https://doi.org/10.46344/JBINO.2020.v09i05.36

OXIDATIVE STRESS AND HEMATO-BIOCHEMICAL STATUS OF FETOTOMY OPERATED BUFFALOES ON THE DAY OF PARTURITION

A. A. Wani[1*] and P. S. Mavi[2]

1Department of Animal Husbandry Kashmir, Jammu & Kashmir, 2Department of Teaching Veterinary Clinics, Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab, 141004.

Email : aejaz748@gmail.com.

ABSTRACT

The oxidative stress parameters viz malondialdehyde level, reduced glutathione and the activities of antioxidative enzymes (superoxide dismutase, glutathione peroxidase and catalase) and Haemato-biochemical profile were compared between fetotomy operated and normally calved buffaloes. 40 buffaloes were used in the present study and were classified into two groups. Group I comprised of 30 buffaloes subjected to fetotomy operation. Group II comprised of 10 normally calved buffaloes. The levels of malondialdehyde, superoxide dismutase, reduced glutathione and catalase were significantly higher ($p<0.05$) and the activity of glutathione peroxidase was significantly lower ($p<0.05$) in fetotomy operated buffaloes as compared to normally calved buffaloes. Similarly the concentration of haemoglobin, calcium, phosphorus, total protein and packed cell volume were significantly ($p<0.05$) lower in fetotomy operated buffaloes as compared to normally parturiated buffaloes. The findings in the present study indicate that dystocia leads to increased systemic oxidative stress as evidenced by increased malondialdehyde level and reduced glutathione peroxidase activity. Dystocia leads to stress and prolonging the duration of dystocia further aggravates the problem; hence it is suggested that dystocia should be corrected as early as possible to avoid oxidative stress and further complications.

Key words: Fetotomy, buffalo, oxidative stress, dystocia

INTRODUCTION

Dystocia is defined as the prolonged parturitions that require assistance of farmer or veterinarian for the extraction of fetus. In dairy cattle frequency of dystocia varies from 2-23 % (Mee, 2008). The main causes of occurrence of dystocia are the failure of expulsive forces during parturition, birth canal inadequacy, foetal mal-positioning, disproportionate calf size to the dam's pelvic size and uterine torsion (Noakes, 2001). Although parturition is a physiological process, dystocia is of great stress for both the dam and foetus (Prabhakar, 1995). Due to this stress there is generation of free radicals or reactive oxygen species (ROS) during normal birth as well as dystocia. The uncontrolled increase in free radicals leads to damage of cells and tissues through oxidative chain reactions and lipid peroxidation which results in oxidative stress. Enzymatic antioxidants like glutathione-peroxidase, super-oxide dismutase, together with catalase and non-enzymatic antioxidants like glutathione and vitamins like A, E and β-carotene protect living organisms from damages of reactive oxygen species (Miller, 1993). There is little literature regarding oxidative stress in buffaloes subjected to fetotomy operation for relieving dystocia and its comparison with normal parturition. Therefore, keeping these points in view present study was designed to assess the levels of antioxidant enzymes and extent of oxidative stress in fetotomy operated buffaloes compared to normally calved buffaloes.

Materials and Methods

Selection of animals: This study was conducted on 30 Murrah buffaloes, presented to Teaching Veterinary Clinics of Guru Angad Dev Veterinary & Animal Science University (GADVASU) Ludhiana. Complete history regarding gestation, duration of labour and previous handling or medication of the buffaloes, was recorded. In all the buffaloes, dystocia was corrected by fetotomy once mutations failed to deliver the foetus. For comparison 10 Murrah buffaloes maintained at GADVASU Dairy Farm served as the control group. These buffaloes were having normal parturition.

Sampling: 5 ml blood samples were collected aseptically in heparinised vials. In addition 1 ml blood was collected in sodium fluoride vial and was used for blood glucose estimation. Immediately after collection, all the blood samples were brought to the laboratory in an ice box. Thereafter, the samples were centrifuged for 15 minutes at 3000 rpm; upper meniscus was marked, blood plasma was separated and stored immediately at -20°C till further analysis.

