

## SERUM ENZYMATIC AND ENDOCRINE PROFILE IN UTERINE TORSION AFFECTED BUFFALOES

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### ABSTRACT

This study was conducted to investigate the endocrinal and enzymatic alterations in buffaloes suffering from uterine torsion (Gr-I, n=10) in comparison to normal parturient buffaloes Gr-II, n=6) just before as well as 1 hr, 1 day and 1 week after parturition. Serum enzymatic profile revealed that the mean values of serum AST and CPK were significantly higher in torsion affected buffaloes than normal parturient buffaloes at all peripartum intervals, whereas, the mean values of serum ALT were found significantly ( $p < 0.05$ ) elevated in affected buffaloes only during prepartum period. Serum endocrine profile revealed apparently lower serum estradiol  $17\beta$  concentrations in uterine torsion affected than normal parturient buffaloes at most periparturient periods, whereas circulatory progesterone and cortisol levels were significantly higher in torsion affected buffaloes than normal ones, before and 1 hr after parturition only.

**Key words:** Uterine torsion, Buffaloes, ALT, AST, CPK, Cortisol, Estradiol, Progesterone.

### INTRODUCTION

Uterine torsion is considered to be a more frequent maternal cause of dystocia in buffaloes compared to cattle (Purohit *et al.*, 2012) and it is among those reproductive disorders that result in deadly outcomes if not attended timely (Tripathi and Mehta, 2016). Following uterine torsion and after its correction by detorsion or surgical treatment, the activities of aspartate amino transferase (AST), alanine amino transferase (ALT), glutamate dehydrogenase (GLDH), creatine phosphokinase (CK) and gamma glutamyl transferase (GGT) are increased (Purohit and Gaur, 2014), which usually become stabilized within 10 days after surgical treatment of uterine torsion (Schönfelder *et al.*, 2007). During recent years it has been mentioned that due to uterine circulatory disturbances and muscle exhaustion, blood parameters evaluating liver and kidney functions can be used as prognostic indicators for the future outcome of uterine torsion affected buffaloes (Purohit and Gaur, 2014). Hence, the present study was conducted to investigate the enzymatic and hormonal alterations associated with the uterine torsion in buffaloes.

### MATERIALS AND METHODS

The present study was conducted during July to December 2018 in Amul milk shed area on torsion affected buffaloes (n=10) in relation to normal parturient buffaloes (n=6). The buffaloes with uterine torsion presented to Veterinary Clinical Complex, Anand and normal parturient buffaloes from field as well as university

farm were included in the investigation. Diagnosis of the uterine torsion was made by history and trans-rectal followed by trans-vaginal examination. All the buffaloes with uterine torsion were treated by Schaffer's method. Four time blood samples were collected from jugular vein of each of 16 animals, *i.e.* before parturition as well as 1 hr, 1 day and 1 week after parturition in serum activator vials to investigate serum enzymatic and hormonal alteration in torsion affected and six normal parturient buffaloes.

The levels of serum enzymatic parameters, *viz.*, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine phosphokinase (CPK) were determined using Diagnostic kits procured from Coral Clinical Systems, Goa, on Chemistry Analyzer (Mindray, Model No: BS-120). The concentrations of serum progesterone, estradiol and cortisol were determined by employing standard RIA techniques of Kubasic *et al.* (1984), Robertson and King (1979) and Brock *et al.* (1978), respectively. Labelled antigen ( $I^{125}$ ), antibody coated tubes and standards were procured from Immunotech-SAS, Marseille Cedex, France. The sensitivity of the progesterone, estradiol and cortisol assays was 0.1 ng/ml, 9.58 pg/ml and 0.1 ng/ml, respectively. The intra- and inter-assay coefficients of variation were 5.4 and 9.1 % for progesterone, 14.4 and 14.5 % for estradiol  $17\beta$ , and 5.8 and 9.2 % for cortisol, respectively. The data on serum enzymatic and hormonal profile were analysed using standard statistical procedures of ANOVA, DMRT and 't' test on SPSS software version 20.00 to know the variations within and

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between the groups for each trait (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

In the present study, the mean values of enzymatic and hormonal constituents in uterine torsion affected and normal parturient buffaloes just before parturition, and at 1 hr, 1 day and 1 week after parturition are presented in Table 1.

