

CONTENTS

	Page
ABSTRACT IN THAI.....	I
ABSTRACT IN ENGLISH.....	III
ACKNOWLEDGMENT.....	V
CONTENTS.....	VI
LIST OF TABLES.....	VIII
LIST OF FIGURES.....	IX
LIST OF ABBREVIATIONS.....	X
 CHAPTER	
I INTRODUCTION	1
1.1 Background.....	1
1.2 Research objectives.....	2
1.3 Hypothesis.....	2
II LITERATURE REVIEWS	3
2.1 Assisted reproductive technologies (ART).....	3
2.2 In vitro embryo production (IVP) of bovine is composed of 3 steps.....	3
2.2.1 <i>In vitro</i> maturation (IVM).....	3
2.2.2 <i>In vitro</i> fertilization (IVF)	3
2.2.3 <i>In vitro</i> culture (IVC)	4
2.3 The environment of culture bovine embryo.....	4
2.4 Evaluation and classification of bovine embryos.....	5
2.5 Cryopreservation	8
2.5.1 Cryoprotectant (CPAs).....	8
2.5.2 Slow freezing	9
2.5.3 Vitrification.....	10
2.6 Bovine blastocyst vitrification.....	13
2.6.1 Vitrification and Warming solution	15

CONTENTS (Continued)

	Page
2.7 Oxidative stress by reactive oxygen species (ROS) and apoptosis	16
2.8 Resveratrol.....	17
III RESERCH METHODOLOGY	20
3.1 Chemicals	20
3.2 In vitro maturation (IVM).....	20
3.3 Preparation of sperm for in vitro fertilization (IVF).....	21
3.4 In vitro fertilization (IVF).....	21
3.5 In vitro embryo culture (IVC)	21
3.6 Cryopreservation of embryos using vitrification technique.....	22
3.6.1 Vitrification procedure	22
3.6.2 Warming technique	23
3.7 Blastocyst staining.....	23
3.8 Evaluation of gene expression in blastocyst.....	24
3.9 Statistical analysis	25
IV RESULTS	26
4.1 Embryo development.....	26
4.2 The survival of vitrified blastocysts.....	26
4.3 The quality of blastocysts.....	29
4.4 The gene expression of fresh and vitrified bovine blastocysts.....	30
V DISCUSSION AND CONCLUSION	33
5.1 Discussion	33
5.2 Conclusion.....	38
REFERENCES.....	40
BIOGRAPHY.....	53

LIST OF TABLES

Table	Page
2.1 Stages of Embryonic Development	7
2.2 Quality grades of embryo	8
3.1 Primer for qPCR.....	25
4.1 The development of blastocysts.....	26
4.2 Treated resveratrol on developmental competency of vitrified embryos	28
4.3 Treated resveratrol on cell number of vitrified embryos	29

LIST OF FIGURES

Figure	Page
2.1 Normal embryonic development of bovine embryos.....	6
2.2 Cryopreservation method.....	11
2.3 Cryotop used for vitrification.....	12
2.4 The mechanism of vitrification and warming procedure.....	13
2.5 The production of ROS.....	16
2.6 The chemical structure of resveratrol.....	17
3.1 Oocytes culture in IVM medium.....	20
3.2 The development of the embryos after culture in IVC medium.....	22
3.3 Expanded hatching and hatched embryos after 24h warm.....	23
3.4 TE and ICM cells of blastocyst after PI and Hoechst 33342 staining.....	24
4.1 The expression of <i>Bax</i> and <i>Bcl2</i> genes in bovine blastocysts.....	31
4.2 The expression of <i>SIRT1</i> , <i>PNPLA2</i> and <i>FoxO3A</i> genes in bovine blastocysts.....	32

LIST OF ABBREVIATIONS

ARTs	=	Assisted reproductive technologies
ATP	=	Adenosine triphosphate
BM	=	Base medium
BSA	=	Bovine serum albumin
cDNA	=	Complementary DNA
CPAs	=	Cryoprotectant
Ct	=	Cycle threshold
DMSO	=	Dimethylsulphoxide
EG	=	Ethylene glycol
ES	=	Equilibration solution
FBS	=	Fetal bovine serum
H ₂ O ₂	=	Hydrogen peroxide
hCG	=	Human chorionic gonadotropin
IU	=	International unit
RT	=	Room temperature
IVC	=	<i>In vitro</i> culture
IVF	=	<i>In vitro</i> fertilization
IVM	=	<i>In vitro</i> maturation
IVP	=	<i>In vitro</i> embryo production
LN ₂	=	Liquid nitrogen
mtDNA	=	Mitochondrial DNA
PBS	=	Phosphate-buffer saline
ROS	=	Reactive oxygen species
RT	=	Reverse transcription

LIST OF ABBREVIATIONS (Continued)

TCM-199	=	Tissue culture medium-199
M	=	Molar
mM	=	Millimolar
mg	=	Milligram
°C	=	Degree Celsius
μM	=	Micromolar
μl	=	Microliter