

Unlocking physiological transformations in riverine buffaloes following uterine torsion correction

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Received: 30 August 2023; Accepted: 11 October 2023

ABSTRACT

This study aimed to examine the physiological alterations in riverine buffaloes with incompletely dilated cervix after the uterine torsion correction and the main objective was to understand the hormonal changes that lead to complete cervical dilatation following detorsion, facilitating per-vaginal birth. Torsion characteristics in buffaloes (n=24) were examined, and detorsion was performed using Sharma's Modified Schaffer's approach. Blood samples were collected pre-detorsion, post-detorsion, and at parturition. The average cervical dilation rate was 1.22 ± 0.16 cm/h, and detorsion-to-parturition duration averaged 17.38 ± 1.44 h. There were no significant differences between haematological and biochemical parameters at three-time intervals except serum calcium. Progesterone levels decreased progressively (1.43 ± 0.01 ng/mL, 1.37 ± 0.01 ng/mL, 1.34 ± 0.01 ng/mL), while estradiol levels increased significantly (65.86 ± 2.05 pg/mL, 77.08 ± 2.05 pg/mL, 91.8 ± 2.55 pg/mL) from pre-detorsion to post-detorsion and parturition. The levels of cortisol were significantly higher pre-detorsion (24.3 ± 2.07 ng/mL) and post-detorsion (27.1 ± 2.5 ng/mL) than at parturition (18.31 ± 1.76 ng/mL). In conclusion, elevated estradiol levels, coupled with reduced progesterone, post-detorsion, trigger complete cervical dilatation, facilitating per-vaginal birth. This study sheds light on the hormonal dynamics driving the birthing process in buffaloes after uterine torsion correction.

Keywords: Buffalo, Cervical dilatation, Endocrine profile, Uterine torsion

Uterine torsion, the twisting of the gravid uterus along its longitudinal axis, culminates in birth canal stenosis and dystocia (Balasopoulou *et al.* 2022, de Carvalho *et al.* 2022). This phenomenon primarily affects pluriparous animals during the final month of gestation or parturition (Dhami *et al.* 2019). Swift intervention in cases of uterine torsion is essential to enhance dam and fetal survival chances. This is due to the propensity of torsion which often leads to partial cervical dilation and uterine inertia, thereby necessitating prompt rectification (Yadav *et al.* 2021). However, rectifying uterine torsion can result in insufficient cervical dilation, posing a substantial impediment to vaginal delivery (Sahu *et al.* 2019). An intriguing observation is that following the correction of uterine torsion, approximately 76% of buffalo cases manifest challenges related to cervical dilation (Chaudhari *et al.* 2019). Incomplete cervical dilation has been closely associated with high collagen levels in cervical and uterine tissues, along with reduced VEGF (Vascular Endothelial Growth Factor) levels due to impaired angiogenesis (Ibrahim *et al.* 2022).

Doppler indices can be used to predict how well dilatation treatment would work in incomplete cervical

dilatation with uterine torsion, together with other factors such as disease duration, uterine torsion location, cervix consistency, and udder engorgement (Chaudhari *et al.* 2023). Furthermore, endocrine fluctuations and hormonal imbalances during calving have been linked to cervical dilation failure (Noakes *et al.* 2019, Yadav *et al.* 2022a). Molecular pathways governing cervical remodeling involve hormonal changes, inflammatory cytokines, and enzymatic degradation, all of which impact cervical softening and vascularization (Ibrahim *et al.* 2022). This study delves into the endocrine, haematological, and biochemical aspects of partially dilated cervix after uterine torsion correction in riverine buffaloes (*Bubalus bubalis*). With incomplete cervical dilation being a common issue after uterine torsion, and the underlying endocrine mechanisms, this research seeks to illuminate the factors influencing cervical dilation.

MATERIALS AND METHODS

Animals: The buffaloes (n=24) affected by uterine torsion were selected for this study. All the animals included in the study were female buffaloes of first to fourth parity. The ethical approval from the Institutional Animal Ethics Committee (1669/GO/ReBiBt-S/Re-L/12/CPCSEA) was taken at the College of Veterinary Sciences, LUVAS, Hisar.

Detorsion was done by Sharma's modified Schaffer's method. Detorsion of uterine torsion was

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followed by a per-rectal and per-vaginal examination of animals. Dexamethasone [Dexona® Vet Injection-Dexamethasone Sodium Phosphate Injection I.P. (Vet.)], Calcium borogluconate [Mifex®-Calcium Magnesium Borogluconate Injection I.P. (Vet.)], and cloprostenol [Vetmate™- Cloprostenol Sodium IP (Vet)] was administered just after detorsion in all animals. The cervical diameter was measured per-vaginally with the help of a cervicometer and cervical dilatation rate was calculated (Yadav *et al.* 2022b).