### Serum Alanine Aminotransferase (ALT):

The mean serum ALT values in torsion affected buffaloes before and 1 hr after parturition were non-significantly and significantly ( $p < 0.05$ ) higher than at 1 day and 1 week after parturition, respectively, whereas no such significant difference was observed in normal

parturient buffaloes. Moreover, the value of ALT just before parturition was significantly ( $p < 0.05$ ) higher, while the values at 1 hr, 1 day and 1 week after parturition were non-significantly higher in uterine torsion affected buffaloes as compared to normal parturient animals. The increase in serum levels of ALT in uterine torsion affected buffaloes were analogues with previous observations of Jeengar *et al.* (2015) and Satish *et al.* (2018). Higher levels of ALT noticed in torsion affected buffaloes may be attributed to uterine hypoxia and increased release of catecholamines from the adrenal medulla associated with uterine torsion and its stress. The elevated prepartum ALT concentration in affected buffaloes was decreased and reached to nearly normal level after 24 hr of fetal delivery.

**Table 1 :** Serum enzymatic and endocrine profile at different peripartum periods in uterine torsion affected (n=10) and normal parturient (n=6) buffaloes (Mean  $\pm$  SE)

Serum Parameter	Group	Before parturition	1 hr after parturition	1 day after parturition	1 week after parturition
ALT (U/L)	Affected	44.44 $\pm$ 2.16 <sup>b</sup>	42.97 $\pm$ 2.20 <sup>b</sup>	40.65 $\pm$ 2.02 <sup>ab</sup>	35.94 $\pm$ 1.68 <sup>a</sup>
	Normal	37.68 $\pm$ 1.42 <sup>*</sup>	40.77 $\pm$ 1.39	38.03 $\pm$ 3.77	33.99 $\pm$ 1.96
AST (U/L)	Affected	94.21 $\pm$ 5.72 <sup>c</sup>	86.43 $\pm$ 5.67 <sup>bc</sup>	78.37 $\pm$ 4.42 <sup>b</sup>	65.85 $\pm$ 1.22 <sup>a</sup>
	Normal	67.56 $\pm$ 3.92 <sup>b**</sup>	68.75 $\pm$ 2.63 <sup>b*</sup>	60.72 $\pm$ 1.65 <sup>ab**</sup>	58.73 $\pm$ 1.86 <sup>a**</sup>
CPK (U/L)	Affected	200.64 $\pm$ 11.40 <sup>b</sup>	204.54 $\pm$ 17.37 <sup>b</sup>	172.45 $\pm$ 25.15 <sup>b</sup>	118.09 $\pm$ 7.76 <sup>a</sup>
	Normal	40.13 $\pm$ 2.80 <sup>b**</sup>	52.77 $\pm$ 3.50 <sup>c**</sup>	34.63 $\pm$ 4.04 <sup>ab**</sup>	27.77 $\pm$ 2.64 <sup>a**</sup>
Estradiol (pg/ml)	Affected	148.90 $\pm$ 11.30 <sup>b</sup>	128.90 $\pm$ 14.70 <sup>b</sup>	89.90 $\pm$ 4.84 <sup>a</sup>	75.90 $\pm$ 5.34 <sup>a</sup>
	Normal	162.17 $\pm$ 11.36 <sup>b</sup>	146.17 $\pm$ 12.11 <sup>b</sup>	92.33 $\pm$ 4.93 <sup>a</sup>	67.83 $\pm$ 5.58 <sup>a</sup>
Progesterone (ng/ml)	Affected	1.33 $\pm$ 0.07 <sup>b</sup>	1.23 $\pm$ 0.06 <sup>b</sup>	0.57 $\pm$ 0.07 <sup>a</sup>	0.50 $\pm$ 0.05 <sup>a</sup>
	Normal	1.12 $\pm$ 0.06 <sup>c*</sup>	0.95 $\pm$ 0.07 <sup>b**</sup>	0.46 $\pm$ 0.02 <sup>a</sup>	0.42 $\pm$ 0.04 <sup>a</sup>
Cortisol (ng/ml)	Affected	110.20 $\pm$ 5.22 <sup>b</sup>	130.60 $\pm$ 8.32 <sup>b</sup>	74.40 $\pm$ 7.87 <sup>a</sup>	66.10 $\pm$ 7.99 <sup>a</sup>
	Normal	73.00 $\pm$ 6.23 <sup>a**</sup>	96.67 $\pm$ 8.66 <sup>b*</sup>	60.33 $\pm$ 2.70 <sup>a</sup>	55.17 $\pm$ 6.00 <sup>a</sup>

