



Cairo University



Faculty of Veterinary Medicine
Department of Theriogenology

Some Studies on Ovarian Inactivity in Cattle

A thesis presented by

Yasser Hussein Ali Saber

(B.V.Sc., Benisuef University, 2011)

Submitted to

Faculty of Veterinary Medicine, Cairo University

In partial fulfillment of the requirements for the degree of

**M.V.Sc. in Veterinary Medical Sciences
(Theriogenology)**

Under supervision of

Dr. Adel Attia Mohamed Seida

Professor of Theriogenology

Faculty of Veterinary Medicine

Cairo University

Dr. Refaat Sobhy Ahmed Ragab

Professor of Theriogenology

Faculty of Veterinary Medicine

Cairo University

Dr. Wahid Mohamed M. Ahmed

Research Professor of Animal Reproduction

and AI, Veterinary Research Division,

National Research Centre

(2017)



Cairo University



Faculty of Veterinary Medicine
Department of Theriogenology

Supervision sheet

Dr. Adel Attia Mohamed Seida

Professor of Theriogenology
Faculty of veterinary Medicine
Cairo University

Dr. Refaat Sobhy Ahmed Ragab

Professor of Theriogenology
Faculty of Veterinary Medicine
Cairo University

Dr. Wahid Mohamed M.Ahmed

Research Professor of Animal Reproduction
and AI, Veterinary Research Division,
National Research Centre

(2017)



Cairo University



Faculty of Veterinary Medicine
Department of Theriogenology

Name: Yasser Hussein Ali Saber
Date and place of birth: January 24, 1989, Benisuef, Egypt
Nationality: Egyptian
Degree: Master Degree in Veterinary Medical Sciences
Specialty: Theriogenology
Thesis Title: Some studies on ovarian inactivity in Cattle
Supervisors:
Dr. Adel A. M. Seida
Professor of Theriogenology, Faculty of Veterinary Medicine, Cairo University
Dr. Refaat S. A. Ragab
Professor of Theriogenology, Faculty of Veterinary Medicine, Cairo University
Dr. Wahid M. M. Ahmed
Research Professor of Animal Reproduction and AI, National Research Centre

ABSTRACT

Ovarian inactivity is one of the most important causes of low fertility in farm animals in most of developing countries with high economic losses due to low calf and milk production. The present study was carried out to investigate the present status of ovarian inactivity in cows at Upper Egypt. Special interest was given to monitor the most important predisposing factors, the relationship with oxidant/ antioxidant status and polymorphism of CYP19 gene. The incidence of ovarian inactivity was traced in records of 11361 cows admitted to veterinary clinics at different cities at Benisuef governorate during 2015. Moreover, a total number of 260 crossbred cows at Benisuef city was assigned for the current study. These animals were examined for ovarian activity by rectal palpation. Progesterone assay was used to confirm the activity. Cows were categorized into a group having ovarian structure and a group showing bilateral smooth inactive ovary. Blood samples were used to determine plasma levels of Malondialdehyde (MDA), Nitric oxide (NO), Total antioxidant capacity (TAC), Superoxide Dismutase (SOD), Zinc (Zn) and Copper (Cu). The allelic patterns of the PCR product of exon 2 of CYP19 gene was analyzed and the 351bp, containing exon was amplified followed by sequence analysis. Results of case records analysis revealed that the incidence of ovarian inactivity at Benisuef governorate during 2015 average 17.13% from animals suffered from reproductive disorders. Alwasta city showed the highest incidence while Beba city showed the lowest incidence. For examined animals at Benisuef city, the incidence average 52.31% from anestrus animals and it was high in winter and in animals with poor body condition score (BCS). Blood analysis indicated that MDA ($P < 0.01$) and NO ($P < 0.05$) increased while, TAC, SOD, Zn and Cu decreased ($P < 0.01$) in affected as compared to normal cyclic cows with no polymorphism in all examined cows. It was concluded that ovarian inactivity is predisposing for low fertility in local cows and it is associated with disturbed oxidant/antioxidant status with no polymorphism in CYP19 gene.

Keywords: Ovarian inactivity - Cows - Benisuef Governorate - Oxidant/Antioxidant Status - CYP19 gene.

DEDICATION

*I would like to dedicate this humble
dissertation with lots of love and respect to*

My father,

My mother,

My wife and

My daughter

*Without their support, love and care, I would
not have realized my dreams in life.*

ACKNOWLEDGEMENTS

*I offer my humble thanks to almighty **ALLAH**, the most merciful and most compassionate and the entire source of all knowledge and wisdom. I am indeed humbly greatfull the holy prophet **Mohammad** (peace be upon him) who is forever a torch of guidance and ideal for all mankind.*

*I feel much pleasure to express my sincere thanks to my supervisor **Dr. Adel Attia Mohamed Seida**, Professor of Theriogenology, Faculty of Veterinary Medicine, Cairo University, for his keen interest, expert guidance, indispensable advice, enthusiasm in reading and criticizing the manuscript and valuable suggestions during entire period to enable me to prepare this work in the present form.*