Haematological, biochemical and endocrine analysis: Blood samples were collected from jugular venipuncture pre-detorsion, post-detorsion, and after per-vaginal delivery of the foetus. Haematological parameters were estimated in automatic analyzer MS4 (JLS380 Melet Schloesing Laboratoires, France) after collecting blood samples in vials containing EDTA. For biochemical analysis, serum was harvested by centrifugation at 3000 rpm for 20 min and then stored at -20°C and parameters were analysed with EM 200™ (Automated random access clinical chemistry analyzer, Erba Mannheim, Germany) analyzer using commercially available TransasiaXL system pack kits. Serum cortisol, progesterone, and estradiol levels were quantified using commercial ELISA kits following specific assay protocols. For progesterone, the assay sensitivity was determined to be 0.11 ng/mL, for estradiol, it was 3.9 pg/mL, and for cortisol, it was 0.39 ng/mL. The ELISA procedures were performed on a microplate reader (Multiskan FC, Thermo Fisher Scientific) according to the manufacturer's instructions.

Statistical analysis: Data expressed as mean±standard error of the mean (Mean±S.E.M.) were analyzed using SPSS software (IBM Inc, USA). All results were considered statistically significant at the 95% confidence level ($p < 0.05$). Post hoc Dunn's multiple comparison tests determined the differences between parameters.

RESULTS AND DISCUSSION

Signalment and clinical observation: Most of the buffaloes affected with uterine torsion belonged to the age group 5-7 years and second parity. Out of 24 animals, 21 did not have a history of wallowing, and three wallowed in the last trimester. All animals were in an advanced stage of gestation (300.5 ± 1.68). While 22 animals were affected with uterine torsion of the right side (Table 1). The most common site of occurrence of uterine torsion was post-cervical. The degree of uterine torsion was moderate in most of the animals (17), followed by mild degree (5) and high degree (2). Sharma's modified Schaffer's method was used for detorsion, and most of the animals were detorted in one and two rollings followed by three rollings. Only six live calves were delivered out of 24 uterine torsion-affected animals.

In this study, among the group of researched animals, the maximum percentages flagged as affected buffaloes belonged to the second parity (45.8%), followed by the first parity (25%), third parity (16.7%), and fourth parity

Table 1. Signalment and clinical observation recorded in buffaloes under study

Parameter	Observation
Gestation length (days) (Mean ± S.E.)	300.5 ± 1.68
Parity	1 st (n=6); 2 nd (n=11); 3 rd (n=4); 4 th (n=3)
Site of uterine torsion	Post-cervical (n=22); Pre-cervical (n=2)
Direction of uterine torsion	Right (n=22); Left (n=2)
Degree of uterine torsion	Mild (n=5); Moderate (n=17); High (n=2)
Rollings for detorsion	One (n=10); Two (n=9); Three (n=5)
History of wallowing	Yes (n=3); No (n=21)

N, Number of animals; S.E.M., Standard error of mean; Mild, 90-180 degree; Moderate, 180-270 degree; High, >270 degree; Yes/No, Animals were wallowed in third trimester/Not wallowed.

(12.5%). The average gestation length of affected animals was 300.5 ± 1.68 days indicating that animals were at full term as observed in previous studies (Mahmoud *et al.* 2020). The suggested reason behind this is the righting reflex which accounts for the active movements of the foetus in the uterus during late gestation and early stage of labour (Noakes *et al.* 2019). The location of uterine torsion was on the right side in 92% of affected cases as flagged in various researchers' reports (Hussein *et al.* 2013, Mahmoud *et al.* 2020, Jeengar *et al.* 2015). The location of uterine torsion was post-cervical in 92% of the affected animals. Ali *et al.* (2011) reported similar results, with 98.4% of cases occurring at post-cervical locations. They suggested that fetal size and amount of fetal fluids might be determining factors for the location of uterine torsion in buffaloes. The calf survivability rate was 25%, which highly depends on the degree and duration of uterine torsion. A moderate degree of uterine torsion was present in 70.8% of cases. The main clinical symptoms observed were a history of colicky signs, kicking at the abdomen, reduced feed and water intake, and restlessness. However, the most common sign was the failure of parturition.

