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The effect of short versus long term elevated non-esterified fatty acid concentrations during murine *in vitro* follicle growth on oocyte developmental competence

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Metabolic disorders, like obesity and type 2 diabetes are characterized by lipolysis-linked elevated non-esterified fatty acid (NEFA) concentrations. Exposure to high NEFA concentrations during the final phase of bovine *in vitro* oocyte maturation (24h) impairs oocyte developmental competence and subsequent embryo quality. However, because elevated NEFA concentrations *in vivo* are often present for a longer period of time, our recent research focused on a more *in vivo*-like long term NEFA exposure (12d) of whole murine follicles *in vitro*. The model covers follicular growth from the early secondary to the antral stage *in vitro*, including final oocyte maturation after an ovulatory stimulus (OS). Results showed an altered follicular growth and physiology (steroid synthesis, gene expression) and a subsequent reduced oocyte developmental competence. However, it remains unclear what specific time frame in follicular development is the most sensitive to such metabolic insult. Therefore, we hypothesized that chronic elevated NEFA concentrations throughout follicle growth affect oocyte developmental competence more severely, than short term NEFA exposure limited to the final phase of oocyte maturation. The aim was to study the effect of elevated NEFA concentrations 1) during the final phase of follicular oocyte maturation (after the OS) and 2) during the whole period of *in vitro* murine follicle growth until the antral stage, including final oocyte maturation.

Early secondary follicles, isolated from the ovaries of 13-day old B6CBAF1 mice, were cultured *in vitro* until the antral stage (3 replicates). Follicles were exposed to: BASAL NEFA mix for 12d (BASAL-BASAL, control); HIGH stearic acid (SA) for 12d (SA-SA); HIGH NEFA mix for 12d (NEFA-NEFA); HIGH SA after the OS (BASAL-SA) and HIGH NEFA mix after the OS (BASAL-NEFA). Oocytes were isolated out of antral follicles 20h after the OS, routinely fertilized and presumptive zygotes cultured. Cleavage (n. cleaved zygotes/n. oocytes) and blastocyst (n. blastocysts/n. oocytes) rates were documented and analyzed by means of binary logistic regression.

Cleavage rate was reduced for BASAL-BASAL (37%) compared to BASAL-NEFA embryos (52%, P=0.045). The BASAL-NEFA treatment (43%) presented with a higher blastocyst percentage than BASAL-BASAL (23%, P=0.004), NEFA-NEFA (26%, P=0.037) and SA-SA (15%, P=0.001) treatments. The BASAL-SA (30%) treatment performed better than the SA-SA treatment (P=0.049).

Even though BASAL-BASAL (control) embryo development was surprisingly low, the results indicate that long-term NEFA exposure during follicle growth *in vitro* affects oocyte developmental competence more severely than an exposure limited to the final phase of maturation after the OS. They thus emphasize that the maternal micro-environment throughout follicular growth and not only during final oocyte maturation is essential for optimal oocyte quality.