The capability of coenzyme Q10 to enhance heat tolerance in male rabbits: evidence from improved semen quality factor (SQF), testicular oxidative defense, and expression of testicular melatonin receptor MT1

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ABSTRACT

Heat stress (HS) has a great influence on the etiology of male infertility. Coenzyme Q10 (CoQ10), known to have powerful antioxidant effects, has been reported to have such actions that are effective to treat infertility caused by HS. The aim of the present study was to investigate the antioxidative effect of CoQ10 on sperm quality, testicular antioxidant activities, and male fertility under HS. For this purpose, 18 mature male rabbits (aged 22 wk) of the Sinai Gabali breed were equally divided into 3 groups and placed at temperature-humidity index of 29 for 8 wk at a farm. The supplementation of CoQ10 at 0, 10, and 20 mg/kg of body weight was done in the first, second, and third groups, respectively. The results showed that the supplementation of CoQ10 had significant ($P < 0.05$) effect on semen quality factor (SQF) and testicular antioxidant activities by the supplementation of CoQ10. Moreover, a significant improvement in the concentration of testosterone, integrity of testicular DNA, and the expression of melatonin receptors was also observed, which were consistent with a significant improvement in buck fertility. The prolificacy was significantly increased ($P < 0.05$) in females when inseminated from bucks that were treated with CoQ10. Our results suggest that CoQ10 tends to decrease oxidative stress by enhancing testicular antioxidant activities, which are considered the most important factors for a buck's fertility. Hence, CoQ10 could be a suitable feed supplement to increase fertility, through enhancing the semen quality, in male rabbits and reducing the harmful effects of HS.

1. Introduction

Heat stress (HS) has been known as an important growth limiting factor that reduces the fertility and growth in domestic animals [1], especially rabbits which are more susceptible to high environmental temperature. Rabbits are less efficient to combat high stress because of fewer sweat glands [2]. Heat stress adversely affects libido, fertility, and embryonic survival in animals, by increasing the accumulation of reactive oxygen species (ROS) and lipid peroxidation (LP) [3,4] that negatively affect mitochondrial function and oxidative phosphorylation [5]. Spermatozoa are especially susceptible to oxidative stress (OS) because of the high

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levels of unsaturated fatty acids within the plasma membrane and inactive scavenging enzymes in their membranes. It makes these organisms readily sensitive to LP in the presence of elevated levels of ROS in the seminal fluid [6]. There exists a clear correlation between HS conditions and ROS levels in the seminal plasma [7], as well as between ROS, LP, and the perturbations of spermatogenesis [8]. Indeed, spermatozoa have a low amount of cellular ROS defense systems (such as catalase [CAT], reduced glutathione [GSH], glutathione peroxidase (GSH-PX), and vitamin E) as compared with seminal plasma, which is well endowed with antioxidant buffering capacity [3]. Moreover, there is a clear evidence that ROS are hostile to integrity of the spermatozoa membrane, altering its permeability, resulting in impaired motility and function of acrosome, as well as increased DNA damage [4,6]. Likewise, the effects of HS on male fertility or on sperm quality parameters have been studied in detail [5,9]. Testicular antioxidant capacity can be increased by nutrient supplementation from natural antioxidants such as coenzyme Q10 (CoQ10), L-carnitine, arginine, ascorbic acid, and α-tocopherol [10,11].

Melatonin is a multifunctional molecule that seems to be protected against free-radical damage, and in mammals, it has an extensive range of functions including the regulation of circadian rhythms, immunomodulation, cardiovascular regulation, testicular protection, and seasonal reproduction. Melatonin can exert many of these functions because of its antioxidant properties [12]. Therefore, it can be used as a testicular biological marker against OS.

Coenzyme Q10 is a fat-soluble vitamin that is present endogenously in the inner mitochondrial membrane of mammals and plants. It is basically a lipid-soluble component and contains 10 isoprene units in its side chain. It is also known as a regulating cofactor of mitochondrial electron transport in the respiratory chain for ATP production [13]. In addition, CoQ10 present in the sperm mitochondria is involved in energy metabolism for motility and acts as an antioxidant to prevent LP of sperm cell membranes [14,15]. Furthermore, it is essential for physiological mechanisms, including mitochondrial oxidative phosphorylation, membrane integrity, and scavenging of ROS to suppress the lipid peroxidation [16]. Moreover, it is necessary for the immune response and ATP biosynthesis during electron transport into the mitochondrial matrix [17,18]. Therefore, there is growing interest in its use as a natural antioxidative agent, which has facilitated the research of infertile gametes and led to the development of novel biotherapeutics for treating associated disorders. Melatonin receptor MT1 is also a multifunctional molecule used for the protection against cellular damage by free radicals, including the regulation of circadian rhythms, immunomodulation, cancer inhibition, gonadal protection, cardiovascular regulation, and helps regulate seasonal reproduction [19,20]. It can perform many of these functions because of its antioxidative actions, including within the reproductive system [12].

