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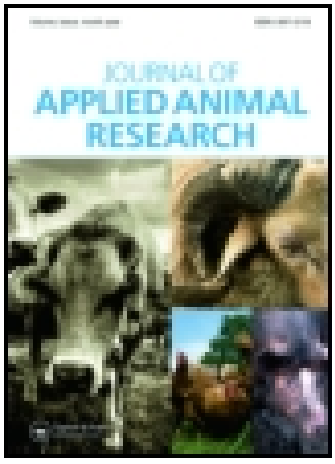

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Comparative evaluation of neutrophil competence and activity of cows and buffaloes around peripartum

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To compare immune activities of neutrophils, blood samples were collected from 12 cows and 12 buffaloes on −15, −7, −5, −3, −2 and −1 days prepartum at calving and on 1, 2, 3, 5, 7 and 15 days postpartum. Plasma cortisol levels, phagocytic activity (PA), enzyme (elastase, collagenase and cathepsin G) levels and expression of TLR-2, TLR-4 and IL-8 were also studied. Blood total leukocyte counts and neutrophil percentage increased at calving and decreased faster in buffaloes. Neutrophil PA was significantly ($P < 0.01$) lower even up to two days post calving in cows. Cortisol levels were significantly ($P < 0.01$) higher in cows and negatively correlated with neutrophilic functions. Elastase was significantly ($P < 0.01$) higher in buffaloes. Collagenase levels were significantly ($P < 0.01$) higher in buffaloes at three days precalving. At 15 days precalving and 7 and 15 days postcalving, expression of TLR-2 gene was significantly ($P < 0.01$) higher in buffaloes. Expression of TLR-4 gene was significantly ($P < 0.01$) higher on days 7 and 15 postcalving in buffaloes. Expression of IL-8 was significantly ($P < 0.01$) higher on day 15 of postcalving in buffaloes. Increased blood neutrophilic function in buffaloes provides disease resistance to them around peripartum.

Keywords: buffaloes; cows; neutrophil activity; peripartum; gene expression; cortisol

1. Introduction

Buffaloes are found mostly in the Indian subcontinent and some parts of South America, Southern Europe, Middle East and Northern America. In Asia, they contribute significantly towards milk, meat and draft purposes (FAO 2009). Buffaloes are considered to be more resistant to number of infections as compared to the cows. This may be ascribed with number of genetic and non-genetic factors (El-Wishy 2007). The immunological basis behind this has not been completely elucidated in buffaloes in specific during peripartum period (Nanda et al. 2003). The present study was therefore formulated to understand the immune physiological basis of resistance of buffaloes to peripartum infections as compared to the cows by taking neutrophil as the cell of interest and one of the major components of immune axis. Neutrophils were taken as they are considered to be the first line of defence and mediate killing of bacterial pathogens by phagocytosis that is mediated by a cascade of proteases, antimicrobial peptides and free radicals (Segal 2005). Eventually, neutrophils undergo apoptosis in the tissues or at the sites of inflammation. Primary role of neutrophils is the participation in inflammatory response by producing cytokines, eicosanoids and cell signalling molecules. The complex interplay between all these chemokines lead to neutrophilic activity causing host cell protection (Serhan & Savill 2005).

The critical period of calving was selected as neutrophils play a critical role in the onset of disease around parturition (Kehrli et al. 1989). Phagocytic and respiratory burst activity get reduced around parturition (Hoeben et al. 2000) This makes the animals susceptible to mastitis, metritis and retained placenta (Kehrli & Harp 2001). According to Burton et al. (2005), blood neutrophils of cattle exhibit expression of glucocorticoids receptors and these receptors respond to high plasma cortisol concentrations in bringing out altered neutrophil signalling and functioning around parturition. Literature is scanty in indigenous cows and buffaloes regarding the activity of neutrophils in terms of phagocytosis (Dang et al. 2009, 2012). Also, there are no reports on the enzymatic activity and differential expression of neutrophilic genes around calving period in these animals. With this overview, the present study was undertaken to elucidate and compare the neutrophilic activities in both cows and buffaloes around parturition.

