VEIN VARICOSIS IN A PANDHARPURI BUFFALO - A CASE REPORT

M.D. Kulkarni, A.S. Kadam, A.V. Khanvilkar, and O.N. Ladukar

Varicose vein is a condition in which veins are markedly dilated as well as elongated to follow an irregular tortuous course to accommodate their excess length. Incidence of varicose veins in animals is low, as compared to human beings. In ruminants mostly lower limb veins are involved; however, the radial, cephalic, saphenous, mammary, scrotal and coccygeal vein involving inferior aspect of the trunk is also observed in cattle and buffaloes (Tyagi and Jit Singh, 2001), while distended metatarsal vein in horse is known as “blood spavin” and incidence is fairly often recorded (O’connor, 2001). Vein varicosity may either be congenital or acquired in origin due to repeated vascular trauma resulting in thinning of the vessel wall.

A case history: The present case of vein varicosity was recorded during the visit of the college purchase committee of Pandharpuri buffalo at Pandharpuri market on dated December 20, 2003. The buffalo belonging to a farmer named Mr. Dilip Ramchandra of Godse Village Kasegaon, Tal, Padharpur, Dist-Solapur was found to be suffering from vein varicosity (Figures 1 and 2) of the coccygeal vein. After anamnesis it can be concluded that condition was due to dilatation of the vein due to trauma and proximal occlusion or insufficiency of the valves as a sequel to arteriovenous fistulaor shunt. The coccygeal vein became engorged with blood due to poor venous drainage and loss of elasticity, became dilated, became tortuous and elongated resulting ultimately in ischaemia, hypoxia, malnutrition. In this case there was alopecia, preliminary stage of necrosis and gangrene with tail hairs at the end.

The extensive swelling of the varicose vein was treated by compression with bandage, firing and legation of vein above and below the swelling by the local livestock development officer without success but leading to exaggeration of the lesion. But obliteration with quinine hydrochloride and urethane or excising the vein with drainage of the contents was not tried due possibility of extensive hemorrhage and shock in the animal.

SUMMARY AND CONCLUSION

A unique case of varicose vein in Pandharpuri buffalo is put up on the record for academic and field veterinarians as the condition is very rare in animals and particularly in buffaloes.

REFERENCES


Krantisinha Nana Patil College of Veterinary Science, Shirwal, Dist-Satara (Maharashtra State) 412 801, India
PERFORMANCE OF SWAMP BUFFALOES OF ASSAM IN RESPECT OF SOME ECONOMICALLY IMPORTANT TRAITS OF REPRODUCTION UNDER FARM CONDITION

Arpana Das, D. Das, R.N. Goswami, G.C. Das and D. Bhuyan

ABSTRACT

A total of 500 lactation records that pertain to 164 swamp buffaloes maintained at the Livestock Research Station, Assam Agricultural University, Assam, India were utilized to study some of the economically important traits of reproduction viz., age at first calving, dry period, service period, gestation period and intercalving period. The effect of different non-genetic factors viz., lactation order, season of calving, period of calving and sex of calf on the traits were investigated. The least squares means for age at first calving, dry period, service period, gestation period and intercalving period were found to be 56.63±0.47 months, 193.41±4.68 days, 187.30±4.57 days, 323.41±0.10 days and 510.68±4.53 days, respectively. Period of calving had highly significant effect (P<0.01) on dry period, service period and intercalving period. Age at first calving, dry period, service period and intercalving period differed significantly (P<0.01) due to different seasons of calving. None of the traits were significantly affected by order of lactation. Effect of sex of calf on gestation period was, however, highly significant (P<0.01), females bearing male calves showing longer gestation periods.

INTRODUCTION

In India, buffalo husbandry is an important component of the livestock production system, being the principal provider of milk and work power. The scenario is the same in Assam, which is located in the north eastern part of India.

MATERIALS AND METHODS

Collection of data

The data utilized in the study pertain to 500 lactations from 164 swamp buffalo cows maintained at the Livestock Research Station, Assam Agricultural University, Assam, India. The traits considered were the age at first calving, dry period, service period, gestation period and intercalving period.

Classification of data

Data were classified as per lactation order, season of calving, period of calving and sex of calf. There were five lactation orders from 1st to 5th (L1 to L5), two seasons of calving viz., most calving season (S1) from August to January and least calving season (S2) from February to July, two periods of calving viz., P1 from 1989 to 1993 and P2 from 1994 to 1999 and two sexes of the calf were male (S’1) and female (S’2).
Statistical analysis

To study the effect of non-genetic factors least squares technique (Harvey, 1975) was employed. Duncan’s Multiple Range Test (DMRT) as modified by Kramer (1957) was carried out for pair wise comparison of sub class means.

RESULTS AND DISCUSSION

The least squares means for age at first calving, dry period, service period, gestation period and intercalving period are presented in Table 1.

The mean age at first calving was found to be 56.63±0.47 months, which compares favourably with the findings of Konanta et al. (1992) in swamp buffaloes of Thailand (56.00 months). However, Gogoi (1994) and Zaman et al. (2000) recorded higher and Das (1988) recorded relatively lower ages at first calving in swamp buffaloes of Assam.

The average dry period observed in the present study (193.41±4.68 days) closely corroborated with the findings of El-Kimary (1996) in Egyptian buffaloes. On the other hand, relatively longer dry periods in swamp buffaloes of Assam were reported by Amonge (1993), Gogoi (1994) and a shorter dry period was recorded by Joshi et al. (1993) in local buffaloes of Nepal.

The mean service period was found to be 187.30±4.57 days in the present study; this was found comparable with the findings of Zaman et al. 2000 (182.99±3.02 days) in swamp buffaloes.

The overall gestation period and intercalving period were recorded as 323.41±0.10 and 510.68±4.53 days, respectively, in the present study. This agrees well with Zaman, 1996 (324.40±0.21 and 507.54±3.00 days, respectively).

Effect of lactation order

The effect of lactation order on the dry period, service period, gestation period and intercalving period were not found to be significant (Table 1). Gogoi (1994) and Das (1988) also observed non-significant effects of lactation order on service period and intercalving period, respectively. Zaman (1996), on the other hand, observed that, in swamp buffalo, the variation due to lactation order were non-significant in regards to dry period and gestation period, but significant for service period and intercalving period.

Effect of season of calving

It was observed that except for gestation period, all the traits under study viz., age at first calving, dry period, service period and intercalving period differed significantly (P<0.01) due to season of calving; most calving season registered lower values for all these four traits (Table 1). Shorter dry period, service period and intercalving period in most calving season in due to early resumption of post partum heat and pregnancy (Ahmad et al., 1998). Similar seasonal influences on reproductive performance of swamp were also reported by Gogoi (1994) and Zaman (1996).

