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Association between maternal dystocia and both the oxidant/antioxidant biomarkers and blood lactate in parturient Egyptian buffaloes (*Bubalus bubalis*)

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Abstract

Dystocia is a critical obstetrical problem in farm animals and is classified into maternal or fetal dystocia. The maternal causes of dystocia include different causes such as uterine torsion (UT), failure of cervical dilation (FCD), and uterine inertia (UI). Maternal dystocia induces massive oxidative stress due to increased production of free radicals with high levels of malondialdehyde (MDA). Little information is available on oxidants and antioxidant biomarkers and blood lactate (bLac) in parturient buffaloes with maternal dystocia. The current research aimed to clarify different causes of maternal dystocia in buffaloes and the association between the oxidative stress of parturient buffaloes and different types of maternal dystocia. Thirty-five parturient buffaloes were included in this study. Parturient buffaloes included normal parturition (NP; n=7), UT (n=23), FCD (n=4), and UI (n=1). Ten-ml blood samples were collected via jugular vein puncture into two tubes; plain vacutainer tubes for separation of serum to measure bLac and heparin coated vacutainer tube for separation of plasma for determination of MDA, glutathione peroxidase (GPx), and total antioxidant capacity (TAC). The results revealed that the major cause of maternal dystocia in the included buffaloes was UT. The UT was commonly post-cervical, clockwise, and > 180°. UT negatively affected both dam survival and calf viability. In addition, evaluation of oxidants and antioxidants biomarkers indicated that maternal dystocia causes more oxidative stress added to physiological stress of calving. This study found that, 4-days post-treatment period was not sufficient to resume antioxidant defense mechanism in UT buffaloes as no significant changes had been detected throughout the sampling period after treatment of UT. Moreover, evaluation of both GPx and bLac indicated their possible detection as indicators for prognosis of UT cases, where the concentration of GPx was significantly lower and the concentration of bLac was significantly higher in dead/culled buffaloes with UT.

Keywords: Calving, Failure of cervical dilatation, Uterine inertia, Uterine torsion.

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Competing interest: The authors have declared that no competing interest exists.

Introduction

Buffaloes are one of the most important livestock in many countries including Egypt. Maintenance of the optimum reproductive performance is one of the most important factors that maintain maximum productivity (Abdela and Ahmed, 2016). Dystocia in bovine, defined as prolonged or difficult parturition during which an assistance is required, causes considerable losses from the agricultural economic point of view (Abdela and Ahmed, 2016; Uematsu et al., 2013). Buffaloes and cows are considered the species in which the incidence of dystocia appears to be the highest amongst all domestic species. Buffaloes are like cows in the calving process, with some differences in the functional anatomy of both the soft and boney birth way (Purohit et al., 2011).

Dystocia is a multifaceted problem and is generally classified into maternal or fetal dystocia (Abera, 2017). The maternal causes of dystocia included uterine torsion as the most frequent maternal cause of dystocia in buffaloes whereas failure of cervical dilation appears to be more frequent maternal cause of dystocia in cows, and other causes of maternal dystocia such as uterine inertia and soft birth way affections which are commonly seen in cows but less frequent in buffaloes (Purohit et al, 2011).

Uterine torsion (UT) is the spiral twisting of gravid uterus on its longitudinal axis. It has been reported in various species like buffaloes (Prabhakar et al., 1994), cows (Cergolj et al., 1999), does (Dhaliwal et al., 1986), ewes (Ijaz and Talafha, 1999), llamas (Hopkins et al., 1991), camels (Cebra et al., 1997), mares (Jung et al., 2008), and pet animals (Brown, 1974; Thilagar et al., 2005) with maximum incidence rate in buffaloes (Purohit and Gaur, 2014). The anatomical difference of genital tract and alignment of gravid uterus may predispose buffaloes to uterine torsion (Kumar et al., 2018). The gravid horn may rotate to its right (clockwise) or left side (anti-clockwise) with degree of rotation varying between ≤90°, 90-180° and ≥180°. The point of rotation can be posterior to the cervix (post-cervical), including the cervix (cervical) and/or just anterior to the cervix (pre-cervical) (Deori et al., 2009).

