Indian Journal of Animal Sciences **81** (3): 235–37, March 2011

Level of cortisol in placental tissue vis-à-vis oxidative stress in dystocia affected buffaloes

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Received: 12 August 2010; Accepted: 23 December 2010

ABSTRACT

The study was planned to evaluate the oxidative stress vis-à-vis inflammation in placental tissue and umbilical cord of normally calved and dystocia affected buffaloes. MDA and total protein were estimated in the placental tissue and umbilical cord of all the animals, whereas cortisol was estimated in the placenta and blood plasma of normally calved and dystocia affected buffaloes. The levels of total protein in placental tissue and umbilical cord were significantly higher in normally calved (15.61±1.43 mg/ml and 14.42±1.58 mg/ml) as compared to the buffaloes suffering from fetal dystocia (2.87±1.04 mg/ml and 6.81±1.08 mg/ml) and uterine torsion (3.57±0.85 mg/ml and 4.66±0.47 mg/ml), respectively. On the contrary, the levels of MDA and cortisol were low in the placenta of normally calved buffaloes (0.17±0.02 µmoles MDA/mg protein ml^{-1} and 43.8±3.2 ng/ml) than in buffaloes with fetal dystocia (4.0±1.18 µmoles MDA/mg protein ml^{-1} and 49.2±1.2 ng/ml) torsion affected ones (2.63±0.87 µmoles MDA/mg protein ml^{-1} and 49.3±1.3 ng/ml), respectively. Significant difference in cortisol level was observed in blood plasma of normally calved (39.3±2.0 ng/ml), fetal dystociac (64.3±10.1 ng/ml) and torsion affected buffaloes (46.2±4.5 ng/ml). Thus it appears that increase in levels of MDA and cortisol in tissue and blood plasma following dystocia and uterine torsion were indicative of stress and may lead to severe postpartum uterine inflammation.

Key words: Buffalo, Cortisol, Dystocia, Oxidative stress

Difficulty in birth such as dystocia in buffalo pregnancies is the major cause of decline in total performance and consequently economic loss (Yokus *et al.* 2007). Stress of any origin is capable of depleting the body's antioxidant resources (Sconberg *et al*. 1993). It affects postpartum reproduction and develops when there is an imbalance between the generation of reactive oxygen species (ROS) and scavenging capacity of antioxidants in reproductive tract (Agarwal and Gupta 2005). Oxidative stress or excessive production of ROS in cows is a contributory factor to increase disease susceptibility, since metabolic demand associated with late pregnancy, parturition and initiation of lactation would be expected to decrease the body's antioxidant resources (Agarwal and Allamaneni 2004). The process of parturition though physiological is a stressful event and abnormal parturition (dystocia) further adds to normal stress of calving (Bondurant 1999). Oxidative stress can be assessed by several means and malondialdehyde (MDA), the end product of lipid peroxidation (LPO), protein peroxidation and cortisol are important markers of it (Sathya *et al.* 2007, Bansal and Bilaspuri 2008). Very little information is available on MDA and activities of cortisol in buffaloes approaching parturition at the level of placenta and its associated tissues. The most immediate contact of fetus with dam is through umbilicus and placenta. In primates, any change in the level of these metabolites could most be reflected in placental tissues (Hodgen *et al.* 1975, Karalis *et al.* 1996). Therefore, the present study was undertaken to ascertain the level and extent of oxidative stress/MDA in placenta and umbilical cord and cortisol in blood plasma of normally calved and dystocia affected buffaloes.

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MATERIALS AND METHODS

Experimental animals: The oxidative stress was assessed in 2 main categories i.e. normally calved and dystociac

animals (fetal dystocia and uterine torsion affected) of Murrah buffaloes (18). While the former group included 6 animals, the latter ones were further divided into 2 groups of 6 animals each. The normally calved buffaloes (unassisted 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 GADVASU Veterinary Clinics for the treatment from various parts of Punjab, India within 24–36 h of onset of parturition. Complete history with regard to their stage of gestation, duration of labour and previous handling or medication of the buffaloes, if any, were recorded. All the buffaloes were in their second to fifth parity. The buffaloes having dystocia due to fetal malpresentation had their fetus removed by mutation through traction after correction as per the standard methods (Roberts 1971). Distortion of uterus in buffaloes with dystocia due to uterine torsion was achieved by Sharma's modified Schaffer's method (Singh and Nanda 1996). All dystocia affected animals had singleton dead fetuses while the normally calved buffaloes had live fetuses.

Blood collection: Blood was collected by the jugular venipuncture using heparinized glass vials (10 IU heparin/ml blood) in all animals. Initial sampling was done in dystocia affected animals after resting them for 30 min to minimize the affect of transportation stress. The dystocia affected buffaloes were presented to Veterinary Clinics within 1 h from their respective places, although the process of parturition had started by that time. In order to minimize the differences, if any, the samples from freshly calved buffaloes were collected immediately after the appearance of first water bag. All the samples were carried to the laboratory on ice and centrifuged at 500 g for 15 min; the supernatant (plasma) was frozen at –20°C.