Effect of period of calving

The period of calving had a significant effect (P<0.01) on the dry period, service period and intercalving period, but age at first calving and gestation period were free from period effect. Dutt and Yadav (1988) and Singh et al. (1992) also found significant effects of period of calving on dry period, service period and intercalving period in riverine buffaloes, whereas the effect was found non-significant in Murrah buffaloes by Gogoi (1994). At variance with the present findings, Gogoi (1994) observed non-significant effects of period of calving on age at first calving and gestation period in swamp buffaloes of Assam.

Effect of sex of calf

The gestation period was found to be significantly (P<0.01) affected by the sex of calf. The animals bearing male calves had significantly longer gestation periods than those bearing female calves (Table 1). The findings of Misra et al. (1970) and Zaman (1996) in buffaloes corroborate the present observation. However, Sarvaiya et al. (1992) failed to see any significant effect of the sex of calf on the gestation period.
Table 1. Least squares means and their standard errors for age at first calving (AFC), Dry period (DP), service period (SP), gestation period (GP) and intercalving period (ICP) along with the results of DMRT.

<table>
<thead>
<tr>
<th>Sub class description</th>
<th>AFC(months)</th>
<th>DP(days)</th>
<th>SP(days)</th>
<th>GP(days)</th>
<th>ICP(days)</th>
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<td>µ</td>
<td>56.63±0.47</td>
<td>193.41±4.68</td>
<td>187.30±4.57</td>
<td>323.41±0.10</td>
<td>510.68±4.53</td>
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<tr>
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<td>-</td>
<td>188.49±6.19</td>
<td>180.64±6.05</td>
<td>323.48±0.14</td>
<td>504.22±6.00</td>
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<td>-</td>
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<td>Season of calving</td>
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<td>54.96±0.49</td>
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<td>S2</td>
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<td>P1</td>
<td>56.09±0.85</td>
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<tr>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>324.70±0.21</td>
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<tr>
<td>S2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>322.12±0.12</td>
</tr>
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</table>

N.B. Within parenthesis is the number of observations. Sub class means in a column with different superscripts differ significantly (P<0.01) from each other.
REFERENCES


DETECTION OF GROUP AND SEROTYPE SPECIFIC ANTIBODIES TO BLUETONGUE VIRUS IN BUFFALOES IN GUJARAT, INDIA


ABSTRACT

A serological survey of bluetongue was conducted in bovine, employing c-ELISA and BT-AGID test. A total of 168 serum samples were screened for the presence of BTV group specific antibodies. Out of these, 66 (39.29%) and 98 (58.33%) of the samples were positive by BT-AGID and c-ELISA respectively. The prevalence of the infection with respect to epidemiological factors such as breed, age, sex and health status of the animals is discussed. Prevalence was highest among aborted animals compared to other sick and apparently healthy animals. BTV serotype specific neutralizing antibodies against BTV serotype 1, 2, 3, 4, 8, 10, 12, 14, 16, 23 and 24 were detected.

INTRODUCTION

Bluetongue is an infectious, a non-contagious, insect-transmitted disease of domestic and wild ruminants. BT has a broad host range and it is a cause for serious concern to the livestock industry due to staggering direct and indirect economic losses mainly attributed to high morbidity, mortality, abortions, foetal deaths and fleece losses. BTV is listed under category “A” of the Office International des Epizootics (OIE) and consequently restrictions are imposed on movement of ruminants from bluetongue endemic regions to bluetongue free zones and so is of major importance to international trade (Alexander et al., 1996).

Clinical signs of disease are uncommon in domestic animals and are generally found only in sheep and some wild ruminants. Though cattle, buffaloes, goats, camels and certain wild animals are also susceptible to BTV infection, the clinical form of disease is not frequently encountered. However, these ruminants provide a reservoir for maintenance as carriers of virus. The infected bovines exhibit prolonged viraemia compared to sheep and may act as reservoir hosts for BT virus (Gard and Melville, 1992).

Definitive diagnosis of BTV infection relies on laboratory techniques for isolation and demonstration of BTV antigens, viral nucleic acid and antibodies (Afshar, 1994). Since the BTV infection is often inapparent in cattle and buffaloes, the detection of infected animals becomes difficult on the basis of clinical profiles or isolation of the virus. However, presence of BTV antibodies in a herd indicates the presence of viral infection. Hence, it is generally necessary to screen the sera for the presence of group and serotype specific antibodies (Jain et al., 1992). Therefore, to study the distribution and gravity of problem, seroepidemiology has been considered as important tool, and in the epidemiology of BT, serological surveys are used to analyse infection status of the ruminants in the area (Sreenivasulu and Subba Rao, 1999). The serological tests used to detect BTV antibodies are of two types, viz., group specific and serotype specific. The Serum Neutralization Test (SNT) and the Haemagglutination Inhibition (HI) test are serotype
specific. Of the two, the SNT is the most widely used test (Appleton and Letchworth, 1983). Tests devised for the detection of group specific antibodies include the agar gel precipitation test (AGPT) (Jochim and Chow, 1969), complement fixation test (CFT), dot-Immunobinding assay (DIA). ELISA and c-ELISA (Afshar et al., 1987 and Afshar, 1994). The object of this communication is to report the results of a serological survey on the prevalence of group specific and serotype specific antibodies to BTV in buffaloes in Gujarat.

MATERIALS AND METHODS

Test samples:

A total of 168 sera samples were collected from buffaloes from various locations of Gujarat. The separated serums were placed in screw-capped vials and heat inactivated at 56°C for 30 minutes and stored at -20°C until tested.

Serum samples for serotyping:

Eight seropositive buffaloes were selected for further collection of serum for serotyping. Serum samples were collected, processed and finally sent to the World Reference Laboratory, Onderstepoort, South Africa, for serotyping.

