Potential roles of neutrophils in maintaining the health and productivity of dairy cows during various physiological and physiopathological conditions: a review

Mohanned Naif Alhussien & Ajay Kumar Dang

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REVIEW

Potential roles of neutrophils in maintaining the health and productivity of dairy cows during various physiological and physiopathological conditions: a review

Mohanned Naif Alhussien [1,2] · Ajay Kumar Dang [2]

Abstract
Neutrophils represent the first line of innate immunity and are the most prominent line of cellular defence against invading microorganisms. On stimulation, they can quickly move through the walls of veins and into the tissues of the body to immediately attack or monitor the foreign antigens. Neutrophils are highly versatile and sophisticated cells which are endowed with highly sensitive receptor-based perception systems. They were traditionally classified as short-lived phagocytes actively involved during infection and inflammation, but recently, it has been seen that neutrophils are capable of detecting the presence of sperms during insemination as well as an implanting embryo in the female reproductive tract. These specialised phagocytes play a major role in tissue remodelling and wound healing, and maintain homeostasis during parturition, expulsion of placenta, folliculogenesis, corpus luteum formation and luteolysis. Here, we review the role played by neutrophils in maintaining homeostasis during normal and inflammatory conditions of dairy cattle. We have summarised the alteration in the expression of some cell adhesion molecules and cytokines on bovine neutrophils during different physiological and physiopathological conditions. Some emerging issues in the field of neutrophil biology and the possible strategies to strengthen their activity during the period of immunosuppression have also been discussed.

Keywords Neutrophil activity · Expression · Tissue remodelling · Homeostasis · Inflammatory disease

Background

Neutrophils are the primary innate immune cells associated with clearing bacterial infections from the body. Neutrophils are about 200 billion in an adult cow; half of them are always circulating in the blood and the other half stored in the bone marrow. They are the frontline defenders or the first cells to be activated and thus are key contributors to acute inflammation. During steady-state, neutrophils maintain homeostasis, but during an emergency, they show granulopoiesis and interact with other cells of the adaptive immune system [1, 2]. Under inflammatory conditions, their number, phagocytic and chemotactic activity, expression of surface adhesion molecules and pattern recognition receptors get modulated [3–5]. The intracellular granules of neutrophil contain different bactericidal peptides including defensins, myeloperoxidase, and neutral and acidic proteases (e.g. elastase; various types of cathepsin, procathepsins and matrix metalloproteinases), which can efficiently kill a variety of invading pathogens [6, 7]. Moreover, neutrophils secrete many cytokines and chemokines which can influence the whole inflammatory process and the immune response [8, 9]. Although neutrophils were considered as rigid and preprogrammed immune cells, recent studies have suggested extensive plasticity in their functions [10–13]. They have emerged as important mediators between the innate and adaptive immune systems. They are a major player in tissue remodelling and help in homeostasis maintenance by disposing of apoptotic cells and phagocytising foreign particles [14, 15]. The functional versatility of neutrophil is mediated by a vast array of receptors capable of recognising a variety of foreign and endogenous

✉ Mohanned Naif Alhussien
mohannadalhussien@gmail.com

Ajay Kumar Dang
rajadang@rediffmail.com

[1] Animal Production Division, Agricultural College, Aleppo University, Aleppo, Syrian Arab Republic

[2] Lactation and Immuno-Physiology Laboratory, ICAR-National Dairy Research Institute, Karnal, Haryana 132 001, India

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ligands and by stimulating different immune responses based on the activated receptor and the detected ligand [16]. Recent advances in microscopic technologies have improved our understanding of neutrophils trafficking from blood vessels to various organs, their morphology and their role in regulating the overall immune response [17]. In this review, we have focused on some of the latest breakthrough discoveries in the role played by neutrophils in maintaining health, reproduction and milk production of dairy cows during various physiological and physiopathological conditions. Ultimately, a better understanding of the neutrophils response to different challenges experienced by dairy animals during their production cycle will help to develop effective neutrophil-targeted therapies for these animals.

Neutrophil-related molecules, their role and modulation during various physiological and physiopathological conditions

Neutrophils have a large number of receptors on their surface such as G protein-coupled chemokine and chemoattractant receptors, Fc receptors, adhesion receptors, cytokine and innate immune receptors [18]. There is always crosstalk between neutrophils and the surrounding environment which depends primarily on various receptors present on the surface of neutrophils and other inflammatory cytokines released either by them or by other cells. In the present review, the receptors and genes that were reported extensively in bovine neutrophils were addressed. Among the receptors, we have addressed and summarised cell adhesion molecules (CD11b, CD18, CD44, CD62L), pattern recognition receptor (CD14) and interleukin-2 receptor alpha (CD25) (Table 1). Chemokine receptors (CXCR1, CXCR2), Toll-like receptors (TLR2, TLR4) and the glucocorticoid receptor (GRα) have also been discussed (Table 2). Among the genes, we have reviewed several pro- and anti-inflammatory cytokines, including interleukins (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10 and IL-12), tumour necrosis factor (TNF)-α and interferon (IFN)-γ (Table 2). Milk neutrophils are the most abundant somatic cells (SC) during subclinical mastitis, mastitis and around calving [21, 22, 64, 65]. Therefore, we have also included the studies in which the whole milk SC were used to test the expression of these genes and receptors.

There is minor modulation in the expression of both CD11b and CD18 during pregnancy, calving and postpartum [50, 66]. However, their expression dramatically increases in response to inflammatory conditions such as mastitis and metritis [4, 20], but not during retained placenta [28]. This reflects the importance of these adhesion molecules in neutrophil migration during a pathogenic infection. Zoldan et al. [41] reported promising results about the expression of CD25 which was positively correlated with disease severity during postpartum and early lactation. During the normal course of lactation and pregnancy, there is no alteration in the expression of CD62L. Unlike CD11b, the expression of CD62L is independent of pathogen presence and gets downregulated during stressful physiological or physiopathological conditions such as calving, retained placenta and mastitis [21, 28, 67]. Several studies have reported an inverse relationship between plasma cortisol and its ligand during normal physiology as well as inflammatory diseases [4, 38, 50]. The expression of TLRs is low around calving which reflects attenuate immune response and higher chances of cows developing health disorders during this critical period [52]. It is more evident that the expression of both TLR2 and TLR4 is regulated by the type of the infection causing pathogens since TLR2 is associated with gram-positive bacterial infections while TLR4 is associated with gram-negative bacterial infections [34, 55]. The binding between the chemokine ligand and its chemokine receptor is complex since multiple ligands can bind a single receptor and vice versa. This system has been proved to play a critical role in both homeostasis and inflammatory conditions by controlling the activation, migration, differentiation and survival of leukocytes [68]. Homeostatic chemokines are constitutively expressed to mediate basal leukocyte migration, whereas the inflammatory chemokines are upregulated in response to cell stimulation viz., by cytokines or pathogens [69]. Although multiple chemokines can bind to the chemokine receptors (CXCR1 and CXCR2), and there is ample evidence that the preferential engagement of either chemokine receptor leads to functional polarisation, the interaction of these ligands (except IL-8) with the chemokine receptors is poorly investigated in bovine neutrophils.

