

MORPHOLOGY, VIABILITY AND PHAGOCYTIC ACTIVITY OF BLOOD
AND MILK NEUTROPHILS DURING PERIPARTUM AND
EARLY LACTATION OF MURRAH BUFFALOES

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ABSTRACT

A comparative study of number, morphology, viability and activity in blood and milk neutrophils isolated from 10 Murrah buffaloes were carried out during different peripartum and early lactating days. Blood samples were collected from all buffaloes at days 30,15,7 before calving, on the day of calving and on days 7, 15, 30, 45 and 90 after calving. Colostrum and milk samples were collected on days 0, 5, 15, 30, 45, 60 and 90 days of early lactation. Blood total leucocyte counts (TLC), milk somatic cell counts (SCC), viability and morphology of neutrophils were measured microscopically. Blood and milk neutrophils were isolated and their *in vitro* phagocytic activity (PA) was estimated by Nitro Blue Tetrazolium assay. There were significant ($P<0.05$) increase in blood TLC and blood neutrophils percentage on the day of calving. Milk SCC and neutrophils in colostrums were significantly higher ($P<0.05$) than milk. On comparison of the viability of neutrophils isolated from blood, colostrum and milk, blood neutrophils viability was significantly ($P<0.05$) higher than both. Partum blood neutrophils viability was significantly ($P<0.05$) higher than other peripartum days. Similarly colostrum neutrophils had

significantly ($P<0.05$) higher viability than its milk counterparts during different postpartum days. Colostrum neutrophils had a significantly ($P<0.05$) higher PA than milk neutrophils on different early lactation days and also from blood neutrophils on the day of calving. Blood neutrophils on different peripartum days had a more lobed and matured nucleus when compared with the morphology of blood neutrophils observed at calving, where they showed band shaped nucleus. Milk neutrophils had more lobed nucleus as compared to blood neutrophils. Scanning Electron Microscopy revealed more ruffled surface of blood than milk neutrophils on early lactation days. This study shows the changes occurring in the shape, viability and PA of blood neutrophils once they cross the blood mammary barrier to become milk neutrophils. Further understanding the functions of neutrophils around peripartum period and early lactation may help to develop more efficient strategies to combat immunosuppression around this period.

Keywords: *Bubalus bubalis*, buffalo, PMN, blood, milk, morphology, viability, phagocytosis, peripartum period and early lactation, SEM

INTRODUCTION

Milk neutrophils are the key players in providing immediate and sustained protection to mammary glandular cells from the invading pathogens. Milk neutrophils originate from the migrating blood neutrophils at the mammary epithelial interface (Burton *et al.*, 2005). The process of neutrophilic migration into mammary gland is a complex signaling process which is initiated due to invading microbes, localized tissue damage and chemokine mediated chemotaxis. Neutrophils cross the epithelial border to reach mammary cells for carrying out phagocytosis (Pyorala, 2003). During periparturient period, various cytokines and hormone *milieu* of blood and extracellular tissue fluid can influence neutrophil development and immunity related activities. In buffaloes, which are the major milk producers in the Indian subcontinent, the molecular basis of various phenotypic changes and physiological benefits or drawbacks occurring around parturition is poorly understood. Therefore there is need to compare the number, morphology, viability and phagocytic activity of blood and milk neutrophils around parturition.

MATERIALS AND METHODS

Ten clinically healthy Murrah buffaloes approaching parturition were selected. Blood samples were collected from them on days 30, 15 and 7 prior to calving, on the day of calving and on days 7, 15, 30 after calving (early lactation). Colostrum and milk samples were collected on the days 0,5,15,30,45,60 and 90 days of early lactation. Blood and milk samples were taken and neutrophils were isolated and suspended in RPMI

medium and kept in CO₂ incubator at different time intervals (0, 12, 24 and 48 h) and the viability was determined using Trypan Blue after counting the cells using a Hemocytometer. This was followed by the evaluation of *in vitro* phagocytic activity (PA) of blood and milk neutrophils by Nitro Blue Tetrazolium (NBT) assay as described by Dang *et al.* (2010 and 2012). Investigation of ultra structural changes of both blood and milk neutrophils was carried out by employing Scanning Electron Microscopy (SEM) as previously described by Tian *et al.* (2005) in goats with minor modifications.