REFERENCE REAGENTS

The bluetongue virus (BTV) and c-ELISA kits were made available by courtesy of Dr. M.M. Jochim, Veterinary Diagnostic Technology, Incorporated, USA. The kits contained the following diagnostic reagents:

a) BT-AGID kit

i. Bluetongue antigen

ii. Bluetongue antibody positive serum

b) c-ELISA kits

(i) 2x8 well Divida strips pre-coated with BTV antigen
(ii) BTV antibody negative, weak positive and strong positive control sera.
(iii) BTV antigen specific monoclonal antibodies
(iv) 20X washing buffer
(v) 10X diluting buffer
(vi) Peroxidase labeled goat anti-mouse immunoglobulin
(vii) Capsules of phosphate-citrate buffer with sodium perborate
(viii) 2 mg O-phenylenediamine dihydrochloride (OPD) tablets
(ix) 3N H₂SO₄ (stopping reagent)
(x) Frames of holding the 2x8 well strips

Bluetongue agar gel immunodiffusion test (BT-AGID)

The protocol of the AGID test was followed as per Pearson and Jochim (1979). Six ml of molten gel was poured in each petri dish and allowed to solidify on a horizontal plane. A pattern consisting of a center well surrounded by six wells was made using a immunodiffusion template. Each well had a diameter of four mm and the center-to-center distance between wells was 6.4 mm. The central well was charged with known BTV soluble antigen and the peripheral wells with the test sera. Each serum sample was placed next to the well containing reference BTV antiserum so as to ascertain the specificity of the lines developed. The charged petri dishes were incubated at room temperature under humid conditions for 72 hours before pronouncing the sample as negative or positive. In the BT-AGID test, clear positive sera showed sharp precipitating lines that were in continuity with those given by the reference antiserum against BTV.
Competitive-Enzyme linked Immunosorbent Assay (c-ELISA)

The test was performed as per the protocol of Afshar et al. (1987) as follows

(i) The positive, weak positive and negative control sera and all the serum samples to be tested were diluted by adding 50 µl of each sample to 200 µl of diluting buffer.

(ii) The antigen-coated Divida strip wells were washed twice with 1X washing buffer and dried by tapping the strips on a tissue paper.

(iii) Two wells meant for diluent control were filled with 100 µl of 1X diluting buffer and covered with a tape to serve as diluent control only.

(iv) 50 µl of 1:5 diluted positive, weak positive and negative sera and all the test sera were run in duplicate.

(v) A dilution of BTV antigen specific monoclonal antibody was prepared by adding 25 µl of monoclonal antibody solution to 205 ml of diluting buffer and 50 µl this diluted monoclonal antibody was added to all the wells except the diluent control wells.

(vi) The reagents in each well were mixed gently by tapping the edge of the Divida strips and kept for 2 hours at room temperature.

(vii) Meanwhile, a dilution of peroxidase conjugated goat anti-mouse immunoglobulin was prepared by adding 25µl of conjugate to 7.5 ml of 1X diluting buffer.

(viii) Tape was removed from the diluent control wells and all the wells were washed three times with 1x washing buffer and dried by trapping the strips on a tissue paper.

(ix) 100 µl of the diluted conjugate was added to each well.

(x) The plate was kept at room temperature for 1 hour.

(xi) Approximately 10 minutes before the hour ended, the substrate-OPD solution was prepared by adding 1 buffer-substrate capsule to 100 µl of deionized water mixed it and 5 ml of this substrate solution was used to dissolve 1 OPD tablet.

(xii) After washing the wells five times with washing buffer, 100 µl of the substrate was added to the wells and held for 10 minutes in dark chamber for development of colour.

(xiii) After 10 minutes, 50 µl of the stop reagent (3N H₂SO₄) was quickly added to all the wells.

(xiv) Finally, the optical density of each well was recorded in an ELISA microplate reader with a 490 nm wavelength filter.

Interpreting the results:
To calculate the result, the average OD value was determined for all the negative, weak positive and strong positive duplicate wells. The average OD value of the diluent wells represented the background OD was subtracted from the average OD values of the control and test sera to yield their adjusted OD values. Then positive/negative ratios were calculated by dividing the adjusted OD values of the positive control and the test sera by adjusted OD values of the negative control serum. These ratios were multiplied by 100 to express them as a percentage of the negative control and this was subtracted from 100 to calculate the percentage inhibition (PI).

The samples were considered to be positive for BTV antibody if the PI value was above 50 percent.

RESULTS AND DISCUSSION

A total of 168 buffalo sera from six different places were screened. The BT-AGID and c-ELISA were performed, and the rates of seroprevalence recorded were 39.29% and 58.33 %, respectively. Table I represents locationwise, sexwise, breedwise and statuswise seroprevalence of BTV group specific antibodies in buffaloes. The results of serotype specific antibodies in the sera of eight seropositive animals are shown in Table 2. These sera showed the presence of antibodies against BTV serotypes 1,2,3,4,8,10,12,14,16,23 and 24.
Of 168 sera tested from buffaloes, 39.29 and 58.33 percent were seropositive by BT-AGID and c-ELISA, respectively. Similar rates of seroprevalence 34.72 and 37.50 percent by AGID and 60.46 percent by c-ELISA have been reported by Chandel (1996), Oberoi \textit{et al.} (1988) and Naresh and Prasad (1995), respectively. However, contrary to present findings Sharma \textit{et al.} (1981) failed to detect BTV antibodies in buffalo, and lower seroprevalence rates of 4.92 to 20.00 percent have reported by several other worers (Saini \textit{et al.}, 1992; Prasad \textit{et al.}, 1998; Sreenivasulu and Subba Rao, 1999).

All the six places included in this study showed a variable rate of seroprevalence 30.00 to 51.28 percent by BT-AGID and 47.62 to 69.23 percent by c-ELISA. The maximum rate of seroprevalence was recorded in Mehsana (51.28 and 69.23%) followed by Banaskantha (34.04 and 53.19%) and Sabarkantha (38.10 and 47.62%) by both the tests employed in the present study corroborating the findings of Chandel (1996) by BT-AGID test.

The buffaloes from which sera was collected consisted of two breeds \textit{viz.}, Mehsani and non-descript (ND) breeds. The prevalence of BTV antibodies was higher in ND breeds (41.67 and 68.75%) than Mehsani (38.33 and 54.17%) and this might be due to almost half of the samples were from the ND animals. With regard to breed susceptibility, no published report is available, so it was not possible to make any inference on breed susceptibility. However, dayakar \textit{et al.} (2001) reported a higher prevalence rate (57.84 percent) in Murrah buffaloes in Andhra Pradesh.

The sexwise seroprevalence recorded was (38.85 and 56.69%) in females and (45.45 and 81.82%) in males by BT-AGID and –ELISA, respectively. Because only a very small number (II) of sera from males were tested, it is difficult to interpret or reach any valid conclusion. However, Jain \textit{et al.} (1992) observed a similar prevalence rates of 12.72 percent in adult male and breeding bulls and 9.52 percent in adult females indicating that both male and female may be equality susceptible to BTV infection.

The total of 168 sera were obtained from four age groups, ≥1, 1-3, 3-5 and >5 years of age, and the seroprevalence in these groups recorded was highest in the >5 years of age group (41.05 and 63.15%) followed by the 1-3 years of age group (40.00 and 60.00%), the 3-5 years (36.58 and 48.78%) and the ≥1 year (33.33 and 50.00%) by BT-AGID and c-ELISA, respectively. This is in accordance with the observations of Jain \textit{et al.} (1992).

