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Status of oxidative stress and antioxidant enzymes in normally calved and dystocia affected buffaloes

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ABSTRACT

The present study was undertaken to assess the activity of antioxidant enzymes, viz. superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase; and extent of oxidative stress/lipid peroxidation (LPO) in the blood plasma and uterine fluid of normally calved and dystocia affected Murrah buffaloes (fetal dystocia, dystocia due to uterine torsion. The results showed that both in the blood plasma and uterine fluid of dystocia affected animals (fetal dystocia and uterine torsion), malondialdehyde (MDA) production was significantly higher than in normally calved buffaloes. Significant changes were observed in the enzymatic antioxidant status (GPx, GR and SOD) of normally calved buffaloes and those suffering from dystocia in the 2 types of fluid, which could be used as a marker to assess the level of oxidative stress in the latter ones during immediate calving. An alternative diagnostic substrate which appeared to be effective in estimating the quantum of oxidative stress was uterine fluid. In conclusion, severe damage in the cases of fetal dystocia and uterine torsion indicated progression towards deteriorative action of various reactive oxygen species and possibly severe oxidative stress which have been generated during the time of parturition.

Key words: Antioxidant enzymes, Buffalo, Dystocia, Oxidative stress

Buffalo is the backbone of animal production in India especially calves which are the future of animal wealth. Dystocia and uterine torsion in bovine pregnancies are the major cause of decline in total performance and consequently economic loss (Yokus *et al.* 2007). Imbalances between the generation of reactive oxygen species (ROS) and scavenging capacity of antioxidants in reproductive tract (Bansal and Bilaspuri 2008) may result in oxydative stress. It is capable of depleting the body's antioxidant resources. The process of parturition though physiological is a stressful event and abnormal parturition (dystocia and torsion of uterus) add to normal stress of calving (Nakao and Grunet 1990). Lipid peroxidation (LPO) and protein peroxidation are the important markers of oxidative stress that can be reduced by using antioxidants (Bansal and Bilaspuri 2008). Common enzymatic antioxidant defenses include superoxide dismutase, glutathione peroxidase and glutathione reductase (Agarwal *et al.* 2005). The present study was designed keeping in view the existing knowledge about the damaging effect of oxidative stress in late gestation dams. The allantois forms the single most important tissue of the fetal membranes in mediating the attachment of developing embryo with endometrium. The information available on LPO and antioxidant enzymatic activities is still obscure in advanced pregnant buffaloes. Therefore, the current investigation was undertaken to ascertain the level of antioxidant enzymes and extent of oxidative stress/LPO in blood plasma and uterine fluid of normally calved and dystocia affected buffaloes.

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MATERIALS AND METHODS

Selection of animals: The oxidative stress was assessed in 2 main categories i.e. normally calved (n=6) and dystocia (fetal dystocia and uterine torsion affected, n=6) each. Murrah buffaloes. The normally calved buffaloes (natural delivery) were maintained at the dairy farm of the University. The animals were housed in individual calving pens for 10-15 days before parturition and were maintained at uniform management and feeding conditions. The fetal dystocia

(abnormally straining) and uterine torsion (twisting of uterus along its longitudinal axis) affected buffaloes were brought to the veterinary clinics of GADVASU for the treatment within 24–36 h of onset of parturition. Complete history with regard to their stage of gestation, duration of labor and previous handling or medication of the buffaloes, if any, were recorded. All the animals were in their second to fifth parity. The buffaloes having dystocia due to fetal malpresentation had their fetus removed by obstetrical mutational operation and traction. Detorsion in buffaloes with dystocia due to uterine torsion was achieved by Sharma's modified Schaffer's method (Singh and Nanda 1996). All the dystocia affected animals had singleton dead fetuses while the normally calved ones had live fetuses.

Blood collection: Blood was collected by the jugular venipuncture using heparinized glass vials (10 IU heparin/ml blood). The buffaloes suffering from dystocia were presented to veterinary clinics within 1h from their respective places, although the process of parturition had started by that time. In dystocia affected animals sampling was done after resting them for 30 min to reduce the transportation stress. In order to minimize the differences, if any, blood from freshly calved buffaloes was collected immediately after the emergence of fetal parts. All the samples were carried to the laboratory on ice and centrifuged at 3000 rpm for 15 min, plasma harvested and stored at –20°C until assayed. Likewise, the uterine (allantoic) fluid of all animals was collected at the time of calving and subsequently stored at –20°C, pending analysis.

