The Haryana Veterinarian (June, 2025) **64(1),** 71-76 ISSN 0033-4359 (P) **Research Article**

INCIDENCE AND EFFECT OF NON-ENZYMATIC ANTIOXIDANTS ON HEMATOLOGICAL PROFILE IN UTERINE TORSION AFFECTED BUFFALOES

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SUMMARY

In the present study, a total of 178 buffaloes suffering from dystocia reported at the Veterinary Clinical Complex Section (VCC) of the Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Bikaner and were also observed for incidence of uterine torsion for a period of two years (January 2022-December 2023). A total of eighteen uterine torsion affected buffaloes were randomly divided in three groups (n=6 animals/group) to evaluate the effects of the combination of vitamin E with Se and vitamin C injections on blood hematological profiles. The buffaloes of group-I were received only routine supportive therapy and considered as control. Animals of group-II were administered intramuscular injection of vitamin E-selenium (α-tocopherol acetate 50 mg and sodium selenite 0.3 mg) whereas animals of group-III were injected vitamin C (250 mg sodium ascorbate/ml) intravenously along with supportive therapy just prior to detorsion. For assessment of the hematological parameters, blood samples were taken from each animal by the jugular vein puncture at the time of reporting, one hour after detorsion and 24 hours of correction of uterine torsion. Uterine torsion was validated as a common cause of dystocia in buffaloes with higher incidence among all the responsible causes. Parenteral regime of both the antioxidants significantly affected ($p \leq 0.05$) the hematological (RBC, Hb, HCT, WBC, neutrophils & monocytes) and whereas blood constituents like MCH, MCHC, MCV, lymphocytes, eosinophils, were remain unaffected by the administration. The results of the study have demonstrated a positive effect of administration of vitamin E with Se and ascorbic acid on hematological profile in uterine torsion affected buffaloes.

Keywords: Hematological, Uterine torsion, Vitamin C, Vitamin E-selenium

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Dystocia can be defined as the delayed or difficult calving which often needs significant human assistance (Jeengar *et al.*, 2015). Cattle and buffalo are considered to have the highest incidence of dystocia amongst all the domestic animals. The maternal causes of dystocia may be considered as uterine inertia, inadequate size of the birth canal whereas as fetal factors include as oversized fetus, abnormal orientation of the fetus in the birth canal (Weldeyohanes and Fesseha, 2020). The higher incidence of dystocia in cattle was reported due to fetal mal-disposition (46.87%) while in buffaloes it noticed by uterine torsion as 41.30% (Juneja *et al.*, 2023).

Uterine torsion is defined as twisting of the pregnant uterine horn on its longitudinal axis (Purohit *et al.*, 2011). However, there is a difference in the prevalence of uterine torsion among different species owing to their difference in the strength of uterine muscular and position and nature of different ligaments supporting the uterus or difference in their mesenteric suspension (Noakes *et al.*, 2019). Uterine torsion may lead to heavy economical losses to the farmers by causing death of either fetus or dam or both beside impairment of lactation (Prakash *et al.*, 2018; Yadav *et al.*, 2021). Incidence of maternal causes and higher contribution of uterine torsion among them evidences to establish the conclusion as maternal dystocia is common in buffaloes and uterine torsion is primary aspect in these (Juneja *et al.*, 2023). Uterine torsion is a highly stressful reproductive disorder in buffaloes (Ghuman, 1995). The alterations in blood parameters are suggestive of deteriorating condition of the dam and thus help to selection of various therapies, *viz*. anti-stress, liver protection and electrolyte therapy (Ghuman, 2010).

