Egyptian Journal of Veterinary Sciences

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

Quercetin Supplementation in Tris Egg Yolk Extender Improves the Post-Thaw Quality of *Zaraibi* Buck Semen

Essam A. Almadaly[1*], Mohamed A. Elbendary[1], Ferial M. Sahwan[2], Ahmed M. Shehabeldin[3], Wael B. El-Domany[4], Ismail I. El-Kon[1], Adel A. Ramoun[1]

[1]*Department of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish Street Kafrelsheikh 33516, Egypt.*

[2]*Animal Breeding and Production, Animal Husbandry and Wealth Development Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt.*

[3]*Biotechnology Research Department, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt.*

[4]*Department of Theriogenology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.*

Abstract

THIS study investigates the effect of adding various concentrations of quercetin (QUE) on the post-thaw semen quality of *Zaraibi* bucks. Semen was collected from seven bucks, examined, and dispensed into five portions, where each portion was diluted with Tris egg yolk extender supplemented with 0 µM as a control, 10 µM, 15 µM, 25 µM, and 40 µM of QUE per mL of semen extender. The diluted portions were loaded into mini-straws, sealed, and frozen in liquid nitrogen. Frozen-thawed straws were evaluated for sperm progressive motility, kinematic parameters, viability, plasma membrane, and acrosome integrity. Also, seminal superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant capacity (TAC) levels and malondialdehyde (MDA) content were assessed. Our results revealed that the extender supplemented with 25 µM QUE has significantly ($P<0.05$) higher CASA sperm progressive motility, curve line, straight line, and average path velocities than the control. The sperm viability, plasma membrane, and acrosome integrity of the 25 and 40 µM QUE groups were markedly higher ($P<0.05$) compared to those of the control group. SOD and GPX levels were substantially higher ($P<0.05$) in semen samples supplemented with 25 µM QUE, while TAC was significantly greater ($P<0.05$) in 15, 25, and 40 µM QUE groups. Conversely, MDA content was markedly lower ($P<0.05$) in 25 µM QUE. In conclusion, adding 25 µM QUE in the freezing extender enhances post-thaw buck semen quality via minimizing reactive oxygen species production and lipid peroxidation, as evidenced by the obtained higher sperm characteristics and enzymatic antioxidant activity.

Keywords: *Zaraibi* buck sperm, Quercetin, Cryopreservation, CASA, Lipid peroxidation.

Introduction

Goats (*Capra hircus*) are an important source of income worldwide especially in developing countries as they can adapt to harsh environments and poor feeding conditions [1]. Artificial insemination (AI) with cryopreserved semen is potent reproductive biotechnology for the conservation of the genetic material of superior males for long periods to improve animal reproduction and production [2]. Cryopreservation is a method to freeze and thaw the sperm cells while keeping their viability. It allows

*Corresponding author: Essam A. Almadaly, E-mail: dr_essam_2005@yahoo.com, Tel.: 01064005262
(Received 31 October 2024, accepted 02 December 2024)
DOI: 10.21608/EJVS.2024.332755.2467

improved fertility when handled *in vitro* as gametes or embryos can be kept in liquid nitrogen for a long time [3]. The sperm undergoes cryodamage throughout the freezing and thawing procedures with subsequent reduced fertilizing capacity due to oxidative stress and lipid peroxidation of the sperm cell membrane [4]. Buck sperm cell membrane has a high content of polyunsaturated fatty acids, so sperm becomes more susceptible to lipid peroxidation in the presence of reactive oxygen species (ROS) during semen cryopreservation [5]. Multiple compounds have recently been added to semen extenders to mitigate lipid peroxidation effects on sperm cells increasing the fertilizing capacity of frozen-thawed sperm cells [6]. Enriching semen extenders with antioxidants mitigates oxidative stress enhancing the post-thaw sperm quality [7]. Quercetin (QUE) is a dietary flavonoid found in many vegetables and fruits and acts as an effective antioxidant in addition to various biological activities that involve anti-inflammatory, antimicrobial, and anticarcinogenic properties [8]. QUE has a polyphenolic structure that prevents oxidation by acting as a scavenger of free radicals, which are responsible for chain reactions [9], and detoxifies oxidative stress-related enzymes, preventing ROS generation [10]. Since QUE contains several double bonds and hydroxyl groups, it can reduce free radicals controlling oxidative stress by donating electrons through resonance [11]. QUE inhibits free radicals' production via iron chelating and inhibits the generation of lipid peroxyl radicals and superoxide ions [12]. Supplementation of semen extenders with QUE improves post-thaw sperm quality in bucks [13], bulls [14], rams [15], and boars [11]. Therefore, this study investigates the possible effects of incorporating different concentrations of QUE into the freezing extender on the post-thaw buck semen quality.

Materials and Methods

Chemicals

Unless otherwise stated, all chemicals used in this study were procured from Sigma-Aldrich Chemical Co., USA.

Ethical statement

The Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine at Kafrelsheikh University, Egypt allowed approval (KFS-IACUC/219/2024) for the present study involving the sampling.

Experimental animals and semen collection

This study was performed at the Riwina Governmental Station, Animal Production Farm, Agricultural Research Center, Kafrelsheikh, Egypt, from October to December 2023. Semen was collected from seven fertile healthy adult *Zaraibi* bucks aged 1 – 2 years with good testicular structure proved by ultrasonographic examination using a 7.5 MHz linear-array transducer and a B-mode scanner (testicular parenchyma was homogenous with moderate echogenicity throughout). The animals were housed in a shed with half walls and wire mesh above with a concrete floor, fed daily with 0.5 Kg concentrate mixture besides green fodder *ad libitum*. The bucks were in good general health condition, free from acquired and congenital abnormalities, and regularly received anthelmintics. After three weeks of acclimatization to semen collection by electroejaculation (EE), one ejaculate was collected from each buck per week for 7 weeks by using an electroejaculator (e320, Minitube, GmbH, Germany), in total 42 ejaculates were collected. The EE device was automated, with a starting voltage of 1 volt and a maximum voltage of 8 volts. It alternated between stimulation and rest for 5 seconds each.

