



بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة / سلوي محمود عقل

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد





Cairo University
Faculty of Veterinary Medicine
Department of Theriogenology



Uncovering the Molecular Aspect of the Pregnancy Maintenance in Dromedary Camels

Thesis presented by
Ola Adel Ahmed Saied
(B.V.Sc. 2018)
As a requirement of master's degree
(Theriogenology)

Under supervision of
Prof. Dr. Adel Attia Mohamed Seida
Professor of Theriogenology
Faculty of Veterinary Medicine
Cairo University

Dr. Karima Gh. M. Mahmoud

Professor of Reproductive Genetics
Veterinary Research Institute
National Research Centre

Dr. Mohamed Fathi Mohamed

Assistant Professor of Theriogenology
Faculty of Veterinary Medicine
Cairo University

(2022)



Cairo University
Faculty of Veterinary Medicine
Department of Theriogenology



Approval Sheet

This is to approve that the dissertation presented by Mrs. **Ola Adel Ahmed Saied** to Cairo University entitled:

Uncovering the Molecular Aspect of the Pregnancy Maintenance in Dromedary Camels

For the degree of M.V.Sc. (Theriogenology) has been approved by the examining committee:

Prof. Dr. Mahmoud Mohamed Hussein

Prof. of Theriogenology
Faculty of Veterinary Medicine
Beni Suef University

Prof. Dr. Khaled Hafez El-Shahat

Prof. of Theriogenology
Faculty of Veterinary Medicine
Cairo University

Prof. Dr. Adel Attia Mohamed Seida (Supervisor)

Prof. of Theriogenology
Faculty of Veterinary Medicine
Cairo University

Dr. Mohamed Fathi Mohamed (Supervisor)

Assistant Professor of Theriogenology
Faculty of Veterinary Medicine Cairo University

Committee Date

16/3/2022



Cairo University
Faculty of Veterinary Medicine
Department of Theriogenology



Supervision sheet
**Uncovering the Molecular Aspect of the Pregnancy
Maintenance in Dromedary Camels**

Thesis presented by
Ola Adel Ahmed Saied
(B.V.Sc. 2018)
As a requirement of Master's Degree
(Theriogenology)

Under supervision of

Prof. Dr. Adel Attia Mohamed Seida

Professor of Theriogenology
Faculty of Veterinary Medicine
Cairo University

Dr. Karima Gh. M. Mahmoud

Professor of Reproductive Genetics
Veterinary Research Institute
National Research Centre

Dr. Mohamed Fathi Mohamed

Assistant Professor of Theriogenology
Faculty of Veterinary Medicine
Cairo University

(2022)

Name : Ola Adel Ahmed Saied

Nationality : Egyptian

Date of birth : 02/08/1994

Scientific degree : M.V.Sc.

Specification : Theriogenology

Title of thesis : Uncovering the molecular aspect of the pregnancy maintenance in dromedary camels

Supervisors:

Prof. Dr. Adel Attia Mohamed Seida

Professor of Theriogenology

Faculty of Veterinary Medicine

Cairo University

Dr. Mohamed Fathi Mohamed

Assistant Professor of Theriogenology

Faculty of Veterinary Medicine

Cairo University

Dr. Karima Gh. M. Mahmoud

Professor of Reproductive Genetics

Veterinary Research Institute

National Research Centre

Abstract

A proper regulated dialogue between the endometrium and conceptus (embryonic disc and extra-embryonic tissues) is required for successful implantation and pregnancy. The present thesis aimed to (I) investigate the dynamic expression pattern of genes related to implantation and vascularization, and (II) shed light on the molecular interactions among left & right uterine horns, as well as uterine body in pregnant dromedary camels. For this, 40 genital tracts and blood samples were collected from slaughterhouse during the breeding season (November, 2020 to March, 2021). Moreover, total RNA isolation, RT-PCR for candidate genes, and measuring of steroid hormones in serum was performed. Results showed that insulin like growth factor (IGF1), vascular endothelial growth factor A (VEGFA), FOS as well as JUN proto-oncogenes mRNAs were up-regulated ($P<0.001$) in the pregnant groups compared to non-pregnant ones. Interestingly, integrin subunit beta 4 (ITGB4), FOS, IGF1 and placental growth factor (PGF) transcripts were increased ($P<0.001$) in the right uterine horn of the pregnant groups compared to non-pregnant. Moreover, JUN, solute carrier organic anion transporter family member 2A1 (SLCO2A1), VEGFA and phosphatase and tensin homolog (PTEN) genes were highly up-regulated ($P<0.001$) in uterine body, left and right uterine horns of the pregnant groups vs. non-pregnant. Platelet derived growth factor subunit A (PDGFA) was up-regulated ($P<0.001$) in left and right uterine horns of the pregnant groups vs. non-pregnant. Apoptosis regulator (BCL2) increased ($P<0.001$) in the uterine body of the pregnant groups vs. non-pregnant. In serum, the levels of progesterone and estradiol 17- β increased in pregnant she-camels compared to non-pregnant ones. In the pregnant she-camels the histological examination revealed hypertrophy of the uterine myometrium, abundant of dilated blood vessels and thickening of the walls. In addition, right and left uterine horn of pregnant she-camels showed normal increasing glands especially in the left one and less fibrous stroma vs. the non-pregnant. Taken together, a precise regulation of genes associated with endometrial receptivity and placental formation is required for a proper embryonic implantation in pregnant she-camels.

Keywords: Camel, Pregnancy, Uterine tissue, Gene expression, Serum, Steroids

Dedication

I dedicate this study to my parents and my brother for their, understanding, supporting and helping to succeed.

Acknowledgement

In the name of Allah the almighty who taught man about matter that he does not know and prayers and peace be upon our holy prophet Muhammed and his good followers till the Day of Judgment.

*First of all I would like to extend my thanks to **Prof. Dr. Adel Attia Seida**, Professor of Theriogenology, Faculty of Veterinary Medicine, Cairo University, for his excellent supervision, encouragement, guidance and valuable instruction that he offered to me while performing this work. His intensive comments have helped me step by step throughout this study and his great help in revising and finishing this thesis indeed, I am very honored to work under his supervision.*

*I express my deep gratitude, sincere and heartfelt thanks to my supervisor **Prof. Dr. Karima Gh. M. Mahmoud**, Professor of Reproductive Genetics, Veterinary Research Institute, National Research Centre, for her excellent supervision, constant support, untiring patience, stimulating suggestions, encouragement, guidance and valuable instruction helped me in both, all the time of research work and the writing of this thesis.*

*I pay my greatest respect and gratitude to **Dr. Mohamed Fathi Mohamed**, Assistant professor of Theriogenology Faculty of Veterinary Medicine, Cairo University for his valuable supervision, sincere attitude and cooperation.*

*I have to give the greatest thanks to **DR. Sally Rashad Elsaid Ibrahim**, Assistant Professor of Reproductive Genetics, National Research Centre, for her planning for the work, practical learning, continuous support, friendship, enormous effort, guidance as well as revising my thesis.*

*I would like to thank sincerely **DR. Samaa Galal** for her continuous encouragement and support.*

*My thanks and appreciation to **all staff members of Theriogenology Department**, Faculty of Veterinary medicine, Cairo University, for their cooperation and encouragement.*