In bovines, methods for correction of uterine torsion may include per-vaginal rotation of fetus, rolling of dam and leparo-hysterectomy (cesarean section)which can be opted as per expertise of veterinarian, stage of pregnancy, severity of uterine torsion as well as condition of dam, uterus and fetus. During correction of uterine torsion by Schaffer's method, rotate of dam to same degree and direction to which the uterus has twisted, keeping the fetus fixed by fixing uterus with a wooden plank (Schaffer, 1946). The correction of uterine torsion by cesarean section had a higher mortality rate and also a longer interval from first service to conception as compared to mutation (Singh *et al.*, 2013); however, the delayed (>72 h) cases of uterine torsion may be directly subjected to caesarean in order to avoid the undue stress of rolling (Prabhakar *et al.*, 1995). Abdominal surgery should be avoided in animals having friable and septic uterus containing an emphysematous fetus (Amer *et al.*, 2008).

Parturition is a stressful physiological event and

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furthermore, abnormal parturition (dystocia) stimulates the stress condition (Nakao and Grunert, 1990). Non-enzymatic antioxidants like vitamin E, vitamin C and Se may be considered as promising agents protecting tissues against oxidative damage with their potent free radical-scavenging and antioxidant properties (Yanardag *et al.*, 2007).

MATERIALS AND METHODS

Experimental animals: A total of eighteen uterine torsion affected buffaloes were randomly divided in three groups (n=6 animals/group). The animals of group-I were received only routine supportive therapy and grouped as control (C group). Animals of group-II were administered 10 ml intramuscular injection of vitamin E-selenium (VES group; Selexidant, containing tocopherol acetate 50 mg and sodium selenite 0.3 mg; Intracin Pharmaceutical Private Limited, Nadiad, Gujrat, India) whereas animals of group-III were injected 30 ml vitamin C (VC group; Ascorvet 250 mg/ml; Phoenix Life Science Private Limited, Rohtak, Haryana, India) intravenously along with supportive therapy just prior to detorsion.

Table 1. Experimental regime of research work

Group-I (C)	Group-II (VES)	Group-III (VC)
Only supportive therapy	Vitamin E-Se+ Supportive therapy	Vitamin C+ Supportive therapy

Clinical examination and diagnosis: The uterine torsion was diagnosed on the basis of anamnesis, transvaginal and transrectal examination of affected buffaloes and also determined the site (Pre and Post-cervical), direction (Clockwise and anticlockwise), degree (≤ 90º, 90º-180º, 180º- 360º, ≥ 360º) of this clinical condition. In this clinical condition, the location of broad ligaments or the twisting of vagina was accessed to encounter the degree, direction and site of uterine torsion as described previously (Jeengar *et al.*, 2015).

Blood sampling schedule: A total of three blood samples (10 ml/animal) were taken from each animal at the time of reporting (AR), one hour after correction (AC) and 24 hours of correction of uterine torsion to access the hematological parameters. Blood samples were collected from each animal from the jugular vein puncture one in 7 ml plain vacutainer tube and the other in 3 ml EDTA added vacutainer tube.

Parameters investigated:

Incidence of uterine torsion: In the current experiment, the incidence of uterine torsion affected buffaloes was calculated by the records for the period of last two years (2022-23) according to total reported cases of dystocia having both the maternal and fetal causes

$$= \frac{\text{Cases of Uterine Torsion}}{\text{Total reported case of Dystocia}} \times 100$$

Estimation of hematological parameters: The whole blood sample was used for evaluating the hematological parameters *viz*. total red blood cells (RBCs), haemoglobin concentration (HGB), mean corpuscular volume (MCV), Haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cells (WBC) and differential leucocytic counts using automated haematology analyser, model MEK- 6550 (Nihon Kohden Corporation, Japan).

Statistical analysis: Data obtained were subjected to analysis by one-way analysis of variance (ANOVA) technique (Snedecor and Cochran 2004) using the statistical package SPSS software version 21. The means of different experimental groups were interpreted for statistical significance ($P<0.05$) by Duncan's Multiple Range Test as described by Duncan (1955).