Quercetin preparation

QUE was prepared as described by Diniz et al. [16]. Briefly, 40 mg of quercetin hydrate was dissolved in 400 µL of 1M NaOH in an Eppendorf 1.5 mL capacity with the help of a vortex (VM-S, Taisite, New York, USA) and then transferred into a falcon tube containing 36 mL of Milli-Q water. pH was adjusted to 8.0 with 70% orthophosphoric acid. Finally, the stock solution (1 mg/mL) is obtained by adding Milli-Q water to get the volume up to 40 mL.

Semen dilution, freezing, and thawing

Ejaculates of more than 80% individual motility and more than 3×10^9/mL sperm cell concentration were pooled and divided into 5 aliquots. Each aliquot was diluted with the Tris egg yolk extender, which was freshly prepared by dissolving 3.07 gm Tris, 1.64 gm citric acid, 1.26 gm fructose, 100 mg streptomycin, 15% egg yolk, and 5% glycerol in100 mL Milli-Q water and then adjust its pH to 6.8–7.2 [17]. Diluted aliquots containing 0 µM as a control (CTRL), 10 µM (10 µM QUE), 15 µM (15 µM QUE), 25 µM (25 µM QUE), 40 µM (40 µM QUE) of QUE were prepared to a final sperm cell concentration 400 x 10^6 sperm/mL [4]. The extended semen samples were equilibrated at 4 °C for 4 hours, aspirated into 0.25 mL mini-straws (IMV, L'Aigle, France), and sealed with polyvinyl alcohol powder. The loaded straws were kept in liquid nitrogen vapor 4 cm above the nitrogen surface for 10 min then dipped into liquid nitrogen (−196 ºC) for freezing. After one week of storage, the frozen straws were thawed at 37 °C for 30 seconds before evaluation [18].

Semen evaluation

The thawed straws were evacuated in a 0.6 mL Eppendorf to assess the following seminal parameters:

Progressive motility and kinetic parameters

Sperm progressive motility and motion parameters were assessed using a computer-assisted sperm motility analysis (CASA, Version 12 IVOS, Hamilton-Thorne Biosciences, Beverly, MA, USA). A 10 µL of frozen-thawed semen sample was transferred to a chamber, which was placed in the microscope's warm stage (37 °C). Three microscopic fields were selected randomly for each concentration and scanned 5 times for each field [19]. Sperm kinetic parameters include curve line velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity % (LIN = (VSL/VCL) × 100), straightness % (STR = (VSL/VAP) × 100), wobble % (WOB = (VAP/VCL) × 100) coefficient were evaluated for control and treatment groups.

Sperm viability

The viability of spermatozoa was evaluated using semen smears stained with eosin-nigrosin stain as described by Gangwar et al. [20]. Two hundred sperm cells were examined using a 400× objective lens. Unstained sperm cells were considered viable sperms while dead sperms were pink-stained.

Plasma membrane integrity

The plasma membrane of sperm has been examined for functional integrity using the hypo-osmotic swelling test (HOST) using HOS solution (0.75 g tri-sodium citrate + 1.351 g fructose dissolved in 100 mL Milli-Q water). A 50 µL of each semen sample was added to 500 µL of HOS solution and incubated at 37 °C for 30 min. After incubation, 2 µL of the prepared well-mixed sample was examined on a pre-warmed slide (37 °C), covering it with a pre-warmed coverslip under a phase-contrast microscope (400×). At least, 200 sperm cells were examined to determine their capacity for swelling in HOS solution. Sperm cells with coiled or swollen tails of various forms were considered to have functional plasma membranes [21]. The true HOST-positive sperm cell % was calculated by subtracting the % of abnormal tail morphology before exposure to HOST from the total % of HOST-positive spermatozoa [22].

Acrosomal integrity

Five µL of frozen-thawed semen was smeared on a clean glass slide and dried on a warming plate at 38.5 °C. The semen smears were fixed in methanol for at least 10 min before being washed under the tap water. The fixed semen smears were immersed into a staining jar containing 7.5% buffered Giemsa stain solution (3 mL Giemsa stock solution mixed with 2 mL phosphate buffer saline, and 35 mL Milli-Q water of pH 7) for 90 min. Furthermore, the slides were immediately washed from the excess stain and dried. The stained smears were examined using an oil immersion lens, and 200 sperms were evaluated. Acrosome-positive sperms were defined as having uniformly purple-stained acrosomal caps, whereas acrosome-negative sperm cells had disrupted and/or unevenly stained or lost acrosomal caps [22].

Activity of enzymatic antioxidants and lipid peroxidation

The frozen-thawed straws were centrifuged at 1000 g for 10 min and the resulting supernatants were examined for the following analyses:

Superoxide dismutase activity (SOD)

SOD activity (U/mL) was measured by using a Cayman SOD assay kit (706002, Cayman Chemicals Co, Colorado, USA) following the methodology of Kumar et al. [23]. In each well, 200 µL of diluted radical detector was mixed with 10 µL of standards/samples and 20 µL of diluted xanthine oxidase. The plate was incubated with gentle shaking for 20 min at room temperature. Following that, SOD standards were used to establish standard curves, and sample SOD activity was estimated.

