinism in bovines. In cattle, there are many reports on chimaerism and freemartinism (Marcum, 1974), while it is not so in the buffaloes. However, Balakrishnan *et al.* (1981) reported chimaerism in one set of heterosexual triplets of Murrah buffalo.

Jost *et al.* (1972) consider the formation of blood vascular anastomosis between the vessels of blood supply of developing foetuses and hence exchange of blood/tissues as the cause of admixture of sex cells in cattle. Recent reports indicate that the H–Y secreted by cells of the bull twin may be responsible for masculinization of the freemartin gonad

in cases of male-female twinning in cattle. It is likely that the same phenomenon operates in buffaloes also.

In cattle there is a close parallelism of XX/XY cells (Basrur and Kanagawa, 1969; Marcum *et al.* 1972, Yadav and Balakrishnan, 1985, b). However, irrespective of large individual variations, average of XX and XY cells in all chimaeric calves has been found to be 1 : 1. A similar pattern was observed in buffalo calves in the present investigation.

Similarity in sex chromosome chimaerism and parallelism among cattle and buffaloes is fairly indicative of the existence of freemartinism in buffaloes, a phenomenon very common in heterosexual multiple births in cattle.

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TABLE 1 : Sex Chromosome Chimaerism in Twins and Triplets.

Twin/ Triplet No.	Age at study in days	Sex of the calf	No. of cells examined	Percentage of XX	Percentage of XY
1	7	F	145	57·90	42·10
	7	M	111	61·30	38·70
	–	M	N.A.		
2	60	F	81	40·74	59·26
	60	M	95	41·05	58.95
3	7	F	134	50·75	49·25
	7	M	87	51·73	48·27
4 *	240	F	100	13·00	87·00
		M	N.A.		
		M	N.A.		
5	60	F	114	33·00	67·00
		M	N.A.		
6	630	F	121	73·55	26·45
		M	N.A.		

* = Members of triplet set;

F = Female; M = Male; N.A. = Not Available.

TABLE 2 : Parallelism of XX/XY Chimaerism.

Twin/ Triplet No.	No of cells examined		Percentages XX cells		Percentages XY cells	
	Male	Female	Male	Female	Male	Female
1 *	111	145	61·30	57·90	38·70	42·10
2	95	81	41·05	40·75	58·95	59·27
3	87	144	51·73	50·75	48·27	49·25
Average			51·36	49·80	48·64	50·20
Average 17 pairs of cattle **			49·40	49·50	50·60	51·50

* = Two members of a triplet set ;

* * = Yadav and Balakrishnan (1985, b).

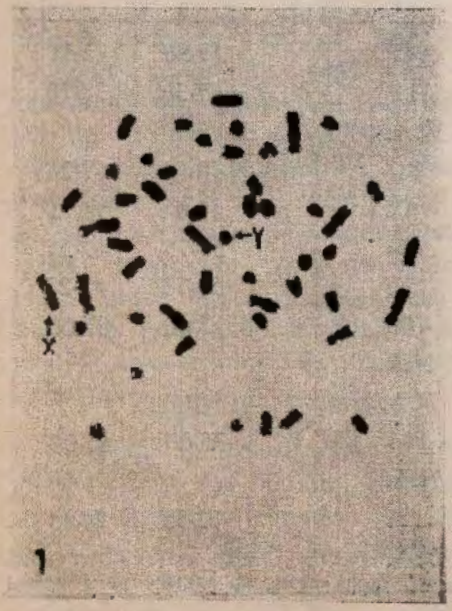

Fig. 1. Giemsa stained metaphase plate with male complement (50, XY)

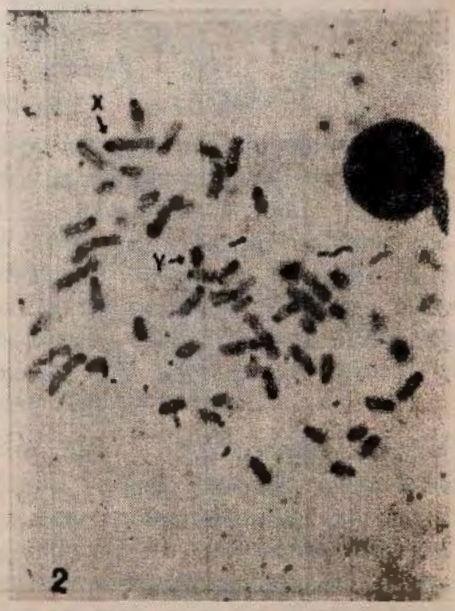

Fig. 2. C-band plate showing 50, XY

Fig. 3. Giemsa stained metaphase plate with female complement (50, XX)

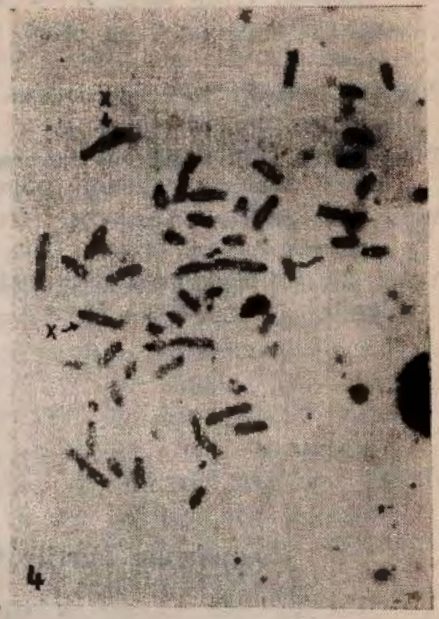

Fig. 4. C-band plate showing 50, XX

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Postpartum Fertile Oestrus Interval In Rural Bovines

S. KUMAR and L.N. PURBEY

Indian Veterinary Research Institute, Izatnagar–243 122.

ABSTRACT

Studies have been recorded on post partum fertile oestrus interval in a sample of rural bovine population (nondescript cows and buffaloes) at ORP, IVRI, Izatnagar. The average duration of post partum fertile oestrus interval was found to be 11·93±0·70 months, 8·32±0·63 months 8·98 ± 0·91 months, 10·13 ± 1 74 months, (overall 9·70±0·98 months) respectively after first to fourth calving in cows and 10·00±0 63 months, 10·78±1·10 months, 9·53±0·71 months, 11·71±1·84 months and 8·35 ± 0·96 months, (overall 10·25±1·24 months) respectively after first to fifth calving in buffaloes. Seasons and parity of calving were found to have no significant effect on this parameter.

Regular calving in cattle and buffalo is the prerequisite for obtaining the optimum gain in bovine industry. Postpartum fertile oestrus interval (service period) is the trait which controls the calving interval and thus is directly related to economy. Reports on this reproductive parameter are available in recognised breeds under farm conditions viz. Tharparkar (Prasad, 1958), Haryana (Luktuke and Subramaniam, 1961). Malvi and Ongole (Rao, 1966), Khillar (Patke, 1967). Nagauri (Bhasin, 1968), Gir (Ponkshe, 1969), Rathi (Sonawane, 1969) and Dangi (Purbey and Sane, 1979) and in Murrah (Venkaya and Anant Krishnan, 1957, Kohali and Mallick 1960 Rai, 1966, Gudi *et al.* 1969, Singh *et al.* 1972). and Surti (Sane *et al.* 1968) buffaloes. Information on Postpartum fertile oestrus interval in rural cows and buffaloes,

however is scanty. In the present paper, studies in a sample of rural bovine population on this parameter is reported.

Materials and Methods

The present study was conducted in a sample of nondescript rural bovine population of the villages under Operational Research Project (ORP) IVRI Izatnagar. Observations were recorded on post partum fertile oestrus interval (service period) in 186 cows and 138 buffaloes which on follow up were diagnosed to have conceived with artificial insemination after calving. The period between calving and post partum fertile oestrus was considered for this parameter. The data was subjected to statistical analysis. Season was divided into Winter (December to February), Spring (March & April), Hot Dry Summer (May & June), Wet summer (July to September) and Autumn (October to November), as per Ahuja (1958).

Results and Discussion

Post partum fertile oestrus interval was calculated to be $11 \cdot 93 \pm 0 \cdot 70$, $8 \cdot 32 \pm 0 \cdot 63$, $8 \cdot 98 \pm 0 \cdot 91$ and $10 \cdot 13 \pm 1 \cdot 74$ months (overall $9 \cdot 70 \pm 0 \cdot 98$ months) from first to fourth calvings respectively, in nondescript rural cows. It was $10 \cdot 00 \pm 0 \cdot 63$, $10 \cdot 78 \pm 1 \cdot 10$, $9 \cdot 53 \pm 0 \cdot 71$, $11 \cdot 71 \pm 1 \cdot 84$ and $8 \cdot 35 \pm 0 \cdot 96$ months (overall $10 \cdot 25 \pm 1 \cdot 24$ months) from first to fifth calving respectively in rural buffaloes (Table 1).

Studies on the effect of lactation orders and seasons of calvings on post-partum fertile oestrus interval have been reported by few workers. Prasad (1958) recorded the period significantly higher after first calving than subsequent calvings but calving season had no significant effect. In Indian buffaloes, in general, Goswami and Kumar (1968) observed significant effect of lactation order and seasons of calving on service period. However, seasons and parity of calving were not found to have significant effect on post partum fertile oestrus interval (Tables 2 and 3).

The present study, however, apparently reveals that the average post partum fertile oestrus interval and hence the intercalving period is considerably longer in rural bovine population. This may be due to their assorted genetic make up, poor nutrition and management under field conditions affecting the economy of the farmers by decreasing the number of calf crops and quantity of milk in the total productive life span of animals. The problem, therefore, needs attention for improvement in nutrition, management and sexual health control measures. The farmers need to be educated in animal husbandry practices to get the maximum gain from their animals. A common tendency observed in some of the farmers is that they do not care much for heat detection and A. I. service in their animals when they yield considerable quantity of milk. The farmers also need to be educated in this respect.

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TABLE 1. Post Partum Fertile Oestrus Interval in Rural Bovine.

Parity Number	Average Post Partum Fertile Oestrus Interval (Months) Cows	Buffaloes
First	11·93±0·70	10 00±0·63
Second	8·32±0·63	10·78±1·10
Third	8·98±0·91	9·53±0·71
Fourth	10·13±1·74	11·71±1·84
Fifth	–	8·35±0·96
Overall	9·70±0·98	10·25±1·24
SEASON		
Winter (December to February)	8·71±0·69	9·85±1·10
Spring (March to April)	9·48±1·13	12·00±6·00
Hot Dry Summer (May to June)	11·43±2·79	12·00±0·00
Wet Summer (July to September)	10·45±1·34	11·34±1·12
Autumn (October to November)	9·83±1·00	8·92±0·85

TABLE 2. Effect of Parity of Calving on Post Partum Fertile Oestrus Interval.

ANOVA

Sources of variation	d. f.	M. S. S.	F. Value	Significance
		Buffaloes		
Between sequence of calving	4	19·62	0·84	Not significant
Error	134	23·49		
Total	138			
		Cows		
Between sequence of calving	3	122·54	0·1754	Not significant
Error	183	698·61		
Total	186			

TABLE 3. Effect of seasons of calving on Post Partum Fertile Oestrus Interval

ANOVA

Sources of variation	d. f.	M. S. S.	F. Value	Significance
		Buffaloes		
Between seasons	4	18·05	0 81	Non-significant
Error	61	22·21		
Total	65			
		Cows		
Between seasons	4	13·92	0·52	Non-significant
Error	93	26·89		
Total	97			

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Circulatory Levels Of Progesterone And Prostaglandin $F_{2\alpha}$ During Estrous Cycle In Buffalo Heifers

G.C. JAIN and R.S. PANDEY

National Dairy Research Institute, Karnal-132 001, INDIA

ABSTRACT

Seven buffalo heifers (nulli-parous) were monitored two times on the day of estrus, on alternate-days from day 1 to 15 and daily from 18 to 22 days of oestrous cycle for prostaglandin $F_{2\alpha}$ and progesterone profiles in the peripheral blood plasma by RIA. Progesterone concentration was minimum on the day of estrus, which gradually increased from day 3 onwards to a maximum level of 4·0 ng./ml. on day 11 of the estrous cycle. The levels then suddenly dropped to less than 1 ng./ml. on day 22 of the cycle. A similar trend in the $PGF_{2\alpha}$ levels, which increased non-significantly from day 0 to day 16, was observed. The study suggested that an increase in $PGF_{2\alpha}$ levels on around days 7 to 11 is required to cause luteolysis of CL during mid luteal phase of the estrous cycle in buffalo heifers.

* * *

Materials and Methods

Selection, management, schedule of blood sampling and estrus detection in buffalo heifers was followed as reported earlier (Jain and Pandey, 1983).

Radioimmuno assay (RIA) of prostaglandin $F_{2\alpha}$ and progesterone : The prostaglandin $F_{2\alpha}$ and progesterone levels in the peripheral blood plasma were estimated as per the procedure reported earlier (Jain and Pandey, 1984).

Statistical Analysis : The prostaglandin $F_{2\alpha}$ and progesterone hormone levels in buffalo heifers for different collection intervals were studied by the least square technique (Harvey, 1979). The significant difference in the means of each estimation for each effect was studied as per the procedure of Duncan (1955).

Results and Discussions

Prostaglandin $F_{2\alpha}$ levels during estrous cycle period : The variations in the levels were not significantly different at any stage of cycle and the levels fluctuated from 0·2 ng/ml. to 0·6 ng/ml. with peak levels on days 7-16. However, the $PGF_{2\alpha}$ levels increased gradually from day 0 to day 16 and then subsequently decreased to 0·2 ng/ml. on day 20 of the estrous cycle.

Progesterone levels during estrous cycle : The progesterone concentration was found very low on the day of estrus (day 0). The levels then rose gradually from day 1 to a peak value of 4 ng/ml. on day 11. It then was maintained at a plateau with slight fluctuations up to day 15 and thereafter started declining from day 16 onwards (Table 1).

