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P-159 Single-cell high-throughput methylation and transcriptomic analysis indicates variations in sensitivity to Folate-mediated one-carbon metabolism in mouse preimplantation embryos

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Study question: Do Folate-mediated one-carbon metabolism (FOCM) supplements impose methylating conditions that influence methylation and transcriptomic profiles during preimplantation period of mouse embryos?

Summary answer: Subtle differences in embryo methylation profiles were induced by FOCM supplements, despite transcriptomic signatures remained stable and selective genes related to metabolism were activated.

What is known already: Previous human studies of populations affected by seasonal variations in diet including folate availability have shown methylation changes in specific loci known as metastable epialleles (MEs). MEs exhibit stability across tissues, indicating these could be potential biosensors for heightened sensitivity to external methylating influences in early embryonic stages. Despite significant progress in IVF embryo culture conditions, the sensitivity to the outer metabolic environment during the extensive genomic transformation of embryo reprogramming remains inadequately understood.

Study design, size, duration: This is a prospective experimental study conducted from January 2023 to December 2023. A total of 111 fresh mouse zygotes (F1 hybrid (B6/CBA)) were subjected embryo culture conditions with different FOCM supplies of 5-MTHF and betaine up to expanded blastocyst stage (105 hours). At different timepoints, individual embryos were collected and disaggregated in individual cells for downstream single-cell analysis.
Participants/materials, setting, methods: Mouse zygotes were collected from the oviduct, washed and cultured in KSOM supplemented with 5-MTHF and betaine at respectively concentrations of 50µM and 50µg/ml (THF/bet(50)), 10µM and 10µg/ml (THF/bet(10)) and no supplement till blastocyst stage at 37 °C, 6%CO₂ and 5%O₂. Individual embryos were collected at 2 cells, 4 cells, 8 cells, Morula and Blastocyst stage and disaggregated into single cells for subsequent single-cell RNAseq (SmartSeq2) and single-cell methylome analysis through Splinted Ligation Adapter Tagging (scSPLAT).

Main results and the role of chance: The methylation analysis conducted though scSPLAT revealed a comprehensive reduction in global methylation levels throughout developmental stages. Notably, zygotes exhibited a maximal proportion of methylated cytosines at CpG sites, while blastocysts displayed minimal methylation levels, with sporadic increases in isolated cells. Quantitative profiling indicated lower methylation intensities in treated embryos from zygote to 4 cells, followed by a reversal trend from 8 cells to blastocyst, suggestive of an enhanced assimilation of one-carbon metabolism (FOCM) supplements.

Transcriptomic profiles obtained through scRNAseq exhibited correlation with developmental trajectories from zygote to blastocyst. However, no discernible variations were attributed to the combined exposure of Betaine and 5-MTHF. Gene enrichment analysis of differentially expressed genes at various timepoints highlighted specific metabolic responses, including mitochondrial oxidative stress and variations in glutathione metabolism. This suggests that the folate forms utilized in this study may possess a protective role as antioxidants, contributing to the observed effects on gene expression during embryonic development.

Limitations, reasons for caution: The methylation analysis shown in this study was a pilot carried out with low coverage of sequencing depthness, further analysis are ongoing to increase reads coverage.

Wider implications of the findings: Exposure of mouse embryos to FOCM enhanced global methylation during cell division from 8 cells to blastocyst, indicating heightened assimilation during DNA synthesis. While the transcriptomic signature remained stable, increased expression of genes linked to mitochondrial oxidative stress and glutathione metabolism suggests FOCM’s potential antioxidant role.

Trial registration number: not applicable