Glutathione peroxidase activity (GPX)

The activity of GPX was detected using a Cayman GPX assay kit (703102, Cayman Chemicals Co, Colorado, USA). 100 µL of assay buffer was added in each well on the plate then mixed with 50 µL of the co-substrate mixture and 20 µL standards/samples. The addition of 20 µL of cumene hydroperoxide initiated the reaction. Using the colorimetric plate, the absorbance was determined at wavelength 340 nm every 1 min for 5 min. The standard curve was created using the GPX standards and GPX values were reported as nmol/min/mL [24].

Total antioxidant capacity (TAC)

Benzine and Strain's technique was utilized to estimate the TAC in seminal plasma as described by Mayasula et al. [25]. TAC kits (TA 2513, Biodiagnostic, Cairo, Egypt) were used in this method. Briefly, with the presence of antioxidants which donate electrons, the colorless complex transforms into a bluish color. The colored complex absorbance at 593 nm is used to calculate the antioxidant capacity of the seminal plasma. The ferric-reducing antioxidant power (FRAP) reagent was prepared and mixed thoroughly with 5 µL of seminal plasma and incubated at 37 °C for 30 min. In addition to the sample, a blank was prepared using 5 µL of double-distilled water and the FRAP reagent. The standard curve was plotted using various concentrations of $FeSO_4$.

Malondialdehyde content (MDA)

MDA level was determined to evaluate lipid peroxidation via the Thiobarbituric acid reaction method (TBA) according to Banday et al. [26] by using commercial kits (MD 2529, Bio diagnostic, Cairo, Egypt). Briefly, in 15 mL centrifuge tubes, 0.1

mL of seminal plasma was diluted with 0.9 mL of distilled water to yield a volume of 1 mL. Then 2.5 mL of TBA reagent was mixed with the sample and kept in a boiling water bath for 1 hour. Following boiling, the mixture was cooled down at room temperature and then centrifuged for 10 min at 1500 g in a cooling centrifuge. Finally, the formed supernatant was separated, and a spectrophotometer was used to measure the absorbance at 535 nm. MDA values were calculated by absorbance coefficient e ($1.56\times10^{5}mol^{-1}cm^{-1}$) and expressed as nmol/mL.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and followed by Tukey's test for pairwise comparison between the control group and treatment means using GraphPad Prism version 5.0.0 (GraphPad Software Inc., San Diego, USA). $P < 0.05$ value for differences were regarded as statistically significant. The results were presented as mean ± standard error of the mean (SEM).

Results

Progressive motility and kinetic parameters

Data about the effect of QUE on sperm progressive motility and kinetic parameters are presented in Table 1. It was noticed that sperm progressive motility was significantly higher ($P<0.05$) in the extender supplemented with 25 µM QUE (66.94 ± 1.85%) than in control (54.41 ± 2.28%) and 10 µM QUE (56.84 ± 1.76%). Similarly, VCL was significantly greater ($P<0.05$) in 25 µM QUE (58.38 ± 2.33 µm/s) than in control (35.40 ± 1.89 µm/s) and other groups. In addition, VSL was significantly higher ($P<0.05$) in 25 µM QUE (53.65 ± 2.04 µm/s) than in control (26.74 ± 1.34 µm/s) and other groups. Moreover, VAP was significantly higher ($P<0.05$) in 25 µM QUE (74.12 ± 2.83 µm/s) than control (43.19 ± 2.11 µm/s) and other groups except 40 µM QUE (65.57 ± 2.20 µm/s). The highest proportions of LIN (91.90 ± 2.39) and STR (72.50 ± 2.77) were recorded in 25 µM QUE while WOB % was greater ($P<0.05$) in 15 µM QUE (136.1 ± 3.33) than in control (123.1 ± 4.33%) and other treatment groups except for 25 µM QUE (127.2 ± 2.92) as shown in Table 1.

Sperm viability, plasma membrane, and acrosome integrity

The effect of QUE on sperm viability, plasma membrane, and acrosome integrity is shown in Table 2. The proportion of sperm viability was higher ($P<0.05$) in 25 (75.00 ± 2.08) and 40 (72.67 ± 2.03) µM QUE compared with the control (62.17 ± 1.74) group. Further, there was a significant increase ($P<0.05$) in the functional sperm plasma membrane integrity in semen samples cryopreserved with 25 (70.50 ± 2.84%) and 40 µM (68.17 ± 2.49%) QUE in comparison with the control group (57.33 ± 1.88%). Moreover, acrosome integrity was found to be higher ($P<0.05$) in extender supplemented with 25 (78.83 ± 2.17%), 40 (77.33 ± 2.32%), and 15 (75.67 ± 1.92%) µM QUE as compared to the control group (65.50 ± 1.73%, Table 2) without significant difference among the four treatment groups.

Activity of enzymatic antioxidants and lipid peroxidation

Data concerning the effect of QUE on the activity of enzymatic antioxidants (SOD, GPX, and TAC) and lipid peroxidation (MDA) are depicted in Table 3. Frozen-thawed buck semen supplemented with 25 µM QUE showed significantly ($P<0.05$) increased SOD activity (32.52 ± 1.49 U/mL) as compared to control (17.89 ± 1.11 U/mL) and other treatment groups, except 40 µM (26.13 ± 1.54 U/mL) QUE. Similarly, 25 µM QUE markedly increased GPX activity (20.76 ± 1.33 nmol/min/mL, $P<0.05$) compared to control (12.53 ± 0.94 nmol/min/mL) and other groups except 40 µM (18.84 ± 1.09 nmol/min/mL) QUE. Furthermore, TAC activity was significantly higher ($P<0.05$) in 15 (2.58 ± 0.04 mM/L), 25 (2.74 ± 0.06 mM/L), and 40 (2.62 ± 0.05 mM/L) µM QUE compared to the control (1.56 ± 0.03 mM/L) and 10 (2.19 ± 0.03 mM/L) µM QUE. Moreover, MDA levels were significantly decreased ($P<0.05$) in semen samples cryopreserved in the presence of 25 µM QUE (15.23 ± 1.05 nmol/mL) as compared to the control (28.41 ± 1.71 nmol/mL) and the other treatment groups as shown in Table 3.

