

## TABLE OF CONTENTS

	<b>Page</b>
ABSTRACT (IN THAI)	i
ABSTRACT (IN ENGLISH)	iv
DEDICATION	vii
ACKNOWLEDGEMENTS	viii
TABLE OF CONTENTS	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiv
TABLE OF ABBREVIATIONS	xvi
CHAPTER I INTRODUCTION	1
1.1 Background and Justifications	1
1.2 Scientific Hypothesis	3
1.3 Objectives	4
1.4 Scope and Limitations	4
1.5 Anticipated outcomes	5
CHAPTER II LITERATURE REVIEW	6
2.1 Follicular growth and development	6
2.2 Angiogenesis and angiogenic factors in the ovary	12
2.3 Vascular function in the ovary	16
2.4 Measurements of follicular vasculature	19
2.5 Follicle-luteal transition: a period of intense angiogenesis	20
2.6 Corpus luteum development	24
2.7 Regulatory proteins	27
2.8 Animal model of reproductive biology	29
2.9 Oocyte and follicle cell interactions	31
2.10 Summary	33

## TABLE OF CONTENTS (Cont.)

		<b>Page</b>
CHAPTER III	EXPERIMENT I ASSOCIATION AMONG VASCULARITY, CELL PROLIFERATION, AND ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN BOVINE ANTRAL FOLLICLES OF THE FIRST FOLLICULAR WAVE	34
3.1	Experiment 1.1: Quantitative vascularity of antral follicle in <i>Bos indicus</i> using Factor VIII immunolocalization	34
3.2	Experiment 1.2: Relationships among vasculature, mitotic activity, and endothelial nitric oxide synthase (eNOS) in bovine antral follicles of the first follicular wave	45
CHAPTER IV	EXPERIMENT II INDUCTION OF FOLLICULAR GROWTH AND ATRESIA: GOAT MODEL	65
4.1	Introduction	65
4.2	Materials and Methods	66
4.3	Results	75
4.4	Discussion	82
4.5	Conclusion	86

**TABLE OF CONTENTS (Cont.)**

	<b>Page</b>
CHAPTER V    EXPERIMENT III EFFECTS OF GnRH REPLACEMENT WITH hCG IN TAI PROTOCOL ON OVULATION AND CONCEPTION RATES, CL DEVELOPMENT, AND PROGESTERONE CONCENTRATIONS IN DAIRY COWS	87
5.1 Introduction	87
5.2 Materials and Methods	89
5.3 Results	93
5.4 Discussion	97
5.5 Conclusion	99
CHAPTER VI    SUMMARY AND CONCLUSIONS	101
CHAPTER VII    FUTURE RESEARCH DIRECTIONS	103
REFERENCES	105
APPENDICES	138
RESEARCH PUBLICATIONS	149
CURRICULUM VITAE	150

## LIST OF TABLES

		<b>Page</b>
Table 3.1	Means $\pm$ SEM of capillary area density (CAD), capillary number density (CND), and area per capillary (APC) of small, medium, and large follicles	39
Table 3.2	Means $\pm$ SEM of capillary area density (CAD), capillary number density (CND), and area per capillary (APC) in antral follicle according to health status and follicle size	41
Table 3.3	Size and status of the follicles evaluated	51
Table 3.4	Least squares means ( $\pm$ SEM) of follicular diameter, follicular fluid E2 and P4 concentrations, E2:P4 ratio, vasculature, eNOS expression, and labeling index (LI) of granulosa and theca cells of total antral follicles (healthy and atretic; n=82)	53
Table 3.5	Least squares means ( $\pm$ SEM) of follicular diameter, follicular fluid E2 and P4 concentrations, E2:P4 ratio, vasculature, eNOS expression, and labeling index (LI) of granulosa and theca cells of the healthy small (3 to 6 mm), medium (7 to 10 mm), and large follicles (>10 mm)	54
Table 3.6	Correlation coefficients among variables evaluated for the healthy antral follicles (E2:P4 ratio $\geq$ 1; n=49)	59
Table 4.1	Quantitative RT-PCR primers	74
Table 4.2	Number of visible follicles, total oocyte, healthy oocytes, cleaved oocytes, morula embryos, and blastocyst embryos of goats received no hormone (control day 17) or normal saline (control day 20) or FSH-P and subsequent withdrawal (1 d FSH + 2 d W, 2 d FSH + 1 d W, and 3 d FSH)	76

**LIST OF TABLES (Cont.)**

	<b>Page</b>
Table 4.3	77
Labeling index (LI) of granulosa and theca cells of follicles in goats received no hormone (control day 17) or normal saline (control day 20) or FSH-P and subsequent withdrawal (1 d FSH + 2 d W, 2 d FSH + 1 d W, and 3 d FSH)	
Table 5.1	94
Follicular dynamic, size and number of CL, and synchronized ovulation rate of postpartum dairy cows received GPH (modified Ovsynch), Ovsynch+hCG, and Ovsynch+GnRH protocols.	