RESULTS AND DISCUSSION

Incidence and the type of uterine torsion in buffaloes: A total of 178 buffaloes suffering from dystocia were brought at VCC section of Department of Veterinary Gynaecology & Obstetrics, College of Veterinary and Animal Science, Bikaner over the course of two years (January 2022 - December 2023). Out of the 178 dystocia instances that were reported, 111/178 (62.36%) and 67/178 (37.64%) had maternal and fetal origins, respectively. Out of all the cases of dystocia in buffaloes that were of maternal origin, uterine torsion accounted for 62/178 (34.83%) of the cases. Uterine torsion was identified as a major cause of the total dystocia cases followed by fetal mal-disposition (21.91%), incomplete cervical dilation (16.85%), fetal emphysema (11.79%), inadequate pelvis (5.62%) and other causes of fetal and maternal origin (8.98%).

Uterine torsion affected buffaloes were separated under two categories *viz*. 3-5 years 4/18 (22.22%) and >5 years 14/18 (77.78%). The cases of uterine torsion in buffaloes were also categorized according to parity *viz*. primiparous 4/18 (22.22%) and multiparous 14/18 (77.78%). The location of the uterine torsion in buffaloes was categorized according to the involvement of cervix, post-cervical uterine torsion cases 16/18 (88.89%) were higher in incidence as compared to the pre-cervical cases 2/18 (11.11%). The direction of uterine torsion in buffaloes was diagnosed as right side (clockwise) in 15/18 (83.33%) of the cases, which was higher than left side (counter

clockwise) 3/18 (16.67%). Torsion degrees of less than or equal to 90º, 90º-180 º, 180º-360º and greater than 360º were found to occur in 16.67, 50%, 22.22 and 11.11% cases, respectively. Out of the reported cases of uterine torsion, 2/18 (11.11%) were at pre term and most of the cases 18/15 (88.89%) were reported being at full term.

The present study evaluates the incidence of maternal causes and higher contribution of uterine torsion among them which supports the finding as maternal dystocia is common in buffaloes and uterine torsion is primary aspect in these (Singh *et al.*, 2020; Juneja *et al.*, 2023). Higher incidence of uterine torsion at full term is generally assigned to the vigorous movement of the dam, strong myometrial contractions and righting reflex of the fetus during the first and/or second stage of parturition causing the failure of delivery (Nagaraju, 2018). Similarly, in the current experiment, a higher incidence rate of uterine torsion at term was recorded which observed in accordance with findings of earlier researchers (Thangamani *et al.*, 2019; Selvaraju and Karthick, 2020).

The effect of age on occurrence of uterine torsion stays around controversy, as there is no age predisposition in torsion affected buffaloes and cattle of 2-18 years age (Purohit and Gaur, 2014) which again validated by the current experiment by showing no correlation with age of buffaloes *viz*. 3-5 years and above 5 years. Parity of animal has also relation with uterine torsion (Matharu and Prabhakar, 2001; Purohit *et al.*, 2013). The increased incidence of uterine torsion as per increment of parity supported the findings of current study by observing the higher incidence in multiparous as compared to primiparous buffaloes which might be due to larger abdominal cavity, loosened long broad ligaments together with loosening of uterine tissue, stretching of pelvic ligaments,decreased uterine tone and weaker abdominal muscles in older bovines (Aubry *et al.*, 2008; Jeengar *et al.*, 2014; Tripathi and Mehta, 2016).

In the current investigation, the higher incidence of post-cervical uterine torsion was observed as compared to pre-cervical in uterine torsion affected buffaloes. Similar findings were also obtained by other authors who also observed higher incidence of post cervical uterine torsion (Selvaraju and Karthick, 2020) which could be due to weakening of anterior vagina or may be due to the absence of the muscles at the cervical region of broad ligaments in bovine (Jeengar *et al.*, 2015). Twisting of pregnant uterus in right side has maximum preponderance in buffaloes due to absence of a muscular fold on right broad ligament (Brar *et al.*, 2008) and restricted twisting in left side due to the presence of the rumen (Purohit and Gaur, 2014). Similarly, the consequences of the current study were also infrared higher tendency of right-side uterine torsion which observed at par with the findings of earlier studies on buffaloes (Selvaraju and Karthick, 2020).

