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Elevated non-esterified fatty acid concentrations during *in vitro* maturation affect the transcriptome profile of day 14 bovine embryos 7 days after transfer

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We showed earlier that exposure to elevated non-esterified fatty acid (NEFA) concentrations during *in vitro* oocyte maturation (IVM) affects post-hatching development of day (D) 14 bovine embryos (Desmet *et al.*, Anim. Reprod. 14,p947,2017). Lipotoxic conditions during IVM influence DNA methylation in the D7 embryo and may thus affect its transcriptome during later development. Therefore the aim was to analyse the transcriptome profile of D14 embryos to investigate which mechanisms mediate carryover effects of adverse maturation condition on post-hatching development. Bovine oocytes were matured for 24h under 2 conditions: 1) basal physiological NEFA conditions (BAS; 28 μ M stearic acid (SA), 21 μ M oleic acid (OA), 23 μ M palmitic acid (PA)); and 2) high PA concentration (most predominant in follicular fluid during negative energy balance) (HPA; 150 μ M PA, 28 μ M SA, 21 μ M OA). After fertilization, zygotes were cultured in SOF with serum. 8 blastocysts (normal and expanded, equally distributed per treatment and per replicate) were transferred to healthy non-lactating Holstein Friesian cows at D7 (n = 8, 5 replicates). Each cow was used once for each treatment in a cross-over design. After transcervical recovery, D14 concepti (n = 45) were dissected into embryonic disc (ED) and extra-embryonic tissue (EXT). ED (n = 11BAS/7PA) and EXT (n = 13BAS/8PA) were subjected to RNA sequencing (without RNA amplification). Differential expression was established in a DESeq2 model based on Negative Binomial distribution. Samples were divided by sample type for further analysis. A false discovery rate (FDR) of 10% was used as cut-off for differentially expressed genes (DEG) and *P*-values were Benjamini-Hochberg corrected. Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) were performed. Recovery rate at D14 was not significantly different between treatments. Within ED and EXT datasets, only 14 and 0 DEGs were detected in HPA embryos compared to BAS embryos, respectively. However, when comparing concepti of similar morphological class (spherical/ovoid/tubular) and sex, higher numbers of DEGs could be detected (e.g. in ED dataset up to 6 times more DEGs). Overall, more DEGs were observed in ED compared to EXT at each morphological stage (except male tubular embryos). IPA and GSEA showed that affected pathways were related to cell growth and adhesion, metabolism, endoplasmic reticulum stress, mitochondrial respiratory chain complex and epigenetic mechanisms. To conclude, elevated PA exposure during IVM has carryover effects on the transcriptome profile of D14 concepti although only good quality D7 embryos with the same morphology have been transferred. D14 transcriptome patterns were dependant on morphology (elongation stage) and cell type (ED versus EXT) but common pathways affected were related to cellular development, metabolism and epigenetics. This suggests that metabolic stress during oocyte maturation may have long-lasting effects on embryo development that may lead to reduced fertility in high-yielding dairy cows.