

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.706.396>

Meloxicam Impact on Blood Plasma Cortisol Levels in Dystocia Affected Bovines

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ABSTRACT

Keywords

Cortisol, meloxicam,
caesarean operation

Article Info

Accepted:

20 May 2018

Available Online:

10 June 2018

Cortisol is often called the "stress hormone" because of its connection to the stress response, however, cortisol is much more than just a hormone released during stress. Parturition, although a physiological process, is a great stress for Dam. So In our study we tried to evaluate impact of meloxicam on blood plasma cortisol levels in dystocia affected bovines. Cortisol higher levels were maintained even after delivery in surgically handled cases and in buffaloes without preemptive administration of meloxicam. The cortisol levels were found to be significantly ($P < 0.05$) elevated at the time of presentation in buffaloes which subsequently expired after obstetrical manipulation or caesarean operation.

Introduction

Plasma glucocorticoids has been considered as a reliable end point for evaluating intensity of stress and of subsequent adaptation (Stephens, 1980; Stott, 1981). Cortisol is a hormone, which is mainly released at times of stress and

has many important functions in animal body. Cortisol is a steroid hormone, in the glucocorticoid class of hormones. When used as a medication, it is known as hydrocortisone. Parturition, although a physiological process, is a great stress for Dam. Cortisol is one of the most important member of the steroids from

the stress point of view. The corticosteroid concentration rises in the plasma of the parturient cows at term and this dramatic rise is followed by sudden fall immediately after parturition (Adams and Wagner, 1970, Smith *et al.*, 1973 Hudson *et al.*, 1976, Hunter *et al.*, 1977; Prakash and Madan, 1985).

Prabhakar *et al.*, (2002) assessed the degree of stress due to occurrence of dystocia and its correction by fetotomy and caesarean section by monitoring cortisol levels in dystocia affected buffaloes. Cortisol concentrations in dystocia-affected buffaloes were 3 to 4 folds higher than that in the normally calving counter parts. The cortisol levels further increased during manoeuvring as compared to pre manoeuvring levels. The buffaloes, which ultimately died following manoeuvring, had prolonged elevated cortisol levels as compared to their surviving counterparts.

Although there are several studies on the effect of dystocia on uterine infections, periparturient disorders, milk production, and newborn survival (Jeffrey and Edward; 1988), limited studies looking at the effect of dystocia on blood biochemical parameters in Buffaloes are available. In addition, the interactions among plasma concentrations of cortisol in relation to the difficult labour have not been well established. Therefore, in the present study, we planned to study the effects of dystocia on plasma cortisol concentrations in dystocia affected Buffaloes.

Materials and Methods

The present investigation was carried out on 41 dystocia affected buffaloes brought to the teaching veterinary clinics, CCS HAU, Hisar for treatment. Before attempting to relieve the dystocia, a complete history of the case with regard to parity, stage of gestation, duration of problem and previous handling of the case by the field staff/laymen, was obtained from the

owner. Thorough general and systemic examination of each case was performed. Rectal temperature, respiration rate, heart rate and rumen motility of every animal was recorded on arrival and subsequently at 2 and 24 hours post treatment.

The nature and cause of dystocia was determined on the basis of systemic examination and depending upon the cause of difficulty in birth and the duration and previous handling of the case, obstetrical management was carried out.

All the animals received routine supportive therapy consisting of parenteral antibiotics, dexamethasone, fluid therapy, hemostatic, uterine stimulants and intrauterine antiseptics post-delivery of the foetus.

Experimental groups

The cases of dystocia were randomly assigned to two groups based on different pre-operative treatment schedule Group I comprised of 19 buffaloes in which mutation/forced extraction or caesarean section was performed to relieve dystocia followed by usual post-operative care of the dam whereas Group II consisted of 22 cases in which obstetrical operation was preceded by I/M administration of meloxicam @ 0.5mg/kg B.Wt., 15 minutes prior to surgery / manipulative delivery.

These groups were further subdivided for the purpose of analysis of data and interpretation depending upon the obstetrical operation and survival following treatment.

Sub grouping on the basis of obstetrical operation

Group-I_A consisted of 8 buffaloes in which dystocia was relieved by non-surgical techniques and without pre-emptive administration of meloxicam.

Group-I_B comprised of 11 buffaloes in which foetus was removed by laparohysterotomy without administration of meloxicam prior to surgery.