Failure of cervical dilation (FCD) occurs as improper dilation of the cervix at the time of delivery. Such cervical dilation is critical for the easy passage of the calf. A wide variety of changes in the hormonal environment (Kindahl, 2000), enzymatic softening of the cervix by elevated collagenase (Rajabi et al., 1988) and the physical forces of the uterine contractions and fetal mass are contributed to sufficient dilatation of the cervix during calving in the buffaloes (Mannari, 1969; Deshmukh, and Kaikini, 1976) and cows (Breeveld et al., 2003).

The condition where the uterine expulsive forces fail to deliver a calf is known as uterine inertia (UI) which is classified into primary and secondary uterine inertia (Jackson, 1995; Arthur et al., 1996).

In addition, maternal dystocia such as uterine torsion induces massive oxidative stress due to increased production of free radicals with high levels of the measured malondialdehyde (MDA) (Bansal et al., 2011). Common enzymatic antioxidant enzymes for scavenging free radicals include reduced glutathione and glutathione peroxidase (GPx) (Aggarwal and Prabhakaran 2005). It should be noted that, during oxidative stress likely due to maternal dystocia, the activities of antioxidant enzymes and lipid peroxidation are significantly altered (Kumar et al., 2010). Oxidative stress can be evaluated by different biomarkers as MDA, (the end-product of lipid peroxidation) (Sathya et al., 2007; Bansal and Bilaspuri, 2008).

Blood lactate (bLac) that is formed via anaerobic metabolism is increased in pathological conditions (Bakker et al., 1996; Wittek et al., 2004). High bLac levels were previously reported with a poor prognosis in case of abomasal displacement in dairy cows (Wittek et al., 2004) and gastric dilatation-volvulus in dogs (Zacher et al., 2010). Pathology changes associated with UT, suggesting that bLac would be useful in early diagnosis and likely prognosis of UT (Boulay et al., 2014; Buczinski et al., 2014).

Little information is available on MDA, GPx, total antioxidants capacity (TAC), and bLac in Egyptian parturient buffaloes with maternal dystocia such as UT, FCD, or UI and whether their levels is related with the severity and/or prognosis of maternal dystocia is to be studied. The current research aimed to study different causes of maternal dystocia in buffaloes and the association between the oxidative stress of parturient buffaloes and different types of maternal dystocia. For these purposes, the values of the related oxidant/antioxidant biomarkers; MDA, GPx, and TAC and bLac were measured.

Materials and methods

Ethical approval:

The research protocol of the present study was performed in accordance with the Ethical Research Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (Approval No. 58/18.09.2022). All procedures in the present study were performed in the Teaching Veterinary Hospital belonging to Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Animals

Thirty-five parturient buffaloes were included in this study. Twenty-eight parturient buffaloes with dystocia were introduced to the Teaching Veterinary Hospital, Faculty of Veterinary Medicine, South Valley University. The remaining seven parturient buffaloes which calved normally were owned by local farmers in Qena Province. All animals included in the study were subjected to full clinical and obstetrical examination. The animals included in this study were presented between July 2019 and March 2022.

The parturient buffaloes included in the current study were subdivided into four groups; normal parturition (NP) included 7 buffaloes weighting from 375 kg to 475 kg (403.57±11.79 kg; mean ±SEM) and aged 3-14 years old (7.29±1.49; mean ±SEM), uterine torsion (UT) group included 23 buffaloes weighting from 275 kg to 475 kg (376.25±11.84 kg; mean ±SEM) and aged 3-8 years old (5.5±0.34; mean ±SEM), failure of cervical dilation (FCD) group included 4 buffaloes weighting from 375 kg to 400 kg (381.25±7.22 kg; mean ±SEM) and aged 3-5 years old (4.0±0.67; mean ±SEM) and one parturient buffalo as uterine inertia (UI) weighting 425 kg and aged 14 years old.

Sampling

Ten-ml blood samples were collected via jugular vein puncture into 2 types of vacutainer collecting tubes; the first one was plain vacutainer tubes for separation of serum to measure bovine bLac and the second type was coated with heparin and centrifuged for separating plasma for determination of MDA, GPx, and TAC. Plasma and serum samples were separated and stored at -20 °C till further analysis. Blood samples were collected at day 0 (Day of calving), day 1, and day 4.