The $PGF_{1\alpha}$ profiles observed during the estrous cycle in buffaloes are in agreement to Shemesh *et al*, (1975) in cows, who suggested further that bovine follicular fluid (BFF) from the mid cycle

follicle inhibited $PGF_2\alpha$ synthesis as well as luteinization. The late release of $PFG_2\alpha$ might contribute to the process of luteolysis in the normal cycles. Our observations are also in agreement with Findlay *et al* (1981), who reported an evidence for a localized change in the dynamics of $PGF_2\alpha$ metabolism leading to an increase in $PGF_2\alpha$ contents in the caruncular and intra-caruncular tissues of the endometrium in non-pregnant animals.

The present results further indicate that the peripheral concentration of progesterone during the early days of cycle is proportional to the growth of the CL. The levels were low on the day of ovulation when the corpora haemorrhagicum had formed. It then rose gradually throughout all the stages of CL development (CL_1, CL_2, and CL_3) which subsequently decreased sharply on day 22 of the cycle reflecting the sudden regression of the CL (CL_3, CL_2 and CL_1). This pattern in the progesterone concentration in buffaloes was in agreement as reported earlier (Batra *et al*, 1979). The secretion of progesterone during the follicular phase before ovulation might be from the granulosa cells lining the developing follicles (Short, 1962) or from the follicular wall, thecal wall or granulosa cells (Moor, 1968).

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Table 1 : Plasma levels of prostaglandin $F_2\alpha$ and progesterone (ng/ml.) during estrous cycle in buffalo heifers.

Day of cycle	$PGF_2\alpha$ levels		Progesterone levels	
	Mean	S. Em.	Mean	S. Em.
od_1	0·4	0·1	0·4*	0·1
od_2	0·3	0·1	0·3*	0·1
1	0·2	0·1	0·6*	0·2
3	0·4	0·1	0·9*	0·2
5	0·5	0·1	1·3	0·5
7	0·7	0·1	0·9*	0·2
9	0·5	0·2	1·7	0·4
11	0·3	0·1	4·0	2·6
13	0·6	0·3	2·8	1·4
15	0·6	0·4	3·5	1·5
16	0·6	0·2	1·6	0·5
17	0·3	0·1	2·0	0·7
18	0·4	0·1	1·4	0·5
19	0·5	0·2	1·9	0·8
20	0·2	0·1	1·8	0·9
21	0·3	0·1	1·8	0·6
22	0·3	0·1	0·8*	0·3

od_1 – day of commencement of estrus
od_2 – 12 hours after od_1
* – (P 0·05) from 4·0.

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Levels Of Calcium And Inorganic Phosphorus In Anestrus Buffalo-Heifers

R.K. CHANDOLIA, S.K. VERMA and N.S. BUGALIA

College of Veterinary Sciences, Haryana Agricultural University, HISAR–125 004

ABSTRACT

Non-significant variations were recorded in plasma calcium levels between anestrus and cycling buffalo-heifers. However, plasma inorganic phosphorus profiles were significantly higher in cycling heifers (P 0·01) than anestrus heifers. Wider Ca:P ratio was observed in anestrus heifers (3·7:1) in comparison to cycling heifers (2·7:1).

* * *

Anestrus in buffaloes is the most common condition faced by veterinarian in breeding programme posing economic stress on the farmers. Incidence of anestrus is higher (56·0 percent) in buffalo heifers than cow heifers (36·0 percent) [Luktuke *et al.* 1979].

It is widely claimed that balanced supply of nutrients is essential for reproduction. Nutritional deficiencies and imbalances particularly excess calcium in non-legume rations without supplementation of other minerals can interfere with ovarian cyclic activity in cattle (Bellows, 1966). Morrow (1977) observed that for efficient reproductive processes the ratio of calcium to phosphorus should be between 1·5:1 and 2·3:1. It is not unusual for this ratio to vary. Abnormal calcium-phosphorus metabolism may be a factor in infertility in cattle, since abnormal-metabolism can result from a primary deficiency or an imbalance intake (Carnahan, 1974). Anestrus in heifers maintained at a fair state of nutrition may be attributed to deficiency of phosphorus (Morrow, 1969).

In the present work, biochemical variations in calcium and phosphorus have been studied in cases of anestrus buffalo heifers on organized farm.

Material and Methods

Present study was conducted on thirty-two Murrah buffalo heifers of three and half to four years of age, maintained at Govt. Progeny Testing Farm, Hisar. These heifers had not exhibited behavioural estrus and had normal genitalia and smooth ovaries as revealed by rectal examination conducted at an interval of 7 to 10 days. Vasectomized bull was used during morning and evening for detection of estrus. Experimental animals consisted of two groups.

Group-I– True anestrus of thirtytwo buffalo-heifers with smooth ovaries and normal genitalia.

Group-II– Cycling heifers of twenty-five buffalo–heifers with normal estrous cycle Control group. Blood samples from anestrus animals after rectal examination were collected in heparinized vials by jugular venipuncture. Blood sampling was done in cycling heifers during estrus (Day 0) and diestrus (Day 10) phase of estrous cycle. Plasma samples were stored at – 20°C till biochemical analysis. Plasma calcium and inorganic phosphorus were analysed as per the methods of Webster (1962) and Fiske and Subbarow (1925). Statistical analysis was done by student test (Snedecor and Cochran, 1967).

Results

The mean plasma calcium level in anestrus heifers was $11{\cdot}84 \pm 0{\cdot}42$ mg/100 ml. In cyling heifers, plasma calcium levels were $12{\cdot}54 \pm 0{\cdot}45$ and $12{\cdot}79 \pm 0{\cdot}66$ mg/100 ml. during estrus and diestrus phases respectively. Non-significant variations in plasma calcium levels were observed between anestrus and cycling heifers. Variations in plasma calcium levels were also non-significant between estrus and diestrus phases in cycling-heifers. Plasma calcium profiles were within the normal range in anestrus and cycling heifers.

Plasma inorganic phosphorus profile was $3{\cdot}18 \pm 0{\cdot}15$ mg/100 ml. in anestrus heifers, whereas phosphorus levels were $4{\cdot}58 \pm 0{\cdot}39$ mg/100 ml. and $4{\cdot}73 \pm 0{\cdot}34$ mg /100 ml. during estrus and diestrus phases in cycling heifers. Significant differences were recorded in plasma phosphorus levels between anestrus and cycling heifers, being significantly lower in anestrus heifers ($P < 0{\cdot}01$) than estrus and diestrus phases in cycling heifers.

The Ca:P ratio was wider in anestrus buffalo heifers in comparison to cycling heifers. The ratio was 3·7:1 in anestrus while 2·7:1 during estrus and diestrus phases in cycling heifers.

Discussion

The normal serum Calcium is 6 to 12 mg/100 ml. and normal serum phosphorus is 3 to 6 mg/100 ml. The ratio of serum Ca:P should be between 1·5:1 and 2·5:1 for efficient reproduction (Carnahan, 1974).

Quayam (1979) reported lower level of calcium in anestrus buffaloes, while in our study the level of calcium was not significantly changed in case of anestrus animals. Similar observations were also made by Samad *et al.* (1980). Controversial reports were made by earlier workers on the role of calcium. Quayam (1979) observed lower level of calcium in anestrum buffaloes while Chauhan *et al.* (1981) noticed higher level of calcium.

In the present study, plasma inorganic phosphorus was significantly lower ($P<0.01$) compared with cycling animals. Similar observations have been made by Dindorkar and Kohli (1979). Higher plasma inorganic phosphorus profiles were associated with fertility in cows (Bodai, 1976 and Reddy, 1982).

The observed Ca:P ratio in anestrus buffalo-heifers was wider (3·7:1) compared with cycling buffalo heifers (2·7:1). Similarly, Quayam (1979) reported that Ca:P ratio was much disturbed in anestrus buffaloes as compared to estrus phase of cycling buffaloes. The ratio was 4.1:1 in anestrus buffaloes and 2·6:1 during estrus phase. However, ratio during diestrus phase was significantly higher (4·3:1) in comparison to our finding during diestrus (2·7:1).

Significance of calcium and phosphorus ratio in fertility was reported by Roberts (1971) and Morrow (1980). Morrow (1977) observed that an imbalance between Ca:P prolong the interval to first ovulation and for efficient reproductive processes, the ratio of Ca to P should be between 1·5:1 and 2·3:1. Adult cattle can tolerate a ratio of 3·1 and Ca:P ratio beyond 3:1 was considered to impair fertility in cattle (Herriek, 1977). Phosphorus deficiency disturbs hypophyseal and gonadal functions and carbohydrate metabolism, causing infertility (Palmer *et al.* 1941 and Morrow 1977). Phosphorus deficiency hinders the Vitamin A synthesis from β carotene (Morrow, 1980).

Thus, disturbed Ca:P and phosphorus deficiency could be accounted for pituitary and gonadal dysfunction in anestrus buffalo-heifers. It may be concluded that either anestrus animals have low level of phosphorus or there might be interference with phosphorus absorption and metabolism, since abnormal metabolism can result from a primary deficiency or an imbalance intake (Carnahan, 1974). Further study is necessary to clarify these two factors.

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Prepartum And Immediate Postpartum Blood Plasma Progesterone, Estradiol-17 β, LH And FSH Profile Of Murrah Buffaloes (Bubalus bubalis)

I.S. LOHAN, M.L. MAKER and M.N. RAZDAN

Department of Animal Production Physiology, Haryana Agricultural University, Hisar–125 004

ABSTRACT

Blood plasma samples collected daily from five Murrah buffaloes over varying periods of time prepartum and upto two days after parturition were analysed for progesterone, estradiol-17 β, LH and FSH concentration. By day 3 to day 2 before parturition, the progesterone level fell considerably. Estradiol-17 β level rose considerably on the day of parturition, followed by a steep and significant fall. LH and FSH levels were low and fluctuated within a narrow range. The results indicated that a high level of estradiol associated with very low level of progesterone appears to play a significant role in the process of parturition in buffaloes.

* * *

Physiology of various reproductive events in buffalo remains poorly under-

stood. Only recently a few reports on the hormone levels in relation to reproductive processes in buffaloes have started appearing in the literature (Arora and Pandey, 1982). The present investigation was carried out to study circulating progesterone, estradiol–17β, LH and FSH profile of Murrah buffaloes during prepartum and following parturition, to understand the interaction of hormones around parturition.

Materials and Methods

Five Murrah buffaloes at different stages of gestation, one at day 79 prepartum and the rest four in last stages of pregnancy were available for this investigation. Plasma was separated at 5°C from heparinized blood samples collected by jugular venipuncture. These were assayed for estradiol–17 β and progesterone by the RIA procedure as described by Dobson and Dean (1974) and Kanchev *et al.* (1976).

LH and FSH in plasma samples were estimated by a homologous bovine double antibody RIA system as used by Kaker *et al.* (1980) and Razdan *et al.* (1982).

Validation of RIA for different hormones

Hormones	Recovery	Sensitivity	Coefficient of variation (percent)	
			Intraassay	Interassay
Estradiol–17β	80–84	3·125 pg	8·82	17·22
Progesterone	81–86	20·0 pg	6·70	10·13
LH (direct assay)	–	0·125 ng	5·00	13·00
FSH (direct assay)	–	1·25 ng	8·00	8·20

The mean values of each day's observations for each of the hormones were calculated using data collected from 20 days prepartum upto day 2 postpartum (Table 1). Sharp increase or decrease especially in sex steroids did not occur simultaneously in all the animals with reference to the day of parturition, resulting in a relative de-emphasis on averaging the data of the animals, hence results of individual animal have been discussed.

Progesterone : Progesterone levels of buffalo S–33 fluctuated between 0·59 and 2·93 ng/ml from day 79 to day 2 prepartum. Then, there was a sharp decline in the level upto 0·35 ng/ml on day 1 prepartum, followed by further decrease to 0·14 ng/ml on the day of parturition. Progesterone level recorded upto day 2 postpartum was low (Fig. 1). In buffalo S–26, progesterone level fluctuated between 0·79 and 1·89 ng/ml from day 20 to day 4 prepartum. Level remained low till day 1 prepartum, followed by a further decline (0·32 ng/ml) on the day of parturition. The hormone decreased further and remained low (0·12 to 0·28 ng/ml) till day 2 postpartum. In case of buffalo 180, progesterone concentration was maintained within the range of 0·80 to 1·26 ng/ml between day 18 and day 2 prepartum. The hormone level fell on day 1 prepartum which decreased markedly on the day of parturition, followed by a further decline on day 2 postpartum (Fig. 1). Plasma progesterone level of buffalo 217 was 1·04 ng on day 2 prepartum and

decreased to 0·56 ng on day 1, the level further declined markedly to 0·23 ng/ml on day of parturition. Very low level was observed upto day 2 postpartum. In buffalo 373, progesterone level was high upto day 2 prepartum and showed a sharp decline on the day of parturition, followed by a further decrease in level after parturition (Fig. 1).

Estradiol - 17 β : In buffalo S–33, circulating estradiol level fluctuated with a range of 8·00 and 34·00 pg/ml, between day 79 and 10 prepartum. Thereafter, a gradual increase in concentration was observed and the level reached to its maximum (70·00 pg/ml) on the day of parturition (Fig. 1). Estradiol level of buffalo S-26 fluctuated between 10·8 and 38·00 pg/ml from day 20 to day 7 prepartum. From day 6, there was a gradual increase in estradiol level and the level reached 80·00 pg/ml on day 1 pre-partum, followed by a peak level of 120 pg/ml on day of parturition (Fig. 1). In buffalo 180, estradiol concentration varied from 15 to 42 pg/ml between day 18 and 5 prepartum. Thereafter, the level increased sharply and a peak of 80 pg/ml was observed on the day of parturition. After parturition, estradiol level was undetectable till day 2 postpartum in three out of five buffaloes (Fig. 1). Plasma estradiol level of buffalo 217 was around 80 pg/ml during the last 2 days before parturition (Fig. 1). In buffalo 373, plasma estradiol level observed was 66 pg/ml and 70 pg/ml on day 3 and day 2 prepartum, respectively. The level increased to 104 pg/ml on the day of parturition (Fig. 1). In three out of five buffaloes, the level of estradiol was undetectable on the day after parturition but in the other two buffaloes the level observed was 8 to 14 pg/ml.