2. Materials and methods

2.1. Selection of animals

Twelve indigenous Sahiwal (SW) cows and 12 Murrah (MU) buffaloes in their advance stage of gestation, i.e.,

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at 15 days before the expected date of calving were selected from experimental herd of the National Dairy Research Institute, Karnal, Haryana, India. The cows and buffaloes both were having average milk production of 2000 litres of milk per lactation. All the animals were offered ad lib green fodder and calculated amount of concentrate mixture. Fresh tap water was also made available ad lib at all times of the day. All the experimental animals were healthy and free from any anatomical, physiological and infectious disorders.

2.2. Collection of samples and analysis

Blood samples were collected from all the cows during −15, −7, −5, −3, −2 and −1 days prepartum, on the day of calving and 1, 2, 3, 5, 7 and 15 days postpartum in vacutainers. Calving in all the animals occurred within ± 5 days of the expected date of calving.

Blood total leukocyte counts (TLC) and differential neutrophil counts were estimated microscopically from all the group of animals. In vitro phagocytic activity of blood neutrophils by nitro blue tetrazolium (NBT) assay (Dang et al. 2012) and plasma cortisol levels were also estimated by ELISA (Endocrine Technologies, USA) during both the pre- and postpartum days as indicated above. The minimum detectible concentration of cortisol by this assay was 0.1ng/ml. Coefficient of variation (CV) was calculated from the calculated concentrations. Inter-assay %CV was found to be 2.63 and intra-assay %CV was found to be 0.06.

Activities of enzymes elastase 2, collagenase and cathepsin G were measured by ELISA kits (Wuhan Eiaab Science Co., Ltd., China) from blood samples collected from −7, −3 days prepartum, on the day of calving and on 3 and 7 days postpartum. For preparing lysate of neutrophils, the isolated neutrophils were dissolved in 1 ml PBS. Glass beads were added to neutrophil suspension and shock was given for 25 seconds by Bead beater (Unigenetics Instrument Pvt. Ltd., India). The neutrophil suspension was kept in the ice for 1 minute and then again shock was given for 25 seconds. The suspension was centrifuged at 1000 × g for 10 minutes. Supernatant was taken in 2-ml eppendorf tubes and were stored at −20°C till further estimation. Percent CV was calculated from the calculated concentrations. Inter-assay %CV was found to be 5.38, 4.64, 5.03 and intra-assay %CV was found to be 3.77, 1.08 and 2.69 for elastase, collagenase and cathepsin G, respectively.

2.3. Relative expression of neutrophilic genes

All solutions were prepared using DEPC-treated RNase free plastic wares and water. Total RNA from the blood neutrophils was extracted using Trizol method as per Chonczynski and Sacchi (1987). The RNA pellet was air dried for 15–30 minutes and dissolved in 25 µl of RNA storage solution and stored at −80°C till further use. Quality of RNA was checked by agarose gel electrophoresis using 0.8% gel (in 1× TAE buffer, pH 8.0) of high-quality molecular biology grade agarose (Sigma, USA). Ethidium bromide was used as fluorescence dye at the rate of 0.5 µg/ml of gel, whereas, bromophenol blue was used as tracking dye at the rate of 3 µl mixed with RNA during time of loading of sample into well of the gel. Electrophoresis was carried out at 8 V/cm for half an hour. After completion of electrophoresis, the gel was examined under UV transilluminator. DNase treatment was done by using DNA free Kit (Ambion, UK) according to manufacturers' instructions. Total RNA was quantified, and OD_{260nm}/OD_{280nm} was determined with ND-3300 flurospectrophotomer (NanoDrop Technologies, UT) and purity of RNA was judged on the basis of optical density ratio at 260:280 nm. Reverse transcription was performed from 1µg of RNA using Novagen first strand cDNA synthesis kit (La Jolla, CA).