Neutrophils have also been reported to exhibit reverse transmigration and can re-enter the circulation [70]. Sagiv et al. [11] hypothesised that the exact origin of recruited neutrophils is unknown. They reported two subsets of neutrophils: N1, i.e. tumour-associated neutrophils (TANs) originating from high-density circulating neutrophils during early tumour formation, whereas N2 (TAN) are low-density neutrophils which are immunosuppressive and accumulate with tumour progression. Takashima and Yao [71] found that neutrophils acquire surface expression of class II major histocompatibility complex (MHC II), costimulatory molecules and other surface markers of dendritic cells when cultured in the presence of specific cytokines such as granulocyte/macrophage colony-stimulating factor, TNF-α and IL-4. To check the plastic nature of neutrophils, Silva et al. [72] challenged the neutrophils with *Mycobacterium tuberculosis* and found that the N1 group shows an increase of IL-8, IL-1-β, INF-γ and the formation of neutrophil extracellular traps (NETs). On the other hand, N2 profile revealed a decline of these inflammatory cytokines, an increase of IL-4 and transforming growth factor beta (TGF-β) and do not form NETs. According to Hong [73], subsets of neutrophils might

Table 1 Alteration in the expression of various cell adhesion molecules (CD11b, CD18, CD44, CD62L), pattern recognition receptor (CD14) and interleukin-2 receptor alpha (CD25) on bovine neutrophils during different physiological and physiopathological conditions

Receptor	Physiological stage	Cell	Exp	References	Physiopathological stage	Cell	Exp	References
CD11b	Calving and postpartum	B PMN	NA	Diez-Fraile et al. [19]	*Escherichia coli* (*E. coli*) and *Arcanobacterium pyogenes* (*A. pyogenes*) endometritis	B PMN	Up	Zerbe et al. [20]
	Pregnancy	B PMN	Down	Alhussien et al. [21]	*Streptococcus uberis* (*S. uberis*) mastitis	B PMN	Up	Smits et al. [22]
	Pregnancy	M PMN	NA	Alhussien et al. [21]	*E. coli* mastitis	B,M PMN	Up	Diez-Fraille et al. [23]
	Embryo implantation	B PMN	Down	Bhat et al. [24]	Muramyldipeptide and lipopolysaccharide (LPS) induced mastitis	M PMN	Up	Langrova et al. [25]
	Embryo implantation	B PMN	NA	Manjari et al. [26]	*Streptococcus dysgalactiae* (*S. dysgalactiae*) mastitis	M PMN	Up	Blagitz et al. [27]
	Retained foetal membrane	B PMN	NA	Pathak et al. [28]	Bovine leukaemia virus (BLV) infection	M PMN	NA	Della Libera et al. [29]
					Staphylococcus aureus (*S. aureus*) mastitis	B,M PMN	Up	Alhussien et al. [21]
CD14	Calving and postpartum	B PMN	Up	O'Driscoll et al. [30]	*E. coli* mastitis	B,M PMN	Down	Paape et al. [31]
	Calving and postpartum	B PMN	NA	Crookenden et al. [32]	*S. aureus* and *S. uberis* mastitis	B PMN	Up	Sladek and Rysanek [33]
					Muramyldipeptide and LPS-induced mastitis	M PMN	Down	Langrova et al. [25]
					E. coli mastitis	B PMN	NA	Worku and Morris [34]
					Corynebacterium bovis (*C. bovis*) mastitis	M PMN	Down	Blagitz et al. [35]
CD18	Calving/postpartum	B PMN	Up/NA	Lee and Kehrli [36]	*E. coli* mastitis	B PMN	Up	Shuster et al. [37]
	Calving	B PMN	Down	Burton et al. [38]	*S. uberis* mastitis	B PMN	Up	Smits et al. [39]
	Calving and postpartum	B PMN	NA	O'Driscoll et al. [30]	*S. aureus* mastitis	B PMN	NA	Nagahata et al. [40]
					S. aureus mastitis	M PMN	Up	Nagahata et al. [40]
CD25	Calving	B,M PMN	Up	Zoldan et al. [41]	Metritis, mastitis	B,M PMN	Up	Zoldan et al. [41]
	Retained foetal membrane	B,M PMN	Up	Zoldan et al. [41]	*Mycobacterium bovis* infection	B L (PMN:?)	Up	Waters et al. [42]
					S. dysgalactiae mastitis	M L (PMN:?)	NA	Blagitz et al. [27]
CD44	Calving/postpartum	B PMN	Up/NA	Crookenden et al. [32]	Mastitis	M PMN	Up	Gonen et al. [43]
					S. aureus mastitis	B PMN	Up	Swain et al. [44]
					S. aureus mastitis	M PMN	Down	Swain et al. [44]
					BLV infection	M PMN	Down	Della Libera et al. [29]
CD62L	Calving/postpartum	B PMN	Down/NA	Lee and Kehrli [36]	*Mycobacterium bovis* infection	B L (PMN:?)	Down	Waters et al. [42]
	Calving/postpartum	B PMN	Down/Up	Weber et al. [45]	*E. coli* mastitis	B,M PMN	Down	Diez-Fraille et al. [23]

Table 1 (continued)

Receptor	Physiological stage	Cell	Exp	References	Physiopathological stage	Cell	Exp	References
	Postpartum	B PMN	Down	Li et al. [46]	*S. aureus* mastitis	B PMN	NA	Nagahata et al. [40]
	Embryo implantation	B PMN	Down	Bhat et al. [24]	*S. aureus* mastitis	M PMN	Up	Nagahata et al. [40]
	Embryo implantation	B PMN	NA	Manjari et al. [26]	*S. aureus* mastitis	M PMN	NA	Alhussien et al. [47]
	Pregnancy	B,M PMN	NA	Alhussien et al. [21]	BLV infection	M PMN	NA	Della Libera et al. [29]
	Retained foetal membrane	B PMN	Down	Pathak et al. [28]				

Expression of neutrophils during calving and postpartum was compared with prepartum period, lactation stages were compared among each other, embryo implantation was compared with the day of artificial insemination (AI: day 0) and various physiopathological diseases are compared with healthy disease-free groups. Wherever the mastitis-causing pathogen is not mentioned, it means that multiple mastitis-causing pathogens were involved in the study. M, milk; B, blood; PMN, polymorphonuclear neutrophils; L, lymphocyte; Exp, expression; Up, upregulated; Down, downregulated; NA, not altered

merely be a reflection of physiological changes in neutrophils during pathological conditions rather than distinct subsets capable of de novo synthesis of cytokines and recirculate through different tissues and organs. In humans and mice, it is clear that neutrophils response to the external cues by adhesion, de-adhesion, change in their shape, transmigration, effector functions of cell activation, pro-inflammatory mediator release and target cell killing. But these receptors, genes and their complex, intracellular signal transduction pathways are poorly addressed in bovine neutrophils and understanding these receptors and their activation steps may help to develop novel therapeutic strategies for controlling inflammation in the bovines.

