

## Elevated non-esterified fatty acid concentrations during bovine oocyte maturation influences DNA methylation in blastocysts

**Implications** Oocyte maturation under elevated concentrations of non-esterified fatty acids leads to changes in DNA methylation patterns of genes related to metabolic and cell survival pathways. These epigenetic alterations may contribute to a deviating foetal growth and altered postnatal health.

**Introduction** Metabolic stress associated with negative energy balance (NEB) is a risk factor for decreased fertility in high producing dairy cattle. Non-esterified fatty acids (NEFA) are implicated in this pathogenesis as they are present in the oocyte's micro-environment during final maturation. Elevated NEFA's hamper the granulosa cell function (steroidogenesis) and jeopardize oocyte maturation leading to reduced developmental competence (Leroy *et al.*, 2005). More specifically, Van Hoeck *et al.* (2013) showed that oocyte maturation in the presence of elevated NEFA concentrations is subject to alterations in the gene transcription profile of the resultant blastocysts. Even the expression of genes essential for the establishment of epigenetic markers (*DNMT3A*, *HIST1H2BN*) was altered in response to elevated NEFA levels during final oocyte maturation. Changes in these epigenetic markers may induce pertinent changes in gene expression: effects which only become evident after birth or even in later life. In this study, we hypothesized that the maternal lipolytic conditions around conception (such as NEB) alter the epigenetic regulation. Therefore, we matured oocytes under physiological and elevated NEFA concentrations and used the EmbryoGENE Bovine methylation microarray to define the DNA methylation profile in the resultant blastocysts.

**Material and methods** *In vitro* embryo production was performed as described by Van Hoeck *et al.* (2011). A total of 1039 oocytes were collected and equally assigned to 2 treatments during 24 hours of *in vitro* maturation (4 replicates): 1) physiological NEFA conditions (mixture of 23  $\mu$ M palmitic acid (PA), 28  $\mu$ M stearic acid (SA) and 21  $\mu$ M oleic acid (OA)) and 2) elevated NEFA concentrations as under lipolytic conditions (mixture of 150  $\mu$ M PA, 75  $\mu$ M SA and 200  $\mu$ M OA). Cleavage and blastocyst rates were determined at 48 hours and 7.5 days after fertilization, respectively. A total of 80 day 7.5 blastocysts were used for DNA and RNA extraction and the DNA was analysed for methylation patterns using the EmbryoGENE Bovine methylation microarray platform. Images were generated with the Tecan PowerScanner microarray scanner and converted into intensity data files using the ArrayPro 6.4 Analyzer software. Data were normalized and fitted to a linear model using the Limma package (Linear Models for Microarray Data). The significance threshold was set to an absolute fold-change greater than 1.5 and a p-value smaller or equal to 0.05. Differences in affected pathways were identified using Ingenuity Pathway Analysis.

**Results** The microarray data reveal a significant difference in DNA methylation of 210 genes of which 119 genes were hyper- and 91 genes were hypomethylated in the blastocysts originating from oocytes matured under elevated NEFA concentrations compared to the blastocysts originating from oocytes matured under physiological NEFA concentrations. Significant differences in methylation patterns were present in genes involved in the process of apoptosis with almost the same number of genes being hyper- and hypomethylated. Similar distribution patterns between hyper- and hypomethylated genes were observed in the lipid metabolism pathways. Also methylation status of genes involved in the regulation of inflammation was affected suggesting a decrease of inflammatory response as there were more genes hypermethylated than hypomethylated. The methylation pattern of genes related to gene transcription was changed with more hypermethylated than hypomethylated genes. At the same time, hypermethylation of a gene important for histone methylation (*JARID2*) was observed. Alterations in this gene can lead to changes in other epigenetic markers. Also, significant changes in methylation of genes associated with diabetes and obesity in humans were observed.

**Conclusion** Maturation of oocytes in the presence of elevated NEFA concentrations causes changes in the methylation patterns of different genes in the resultant blastocysts. These epigenetic changes could influence the embryonic development and even the onset of disease in later life. Evaluation of the gene-expression of the differently methylated genes through qRT-PCR is necessary to confirm these results.

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### References

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