Optimal blood anti-oxidant concentrations at the time of breeding may enhance preovulatory granulosa cell functions after negative energy balance in dairy cows. A transcriptomic insight.

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Early postpartum metabolic stress in high-yielding dairy cow s is strongly linked with reduced fertility. We have recently demonstrated that negative energy balance (NEB) and the increase in non-esterified fatty acids (NEFAs) at week 2 postpartum (pp) are associated with long-term changes in the granulosa cell (GC) transcriptome in the preovulatory follicles at the time of breeding (w8 pp) (Marei et al. 2022, *JDS*). Genes involved in cellular stress and inflammatory responses, and responses to lipids and ketones were upregulated in the GCs of NEB cows compared to those with basal blood NEFAs at w2. In the present study we aimed to evaluate the potential interaction between blood antioxidants (AO) and NEFAs on GC functions. We hypothesized that an optimal AO status may attenuate the long-term effects of NEFAs on the ovarian follicle.

To test this hypothesis, we used our RNAseq database of w8 preovulatory (pre-LH surge) follicle GCs collected from cows with known w2 and w8 blood AOs (βCarotene, βC; Vitamin E, VitE) and NEFA concentrations (n=16). For this study, we selected the cows with above median w2 blood NEFAs (0.78±0.19 mM, n=10). These cows were then split into 2 subgroups (n= 4-6) based on median values of blood βC and VitE, either at w2 (High AOs: 3.0±0.9 mg/dL βC and 2.9±0.4 mg/dL VitE *vs.* Low AOs: 1.3±0.3 mg/dL βC and 1.7±0.3 mg/dL VitE) or at w8 (High AOs: 5.5±1.9 mg/dL βC and 6.5±1.4 mg/dL VitE *vs.* Low AOs: 2.3±0.6 mg/dL βC. and 3.0±1.0 mg/dL VitE). The GC transcriptomic profiles of these subgroups were compared using a DESeq2 analysis at each timepoint to determine the differentially expressed genes (DEGs: *P*-adj<0.05, 5% FDR). DEGs were functionally annotated using Bioconductor packages in R.

Only 3 DEGs (3↑, 0↓) could be detected in the w2 comparison, whereas 194 DEGs (48↑, 146↓) were detected in the w8 comparison. The enriched upregulated pathways of the w8 comparison are related to activation of meiosis, MAPK signaling, IGF and EGF receptor signaling, as well as genes involved in fertilization. These are indicators of a better oocyte supportive capacity. High AOs also appear to increase active RNA biosynthetic processes, amino acid and carbohydrate metabolism, and mitotic activity (cell proliferation) as indicated by the upregulated pathways, suggesting better cell viability and follicle quality. The downregulated pathways indicate lower levels of inflammation and cellular stress because genes related to mitochondrial fragmentation, DNA breakdown, sphingomyelin biosynthesis and apoptosis were downregulated.

In conclusion, these results strongly suggest that GCs from follicles exposed to elevated NEFA during their early growth phases exhibit reduced cell stress levels and lower oxidative damage when the final follicle preovulatory development takes place under optimal antioxidant concentrations. In other words, a high blood antioxidant profile in dairy cows at the time of breeding may alleviate, at least in part, the impact of NEB on GC functions.

Keywords: antioxidants, granulosa cell transcriptome, metabolic stress