The aim of the present study is to assess the effects of oral ingestion of CoQ10 during long exposure (8 wk) to in vivo summer circadian HS cycles on semen quality factor (SQF), testicular antioxidant activity, and MT1 expression in relation to the fertility and proficiency of rabbit bucks.

2. Materials and methods

2.1. Ethics statement

The study was conducted at Rabbitry Farm of the Animal Production Department, Faculty of Agriculture at Benha University and the Laboratory of National Organization for Drug Control, Egypt, and in collaboration with the College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, PR. China. All experimental procedures were reviewed and approved by Animal Production Department, Faculty of Agriculture, Benha University, Egypt, with approval number BUAPD-18127. Animals were handled in accordance with the guidelines described by the Animal Care Committee of Qalyubiya Province, Egypt.

2.2. Experimental design, management, and ambient temperature-humidity index

Eighteen mature male rabbits of the Sinai Gabali (SG) breed and of the same age (22 wk) and weight (2.5 ± 0.20 kg) were selected. The bucks were divided randomly into 3 groups (n = 6). The control group (CoQ10–0) was fed on a standard diet (2,700 kcal/kg and 21% protein) without any supplementation, whereas the second (CoQ10–10) and third (CoQ10–20) groups were fed the same diet with daily oral dosages of 10 and 20 mg of CoQ10/kg body weight, respectively, for 8 wk (Fig. 1A). All bucks were individually housed in cages ($60 \times 40 \times 35$ cm) and inside an open-system-type farm (uncontrolled system) and all management processes were fixed for all groups of animals. To verify the exposure of bucks to natural thermal stress, the temperature-humidity index (THI) was calculated inside the farm based on a natural climate of thermal stress in Qalyubia Province, Egypt, according to Dikmen, Hansen [21]. The ambient temperature and humidity were 37°C and 60% before the start of the study, respectively. The value of THI inside the farm was measured as more than 29, higher than the maximum livestock safety index value (optimum THI value of 23).

2.3. Semen collection and evaluation

Bucks were trained for the semen collection 2 wk before the start of experiment, and then samples were individually collected from each buck twice a week for 8 wk. The reaction time (in seconds) was the interval from the introduction of the "teaser" doe into the male's cage for sniffing, grooming, mounting, and ejaculation in artificial vagina and was considered an indication of libido. The samples were collected in the early morning by artificial vagina method [22]. The healthy ejaculates (we discarded the urinary or bloody ejaculates) were evaluated after removing the clot of gel. Ejaculate volume was assessed using a graduated conical tube and the pH value of semen was visually measured using a Whatman pH Indicator Papers. In addition, the sperm concentration was measured by a spectrophotometer. The percentages of sperm variability and normality were estimated using aniline blue stain [23] and graded for individual motility and percentage of mass motility [24]. Finally, seminal quality indicators
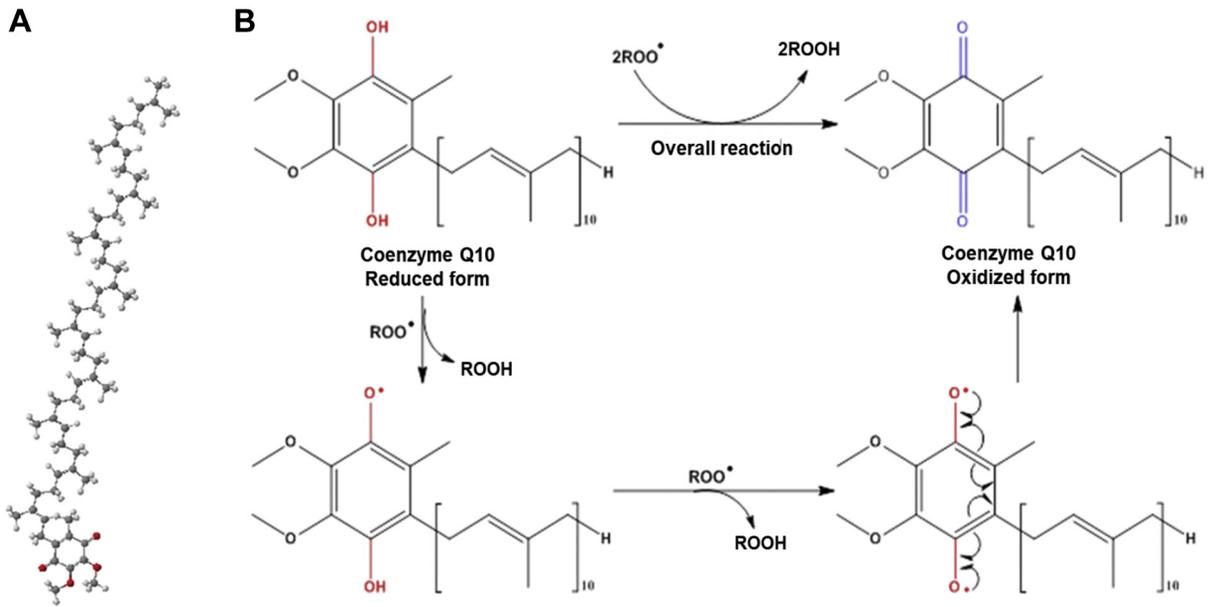
Fig. 1. (A) Three-dimensional structures of coenzyme Q10. (B) The theoretical mechanism for coenzyme Q10 scavenging reactive oxygen species (ROS) during the perturbations of a hostile environment to prevent lipid peroxidation in testicular tissue.

were assessed in terms of sperm concentration, volume, and livability, by SQF, as reported by Liu et al [25].