Assessment of oxidant/antioxidant biomarkers:

Assessment of lipid peroxide (malondialdehyde, MDA):

Plasma MDA (nmol/mL) determination was performed by using lipid peroxide (MDA) kits (CAT. No. MD 2529) using a spectrophotometer at a wavelength of 534 nm according to (Ohkawa et al., 1979)

Assessment of Glutathione Peroxidase (GPx):

Plasma GPx (mU/mL) determination was performed by using GPx kits (CAT. No. GP 2524) using a spectrophotometer at a wavelength of 340 nm according to (Paglia and Valentine, 1967).

Assessment of total antioxidant capacity (TAC):

Plasma TAC (mM/L) determination was performed by using TAC kits (CAT. NO. TA 2513) using a spectrophotometer at a wavelength of 505 nm according to (Koracevic et al., 2001).

Assessment of blood lactate (bLac):

Serum lactate (bLac; mg/dL) determination was performed by using a spectrophotometer kits at a wavelength of 340 nm (Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.)

Statistical analysis

All results were expressed as means ± standard mean of error (means ± SEM). Data were analyzed statistically using one-way ANOVA with Tukey Comparison Test as a post-test using the computer statistics Prism 6.0 package (GraphPad Software, Inc.). *P*-values less than 0.05 were considered statistically significant. *$P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Results

In the studied cases in the present study, the common causes of maternal dystocia in buffaloes were UT, FCD, and UI with incidence of 82.14 % (23/28), 14.29 % (4/28), and 3.57 % (1/28), respectively.

Descriptive analysis of UT showed that the UT types were post-cervical and pre-cervical/cervical UT with incidence of 86.70% and 13.04%, respectively. The directions of UT were clockwise and anti-clockwise with incidence of 95.65% and 4.35%, respectively. The degrees of UT were < 90, 90-180 and > 180 with incidence of 13.04%, 13.04% and 73.91%, respectively (Table 1).

Table 1. Descriptive analysis of uterine torsion in the parturient buffaloes with uterine torsion as a cause of maternal dystocia.

Type; n (%)		Direction of torsion; n (%)		Degree of torsion; n (%)		
Post-cervical	**Pre-cervical and cervical**	**Clockwise (Right)**	**Anti-clockwise (Left)**	**< 90**	**90-180**	**> 180**
20/23 (86.70%)	**3/23 (13.04%)**	**22/23 (95.65%)**	**1/23 (4.35%)**	**3/23 (13.04%)**	**3/23 (13.04%)**	**17/23 (73.91%)**

The parturient buffaloes with UT in this study mainly pluriparous (82.61%) with parity of two (26.61%), three (34.78%) and four (21.74%) while 17.39% of buffaloes with UT had 0-1 previous calving (Table 2). The survival of the parturient buffaloes with UT in the present study was reported as 47.83% alive and 52.17% dead/culled (Table 2)

Parturient buffaloes with severe UT in the present study gave birth to male and female calves with ratio of 65.22% and 34.48% respectively. The viability of calves in the present study was reported as 21.74% alive and 78.26% dead/stillbirth (Table 3)

Table 2. Descriptive data of the parturient dams with severe uterine torsion as a cause of maternal dystocia.

Parity; n (%)				Survival; n (%)	
0-1	Two	Three	≥ Four	Survived	Dead/culled
4/23 (17.39%)	6/23 (26.61%)	8/23 (34.78%)	5/23 (21.74%)	11/23 (47.83%)	12/23 (52.17%)

Table 3. Viability and gender of the calves that were born to the parturient buffaloes with severe uterine torsion as a cause of maternal dystocia.

Gender; n (%)		Viability; n (%)	
Male	Female	Alive	Dead/stillbirth
15/23 (65.22%)	8/23 (34.48%)	5/23 (21.74%)	18/23 (78.26%)

Oxidants and antioxidants biomarkers and bLac in parturient buffaloes with normal parturition (NP), uterine torsion (UT), or failure of cervical dilatation (FCD):

The levels of plasma MDA (nmol/mL) were significantly lower (P< 0.001) in NP parturient buffaloes in compare to FCD with values (mean ± SEM) of 5.38 ±0.53, 5.36 ±0.48, and 13.19 ±2.91 nmol/mL in NP, UT, and FCD groups, respectively (Fig. 1A). The parturient buffalo with UI showed a plasma TAC level of 9.04 nmol/mL.

