

ASSESSMENT OF PHYSIOLOGICAL STRESS IN PERIPARTURIENT COWS AND NEONATAL CALVES

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Abstract : Pregnancy is considered to be one of the physiological stressors. The stress hormone, cortisol is significantly involved in various events during periparturient period including initiation of parturition. The study was conducted to estimate the serum cortisol concentration in cows and the neonatal calves in order to correlate the effect of cortisol on certain haematological and biochemical parameters such as blood glucose level (BGL), total plasma protein (TPP), lymphocyte:neutrophil ratio and mitogen induced lymphocyte proliferative response. Blood samples were collected from six cows in four periods, namely, 3 days prior to parturition, on the day of parturition, and 7 days after parturition. Blood samples were also collected from neonatal calves in the periods 0, 7 and 14 days of age. Calves above two months of age and non-pregnant dry cows were considered as the controls. The serum cortisol concentration in cows on the day of parturition was significantly higher ($P < 0.01$) than controls and the value in calves was also significantly higher ($P < 0.01$) at 0 day than their controls. On the day of parturition BGL level of the dam and calves were significantly higher ($P < 0.01$), whereas the proliferative response of lymphocytes to mitogen was significantly lower ($P < 0.01$) than controls. However TPP levels did not differ significantly. This confirmed that the dam at the time of parturition and neonatal calf before taking colostrum are under a high risk of infection because of the low profile of immune status. The lymphocyte: neutrophil ratio also justified the above suggestion.

Key words : cortisol
neonatal calves

lymphoblastogenesis
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INTRODUCTION

Various factors such as management, environment and physiological conditions are responsible for the stressful conditions in livestock and it is generally approved that

these conditions may result in alteration of the immune status of the body. The immune system plays a key role in the maintenance of the physiological homeostasis of animals. High physiologic concentration of corticosteroids during stressful situations

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was found to suppress the porcine lymphocyte proliferative response to mitogen (1,2) in correlation with the suppressed immune function in pigs. Early reports proved that immunological functions are suppressed by application of stressors such as shipping (3), acute exertion (4), thermal stress (5) and restraint (6). Restraint stress in mice made them susceptible to mycobacterial infections (7) whereas exercise stress in mice reduced the number of T and B lymphocytes in spleen (8). In short, the productivity and well-being of animals can be substantially affected by stress.

Physiologically pregnancy is considered to be one of the stressors. Increased cortisol concentration during stress has been implicate as one of the predisposing factors in the pathogenesis of infections diseases in cattle. The increase in the level of serum cortisol suppresses the antibody response to non-replicating antigens, lymphogenic response to mitogens and certain aspects of neutrophil function (9).

The objectives of the study were to; (a) quantify the serum cortisol concentration during periparturient period in crossbred cows and neonatal calves, (b) correlate the effect of cortisol on certain haematological and biochemical parameters like blood glucose level (BGL), total plasma protein (TPP), lymphocyte: neutrophil ratio and mitogen induced lymphocyte proliferative response. The present study was also aimed to find out whether the dam or neonatal calves are facing any risk of reduced profile of immune system because of the dominance of cortisol.

METHODS

Animals: Four groups of animals were used for the present study: Group I comprising of six crossbred cows in advanced stage of pregnancy (plus 265 days); Group II comprising of six neonatal calves born to group I; Group III comprising of six non-pregnant dry crossbred cows (control) Group IV comprising of six calves of two months of age (control). Blood samples (10 ml each) with and without anticoagulant (Heparin, 20 units/mL of blood) were collected from animals of Group I in four different periods i.e. 3 days prior to parturition, on day of parturition and 7 days after parturition and from animals of Group II in the periods of 0, 7 and 14 days of age. Blood samples were also collected from the other two control groups. Serum was separated from the blood samples by centrifuging at 3000 rpm for 10 min and transferred into clean, dry labeled vials and stored vials and stored at -20°C until the analysis was carried out. Blood smears were prepared on dry clean slides.

Hormone assay: Serum cortisol concentration was determined using antibody-coated tubes and ¹²⁵I-labeled cortisol, an assay system described and validated by (10). Commercial kits (Diasorin, Stilwater, Minnesota, USA) were used for cortisol estimation by RIA method. A semi-logarithmic graph was plotted against counts obtained for 6 number of cortisol serum standards ranging from 0-60 µg/dl in concentration. The corresponding counts obtained from gamma counter for different serum samples were referred in the plotted area and the concentration was calculated and expressed as µg/dl.

BGL: On the day of blood collection, the level of blood glucose was determined by glucose oxidase method (11).

TPP: Level of total plasma protein was determined using phenol reagent (Folin-ceacaltea) (12).

