Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Effect of Cysteamine on Maturational Competence, Molecular and Functional Responses Underlying Mitochondrial Activity and Apoptosis of Heat-stressed Egyptian Buffalo Oocytes *in vitro*.

Marwa S. Faheem[1]*, Ehab S. Abdel-Aal[2], Salah El-asaal[3], Dalia A. Ahmed[1], Manar Moustafa[1] and Ahmed Amin[1]

[1]*Department of Animal Production, Faculty of Agriculture, Cairo University, Cairo, Egypt.*

[2]*Animal Production Research Institute (APRI), Agriculture Research Centre, Dokki, Egypt.*

[3]*Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt.*

Abstract

GLOBAL warming threatens animal health, performance and reproductive capacity by disrupting their natural physiological processes. Therefore, incorporating antioxidants into biological systems becomes crucial to mitigate the detrimental impacts of heat-induced stress. The present study aimed to assess the impact of cysteamine supplementation as an exogenous antioxidant on buffalo oocytes maturation in vitro under heat stress conditions as well as monitoring the oocytes competence post maturation in relation to mitochondrial activity, ROS generation levels and their related molecular responses. Buffalo immature oocytes were subjected to cysteamine supplementations in four concentrations (100, 200, 300 and 400 µM) compared to control (0 µM) under two different culture conditions, 1) heat stress (41°C for the first hour of maturation followed by 38.5°C till the end of culture, 2) normal physiological temperature condition at 38.5°C for 24 hrs. Our findings revealed that there were no significant differences between the oocytes matured under heat stress conditions and normal physiological temperature with a relative advantage at 200 µM concentration of cysteamine. Under heat stress, cysteamine-treated oocytes demonstrated enhanced mitochondrial activity, reduced ROS production levels along with increased SOD1 and decreased BAX expression levels particularly at concentrations above 100 µM. In contrast, under normal temperature conditions, higher cysteamine concentrations resulted in elevated ROS production. In conclusion, cysteamine as an exogenous antioxidant, may improve cellular redox homeostasis and serve as a protective mechanism against reactive oxygen species (ROS) generation.

Keywords: Cysteamine, Buffalo oocytes, In vitro maturation, Mitochondria activity, ROS level, Gene expression.

Introduction

Physiological animal responses are susceptible to numerous threats under thermal stress conditions. The anticipated cellular and physiological changes might likely impair the optimal functioning of several biological systems that eventually affect animal performance and fertility. Thermal stress reduces the reproductive efficiency of different farm animals through inducing hormonal disruptions, affecting follicular characteristics, steroid production and uterine environment, while causing a direct negative impact on oocyte quality, function and developmental competence, compromising subsequent embryo developmental potential. Thus, the acquisition of the required embryonic capacity for development is effectively initiated from the oocyte maturation environment. It has been revealed that oocytes at germinal vesicle (GV) and early embryonic development stages, before genome activation, are functionally sensitive to different stressors including high temperature, while embryos become more resistant with the developmental progress [1]. The crucial maturation events for GV stage oocytes, including cytoplasmic, nuclear and

*Corresponding authors: Marwa S. Faheem, Email: marwasf@agr.cu.edu.eg, Tel: 01001521316
(Received 23 June 2025, accepted 09 November 2025)
DOI: 10.21608/ejvs.2025.397057.2921

molecular changes, are severely compromised due to the adverse effect of thermal stress. Excessive reactive free radicals' production, enzymatic defense mechanism disorders and oocytes' mitochondrial dysfunction are suggested to be the underlying mechanisms by which thermal stress impairs oocyte maturation. In a consequence oocyte capacity to undergo fertilization and further embryonic development up to blastocyst is weakened. Moreover, the intrinsic mechanisms leading to apoptosis might probably triggered [2]. Thus, providing an optimal in vitro culture environment for oocyte maturation leads to strengthening the oocyte developmental potential, mainly under stress stimuli. Antioxidants act to alleviate the deleterious effect of the intracellular ROS accumulation, suppressing the oxidative stress series which can consequently affect oocyte cytoplasmic and meiotic maturation [3]. In addition, antioxidants support oocyte to activate their enzymatic antioxidant defense mechanisms in order to neutralize ROS levels to the physiological limit, particularly through the supplementation of non-enzymatic antioxidants [4]. Cysteamine, non-enzymatic thiol antioxidant, has been shown to significantly suppress oxidative damage by promoting the intracellular uptake of cysteine and ameliorating the destructive effects of reactive oxygen species (ROS) through its scavenging potential in different animal species [5]. In addition, the effective action of cysteamine in enhancing the cellular glutathione content has been well acknowledged [6]. The present study, therefore, was conducted to assess the impact of cysteamine supplementation, as an exogenous antioxidant, in different concentrations on buffalo oocytes maturation in vitro under heat stress conditions as well as monitoring the oocytes competence post maturation in relation to mitochondrial activity, ROS generation and their related molecular responses.

Material and Methods

Chemicals and reagents

All the chemicals and reagents used for in vitro maturation were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. Disposable plastic dishes and ware were obtained from Nunc, Roskilde, Denmark.

Experimental design

Buffalo immature oocytes were subjected to in vitro maturation experiments to determine the impact of cysteamine supplementations in four concentrations (100, 200, 300 and 400 µM) compared to control group (0 µM) under two different culture conditions: 1) heat stress at 41°C for 60 min of maturation commence followed by 38.5°C till the end of culture and 2) normal physiological temperature at 38.5°C. To assess the competence of buffalo oocytes, post 24 h of maturation, cytoplasmic and nuclear oocytes maturation rates were recorded, and the mitochondria and ROS activities were evaluated. At the molecular level, the expression pattern of different target genes regulating antioxidant activity (SOD1) and apoptosis (BAX) were assessed. Each experiment was conducted in at least three biological replicates per condition.

Ovaries collection and oocytes retrieval

Ovaries were harvested from apparently normal estrus cyclic buffaloes from local slaughterhouses and transported to the lab in a warm (34 - 37°C) normal physiological saline solution (0.9% NaCl) supplemented with 50 µg/ml gentamycin sulfate. Upon lab arrival, ovaries were washed twice with pre-warmed 0.9% NaCl, rinsed in 70% ethanol for 30 seconds, followed by final wash with fresh pre-warmed saline solution. Ovarian antral follicles with a diameter of 2-8 mm were subjected to follicular fluid aspiration using an 18-gauge sterile disposable needle. The follicular fluid contents and cumulus oocyte complexes (COCs) were allowed to settle in a water bath at 37°C for 15 min. Based on microscope examination, COCs were retrieved and classified according to their morphological features into four categories based upon the ooplasm homogeneity and cumulus layer compaction [7]. Briefly, Grade A oocytes with homogeneous evenly granular ooplasm and surrounded by ≥ 5 layers of compact cumulus cell; Grade B oocytes with homogeneous ooplasm and 1-2 layers of compact cumulus cells; Grade C oocytes with less homogeneous ooplasm and free of cumulus cells and Grade D oocytes with fragmented, vacuolated or degenerated ooplasm and damaged cumulus cells [8].

