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# LONG TERM ELEVATED NEFA CONCENTRATIONS DURING *IN VITRO* MURINE FOLLICLE GROWTH REDUCE OOCYTE DEVELOPMENTAL COMPETENCE AND ALTER SUBSEQUENT EMBRYO METABOLISM

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Metabolic disorders, such as a negative energy balance in dairy cows or obesity and type 2 diabetes in human, are characterized by elevated serum and follicular fluid non-esterified fatty acid (NEFA) concentrations, due to an increased lipolysis. Such high NEFA concentrations during the final phase of oocyte maturation *in vitro* (24h) impair bovine oocyte developmental competence and subsequent embryo quality and metabolism. We recently showed that long-term elevated NEFA concentrations during murine whole follicle growth, more closely mimicking the *in vivo* situation, only moderately affect antrum formation, but substantially alter granulosa cell gene expression patterns and steroidogenesis. How this may affect the oocyte and subsequent embryo is unknown. Therefore, we hypothesized that long-term elevated NEFA concentrations may hamper oocyte developmental competence and subsequent embryo quality through an altered follicular physiology (indirect) or via direct effects at the oocyte level. The specific aim was to study the effect of elevated NEFA concentrations during murine *in vitro* follicle growth, on oocyte developmental competence and embryo metabolism, as a marker for embryo quality.

Murine early secondary follicles were cultured individually until the antral stage (12days), under the following conditions: BASAL NEFA [72µM palmitic acid (PA), stearic acid (SA) and oleic acid (OA) mix], HIGH SA (280µM SA) and HIGH NEFA (720µM NEFA mix). After a Day 12 ovulatory stimulus (hCG, EGF), oocytes from all antral follicles were isolated, fertilized and presumptive zygotes were cultured following standard laboratory procedures. Cleavage rate (Day 1 p.i.) and blastocyst formation (Day 5 p.i.) were quantified (4 replicates). Furthermore, on Day 2 p.i., 4- to 8-cell stage embryos were selected and cultured in groups of 10 in 5µl homemade ASSAY medium drops (specific supplementation of glucose and amino acids) under a mineral oil overlay. Embryos were allowed to grow for exactly 24h, after which the (mostly) morula stage embryos were removed from the culture drops. ASSAY medium droplets were then analyzed for glucose (ultrafluorometry) and amino acid composition (HPLC) (4 replicates). Data were analyzed with binary logistic regression (embryo development) or non-parametric Kruskal-Wallis tests (amino acids, glucose).

Cleavage rate was reduced for HIGH NEFA embryos (53%), compared to BASAL embryos (69%, $P<0.01$). Blastocyst formation was impaired in HIGH SA, HIGH OA and HIGH NEFA embryos (32%, 33% and 42% respectively), compared to the BASAL treatment (63%, $P<0.01$). Furthermore, HIGH SA embryos consumed significantly less glucose compared to BASAL and HIGH NEFA embryos ($P<0.01$). Amino acid analyses only showed a trend ($P=0.097$) for an increased overall amino acid production in HIGH NEFA embryos.

In conclusion, our results indicate that long-term elevated NEFA concentrations during follicular growth, alter follicular physiology, ultimately leading to an impaired oocyte developmental competence and embryos with an altered ('glucose intolerant') metabolism.