LH : The LH level of buffalo S–33 showed wide variations and fluctuated between 1·70 and 4·80 ng/ml from day 79 to day 6 prepartum. The level declined to 2·00 ng on day 5 prepartum, with very narrow fluctuations. Low level was maintained till day 2 postpartum. Plasma LH level of buffalo S–26 varied between 1·03 and 2·08 ng/ml from day 20 to 12 prepartum. Thereafter, the level declined slightly and fluctuated between 0·49 to 0·89 ng/ml. Around parturition, the level varied within a narrow range. Plasma LH level of Buffalo 180, during prepartum period ranged with narrow fluctuations (1·32 to 2·80 ng/ml). In buffalo 217, LH concentration remained undetectable ($\angle$0·125 ng/ml) around parturition, but on the day of parturition the level of LH was 0·68 ng/ml. LH level of Buffalo 373 fluctuated within 1·00 ng/ml before calving and decreased to undetectable level on the day after parturition upto day 2 postpartum.

FSH : Plasma FSH level of buffalo S–33 varied (12·08 to 32·97 ng/ml) with large and wider fluctuation from day 79 prepartum through day 2 postpartum. Similarly, FSH level of buffalo S–26 also fluctuated but had wider variations (3·17 to 12·64 ng/ml) from day 20 prepartum till day 2 postpartum. Plasma FSH level of buffalo 180 fluctuated between 5·00 to 11·16 ng/ml until day 5 prepartum, followed by a decrease. A relatively low level was reached during the last five days before parturition through 2 days postpartum. FSH level of buffalo 373 also exhibited fluctuation within a narrow range (5·47 to 12·76 ng/ml) from day 5 prepartum till day 2 postpartum.

Discussion

Circulating progesterone levels in pregnant buffaloes were high but declined

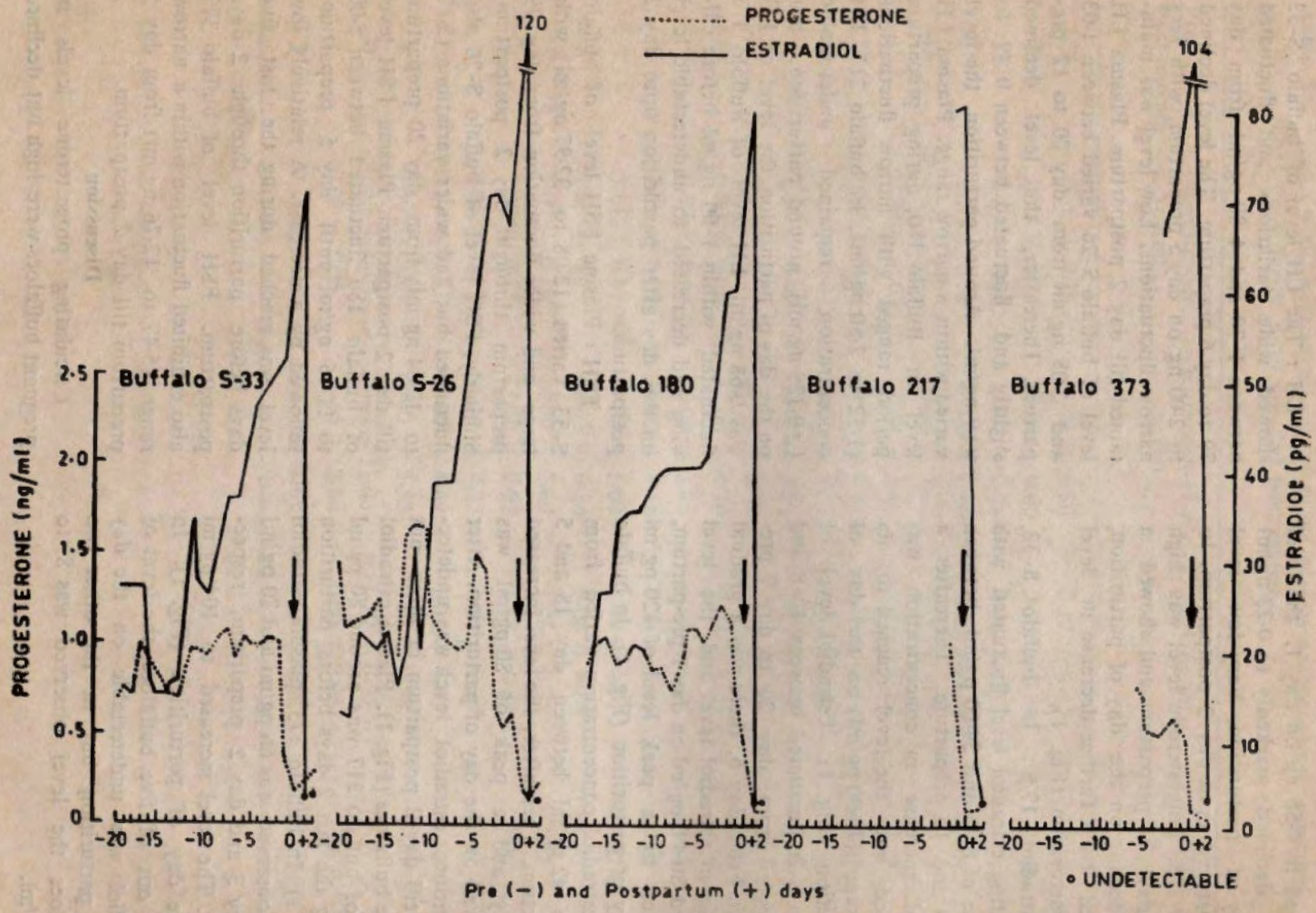

FIG. 1. DAILY PLASMA PROGESTERONE AND ESTRADIOL-17 β LEVELS OF BUFFALOES DURING PRE AND IMMEDIATE POSTPARTUM PERIOD

sharply through the last 72 to 24 hours before parturition. Progesterone concentration was observed to be very low in all the five animals on the day of parturition. Buffaloes have a relatively lower progesterone profile during gestation as well as on the day of parturition in comparison to cattle (Arije *et al.* 1974).

Estradiol level showed a gradual increase between 12 and 14 days prepartum. A sharp increase was observed from day 2 onwards reaching a maximum on the day of parturition. Gradual decrease in progesterone concentration prior to parturition, coinciding with increasing estradiol level thus reflects possible utilisation of progesterone as a precursor for increased synthesis of estradiol. Increased level of estrogens around parturition might be involved in triggering prostaglandin release which in turn would cause myometrial contraction (Fairclough *et al.* 1975). Low profile of gonadotrophins (LH and FSH) observed during advanced prepartum period might be due to the suppressive effect of high levels of pregnancy sex steroids at hypothalamic level. It is evident from the study that in buffaloes very high levels of estradiol accompanied by very low levels of progesterone are responsible for initiation of parturition.

Acknowledgements

Authors are thankful to the Department of Livestock Production and Management HAU for providing buffaloes. Technical help received from Dr. M. M. Galhotra, Assistant Scientist in the initial stages of the investigation, is highly appreciated. Authors are also grateful to NIAMDD, Bethesda for providing standard gonadotrophins and to Drs. M. Mouren, Roussel Uclaf, France and H. Dobson, University of Liverpool, U. K. for generous gift of sex steroids antisera.

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Table 1 : Mean (±SE) concentrations of blood plasma progesterone, estradiol, LH and FSH on different days pre and post partum.

Pre/ Post partum day	Progesterone ng/ml	Estradiol–17 β pg/ml	LH ng/ml	FSH ng/ml
–20	0·88 ± 0·26	19·20 ± 6·80	2·17 ± 0·83	17·20 ± 12·14
–19	0·95 ± 0·25	10·80 ± 0·00	2·25 ± 0·87	15·50 ± 10·92
–18	0·89 ± 0·16	19·00 ± 2·08	1·98 ± 0·37	10·31 ± 4·08
–17	1·10 ± 0·11	26·00 ± 0·00	1·73 ± 0·22	11·39 ± 4·70
–16	0·89 ± 0·08	20·00 ± 3·21	2·10 ± 0·73	8·16 ± 2·43
–15	0·89 ± 0·02	23·00 ± 5·81	1·87 ± 0·17	7·22 ± 3·68
–14	0·85 ± 0·07	21·66 ± 6·61	1·69 ± 0·12	10·37 ± 3 68
–13	1·10 ± 0·27	23·33 ± 5·81	2·24 ± 0·61	9·70 ± 5·01
–12	0·96 ± 0·03	32·00 ± 3·28	2·19 ± 0·50	14·12 ± 3·46
–11	1·17 ± 0·57	28·00 ± 5·80	2·18 ± 0·85	8·68 ± 2·41
–10	0·99 ± 0·08	34·00 ± 4·70	2·24 ± 0·91	7·50 ± 1·80
– 9	0·98 ± 0·06	36·00 ± 3·06	1·96 ± 0·67	14·08 ± 7·57
– 8	1·02 ± 0·07	38·00 ± 1·16	1·78 ± 0·69	16·89 ± 6·32
– 7	1·01 ± 0·07	37·00 ± 1·00	2·09 ± 0·57	11·03 ± 4·64
– 6	1·09 ± 0·03	47·33 ± 6·36	2·08 ± 0·54	11·70 ± 5·31
– 5	1·09 ± 0·15	44·00 ± 2·00	1·28 ± 0·35	13·42 ± 4·28
– 4	1·19 ± 0·13	61·00 ± 9·02	1·48 ± 0·35	9·90 ± 4·54
– 3	0·86 ± 0·17	61·50 ± 4·35	1·08 ± 0·39	10·47 ± 5·84
– 2	0·87 ± 0·13	65·60 ± 4·69	1·04 ± 0·45	10·34 ± 4·43
– 1	0·60 ± 0·19	77·00 ± 3·52	0·87 ± 0·58	6·30 ± 3·11
0	0·23 ± 0·04	86·80 ± 11·03	1·22 ± 0·27	10·57 ± 3·52
+ 1	0·09 ± 0·01	4·40 ± 2·84	0·90 ± 0·06	7·36 ± 2·33
+ 2	0·17 ± 0·04	Undetectable	0·69 ± 0·38	15·34 ± 5·96

IJAR 8 : 2 : 127-128, 1987

Serum Progesterone Level During Oestrous Cycle In Goat

R.N. BARUAH, J.C. DUTTA[1], LEENA DUTTA, J. GOSWAMI and S.C. TALUKDAR

Department of Animal Physiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781 022

ABSTRACT

The mean serum progesterone concentration on various days of oestrus cycle in indigenous goat of Assam varied between 0·47 ± 0·04 to 5·56 ± 0·12 ng/ml. The average concentrations on the day of oestrus and 4th, 8th, 12th, 16th and 20th day after oestrus were found to be 0·47 ± 0·04, 1·81 ± 0·07, 4·63 ± 0·17, 5·56 ± 0·12, 3·82 ± 0·13 and 0·50 ± 0·03 ng/ml, respectively. The lowest and highest levels were observed on the day of oestrus, and 12th day of the oestrus cycle respectively.

* * *

Reproductive efficiency is one of the important factors in determining the economy of animal industry. In adult female animals, the reproductive efficiency depends upon the optimum levels of steroid hormones viz. oestrogens and progesterone liberated from the ovary. A disturbance of the secretion of these steroid hormones may lead to infertility or even sterility. Thus it is necessary to know about the level of these hormones in animals during various phases of reproduction. Adequate studies have not been conducted on the level of ovarian steroids in goats during various phases of reproductive cycle (Heap and Linzell, 1966; Thorburn and Schncider, 1972; Jain *et al.* 1980). The present study reports the serum progesterone level on various days of oestrus cycle in indigenous goat (Capra hircus) of Assam.

Materials and Methods

Nine regularly cyclic indigenous goats of Assam were taken for the present study. The animals were 1 to 2 years of age having 15 to 20 kg body weight. All the animals were maintained under standard nutrition and managerial conditions. Blood samples (10 ml) were collected from the jugular vein in clean glass tubes and allowed to clot. From each of the animals, 6 blood samples were collected on day 0, 4, 8, 12, 16 and 20 days post estrus. The serum was separated from the clotted blood samples and stored at -20°C till the assay was carried out.

The serum progesterone was determined by RIA technique, as per the method of Biodata progesterone 125 I/ter kit Code 11274 (Biodata laboratories, Via Tiburtina Valeria Km 19,600 00012 Guidonia Montecelio, Rome, Italy.) The sensitivity of the assay was found to be 0·1 ng/ml. The intra-assay variation ranged from 5·0 to 9·0 and recovery was 106·3%.

Results and Discussion

The lowest (0·47 ± 0·04 ng/ml) and highest (5·56 ± 0·12 ng/ml) levels of serum progesterone were recorded on the

1. Department of Gynaecology & Obstetrics.

day of oestrus and 12th day of the oestrous cycle (Table 1).

Thorburn and Schneider (1972) reported the plasma progesterone levels in goat on the day of oestrus and 10th day of the 21 day cycle as 0·2 ng/ml and 4 ng/ml, which corroborate with our findings. There was an increasing trend of serum progesterone level from the day of oestrus through 12th day of the oestrous cycle. From day 16th through 20th of the cycle, the serum progesterone level continued to decline. This decline of progesterone level in circulation towards the end of oestrus cycle and a peak level during the time of CL development (mid-cycle) indicates that ovarian functions can be monitored by estimating the peripheral serum progesterone level in goats.

Table 1 : Serum progesterone level (ng/ml) in goat.

