

HEMATO-BIOCHEMICAL CHANGES IN RELATION TO CERVICO VAGINAL PROLAPSE IN BUFFALOES

**Ashwini Upadhyay^{1,*}, Sant Prasad Nema¹, Madhu Shivhare¹,
Hemant Kumar Mehta² and Sudarshan Kumar¹**

ABSTRACT

A study was carried out to 24 buffalos, comprising 18 buffaloes having Cervico-Vaginal Prolapse and 6 normal pregnant buffaloes (above 7 months) on clinical cases brought to the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Mhow, College dairy farm and Villages in and around Mhow to compare haemato-biochemical profile in normal and Cervico vaginal prolapsed buffaloes. Blood samples were collected initially before treatment (Day 0) and after treatment on 7th day. Haemato-biochemical parameters (haemoglobin, total leukocyte count, differential leukocyte count, serum calcium, and serum phosphorus, Serum magnesium (Mg)) were estimated and compared between control and treatment group animals. The mean values of serum calcium and inorganic phosphorus were significantly ($P<0.01$) lower in cervico vaginal affected buffaloes on day of prolapse (Day 0) which restored to nearly normal on the 7th day after treatment, though statistically significant variation was not observed.

Keywords: *Bubalus bubalis*, buffaloes, cervico vaginal prolapse, haemato-biochemical, serum

calcium, inorganic phosphorus

INTRODUCTION

According to Nineteenth Livestock Census 2012, the total numbers of buffaloes in India are approximately 108.08 million contributing 54% of total Indian milk production, Today India is the oyster of global dairy industry. Cervico vaginal prolapse is a serious and occasionally fatal obstetrical malady in cattle and buffaloes herds, especially during calving season. Pre-partum vaginal prolapse in buffaloes is responsible for heavy morbidity (Rai and Prabhakar, 2000) that leads to high economic loss in terms of reduced milk yield, high veterinary expenses, loss of calf crop and delayed post partum conception.

The exact etiology of prolapse is still unclear (Noakes *et al.*, 2001). Ahmed *et al.* (2005) reported significant increase in neutrophil, while a significant decrease in lymphocyte and monocyte count and unchanged Basophil and eosinophil count in prolapsed animals as compared to normal animals. Hypocalcaemia results in myometrial fatigue that delays cervical involution, both of which could predispose to uterine prolapse (Roberts, 2004). This condition places considerable

¹Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, India, *E-mail: drmadhushivhare@gmail.com

²Department of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, India

stress on the animal. Delayed cases may develop fatal septicemia (Bhattacharyya *et al.*, 2007).

MATERIALS AND METHODS

24 buffaloes were selected, consisting of total 18 buffaloes with Cervico vaginal prolapse and 6 buffaloes above 7 month pregnant served as control group on clinical cases brought to the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Mhow, college dairy farm and villages in and around Mhow including ambulatory clinics. The experiment was carried out on 24 buffaloes, comprising 18 buffaloes having cervico-vaginal prolapse and 6 normal pregnant buffaloes.

Grouping of animals

Control group

This group consisted of six normal advanced pregnant buffaloes (above 7 months) and has not suffered with cervico vaginal prolapse.

Treatment group

These animals were selected by degree of prolapse. The animals which have 2nd (vaginal floor or wall protruding out of vulval aperture) and 3rd degree prolapse (complete protrusion of vagina exposing the cervix may or may not be lacerated) comprised of this group.

All the animals in this group were subjected to:

- i. Caudal epidural anesthesia using 2% Xylocain hydrochloride 5 to 7ml.
- ii. The prolapsed cervico-vaginal mass was cleaned with povidone iodine solution.

iii. Buffaloes with cervico-vaginal prolapse were treated with Herbal spray locally and after 10 minutes the prolapsed mass was reposed manually.

iv. Rope truss was applied for retention, which was removed after 24 h.

Buffaloes of treatment group were divided into 3 sub groups consisting of 6 animals in each group after repositioning, for further treatment.

Sub Group A

Buffaloes in this group was administered inj. Enrofloxacin 5 mg/kg b.wt. only, daily for 3 days after repositioning.