*I must extend deep emotions of appreciation to **Dr. Refaat Sobhy Ahmed Ragab**, Professor of Theriogenology, Faculty of Veterinary Medicine, Cairo University, for his religious manner, heartening, kind supervision, continuous encouragement and inexhaustible inspiration throughout my course and research work,*

*I wish to express my thanks and the deepest gratitude to **Dr. Wafid Mohamed Ahmed**, Researcher Professor of Animal Reproduction and AI at Veterinary Research Division, National Research Centre, for providing me enthusiastic guidance, constructive criticism, great advice and effort he exerted all over this work, valuable suggestions during entire period and financing support*

*I wish to express my deep appreciation to **Dr. Emtenan Mohamed Hanafi**, Professor of Animal Reproduction & A.I., National Research Centre, for her scientific cooperation, helpful suggestion, guidance, constant supervision and great help throughout the whole work,*

I wish to express my thanks to Dr. Esraa Ali Mohamed, Researcher in Cell Biology Department, Genetic Engineering Division for her support and helpful to me in genetic part of thesis.

I wish to express my thanks to all the staff members, Department of Animal Reproduction and AI, National Research Centre and Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, for their valuable help, support and encouragement.

CONTENTS

Item	Page
List of contents.....	I
List of tables.....	II
List of figures.....	III
Introduction.....	1
Review of literature.....	4
Materials and Methods.....	29
Results.....	38
Discussion.....	55
Conclusions.....	68
Summary.....	69
References.....	72
Abbreviations.....	92
الملخص العربى.....	
المستخلص العربى.....	

LIST OF TABLES

Table No	Title	Page
A	Information of PCR primer used in the present study	35
B	PCR program for amplification of the studied gene	35
1	Incidence of ovarian inactivity in cows at different cities of Benisuef governorate during 2015 (%)	41
2	Monthly variations in incidence of ovarian inactivity in examined anestrus cows (%)	43
3	Seasonal variations in incidence of ovarian inactivity in examined anestrus cows (%)	45
4	Incidence of ovarian inactivity in relation to body condition score (BCS) in examined anestrus cows (%)	47
5	Incidence of ovarian inactivity in relation to parity in examined anestrus cows (%)	49
6	Progesterone values in normal cyclic cows and cows suffering from ovarian inactivity	51
7	Oxidants and antioxidants values in normal cyclic cows and cows suffering from ovarian inactivity	52

LIST OF FIGURES

Figure No	Title	Page
1	Incidence of ovarian inactivity in cows at different cities of Benisuef governorate during 2015 (%)	42
2	Monthly variations in incidence of ovarian inactivity in examined anestrus cows (%)	44
3	Seasonal variations in incidence of ovarian inactivity in examined anestrus cows (%)	46
4	Incidence of ovarian inactivity in relation to body condition score (BCS) in Examined Anestrus Cows (%)	48
5	Incidence of ovarian inactivity in relation to parity in examined anestrus cows (%)	50
6	Agarose gel stained with ethidium bromide showing the PCR product of Cyp19 gene in cow	53
7	The sequence alignment of Cyp19 gene in two cows during cyclicity and ovarian inactivity (alignment by ClustalW2)	54

Introduction

Reproductive disorders generally and inactive ovaries, especially are the main cause of low reproductive efficiency in farm animals in most of developing countries, especially Egypt (**Ahmed, Hanafi and Zaabal, 2012**).

Ovarian inactivity is mainly manifested as late maturity or long postpartum anestrus and is directly or indirectly attributed to managerial, pathological and other external influences with consequent high economic losses (**Perera, 2011 and Akhtar, Lodhi, Ahmad, Qureshi and Muhammad, 2012**). This condition induces high economic losses due to decreased milk production, cost of treatment and decreased number of calves output during the animal life span, besides it predispose for infection of the genital system (**Ahmed et al ., 2012**).

Many factors have been reported to affect the incidence of ovarian inactivity including geographical environment, species, breed, season, level of nutrition, parity and managerial conditions (**Kumar, Singh, Kharche, Govindaraju, Behera, Shukla, Kumar, Agarwal, 2014**). The last available record of the General Egyptian organization for veterinary services (GEOVS) indicated that 23.56% of the total examined cows showing ovarian inactivity with high incidence (23.86%) in cows suffering from genital disorders and it is higher in upper (24.28%) than lower (23.66) Egypt (**GEOVS, 2014**).

Following physiological activities of living organisms, reactive oxygen species (ROS) are produced and soon will be neutralized by oxidant / antioxidant system. Over accumulation of ROS leads to oxidative stress. The condition was recorded to negatively affect the prevalence and severity of several health and reproductive disorders in cows, whereas the accumulated reactive oxygen metabolites (ROM) are toxic to cells (**Ahmed, Nabile, El Khadrawy, Hanafi, Abd El-Moez and Abd El Hameed, 2010**).