2.4. Measurements of testosterone and testicular antioxidant activities

Blood samples (n = 12) were collected from the marginal ear vein at the fourth and eighth week, in non-heparinized tubes (10 mL). The testosterone content of serum was detected by ELISA using commercial kits (Fortress Diagnostics Ltd, UK and North Ireland) according to the manufacturer's instructions. Malondialdehyde (MDA) concentration was measured using tetraethoxypropane [26]. In addition, nitric oxide (NO), oxidized glutathione (GSSG), and GSH were detected by HPLC (Agilent HP 1200 series HPLC apparatus). All standards for HPLC were obtained from Sigma Chemical Co, according to Jayatilleke and Shaw [27].

2.5. Artificial insemination and fertility calculation

To verify the effect of CoQ10 supplementation on male fertility, 18 SG doe of similar age (24 wk) and weight (2.6 ± 0.15 kg) were equally divided into 3 groups (n = 6). The first group was artificially inseminated using semen from the CoQ10–0 group. The second and third groups were inseminated by CoQ10–10 mg/kg of body weight and CoQ10–20 mg/kg of body weight groups, respectively. The receptive doe (identified by red vulvar lips) was inseminated using a curved glass pipette (0.5 cm diameter), consisting of 0.5 mL of fresh and pure semen according to Daniel and Renard [28]. The reproductive variables (fertility rate, number of services per conception, percentage of conception rate) for each buck were recorded after pregnancy diagnosis on the

14th day of insemination. Litter size at birth (LSB) for each doe was recorded and prolificacy was defined as the total number of bunnies born per each of 3 parities. According to the duration of spermatogenesis in rabbits (52 d), the fertility rate and its dependencies from LSB of the third parity was included in the period of experimental effect, although it lasted longer than 8 wk [29].

2.6. Detection of testicular DNA damage using comet assay technology

To detect testicular DNA damage, the comet assay technique was applied to reveal DNA single-strand breaks [30]. Briefly, the testicles were collected immediately after slaughtering the animals and preserved at −80°C until the final experiment was carried out. The testicular cells were embedded in agarose gel on microscopic slides, lysed by detergents and concentrated salts, and then electrophoresed for a short period under alkaline conditions. During electrophoresis, broken DNA fragments migrated toward the anode before undamaged DNA. After staining with ethidium bromide, the DNA resembled a comet-like appearance with a bright fuorescent head (nuclear intact material) and a tail (containing damaged DNA fragments) whose length and intensity were determined by the level of DNA strand breakage produced within the cells. The DNA migration patterns of 100 cells (50 cells from each of 2 replicate slides) for each sample were observed using an Olympus Fluorescence Microscope (BX51) and images were captured by a CCD camera (Exwave HAD, Sony, Tokyo, Japan). The extent of DNA damage tail length (μm) and tail moment in the cells were estimated using CASP software (Comet Assay Software).

2.7. Immunohistochemical localization and analysis of testicular melatonin receptor MT1

MT1 immunohistochemistry analyses on a total of 9 bucks (3 in each group) was performed at the end of the experiment according to previously reported studies [31,32]. In brief, the tissue sections were incubated overnight at 4°C with primary rabbit polyclonal MT1 antibody (Cat: 17172-1-AP) according to the manufacturer instructions (Proteintech, Wuhan Sanying Biological Technology Co, Ltd, Wuhan, Hubei, China). After washing with phosphate-buffered saline (PBS), the tissue sections were incubated at room temperature for 2 h with secondary goat anti-rabbit antibody (Cat: SA00001–2, http://www.ptgcn.com) for MT1. After washing with PBS, the tissue sections were immersed in diaminobenzidine solution to obtain a brownish yellow color. Hematoxylin was used as a counter stain for staining nuclei. Then, the tissue sections were cleaned in xylene and mounted with a mixture of distyrene, a plasticizer, and xylene. A Zeiss light microscope was used for taking photos. The serial sections were examined under a light microscope (BH-2, Olympus, Japan) with a digital camera (DP72; Olympus). The distribution and expression level of different proteins were measured in high-power fields which were randomly selected. All of the images were taken using the same microscope and camera. Image-Pro Plus 6.0 software (Media Cybernetics) was used to calculate the mean density for positive staining. Background lighting of images was calibrated to measure the integrated optical density (IOD) per area, which was used to determine the concentration of antigen of interest. The results of IOD were expressed as a percentage of staining intensity and the area of staining.