Sub Group B

Buffaloes of this group was administered with inj. Enrofloxacin 5 mg/kg b.wt. + inj. Chlorpheniramine maleate 0.08 mg/kg b.wt and inj. Meloxicam 0.5 mg/kg b.wt. intramuscularly daily for three days after repositioning.

In addition to above, inj. Calcium magnesium borogluconate 200 to 350 ml. was also administered by intravenous route once only.

Sub Group C

Buffaloes of this group were treated with herbal drug (*Mimosa pudica*) 100 g orally and inj. Enrofloxacin 5 mg/kg b.wt daily for 3 days after repositioning.

Blood samples were collected from jugular vein just before initiation of treatment and on 7th day post partum. A total of 7 ml blood from each buffaloes was collected in 10 ml sterilized glass tube. Out of which 2 ml blood was kept in vial with anticoagulant (EDTA 2 mg per ml of blood) for assessing values of haematological parameters and the other 5 ml blood was kept in a vial without anticoagulant for separation of serum and separated serum was stored in a sterilized vial

at -20°C for biochemical analysis.

Haematological parameters

Following haematological parameters were estimated as per methods described by Jain (1986).

Haemoglobin (Hb)

Haemoglobin was determined by Sahle's acid hematin method using haemoglobino-meter and values are expressed in g/dl.

Total leukocyte count (TLC)

Counting of the white blood cells was done in a haemocytometer consisting of Neubauer's chamber. The values are expressed in thousand/cu.mm.

Differential leukocyte count (DLC)

The differential leukocytes count was done by preparing thin blood smears on glass slide stained with Leishman's stain and examined under 100X magnification of microscope using oil immersion lens. One hundred cells were counted and values are expressed in percentage.

Biochemical parameters

Serum calcium (Ca)

Serum calcium was estimated by Optical Cresol Pthalein Complexon (OCPC) method using Ark diagnostic kit as described by Moorhead and Briggs (1974). The values are expressed in mg/dl.

Serum inorganic phosphorus (P)

Serum inorganic phosphorus was estimated by modified Metol method using Ark diagnostic kit as described by Gomori (1942). The values are expressed in mg/dl.

Serum magnesium (Mg)

By colorimetric method using Ark diagnostic kit as described by Gindler (1971) and values are expressed in mg/dl. Means and standard errors were calculated as per standard formulas. Data were analyzed by applying completely randomized design (CRD) and student's 't' test to work out statistical significance between various treatments as detailed by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Haemoglobin

The results of haemoglobin concentration is presented in Table 11, which reveals that the overall mean haemoglobin (g/dl) level in prolapsed and control group animals was found to be 10.52 ± 0.25 and 10.58 ± 0.38 on day 0 and the corresponding level on day 7 was 10.39 ± 0.26 and 10.91 ± 0.39 (g/dl) respectively. The difference in mean haemoglobin (g/dl) level between these groups on both the days was statistically non-significant.

The group wise means levels of haemoglobin (g/dl) were studied and their mean values are presented and depicted in Table 1, On the day of prolapse (0th) in sub group A, B, and C they were 9.86 ± 0.30 , 10.38 ± 0.45 , 11.33 ± 0.40 and on day 7th 9.70 ± 0.19 , 10.51 ± 0.49 , 10.96 ± 0.50 respectively. The difference in group wise mean haemoglobin (g/dl) level between these groups on both the days was also statistically non-significant.

Total leukocyte count

The mean total leukocyte counts (thousand/cu.mm.) data is presented in Table 11. The overall mean total leukocyte count was 8.00 ± 0.39 in control animals whereas in prolapsed

animals it was 9.73 ± 0.24 on day 0th. The difference in the count was significantly higher ($P \leq 0.01$) in prolapsed group buffaloes. The corresponding count on day 7th was 8.23 ± 0.40 in control group animals and 7.43 ± 0.17 (thousand/cu.mm.) in prolapsed animals respectively. Statistically, the difference in the count was significantly lower ($P \leq 0.05$) in prolapsed group buffaloes.