The levels of plasma GPx (mU/mL) were significantly higher (P< 0.05) in NP parturient buffaloes in compare to UT with values (mean ± SEM) of 0.54 ±0.01, 0.40 ±0.02, and 0.65 ±0.09 mU/mL in NP, UT, and FCD groups, respectively (Fig. 1B). The parturient buffalo with UI showed a plasma TAC level of 0.40 mU/mL.

The levels of plasma TAC (mM/L) were significantly higher (P< 0.001) in NP parturient buffaloes in compare to both common causes of maternal dystocia; UT and FCD with values (mean ± SEM) of 1.93 ±0.03, 1.48 ±0.01, and 1.55 ±0.02 mM/L in NP, UT, and FCD groups, respectively (Fig. 1C). The parturient buffalo with UI showed a plasma TAC level of 1.51 mM/L.

No significant changes (P> 0.05) were reported between the levels of serum bLac (mg/dL) in NP, UT, or FCD parturient buffaloes with values (mean ± SEM) of 23.61 ±4.32, 52.26 ±5.42, and 62.31 ±31.16 mg/dL in NP, UT, and FCD groups, respectively (Fig. 2). The parturient buffalo with UI showed a serum bLac level of 89.27 mg/dL.

Oxidants and antioxidants biomarkers and bLac in parturient buffaloes with uterine torsion (UT) at day 0, day 1, and day 4:

No significant changes (P> 0.05) were reported between the levels of the plasma MDA (nmol/mL) in parturient buffaloes with UT at all sampling days with values (mean ± SEM) of 5.37 ±0.67, 7.51 ±1.32, and 4.36 ±1.08 nmol/mL in day 0, day 1, and day 4, respectively (Fig. 3A).

No significant changes (P> 0.05) were reported between the levels of the plasma GPx (mU/mL) in parturient buffaloes with UT at all sampling days with values (mean ± SEM) of 0.45 ±0.03, 0.42 ±0.01, and 0.40 ±0.003 mU/mL in day 0, day 1, and day 4, respectively (Fig. 3B).