Hormonal assay study: There was significant decrease ($p < 0.05$) in serum progesterone concentration and a significant increase in the concentration of estradiol post-detorsion ($p < 0.05$) (Table 2). Serum cortisol levels were significantly higher ($p < 0.05$) pre-detorsion and post-

Table 2. Endocrine profile of buffaloes (Mean±S.E.M.) under study

Hormone	Pre-detorsion (n=24)	Post-detorsion (n=24)	At parturition (n=24)
Estradiol - 17β (pg/mL)	65.86±2.05 ^a	77.08±2.05 ^b	91.8±2.55 ^c
Progesterone (ng/mL)	1.43±0.01 ^c	1.37±0.01 ^b	1.34±0.01 ^a
Cortisol (ng/mL)	24.3±2.07 ^b	27.1±2.5 ^b	18.31±1.76 ^a

Means with different superscripts (a/b/c) in a column show significant differences at different time intervals ($p < 0.05$).

detorsion than at parturition.

The existing hormonal imbalance may be a cause of incomplete cervical dilation after detorsion in affected cases. This may be indicating that the non-conversion of progesterone to estrogen by the non-functional placenta as blood supply to the uterine artery is reduced due to stenosis and partial degeneration of the corpus luteum of pregnancy (Nanda and Sharma 1986) as the stressed foetus release prostaglandins (PGE_2) which have luteotropic effects (Hafez 2000). The critical finding of this study was that there was a significant decrease ($p<0.05$) in serum progesterone concentration after detorsion and a significant increase in the concentration of estradiol after detorsion ($p<0.05$) similar to various researchers (Abdel-Ghaffar and Abou-El-Roos 2002, Amer *et al.* 2008). This suggested that either there might be restoration of the progesterone conversion potential to the placenta's estrogen after detorsion in buffaloes or it might be due to partial luteolysis occurring during this time period. It needs further investigation. Here it has been demonstrated that the current study was only done on a small number of animals, and this should also be considered regarding the viability of the number of fetuses. Numerous investigations using Doppler indices have demonstrated that blood flow returns to normal following detorsion in the uterus. This may be related to the restoration of the placenta's conversion potential. Serum cortisol levels were increased, showing a picture of existing stress conditions in all affected animals. Similar findings have been observed by Ghuman *et al.* (1997) and Amer *et al.* (2008). Ghuman *et al.* (1997) attributed the increased cortisol level to stress conditions in buffaloes affected by 180° to 360° uterine torsion. The lower concentration of cortisol in parturition compared to the post-detorsion period can be attributed to several physiological factors. During parturition, there is a natural surge in various hormones, such as oxytocin and prostaglandins, which play crucial roles in initiating and regulating the birthing process in mammals, including buffaloes. In the context of uterine torsion correction and subsequent pregnancy, cortisol levels might have been elevated during the post-detorsion period due to the stress and physiological challenges associated with the torsion and detorsion with the restoration of proper uterine function. However, as the pregnancy progresses and parturition approaches, the body shifts its focus towards the birthing process, and the hormonal milieu changes accordingly. The maternal adrenal gland is activated during the last days of pregnancy and is further stimulated during torsion or parturition to give high cortisol levels to the mother during and after birth (Amer *et al.* 2008).

Cervical dilatation rate: The time taken for parturition after detorsion was 17.38 ± 1.44 h. The cervical dilatation rate of buffaloes is 1.22 ± 0.16 cm/h (Fig. 1).

The prolonged parturition causes the mother to become exhausted and the foetus to become stressed. One of the efficient outcomes of the straining forces of parturition is complete cervical dilation. Incomplete cervix dilatation,

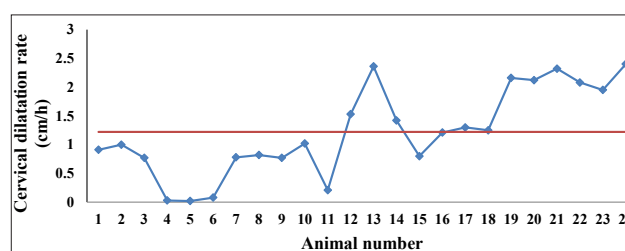


Fig. 1. Depicting cervical dilatation rate of detorted buffaloes. (Blue line: Depicting cervical rate of buffaloes under study. Red line: Mean of cervical dilatation rate of all buffaloes under study).

which is frequent in buffaloes, might be due to the fewer cervical rings in the cervix (Purohit *et al.* 2011). No reference studies are available in the literature for cervical dilatation rate in torsion-detorted buffaloes. However, studies regarding duration of complete cervical dilatation after detorsion and drug administration are available (Das *et al.* 2008).

Haematological and biochemical changes: No significant difference ($p<0.05$) in any of the haematological parameters was observed at different time intervals during the entire observation period (Table 3).