Animal No.	Days of oestrous cycle					
	0 day	4th day	8th day	12th day	16th day	20th day
1.	·50	1·8	4·5	5·7	3·7	·52
2.	·70	2·2	5·8	6·2	4·2	·68
3.	·39	1·5	4·2	5·4	4·0	·42
4.	·45	2·0	4·0	5·1	3·8	·40
5.	·62	1·9	4·6	6·0	3·5	·50
6.	·42	1·6	4·9	5·4	4·5	·49
7.	·51	1·8	4·6	5·6	3·9	·50
8.	·29	1·8	4·4	5·4	3·2	·53
9.	·32	1·7	4·7	5·2	3·6	·43
Mean ± SE	0·47 ± 0·04	1·81 ± 0·07	4·63 ± 0·17	5·56 ± 0·12	3·82 ± 0·13	0·50 ± 0·03

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Studies On Embryo Transfer In Indigenous Goats Under Tropical Climate : Synchronization Of Oestrus By Melengestrol Acetate

K.P. AGRAWAL*

Division of Physiology & Climatology, Indian Veterinary Research Institute, Izatnagar (U. P.)

ABSTRACT

26 nullipara female Barbari goats (10 to 15 months age) in various stages of the oestrous cycle, were administered melengestrol acetate (MGA) with concentrate mixture @ 0·15 mg/animal for 16 days. 32 other animals of similar age and para were also studied to compare duration of oestrus and oestrous cycle length, percent conception, gestation length, litter size, weight of the placenta, time of placental expulsion, birth weight and subsequent growth of the kids. 25 out of 26 (96·12 %) animals exhibited oestrus within 4 days of treatment. The percent conception at first synchronized and subsequent heats was lower as compared to that of control animals but the differences were statistically insignificant ($P > 0·05$). Though there was no significant difference ($P > 0·05$) in the duration of first synchronized oestrus in treated and control animals, the subsequent post-synchronized duration of oestrus and oestrous cycle length in animals not conceived to first synchronized heat were significantly reduced ($P < 0·01$). The treatment with MGA seemed to cause no change in litter size, gestation length, weight of the placenta, time of placental expulsion, birth weight and subsequent growth of the kids ($P > 0·05$) in Barbari breed.

* * *

The oviduct and uterine environment changes in response to fluctuating hormonal levels during different phases of the oestrous cycle. For satisfactory pregnancy rates on embryotransfer, the embryo must be placed in an environment that simulates that from which it is removed. Close synchronization of oestrus in donor and recipient individuals is considered essential in a number of species : sheep (Moore and Shelton, 1964; Rowson and Moor, 1966), pig (Webel *et al.* 1970) and cow (Rowson *et al.* 1972).

The objective of the present study is to investigate the use of MGA oral therapy for synchronization of oestrus and subsequent fertility in indigenous Barbari goats.

Materials and Methods

Normally cycling 58 nullipara female Barbari goats (10–15 months of age) were used. 26 goats (experimental) were administered MGA orally in concentrate mixture @ 0·15 mg/animal for 16 days. 32 other goats of similar age and para were served as control.

* Present Address :
Sr. Scientist & Incharge, Division of Goat Reproduction & Physiology, Central Institute For Research on Goats, Makhdoom, P.O, Farah, Mathura-281122 (U.P.)

Oestrus was detected in the morning and evening by parading a vasectomized buck. The animals in oestrus were subjected to artificial insemination twice : one at first appearance and second 12 hrs. later. The animals not conceived in the first oestrus were re-inseminated in subsequent second and third oestruses.

Observations on oestrous synchronization rate, duration of oestrus, oestrous cycle length, conception rate, gestation length, litter size, weight of the placenta, time of discharge of placenta, birth weight and postnatal growth of the kids upto the age of 4 months were taken. The difference in value between control and experimental groups were statistically tested.

Results and Discussion

Out of 26 goats administered MGA, 25 exhibited oestrus within 4 days of treatment. The duration of synchronized oestrus was $33{\cdot}12 \pm 2{\cdot}79$ hr. and did not differ significantly ($P > 0{\cdot}05$) from that of the control. The duration of post-synchronized oestruses was, however, found to be reduced ($P < 0{\cdot}01$). Post treatment oestrus cycles length was observed to be significantly lower ($P < 0{\cdot}01$) (Table 1).

In the experimental group, out of 25 animals bred, 14 conceived at synchronized heat and 5 each at second and third post synchronized heats. All the 32 goats in control group were conceived : 24 at first, 7 at second and 1 at third heat. The differences between two groups were not significant ($P > 0{\cdot}05$). There was no significant difference ($P > 0{\cdot}05$) in litter size between the control and experimental groups (1·18 vs 1·27) (Table 2).

The MGA treated pregnant goats carried the foetuses to full term of $146{\cdot}00 \pm 0{\cdot}31$ days and did not differ significantly ($P > 0{\cdot}05$) than that of control group. The average birth weight of tne kids, irrespective of their sex and litter size, were $1{\cdot}81 \pm 0{\cdot}07$ and $1{\cdot}73 \pm 0{\cdot}03$ kg. in control and experimental groups respectively. Only female kids were used to study the postnatal growth rate upto 4 months of age. The average gain per day was $47{\cdot}42 \pm 3{\cdot}07$ and $42{\cdot}47 \pm 1{\cdot}53$ g. in control and experimental groups respectively. The difference between the two groups was insignificant ($P > 0{\cdot}05$, Table 3). The values in the weight and time of discharge of placenta between the two groups did not differ significantly ($P > 0{\cdot}05$).

The oestrus was synchronized to the extent of 96·12% within 4 days of the last feeding. Similar observations using synthetic progestogens orally were also recorded by several other workers in goats (Rahaman *et al.* 1978; Sanwal *et al.* 1978).

Present study reveals that the synchronization of oestrus with MGA causes a decline in conception rate. However, the differences were found to be insignificant ($P > 0{\cdot}05$). A decline in percent conception and fertility at the synchronized heat due to progestogen treatment has also been reported by other workers (Burnner *et al.* 1964; Deweese *et al.* 1970)

The gestation period, litter size, birth weight of the kids, postnatal growth and survivality of the kids, weight of the placenta and time of their discharge in treated goats are not significantly different ($P > 0{\cdot}05$) from the values of control animals and are similar to the

values reported by other workers for this breed (Prasad *et al.* 1971).

The present investigation indicates that MGA may be successfully utilized in Barbari nannies for synchronization of oestrus, without significantly impairing subsequent fertility.

Acknowledgements

Gratitude is expressed to Dr. N. K. Bhattacharyya, Head, Division of Physiology and Climatology and Dr. C.M. Singh, Director of the Institute for providing the necesary facilities.

Table 1 : The effect of MGA feeding on duration of oestrus and oestrous cycle length in goats.

Attributes	Control*	Experimental		
		First	Second	Third
Duration of Oestrus (h)	28·42 ± 2·10[a] (19)	33·12 ± 2·79[a] (25)	19·63 ± 2·92[b] (11)	22·00 ± 2·00[b] (6)
Length of Oestrous Cycle (days)	22·76 ± 3·15[a] (25)	3·08 ± 0·10[b] (25)	13·09 ± 2·61[c] (11)	10·50 ± 2·53[c] (6)

* The data on the duration of oestrus and oestrous cycle length is based on study during one cycle only.

The differences in values marked with common superscript are statistically insignificant ($P > 0·05$).

Figures in parentheses are number of observations.

Table 2 : Conception rate of goats fed with MGA.

	Control			ND	Experimental		
	No. Obs.	Conceived No.	Conceived %	Diff.	No. Obs.	Conceived No.	Conceived %
First Oestrus	32	24	75·0	1·51	25	14	56·0 NS
Second Oestrus	8	7	87·5	1·87	11	5	45·45 NS
Third Oestrus	1	1	100·0	0·44	6	5	83·33 NS
Total	32	32	100·0	0·30	25	24	96·0 NS

NS – $P > 0·05$

Table 3 : **Influence of MGA feeding on gestation length, birth weight and postnatal growth of the kids, weight and time of discharge of the placenta.**

Attributes	Control	Experimental	Value
Gestation length (days)	144·85 ± 0·60 (21)	146·00 ± 0·31 (22)	1·68 NS
Birth weight of the kids (KG)	1·81 ± 0·07 (31)	1·73 ± 0·03 (28)	1·00 NS
Weight gain/days (g) during first 4 months (only female kids)	47·42 ± 3·07 (12)	42·47 ± 1·53 (18)	1·44 NS
Weight of the placenta (g)	238·00 ± 14·51 (14)	220·00 ± 18·41 (17)	0·76 NS
Time of discharge of placenta (mt).	114·28 ± 5·90 (14)	111·92 ± 8·40 (13)	0·22 NS

Figures in parentheses are number of observations.
NS – $P > 0·05$

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Variation In The Metabolic Constituents Of Caprine Foetal Fluids During Different Stages Of Gestation

R.A. LUTHRA, R.C. GUPTA, S.K. KHAR and P. BARU

Department of Gynaecology and Obstetrics, College of Veterinary Sciences Haryana Agricultural University, HISAR-125 004

ABSTRACT

The changes in the metabolic constituents of foetal fluids and maternal blood plasma were studied in 12 pregnant goats during different stages of gestation. Total protein concentration was generally low in both fluids. Glucose and fructose levels increased in allantoic fluid with glucose concentration steadily registering lower values till about 3½ months of gestation, followed by a subsequent increase. Creatinine, urea and uric acid increased significantly with advancing gestation in allantoic fluid whereas in the amniotic fluid only urea levels increased significantly.

* * *

The constituents of the foetal fluids are in a dynamic state of equilibrium with foetus, membranes, placenta, uterus and the maternal organism. The different constituents, exchanging at different rates with different compartments, undergo constant changes during the entire gestational length. Such changes have been reported for bovines (Reeves *et al.* 1972; Baetz *et al.* 1976) and for ovines (Mellow and Slater, 1971, 1972). The present work was undertaken to study the normal range and changes in the metabolic constituents of foetal fluids and maternal plasma during different stages of gestation in conscious goats.

Material and Methods

The present study was conducted on twelve pregnant goats (Local breed), kept under isomanegerial conditions during four stages of gestation, thus :

Stage-I comprised of three animals between 1 to 2 months of gestation,

Stage-II comprised of three animals between 2 to 3 months of gestation,

Stage-III comprised of three animals between 3 to 4 months of gestation and

Stage-IV comprised of three animals between 4 to 5 months of gestation.

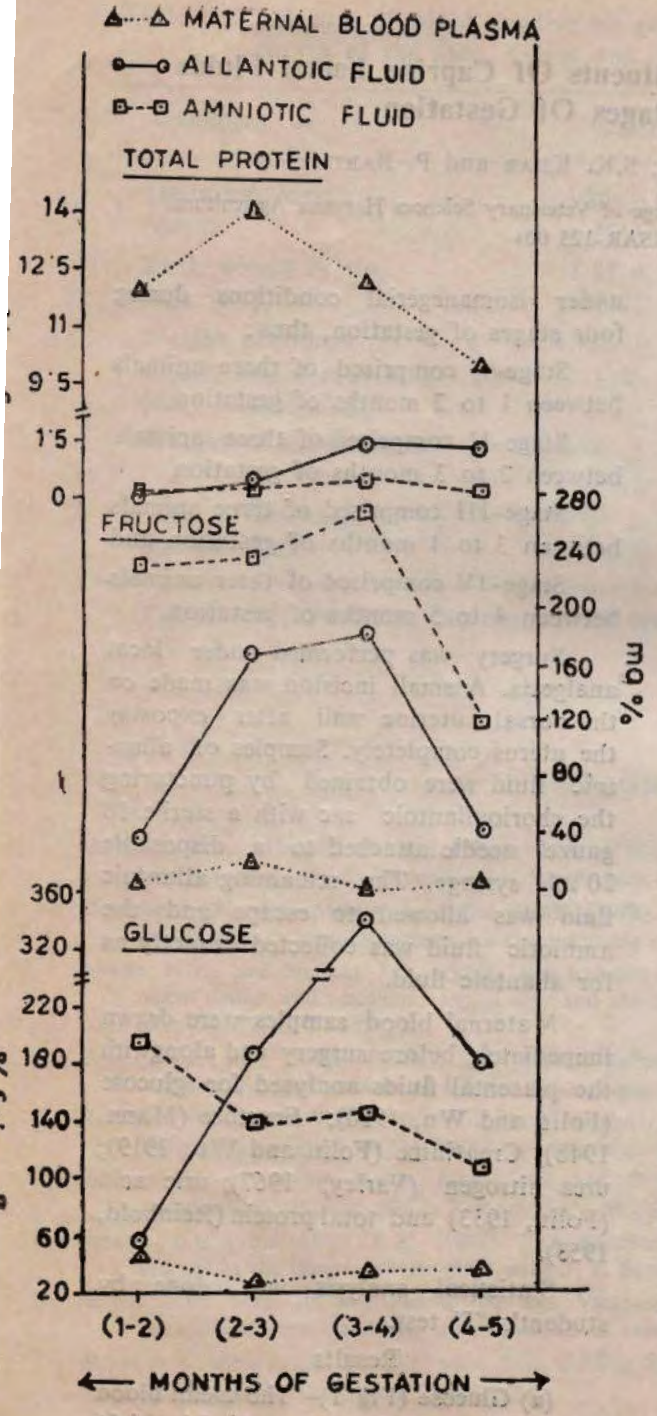
Surgery was performed under local analgesia. A small incision was made on the dorsal uterine wall after exposing the uterus completely. Samples of allantoic fluid were obtained by puncturing the chorioallantoic sac with a sterile 18 gauze needle attached to a disposable 20 ml syringe. The remaining allantoic fluid was allowed to escape and the amniotic fluid was collected exactly as for allantoic fluid.

Maternal blood samples were drawn immediately before surgery and alongwith the placental fluids analysed for glucose (Folin and Wu, 1920); Fructose (Mann, 1948); Creatinine (Folin and Wu, 1919); urea nitrogen (Varley, 1967); uric acid (Folin, 1933) and total protein (Reinhold, 1953).

Statistical analysis was done by student's 'T' test.

