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Lymphocyte Proliferation Response of Blood and Milk Lymphocytes of Crossbred Cows During Peripartum Period

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Abstract

Six crossbred cows (Holstein Fresian × Kankrej) were selected in their advanced stage of gestation. The study was conducted on the selected animals from 30 days before calving to 30 days after calving. From each experimental animal 9 ml Blood was drawn in sterile heparinized vacutainer tube from jugular vein, on days -30, -15, -7 and -3 before calving, on the day of calving and on days 3, 7, 15 and 30 after calving. From each experimental animal 25ml milk sample was also collected on the day of calving and 3, 7, 15, 30 days after calving. Highest lymphocyte count observed at 30 day before calving, whereas significant (p<0.05) reduction was observed on the day of calving. Lowest lymphocyte proliferation response (LPR) of blood was observed at 3rd day before calving while LPR of milk was observed highest on the day of calving. Highest blood plasma cortisol and prolactin was observed at calving.

Keywords: Cow, LPR, Lymphocyte, Peripartum Period

Introduction

Peripartum period which may also be called as transition period i.e. the period 3 weeks before to 3 weeks after parturition is generally accepted as the most critical period with respect to mammary health in a dairy cow (Abuelo *et al.,* 2019). Compared with other stages of the lactation cycle, relatively little is known about fundamental biological processes during the peripartum period. During this period energy demand of animal increases tremendously as the nutrient requirement of the foetus increases greatly and the body of animal has to be prepared for calving events (Sundrum, 2015). In this critical period, the mammary gland undergoes marked biochemical, cellular and immunomodulatory changes to accommodate involution, to prepare for parturition, to withstand the stress of parturition, to transform colostrum into milk, and then for the attainment of peak milk production. These periods also coincide with impaired lymphocyte and neutrophil function. These immunological dysfunctions not only increase the risks of udder infection, mastitis, retained placenta and metritis but also lead to suppressed growth of mammary cells and subsequent milk production as energy and nutrients go preferentially to immune and homeostatic pathways (Kehrli *et al*., 1989).

Defects in lymphocyte function also may contribute to the dairy cow's increased susceptibility to mastitis during peripartum period (Ohtsuka *et al.,* 2010). Lymphocytes' role in bovine mammary gland immunity have not been established clearly. Antibody secreted by lymphocytes facilitates phagocytosis. Like macrophages, bovine lymphocytes can be activated to have enhanced activity by antigen induced lymphokines (Lukacs *et al.,* 1985). Suppressed lymphocytic blastogenic values after calving, compared with values observed during gestation, have been reported (Wells *et al.,* 1977). In view of the above, the present study was carried out to determine alteration in bovine lymphocyte function during the peripartum period.

Materials and Methods

Six crossbred cows (Holstein Friesian × Kankrej) were selected in their advanced stage of gestation (eight months pregnant) with different parity from the Livestock Farm Complex (LFC), Department of Livestock Production Management at College of Veterinary Science and A.H., Anand. The study was conducted on the selected animals from 30 days before calving to 30 days after calving. The experimental animals were reared in semi-open housing system, which is made up of concrete floor under asbestos roofed housing system constructed east-west direction and well covered with trees.

From each experimental animal 9 ml Blood was drawn in sterile heparinized vacutainer tube from jugular vein, posing minimum disturbance to the animal during collection on days -30, -15, -7 and -3 before calving, on the day of calving and on days 3, 7, 15 and 30 after calving. Blood samples were obtained as soon as possible after parturition and always within the first 24 hr. Immediately after collection, the samples were transported to the laboratory over ice for further processing. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20$^{\circ C}$ in deep freeze until analyzed for cortisol and prolactin. Whereas, the white Buffy coat zone was utilized for estimation of lymphocytic proliferation activity. From each experimental animal 25ml milk sample was also collected on the day of calving and 3, 7, 15, 30 days after calving. Milk lymphoytes were isolated as per method described by Mehrzad *et al.* (2001). Estimation of Lymphocytic proliferation activity of blood and milk lymphocytes were done by MMT assay. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue formazan crystal which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilisation of the cells by the addition of a detergent (DMSO) results in the liberation of the crystals which are solubilized. The number of surviving cells is directly proportional to the level of the formazan product created. The results can be read on a multiwell scanning spectrophotometer (ELISA reader) (Mosmann, 1983). The 25 μl MTT solution was added to each well after culturing lymphocytes for 24h. The plates were further incubated for 4 h at 37°C in humidified CO_2 incubator. During this period, formazon crystals were formed at the bottom of each well. The residual supernatant along with the suspended cells were pipetted out without disturbing formazon crystal layer. Then 100 μl of DMSO was added to each well and mixed thoroughly to dissolve the dark blue crystals. After incubating the plate at room temperature for 15 min., the optical density was read using ELISA reader in dual wavelength measuring system, at a test wavelength of 503 nm and a reference wavelength of 630 nm. Plates were normally read within 1 h of adding the DMSO. The data were analyzed using two ways ANOVA. The results were expressed as mean (± SEM). Significance was tested by Duncan's New Multiple Range Test (DNMRT) (Snedecor & Cochran, 1994).

