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Gene expression profile analysis of Bcl-2 in extracorporeal udders in response to Streptococcus agalactiae

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Mastitis is one of the most prevalent and costly diseases affecting the dairy industry worldwide. It causes considerable economic losses due to decreases in the quality and quantity milk production, increases in the cost of treatment and veterinary services and discarded milk. The development of innovative solutions to combat and prevent bovine mastitis is of great importance to maintain herd health as well as become more competitive milk yield in the country and produce better quality products. Therefore, for further understanding of the biological processes involved in the response to this disease and to avoid the use of live animals, we evaluated the expression profile of the *BCL2* gene in the mammary alveolar tissue in response to inoculation with *Streptococcus agalactiae* through the extracorporeal mammary glands model of crossbreed Holstein x Gyr cows. Four udders were collected from cows with healthy mammary gland ready for culling. These were cooled at 8°C and taken into cool boxes to the Nanotechnology Laboratory for Animal Healthy 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 teats were inoculated with strain of *S. agalactiae* and the others two teats were infused with sterile 1X PBS and used as control. The alveolar tissue were collected before inoculation (time 0), 3 and 6 hours after inoculation. 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). After that, the first strand cDNA was synthesized with the SuperScript III First-Strand Synthesis System for RT-PCR (ThermoFisher). The contrasts of gene expression evaluated by Real time PCR were analyzed with the REST2009 program. We noted an increase in the expression of BCL2 by 2,5 times at 3 h after inoculation compared to non-inoculated quarters ($P<0.05$). Bcl2 is found in the mitochondrial membrane; among other functions, blocks the release of cytochrome C from mitochondria, making it difficult to caspase activation leading to apoptosis. Because it is an experiment carried out in extracorporeal udder, this is an important result, as it may indicate that the cells of mammary alveolar tissue were not in a state of programmed death. There was no difference in the expression level of this gene after 6 hours of infection by *S. agalactiae*. The gene expression profile observed in this study suggests that this gene plays important roles in the response mechanisms to bovine mastitis in crossbreed Holstein x Gyr animals. Other genes will be analyzed to complement this information.

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