Role of neutrophils under normal physiological conditions

Under normal physiological conditions, neutrophils are capable of exhibiting a vast number of specialised functions which have been discussed under various subheadings below:

Ovarian function (folliculogenesis, corpus luteum formation and regression)

Folliculogenesis describes the growth and development or atresia of follicles. It involves a series of morphological and functional stages that can be seen during the progression of primordial follicles into large preovulatory follicles. After ovulation, the ovulatory follicle undergoes morphological changes and forms the corpus luteum (CL) which acts as a source of progesterone if pregnancy occurs otherwise it will undergo luteolysis [74]. Initially, it was thought that immune cells are involved in the destruction of the luteal tissue during regression of CL through a variety of defence mechanisms, including phagocytosis [75]. Later on, it was revealed that various immune cells have an essential role in maintaining the health and functionality of both ovarian follicles and CL via the local secretion of modulating cytokines [14, 15, 76]. Moreover, the CL has been suggested as an excellent model for a better understanding of immune cell regulation of tissue homeostasis [77]. High concentrations of various cytokines and chemokines are found in the follicular fluid during the preovulatory phase which causes massive infiltration of neutrophils in the preovulatory follicle at the time of ovulation in humans [78]. The possible role of neutrophils during ovulation was studied by depletion of neutrophils in rats by administration of a monoclonal antibody against neutrophils, and it reduced ovulation in them [79].

Neutrophils have been reported as a potential regulator of angiogenesis in developing CL of the cow [80]. The extracellular matrix (ECM) is essential for angiogenesis and tissue remodelling and can be cleaved by matrix metalloproteinases

Table 2 Alteration in the expression of various chemokines, toll-like and glucocorticoid receptors as well as cytokines on bovine neutrophils during different physiological and physiopathological conditions

Receptor/ gene	Physiological stage	Cell	Exp	References	Physiopathological stage	Cell	Exp	References
CXCR1	Calving/postpartum	B PMN	NA/Down	Crookenden et al. [32]	*E. coli* mastitis	B PMN	Up	Leyva-Baca et al. [48]
	Calving	B,M PMN	Up	Alhussien et al. [21]	*S. dysgalactiae* mastitis	M SC	Up	Beecher et al. [49]
	Early and late lactation	M PMN	Up	Alhussien and Dang [50]	*Staphylococcus chromogenes* mastitis	M SC	Up	Verbeke et al. [51]
	Mid-lactation	M PMN	Down	Alhussien and Dang [50]	*S. aureus* mastitis	B,M PMN	Up	Alhussien et al. [21]
	Pregnancy	B,M PMN	NA	Alhussien et al. [21]	*Streptococcus agalactiae* (*S. agalactiae*) mastitis	M PMN	Up	Alhussien and Dang [4]
CXCR2	Calving	B PMN	Down	Burton et al. [38]	*S. aureus* mastitis	B,M PMN	Up	Alhussien et al. [21]
	Calving and postpartum	B PMN	NA	Crookenden et al. [32]	*E. coli* mastitis	M PMN	Up	Alhussien and Dang [4]
	Early and late lactation	M PMN	Up	Alhussien and Dang [50]				
	Pregnancy	B,M PMN	NA	Alhussien et al. [21]				
TLR2	Calving/postpartum	B PMN	Down/NA	Pathan et al. [52]	*S. aureus* mastitis	B,M PMN	Up	Swain et al. [53]
	Calving/postpartum	B PMN	Up/Down	Crookenden et al. [32]	*E. coli* mastitis	B PMN	NA	Moyes et al. [54]
	Early lactation	M PMN	Up	Alhussien and Dang [50]	*C. bovis* mastitis	M PMN	Down	Blagitz et al. [35]
	Mid and late lactation	M PMN	Down	Alhussien and Dang [50]	*S. agalactiae* mastitis	M SC	Up	Fonseca et al. [55]
TLR4	Calving/postpartum	B PMN	Down/NA	Pathan et al. [52]	*E. coli* mastitis	B PMN	Up	Worku and Morris [34]
	Postpartum	B PMN	Down	Li et al. [46]	*S. dysgalactiae* mastitis	M SC	Up	Beecher etal. [49]
	Calving and postpartum	B PMN	NA	Crookenden et al. [32]	*S. aureus* mastitis	B,M PMN	Up	Swain et al. [53]
	Early and late lactation	M PMN	Down	Alhussien and Dang [50]	*C. bovis* mastitis	M PMN	NA	Blagitz et al. [35]
	Mid-lactation	M PMN	Up	Alhussien and Dang [50]	*S. agalactiae* mastitis	M SC	Up	Fonseca et al. [55]
GRα	Calving/postpartum	B PMN	Down/NA	Preisler et al. [56]	*E. coli*, *S. agalactiae* and *S. aureus* mastitis	M PMN	Down	Alhussien and Dang [4]
	Early lactation	M PMN	Down	Alhussien and Dang [50]				
	Mid and late lactation	M PMN	Up	Alhussien and Dang [50]				
	Retained foetal membrane	B PMN	Down	Pathak et al. [28]				
IL-1β	Postpartum	B PMN	Up	Zhou et al. [57]	*S. aureus* mastitis	M SC	Up	Riollet et al. [58]
					S. dysgalactiae mastitis	M SC	Up	Beecher et al. [49]
					S. agalactiae mastitis	M SC	Up	Fonseca et al. [55]
IL-2	Calving and postpartum	PBMC;PMN?	Up	Patra et al. [59]	Mastitis and postpartum metritis	B leucocytes	Down	Sato [60]
					S. aureus mastitis	M SC	NA	Riollet et al. [58]
					Mastitis	M SC	NA	Fonseca et al. [61]
					Metritis, endometritis	PBMC;PMN?	Down	Patra et al. [59]
IL-4	Calving and postpartum	PBMC:PMN?	Up	Patra et al. [59]	Metritis, endometritis	PBMC:PMN?	Down	Patra et al. [59]
					S. aureus mastitis	M SC	NA	Riollet et al. [58]

Table 2 (continued)

Receptor/ gene	Physiological stage	Cell	Exp	References	Physiopathological stage	Cell	Exp	References
					Mastitis	M SC	NA	Fonseca et al. [61]
IL-6	Calving/postpartum	B PMN	Up/NA	Crookenden et al. [32]	*S. aureus* mastitis	M SC	Up	Riollet et al. [58]
					E. coli or *S. aureus* mastitis	M SC	Up	Lee et al. [62]
					Mastitis	M SC	NA	Fonseca et al. [61]
IL-8	Calving/postpartum	B PMN	Down/NA	Pathan et al. [52]	*E. coli* mastitis	B PMN	Up	Worku and Morris [34]
	Calving	B,M PMN	Up	Alhussien et al. [21]	*S. aureus* mastitis	B,M PMN	Up	Alhussien et al. [21]
	Embryo implantation	B PMN	NA	Manjari et al. [26]	*S. dysgalactiae* mastitis	M SC	Up	Beecher et al. [49]
	Pregnancy	B,M PMN	NA	Alhussien et al. [21]	*S. agalactiae* mastitis	M PMN	Up	Alhussien and Dang [4]
IL-10	Calving/postpartum	B PMN	Up/NA	Crookenden et al. [32]	*S. aureus* mastitis	M SC	NA	Riollet et al. [58]
	Embryo implantation	B PMN	NA	Shirasuna et al. [63]	Mastitis	M SC	NA	Fonseca et al. [61]
IL-12	Calving and postpartum	B PMN	NA	Crookenden et al. [32]	*E. coli* or *S. aureus* mastitis	M SC	Up	Lee et al. [62]
					S. agalactiae mastitis	M SC	Up	Fonseca et al. [55]
TNF-α	Postpartum	B PMN	NA	Zhou et al. [57]	*E. coli* or *S. aureus* mastitis	M SC	Up	Lee et al. [62]
	Calving/postpartum	B PMN	Down/Up	Crookenden et al. [32]	*S. dysgalactiae* mastitis	M SC	Up	Beecher et al. [49]
	Embryo implantation	B PMN	NA	Shirasuna et al. [63]				
IFN-γ	Calving/postpartum	B PMN	Down/NA	Crookenden et al. [32]	Metritis, endometritis and delayed uterine involution	PBMC;PMN?	Down	Patra et al. [59]
					S. aureus mastitis	M SC	NA	Riollet et al. [58]
					E. coli mastitis	M SC	Up	Lee et al. [62]

