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Effect of dexamethasone administration on cortisol concentration and biochemical profile in buffaloes suffering from dystocia

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Abstract

The present study was undertaken to evaluate adrenal response and biochemical changes in the blood following dexamethasone (DEX) administration at the time of parturition and during the immediate postpartum period in buffaloes suffering from dystocia. Plasma cortisol concentration, blood glucose, plasma total proteins, and non-esterified fatty acids (NEFA) were evaluated. Plasma cortisol concentration was higher ($P < 0.01$) in dystocia groups than the eutocia group on the day of parturition. There was a significant reduction ($P < 0.0001$) in plasma cortisol concentration on Day 1 postpartum in the DEX-treated group. Dexamethasone treatment maintained higher levels of blood glucose ($P < 0.05$) and NEFA ($P < 0.0001$ to $P < 0.05$) and marginally lowered the total protein level. This study demonstrated that the hypothalamo-pituitary-adrenal axis was responsive to DEX suppression during the immediate postpartum period following dystocia, and DEX treatment was found to be beneficial in promoting metabolism to meet energy demands during stress.

Keywords: adrenal response, buffaloes, cortisol, dexamethasone, dystocia.

Introduction

The process of parturition, though physiological, is a stressful event (Guidry *et al*., 1976). Abnormal parturition further adds to the stress of calving (Nakao and Grunert, 1990, Prabhakar *et al*., 1999). Endogenous glucocorticoids help in the survival of animals following stress. In addition, supplementation of exogenous glucocorticoids is recommended to support the adrenal response to prevent adrenal exhaustion due to over stimulation during a stressful period (Ferguson and Hoenig, 2001).

Activity of the hypothalamo-pituitary-adrenal (HPA) axis is regulated by the levels of glucocorticoid secretion that varies with the presence or absence of stressors (Dallman *et al*., 1987). Pregnancy in ewes and the postpartum period in ewes and cattle were found to be stress-hyporesponsive periods (Przekop *et al*., 1985; Nanda *et al*., 1989; Smart *et al*., 1994; Altemus *et al*., 1995; Windle *et al*., 1997; Lightman *et al*., 2001). Enhanced glucocorticoid negative feedback (Schmidt *et al*., 2005) and elevated progesterone during pregnancy (Keller-Wood *et al*., 1986) were implicated as mediators in the hyporesponsiveness of the HPA axis. On the contrary, reduced forward drive rather than enhanced negative feedback (Johnstone *et al*., 2000) and reduced vasopressin release from the paraventricular nucleus (Douglas *et al*., 2003; Ma *et al*., 2005) were also found to mediate the hyporesponsiveness. However, Smith *et al.* (1987) and Owens *et al.* (1987) observed reduced dexamethasone (DEX) suppressibility of plasma cortisol and adrenocorticotropic hormone in late pregnant and early postpartum periods in women, respectively. Stressors maintain the HPA axis in an activated state by attenuating glucocorticoid negative feedback until the stressful condition subsides (Asaba *et al*., 2004). Hence, the effectiveness of glucocorticoid feedback varies with the physiological stage of reproduction and presence of stressors.

The adrenal response to glucocorticoid negative feedback has not been reported at the time of parturition associated with higher degree of physical, inflammatory, or immunological stress (i.e. dystocia). Moreover, the persistence of elevated plasma cortisol concentrations for longer periods in buffaloes suffering from dystocia (Prabhakar *et al*., 1999) questioned the effectiveness of negative feedback associated with a difficult parturition. The present study was conducted to evaluate the negative feedback effects of DEX in buffaloes suffering from dystocia. In addition, the biochemical alterations following DEX treatment in buffaloes suffering from dystocia were also studied.