In the current experiment, higher incidence of uterine torsion was observed between 90º and 180º in buffaloes which is in consonance with the findings of earlier workers (Jeengar *et al.*, 2015; Tripathi and Mehta, 2016) who also recorded the higher proportion of uterine torsions between 90º and 180º (Ramteke and Razzaque, 2019).

Haematological parameters of uterine torsion affected buffaloes supplemented with antioxidants

Haematological parameters: The various haematological changes in buffaloes affected with uterine torsion and treated with vit E-selenium and vitamin C are presented in table 2.

Effect of administration of antioxidants on red cell indices: In the current study, parenteral administration of antioxidants showed the significant ($p<0.05$) effect on blood RBC, Hb and HCT among the groups after 24 hrs of correction in uterine torsion affected buffaloes. After 24 hrs of correction, intravenous administration of vitamin C exhibited significantly the highest blood HCT level followed by vitamin E+Se group. In the current investigation, the mean blood RBC and Hb level was observed significantly higher in both the antioxidant groups after 24 hrs of detorsion.

The observations are also in agreement with earlier reports that also recorded significant increase in mean TEC during early lactation compared to advanced gestation in transition dairy cows, suggesting supporting role of Se alone and supplementation of combination of vitamin E+Se during elevated level of oxidative stress (Ambily *et al.*, 2019). According to Shokrollahi *et al.* (2013) blood parameters such as RBC and Haemoglobin were found the highest ($p<0.01$) supplemented byvitamin E and Se enriched milk to newborn goat kids. Similarly, significantly increase ($p<0.05$) in mean haemoglobin and TEC values were also observed by parenteral administration of vitamin E and Se along with Buparvaquone + Marbofloxacin in cattle affected with tropical theileriosis (Nayak *et al.*, 2018). Mohri *et al.* (2011) observed that calves fed with vitamin E and selenium in the age of 3rd and 4th weeks had higher HCT and haemoglobin levels.

The results recorded during the current study are in accordance with the findings of Kassab and Mohammed (2014) who observed that ascorbic acid group had positive effect on red blood cell, Haemoglobin and HCT values in

Table 2. The impact of vit E+Se and vit. C administration on Haematological profile in uterine torsion affected buffaloes