Expression of neutrophils during calving and postpartum was compared with prepartum period, lactation stages were compared among each other, embryo implantation was compared with the day of artificial insemination (AI: day 0) and various physiopathological diseases are compared with healthy disease-free groups. Wherever the mastitis-causing pathogen is not mentioned, it means that multiple mastitis-causing pathogens were involved in the study. M, milk; B, blood; SC, somatic cell; PMN, polymorphonuclear neutrophils; PBMC, peripheral blood mononuclear cell; L, lymphocyte; Exp, expression; Up, upregulated; Down, downregulated; NA, not altered

(MMPs). MMP-9, which is presented abundantly in bovine neutrophils, is involved in many biological functions, including wound healing, angiogenesis and inflammation [81, 82]. MMP-9 is localised in neutrophils in vascular areas and have a strong pro-angiogenic effect [82]. The mRNA expression of MMP-9 in the developing CL increases markedly just after ovulation in cows [81]. In another study, Shirasuna et al. [14] hypothesised that the recruited neutrophils might differentiate to anti-inflammatory N2-type neutrophils under the influence of TGF-β, IL-10 and prostaglandin E2 (PGE2) in the developing CL. After that, N2-type neutrophils release MMP-9 which acts as a potent promoter of angiogenesis. Progesterone is secreted by the ovarian CL and is the unequivocal hormone of pregnancy. Interferon-tau (IFNT) is a type 1 interferon and is released from the bovine conceptus and aids in the maternal recognition of pregnancy (MRP). It not only acts within the uterus to ensure successful implantation but it also induces refractory ability on the CL and prevents the luteolytic action of prostaglandin F2α (PGF2α) [15]. The antiluteolytic effects of IFNT inhibit transcription of oxytocin receptor in the endometrial luminal epithelium of cattle which prevents the release of PGF2α, thereby ensuring maintenance of the CL and continuous production of progesterone [83]. The interferon-stimulated gene 15 (ISG15) gets upregulated in the blood neutrophils [26], and in the CL during the MRP in the pregnant cows [84]. These observations were recently justified by Shirasuna and Miyamoto [15] who observed that IFNT enhances the number of neutrophils and the expression of IL-8 on the luteal cells in the CL during the MRP in cows. Thus, IFNT-activated neutrophils and IL8 are essential for CL functionality during pregnancy establishment through increased secretion of progesterone from the luteal cells.

During the regression of CL, there is a higher expression of class II MHC antigens in bovine luteal cells [85]. Although luteal cells are non-lymphoid tissues, elevated MHC II expression in such tissues may also lead to autoimmunity. This is because higher expression of MHC II on non-lymphoid tissues may confer the property of antigen presentation to these cells, thus allowing the target cells to present previously unrecognisable autoantigens to lymphocytes causing an initiation and enhancement of immune responses against the target tissue [86, 87]. Moreover, various inflammatory cytokines including TNF-α, IL-1β, IL-8 and IFN-γ and the chemokine receptor CXCR1 are reported to be critical for the regression of CL [88, 89], which occurs mainly through apoptosis [90], suggesting that the regression of CL is an inflammatory-like immune response in cows. Similarly, Shirasuna et al. [89] reported that luteolytic cascade by PGF2α involves an acute inflammatory-like response which enhances the expression of P-selectin in the luteal endothelial cells and causes rapid infiltration of neutrophils. Further, migrated neutrophils also have the potential to recruit other immune cells including lymphocytes and macrophages to boost luteolytic cascades through inflammatory and immune responses in the CL tissue.

During fertilisation

At the time of natural mating or artificial insemination (AI), 5–40 millions of sperms are deposited into the female reproductive tract (FRT). During this process, not only semen but microorganisms which are originating from the penis or the vagina are also transported either into the vagina or the cervix and therefore need to be eliminated [91]. At this time, the female activates an immune response against the male antigens present in seminal fluid. Several researchers have reported that neutrophils are recruited to the FRT following insemination and remove excess sperm, mainly via phagocytosis [92–94]. The presence of a fertility-promoting factor in seminal plasma with homology to DNAse I and its ability to reduce the sperm phagocytosis by neutrophils have been reported in bovines [95]. During natural breeding, bovine sperm cells are deposited in the vagina and move through the cervix into the uterus leaving most of the seminal fluid behind [93]. Moreover, the essential factors of seminal plasma bind to sperm cells in the vagina and protect them against various immune reactions occurring in the uterus and oviduct. However, a variable amount of seminal plasma (that naturally remains in the vagina) is introduced into the uterus during AI. Addition of seminal plasma to various semen extenders increased sperm–neutrophil binding and NETs formation over time [93]. Moreover, the most commonly used extender in AI viz. egg yolk prevents bovine sperm–neutrophil binding regardless of the presence or absence of seminal plasma. These findings highlight the importance of re-evaluating the composition of various semen extenders and the semen-processing procedures to improve fertility from an immunological perspective.

Neutrophils can trap and kill any foreign materials by releasing neutrophil extracellular traps [96, 97]. Neutrophils respond and remove spermatozoa just like bacteria either by phagocytosis or by the formation of NETs only after about 3 h of natural or artificial insemination. So, the less mobile bovine spermatozoa are ensnared by NETs directly in the vagina due to the action of seminal plasma, whereas vigorous and highly motile spermatozoa enter the uterus and thereby evade interaction with neutrophils which increases the chance of conception. Using scanning electron microscopy, Marey et al. [98] found that incubation of neutrophils with either PGE2 or LH-stimulated bovine oviduct epithelial cells (BOECs) supernatant prevent sperm entanglement via impairment of neutrophil extracellular traps formation and phagocytosis. Recently, it has been reported the binding of sperm to BOECs stimulate various anti-inflammatory mediators such as IL-10, TGF-β, PGE2 and bovine serum albumin which protect sperm from being phagocytised by neutrophils to ensure successful fertilisation [99, 100]. Moreover, the angiotensin-endothelin-PGE2 system directs the oviduct contraction to alter the neutrophil phagocytic behaviour to sperm

in the oviduct. However, under a pathologic condition, the oviduct signals the neutrophils to attack and clear the sperm [99]. These studies indicate that neutrophils in the FRT represent a unique immunologic challenge which requires a delicate balance between protecting the system from invading microorganisms and simultaneously maintaining a favourable environment for fertilisation and survival of the allogeneic sperms. More recently, Hong et al. [101] have identified nine inhibitors against phosphatidylinositol 3-kinase which can strongly reduce neutrophil–sperm interaction without affecting sperm motility or in vitro fertilisation. These inhibitors may reduce the dose of sperm required for AI in cattle.