Results and Discussion

In vitro lymphocyte proliferation response of blood and milk lymphocytes have been presented in Table 1. This study was performed to evaluate one of the measures for studying the immune status of crossbred cows on different days of peripartum periods. Six crossbred cows were taken as lymphocyte donors. The lymphocytes after washing were subjected to *in vitro* culture for 24h with mitogen (Con A and LPS) in triplicate. The lymphocyte proliferation activity at the end of culture period was compared between different days.

Table 1: Mean (± SE) values of blood lymphocyte, LPR, cortisol and prolactin of crossbred cows (n=6) during peripartum period

Days	Lymphocyte (%)	T-LPR (ConA)	B-LPR (LPS)	Cortisol (ng/ml)	Prolactin (ng/ml)
-30	55.60^{a}±3.28	0.13^{bc}±0.02	0.14^{ab}±0.03	27.69^{ab}±5.22	188.97^{c}±24.53
-15	50.38^{ab}±4.90	0.09^{cd}±0.04	0.10^{abc}±0.03	25.04^{ab}±6.20	268.44^{b}±27.69
-7	48.92^{b}±4.43	0.09^{cd}±0.03	0.09^{bc}±0.03	26.67^{ab}±3.48	309.00^{ab}±29.65
-3	46.30^{b} ±3.91	0.02^{e}±0.01	0.08^{c}±0.03	28.60^{ab}±5.15	306.75^{ab}±14.75
0	48.27^{b}±4.20	0.03^{de}±0.01	0.08^{c}±0.02	44.04^{a}±8.87	333.28^{a}±4.27
3	52.68^{ab}±3.26	0.13^{bc}±0.04	0.14^{ab}±0.03	38.61^{ab}±8.73	287.43^{ab}±32.05
7	50.55^{ab}±1.90	0.20^{b}±0.02	0.16^{a}±0.02	32.28^{ab}±7.99	298.42^{ab}±13.66
15	49.68^{b}±3.87	0.07^{cde}±0.03	0.09^{bc}±0.02	22.99^{b}±2.63	312.25^{ab}±20.11
30	52.98^{ab}±3.52	0.27^{a}±0.02	0.15^{ab}±0.04	35.66^{ab}±6.18	305.35^{ab}±20.66
Overall Mean	**50.60 ± 3.70**	**0.12 ± 0.02**	**0.11±0.02**	**31.29±5.84**	**289.99±17.13**
CV%	**19.3**	**47.82**	**49.14**	**45.72**	**14.47**
$CD_{0.05}$	**-**	**0.06**	**-**	**-**	**48.97**

**Values having different superscripts differed significantly (p<0.05) within column*

The effect of Con A and LPS induced T and B lymphocytic proliferation response have also been presented in Table 1. The mean (±SEM) of blood T and B lymphocytic proliferation were ranged from 0.02±0.01 to 0.27±0.02 and from 0.08±0.02 to 0.16±0.02 respectively, which ware significant (p<0.05). Con A induced lymphocytic proliferation response (LPR) of blood decreased non significantly on days 15 and 7 before calving, sharp depression in T lymphocytic activity was observed between 3 days prepartum to the day of calving as compared to 30 day before calving. T-lymphocyte proliferation response was increased significantly (p<0.05) on 3rd day after calving as compared to the day of calving. Significant (p<0.05) reduction in T- LPR was also observed at 15 days after calving as compared to 7 day after calving, which was non-significant with T-LPR found on the day of calving. The T-LPR then increased significantly (p<0.05) on 30 days post calving and became the highest as compared to other peripartum days. Similar results and trend was also observed for LPS induced B- lymphocyte proliferation response in present study**.**

The mean (±SEM) of Milk T and B lymphocytic proliferation were ranged from 0.27±0.03 to 0.10±0.01 and 0.58±0.09 to 0.10±0.06 which ware significant (p<0.05). Con A induced LPR of milk was higher on the day of calving and decreased significantly (p<0.05) on 3, 7, 15 and 30 days postpartum. Highest value was observed on the day of calving, thereafter significant (p<0.05) reduction was observed after calving to 30-day post-partum. Similar trend was also observed for LPS induced B- lymphocyte's LPR. During the peripartum period, blood plasma cortisol (ng/ml) level was ranged from 44.04±8.87 to 22.99±2.63. Continuous non-significant increase in level of cortisol was observed from 30 day before calving to on the day of calving, which become highest on the day of calving. After calving non-significant reduction in cortisol was observed till 15 days of calving, which was again increased non-significantly on 30th day of calving. During the peripartum period, blood plasma cortisol (ng/ml) level was ranged from 44.04±8.87 to 22.99±2.63. Continuous non-significant increase in level of cortisol was observed from 30 day before calving to on the day of calving, which become highest on the day of calving. After calving non-significant reduction in cortisol was observed till 15 days of calving, which was again increased non-significantly on 30th day of calving.

