ELUCIDATION OF THE IDENTIFICATION MECHANISM OF MURINE EMBRYONIC CRYOTOLERANCE THROUGH METABOLOMIC ANALYSES AND THE STUDY OF CELLULAR ULTRASTRUCTURES

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1.0 INTRODUCTION

Cryopreservation of embryos is an essential procedure in all assisted conception units. Prediction of embryonic cryotolerance and developmental capacity after cryopreservation has previously relied on morphological assessment and observation on developmental capacity (Kuleshova *et al*., 2001; Han *et al*., 2003). Technological advancements in recent years has allowed for high-resolution microscopy in which details of ultrastructure at the organelle level may be used to select viable embryos for transfer (Dailey *et al.,* 2006; Yamagata *et al.,* 2009). In these studies, disruption to cytoskeletal components and mitochondrial distribution are observed and used in the selection of viable embryos.

Although selection done based on qualitative morphological criteria produces valuable information on the physical damages after cryopreservation, the use of quantitative methods has proven to be more accurate in predicting survivability (Stokes, *et al*., 2007). Metabolomics is a useful quantitative tool to predict viability of embryos for transfer (Katz-Jaffe *et al*., 2009). With regard to metabolomics, amino acid turnover in the culture media (Houghton *et al.* 2002: Brison *et al*. 2007) and measurements of key functional groups using near-infrared spectroscopy (NIR) (Vergouw *et al.* 2009) has been used effectively to predict embryo developmental capacity.

It has been shown that the addition of metabolomic profiling by NIR to morphological assessment techniques allows greater discrimination for selection of human embryos for transfer and has the potential to improve IVF outcomes (Sakkas *et al.* 2008). However, to date no studies have reported the combined use of metabolic profiling by amino acid analyses and morphological assessment to predict murine embryo cryotolerance. Hence, this study is designed to assess whether the combined

use of amino acid metabolomic analyses and morphological assessment would be an efficientl predictor of cryotolerance. The accurate selection of cryotolerant embryos would reduce the cost of embryo transfers by eliminating the transfer of unnecessary non-viable embryos.

1.1 Research questions

The study was designed to address the issues listed below:

a) What is the effect of cryopreservation on cytoskeletal organizations, mitochondrial distributions, nuclear damage and survivability of the *in vivo* and *in vitro* embryos?

b) What is the effect of cryopreservation on amino acid metabolomic analyses of *in vivo* and *in vitro* produced 2-cell, 8-cell and morula stage embryos?

c) Is there any improvement in the assesment of cryotolerance from the combination of amino acid metabolomic analyses and morphological evulation?

1.2 Study plan

The study was conducted to investigate the effect of vitrification procedures on the ultrastructure of *in vitro* and *in vivo* produces embryos. The most cryotolerant embryonic stages of *in vitro* and *in vivo* produces embryos were also being determined in this study. The Confocal Laser Scanning Microscope (CLSM) was used to investigate ultrastructural changes/damages to vitrified and non-vitrified embryos. The cytoskeletal quality, cellular damage, survivability and development of the embryos were also observed at the end of this study in order to select the best embryonic stage for vitrification.

1.3 Objectives of the study

The objectives of this study are:

1. To investigate the effect of cryopreservation on *in vivo* and *in vitro* produced 2-cell, 8-cell and morula stage embryos by assessment of ultrastructures using confocal microscopy.

2. To investigate the effect of cryopreservation on *in vivo* and *in vitro* produced 2-cell, 8-cell and morula stage embryos based on amino acid metabolomic analyses.

3. To assess whether the combined use of amino acid metabolomic analyses and morphological assessment would be an efficient predictor of cryotolerance.

1.4 Significance of the study

The information obtained from this study would contribute to the selection of embryos for transfer after cryopreservation. With improved embryo quality which translates to better survivability, not as many embryos need be cryopreserved. This reduces the cost of long term storage by eliminating unnecessary storage of non-viable embryos.