



***In vitro* phagocytic activity of blood and milk neutrophils against *Saccharomyces cerevisiae* in primiparous and multiparous Karan Fries crossbred cows throughout the dry period and lactation cycle**

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ABSTRACT

The differences between *in vitro* phagocytic activity (PA) of neutrophils isolated from first lactating i.e., primiparous (PP) and multiparous (MP) Karan Fries (Holstein Fresian × Tharparkar) cows, were studied. Blood and milk samples were collected from 12 PP and 12 MP cows during different days of production cycle (dry period and lactation). Blood total leukocyte counts (TLC) and milk somatic cell counts (SCC) were significantly lower in PP as compared to MP cows during all stages of production cycle. Blood TLC was significantly higher at calving and then decreased towards the end of lactation in both the groups. Milk SCC was highest in colostrums, decreased significantly around mid lactation and again increased towards the end of lactation. Phagocytic activity (PA) of blood neutrophils was significantly higher in PP as compared to MP cows. Suppression of PA around parturition was less and also restored earlier in PP as compared to MP cows. PA of colostrums was higher in MP as compared to PP cows; however, PA of milk neutrophils was always higher in PP as compared to MP cows throughout the lactation period. Less PA of neutrophils make the MP cows more prone to infections during the production cycle. Therefore, proper care and nutrition should be provided to them throughout the production cycle.

Key words: Crossbred cows, Neutrophils, Phagocytic activity, Phagocytic index, Production cycle, Somatic cell counts

Neutrophils are the first ones to respond (Jain *et al.* 1986) and migrate from blood circulation to an inflamed area where they phagocytose and kill bacteria by two distinct mechanisms, i.e. respiratory burst and by using lysosomal enzymes. Neutrophils travel from blood to the mammary gland in response to inflammatory mediators, such as cytokines, complement and prostaglandins (Janeway *et al.* 2001). Phagocytic activity (PA) of the neutrophils is affected by age (Hurley *et al.* 2002), presence of antibodies opposing the bacteria (Rainard and Riollet 2003), calving; and primiparous (PP) approaching their first calving behave differently than multiparous (MP) cows. This difference is due to the different nutrient requirements, PP cows require nutrients for their continued growth in addition to that of

their developing self (Wathes *et al.* 2006). MP cows produce more milk hence more nutrients are diverted towards their mammary gland. These differences lead to varying degree of tissue mobilization between PP and MP cows which may promote nutrient partitioning into growth, milk production and immunity. Clinical mastitis occur more at calving in MP cows (Kalmus *et al.* 2006) because their neutrophils has less viability and free radical production capacity as compared to heifers (Mehrzaad *et al.* 2009). Changes occurring in the PA of neutrophils isolated from both PP and MP cows throughout the production cycle may affect the incidence of disease in these cows during their production cycle. There is no such study which compares the activity of neutrophils throughout the production cycle. Therefore, the present study was initiated to study the difference in between the PA of blood and milk neutrophils isolated from both PP and MP crossbred cows throughout the dry and lactation period.

MATERIALS AND METHODS

Pregnant crossbred, Karan Fries cattle (12 primiparous and 12 multiparous) were kept in a loose housing system with brick flooring and were managed as per the practices followed in the institute's herd. They were offered *ad lib.*

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green fodder and calculated amount of concentrate mixture based on milk production was offered only at the time of milking. Fresh tap water was available *ad lib.* at all the time of the day.

Blood samples from all the cows were collected on different days of production cycle (i.e. 45, 30, 15 day prepartum, on the day of calving and 30, 60, 90, 120, 150, 180, 210, 240, 270 days postpartum). Milk samples were also collected twice from these cows on the day of calving, and on days 30, 60, 90, 120, 150, 180, 210, 240 and 270, respectively, after calving. Milk yield of the animals were recorded daily.