Real-time PCR for TLR-2, TLR-4 and IL-8 and two housekeeping genes (glyceraldehydes 3-phosphate dehydrogenase [GAPDH] and β-actin) was carried out using Roche Light Cycler-480, Germany. The above housekeeping genes were selected as they had been shown to be the most stably expressed in the neutrophils (Robinson et al. 2007). The sequence information of gene was retrieved from NCBI database and suitable primers were designed using primer-3 web interfaces. Details of primer specification are given in the Table 1. Broadly for each real-time quantitative PCR (qPCR), 1 µg cDNA was added to a 20µl mix containing primers, IQ SYBER-green supermix (Bio-Rad) and nuclease free water. PCR conditions were 300s at 95°C, 45 cycles of 20s at 95°C, 20s at appropriate annealing temperature (Table 1) and 20s at 72°C. A melting curve for each qPCR with a single peak at the correct melting temperature was indicative of reliable and desired PCR product. mRNA abundance on 0 day (a day of parturition) was taken as calibrator to whom relative expression was seen. Calculation was done using the $2^{-\Delta\Delta Ct}$ method (Livak Kenneth & Schmittgen 2001).

2.4. Statistical analysis

Statistical analysis was performed using least square model through SYSTAT software (sigma plot 11.0, Chicago, IL, USA). The model used for analysis was $Y_{ij} = \mu + G_i + D_j + T_i\ (D_j) + E_{ij}$, where Y_{ij} was an observation of dependent variable; μ was the population mean for the variable; G_i was the effect of the group; D_j was the effect days; $G_i\ (D_j)$ was the interaction between the group and days and E_{ij} was the random error associated with observation. The means were separated

Table 1. Specifications for qPCR.

Genes	Sequence (5′→3′)	Acc no.	Size (bp)	Annealing temp (°C)
TLR 2	**F** GCCTTGACCTGTCCAACAAT	NM174197.2	199	59
	R GACCTGAACCAGGAGGATGA			
TLR 4	**F** GGCATCATCTTCATCGTCCT	AY634630.1	178	59
	R CTGGACTCTGGGGTTTACCA			
IL-8	**F** TGCTCTCTGCAGCTCTGTGT	EU276073.1	190	59
	R CAGACCTCGTTTCCATTGGT			
β-actin	**F** TCCCTGGAGAAGAGCTACGA	NM_173979.3	179	59
	R TAGAGGTCCTTGCGGATGTC			
GAPDH	**F** GGGTCATCATCTCTGCACCT	NM_001034034.1	176	59
	R GGTCATAAGTCCCTCCACGA			

and compared using Tukey test as post hoc test, because this test is able to control the errors of multiple comparisons simultaneously. Further, the effect of different treatments on 15 days of prepartum was not used as covariate for subsequent analysis as our main interest was to differentiate the effect of two different treatments.

3. Results

Blood TLC was carried out in cows and buffaloes during both the pre- and postpartum periods. Values of blood TLC increased during the prepartum from day 7 in both the groups of animals with peak observed at calving and then it started decreasing after calving (Table 2). The degree of increase in the TLC level was non-significantly higher in the MU buffaloes as compared to the SW cows. However, MU buffaloes exhibited a rapid reduction in the TLC level after calving. Blood neutrophils percentage also showed an increase on the day of calving. After calving, the neutrophil count decreased rapidly in MU buffaloes and attained the normal basal levels. We also observed an increase in percentage of band neutrophils on the day of calving in both the groups of animals at calving.

Neutrophil PA was estimated in SW cows and MU buffaloes during peripartum period (−15 to +15 days). The PA was represented in terms of formation of formazan crystals and their optical density (Table 3). The results revealed a 2–3 times significant ($P < 0.01$) increase in PA of neutrophils in MU buffaloes as compared to SW cows both during the pre- and postpartum periods. The neutrophilic PA gradually decreased significantly ($P < 0.01$) in both the group of animals at calving. Minimum neutrophilic PA was found to be lowest on the day of calving in MU buffaloes, whereas, PA remained lower even up to two days of postcalving in SW cows.