Discussion

Cryopreservation is a process of great potential for goat breeders as it improves animal productivity despite this, freezing-thawing procedures cause inevitable cryodamage to the plasma membrane, acrosome, and DNA of spermatozoa with subsequent reduction in the number of forward motile sperms post-thawing [27]. Overproduction of ROS during the cryopreservation process can result in oxidative stress causing damage to various constituents of sperm cells involving lipids, sugars, proteins, and nucleic acid [28]. Endogenous antioxidants such as GPX, SOD, and catalase protect sperm cells against ROS. However, their activities may not be enough during the freezing-thawing protocol [29]. Antioxidants can be used in semen extenders to limit ROS generation during the freezing and thawing process improving the structural and functional quality of sperm cells [30].

QUE is one of the polyphenolic flavonoids. It is found in many plants and foods such as cherries, tea, broccoli, kale, onion, berries, apples, and red grapes [31]. It diminishes the levels of certain enzymes including NADPH oxidase and NADH-dependent oxidoreductase and improves enzymatic antioxidant activity [32]. QUE is a stronger antioxidant than vitamins E and C, having an inhibitory effect on lipid peroxidation [33]. The current study was performed

on frozen-thawed buck semen to detect the impact of QUE on sperm quality (progressive motility, CASA kinetics, viability, plasma membrane, and acrosome integrity) and activity of antioxidant enzymes (SOD, GPX, and TAC) in addition to MDA content as an indication for lipid peroxidation.

Sperm CASA motility indices are widely regarded as one of the most important sperm characteristics associated with its ability to fertilize an oocyte. In addition, CASA provides accurate details about different sperm kinetics that are useful in predicting the fertilizing ability of the sperm cells [34]. This study showed that the overall quality of post-thawed buck semen including sperm progressive motility and sperm kinetics was improved by adding QUE in Tris egg yolk extender. 25 μM QUE yielded the best results of post-thawed progressive motility and velocity parameters (VCL, VSL, VAP, LIN, STR, and WOB) of buck spermatozoa. In line with our results, Batool et al. [35] observed that an extender supplemented with 5 μM QUE improved the total, progressive motility and kinetic parameters of post-thawed buck spermatozoa. Furthermore, the post-thawed motility (total and progressive) and motion kinetics were improved in the report of Khan et al. [10] by adding 20 μM QUE to the semen extender of the ram. QUE enhances antioxidant activity when combined with other additives, protecting the plasma membrane from ROS damage and improving the motility and kinetic parameters of sperm cells [13].

Viability refers to the percentage of viable sperm measured by cell assessment reduced during cooling and/or freezing in addition to multiple factors during semen processing [36]. In the present study, greater values for sperm viability after thawing were found when the semen extender was supplemented with 25 and 40 μM QUE. Following these results, Siari et al. [9] reported that 10 mM QUE increased post-thawed viability of rooster sperm. Moreover, Tvrdá et al. [37] observed that 50 and 100 μmol/l of QUE improved the viability of bovine spermatozoa.

Sperm plasma membrane integrity is mainly reduced due to lipid peroxidation, especially in goats due to the high concentration of phospholipid-rich unsaturated fatty acids in the sperm plasma membrane [38]. Obtaining a high proportion of sperm cells with intact plasma membranes after semen processing and preservation is crucial for proper sperm function as sperm metabolism is related to its plasma membrane integrity [39]. Our study demonstrated that QUE supplementation at doses of 25 and 40 μM in a cryopreservation medium greatly enhances the post-thawed functional plasma membrane integrity of buck spermatozoa. Similarly, previous studies proved that QUE improves sperm plasma membrane integrity after thawing in the semen of goat [40], buffalo bull [41], and jungle fowl [42].

Acrosome integrity plays a critical role in sperm cell fertility and is essential for the process of sperm-ovum penetration [43]. The current study indicated that post-thawed acrosome integrity of buck sperm was higher in semen samples cryopreserved with 15, 25, and 40 μM QUE. In agreement with these findings, Ahmed et al. [44] reported that an extender supplemented with 150 and 200 μM QUE maintains post-thawed sperm acrosomal integrity in buffalo bulls. Furthermore, Bang et al. [45] found that the post-thawed sperm acrosome integrity of the boar sperm was increased in extender supplementing with 50 μM QUE. Moreover, Gibb et al. [46] reported that incorporating 150 μM QUE in semen extender improved the acrosomal integrity of sex-sorted cryopreserved stallion spermatozoa in comparison to control and other additives (200 U/mL catalase or 0.2 mg/mL cysteine).

Excessive generation of ROS impaired the quality of semen and even led to infertility. Therefore, minimizing the damaging impacts of ROS on sperm cells depends on the adequate activity of enzymatic antioxidants [47]. Results of the current study revealed that SOD and GPX levels were higher in semen samples cryopreserved in the presence of higher (25 and 40 μM) concentrations of QUE than the control group and lower (10 and 15 μM) concentrations of QUE. As a result, all treatment groups had higher TAC compared with the control group. On the other hand, MDA was markedly reduced in 25 μM QUE post-thawing. In concordance with our results, Appiah et al. [8] showed that casein-based extender cryopreserved with 0.01 mg/mL QUE improved SOD and GPX activities in rooster semen. Kumar et al. [40] reported that the addition of QUE at a concentration of 20 μmol/mL increased SOD and GPX levels and decreased the MDA content of post-thawed buck semen. Furthermore, Batool et al. [35] found that cryopreservation of buck semen enriched with 5 μM QUE results in higher TAC activity and lower MDA content. Moreover, El-Khawagah et al. [41] reported that QUE supplementation at a dose of 10 μM decreased the MDA content of post-thawed buffalo bull semen.