Preparation of Haemolysate: For the preparation of haemolysate, the erythrocyte pellet was washed thrice with normal saline. Thereafter ice cooled distil water was added to the pellet with constant stirring up to the marked level to prepare haemolysate.

Estimation of oxidative stress: Oxidative stress parameters Malondialdehyde (MDA), reduced glutathione (GSH) levels, superoxide dismutase (SOD), Glutathione peroxidase (GPx) and catalase (CAT) activity were analysed as per the

methods (Shafiq-u-Rehman. 1984, Hafeman *et al.*, 1974, Nishikimi *et al.*, 1972, Hafeman *et al.*, 1974 and Aebi, 1983). Haemoglobin (Hb), Packed Cell Volume (PCV), Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC) was estimated by using Automatic Analyzer (SIEMENS-ADVIA 2120 HEMATOLOGY SYSTEM). VITROS DT- II Chemistry system (Ortho-Clinical Diagnostics, Johnson & Johnson Company) was used for estimation of serum calcium, phosphorus, total protein and blood glucose concentrations were estimated by using VITROS DT Slides.

Statistical analysis: All data was expressed as mean values with standard error (±SE). Comparison between the two groups was evaluated by student's t-test. P value of 0.05 was selected as a criterion for statistically significant differences.

Result and Discussion

The present study was conducted in animals subjected to fetotomy operation. The animals with dystocia were anesthetised with epidural anaesthesia. The majority of dystocia cases were caused by head and limb deviations, carpal and hock flexion, foetal emphysema and foetal monstrosities.

Changes in oxidative stress parameters viz lipid peroxidation level, reduced glutathione level, superoxide dismutase, glutathione peroxidase and catalase activity are shown in Table1. MDA level, SOD activity, GSH level and erythrocytic catalase activity were significantly ($P < 0.05$), higher in buffaloes subjected to fetotomy than the normally calved buffaloes. Similarly GPx level was significantly ($P < 0.05$), lower in buffaloes subjected to fetotomy than the normally parturiated buffaloes. Haematological and biochemical values are presented in Table 2 for fetotomy operated and normally parturiated buffaloes. The mean value of Hb and PCV were significantly ($P<0.05$) lower in buffaloes subjected to fetotomy, than the eutocic buffaloes on the day of parturition. TEC and TLC were found to be non-significant in both groups. The serum concentrations of calcium, inorganic phosphorus and total protein was significantly ($p<0.05$), lower whereas serum glucose level was significantly ($p<0.05$) higher in dystocia affected buffaloes as compared to normally parturiated group on the day of parturition.

Malondialdehyde level and Superoxide dismutase activity: In the present study MDA levels were found to be increased which might be due to physical efforts of calving. . These results are in consonance with that of (Bansal *et al.*, 2011), who also observed higher levels of MDA in dystocia affected buffaloes. The problems of dystocia and the obstetrical operations like rolling, mutations and fetotomy enhance the level of stress (Noakes, 2001). This leads to generation of (ROS) which causes peroxidation of placental membrane lipids especially polyunsaturated fatty acids thus culminating into lipid peroxidation/oxidative stress (Anand and Kumar, 2001). So a high level of MDA in dystocia affected animals could be used as an indicator for oxidative stress owing to abnormalities in birth as in the present study. In the present study dystocia affected buffaloes had higher SOD

activity than eutocic buffaloes, which is supported by that of (Ahmed *et al.*, 2009). Stress leads to oxidation of oxyhaemoglobin to methaemoglobin leading to generation of superoxide ions (O_2^-), which in turn enhances the activity of SOD (Jens and Ove, 2006). In the present study this stands true for the dystocia affected buffaloes both due to high degree of inflammation in the reproductive tract at the time of calving as well as higher degree of stress due to difficulty in birth.

