KAMOLCHANOK TONEKAM: EFFECT OF RESVERATROL SUPPLEMENTATION INTO *IN VITRO* CULTURE MEDIUM ON DEVELOPMENTAL COMPETENCE, CRYOTOLERANCE AND GENE EXPRESSION OF *IN VITRO* PRODUCED BOVINE EMBRYOS. THESIS ADVISOR: RANGSUN PARNPAI, Ph.D., 67 PP.

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In assisted reproductive technology (ART), embryo cryopreservation, particularly via vitrification, is essential for preserving the quality of ruminant embryos. However, vitrification can adversely affect embryo viability post-thawing due to oxidative stress induced by reactive oxygen species (ROS). The present study aimed to evaluate the effects of resveratrol, a known antioxidant, on the survival and developmental competence of bovine embryos cultured *in vitro*. In Experiment 1, *in vitro*-produced embryos were divided into two groups: (1) supplemented with 0.5 μM resveratrol in embryo culture medium (+R) and (2) without resveratrol in embryo culture medium (-R) which served as control group. Results indicated that cleavage and blastocyst developmental rates in the +R group (81.70% and 37.75%, respectively) were significantly higher ($P < 0.05$) than the -R group (75.13% and 29.82%, respectively). Experiment 2, investigated the developmental outcomes of blastocysts from the +R and -R groups subjected to vitrification, warmed in media without resveratrol, and then subsequently cultured *in vitro* with or without resveratrol. The results found that supplemented resveratrol only in culture medium (not in post-warming, +R/-R) exhibited significantly higher ($P < 0.05$) hatching rates (71.50%) compared to those supplemented only in post-warming culture medium (-R/+R, 45.03%). However, resveratrol supplementation did not affect the number of trophectoderm (TE), inner cell mass (ICM), or total cell numbers in both fresh and vitrified embryos. Gene expression analysis revealed that resveratrol enhanced expression of antioxidant-related genes (*SOD1*, *CAT*), stress resistance (*SIRT1*), mitochondrial function (*TFAM*), anti-apoptosis (*BCL2*), epigenetic regulation (*DNMT1*, *DNMT3A*), pluripotency (*OCT4*), and pregnancy signaling (*IFN-tau*) in fresh embryos. In vitrified embryos, resveratrol maintained high levels of *GPX4* expression, particularly

when administered during embryo culture. Moreover, resveratrol significantly reduced the expression of the pro-apoptotic gene (*BAX*) in embryos cultured *in vitro*, suggesting improved cell viability. In conclusion, the findings demonstrate the beneficial effects of resveratrol supplementation during embryo culture, enhancing cryotolerance, regulating gene expression, and promoting developmental competence of bovine embryos.

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