Around the time of embryo implantation

The immune cells of the reproductive tract of cows have unique capabilities in dealing with bacterial and viral infections, and the semi-allogeneic embryo [99, 102]. Recently, neutrophils have been reported to detect the implantation of a semi-allogeneic embryo in the uterus [24, 103]. This hypothesis has been verified in our laboratory [26, 66, 104] in which we found that a difference existed between the role played by the neutrophils and the inflammatory cytokines in both pregnant and non-pregnant cows. According to Hannan and Salamonsen [105], implantation process encompasses a highly regulated temporal and spatial expression of chemokines in the endometrium. This leads not only to specific recruitment and activation of neutrophils but also coordinates proper placentation and angiogenesis. Ssemaganda et al. [106] identified two phenotypically different populations of neutrophils in the maternal and cord blood in healthy human pregnancies. Dempsey [107] found that depletion of neutrophils in pregnant mice not only cause defective placental development but also decrease the number of viable foetuses. Upregulation in the activity of CD62L, CD11b and IL-8 in non-pregnant cows during the initiation of implantation indicates a hostile condition for implanting embryo and thus may influence the outcome of embryo implantation [26]. IFNT is secreted from the mononuclear trophoblast cells of the ruminant conceptus during the peri-implantation period of pregnancy. Paracrine and endocrine actions of IFNT contribute to survival, elongation, implantation and establishment of pregnancy in ruminants [108]. We have observed higher expression of IFNT-stimulated genes on days 16–21 post AI in blood neutrophils of pregnant cows as compared to non-pregnant cows [66].

Neutrophils also exhibit cell to cell crosstalk with other immune cells during the initiation of pregnancy. Nadkarni et al. [109] found that when human neutrophils are exposed to pregnancy hormones, they induce a specific population of T cells that have regulatory-like and proangiogenic phenotypes which help in normal placental vascularization and foetal growth during the allogeneic pregnancy. Moreover, neutrophils facilitate embryo implantation and pregnancy establishment via CL regulation [110]. Oviductal epithelial cells maintain immune homeostasis using ovarian steroids and luteinizing hormone to ensure successful fertilisation via downregulation of pro-inflammatory responses to the semi-allogeneic embryo in the bovine oviduct [99, 111]. A better understanding of the crosstalk between zygote, neutrophils and reproductive organs during early pregnancy is required as it may increase the chance of embryo implantation and improve fertility rate in cattle.

Over the transition period (calving)

The transition period in dairy cows is marked with an extensive change in the metabolic, physiological and immunological status thus making the animal more vulnerable to immunosuppression. During this critical period, there is a shift in the energy demand as more and more nutrients are partitioned towards the growing foetus and colostrum production. According to LeBlanc [112], about 30 to 50% of the cows experience health disorders immediately post-calving. During periparturient immune suppression, leukocytes exhibit impaired inflammatory responses associated with leukocytosis, and there is an increased susceptibility of the cows to opportunistic bacteria such as gram-negative coliforms that cause mastitis [113]. Respiratory burst is one of the major killing mechanisms used by neutrophils when appropriately stimulated. Neutrophils activate their NADPH-oxidase complex to produce large amounts of superoxide which acts as a precursor of other reactive oxygen species (ROS) necessary to damage bacteria during phagocytosis [114]. Burton and Erskine [115] observed impairment in the neutrophil adhesion, migration and phagocytosis-induced respiratory burst activities in parturient cows. Measuring the total number of blood neutrophils, the proportion of immature neutrophils along with an alteration in the genes involved in neutrophil adhesion, chemotaxis and phagocytosis on the day of calving can be used as a diagnostic tool for monitoring the health status of dairy cattle [21, 28, 65, 116]. These changes are likely a normal adaptation to calving that can affect the inflammatory response of neutrophils and, in some animals, make them more prone to infections [32, 117].

Stress around calving activates the hypothalamic-pituitary-adrenal axis, which increases plasma corticosteroids and causes immunosuppression [118]. Madsen et al. [119] reported that the key genes, bovine mitochondrial cytochrome b and ribosomal protein S15 which mediate respiratory metabolism and translation in bovine neutrophils are impaired during parturition, possibly due to influence of some steroid hormones. Modulation of neutrophil activity by cortisol in cattle is mediated by GRα [38, 56]. Preisler et al. [56] showed that activation of GRα in neutrophil is associated with acute and pronounced changes in the expression of selectin resulting in the reduced migratory activity of neutrophils. A dramatic

impairment in random migration, adhesion and ROS production of blood neutrophil was seen during the first week after parturition in association with cortisol hormone elevation [120]. We have reported impairment in the phagocytic activity, chemotactic activity and relative mRNA expression of some essential receptors for the functions of neutrophils with a concomitant increase in plasma cortisol in parturient cows and during early lactation [21, 50, 121].

Two genes mainly galectin-8 and talin 2 have independent and synergistic functions in cell-extracellular matrix adhesion and play a major role in the migration of neutrophils into the target tissue. Downregulation of these genes on the day of parturition reduces the capacity of neutrophil to undergo diapedesis into the inflammatory site, reduce their ability to kill pathogens and ultimately increase the possibility of these animals to develop health disorders [122]. Expression of Fas and caspase-3 genes in blood neutrophils were found to be downregulated on the day of calving compared to day seven prepartum, which then again was upregulated on day two postpartum indicating immunosuppression at calving [28]. Crookenden et al. [32] have extensively studied the changes in the circulating neutrophil expression of different genes around calving. They found differential expression in the genes related to neutrophil adhesion (selectin, ITGB2 and ITGBX), mediation of the immune response, maturation, cell cycle progression and apoptosis (MCL1, BCL2, FASLG and RIPK1). They have also reported lower gene expression of pro-inflammatory cytokines and higher expression of the anti-inflammatory cytokine and antimicrobial peptides (BNBD4, DEFB10 and DEFB1) on the day of calving.

Expulsion of foetal membrane

Foetal membranes line the uterine cavity and surround the developing foetus. At the onset of parturition, neutrophils play a major role in foetal membrane separation since this membrane is recognised as 'foreign' tissue and have to be eliminated by the immune system. Increased apoptosis, degradation of the ECM and production of the powerful neutrophil chemoattractant IL-8 around calving cause a massive influx of neutrophil into the uterus and the cervix of cows that expel their placenta normally [123]. MMP9 is present in the tertiary granules of neutrophils and is released at the initiation of uterine contractions in response to chemokine receptors activation by the IL-8. It also helps in the degradation of collagen types IV and V, and stimulates the release of the inflammatory cytokine TNF-α [124, 125]. These observations are in agreement with earlier reports in which impaired transmigration, chemotaxis and lower superoxide anion production of neutrophils have been found to be positively correlated with the incidences of retention of foetal membranes, RFM [123, 126]. Retention of foetal membranes also known as retained placenta is a condition in which there is a failure to expel foetal membranes within 24 h after parturition. Cows with RFM are at increased risk of metritis, displaced abomasum and mastitis [125, 127].

