Non-esterified fatty acids affect sperm binding capacity of Bovine Oviduct Epithelial Cells in two *in vitro* culture systems

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Early post-partum negative energy balance in high-yielding dairy cows is characterized by up-regulated lipolysis and a rise of non-esterified fatty acids (NEFAs) in blood as well as in follicular fluid (FF). This has been associated with poor reproductive performance, as it affects oocyte and pre-implantation embryo quality. However, the effects of elevated NEFAs on the oviductal micro-environment and physiology remain largely unknown. To study Bovine Oviduct Epithelial Cell (BOEC)-quality and -functionality *in vitro,* their affinity for sperm attachment can be used. In order to observe the effect of NEFAs on *in vitro* BOEC-sperm binding, and to establish a BOEC-culture that mimics the *in vivo* oviduct-sperm interactions most closely, we studied BOEC-sperm binding in two specific cell culture systems: 1) a short term suspension culture of BOEC-explants (BOECe), and 2) a 2-compartment (apical and basolateral) polarized cell culture system (PCC) with BOEC-monolayers (BOECm) in hanging inserts.

Therefore, 4 ipsilateral bovine oviducts at day 3-5 of the estrous cycle were selected in a local slaughterhouse. After mechanical isolation, BOECs were pooled and transferred into their respective culture systems. In **experiment 1,** BOECe were selected according to ciliary activity and size (surface area < 20 000μm^2) and divided in 4 treatment groups (10 BOECe per treatment): Control (0µM NEFA), Basal (72µM NEFA), Moderate (360µM NEFA) and High (720µM NEFA). In **experiment 2,** BOECm confluency was recorded (TER > 700$\Omega.cm^2$ at day 9) after which NEFAs were added unilaterally (by alternating 72µM and 720µM NEFA-medium between the 2 compartments) or bilaterally at both concentrations. In the 2 experiments, NEFAs (NEFA combi of 3 predominant NEFAs in blood and FF: Oleic, Palmitic and Stearic Acid) were added for 24h. Subsequently the NEFA-medium was discarded, the cells were washed and coincubated with $1x10^6$ spermatozoa/ml in Sperm-TALP. After 30 minutes, unbound sperm cells were washed away and BOECs with bound sperm cells were fixed in 4% PFA. The bound sperm cells per surface area (sp/0.05mm^2±SD) were counted using light microscopy.

Results in **experiment 1** showed a significantly reduced BOECe-spermbinding in the Moderate and High NEFA treatment groups, of 59.19% and 68.05% respectively, when compared to binding in the control group (*P<0.05*). In **experiment 2**, bilateral NEFA-exposed BOECm (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 unilateral exposed monolayers tended to be more affected by apical (39.95±19.30 sp/0.05mm^2) than by basolateral NEFA-contact (68.55±15.38 sp/0.05mm^2; *P=0.051*).

BOECe sperm binding due to *in vivo*-like sperm-cilia interactions was negatively affected by elevated NEFAs, indicating a diminished BOEC-functionality. BOECm tended to dedifferentiate during culture and sperm binding was established by microvilli rather than cilia. Interestingly, the PCC allowed a more physiologically relevant BOECm-NEFA contact, in which BOECm tended to be affected more by apical than by basolateral NEFA-exposure. This suggests that BOECm are capable of partially buffering the effects of basolaterally administered NEFAs and thereby guard over the oviductal lumen and the processes taking place therein. Though, this needs further investigation, in an optimized experimental setting combining the advantages of both BOECe- and BOECm-culture.