2.8. Statistical analysis

The data were analyzed by using the GLM function of R software version 3.2.2, R Core [33]. The results were expressed as least square means (LSM ± SEM), and Duncan's multiple range test was used to compare the differences between means. All data were evaluated for conformation to a normal distribution with the Kolmogorov-Smirnov test, and all percentage data were also normalized through arcsine transformation. Two statistical models (repeated measurements and one-way ANOVA) were used to estimate the significance of fixed factors according to the following models; $Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + e_{ijk}$, repeated measurements for parameters of seminal variables, and $Y_{ij} = \mu + T_i + e_{ij}$ ANOVA for other reproductive parameters of the data set, where Y_{ijk} and Y_{ij} are observations; μ is the overall mean; T_i is the effect of ith treatment, i = (CoQ10–0, CoQ10–10, and CoQ10–20); W_j is the effect of jth time by weeks, j = 1 to 8; $(TW)_{ij}$ is the effect of the interaction between treatment and time, and e_{ij} and e_{ijk} are the experimental error assumed to be randomly distributed (0, σ^2_e). The individual rabbit was considered as the experimental unit and included for one fixed effect of the male reproduction activities in the statistical model. All figures were generated by GraphPad Prism Software (version 7.01). In addition, PC program CS ChemBioDraw Ultra 14.0 (CambridgeSoft, Cambridge, MA) was used to construct the mechanism of CoQ10/ROS interaction.

3. Results

3.1. Effects of coenzyme Q10 on reaction time, testosterone level, and semen quality factor

The characteristics of semen are presented in Table 1 according to treatment groups (CoQ10–0, CoQ10–10, and CoQ10–20). There were statistically significant differences among the groups with respect to ejaculate volume, sperm concentration, total motility, progressive motility, and normal morphology ($P < 0.001$), as well as a tendency for total motility ($P < 0.075$). No significant difference was observed in the mean seminal pH of the 2 study groups. Significant differences were found among the groups in the calculation of SQF and male fertility ($P < 0.01$). It was found that the intake of CoQ10 (CoQ10–10 and CoQ10–20) was better for SQF and male fertility than CoQ10–0, as shown in Table 1.

3.2. Effects of coenzyme Q10 on testicular antioxidant activities and the possibility of a theoretical mechanism

The testicular antioxidant parameters of the bucks are presented in Table 2. Testicular GSH activity was significantly increased ($P < 0.05$) in CoQ10–20 group compared

Table 1
Effect of coenzyme Q10 on semen parameters of male Sinai Gabali rabbits.

Semen parameters	Treatment (coenzyme Q10 supplementation) (LSM ±SEM)			SEM	P-value			
	CoQ10–0	CoQ10–10	CoQ10–20		Treatment (Trt)	Time (tim.)	Trt. [d]Tim.	Animal [d]Trt.
Reaction time (s)	14.37[b]	14.81[ab]	15.27[a]	0.25	0.073	0.188	0.056	0.009
Ph grade	7.43	7.58	7.45	0.07	0.327	0.573	0.968	0.794
Ejaculate volume (mL)	0.51	0.43	0.51	0.03	0.193	0.367	0.001	0.275
Concentration ([d]10^6/mL)	378.43[c]	395.06[b]	415.31[a]	3.19	0.001	0.001	0.001	0.001
Mass motility grade	3.49[b]	3.94[a]	3.97[a]	0.06	0.001	0.183	0.072	0.052
Total motility (%)	69.25[b]	71.93[a]	72.78[a]	0.99	0.001	0.027	0.031	0.202
Viability (%)	74.50[b]	79.06[a]	80.31[a]	0.80	0.001	0.053	0.001	0.042
Normality (%)	73.25[b]	80.93[a]	80.57[a]	1.33	0.001	0.265	0.026	0.001
Semen quality factor (SQF)	144.23[b]	160.08[a]	170.46[a]	11.09	0.001	0.035	0.001	0.001

CoQ10–0 is no addition of CoQ10; CoQ10–10 and CoQ10–10 are addition of CoQ10 at 10 and 20 mg/kg body weight, respectively.
Male/group n = 12; ejaculate/male n = 8.
[a,b,c]LSM within a row with different superscripts differ significantly among groups ($P < 0.05$).
[d] Interaction.

Table 2
Effect of coenzyme Q10 on serum testosterone content and testicular antioxidant parameters of male Sinai Gabali rabbits.