Plasma cortisol levels were always found to be significantly ($P < 0.01$) higher in SW cows as compared to MU buffaloes during the peripartum period (Table 4). A steady rise in plasma cortisol level was observed in both the group of animals with peak observed on the day of calving. During postpartum period, plasma cortisol levels declined significantly ($P < 0.01$) in both the group of animals on day 1 postcalving. A non-significant increase was seen on day 5 in both the groups. In buffaloes, the cortisol levels then remained same whereas a non-significant increase was again observed even up to day 15 in cows.

The enzymes, elastase and cathepsin G are found in the azurophilic or primary granules, whereas, collagenases are part of specific granules present in the neutrophils (Table 5). The levels of elastase were significantly higher ($P < 0.01$) in MU buffaloes as compared to SW cows. Collagenase levels were non-significantly higher in buffaloes but their levels were found to be significantly higher ($P < 0.01$) at 3 days before calving. Thereafter, these levels remained similar to up to 7 days postpartum. Levels of Cathepsin G, however, were found to be non-significantly higher in SW cows throughout the prepartum period. Levels of all the enzymes decreased significantly ($P < 0.01$) at calving but then came back or even increased than their prepartum levels.

The results of the relative expression of the important neutrophilic genes TLR-2, TLR-4 and IL-8 have been presented in Table 6. At day 15 before calving and on days 7 and 15 after calving, expression of TLR-2 gene were significantly ($P < 0.01$) higher in MU buffaloes as compared to SW cows. Expression of TLR-4 gene were significantly ($P < 0.01$) higher on days 7 and 15 after calving in buffaloes. Expression of IL-8 was non-significantly higher in MU buffaloes at days 15 and 7 of precalving and on the day 15 of postcalving. Expression of all these genes was found to be significantly ($P < 0.01$) lower at calving in both cows and buffaloes.

Table 2. Blood TLC × 10^3 cells/μl in SW and MU buffalo during the pre- and postpartum period.

	Prepartum days							
Cows	−15	−7	−5	−3	−2	−1	0	Overall mean ± SE
SW	7.50 ± 0.16^{a}	7.14 ± 0.26^{c}	7.23 ± 0.24^{dc}	7.70 ± 0.10^{e}*	8.03 ± 0.13^{f}*	8.36 ± 0.14^{g}*	8.81 ± 0.09^{b}*	7.82 ± 0.16*
MU	7.52 ± 0.17^{AG}	7.31 ± 0.15^{AG}	7.38 ± 0.12^{AE}	7.95 ± 0.16^{BE}	8.29 ± 0.13^{BCF}	8.70 ± 0.12^{CD}	9.00 ± 0.12^{D}	8.02 ± 0.14
	Postpartum days							
	0	1	2	3	5	7	15	
SW	8.81 ± 0.09^{a}*	8.50 ± 0.13^{c}*	8.10 ± 0.17^{d}*	7.85 ± 0.16^{e}*	7.69 ± 0.46^{bef}	7.61 ± 0.13^{f}	7.45 ± 0.15^{b}*	8.00 ± 0.18
MU	9.00 ± 0.12^{D}	8.83 ± 0.11^{D}	8.51 ± 0.14^{BD}	8.02 ± 0.12^{EF}	7.69 ± 0.11^{EG}	7.39 ± 0.13^{AG}	7.23 ± 0.13^{A}	8.09 ± 0.12

Values within a row having different superscript are significantly ($P < 0.05$) different from each other.
Values within a column having (*) are significantly ($P < 0.05$) different from each other.

Table 3. Optical density of formazan crystals of blood neutrophils isolated from SW and MU buffalo during the pre- and postpartum period.

