ULTRA- STRUCTURE, TRANSCRIPTIONAL PROFILE AND EMBRYONIC DEVELOPMENT OF EGYPTIAN BUFFALO OOCYTES MATURED *IN VITRO* UNDER OXIDATIVE STRESS

By

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B.Sc. Agric. Sci. (Animal Production), Fac. Agric., Cairo Univ., 2005
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Title of Thesis: Ultra-Structure, Transcriptional Profile and Embryonic Development of Egyptian Buffalo Oocytes Matured *In Vitro* Under Oxidative Stress
Supervisors: Dr. Nasser Ghanem Osman
Dr. Sherif Mohammed Dessouki
Department: Animal Production **Branch:** Animal Physiology
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ABSTRACT

This study was performed to investigate cellular and molecular changes of buffalo cumulus-oocyte complexes (COCs) cultured under oxidative stress, and to assess developmental competence after maturation through, *in vitro* fertilization and early embryonic development up to the blastocyst stage. Morphologically good quality COCs (n=2672) were screened with brilliant cresy- blue (BCB) staining and then graded into three groups (BCB+, BCB - and control). Oocytes from the different groups were evaluated under low (5%) and high oxygen level (20 %), The progress of the nuclear maturation stage and the rate of cumulus expansion were assessed after 24 hrs of *in vitro* maturation. Ultra-structure of oocytes have been monitored using transmission electron microscope. The fluorescent intensity of lipid, mitochondria, and reactive oxygen species (ROS) were measured using different molecular probes. Transcript abundance of genes regulating different molecular pathways during oocyte maturation was profiled using Real-time PCR. The cumulus expansion of all oocyte groups under low oxygen tension (5%) was higher ($P < 0.05$) than those cultured under high oxygen tension (20%). Under high oxygen tension, low-competent oocytes (BCB -) showed the lowest maturation rate ($P < 0.05$). This was associated with an increase in ROS, which was against the BCB+ oocyte pattern. Ultra-structure examination indicated that under low oxygen tension the competent BCB+ oocytes had a higher rate of migration of cortical granules than BCB -. In parallel, the MAPK14 and CPT2 gene expression profile ($P < 0.05$) was increased under low compared to high oxygen tension. There were no significant differences in cleavage, morula and transferable embryos rates among BCB+, BCB- and control groups under low and high oxygen tension. Blastocyst rate was significantly greater in control group than BCB- COCs. According to oocyte quality, there were no significant differences in cleavage, morula and transferable embryos rates between BCB+, BCB - and control groups. The rate of morula and transferable embryos were increased in buffalo COCs developed under low oxygen tension compared to high oxygen tension group. In addition, cleavage, morula, blastocyst and transferable embryos rates were greater in BCB+ under low than high oxygen tension group. In conclusion, the results of this study demonstrated that low tension of oxygen provides favorable conditions for oocytes to carry out all the cellular and molecular activities necessary for progression of maturation. The low competent oocytes (BCB -) could not cope with oxidative stress. BCB staining might be not an effective tool for assessment developmental competence of buffalo COCs. Buffalo morula and transferable embryos prefer low oxygen tension for early development, which could be applied during *in- vitro* embryo production in Buffalo.

Keywords: Oxidative stress, BCB, Oocytes, Ultrastructure, Gene expression

DEDICATION

I dedicate this work to my late mother and to my father and sister for their endless love, support and encouragement. My dedication is also extended to my husband and my precious son and daughter, for their support and patience to finish this study

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LIST OF ABBREVIATIONS

µg/ml	Microgram per milliliter
µl	Micro litter
µm	Micrometer
ART	Assisted reproductive technologies
ATP	Adenosine triphosphate
BCB-	Brilliant cresyl blue -
BCB+	Brilliant cresyl blue +
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
CG	Cortical granules
COCs	Cumulus - oocytes complexes
CPT2	Carnitine palmitoyl transferase II
DCHFDA	2´,7´- dichlorodihydro fluorescein diacetate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EAA	Essential amino acid
EDTA	Ethylene diamine tetra-acetic acid
ET	Embryo transfare
FAO	Fatty acid oxidation
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase
GV	Germinal vesicle
GVBD	Germinal vesicle breakdown
H_2O_2	Hydrogen peroxide
ICM	Inner cell mass
IVC	*In vitro* culture
IVEP	*In vitro* embryo production
IVF	*In vitro* fertilization
IVM	*In vitro* maturation
LCFA	Long-chain fatty-acid
MAPK14	Mitogen activated protein kinase 14

LIST OF ABBREVIATIONS (continued)

mDPBS	Modified Dulbecco phosphate buffer saline
MI	Metaphase I
MII	Metaphase-II
Min	Minutes
Ml	Milliliter
Mm	Millimeter
MMP	Mitochondrial Membrane Potential
MOET	Multiple Ovulation and Embryo Transfer
MPF	Maturation-Promoting Factor
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NEAA	Non-essential amino acid
NFE2L2 (NRF2)	Nuclear factor erythroid 2-related factor 2
nm	Nanometer
OS	Oxidative stress
OSO4	Osmium tetroxide
OXPHOS	Oxidative phosphorylation
PCR	Polymerase chain reaction
PVP-PBS	Polyvinylpyrrolidone phosphate buffer saline
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SCNT	Somatic cell nuclear transfer
SOD2	Superoxide dismutase2
TAB1	Transforming Growth Factor-beta activated kinase-1 and MAP3K7-binding protein 1
TALP	Tyrode's albumin lactate pyruvate
TCM-199	Tissue culture medium-199
TE	Trophectoderm
TEM	Transmission electron microscopy
ZP	Zona pellucida