Sera collected from buffaloes of different status were screened, and the highest seroprevalence was found in aborted buffaloes (48.72 and 61.54%) followed by stiffness (41.18 and 58.82%), apparently healthy (36.54 and 58.65%), retention of placenta (20.00 and 40.00%) and dystocia (33.33 and 33.33%). The present findings in aborted buffaloes corroborates with the findings of Chandel (1996) and Chandel \textit{et al.} (2001), who reported higher rates of seroprevalence (36.48 and 29.83%). Respectively, by the AGID test, whereas Tongaonkar \textit{et al.} (1983) reported the lower prevalence of 15.85 percent in aborted and apparently healthy buffaloes. One Mehsani buffalo gave birth of a calf with congenital deformities of legs and head. The was seropositive, but the calf could not be tested for the presence of BTV antibodies as it died immediately after birth. This finding corroborates the findings of Chandel (1996), who reported a seropositive dam with a calf having congenital deformities of the lower jaw in Jafrabadi buffalo. Congenital deformities in the calf of a seropositive dam was also reported by Luedke \textit{et al.} (1970).

Serotyping based on neutralizing antibodies revealed the presence of multiple serotypes in buffaloes in Gujarat State. Neutralizing antibodies against BTV serotypes 1,2,3,4,8,10,12, 14,16,23 and 24 were adduced. The most prevalent
of these were serotypes 10 and 12. There were two animals infected with one BTV serotype, one with two, one with three, three with four, and one animal showed serotype specific neutralizing antibodies against seven different BTV serotypes simultaneously. To date, 24 BTV serotypes have been recorded worldwide, 21 of which have been reported in India (Wilson et al., 2000). The presence of multiple BTV serotypes in sheep has also been reported by Harbola et al. (1982) from Maharashtra, Janakiraman et al. (1991) from Tamilnadu; Chandel (1996) in Gujarat and Sreenivasulu and Subba Rao (1999) in Andhra Pradesh. There were similar reports of multiple serotypes present, not only in cattle herds or flocks of sheep, but also multiple BTV and EHDV serotypes in a single animal (Stott et al., 1982).

Animals exposed to BTV serotypes repeatedly in nature will have neutralizing antibodies to multiple serotypes because of cross reactions among the BTV serotypes (Jeggo et al., 1983). Hence, it is difficult to assess the prevailing BTV serotypes in an area based on neutralizing antibodies in adults which have been exposed to BTV serotypes previously.

Clinical BT has not yet been noticed in cattle and buffaloes in this country, although BTV antibodies have been reported from some states of India. Though the present study is based on a limited number of sera, it is difficult to reach at any meaningful conclusion. However, it appears that BTV infection occurs in buffaloes in Gujarat, which is indicative of either previous exposure of these animals to the bluetongue virus or inapparent infection. Therefore, further studies to understand the dynamics of BTV infection in buffaloes are warranted.

ACKNOWLEDGEMENTS

We thank Dr. V.P. Vadadaria, Dean, College of Veterinary Science and AH for providing necessary facilities. The authors are grateful to Dr. M.M. Jochim, President, Veterinary Diagnostic Technology, Inc. USA for supplying the BT-AGID and BTV c-ELISA test kits. We are also highly grateful to Dr. (Mrs.) T. Geredes, Head, Deptt. Virology, World Reference Laboratory, Onderstepoort, South Africa for serotyping. We also gratefully acknowledge the help of Dr. D.R. Mewada, Dr. D.C. Raval, Dr. M.A. Patel and field veterinarians for sera collection.
Table 1. Seroprevalence BTV group specific antibodies in buffaloes.

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<td>BT-AGID</td>
<td>Percent positive</td>
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<td>Total</td>
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<td>66</td>
<td>39.29</td>
<td>98</td>
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Table 2. Antibody clusters to different serotypes of BTV in buffaloes in Gujarat.

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<td>F</td>
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REFERENCES


**FEEDING AND NUTRITION**


An experiment was conducted to study the effect of feeding ammoniated wheat straw treated with HCl on blood biochemical profiles in growing male buffalo (*Bubalus bubalis*) calves. Twenty-four growing male buffalo calves (one year of age, 88.54 plus or minus 3.81 kg average body weight) were divided into three groups in a completely randomized design on the basis of their body weight. Animals in all the three groups were fed on concentrate mixture. In addition, they were offered wheat straw, ammoniated wheat straw (4% urea at 50% moisture level) and HCl treated ammoniated wheat straw (4% urea at 50% moisture level and HCl added to trap 30% of the NH3 evolved) in groups I, II and III, respectively for a period of 180 days, as per Kearl (1982) for body weight gain of 500 g/d. In all diets, concentrate:roughage ratio was fixed at 50:50 and were made isonitrogenous by adjusting CP levels of conc. Mixtures. Blood was collected from jugular vein of each buffalo calf at the beginning and subsequently at two months interval of experimental feeding. Due to urea-ammoniation, the CP content of wheat straw increased from 2.90 to 6.96% and addition of HCl along with urea further increased the CP content to 10.09%. In all the three groups, the mean values of plasma glucose (mg %) and serum globulin (g %), showed a decreasing trend, while the mean value of serum TP (g %), serum A:G ratio, serum urea (mg %), serum creatinine (mg %), serum ALP (KA units), SGOT (units/ml), SGPT (units/ml), serum T3 and T4 (ng/ml) showed an increasing trend with the advancement of feeding period. The cumulative period mean values of serum TP (6.15 to 6.20 g%), serum albumin (3.07 to 3.18, g %), serum globulin (2.98 to 3.09, g %), serum A:G ratio (1.03 to 1.10), serum ALP (23.15 to 23.63, KA units), serum T3 (1.20 to 1.23 ng/ml) and serum T4 (21.33 to 21.88 ng/ml) were comparable among the groups. The cumulative period mean plasma glucose (mg%) in group III (57.28) was similar to groups I (55.31) and II (59.41), however, the cumulative period mean plasma glucose in group II was significantly (p<0.01) higher than group I. The cumulative period mean serum urea (mg%) in group III (47.34) was significantly (p<0.001) higher than group I (38.38) and II (42.24), which were statistically alike. However, the cumulative period mean serum creatinine values (mg%) in groups II (1.43) and III (1.52) were similar and were significantly (p<0.01) higher than group I (1.24). The cumulative period mean SGOT (unit/ml) in groups I, II and III was 91.71, 96.64, respectively. Similarly the cumulative period mean SGPT (units/ml) was 19.00, 19.93 and 20.01 in groups I, II and III, respectively. The cumulative period mean values of SGOT (P<0.05) and SGPT (P<0.001) in groups II and III were similar and were significantly higher than group I. The cumulative period mean serum T3 and T4 values in groups I (1.21 and 21.81), II (1.23 and 21.42) and III (1.20 and 21.33) were comparable. From the present study it may be concluded that feeding of AWS treated with and without HCl to growing male buffalo calves for 180 days had no significant adverse effect on blood biochemical profile.
An experiment was conducted on 3 male rumen fistulated adult buffaloes fed on wheaten straw and concentrate mixture in a Latin square design to study the impact of niacin supplementation on rumen metabolites. Three animals were fed wheaten straw+concentrate mixture (group I, control), wheaten straw+concentrate mixture+100 ppm niacin (group II) and wheaten straw+concentrate mixture+200 ppm niacin (group III). After 21 days feeding, rumen liquor was drawn for 3 consecutive days at different time intervals (0, 2, 4, 6 and 8 h) to study the various rumen metabolites i.e., rumen pH, ammonia-N, total-N, trichloroacetic acid precipitable-N, non-protein nitrogen, total volatile fatty acids, their fractions and number of protozoa. Mean pH values in strained rumen liquor (SRL) of animals in 3 groups were 6.64, 6.71 and 6.67, indicating no statistically significant difference. Results revealed a significant (p<0.01) increase in TVFA concentration among the supplemented groups (group II and III) in comparison to control group. Mean TVFA concentration (meq/dl) was 9.75, 10.97 and 11.44 in 3 groups, respectively. The highest concentration of TVFA was observed at 4 h and minimum at 0 h in all the 3 groups. The percentage of acetic, propionic, butyric and isobutyric was statistically similar among the three groups. The mean ammonia-N concentration (mg/dl SRL) was significantly (p<0.01) lower in group II (16.38) and group III (15.42) than group I (18.14). Ammonia-N concentration was higher (p<0.01) at 4 h as compared to all the time intervals. The mean total-N concentration (mg/dl SRL) was higher (p<0.01) at 4 h as compared to other time intervals and lowest value was recorded at 0 h. Concentration of TCA-ppt-N (mg/dl SRL) was significantly (p<0.01) lower in control group as compared to niacin supplemented groups. Mean value of NPN (mg/dl SRL) was significantly (p<0.01) lower in group III (23.21) as compared to group I (25.71), whereas groups I and II and groups II and III were similar to each other. Total protozoa number (x104/ml SRL) ranged from 18.06 to 27.41 in group I, 20.89 to 38.44 in group II and 27.61 to 39.45 in group III. The mean protozoa number was significantly (p<0.01) higher in SRL of group II (27.60) and III (30.59) as compared to group I (22.48). It can be concluded from the study that supplementation of niacin in the diet of buffaloes had improved the rumen fermentation by decreasing the concentration of ammonia-N and increasing protein synthesis.