Testicular antioxidant parameters	Coenzyme Q10 supplementation (LSM ±SEM)			SEM	F-value	P-value
	CoQ10–0	CoQ10–10	CoQ10–20			
Testosterone (ng/mL)	2.75[b]	3.09[a]	3.13[a]	0.048	18.03	0.001
GSH (µmol/g)	20.49[b]	22.77[b]	26.88[a]	1.56	4.27	0.020
MDA (µmol/g)	28.45[a]	25.34[a]	21.54[b]	1.11	9.69	0.005
GSSG (µmol/g)	0.357	0.404	0.420	0.028	0.98	0.385
NO (µmol/g)	0.380[b]	0.450[b]	0.563[a]	0.033	7.59	0.001

Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; NO, nitric oxide.
CoQ10–0 is no addition of CoQ10; CoQ10–10 and CoQ10–10 are addition of CoQ10 with 10 and 20 mg/kg body weight, respectively.
Samples/group n = 12.
[a,b]LSM means within a column with different superscripts differ significantly among groups ($P < 0.05$).

with CoQ10–10 and CoQ10–0 groups. Similarly, no activity in CoQ10–20 group was significantly higher ($P < 0.001$) than those of both the CoQ10–10 and CoQ10–0 groups. Yet, no difference could be demonstrated among the groups with respect to testicular GSSG activity that did not differ significantly among the study groups. However, the testicular MDA concentration in the CoQ10–20 group was significantly lower ($P < 0.001$) than those in both the CoQ10–10 and CoQ10–0 groups, as shown in Table 2. In addition, a mechanism for the possible interaction between CoQ10 and ROS may include 3 steps: First, one molecule of ROS attacks the hydroxyl proton in the reduced form of CoQ10 which results in the formation of oxygen radical; this step is repeated by another molecule of ROS to generate a diradical. Finally, the rearrangement of such a diradical restores the oxidized form of CoQ10. Hence, each molecule of CoQ10 consumes 2 molecules of ROS as shown in Figure 1B.

3.3. Effects of coenzyme Q10 on buck fertility, insemination variables, and doe prolificacy

The effect of CoQ10 on male fertility, services per conception, conception rate or pregnancy percentage, and average LSB for 2 parities of doe prolificacy have been summarized in Table 3. As evident from the results, the addition of CoQ10 significantly improved ($P < 0.05$) buck fertility compared with the control group; however, there were no significant differences for services per conception and conception rate among groups in doe insemination (Table 3). The doe prolificacy showed significant differences ($P < 0.05$) among groups in LSB. The LSB significantly increased the 3rd parity of prolificacy in CoQ10–10 and CoQ10–20 compared with CoQ10–0 as show in Table 3.

3.4. Effects of coenzyme Q10 on testicular localization of melatonin receptor MT1

Immunostaining observation parameters of testes from bucks are presented in Figure 2. It was observed that immuno-positive signals of MT1 were increased in the seminiferous tubules in CoQ10–10 and CoQ10–20 groups and almost all seminiferous tubules were undergoing complete spermatogenesis compared with CoQ10-0. MT1 was strongly concentrated in the spermatogonia, spermatozoa, and Sertoli cells in CoQ10–10 and CoQ10–20 bucks compared with CoQ10–0 animals. Generally, immuno-positivity for MT1 appeared as dark brown spots in the intracytoplasm of spermatocytes inside the seminiferous tubules, while the signal in Leydig cells appeared with less intensity. The immuno-positive signals for MT1 were highest in CoQ10–20 and CoQ10–10 bucks (Fig. 2A). These changes were significantly attenuated in the testicular tissues of CoQ10–10 and CoQ10–20 bucks compared with CoQ10–0 group. Significant differences ($P < 0.05$) in the IOD of both CoQ10–10 and CoQ10–20 bucks were also observed compared with the CoQ10–0 group (Fig. 2B). The highest average of IOD for MT1 was recorded for CoQ10–20 bucks as shown in Figure 2B.

3.5. Effects of coenzyme Q10 on testicular histology and morphometric traits

The histological observations of testes in the studied groups are presented in Figure 2C. Rabbits treated with CoQ10 for 8 wk showed distinct histological differences when compared with the control group. Testicular sections from CoQ10–20 and CoQ10–10 groups showed normal seminiferous structure and their compositions of

Table 3
Effects of coenzyme Q10 on buck fertility, insemination variables, and doe prolificacy of Sinai Gabali rabbits.

Reproductive parameters	Coenzyme Q10 supplementation (LSM ±SEM)			SEM	F-value	P-value
	CoQ10–0	CoQ10–10	CoQ10–20			
Male fertility (%)	61.11[b]	76.22[a]	83.33[a]	0.07	5.94	0.04
Services per conception	2.33[a]	1.83[b]	1.50[c]	0.36	5.94	0.04
Conception rate (%)	30.55[c]	36.11[b]	41.66[a]	0.03	6.36	0.02
Litter size at birth	5.12[c]	5.62[b]	6.71[a]	0.05	8.52	0.01

