



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



Faculty of Science
Zoology Department



Molecular characterization of some immunity genes related to endometritis in Egyptian buffalo

A Thesis

Submitted for the award of Ph.D. degree of Science in Zoology

By

Dalia Ahmed Abdul Kader Ali Taha

(B.Sc. Zoology - M.Sc. Zoology)

Assistant Researcher

Cell Biology Department- Genetic Engineering and Biotechnology Division

National Research Centre

Supervised by

Prof. Dr. Nagwa Hassan Ali Hassan

Professor of Cytogenetics

Zoology Department - Faculty of Science

Ain Shams University

Prof. Dr. Othman El-Mahdy Sayed Othman

Professor of Molecular Genetics and Biotechnology

Cell Biology Department - Genetic Engineering and Biotechnology Division

National Research Centre

Prof. Dr. Eman Roshdy Mahfouz

Professor of Molecular Genetics and Biotechnology

Cell Biology Department - Genetic Engineering and Biotechnology Division

National Research Centre

**Faculty of Science
Ain Shams University**

2021

﴿بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ﴾

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا
عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

﴿صدق الله العظيم﴾

سورة البقرة (٢٢)



Approval Sheet

Student Name: **Dalia Ahmed Abdel Kader Ali Taha**

Thesis Title: **“Molecular characterization of some immunity genes related to endometritis in Egyptian buffalo”**

Degree: **Ph.D. of science in Zoology**

Supervisors Committee

1	Prof. Dr. Nagwa Hassan Ali Hassan	Professor of Cytogenetics Zoology Department - Faculty of Science- Ain Shams University
2	Prof. Dr. Othman El-Mahdy Sayed Othman	Professor of Molecular Genetics and Biotechnology- Cell Biology Department- National Research Centre
3	Prof. Dr. Eman Roshdy Mahfouz	Professor of Molecular Genetics and Biotechnology- Cell Biology Department- National Research Centre

Approval Committee

1	Prof. Dr. Sobhy El-Sayed Hassab El-Nabi	Professor of Genetics & Molecular Biology -Faculty of Science – Menoufiya University
2	Prof. Dr. Nadia Hussien Mohamed sayed	Professor of cytogenetics & Molecular genetics- Faculty of Girls – Ain shams University
3	Prof. Dr. Nagwa Hassan Ali Hassan	Professor of Cytogenetics Zoology Department - Faculty of Science- Ain Shams University
4	Prof. Dr. Othman El-Mahdy Sayed Othman	Professor of Molecular Genetics and Biotechnology- Cell Biology Department- National Research Centre

Thesis decision 28 /8/ 2021

Approval Stamp

Thesis Approved date / /2021

Faculty council approval

University council approval

ACKNOWLEDGMENT

First and for most, I feel indebted to **ALLAH**, most graceful, who gave me the strength to complete this work and helping me in all my life.

I would like to express my deepest gratitude and thankful to, **Prof. Dr. Nagwa Hassan Ali Hassan**, Professor of Cytogenetics, Zoology Department, Faculty of Science, Ain Shams University, for her valuable help, guidance, generous support, encouragement and continuous supervision. It's been very pleasant to work with her.

Words are few to express my deepest gratitude and appreciation to **Prof. Dr. Othman El-Mahdy Sayed Othman**, Professor of Molecular Genetics and Biotechnology, Cell Biology Department, National Research Centre, for his continuous encouragement, and close supervision throughout this work and for his precious time and valuable advice and effort that made this work possible. I was lucky to work with him.

My profound gratitude and deepest thanks are to **Prof. Dr. Eman Roshdy Mahfouz**, Professor of Animal Genetics, Cell Biology Department, National Research Centre for her guidance, kind supervision, valuable advices, time, encouragement and patient throughout my research work.

I would like to express my deep appreciation and gratitude to my Interior supervisor at National Research Center **Prof. Dr. Mona Ahmed Bibars**; Professor of Animal Genetics, Cell Biology Department, National Research Centre; for her encouragement, continuous advice, infinite patience and her keen supervision.

My appreciation is extended to **my colleagues** in the National Research Centre for their distinguished help and support.

Last but not least, I would like to express my endless gratitude to **All My Family** for their great encouragement and support.

DEDICATION

Words can not express how grateful I am to my dear **Family** thank you for your endless love and support throughout my life.

My dearest **Parents** for all sacrifices that you've made on my behalf. My loving Dad **Ahmed**, for encourage me, inspiration and wisdom. To my amazing Mom **Eman**, for raising me to be successful, independent and strong.

My heart felt regard goes to my **Parents-in-law**, for their love, help and moral support.

To my beloved partner and friend my Husband **Mostafa**, for his continued and unfailing love, for always being there, believe in me and support me to reach my goals. I can't thank you enough for encouraging me throughout this experience.

My handsome Sons **Mazen & Ahmed**, I would like to express my thanks for being such good boys always cheering me up and helping me. I am so grateful to Allah to have both of you.

To my precious, sister **Nancy** & brother **Mohamed**, for their selfless love, caring, standing beside me and support me for everything. Also, my thanks to my sister in-law, **Aliaa** & brothers-in-law, **Khaled & Omran**, for their kind help, support and constant love.