Blood TLC was counted in a hemocytometer under a microscope as per standard procedure. Isolation of polymorphonuclear neutrophils (PMN) from peripheral blood was performed as per Mehrzad *et al.* (2001). somatic cell counts (SCC) of each original milk sample was determined in duplicate within 2 h post collection and measured microscopically (Dang *et al.* 2008). Isolation of PMN from milk was performed as per Dang *et al.* (2010). Trypan blue exclusion method was used to determine the proportion of viable cells in the separated PMN from blood and milk. The cell viability in different experiments ranged between 90 and 93% within 2 h of processing and then decreased afterwards.

In vitro phagocytosis assay was performed as per Hay and Westwood (2002). Yeast cells (*Saccharomyces cerevisiae*) were used for determining phagocytosis. Percentage of phagocytosing neutrophils was recorded as percentage activity (PA) and average number of yeast cells per neutrophil was recorded as mean phagocytosis or phagocytic index (PI) as described by Guidry *et al.* (1976).

The results were expressed as mean standard error of mean. Significance was tested by employing two ways ANOVA with the help of software SYSTAT.

RESULTS AND DISCUSSION

Changes occurring in the blood TLC of both PP and MP cows during different days of production cycle are presented in Fig. 1. TLC was 8,625 and 8,342/ μ l of blood at 45 days prepartum in multiparous and primiparous cows respectively. TLC increased significantly ($P<0.01$) in multiparous cows at 30 days before calving and again on the day of calving, whereas, TLC increased significantly ($P<0.01$) only on the day of calving in primiparous cows. TLC remained unchanged in the multiparous cows till days 120 and then decreased significantly ($P<0.01$) afterwards during the lactation cycle. In PP cows, TLC increased nonsignificantly up to 90 days and then decreased nonsignificantly throughout the lactation period.

Total leukocyte counts may indicate the inflammatory status of an animal. TLCs in crossbred KF cows during this study were within normal range (Jain *et al.* 1986). MP cows had higher TLC during the dry period till mid lactation. TLC was significantly ($P<0.01$) higher around parturition, which may be due to the stress caused by hormonal and metabolic adjustments as well as by differences in

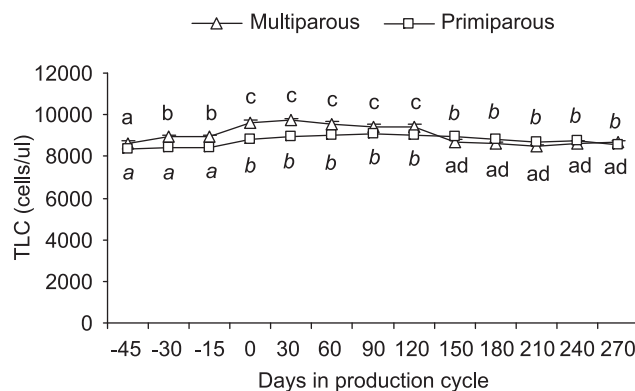


Fig. 1. Blood TLC in primiparous and multiparous KF cows during different days of production cycle. Values expressed as mean $\pm$ SEM. Values with different superscript are significantly ($P<0.01$) different.

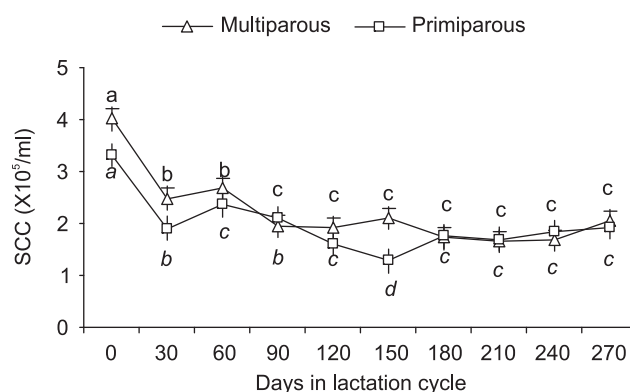


Fig. 2. Milk SCC in primiparous and multiparous KF cows during different days of lactation cycle. Values expressed as mean $\pm$ SEM. Values with different superscript are significantly ($P<0.01$) different.

management observed around calving (Mallard *et al.* 1998).