The groups wise mean total leukocyte counts (thousand/cu.mm) were studied and their values are presented and depicted in Table 2, on the day of prolapse (0th) belonging to sub Group A, B and C were 10.28 ± 0.25 , 9.61 ± 0.39 , and 9.30 ± 0.54 , whereas on day 7 the count in respective sub groups was 7.93 ± 0.21 , 7.60 ± 0.16 and, 7.65 ± 0.54 . The analysis of variance revealed highly significant ($P \leq 0.01$) variation in the mean total leukocyte count between treatment and control group on 0th day, whereas there was reduction in the leukocyte count in all the treatment sub groups on 7th day as compared to their corresponding value on 0th day.

Differential leukocyte count

Neutrophil

The mean neutrophil count in all the sub-groups is presented and depicted in Table 3, The analysis of variance is presented in Table 8 which reveals that the overall mean neutrophil count (%) in control group animals on 0th day was $27.95 \pm 0.68\%$ against the prolapsed animal groups where it was $31.97 \pm 0.40\%$. Statistically the difference was highly significant. Similarly, on 7th day the count was $29.18 \pm 0.69\%$ in control animals and $27.18 \pm 0.54\%$ in prolapsed animals, respectively. Statistically there was no significant difference between the neutrophil count values of control and treatment group animals on this day. The group wise mean values of neutrophils (%) on the day of prolapse (0th) were 32.16 ± 1.10 , 31.68 ± 0.49 , 32.10 ± 0.41

and on day 7 these were 29.83 ± 0.79 , 26.05 ± 0.33 , 25.66 ± 0.40 in sub group-A, B and C, respectively. The variation between all the three treatment sub groups on day 0th was significantly different and on day 7th the variation significantly differed only between sub Groups B and C whereas in sub group A non significant difference in comparison to their corresponding control was noticed.

Lymphocyte

The mean values of lymphocyte count are presented and depicted in Table 4. The analysis of variance is presented in Table 8 the overall mean lymphocytes count (%) were found to be 53.22 ± 0.58 and 48.66 ± 2.44 in prolapsed and control animals on 0th day respectively, and the value on 7th day were 51.38 ± 0.89 and 50.83 ± 2.06 (%) in prolapsed and control animals. The difference was statistically non-significant.

The group wise mean values of lymphocyte (%) on the day of prolapse (0th) were 52.83 ± 0.94 , 53.50 ± 1.83 , 53.33 ± 1.25 and on day 7th were 53.50 ± 1.66 , 54.65 ± 0.71 , 52.16 ± 0.80 in sub Group A, B and C groups, respectively the values were non-significant in all groups on the day 0th and 7th day in comparison to their corresponding control.

Monocyte

The mean values of monocyte count was statistically studied and the analysis of variance is presented in Table 8 which reveals that the overall mean values of monocyte (%) were found to be 3.16 ± 0.40 in control animals and 3.33 ± 0.16 in prolapsed animals on day of prolapse (0th), respectively. The value on 7th day was 2.33 ± 0.49 in control animals and 3.33 ± 0.24 (%) in prolapsed animals respectively. The variation did not differ significantly between treatment groups with control.

The data is presented in Table 5, The group wise mean values of monocyte (%) on the day of prolapse (0th) were found to be 3.50 ± 0.22 , 3.16 ± 0.30 , 3.33 ± 0.40 and on the day 7 were 2.66 ± 0.47 , 3.66 ± 0.42 , 3.16 ± 0.40 in sub Group A, B and C groups, respectively. The variation in values was statistically non-significant among all the three groups on the day 0 and 7th day comparison to their corresponding control.

Eosinophil

The analysis of variance presented in Table 11 for the mean eosinophil counts reveals that the overall mean values of eosinophil (%) were 4.33 ± 0.24 and 4.16 ± 0.40 in prolapsed and control animals on the day of prolapse (0th), respectively and did not differ significantly. The value on 7th day was 3.88 ± 0.29 and 3.83 ± 0.30 (%) in prolapsed and control animals, respectively, which also has non-significant difference with control group values.