	Days prepartum							
Cows	−15	−7	−5	−3	−2	−1	0	Overall mean ± SE
SW	0.23 ± 0.03^{ae}*	0.26 ± 0.05^{ad}*	0.37 ± 0.08^{c}*	0.37 ± 0.06^{c}*	0.28 ± 0.02^{d}*	0.25 ± 0.05^{de}*	0.19 ± 0.02^{b}*,**	0.28 ± 0.04*
MU	0.77 ± 0.08^{ABC}	0.87 ± 0.08^{AB}	0.70 ± 0.06^{BCD}	0.64 ± 0.03^{CD}	0.57 ± 0.04^{CD}	0.53 ± 0.03^{CD}	0.45 ± 0.03^{D}	0.65 ± 0.05
	Days postpartum							
	0	1	2	3	5	7	15	
SW	0.19 ± 0.02^{a}*	0.16 ± 0.04^{a}*	0.16 ± 0.03^{a}*	0.27 ± 0.03^{c}*	0.30 ± 0.02^{c}*	0.39 ± 0.06^{b}*	0.41 ± 0.05^{b}*,**	0.27 ± 0.04*
MU	0.45 ± 0.03^{D}	0.50 ± 0.06^{D}	0.61 ± 0.07^{BD}	0.64 ± 0.04^{BD}	0.73 ± 0.05^{ABC}	0.81 ± 0.05^{AB}	0.96 ± 0.06^{A}	0.67 ± 0.05

Values within a row having different superscript are significantly ($P < 0.05$) different from each other whereas, **indicate difference was highly significant ($P < 0.001$).
Values within a column having (*) are significantly ($P < 0.05$) different from each other.

Table 4. Plasma cortisol levels (ng/ml) in SW and MU buffalo during the pre- and postpartum period.

	Days prepartum							
Cows	−15	−7	−5	−3	−2	−1	0	Overall mean ± SE
SW	7.40 ± 0.73^{a}*	9.05 ± 1.09^{ce}*	9.08 ± 0.88^{df}*	9.53 ± 0.89^{cd}*	7.94 ± 0.90^{a}*	8.12 ± 0.49^{aef}*	12.47 ± 0.75^{b}*,**	9.08 ± 0.82*
MU	3.73 ± 0.22^{AB}	3.97 ± 0.33^{AB}	3.07 ± 0.45^{B}	4.76 ± 0.32^{ABC}	5.34 ± 0.32^{C}	3.77 ± 0.60^{AB}	9.62 ± 0.39^{D}	4.89 ± 0.38
	Days postpartum							
	0	1	2	3	5	7	15	
SW	12.47 ± 0.75^{a}*	6.05 ± 0.60^{c}*	7.29 ± 0.81^{ab}*	8.27 ± .07^{bd}*	8.85 ± 0.77^{d}*	5.63 ± 1.30^{c}	7.31 ± 0.81^{b}*	7.98 ± 0.87*
MU	9.62 ± 0.39^{D}	3.87 ± 0.43^{ACE}	2.91 ± 0.25^{BE}	3.62 ± 0.43^{AB}	4.65 ± 0.37^{AB}	4.23 ± 0.14^{AB}	4.50 ± 0.42^{AC}	4.77 ± 0.35

Values within a row having different superscript are significantly ($P < 0.05$) different from each other whereas, **difference was highly significant ($P < 0.001$).
Values within a column having (*) are significantly ($P < 0.05$) different from each other.

4. Discussion

Values of blood TLC and neutrophil counts observed around the peripartum period in cows and buffaloes were within the normal range as reported by Meglia et al. (2001) in exotic cows and by Abd Ellaha et al. (2013) in midterm pregnant buffaloes. Antepartum rise in cortisol levels increases TLC around calving, whereas, after calving TLC decreases in blood. This is associated with migration and recruitment of blood neutrophils towards uterine lumen and mammary tissues (Preisler et al. 2000). Around calving neutrophils are released in large amounts due to stimulation of red bone marrow by higher concentration of cortisol as also observed in this study.

