# UNIVERSITI TEKNOLOGI MARA

# THE EFFECT OF LEPTIN ON UTERO-PLACENTAL EXPRESSION OF EPHB4, EPHRIN-B1, EPHRIN-B2 AND BLOOD PRESSURE IN SPRAGUE DAWLEY RATS

## NORIZAN BINTI KAMAL BASAH

MSc

July 2017

### **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	:	Norizan Binti Kamal Basah
Student I.D. No.	:	2002673312
Programme	:	Master of Science (Medicine) – MD780
Faculty	:	Medicine
Thesis Title	:	The Effect of Leptin on Utero-Placental Expression of
		EPHB4, Ephrin-B1, Ephrin-B2 and Blood Pressure
		In Sprague Dawley Rats

Signature of Student	:	
Date	:	July 2017

#### ABSTRACT

Background: Ephs and ephrins might play a role in the migration of cytotrophoblast during endometrial invasion. Objective: This study attempts to establish the pattern of expression of EPHB4, Ephrin-B1 and Ephrin-B2 in the utero-placental unit during pregnancy in control and leptin-treated rats. Method: Normotensive female Sprague-Dawley rats, aged 12 weeks in pro-estrous were individually housed overnight with a male. Upon confirmation of mating the rats were randomized into two groups; control group and leptin-treated group. These groups were further divided into 10 subgroups (n=6 per group). Rats in the leptin-treated groups were given daily 60 µg/kg BW/day of leptin subcutaneously from two weeks prior to mating and until the day they were euthanized. Rats were euthanized every 2 days from day 5 of pregnancy and the placentae were collected for RT-PCR and immunohistochemistry. Data were analyzed using two-way ANOVA and expressed as mean  $\pm$  SEM. Result: Results showed that Ephrin-B1 and EPHB4 expressions in the placenta and uterus decreased significantly from early to mid-pregnancy and then increased towards the end of pregnancy in normal rats (p<0.001). Ephrin-B2 expression decreased over the course of pregnancy in both the placenta and uterus (p<0.001). Ephrin-B1 expression was significantly higher in the leptin-treated group at day 21 of pregnancy. It appears that Ephrins-B1, B2 and EPHB4 are expressed by both the placenta and the uterus of the rat and their expression varies along the course of pregnancy. Conclusion: In conclusion, although the expression of Ephrin-B1 is affected by leptin, it might not have a role in leptininduced hypertension during pregnancy in the rats.

### ACKNOWLEDGEMENT

I would like to acknowledge God almighty who gave me strength and good health.

I would like to express my deepest gratitude and thanks to my supervisors, Dr. Gupalo Sergey and Prof. Dr Harbindar Jeet Singh for all the knowledge that their encouragement, constructive advice, criticisms and guidance throughout this project and thesis writing, which kept me going through all the difficulties. My gratitude also goes to Universiti Teknologi MARA for providing the Research grant [600-RMI / DANA 5/3/RIF (16/2012)] that helped fund this study.

Many thanks to Prof. Marina Yurievna Kapitonova and Prof. Dr. Methil Kannan Kutty for their critical comments and valuable insights that helped me in the conduct of the research and preparation of this thesis. I am grateful to them for their guidance and advice that have motivated me to become a better student and researcher.

I also would like to thank my fellow postgraduate students and technicians at institute for Medical and Molecular Biotechnology (IMMB), Laboratory animal care unit (LACU) and Center for Pathology Diagnostic and Research Laboratories (CPDRL).

I would like to express my appreciation to Adipokine Interest Group (AIG) for their suggestions and recommendations in this study. Finally, my appreciation and my utmost gratitude are expressed to my family and my fiancé for their support, great tolerance, love, trust and encouragement that motivated me and kept me going.

## TABLE OF CONTENTS

Page

CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR' S DECLARATION	Iii
ABSTRACT	iv
ACKNOWLEDGMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF SYMBOLS	XV
LIST OF ABBREVIATIONS	xvi

### CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1	Introduction	1	
1.2	Discovery Of Leptin	4	
1.3	Leptin Synthesis And Function		
1.4	Leptin In Puberty		
1.5	Leptin In Normal Pregnancy	11	
1.6	Pregnancy And Hypertension	15	
1.7	Eph Receptors And Ephrins Ligands		
	1.7.1 The Eph Class Of Receptor Tyrosine Kinase	18	
	1.7.2 Ephrins Ligands	19	
	1.7.3 Structure Of Eph Receptor And Ephrin Ligand	20	
	1.7.3.1 Eph Receptor Structure	20	
	1.7.3.2 Ephrin Ligands Structure	20	
1.8	Bidirectional Signaling Between Eph Receptor And Ephrin	22	
	Ligands		
	1.8.1 Binding And Activation Of EPH And Ephrin	22	