CoQ10–0 is no addition of CoQ10; CoQ10–10 and CoQ10–10 are addition of CoQ10 at 10 and 20 mg/kg body weight, respectively.
Male/group n = 12; female/male n = 12; replicate of AI n = 3.
[a,b,c]LSM within a row with different superscripts differ significantly among groups ($P < 0.05$).
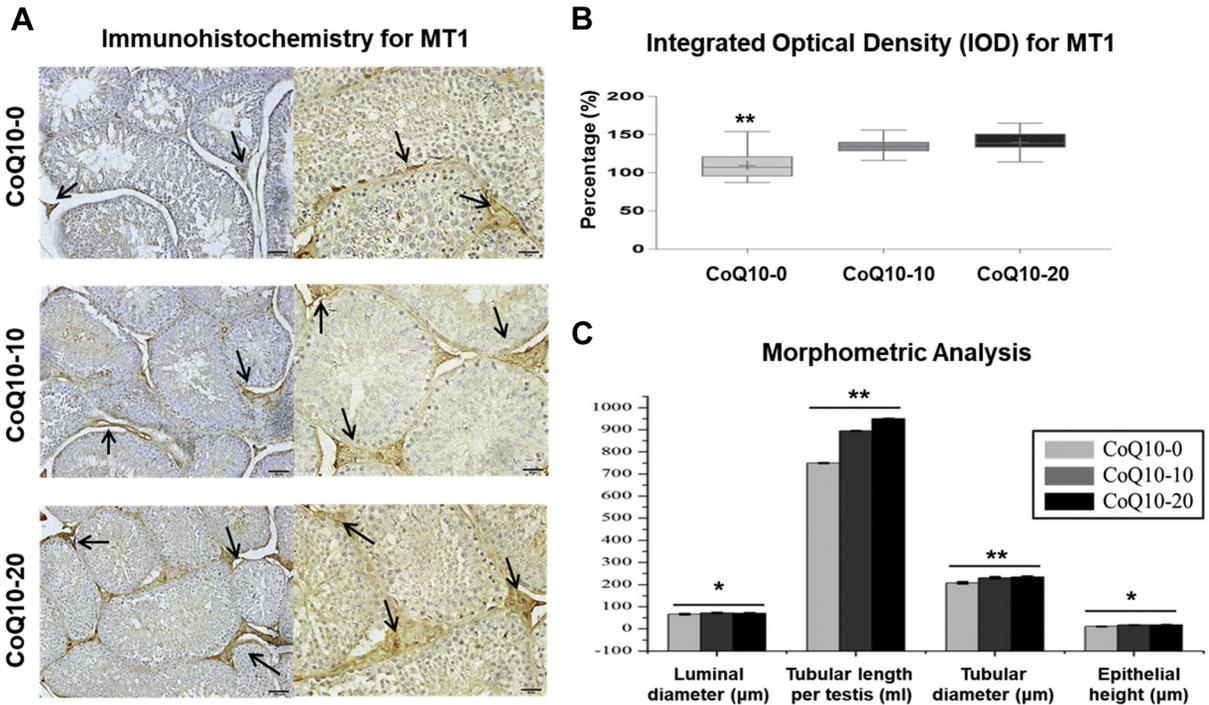
Fig. 2. Immunohistochemistry of melatonin receptors in testicular sections of Sinai Gabali rabbits. (A) Shows melatonin receptors 1 (MT1), the MT1-immunopositive signals shows higher expression in CoQ10–10 and CoQ10–20 bucks compared with CoQ10–0 bucks. (B) Shows the integrated optical density (IOD) percentage for MT1-immunopositive signals. (C) Histological and morphometric measures of testicular groups. ("*" and "**" means significant at $P < 0.05$ and $P < 0.01$, respectively; Sections/groups n = 6; scale bar = 100 μm).

spermatogonia, spermatocytes, spermatids, and spermatozoa and Sertoli cells. Our results showed a increase in the diameter of the seminiferous tubules, enlargement of their lumens, and congestion of the blood vessels. Some of spermatogonia and Sertoli cells were degenerated in the testicular sections of CoQ10–0 group compared with the treated groups. Rabbits treated with CoQ10–20 showed improvement in the seminiferous diameter and in germ cell height as compared with the CoQ10–0 and CoQ10–10 groups. Epithelial heights in testicular sections of CoQ10–20 and CoQ10–10 groups were significantly higher ($P < 0.001$) than those in CoQ10–0 group. On the contrary, the tubular diameter and tubular length per testis were significantly increased ($P < 0.01$) in CoQ10–20 and CoQ10–10 groups.

3.6. Effects of coenzyme Q10 on spermatozoa integrity findings by comet assay

Spermatozoa integrity parameters, as assessed by comet assay in the study groups, are presented in Figure 3. A significant increase in DNA fragmentation was evident as spots indicating damaged and strongly damaged DNA appeared in samples from the CoQ10–0 group (as detected in Fig. 3A). Both CoQ10–10 and CoQ10–20 groups showed significantly increased amounts of intact DNA in heads compared with the number of damaged and strongly damaged spots of spermatozoa. Yet, no difference was detected in the head diameter of spermatozoa among the

groups. On the other hand, head and tail abnormalities (without head, DNA in tail, amorphous, and tail moment shape) were significantly ($P < 0.001$) higher in the CoQ10–0 group as compared with CoQ10–20 and CoQ10–10 groups. Treating rabbits with CoQ10 caused a significant decrease in sperm head abnormalities as shown in Figure 3B.