Results

(a) **Glucose** (Fig 1)- The mean blood glucose concentrations were 44, 26, 34·66


MATERNAL BLOOD PLASMA
ALLANTOIC FLUID
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TOTAL PROTEIN
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1·5
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280
240
200
160
120
80
40
0
mg %
360
320
220
180
140
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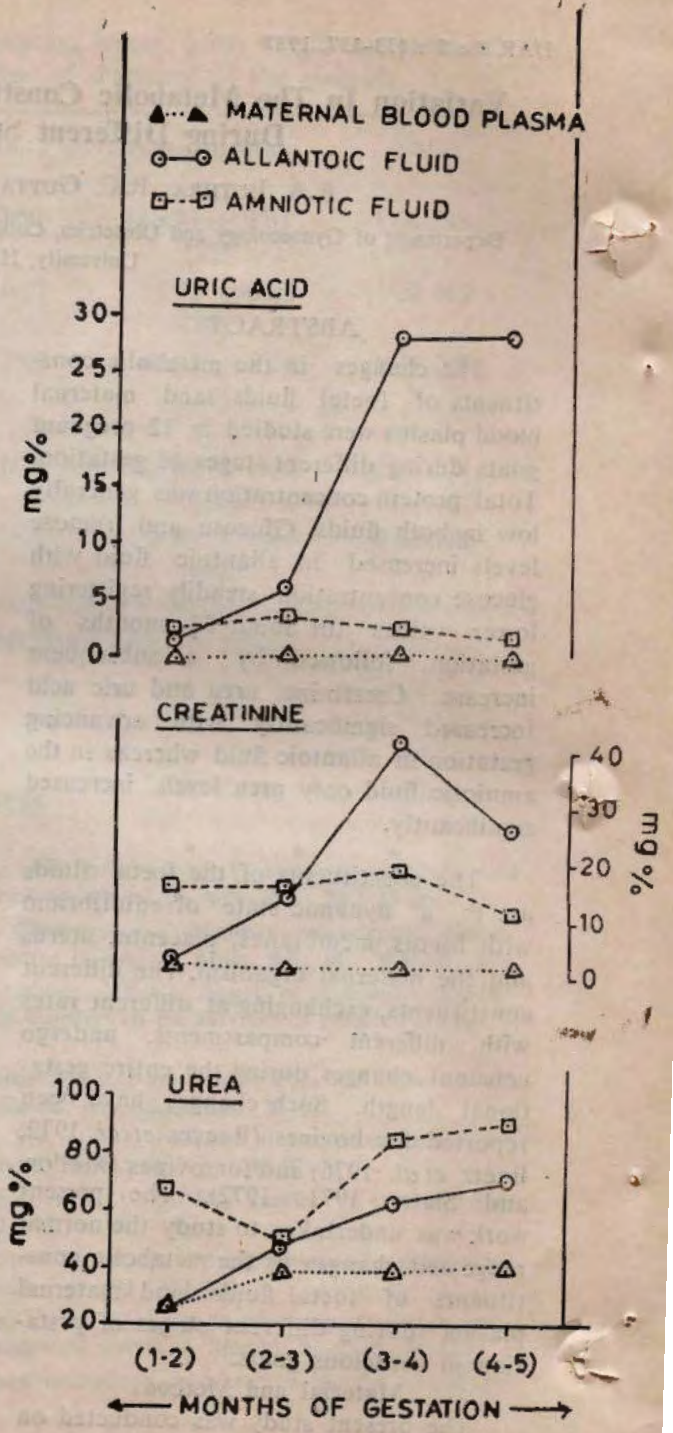
GLUCOSE
(1-2)
(2-3)
(3-4)
(4-5)
MONTHS OF GESTATION

Fig. 1


MATERNAL BLOOD PLASMA
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(1-2)
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(3-4)
(4-5)
MONTHS OF GESTATION

Fig. 2

and 35·33 mg% at the four gestational stages studied. The allantoic fluid glucose concentration at stage I, 55·33 mg% was marginally higher than blood concentration, but the concentrations at stages II, III and IV of 184·66, 339·33 and 178·66 mg% were significantly higher in comparison to corresponding stages except stage III. In the amniotic fluid consistently higher glucose concentrations of 196·66, 136·33 and 144·00 mg% were recorded. These values, however, are significantly different from maternal blood values only at stage I.

(b) Fructose (Fig. 1) – The blood fructose concentrations were either undetectable (stage III) or low (stages I and IV). However, at stage II the highest concentration 20 mg % was obtained. In the allantoic fluid this concentration increased to 166·66 mg% at stage II from an initial value of 36·66 mg % at stage I. This increased further to 182·66 mg % at stage IV. In the amniotic fluid the fructose concentrations were very high (230·66 mg % at stage I), which did not show any change at stage II. At stage III the level reached 270·66 mg % followed by a decrease to 120·66 mg % at stage IV. These differences were not significant.

(c) Total Protein (Fig 1)– The total protein concentrations in the maternal plasma were comparatively much higher than either of the foetal fluids at all gestational stages. The maternal plasma values varied between 9·86 gm % at stage IV to 14 gm % at stage II, whereas in the allantoic and amniotic fluid the average concentrations at most of the stages were below 1 gm % except in the allantoic fluid at stages III and IV.

(d) Creatinine (Fig 2) – The blood creatinine values were low and these decreased further with increasing gestation. The allantoic fluid creatinine concentration at stage I was comparable to the maternal plasma values, followed by a significant, dramatic increase at stages II and III and a sharp decline at stage IV. The amniotic fluid creatinine values at all gestational stages were more or less uniform varying from 10·86 mg % to 18·80 mg %.

(e) Urea Nitrogen (Fig 2) – The maternal plasma urea nitrogen concentration of 25·66 mg % at stage I increased to 39 mg % at stage II with almost no change at stages III and IV. In the allantoic fluid at stage I was 24·33 mg % increasing to 47 mg % at stage II with a further increase in values to 61·33 mg % and 69 mg % at stages III and IV. On the other hand the initial amniotic fluid concentration at stage I was high (67·33 mg %), which decreased to 48 mg % at stage II and registered a sharp increase to 84·66 and 90 mg % at stages III and IV.

(f) Uric Acid (Fig. 2) – The uric acid levels in the maternal blood plasma were either undetectable (Stages I and IV) or very low (Stages II and III). In the allantoic fluid at stage I it was 1·48 mg % which increased to 5·76 mg % at stage II and were constant around 28 mg % at stages III and IV. In the amniotic fluid the stage I value of 2·73 mg % at stage II was followed by a decrease to 2·80 and 1·80 mg % at stages III and IV.

Discussion

Glucose from maternal circulation is considered the main energy source for foetus (Alexander *et al.* 1970). Fructose is not utilised by foetus unless glucose supply is interrupted and even then its

metabolism is low (Bassett and Madill, 1974), since the foetal kidney does not conserve fructose, therefore it is carried through urine to the various foetal fluid compartments (Mellor and Slater, 1972). In the present study the allantoic fluid glucose concentration progressively increased upto stage III, whereas in amniotic fluid it decreased with advancing gestation. Similar findings are reported for cattle (Reeves *et al.* 1972) and Sheep (Mellor and Slater 1971, 1972). The amnion appears relatively impermeable to solutes (Mellor, 1970; Mellor and Slater, 1971) although passage of glucose between foetal plasma and amniotic fluid have been demonstrated. The greater permeability of chorioallantoic membrane (Mellor, 1970) suggests that the foetal blood in chorioallantois and maternal blood in the endometrium are potential source of foetal fluid glucose.

The foetal fluid fructose concentrations are dependent on the foetal urine fructose concentrations and these are reported to decrease after 110 days of gestation in sheep (Pearson and Mellor, 1977). The decrease in foetal fluid fructose concentration observed in the present study at the last gestational stage may also be due to similar phenomenon in goat.

The very low total protein concentration observed in the amniotic fluid of goats, with higher allantoic fluid concentration at corresponding stages agree with the findings in bovine (Baetz *et al.* 1976) and sheep (Alexander and Williams, 1968). The low protein concentrations are reported to be compensated by the high chloride concentration (Soliman, 1975).

A definite increase in the allantoic creatinine concentration upto stage III of gestation with no corresponding changes in amniotic fluid were observed. The uric acid changes were on identical lines as in creatinine concentrations. Since a greater quantity of foetal urine during gestation passes into the allantoic cavity, increase in the values of these constituents in the allantoic fluid appears logical. Similar reports are available for sheep (Mellor and Slater, 1971, 1972) and in bovines (Reeves *et al.* 1972). Such metabolic products that are normally excreted in the urine, slowly accumulate in the allantoic and amniotic fluids either due to increased foetal metabolism or insufficient exchange with internal circulation (Baetz *et al.* 1976).

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Certain Biochemical Profiles Of The Oviduct And Vagina Of Ewe (Ovis aries) During Estrous Cycle*

A. Seshagiri Rao, P.N. Rao, S. Govindappa[1] and S.V. Ramachandraiah

Department of Animal Reproduction, College of Veterinary Science, TIRUPATI-517 502, A.P.

ABSTRACT

The fluctuations in the mean glucose, pyruvate, lactate, glycogen, total carbohydrates and activity levels of lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), phosphorylase a and b, acid and alkaline phosphatases (ACP and AKP) and structural, soluble and total proteins, DNA, RNA and total lipids in the oviduct and vaginal tissue homogenate of ewe during various phases of estrous cycle were studied. The changes in the levels of substrate and enzyme activities were interpreted in the light of fluctuating levels of gonadal hormones or on the basis of physiological requirement.

* * *

An attempt was made to elucidate the biochemical profiles in the oviduct and vaginal tissue homogenate of ewe during various phases of estrous cycle and to interpret in the light of fluctuating levels of gonadal hormones or on the basis of physiological requirement.

Materials and Methods

The genitalia of adult Nellore breed ewes obtained from local abattoir, were transported to laboratory in ice. The reproductive organs were classified into various phases of estrous cycle as per Grant (1934). Both the oviducts and vaginal tissues from the reproductive tract were separated and dried quickly by rolling in a filter paper and weighed to the nearest milligram. The enzymatic and protein assays were undertaken first in preference to the rest of the parameters.

Glucose (Mendel *et al.* 1954) pyruvate (Friedmann and Hanger, 1942), Lactate (Barker and Summerson, 1941), Glycogen (Kemp and Van Heijningen, 1954), total carbohydrates (Caroll *et al.* 1956) and the activity levels of Lactate dehydrogenase (LDH) (Reddanna and Govindappa, 1979), Glucose-6-phosphate-dehydrogenase (G-6-PDH) (Bergmeyer and Bruns, 1965), phosphorylases (Cori *et al.* 1955), total lipids (Overturb and Dryer, 1969), proteins (Lowry *et al.* 1951), DNA (Giles and Myers, 1965), RNA (Munro and Fleck, 1966) and the activity levels of acid and alkaline phosphate ACP and AKP (Taussky and Shorr, 1953) were estimated in the oviduct and vaginal tissue homogenate.

Results and Discussion

The significant increase ($P < 0{\cdot}1$) in the levels of oviducal and vaginal glucose from proestrus to metestrus through estrus when compared to anestrus (Tables

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1. Department of Zoology, S. V. University, TIRUPATI 517 502. A. P.

1 and 3) indicated an enhanced tissue metabolism during the follicular phase which is in agreement with the increased oviducal glucose content in ewe and sow as observed by Iritani *et al.* (1969; 1974). The increased oviducal and vaginal metabolism during the above phases might be under the influence of higher circulating levels of estradiol-17 β as recorded by Niswender *et al.* (1974). A gradual, non-significant increase in the pyruvate levels noticed from anestrus to estrus indicated a gradual increase in the glycolytic activity of the tissues leading to accumulation of pyruvate. The increased pyruvate levels in the tubal fluid might be intended for the metaholic support of fertilized ovum (Salisbury and Van Demark, 1961). The elevation of pyruvate during diestrus might be due to conversion of lactate to pyruvate under the significant increased activity of LDH (Table 1). The significant decrease ($P < 0{\cdot}01$) in the lactate levels observed during proestrus, estrus and metestrus when compared to anestrus levels might be due to its secretion into the oviducal fluids as reported by Iritani *et al.* (1969) who recorded a higher lactate concentration in the oviducal fluid of ewe, from Day 5 to 16. The elevation in the glycogen levels recorded during proestrus and estrus in the present study might have been due to the glycogen synthesis activity under the influence of estrogen stimulation. The decline in glycogen level during metestrus might be due to non-significant increase of phosphorylase-a activity observed during estrus in the present investigation and also due to the increased breakdown of glycogen to glucose during the transition period from estrus to metestrus. The significant ($P < 0{\cdot}05$) glycogen accumulation during diestrus might be due to resorption of estrogen induced imbibed water, consequently increasing the total quantity of tissue per gram weight. The rise in G 6-PDH activity levels during metestrus might help in the production and secretion of pentoses into the fluids of oviduct to support the metabolic activity of the embryo.

The significant increase ($P < 0{\cdot}01$) in the total lipids observed during proestrus and metestrus when compared to anestrus (Table 2) is in agreement with the report of Iritani *et al.* (1969) who noticed increased lipid aldehyde and lipid choline content of ewe oviducal secretions between Day of estrus and Day 4 when compared to the levels recorded from Day 5 to Day 16 of the estrous cycle. The accumulation of total lipids in the vaginal tissue noticed during proestrus to metestrus (Table 4) might have been due to increased uptake of lipid material as a consequence of estrogen stimulated increased vascularity of the vagina. The significant increase ($P < 0{\cdot}01$) in the mean level of structural, soluble and total proteins observed during follicular phase in the oviducal and vaginal tissue homogenate (Tables 2 and 4) might have been induced by the increased levels of circulating estradiol-17 β. A rise in the oviduct and vaginal DNA content was noticed from anestrus to estrus (Tables 2 and 4) and this elevation might be due to replication of cells associated with estrogen induced mitosis (McDonald, 1980). The maximum level of RNA recorded during proestrus is in agreement with the observations of Miller (1976) showing that the mean ewe oviduct cell content of RNA to have rapidly increased at proestrus under the influence of estradiol-17 β. The increased RNA might have contri-

buted to the increased protein levels observed in this experiment. A non-significant increase in the activity levels of ACP from anestrus to proestrus recorded in this investigation finds support from the observations of Szego (1971) who demonstrated that estrogen induced localization of lysosomal membranes in the cytosol fraction of target tissue cells causes an increase in the ACP levels. The elevation in the AKP activity during metestrous and diestrous phases is in line with the observations of Rangaswamaiah (1981) who observed higher enzyme activity during luteal phase than in follicular phase of the buffalo oviduct.

