# UNIVERSITI TEKNOLOGI MARA

# THE EFFECT OF TOCOTRIENOL-RICH FRACTION (TRF) SUPPLEMENTATION IN DELAYING THE CONSEQUENCES OF AGING ON THE QUALITY OF OOCYTES AND EMBRYOS IN MICE

## NORERLYDA BINTI HAMDAN

MSc

**June 2018** 

### **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.

Name of Student	:	Norerlyda Binti Hamdan
Student I.D. No.	:	2014626172
Programme	:	Master of Science (Physiology) – MD754
Faculty	:	Medicine
Thesis Title	:	The Effect of Tocotrienol-rich Fraction (TRF) Supplementation in Delaying the Consequences of Aging on the Quality of Oocytes and Embryos in Mice
Signature of Student	:	

Date : June 2018

### ABSTRACT

Aging is one of the major contributors of female infertility. In excess, reactive oxygen species (ROS) can eventually causing oxidative stress which will directly impact the reproductive system. Fortunately, tocotrienol is proven to play a role in protecting the damaging effect of ROS in female reproductive system. Accordingly, the objectives of this study were to determine the effect of tocotrienol-rich fraction (TRF) supplementation in aging mice on the i) oocytes and embryos quality together with the embryonic development and ii) telomere length, telomerase activity and estradiol level. This study was divided into Experiment 1 and Experiment 2: young female mice at the age of six weeks old (Group A) and aging mice of six months old were used. Aging mice groups were divided into 5 subgroups; one group was kept without any supplementation (Group B1), one group was given tocopherol-stripped corn oil as vehicle control (Group B2), three groups were given TRF supplementation orally at the dose of 90, 120 and 150 mg/kg BW (Group B3, B4 and B5) respectively for two months. After two months duration, mice were superovulated and euthanized to collect the oocytes and embryos for quality assessment. Oocytes and embryos retrieved were further analysed using comet assay to determine the degree of deoxyribonucleic acid (DNA) damages. In addition, in Experiment 2, at the end of TRF-supplementation period, mice were superovulated and euthanized to collect the plasma for estradiol levels analysis, ovaries for telomere length and telomerase activity. Results showed that aging negatively affected the oocytes as well as embryo quality. Aging also shorten the telomere length and increased the telomerase activity. The TRF supplementation was able to improve the oocytes quality and embryonic development at the dose of 150 mg/kg BW. Furthermore, TRF was able to improve the telomere length and telomerase activity. Consequently, it is concluded that TRFsupplementation delay the consequences of aging that lead to infertility by protecting the reproductive organs from further deterioration.

#### ACKNOWLEDGEMENT

In the name of Allah, the Beneficent, the Merciful.

الحمد لله رب العالمين، والصلاة والسلام على رسوله محمد وعلى أله وصحبه اجمعين

First of all, I would like to express my grateful to Allah SWT for the blessing, where I finally completed this research and able to submit the report on time. I would like to take this opportunity to express my sincere appreciation to individuals, who have made important contributions to this thesis. Hence, I would like to state my special and greatest gratitude to lecturers, friends and families, who have been very enduring and appreciative throughout of my research time. For their assistance and supports, I am especially grateful to the following person:

- Associate Professor Dr. Nuraliza Abdul Satar (Main supervisor), Dr. Mudiana Muhamad (Co-supervisor), Dr. Nasibah Azme and Dr. Fathimah Mohamad for their encouragements, advices and supports from the beginning until the completion of this thesis.
- Mr. Hamdan Mohamad and Mrs. Roshada Mohd Tahir (parents) and my siblings (Mohd Ilham, Norerny Shuhada and Mohd Izham), for their encouragement, moral support, patience, motivation, pray and understanding of my needs and requirements while completing this research.
- Nor Ain Manap (Medical Laboratory Technologist), for her help and guidance during experimental work at the laboratory animal care unit (LACU).
- The Institute of Medical Molecular Biotechnology (IMMB) staffs, Norita Salim, Nor Shahida Abdul Rahman, Abu Thalhah Abdul Aziz and Salina Othman for their aid and cooperation during the progress of this study.
- All of my friends for their support, encouragement, ideas and opinion.

Thank you very much and may Allah bless you all.

### **TABLE OF CONTENT**

Page

CON	ii						
AUTHOR'S DECLARATION ABSTRACT ACKNOWLEDGEMENT							
				ТАВ	LE OF	CONTENT	vi
				LIST	Г <mark>OF</mark> ТА	ABLES	X
LIST OF FIGURES			xii				
LIST	Г <mark>OF PL</mark>	ATES	xiv				
LIST	Г <mark>OF</mark> SY	<b>MBOLS</b>	XV				
LIST	Г OF AE	BBREVIATIONS	xvii				
СНА	APTER	ONE: INTRODUCTION	1				
1.1	Gener	al Background	1				
1.2	Proble	em Statement	5				
1.3	Objec	ectives					
1.4	Signif	Significance of Study					
СНА	APTER 7	<b>FWO: LITERATURE REVIEW</b>	7				
2.1	Preva	Prevalence of Infertility					
	2.1.1	Infertility Rates Worldwide	7				
	2.1.2	Infertility Rates in Malaysia	8				
2.2	Femal	Female Reproductive System					
	2.2.1	The Anatomy of Female Reproductive System	9				
	2.2.2	The Structure of Ovary and Ovarian Cycle	12				
	2.2.3	Hormones of Female Reproductive System	19				
	2.2.4	The Embryonic Development in Mammals	20				
	2.2.5	The Structure of Developing Oocytes and Embryos	23				
2.3	The T	The Theories of Aging					
	2.3.1	The Free Radical Theory	27				
	2.3.2	The Somatic Deoxyribonucleic Acid (DNA) Theory	27				
	2.3.3	The Cellular Senescence Theory	28				