Conclusion

It is concluded that QUE improves post-thawed sperm progressive motility, motion kinetics, viability, plasma membrane, and acrosome integrity in addition to enhancing antioxidant status and suppressing lipid peroxidation of cryopreserved buck semen. Suggesting supplementation of buck semen extender during cryopreservation with QUE, especially 25 μM QUE, due to its protective effect against oxidative stress.

Acknowledgments

The authors express their gratitude to the entire team of the Riwina Governmental Station, Animal

Production Farm, Kafrelsheikh, Egypt, and the International Livestock Management Training Center, Sakha, Kafrelsheikh, Egypt, for their technical assistance during the experiment.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Conflict of Interest

The authors declared no conflicts of interest in the publication of this research article.

TABLE 1. Effect of different concentrations of quercetin (QUE) on sperm progressive motility and kinetic parameters of frozen-thawed buck semen (mean ± SEM) *

Parameter	CTRL	Quercetin (µM)			
		10	15	25	40
PM (%)	54.41 ± 2.28^{c}	56.84 ± 1.76^{bc}	62.77 ± 2.17^{ac}	66.94 ± 1.85^{a}	65.88 ± 2.02^{ab}
VCL (µm/s)	35.40 ± 1.89^{d}	37.35 ± 1.21^{d}	42.19 ± 1.40^{c}	58.38 ± 2.33^{a}	53.66 ± 1.95^{b}
VSL (µm/s)	26.74 ± 1.34^{c}	28.87 ± 1.51^{c}	33.88 ± 1.86^{bc}	53.65 ± 2.04^{a}	38.85 ± 1.97^{b}
VAP (µm/s)	43.19 ± 2.11^{c}	40.95 ± 1.41^{c}	57.40 ± 2.71^{b}	74.12 ± 2.83^{a}	65.57 ± 2.20^{ab}
LIN (%)	75.83 ± 4.23^{ab}	77.23 ± 1.58^{ab}	80.33 ± 4.05^{ab}	91.90 ± 2.39^{a}	73.83 ± 2.97^{b}
STR (%)	62.07 ± 3.30^{ab}	70.57 ± 3.41^{ab}	59.43 ± 2.02^{b}	72.50 ± 2.77^{a}	59.23 ± 1.82^{b}
WOB (%)	123.1 ± 4.33^{b}	109.8 ± 2.60^{c}	136.1 ± 3.33^{a}	127.2 ± 2.92^{ab}	124.6 ± 2.46^{b}

*Within the same row, means bearing one common superscript were non-significantly (P≥0.05) different. CTRL = Control, PM = Progressive motility, VCL = Curve line velocity, VSL = Straight line velocity, VAP= Average path velocity, LIN = Linearity, STR = Straightness, WOB = Wobble.

TABLE 2. Effect of different concentrations of quercetin (QUE) on sperm viability, plasma membrane, and acrosome integrity of frozen-thawed buck semen (mean ± SEM) *

Parameter (%)	CTRL	Quercetin (µM)			
		10	15	25	40
Viability	62.17 ± 1.74^{b}	67.83 ± 2.17^{ab}	70.50 ± 1.80^{ab}	75.00 ± 2.08^{a}	72.67 ± 2.03^{a}
Plasma membrane integrity	57.33 ± 1.88^{b}	61.50 ± 2.18^{ab}	66.00 ± 1.73^{ab}	70.50 ± 2.84^{a}	68.17 ± 2.49^{a}
Acrosomal integrity	65.50 ± 1.73^{b}	71.17 ± 2.21^{ab}	75.67 ± 1.92^{a}	78.83 ± 2.17^{a}	77.33 ± 2.32^{a}

*Within the same row, means bearing one common superscript were non-significantly (P≥0.05) different. CTRL = Control

TABLE 3. Effect of different concentrations of quercetin (QUE) on enzymatic antioxidants and lipid peroxidation of frozen-thawed buck semen (mean ± SEM) *

Parameter	CTRL	Quercetin (µM)			
		10	15	25	40
SOD (U/mL)	17.89 ± 1.11^{c}	21.51 ± 1.28^{bc}	24.07 ± 1.45^{bc}	32.52 ± 1.49^{a}	26.13 ± 1.54^{ab}
GPX (nmol/min/mL)	12.53 ± 0.94^{c}	13.58 ± 0.96^{c}	15.65 ± 1.11^{bc}	20.76 ± 1.33^{a}	18.84 ± 1.09^{ab}
TAC (mM/L)	1.56 ± 0.03^{c}	2.19 ± 0.03^{b}	2.58 ± 0.04^{a}	2.74 ± 0.06^{a}	2.62 ± 0.05^{a}
MDA (nmol/mL)	28.41 ± 1.71^{a}	24.87 ± 1.51^{b}	20.96 ± 1.50^{c}	15.23 ± 1.05^{d}	19.58 ± 1.29^{c}

*Within the same row, means bearing one common superscript were non-significantly (P≥0.05) different. CTRL = Control, SOD = Superoxide dismutase, GPX = Glutathione peroxidase, TAC = Total antioxidant capacity, MDA = Malondialdehyde.