Changes occurring in the milk SCC of primiparous and multiparous cows during different days of production cycle are presented in Fig. 2. SCC was highest in colostrums and then decreased significantly ($P<0.01$) in both the groups of cows till day 30 of lactation cycle. An increase in milk SCC was seen in both the groups with the multiparous cows showing a significant increase ($P<0.01$) in milk SCC. It then decreased sharply, with primiparous showing a sharper decline around day 150 of the lactation cycle. Milk SCC then increased nonsignificantly towards the end of the lactation cycle.

Milk somatic cells are used as an index of udder health and milk quality and any mammary disturbance significantly increases milk SCC (Harmon 1994). SCC observed in both the group of cows was lower than that reported by Barkeman *et al.* (1998) for exotic cows but were within range. Less SCC in primiparous cows indicated less amount of mammary stress faced by these animals.

Changes occurred (Figs 3, 4) in the PA and PI of blood neutrophils in primiparous and multiparous crossbred cows during different days of lactation cycle. PA decreased

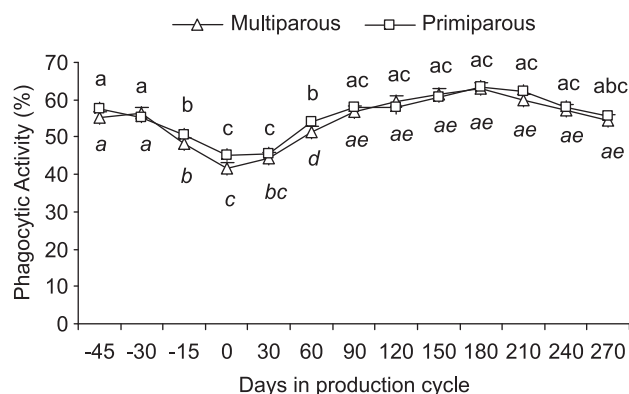


Fig. 3. Phagocytic activity of blood neutrophils in primiparous and multiparous KF cows during different days of production cycle. Values expressed as mean $\pm$ SEM. Values with different superscript are significantly ($P<0.01$) different.

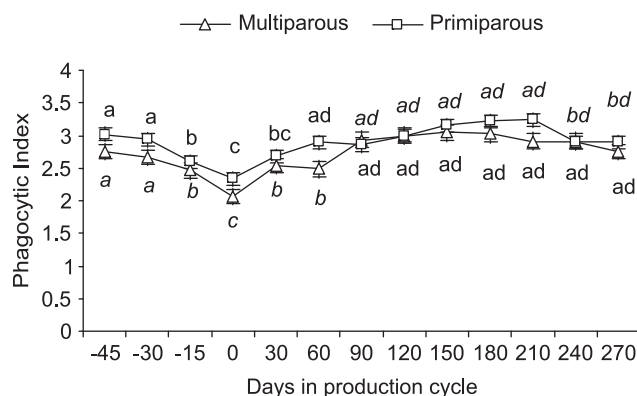


Fig. 4. Phagocytic index of blood neutrophils in primiparous and multiparous KF cows during different days of production cycle. Values expressed as mean $\pm$ SEM. Values with different superscript are significantly ($P<0.01$) different.

significantly ($P<0.01$) in both the group of cows, first on the day 15 before calving and then again on the day of calving, with the multiparous cows showing a greater decline. At 15 days before calving, blood PA decreased by 14.3 and 8.4% in MP and PP cows respectively. Whereas, at calving this PA further declined with MP showing a 14% decrease as compared to only 11% in PP cows. PA increased significantly around day 60 in both the groups. PA then increased nonsignificantly till 180 days of lactation, and decreased nonsignificantly towards the end of lactation in both the groups. PI also decreased significantly around 15 days prepartum and on the day of calving in both the groups of cows. PI increased in both the groups till day 210 and again decreased nonsignificantly towards the end of lactation.