Higher expression of inflammatory cytokines and various surface receptors is essential to mediate neutrophil role in the normal expulsion of placenta. The lower relative expression of various inflammatory cytokines including IL-1, IL-6, IL-8 and TNF-α in the uteroplacental tissues of cows that developed RFM compared to healthy cows could be attributed to impaired neutrophil migration and suppression of inflammatory process which is essential for tissue remodelling and successful expulsion of placenta [128]. They also observed downregulation of ICAM-1 (intercellular adhesion molecule-1) and PECAM-1 (platelet/endothelial cell adhesion molecule-1) in the RFM cows. This may also result in incomplete collagenolysis of the ECM and insufficient neutrophil infiltration into the placentome [123]. The impaired transmigratory activity of neutrophils in RFM cows can be attributed to reduced expression of selectin molecules [28], which might also be responsible for reduced concentrations of certain pro-inflammatory cytokines in RFM cows [129]. Significantly higher plasma cortisol level between 12 and 24 h after calving has been reported in RFM cows as compared to healthy cows [130]. The increased plasma cortisol levels are negatively correlated with the expression of GRα and Fas gene of neutrophils in these cows [28]. These changes contributed to the longevity of neutrophils along with an increased number of immature neutrophils but reduced their chemotactic and phagocytic activity leading to higher incidence of RFM.

Role of neutrophils during postpartum pathophysiological conditions

During early lactation, most of the high yielding dairy cows are under stress and at an increased risk of developing uterine and mammary infections which can influence the incidence of each other. Hossein-Zadeh and Ardalan [131] found that the risk factor of metritis may indirectly lead to mastitis. Bacha and Regassa [132] reported that subclinical mastitis could directly influence the incidence of subclinical endometritis at 30 and 60 days postpartum. This is because the inflammation of uterus is associated with decreased leukocyte function, increased probability of translocation of various bacteria from the uterus to the udder and vice versa, which may be a common cause for both endometritis and mastitis [132, 133]. Similarly, Schukken et al. [134] found an association between RFM and mastitis and speculated that there is a common defence mechanism in both diseases. Their mode of action is also similar as they release an increased amount of elastase and myeloperoxidase in both mastitis and metritis [135]. The mechanism of pathogens identification, immune stimulation and subsequent neutrophil recruitment during an

inflammatory condition has been explained in Fig. 1. Here, we are discussing the role played by neutrophils during the infection of both uterus and udder separately.

During infection of the uterus (metritis and endometritis)

Metritis is characterised by the inflammation of the walls of the uterus that occurs within the first 3 weeks postpartum mainly during the first 10 days post-calving. However, endometritis is caused by the inflammation of the functional lining of the uterus within the first 21 days postpartum or afterwards [136, 137]. After parturition, the uterine lumen is usually contaminated with a wide range of bacteria which sometimes develops into clinical disease. This occurs when the pathogenicity of the bacteria exceeds the host immune tolerance [136]. Gram-negative bacteria, especially *Escherichia coli* (*E. coli*) and *Trueperella pyogenes* (*T. pyogenes*) seem to dominate the uterus within the first days after parturition followed by a range of anaerobic bacteria such as *Prevotella* species, *Fusobacterium necrophorum*, *Fusobacterium nucleatum* and *Bacteroides* [138, 139]. The initial defence mechanism of the cow endometrium against various microbes is dependent on the innate immunity, including TLRs and inflammatory cytokines which are highly expressed on the endometrium of dairy cows [140, 141]. Detection of the bacteria is mediated by the TLRs which bind to components of bacteria like lipopolysaccharide (LPS), lipopeptides and nucleotides and initiate signalling cascades, causing the release of pro-inflammatory cytokines and chemokines that recruit phagocytic cells into the uterus [141, 142].

Neutrophils are the first and an essential phagocytic cell type recruited to the uterine lumen in response to a pathogen challenge [143]. A cutoff point of neutrophils proportion can be used to diagnose cytological endometritis at the time of AI [144], and enhanced expression of distinct genes encoding for inflammatory mediators in blood leukocytes reflects the subclinical uterine inflammatory process in cows [145]. Neutrophils were higher in cervical-vaginal mucus (CVM) of cows with clinical endometritis from 7 to 21-day postpartum compared with healthy cows [146]. Usually, *E. coli* appears in the uterus first and causes pronounced functional depression of neutrophils which facilitate the co-infection of this organ by *T. pyogenes* at later times [20]. The function of neutrophils in the uterine lumen of infected cows is also severely depressed by soluble factors in lochial secretions which reduce ROS release as compared to the healthy cows [20]. Neutrophil glycogen stores get reduced during early postpartum which is more pronounced in cows that experience uterine infections [147]. Whenever there is less availability of glucose or glutamine at the endometrial tissue, it impairs the secretion of inflammatory cytokines in response to pathogenic bacteria and LPS thus leading to higher risks of uterine infections [148]. Calcium has been found to be an important second messenger for neutrophil activation and is also significantly lower in cows that developed uterine diseases as compared to healthy cows [149]. Neutrophils isolated from cows experiencing uterine infection have displayed reduced phagocytosis, myeloperoxidase activity and oxidative burst [150, 151]. The proportion of neutrophil in the total number of endometrial cells is considered to be a good indicator of subclinical endometritis, and multiple threshold values for the proportion of neutrophil have been suggested, varying from 5 to 18% [137, 152]. Recently, Jeon et al. [151] have reported decreased neutrophil activity and lower production of inflammatory cytokines in metritis cows without a fever compared to metritis cows with a fever despite a similar bacterial challenge in both groups of cows. These results reflect the necessity to address various factors involved in the initiation of the inflammatory cascade such as TLRs, nuclear factor-κB and others which are essential for neutrophil diapedesis and bacterial clearance during the infection of the uterus.

During infection of the udder (mastitis)

Neutrophils are always present in sufficient number in a healthy mammary gland. They get removed during every milking and are replaced by other neutrophils coming from the bone marrow. Mastitis usually occurs when bacteria invade the mammary gland via the teat orifice and establishes intramammary infection, which provokes an inflammatory response manifesting itself in either subclinical or clinical mastitis [153, 154]. This leads to a massive influx of neutrophils which migrate from the blood into milk to fight the bacteria and employ cascades of reactions including both oxidative and nonoxidative mechanisms [18]. Several pathogens are involved in the udder infection including *Staphylococcus aureus* (*S. aureus*), *E. coli*, *Streptococcus agalactiae* (*Strep. agalactiae*), *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Mycoplasma* spp. [4, 27, 33, 39]. The time required for recovery and neutrophil response varies depending on the mastitis-causing pathogen. For example, *E. coli* intramammary infections often result in acute mastitis, strong immune response and resolves within a shorter period compared to symptoms induced by *S. aureus* infection which are comparatively less severe, attenuate an immune response, and the infection can persist for a long duration [4, 155]. Recently, we reported attenuated activity of milk neutrophils and lowered inflammatory cytokines response during *Strep. agalactiae* and *S. aureus* bacterial infections compared to *E. coli* infections [4]. Moreover, udder infected with *Strep. agalactiae* showed significantly lower milk quality as compared to other types of bacterial infections.