References

1. Akintunde, A. O., Mustofa, I., Ndubuisi-Ogbonna, L. C., Oyekale, O. O. and Shobo, B. A. Exploring the Genetic Diversity: A Review of Germplasm in Nigerian Indigenous Goat Breeds. *Small Ruminant Research*, 107236 (2024).
2. Seifi-Jamadi, A., Kohram, H., Zareh-Shahne, A., Dehghanizadeh, P. and Ahmad, E. Effect of various concentrations of butylated hydroxyanisole and butylated hydroxytoluene on freezing capacity of Turkman stallion sperm. *Animal Reproduction Science*, **170**, 108-113 (2016).
3. Raeeszadeh, M. and Akbari, A. The effects of broccoli and caraway extracts on serum oxidative markers, testicular structure and function, and sperm quality before and after sperm cryopreservation. *Cryobiology*, **99**, 11-19 (2021).
4. Falchi, L., Pau, S., Pivato, I., Bogliolo, L. and Zedda, M. T. Resveratrol supplementation and cryopreservation of buck semen. *Cryobiology*, **95**, 60-67 (2020).
5. Heidari, M., Qasemi-Panahi, B., Moghaddam, G., Daghigh-Kia, H. and Masoudi, R. L-carnitine improves quality parameters and epigenetic patterns of buck's frozen-thawed semen. *Animal Reproduction Science*, **247**, 107092 (2022).
6. Alcay, S., Aktar, A., Koca, D., Kilic, M. A., Akkasoglu, M. and Sagirkaya, H. Positive effect of autologous platelet rich plasma on Saanen buck semen cryopreservation in non-breeding season. *Cryobiology*, **103**, 45-48 (2021).
7. Yousefian, I., Emamverdi, M., Karamzadeh-Dehaghani, A., Sabzian-Melei, R., Zhandi, M. and Zare-Shahneh, A. Attenuation of cryopreservation-induced oxidative stress by antioxidant: Impact of Coenzyme Q10 on the quality of post-thawed buck spermatozoa. *Cryobiology*, **81**, 88-93 (2018).
8. Appiah, M. O., Li, W., Zhao, J., Liu, H., Dong, Y., Xiang, J. and Lu, W. Quercetin supplemented casein-based extender improves the post-thaw quality of rooster semen. *Cryobiology*, **94**, 57-65 (2020).
9. Siari, S., Mehri, M. and Sharafi, M. Supplementation of Beltsville extender with quercetin improves the quality of frozen-thawed rooster semen. *British Poultry Science*, **63**(2), 252-260 (2022).
10. Khan, M. T., Khan, M. I. U. R., Ahmad, E., Yousaf, M. R. and Oneeb, M. Synergistic effect of extracellular adenosine triphosphate and quercetin on post-thaw quality and fertilization potential of Lohi ram sperm. *Cryobiology*, **113**, 104593 (2023).
11. Winn, E. and Whitaker, B. D. Quercetin supplementation to the thawing and incubation media of boar sperm improves post-thaw sperm characteristics and the in vitro production of pig embryos. *Reproductive Biology*, **20**(3), 315-320 (2020).
12. Bardestani, A., Ebrahimpour, S., Esmaeili, A. and Esmaeili, A. Quercetin attenuates neurotoxicity induced by iron oxide nanoparticles. *Journal of Nanobiotechnology*, **19**, 1-33 (2021).
13. Seifi-Jamadi, A., Ahmad, E., Ansari, M. and Kohram, H. Antioxidant effect of quercetin in an extender containing DMA or glycerol on freezing capacity of goat semen. *Cryobiology*, **75**, 15-20 (2017).
14. Tironi, S. M. T., Seixas, F. A. V., de Moraes, G. V., Stefanello, T. F., Akashi, K. T., Marques, N. F. S. and Martinez, A. C. Effects of treatment with quercetin on the quality of cryopreserved bovine semen. *Acta Scientiae Veterinariae*, **47**(1), (2019).
15. Ardeshirnia, R., Zandi, M. and Sanjabi, M. R. The effect of quercetin on fertility of frozen-thawed ram epididymal spermatozoa. *South African Journal of Animal Science*, **47**(2), 237-244 (2017).
16. Diniz, W., Modi, L., Chaudhari, N., Chaudhary, S., Pandor, M. and Kumar, D. Antioxidant effect of quercetin in tris egg yolk citrate extender on Surti buck semen preserved at refrigerated temperature. *International Journal of Livestock Research*, **11**(1), 87-92 (2021).
17. Naijian, H. R., Kohram, H., Shahneh, A. Z., Sharafi, M. and Bucak, M. N. Effects of different concentrations of BHT on microscopic and oxidative parameters of Mahabadi goat semen following the freeze–thaw process. *Cryobiology*, **66**(2), 151-155 (2013).
18. Lv, C., Larbi, A., Wu, G., Hong, Q. and Quan, G. Improving the quality of cryopreserved goat semen with a commercial bull extender supplemented with resveratrol. *Animal reproduction science*, **208**, 106127 (2019).
19. Salmani, H., Nabi, M. M., Vaseghi-Dodaran, H., Rahman, M. B., Mohammadi-Sangcheshmeh, A., Shakeri, M. and Zhandi, M. Effect of glutathione in soybean lecithin-based semen extender on goat semen quality after freeze-thawing. *Small ruminant research*, **112**(1-3), 123-127 (2013).
20. Gangwar, C., Kharche, S. D., Ranjan, R., Kumar, S., Goel, A. K., Jindal, S. K. and Agarwal, S. K. Effect of vitamin C supplementation on freezability of Barbari buck semen. *Small Ruminant Research*, **129**, 104-107 (2015).
21. Ahmad, M., Nasrullah, R. and Ahmad, N. Effect of cooling rate and equilibration time on pre-freeze and post-thaw survival of buck sperm. *Cryobiology*, **70**(3), 233-238 (2015).
22. Almadaly, E., Farrag, F., Shukry, M. and Murase, T. Plasma membrane integrity and morphology of frozen-thawed bull spermatozoa supplemented with desalted and lyophilized seminal plasma. *Global Veterinaria*, **13**, 753-766 (2014).
23. Kumar, P., Kumar, D., Sikka, P. and Singh, P. Sericin supplementation improves semen freezability of buffalo bulls by minimizing oxidative stress during cryopreservation. *Animal reproduction science*, **152**, 26-31 (2015).
24. Almadaly, E. A., Ibrahim, I. M., Salama, M. S., Ashour, M. A., Sahwan, F. M., El-Kon, I. I. and El-Domany, W. B. Effect of platelet-rich plasma (PRP) on post-thaw quality, kinematics and in vivo fertility of fertile and subfertile buffalo (Bubalus bubalis)

