Enhancing developmental competence and post-thaw quality of *in vitro* bovine embryos through BOEC-derived extracellular vesicles.

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Abstract

This study investigated the impact of extracellular vesicles derived from bovine oviductal epithelial cells (BOEC-EVs) on the development and quality of *in vitro* produced bovine embryos, with particular emphasis on their resilience following vitrification and warming. Presumptive zygotes were cultured in CR1aa medium supplemented with depleted FBS, while the treatment group received additional supplementation of 4×10^6 particles/mL BOEC-EVs. The results showed that the fresh control (+)BOEC-EVs group significantly enhanced embryonic development, with higher cleavage (78.35±0.90%) and blastocyst rates (40.30±1.12%) compared to the fresh control (-)BOEC-EVs group (72.00±1.20% and 26.18±0.95%, respectively; $P<0.05$). In terms of embryo quality, differential staining revealed that the fresh control (+)BOEC-EVs groups exhibited significantly higher numbers of trophectoderm (TE), inner cell mass (ICM) and total cells than the fresh control (-)BOEC-EVs groups. Although vitrification and warming reduced cell numbers across all groups, the vitrified-warmed (+)BOEC-EVs groups maintained higher TE, ICM and total cell counts. Notably, the vitrified-warmed (+)BOEC-EVs groups showed partial recovery of embryo quality, approaching levels observed in fresh control groups. These findings suggest that BOEC-EVs not only enhance *in vitro* embryo development but also mitigate the adverse effects of cryopreservation, representing a practical strategy to improve the efficiency of bovine assisted reproductive technologies.

Keyword: bovine, embryo, *in vitro* fertilization, oviduct epithelial cells, extracellular vesicles