The migration of blood neutrophils into the mammary tissue occurs within 2 to 4 h post infection, and 10 h later the migration process reaches its maximum level as the release of

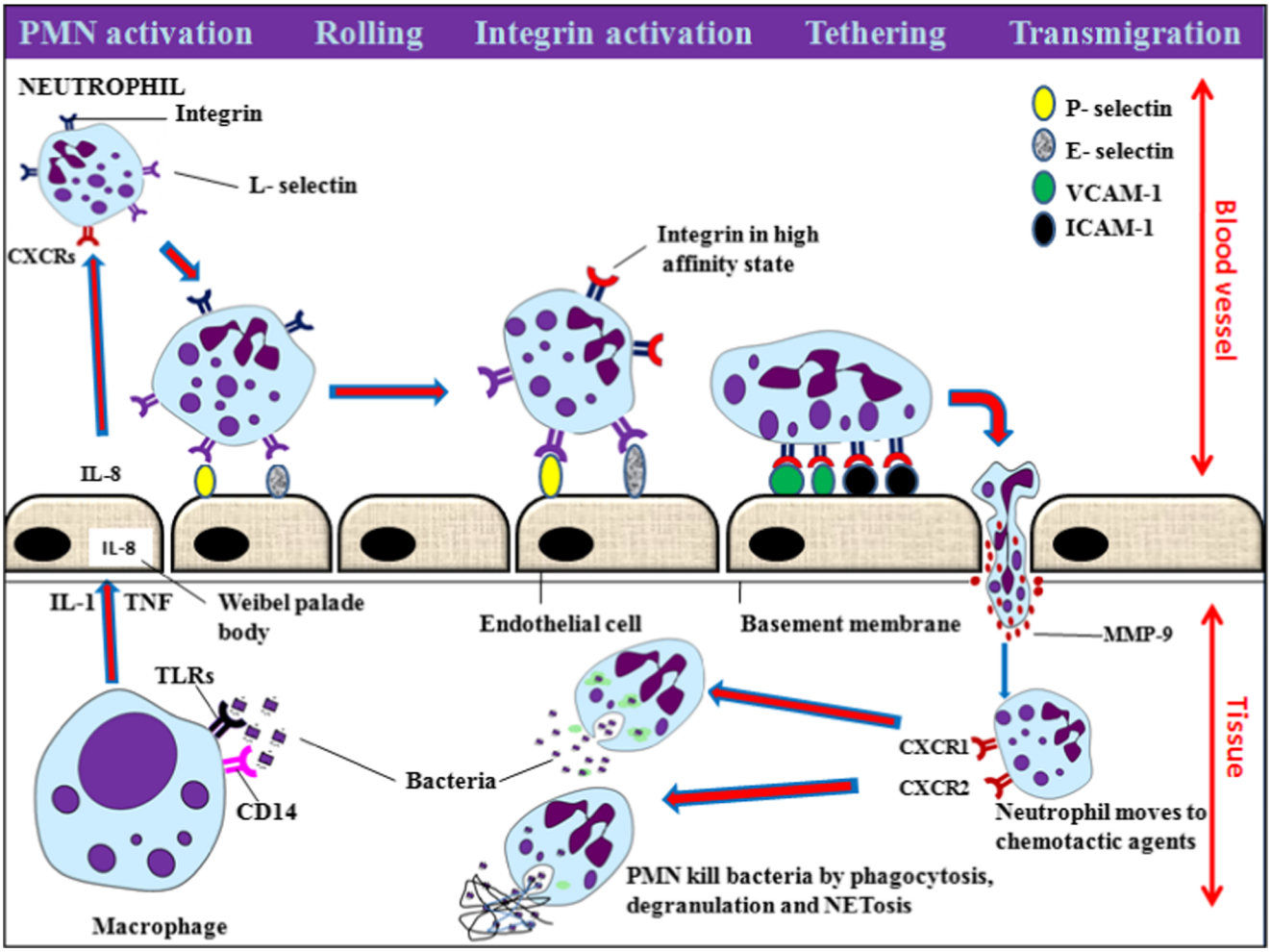

Fig. 1 Diagrammatic representation of phagocytic cells (neutrophil and macrophage) activation and migration at the site of inflammation. The main phagocytic cells are macrophages in the tissues and neutrophils in the bloodstream. Initially, macrophages in the tissue sense the presence of invaders through CD14 and TLRs, and release pro-inflammatory cytokines (IL-1 and TNF) which act on the endothelial cells (ECs) and cause the release of interleukin (IL-8) from Weibel-Palade bodies as well as expression of both P-selectin and E-selectin on the surface of ECs. IL-8 binds to the chemokine receptors (CXCR1, CXCR2) on neutrophil surface causing neutrophil activation. Activated neutrophil uses L-selectin for slowing down and integrin for tethering to the ECs, matrix metalloproteinases (MMP-9) helps in transmigration. Chemokine receptors help neutrophils to move towards chemotactic gradients and after reaching its target, they attach, ingest and kill the foreign agent by various mechanisms

cytokines peaks [156, 157]. Mutations in the CD18 gene (Integrin) lead to bovine leukocyte adhesion deficiency (BLAD). This phenomenon has been linked to an autosomal recessive granulocytopathy syndrome of young Holstein calves characterised by recurrent bacterial infections, progressive periodontitis, delayed wound healing and impaired inflammatory responses [158]. These animals cannot mobilise blood neutrophils into inflammatory sites and die at an early age due to the complications of infection [158, 159]. Heterozygous carriers seem to be clinically normal, but heterozygous cows and bulls have a 25% probability of producing homozygous calves that can be affected by BLAD [5, 159]. Therefore, heterozygous carriers remain healthy and have no immune dysfunction which means that BLAD is not involved in inflammatory diseases (mastitis, metritis, etc.) of adult dairy cows. Milk neutrophils exhibited short half-life time ($t_{1/2} = 8$ h) and have lesser phagocytic activity as compared to blood neutrophils which may be due to exhaustion of their intracellular glycogen reserve, the potential induction of apoptosis and decreased ROS generation by them after diapedesis [18].

CD14 is an essential co-receptor required for bacterial recognition by TLR2 and TLR4. It is stored inside bovine neutrophils and can be translocated to the cell membrane where it gets shed in soluble form during bacterial infections [31]. Soluble CD14 plays a critical role in neutralising LPS and maintaining homeostasis condition of the mammary gland during acute coliform mastitis [160]. Injection of recombinant bovine CD14 prior to intramammary challenge with *E. coli* caused early neutrophil recruitment and stronger activity with

milder signs of infection compared to calves received saline only prior to the challenge [160]. We have also observed lower expression of CD14 on blood neutrophils prior to udder and uterine infection in dairy cows compared to healthy animals (unpublished data). Huang et al. [161] studied the polymorphisms of the CD14 gene in bovine neutrophils and suggested this gene as a functional biomarker for mastitis resistance in dairy cows.