Materials and Methods

Animals and dexamethasone administration

Twenty-one buffaloes, divided into three groups, were included in the present study. The normally calving or eutocia group (EG) included 6 normally-calving Murrah buffaloes from the Punjab Agricultural University (PAU) dairy farm. The animals were housed in individual calving pens 10-15 days before parturition and were maintained under uniform management and feeding conditions. The dystocia control group (DCG, n=5) and dystocia treatment group (DTG, n=10) included parturient Murrah buffaloes brought into the Veterinary Clinics of PAU for treatment of dystocia from various parts of the state of

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Punjab, India, within 24-36 hours of onset of parturition (i.e. abdominal straining in cases of dystocia due to torsion of uterus or rupture of allantoin without further progress in delivery in other cases was taken as the time of onset of parturition). These animals were given the necessitated obstetrical [included manipulative delivery (DCG: n=1; DTG: n=2), fetotomy (DCG: n=1, DTG: n=2), uterine detorsion (DCG: n=2; DTG: n=3), or cesarean section (DCG: n=1; DTG: n=3)] and supportive [included equal amounts of the same antibiotics, saline, and ecbolics] treatments. In addition to these treatments, DTG buffaloes were administered DEX (40 mg, i.v.; Dexona, Sarabhai-Zydus, India) before relieving dystocia (Day 0) and subsequently once daily for two days postpartum (Days +1 and +2). Buffaloes in the DCG group were not given DEX treatment.

Blood sampling schedule

Blood samples were collected daily from EG buffaloes at 0900 h from Day 3 prepartum (Day -3) to Day 3 postpartum (Day +3). Buffaloes suffering from dystocia were sampled thrice on Day 0 (before, during and immediately after obstetrical treatment on the day of assisted delivery) and subsequently once daily for three days. In all the groups, blood samples (5 ml) were collected in heparinized glass vials (50 IU heparin/vial) by jugular venipuncture before giving any treatment. An aliquot of 2 ml of blood was used for preparation of protein-free filtrate for blood glucose estimation and the rest was centrifuged at 500 g for 15 min for separation of plasma, which was stored at -20 ºC until assayed for cortisol, total proteins, and non-esterified fatty acids (NEFA).

Assay procedures

Plasma cortisol concentrations were determined by radioimmunoassay as described previously (Prakash and Madan, 1984). Standard dilutions of cortisol from 0.2 - 8.0 ng/ml were used in each assay for plotting the standard curve. The assay sensitivity was 0.2 ng/ml. The intra- and interassay coefficients of variation were 9.2% and 10.7%, respectively, and were determined from quality controls in the range of 0.4 to 2 ng/ml. There was no cross-reactivity with DEX in the assay.

Blood glucose (mg/dl), total plasma proteins (g/dl), and NEFA (mg/L) were estimated by Haden's modification of the Folin-Wu method (Goldenberg and Frankel, 1970), Biuret method (Wootton, 1964), and spectrophotometric method (Lowry and Tinsley, 1976), respectively.

Statistical analyses

Data are expressed as the mean ± SEM. Two-way ANOVA was done to test for the effects of group and time and their interaction. When fixed effects were found to be significant, differences were tested using Duncan's multiple range test. Pearson's correlation was calculated between plasma cortisol and NEFA and the significance was tested using a t-test. Analyses were performed using the Statistical Package for Social Sciences software programme (SPSS 12.0, Chicago, IL; 2003), and $P < 0.05$ was considered statistically significant.

Results

Plasma cortisol

In the eutocia group, the plasma cortisol concentration on Day 0 was higher ($P < 0.01$) compared to all of the pre- and post-partum days of observation (Fig. 1A). In both the dystocia groups on Day 0, plasma cortisol was higher ($P < 0.01$) compared to the eutocia group (Fig. 2A). The effect of time was significant ($P < 0.001$) and a decline in cortisol concentration was observed in all the groups during the immediate postpartum period. In the DTG, plasma cortisol concentration decreased ($P < 0.0001$) from Day 0 to Day +1, while the reduction observed in the DCG was not significant. On Day 0, plasma cortisol concentrations were not different between the DCG and DTG but were lower ($P < 0.05$) on Days +2 and +3 in the DTG. The percentage decrease in plasma cortisol concentration from Day 0 (after obstetrical treatment in DCG and DTG) to Day +3 was 24%, 41%, and 77% in the EG, DCG, and DTG, respectively.

Blood glucose, plasma total proteins, and non-esterified fatty acids

In the eutocia group, blood glucose levels on Day 0 were higher ($P<0.05$) than those on Days -2 and -3 (Fig. 1B). In the DTG, the blood glucose level on Day 0 after delivery by obstetrical manipulation was higher ($P < 0.05$) than that on Day 0 in the EG (Fig. 2B). In the DCG, the blood glucose level decreased ($P < 0.05$) on subsequent days postpartum to levels almost similar to those of the EG. In the DTG, the decrease in blood glucose level was not significant, and a higher ($P < 0.05$) level was maintained compared to the EG and DCG.