Lymphocyte: neutrophil (L:N) ratio: Wright's stain was used to stain the prepared blood smears and the proportion of lymphocytes and neutrophils in 100 leucocytes counted were noted and the ratio was calculated.

Mitogen induced lymphocyte proliferative response: Six ml of blood sample was cautiously layered over 3 ml of ficoll-hypaque plus solution. It was then centrifuged at 3000 rpm for 20 minutes at room temperature. Mononuclear cells at the plasma-ficoll interphase were collected and was transferred to another 5 ml of complete medium (RPMI 1640, 10% fetal calf serum, 100 units of penicillin and 100 µg of streptomycin) and centrifuged at 3000 rpm for 10 min. Two more washings in complete medium were done in order to remove all traces of ficoll. The cell pellet was then suspended in 1 ml of complete medium and

the cell concentration was determined using hemocytometer. Then the concentration was adjusted to get 2×10^6 cells/ml of complete medium. Viability of cells were checked by trypan-blue dye exclusion test and those with >98% viability were only used for culturing. Then 1×10^6 cells (0.5ml) were incubated in 7 ml of complete medium containing 200 µL of 1% phytohemagglutinin-M (PHA) (Difco Labs, Detroit, Michigan, USA) and 2 ml autologous serum for 72 hrs at 37°C with periodical shakings (13). After incubation lymphocyte population was determined using hemocytometer and counts were expressed in percentage.

Statistical analysis of the data were conducted using Student's 't' test and completely randomised design as per methods (14).

RESULTS

The data for blood levels of cortisol, glucose, total plasma protein, lymphocyte: neutrophil ratio and mitogen induced lymphocyte proliferative responses of the control and periparturient cows are shown

TABLE I: Changes in concentration of certain blood biochemical parameters in periparturient cows (Mean ± SE).

Group (n = 6)	Cortisol (µg/dl)	Blood glucose level (mg/dl)	Total plasma protein (g/dl)	Lymphocyte: Neutrophil ratio	Lymphocyte proliferation (%)
Control	6.33±0.35 ^a	64.83±1.88 ^a	6.5±0.06 ^a	2.34±0.07 ^a	88.16±8.43 ^a
3 days before parturition	9.83±0.33 ^b	66.16±1.86 ^a	6.46±0.08 ^a	2.06±0.04 ^b	66.33±5.99 ^a
On the day of parturition	12.16±1.53 ^b	145±4.63 ^b	6.65±0.04 ^a	1.54±0.04 ^b	61±4.59 ^b
7 days after	10.16±0.33 ^b	67.66±3.12 ^a	6.54±0.08 ^a	2.21±0.06 ^a	68.33±5.48 ^a

Values bearing similar superscripts in the column did not differ significantly (P<0.01).

TABLE II: Changes in the concentration of certain blood biochemical and related parameters of neonatal calves (Mean \pm SE).

Group (n = 6)	Cortisol ($\mu\text{g/dl}$)	Blood glucose level (mg/dl)	Total plasma protein (g/dl)	Lymphocyte: neutrophil ratio	Lymphocyte proliferation (%)
Control	10.33 \pm 0.69 ^a	69 \pm 0.89 ^a	6.41 \pm 0.03 ^a	1.94 \pm 0.04 ^a	56.66 \pm 8.36 ^a
On the day of birth	22.66 \pm 0.76 ^b	149.16 \pm 5.6 ^b	6.03 \pm 0.03 ^b	1.38 \pm 0.04 ^b	6.5 \pm 5.92 ^b
7 days of age	11.5 \pm 0.6 ^a	82 \pm 2.92 ^c	6.72 \pm 0.07 ^c	2.22 \pm 0.06 ^b	39 \pm 11.3 ^a
14 days of age	10.58 \pm 0.3 ^a	74.33 \pm 3.67 ^a	6.50 \pm 0.10 ^a	2.07 \pm 0.04 ^a	43.66 \pm 8.81 ^a

Values bearing similar superscripts in the column did not differ significantly ($P < 0.01$).

in Table 1, and the corresponding values of neonatal calves are shown in Table 2. On the day of parturition the cows had a significantly higher ($P < 0.01$) levels of cortisol ($12.16 \pm 1.53 \mu\text{g/dl}$), blood glucose ($145 \pm 4.63 \text{ mg/dl}$) as well as total plasma protein ($6.65 \pm 0.04 \text{ gm/dl}$) than their controls which later returned to the basal value. Similarly, the neonatal calves on the day of their birth showed significantly higher ($P < 0.01$) level of cortisol ($22.66 \pm 0.76 \mu\text{g/dl}$) with a significantly lower ($P < 0.01$) level of total plasma protein ($6.03 \pm 0.03 \text{ g/dl}$) than their controls, which later returned to their basal levels by two weeks of age.