Glutathione Peroxidise, Reduced Glutathione and Catalase activity: GPx activity was significantly lower ($p<0.05$) in fetotomy operated buffaloes than the normally calved buffaloes. The results of the present study are in consonance with that of (Bansal *et al.*, 2011) who also observed lower levels of GPx in dystocia affected buffaloes on the day of parturition. GPx is a selenium containing enzyme that acts as an antioxidant in reducing the oxidative damage caused by dystocia (Sathya *et al.*, 2007). ROS production causes reduction in selenium intake by the buffalo erythrocytes which may lead to relative deficiency of GPx thereby resulting into increased oxidative stress (Erisir *et al.*, 2006).

The findings of the present study are in concurrence with that of (Bansal *et al.*, 2011 and Hamit *et al.*, 2011), who also observed lower levels of GSH in dystocia affected buffaloes. Glutathione is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds. It annihilates oxygen toxicity by interrupting the reaction leading to superoxide formation (Yoda *et al.*, 1986).

In the present study the activity of catalase was significantly higher in fetotomy operated buffaloes than the normally parturiated buffaloes which is in consonance with that of (Bansal *et al.*, 2011 and Hamit *et al.*, 2011). Catalase detoxifies H_2O_2 produced during different metabolic processes/stressful conditions by reducing it to H_2O and O_2 (Kumar *et al.*, 2010 and Switala and Loewen., 2002). It is clear that higher the generation of free radicals, higher is the production of H_2O_2, which in turn leads to increased catalase activity to detoxify the peroxide radicals.

Haematology: Hb and PCV were lower in fetotomy operated buffaloes on the day of parturition. These findings are in consonance with that of (Ahmed *et al.*, 2009). The lower levels of Hb and PCV in dystocia affected animals could be due to the higher levels of ADH due to stress causing increased retention of fluid (Moran *et al.*, 1964 and Kinney, 1967). In the present study lower serum levels of Calcium (7.26±0.19 mg/dl) and phosphorus (3.07±0.24 mg/dl) in dystocia affected animals as compared to normally calving animals (7.26±0.19 mg/dl and 3.07±0.24 mg/dl) have also been reported by (Husnain *et al.*, 2001, Mulle *et al.*, 1983 and Ciani *et al.*, 1964). Since Ca plays an important role in neuromuscular excitability, cell membrane permeability, muscle contraction and nerve impulse transmission, its deficiency may lead to reduced vaginal and uterine muscle tone which predisposes the animals to dystocia as proposed by (Roberts, 1980). Rise in blood glucose concentration in dystocia affected buffaloes (137.70±17.50

mg/dl) has also been reported by (Varshney *et al.*, 1992, Prabhakar, 1995, Sathya *et al.*, 2007 and Wani *et al.*, *2018*). The elevated blood glucose level could be attributed to elevated cortisol and catecholamines, which occurs following various obstetrical procedures in animals, as a response to stress (Singh, 2002 and Breazile, 1987). The lower total protein concentration in fetotomy operated animals could be because of manipulation or haemorrhage during obstetrical manoeuvre, with subsequent extraction of intestinal fluid into the circulation as concluded by (Nakao J, Grunter, 1997).

From the above observations it is clear that dystocia leads to stress and prolonging the duration of dystocia further aggravates the problem which leads to increased systemic oxidative stress as evidenced by increased malondialdehyde level and reduced glutathione peroxidase activity as compared to normal parturition. Hence it is suggested that dystocia should be corrected as early as possible to avoid oxidative stress and further complications. In addition to this measurement of oxidative stress must be a keystone in obstetrical research because it yields quantitative information which gives better understanding of the causes of the oxidative stress and help in devising the necessary corrective measures to ensure sustained productivity.

Table 1. Oxidative stress parameters in fetotomy operated buffaloes (Mean±SE) in comparison with normally calved buffaloes.