High quality oocytes from grades A and B were selected and washed twice in the oocytes washing medium consisting of TCM-199 HEPES medium supplemented with 10% (v/v) fetal bovine serum (FBS), 0.3 mg/ml glutamine, and 50 µg/ml kanamycin.

Cytoplasmic and nuclear oocytes maturation

Cumulus oocytes complexes were randomly assigned into five groups of cysteamine supplementation (0 µM as a control, 100, 200, 300 and 400 µM) in the oocytes maturation medium consisting of TCM-199 HEPES medium supplemented with 10% FBS, 1 µg/ml estradiol-17β, 5 µg/ml FSH, 22 µg/ml Na-pyruvate, 0.15 mg/ml glutamine and 50 µg/ml kanamycin. All oocyte treated groups were then matured under 5% CO_2 in air and 95% relative humidity at 38.5 °C. After 22-24 h of maturation either under heat stress or normal physiological temperature, cumulus oocyte expansion rate and oocyte meiotic status were recorded.

For nuclear oocytes maturation assessment, oocytes were mechanically freed from cumulus by a gentle repeated pipetting then fixed with glacial

acetic acid: methanol (1:3 v/v) for 24 h at room temperature in a sealed petri dish. After fixation, the oocytes were uploaded on a glass slide, gently covered with a glass cover slip to avoid oocytes damage and stained with 1% aceto-orcein (1g Orcein stain/ 100 ml of 45% boiling glacial acetic acid and filtered twice with filter paper) for 5-8 min. The stained oocytes were examined under inverted microscope (Leica, Germany) for their meiotic stages development and classified according to Roeles and Tsiavaliaris[9] as followed: Germinal vesicle (GV), oocytes with slightly condensed chromatin and with very distinct and intact nuclear membrane; Germinal vesicle breakdown (GVBD), oocytes with filamentous chromatin, and with partial or complete dissolution of the nuclear envelope; Metaphase I (MI), oocytes with chromosomes arranged in the metaphase plate and the diploid sets of chromosomes (2n) are fully condensed; Anaphase I (AI), oocyte with separate bivalent pairs of chromosomes; Telophase I (TI), oocyte with two completely separate set of chromosomes lined up to the both poles; and Metaphase II (MII), oocytes with an extruded polar body or two shiny chromatin spots reached the pole [9] .

Analysis of oocytes mitochondrial activity

Mitochondrial activity of oocytes, post maturation, was evaluated using MitoTracker Red (M7512, Invitrogen, Karlsruhe, Germany) according to the manufacturer instructions with some modifications [10]. The cumulus-free oocytes, post denudation, were washed 2 to 3 times with DPBS. At least ten oocytes from each treatment groups were then incubated with 20 µL of 200-nM MitoTracker red dye for 45 min at 38.5°C followed by washing twice with PBS-PVA. Oocytes were then fixed with 4% formaldehyde in 4°C for overnight and mounted in 10 µl droplet of ProLong Diamond antifade mountant (Life Technologies) on the slide in groups. Mitochondrial activity levels in the tested samples were then observed under fluorescence light microscope (Leica DM LB2, Leica Microsystems, Wetzlar, Germany) by using the red-fluorescence filter. Florescent images were acquired by LAS Core software and fluorescence intensity was measured using Image J software.

Analysis of oocytes ROS level

Intracellular ROS levels were measured by 6-carboxy-2, 7-dichlorodihydro fluorescein diacetate kit (H2DCFDA, Lite-technologies, USA) according to the manufacturer instructions with some modifications [11] .The cumulus-free oocytes, post denudation, were washed 2 to 3 times with DPBS. Then, samples from the same previous treatment groups were incubated with 200 µL of 15 µM H2DCFDA dye for 20 min at 38.5°C in the dark, followed by washing twice with PBS-PVA. ROS levels in the tested oocytes were visualized under fluorescence light microscope (Leica DM LB2, Leica Microsystems, Wetzlar, Germany) using a green fluorescence filter, and images were acquired by LAS Core software. Oocytes fluorescence intensity was quantified using Image J software.

Expression analysis of BAX apoptotic gene and SOD1 antioxidant gene

RNA extraction and cDNA Synthesis

Total RNA was extracted from buffalo oocytes of three different biological replicates of each experimental group (70 – 80 oocytes/ group) using the PicoPure RNA isolation kit (Arcturs, Munich, Germany) following the manufacturer's instructions. DNA was digested using on-column DNase treatment (Qiagen GmbH, Hilden, Germany).

Total RNA concentration and purity were determined using a NanoDrop 2000C (Thermo Scientific, Wilmington, DE, USA) at A260/280 nm ratio. Samples with ≥1.8 only were used for further analysis. After adjusting the RNA concentration of all samples (1 µg total RNA), RNA samples from each treatment group were reverse transcribed to cDNA in a total volume 20 µL using oligo (dT)-25 and random primers (Promega, Madison, WI) and Superscript reverse transcriptase II (Invitrogen, Karlsruhe, Germany).

Real-time polymerase chain reaction

Primers for the studied genes were designed using Primer3 software (http://primer3.wi.mit.edu//) based on sequences described in the GenBank database (www.ncbi.nlm.nih.gov) as shown in Table 1. The reaction was performed in a 20 µL total reaction mixture consisting of 10 µL of SYBR green with ROX (Thermofisher Scientific, California, USA), 0.2 µL of specific-reverse primer, 0.2 µL of specific-forward primer, 7.6 µL of nuclease-free water, and 2 µL of the cDNA sample. The thermal cycling reaction conditions were as follow: 10 min at 95 ◦C as an initial denaturation step, then 40 cycles of 15 s at 95 ◦C, 20 s at 60 ◦C, and 30 s at 72 ◦C, followed by a final step of 1 min at 60 ◦C. The qRT-PCR was performed using a StepOnePlus™ Real-Time PCR instrument (Applied Biosystems, California, USA). Results were quantitatively analyzed using 2^(delta delta CT) method and stated as the relative abundance of SOD1 and BAX mRNA transcripts after normalization of the target transcript to reference gene (GAPDH) according to Khan et al. [12] .

Statistical Analysis

A minimum of three biological replicates were used in each experiment. The normality of the distribution of the data was examined. Data were statistically analyzed using the general linear univariate model procedure (SPSS 16.0) including interaction. version 9.1 (SAS Institute Inc., Cary,

NC). Maturation rates across different treatment groups of oocytes were expressed as mean ± standard error of the mean (SEM). A P-value of less than or equal 0.05 ($P \leq 0.05$) was considered statistically significant. Relative mRNA expression data were analyzed using General Linear Model (GLM) SAS version 9.1 (SAS Institute Inc., Cary, NC). Differences in mean values among treatments were assessed using ANOVA. Duncan's multiple range test was used to detect when there were differences among the means.