The plasma total protein level around calving did not vary significantly in the EG (Fig. 1C). The levels in the DCG and DTG were apparently lower on all the days of observation although they did not vary significantly from the EG (Fig. 2C). The protein level on Day 0 before obstetrical treatment was significantly higher ($P < 0.05$) in the DCG as compared to the DTG due to random selection of animals. Although the protein level in the DCG decreased from Day 0 to Day +1, it was not statistically significant.

In the EG, the NEFA level on Day -3 gradually increased to reach a higher ($P < 0.05$) level on Day 0 and then declined by Day +3 ($P < 0.05$). The level on Day 0 was higher ($P < 0.05$) than all pre and post-partum days (Fig. 1D). Concentration of plasma cortisol and NEFA were lowly correlated ($r = 0.307$; $P < 0.05$). In the DTG,

the NEFA level on all the postpartum days remained higher ($P < 0.05$) than that in the EG (Fig. 2D). But in the DCG, the NEFA level was higher ($P < 0.05$) only on Day +3 as compared to that in the EG. The level in the DTG on Day +2 and +3 was higher ($P < 0.05$) than in the DCG

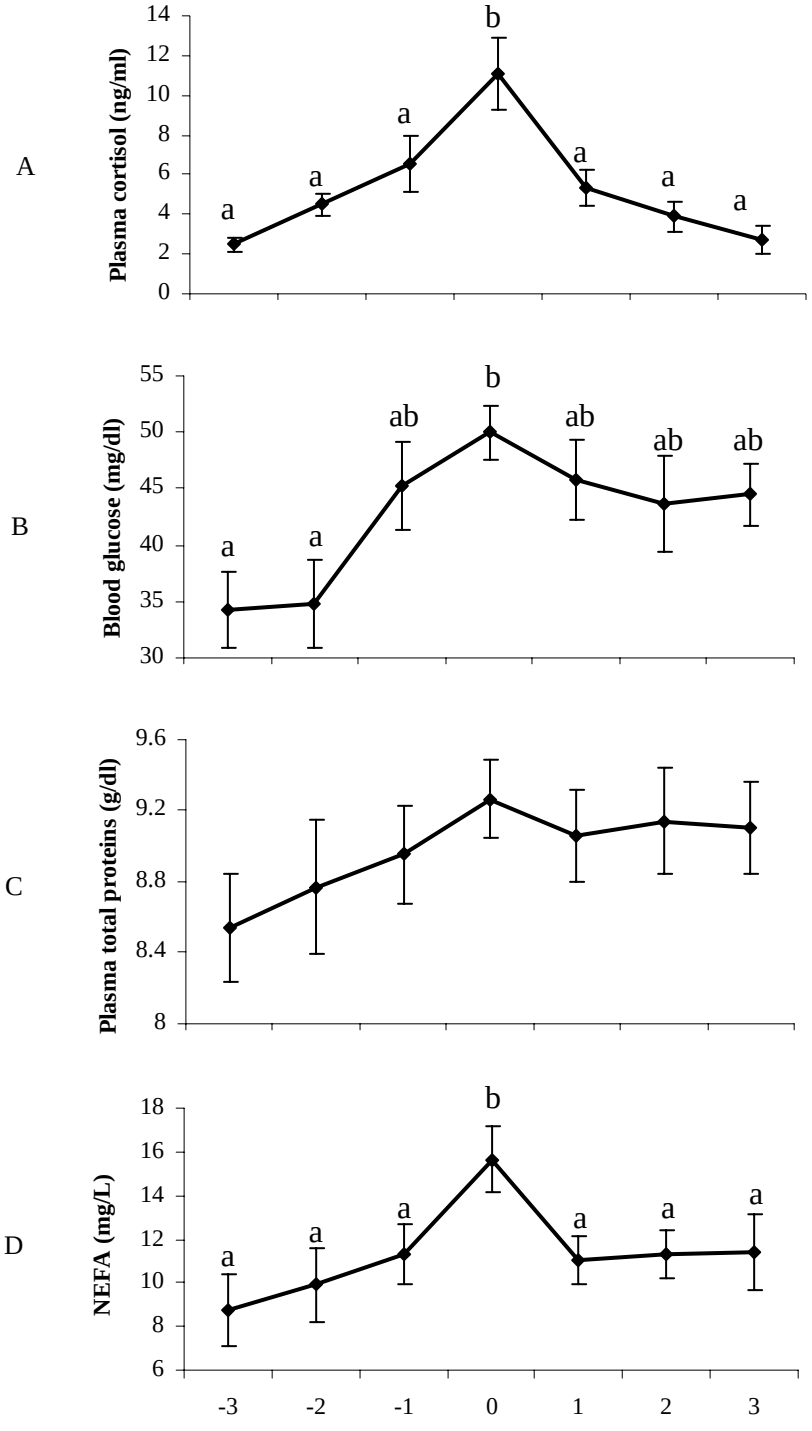

Figure. 1. Plasma cortisol (ng/ml; A), blood glucose (mg/dl; B), plasma total proteins (g/dl; C), and non-esterified fatty acids (mg/L; NEFA; D) in normally-calving buffaloes (eutocia) from Days -3 to 3 relative to parturition. Different letters (a, b) indicate significant differences (Plasma cortisol: $P < 0.01$; Blood glucose and NEFA: $P < 0.05$).