The treatment groups wise mean values of eosinophil (%) are depicted in Table 6. The value of eosinophil on the day of prolapse (0th) were found to be 4.33 ± 0.33 , 5.00 ± 0.51 , 5.33 ± 0.41 and on the day 7 were 4.00 ± 0.57 , 3.83 ± 0.60 , 3.97 ± 0.40 in sub Group A, B and C groups, respectively. The variation between values was non-significant in all the three groups on day 0th and 7th day.

Basophil

The analysis of variance for basophil count is presented in Table 11 which narrates that the overall mean values of basophil (%) were found to be 0.50 ± 0.12 and 0.66 ± 0.21 in prolapsed and control animals on the day of prolapse (0th), and on 7th day were 0.33 ± 0.11 and 0.33 ± 0.21 (%) in prolapsed and control animals, respectively. The variation in the count was also non-significant with

control group animals on both the periods.

The group wise means values of basophil count (%) were studied and their values are presented and depicted in Table 7. The values were found to be 0.50 ± 0.22 , 0.33 ± 0.22 , 0.66 ± 0.22 on the day of prolapse (0th) and on the 7th day were 0.33 ± 0.21 , 0.33 ± 0.21 , 0.33 ± 0.21 in sub Group A, B and C respectively. The differences in values were non-significant in all three groups on the day 0 and 7th day.

Serum calcium

The overall mean values of serum calcium are presented in Table 11. The overall mean value of serum calcium (mg/dl) was found to be 9.26 ± 0.38 in control group animals and 7.62 ± 0.25 in prolapsed animals on 0th day. Statistically the values highly significantly differed ($P\leq 0.01$) than control group animals. The values on 7th day were 9.78 ± 0.41 in control group animals and 8.58 ± 0.28 in prolapsed buffaloes of sub Group A, B and C. Statistically the values of serum calcium were found significantly lower than the control group animals.

The group wise mean level of serum calcium (mg/dl) are presented and depicted in Table 8. The values were found to be 7.16 ± 0.07 , 7.18 ± 0.09 and 6.88 ± 0.27 in buffaloes of sub Group A, B and C respectively on 0th day while 8.90 ± 0.39 , 9.41 ± 0.31 and 7.43 ± 0.40 in 7th day respectively. Statistical analysis revealed that the values were significantly different ($P\leq 0.05$) in sub Group A and B than sub Group C on 7th day.

The values were highly significantly ($P\leq 0.01$) lower in all the groups on 0th day and significantly ($P\leq 0.05$) lower with the values of 7th day, in comparison to their corresponding control.

Serum inorganic phosphorus

The mean values of serum inorganic phosphorus is presented in Table 11 and the mean value of serum inorganic phosphorus (mg/dl) was 5.81 ± 0.27 in control group and 5.45 ± 0.1 in CVP affected buffaloes on day 0th. Statistically the values highly significantly differed ($P \leq 0.01$) than control group animals. The values on 7th day were 5.82 ± 0.14 in control group animals and 4.69 ± 0.20 in CVP affected buffaloes. Statistically the values highly significantly differed ($P \leq 0.01$) than control group animals.

The group wise mean values are presented and depicted Table 9. the mean level of serum inorganic phosphorus (mg/dl) on 0th day was 4.05 ± 0.30 , 4.78 ± 0.44 and 4.53 ± 0.15 in sub Group A, B and C. while on day 7th the mean values was and 5.17 ± 0.17 , 5.86 ± 0.32 and 4.45 ± 0.36 in buffaloes of sub Group A, B and C, respectively. Statically the values of sub Group A were significantly ($P \leq 0.05$) differed with sub Group C while non significant with sub Group B as compared to control group animals.

The mean values were significantly differed ($P \leq 0.05$) in sub Group A and B on day 0th in comparison to day 7th while non significant in sub Group C.

Serum magnesium

The overall mean values of serum magnesium are presented in Table 11 and group wise mean are presented and depicted Table 10. The data reveals that overall mean values of serum magnesium (mg/dl) was 2.23 ± 0.21 in control group and 2.71 ± 0.08 in prolapsed animals on 0th day the values were highly significant. Group wise mean values were found 2.81 ± 0.10 , 2.53 ± 0.19 and 2.79 ± 0.13 in prolapsed animals of sub Group A, B and C respectively, which were significantly

($P \leq 0.01$) higher than control.

