UNIVERSITI TEKNOLOGI MARA

INTRACELLULAR CALCIUM RELEASE DURING IN VITRO MAMMALIAN EMBRYONIC DEVELOPMENT IN THE PRESENCE OF Kalanchoe pinnata EXTRACT

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Thesis submitted in the fulfillment of the requirements for the degree of Master of Science

Faculty of Applied Sciences

October 2014
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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ABSTRACT

Medium composition plays an important role in mammalian in vitro culture. The availability of commercial culture media with different formulations and chemical constituents indicates the specific need and application of using medium for in vitro culture. WM was selected in this study for rabbit and mouse embryo cultures. WM+1 mg/ml and WM+2.5 mg/ml of K. pinnata extract were used to investigate the efficacy of the extract on intracellular calcium release in relation to cleavage development of embryos. The embryos were cultured in the respective medium and the cleavage developments were observed daily for six consecutive days. The intracellular calcium release was measured from the 2- to 6/8-cell embryos and fluorescence imaging of nuclear materials, microtubules and mitochondria were obtained. Rabbit embryos cultured in WM, WM+1 mg/ml and WM+2.5 mg/ml of K. pinnata extract had developed to morula stage with 6.35 ± 6.32%, 4.44 ± 4.17% and 5.13 ± 2.92%, respectively. There was a significantly higher percentage of development at 3/4-cell stage (p<0.05) in WM+2.5 mg/ml of K. pinnata extract. The intracellular calcium release in 3/4-cell and 6/8-cell stages were in the range of 3.95-50.10 μM and 24.50-782.00 μM, respectively. Embryos retrieved at 48 hours post hCG survived until blastocyst (0.84 ± 0.47%), 8-cell (2.01 ± 0.97%) and morula stage (2.20 ± 1.17%) in WM, WM+1 mg/ml and WM+2.5 mg/ml of K. pinnata extract, respectively. Low intracellular calcium release caused fragmented nuclei and the mitochondria and microtubules were aggregated only at the center part of blastomeres. The presence of K. pinnata extract as supplement for the culture medium had significantly enhanced the development of rabbit embryos at 3/4-cell stage and mouse embryos at 2-cell and 8-cell stage (p<0.05). Different formulations of herbal extract as supplement are required to improve the embryo development at each cell stage.
ACKNOWLEDGMENTS

In The Name of Allah, The Most Gracious and The Most Merciful

All the praises to Allah, The Almighty God for His mercy and blessing for giving me strengths and courage to complete this study successfully.

I would like to give my biggest appreciation to my main supervisor, Dr Nooraain binti Hashim for her continuous guidance and support. All of her comments and suggestions regarding the experimental works and thesis writing have contributed to the completion and successful of this study. I would also like to dedicate my gratitude to my co-supervisor, Prof Madya Dr Hjh Farida Zuraina binti Mohd Yusof for her support and knowledge regarding this research.

I would also like to thank the Ministry of Higher Education for the research funds (FRGS 1245) used during this research and also to Ministry of Science, Technology and Innovation (MOSTI) for the financial support during my two years of study. Not forgetting, many thanks to the Faculty of Applied Sciences, Universiti Teknologi Mara for my financial support to attend conferences. Special thanks to my lab assistants, Pn Zaiemah binti Sangit, En Rozali, and En Ahmad Kambali for their kindness, helping me during my research works.

My deepest gratitude to my beloved parents and family for their continuous moral and financial supports, love, prayers and encouragement. Last but not least to those who had directly or indirectly contributed to this research. Thank you very much.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTHOR’S DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xi</td>
</tr>
</tbody>
</table>

## CHAPTER ONE: INTRODUCTION

1.1 Background of Study  
1.1.1 *In Vitro* Embryonic Development  
1.1.2 *Kalanchoe pinnata* Extract  
1.1.3 Calcium Induced Fertilization and Embryo Development  
1.2 Problem Statement  
1.3 Significance of Study  
1.4 Objectives of Study  
1.5 Scope and Limitations of Study

## CHAPTER TWO: LITERATURE REVIEW

2.1 *Kalanchoe pinnata* sp.  
2.1.1 Taxonomy of *Kalanchoe pinnata* (Lam.) Pers.  
2.1.2 Bioactive Molecules in *K. pinnata* Leaves  
2.1.3 Plant Extracts  
2.2 Superovulation  
2.3 Embryonic Development *In Vitro*  
2.3.1 *In Vitro* Culture Conditions  
2.3.2 Factors Influencing the Embryonic Development *In Vitro*  
2.3.2.1 Oocytes and Follicular Developmental Factors