Results

Oocytes cytoplasmic and nuclear maturation

The oocytes competency for maturation was stated in Table 2 and 3. For oocytes maturation, assayed by cumulus-oocyte expansion rate, there were no significant differences between the oocytes matured under heat stress condition and normal physiological temperature. However, a decline trend was observed within heat treated groups when compared with their counterpart groups under normal temperature except for 400 µM concentration of cysteamine. The 200 µM of cysteamine showed a higher expansion rate under the two culture conditions compared to the other studied groups, being significantly different with 400 µM group of cysteamine under normal physiological temperature.

The meiotic competence of the oocytes was depicted in Table 3 and Figure 1. Under heat stress conditions, higher nuclear maturation rate was observed within cysteamine-treated oocytes groups for the concentrations of 200, 300 and 400 µM, respectively. While under normal physiological condition, the higher meiotic developmental rate was stated for 300 µM of cysteamine followed by 200 and 400 µM.

Mitochondrial activity

The pattern of oocytes mitochondrial activity was affected by the culture conditions as shown in Figures 2 (a and b) and 3 (a and b). Under normal physiological temperature, cysteamine supplemented groups revealed higher ($P \leq 0.05$) mitochondrial fluorescent intensity when compared to the control group, while with a descend pattern with the increase of cysteamine concentrations, without significant difference between 100 and 200 µM groups (Fig. 2 a&b). Under heat stress conditions, the higher concentrations of cysteamine (200, 300 and 400 µM) significantly enhanced mitochondrial activity when compared to the control group and the lowest concentration of cysteamine (Fig. 3 a&b).

Reactive oxygen species level (ROS)

Buffalo oocytes, under normal physiological temperature, showed a raising pattern ($P \leq 0.05$) of ROS levels along with the increase of cysteamine concentrations, being significantly different with the control group (Fig 4 a&b). Moreover, there were significant differences within cysteamine supplemented groups. However, under heat stress conditions, the higher levels ($P \leq 0.05$) of ROS were observed in the control group and 100 µM cysteamine concentration, without significant differences. Whereas 200, 300 and 400 µM of cysteamine concentrations interestingly showed significantly lower ROS levels as shown in Fig 5 a&b.

Gene expression

To assess the molecular response of mature buffalo oocytes, the qRT-PCR analysis was performed to evaluate the relative abundance of mRNA transcripts for genes regulating stress resistance (SOD1) and apoptosis related gene (BAX) for all groups of the study as depicted in Figures 6 and 7. The results revealed that under normal physiological temperature the relative abundance of SOD1 transcripts were significantly increased in 200 and 300 µM of cysteamine compared to 100 and 400 µM groups, respectively. While under heat stress, the groups of 200, 300 and 400 µM of cysteamine showed higher ($P \leq 0.05$) expression of SOD1 compared to 100 µM and control groups (Fig 6 a&b). For apoptosis related gene, the relative abundance of BAX transcripts, under normal physiological temperature, was higher in 200, 300 and 400 µM groups of cysteamine compared to 100 µM and control, respectively. However, a contrary pattern was observed for the heat-treated groups of oocytes as shown in Fig 7 (a&b).

Discussion

Oocytes quality is an intrinsic factor determining oocytes maturation competence and their subsequent embryonic developmental potential. The cytoplasmic, nuclear and molecular events that take place during oocyte maturation are accomplished in accordance with the surrounding culture environment, implicating serious consequences coincided with stressful insults. Moreover, in vitro culture conditions are not exempt from being a cause for the emergence of stress. Therefore, the present study was designed to evaluate the effect of cysteamine supplementation during in vitro maturation of buffalo oocytes in an attempt to adjust the culture conditions to reduce stress harmful consequences and enhance the maturity competence of the oocytes *in vitro*. The findings of the current study indicated that heat stress impaired the cytoplasmic maturation rate of oocytes; however, this adverse effect was mitigated within cysteamine treated groups. Mietkiewska [1] acknowledged that exposure of oocytes to heat stress, even with variations in the duration and intensity of exposure, often compromises their maturational and developmental capabilities and may result in impaired oocyte function [1]. Noteworthy, germinal

vesicle (GV) stage oocytes are more susceptible to heat stress insults [6]. Exposure of GV stage oocyte to heat stress induces perturbations in both nuclear and cytoplasmic maturation events, such as the resumption of meiosis, metaphase II plate formation, and translocation of cortical granules [13] . Furthermore, excessive free radical formation was stated under heat stress leading to mitochondrial function impairment and apoptosis [14] . In the current study, under normal physiological temperature conditions, cysteamine supplementation led to a dose-dependent elevation in reactive oxygen species (ROS) levels. As a result, the anticipated benefit of cysteamine supplementation was not observed, as indicated by the cytoplasmic maturation rates attained except for 200 µM of concentration. However, the intended benefit of cysteamine was revealed under heat stress conditions when compared to control group. Heat stress diminished mitochondrial activity in the matured oocytes; however, cysteamine supplementation at concentrations above 100 µM significantly enhanced this activity. Furthermore, while heat stress triggered excessive ROS accumulation, cysteamine at these doses significantly reduced ROS levels, correlating with a comparably high oocyte cytoplasmic and nuclear maturation rates. Consistent with our findings, it was asserted that mitochondrial function and activity significantly influence cellular responses to heat stress. This occurs through their role in energy production—critical during oocyte maturation—and their regulation of reactive oxygen species (ROS) levels [15,16] . In this context, cysteamine's role as a precursor to glutathione synthesis was acknowledged [4, 6,17] .Several studies indicated that cysteamine enhances cellular cysteine uptake, which increases the substrate available for GSH synthetase. This accordingly leads to an increase in glutathione synthesis and content in the oocytes [5,18] . Elevated intra-oocyte glutathione levels are thought to support mitochondrial function, as mitochondria lack intrinsic enzymatic machinery for glutathione synthesis [19] . Therefore, under heat stress conditions, cysteamine-mediated glutathione accumulation may improve cellular redox homeostasis and serve as a protective mechanism against reactive oxygen species (ROS) generation as our results revealed. While the increase in mitochondrial activity, under normal temperature conditions, coincided with a decrease in the concentrations utilized of cysteamine, suggesting that higher concentrations of cysteamine may exert toxic effects [5,6]. On the molecular level, SOD1 as an antioxidant capacity regulating gene, plays a potential role in inactivating superoxide radicals. The relative abundance of SOD1 gene transcripts showed significant increase at cysteamine concentrations higher than 100 µM under heat stress conditions, with this elevation remaining consistently observed across treatment groups, while these increases exhibited declining rate with the rise in cysteamine concentrations used under normal temperature conditions. Emerging research indicates that elevated SOD1 levels are associated with increased mitochondrial function, reduced apoptosis, and improved cytoplasmic maturation, contributing subsequently to higher fertilization capacity and greater embryo developmental potential [3]. This observation might account for the elevated maturation rate observed in the 200 µM concentration group under normal temperature conditions, which displayed pronounced expression of this antioxidant and stress-resistance-related gene compared to the other groups of cysteamine, along with, improved mitochondrial function and reduced free radical generation, resulting in a relative advantage at a concentration of 200 µM of cysteamine. In line with a comparable study conducted on buffalo granulosa cells treated with the same cysteamine concentrations, the 200 µM of cysteamine demonstrated relatively higher cell viability rate while under oxidative stress conditions [20].Concerning apoptosis-regulating gene, interestingly, pro-apoptotic BAX transcripts displayed elevated expression within cysteamine treated groups under normal conditions, while this may be attributed to cysteamine's potential toxic effects rather than its antioxidant properties. In contrast, under heat stress, cysteamine exerted protective effects against oxidative damage and apoptosis, as evidenced by suppressed BAX and heightened SOD1 expressions, particularly at concentrations above 100 µM.