Group II_A included 8 meloxicam administered dystocia affected buffaloes treated non surgically.

Group II_B was comprised of 14 caesarean operated buffaloes treated preemptively with meloxicam.

Sub grouping on the basis of survival of dam following obstetrical management

Group S consisted of 24 buffaloes which survived following treatment.

Group D included 17 buffaloes which died during or after obstetrical operation.

Groups S_I and S_{II} consisted of 13 and 11 buffaloes respectively which survived without/with pre emptive administration of meloxicam.

Groups D_I and D_{II} were composed of six and 11 buffaloes respectively which died following obstetrical intervention without/with administration of meloxicam.

Groups S_B and D_B included 12 and 13 caesarean operated buffaloes respectively which survived or died following operation. Irrespective of treatment with Meloxicam.

Sampling Schedule

Jugular blood samples were collected by standard venipuncture technique from all the cases before treatment (0hour) and then at 2 hours and 24 hours post-manuevering. Two ml of blood was drawn in vials containing sodium oxalate for hematological investigations and 20ml was collected in

heparinised glass tubes. Five ml of blood was kept in refrigerator for histamine assay and the remaining blood was centrifuged at 3000 rpm for 10 minutes for separation of plasma. Blood plasma was stored at -20°C for subsequent blood biochemical and endocrinological studies.

Hormone Estimation

Blood plasma cortisol levels were estimated by implying solid phase Radio immuno assay technique using Gamma Coat ^{125I} RIA Kits supplied by Diasorin Inc. Minnesota U.S.A. The procedure followed for assay was as suggested by the manufacturer of the Kit.

Performance Data of the Kit

Sensitivity

The minimal detectable concentration (sensitivity) of the kit used in the present study was reported as 0.21 ug/dl.

Specificity

The data on cross-reactivity of the antiserum used in the kit has been depicted in Annexure-I. The antiserum had very low cross reactivity to compounds other than cortisol.

Precision

The intra-run and inter-run Precision was determined by the manufacturer on the basis of 20simultaneous assays per serum sample for the former or 20 separate runs for the latter and observed to be 6.6-7.7 percent and 8.8 to 9.8 percent respectively.

Survivability / Recovery of the Animal

The cases were followed and data was collected on recovery and survival of dam following treatment by interaction with the

owner of the animal in order to correlate the observations on different parameters studied with survivability to arrive at possible prognostic indices.

Statistical analysis

The data was analyzed statistically using Duncan's Multiple Range test (Duncan 1955).

Results and Discussion

The circulating cortisol concentrations in different groups of dystocia affected buffaloes in the present study have been depicted in Table 40. Pre-emptive administration of meloxicam in dystocia affected buffaloes prior to obstetrical intervention had no influence on the circulating plasma cortisol levels. Significant difference was not recorded in cortisol concentrations among different intervals in either meloxicam treated or untreated buffaloes and also between the two groups (Meloxicam treated v/s untreated). Wide individual variation was reflected in blood plasma cortisol concentration in all the groups.

No specific trend was recorded in post-treatment levels of circulating cortisol. The levels at 2 and 24 hours post-treatment were found to be significantly ($P < 0.05$) higher in caesarean operated buffaloes when compared with the non-surgically treated buffaloes without preemptive administration of meloxicam (Table 1). However, there was no significant difference in plasma cortisol concentration between surgically and non-surgically handled dystocia cases when meloxicam was administered prior to obstetrical manipulation.

The cortisol levels were found to be significantly ($P < 0.05$) elevated at the time of presentation in buffaloes which subsequently expired after obstetrical manipulation or

caesarean operation (Table 2). These higher levels were maintained even after delivery in surgically handled cases and in buffaloes without preemptive administration of meloxicam. However, significant difference was not observed in cortisol concentration of meloxicam treated animals which survived or died following obstetrical management.

Survivability V/S treatment

The data on survival of dystocia affected buffaloes following preemptive administration of meloxicam has been shown in table 4. Only one half of the buffaloes suffering from difficulty in birth survived when the buffaloes had been administered meloxicam intramuscularly prior to handling of dystocia. The survival rate of the dam was much better (68.42%) when meloxicam was not administered to the dystocia affected buffaloes.