## LIST OF FIGURES

		<b>Page</b>
Figure 2.1	Dynamics of ovarian follicular development and gonadotropin secretion during two- and three-wave estrous cycles in cattle.	8
Figure 2.2	Schematic representation of the temporal changes in the levels of angiogenic factors during the bovine follicular–luteal transition.	23
Figure 3.1	Representative fluorescent micrographs of Factor VIII positive staining (green) in antral follicles of bovine ovaries.	40
Figure 3.2	Representative fluorescent micrographs of positive staining for Factor VIII and eNOS (green) in the section of healthy small, healthy medium, and healthy large follicles in cattle ovaries from the first follicular wave.	55
Figure 3.3	Representative fluorescent micrographs of positive staining for Factor VIII and eNOS (green) in the section of atretic small, atretic medium, and atretic large follicles in cattle ovaries from the first follicular wave.	56
Figure 3.4	Representative micrographs of positive staining for PCNA in the section of healthy small, atretic small, healthy medium, atretic medium, healthy large, and atretic large follicles in cattle ovaries from the first follicular wave.	57
Figure 4.1	Description of timing of injections for the five treatment protocols.	68
Figure 4.2	Representative micrographs of positive staining for PCNA in the section of follicles collected from the control (day 17), control (day 20), 1 d FSH + 2 d W, 2 d FSH + 1 d W, and 3 d FSH groups.	78

### LIST OF FIGURES (Cont.)

		<b>Page</b>
Figure 4.3	Mean ( $\pm$ SEM) plasma progesterone (P4) concentration in goats received no hormone (control day 17) or normal saline (control day 20) or FSH-P and subsequent withdrawal (1 d FSH + 2 d W, 2 d FSH + 1 d W, and 3 d FSH).	79
Figure 4.4	Relative abundance of Cx43 in morula embryos (black bars) and blastocyst embryos (white bars) developed from oocytes collected from follicles in goats received no hormone (control day 17) or normal saline (control day 20) or FSH-P and subsequent withdrawal (1 d FSH + 2 d W, 2 d FSH + 1 d W, and 3 d FSH).	80
Figure 4.5	Relative abundance of Bcl-2 in morula (black bars) and blastocyst embryos (white bars) developed from oocytes collected from follicles in goats received no hormone (control day 17) or normal saline (control day 20) or FSH-P and subsequent withdrawal (1 d FSH + 2 d W, 2 d FSH + 1 d W, and 3 d FSH).	81
Figure 5.1	Description of timing of injections for the three synchronization protocols.	92
Figure 5.2	Sonogram images of a CL on day 5 after AI and two CL side by side on the ovary of a hormone-treated cow on day 12 after AI.	95
Figure 5.3	Mean plasma P4 concentrations (ng/ml) determined in dairy cows after receiving GPH (black bars), Ovsynch+hCG (white bars), or Ovsynch+GnRH (hatched bars).	96

## TABLE OF ABBREVIATIONS

ADP	Adenosine diphosphate
AI	Artificial insemination
ANGPT	Angiopietin
APC	Area per capillary
ART	Assisted reproductive technology
ATP	Adenosine triphosphate
Bcl-2	B cell lymphoma-2
BCS	Body condition score
bFGF	Basic fibroblast growth factor
BW	Body weight
°C	Degree Celsius
CAD	Capillary area density
CL	Corpus luteum
CND	Capillary number density
CO <sub>2</sub>	Carbon dioxide
COCs	Cumulus-oocyte complexes
Cx43	Connexins43
DAB	Diaminobenzadine
DAPI	4',6-diamidino-2-phenylindole
DIM	Day in milk
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
E2	Estradiol
EA	Estrogen-active
eCG	Equine chorionic gonadotrophin
ECM	Extra-cellular matrix
EGF	Endothelial growth factor
EI	Estrogen-inactive
ELISA	Enzyme-linked immunosorbent assay

**TABLE OF ABBREVIATIONS (Cont.)**

eNOS	Endothelial nitric oxide synthase
FF	Follicular fluid
FGF2	Fibroblast growth factor 2
FMD	Foot and mouth disease
FSH	Follicle-stimulating hormone
FSHR	Follicle-stimulating hormone receptor
G	Granulosa cell layers
GnRH	Gonadotropin-releasing hormone
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
h	hour
hCG	Human chorionic gonadotropin
HIF1A	Hypoxia-induced factor 1 $\alpha$
HS	Hemorrhagic septicemia
IGF	Insulin-like growth factor
IgG	Immunoglobulin G
INF- $\tau$	Interferon tau
iNOS	Inducible nitric oxide synthase
IVC	In vitro culture
IVF	In vitro fertilization
IVP	In vitro production
IVM	In vitro maturation
kg	kilogram
LH	Luteinizing hormone
LHR	Luteinizing hormone receptor
LI	Labeling indices
LPO	Lipid peroxidation
mg	Milligram
MgCl <sub>2</sub>	Magnesium chloride
mm	Millimeter

**TABLE OF ABBREVIATIONS (Cont.)**

mM	Millimole
mRNA	Messenger ribonucleic acid
ng	Nanogram
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
OR	Oxytocin receptors
P4	Progesterone
P450 arom	P450 aromatase
PB	Polar body
PBS	Phosphate buffer solution
PCNA	Proliferating cell nuclear antigen
PDGF	Platelet-derived growth factor
PGF <sub>2α</sub>	Prostaglandin F <sub>2α</sub>
PMSG	Pregnant mare serum gonadotropin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
PR	Progesterone receptor
RT	Reverse transcription
RT-PCR	Reverse transcription polymerase chain reaction
s	Second
SEM	Standard error of mean
StAR	Steroidogenic acute regulatory protein
T	Theca cell layers
TAI	Time artificial insemination
μl	Microliter
μm	Micrometer or micron
v/v	volume/volume
VEGF	Vascular endothelial growth factor
vSMC	Vascular smooth muscle cells