The experiment was conducted in five male growing Thai swamp buffaloes (1 year old) with an average initial weight of 172±4.8 kg to study the effects of complete diets containing different N sources on urinary purine derivatives excretion, rumen microbial production, some digestive and blood metabolite parameters. The 4th Chulalongkorn University Veterinary Annual Conference (2005). 134.
Influence of berseem and lucerne silages on feed intake, nutrient digestibility and milk yield in lactating Nili buffaloes

M. Sarwar, M.A. Khan, Mahr-un-Nisa, N.A. Touqir. 


This study was conducted to evaluate the feeding value of berseem and lucerne silage as a replacement for conventional fodder (berseem fodder) in lactating Nili buffaloes. Fifteen early lactating multiparous Nili buffaloes, five buffaloes in each group, were allotted to three experimental diets. Berseem and lucerne fodders were ensiled at 30% DM (wheat straw was used to adjust the DM of fodders) with molasses (at the rate of 2% of fodder DM) in two bunker silos for 30 days. The diets contained 75% DM from berseem fodder (BF), 75% DM from berseem silage (BS) and 75% DM from lucerne silage (LS). Each diet contained 25% concentrate DM. Diets were mixed daily and fed a day at ad libitum intakes. Dry matter intake (DMI) was significantly higher (13.8 kg/day) in buffaloes fed BF diet than those fed LS (12.5 kg/day) and BS (11.9 kg/day) diets. The differences in digestible DMI and DMI as percent body weight were significant between fodder and silage based diets but non-significant when BS and LS were compared. Lower DMI with silage-based diets was probably because of low silage pH. Intake of NDF (NDFI) was higher (5.68 kg/day) in buffaloes fed BF diet followed by those fed LS (5.50 kg/day) and BS (5.00 kg/day) diets. The difference was significant (p<0.05) across fodder and silage based diets but NDFI was non-significant across both silage-based diets. The apparent DM digestibility was significantly different (p<0.05) between fodder and silage-based diets but was non-significant between LS and BS diets. Four percent fat corrected milk yield was significantly different (p<0.05) between fodder and silage-based diets but was non-significant between LS and BS diets. Higher milk yield with fodder based diet was because of more digestible nutrient intake compared with silage based diets. Milk CP, TP and NPN and SNF did not show any treatment effects. The present results indicated that the berseem and lucerne fodder ensiled at 30% DM level with 2% molasses could safely replace (75% DM) the conventional leguminous fodder in the diets of lactating Nili buffaloes.
HEALTH AND DISEASES


The pharmacokinetics and dosage regimen for cefoperazone were investigated in 5 male buffalo calves following a single intramuscular dose at 15 mg/kg body weight. The absorption and elimination half lives were 0.8 plus or minus 0.393 and 5.65 plus or minus 0.30 h, respectively. Based on the pharmacokinetic parameters, the intramuscular dosage regimen for cefoperazone in buffalo calves would be 12 mg/kg followed by 9 mg/kg body weight dosages at 12-h intervals.


The prevalence of F. gigantica infection, faecal egg counts, worm counts and carcass and liver weights were determined in 32 buffaloes and 250 cattle slaughtered in Cotabato, Mindanao, Philippines (date not given). It was shown that the prevalence of infection was higher in buffaloes than in cattle, and was higher in the females of both species compared to males. The highest prevalence was observed in cattle and buffaloes more than 6 years of age, followed by those aged more than 3-6 years. The lowest prevalence was in animals aged 3 months-3 years. The number of mature and immature F. gigantica and faecal egg counts were higher in cattle compared to buffaloes. Animals aged >6 years also had a higher number of mature and immature parasites compared to younger ones. The number of adult liver flukes and faecal egg counts were significantly correlated (P<0.01) but faecal egg counts and age of the animals were not (P=0.88). Carcass weight was significantly lower in affected buffaloes than in heathones at more than 6 years of age. However, affected cattle also had higher carcass weights compared to non-infected ones in all age groups. Liver weight was also significantly higher in infected animals compared to healthy ones. The lower numbers of mature and immature F. gigantica and faecal egg counts in buffaloes may indicate that this species is more resistant to liver fluke infection compared to cattle, and that older animals of both species may also be more resistant to infection compared to younger ones.


