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An increase of intracellular cyclic AMP improves mitochondrial function of bovine immature oocytes.

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Study question: How does a transient increase of cAMP prior to in vitro maturation (IVM) improves oocyte competence?

Summary answer: An increase of intracellular cAMP improved oocyte quality by enhancement of its mitochondrial function. On the other hand, nuclear maturation did not proceed during treatment.

What is known already: IVM of oocytes is an important technology for assisted reproduction (ART) with a wide range of research and clinical applications. However, it is generally accepted that the development of embryos produced using IVM oocytes are lower than their in vivo counterparts probably due to inappropriate status of cytoplasm. It has been also shown that an artificial increase of intracellular cAMP before IVM significantly improves oocyte developmental competence in cattle and mice. However, the precise mechanism by which cAMP improves oocyte competence during an increase of cAMP remains to be elucidated.

Study design, size, duration: The changes of gene expression in bovine oocytes and surrounding somatic cells following FSK and IBMX treatment were investigated by transcriptome analyses. A total of 458 COCs were exposed to pre-IVM treatment. As references, 491 COCs without culturing to pre-IVM were also used. Time-dependent change of nuclear maturation of oocytes and mitochondrial function in oocytes following FSK and IBMX treatment were also assessed.

Participants/materials, setting, methods: Total RNA was extracted using the RNAqueous-Micro Kit (Thermo Fisher Scientific, CA, USA) from oocytes or cumulus cells separately after isolations of oocytes and cumulus cells. RNA of Clusters were generated on a cBot (Illumina), and two lanes for the four groups were sequenced as

50-bp reads (single end) on a HiSeq 2500 (Illumina). The oxygen consumption rates (OCRs), cytochrome c oxidase (CCO) activity in mitochondria and ATP contents of oocytes were also measured.

Main results: Although the expression of several genes related with the progress of meiotic maturation was up-regulated, the key gene for MPF activation, Cdc25 was down-regulated ($p < 0.01$). As a result, the duration required for meiotic maturation of oocytes treated with FSK and IBMX was the same as that of control oocytes. Meiotic resumption was arrested during FSK and IBMX treatment.

The expression of genes encoding proteins which compose beta-oxidation, glycolysis, mitochondrial electron transport system, lipase maturation, and transportation of fatty acids significantly increased in oocytes following FSK and IBMX treatment ($p<0.01$). The OCRs, the proportion of mitochondria with high CCO activity and ATP content in oocytes significantly increased ($p<0.01$).

Genes involved in glycolysis and ovarian steroidogenesis were significantly upregulated in cumulus cells following FSK and IBMX treatment ($p<0.01$).

Limitations, reason for caution: Further studies should be required to assess whether the data obtained from bovine oocytes is applicable to human ART.

Wider implications of the findings: The data of the present study revealed that FSK and IBMX treatment at prophase stage induced the gene expression of glycolysis, fatty acid degradation and mitochondrial functions in oocytes, and accordingly improved mitochondrial functions and ATP levels in oocytes.

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