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Non-esterified fatty acid supplementation negatively affects *in vitro* bovine oviduct epithelial cell metabolism and transcriptome

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Elevated non-esterified fatty acids (NEFAs) have been recognized as an important link between lipolytic metabolic conditions and impaired fertility in high yielding dairy cows. However, NEFA-effects on the oviductal micro-environment currently remain unexplored despite the oviduct's involvement in many important reproductive events. Therefore, we hypothesize that elevated NEFAs may contribute to the complex pathology of subfertility by exerting a negative effect on Bovine Oviductal Epithelial Cell (BOEC)-metabolism. Hereto, a polarized cell culture system (PCC) was used to study changes in the fatty acid (FA) composition of the spent medium and in BOEC-metabolism related gene expression patterns.

BOECs were seeded in a PCC system with hanging inserts (4 replicates), thus creating two very distinct compartments: apical and basal. Confluency was confirmed at D9 by Transepithelial Electric Resistance, after which BOECs were NEFA-exposed (720µM NEFA-combi: 230µM PA, 280µM SA and 210µM OA) for 24h in 4 groups: 1) control (0µM NEFA + 0%EtOH), 2) solvent control (0µM NEFA + 0.45%EtOH), 3) basal NEFA (720µM NEFA + 0.45%EtOH in the basal compartment), 4) apical NEFA (720µM NEFA + 0.45%EtOH in the apical compartment). Subsequently, spent medium was photometrically assessed for total FA-concentration and subjected to gas chromatography for the measurement of individual FA-concentrations in different lipid-fractions. Furthermore, BOEC RNA was submitted to RT-qPCR, targeting genes related to FA and carbohydrate metabolism.

Spent medium analyses showed a 19.5% NEFA-decrease in the supplemented compartment of the basal NEFA group, with integral passage to the non-supplemented, apical compartment of PA (56.0%↑), SA (60.0%↑), OA (33.5%↑) as free FAs. However, in condition 4 (apical NEFA) 53.4% of FA-decrease was observed in the supplemented compartment, while no FA-increase was apparent at the non-supplemented side. This indicates intracellular lipid storage or consumption. Upregulated transcript abundance for *CPT1* and *ACSL1* (both FA-oxidation), combined with downregulated *ACACA* (FA-synthesis) and *G6PD* (glucose consumption) is indicative for altered BOEC-metabolism in response to apical NEFAs *($P<0.05$)*.

In conclusion, these data show that the impact of NEFAs in a polarized BOEC-model on the FA profile of the non-supplemented compartment, significantly differs depending on the exposure side. Moreover, mainly apical NEFA-exposure prompts the BOECs towards altered gene expression patterns, involving metabolic pathways, suggesting FAs rather than glucose to be the prime energy source in apically exposed cells. These data, therefore, raise the concept of the oviduct as a possible gate-keeper that shields its micro-environment from detrimental metabolites, such as elevated NEFAs.