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, CPK = Creatine phosphokinase. Means bearing uncommon superscripts within the row (a,b,c) differ significantly ( $p < 0.05$ ). \* $p < 0.05$  and \*\* $p < 0.01$  between affected and normal control groups.

### Serum Aspartate Aminotransferase (AST):

The prepartum serum AST value in uterine torsion affected buffaloes was reduced significantly ( $p < 0.05$ ) at 1 day and 1 week after parturition. But, in normal parturient animals the prepartum serum AST concentration was reduced significantly ( $p < 0.05$ ) only at 1 week after parturition. Uterine torsion affected buffaloes evinced higher serum AST values as compared to normal parturient buffaloes with highly significant ( $p < 0.01$ ) differences before parturition, and after 1 day and 1 week of parturition and significant ( $p < 0.05$ ) difference at 1 hr after parturition. These findings were in close harmony with previous reports of Amer *et al.* (2008), Arora *et al.* (2013) and Satish *et al.* (2018), who observed elevated AST levels at the time of presentation of uterine torsion affected buffaloes compared to normal parturient buffaloes. Amer *et al.* (2008) opined that the increase of AST at term was necessary for accelerating the rate of

metabolism and protein biosynthesis needed for fetal growth as well as milk production. In addition, the increase in AST levels may be due to great muscular effort which is exerted during uterine twisting and the process of calving (Farrag *et al.*, 1984). At the same time, the nutritive differences such as imbalance in proteins and carbohydrates or insufficient crude fiber content resulted in upsetting the proper function of the rumen, causing more or less harmful energetic deficiency and damage of liver leading to increase of serum AST activity (Younis, 1990). In the previous studies (Amer *et al.*, 2008), the AST levels decreased after 24 hr of fetal delivery. In our study however, the AST concentration was significantly higher even after 1 week of parturition in torsion affected buffaloes as compared to normal parturient buffaloes.

### Serum Creatine Phosphokinase (CPK):

The mean serum level of CPK at 1 hr after parturition was non-significantly higher than before parturition in

uterine torsion affected buffaloes, however, the concentration of CPK was reduced non-significantly at 1 day postpartum than before and 1 hr after parturition and further reduced ( $p < 0.05$ ) at 1 week after parturition. Similarly, in normal parturient buffaloes also, the mean value of CPK was significantly ( $p < 0.05$ ) higher at 1 hr after parturition than before parturition, and further significantly ( $p < 0.05$ ) reduced at 1 week after parturition. The values of CPK were significantly ( $p < 0.01$ ) higher in uterine torsion affected than normal parturient buffaloes at all four stages/periods. Present findings gained support from previous observations of Amin *et al.* (2011) and Abd Allah *et al.* (2014), who found increased levels of CPK in uterine torsion affected buffaloes than normal parturient buffaloes, irrespective of duration and degree of uterine torsion. The increase in serum CPK activities may be attributed to muscle exertion produced by strong uterine and abdominal contractions due to uterine torsion (Abd Allah *et al.*, 2014).