Milk composition pertaining to somatic cell count (SCC), electrical conductivity (EC), lactose and pH was compared in three quarter milk fractions (foremilk, mid-milk and strippings) and one udder composite milk from healthy and mastitic buffaloes. A total of 225 quarters from 57 lactating buffaloes were studied. All the components except pH showed a significant variation over the milk fraction and udder health. In general, EC and lactose decreased while SCC increased in strippings as compared to that in foremilk. The levels of variation over the milk fractions were different for healthy and mastitic quarters. The increase in SCC over the milking was much higher in specific mastitis quarters than that in healthy quarters. Mid-milk
managed significantly lower SCC than that of foremilk in healthy but not in mastitic quarters. The pH of foremilk and strippings did not differ significantly, neither in healthy nor in mastitic quarters. The decrease in the EC and lactose content of milk in strippings from that of foremilk was observed to be more in healthy quarters than in mastitic quarters. The difference between foremilk and mid-milk for SCC, EC, lactose was significant in healthy quarters only. Mastitis at its all levels i.e. specific, specific, non-specific and latent resulted in a significant alteration in one or more of the parameters studied. The comparison of various parameters in udder composite milk samples revealed significance of effect for milk SCC and lactose but not for EC and pH. The evaluation of selected parameters in diagnosis of mastitis with respect to milk fraction showed that highest level of discrimination was obtained for EC in strippings (66.0%) and for lactose in foremilk fraction (85.65%).

**MANAGEMENT AND PRODUCTION**

M.A. Brescia, M. Monfreda, A. Buccolieri, C. Carrino, Dipartimento di Chimica, Universita degli Studi di Bari, Via Orabona 4, 70126 Bari, Italy. *Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations.* Food Chemistry (2005), 89(1): 139-147.

Appreciation of qualified national products, together with a guaranteed reference for consumers, has become necessary in the field of dairy products. Indeed, the Protected Designation of Origin (PDO) trademark has been assigned to numerous cheeses, such as buffalo milk mozzarella. In order to receive this designation, the raw materials have to be produced and processed in the specified region from which the product gets its name. Therefore, in order to determine the authenticity of typical dairy products it is necessary to determine the geographical origin of the milk and of the finished product obtained from it. Classical techniques, high performance ion chromatography (HPIC), inductively coupled plasma emission spectroscopy (ICP-AES), nuclear magnetic resonance (NMR) and isotope ratio mass spectrometry (IRMS) were used for determining different compounds in combination with chemometric methods for the geographical characterization of buffalo milk mozzarella cheeses originating from two areas of Southern Italy. Isotopic ratios (13C/12C and 15N/14N) and other variables were affected by the specific area of origin of milk samples, while NMR data, together with isotopic ratios, were useful for the discrimination of mozzarella samples.


Milking-related release of oxytocin, prolactin, and cortisol was studied following three pre-milking treatment. Six Murrah buffaloes were treated with direct application of milking cluster (o), a 1-minute pre-stimulation (M), and combined feeding and pre-stimulation (MF). Machine milk yield, stripping yield and milk composition were recorded. Milk ejection occurred significantly earlier with MF than M and O (P<0.25; 2.50, 5.10 and 6.33 minutes, respectively). In all treatments, milk ejection occurred with small increases >3-5 ng/l in oxytocin concentration over a threshold level and milk ejection occurred simultaneously and were closely correlated (r=0.83, P<0.05). There was a positive correlation between total time oxytocin
concentration remained elevated over threshold levels and machine yield ($r=0.86$, $P<0.05$). For treatment O, milk ejection was inhibited during machine milking, while a marked increase in oxytocin occurred during hand stripping (6 and 16 ng/l, respectively). For treatment M, mean oxytocin concentrations remained unchanged during pre-stimulation but increased during subsequent machine milking and hand stripping (6.38, 18.06 and 12.36 ng/l, respectively). For treatment MF, although there was a 3.6-fold increase during pre-stimulation, oxytocin increased by 10-fold and 3-fold during machine milking and hand stripping, and was significant for machine milking ($P<0.05$, 17.32, 47.86, 18.13 ng/l, respectively). Milk-ejection-related cortisol release was visible only in treatment MF. For treatments O and M, prolactin concentration increased prior to the increase in oxytocin. The stripping yield was higher, and fat content in the stripping yield significantly lower, for treatment O indicating incomplete milking. Thus buffaloes are easily disturbed even by small changes in milking routines.


Influence of herd size and milking management on the udder health status of buffaloes was studied. 749 quarter milk samples from 190 animals in two herd sizes and three milking management systems: calf suckling and hand milking (CH), manual pre-stimulation and hand milking (MH), and manual pre-stimulation and machine milking (MM) were analysed for somatic cells (SCC), California Mastitis Test (CMT), proportion of neutrophils (NEU), and bacterial infection. Among the total quarters 97.5% had no clinical signs of mastitis. The correlation between CMT, SCC, and NEU appeared weaker than observed in bovine milk. Milking management significantly influenced occurrence of mastitis while influence of herd size was weaker. Small CH-herds had lowest prevalence while large MM-herds had highest. However, among small hers with manual pre-stimulation, the machine milked and hand milked herds had similar prevalence of mastitis, indicating that machine milking per se did not negatively influence udder health. Prevalence on animal basis was, based on CMT 37%, SCC32%, increased NEU 21% and infection 33%, while the corresponding figures on quarter basis were: 15%, 14%, 8% and 12%. Among infected quarters environmental bacteria were predominant with large MM-herds having the highest prevalence. The results indicate that mastitis was mainly due to poor environment. Calf suckling had a positive effect on udder health.

**PHYSIOLOGY**


The haematobiochemical effects of epidural ketamine, xylazine and their combination in buffaloes were evaluated to examine the safety of these drugs. 12 clinically healthy male buffaloes were divided into 3 groups of 4 animals each. In animals of groups A, B and C, xylazine (0.05 mg/kg), ketamine (2 mg/kg) and a combination of xylazine
(0.05 mg/kg) and ketamine (2 mg/kg), respectively, were administered at the first intercoccygeal epidural space. Blood samples were collected for the estimation of haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), plasma glucose, total proteins, albumin, globulin, A:G ratio, urea nitrogen, creatinine and gamma-glutamyltransferase (GGT) up to 48 h after injection of the drug(s). Reduction in Hb, PCV and TLC was observed from 30 to 120 minutes in animals belonging to all groups. A significant hyperglycaemia was recorded in all the groups. This was slightly longer in group C. A significant (P<0.05) decrease in plasma proteins was recorded in animals of group A. In animals of groups B and C, an insignificant change in the total plasma proteins was recorded. An insignificant decrease in plasma albumin and an increase in plasma globulin were recorded in all groups. A transient but significant (P<0.05) increase in urea nitrogen, creatinine and GGT was recorded in all the groups. The values for these parameters, however, remained within the normal reference range. Since the changes in various haematobiochemical parameters were only transient and reversible, these drugs could be considered safe for epidural analgesia in buffaloes. However, the combination of xylazine and ketamine was better than xylazine alone.