RESULTS AND DISCUSSION

There was a significant ($P<0.05$) increase of TLC on the day of calving from 15 days prior to calving (Figure 1). A significant ($P<0.05$) decrease in TLC was observed at 7 days post calving. The results of colostrums and milk SCC during postpartum days of Murrah buffaloes have been presented in Figure 2. Colostrum SCC was about 2, 80,000 per ml of colostrum. The SCC counts decreased significantly ($P<0.05$) at 7 days postpartum in milk. The rise in neutrophil count may be associated with an excessive rise in cortisol on the day of calving (Burton *et al.*, 2005), whereas, postpartum decrease in TLC is associated with the increased migration of leucocytes to uterine lumen followed by migration to mammary alveolar lumen (Preisler *et al.*, 2000). The results of blood, colostrum and milk PA during pre and postpartum days of Murrah buffaloes have been presented in Figure 3. Reduced PA of milk neutrophils as observed in our study may be associated with exhaustion of neutrophils, spontaneous ageing, depletion of glycogen reserves, degranulation, engulfment of casein and fat, masking of surface

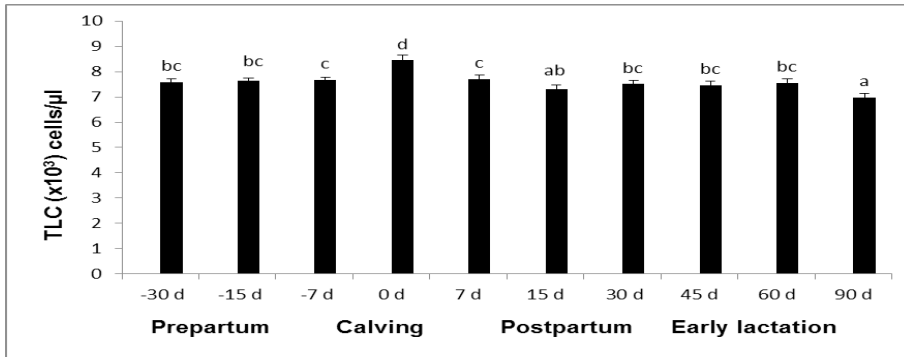


Figure 1. Blood total leucocyte counts (TLC) in periparturient and early lactating Murrah buffaloes.

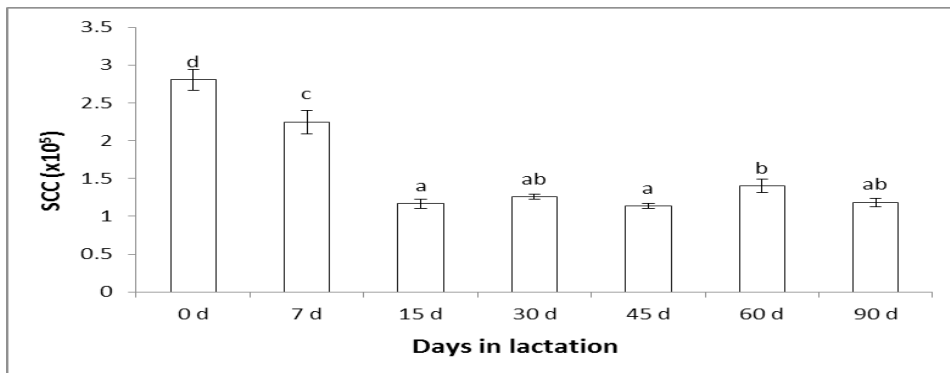


Figure 2. Colostrum and milk somatic cell counts ($\times 10^5$)/ml in early lactating Murrah buffaloes.

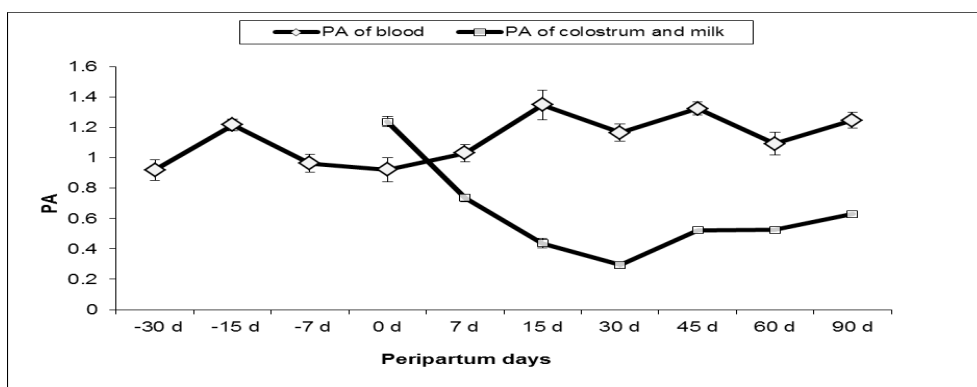


Figure 3. Comparisons of PA of blood, colostrum and milk neutrophils of Murrah buffaloes.