spermatozoa. *Veterinary Research Communications*, **47**(1), 61-72 (2023).

25. Mayasula, V. K., Arunachalam, A., Sellappan, S., Guvvala, P. R., Naidu, S. J., Dintaran, P. and Bhatta, R. Organic Zn and Cu supplementation imprints on seminal plasma mineral, biochemical/antioxidant activities and its relationship to spermatozoal characteristics in bucks. *Reproductive Biology*, **20**(2), 220-228 (2020).
26. Banday, M. N., Lone, F. A., Rasool, F., Rashid, M. and Shikari, A. Use of antioxidants reduce lipid peroxidation and improve quality of crossbred ram sperm during its cryopreservation. *Cryobiology*, **74**, 25-30 (2017).
27. Alcay, S., Gokce, E., Toker, M. B., Onder, N. T., Ustuner, B., Uzabacı, E. and Cavus, S. Freeze-dried egg yolk based extenders containing various antioxidants improve post-thawing quality and incubation resilience of goat spermatozoa. *Cryobiology*, **72**(3), 269-273 (2016).
28. Qamar, A. Y., Fang, X., Bang, S., Kim, M. J. and Cho, J. Effects of kinetin supplementation on the post-thaw motility, viability, and structural integrity of dog sperm. *Cryobiology*, **95**, 90-96 (2020).
29. Sharafi, M., Zhandi, M., Shahverdi, A. and Shakeri, M. Beneficial effects of nitric oxide induced mild oxidative stress on post-thawed bull semen quality. *International journal of fertility & sterility*, **9**(2), 230 (2015).
30. Ariyan, F., Farshad, A. and Rostamzadeh, J. Protective effects of Tribulus terrestris and Cinnamomum zeylanicum extracts and trehalose added to diluents on goat epididymal sperm freezability. *Cryobiology*, **98**, 172-180 (2021).
31. Xu, D., Hu, M. J., Wang, Y. Q. and Cui, Y. L. Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules*, **24**(6), 1123 (2019).
32. Azadi, L., Tavalaee, M., Deemeh, M. R., Arbabian, M. and Nasr-Esfahani, M. H. Effects of tempol and quercetin on human sperm function after cryopreservation. *CryoLetters*, **38**(1), 29-36 (2017).
33. Azimi, G., Farshad, A., Farzinpour, A., Rostamzadeh, J. and Sharafi, M. Evaluation of used Purslane extracts in Tris extenders on cryopreserved goat sperm. *Cryobiology*, **94**, 40-48 (2020).
34. Ahmed, H., Andrabi, S. M. H. and Jahan, S. Semen quality parameters as fertility predictors of water buffalo bull spermatozoa during low-breeding season. *Theriogenology*, **86**(6), 1516-1522 (2016).
35. Batool, I., Fayyaz, M. H., Hameed, A., Andrabi, S. M. H., Kausar, R., Shahzad, M. and Hussain, T. Quercetin in semen extender improves frozen-thawed spermatozoa quality and in-vivo fertility in crossbred Kamori goats. *Frontiers in Veterinary Science*, **11**, 1385642 (2024).
36. Amini, S., Masoumi, R., Rostami, B., Shahir, M. H., Taghilou, P. and Arslan, H. O. Effects of supplementation of Tris-egg yolk extender with royal jelly on chilled and frozen-thawed ram semen characteristics. *Cryobiology*, **88**, 75-80 (2019).
37. Tvrdá, E., Tušimová, E., Kováčik, A., Paál, D., Libová, Ľ. And Lukáč, N. Protective effects of quercetin on selected oxidative biomarkers in bovine spermatozoa subjected to ferrous ascorbate. *Reproduction in Domestic Animals*, **51**(4), 524-537 (2016).
38. Ren, F., Fang, Q., Feng, T., Li, Y., Wang, Y., Zhu, H. and Hu, J. Lycium barbarum and Laminaria japonica polysaccharides improve Cashmere goat sperm quality and fertility rate after cryopreservation. *Theriogenology*, **129**, 29-36 (2019).
39. Makarevich, A. V., Spalekova, E., Olexikova, L., Lukac, N., Kubovicova, E. and Hegedusova, Z. Functional characteristics of ram cooling-stored spermatozoa under the influence of epidermal growth factor. *General Physiology and Biophys*ics, **30**, S36-S43 (2011).
40. Kumar, A., Prasad, J. K., Kumar, N., Anand, M., Verma, S., Dhariya, R. and Gattani, A. Quercetin in semen extender curtails reactive oxygen and nitrogen species and improves functional attributes of cryopreserved buck semen. *Cryobiology*, **116**, 104931 (2024).
41. El-Khawagah, A. R., Kandiel, M. M. and Samir, H. Effect of quercetin supplementation in extender on sperm kinematics, extracellular enzymes release, and oxidative stress of egyptian buffalo bulls frozen–thawed semen. *Frontiers in Veterinary Science*, **7**, 604460 (2020).
42. Rakha, B. A., Qurrat-ul-Ain, Ansari, M. S., Akhter, S., Akhter, A., Awan, M. A. and Santiago-Moreno, J. Effect of quercetin on oxidative stress, mitochondrial activity, and quality of Indian red jungle fowl (Gallus gallus murghi) sperm. *Biopreservation and Biobanking*, **18**(4), 311-320 (2020).
43. Zheng, Y., Zhang, N., Liu, S., Li, Q. and Jiang, Z. Effects of water-soluble Laminaria japonica polysaccharide 3 (LJP-P3) on bull cryopreservation sperm. *Cryobiology*, **79**, 50-55 (2017).
44. Ahmed, H., Jahan, S., Salman, M. M. and Ullah, F. Stimulating effects of Quercetin (QUE) in tris citric acid extender on post thaw quality and in vivo fertility of buffalo (Bubalus bubalis) bull spermatozoa. *Theriogenology*, **134**, 18-23 (2019).
45. Bang, S., Tanga, B. M., Fang, X., Seong, G., Saadeldin, I. M., Qamar, A. Y. and Cho, J. Cryopreservation of pig semen using a quercetin-supplemented freezing extender. *Life*, **12**(8), 1155 (2022).
46. Gibb, Z., Butler, T. J., Morris, L. H. A., Maxwell, W. M. C. and Grupen, C. G. Quercetin improves the postthaw characteristics of cryopreserved sex-sorted and nonsorted stallion sperm. *Theriogenology*, **79**(6), 1001-1009 (2013).
47. Ahmed, H., Jahan, S., Ullah, H., Ullah, F. and Salman, M. M. The addition of resveratrol in tris citric acid extender ameliorates post-thaw quality parameters, antioxidant enzymes levels, and fertilizing capability of buffalo (Bubalus bubalis) bull spermatozoa. *Theriogenology*, **152**, 106-113 (2020).