In this study, bubaline ovarian follicular fluid collected from different size categories of follicles (small, medium and large) was fractionated with ammonium sulphate precipitation at different saturation levels in to different protein fractions (A to G). The amount of protein in each fraction and relative percentage of total protein partitioned into different protein fractions was estimated. The amount of protein partitioned in to different fractions of follicular fluid varied significantly in any particular category of follicles. There was a significant difference among different categories of follicles with respect to the peptide level/relative percentage of protein partitioned into a particular fraction. By 95% of ammonium sulphate saturation, 81 and 78% of the protein quantity in the follicular fluid of small and large follicles, respectively, got precipitated but in the medium size follicles, only 45% of the protein quantity got precipitated. It may be because of the variation in the type of proteins or quantity of specific protein present in the different size categories of follicles. The data presented may give an idea about the quantity of protein that can be obtained from different protein fractions of the fluid that facilitates effective planning of the strategies for the isolation and purification of specific peptides of ovarian follicular fluid in buffaloes.


Follicular fluid consists of many proteins that influence oocyte maturation. Fractionation of ovarian follicular fluid into different protein fractions is the first step in the isolation of peptides/proteins.


A specific protein from buffalo follicular fluid was isolated which exerted a stimulatory effect on progesterone secretion by granulosa cells (GC) during culture and was termed as “Steroidogenesis Stimulating Protein” (SSP). The present study describes the purification and characterization of this
protein. Purification of this protein from buffalo follicular fluid involved ammonium sulphate precipitation, gel permeation chromatography and ion-exchange chromatography. The molecular characterization of the protein revealed a molecular weight of 66 kDa, glycoprotein nature, an iso-electric point of 5.3 and amino acid composition with glutamate, phenylalanine and aspartate as the major amino acids. This protein also showed strong cross-reactivity (>80%) with anti-BSA. The pooled buffalo GC were cultured in plates (400 μl/well) using Medium 199 supplemented with antibiotics and FCS. The effect of SSP and BSA on progesterone secretion by GC was studied in serum free medium. There was significant (p<0.001) stimulatory effect of SSP and BSA on progesterone secretion by GC and maximum stimulation was obtained at a dose of 50μg/ml on day 4 and day 6 of culture. A novel finding was that the ammonium sulphate (60-80%) precipitated protein fraction was bilirubin-rich, indicating that its stimulatory effect might be due to bilirubin bound to protein. The purified protein (SSP) was found without any mitogenic effect on granulose cells and it did not enhance progesterone secretion by granulose cells in the presence of FSH and LH. It is concluded that SSP is molecule showing considerable difference from BSA. The relationship of SSP with buffalo serum albumin may be established only after studing the molecular properties of buffalo albumin.


The study of amniotic and allantoic fluid composition can be important to reflect the foetal metabolism and development. Like this bubaline may be an important experimental model to study the physiology of molecule transport between placental compartments. The objective of this study was to evaluate total protein concentration and to describe electrophoretic profile of amniotic and allantoic fluids correlating them with length and sex of bubaline fetuses. The fluids were collected from 17 bubaline genitals by puncture of amniotic and allantoic sacks. Total protein concentration was determined using bicinechonic acid method and the protein profile by SDS polyacrilamide gel electrophoresis. The gels were stained with silver nitrate and digital images were analysed. Total protein concentration was significantly lower (p=0.0002) in the allantoic fluid. There was a positive correlation (r=0.77; p=0.0003) between its total protein concentration and foetal crown-rump length. A total of 39 and 44 bands were found in the allantoic and amniotic fluids, respectively. There was no correlation between foetus sex and the other variables. We concluded that protein composition I the amniotic and allantoic fluid is different, and allantoic fluid proteins correlate with foetal development.

REPRODUCTION


Twelve buffalo heifers of similar age (21-25 month) and body weight (325-385 kg) were superovulated during mid luteal phase using pFSH (total 65 NIH unit Super-Ov divided into 6 equal dose, 1.4 ml each, for 3 consecutive days) and lutealise (25mg injected with the 5th injection). To improve ovarian response variable doses of LH (0, 2,4,5,7 and 10 thousands USP unit, Steris, Lab. Inc
Phoenix, Arizona) were injected at the morning of the 4th day of the treatment in 6 trials (n=2). Fertile bulls were allowed to mount heifers frequently after 24 h from onset of estrus. Heifers were classified into 3 equal groups (2 trials/each) which slaughtered at various time intervals from the onset of estrus: 72-89, 100-106 and 118-120 h. After slaughter, the intact genitalia were dissected free and transported to the lab in a thermos container at 4°C. The number of newly formed corpora lutea (CL) and unovulated large follicles (UF) in both ovaries were done. Flushings of the oviduct and uterine horn were performed separately using phosphate buffer saline to identify the numbers and locations of embryos. The duration of estrus (h), numbers of CL and UF were 41.5±11.2, 3.1±1.3 and 1.1±0.76 respectively. The overall ovulation and embryo recovery rates were 72.5 and 54% respectively. Group without LH gave low response (50 and 0%). The higher ovulation rate (66-100%) were recorded for heifers supplemented with 4000 and more USP unit LH while the higher embryo recovery rates (50-100%) were associated with the doses of 4000-7000 unit.