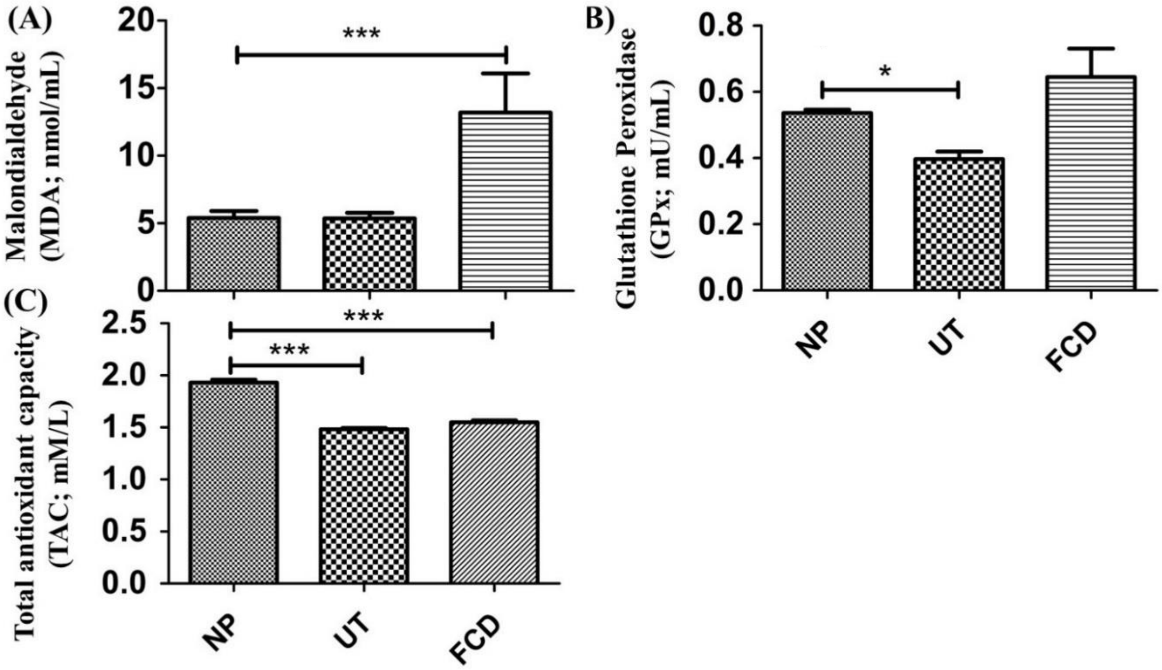

Fig. 1. Oxidants and antioxidants biomarkers in parturient buffaloes with normal parturition (NP), uterine torsion (UT), and failure of cervical dilatation (FCD); (A) Malondialdehyde (MDA; nmol/mL), **(B)** Glutathione peroxidase (GPx; mU/mL), and **(C)** Total antioxidant capacity (TAC; mM/L). * = $P < 0.05$, *** = $P < 0.001$. Values are expressed as mean ± SEM.

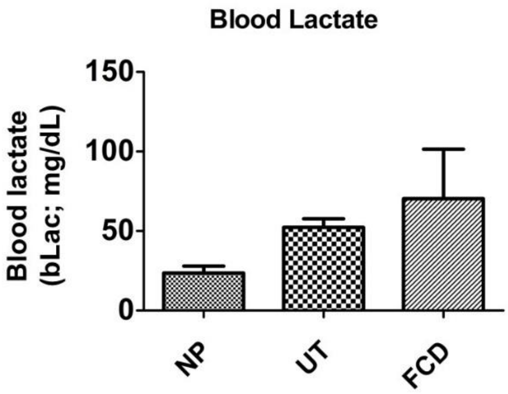

Fig. 2. Blood lactate levels (bLac; mg/dL) in parturient buffaloes with normal parturition (NP), uterine torsion (UT), and failure of cervical dilatation (FCD). Values are expressed as mean ± SEM.

No significant changes ($P > 0.05$) were reported between the levels of the plasma TAC (mM/L) in parturient buffaloes with UT at all sampling days with values (mean ± SEM) of 1.50 ±0.02, 1.54 ±0.03, and 1.46 ±0.06 mM/L in day 0, day 1, and day 4, respectively (Fig. 3C).

No significant changes ($P > 0.05$) were reported between the levels of serum bLac (mg/dL) in parturient buffaloes with UT at all sampling days with values (mean ± SEM) of 63.78 ±11.05, 69.76 ±9.19, and 36.67 ±7.34 mg/dL in day 0, day 1, and day 4, respectively (Fig. 4).

