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An *ex vivo* model of heifer udder to study the innate immune response to bacterial infections.

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Abstract

Mastitis is the major concern for the dairy industry. Intramammary infections cause a complex signaling network that activates the innate immune response, leading to inflammation symptoms. Several types of cells, including endothelial cells, epithelial cells, resident macrophages and other leucocytes, are involved in the immune response. Therefore we aimed to set up an *ex vivo* model of the mammary gland, where the three-dimensional structure is maintained, assuming that the results were more comparable to the *in vivo* response. Two mm³ -sections were taken from the mammary gland of a heifer after slaughter and then incubated with either *S. aureus* lipoteichoic acid (LTA) or with *E. coli* lipopolysaccharide (LPS) for 1, 3, 6, and 18h. These molecules are constituent of the cell walls of Gram-positive or -negative bacteria, respectively, and are applied as an inflammatory stimulus in the research of mastitis pathogenesis. Quantitative real-time PCR was applied to quantify the mRNA expression of tumour necrosis factor (TNF- α), interleukin (IL)-1 β , IL-6, IL-8 (Griesbeck-Zilch 2008), Pentraxin 3 (PTX3; Lutzow, 2008), lingual antimicrobial peptide (LAP) (Günther, 2010;) and of interleukin-1 receptor 8 (IL1-R8; Riva, 2012) and Toll-like receptor 4 (TLR4; Ibeagha-Awemu, 2008). These molecules have been chosen as key factors of the innate immune response. Preliminary results showed that In LPS-treated cells, cytokine mRNA expression increased between 1-3h, while TLR4, PTX3 and IL1-R8 peaked at 3h and then decreased. LAP displayed a different pattern, with the highest values at 3h, slightly increasing up to 18h observation. These data suggest that such *ex vivo* model could be a valid approach to study the mammary immune response to bacteria.

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