Neutrophil extracellular traps (NETs) are structures composed of DNA, histones, and antimicrobial proteins that are released extracellularly by neutrophils and other immune cells as a means for trapping and killing invading pathogens. Pisanu et al. (2015) reported the formation of NETs in mammary alveoli of sheep suffering bacterial mastitis, providing detailed information for understanding pathogenic mechanisms enacted by bacteria to survive the action of the innate immune system in the mammary gland. Xie et al. (2022) carried out research on the neutrophil extracellular traps (NETs) and reactive oxygen species (ROS) formation capacity of polymorphonuclear cells (PMN) during different lactational stages to Holstein cows and found it an excellent model to mimic inflammation and study fundamental aspects of the production of NETs and ROS in vitro.

Camels may be affected by all types of mastitis similar to other dairy animals (Al-Ashqar et al., 2015; Alebie et al., 2021; Geresu et al., 2021; Ranjan et al., 2021). Bovine milk somatic cells are typically represented by epithelial cells, neutrophils, macrophages, and lymphocytes as the major cell types (Riollet et al., 2000). Kaskous et al. (2021) suggested that 150 x 10³ cells/ml in milk is a limit value for healthy camel milk. If the somatic cell count (SCC) exceeds this limit, subclinical or clinical mastitis of the udder may occur and the milk may be contaminated with microbes. Aljumaah et al. (2020) found that the objective SCC test possesses considerable diagnostic merit for early detection of subclinical mastitis in camels. Subclinical mastitis is associated with huge economic losses due to the reduced milk yield and quality and high treatment costs. In addition, it is a public health concern for camel milk consumers (Osman et al., 2014).

Milk phagocytes, including macrophages and neutrophils, are the primary effector cells of the mammary gland innate immune system with a key role during mammary gland infections (Alhussien et al., 2021). They contribute to the early elimination of bacterial pathogens by several antimicrobial functions, including phagocytosis, production of

**NEUTROPHILS EXTRACELLULAR TRAPS FORMATION AND REACTIVE OXYGEN SPECIES (ROS) PRODUCTION BY MILK IMMUNE CELLS FROM CAMELS WITH SUBCLINICAL MASTITIS**

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**ABSTRACT**

Subclinical mammary gland infections are within the most important infectious diseases in dromedary camels with high impact on milk production and animal health. Using flow cytometry, the present study analysed the capacity of milk phagocytes to form neutrophil extracellular traps (NETs) and to produce reactive oxygen species (ROS) in vitro. Based on the California mastitis test, clinically healthy camels were divided into animals with subclinical mastitis (n = 5) and camels with healthy mammary gland (n = 5). The ex vivo ROS production and the NETs formation activity of milk phagocytes were compared between healthy and affected animals. A basic fraction of phagocytes (10.0 ± 1.7 % of total cells) with positive staining with the NETs-sensitive dye SYTOX™ Green was detected in milk samples from healthy camels. The NETs-positive fraction was significantly lower in milk from camels with subclinical mastitis (4.8 ± 1.5 % of total cells) compared to milk samples from healthy camels. Stimulation of milk cells with the gram-negative bacteria *E. coli* resulted in enhanced ROS production in milk phagocytes from both healthy and affected camels. The two groups, however, did not differ in the ROS level in their unstimulated or stimulated phagocytes. In conclusion, the present study identified basic levels of NETs formation by milk phagocytes separated from healthy camels. The reduced NETs formation by cells from infected camels may play a role in the pathogenesis of subclinical mammary gland infections in camels.

**Key words:** Camel, flow cytometry, immune cells, milk, Neutrophils NETosis, ROS