

TITLE: *IN VITRO* INTERACTION BETWEEN *Mycobacterium avium* subsp. *paratuberculosis* AND BOVINE SPERM CELLS

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ABSTRACT

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the etiologic agent of paratuberculosis, a disease that primarily affects ruminant species. Animals affected by the agent eliminate the microorganism through feces, milk and semen. The economic impact caused by paratuberculosis occurs mainly in dairy herds and is related to the decrease of milk production, low reproductive efficacy, premature slaughter and reduction of carcass value at slaughter. MAP has already been isolated from semen and reproductive organs of infected bulls and rams, and there is evidence that MAP can survive semen conservation procedures containing antibiotic in liquid nitrogen. The aim of this study was investigating the interaction between MAP and frozen bovine semen. One straw of frozen semen was thawed in a 37 °C water bath for 30 s before the entire medium has been removed by centrifugation. The sperm cells were washed and transferred to a 199 culture medium supplemented with 10% fetal bovine serum, MAP K-10 strain was added in a cell ratio of 1:1 (bacteria:sperm cells). This mixture (bacteria-sperm cells) was maintained at 37 °C for 1, 4 and 6 hours. At each moment, this mixture was fixed and multiple fields were analyzed by Scanning Electron Microscopy. MAP k10 and sperm cells alone were used as controls. At all analyzed time, MAP attached on spermatozoa by tail midpiece, there was no attachment elsewhere. In addition, at 4 and 6 hours, a greater amount of MAP attached was observed in tail midpiece compared with previous time. The midpiece of spermatozoa is a region of the sperm cell fibronectin protein-rich and has a central filamentous core with many mitochondria spiralled around it, used to produce ATP for the travel through the cervix of uterus, uterus and uterine tubes. In this way, bacteria attached in this part could alter this journey, decreasing the reproductive potential. More studies are necessary to investigate possible damages to the sperm cells in the presence of MAP.

Keywords: Bovine; MAP; Paratuberculosis; Reproduction

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