

## LIST OF CONTENTS

Chapter	Page
<b>I INTRODUCTION</b> .....	1
Rationale and significance of the study.....	1
Purpose of the study.....	2
Hypothesis .....	3
Scope of this study.....	3
Keywords.....	3
Outcome expectations.....	3
Conceptual framework.....	4
<b>II LITERATURE REVIEWS</b> .....	5
Methamphetamine .....	5
Methamphetamine and behavior.....	6
Methamphetamine and neurotoxicity .....	6
Methamphetamine and reproductive system .....	7
Male reproductive system.....	8
Spermatogenesis .....	9
The stage of the cycle of the seminiferous epithelium in the rat .....	10
Sertoli cell.....	12
Leydig cell .....	12
Semen analysis.....	13
Sperm motility .....	13
Sperm morphology .....	14
Rat sperm morphology.....	15
Sperm concentration .....	16
The hypothalamic pituitary gonadal axis.....	17
Hormonal regulation of spermatogenesis .....	18
Androgen .....	20
Androgen receptor .....	22

## LIST OF CONTENTS (CONT.)

Chapter	Page
<b>III RESEARCH METHODOLOGY</b> .....	24
Animals.....	24
Chemical.....	24
Methamphetamine administration.....	24
Sperm collection.....	28
Sperm motility.....	29
Sperm morphology.....	29
Sperm concentration.....	29
Tissue preparation.....	30
Immunohistochemistry study.....	31
Evaluation of immunoreactive cell.....	33
Blood collection and preparation.....	34
Electrochemiluminescence immunoassay.....	34
Statistical analysis.....	35
Research place and acknowledgement.....	35
<b>IV RESULTS AND DISCUSSION</b> .....	36
Sperm motility.....	37
Sperm morphology.....	38
Sperm concentration.....	39
Androgen receptor expression in the testis.....	40
Androgen receptor expression in Leydig cells.....	41
Androgen receptor expression in Sertoli cells.....	42
Androgen receptor expression in round spermatids.....	43
Androgen receptor expression in elongated spermatids.....	44
Plasma testosterone levels in male rats after treated with METH.....	45

## LIST OF CONTENTS (CONT.)

Chapter	Page
Discussion .....	46
Effect of METH administration on sperm motility in male rat .....	46
Effect of METH administration on sperm morphology in male rat .....	46
Effect of METH administration on sperm concentration in male rat .....	47
Effect of METH administration on AR in the testis in male rat.....	47
Effect of METH administration on plasma testosterone levels in the testis .....	48
<b>V CONCLUSION</b> .....	50
<b>REFERENCES</b> .....	51
<b>APPENDIX</b> .....	60
<b>BIOGRAPHY</b> .....	69

## LIST OF TABLES

Table		Page
1	Normal criteria for human semen analysis of WHO in 1992.....	13
2	Schedule of saline administration in AB-METH group on days 1-14 and a high dose binge of METH on day 15.....	26
3	Schedule of METH administration in ED-METH group on days 1-14 and a saline on day 15.....	27
4	Schedule of METH administration in ED-METH binge group on days 1-14 and a high dose binge on day 15 .....	28

## LIST OF FIGURES

Figures	Page
1 Conceptual framework of this study .....	4
2 Differentiation of chemical structure between amphetamine and methamphetamine.....	5
3 Methamphetamine causes release of monoamine neurotransmitters such as dopamine and blocks re-uptake of dopamine in pre-synaptic neuron .....	7
4 Seminiferous tubule and relative duct of male reproductive system.....	8
5 Seminiferous tubule.....	9
6 Spermatogenesis of seminiferous tubule.....	10
7 The stage of the seminiferous epithelium in the adult male rat.....	11
8 Leydig cells and Sertoli cells.....	12
9 Human spermatozoon.....	14
10 Schematic drawing of some abnormal human spermatozoa .....	15
11 Normal rat sperm morphology .....	16
12 Sperm were counted by hemacytometer under bright field microscope. The number is counted sperm and asterisk is uncounted sperm.....	17
13 The hypothalamic pituitary gonadal axis .....	18
14 Testosterone synthesis.....	20
15 Classical (left) and non-classical (right) testosterone pathways in spermatogenesis.....	22
16 Animals were treated with METH or saline in 4 groups (Control group, AB-METH group, ED-METH group and ED-METH binge group).....	25
17 Five small squares of large center square were used for sperm count .....	30
18 Indirect immunohistochemistry method.....	31

