شبكة المعلومات الجامعية

بسم اللّه الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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

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شبكة المعلومات الجامعية

بالرسالة صفحات لم ترد بالأصل

IMPACT OF MELATONIN AND CRYOPROTECTANTS ON BUCK FROZEN SEMEN DURING HOT AND COLD MONTHS

By

ELIAS MICHAEL GABRIEL KODI

B.Sc. NRES. (Animal Production), College of Natural Resources and Environmental Studies. Juba Univ., 2009

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Animal Production)

Department of Animal Production
Faculty of Agriculture
Cairo University
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APPROVAL SHEET

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Date: / /

SUPERVISION SHEET

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Name of Candidate: Elias Michael Gabriel Kodi **Degree:** M.Sc
Title of Thesis: Impact of Melatonin and Cryoprotectants on Buck Frozen Semen During Hot and Cold Months.
Supervisors: Dr. Gamal Ashour Hassan
Dr. Sherif Mohamed Dessouki
Dr. Moataz Ahmed El-Gayar
Department: Animal Production **Branch:** Animal Breeding
Approval: 18 /3/2020

ABSTRACT

This research was designed to assess the improvement of the freezability of buck semen using two different types of cryoprotectants supplemented with melatonin as antioxidant in cold and hot months. Pooled samples from four sexually mature Egyptian Baladi Bucks were used in this experiment. Semen was extended (1:8) with Tris-fructose-citric containing egg yolk using glycerol and dimethyl sulfoxide (DMSO) supplemented with two doses of melatonin (10^{-6}M and10^{-3}M) in addition to control group. Computer assisted semen analysis (CASA) was used to evaluate semen after cryopreservation. While, enzymatic activity was measured using spectrophotometer technique. Real-time PCR was used for expression profile of selected genes. The results revealed that the progressive motility percentage was higher ($P<0.05$) in samples supplemented with low dose of melatonin (10^{-6} M) compared to high dose (10^{-3}M) in glycerol (74.4±2.4 *vs.* 64.4±2.5) and DMSO based extender (35.5 ±2.4 *vs.* 32.9 ±2.5) in cold months. The same trend was found in samples cryopreserved with glycerol (75.1±2.2 *vs.* 53.5±2.2) and DMSO (32.1±1.9 vs. 22.0±1.8) in hot months. The results also demonstrated that CASA parameters (VAP and VCL) were significantly increased in low compared to high melatonin doses in glycerol based extender during cold and hot months. The activity of total antioxidant capacity (TAC) was significantly higher in samples supplemented with low melatonin dose (0.49 mM/L ±0.09) than high melatonin dose (0.16 mM/L±0.09) in DMSO extender. Transcript abundance of CPT2, ATP5F1A and SOD2 genes was increased significantly in glycerol based extender groups and this was more apparent in low melatonin dose compared with all other glycerol based extender groups in cold months. On the other hand, NFE2L2 gene was up-regulated in groups cryopreserved with DMSO compared with those cryopreserved in glycerol based extender in both cold and hot months. It could be concluded that the type of extender and season of collection represent the main factors affecting semen quality, antioxidant defense and molecular activities. Furthermore, melatonin supplementation to extender enhances antioxidant enzymes and genes regulating mitochondrial activity during cold period, which may maintain the post-thaw fertilizing ability of buck semen.

Key words: Buck semen, melatonin, cryoprotectants, enzymes activities, genes profile, cold and hot months.

DEDICATION

I dedicate this work to

My mother, Aunt Terezina Henry Bilal and my late father and to all those

Who have encouraged me

To fulfill

My dreams and aspirations.

Thank you all for you challenged me to

Realize my dream of childhood.

ACKNOWLEDGEMENT

I would like to express my genuine gratitude to my major supervisor ***Professor Dr. Gamal Ashour Hassan,*** *the former Head Department of Animal Production, Faculty of Agriculture, Cairo University for suggesting and designing this research, his guidance and moral support, valuable suggestions, constant encouragement, constructive criticism and continuous follow up and monitoring throughout the whole of my study. It is a great pleasure and pride for me to have an opportunity of working with this affectionate and benevolent scientist.*

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My appreciation to ***Professor Dr. Moataz Ahmed El-Gayar****, my supervisor who also contributed in designing and availing me access to the farm and animals where I was able to get first hand training on semen collection and freezing. He allowed me to use Animal Physiology laboratory to do all the Pre-thaw semen analysis and freezing procedures until transportation of cryopreserved semen to main laboratory in the Faculty of Agriculture Cairo University.*

I sincerely acknowledge the valuable contributions, scholarly guidance and all possible support extended by ***Associate Professor Dr. Nasser Ghanem.*** *He was part of the research designing, carried out transcript abundance analysis, and supported me in writing the manuscript for publication and production of this thesis. He is the corresponding author for the publication and an invisible man whose efforts led to the production of this thesis at an exceptional time.*

I extend my heartfelt appreciation to ***Associate Professor Dr. Fakhri El-Hadi El-Azzazi*** *member of the team. He contributed in design of the research, trained me on semen collection methods and procedures of freezing and would visit me at midnight when I was working in Animal Physiology laboratory, Faculty of Agriculture, Suez Canal University in Ismailia.*

Mr. Sherif Helmi is acknowledged, the technician in the Artificial Insemination Laboratory and Ultrasonography Unit Animal Production Department Faculty of Agriculture Cairo University and all the staff of animal farm in Faculty of Agriculture and Faculty of Veterinary Medicine Suez Canal University.

LIST OF ABBREVIATIONS

µ	Micro/Micron
µl	Microliter
ALH	Amplitude of Lateral Head Displacement
ART	Assisted Reproductive Technology
ATP5F1A	ATP 5 synthase F1 subunit Alpha
BCF	Beat Cross Frequency
bp	Base pair
CASA	Computer Assisted Semen Analysis
CAT	Catalase
cDNA	Complementary Deoxyribonucleic acid
Conc.	Concentration
CPT2	Carnitine Palmitoyl Transferase 2
DAP	Distance Average Path
DCL	Distance Curved Line
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acids
DSL	Distance Straight Line
F	Forward
g/dl	Gram per deciliter
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLM	General linear model
g	Gram
GPX	Glutathione peroxidase
HOST	Hypo-Osmotic Swelling Test
H_z	Hartz
ICSI	Intracytoplamic Sperm Injection
IMV	Instruments de Medecine Veterinaire
IU	International unit
IVF	*In vitro* Fertilization
LIN	Linearity
MDA	Malondialdehyde
ml	Milliliter
mM/L	Millimole per liter
mu/mL	Micro mole per milliliter
NFE2L2	Nuclear Factor erythroid-derived 2-like 2
nM	Nano mole
nmol/mL	Nano mole per milliliter

NRF2	Nuclear factor erythroid 2–related factor 2
PCR	Polymerase chain reaction
pM	Picomolar
qRT-PCR	Quantitative real time polymerase chain reaction
R	Reverse
RCF	Relative Centrifugal Force
RNA	Ribonucleic acid
rpm	Revolutions per Minute
SE	Standard error
SAS	Statistical analysis software
SOD2	Superoxide dismutase 2
STR	Straightness
TAC	Total antioxidant capacity
U/L	Micro per liter
VAP	Velocity Average Path
VCL	Velocity Curved Line
VSL	Velocity Straight Line
WOB	Wobble
μM	Micromole
μm/s	Micrometer per second

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