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Table 1 : Mean levels of certain substrates and enzymes involved in ewe oviducal carbohydrate metabolism during oestrous cycle.

Substrate/enzyme	Anoestrus (n = 12)	Pro-estrus (n = 12)	Oestrus (n = 12)	Metestrus (n = 10)	Diestrus (n = 12)
Glucose** (mg/g wet wt)	0·09±0·01^{a}	0·13±0·01^{b}	0·13±0·01^{b}	0·16±0·01^{bc}	0·19±0·01^{c}
Pyruvate (μ moles/g wet wt)	3·68±0·30	3·81±0·39	4·54±0·41	3·87±0·23	4·87±0·28
Lactate** (mg/g wet wt)	23·15±1·38^{c}	16·70±1·45^{ab}	18·60±0·92^{b}	16·51±0·82^{ab}	14 55±2·05^{a}
Glycogen* (mg/g wet wt)	0·15±0·01^{a}	0·24±0·01^{b}	0·32±0·01^{d}	0·22±0·02^{b}	0·28±0·02^{c}
Total carbohydrates (mg/g wet wt)	0·74±0·07	0·92±0·07	1·06±0·07	0·77±0·11	0·80±0·07
Lactate dehydrogenase** (μ moles of formazan formed/mg protein/hr)	0·50±0·08^{a}	0·49±0·05^{a}	0·56±0·05^{a}	0·46±0·06^{a}	0·77±0·07^{b}
Glucose-6-phosphate dehydrogenase** (μ moles of formazan formed/mg protein/hr)	0·33±0·03^{a}	0·33±0·03^{a}	0·30±0·02^{a}	0·42±0·03^{b}	0·53±0·04^{c}
Phosphorylase-a (μ moles of Pi formed/ mg protein/hr)	1·58±0·17	1·96±0·55	2·15±0·29	1·81±0·33	1·90±0·56
Phosphorylase-b (μ moles of Pi formed/ mg protein/hr)	1·19±0·23	0·67±0·21	1·47±0·88	1·03±0·24	0·67±0·17

Values are mean ± S.E.

* Values having different superscripts are significantly different at P 0·05

** Values having different superscripts are significantly different at P 0·01

Table 2 : Levels of total lipids, structural, soluble and total proteins, DNA, RNA, ACP and ALP in ewe oviducal tissue homogenate during oestrous cycle.

Substrate/enzyme	Anoestrus (n=12)	Pro-estrus (n=12)	Estrus (n=12)	Met-estrus (n=10)	Di-estrus (n=12)
Total lipids** (mg/g dry wt)	94·78±9·43^a	170·24±3·95^c	180·45±10·30^c	149·28±10·98^b	139·09±7·28^b
Structural protein** (mg/g wet wt)	55·17±2·79^a	72·14±2·74^b	100·37±3·56^c	72·55±3·51^b	75·14±3·42^b
Soluble proteins** (mg/g wet wt)	67·41±2·80^a	88·65±3·35^b	117·76±3·05^c	84·86±2·70^b	86·92±3·58^b
Total proteins** (mg/g wet wt)	122·68±5·15^a	160·18±6·84^b	218·16±6·93^c	158·52±4·50^b	161·98±5·63^b
DNA** (mg/g wet wt)	2·50±0 30^a	4·59±0·39^b	4·98±0·56^b	5·14±0·69^b	4·16±0·35^b
RNA** (mg/g wet wt)	0·63±0·07^a	0·92±0·06^b	0·88±0·10^{ab}	0·76±0·10^a	0·69±0·11$_a$
ACP (μg of Pi liberated/mg protein/hour)	0·96±0·09	1·48±0·40	1·19±0·23	1·17±0·15	1·34±0·19
ALP** (μg of Pi liberated/mg protein/hr)	1·54±0·10^b	1·10±0·10^a	1·06±0·13^a	1·62±0·18^b	1·92±0·23^b

Values are mean ± S.E.

** Values with different superscripts are significantly different at $P < 0·01$

Table 3 : Mean levels of certain substrates and enzymes involved in ewe vaginal tissue carbohydrate metabolism during oestrous cycle.

Substrate/enzyme	Anoestrus (n=12)	Proestrus (n=12)	Estrus (n=12)	Metestrus (n=10)	Diestrus (n=12)
Glucose** (mg'g wet wt)	0·09±0·01[a]	0·13±0·01[b]	0·11±0·01[b]	0·17±0·01[c]	0·22±0·01[d]
Pyruvate** (μ moles/g wet wt)	3·12±0·23[a]	4·46±0·40[b]	4·92±0·34[b]	3·72±0·30[a]	4·81±0·26[b]
Lactate** (mg/g wet wt)	21·61±1·37[c]	15·31±1·23[ab]	17·84±0·89[b]	13·70±1·17[a]	13·85±0·94[a]
Glycogen** (mg/g wet wt)	0·17±0·02[a]	0·28±0·02[c]	0·41±0·01[d]	0·23±0·02[b]	0·17±0·08[a]
Total carbohydrates** (mg/g wet wt)	0·75±0·09[a]	0·93±0·06[ab]	1·08±0·08[b]	0·74±0·08[a]	1·06±0·08[b]
Lactate dehydrogenase (μ moles of formazan formed/mg protein/hour)	0·30±0·02	0·37±0·06	0·43±0·05	0·41±0·10	0·69±0·09
Glucose-6-phosphatate dehydrogenate** (μ moles of formazan formed/mg protein/hour)	0·31±0·04[a]	0·31±0·03[a]	0·31±0·02[a]	0·38±0·04[a]	0·50±0·07[b]
Phosphorylase-a (μ moles of Pi formed/ mg protein/hour)	1·65±0·77	1·89±0·38	1·46±0·26	1·74±0·33	1·37±0·30
Phosphorylase-b (μ moles of Pi formed/ mg protein/hour)	0·93±0·20	0·68±0·46	0·92±0·20	0·78±0·32	1·06±0·30

Values are mean ± S.E.

** Values with different superscripts are significantly different at $P < 0.01$

Table 4 : Levels of total lipids, structural, soluble and total proteins, DNA, RNA, ACP and ALP in ewe vaginal tissue homogenate during oestrus cycle.

Substrate/enzyme	Anestrus (n=12)	Proestrus (n=12)	Estrus (n=12)	Metestrus (n=10)	Diestrus (n=12)
Total lipids** (mg/g dry wt)	71·11±3·35^{a}	113·17±7·27^{b}	150·85±11·42^{d}	131·42±10·42^{c}	130·62±7·11^{c}
Structural proteins** (mg/g wet wt)	55·32±1·97^{a}	68·99±2·04^{b}	90·33±3·19^{c}	64·41±1·77^{b}	70·95±3·58^{b}
Soluble proteins** (mg/g wet wt)	64·54±1·92^{a}	80·06±1·79^{b}	105·52±3·53^{c}	77·68±1·97^{b}	84·40±3·12^{b}
Total proteins** (mg/g wet wt)	118·95±3·89^{a}	148·97±3·59^{b}	193·74±5·81^{c}	144·97±3·61^{b}	155·76±6·02^{b}
DNA** (mg/g wet wt)	3·51±0·31^{a}	4·65±0·24^{ab}	5·58±0·64^{b}	5·82±0·96^{b}	4·79±0·55^{ab}
RNA** (mg/g wet wt)	0·43±0·05^{a}	1·03±0·07^{c}	0·98±0·08^{c}	0·85±0·11^{bc}	0 77±0·08^{b}
Acid phosphatase (μg of Pi liberated/mg protein/hour)	0·92±0·11	1·56±0·33	1·16±0·24	0·90±0·12	1·07±0·12
Alkaline phosphatase (μg of Pi liberated/mg protein/hour)	1·13±0·07	1·03±0·09	1·05±0·10	1·56±0·30	1·63±0·30

Values are mean ± S.E.

** Values having different superscripts are significantly different at $P < 0.01$.

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Serum Level Of Minerals In Crossbred Cows And Buffaloes During Retained Placenta And Post-Partum Vaginal Prolapse

Y.P.S. DABAS[1] S.P. SINGH[2] and O.P. SAXENA[3]

College of Veterinary Science, G. B. Pant University of Agriculture & Technology, Pantnagar-263145

ABSTRACT

Serum concentration of calcium, inorganic phosphorus, magnesium, sodium, potassium, copper and zinc were recorded in the crossbred cows and Murrah buffaloes during retained placenta and vaginal prolapse. While serum values of calcium and inorganic phosphorus in cows and buffaloes were found to be singnificantly lower in both the retained placenta and vaginal prolapse, serum copper level was significantly lower in both the species only during retained placenta.

* * *

Retained placenta and post-partum vaginal prolapse are common reproductive disorders in cattle and buffaloes which cause great economic losses by reducing reproductive efficiency (Kaikini *et al.* 1976). Few investigators have shown that mineral deficiencies of sodium (Kerk, 1968), Zinc (Hoekstra *et al.* 1967) are associated with premature calving and retained placenta. In the present study concentration of a few minerals have been estimated in cows and buffaloes reporting to the Veterinary hospitals with conditions of retained placenta and vaginal prolapse.

Materials and Methods

The investigation was carried out on 52 Murrah buffaloes and 58 crossbred cows brought to college clinics and neighbouring Government Veterinary Hospital, Rudrapur. The animals were classified into 3 groups thus :

Gr. I– Retained placenta group : Consisting of 27 Murrah buffaloes and 31 crossbred cows which failed to expel their placenta within 12 hrs of parturition and were treated as cases of retained placenta.

Gr. II– Vaginal prolapse group : Comprising 15 Murrah buffaloes and 17 crossbred cows which were having post-partum vaginal prolapse.

Gr. III– Control group : Consisting of 10 Murrah buffaloes and 10 crossbred cows which calved normally without any complications.

Jugular blood was collected from all the animals between 48–72 hrs of parturition depending upon the case, and serum separated. The serum samples were analysed for calcium (Spandrio, 1964), inorganic phosphorus (Goldenberg and Fernandez, 1966), magnesium (Basinaski, 1965) sodium and potassium flame photo-

1. Asstt. Prof., Department of Vety. Clinics.
2. Prof. & Head, Vety. Physiology & Pharmacology Himachal Pradesh University of Agriculture, Palampur (H P.) – 176062.
3. Incharge, Vety. Clinics.

metrically, copper (Gubler *et al.* 1952) and zinc (Devies, 1968). The data were analysed statistically (Snedecor and Cochran, 1967).

Results and Discussion

The values of serum sodium and potassium in cases of retained placenta and vaginal prolapse in both the species were in the normal range and did not differ significantly from the values recorded in normal calving animals. Similarly, no difference in the serum values of magnesium and zinc was recorded in either species in cases of retained placenta and vaginal prolapse compared to the animal which calved normally. In general, however, the serum magnesium values were of lower order in crossbred cows compared to the Murrah buffaloes. The values of serum zinc in crossbred cows were also found to be lower than that of buffaloes. (Table 1) The serum values of calcium and phosphorus recorded in cows as well as buffaloes with conditions of retained placenta and vaginal prolapse were found to be significantly lower compared to the animals which calved normally. Although the role of calcium and inorganic phosphorus in the conditions of retained placenta and vaginal prolapse is not very clear, there is enough evidence to suggest that these elements are associated with fertility.

Delayed conception causing temporary infertility has been observed in dairy cows with subnormal inorganic phosphate contents in their blood (Snook, 1958). The fertility of dairy cows with subnormal serum phosphate, was found improved when defluorinated super phosphate was added to their drinking water (Scharp, 1979). In addition, a decline in serum calcium and inorganic phosphorus values with advancing parturition has been observed by Allcroft and Godden (1934). This decline was believed to occur in serum due to excessive mammary intake of these elements and their release into colostrum The cows and buffaloes with retained placenta were found to have lower serum copper values as compared

Table 1 : Serum concentration of certain minerals in retained placenta, vaginal prolapse and normal calving buffaloes and crossbred cows (Mean ± S.E)

Minerals	Retained Placenta		Vaginal Prolapse		Normal Parturition	
	Buffalo	Cow	Buffalo	Cow	Buffalo	Cow
Calcium (mg/dl)	8·25±0·35	8·40±0·25	8·3±0·2	8·54±0·35	9·6±0·4	9·25±0·3
Inorganic Phosphorus (mg/dl)	4·25±0·25	4·16±0·2	4 0±0·45	4·25±0 15	5·0±0·5	5·15±0 09
Magnesium (mg/dl)	4·15±0·37	2·80±0·20	4·08±0·32	2 82±0·22	4·25±0·25	2·75±0·3
Sodium (mEq/l)	136±4·0	142±5·5	138±4 0	140±5·0	135±5·0	138±4·0
Potassium (mEq/l)	4·50±0 3	4·25±0·12	4·45±0·25	4·35±0·21	4·25±0·2	4·1±0·16
Copper (μg/dl)	177±9·0	176±10 0	179±6·0	183±9·5	181±5·0	182±8·0
Zinc (μg/dl)	308±6·0	294±9·0	306±5·0	295±8·5	310±8·0	298±7·0

to normal calved animals. This element is an integral part of metalloenzymes like cytochrome oxidase, ceruloplasmin etc. which play important role in reproduction (Vallee, 1971). The low serum copper values recorded in retained placenta probably may interfere with normal detachment process of the placenta after parturition. More precise studies involving experimental animals under controlled dietary intakes of these elements may probably point out specific role of these elements in retained placenta and vaginal prolapse.