The overall mean values of serum magnesium (mg/dl) was 2.85 ± 0.23 in control group and 2.60 ± 0.08 in prolapsed animals on 7th day statistically the values did not differ significantly than control group animals. Group wise mean values were found 2.48 ± 0.10 , 2.93 ± 0.05 and 2.45 ± 0.13 in prolapsed animals of sub Group A, B and C respectively, there was significant variation with sub Group B while no variation found with sub Group A and C in comparison to their corresponding control.

The values were significantly higher ($P \leq 0.01$) in all groups on the day 0th as compared with the value of 7th day.

In present study the mean values of hemato-biochemical norms are presented in Table 1. The mean haemoglobin concentration did not differ significantly between normal and Cervico vaginal prolapsed buffaloes on day 0 and day 7. Similar finding were reported by Pandit *et al.* (1982); Pandey (2006). The mean total leukocyte count was significantly higher ($P < 0.01$) in buffaloes suffering from Cervico vaginal prolapse on day 0 when compared to control group animals and day 7th. However there was no significant variation found in the values of the 7th day as compared to control group animals. Significantly higher leukocyte count in buffaloes with cervico vaginal prolapse may be due to secondary bacterial infection and urinary tract infection as also mentioned by, Sharma *et al.* (1977) which may be due to urinary tract infection. Similar finding were also reported by Pandit *et al.* (1982) and Pandey (2006).

The mean serum calcium and inorganic phosphorus level were significantly lower ($P < 0.01$) in buffaloes with cervico vaginal prolapse on the day of prolapse (day 0) in comparison to the level on the seventh day and to control group animals.

Table 1. Group wise haemoglobin concentration g/dl (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	10.58±0.38	10.91±0.39
2	Sub A	6	9.86±0.30	9.70±0.19
3	Sub B	6	10.38±0.45	10.51±0.49
4	Sub C	6	11.33±0.40	10.96±0.50

Table 2. Group wise Total leukocyte count thousand/cu.mm (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	8.00±0.39 ^b	8.23±0.40
2	Sub A	6	10.28±0.25 ^{aA}	7.93±0.21 ^B
3	Sub B	6	9.61±0.39 ^{aA}	7.60±0.16 ^B
4	Sub C	6	9.30±0.54 ^{aA}	7.65±0.54 ^B

Means having different superscript (a- b in a column; A- B in a row) differ significantly.

Table 3. Group wise Neutrophil count % (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	27.95±0.68 ^b	28.18±0.69 ^a
2	Sub A	6	32.16±1.10 ^{aA}	29.83±0.79 ^{aB}
3	Sub B	6	31.68±0.49 ^{aA}	26.05±0.33 ^{bB}
4	Sub C	6	32.10±0.41 ^{aA}	25.66±0.40 ^{bB}

Note: Means having different superscript (a- b in a column; A- B in a row) differ significantly.

Table 4. Group wise Lymphocyte count % (Mean±SE).

S. No.	Groups	No. of animals	Day-0	Day-7
1	Control	6	48.66±2.44	50.83±2.06
2	Sub A	6	52.83±0.94	53.50±1.66
3	Sub B	6	53.50±1.83	54.65±0.71
4	Sub C	6	53.33±1.25	52.16±0.80

Table 5. Group wise Monocyte count % (Mean±SE).

S. No	Groups	No. of animals	Day 0	Day 7
1	Control	6	3.16±0.40	2.33±0.49
2	Sub A	6	3.50±0.22	2.66±0.47
3	Sub B	6	3.16±0.30	3.66±0.42
4	Sub C	6	3.33±0.40	3.16±0.40

Table 6. Group wise Eosinophil count % (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	4.16±0.40	3.83±0.30
2	Sub A	6	4.33±0.33	4.00±0.57
3	Sub B	6	5.00±0.51	3.83±0.60
4	Sub C	6	5.33±0.41	3.97±0.40

Table 7. Group wise Basophil count % (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	0.66±0.21	0.33±0.21
2	Sub A	6	0.50±0.22	0.33±0.21
3	Sub B	6	0.33±0.22	0.33±0.21
4	Sub C	6	0.66±0.22	0.33±0.21

Table 8. Group wise serum calcium concentration (mg/dl) (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	9.26±0.22 ^{aA}	9.78±0.41 ^{aA}
2	Sub A	6	7.16±0.07 ^{bA}	8.90±0.39 ^{aB}
3	Sub B	6	7.18±0.09 ^{bA}	9.41±0.31 ^{aB}
4	Sub C	6	6.88±0.27 ^{bA}	7.43±0.40 ^{bA}

Means having different superscript (a- b in a column; A- B in a row) differ significantly.