Neutrophils are first cell to migrate to the site of inflammation and carry out phagocytosis through a chain of events that mediate the intracellular digestion by lysosomal enzymes and the key events like respiratory burst (Jain 1986). We observed decreased PA of neutrophils around calving. Diminished neutrophil functions and compromised host resistance mechanisms during peripartum period in dairy cows have also been observed by Meglia et al. (2001) and Dang et al. (2012). Poor activity of neutrophils may be due to more numbers of immature neutrophils, which are coming in circulation and have no proper machinery to fight against phagocytose infection.

Parturition reflex causes higher plasma cortisol level that causes hyper stimulation of red bone marrow for the faster release of neutrophils (Table 7). As a result of this, there is release of more number of immature band neutrophils and a less number of matured segmented neutrophils. That is why the phagocytic activity of neutrophils decreases as evident in our study (Paape et al. 2003). During prepartum period (15 day before calving) animals are in dry stage, so there is no stress of milk production but at parturition animals have to face stress of calving, synthesise colostrum (up to 3 days) and milk. According to Burton et al. (2005), glucocorticoids are a class of steroid hormones that bind to the glucocorticoid receptor, and are part of the feedback

Table 5. Elastase, collagenase and cathepsin G enzymes levels (ng/ml) of blood neutrophils isolated from SW and MU buffalo during the peripartum period.

		Days peripartum					
Enzymes (ng/ml)	Cows	−7	−3	0	3	7	Overall mean ± SE
Elastase 2	SW	34.06 ± 2.10^{a}*	53.61 ± 5.93^{b}*	31.03 ± 2.36^{a}*	50.24 ± 9.44^{b}	35.21 ± 4.32^{a}*	40.83 ± 4.83*
	MU	68.47 ± 7.35^{A}	72.29 ± 7.41^{A}	43.26 ± 2.80^{B}	78.19 ± 15.77^{A}	61.61 ± 9.49^{A}	64.76 ± 8.56
Collagenase	SW	6.58 ± 0.59^{a}	5.69 ± 0.68^{a}*	2.97 ± 0.39^{b}*	9.23 ± 0.41^{c}*	15.15 ± 0.50^{d}*	7.92 ± 0.52
	MU	6.86 ± 0.71^{A}	10.38 ± 1.08^{BD}	4.02 ± 0.67^{C}	10.50 ± 0.88^{D}	15.17 ± 0.67^{E}	9.39 ± 0.80
Cathepsin G	SW	14.27 ± 1.01^{ab}*	11.92 ± 0.64^{bd}	6.29 ± 0.97^{c}	10.34 ± 0.74^{d}	16.82 ± 0.45^{a}	11.93 ± 0.76
	MU	12.00 ± 1.60^{AB}	11.22 ± 0.66^{B}	5.05 ± 0.46^{C}	10.46 ± 0.84^{B}	15.65 ± 0.85^{A}	10.88 ± 0.88

Values within a row having different superscript are significantly ($P < 0.05$) different from each other.
Values within a column having (*) are significantly ($P < 0.05$) different from each other.

Table 6. Relative expression of TLR- 2, TLR-4 and IL-8 genes of blood neutrophils isolated from SW and MU buffalo during the peripartum period.

		Peripartum days				
Genes	Cows	−15	−7	0	7	15
TLR-2	SW	5.26 ± 0.44^{a}	4.31 ± 0.36^{ac}*	1.09 ± 0.16^{b}	2.70 ± 0.49^{c}*	4.90 ± 0.38^{a}*
	MU	6.35 ± 0.49^{A}	3.62 ± 0.40^{B}	1.01 ± 0.10^{C}	4.10 ± 0.42^{B}	6.75 ± 0.78^{A}
TLR-4	SW	5.61 ± 0.45^{a}	5.78 ± 0.63^{a}*	1.04 ± 0.10^{b}	1.77 ± 0.47^{b}*	4.80 ± 0.60^{a}*
	MU	6.11 ± 0.59^{A}	3.67 ± 0.71^{A}	1.03 ± 0.15^{B}	4.45 ± 0.52^{A}	6.10 ± 0.72^{A}
IL-8	SW	4.01 ± 0.62^{ab}	2.90 ± 0.33^{bd}	1.07 ± 0.12^{c}	3.60 ± 0.45^{ad}*	5.22 ± 0.42^{a}*
	MU	4.39 ± 0.52^{A}	3.37 ± 0.43^{A}	1.07 ± 0.16^{B}	2.28 ± 0.36^{B}	6.09 ± 0.42^{A}