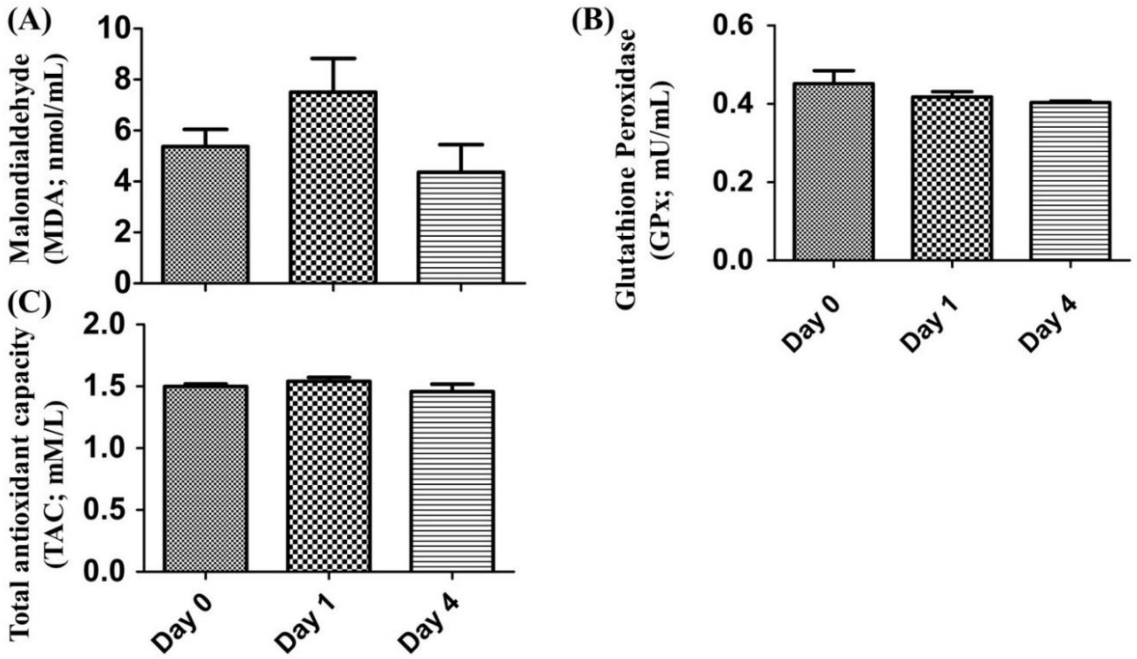

Fig. 3. Oxidants and antioxidants biomarkers in parturient buffaloes with uterine torsion (UT) at Day 0, Day 1, and Day 4; (A) Malondialdehyde (MDA; nmol/mL), **(B)** Glutathione peroxidase (GPx; mU/mL), and **(C)** Total antioxidant capacity (TAC; mM/L). Values are expressed as mean ± SEM.

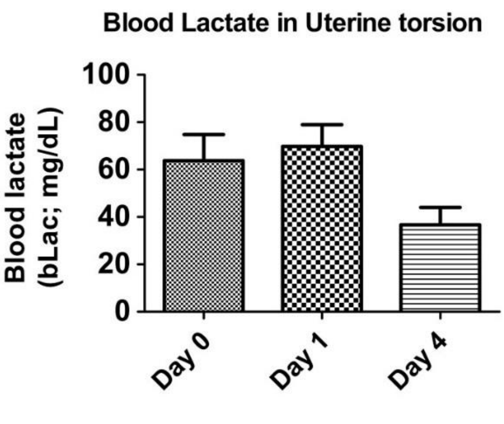

Fig. 4. Blood lactate levels (bLac; mg/dL) in parturient buffaloes with uterine torsion (UT) at Day 0, Day 1, and Day 4. Values are expressed as mean ± SEM.

Oxidants and antioxidants biomarkers and bLac in parturient buffaloes with NP, survived UT, or dead/culled UT:

No significant changes ($P > 0.05$) were reported between the levels of the plasma MDA (nmol/mL) in NP parturient buffaloes and both survived UT, or dead/culled UT with values (mean ± SEM) of 5.37 ±0.67, 4.50 ±0.71, and 5.68±0.49 nmol/mL in NP, survived UT, and dead/culled UT, respectively (Fig. 5A).

The levels of plasma GPx (mU/mL) were significantly higher ($P < 0.01$) in NP parturient buffaloes in compare to dead/culled UT with values (mean ± SEM) of 0.54 ±0.01, 0.42 ±0.04, and 0.36 ±0.02 mU/mL in NP, survived UT, and dead/culled UT, respectively (Fig. 5B).

The levels of plasma TAC (mM/L) were significantly higher ($P < 0.001$) in NP parturient buffaloes in compare to both survived UT, or dead/culled UT with values (mean ± SEM) of 1.93 ±0.03, 1.50 ±0.02, and 1.46 ±0.009 mM/L in NP, survived UT, and dead/culled UT, respectively (Fig. 5C).

The levels of serum bLac (mg/dL) were significantly lower ($P < 0.05$) in NP parturient buffaloes in compare to dead/culled UT. Moreover, the levels of serum bLac were significantly lower in survived UT in compare to dead/culled UT with values (mean ± SEM) of 36.97 ±8.72, 44.76 ±5.56, and 76.49 ±10.51 mg/dL in NP, survived UT, and dead/culled UT, respectively (Fig. 6).