Table 3. Haematological profile of buffaloes (Mean $\pm$ S.E.M) under study

Parameter	Before detorsion	After detorsion	At parturition
Haemoglobin (g/dl)	11.93 $\pm$ 0.44	11.41 $\pm$ 0.40	11.12 $\pm$ 0.40
Packed cell volume (%)	41.24 $\pm$ 1.88	38.20 $\pm$ 1.61	36.86 $\pm$ 1.49
Total leucocyte count ($\times 10^3$ cells/mm 3)	8.43 $\pm$ 0.52	8.35 $\pm$ 0.51	8.33 $\pm$ 0.49
Granulocytes (%)	73.82 $\pm$ 1.88	76.60 $\pm$ 1.52	77.13 $\pm$ 1.32
Mean corpuscular haemoglobin (pg/cell)	20.79 $\pm$ 0.43	20.99 $\pm$ 0.38	21.08 $\pm$ 0.37
Mean corpuscular volume (fL)	71.46 $\pm$ 1.25	70.94 $\pm$ 1.25	70.83 $\pm$ 1.24

The uterine torsion-affected buffaloes suffer from normocytic normochromic anemia (Amer and Hashem 2008). The leukogram of torsion-affected buffaloes revealed lymphocytopenia, neutrophilia, and monocytosis is associated with eosinopenia (Amer and Hashem 2008). Increased values of haemoglobin and PCV were reported with increased degree and duration of torsion (Ali *et al.* 2011, Nagaraju 2018), which may be due to acute dehydration as a consequence of reduced water and feed intake and oozing of fluids from congested uterine blood vessels. However, the present study did not represent such haemo-concentration; it is attributed to the timely attending of the cases, which prevented severe dehydration and toxemia in affected buffaloes. Pateluya *et al.* (2019) also reported that timely treatment of uterine cases in buffaloes prevents haemo-concentration and dehydration. The increased TLC might be attributed to the stress exerted on the affected animals (Amer *et al.* 2008). Karthick *et al.* (2016) and Pateluya *et al.* (2019) reported that TLC levels after parturition decrease sharply in detorted

Table 4. Biochemical profiles of buffaloes (Mean±S.E.M) under study

Parameter	Pre-detorsion	Post-detorsion	At parturition
AST/SGOT (U/L)	137.68±5.26	135.33±4.89	149.70±6.67
Phosphorus (mg/dL)	3.91±0.18	3.77±0.12	4.22±0.32
Urea (mg/dL)	19.30±1.44	20.86±1.41	21.25±1.76
Creatinine (mg/dL)	1.54±0.09	1.61±0.11	1.63±0.13
Calcium (mg/dL)	8.92±0.17 ^a	16.68±0.74 ^b	10.29±0.37 ^a
Albumin (g/dL)	2.07±0.07	1.97±0.06	2.03±0.06
Total proteins (g/dL)	5.97±0.22	5.91±0.16	6.06±0.15
ALT/SGPT (U/L)	63.49±1.40	62.67±1.61	65.91±1.78

Means with different superscripts (a/b/c) in a column show significant differences at different time intervals (p<0.05).

buffaloes. The suggested reason behind this reduction in TLC levels after parturition is the migration of neutrophils toward the uterine lumen and mammary gland to alleviate stress and infection (Karthick *et al.* 2016). The results of the haematological parameters of this study are analogous to Amer *et al.* (2008), Ali *et al.* (2011), Karthick *et al.* (2016), and Pateliya *et al.* (2019). The long-standing uterine infection followed by toxemia resulted in monocytosis in torsion-affected buffaloes (Ali *et al.* 2011).

The mean serum calcium levels exhibited a significant rise (p<0.05) post-detorsion in comparison to calcium levels both before detorsion and during parturition (Table 4). The increased calcium level was due to the administration of calcium borogluconate. Total protein levels in the torsion-affected buffaloes are lowered. Similar observations have been made in earlier studies (Arora *et al.* 2013, Pateliya *et al.* 2019). The present findings on serum urea and creatinine are analogous to the previous reports of Satish *et al.* (2018) and Pateliya *et al.* (2019). High levels of biochemical constituents LDH, AST, glucose, BUN, creatinine, CPK, and gamma-glutamyl transferase have been reported in pregnant cows and buffaloes affected by various degrees of uterine torsion (Kuhad *et al.* 1996, Amer and Hashem 2008). There is great destruction of the muscular cells of the uterus, and this destruction results in the increased enzymatic activities of AST and CPK (Kraft and Dürr 2005).

In summary, this study has provided significant insights into the hormonal dynamics of buffaloes experiencing uterine torsion and subsequent detorsion. Notably, a substantial decline in blood progesterone levels and a marked rise in serum estradiol concentrations were observed after detorsion. While these changes might suggest a potential role in the restoration of placental function and hormone conversion following detorsion, further research is needed to elucidate the exact mechanisms involved in this observed hormonal shift.

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