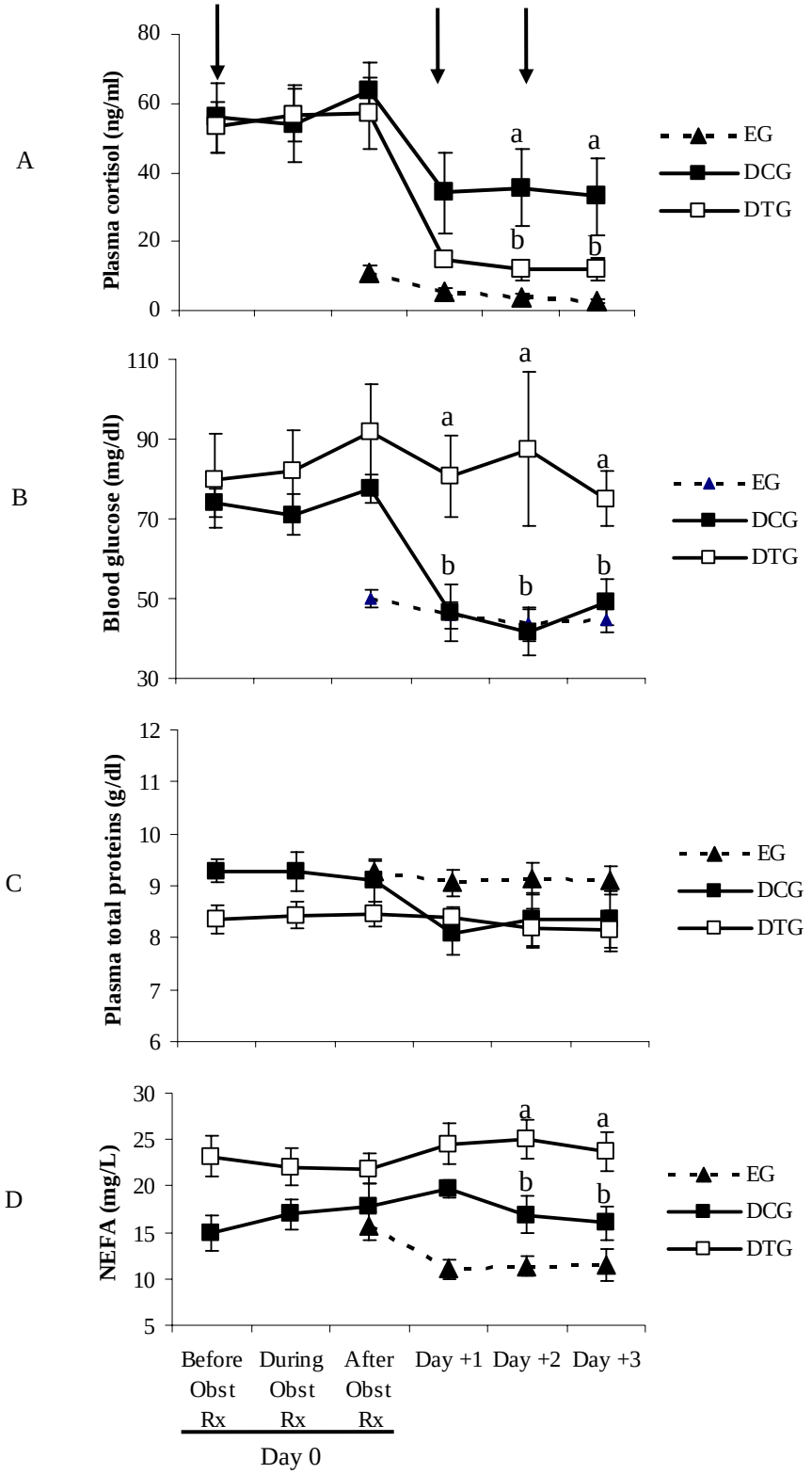

Figure. 2. Plasma cortisol (ng/ml; A), blood glucose (mg/dl; B), plasma total proteins (g/dl; C), and non-esterified fatty acids (mg/L; NEFA; D) in normally-calving buffaloes (eutocia) and buffaloes suffering from dystocia (control and treatment) before, during, and after parturition or obstetrical treatment. Downward arrows indicate dexamethasone treatments on Days 0, +1 and +2 in dystocia treatment buffaloes. "Obst Rx" refers to obstetrical treatment. EG: Eutocia group; DCG: Dystocia control group; DTG: Dystocia treatment group. Different letters (a, b) indicate significant differences ($P < 0.05$).

Discussion

Calving, though a physiological process, is stressful for both the dam and the fetus even in uncomplicated cases (Hudson *et al.,* 1976). In EG buffaloes, the gradual increase in plasma cortisol concentration during the prepartum period that reached to a peak on the day of calving could be explained by prepartum anxiety and myometrial contractions associated with the stress of parturition (Gillette and Holm, 1963; Noakes *et al*., 2001). Furthermore, parturition is an inflammatory process involving the release of cytokines and prostaglandins, which stimulate the HPA axis (Halgunset *et al*., 1994; Kelley, 1996; Rivest, 2001). Therefore, the increase in plasma cortisol concentrations during the immediate prepartum period could also be related to increasing concentrations of inflammatory mediators.