Contents

Abstract.....	
Dedication.....	
Acknowledgement.....	
Table of contents.....	
List of tables.....	I
List of figures.....	II
List of abbreviations.....	III-IV
Chapter (1): Introduction.....	1
Chapter (2): Review of literature.....	4
1. Camel	4
2. Pregnancy in camel	5
2.1. Site of pregnancy	5
2.2. Embryonic migration	5
2.3. Multiple ovulation and early embryonic death	6
2.4. Follicular activity during pregnancy	6
2.5. Placentation	7
3. Maternal recognition of pregnancy	8
4. Steroid hormones during pregnancy and corpus luteum vascularization	9
5. Determination of the fetal age	11
6. Genes regulate pregnancy	12
6.1. Candidate genes	13
6.1.1. Genes related to implantation and matrix formation	13
6.1.1.1. Integrin subunit beta 4 (ITGB4)	13
6.1.1.2. Solute carrier organic anion transporter family member 2A1 (SLCO2A1)	14

6.1.1.3. FOS and JUN proto-oncogenes	15
6.1.2. Genes related to vascularization and placental formation	15
6.1.2.1. Vascular endothelial growth factor A (VEGFA)	16
6.1.2.2. Placental growth factor (PGF)	16
6.1.2.3. Platelet derived growth factor subunit A (PDGFA)	16
6.1.3. Genes related to growth and development	17
6.1.3.1. Insulin like growth factor 1 (IGF1)	17
6.1.3.2. Phosphatase and tensin homolog (PTEN)	17
6.1.4. Apoptosis regulator gene (BCL2)	18
6.2. Other genes	18
6.2.1. Interferons (IFNs) and Interferon stimulated genes (ISGs)	18
6.2.1.1. 2'-5' oligoadenylate synthetase (OAS1)	19
6.2.1.2. Myxovirus resistance protein (MX)	20
6.2.1.3. Suppressor of cytokine signaling (SOCS)	20
6.2.1.4. Radical S-Adenosyl Methionine Domain Containing 2 (RSAD2)	22
6.2.2. Signal transducer and activator of transcription 1 (STAT1)	23
6.2.3. Fork head box L2 (FOXL2)	23
6.2.4. Mucin 1 (MUC1)	24
6.2.5. Tissue-derived matrix metalloproteinase inhibitors (TIMPS)	25
6.2.6. Progesterone receptor (PGR)	26
6.2.7. Estrogen receptors (ER)	27
6.2.8. Oxytocin receptor (OTR)	29
6.2.9. Cyclooxygenase 2 (COX-2)	30
Chapter (3): submitted and published paper (S)	
3.1. Dynamic expression pattern of genes related to implantation and vascularization in pregnant dromedary camels	31
1. Abstract	32
2. Introduction.....	33

3. Materials and Methods.....	34
3.1. Collection of genital tracts and blood samples	34
3.2. RNA isolation and cDNA synthesis	35
3.3. Quantitative real-time PCR analysis	35
3.4. Assay of the progesterone concentrations	36
3.5. Histological examination	36
3.6. Data Analysis	36
4. Results.....	36
4.1-The gene expression pattern related to implantation, vascularization and placental formation	36
4.2- The serum level of progesterone in pregnant and non-pregnant she camel	36
4.3-Histological examination of the uterine tissue of the pregnant and non-pregnant she camel	37
5. Discussion	37
6. Conclusion	39
7. References	39
3.2- Molecular regulation of pregnancy in she-camels: Influence of corpus luteum contralateral to gravid uterine horn	47
1. Introduction.....	49
2. Materials and Methods.....	51
2.1. Samples collection	51
2.2. RNA isolation and cDNA synthesis	52
2.3. Quantitative real-time PCR analysis (qRT- PCR)	53
2.4. Evaluation the steroid concentrations (P4 and E2)	53

2.5. Histological examination	54
2.6. Data Analysis	54
3. Results.....	54
3.1. The expression pattern of genes related to implantation and matrix formation	54
3.2. The relative abundance of genes related vascularization and placental formation	55
3.3. The expression profile of genes related to growth, development and cell death regulation	55
3.4. The dynamic levels of steroid hormones (P4 and E2) in serum of pregnant and non-pregnant she-camels	56
3.5. Histological examination	56
4. Discussion	56
5. References	60
Chapter (4): Discussion	73
Chapter (5): Conclusion and Recommendations	78
Chapter (6): Summary	79
Chapter (7): References	81
Appendix	107
الملخص العربي	
المستخلص العربي	