Table 9. Group wise serum phosphorus concentration (mg/dl) (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	5.81±0.27 ^{Aa}	5.82±0.14 ^{aA}
2	Sub A	6	4.05±0.30 ^{bA}	5.17±0.17 ^{abB}
3	Sub B	6	4.78±0.44 ^{bA}	5.86±0.32 ^{bcB}
4	Sub C	6	4.53±0.15 ^{Ba}	4.45±0.36 ^{cA}

Means having different superscript (a-b in a column; A-B in a row) differ significantly.

Table 10. Group wise serum magnesium concentration mg/dl (Mean±SE).

S. No.	Groups	No. of animals	Day 0	Day 7
1	Control	6	2.23±0.21 ^b	2.85±0.23 ^{ab}
2	Sub A	6	2.81±0.10 ^a	2.48±0.10 ^b
3	Sub B	6	2.53±0.19 ^{abB}	2.93±0.05 ^{aA}
4	Sub C	6	2.79±0.13 ^a	2.45±0.13 ^b

Means having different superscript (a-b in a column; A-B in a row) differ significantly.

Table 11. Haemato-biochemical constituents in normal and cervico-vaginal prolapsed buffaloes (Mean±SE).

S. No.	Parameters	Day 0			Day 7		
		Control (n=6)	CVP (n=18)	t-value	Control (n=6)	CVP (n=18)	t-value
1	Haemoglobin (g/dl)	10.58±0.38	10.52±0.25	0.11	10.91±0.39	10.39±0.26	1.02
2	Total Leukocyte Count (Thousand/cu.mm)	8.00±0.39	9.73±0.24	3.56**	8.23±0.40	7.43±0.17	2.29*
3	Neutrophil (%)	27.95±0.68	31.97±0.40	4.96**	29.18±0.69	27.18±0.54	1.93
4	Lymphocyte (%)	48.66±2.44	53.22±0.58	2.69	50.83±2.06	51.38±0.89	0.28
5	Monocyte (%)	3.16±0.40	3.33±0.16	0.46	2.33±0.49	3.33±0.24	1.97
6	Eosinophil (%)	4.16±0.40	4.33±0.24	0.34	3.83±0.30	3.88±0.29	0.40
7	Basophil (%)	0.66±0.21	0.50±0.12	0.68	0.33±0.21	0.33±0.11	0.00
8	Serum calcium (mg/dl)	9.26±0.38	7.62±0.25	6.47**	9.78±0.41	8.58±0.28	2.17*
9	Serum inorganic phosphorus (mg/dl)	5.81±0.27	4.45±0.19	3.69**	5.82±0.14	4.69±0.20	5.37**
10	Serum magnesium (mg/dl)	2.23±0.21	2.71±0.08	3.18**	2.85±0.23	2.60±0.08	

Figure In parenthesis (n=) indicate number of animals

*Significant (P≤0.05), ** Significant (P≤0.01).

The mean level of serum calcium and inorganic phosphorus rose to nearly normal on the seventh day however, statistical variation was non significant. Similar finding were reported by Pandit *et al.* (1982); Mandali *et al.* (2002). It seems that calcium is required for cell membrane permeability, muscle contraction and nerve impulse transmission, and its deficiency may result in reduced muscle tone and resultant Cervico vaginal prolapse (Herrick, 1977; Roberts, 1986). The mean level of serum magnesium was significantly higher in buffaloes with cervico vaginal prolapsed on the day of prolapse (day 0) in comparison to the level on the seventh day and to control group animals. The values rose to nearly normal on the seventh day as also reported by Pandit *et al.* (1982); Choudhury *et al.* (1987).

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