Table 2: Mean (± SE) values of milk lymphocytic proliferation of crossbred cows (n=6) during peripartum period

Days	T-LPR (ConA)	B-LPR (LPS)
0	0.27^a±0.03	0.58^a±0.09
3	0.15^b±0.01	0.19^b±0.05
7	0.15^b±0.01	0.23^b±0.05
15	0.14^b±0.01	0.10^b±0.06
30	0.10^b±0.01	0.16^b±0.04
Overall Mean	**0.16 ± 0.02**	**0.25±0.06**
CV%	**23.96**	**56.02**
$CD_{0.05}$	**0.05**	**0.17**

**Values having different superscripts differed significantly (p<0.05) within column*

Blood plasma prolactin (ng/ml) level was ranged from 188.97±24.53 to 333.28±4.27 which was significant (p<0.05). Continuous increase in level of prolactin was observed from 30 day before calving to on the day of calving, which became highest on the day of calving. After calving non-significant reduction observed in prolactin till 30 day after calving (Table 1).

Mukherjee *et al.* (2015) reported that Mitogen-induced lymphocyte blastogenic response is used to measure the immune competence of lymphocytes. In the present investigation, Con A and LPS were used as mitogen for stimulating T and B lymphocytes, respectively. In the present study T and B-LPR decreased at calving. Lymphocyte per cent was also decreased at calving. So, it can be said that the lowered LPR might be due to lower number of lymphocytes at calving. Plasma concentration of corticosteroids in parturient cattle have been shown to be elevated (Pathan *et al.*, 2015) and may be responsible for this reduction in immunological reactivity since corticosteroids are known to be capable of inhibiting the manifestations of cell mediated immunity (Pathan *et al.*, 2017). Depression in responsiveness of blood lymphocytes upon stimulation with Con A around parturition and immediate postpartum period of the cow has been observed by Dang *et al.* (2013). Negative correlation between T & B- LPR and cortisol was also observed during the study. A significant negative correlation was observed between T, B - LPR and prolactin. So, there might some negative effect of hormone over LPR. Agricola *et al.* (2007) reported that reduced physiological immune response occurring in mares during gestation might be due to T-helper and T-cytotoxic lymphocytes reduction. Lymphocyte trafficking patterns also changed in periparturient cows because the percentage of T cells declines from 45% of circulating lymphocytes in mid lactating cows to 20% in periparturient cows (Shafer-Weaver *et al*., 1996). Mukharjee *et al*. (2015) observed the Con A Mitogen-induced milk LPR was highest on day (in colostrum) which was decreased significantly (p<0.01) on the 15^{th} day postpartum. We had also found the same trend this study. During this time, cortisol secretion rate and plasma cortisol concentrations are significantly higher as compared with non-lactating cows or cows in established lactation (Paterson & Linzell, 1974). Glucocorticoids are necessary for the formation of milk components (Ray *et al.*, 1996) and to induce differentiation of the lobule-alveolar system (Tucker *et al.,* 1981). Lymphocyte proliferation response was higher in colostrum in accordance with the reports of Ogra *et al.* (1978) in humans. Kehrli *et al.* (1989) reported diminished leukocyte functions during lactogenesis.

Table 3: Correlation between various blood parameters of crossbred cows (n=6) during peripartum perio

	Cortisol	Prolactin	T-LPR of blood	B- LPR of Blood
Cortisol	1	-0.019	-0.006	0.058
Prolactin		1	-0.310^{*}	-0.365^{**}
T-LPR of Blood			1	0.622^{**}
B- LPR of Blood				1

*** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)*

Cortisol is released in response to stress and a low level of blood glucocorticoids. Its primary functions are to suppress the immune system and increase blood sugar through gluconeogenesis. The cows during peripartum period are under various types of stressful conditions viz. stress of providing nutrition to its growing calf, stress of labour, to synthesize colostrum and then milk. A social stress of being isolated is also there. Overall effects of stress increased cortisol level, produced lymphopenia with decreased functional capacity of neutrophils lymphocytes which leads to, immunosupression and ultimately the cows become more prone to mastitis and other infections. (Kehrli *et al.*, 1991). High levels of cortisol at calving have also been reported (Goff and Horst, 1997) to act as powerful immunosuppressive agent. The result of present study also collaborated with the findings of Prakash and Madan (1985) in buffaloes, Goff and Horst, (1997) in cows and Khan and Ludri (2002) in crossbred goats.

Kodagali *et al.* (1980) reported that the prolactin concentration varied with each stage of lactation; being high in early, middle in mid lactation, and low in late lactation in Gir cows. The present study also accordance with Akasha *et al.* (1987), where serum prolactin concentration declined as lactation progressed. The role of prolactin in the immune system has contradictory findings (Segei *et al.*, 1980). A series of *In Vitro* and *In Vivo* studies showed the immunostimulatory effects of prolactin (Yu-Lee, 1997).

Conclusion

Highest lymphocyte count observed at 30 day before calving, whereas significant ($p<0.05$) reduction was observed on the day of calving. Lowest LPR of blood was observed at 3rd day before calving while LPR of milk was observed highest on the day of calving. Highest blood plasma cortisol and prolactin was observed at calving. lymphocytic proliferation response was negatively correlated with blood plasma cortisol levels. Effect of prolactin on blood neutrophilic and lymphocytic activity was non-significant.

Conflict of Interests

There is no conflict of interest.

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