List of tables

No.	Table	Page
<u>Chapter 3.1</u> Table (1)	List of primers used for qRT-PCR analysis	44
<u>Chapter 3.2</u> Table (1)	List of primers used for qRT-PCR analysis	65
Table (2)	The level of steroid hormones (P4&E2) in serum of pregnant and non-pregnant she camels	66

List of figures

No.	Figure	Page
<u>Chapter 2</u>	Maternal recognition steps during pregnancy.	9
Figure (1)		
Figure (2)	A schematic representation of an implanting embryo.	13
<u>Chapter 3.1.</u>	The relative abundance of IGF1, VEGFA, FOS and JUN genes in pregnant and non-pregnant she camels.	45
Figure (1)		
Figure (2)	Progesterone concentrations between the pregnant and non-pregnant she camels.	46
Figure (3)	Histological examination of the uterine tissues of the pregnant and non-pregnant she-camels.	46
<u>Chapter 3.2</u>	The relative abundance of ITGB4, SLCO2A1, FOS and JUN proto-oncogenes between pregnant and non-pregnant she camels.	67
Figure (1)		
Figure (2)	The expression level of VEGFA, PGF and PDGFA in pregnant and non-pregnant groups.	68
Figure (3)	Fold change of IGF1, PTEN, BCL2 genes in pregnant and non-pregnant she-camels	69
Figure (4)	Histological examination of uterine body, right and left uterine horns in pregnant and non-pregnant she camels.	71
Figure (5)	Schematic diagram showing the precise regulation among genes related to endometrium receptivity, vascularization and placental formation, growth and development as well as cell death regulation that are necessary for successful implantation and pregnancy maintenance.	72

List of abbreviations

AI	Artificial Insemination
BCL2	Apoptosis Regulator Gene
CISH	Endometrial cytokine Inducible SH2 Containing Protein
CL	Corpus Luteum
COX-2	Cyclooxygenase2
CVRL	Crown Vertebral Rump Length
E2	Estrogen
EMMPRIN	Extracellular Matrix Metalloproteinase Inducer
ER	Estrogen Receptor
FAO	Food and Agriculture Organization
FOXL2	Fork Head Box L2
GE	Glandular Epithelium
IFN	Interferon
IGF1	Insulin like Growth Factor 1
IRF	Interferon Regulatory Factor
ISGs	Interferon Stimulated Genes
ITG	Integrin
ITGB4	Integrin Subunit Beta 4
LE	Luminal Epithelium
LGMN	Legumain
LUH	Left Uterine Horn
MMP	Matrix Metalloproteinase
MRP	Maternal Recognition of Pregnancy
MUC1	Mucin 1
MX	Myxovirus Resistance Protein
OAS1	Oligoadenylate Synthetase

OTR	Oxytocin Receptor
P4	Progesterone
PDGFA	Platelet Derived Growth Factor Subunit A
PGE2	Prostaglandin E2
PGES	Prostaglandin E2 Synthase
PGF	Placental Growth Factor
PGF _{2α}	Prostaglandins F2 Alpha
PGH2	Prostaglandin G/H Synthases 2
PGR	Progesterone Receptor
PGS	Prostaglandins
PTEN	Phosphatase and Tensin Homolog
PTGFR	Prostaglandin F Receptor
RSAD2	Radical S-Adenosyl Methionine Domain Containing2
RUH	Right Uterine Horn
SACs	South American Camelids
SLCO2A1	Solute Carrier Organic Anion Transporter Family Member 2A1
SOCS	Suppressor of Cytokine Signaling
STAT1	Signal Transducer and Activator of Transcription1
TIMPS	Tissue-Derived Matrix Metalloproteinase Inhibitors
VEGFA	Vascular Endothelial Growth Factor A
Vs.	<i>Versus</i>