UNIVERSITI TEKNOLOGI MARA

GENE EXPRESSION OF BIOPSIED MURINE PREIMPLANTATION EMBRYOS

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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

Biopsy of embryo is a prerequisite technique employed in Preimplantation Genetic Diagnosis (PGD). Removal of one or two intact blastomeres can be carried out without detriment to embryonic development. However, the effect of piezo-assisted biopsy on gene expression of embryos remains to be elucidated. This study was conducted to elucidate the effect of piezo-assisted biopsy on the 8-cell, morula and blastocyst stages on embryonic development, global gene expression and their functional pathways. Following zona breaching by piezo-drilling, single blastomeres were removed from *in vivo*-derived 8-cell ICR mouse embryos. Biopsied embryos were allowed to develop further in M16 medium, and collected for RNA extraction after 1 h (8-cell), 16 h (morula) and 42 h (blastocyst). cDNA were converted, amplified, labelled and hybridized on the GeneChip®. Microarray data were analysed using GeneSpring GX 12. Gene expression of non-biopsied and post-biopsied embryos at the 8-cell, morula and blastocyst stages were compared using the t-test with $p<0.05$, including fold change of ≥ 1.5 for each gene. Results showed a significant difference in blastocyst development after piezo-assisted biopsy. A total of 82 genes, 75 genes and 66 genes were significantly different at the 8-cell, morula and blastocyst stages respectively. The differentially expressed genes were annotated using DAVID software for Gene Ontology and GO Metacore for pathways analyses. Several enriched genes at each stage were validated using RT-qPCR. The enriched pathways involved were energy metabolism, cell cycle, immune response and fatty acid metabolism. Elucidation of these changes provides a greater understanding of molecular alterations after piezo-assisted biopsy.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF BIOPSY AND PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

Biopsy of *in vitro* fertilization (IVF) embryos is an essential component of Preimplantation Genetic Diagnosis (PGD) procedures. PGD is used to help IVF patients who are at risk of transmitting inherited diseases to their children to select embryos which are free of genetic mutations and chromosomal abnormalities, and also to improve pregnancy (Harper *et al.*, 2004). In recent years however, the use of ART and PGD have been linked to potential genetic problems through its effect on genetic imprinting (Lawrence and Kelle, 2008).

One of the prerequisites of PGD protocol is biopsy which is the successful removal of one or two intact blastomeres without adversely affecting subsequent development of the embryo (Wang *et al.*, 2008). The most commonly used technique for blastomere biopsy is direct aspiration of one blastomere through an opening in the zona pellucida by laser drilling, acid breaching and mechanical interaction. Piezo drill was developed successfully in the procedure of Intracytoplasmic Sperm Injection (ICSI) of horse oocytes. This method produced increasing cleavage rates (Hinrichs, 2005). In this study single micropipette for drilling and aspiration was employed on biopsy procedure on cleavage-stage mouse embryos. Chen *et al.* (1998) demonstrated that a simplified two-pipette technique is more efficient than the conventional three-pipette method for blastomere biopsy procedure in human embryos, in which a single pipette is used for both drilling and blastomere aspiration. However, these methods require precise skills, which would otherwise possibly cause rupture to the biopsied blastomere and damage to the embryo (Wang *et al.*, 2008).

Blastomere biopsy of embryos at six to eight cell stage combined with molecular techniques have been used successfully in human PGD to select embryos free of hereditary diseases (Grifo *et al.*, 1994). Biopsy of embryos in PGD platform is normally performed at 8-cell stage as it exerts less detrimental effect on subsequent embryonic development compared to at 2- and 4-cell stage biopsies (Krzyminska *et*