

## Identification of Transcriptomic and Associated DNA Methylation Changes Induced by the Oocyte *In Vitro* Maturation in Pig

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In humans, oocyte *in vitro* maturation (IVM) is an assisted reproductive technique used for patients with a high antral follicle count, polycystic ovary syndrome, and fertility preservation in cancer. In other cases, controlled ovarian hyperstimulation allowing *in vivo* maturation is the preferred method since it yields higher success rates. However, increasing evidence has revealed that hyperstimulation can lead to perturbed genomic imprinting and DNA methylation, which may impair offspring health. Therefore, IVM should be considered an alternative technique, but it is yet to be determined if similar epigenetic alterations also occur. Here we used the pig as a model to investigate the potential impacts of IVM on gene expression and DNA methylation in oocytes. To account for such variation, we used single-cell bisulphite sequencing and single-cell RNA sequencing techniques. The study was performed on 20 porcine oocytes obtained after IVM and 20 *in vivo* matured oocytes obtained by ovum pick-up from *ex vivo* ovaries. Differentially expressed genes (DEGs:  $P < 0.05$ , FC  $> 1.5$ ) and differentially methylated regions (DMRs,  $P < 0.05$ , FC  $> 0.1$ ) were determined and functionally annotated using Bioconductor packages in R. For the integration of both omics datasets, the single-cell aggregation and integration tool was used and a  $P < 0.05$  and FC  $> 0.25$  was applied to consider the interaction significant. Using the *in vivo* group as a reference, 1297 and 476 DEGs were down-regulated and up-regulated, respectively, in the IVM group. The up-regulated DEGs in IVM oocytes were mainly associated with the regulation of organelle organization, DNA methylation, and cell cycle processes; down-regulated genes were mainly enriched in ribosomal RNA processing, protein synthesis, oxidative phosphorylation and metabolomic processes such as glycosyl and aldehyde compound pathways. The global percentage of methylation was similar between the IVM and *in vivo* groups, but 321, 344, and 843 DMRs were detected ( $P < 0.05$ ) in CpG islands, promoters and coding regions, respectively. Notably, the germline differentially regions of imprinted genes were

appropriately methylated irrespective of IVM. Integrative analysis of DNA methylation and RNA-seq data identified the main methylated regions and genes that define each group. A total of 236 loci and 296 genes defined the IVM group, while 856 loci and 688 genes were related to the *in vivo* group. In addition, in the IVM group, we found a higher number of negative correlations between gene expression and DNA methylation, while the *in vivo* group showed higher number and stronger positive correlations. Taken together, these results indicate a discrete effect of IVM on the DNA methylation landscape in mature porcine oocytes, but these changes seem to have a greater impact on gene expression regulation.

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