Genetic evaluation of animal reproductive performance depends on molecular technology for identifying genes and analysis of their polymorphism (**Beuzen, Stear and Chang, 2000**). The biosynthesis of estrogens from androgen precursors needs enzymatic complex of proteins known as cytochrome P450 aromatase (**Kowalewska-luczak, Michniewicz and Kulig, 2013**). Aromatase, the protein product of CYP19 gene is the main enzyme in estrogen biosynthesis (**Jędrzejczak, Grzesiak, Szatkowska, Dybus, Muszyńska and Zaborski, 2011**).

Aim of the work: This work aimed to:

- 1- Investigate the present status of ovarian inactivity in cows raised at Benisuef governorate as a model for Upper Egypt.
- 2- Analysis of progesterone in relation to ovarian inactivity.
- 3- Determination of some oxidant / antioxidant values in relation to ovarian inactivity including:

- Oxidants as Malondialdehyde and Nitric oxide.
 - Antioxidants as Total antioxidant capacity, Superoxide Dismutase, Zinc and Copper.
- 4- Monitoring polymorphism of CYP19 gene.

Review of literature

1. Incidence of ovarian inactivity:

Despite the advance in reproductive management techniques such as estrous synchronization and artificial insemination, the reproductive activity of cows has not improved. The last fifty years have seen a significantly increase in the incidence of reproductive problems and infertility of cows **(Butler, 2000)**.

In Egypt, ovarian inactivity was detected as an important component of infertility in native and native x frisian cows rose at Elmenya, Assuit and Sohag governorates. The overall average represented 78.9% from total examined anestrus animals followed by endometritis and silent heat **(Ali, Abdel-Razek, Derar, Abdel-Rheem and Shehata, 2009)**.

In Lower Egypt, ovarian inactivity is the main reason of postpartum anestrus in dairy cows. It represented 41.0% from total examined anestrus dairy cows during the period from May 2006 to August 2009 in Kafrelsheikh governorate **(Karen, Heleil, Nasef and Serur, 2010)**.

The last available records indicated that 23.56% of the total examined cows showed ovarian inactivity while, the incidence was 23.86% out of the number of cows suffering from

genital disorders. Moreover, the incidence was higher in Upper (24.28%) than Lower (23.66) Egypt (**GEOVS, 2014**).

In Iraq, ovarian inactivity represented nearly one quarter (23.6 %) of the total reproductive disorders in the examined dairy cows (**Al-Dahash and Bensassi, 2009**). Furthermore, in an applied study on lactating cows located in Sulaimania region in Boghdad, 26.62% of examined cows suffered from ovarian disorders and inactive ovaries represented 19.51% from these disorders (**Ali and Al-Timimi, 2011**).

Cows slaughtered at the Maiduguri abattoir in northeastern Nigeria and neighboring parts of Niger, Chad and Cameroon Republics that did not show visible follicles or corpora lutea on any of their ovaries represented 11.8% from the total slaughtered animals (**Maina, Furo and Adamu, 2010**).

In Poland, the incidence of ovarian inactivity was recorded as 15.9% from the total number of examined animals and represented 45.9% from the total number of anestrus Polish black and white dairy cows (**Mwaanga, Zduńczyk, Janowski and Kotowski, 2003**). In Holstein-Friesian cows raised at Poland, ovarian inactivity represented 4.6% from examined animals (**Zbylut, Gehrke and Malinowski, 2012**).

For a 10 year retrospective epidemiological survey, using data from a reproductive management program in high producing Holstein-Friesian dairy cows in north-eastern Spain,

the incidence of ovarian inactivity was 7.0% from the total examined animals and this incidence increased significantly with time (**Lopez-Gatius, 2003**).

In a geographical area experiencing both warm and cool conditions, the overall rate of cows suffering from ovarian inactivity was 9.3% of examined lactating cows in north-eastern Spain (**Yaniz, Lopez-Gatius, Bech-Sabat, Garcia-Ispuerto, Serrano and Santolaria, 2008**). Out of the postpartum ovarian dysfunctions, ovarian inactivity recorded 49.2% from anestrus dairy cows (**Peter, Vos and Ambrose, 2009**).

Thirty-three percent of the slaughtered cows (aged two to 14 years) at the Ngaoundere Municipal slaughter house in Cameroon were infertile and the common observed disorder was ovarian inactivity (24.8%) (**Bah, Ebangi, Niba, Manchang, Messine and Achukwi, 2010**).

Xiuli Peng, Cao, Deng, Li, Ye and Yu (2011) found ovarian abnormality in about 60% of dairy cows fed at Yangtze dairy cow farm (Wuhan, China) from 2004 to 2006 and ovarian quiescence represented 18.9% from total ovarian abnormalities.

In Kashmir, India, **Shah and Andrabi (2012)** reported that after three consecutive per rectum and clinical examinations conducted at 7 days interval, out of total examined anestrus crossbred cows, 76.19% showed ovarian inactivity and had not display oestrus for at least 2-4 months.