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Serum Proteins, Ascorbic Acid And Total Cholesterol In Anoestrus Kankrej Heifers

F.S. KAVANI[1], V.K. SHARMA[2], G.M. SIDDIQUEE[3], V.P. VADODARIA[4]

College of Veterinary Science & Animal Husbandry, Sardar Krushinagar-385 506

ABSTRACT

The serum proteins, total cholesterol and ascorbic acid levels in normal cycling and anoestrus Kankrej heifers were studied. The levels of total proteins (5·65± 0·23 g%), albumin (3·47±0·06 g%) and total cholesterol (169·54±6·08 mg%) were significantly lower in anestrus heifers. The level of total proteins was found to be positively correlated with globulin and cholestrol contents in normal cycling heifers whereas there was a negative correlation between total proteins and globulin in anoestrus heifers.

* * *

Commencement of estrous cycle and conception are events influenced by female sex steroid hormones, bioclimatic factors as well as managerial conditions. The present investigation has been carried out to estimate serum proteins, total cholesterol and ascorbic acid serum levels to study the relationship of these constituents amongst normal and anestrus heifers and variations in them.

Materials and Methods

Twenty-four heifers examined gynaecologically were categorized as normal cyclic heifers (n = 10) and anestrus heifers (n = 14). Blood samples were collected to harvest serum which was centrifuged to get clear serum samples which were stored in deep freeze until assayed. Standard methods were used for estimations of total proteins, albumen, globulin (Wootton and Freeman, 1982), total cholesterol (Span diagnostic kit) and ascorbic acid (Varley, 1976). The results were analysed for student's 't' test and for correlation coefficient (Snedecor and Cochran, 1967).

Results and Discussion

The mean ± SE values of biochemical estimates are presented below:

Sr. No.	Characteristics	Normal cyclic heifers $\bar{X}$ ± SE	Infertile heifers $\bar{X}$ ± SE
1.	Total proteins (g%)	7·14 ± 0·22	5·65 ± 0·23**
2.	Albumin (g%)	3·98 ± 0·10	3·47 ± 0·06*
3.	Globulin (g%)	3·20 ± 0·13	2·61 ± 0·22
4.	A : G ratio	1·27 ± 0·097	1·56 ± 0·19
5.	Total cholesterol (mg%)	269·10 ± 22·20	169·54 ± 6·08*
6.	Ascorbic acid (mg%)	0·084 ± 0·02	0·13 ± 0·02

* $P < 0·05$ and ** $P < 0·01$

1. Associate Professor of Gynaecology.
2. Assistant Professor of Gynaecology.
3. Assistant Research Scientist.
4. Associate Professor of Biochemistry.

Perusal of results revealed significantly ($P < 0.01$) higher concentration of total proteins (7.14 ± 0.22 g%) in normal cyclic heifers. Association of lower protein content with anestrous condition, could be due to resultant lesser functioning of pituitary and reproductive organs (Maynard *et al.* 1979). Further, significant correlation (+ 0.650) was observed between total protein concentration and total cholesterol in normal heifers but correlated negatively (–0.372) in anestrous heifers. It may be due to the fact that steroid synthesis is related to energy status of animals (Hafez, 1980).

Further, proteins content revealed significant positive correlation with globulin (0.615) in normal heifers, whereas contrary relationship (–0.762) of these estimates was observed in anestrous heifers. Of the higher mean values obtained for albumin and globulin, only albumin levels differed significantly ($P < 0.05$), in normal heifers. The findings corroborate with that of Sharma *et al* (1984).

Steroid hormones have a direct relationship with cholesterol metabolism (Hafez, 1980). Pituitary and corpus luteum activity in buffalo heifers is related to high cholesterol concentration (Zala *et al.* 1972) and precursor compounds for steroid synthesis are derived from cholesterol. Present findings of significantly low concentration of serum cholesterol (169.54 ± 6.08 mg %) in infertile heifers support this view. Vadodaria *et al.* (1976) reported higher cholesterol content in estrogen dominating phase of estrous cycle of buffalo. Thus, low cholesterol level might have resulted in inadequate synthesis of sex steroid hormones leading to anestrous condition.

Ascorbic acid level differed non-significantly between the two groups of animals. Philips (1941), did not report any difference between good and poor breeder with respect to their peak level of ascorbic acid during estrus. However, higher ascorbic acid level has been reported to be associated with better fertility status in cattle (Baghi, 1981).

The present findings reveal that significantly low levels of total proteins, cholesterol, albumen and absence of positive correlationship between total proteins and cholesterol are related to anoestrous condition which could have resulted due to inadequate energy supply and output of steroid and proteinous hormones from gonads and pituitary gland, respectively.

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SHORT COMMUNICATIONS

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Sporadic Abortions Due To Escherichia coli In Buffaloes And Cows

A.M. DAS and V.L. PARANJAPE

Department of Bacteriology, Bombay Veterinary College, Parel, Bombay-440 012

Specific reports on *E. coli* associated bovine abortion are on record (Savov and Buchvarova, 1977; Habiballa and El-Zubeir, 1978; Rowe and Smithies, 1978 and Moorthy, 1985). This is an amalgamated information on bovine and bubaline abortion due to *E. coli* infection.

During the year 1983-85, a total of 126 buffaloes and 55 cows were examined. Of these, five buffaloes (EB_1-EB_5) and four cows (EC_1-EC_4) were considered positive for *E. coli* associated abortion. Aborted foetuses and placentae were collected for bacteriological examination and the convalescent phase sera, for serological examination. Smear examination, multiple isolation approach and biochemical identification were made as per the previous protocol (Das and Paranjape, 1987). Serotyping was done by Frits and Ida Orskov at the International *Escherichia* and *Klebsiella* centre, Copenhagen, Denmark. Antibiotic sensitivity test with common antibiotics was performed according to Cruickshank *et al.* (1975). Newer antibiotics were tested on nutrient agar plates using Disc Cartridges (Oxoid) and Disc Dispenser MK II (Oxoid). Biological test was conducted in pregnant Guinea pigs. One was injected with 1 ml of 18 hour broth culture of a buffalo strain (EB_1) and the other with a cow strain (EC_1) by the intraperitoneal route. The control received the sterile broth. The serology included standard tube agglutination test and macroscopic

Taken in part from the Ph.D. thesis submitted by the senior author to the Konkan Agricultural University, Dapoli-415712.

agglutination test for brucellosis and leptospirosis respectively. Serum agglutinins against *E coli* were detected in a tube agglutination test by using somatic (O) antigens of strains EB_1 and EC_1 (Sojka, 1965). The sera of nonaborting healthy buffaloes and cows with cervical mucus negative and positive for *E. coli* isolation were employed as negative controls. Two cows (EC_1, EC_4) were treated with chloramphenicol at the rate of 20 mg / kg. body weight by intramuscular route and 1000 mg by intrauterine route for 5 days. Cervical mucus was culturally examined 3 days prior to and 5 days after the treatment schedule.

All buffaloes aborted at the 8th month and cows, between 4½–6 months. All had retained placenta and post-abortion endometritis not responding to strepto-penicillin or tetracycline. Gross lesions revealed necrotic placentitis and septicemic lesions in the foetal organs. Lesions were milder in cows than in buffaloes. *E coli* was isolated in pure culture from most of the foetal and placental specimens. No other bacterial entity was detected by multiple isolation approach. All buffalo strains were hemolytic and all cow strains were nonhemolytic on blood agar. Buffalo strains belonged to one particular serotype 0:8 whereas cow strains belonged to different serotypes i. e. 0:7, 0:NT, 0:8 and 0:96. All strains were resistant to common antibiotics like penicillin, ampicillin, oxytetracycline, chloramphenicol (except EC_1 and EC_4), erythromycin, furadantin, co-trimoxazole, carbenicillin (except EC_1, EC_3 and EC_4) neomycin, gentamycin (except EC_1, EC_2 and EC_4), kanamycin and streptomycin. Among newer antibiotics, all strains were sensitive to amikacin, netilmycin, cefotaxime and ceftazidime and resistant to tobramycin, cefsulodin and spectinomycin. Variable sensitivity pattern was observed against azlocillin and piperacillin (EC_1, EC_3 and EC_4=S; EC_2 and EB_1–EB_5=R). All serum samples were negative for antibodies against *Brucella* and *Leptospira* and positive for antibodies against *E coli* (EB_1 = 1280, EB_2 = 320, EB_3 = 320, EB_4 = 640, EB_5 = 1280, EC_1 = 80, EC_2 = 320, EC_3 = 320, EC_4 = 160). Negative control sera were devoid of agglutinins against *E coli* except that of the diagnostically nonsignificant titres (i. e. 4, 8, 16) in animals positive for *E. coli* in cervical mucus. The pregnant Guinea pig inoculated with buffalo strain (EB_1) aborted on the 3rd day and the one inoculated with cow strain (EC_1) aborted on the 5th day. Fetal lesions were more severe in the Guinea pig inoculated with the buffalo strain (EB_1). Foetal organs were culturally positive for *E. coli* in both the test animals. The serum antibody titres of aborted Guinea pigs inoculated with EB_1 and EC_1 strains against *E. coli* were 160 and 80 respectively. The cows (EC_1, EC_4) responded to the treatment with chloramphenicol. Cervical mucus were culturally positive three days prior to and negative five days after the treatment schedule. Per rectal examination revealed normalcy of the uterus following a normal cycling. Insemination induced conception at the first service.

Association of *E. coli* in a colonized infection should be examined with caution because of the ubiquitous nature of this organism. It is emphasized on the basis of the present and earlier observations (Habiballa and El-Zubeir, 1978 and Moorthy, 1985) that simultaneous isolation of *E. coli* from the internal organs of the fetus and from the maternal

specimens, in absence of established abortifacients, is suggestive of *E. coli* induced abortion. Demonstration of maternal antibodies against the agent as shown here and elsewhere (Savov and Buchvarova, 1977) may be of added advantage. Healthy bovine / bubaline sera may contain, but too low residual titres to interfere with the diagnosis. Epidemiological pattern revealed the occurrence in a sporadic form which concurred with that of Rowe and Smithies (1978). The interesting seroepidemiological type identification results explained that only one particular serotype caused abortion in buffaloes whereas variable serotypes did so in cows. The buffalo strains appeared more virulent and expressed a more pronounced 'multiple drug resistance pattern' as compared to cow strains.

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A Note On Congenital Defects In Corriedale Lambs

DAS KORNEL

Central Sheep Breeding Farm, Hissar.

Leipold *et al.* (1972) have defined congenital defects and abnormalities of structures of function present at birth. It excludes causes. The definition where congenital also is held synonymous to hereditary is no longer tenable. Many, but not all congenital defects are caused by genetic factors.

Congenital defects observed out of 3,241 corriedale lambs born at the Central Sheep Breeding Farm, Hissar, during the period 1973 - 74 are reported. Dennis (1965) has reported the congenital abnormalities of lambs from West Australia.

Present Address : Deptt. of Animal Breeding & Genetics, Orissa Veterinary College Bhubanewar-751002

1. **Specific defects observed in the present study :**

(a) Face–

(i) Agnathia : Only three lambs with agnathia have been observed. All the agnathia lambs were dead before birth. The lambs had absence of Craniofacial skeleton with absence of pharynx and larynx (Figs. 1 and 2). Dennis (1970) has reported otognathia in a lamb.

Fig. 1 Agnathia ventral view in a lamb.

Fig. 2 Agnathia lateral view in a lamb.

(ii) Superior brachygnathia (Hog mouth) and inferior brachygnathia (short lower jaw, Parrot mouth) – These defects are observed less frequently during weaning and are culled.

2. Vertebral column :

(i) Lordosis (Ventral deviation) : Six cases of lordosis at the cervical vertebral column were recorded. These lambs find difficulty in following the mother as well as for running. No lamb with lordosis was recorded during weaning at the farm.

(ii) Scoliosis (Lateral deviation or Torticol or Wry neck) : Eighteen lambs with scoliosis were recorded. They behave similar to lordosis lambs. Only one lamb with lateral deviation of lumbar vertebral column was recorded at the farm.

(iii) Wry tail– In a set of triplets, one lamb with wry tail was recorded.

3. Muscular system–

(i) Arthrogryposis (permanent flexor of a joint or constricture tendon) : It is one of the most common defects observed among the Corriedale lambs. Lambs with this defect are either born dead or die soon after birth. The forelegs are commonly affected (Fig. 3). One ram lamb was born as a twin with the defect survived by hand feeding and care during

Fig. 3 Arthrogryposis in a lamb.

1974. It started walking on the knees and after two months it started walking on feet with the constricture. Fifteen lambs with arthrogryposis of forelegs have been recorded during the study period.

(ii) Slippery tendon : Superficial flexor tendon is always involved in the lambs. The hind legs look like bow. Six lambs during the study have been recorded.

4. Organs of special senses :

(a) Eye–

Entropion : It is a common defect observed in the Corriedale lambs. Out of 7,000 lamb births 1·35 percent showed the incidence during 1974 (Kornel, 1976). It is quite evident soon after the birth and leads to keratitis with subsequent blindness. It is observed in both the sexes, one or both eyes are affected, and commonly the low eyelid incidence is high.

5. Monsters :

Three lambs weighing 6·8 to 8·6 kg. at birth have been recorded during 1974. All had dystocia, of which two ewes died.

6. Reproductive organs :

(i) Harmophrodite : Only one lamb has been recorded at the farm during the study. (ii) Cryptorchidism : It is observed during the time of weaning only but actual figures are not available.

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A Note On The Occurrence Of Adenitis Of Cowper's Gland In a Hissardale Ram

*D.L. PAIKNE and D.S. KALRA

Haryana Agricultural University, Hissar-125 004

Although orchitis, epididymitis, seminal vesciculitis and prostatitis have been frequently reported, there are few records of inflammatory conditions affecting Cowper's gland. Authors are therefore, placing on record adenitis of Cowper's gland which was encountered during experimental study with *Brucella abortus* biotype 1 (aerobic strain) in a Hissardale ram.