## LIST OF FIGURES (CONT.)

Figures	Page
<p>19 Relative optical density (ROD) per area of AR expressions in seminiferous tubule stage VII-VIII in each dilution was examined. The standard curve was generated by plotting the ROD/area of AR staining against the dilution of AR staining.....</p>	33
<p>20 Electrochemiluminescence immunoassay.....</p>	34
<p>21 Percentage of normal sperm motility in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, ** <math>p &lt; 0.01</math> vs control group and *** <math>p &lt; 0.001</math> vs control group (ANOVA post-hoc Dunnett test).....</p>	36
<p>22 Sperm morphology in male rats in the control group (A), acute dose-METH binge (AB-METH) group (B), escalating dose- METH (ED-METH) group (C) and escalating dose-METH binge (ED-METH binge) group (D). Blue arrow = normal sperm, green arrow = tailless sperm, red arrow = headless sperm, arrow head = bent tail sperm. Scale bar = 25µm. Objective lens = 20x.....</p>	37
<p>23 Percentage of normal sperm morphology in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, * <math>p &lt; 0.05</math> vs control group and ** <math>p &lt; 0.01</math> vs control group (ANOVA post-hoc Dunnett test).....</p>	38

## LIST OF FIGURES (CONT.)

Figures	Page
<p>24 Epididymal sperm number (<math>\times 10^6</math> cells/ml) in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean<math>\pm</math>SEM, n = 6 (ANOVA post-hoc Dunnett test).....</p>	39
<p>25 Sections of rat testis stained with anti-androgen receptor primary antibody. There are the expression of AR in Leydig cell (arrow head), Sertoli cell (red arrow), round spermatid (green arrow) and elongated spermatid (black arrow). Figure A showed the AR immunostaining with hematoxylin. Figure B showed the AR immunostaining without hematoxylin. The small block is negative staining. Scale bar = 25<math>\mu</math>m. Objective lens = 20x.....</p>	40
<p>26 Percentage of AR expression in Leydig cells in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean<math>\pm</math>SEM, n = 6, * <math>p &lt; 0.05</math> vs control group and *** <math>p &lt; 0.001</math> vs control group (ANOVA post-hoc LSD).....</p>	41
<p>27 Percentage of AR expression in Sertoli cells in the control group, acute dose- METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean<math>\pm</math>SEM, n = 6, (ANOVA post-hoc LSD).....</p>	42
<p>28 Percentage of AR expression in round spermatids in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean<math>\pm</math>SEM, n = 6, * <math>p &lt; 0.05</math> vs control group (ANOVA post-hoc LSD).....</p>	43

## LIST OF FIGURES (CONT.)

Figures	Page
29 Percentage of AR expression in elongates spermatids in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, *** $p < 0.001$ vs control group (ANOVA post-hoc LSD) .....	44
30 Plasma testosterone levels in the control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and Escalating dose-METH binge (ED-METH binge) group. Data were presented as mean±SEM, n = 6, (ANOVA post-hoc LSD) .....	45

## ABBREVIATIONS

ABC	=	Avidin-biotinylated horseradish peroxidase complexes
AR	=	Androgen receptor
°C	=	Degree Celsius
DAB	=	3,3'-Diaminobenzidine
ECLIA	=	Electrochemiluminescence immunoassay
H <sub>2</sub> O <sub>2</sub>	=	Hydrogen peroxide
i.p.	=	Intraperitoneal
kg	=	Kilogram
METH	=	Methamphetamine
mg	=	Milligrams
PBS	=	Phosphate buffer saline
ROD	=	Relative optical density
rpm	=	Revolutions per minute
SEM	=	Standard error mean
μl	=	Microlitre
μm	=	Micron