Groups	C			VES			VC		
Time interval	AR	AC	24 hrs	AR	AC	24 hrs	AR	AC	24 hrs
RBC ($\times10^6/\mu L$)	$5.34^{Aa}\pm0.11$	$5.45^{Aa}\pm0.11$	$5.73^{Aa}\pm0.15$	$5.29^{Aa}\pm0.20$	$5.54^{Aa}\pm0.22$	$6.63^{Bb}\pm0.28$	$5.23^{Aa}\pm0.18$	$5.37^{Aa}\pm0.19$	$6.86^{Bb}\pm0.17$
Hb (gm/dl)	$8.13^{Aa}\pm0.12$	$8.23^{Aa}\pm0.11$	$8.31^{Aa}\pm0.11$	$7.98^{Aa}\pm0.15$	$8.42^{Aa}\pm0.19$	$9.53^{Bb}\pm0.25$	$8.08^{Aa}\pm0.11$	$8.55^{Aa}\pm0.18$	$9.88^{Bb}\pm0.21$
HCT (%)	$27.39^{Aa}\pm0.61$	$28.37^{Aa}\pm0.57$	$29.16^{Aa}\pm0.57$	$28.16^{Aa}\pm0.75$	$28.84^{Aa}\pm0.62$	$33.95^{Bb}\pm0.61$	$29.02^{Aa}\pm0.90$	$29.54^{Aa}\pm0.89$	$36.68^{Cb}\pm0.89$
MCV (fL)	42.24±0.77	42.58±0.77	43.41±0.82	43.14±0.85	43.65±0.81	44.75±0.76	41.94±0.93	42.46±0.74	43.38±0.79
MCH (pg)	13.05±0.46	13.18±0.47	13.26±0.48	12.98±0.49	13.04±0.49	13.29±0.55	13.12±0.49	13.21±0.48	13.28±0.48
MCHC (gm/dl)	29.82±1.08	29.09±0.94	27.08±0.69	28.42±0.78	27.92±0.59	26.19±0.69	27.98±0.93	26.75±1.08	26.18±0.74
WBC($\times10^3/\mu L$)	$13.12^{Aa}\pm0.39$	$13.03^{Aa}\pm0.39$	$12.92^{Ba}\pm0.38$	$13.03^{Ab}\pm0.49$	$13.14^{Ab}\pm0.48$	$9.22^{Aa}\pm0.30$	$12.95^{Ab}\pm0.40$	$12.86^{Ab}\pm0.39$	$10.13^{Aa}\pm0.25$
LYM (%)	58.59±1.19	59.14±1.23	60.26±1.21	60.22±1.08	60.86±1.11	61.29±1.12	59.32±1.54	59.84±1.45	60.95±1.39
NEU (%)	$71.82^{Aa}\pm2.78$	$68.64^{Ba}\pm2.65$	$64.83^{Ba}\pm2.75$	$69.64^{Ab}\pm1.90$	$64.64^{Ab}\pm1.92$	$49.17^{Aa}\pm2.24$	$74.87^{Ac}\pm1.73$	$59.53^{Ab}\pm2.04$	$50.92^{Aa}\pm2.53$
MONO (%)	$11.11^{Aa}\pm0.37$	$10.88^{Ba}\pm0.37$	$9.92^{Ba}\pm0.49$	$10.87^{Ab}\pm0.38$	$9.95^{Bb}\pm0.33$	$7.23^{Aa}\pm0.29$	$9.96^{Ac}\pm0.46$	$8.42^{Ab}\pm0.42$	$6.93^{Aa}\pm0.39$
EOS (%)	$0.99^{a}\pm0.03$	$1.07^{b}\pm0.03$	$1.11^{b}\pm0.02$	$1.08^{a}\pm0.04$	$1.12^{a}\pm0.03$	$1.13^{a}\pm0.03$	$1.06^{a}\pm0.02$	$1.08^{a}\pm0.02$	$1.12^{a}\pm0.02$

Mean values bearing different superscripts in a column (Capital letters) differed significantly ($p\leq0.05$)
Mean values having different superscripts in a row (Small letters) differed significantly ($p\leq0.05$)
AR- At reporting of case; AC-At one hr after correction of UT; 24 hrs- At 24 hrs of UT correction

sheep having transportation stress. Likewise, Men-Kin *et al.* (1994) also observed increased RBC count and haemoglobin level after vitamin C supplementation in broiler chickens.

Hematological responses to vitamin E could mediate its enhancing for erythropoiesis and decreasing the premature erythrocyte hemolysis by reducing the fragility of them (Jiliani and Iqbal, 2011). Vitamin C has been considered to be a chain breaking antioxidant, contributes in the prevention and limitation of free radical chain formation and also propagation, consequently, protecting blood cells (Powers and Jackson, 2008; Urban-Chmiel *et al.*, 2009).

In the current experiment, parenteral administration of antioxidants showed the non-significant effect on blood MCH, MCHC and MCV level in uterine torsion affected buffaloes. The present results are corroborated by showing non-significant difference on MCH, MCHC and MCV in dietary supplementation of vitamin E and Se group having in heat stressed HF cows (Sultana *et al.*, 2022). The present results stand at par with findings of Adenkola *et al.* (2009) who observed non-significant difference on MCH level by oral administration of ascorbic acid in pigs during transportation. These results stand at par with findings of Kassab and Mohmmed (2014) who noted non-significant difference in MCH, MCHC and MCV values after oral administration of ascorbic acid in transportation stressed sheep. Non-significant effect of dietary supplementation of vitamin C and vitamin E on MCH, MCHC and MCV values was also reported in heat stressed rabbits (Abdelhamid *et al.*, 2019). Likewise, Fujihara and Orden (2014) conducted a similar study to validate the effect of vitamin E administration in rats and didn't observe any significant effect on MCH, MCHC and MCV values.