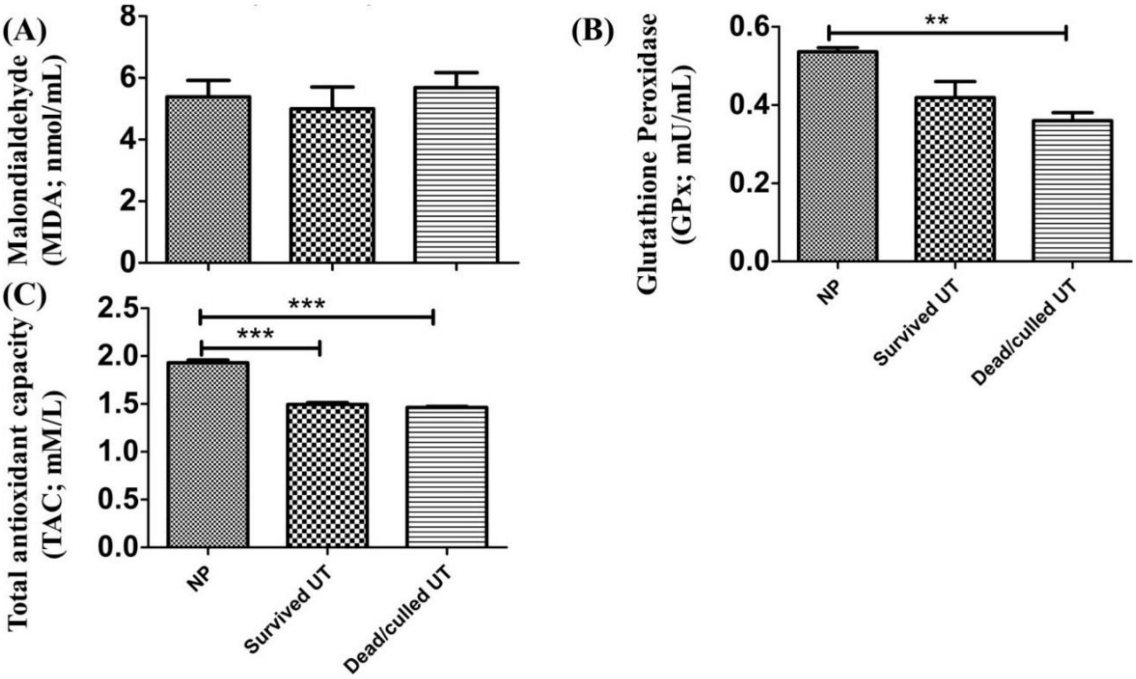

Fig. 5. Oxidants and antioxidants biomarkers in parturient buffaloes with normal parturition (NP), survived buffaloes after correction of uterine torsion (Survived UT), or dead/culled buffaloes after correction of uterine torsion (Dead/culled UT) (A) Malondialdehyde (MDA; nmol/mL), **(B)** Glutathione peroxidase (GPx; mU/mL), and **(C)** Total antioxidant capacity (TAC; mM/L). ** = $P < 0.01$, *** = $P < 0.001$. Values are expressed as mean ± SEM.

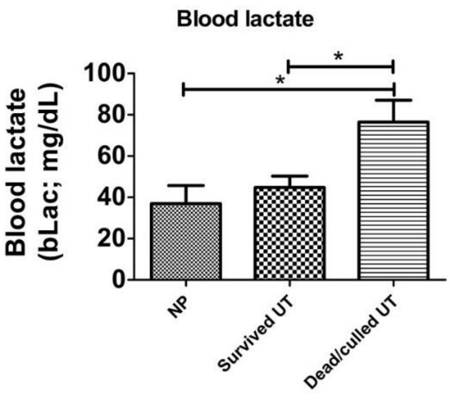

Fig. 6. Blood lactate levels (bLac; mg/dL) in parturient buffaloes with normal parturition (NP), survived buffaloes after correction of uterine torsion (Survived UT), or dead/culled buffaloes after correction of uterine torsion (Dead/culled UT). * = $P < 0.05$. Values are expressed as mean ± SEM.

Discussion

The present study successfully reported detailed descriptive data about the common maternal causes of dystocia and the associated oxidants and antioxidants biomarkers and bLac in parturient buffaloes. Several studies previously reported that dystocia causes significant changes in the hormonal, biochemical and oxidant/antioxidant profiles in different animal species (Amin et al., 2020; Ghoneim et al., 2016; Thangamani et al., 2019)

In the current study, three causes of maternal dystocia were reported; UT as the most common one, then FCD as the second major cause of maternal dystocia and finally UI. The high incidence of UT as a major cause of maternal dystocia reported in the present study (77.27%) was similar to that previously reported by Purohit and Gaur (2014). Another recent study reported that UI, FCD, and UT in addition to narrow pelvis were the main causes of maternal dystocia in buffaloes (Rahawy, 2019), while the prevalence of each cause was different from the current study. Regarding UT as the most common cause of maternal dystocia, the current study found that, UT in parturient buffaloes with maternal dystocia is commonly post-cervical, clockwise, and > 180°. These results agreed with previous reports (Ghosh et al., 2013; Jeengar et al., 2015). High percentage of male calves reported in this study increase the attention for more research about a possible relation between the gender of the calf and the occurrence of UT. Moreover, UT negatively affected both the dam survival and the calf viability.