Tissue collection and preparation: The placenta and umbilical cord of each buffalo was collected immediately after delivery in phosphate buffer saline solution (PBS) with pH 7.4. Respective tissues were thoroughly washed with PBS pH 7.4, dried between folds of paper, weighed and about 1 g of tissue was suspended in 5 ml of Tris-HCl buffer, pH 7.5 + Dithiothretol + EDTA + 2% SDS + protease inhibitors and sonicated at 20 w for 2 min. The sonicated samples were centrifuged at 16000 g for 30 min and tissue extracts were stored at –20°C till further use.

Total protein, MDA and cortisol analysis: Total protein (Lowry *et al.* 1951) and MDA (Buege and Steven 1978) were estimated in the placental and umbilical cord tissue extracts. Cortisol was estimated in placental tissue extracts and blood plasma using ELISA kit as per kit's instruction.

Statistical analysis: Analysis of Factorial Experiment in CRD (software program written by Department of Mathematics, Statistics and Physics, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, India.) or two-way analysis of variance was used to evaluate the significant levels between the parameters studied. The significant interactions were tested using Duncan's multiple range test. Differences in mean (±SEM) MDA, total protein and cortisol in the 2 groups were subjected to Students 't' test (Zar 2008). A P value of 0.05 was selected as a criterion for statistically significant differences.

RESULTS AND DISCUSSION

Malondialdehyde: In the placental tissue of buffaloes suffering from fetal dystocia and uterine torsion malondialdehyde (MDA), the end product of LPO was significantly ($P \leq 0.05$) higher as compared to normally calved buffaloes. Nonsignificant differences were observed between the 2 groups of dystocia affected buffaloes. Although nonsignificant, MDA production in the umbilical cord was lowest in normally calved buffaloes. An increased lipid peroxidation in dystocia is expected due to physical efforts of calving. The problem of dystocia and the obstetrical treatment such as rolling, mutations, and/or fetotomy procedures are highly stressful (Naokes 2001). Under these stressful conditions, levels of adrenaline and glucocorticoids rise and ROS are produced in excess (Freeman and Crapo 1982). Subsequently, ROS cause peroxidation of placental membrane lipids especially polyunsaturated fatty acids (PUFA's) which leads to disturbance in membrane structure and functions and results in LPO/oxidative stress (Anand and Kanwar 2001).

Total protein: The total protein content both in placenta and umbilical cord in the 2 groups of dystocia affected buffaloes was significantly ($P < 0.05$) less than in normally calved buffaloes (Table 1). This decrease in dystociac buffaloes may be due to increased oxidative stress. To combat oxidative damages generated during parturition, requirement of protein increases for repairing the damaged tissue. This leads to depletion of protein reserves in placenta and umbilical cord of dystociac animals which are the target tissues for passive diffusion of metabolic nutrient to the neonates. Ferguson and Hoenig (2001) reported that a decrease in the level of total protein may reflect high level of glucocorticoid generation during oxidative stress in dystociac buffaloes.

Cortisol: A significant ($P < 0.05$) difference was observed in plasma cortisol profiles of normally calved (39.3±2.0 ng/ml), fetal dystociac (64.3±10.1 ng/ml) and torsion affected buffaloes (46.2±4.5 ng/ml). Even the placental cortisol was significantly ($P < 0.05$) high in dystocia affected buffaloes than in their normal counterparts (Table 1). The increase in the level of cortisol on the day of calving observed in the normally calved buffaloes was due to prepartum anxiety and parturition associated muscular activity (Naokes 2001). Since parturition is an inflammatory process involving the rise of

cytokines and prostaglandins, which stimulate the stress axis, the rise in cortisol level in the immediate prepartum period is also due to rising levels of these compounds (Rivest 2001). In dystocia affected buffaloes, higher degree of anxiety and muscular activity and pain results in significantly higher cortisol concentration on the day of delivery. Further increase in plasma cortisol concentration during obstetrical manipulations is the result of stressful procedures such as fetotomy and/ or cesarean section (Prabhakar *et al.* 1999) as reported earlier. Inflammation, tissue damage and bacterial infections are the common outcomes in dystocia affected buffaloes and attenuation of glucocorticoid negative feedback by the pro-inflammatory molecules, reactive oxygen metabolites and endotoxins prevents the rapid decline of cortisol in animals under stress (Asaba *et al.* 2004).

From the above study, it can be summarized that increase in levels of MDA and cortisol following dystocia and uterine torsion in placental tissue reflects the status of oxidative stress and were indicative of progression towards severe inflammation. It is therefore recommended to handle dystocias as early as possible to avoid tissue damage and stress to the animals.

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