إضافة الكوريستين فى مخفف صفار البيض تريس يحسن من جودة السائل المنوى المجمد لذكور الماعز الزرايب

عصام عبدالسلام المعدلي[1] ، محمد أحمدالبنداري[1] ، فريال محمد صهوان[2] ، أحمد محمد شهاب الدين[3] ، وائل بدير الدوماني[4] ، إسماعيل إسماعيل القن[1] وعادل عبدالفتاح رامون[1]

[1] قسم التوليد والتناسل والتلقيح الاصطناعي - كلية الطب البيطري - جامعة كفرالشيخ - كفرالشيخ - مصر.

[2] قسم الرعاية وتنمية الثروة الحيوانية - كلية الطب البيطرى - جامعة الأسكندرية - الأسكندرية - مصر.

[3] معهد بحوث الإنتاج الحيوانى - مركز البحوث الزراعية - وزارة الزراعة واستصلاح االأراضى - القاهرة - مصر

[4] قسم التوليد والتناسل والتلقيح الاصطناعي - كلية الطب البيطري - جامعة المنصورة - المنصورة - مصر.

الملخص

أجريت هذه الدراسة لمعرفة تأثير إضافة تركيزات مختلفة من مادة الكوريستين على جودة السائل المنوي بعد الذوبان في ذكور الماعز الزرايبي. تم تخفيف وتقسيم السائل المنوى المجمع من 7 من ذكور الماعز الزرايبي الى 5 أجزاء متساوية مزودة بخمسة تركيزات المجموعة الضابطة [10µM , 15µM, 25µM, 40µM] من مادة الكوريستين ثم تم التبريد والتجميد وبعد ذلك تم تقييمها من حيث الحركة التقدمية للحيوانات المنوية بعد الذوبان، والمعايير الحركية، وحيوية الحيوانات المنوية، ونسب سلامة الغشاء البلازمي، والأكروسوم. وأيضا تم تقييم نشاط كل من SOD وGPX وTAC بالإضافة إلى محتوى MDA. كشفت نتائجنا أن المخفف المزود بـ 25 µM من مادة الكوريستين أدى إلى حركة تقدمية وسرعات الخط المنحني والخط المستقيم والمسار المتوسط للحيوانات المنوية أعلى بكثير مقارنةً بالمجموعة الضابطة كما أدت كل من مجموعات 25 µM و 40 µM من مادة الكورستين إلى زيادة كبيرة في حيوية الحيوانات المنوية وسلامة الغشاء البلازمي والأكروسوم مقارنةً بالمجموعة الضابطة. كما تمت زيادة مستويات كل من انزيم SOD وGPX بشكل ملحوظ في عينات السائل المنوي المزودة بـ 25 µM بينما كان TAC أكبر بكثير في 15 µM و25 µM و40 µM من مادة الكوريستين. على العكس من ذلك، وجد أن مستوى MDA أقل بشكل ملحوظ في المجموعة المزودة 25 µM . الخلاصة ان إضافة 25 µM من مادة الكوريستين الى المخفف يحسن جودة السائل المنوي لذكور الماعز بعد إذابته عن طريق تقليل كل من إنتاج ROS وأكسدة الدهون كما ثبت من خلال تحسن قيم خصائص الحيوانات المنوية ونشاط مضادات الأكسدة الأنزيمية.

الكلمات الدالة: الحيوان المنوى للماعز الزرايبى ، الكوريستين ، الحفظ بالتجميد ، جهاز تحليل السائل المنوى المحوسب، أكسدة الدهون.