

**EFFECTS OF EXPOSURE TIME USING PROPANEDIOL  
CRYOPROTECTANT ON VITRIFICATION METHOD  
TOWARD VIABILITY OF MICE EMBRYO  
IN VITRO CULTURED**

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**ABSTRACT**

Vitrification was regarded as a possible alternative to the cryopreservation method. This record investigated the effects of exposure time using propanediol to maintain viability of developmental mice embryo from two stage cell until blastocyst stage when cultured in vitro. The collected embryos were washed using Phosphate Buffer Saline (PBS) then placed in vitrification medium containing 30% propanediol + 0,5 M sucrose. Exposure time used 5, 10, and 15 minutes. Embryos were kept in mini straw 0,5 ml, and then plunged into liquid nitrogen rapidly. The frozen embryos thawed in a 30°C water and washed twice in 0,5 M sucrose. Viability of embryos examined using inverted microscope. Embryos that have good qualification cultured in MEM medium + BSA 3%. Developmental viability of embryos then assessed in every 24 hours during four days to destinate and calculate embryos lived. The viability of embryos was assessed the basis of the intact morphology, blastomere compact and dense, zone pellucida doesn't shrink or ripped, and there are no debris of cells. The result showed that there were significantly different ( $p < 0,05$ ) for survival embryos among treatment of exposure time 5, 10, and 15 minutes respectively in each developmental stage, except in blastocyst stage. The result showed that by exposing 5 minutes gave the best effect in protecting embryos viability in every developmental stage than 10 and 15 minutes (two cell stage:  $88,54\% \pm 10,04$ , four cell stage:  $76,56\% \pm 9,69$ , morula stage:  $56,11\% \pm 56,11$ , blastocyst stage:  $3,52\% \pm 5,46$ ) respectively.

**Keyword:** mice embryo, vitrification, propanediol, exposure time, viability.