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Expression of TLR2 gene in the mammary gland perfused and inoculated with Streptococcus agalactiae

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In recent years dairy cattle's farming was the fastest growing in Brazilian agricultural industry; however, this progress in the milk production chain could be even higher if some obstacles were resolved. Among these barriers include animal health problems, infectious-contagious diseases stand out the most, and mastitis is the main such disease afflicting dairy cattle from an economic standpoint. The milk produced by cows with subclinical mastitis can be consumed and used in the manufacture of derivatives, however, may occur significant reductions in milk production and quality. Studies to better understand the biological processes involved in this disease are essential to develop innovative solutions in our country. To contribute to this effort, the studies of gene expression and the identification of genes associated with immune response to this infection can help in the selection of more resistant animals. Besides that, due to ethical issues the use of in vivo models represents an important limitation in animal studies and the isolated perfused bovine udder approach is great alternative. Therefore, we evaluated the expression profile of the *TLR2* gene by real time PCR in alveolar tissue collected from extracorporeal udders of crossbreed Holstein x Gyr cows at two times: before inoculation (time 0) and 6 h after inoculation with *S. agalactiae*. To meet this purpose, four cows with healthy mammary gland, were selected among others scheduled for culling and their udders were collected. The udders were cooled at 8°C immediately after the collection and taken into isothermal boxes to the Nanotechnology Laboratory for Animal Healthy and Production of Embrapa Dairy Cattle, which they were attached on a metal support mimicking the standing position. All four udders were then perfused with Tyrode´s solution in order to avoid clot formation insides of the vessels. The left anterior and posterior quarters were inoculated with strain of *S. agalactiae* and the others two quarters were infused with sterile 1X PBS and used as control. Total RNA was extracted with the RNeasy mini Kit (Qiagen), quantified by spectrophotometry (Nanodrop®) and the quality of the RNA was evaluated by RIN index after analyses in Bioanalyzer 2100 (Agilent). The first strand cDNA was synthesized with the SuperScript III First-Strand Synthesis System for RT-PCR (ThermoFisher). Real time PCR data were analyzed with REST2009 software. The *TLR2* gene showed a significant decrease in the expression level (about four-fold) in the inoculated compared to non- inoculated quarters 6 h after inoculation. According literature, TLR2 recognizes lipopolysaccharides acid and peptidoglycan present in gram-positive bacteria, such as *S. agalactiae*. The activation of TLR2 can trigger pro-apoptotic signals and lead to cell death, so this result should be further investigated in the future together to apoptosis-related genes.

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