Regarding the MDA as a biological marker for oxidative stress, the present study reported significantly higher levels in plasma MDA in maternal dystocia due to FCD than in the NP parturient buffaloes. MDA is a highly reactive byproduct of polyunsaturated fatty acid peroxidation that comes from polyunsaturated lipids after degradation by reactive oxygen species (ROS). Although, a physiological increase in ROS is essential for parturition due to their critical role in cervical ripening likely because they modulate the synthesis and release of prostaglandins (Jenkin and Young, 2004; Mocatta et al., 2004), oxidative stress, indicated by significantly high MDA levels reported in FCD in this study, may promote cell death and induced necrosis in cervical cells which interfere with normal cervical ripening and dilatation (Tantengco et al., 2021). This notion is supported by our present findings showed that lower TAC levels indicate consumption and exhaustion of the antioxidant defenses mechanism earlier than the highly significant increase in the MDA (Singh et al., 2013) especially with prolonged dystocia. Absence of significant difference in MDA level between NP and UT reported in the current study would be because of naturally high MDA levels around calving due to physiologically increase in lipid peroxidation as a result of parturition-associated oxidative stress (Mohamed et al., 2021; Walsh, 1994). The present results indicated no significant difference of MDA levels throughout the 4-days posttreatment period of UT. A possible reason for such sustained levels of MDA throughout this sampling period is that the 4-days post-treatment duration was not enough to the dam animals to relieve the oxidative stress due to UT.

Our findings suggested that maternal dystocia impairs the antioxidant defenses mechanism. The present study reported significantly lower TAC levels in both UT and FCD in compare to NP parturient buffaloes and lower GPx levels in UT in compare to NP parturient buffaloes. This indicates that maternal dystocia especially UT increase the oxidative stress in the parturient buffaloes than the physiological stress of calving (Nakao and Grunet, 1990). This is also supported by the findings of Thangamani et al., (2019) who reported increased levels of oxidative stress biomarkers in case of both the maternal and fetal dystocia. In addition, it was reported that increase ROS production during dystocia causes reduction in selenium intake by the buffalo erythrocytes that result in relative deficiency of GPx (Erisir et al., 2006). This is also supported by the findings of Bansal et al., (2011) who found that GPx level was significantly higher in NP as compared to those suffering from maternal or fetal dystocia. Moreover, the lower TAC and GPx levels indicate early consumption of the antioxidant defenses mechanism (Singh et al., 2013). Interestingly, our results indicated that there was a highly significant difference in GPx levels between the NP and survived UT, but not dead/culled UT buffaloes indicates a possible prognostic value of GPx levels. In addition, our results indicated that 4-days period is not enough to resume the antioxidant defense mechanism in UT because there were no significant differences in both TAC and GPx levels throughout the 4-days post-treatment period of UT.

Despite the present finding of bLac levels in maternal dystocia indicated that there was no significant difference of bLac levels between NP and both causes of maternal dystocia, the current study reported significantly higher levels of bLac in case of dead or culled UT in compare to both NP and survived UT buffaloes. This finding supported the importance of determination of bLac as an indicator for prognosis in case of UT in buffaloes. Association of high bLac levels and bad prognosis conditions was previously reported as high bLac levels were associated with bad prognosis abomasal displacement surgery in dairy cows (Constable et al., 1991 and Figueiredo et al., 2006), and poor prognosis colic in horses (Delesalle et al., 2007). Moreover, Murakami et al., (2017) found that bLac level in dead/culled UT dams was significantly higher than that in survived UT dams with values of 10.2 and 3.1 mmol/l, respectively.

Conclusion

The present study concluded that the major cause of maternal dystocia in buffaloes is UT, which is commonly post-cervical, clockwise, and > 180°. Sever UT negatively affected both dam survival and calf viability. In addition, evaluation of oxidants and antioxidants biomarkers indicates that maternal dystocia causes more oxidative stress added to physiological stress of calving, and 4-days post-treatment period was not enough to resume antioxidant defense mechanism in UT buffaloes. Moreover, evaluation of both GPx and bLac indicated possible importance for prognosis of UT because of significantly lower GPx and higher bLac reported in dead/culled UT buffaloes.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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