THE EFFECT OF NON-ESTERIFIED FATTY ACIDS DURING IN VITRO CULTURE ON DNA METHYLATION OF BOVINE BLASTOCYSTS

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High producing dairy cattle undergo a period of negative energy balance during which the concentration of non-esterified fatty acids (NEFA) increases in serum and follicular fluid. This metabolic disorder is simultaneously associated with subfertility. Elevated NEFA concentrations during embryo culture have adverse effects on the developmental competence of the embryo and alter gene-expression. Some of these differentially expressed genes (*HIST1H1C*, *HIST1H2BN*) are related to the compaction of chromatin and thus to epigenetic mechanisms. Changes in these epigenetic markers may induce pertinent changes in gene expression influencing further embryonic development or even later life. We hypothesized that high NEFA concentrations during early pre-implantation growth alter DNA methylation in embryos.

A total of 1412 bovine oocytes (4 replicates) were matured and fertilized following standard procedures. Zygotes were cultivated for 6.5 days under 1) physiological NEFA conditions (mixture of 23 µM palmitic acid (PA), 28 µM stearic acid (SA) and 21 µM oleic acid (OA)) (BASAL) or 2) elevated NEFA concentrations as under lipolytic conditions (mixture of 230 µM PA, 280 µM SA and 210 µM OA) (HIGH COMBI). Cleavage and blastocyst rate were determined at day 2 and day 7,5 after fertilization, respectively. A selection of 10 blastocysts per treatment per replicate was analyzed for DNA methylation patterns using the EmbryoGENE Bovine microarray platform (fold-change > 1.5 and P-value ≤ 0.05). Epigenetically modified pathways were examined by Ingenuity Pathway Analysis.

The cleavage and blastocyst rate were significantly decreased due to elevated NEFA concentrations ($P < 0.01$). The microarray data revealed a total of 4671 differentially methylated genes of which 1912 hypermethylated genes and 2759 hypomethylated genes in blastocysts under BASAL conditions compared to HIGH COMBI conditions. The five most important altered pathways were cell death and survival, lipid metabolism, carbohydrate metabolism, molecular transport and embryonic development. Previous research revealed that maturing oocytes under high NEFA conditions for 24 hours resulted in a twenty times lower number of genes with an altered DNA methylation pattern.

We conclude that embryonic exposure to elevated NEFA concentrations not only reduce the embryo development but also alter the DNA methylation profile in embryos that do survive. Especially genes associated with metabolism and cell fate are affected which may lead to an altered embryonic or fetal development or even postnatal health. The developmental stage during and/or the duration of the NEFA exposure could influence the number of genes that were differentially methylated.