UNIVERSITI TEKNOLOGI MARA

ELUCIDATION OF CRYOTOLERANCE IN PREIMPLANTATION MURINE EMBRYOS USING CONFOCAL MICROSCOPY AND DEVELOPMENTAL STUDY

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of the requirements for the degree of
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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 result 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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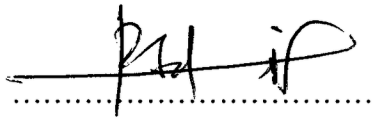
Signature of Student	:	
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ABSTRACT

The early stages of embryo development are highly unique, very predictable developmental transitions that begin with fertilization and continue throughout embryogenesis. The organization of the structural elements such as actin and tubulin undergoes dramatic architectural changes which is associated with the distribution of mitochondria and nucleus. Cryopreservation of preimplantation embryos is a valuable technique in Assisted Reproductive Technology (ART) because it can preserve the embryos for extended times. However, to date, limited information is available about the ultrastructural changes and damages in the cytoskeletal elements of the preimplantation embryos after being cryopreserved with different cryopreservation techniques namely vitrification and slow freezing. The information is needed and essential in the cryopreservation programme to select the best cryotolerant stages that can be a potential candidate for embryonic transfer and IVF. The Confocal Laser Scanning Microscope (CLSM) is used to analyze the distributions of each cytoskeletal elements. The objectives of this research was to determine the most cryotolerant stage of embryonic development in *in vivo* and *in vitro* produced embryos and to establish whether a correlation exists between the structural changes and developmental capacity of vitrified embryos. The results demonstrate that *in vivo* embryonic survival until the hatched blastocyst stage was more adversely affected by slow freezing compared to control and vitrified embryos. However, in the *in vitro* study, although the pattern were similar, the difference between the slow freezing and vitrification were not significant. The 8-cell stages were found to be the best embryonic stage with the best embryonic grading scores both in vitrified and slow freezing techniques as compared to the other stages. In terms of cytoskeletal alterations, vitrification and slow frezing method caused highly significant changes in cytoskeletal structures of the 2-, 4-, and 8-cell stage embryos especially in the tubulin, actin, nucleus and mitochondria. The lowest intensities of tubulin, actin, nucleus and mitochondria were shown in the slow frozen embryos as compared to the normal and vitrified embryos both in *in vivo* and *in vitro* group.

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