Higher percentage of buffaloes with dystocia survived following treatment (68.75%) when difficult birth was managed by non-surgical manoeuvring as compared to the buffaloes in which foetus was delivered by laparohysterotomy. Only 52% of the caesarean operated buffaloes survived in the present investigation (Table 3). Overall, 24 of the 41 cases treated in this study could survive the obstetrical management of dystocia irrespective of the treatment adopted or procedure followed by the relief of dystocia (Table 3).

Plasma cortisol concentration in dystocia affected buffaloes were observed to be highly elevated as compared to the levels reported by other workers (Dahiya, 1998, Mehta *et al.*, 1991) in normal parturient buffaloes. Similar observation have been recorded earlier by Manju (1984), Siddiquee (1988), Ghuman (1995) and Prabhakar *et al.*, (1999b) in cases of uterine torsion/dystocia in buffaloes.

Table.1 Effect of Obstetrical operation on plasma Cortisol concentration in Dystocia affected Buffaloes

Group	Sampling interval Cortisol (ng / ml)		
	0 hour	2 hours	24 hours
I A (n=8)	21.812 ± 3.620	20.147 ± 5.029	19.975 ± 4.202
I B (n=11)	31.409 ± 3.656	32.600 ± 8.073*	26.636 ± 7.189*
II A (n=8)	19.687 ± 5.802	25.687 ± 7.850	30.300 ± 7.391
II B (n=14)	24.129 ± 4.015	26.523 ± 5.264	20.077 ± 4.102

* Significantly (P<0.05) different between groups at same interval

Table.2 Observation on plasma Cortisol concentration and survivability in different groups of Dystocia affected buffaloes

Group	Sampling interval Cortisol (ng / ml)		
	0 hour	2 hours	24 hours
S(n=24)	20.70 ± 2.177	23.195 ± 4.244	19.783 ± 2.58
D(n=17)	28.852 ± 3.74	29.893 ± 4.804	27.273 ± 5.620
S I (n=13)	22.30 ± 2.51	21.43 ± 6.42	17.061 ± 2.67
D I (n=6)	38.33 ± 4.43*	40.16 ± 6.63	38.50 ± 11.36
S II (n=11)	18.80 ± 3.76	25.27 ± 5.57	23.00 ± 4.641
D II (n=11)	23.68 ± 4.65	23.73 ± 5.97	19.78 ± 4.56
S B (n=12)	18.358 ± 2.585	22.00 ± 6.668	15.41 ± 1.932
D B (n=13)	33.461 ± 3.617**	33.400 ± 5.563	28.083 ± 6.624

* Significantly (P<0.05) different between groups at same interval

Table.3 Effect of obstetrical operation on survival of dystocia affected buffaloes

Obstetrical operation	No. of animals	
	Survived	Dead
Caesarean operation (25)	13 (52%)	12 (48%)
Non-surgically treated	11 (68.75%)	5 (31.25%)
Total (41)	24 (58.53%)	17 (41.46%)

Table.4 Effect of preemptive administration of meloxicam on survival of Dystocia affected buffaloes

Group	No. of animals	
	Survived	Dead
Meloxicam treated (22)	11 (50%)	11 (50%)
Meloxicam untreated (19)	13 (68.42%)	6 (31.58%)
Total (41)	24 (58.53%)	17 (41.47%)

The elevated concentration of cortisol in dystocia affected buffaloes could be due to stress of dystocia which stimulated adrenal cortex leading to hypercortisolaemia (Manju *et al.*, 1985, Prabhakar, 1995). The variation observed in the cortisol levels in different buffaloes in the present study may be attributed to the variation in individual sensitivity to stress. The high levels of plasma cortisol were maintained upto 24 hours post treatment. Various workers have reported the maintenance of higher levels for periods ranging from day 1 to day 5 post treatment in buffaloes affected with torsion of uterus (Siddiquee, 1988; Manju, 1984; Dahiya, 1998).

Preemptive administration of meloxicam in dystocia affected buffaloes had no influence on the circulating plasma cortisol levels. No such study has been carried out by any other worker in large ruminants. However, Mondal *et al.*, (2006) while investigating the post-operative analgesic effects of different NSAIDS in bitches undergoing ovariohysterectomy, recorded no significant difference in serum cortisol levels in ovariohysterectomised bitches receiving meloxicam @ 2 mg/kg I/M after completion of surgery. Contrary to this, Fazili (2005) reported significant decline in plasma cortisol concentration within 20 hours of management of fracture in dogs administered meloxicam or rofecoxib prior to surgery. Different analgesics such as Ketamine, pethidine, Ketoprofen and tramadol have been shown to have a suppressive effect on post-operative cortisol response in dogs (Raj *et al.*, 2002; Mondal *et al.*, 2006). Whether meloxicam administration is equally effective in pain management in large ruminants as in canines needs further investigation.