Note: Day of parturition was taken as calibrator.
Values within a row having different superscript are significantly ($P < 0.05$) different from each other.
Values within a column having (*) are significantly ($P < 0.05$) different from each other.

mechanism in the immune system that down regulate the immune activity. We observed a significant increase in the level of cortisol on the day of calving as compared to 15 days pre- and postpartum. High levels of cortisol at calving have also been reported (Goff & Horst 1997) to act as powerful immunosuppressive agent. According to Kehrli et al. (1991), overall effects of stress increased cortisol level, produced neutrophilia with decreased functional capacity of neutrophils and immunosuppression. As a result the cows become less resistant to mastitis and other infections around calving.

Neutrophils mediate phagocytosis through a complex cascade of enzymes and their interrelated pathways. The release of enzymes is specifically regulated by cytokine network and their signalling to neutrophils via cytokine receptors. Elastase 2, collagenase and cathepsin G are granular enzymes that are stored in neutrophil cytoplasm. The timely and net release of these enzymes determines the ultimate fate of neutrophiil activities in terms of phagocytosis and resolution of inflammatory cascades. Granules are present in neutrophils and are store house of variety of enzymes that are released to extra cellular space during inflammation and mediate pathogen inactivation and killing. Soluble agents like fMLP (N-formyl-methionine-leucine-phenylalanine), chemotaxins and C5a are the regulators of granule release from neutrophils. Neutrophils release elastase and cathepsin, which are serine proteases during inflammation to bring out destruction of pathogens (Belaaouaj et al. 2000). Decreased neutrophil enzyme levels observed in this study may be because at calving more immature neutrophils are released that are poor in synthesis of granular enzymes as well as due to high cortisol are not able to release granular enzymes.

We observed the expression of toll-like receptor 2 and 4 in neutrophils. These receptors detect pathogen by a variety of pattern-recognition systems and are likely to have important roles in the regulation of neutrophil function (Sabroe et al. 2003; Parker et al. 2005). TLR-2 and TLR-4 are commonly known as pattern recognisation molecules/receptors. These are key in cytokine-modulated signalling after antigenic exposure and expression of these genes were also up-regulated during diseases conditions like endometritis (Patraab et al. 2013). All together, these mediate phagocytic activities of neutrophils. Similar role is played by IL-8, which is considered as the central regulator of neutrophil signalling (Burton et al. 2005). During the study, we noticed that buffalo blood neutrophils are more immuneocompetent as compared to cow blood neutrophils. The relative phagocytic activity of MU buffaloes was higher both morphologically and also at molecular levels. Increased neutrophilic activities are associated with consequent reduction in buffalo susceptibility to postparturient infections. This also makes MU buffaloes more resistant to infections as compared to SW cows.

We are the first to report about the enzyme concentrations and activities of neutrophils during peripartum in Sahiwal cows and MU buffaloes. It is usually observed that, high-producing dairy animals undergo a severe immune suppression due to a multifactorial regime of metabolic and physiological alterations during pregnancy and in specific during parturition. The internal milieu of hormones, metabolites, cell receptors all get compromised that is either elevated or diminished. These are the indirect predictors of future infections during peripartum. Our study focused on some of the key factors of neutrophils both in terms of their activities and relative gene expressions. Study concluded that MU buffaloes are less exposed to parturition associated stress; secondly immunocompetency of MU blood neutrophils is higher as compared to SW blood neutrophils and that is why MU buffaloes are less prone to infections after parturition.

