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PROTEIN EXPRESSIONS OF PDK1, XIAP, S6K1, MEK1/2 AND ERK1/2 WHICH FUNCTION IN GROWTH, DIFFERENTIATION AND SURVIVAL OF NON-VITRIFIED AND VITRIFIED MURINE PREIMPLANTATION EMBRYOS

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ABSTRACT

Embryo development is closely related to the proteins which are differentially expressed in each developmental stage. Proteins are crucial in regulating biological processes such as growth development, migration, proliferation, differentiation, and survivability of preimplantation embryos. Therefore, the objectives of this study are to analyse the expression of PDK1, XIAP, S6K1, MEK1/2 and ERK1/2 proteins at the 2-cell and blastocyst stages and investigate the effect of vitrification on the expression of these proteins. Murine oocytes were superovulated with 10 i.u PMSG, followed 48 hours later with 10 i.u hCG. Vitrification of the embryos at the blastocyst stage was carried out using ESF40 as a cryoprotectant. Proteins expression was observed by Western blot analysis. Experiments were carried out in triplicates. Results showed that five selected proteins; PDK1, XIAP, S6K1, MEK1/2 and ERK1/2 were predominantly expressed in embryo cytoplasm and exhibited notable involvement of PI3K and MAPK at the 2-cell stage. Vitrified blastocysts showed a decrease in the expression of ERK1/2, S6K1 and XIAP compared to non-vitrified blastocyst. This study showed that the expression of these proteins in embryos was developmental stage dependent. Each stage of development is responsible for specific biological processes in cell growth. Vitrification was shown to modulate the protein expression involved in embryonic developmental competence, associated with the activation of the apoptotic pathway through regulation of the expressions of PDK1, XIAP, S6K1, MEK1/2 and ERK1/2.

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INTRODUCTION

1.1 INTRODUCTION

Preimplantation development is the period extending from fertilization to implantation at the uterine wall. During preimplantation development, the embryo undergoes several molecular and morphological events, including compaction and blastocyst formation and cavitation, prior to implantation. These processes are complex and depend upon the regulation and expression patterns of numerous proteins. Studies of mammalian embryonic development *in vitro*, especially in the mouse have provided key insight into early mammalian developmental pathways. The growth and development of preimplantation embryos involve several stages and the developmental process from 1-cell stage to the blastocyst stage takes about five days in human embryos (Hardarson *et al*., 2012)

1.2 THE IMPORTANT OF SELECTION OF CULTURE MEDIA

Assessment of embryonic development is a useful platform to select the most viable embryos for transfer. However, the survivability of embryos in culture media depends on several factors, including sufficient nutrient supply to maintain the growth of embryos at every stage of development. Various studies have been conducted to evaluate culture systems with respect to developmental competence and embryo grading in human (Quinn *et al*., 1985 and Cossiello *et al*., 2012) and mice (Naomi *et al*., 2010 and Dai *et al*., 2012). Preimplantation stages of murine embryonic development are normally characterized by morphology of the developing cells and specific alteration in gene transcription, followed by messenger ribonucleic acid (mRNA) and protein synthesis (Latham and Solter, 1991). The primary goal in culturing preimplantation embryos is to allow growth of embryos in artificial environment in order to assist in the development of embryos. Media such as M16, HTF and KSOM used in this study contain similar metabolites, such as glucose,