The MCH, MCHC and MCV values didn't differ significantly among the treatment groups whereas were numerically higher in antioxidants supplementation groups which may be due to less oxidative stress and better adaptation to the environment.

Effect of antioxidants administration on total and differential leucocyte count:

In the current study, the mean blood WBC, neutrophils and monocyte value was noted significantly lower ($p \leq 0.05$) by parenteral administration of vitamin C and vitamin E+Se after 24 hours of correction of uterine torsion. After 1 hour of detorsion, the mean value of blood monocytes also observed significantly lower ($p \leq 0.05$) by intravenous administration of ascorbic acid. The parenteral administration of antioxidants didn't affect the blood lymphocytes and eosinophills values in uterine torsion affected buffaloes.

Findings of the present study are in agreement with observations of Nayak *et al.* (2018) who also observed significantly lower ($p\leq0.05$) TLC values after supplemented of vitamin E and Se in tropical theileriosis affected cattle. In accordance of the present study, significantly reduced ($p \leq 0.05$) total leucocyte and neutrophils count and also non-significant effect of dietary supplementation of vitamin E and C on lymphocyte count was observed in heat stressed rabbits (Abdelhamid *et al.*, 2019).

The current data are in accordance with the obsrvations of Faixova *et al.* (2007) who recorded lower level of WBC in lambs supplemented by Se enriched yeast

for 3 months. Mohri *et al.* (2005) reported non-significant effect on eosinophil values even after supplementation with vitamin E and Se in lambs and calves, respectively. The present results are corroborated by the non-significant difference in lymphocyte level by dietary administration of vitamin E and Se in HF cows under heat stress condition (Sultana *et al.*, 2022). These results stand in accordance with findings of Kassab and mohammed (2014) who also infrared non-significant effect on lymphocytes and eosinophils values by oral supplementation of ascorbic acidin transportation stressed sheep.

Vitamin E and Se are considered as potent antioxidants and free radical scavengers which plays a significant role to compensate free radical burden and increase the neutrophil activity (Bergamini *et al.*, 2004). Also, vitamin C has been considered as a chain breaking antioxidant, involved in the prevention and limitation of free radical chain formation and propagation which also helps to protect from oxidative damage to blood cells, including neutrophils and lymphocytes (Powers and Jackson, 2008 and Urban-Chmiel *et al.*, 2009). Supplementation of vitamin C and vitamin E diminishes the total leucocyte and neutrophils count by reducing the cortisol level (Sahin *et al.*, 2002). Antioxidant effect of vitamin E enables the protection of organelles and cell membranes by affecting the cell numbers of lymphocyte (Moeini and Jalilian, 2014).

CONCLUSION

In conclusion, higher incidence of uterine torsion was observed between 90º and 180º in buffaloes. The results of our study demonstrated a clear positive effect of parenteral administration of vitamin E with Se and ascorbic acid on hematological profile in uterine torsion affected buffaloes.

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RETRACTION OF ARTICLE

This article earlier available at https://www.luvas.edu.in/haryana-veterinarian/download/harvet2016-dec/1.pdf entitled *"Occurrence of some organochlorine pesticide residues in poultry feed and meat"* has been retracted by the authors because of some error made during the data analysis process of the experimental observations due to counting the number of samples showing the concentration of pesticide below its corresponding Limit of Detection. All authors take full responsibility for this mistake and sincerely apologize for any inconvenience it may cause.

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