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Elevated Non-Esterified Fatty Acid concentrations hamper *in vitro* Bovine Oviductal Epithelial Cell Physiology

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Elevated non-esterified fatty acids (NEFAs) have been recognized as an important link between lipolysis based metabolic conditions and impaired fertility in high yielding dairy cattle. However, NEFA-effects on the oviductal micro-environment currently remain unknown. We hypothesize that elevated NEFAs may contribute to the complex pathology of sub- and infertility by exerting a negative effect on Bovine Oviductal Epithelial Cell (BOEC)-physiology. Therefore, the objectives of this study were to elucidate NEFA toxicity effects on BOECs, both qualitatively and morphologically, by assessing BOEC-sperm binding affinity, monolayer integrity, proliferation capacity and morphology.

BOECs of 4 bovine oviducts (4 replicates) at day 3-5 of the estrous cycle from a local slaughterhouse were mechanically isolated, pooled and cultured in a polarized cell culture system (ThinCert) with DMEM/F12-based culture medium for 9 days until Transepithelial Electric Resistance (TER) reached at least 700Ωcm^2 to prevent leakage between the 2 compartments. At day 10 monolayers were exposed to a 720µM NEFA-mixture of OA, SA and PA, for 24h in 4 treatment groups according to exposure side: Control, Basal (B), Apical (A), A+B. BOECs were washed and monolayer quality was assessed by means of a sperm binding assay (30 minute co-culture of BOEC monolayer and 10^6 spermatozoa/ml), TER-measurements (both pre- and post-exposure) and a wound healing assay (8h observation of BOEC proliferation capacity after over an artificial gap). BOEC morphology was assessed by scanning electron microscopy on cell polarity, presence of microvilli and cilia, and monolayer integrity. Data (mean±SD) were analyzed by mixed model ANOVA.

In A+B, monolayers (31.28±6.16 sp/0.05mm^2) showed a significantly reduced **sperm binding affinity** compared to the control (97.90±10.76 sp/0.05mm^2; *P<0.05*), and treatment A tended to be more affected (39.95±19.30 sp/0.05mm^2) than treatment B (68.55±15.38 sp/0.05mm^2; *P=0.051*).
The absolute **TER**-increase post-NEFA-exposure in the control (110±11 $\Omega.cm^2$) was significantly higher than in all the other treatments. Also, the TER-increase differed significantly depending on the exposure side: in treatment A (3±6 $\Omega.cm^2$) the TER-increase was lower than in treatment B (29±8 $\Omega.cm^2$), and monolayers in treatment A+B were even associated with a significant TER-reduction (-15±10 $\Omega.cm^2$) (*P<0.05*).
Cell **proliferation capacity** showed a significant closure of the gap in all treatments, but only the control group (41.64% closure) differed significantly (*P<0.05*) from the other groups (B=28.3%, A=31.62%, A+B=30.9% closure) irrespective of the exposure side.
BOEC **morphology** was not affected.

Depending on the exposure side, elevated NEFAs exert a negative effect on BOEC physiology but not morphology. Ultimately, these physiological alterations in its micro-environment may result in suboptimal development of the pre-implantation embryo and a reduced reproductive outcome.