#### **Serum Estradiol-17 $\beta$ and Progesterone:**

The observed prepartum mean values of serum estradiol-17 $\beta$  in both uterine torsion affected and normal parturient buffaloes, though statistically at par with those of 1 hr postpartum, were significantly ( $p < 0.05$ ) higher than the values at 1 day and 1 week after parturition. The uterine torsion affected buffaloes showed apparently lower values of serum estradiol-17 $\beta$  than normal parturient buffaloes at most of the peripartum intervals, except at 1 week after parturition, where it was found non-significantly inverted.

In uterine torsion affected buffaloes, the progesterone level was highest before parturition, which then reduced gradually and non-significantly at 1 hr after parturition and further declined significantly ( $p < 0.05$ ) at 1 day and 1 week after parturition. In normal parturient buffaloes, the mean serum progesterone concentration observed before parturition decreased significantly ( $p < 0.05$ ) at 1 hr after parturition and further reduced significantly ( $p < 0.05$ ) at 1 day and 1 week after parturition, where the later two values were statistically similar. The mean values of progesterone were higher in uterine torsion affected buffaloes than the normal parturient buffaloes at all four intervals, but the differences were significant ( $p < 0.05$ ) only before parturition and at 1 hr after parturition ( $p < 0.01$ ).

These findings were in analogues with the study of Amer *et al.* (2008), who reported significantly ( $p < 0.01$ ) higher progesterone levels in torsion affected buffaloes before and after detorsion, and immediately after birth. They opined that highly significant decrease in estradiol-17 $\beta$  and increase in progesterone levels associated with uterine torsion in buffaloes could be attributed to the live fetus, which might delay the onset of luteolysis and

maintain high progesterone output and thereby low levels of estrogen, because the fetus release prostaglandins (PGE<sub>2</sub>) which have luteotropic effects. The reason for hormonal imbalance could be non-conversion of progesterone to estrogen by non-functional placenta (Siddique, 1988) and partial degeneration of CL of pregnancy (Nanda and Sharma, 1986).

#### **Serum Cortisol:**

In uterine torsion affected buffaloes, cortisol level was observed to be highest at 1 hr after parturition, which was non-significantly higher than the mean level before parturition. It was then reduced significantly ( $p < 0.05$ ) at 1 day after parturition reaching the lowest level at 1 week after parturition, however, the difference between the values at 1 day and 1 week postpartum was non-significant. In normal parturient buffaloes also, the mean serum cortisol level was significantly ( $p < 0.05$ ) higher at 1 hr after parturition than remaining three stages for which the values were statistically similar though apparently reduced at 1 week postpartum compared to prepartum level. Uterine torsion affected buffaloes showed higher serum cortisol levels than normal parturient buffaloes at all four periods with highly significant ( $p < 0.01$ ) difference before parturition and significant ( $p < 0.05$ ) difference at 1 hr after parturition. The results of the present study were in accordance with the previous studies of Garg and Chander (1997) and Amer *et al.* (2008), who reported the higher levels of cortisol in uterine torsion affected buffaloes than the normal parturient buffaloes. The present higher cortisol level found at 1 hr after parturition than before concurred with the findings of Ghuman *et al.* (1997). The afferent stimulation due to the stress caused by the torsion of uterus and trauma associated with disease could lead to the increased secretion of ACTH and subsequently higher plasma cortisol levels (Garg and Chander, 1997). Moreover, higher cortisol level may be attributed to the fact that the maternal adrenal gland is activated during the last days of pregnancy and is further stimulated during torsion or parturition to produce high cortisol levels for gluconeogenesis by the mother during and after birth (Amer *et al.*, 2008).

From the study, it was concluded that the serum levels of ALT, AST, and CPK at the time of presentation of a case of uterine torsion can be used as the prognostic indicators, moreover, high progesterone and low estradiol immediate prepartum period makes the uterus flaccid, and predisposes to increased chances of its torsion. However, studies with more number of animals is warranted to conform these findings.

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