In buffaloes suffering from dystocia, severe hypercortisolemia observed on the day of calving could be related to prolonged stress, muscular activity, and pain. However, the removal of the fetus leads to a decrease in plasma cortisol concentration during the postpartum period. This decrease in plasma cortisol concentration could be due to the removal of the stress of dystocia and the negative feedback effect of plasma cortisol (Miller and O'Collaghan, 2002). But during the immediate postpartum period, plasma cortisol concentrations in dystocia control buffaloes remained higher than those in EG buffaloes. A higher degree of inflammatory response and stress-response mediators maintain higher plasma cortisol concentration despite the negative feedback effect (Asaba *et al*., 2004).

In buffaloes suffering from dystocia, during the postpartum period, the greater decrease in plasma cortisol concentration in the DEX-treated group compared to the untreated group could be attributed to the negative feedback effect of DEX because it is capable of more pronounced suppression of the HPA axis than endogenous cortisol (Ferguson and Hoenig, 2001). Hence in this study, DEX treatment constrained the stress-induced HPA activity in buffaloes suffering from dystocia. Such results were also obtained in laboratory animals, and a delayed negative feedback mechanism, which occurred 30-45 min after DEX treatment, was proposed as a cause (DeKeyser *et al*., 2000; Ginsberg *et al*., 2003). However, in the present study, DEX pretreatment did not suppress the plasma cortisol concentration during and after obstetrical manipulations, which varied in duration from 30 min to 2 h. This could be due to strong adrenal stimulation by the stress of dystocia and obstetrical manipulations, thus overcoming the immediate potent negative feedback effects of DEX.