4. Discussion

In the present study, it was found that the addition of 10 and 20 mg/kg/d of CoQ10 for 8 wk helped to prevent OS by enhancing heat tolerance, which induced improvements to male fertility. This beneficial effect enhanced blood metabolites molecules associated with heat tolerance and activated the synthesis of testosterone under HS conditions. In addition, CoQ10 effectively decreased MDA concentration and raised major antioxidant enzyme activity, which resulted in a decreased accumulation of LP and ROS, mainly induced by HS. These results suggest that the protective action of CoQ10 was due to the supportive heat tolerance and antioxidant effect, hence resulting in an improved SQF and male fertility.

HS has harmful effects on male fertility leading to temporary or permanent sterility. It plays an important role in physiological disorders, especially in reproductive failure and various gonadal diseases [34]. In addition, previous studies have shown that HS evokes negative effects by inducing LP, generating ROS, DNA degradation,
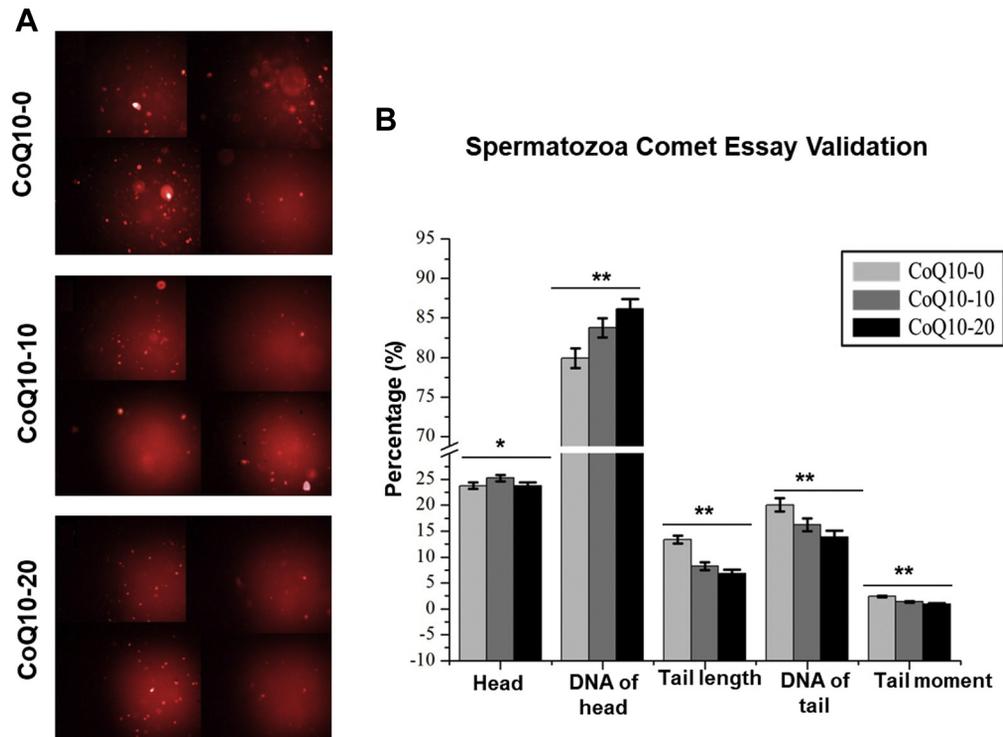
Fig. 3. (A) Micrographs of testis sections using the comet assay (single-cell gel electrophoresis) to detect DNA degradation. High levels of degradation and migration from cells was noted for CoQ10–0 bucks but was less in CoQ10–10 and CoQ10–20 bucks. (B) Spermatozoa integrity in groups enrolled in this study. ("*" and "**" means significant at $P < 0.05$ and $P < 0.01$, respectively; sections/groups n = 6; scale bar = 100 μm).

mitochondrial dysfunction, cell membrane damage, and inactivating transport enzymes [7]. Some reproduction studies have investigated the role of natural antioxidative compounds, which have facilitated the research on infertile gametes and the development of novel biotherapeutics, including CoQ10, carnitine, arginine, ascorbic acid, and α-tocopherol [10,35]. These antioxidant treatments have been shown to have protective effects in testicular tissue against OS and to improve reproduction in HS conditions [7,9].

The integrity of spermatogenesis was used as an indicator of semen quality. It is the downstream target for improving spermatogenesis to regulate the division of spermatogonia and spermatocyte maturity, as well as the activation of antioxidants in the face of HS. The effect of CoQ10 on sperm quality involves an essential cofactor in mitochondrial oxidative phosphorylation to produce ATP spermatozoa and their motility [15,36,37]. In addition, CoQ10 acts as a mobile electron carrier, transferring electrons to NADH coenzyme matrices, enhancing the resistance of spermatozoa to stress disorders [13,14]. Recently, CoQ10 appeared to be a possible supplement to counteract several metabolic disturbances associated with semen quality and male fertility in vivo as well as in in vitro [38]. In addition, the oral supplementation of CoQ10 improved SQF, decreased testicular peroxides, and improved the recycling of long-chain fatty acid into the mitochondrial matrix for the production of ATP [37,38].

