O-223 Fatty acid supplementation into warming solutions improve the developmental competence of mouse, bovine, and human oocytes and embryos after vitrification

K. Ohata[1], K. Ezoe[1], T. Miki[1], S. Kouraba[2], N. Fujiwara[1], A. Yabuuchi[1], K. Kato[3]

[1]Kato ladies clinic, R&D division, Tokyo, Japan ;
[2]Towako Medical Research Center, R&D division, Ishikawa, Japan ;
[3]Kato ladies clinic, Gynecology, Tokyo, Japan

Study question: Does fatty acid (FA) supplementation into vitrification and warming solutions influence the developmental competence of oocyte and embryo after vitrification and warming?

Summary answer: FA supplementation during the warming process improves the developmental competence of vitrified-warmed mouse oocytes and embryonic-morphologies after vitrification at the cleavage-stage in bovines and humans.

What is known already: Vitrified metaphase II stage oocytes exhibit a diminished ability to develop into blastocysts and live births. Previous studies have shown reduction in intracellular lipid content as one of the factors associated with reduced developmental competence of oocytes after vitrification as the intracellular lipid content of oocytes is affected by vitrification. FAs derived from break down of lipids are primarily transferred to the mitochondria, where it plays a crucial role in cellular metabolism. However, the effects of FA supplementation in warming solutions on the cytoplasmic lipid content and subsequent embryo development are unknown.

Study design, size, duration: A chemically defined FA mixture was added to the vitrification and/or warming solutions. Oocytes collected from C57BL6/N (n=80) were randomly divided into three groups (fresh, n=634; non-FA (control), n=961; FA, n=1,686), and were vitrified-warmed with/without FA. Lipid composition, developmental competence, and gene expression levels were compared among the groups. Bovine embryos (fresh, n=420; control, n=524; FA, n=492) and discarded human day-2 embryos (control, n=87; FA, n=92) were used to examine the developmental competence of embryos.

Participants/materials, setting, methods: Lipids in the ooplasm were stained with Nile red and the fluorescence intensity was analysed. The developmental competence of mouse oocytes was examined by performing intracytoplasmic sperm injection. Expressions of FA metabolism-related genes were measured. The bovine embryos were vitrified at the four-cell stage and cultured to the blastocyst stage after warming. Cryopreserved discarded human embryos were warmed and cultured. The obtained blastocysts were then placed on fibronectin-coated dishes to examine the outgrowth formation.

Main results and the role of chance: Lipid content of mouse oocytes was significantly lower in the control group compared to that in the fresh group ($P<0.05$). On the contrary, lipid contents of FA and fresh groups were comparable ($P=0.24$). Blastocyst formation rate was significantly higher in the FA group than that in the control group (55.7% and 44.8%, respectively; $P<0.05$). To examine the optimal timing for FA supplementation, FA was added to the vitrification solution (FAvit), warming solution (FAthaw), and/or both solutions (FAvit-thaw). Blastocyst formation rate was significantly higher in the FAthaw group than that in the control group (59.8% and 50.0%, respectively; $P<0.05$). The mRNA expressions of *Acaa2* and *Hadha* in mouse embryos were significantly higher in the FAthaw group compared to that in the control group ($P<0.05$). Moreover, FA supplemented warming solutions significantly improved the blastocyst formation rate in bovines (control, 53.5%; FAthaw, 64.5%; $P<0.05$). Developmental rate to the expanded blastocyst stage was slightly improved in human embryos (control, 53.7%; FAthaw, 63%; $P=0.38$) and the proportion of Grade A in inner cell mass and trophectoderm was significantly higher in the FAthaw group than that in the control group ($P<0.05$). There were no differences in the outgrowth abilities between the control and FAthaw groups.

Limitations, reasons for caution: Since the experiments of the current study on human embryos were performed *in vitro* using discarded embryos, in vivo developmental ability was not evaluated. Therefore, to validate the application of our findings in human assisted reproductive technologies, further clinical trials (ART) are warranted.

Wider implications of the findings: FA supplementation into the warming solutions improved the developmental competence of vitrified–warmed oocytes and cleaved embryos by activating the β-oxidation pathway. These results indicate that FA supplementation into warming solutions is a potential strategy to improve clinical outcomes in human ART.

Trial registration number: not applicable