Changes occurring in the PA and PI of milk neutrophils in primiparous and multiparous crossbred cows during different days of lactation cycle are presented in Figs 5 and 6, respectively. PA and PI of milk colostrums was 14% higher in multiparous as compared to primiparous cows. PA decreased significantly ($P<0.01$) in the milk on day 5 in

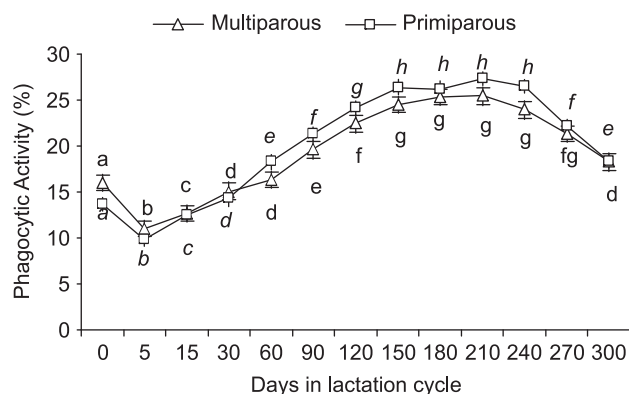


Fig. 5. Phagocytic activity of milk neutrophils in primiparous and multiparous KF cows during different days of lactation cycle. Values expressed as mean $\pm$ SEM. Values with different superscript are significantly ($P<0.01$) different.

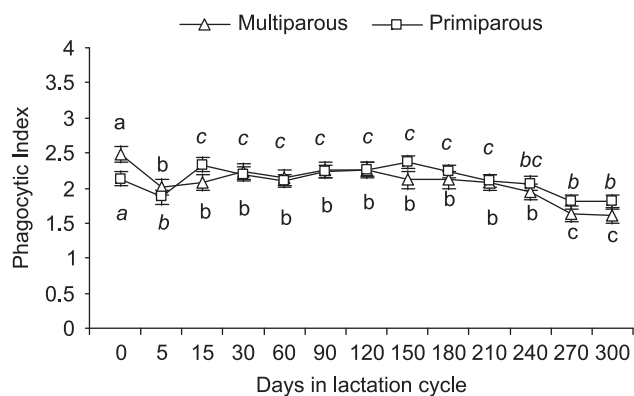


Fig. 6. Phagocytic index of milk neutrophils in primiparous and multiparous KF cows during different days of lactation cycle. Values expressed as mean $\pm$ SEM. Values with different superscript are significantly ($P<0.01$) different.

both the groups of cows, with MP cows showed a decline of 31% as compared to 28% in PP cows. PA increased significantly till day 150 of the lactation cycle. PA of milk neutrophils remained unchanged around days 180 to 210 days of lactation and then decreased nonsignificantly towards the end of lactation. Milk PI decreased significantly in the milk samples collected on the day 5 of lactation cycle. PI increased significantly in primiparous cows on the day 15 and then remained unchanged till 210 days of lactation cycle, whereas, milk PI remained unchanged till days 240 of the lactation cycle. PI decreased significantly in cows towards the end of the lactation cycle.

Phagocytic activity and index in both the group of cows started decreasing from 15 days prepartum and maximum depression was observed at calving. This indicated that immunosuppression of the neutrophilic activity starts from 2 weeks before parturition and remained low until day of lactation. Further, PA of neutrophils separated from the blood of crossbred cows in this study was less than that reported by Guidry *et al.* (1974) who found 100% phagocytic activity while incubating whole blood with yeast

cells. Yeast per phagocytosing cell was less in this study than reported by Wise *et al.* (2003) in phagocytosing neutrophils for *E. coli*. Phagocytosis both by blood and milk neutrophils was also significantly lower than that reported by Dosogne *et al.* (2001) when neutrophils were incubated with *S. aureus*. However, killing activity of blood neutrophils against *S. aureus* was only 42% in primiparous cows and 23% in multiparous cows during early lactation (Mehrzhad *et al.* 2009). The reason for this difference may be the large sizes of yeast particles used in this study, hence their engulfment by both blood and milk neutrophils was less.