It was also recorded that, on the day of parturition, cows exhibited lower circulating lymphocyte number as evidenced by significantly lower ($P < 0.01$) L:N ratio (1.54 ± 0.04) when compared to the control group (2.34 ± 0.07) and a significantly lower ($P < 0.01$) response to mitogen induced lymphoblastogenesis as only by $61 \pm 4.59\%$ increase in proliferation

compared to $88.16 \pm 8.43\%$ observed in controls (Fig. 1).

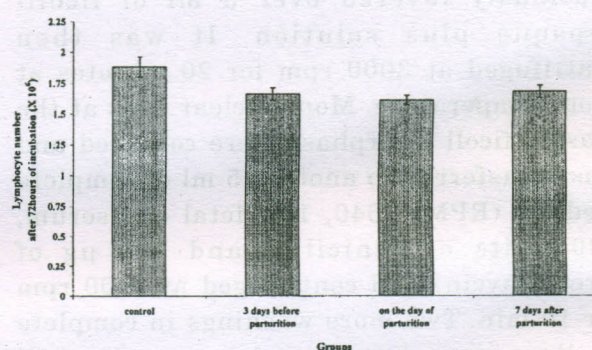


Fig. 1: Lymphocyte's proliferative response to mitogen in periparturient cows.

In the neonatal calves, it was observed that circulating lymphocytes number was reduced, when the cortisol concentration of the system was high (L:N ratio became 1.38 ± 0.04 compared to control value of 1.94 ± 0.04) and circulating lymphocytes did not respond well ($6.5 \pm 5.92\%$) to mitogen induced lymphoblastogenesis as compared to control ($56.66 \pm 8.36\%$) (Fig. 2).

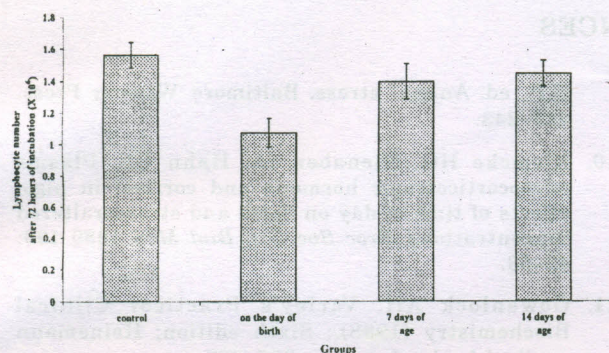


Fig. 2: Lymphocyte's proliferative response to mitogen in neonatal calves.

DISCUSSION

Corticosteroids are well known to suppress several functional capabilities of the immune system (15). It is also well established that high physiologic concentrations of glucocorticoids do alter some of the biochemical parameters of the system. In this study, it was observed that significantly higher cortisol concentration was encountered in the dam during periparturient period which closely agreed with earlier reports (16, 17) suggesting that cows and neonatal calves at the time of parturition experienced a severe stress which resulted in hyper-adrenocortical activity with higher levels of circulating steroids like cortisol. It is well known fact that cortisol is a gluconeogenic hormone thereby favouring hyperglycemia at the expense of body proteins. In this study it was recorded that there were parallel increase in the levels of cortisol and blood glucose with a drop in the total plasma proteins and this is in agreement with earlier report (18).

In this study it was also observed that there occurred a marked lymphocytopenia and neutrophilia when cortisol level in the system was very high, and this observation corroborates well with an earlier report (19). It was also found that an increase in serum cortisol concentration resulted in a depressed *in vivo* and *in vitro* immune responses, the lymphocytes isolated and grown in medium containing higher cortisol concentration responded poorly to the mitogen induced proliferation. Furthermore, the sensitivity of bovine lymphocytes to corticosteroids lowered with the age of animal as the cells of adult animals during stress proliferated much better than the cells from neonatal calves with manifested less steroid resistance.

To summarise, the pregnancy and parturition which are considered to be physiological stressors resulted in hyperadrenocortical activity in cows especially during periparturient period are highly susceptible for various infections since *in vitro* immune response stimulated by mitogens was poor, and possibly *in vivo* antigen stimulated activation would also be of the same kind. The neonatal calves especially on the day of parturition are subjected to severe stress as they have higher levels of blood cortisol which is required for initiation of parturition. Thus, they are also highly susceptible for various infections especially before the ingestion of colostrum. Therefore, high degree of care and management measures for the newborns must be taken in order to avoid various infections.

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