Conclusion

The findings of the current study revealed that cysteamine supplementation as an antioxidant during in vitro oocytes maturation, improved mitochondrial function and reduced free radical generation and their associated regulatory genes, and ultimately enhancing oocyte maturation rates, especially under heat stress conditions. Furthermore, under normal physiological conditions, 200 µM of cysteamine exerted positive impact on buffalo oocytes maturation in vitro, however further studies need to be conducted to establish the optimal concentration of cysteamine that maximizes efficacy while minimizing oxidative risks associated with toxicity.

Ethics approval and consent to participate

Institutional Animal Care and Use Committee (IACUC) at Cairo University, Egypt approved the experimental protocol of the present study (Protocol # CU-II-F- C-52-23).

Conflict of Interest

There is no conflict of interest

TABLE 1. Oligonucleotide primers used for quantitative real-time polymerase chain reactions (qRT-PCR)

Gene name	Gene bank accession number	Primer sequence	Fragment size (bp)
SOD1	NM_001290973.1	F: 5´- CACTTCGAGCAGAAGGGAAC -3´ R: 5´- CGTGCCTCTCTTCATCCTTC -3	355
BAX	XM_025269476.3	F: 5′ GTGCACCAAGGTGCCGGAACTG -3′ R: 5 ATCTGAAGATGGGGAGAGGGGACC -3′	253
GAPDH	NM_001034034.2	F: 5′AGGTCGGAGTGAACGGATTC -3′ R: 5′GGAAGATGGTGATGGCCTTT -3′	219

TABLE 2. Cytoplasmic cumulus-oocytes maturation rate (No.)

Treatment	Control	Cysteamine concentrations			
		100 µm	**200 µm**	**300 µm**	**400 µm**
Normal physiological temperature	78.2 ± 5.6 ^{ab} (96)	77.9 ± 5.2 ^{ab} (105)	82.9 ± 5.6 ^{a} (102)	72.1 ± 5.6 ^{ab} (113)	66.8 ± 5.6 ^{b} (101)
Heat stress	71.1 ± 5.0 ^{ab} (99)	74.4 ± 5.0 ^{ab} (86)	80.9 ± 5.0 ^{ab} (96)	70.7 ± 5.7 ^{ab} (84)	77.3 ± 5.0 ^{ab} (97)

TABLE 3. Meiosis developmental stages for in vitro matured buffalo oocytes.

Treatment	Cysteamine groups	Meiosis developmental stages (%)						
		GV	GVBD	MI	AI	TI	MII	**Nuclear maturation rate** (TI+MII)
Normal physiological temperature	**Control**	0.0	56.5	8.7	0.0	21.7	17.4	39.1
	100	18.6	25.6	11.6	14.0	11.6	18.6	30.2
	200	3.0	20.6	8.8	23.5	29.4	14.7	44.1
	300	4.3	4.3	17.4	4.3	30.4	39.1	69.6
	400	5.0	25.6	15.4	15.4	17.9	20.5	38.5
Heat stress	**Control**	16.7	27.8	5.6	11.1	16.7	22.2	38.9
	100	11.4	22.8	5.7	22.8	11.4	25.7	37.1
	200	4.3	4.3	4.3	30.4	30.4	26.1	56.5
	300	18.7	12.5	18.7	0.0	25.0	25.0	50.0
	400	0.0	35.7	7.1	14.3	10.7	32.1	42.9

GV = germinal vesicle; GVBD = germinal vesicle breakdown; MI = metaphase I; AI = anaphase I; TI = telophase I; MII = metaphase II.

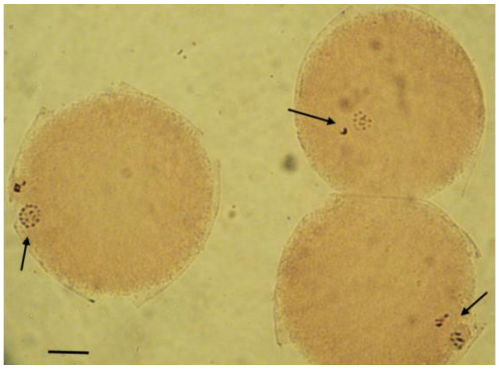

Fig. 1. Buffalo matured oocytes (nuclear maturation) at Metaphase II (MII) plate (Arrow). Original magnification X200.

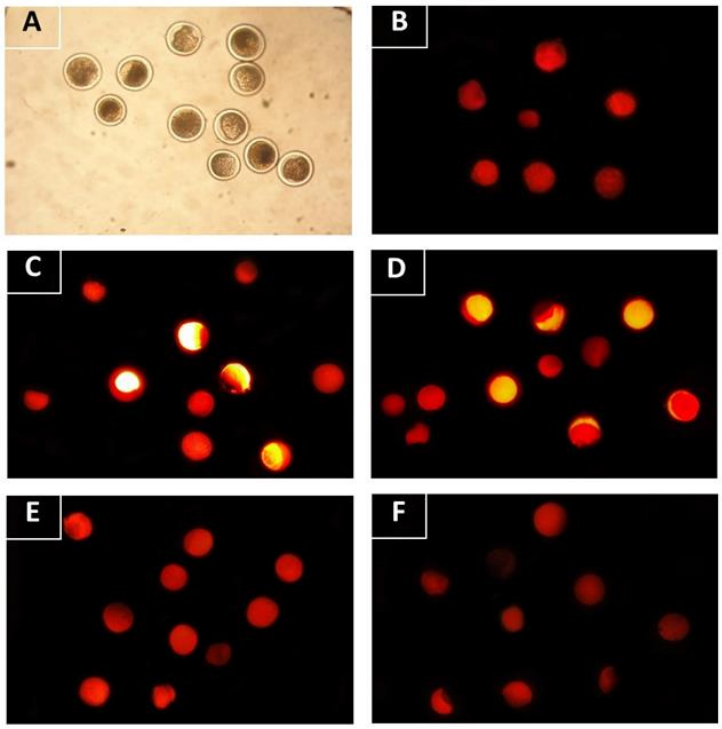

Fig. 2a. Mitochondrial activity of buffalo oocytes cultured under normal physiological condition for the control group (A and B) and cysteamine supplemented groups; 100 µM (C), 200 µM (D), 300 µM (E) and 400 µM (F).