Mukherjee et al. [162] reported a higher in vitro phagocytic index, and inflammatory response of milk neutrophils isolated from Karan Fries crossbred cows during mid-lactation as compared to early and late lactation. Recently, we have extensively investigated the activity and receptors expression of milk neutrophils in indigenous Sahiwal cows throughout the lactation cycle under different seasons and found a higher chance of mammary infection during early lactation of the hot-humid season and stronger neutrophil activity around mid-lactation [50]. Blood neutrophils isolated from early lactating cows showed higher apoptosis compared to those obtained from cows in their mid-lactation [163]. These findings indicate depressed neutrophil functions and greater risk of developing udder infection during early lactation as compared to other stages of lactation. Variations in the activity of milk neutrophils during heat stress were reported in three native Indian breeds of cows [164]. By studying phagocytic activity and expression of different cell adhesion molecules and heat shock proteins, it was revealed that Tharparkar was more heat resilient and displayed lower chances of udder infection followed by Gir and Sahiwal cows, respectively. There are many reports about the ability of milk neutrophils to use NETs to trap and kill mastitis pathogens in the udder during infection [53, 165]. Once milk neutrophils perform their functions, they undergo apoptosis and are removed by macrophages [18]. A positive correlation between delayed neutrophil apoptosis and formation of NETs as strategies to fight the invading pathogens in the mammary gland during *Staphylococcal* mastitis has been observed [53].

Strengthening the activity of neutrophils

Ensuring an adequate and well-balanced diet is necessary for the maintenance of health and productivity of cows. Supplementation of antioxidants including vitamins and trace minerals is the most common and satisfactory strategy to strengthen the immunity of dairy cattle during immunosuppressive conditions. Antioxidants are molecules that delay, prevent and remove oxidative damage to a target molecule. They help in maintaining cell homeostasis in various ways: as preventive antioxidants, as free radical scavengers, sequestration of elements by chelation and also quench active oxygen species [166]. Antioxidants and their associated enzymes also play an important role in neutralising oxygen metabolites after the neutrophil kills invading bacteria through respiratory bursts. They prevent damage to tissues and cells in the host including protecting the neutrophils from self-destruction or damage before bacterial clearance [167]. According to Maggini et al. [168], antioxidants contribute to the body's natural defences by supporting physical barriers like the skin and mucosa and are also involved in antibody production. Inadequate levels of antioxidants and trace elements have been found to decrease the neutrophil functions and increase the incidence of mastitis and retention of placenta [166, 169]. However, dietary supplementation of various antioxidants resulted in a more rapid influx of these phagocytes to the affected area and increased killing of ingested pathogens by the neutrophils [170].

Supplementation of vitamin E to periparturient dairy cows improved the ability of blood neutrophils to kill ingested bacteria [171], prevented a decline in neutrophil superoxide anion production, and chemotactic responsiveness [172] and enhance chemotaxis by increasing receptor-bound urokinase–plasminogen activator in neutrophils [173]. There is even a reduction in the stress hormone cortisol along with increased neutrophil phagocytosis after vitamin E supplementation around calving which helps in keeping the animal healthy and stronger during this critical period [174]. Selenium is one of the trace minerals critical for the antioxidant enzyme glutathione peroxidase and contributes to the maintenance of the redox state of a cell [175]. Selenium also helps in the interaction between activated neutrophils and the adhesion molecules expressed on the endothelial cells, and its deficiency causes tight adhesion between bovine neutrophils and endothelial cells which ultimately hinder neutrophil migration to the infected site [176]. It was seen that selenium deficiency in dairy cows reduced the ability of both blood and milk neutrophils to kill bacteria [171, 177], and in vitro selenium supplementation increases the chemotactic migration, intracellular superoxide and hydrogen peroxide production of neutrophils [178]. The role of copper in affecting neutrophil functionality and enhancing both innate and adaptive immunity is well documented [179, 180]. Copper is involved in the antioxidant system through its role in the enzymes superoxide dismutase which is responsible for the dismutation of superoxide radicals to hydrogen peroxide in the cytosol of neutrophil during respiratory burst process [181]. Mild dietary copper insufficiency has been reported to depress production of superoxide anion, and bactericidal activity of neutrophil and in vitro copper supplementation was seen to enhance their phagocytic activity [179, 182]. Osorio et al. [183] found a positive response in milk yield, milk protein and neutrophil phagocytosis in cows supplemented with zinc, manganese and complex of copper and cobalt during the periparturient period. Riboni et al. [184] reported that in vitro supplementation of methionine and choline can enhance function, recognition capacity and reduce oxidative stress in bovine neutrophils. Niranjan et al. [185]

in an in vitro study found that excess supplementation of micronutrients may be detrimental to the activity of milk neutrophils. This fact was further supported by Sordillo [186], she reported that deficiency or overexposure to macro and micronutrients could contribute to immune dysfunctions and the subsequent development of health disorders. Therefore, there is a need to understand the linkages between nutrients and immunity particularly the role played by the first line of cellular defence, i.e. neutrophils which will help in designing nutritional regimes and reduce disease susceptibility in lactating cows.

Conclusions

Although neutrophils are lesser in number in bovines during normal physiology as compared to humans, they play a critical and important role in maintaining homeostasis, tissue integrity and tissue remodelling which are essential for optimum productivity in cows. This review elaborates the various capabilities of neutrophils in detecting pathogens, the presence of a different genome and in monitoring the entry of sperms into the FRT of cows. But many questions remain unanswered; firstly, what are the mechanisms which regulate their functions at molecular levels? Secondly, how they crosstalk between themselves and with other immune cells? Thirdly, can we use them as potential markers to quantify an inflammatory response in bovines as being done in humans? Further, understanding various mechanisms that modulate or regulate the neutrophil expression of different receptors and inflammatory genes may help to optimise their functions during the expulsion of placenta, fertilisation, implantation and inflammatory diseases like mastitis and metritis. A better understanding of the multifactorial interactions between supplementation of antioxidants and immune response of dairy cows can lead to more effective management strategies to control various health disorders at the time of immunosuppression. How different neutrophil phenotypes change during each physiological and physiopathological condition also needs to be investigated. Integration of all the above changes will open new avenues in the field of diagnostics in dairy cows and improve their productivity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations AI, Artificial insemination; BLAD, Bovine leukocyte adhesion deficiency; BOECs, Bovine oviduct epithelial cells; CL, Corpus luteum; *E. coli*, *Escherichia coli*; ECM, Extracellular matrix; FRT, Female reproductive tract; GRα, Glucocorticoid receptor; ICAM-1, Intercellular adhesion molecule-1; IFNT, Interferon-tau; IL, Interleukin; ISG15, Interferon-stimulated gene 15; LPS, Lipopolysaccharide; MHC, Major histocompatibility complex; MMP, Matrix metalloproteinase; MRP, Maternal recognition of pregnancy; NET, Neutrophil extracellular trap; PECAM-1, Platelet/endothelial cell adhesion molecule-1; PGE2, Prostaglandin E2; PGF2α, Prostaglandin F2α; PMN, Polymorphonuclear neutrophils; ROS, Reactive oxygen species; *S. aureus*, *Staphylococcus aureus*; SC, Somatic cell; *Strep. agalactiae*, *Streptococcus agalactiae*; *T. pyogenes*, *Trueperella pyogenes*; TAN, Tumour-associated neutrophil; TGF, Transforming growth factor; TLR, Toll-like receptor; TNF, Tumour necrosis factor; ECs, Endothelial cells

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