Quantification of antioxidant metabolites: Malondialdehyde (MDA: end product of lipid peroxidation; Buege and Steven 1978), total protein (Lowry *et al.* 1951), superoxide dismutase (SOD, Nishikimi *et al.* 1972), glutathione peroxidase (GPx, Necheles *et al.* 1968), glutathione reductase (GR, Krohne-Ehrich *et al.* 1977) and glutathione-S-transferase (GST, Habig and Jakoby 1981) were analyzed both in the plasma and uterine fluid.

Statistical analysis: Two way analysis of variance was used to evaluate the significant levels between the parameters studied. A P value of 0.05 was selected as a criterion for statistically significant differences. The significant interactions were tested using Duncan's multiple range test. Differences in mean enzymatic profile in the two groups was subjected to Students 't' test.

RESULTS AND DISCUSSION

Changes in antioxidant enzymatic activity and metabolic profile both in plasma and uterine fluid are shown in Tables 1 and 2. Any fluctuation in the antioxidant parameters seemed to indicate the dynamic changes in oxidative stress occurring due to impending calving.

Malondialdehyde: MDA production was significantly ($P < 0.05$) higher in dystocia affected than in normally calved buffaloes in plasma as well as uterine fluid. Nonsignificant

Table 1. Antioxidant profiles in the blood plasma of freshly calved buffaloes (mean±SEM)

Parameter	Unit	Normally calved buffaloes (n = 6)	Dystocia affected buffaloes (n = 12)	
			Fetal dystocia (n = 6)	Torsion affected (n = 6)
MDA	µmoles MDA/mg protein ml^{-1}	0.44±0.05^a	1.57±0.31^b	2.04±0.19^b
Total protein	mg/ml	3.20±0.35^a	2.70±0.29^a	2.76±0.28^a
SOD	unit/µg protein/min	9.69±1.83^a	5.14±1.20^b	5.54±1.54^b
GPx	nmoles of glutathione oxidized/min/mg protein	9.60±2.30^a	1.07±1.24^b	1.59±0.21^b
GR	µmoles NADH/min/mg protein	18.41±0.92^a	12.52±1.18^b	12.60±1.90^b
GST	µmoles/min/mg protein	0.25±0.15^a	0.78±0.21^b	0.54±0.14^b

Values with different superscripts within the rows differ significantly ($P < 0.05$).

Table 2. Antioxidant profiles in the uterine fluid of freshly calved buffaloes (mean±SEM)

Parameter	Unit	Normally calved buffaloes (n = 6)	Dystocia affected buffaloes(n = 12)	
			Fetal dystocia (n = 6)	Torsion affected (n = 6)
MDA	µmoles MDA/mg protein ml^{-1}	0.65±0.25^a	1.45±0.38^b	1.32±0.18^b
Total protein	mg/ml	1.49±0.24^a	1.33±0.35^a	1.37±0.26^a
SOD	unit/µg protein/min	114.52±17.91^a	59.20±10.15^b	59.95±11.25^b
GPx	nmoles of glutathione oxidized/min/mg protein	343.75±29.76^a	108.41±10.61^b	114.91±14.57^b
GR	µmoles NADH/min/mg protein	322.98±24.21^a	164.48±15.22^b	111.16±17.79^c
GST	µmoles/min/mg protein	1.92±1.62^a	2.16±0.85^a	2.58±1.13^a

Values with different superscripts within the rows differ significantly ($P < 0.05$).

($P > 0.05$) difference was observed between the buffaloes suffering from fetal dystocia and uterine torsion. In general, increased lipid peroxidation in dystocia is expected due to physical efforts of calving. The problem of dystocia and the obstetrical operations such as rolling, mutations and fetotomy procedures are highly stressful (Naokes 2001). Under these stressful conditions, level of adrenaline, non adrenaline and glucocorticoids rise resulting in excessive production of ROS (Freeman and Crapo 1982). Subsequently, ROS cause peroxidation of placental membrane lipids especially polyunsaturated fatty acids which lead to disturbances in membrane structure and functions and results in LPO/ oxidative stress (Anand and Kanwar 2001). Eventually, a high level of MDA both in the blood plasma and uterine fluid of dystocia affected animals could be used as an indicator for oxidative stress owing to abnormalities in birth in the present study.