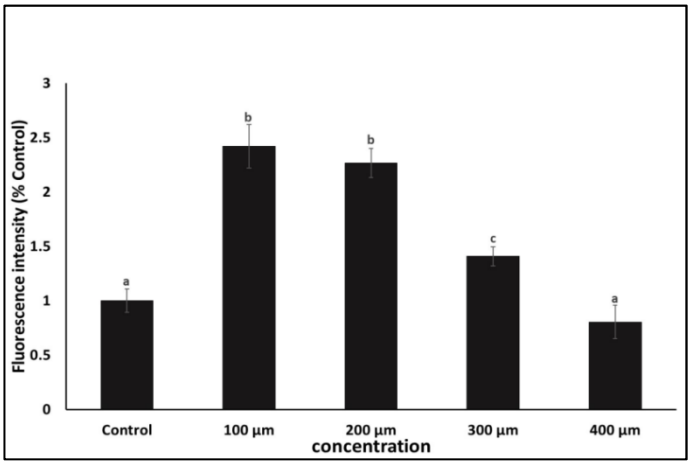

Fig. 2b. Mitochondrial fluorescence intensity of buffalo oocytes cultured with cysteamine supplementations (100, 200, 300 and 400 µM) and without (control group) under normal physiological temperature.

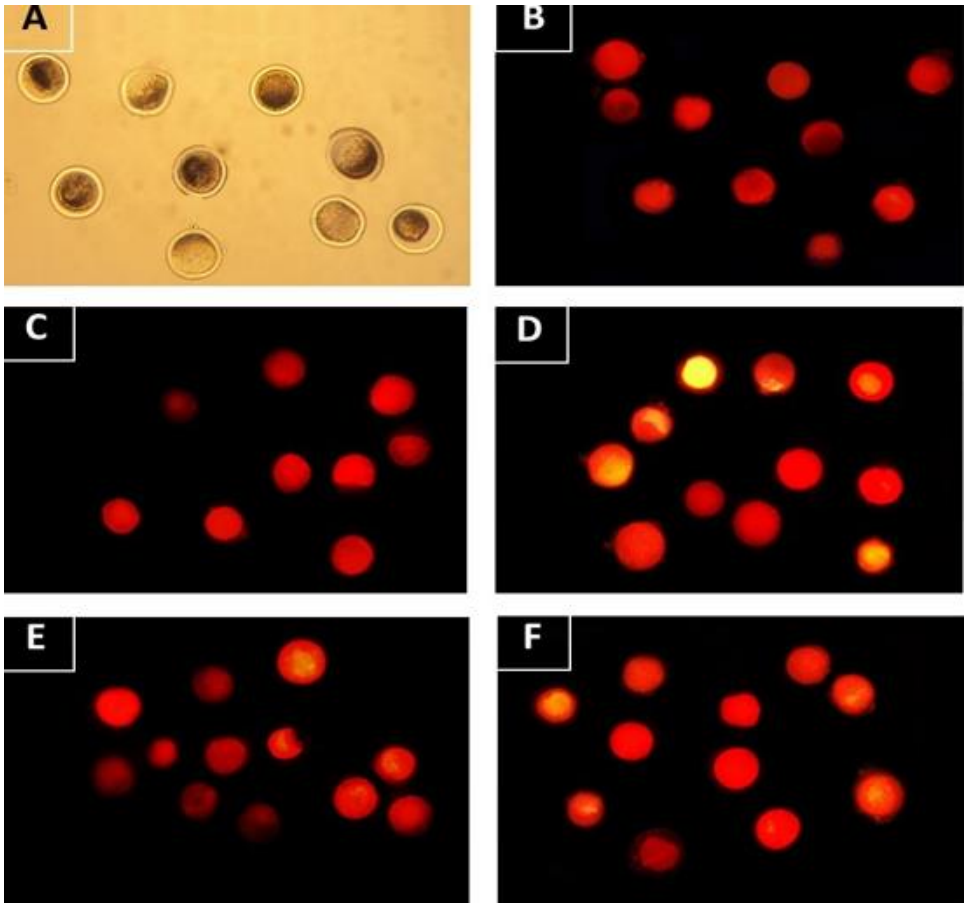

Fig. 3a. Mitochondrial activity of buffalo oocytes cultured under heat stress condition at 41°C for the control group (A and B) and cysteamine supplemented groups; 100 µM (C), 200 µM (D), 300 µM (E) and 400 µM (F).

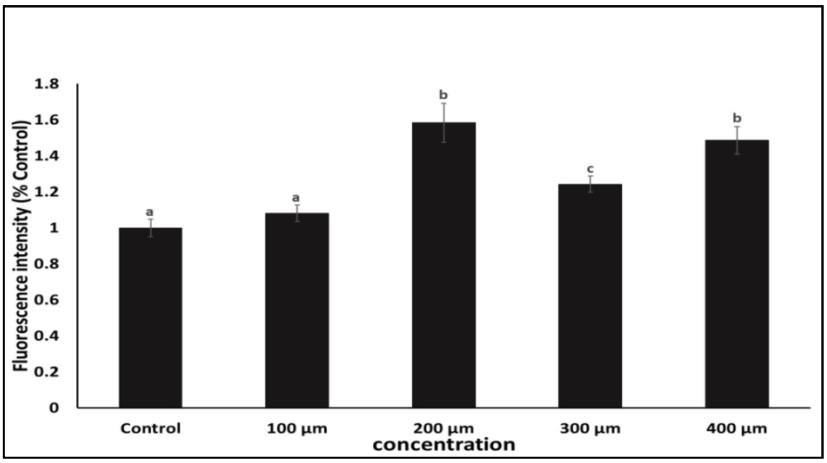

Fig.3b. Mitochondrial fluorescence intensity of buffalo oocytes cultured with cysteamine supplementations (100, 200, 300 and 400 µM) and without (control group) under heat stress conditions.