The ram belonged to group III which was infected by intraprepucial route with 48 hours old cultures of *Br. abortus* biotype 1 (aerobic strain) 7.6×10^9 organisms grown on tryptose agar supended in normal saline solution (NSS). The animals were reinfected 22nd, 24th and 26th days after first infection, as there was no serological evidence 3 weeks after first infection. The animal was sacrificed 126 days post infection (PI).

Clinically, the animal did not show any sign of orchitis or epidydimitis at any time during the experiment. Serologically, the animal was negative to Rose Bengal Plate Test (RBPT) and complement fixation Test (CFT). Response to standard Agglutination Test (STAT) was somewhat erratic. The titre was never higher than 40 I. U. (1 : 20), except on 14 days PI when it was 160 I. U. (1:80). Serological titre of the animal was zero (0) on 3rd, 28th, 49th, 56th, 63rd, 70th, 77th and 126th days PI. It was 20 I. U. (1:10) on 21st, 35th, 42nd, 84th, 98th, 105th, 112th and 119th days PI. The titre on 7th and 91st day was 40 I. U. (1:29).

The animal did not show any gross lesions on viscera as well as reproductive organs, except enlargement of both the Cowper's glands. Both the glands appeared to be 2 cm in diameter. Bacteriological cultures were attempted from heart, lung, liver, spleen, kidney, reproductive organs including Cowper's gland and were found to be negative. Suspension from tissue was made from Cowper's gland and was injected in male Guinea pig. Guinea pig did not show any gross lesion or serological response

Histological sections were prepared from the enlarged Cowper's glands in routine manner and stained by Haematoxylin-Eosin (H. E.) method. There was heavy infiltration of neutrophils and a few mononuclear cells in the interstitial tissue. Sections stained by Brown and Bren's method failed to show evidence of any organism. Etiology of inflammatory condition could not be established.

* Reader in Pathology, Nagpur Veterinary College, Nagpur-440006.

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Dystocia Due To Stenosis Of Pelvic Outlet In A Buffalo

B. MUNILAL DUBAY

Department of Gynaecology and Obstetrics, Veterinary College, University of Agricultural Sciences, Bangalore-560 024

Pelvic fractures and exostosis, as a cause of dystocia are uncommon in large domestic animals, but are occasionally observed in dogs and cats that are injured by moving vehicles (Roberts, 1971). The reduced bisiliac dimensions due to previous fracture of pelvic wall and its displacement, cause dystocia in bitches and sow (Arthur, 1964). There is no literature on stenosis of pelvic outlet causing dystokia in buffaloes.

Case Report and Discussion

A naturally served primiparous buffalo was brought to the college hospital with the history of onset of labour pains since 36 hours without the delivery of the foetus. The water bag had appeared and ruptured at vulval lips, 30 hours earlier. The patient was in debilitated and dehydrated condition. Vulval lips were oedematous and vagina was congested. It was diffult to pass the hand per vaginum into pelvis and it revealed a dead foetus in anterio-dorsosacral position with fetlock and head lying in the vagina. The pelvic cavity resembled 'v' shape with an acute angled pelvic floor. A detailed history revealed that the patient had fallen into a deep gutter at six months of age, with resultant change in its walking gait. The case was diagnosed as dystokia due to stenosis of pelvis outlet. Right flank laparo-hysterotomy was performed under para-vertebral anaesthesia and a dead male foetus was delivered. Placenta was removed and surgical wound closed. Post-operative recovery was uneventful and on the 8th day the buffalo was discharged.

Accidents causing pelvic deformity can cause dystocia. In she buffaloes, the symphisis pubis is not fused completely as in cows (Hadi and Sane, 1965). In the present case, per-vaginal examination revealed a healed bilateral pubic bone fracture near acetabulum with acute angulation of pubis symphisis, resulting in increased sacro-pubic diameter and decreased bis-iliac diameter. The variations in pelvic biometry and the characteristic anatomical features of buffalo pelvis resulting in stenosis of pelvic outlet had caused dystocia in the reported case.

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A Note On The Processing Of Uterine Biopsy Material

K.N. Aravinda Ghosh, T.R. Bharathan Namboothiripad* and
K. Praphakaran Nair

Department of Animal Reproduction, College of Veterinary & Animal Sciences, Mannuthy-680651

The standard procedure for processing autopsy tissue was found to be unsuitable for uterine biopsy material, since the tissue pieces obtained were too small. Hence a modification in the timing for dehydration, clearing, paraffin infiltration and embedding had been made by trial and error on uterine tissues collected with biopsy instrument from abattoir specimens. The standardized method was adopted in processing of uterine biopsy tissue collected by a modified biopsy technique(Ghosh *et al*, 1980)from 26 repeat breeder cows and four normal cows. The size of the endometrial piece obtained in biopsy varied from 3 to 4 mm. These were transferred to physiological saline to remove the clots and mucus. The clean tissues were fixed in Bouine's fluid for a period of 6 to 12 hrs. and then washed with 50% isopropyl alcohol. For dehydration, clearing and paraffin infiltration, the following steps were adopted : (i) 50% Alcohol 1 change 10 mts. (ii) 70% Alcohol 1 change 10 mts. (iii) 80% Alcohol 1 change 10 mts. (iv) 90% Alcohol 1 change 10 mts. (v) 95% Alcohol 1 change 10 mts. (vi) 100% Alcohol 2 changes 100 mts. each (vii) Alcohol xylene mixture 1 change 5 mts. (viii) xylene 2 changes 2 to 3 mts. each and (ix) Melted paraffin 4 changes 5 mts. each.

Embedding was done in paraffin wax having a congealing point of 59° to 60°C. The fragments of endometrium were placed in the paraffin so that the mucosal surface was perpendicular to the plane at which the block was sectioned. Sections of 3 to 5 microns in size were stained by haemetoxylin eosin method. The detailed histopathological studies have already been reported (Ghosh *et al*, 1983).

The processing technique presently employed was different from the routine histological techniques (Humason, 1972), and from the process earlier described for uterine biopsy (Brus 1952, Hallway, 1971). The main modifications employed were with regard to the fixation time and the duration of treatments for dehydration, clearing and paraffin infiltration. Treatment for 10 mts. in each of the ascending series of alcohol and short treatment for 2 to 3 mts. in xylene for clearing and 4 changes in paraffin for infiltration proved to give best results for uterine biopsy materials.

* Since deceased.

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CLINICAL ARTICLES

IJAR 8 : 2 : 158–159, 1987

Dystocia Due to Perosomus Horridus In A Buffalo–A Case Report

A.S. NANDA, R.D. SHARMA and K.S. ROY[1]

Department of Obstetrics & Gynaecology, Punjab Agricultural University Ludhiana-141 004

ABSTRACT

Monstrosities are the result of abnormal or arrested fetal developments. Teratology, the study of neonatal malformations, is of great importance from the genetic transmission of such malformations. This warrants the recording of various malformations in animals. Perosomus horridus is a fairly discussed monster in cattle (Roberts, 1971) which, however, has not yet been reported in buffaloes. The present report records the occurrence of perosomus horridus fetal monster causing dystocia in a buffalo.

* * *

A pluriparous buffalo which had completed gestation period and was in labor pains for the past two days was presented to Veterinary Clinics, PAU for dystocia treatment. Vaginal examination revealed an ankylosed distorted fetus jammed in the birth canal of dam. The previous handling and delay in presentation of the case to the clinics rendered it unfit for fetotomy. Cesarean operation was, therefore, undertaken and a dead male fetus with distorted contour was removed. The monster was not excessively oversized.

It had two bends in the vertebral column. The anterior bend was downwards (concavity) in the cervical region while the posterior was upwards (convexity) in the lumbar region, thereby giving a linear S–shape deformity (Fig. 1) The fetus appeared shorter in length because of bends in the vertebral column. Unlike

1. Associate Professor, Department of Anatomy & Histology.

sharp bend in the vertebral column in the schistosomus reflexus, the bends in this monster were gradual. The sacral and coccygeal vertebrae were fused together. All the other vertebrae were distinct and ankylosed.

Fig. 1 Perosomus horridus–a buffalo fetal monster with double S–shaped twist of vertebral column.

The lower jaw of the fetus was small in size (Micrognathia inferior). The skull was complete, the meninges were present but the brain tissue was almost ill-developed. Similarly, the vertebral canal was also empty. There was agenesis of the nervous tissue. It resembled with perosomus elumbis, where lack of nervous stimulation due to partial agenesis of the spinal cord leads to atrophic changes in bones and muscles (Cohr, 1965). However, it differs from the latter in which vertebral axis ends at caudothoracic region and the lumbar, sacral and coccygeal vertebrae may be absent (Jubb *et al.* 1985). The fetus slightly resembled with Campylorrhachis scoliosa (Nanda *et al.* 1985). However, it had two bends instead of one. The buccal cavity was normal contents except that the tongue was membranous and adhered to the floor of mouth cavity. The forelimbs were free while all the joints of the hind limbs, were ankylosed. The patella was rudimentary. All the viscera were apparently normal. The scrotum was developed but the testis were retained in the abdomen. Cryptorchidism, ankylosis and death of the fetus in last trimester of pregnancy has been reported to be genetically transmitted in cattle (Thomson *et al.* 1957). The same may be true in buffalo also. It is suggested that breeding of parents of such offsprings should be avoided.

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Schistocephalus Fissipalatinus, Schistocephalus Fissilabrus With Single Nostril In A Crossbred Murrha Buffalo Calf

M.R.K. Iyer[1] and K.S. Nair[2]

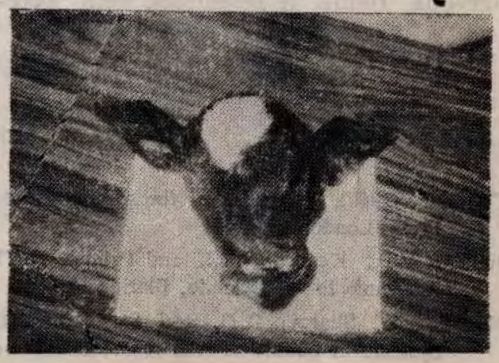
ABSTRACT

Published reports on the occurrence of congenital anomalies among black cattle are not many and hence there is need to document periodic information whenever available. Developmental anomalies have been reported by Jubb and Kennedy (1963) and Christopher (1971). The present report puts on record the occurrence of a fetal monster having schistocephalus fissipalatinus, Schistocephalus fissilabrus with single nostril in an Indian Water Buffalo.

* * *

Clinical History and Findings

A day old Buffalo Calf belonging to the Central Prison was presented with the history that the animal was unable to suckle. On enquiry it was reported that the calf was the fifth one born to a Murrha crossbred dam by natural crossing. It was female, fully grown, alert but weak and weighed 12 kg. It could move freely. Except for the head defects no other abnormalities cculd be seen. The head was comparatively small. The eyes and eye lids were well formed and there was movement of eye balls. The calf had good vision. The nasal bridge was placed obliquely. The upper lip was also flattened with underdeveloped muzzle on the left side, with small cleft in the centre. The mandibular joints were partly ankylosed resulting in lack of sideward, upward and downward movement. Only with force a finger could be inserted in the mouth. The left nostril alone was present and the external nares could be dilated during respiration. Breathing was slightly laboured due to aspiration pneumonia. The palate was clefted and was communicating with the nasal cavity. During force feeding, there was partial dysphagia and postprandial nasal discharge of milk. The nasal cavity was communicating with the pharynx. The tongue was well developed and sucking tendency was absent. The lower jaw was comparatively longer, slightly curved upward resemhling a spoon. There was malocclusion, prognathism of mandible and the inciser buds were smaller in size.



Surgical repair of the cleft palate was not attempted since the prognosis

1. Assistant Director, Directorate of Animal Husbandry, Trivandrum.
2. Senior Veterinary Officer - S.P.C.A. Hospital, Trivandrum.

was grave. It was reported that the calf had died within 72 hours after its birth. No abnormalities could be detected in any of the internal organs on postmortem examination.

Discussion

In mammals congenital defects have been defined as abnormalities of structure or function present at birth and with no clearly established cause, eventhough interaction of environmental factors such as toxic plants, ababane virus, viral diarrhoea, drugs and trace elements may occasionally induce the defects besides genetic factors as the main etiological cause (Leopold *et al.* 1983). As per Shupe *et al.* (1967) cleft palate may be due to hereditary factors. In the present case the contribution of the bull towards this trait cannot be ruled out, because two calves born earlier and sired by this bull had harelip and cleft palate eventhough born to different dams, indicative of a genetic cause. According to Jubb and Kennedy (1963) the development of skull is intimately related to the development of neural groove and hence invariably anomalies of the one frequently accompany that of the other.

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Anthelia In A She Goat

A.K. Gupta[1], B.L. Bishnoi[2], M. Taneja[3] and I.S. Kohli[4]

Department of Obstetrics and Gynaecology, College of Veterinary and Animal Science, Bikaner–334 001

She goats, more often than not, have 2 teats. However, there are reports of the presence of fused and functional supernumerary teats (Kaleff, 1942; Fisher, 1962; Roberts, 1971; Cokrill, 1974) and absence of teats–Anthelia (Fisher, 1856) in buffaloes. There are also reports of partial amastia (Sinha, 1980; Singh *et al.*

1. Assistant Professor, 2. Associate Professor, 3. M. V. Sc. Student, 4. Professor.

1983) in buffaloes. But there are no reports on anthelia in goats, hence this case report.

* * *

A hornless indigenous goat aged 4 years was brought to the College Clinic for pregnancy diagnosis. The goat had only one teat. She had parturated twice before, with good flow of milk through this one teat only. On examination it was found that the animal had only one half of the udder well developed with the other half rudimentary. Demarcation between the two at the base of the udder could be palpated (Fig. 1) No rudimentary teat was seen.

Supernumerary teats and athelia are said to be genetic in origin so this condition anthelia may also be similar to them.

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