List of Contents

Contents	Page
List of Abbreviation	i
List of Figures	iii
List of Tables	v
Abstract	vi
Introduction	1
Aim of Work	4
CHAPTER1: REVIEW OF LITERATURE	5
1.1. Water buffalo	5
1.2. Buffalo in Egypt	8
1.3. Endometritis in Buffalo	11
1.3.1. Diagnosis of endometritis in buffalo	13
1.4. Buffalo immune system	15
1.5. Gene Expression	17
1.5.1. Real-time polymerase chain reaction (RT-PCR)	18
1.5.2. Analysis of Real Time PCR Ct Values	21
1.6. Expression of immunity genes related to buffalo endometritis	24
1.6.1. Interleukin-1 alpha (<i>IL-1α</i>) gene	25
1.6.2. Interleukin-1 beta (<i>IL-1β</i>) gene	25
1.6.3. Interleukin-6 (<i>IL-6</i>) gene	26
1.6.4. Interleukin-10 (<i>IL-10</i>) gene	27
1.6.5. Tumor necrosis factor alpha (<i>TNF-α</i>) gene	27
1.6.6. Transforming growth factor beta (<i>TGF-β</i>) gene	28

1.6.7. Chemokine CXC ligand 5 (<i>CXCL5</i>)	29
1.6.8. Haptoglobin (<i>HP</i>) gene	29
1.6.9. Prostaglandin E2 receptors (<i>PTGER2</i> and <i>PTGER4</i>)	30
CHAPTER 2: MATERIALS & METHODS	32
2.1. Materials	32
2.1.1. Kits	32
2.1.2. Instruments	32
2.2. Methods	33
2.2.1. Collection of samples	33
2.2.2. Bacterial identification	33
2.2.3. RNA extraction	33
2.2.4. RNA concentration and purity measures	35
2.2.5. cDNA synthesis	35
2.2.6. Primers used	36
2.2.7. Real-Time quantitative polymerase chain reaction (RT-qPCR)	39
2.2.8. Statistical data analysis	40
CHAPTER 3: RESULTS	41
3.1. Bacteriological examination of uterine samples	41
3.2. RNA isolation from uteri samples	42
3.3. cDNA synthesis	44
3.4. Gene expression analysis	45
3.4.1. Glyceraldehyde 3-phosphate dehydrogenase (<i>GAPDH</i>) gene	47
3.4.2. Interleukin-1 alpha (<i>IL-1α</i>) gene	49
3.4.3. Interleukin-1 beta (<i>IL-1β</i>) gene	52
3.4.4. Interleukin-6 (<i>IL-6</i>) gene	55

3.4.5. Interleukin-10 (<i>IL-10</i>) gene	58
3.4.6. Tumor necrosis factor alpha (<i>TNF-α</i>) gene	61
3.4.7. Transforming growth factor beta receptor 1 (<i>TGFBRI</i>) gene	64
3.4.8. Haptoglobin (<i>HP</i>) gene	67
3.4.9. Chemokine CXC ligand 5 (<i>CXCL5</i>) gene	70
3.4.10. Prostaglandin E2 receptors (<i>PTGER2</i>) gene	73
3.4.11. Prostaglandin E4 receptors (<i>PTGER4</i>) gene	76
CHAPTER 4: DISCUSSION	82
SUMMARY	100
CONCLUSION	103
CHAPTER 5: REFERENCES	104
Arabic Summary	٣
Arabic Abstract	١

LIST OF ABBREVIATION

APPs	Acute phase proteins
BLV	Bovine leukemia virus
Bp	Base pair
CD4+	Cluster of differentiation 4
cDNA	Complementary DNA
Ct	Cycle threshold
Δ Ct	Delta cycle threshold
<i>CXCL5</i>	Chemokine CXC ligand 5
DEPC	Diethyl pyrocarbonate
E. Coli	Escherichia coli
ELISA	Enzyme-linked immunosorbent assay
<i>GAPDH</i>	Glyceraldehyde 3-phosphate dehydrogenase gene
<i>HKG</i>	House-Keeping gene
<i>HP</i>	Haptoglobin gene
<i>IFN</i>	Interferon gene
<i>IL-1</i>	Interleukin-1 gene
<i>IL-10</i>	Interleukin-10 gene
<i>IL-1α</i>	Interleukin-1 Alpha gene
<i>IL-1β</i>	Interleukin-1 beta gene
<i>IL-6</i>	Interleukin-6 gene
LPS	lipopolysaccharide
M. avium subsp	Mycobacterium avium subspecies
mRNA	Messenger ribonucleic acid
NCBI	National Center for Biotechnology Information
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
pp	Postpartum
PTGER	Prostaglandin E receptors
PTGS2	Prostaglandin-endoperoxide synthase 2
qPCR	Quantitative PCR

qRT-PCR	Quantitative Reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SAA	Serum amyloid A
SCE	Subclinical endometritis
<i>TGF-β</i>	Transforming growth factor beta gene
<i>TNF-α</i>	Tumour necrosis factor alpha
TRAF1	TNF receptor-associated factor 1
VSV	Vesicular stomatitis virus infection
<i>β-actin</i>	Beta-actin gene

List of Figures

Fig.	Title	Page
1	Gene expression steps using real-time PCR.	19
2	The PCR amplification curve's phases. At each reaction cycle, the PCR amplification curve shows the accumulation of fluorescent fluorescence.	21
3	RNA extracted from buffalo uteri shows 28S and 18S rRNA bands indicating the integrity of extracted RNA.	42
4	The electrophoresis of synthesized cDNA on EtBr-stained agarose gel.	45
5	No. of fold change in <i>IL-1α</i> gene expression between healthy and infected animals.	51
6	No. of fold change in <i>IL-1β</i> gene expression between infected and healthy animals	54
7	No. of fold change in <i>IL-6</i> gene expression between infected and healthy animals	57
8	No. of fold change in <i>IL-10</i> gene expression between healthy and infected animals.	60
9	No. of fold change in <i>TNF-α</i> gene expression between healthy and infected animals	63
10	No. of fold change in <i>TGFBRI</i> gene expression between healthy and infected animals.	66