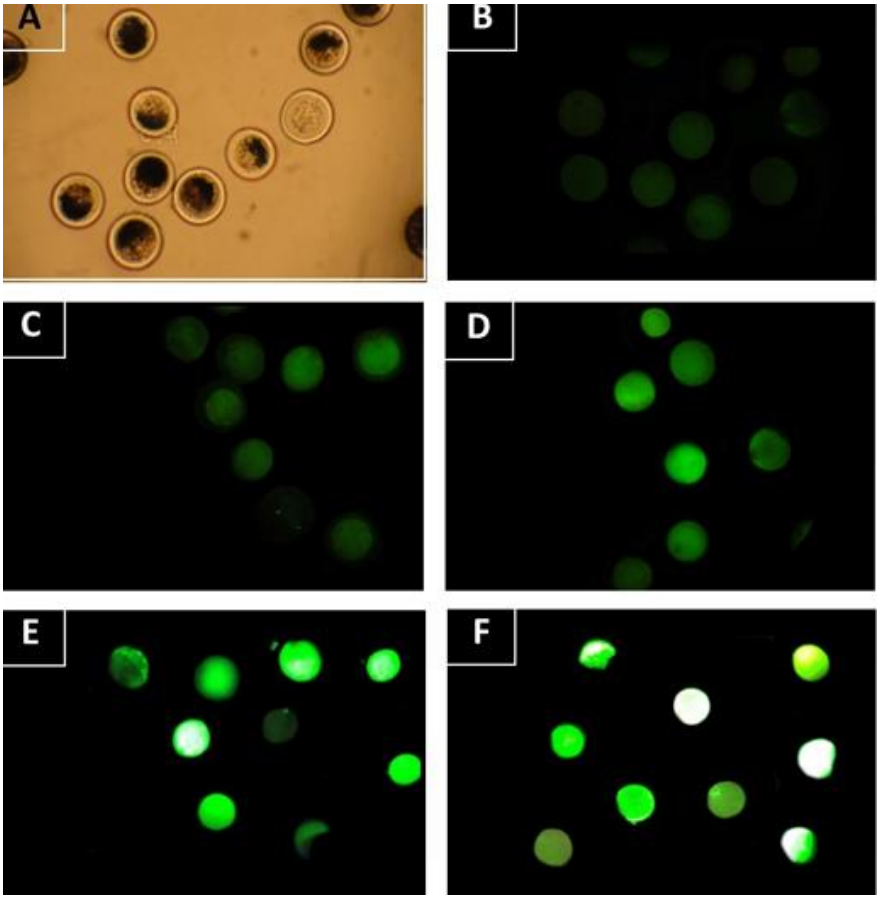

Fig. 4a. Reactive oxygen species (ROS) levels of buffalo oocytes under normal physiological temperature for the control group (A and B) and cysteamine supplemented groups; 100 µM (C), 200 µM (D), 300 µM (E) and 400 µM (F).

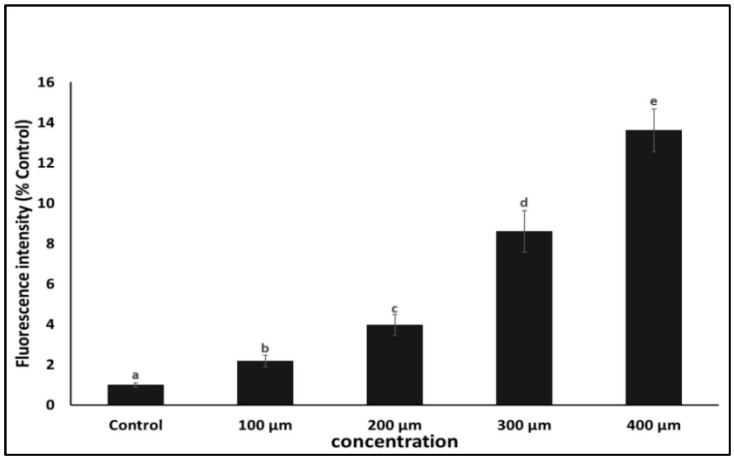

Fig.4b. Reactive oxygen species (ROS) levels of buffalo oocytes cultured with cysteamine supplementations (100, 200, 300 and 400 µM) and without (control group) under normal physiological temperature.

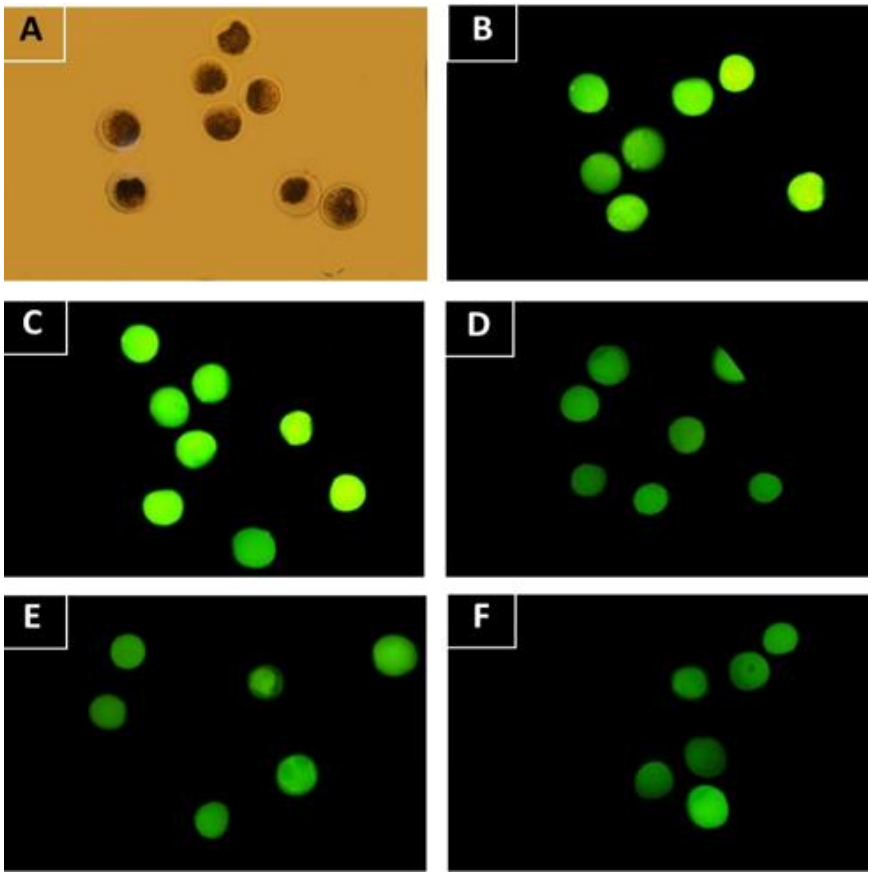

Fig. 5a. Reactive oxygen species (ROS) levels of buffalo oocytes under heat stress condition at 41°C for the control group (A and B) and cysteamine supplemented groups; 100 µM (C), 200 µM (D), 300 µM (E) and 400 µM (F).

