EFFECT OF HEAT ELEVATION ON BUFFALO (*Bubalus bubalis*) OOCYTES' COMPETENCE WITH PARTICULAR REFERENCE TO RELATED GENE EXPRESSION

By

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B.Sc. Agric. Sci. (Animal Production), Fac. Agric., Cairo Univ., Egypt, 2005
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Name of Candidate: Rehab Nagy Ibrahim Ali **Degree:** Ph.D.
Title of Thesis: Effect of Heat Elevation on Buffalo (***Bubalus bubalis***) Oocytes' Competence with Particular Reference to related Gene Expression.
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Department: Animal Production **Approval: 26 / 08 / 2018**

ABSTRACT

This study was performed to investigate the effects of physiologically relevant heat shock during oocyte maturation on the developmental competence of Egyptian buffalo oocytes and the expression of heat stress genes.

This study was designed into three experiments. The first and second experiments were run on three groups, (n=250 oocyte/group), buffalo cumulus oocyte complexes (COCs) were cultured at 38.5° C (T_0, control) or were exposed to 39.5° C (T_1) or 40.5° C (T_2) for the first 6 h of the *in vitro* maturation (IVM). After this period, all groups were incubated at 38.5°C up to the blastocyst stage (7 day). In the 1st experiment, the groups were exposed to the assigned temperatures to study the effect of partial exposure of buffalo oocytes to heat stress (T_1 and T_2) on their maturation rate. The 2nd experiment has studied the ability of *in vitro* fertilized buffalo oocytes to develop until the blastocyst stage after being exposed to partial heat stress (T_1 and T_2). The 3rd experiment was conducted to draw the profile of mRNA expression of selected target genes (HSF-1, HSF-2, HSP-70, HSP-90، BAX, P53, SOD1, COX1, MAPK14) in denuded oocytes and their isolated cumulus cells resulted from control COCs as well as from COCs exposed to 39.5 ° C (T_1). The results indicated that heat shock significantly ($p<0.01$) decreased maturation rate of T_1 and T_2 compared to T_0. After in vitro fertilization (IVF), cleavage rate was lower ($p<0.01$) for oocytes exposed to heat stress, and the percentage of oocytes arrested at two or four-cell stage was higher than ($p<0.01$) in T_1 and T_2 as compared to T_0. Percentage of oocytes that developed to 8-cell, 16-cell or blastocyst stage was lower ($p<0.01$) in both T_1 and T_2 groups compared to the control one. There is no single oocyte that had been exposed to 40.5°C (T_2) was able to develop to the blastocyst stage. The mRNA expression level for the studied genes decreased ($p<0.05$) in treated oocytes (T_1) except for HSP-90 and HSF-1 which up-regulated. In cumulus cells isolated from COCs (T_1), the expression level for the target genes showed up-regulation except for BAX which showed down-regulation.

The obtained results showed that, the expression of all genes was detected in oocyte and their CCs matured under normal conditions. Mature oocytes showed higher transcripts of HSP90, HSP70, and Bax. The relative transcript of COX1 showed down-regulation in oocyte and opposite trend was in CCs. Lower abundance of HSP90 and P53 was detected in CCs.

HSP90 has significant negative correlation (r = -1.000) with HSF1, HSF2, HSP70, P53, COX1, Bax, and SOD1 in IVM oocyte under normal conditions. An opposite trend for a correlation between HSP90 and other genes appeared in CCs.

The present findings, therefore, provide a clear evidence that, the reduction of oocyte quality as a consequence to heat shock in vitro is likely help to understand the early embryonic losses in buffalo following heat stress conditions. Hence, it is put forward that high ambient temperature may after the molecular events occur during maturation and subsequent embryonic development at various cell stages that may ultimately cause a negative impact on buffalo reproduction. In coclusion, the results of this study demonstrate that exposure of buffalo oocytes to elevated temperatures for a given duration of time severely compromises their developmental competence and gene expression.

Key words: Heat stress, Buffalo, Oocyte, Embryo, Cumulus cells, Gene expression.

DEDICATION

I dedicate this work to whom my heartfelt thanks; my parents, husband, sister, brother and my lovely daughter Asya for their patience, encouragement, and help, along with the course of my study.

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LIST OF ABBREVIATIONS

IPCC	Intergovernmental panel on climate change
µg/ml	Microgram per milliliter
AMP	Adenosine mono phosphate
ATP	Adenosine tri phosphate
bp	Base pair
BSA F-V	bovine serum albumins fraction v
BT	Body temperature
CC	cumulus cells
COC	cumulus oocyte complexes
C_t	cycle threshold
dNTP	deoxyribonucleotide triphosphate
DTT	Dithiothreitol
ETC	electron transport chain
FBS	fetal bovine serum
GV	germinal vesicle
GVBD	germinal vesicle breakdown
HS	Heat stress
HSE	heat shock element
HSF	heat shock factor
HSP	heat shock proteins
IU/ml	International Units Per Millilitre
IVC	In vitro culture
IVF	In vitro fertilization
IVM	In vitro maturation
MI	metaphase I
mM	Millimolar
MOM	mitochondrial outer membrane
oligo (dT)	single-stranded sequence of deoxythymine
OMM	outer mitochondrial membrane
PBS	phosphate buffer saline
PCD	programmed cell death

LIST OF ABBREVIATIONS (continued)

PHE	penicillamine–hypotaurine–epinephrine
pmol/μl	Picomol/microliter
ROS	Reactive oxygen species
THI	temperature–humidity index
VDAC	voltage-dependent anion channel

CONTENTS