Parameter	Normally calved buffaloes	Fetotomy operated buffaloes
MDA (nmol/gHb)	4.6±0.59^{a}	6.70±0.65^{b}
SOD (U/mg Hb)	41.91±5.96^{a}	55.77±6.69^{b}
GPx (U/mg Hb)	119.70±3.14^{b}	90.98±18.51^{a}
GSH (µmol/ml)	1.73±0.56^{a}	3.76±1.99^{b}
CAT (K/mg Hb)	290.79±2.39^{a}	336.69±2.83^{b}

The values with different superscripts differ significantly (P≤0.05) within a row.

Table 2. Haematological and biochemical profile in fetotomy operated buffaloes (Mean±SE) in comparison with normally calved buffaloes.

Parameter	Normally calved buffaloes	Fetotomy operated buffaloes
Hb (g/dl)	14.12±0.97[b]	11.28±0.61[a]
PCV (%)	36.86±2.86[b]	29.50±1.26[a]
TLC $X10^3$ (cells/µl)	12.80±0.29[a]	12.95±0.24[a]
TEC $X10^6$ (cells/µl)	6.36±0.21[a]	6.06±0.41[a]
Calcium (mg/dl)	9.52±0.14[b]	7.26±0.19[a]
Phosphorus (mg/dl)	4.84±0.10[b]	3.07±0.24[a]
Glucose (mg/dl)	47.20±3.15[a]	137.70±17.50[b]
Total protein (g/dl)	8.98±0.21[b]	7.56±0.14[a]

The values with different superscripts differ significantly (P≤0.05) within a row.

References

1. Mee, J.F. **(2008)**. Prevalence and risk factors for dystocia in dairy cattle: A review. *Vet. J.* **76:** 93-101.
2. Noakes, D.E. **(2001).** Foetal dystocia: Aetiology and incidence. *In: Arthur's Veterinary Reproduction and Obstetrics.* Noakes DE, Parkinson TJ and England GCW 8th Edn. W B Saunders Company, Philadelphia, p. 121-24.
3. Prabhakar, S. **(1995).** *Studies on modulation of stress of dystocia in buffaloes.* PhD, Punjab Agricultural University, Ludhiana, India.
4. Miller, J.K., Brzezinska-Slebodzinska, E. and Madsen, F.C. **(1993).** Oxidative stress, antioxidants, and animal function. *J. Dairy Sci.***76:** 2812-2823.
5. Shafiq-u-Rehman. **(1984).** Lead induced regional lipid peroxidation in brain. *Toxicol Lett.* **21:** 333-337.
6. Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. **(1974).** Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in rats. *J. Nutr.* **104:** 580-587.
7. Nishikimi, M., Appaji, R.N. and Yagi. K. **(1972).** The occurrence of superoxide ion in the reaction of reduced phenazinemethosulfate and molecular oxygen. *Biochem. Biophys. Res. Comm.* **46:** 849-54.
8. Aebi, H.E. **(1983).** Catalase. In: Bergmeyer, H.U. ed. Methods of Enzymatic analysis. pp. 276-86. New York Academic Press.
9. Anand, R.J.K. and Kumar, U. **(2001).** Role of some trace metal ions in placental membrane lipid peroxidation. *Biol. Trace Elem.Res.* **82:** 61-75.
10. Bansal, A.K, Singh, A.K., Cheema, R.S., Brar, P.S., Gandotra, V.K., Singh, P. and Prabhakar, S. **(2011).** Status of

oxidative stress and antioxidant enzymes in normally calved and dystocia affected buffaloes. *Indian J. Anim. Sci.* **81:** 915-18.

11. Ahmed, W.M., Amal, R., Abd, E., Hameed, H.H. and Emtenan, M.H. **(2009).** Investigations on Retained Placenta in Egyptian Buffaloes. *Global Vet.* **3:** 120-24.

12. Jens, L. and Ove, S. **(2006).** Oxidants and antioxidants in disease: oxidative stress in farm animals. *Vet. J*. **10:** 10-16.