Phagocytic activity and index were maximum in neutrophils obtained from colostrums. Sugisawa *et al.* (2001) found that as the immunoglobulins were very high in colostrums as compared to milk, therefore, it increased the PA of colostrums neutrophils by up to 25% and was dose dependent. Further, PA was higher in the colostrums of multiparous animals because they produce more immunoglobulins in their colostrums (Dang *et al.* 2009). PA decreased between day 30 and 60 i.e. when the animal attains peak yield, which may be due to hormonal and metabolic changes such as glucocorticoids, ketone bodies and pregnancy associated glycoprotein. PA and index were higher around mid lactation (120–150 days) as there was less degree of mammary stress due to lesser milk production as compared to early lactation. PA decreased non-significantly in both multiparous and primiparous cows towards the end of lactation. But this decrease in PA did not have any major effect on the mammary health because milk yield was also less during this period as compared to early lactation, where decrease in PA is accompanied by copious amount of milk secretion thus stressing the mammary gland. However, these results were different than those reported in buffaloes (Dang *et al.* 2010) where a significant decrease in the PA of milk neutrophils was observed during late lactation. Mehrzhad *et al.* (2009) found that PA of milk neutrophils was 20% in primiparous cows and 10% in multiparous exotic cows in early lactation, whereas, no such differences were found in the PA of milk neutrophils of crossbred cows throughout the production cycle in our study.

Comparisons of the phagocytic ability of the neutrophils of milk with that of the neutrophils of blood showed that the number of bacteria killed by milk neutrophils was significantly less ($P < 0.01$) than the number killed by the neutrophils isolated from blood. As milk neutrophils have reduced glycogen stores compared with that of blood, this may limit the availability of energy. Milk neutrophils also have reduced abilities to produce ROS, when compared to that of blood (Goldbery *et al.* 1995, Dosogne *et al.* 2001). An *in vitro* cell culture model (Smits *et al.* 1999) demonstrated that diapedesis of blood PMN through the blood-milk barrier causes a reduction of the phagocytic and oxidative burst activity. Reduction in the PA of blood neutrophils of multiparous cows also results from the profound decrease of PMN activity and free radicals

production capacity in them (Mehrzhad *et al.* 2009).

The ability of neutrophils to phagocytose foreign particles is important for protection of an individual and *in vitro* analysis of neutrophil function provides a very effective tool for the study of natural disease resistance (Macdonald *et al.* 1994). Concept of phagocytosis is crucial for host defence and pathogenecity (Repine and Beehler 1991). This concept forms the fundamental basis for future therapeutic guidelines in immunomodulation. Rivas *et al.* (2002) suggested that estimation of PA of neutrophils can also be used as an effective tool for breeding practices. Therefore, apart from selection of cows with better immunity, this *in vitro* technique can further be exploited to develop specific drugs which may help in decreasing the harmful effects while retaining or enhancing the beneficial effects of neutrophils.

Our results presented the differences obtained in the blood and milk cell counts and PA of neutrophils isolated from the blood and milk samples of PP and MP cows throughout the production cycle. This study indicated that the PA of both blood and milk neutrophils is more in PP cows as compared to MP cows. Further, maximum immunosuppression in terms of PA of both blood and milk neutrophils can be seen around parturition and during late lactation, thus indicating chances of maximum mammary infections during these periods. Therefore our dairy animals require more managerial interventions like better care, nutrition and proper hygiene during early and late lactation.

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