The DEX negative feedback effect was pronounced on Day +1 when the plasma cortisol concentration declined. However, it was not suppressed further on subsequent days despite continuation of DEX treatment at 24 h intervals up to Day +2. Hence, DEX treatment overcame the HPA-stimulating effects of inflammatory mediators to suppress the HPA response on the immediate postpartum day, but it could not suppress the cortisol concentration thereafter. Persisting higher concentrations of inflammatory mediators like $PGF_{2\alpha}$, (Nakao *et al*., 1997), reactive oxygen metabolites as indicated by higher level of lipid peroxidation products (Sathya *et al*., 2005), and endotoxemia as a result of uterine infections following dystocia (Singh, 2002), might be responsible for reduced suppressibility of plasma cortisol by DEX in the early postpartum days, which are capable of attenuating glucocorticoid negative feedback (Asaba *et al*., 2004). Since effects of DEX treatment were not studied in EG buffaloes, the degree of suppression of plasma cortisol by DEX in buffaloes suffering from dystocia could not be compared to them. However, this study demonstrated that glucocorticoid negative feedback is effective around parturition in buffaloes suffering from dystocia, and the HPA axis is responsive to DEX treatment around the immediate postpartum day in these buffaloes but not thereafter.

Cortisol induces the enzymes involved in gluconeogenesis in hepatic tissue and inhibits utilization of glucose in extra-hepatic tissues, thereby elevating the blood glucose concentration (Mayes and Bender, 2003). The blood glucose levels were higher in buffaloes suffering from dystocia reflecting a higher degree of stress. It was also observed that blood glucose level increased following various obstetrical procedures. Obstetrical manipulations have been found to be stressful in the past (Prabhakar *et al*., 1999). The elevated blood glucose level was due to elevated cortisol and catecholamines, which increased following various obstetrical procedures as a response to stress (Breazile, 1987 Nakao and Grunert, 1990). Decreasing levels of plasma cortisol after the removal of the fetus and thus the stress due to dystocia resulted in the decrease of blood glucose levels during the immediate postpartum period. Since DEX is 30 times more potent than cortisol in its gluconeogenic effects (Ferguson and Hoenig, 2001), a higher blood glucose level was maintained in DEX treated buffaloes. This might be beneficial during acute phases of stress to meet the urgent needs of reestablishing homeostasis and fueling the essential organs. However, prolonged DEX treatment may antagonize insulin resulting in poor glucose utilization (diabetes-like condition) and enhanced catabolic effects in lymphoid and connective tissue, muscle, fat, and skin (Ferguson and Hoenig, 2001).

The marginally lower levels of total proteins in buffaloes suffering from dystocia, as compared to EG buffaloes, during the immediate postpartum period were due to a decrease in protein synthesis as a consequence of a higher level of plasma cortisol (Ferguson and Hoenig, 2001). There was no alteration in total protein level due to DEX treatment.

When fuel is needed for the body, triacylglycerols stored in adipose tissue are hydrolysed by hormone-sensitive lipases within the adipocytes to release NEFA (Nelson and Cox, 2005). Epinephrine greatly stimulates the triacylglycerol lipase, and the glucocorticoids have permissive effects for this enzyme activity (Ferguson and Hoenig, 2001). Hence, NEFA are the indicators of an increase in demand for energy. Higher levels of NEFA in EG buffaloes were due to catecholamines and cortisol released at the time of parturition as a stress response (Axelrod and Reisine, 1984). Dexamethasone maintained higher levels of NEFA in DEX-treated buffaloes suffering from dystocia. Increased NEFA levels are needed under severely stressful conditions (Ferguson and Hoenig, 2001). Hence, DEX treatment may be advantageous in directing the fat reserves to meet the enhanced fuel demand of the body during stress.

In summary, the responsiveness of the HPA axis to DEX treatment and effectiveness of glucocorticoid negative feedback following difficult parturition in buffaloes have been documented in the present study. Since continued DEX treatment for 3 days did not suppress the plasma cortisol concentration beyond Day +1 postpartum, it may be ascertained that the DEX suppressibility of plasma cortisol was reduced in buffaloes suffering from dystocia during the postpartum period. Higher blood glucose and NEFA levels in DEX-treated buffaloes suggested that DEX promoted metabolic pathways to meet the required demand for energy. Hence DEX administration could be beneficial during stressful episodes. However, the effects of higher doses of DEX recommended during stressful periods on HPA suppression, adrenal secretory capacity, immunity, and insulin antagonism are yet to be studied in buffaloes during the periparturient period.

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