13. Sathya, A., Prabhakar, S., Sangha, S.P.S. and Ghuman, S.P.S. **(2007).** Vitamin E and Selenium Supplementation Reduces Plasma Cortisol and Oxidative Stress in Dystocia-Affected Buffaloes. *Vet. Res. Comm.* **31:** 809-818.

14. Erisir, M., Akar, Y., Gurgoze, S.Y. and Yuksel, M. **(2006).** Changes in plasma malondialdehyde concentration and some erythrocyte antioxidant enzymes in cows with prolapsed uteri, caesarean section, and retained placenta. *Rev. Med. Vet.* **157:** 80-83.

15. Yoda, Y., Nakazawa, M. and Abe, T. **(1986).** Prevention of doxorubicin myocardial toxicity in mice by glutathione. *Canc. Res.* **46:** 2551-55.

16. Hamit, Y., Halil, S., Nevzat, S. and Murat, Y. **(2011).** Effects of dystocia on lipid peroxidation and enzymatic and non-enzymatic antioxidants in crossbred dairy cows. *Bull. Vet. Inst. Pulawy.* **55:** 135-39.

17. Kumar, B.V.S., Singh, G. and Meur, S.K. **(2010).** Effects of addition of electrolyte and ascorbic acid in feed during heat stress in buffaloes. *Asian Australas. J Anim Sci.* **23:** 880-88.

18. Switala, J. and Loewen, P.C. **(2002).** Diversity of properties among catalase. *Arch. Biochem. Biophys.* **401:** 145-54.

19. Moran, W.H.J., Miltenberger, F.W., Schuayb, W.H. and Zimmerman, B. **(1964).** The relationship of antidiuretic hormone to surgical stress. *Surgery* **56:** 99-108.

20. Kinney, J.M. **(1967).** The effect of injury on metabolism. *Br. J. Surg.* **54:** 435-437.

21. Husnain, Z.U., Ali, C.S, Ahmad, K.M. and Samad, H.A. **(2001).** Studies on the relatuionship between blood mineral level and fertility of buffalo. *Pakistan Vet. J*. **1:** 141-44.

22. Muller, I.C., Gottschild, C., Kolb, E., Seidel, H. and Ziemke, G. **(1983).** Behaviour of various constituents of blood plasma (glucose free fatty acids, insulin, calcium, phosphorus, magnesium, alkaline phosphatise) in three parturient cows after prophylactic use of high dose of vitamin D_3. *Vet. Bull.* **53:** 2575.

23. Ciani, F., Russo, M., Cavaliere, M. and Russo, F. (1994). Changes in the plasma levels of parathyrin and calcitionin in pregnant buffaloes during pregnancy and after

parturition. *Acta Med. Vet.* **40:** 297-303.

24. Roberts, S.J. **(1980).** *Veterinary Obstetrics and Genital Diseases*. 3rd Edn. Edward Brothers, Microghan, USA.

25. Varshney, A.C., Kumar, A., Singh, H., Jadon, N.S. and Verma, M.C. (1992). Studies on haematological and biochemical alterations following caesarean section in cows. *Indian Vet. J.* **69:** 632-36.

26. Singh, S. **(2002).** *Studies on toxaemic status in dystocia affected buffaloes*. MVSc, Punjab Agricultural University, Ludhiana, India.

27. Breazile, J.E. **(1987).** Physiologic basis and consequences of distress in animals. *J. Am. Vet. Med. Assoc.* **191:** 1212.

28. Nakao, J. and Grunter, E. **(1997).** Effects of dystocia on postpartum adrenocortical function in dairy cows. *J. Dairy Sci.***73:** 2801-06.

29. A.A. Wani, P.S. Mavi, M.A. Rafee, P.S. Brar and V.K. Gandotra. (2018). Haemato-Biochemical and inflammatory markers of fetotomy operated buffaloes. *Explor. Anim. Med. Res.*8(1): 85-89.