



Biochemical studies on cryopreservation of ram semen

By

Dina Mahdy Mohamed Rashad Mohamed Shokry
BVSc., Faculty of Veterinary Medicine, Mansoura University, 2009
MVSc., Biochemistry, Faculty of Veterinary Medicine, Zagazig University, 2016

THESIS

**Submitted in partial fulfilment of the
Requirements for the Degree of**

Doctor of philosophy (Ph.D.)

in

Veterinary Medical Science

**Department of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine
University of Sadat City
EGYPT**

2020



SUPERVISION SHEET

Biochemical studies on cryopreservation of ram semen

Ph.D. Thesis

In

**Veterinary Medical Science
(Biochemistry and Chemistry of Nutrition)**

By

Dina Mahdy Mohamed Rashad Mohamed Shokry
BVSc., Faculty of Veterinary Medicine, Mansoura University, 2009
MVSc., Biochemistry, Faculty of Veterinary Medicine Zagazig University, 2016

SUPERVISION COMMITTEE

Dr. Mabrouk Attia Abd Eldaim
Assistant Professor and Head of Biochemistry and
Chemistry of Nutrition Department, Faculty of Veterinary Medicine,
Menofia University

Dr. Sahar Hassan Orabi
Assistant Professor of Biochemistry and Chemistry of Nutrition,
Faculty of Veterinary Medicine, University of Sadat City

Prof. Magdy Ramadan Badr
Head of research, Artificial Insemination and Embryo Transfer Department
and Deputy of Animal Reproduction Research Institute,
Agriculture Research Center, Haram, Giza,

Dr. Hanem Kamal Basuni khalifa
Lecturer of Biochemistry and Chemistry of Nutrition
Faculty of Veterinary Medicine
University of Sadat City

List of abbreviations

Abbreviation	
AI	Artificial insemination
ARRI	Animal Reproduction Research Institute
ARC	Agriculture Research Center
AR	Acrosome reaction
ANOVA	Analysis of variance
ATP	Adenosine Triphosphate
AGs	Accessory sex glands
ACP	Acid phosphatase
ALP	Alkaline phosphatase
AMI	Acrosomal membrane integrity
AV	Artificial Vagina
BW	Body weight
CAT	Catalase
DNA	Deoxyribo Nucleic Acid
EV	Ejaculate volume
GPx	Glutathione peroxidase
GRD	Glutathione reductase
GSSG	Oxidized glutathione
GSH	Reduced glutathione
HOST	Hypo Osmotic Swelling Test
i.m	Intra muscular
LDH	Lactate Dehydrogenase
LH	Luteinizing Hormone
LPO	Lipid Peroxidase
MDA	Malondialdehyde
MOLE	Moringa oleifera leave extract
ODF	Outer Dense Fibres
PM	Plasma Membrane
PMI	Plasma Membrane Integrity
PUFA	Polyunsaturated Fatty Acid
ROS	Reactive oxygen species
r.p.m.	Revolution per minute
SP	Seminal plasma
S.E.	Standard error
SOD	Superoxide dismutase
TAC	Total Antioxidant Capacity
TP	Total Proteins
TCA	Trichloroacetic acid

Contents

	Page
List of tables	I
List of figures	III
1. Introduction	1
2. Review of literature	5
3. Materials and Methods	33
4. Results	50
5. Discussion.....	82
6. Conclusion and recommendation.....	94
7. Summary	95
8. References	98
9. Arabic summary	
10. Arabic abstract.....	

List of tables

Table No.	Title	Page
Table 1	Scores and description for progressive motility	37
Table 2	Effect of MOLE and vitamin E and Se on body weight of adult rams	50
Table 3	Effect of MOLE and vitamin E and Se on reaction time (libido) of adult rams	51
Table 4	Effect of MOLE and vitamin E and Se on semen volume of adult rams	52
Table 5	Effect of MOLE and vitamin E and Se on sperm individual motility (%) of adult rams	53
Table 6	Effect of MOLE and vitamin E and Se on sperm concentration of adult rams	54
Table 7	Effect of MOLE and vitamin E and Se on live dead sperm percentage of adult rams	55
Table 8	Effect of MOLE and vitamin E and Se on sperm abnormality percentage of adult rams	56
Table 9	Effect of MOLE and vitamin E and Se on plasma membrane integrity of adult rams	57
Table 10	Effect of MOLE and vitamin E and Se on DNA fragmentation, tail length and tail moment of adult rams	58
Table 11	Effect of MOLE and vitamin E and Se on seminal plasma glutathione reductase activity of adult rams	59
Table 12	Effect of MOLE and vitamin E and Se on seminal plasma glutathione peroxidase activity of adult rams	60
Table 13	Effect of MOLE and vitamin E and Se on seminal plasma total antioxidant capacity of adult rams	61
Table 14	Effect of MOLE and vitamin E and Se on seminal plasma superoxide dismutase activity of adult rams	62
Table 15	Effect of MOLE and vitamin E and Se on seminal plasma catalase activity of adult rams	63
Table 16	Effect of MOLE and vitamin E and Se on seminal plasma ascorbic acid concentration of adult rams	64
Table 17	Effect of MOLE and vitamin E and Se on seminal plasma alkaline phosphatase activity of adult rams	65
Table 18	Effect of MOLE and vitamin E and Se on seminal plasma acid phosphatase activity of adult rams	66