receptors, decreased pH of neutrophils (Paape *et al.*, 2003; Hurley, 2006). The PA of neutrophils was found to be lesser on the day of calving and highest in colostrum of buffaloes.

At 15 days before calving, neutrophils exhibited a distinct polylobed, multisegmented nucleus. On 0 day, they exhibited band nucleus without distinct segmentation and on 15th day of post calving they are polylobed and multisegmented. Neutrophils isolated from blood samples collected after 15 days of calving shows a multilobed (having 3 to 4 lobes) nucleus and lobes are separated by thin chromatin fibers. Number of immature neutrophils is more on day of calving and hence the net PA of blood neutrophils was less. Viability was mostly constant in blood but was found to be highly variable in milk neutrophils (Table 1). At calving presence of more juvenile neutrophils may be associated with a prolonged lifespan in milk. Secondly, it can be assumed that reduced functional ability of blood neutrophils on

the day of calving may be a factor in the prolonged survival of neutrophils due to less mitochondrial activity. Scanning Electron Microscopy revealed that both blood and milk neutrophils are round in shape. Blood neutrophils exhibited ruffled surface as compared to milk neutrophils. Milk neutrophils appeared smoother, but on higher magnification, milk neutrophils exhibited more ruffled surface as compared to blood neutrophils. Smooth surface of milk neutrophils as observed under SEM is the indication of decreased immune status of the animal which may be due to the peripartum period as well as the stress due to the parturition. To validate our SEM findings, we carried out *in vitro* phagocytic assay of both blood and milk neutrophils. Milk neutrophils isolated and studied during early lactation exhibited a decreased phagocytic activity as compared to the blood neutrophils. A remarkable and significant reduction in phagocytic activities of milk was seen in neutrophils from 10 to 30 after calving.

Table 1. Percentage viability of blood, colostrum and milk neutrophils isolated from periparturient Murrah buffaloes and incubated for various hours.

Incubation (Hours)	Blood				Colostrum	Milk	
	-7d	0d	7d	30d	0d	7d	30d
0	91.00 ±0.5 ^{aD}	94.00 ±1.51 ^{bD}	89.00 ±0.97 ^{aD}	90.83 ±0.91 ^{aD}	70.00 ±1.43 ^{eD}	47.00 ±2.01 ^{cD}	60.00 ±1.61 ^{dB}
12	70.00± 1.66 ^{aC}	77.00 ±1.01 ^{bC}	69.00 ±1.56 ^{aC}	71.83 ±2.26 ^{aC}	44.00 ±1.56 ^{cC}	30.00 ±1.33 ^{cC}	39.00 ±1.99 ^{dC}
24	44.00 ±1.54 ^{bB}	50.00 ±1.83 ^{bB}	36.00 ±2.17 ^{aB}	46.67 ±2.16 ^{bB}	21.00 ±2.19 ^{eB}	10.00 ±0.73 ^{cB}	17.00 ±1.51 ^{dB}
48	16.00 ±1.30 ^{bA}	26.00 ±1.56 ^{cA}	11.00 ±0.98 ^{aA}	17.15 ±1.51 ^{bA}	12.00 ±0.92 ^{eA}	2.00 ±0.54 ^{cA}	8.00 ±1.05 ^{dA}

Values within a row having different superscripts (small letters) are significantly (P<0.05) different from each other. Values within a column having different superscript (capital letters are significantly) (P<0.05) different from each other.

This study indicates various changes that are occurring in the shape, viability and phagocytic activity of blood neutrophils once they cross the blood mammary barrier to become milk neutrophils. Further understanding the functions of neutrophils around peripartum period and early lactation in large number of buffaloes may help to develop more efficient strategies to combat immunosuppression around this period.

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