Post treatment plasma cortisol concentrations were significantly higher in caesarean operated buffaloes than the buffaloes in which

dystocia was handled non surgically. This is in tune with the observation of Heuwiesser *et al.*, (1987) who reported significantly higher glucocorticoid concentrations in cows on which caesarean section had been carried out than those whose calves were extracted by traction. Higher cortisol levels after caesarean section in buffaloes under report is reflective of higher tissue trauma and pain in these animals. Rise in cortisol has been suggested to be in proportion to severity of the tissue trauma (Schmidt and Brooker, 1982). Marked increase in cortisol concentration have been reported after variety of surgical procedures in dogs and cats (Kyles *et al.*, 1998 and Cambridge *et al.*, 2000). According to Tsuji *et al.*, (1983) and Robertson (2003) the level of increase depends upon the magnitude of operative stress.

Buffaloes which could not survive following caesarean section were having significantly higher plasma cortisol concentration at the time of presentation of case and these higher concentration were maintained upto 24 hours post operatively. This indicates that the animals which subsequently died were highly stressed even before the operation was carried out.

Similar observations have been made by Ghuman (1995) in buffaloes with uterine torsion in which detorsion was done by rolling the dam. He concluded that prolonged elevation of cortisol concentration were associated with death of the affected animals. Prabhakar *et al.*, (2002) while assessing the degree of stress in buffaloes with dystocia maintained that the buffaloes that ultimately died following manoeuvring had prolonged elevated cortisol levels as compared to their surviving counterparts. The monitoring of peripheral cortisol levels particularly the pre-treatment concentration may serve as useful index of stress and prognosis in dystocia affected buffaloes.

Nearly fifty percent of the dystocia affected buffaloes survived following caesarean operation in the present investigation. The survival rate following caesarean section in the present investigation is less than that reported by Luthra (1996) and Luthra and Khar (1999) but matches well with the observations of Prabhakar *et al.*, (1997) and Matharu and Prabhakar (2001). The success rate of caesarean section is lower in buffaloes as compared to cows. Frazer *et al.*, (1996) and Schonfelder *et al.*, (2003) reported that 78% and 80% of cows affected with uterine torsion and corrected by caesarean section survived. Thirty four per cent of the animals operated upon for treatment of uterine torsion by Sharma *et al.*, (1995) died during or immediately after surgery.

Several factors influence the success rate of caesarean operation in animals with difficult birth. The most important of these are the duration of dystocia and the previous handling of the case. As the interval between the onset of first stage of parturition and the operation increased. The survival rate decreased (Singh and Dhaliwal, 1998). A drastic reduction in success rate of caesarean section has been recorded by Murthy *et al.*, (1999) as the duration of dystocia increased from 24 to 72 hours (100% v/s 25%). Matharu and Prabhakar (2001) obtained lower survival rate when the buffaloes were subjected to caesarean section either due to failure of detorsion or failure of cervical dilation (50.0%) as compared to those where caesarean section was performed directly without attempted detorsion.

All the cases referred to the vety. Clinics and included and most of these were the delayed cases. This may account for only fifty two per cent of the buffaloes surviving caesarean operation. The higher percentage of buffaloes surviving after obstetrical management of dystocia when non-surgical methods were

employed as compared to the C.S. should be because of cumulative stress of dystocia and surgery in the later. Caesarean section has been found to be highly stressful as reflected by plasma cortisol levels (Prabhakar, 1995; Dahiya, 1998). Higher cortisol levels in caesarean operated buffaloes than the non-surgically handled dystocia in the present study supports the view that caesarean operation adds stress of an already stressed individual and leads to reduction in chances of survival following obstetrical manoeuvring.

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How to cite this article:

Umed Singh Mehra, S. C. Arya, Anupama Deora, Ravidutt Rai, Rajendra Yadav, Pankaj Kumar and Diwakar R. P. 2018. Meloxicam Impact on Blood Plasma Cortisol Levels in Dystocia Affected Bovines. *Int.J.Curr.Microbiol.App.Sci.* 7(06): 3374-3382.
doi: <https://doi.org/10.20546/ijcmas.2018.706.396>