UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF MATERNAL SUPPLEMENTATION OF VITAMIN E ON MURINE EMBRYO CULTURE AND VITRIFICATION OUTCOMES

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Assisted Reproductive Technology (ART), involves *ex vivo* manipulations of embryos. Techniques related to ART cause the generation of reactive oxygen species and oxidative stress which reduces embryonic viability. Vitamin E has been shown to improve *in vitro* culture outcomes, but it has not been used to improve vitrification outcomes. This study investigates the effects of maternal supplementation of 60 mg/kg body weight Vitamin E [alpha-tocopherol and Tocotrienol Rich Fraction (TRF)] on non-vitrified and vitrified murine embryos. Female C57BL/6NTac mice aged 12-16 weeks were divided into the following treatment groups: non-vitrified G1 (no treatment)(control); vitrified G2 (no treatment); non-vitrified G3 treated with palm olein stripped of Vitamin E (vehicle) and vitrified G4 treated with palm olein stripped of Vitamin E (vehicle); non-vitrified G5 treated with 60 mg/kg TRF in vehicle and vitrified G6 treated with 60 mg/kg TRF in vehicle; non-vitrified G7 treated with 60 mg/kg alpha-tocopherol in vehicle and vitrified G8 treated with 60 mg/kg alpha-tocopherol in vehicle. All treatments were administered orally, for seven consecutive days. After superovulation on Day-4 with 5 IU PMSG and Day-6 with 5 IU hCG, the females were mated with fertile males. Two-cell embryos were harvested by oviductal flushing procedure. The G2, G4, G6, and G8 group embryos underwent vitrification. Morphological examination and developmental study showed no significant differences among the non-vitrified groups ($P>0.05$). The vitrified alpha-tocopherol group had significantly higher blastocyst formation compared to the TRF group (48.1% vs 19.6%) ($P<0.05$). Gene expression analysis revealed the upregulation of the *Bax* gene in both non-vitrified TRF (318-fold) and vitrified TRF groups (428-fold), implying apoptosis which was further intensified with vitrification ($P<0.05$). The upregulation of the *Cox4i* gene was observed in the non-vitrified alpha-tocopherol group (7-fold) ($P<0.05$), suggesting energy transduction to promote survivability. Ultrastructural assessment showed the presence of mitochondrial clustering, lipid droplets (LDs) and endoplasmic reticulum (ER) in non-vitrified groups. In the non-vitrified alpha-tocopherol group, vacuolated mitochondria were observed. Swollen mitochondria, fragmented ERs, LDs and lysosomes were evident in vitrified groups. The vitrified TRF group showed mass vesicles associated with apoptosis, relevant to *Bax* overexpression and decreased development. Alteration of these organelles led to cellular transport congestion and accumulation of reactive oxygen species (ROS). Results showed that G6 had the highest ROS levels (11.9 ± 3.3) x 10^3 pixels/embryo ($P<0.05$). In conclusion, 60 mg/kg TRF impaired the viability of both non-vitrified and vitrified C57Bl/6NTac murine embryos. Alpha-tocopherol produced better viability in vitrified embryos. Further investigation of the dose-dependent effect of TRF is recommended to provide better insights into the observed effect.

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