The objective of this study was to evaluate the efficiency of Ovum Pick Up (OPU) in cycling (n=5) and lactating, postpartum, swamp buffaloes (n=6) with and without gonadotropin stimulation. The OPU was performed every two weeks in all groups of animals, for a total of six sessions. Thirty collections were performed in five cycling buffaloes and 36 collections in six lactating postpartum buffaloes. Buffaloes those received hormonal stimulation were given a total of 400 mg, follicle stimulating hormone (FSH), administered twice daily over 3 days in decreasing doses, together with 100 g of GnRH, 24 h after the last FSH injection. Following a resting period of 1 month, the two groups of buffaloes, were subjected to the same OPU regimen, but without any hormonal treatment for an additional six OPU sessions. The number of aspirated follicles recorded from the hormonal stimulated, cycling animals and lactating, postpartum buffaloes was not significantly different, 7.2 ± 3.7 and 9.0 ± 3.2, respectively (p>0.05). Recovered oocytes collected from the two groups of hormonally stimulated animals were also not statistically different: 3.7 ± 2.7 in the cycling and 5.9 ± 3.5 in the lactating postpartum group (p<0.05). In the two groups of buffaloes not receiving hormonal stimulation, the number of aspirated follicles was not significantly different: 2.1 ± 1.4 and 1.4 ± 0.7 in cycling and lactating postpartum buffaloes respectively (p>0.05). Recovered oocytes in the non-treated groups were also similar: 1.4 ± 1.3 vs 0.7 ± 0.8 in cycling and lactating buffaloes (p>0.05). Among stimulated buffaloes, most aspirated follicles were small in size (less than or equal to 5 mm), whereas they were mostly medium and large sizes in the non-treated buffaloes. The oocyte recovery rate in both the groups, cycling and lactating postpartum, were 51.6% and 69.5% in stimulated groups and 55.0% and 53.1% in non-stimulated groups (p>0.05). The majority of recovered oocytes were single- and multi-layered, and the number was greater in the cycling than in the lactating, postpartum buffaloes. The number and quality of recovered oocytes was similar in all groups of buffaloes whether they were received or did not receive hormonal stimulation. Moreover no difference was found in multi- and single-layered oocytes between cycling and lactating, postpartum buffaloes. In conclusion, OPU can be performed successfully in swamp buffalo in buffalo in different reproductive status and FSH administration was shown to increase the number of aspirated oocytes in both cycling and lactating postpartum buffaloes.
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**Effect of oestrus synchronization protocols, lactation yield and body condition score on conception rates in buffaloes.** *Indian Veterinary Journal* (2005), **82**(1):45-47.

The effect of lactation and body condition score (BSC) on the conception rates of buffaloes under the Ovsynch programme was evaluated. During postpartum period (>40 day), 87 buffaloes received 25 mg of Lecirelin i.m. on day 0 and 150 mg of D-cloprostenol i.m. on day 7. On Day 8, a total of 43 buffaloes received 1.0 mg of estradiol benzoate (EB) i.m. (group II). In group I, a total of 44 animals received 25 mg of Lecirelin i.m. on Day 9. All animals were fixed time inseminated 30 h after EB (group II) or 16 h after second GnRH injection (group I). Animals were also divided into 2 groups according to daily milk production (<7.0 or >7.0 litres per day) to evaluate the effect of lactation yield in the treatments. The conception rates did not differ according to BCS. In group I, the lactation yield did not interfere with the conception rate of animals treated with GnRH on day 9 (P>0.05). In group II, animals with higher daily lactation yield had lower conception rate than buffaloes with lower milk production.


Conceptus derived substances are more precise and reliable markers of pregnancy and fetal growth. In the present study, attempts have been made on isolation, purification and characterization of pregnancy-associated protein(s) in buffaloes from uterine and oviduct flushing and uterine scrapings using affinity chromatography. These purified proteins were further analyzed by polyacrylamide gel electrophoresis (SDS-PAGE) and western blot. Results revealed lower OD values for purified fractions than the reproductive fluids from non-pregnant and pregnant genital tracts (NPRF, and elute fractions indicating presence of some specific proteins in the purified fractions, which may be associated with pregnancy. SDS-PAGE analysis of purified fractions revealed presence of seven-polypeptide band of different molecular weights (67,
53, 45, 33, 24, 16 and 11.5 kDa) and only three polypeptides (67, 53 and 24 kDa) were observed immunoreactive in western blot analysis. Results indicated presence of certain proteins secreted by conceptus in the reproductive fluid of pregnant genital tracts and in future its further purification and characterization will be helpful to evolve a specific and sensitive method for early pregnancy diagnosis in buffaloes.


At the time of AI following Ovsynch protocol, a total of 51 buffaloes were randomly divided in a first group (n=30) subjected to conventional AI into the uterine body with 20 million non-sex sorted frozen-thawed spermatozoa, while a second group (n=21) was inseminated near the utero-tubal junction (UTJ) ipsilateral to the ovary carrying the preovulatory follicle with 2.5 million live (4 million total) sex-sorted frozen-thawed spermatozoa. The semen used for flowcytometric sorting was collected and processed on a farm in Italy, and then shipped to a laboratory in Germany. Eleven buffaloes were inseminated with X-chromosome bearing spermatozoa and 10 with Y-chromosome bearing spermatozoa. Conception rates after conventional and UTJ inseminations were 43.3% (n=13) and 42.8% (n=9) respectively (p=0.97). Eight of the nine fetuses obtained after insemination with sexed spermatozoa corresponded to the sex as predicted by the cell sorting procedure (five male and four female fetuses by ultrasound vs six male and three female fetuses by cell sorting). In conclusion, for the first time buffalo semen has been successfully subjected to procedures for flowcytometric sperm sorting and freezing. Low doses of sexed spermatozoa have been deposited near the UTJ giving conception rates similar to those of conventional AI with full dose.


This experiment was conducted at the Buffalo Breeding and Development Farm in Bagerhat to investigate conception rate, service per conception, gestation period, age at first calving, birth weight, postpartum heat period and calving interval of Nili-Ravi and crossbred buffaloes. It was shown that the average conception rate, service per conception, gestation period, age at first calving, birth weight, calving interval and postpartum heat period were 95.24 and 88.46%; 1.05 and 1.13; 301.486 ± 6.72 and 303.200 ± 7.07 days; 61.857 ± 3.44 and 63.048 ± 3.89 months; 33.266 ± 3.49 and 30.508 ± 3.43 kg; 572.633 ± 116.54 and 581.481 ± 94.15 days and 167.800 ± 27.92 and 174.500 ± 41.04 days for Nili-Ravi and crossbred buffaloes, respectively. In conclusion, Nili-Ravi buffaloes have better reproductive performances than crossbred buffaloes.

CONTENTS

Vein varicosis in a Pandharpuri buffalo - a case report.
M.D. Kulkarni, A.S. Kadam, A.V. Khanvilkar, and O.N. Ladukar ........................................ 24

Performance of swamp buffaloes of Assam in respect of some economically
traits of reproduction under farm condition
Arpana Das, D. Das, R.N. Goswami, G.C. Das and D. Bhuyan........................................ 25

Detection of group and serotype specific antibodies to bluetongue virus in buffalo
in Gujarat, India.
H.C. Chauhan, B.S. Chandel, T. Gerdes, K.A. Vasava, A.R. Patel,
K.M. Jadhav, H.N. Kher........................................................................................................... 28

RESEARCH ABSTRACTS................................................................................................. 34