The antioxidant capacity of seminal plasma is affected by many factors, including climate, nutrition, age, and infection [3]. In the present study, CoQ10 enhanced total seminal plasma antioxidants, which act as a protective system against ROS, perhaps exerting some beneficial effects on OS biomarkers in serum and also in the seminal plasma of infertile males [38]. CoQ10 decreased the testicular MDA concentration, resulting from testicular oxidative defense against LP. Indeed, testicular MDA is a biomarker and a direct indicator of LP activity and the mitochondrial integrity of spermatozoa. Based on the normality of spermatozoa, as determined by the integrity of the mitochondrial sheath, it was inferred that integrity of phospholipids were one of its major constituents. Thus, if fatty acids in these phospholipids are oxidized into the LP matrix by free radicals, the motility of spermatozoa will be impaired because of oxidative damage [39]. In addition, the testicular MDA content is a direct indicator of the normality of spermatozoa and its membrane integrity, as the composition of phospholipids is one of the major constituents of the membrane. Consequently, the fatty acids are subject to HS and could increase ROS levels mostly by producing free radicals through the NADPH oxidase pathway [6,40]. The present study showed that the antioxidant capacity of seminal fluid was improved by increasing GSH, GSSG, and NO activities. The testicular tissue of CoQ10 bucks showed more activity of these antioxidant enzymes than the control group. Our findings are in agreement with the studies of Atig et al [41], Sharafi et al [42], and Nadjarzadeh et al [39], who reported that addition of CoQ10 in the diet may represent a novel

biotherapeutic approach for improving male fertility during HS.

For the male fertility and doe prolificacy traits, the results have confirmed the hypothesis that a certain level of HS has a detrimental effect on sperm quality, male fertility, and doe prolificacy, but the supplementation of CoQ10 has beneficial attributes and improve these traits. Similarly, Piles et al [43] found a harmful effect of THI on both sperm quality and male fertility that resulted in decreased doe prolificacy traits. This effect is eliminated with supplementation of CoQ10 [38,44,45]. This indicates that the physiological mechanisms leading to heat tolerance and sperm survival in the oviduct after insemination are not entirely similar among the groups.

The results also showed that DNA damage was significantly decreased in CoQ10 groups compared with the control group. Accordingly, a significant increase in DNA damage was detected in testicular tissue exposed to HS of the control group compared with the other groups. This is in agreement with a previous study, which reported decreased DNA damage after the supplementation of CoQ10 against OS [46]. Hence, the significant correlation between the findings from the comet assay and the testicular antioxidant capacity (concentrations of MDA and activities of GSH and NO) suggests that DNA damage is due to OS, which is rescued by CoQ10 activity. These results are consistent with Carneiro et al [36] and Crane [47], who detected significantly lowered plasma antioxidant status under HS when compared with CoQ10 administration.

As shown in the present work, the administration of CoQ10 caused an increase in immuno-positive signal for MT1. This protein scavenges free radicals and stimulates cellular antioxidant defenses by increasing the expression of major antioxidant enzymes [48]. In addition, MT1 expression leads to the maintenance of high testicular contents of CAT, GSH, and GSH-PX, and reduces MDA by preventing LP [49]. Comet assay results showed that exposure to CoQ10 prevented DNA damage because of the activity of MT1, likely by increasing a cellular antioxidant defense. The higher testes integrity in the comet assay can be explained by the fact that CoQ10 mediated the synthesis and expression of MT1 in testes [50]. In addition, the potential mediation by MT1 not only alleviates DNA damage but also recovers the impaired mitochondrial function of spermatozoa through cellular antioxidant defenses [48]. Interaction between MT1 and G-proteins contributes to the synthesis of testosterone by regulating cAMP signal transduction cascades and also affects the stimulators of gonadotropin-releasing hormone that regulates the secretion of follicle stimulating hormone and luteinizing hormone [51]. In addition, MT1 regulates Leydig cell secretion of androgen through a melatonin membrane receptor [52].

In conclusion, the supplementation of 10 or 20 mg/kg/d of CoQ10 for 8 wk significantly helped to alleviate the increase of ROS accumulation and lipid peroxidation that activates the major testicular antioxidant enzymes (GSH and OX) and decreased the MDA concentration in terms of the responses of cellular antioxidant defense against HS. Moreover, addition of CoQ10 improved SQF, male fertility, and testicular MT1 expression as well as alleviated spermatozoa DNA damage and histological changes in the testes. Therefore, on the basis of the present results and the aforementioned reports, it is likely that CoQ10 can be used as a biotherapy component for the improvement of semen quality and male fertility in the global climate of HS.

CRediT authorship contribution statement

A.I. El-Sayed: Conceptualization, Project administration, Funding acquisition, Resources, Visualization, Supervision, Writing - original draft, Writing - review & editing. **O. Ahmed-Farid:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Resources. **A.A. Radwan:** Conceptualization, Project administration, Visualization, Supervision, Funding acquisition. **E.H. Halawa:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Resources. **A.A. Elokil:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.

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