Our study also indicated a down regulation of blood PMN expression of immune genes due to increased endogenous blood plasma cortisol during peripartum period in both crossbred and indigenous breed of cows. We observed a significantly higher expression of TLR-2,

Table 7. Blood neutrophil counts (%) in SW and MU buffalo during the pre- and postpartum period.

	Prepartum days								
Cows	−15	−7	−5	−3	−2	−1	0s	Overall mean ± SE
SW	29.25 ± 0.72^{a}	29.75 ± 0.40^{a}	30.67 ± 0.52^{c}	30.84 ± 0.47^{d}*	31.25 ± 0.40^{de}*	33.17 ± 0.70^{f}*	35.67 ± 0.46^{b}*	31.51 ± 0.52*
MU	29.18 ± 0.70^{AB}	30.45 ± 0.55^{ABC}	31.97 ± 0.41^{AE}	32.83 ± 0.50^{AB}	32.25 ± 0.38^{BCE}	34.77 ± 0.80^{CE}	37.31 ± 0.49^{D}	32.68 ± 0.54
	Postpartum days							
	0	1	2	3	5	7	15	
SW	37.63 ± 0.46^{a}*	33.92 ± 0.37^{c}*	32.75 ± 0.62^{de}*	32.34 ± 0.37^{e}*	30.33 ± 0.65^{f}*	30.25 ± 0.64^{gf}*	30.92 ± 0.51^{bef}	32.45 ± 0.52*
MU	37.77 ± 0.49^{D}	32.72 ± 0.43^{E}	32.05 ± 0.40^{AE}	31.13 ± 0.53^{AB}	29.23 ± 0.65^{ABC}	29.43 ± 0.50^{A}	28.12 ± 0.75^{A}	32.45 ± 0.56

Values within a row having different superscript are significantly ($P < 0.05$) different from each other.
Values within a column having (*) are significantly ($P < 0.05$) different from each other.

TLR-4 and IL-8 on 15 days before and after calving as compared to the day of calving. During periparturient period, animal experiences negative energy balance from 3 days before to 3 days after calving (Ingvartsen & Andersen 2000). The higher level of cortisol helps to provide energy demand by increasing lypolysis and gluconeogenesis, which results in an increase in the ratio of unsaturated fatty acid to saturated fatty acid. Saturated fatty acid induces the activation of TLR-2 and 4, whereas, unsaturated fatty acids inhibits it (Lee Joo & Hwang Daniel 2006). Decreased expression of these genes during calving might be due to increased levels of unsaturated fatty acids. Further, our results also showed that cortisol is negatively correlated with all neutrophilic functions like PA, enzymatic activity and expression levels of TLR-2, TLR-4 and IL-8.

5. Conclusions

The current study was designed to evaluate the relative competency of blood neutrophils in SW cows and MU buffaloes during peripartum period. The range experiments conducted during the period were mainly confined to understand some of the basic features of blood neutrophils in terms of their activities and gene expression that are associated with the regulation of immune physiological responses. We observed an increase in the PA of buffalo blood neutrophils as compared to SW cows. The relative lower circulating concentration of cortisol is another determining factor that regulated higher PA of buffalo neutrophils. Higher content of neutrophilic enzymes in the MU buffalo neutrophils strongly supported that immunocompetency of MU neutrophils is more than that of SW cows. Eventually, we also reported an increase in the mRNA expression of TLR-2, TLR-4 and IL-8 genes. Altogether, these findings make us to frame a conclusion that the buffalo neutrophils are more potent as compared to the SW cow neutrophils. It can be a probable explanation behind the fact that buffaloes are more resistant to infections during transition period as compared to the cows. This study although carried out on some of the neutrophilic functions, clearly indicated the degree of immune suppression occurring in two different species around the peripartum. These results will help in understanding the physiology of neutrophils at calving and help to develop strategies to improve the immune functions around this period. Also, further studies are required to employ genetic and proteomic tools to find out the exact mechanism of neutrophil action in buffaloes.

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