Total protein: Although non significant, the total protein content was low in dystocia affected animals as compared to normally calved ones in blood plasma and uterine fluids. A slight decrease in the protein content reflects the status of oxidative stress in late pregnant animals (Castillo *et al.* 2005). A similar response evoked within the sub-groups of dystocia affected buffaloes. The results are in consonance with the findings of Yokus *et al.* (2007) who reported that increased oxidative damages generated during parturition disturb the lipid-protein-interactions and block the protein dependent transport mechanisms, thereby resulting in depletion of protein content. In a state of impaired metabolite fluidity, the transport of protein across the maternal-fetal circulation will be disturbed causing alteration in protein concentration of the uterine fluid as observed in the current set of experiment.

Superoxide dismutase: While a nonsignificant difference in the SOD profile was observed between the buffaloes suffering from fetal dystocia and uterine torsion, the enzymatic antioxidant activity was significantly ($P<0.05$) lower than those delivered normally in both types of fluids. Eventually, a similar variation was observed in the uterine fluid with significant ($P<0.05$) difference between the normally calved and fetal dystociac buffaloes as well. Dystocia affected animals have lower SOD activity than those calved normally (Ahmed *et al.* 2009). Under stressful conditions, oxidation of oxyhemoglobin to methemoglobin in the blood plasma generates superoxide ions (O_2^-) which in turn diminish the activity of SOD (Jens and Ove 2006). In the present investigation, this stands true for the dystocia affected buffaloes both due to high degree of inflammation in the reproductive tract at the moment of calving as well as higher degree of stress due to difficulty in birth.

Glutathione peroxidase: GPx level was significantly ($P<0.05$) higher in normally calved buffaloes as compared to those suffering from dystocia in both types of fluid. However, a non significant difference was observed within the two groups of dystociac buffaloes. GPx is a selenium containing enzyme that acts as an antioxidant in reducing the oxidative damages caused by dystocia (Sathya *et al.* 2007). It removes peroxyle (ROO.) radicals from various peroxides like H_2O_2 and converts GSH (glutathione reduced) to GSSG (glutathione oxidized). During dystocia, ROS production causes reduction in selenium intake by the buffalo erythrocytes that result in relative deficiency of GPx concentration thereby leading to occurrence of oxidative stress (Erisir *et al.* 2006). This study suggests that under enhanced stressful conditions generated during dystocia, there is excessive exposure to stimulators of ROS that causes decrease in the production of GPx and in due course of time lead to the development of oxidative stress.

Glutathione reductase: Like GPx, plasma GR was significantly ($P < 0.05$) elevated in the buffaloes delivered normally than in dystocia affected ones. Our results are in consonance with the findings of Sathya *et al.* (2007). Lower levels of GR in the dystocia affected buffaloes compared to normally calved ones may be explained by higher levels of eicosanoids and epinephrine-induced pathways of aerobic energy production associated with immediate parturition, which generates ROS and thus causing lipid peroxidation, thereby reducing GR profiles (Nockels 1996). Thus, GPx and GR can give indication about the antioxidant status in advanced pregnant animals and can be used as a marker for such stressful happenings.

Glutathione-S-transferase: Although non-significant, uterine GST was higher in the buffaloes suffering from fetal dystocia and uterine torsion. Contrarily, significant ($P < 0.05$) difference was observed in plasma between normally calved and dystocia affected buffaloes. Previous studies have shown that GST may be a useful specific biomarker in hepatic diseases and in acute stress (Salinas and Wong 1999). The process of parturition is a stressful episode and dystocia further contributes increase in normal stress of delivery (Nakao and Grunet 1990). An increased enzymatic activity in the latter ones could be due to severe stress owing to abnormal birth.

Certainly, antioxidant estimations must remain the keystone of obstetrical research because it is quantitative information which can give us better understanding of the causes of oxidative stress. Any shift in the antioxidant enzymatic profile along with MDA may be associated with the basic disorder. Uterine fluid appeared to be valuable and practicable extra diagnostic tool in estimating the level of oxidative stress. However, it is unrealistic to expect any single measurement or observation to provide a complete insight into the condition of the oxidative stress. It is therefore recommended to monitor antioxidant parameters as a matter of critical care and handle dystocias as early as possible to avoid oxidative stress and further complications.

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