

***In vitro* monolayer barrier function of bovine oviduct epithelial cells is modified due to high concentrations of non-esterified fatty acids**

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Early post-partum negative energy balance in high yielding dairy cows has considerable repercussions on reproductive ability and economic merit of these animals. Typically, lipolysis is upregulated and the associated rise of non-esterified fatty acids (NEFAs) has been proposed as a key factor in the decline of oocyte and embryo quality. However, the effects on the oviductal micro-environment remain largely unknown. In this study, we hypothesized that elevated NEFAs may modify *in vitro* bovine oviduct epithelial cell (BOEC)-physiology by altering the BOEC-barrier function, and thus may potentially affect overall fertility. Hereto, fatty acid (FA)-transfer was evaluated, monolayer permeability was linked to transepithelial electric resistance (TER), and BOEC *TJP1*-expression and lipid droplet (LD) formation were analyzed.

In 4 repeats, early luteal BOECs were seeded in a polarized cell culture (PCC)-system. After reaching 100% confluency (D9), monolayers were NEFA-exposed (PA+SA+OA) for 24h in 4 groups: 1) CONTROL (0 $\mu$ M NEFA + 0%EtOH), 2) SOLVENT CONTROL (0 $\mu$ M NEFA + 0.45%EtOH), 3) BASAL NEFA (720 $\mu$ M NEFA + 0.45%EtOH in the basal compartment), 4) APICAL NEFA (720 $\mu$ M NEFA + 0.45%EtOH in the apical compartment). Next, spent medium was photometrically assessed for total FA-concentration and subjected to gas chromatography for FA-profiling. Also, a 3h permeability assay using FITC-albumin was performed, and related to pre- and postexposure TER-measurements. BOEC-mRNA was retrieved for qRT-PCR of *TJP1* to assess expression levels of tight junction protein 1. LD-formation was studied using Bodipy<sup>®</sup> 493/503 and confocal imaging. All data were analyzed with one way ANOVA.

Spent medium analyses showed a 19.5% NEFA-decrease in the supplemented compartment of BASAL NEFA, with paracellular passage to the non-supplemented, apical compartment of PA (56.0% $\uparrow$ ), SA (60.0% $\uparrow$ ), OA (33.5% $\uparrow$ ) as free FAs. However, in APICAL NEFA 53.4% of FA-decrease was observed in the supplemented compartment, while no FA-increase was apparent at the non-supplemented side, suggesting intracellular FA-uptake, which was positive for LD-formation in APICAL NEFA. FITC-albumin flux increased significantly (27.59%) in APICAL NEFA, associated with a reduced relative TER-increase (46.85%) during the NEFA-exposure. *TJP1*-expression was not affected by the treatments.

In conclusion, elevated NEFAs in the apical, 'oviductal lumen' compartment may decrease the tightness of cell-cell interactions. BOEC-barrier function was thereby compromised in APICAL NEFA. Also, the PCC allows to observe FA-transfer across BOEC-monolayers and the resulting response strongly depends on cell polarity. These data substantiate the concept of the oviduct as a possible gatekeeper that shields its micro-environment from detrimental metabolites, such as high NEFAs.

**Keywords:**

Bovine oviduct

Maternal metabolism