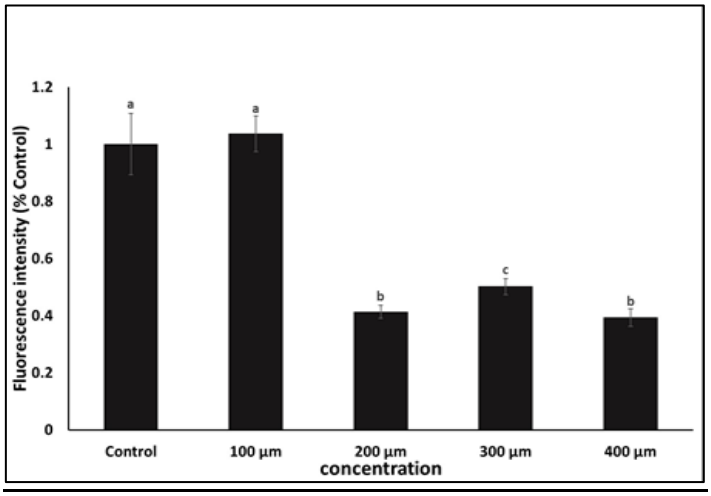

Fig.5b. Reactive oxygen species (ROS) levels of buffalo oocytes cultured with cysteamine supplementations (100, 200, 300 and 400 µM) and without (control group) under heat stress conditions.

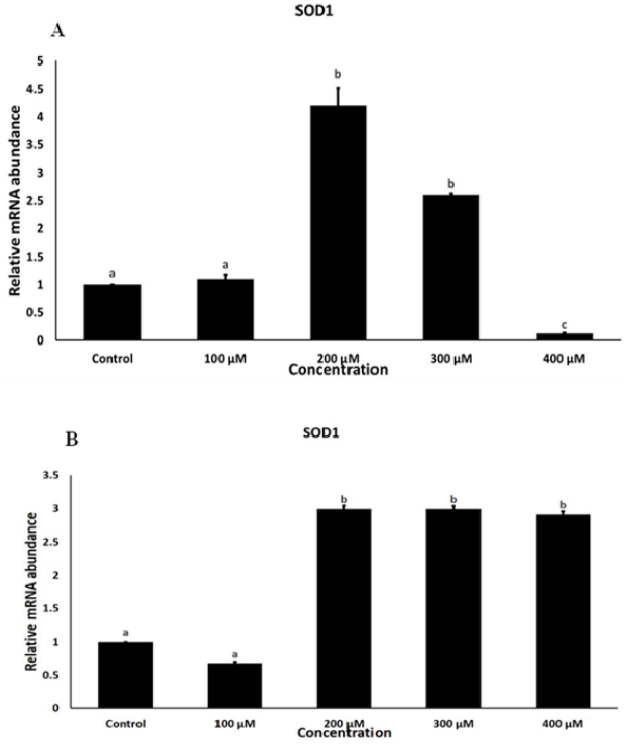

Fig.6. The relative abundance of SOD1 transcript for buffalo oocytes cultured with cysteamine supplementations (100, 200, 300 and 400 µM) and without (control group) under A) normal physiological temperature and B) heat stress conditions.

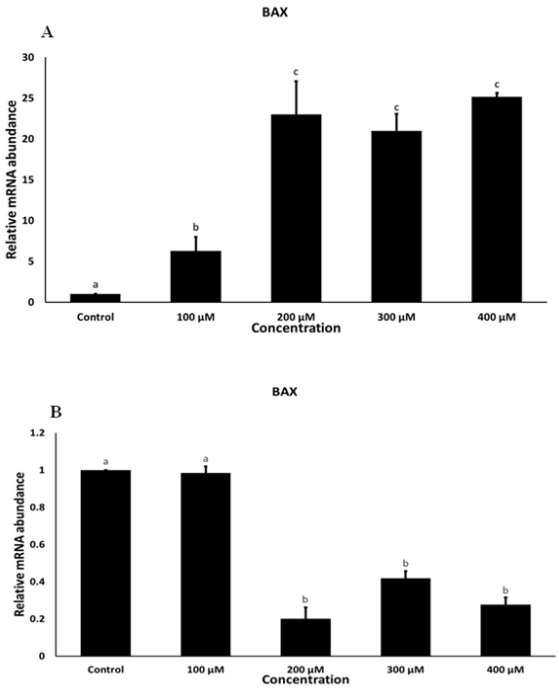

Fig. 7. The relative abundance of BAX transcript for buffalo oocytes cultured with cysteamine supplementations (100, 200, 300 and 400 µM) and without (control group) under A) normal physiological temperature and B) heat stress conditions.