List of tables

Table 19	Effect of MOLE and vitamin E and Se on seminal plasma malondialdehyde concentration of adult rams	67
Table 20	Effect of MOLE and vitamin E and Se on serum level of testosterone of adult rams	68
Table 21	Effect of different doses of MOLE on post thawing sperms motility and viability index of Barki rams	69
Table 22	Effect of different doses of vitamin E and Se combination on post thawing sperms motility and viability index of Barki rams	71
Table 23	Effect of different doses of vitamin E and Se mixture on post thawing sperms motility and viability index of Barki rams	72
Table 24	Effect of selected doses of MOLE and vitamin E and selenium mixture on post thawing sperms motility and viability index of Barki rams	73
Table 25	Effect of selected doses of MOLE and vitamin E and selenium mixture on post thawing membrane integrity, sperm abnormalities and acrosomal defect of Barki rams	74
Table 26	Effect of selected doses of MOLE and vitamin E and selenium mixture on sperm DNA fragmentation, tail length and tail moment	76
Table 27	Effect of selected doses of MOLE and vitamin E and selenium mixture on catalase activity (CAT), ascorbic acid concentration, acid phosphatase activity (ACP) and alkaline phosphatase activity (ALP) in diluted seminal plasma	78
Table 28	Effect of selected doses of MOLE and vitamin E and selenium mixture on total antioxidant capacity (TAC), malondialdehyde concentration (MDA), superoxide dismutase activity (SOD), glutathione peroxidase activity (GPx) and glutathione reductase activity (GR) in diluted seminal plasma	80

List of figures

Figure No.	Title	Page
Figure 1	The design of the study including the number of animals, and the treatment program.	34
Figure 2	Comet assay for evaluation of DNA fragmentation of fresh semen of control (A), MOLE (B) and vitamin E and selenium (C) white arrow show fragmented DNA.	58
Figure 3	Comet assay for evaluation of DNA fragmentation of frozen semen of control (A), MOLE (B) and vitamin E and selenium (C) white arrow show fragmented DNA.	77
Figure 4	Some sperm abnormalities include: 1 normal sperm, 2 coiled tail and 3 detached head	81

Name of Candidate: Dina Mahdy Mohamed Rashad Mohamed Shokry

Degree: Ph.D.

Title of thesis: Biochemical studies on cryopreservation of ram semen.

Supervisors:

- 1- Dr. Mabrouk Attia Abd eldaim**
- 2- Dr. Sahar Hassan Orabi**
- 3- Prof. Magdy Ramadan Badr**
- 4- Dr. Hanem Kamal Basuni khalifa**

Department: Biochemistry and Chemistry of Nutrition

Approval: 24 / 8 / 2020

ABSTRACT

This study was carried out to evaluate the efficacy of *Moringa oleifera* leaves extract (MOLE) in improving the characters of fresh and cryopreserved semen compared to vitamin E and Selenium combination. Twenty-four mature Barki rams (50-70 Kg) were randomly assigned into three groups, eight rams each. The first group was given distilled water orally. The second group was given MOLE orally daily at a dose of 40 mg/kg. The third group was injected with a combination of vitamin E and selenium at a dose of 3 ml (4.5 mg sodium selenite and 204 mg vitamin E)/ head i.m twice a week for 64 days. MOLE increased semen volume, sperm concentration, activities of seminal plasma catalase, glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), alkaline phosphatase (ALP), acid phosphatase (ACP), levels of ascorbic acid and total antioxidant capacity (TAC). In addition, it significantly increased post thawing sperms motility, viability index, membrane integrity, and the activities of post thawing semen antioxidant enzymes. While, it decreased seminal plasma concentration of malondialdehyde (MDA) and acrosomal defects and DNA fragmentation of sperm in cryopreserved semen. Vitamin E and selenium decreased semen volume, sperm concentration, seminal plasma ascorbic acid, TAC concentrations and activities of antioxidant enzymes while its increased sperm abnormalities, DNA fragmentation and MDA concentration in seminal plasma. This study indicated that MOLE improved the characters of the fresh and cryopreserved ram semen via improving seminal plasma antioxidant defense mechanism.

Keywords: *Moringa oleifera*, Rams, Antioxidant, Cryopreservation