Elevated non-esterified fatty acid concentrations during bovine oviduct epithelial cell and zygote coculture hamper early embryo development

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Maternal lipolytic disorders and the associated systemic rise of non-esterified fatty acids (NEFAs) have been suggested to affect oviduct physiology and functionality. An altered oviduct micro-environment may influence early embryo development, however its consequences remain largely unknown. Therefore, we hypothesize that elevated NEFAs in a polarized cell culture system hamper early embryo development. Furthermore, we state that effects will depend on the presence of bovine oviduct epithelial cells (BOECs) and the direction of NEFA exposure.

In 4 repeats, early luteal BOECs were seeded at $1x10^6$ cells/mL in a polarized cell culture system. After reaching 100% confluency (day 7), monolayers were cocultured with 25 zygotes per insert in 100µL SOF with 10% FBS and 0.75% BSA for 96h. Hereto, bovine oocytes were matured and fertilized *in vitro* following standard procedures. During subsequent BOEC/zygote coculture in SOF, NEFA exposure (720µM containing 210µM oleic acid + 230µM palmitic acid + 280µM stearic acid) was implemented in 3 groups: 1) [APICAL NEFA] i.e. 720µM NEFA + 0.45% EtOH in the apical compartment, 2) [BASAL NEFA] i.e. 720µM NEFA + 0.45%EtOH in the basal compartment, 3) [A/B NEFA+] i.e. 720µM NEFA + 0.45%EtOH in both compartments. Treatments were compared to [SOLVENT+] i.e. 0.45% EtOH in both compartments with BOEC coculture, [A/B NEFA-] i.e. 720µM NEFA + 0.45%EtOH in both compartments but without BOEC coculture, and [SOLVENT-] i.e. 0.45%EtOH in both compartment without BOEC coculture. After 96h, all morulae were transferred to SOFmedium in a 96-well plate without BOEC. Embryo development was assessed using cleavage- (48h pi), morula- (120-126h pi), and blastocyst rates (192h pi).

Data were analysed using binary logistic regression with Bonferroni correction in SPSS, and were considered statistically different when $P<0.05$.

Total cleavage rates in A/B NEFA+ (51.63%) and A/B NEFA- (43.19%) differed significantly ($P=0.02$), and were lower compared to other treatments. From the cleaved oocytes APICAL NEFA showed an increased percentage of zygotes in 3-cell stage (17.61%; $P=0.032$). Morula rates were on average 28.05% out of total oocytes and 47% out of cleaved oocytes, and similar between all treatments ($P>0.05$). Blastocyst rates were significantly higher in SOLVENT+ and SOLVENT- (26.11% and 22.67%, resp) compared to NEFA treatments (12.59%; $P<0.001$). In all treatments, day 8 blastocysts were mostly in expanded stage (55.06%), except for APICAL NEFA which showed 48.14% young blastocysts.

In conclusion, NEFAs negatively affect embryo developmental competence. During cleavage, but not at blastocyst level, these effects are limited to bidirectionally exposed groups, and the cocultivation with BOECs seemed to have beneficial effects. Data suggest that elevated NEFAs in the oviduct may attribute to the complex pathogenesis of sub- and infertility during lipolytic disorders, however, more research is required to further elaborate on potential compensatory effects mediated by the oviduct.

Key words: NEFAs, BOEC/zygote coculture, polarized cell culture system