References

1. Miętkiewska, K., Kordowitzki, P. and Pareek, C. S. Effects of heat stress on bovine oocytes and early embryonic development—an update. *Cells*, **11**(24), 4073 (2022). DOI: 10.3390/cells11244073.
2. Khan, M.Z., Khan, A., Chen, W., Chai, W. and Wang, C. Advancements in Genetic Biomarker and Exogenous Antioxidant Supplementation for Safeguarding Mammalian Cells against Heat-Induced Oxidative Stress and Apoptosis. *Antioxidants*, **13**(3), 258 (2024). DOI: 10.3390/antiox13030258
3. Rakha, S.I., Elmetwally, M.A., El-Sheikh Ali, H., Balboula, A., Mahmoud, A.M. and Zaabel, S.M. Importance of antioxidant supplementation during in vitro maturation of mammalian oocytes. *Veterinary Sciences*, **9**(8), 439 (2022). DOI: 10.3390/vetsci9080439
4. Silva, B.R. and Silva, J.R. Mechanisms of action of non-enzymatic antioxidants to control oxidative stress during in vitro follicle growth, oocyte maturation, and embryo development. *Animal Reproduction Science*, **249**, 107186 (2023). DOI: 10.1016/j.anireprosci.2022.107186
5. Guseva, O., Kan, N., Chekmareva, V., Kokorev, D. and Ilyasov, P. The impact of antioxidant supplements on oocytes and preimplantation embryos of humans and mammals, and their potential application for mitigating the consequences of oxidative stress in vitro: a review. *Reproductive and Developmental Medicine*, **8**(4), 252-263. (2024). DOI: 10.1097/RD9.0000000000000100
6. Feng, X., Li, C., Zhang, H., Zhang, P., Shahzad, M., Du, W. and Zhao, X. Heat-stress impacts on developing bovine oocytes: Unraveling epigenetic changes, oxidative stress, and developmental resilience. *International Journal of Molecular Sciences*, **25**(9), 4808 (2024). DOI: 10.3390/ijms25094808
7. Khurana, N.K. and Niemann, H. Energy metabolism in preimplantation bovine embryos derived in vitro or in vivo. *Biology of Reproduction*, **62**(4), 847-856 (2000). DOI: 10.1095/biolreprod62.4.847
8. Ahmed, D.A.E.R., Ghanem, N., Dessouki, S.M., Faheem, M.S., Gad, A.Y. and Barkawi, A.H. Developmental Competence of Buffalo Oocytes Cultured Under Different Oxygen Tensions after Selection with Brilliant Cresyl Blue. *World's Veterinary Journal*, **10**(2), 246-253 (2020). DOI: https://dx.doi.org/10.36380/scil.2020.wvj32
9. Roeles, J. and Tsiavaliaris, G. Actin-microtubule interplay coordinates spindle assembly in human oocytes. *Nature Communications*. **10**(1), 4651 (2019). https://doi.org/10.1038/s41467-019-12674-9
10. Amin, A. Gad, A., Salilew Wondim, D., Prastowo, S., Held, E., Hoelker, M., Rings, F., Tholen, E., Neuhoff, C., Looft, C., Schellander, K. and Tesfaye, D. Bovine embryo survival under oxidative-stress conditions is associated with activity of the NRF2-mediated oxidative-stress-response pathway. *Molecular of Reproduction and Development*, **81**(6), 497–513 (2014). DOI: 10.1002/mrd.22316
11. Lin, J. and Wang, L. Oxidative stress in oocytes and embryo development: Implications for in vitro systems. *Antioxidants & Redox Signaling*, **34**(17), 1394-1406 (2021). DOI: 10.1089/ars.2020.8209
12. Khan, A., Khan, M.Z., Umer, S., Khan, I.M., Xu, H., Zhu, H. and Wang, Y. Cellular and molecular adaptation of bovine granulosa cells and oocytes under heat stress. *Animals*, **10**(1), 110 (2020). DOI: 10.3390/ani10010110
13. Hardy, M.L., Day, M.L. and Morris, M.B. Redox regulation and oxidative stress in mammalian oocytes and embryos developed in vivo and in vitro. *International Journal of Environmental Research and Public Health*, **18**(21), 11374 (2021). DOI: 10.3390/ijerph182111374
14. Gómez-Guzmán, J.A., Parra-Bracamonte, G.M. and Velazquez, M.A. Impact of heat stress on oocyte developmental competence and pre-implantation embryo viability in cattle. *Animals.* **14**(15), 2280 (2024). DOI: 10.3390/ani14152280
15. Payton, R.R., Rispoli, L.A., Nagle, K.A., Gondro, C., Saxton, A.M., Voy, B.H. and Edwards, J.L. Mitochondrial-related consequences of heat stress exposure during bovine oocyte maturation persist in early embryo development. *Journal of Reproduction and Development*, **64**(3), 243-251 (2018). DOI: 10.1262/jrd.2017-160
16. Gallo, A., Boni, R. and Tosti, E. Gamete quality in a multistressor environment. *Environment International*, **138**, 105627 (2020). DOI: 10.1016/j.envint.2020.105627
17. Patel, P.A., Chaudhary, S.S., Puri, G., Singh, V.K. and Odedara, A.B. Effects of β-mercaptoethanol on in vitro maturation and glutathione level of buffalo oocytes. *Veterinary World*, **8**(2), 213-216 (2015). DOI: 10.14202/vetworld.2015.213-216
18. Sandal, A.I., Ozdas, O.B. and Baran, A. *In vitro* maturation of bovine oocytes: beneficial effects of cysteamine. *Journal of Dairy, Veterinary & Animal Research*, **7**(3), 64-65 (2018). DOI: 10.15406/jdvar.2018.07.00191
19. Marí, M., Morales, A., Colell, A., García-Ruiz, C. and Fernández-Checa, J.C. Mitochondrial glutathione, a key survival antioxidant. *Antioxidants & Redox Signaling*, **11**(11), 2685-700 (2009). DOI: 10.1089/ARS.2009.2695
20. Faheem, M., Ahmed, D. and Dessouki, S. Antioxidant capacity of cysteamine on buffalo granulosa cell performance under oxidative stress conditions. *Egyptian Journal of Animal Production*, **61**(1), 31-37 (2024). DOI: 10.21608/ejap.2024.250268.1065

تأثير السيستامين على الكفاءة الإنضاجية والاستجابات الجزيئية والوظيفية الكامنة وراء نشاط الميتوكوندريا وموت الخلايا لبويضات الجاموس المصري المعرضة للإجهاد الحراري معملياً

مروة س. فهيم[1] ، ايهاب ص. عبد العال[2] ، صلاح العسال[3] ،داليا ع . أحمد [1]، منار مصطفى[3] وأحمد أمين[1]

[1] قسم الإنتاج الحيوانى، كلية الزراعة، جامعة القاهرة، مصر.

[2] معهد بحوث الإنتاج الحيوانى، مركز البحوث الزراعية، الدقى، مصر.

[3] قسم الوراثة ، كلية الزراعة، جامعة القاهرة، مصر.

الملخص

يهدد الاحتباس الحراري صحة الحيوان وأدائه وقدرته الإنجابية من خلال تعطيل عملياته الفسيولوجية الطبيعية. لذلك، يصبح دمج مضادات الأكسدة في الأنظمة البيولوجية أمرًا بالغ الأهمية للتخفيف من الآثار الضارة للإجهاد الناجم عن الحرارة. تهدف الدراسة الحالية إلى تقييم تأثير مكملات السيستامين كمضاد أكسدة خارجي على نضج بويضات الجاموس في المختبر تحت ظروف الإجهاد الحراري بالإضافة إلى مراقبة كفاءة البويضات بعد النضج فيما يتعلق بنشاط الميتوكوندريا ومستويات توليد أنواع الأكسجين التفاعلية واستجاباتها الجزيئية ذات الصلة. خضعت بويضات الجاموس غير الناضجة لمكملات السيستامين بخمسة تركيزات (0 و100 و200 و300 و400 ميكرومولار) في ظل ظروف زراعة مختلفة، 1) الإجهاد الحراري (41 درجة مئوية للساعة الأولى من النضج تليها 38.5 درجة مئوية حتى نهاية الزراعة، 2) حالة درجة حرارة فسيولوجية طبيعية عند 38.5 درجة مئوية لمدة 24 ساعة. كشفت نتائجنا عن عدم وجود فروق جوهرية بين البويضات الناضجة تحت ظروف الإجهاد الحراري ودرجة الحرارة الفسيولوجية الطبيعية، مع ميزة نسبية عند تركيز 200 ميكرومولار من السيستامين .

تحت الإجهاد الحراري، أظهرت البويضات المعالجة بالسيستامين نشاطًا معززًا للميتوكوندريا، وانخفاضًا في مستويات إنتاج أنواع الأكسجين التفاعلية (ROS)، بالإضافة إلى زيادة في مستويات SOD1 وانخفاض في مستويات التعبير عن BAX، وخاصةً عند تركيزات أعلى من 100 ميكرومولار. في المقابل، أدت تركيزات السيستامين الأعلى في ظروف الحرارة الطبيعية إلى زيادة إنتاج أنواع الأكسجين التفاعلية (ROS). ختامًا، قد يُحسّن تراكم الجلوتاثيون بوساطة السيستامين من توازن الأكسدة والاختزال الخلوي، ويُشكّل آلية حماية ضد تكوّن أنواع الأكسجين التفاعلية (ROS).

الكلمات الدالة: السيستامين، بويضات الجاموس، النضج في المختبر، نشاط الميتوكوندريا، مستوى أنواع الأكسجين التفاعلية، التعبير الجيني.