

EFFECTS OF COENZYME Q10 SUPPLEMENTATION IN A FREEZING EXTENDER ON THE QUALITY OF CRYOPRESERVED AC CHICKEN SPERM

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Abstract – Cryopreservation is an effective technique for preserving poultry genetic resources; however, oxidative stress during freezing and thawing severely impairs sperm quality. This study evaluated the effects of different concentrations of coenzyme Q10 supplemented in a Beltsville freezing extender on the post-thaw quality of Ac chicken sperm. Semen collected from ten healthy Ac roosters was pooled and cryopreserved in extenders containing 0, 5, 10, 20, 40, or 80 µg/mL coenzyme Q10. The experiment was conducted with four independent replicates, each consisting of a pooled semen sample collected from 10 roosters at Tra Vinh University, Vietnam, from January to May 2026. After thawing, sperm quality was assessed by computer-assisted sperm analysis for motility parameters, Eosin–Nigrosin staining for viability, the hypo-osmotic swelling test for plasma membrane integrity, and the thiobarbituric acid assay for malondialdehyde concentration. Coenzyme Q10 supplementation significantly improved all evaluated sperm quality parameters in a non-linear dose response peaking at 40 µg/mL ($P < 0.05$). The extender supplemented with 40 µg/mL coenzyme Q10 produced the highest total motility (47.12%), progressive motility (44.87%), viability (59.09%), plasma membrane integrity (59.51%), and sperm velocity parameters, while exhibiting the lowest malondialdehyde concentration (25.38 nmol/20 × 10⁶ spermatozoa). In contrast, supplementation with 80 µg/mL coenzyme Q10 significantly reduced these beneficial effects, although sperm quality remained superior to that of the

control group. These findings demonstrate that supplementation of the freezing extender with 40 µg/mL coenzyme Q10 effectively enhances the cryotolerance of Ac chicken sperm by reducing oxidative damage and preserving sperm motility and membrane integrity.

Keywords: Ac chicken, coenzyme Q10, cryopreservation, oxidative stress, sperm quality.

I. INTRODUCTION

Artificial insemination has become an essential reproductive biotechnology in modern poultry production because it improves breeding efficiency, facilitates the dissemination of superior genetic traits, and contributes to the conservation of valuable genetic resources. The successful implementation of artificial insemination relies heavily on the availability of high-quality preserved semen, making semen cryopreservation an important component of poultry breeding programs. However, despite continuous improvements in freezing protocols, cryopreservation still causes substantial deterioration of sperm quality, resulting in reduced post-thaw motility, membrane integrity, viability, and fertilizing capacity.

Chicken spermatozoa are particularly susceptible to cryoinjury because their plasma membranes contain high concentrations of polyunsaturated fatty acids, which are highly vulnerable to oxidative attack. During cooling, freezing, and thawing, excessive production of reactive oxygen species (ROS) disrupts the physiological redox balance, leading to lipid peroxidation, mitochondrial dysfunction, DNA damage, membrane destabilization, and ultimately sperm death. Although endogenous antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, protect physiological condi-

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tions, their activity is markedly reduced during semen dilution and cryopreservation, rendering spermatozoa more vulnerable to oxidative stress. Consequently, oxidative damage has been recognized as one of the major factors limiting the efficiency of poultry semen cryopreservation [1–3].

To minimize cryopreservation-induced oxidative injury, numerous antioxidants have been incorporated into poultry semen extenders. Previous studies have demonstrated that supplementation with dimethylacetamide, vitamins C and E, catalase, ginseng, and various natural antioxidants can improve post-thaw sperm quality by reducing oxidative damage and preserving membrane integrity [4–9]. Nevertheless, the protective effects of these antioxidants are highly dependent on their concentration and antioxidant capacity, highlighting the need to identify more effective compounds capable of maintaining sperm function during cryopreservation.

Coenzyme Q10 (CoQ10) has attracted considerable attention as a multifunctional antioxidant because it serves both as an essential component of the mitochondrial electron transport chain and as a potent lipid-soluble free radical scavenger. Located within the inner mitochondrial membrane, CoQ10 facilitates adenosine triphosphate (ATP) synthesis while simultaneously preventing electron leakage and suppressing ROS generation. In addition, its reduced form (ubiquinol) interrupts lipid peroxidation chain reactions, thereby protecting membrane phospholipids, proteins, and nucleic acids from oxidative damage [10, 11]. High concentrations of CoQ10 have been detected in reproductive tissues, particularly in the testes, where it plays a crucial role in maintaining mitochondrial bioenergetics, spermatogenesis, and sperm function [12, 13].

The beneficial effects of CoQ10 supplementation on semen quality have been demonstrated in several animal species. In poultry, supplementation of freezing extenders with CoQ10 improved post-thaw motility, plasma membrane integrity, antioxidant status, and fertility potential of rooster sperm [14]. Likewise, CoQ10 supple-

mentation during liquid storage enhanced rooster semen quality and reduced oxidative stress [15]. Similar improvements in sperm quality and antioxidant capacity have also been reported in goat bucks and other mammalian species [12]. Collectively, these findings indicate that CoQ10 is a promising antioxidant for protecting spermatozoa against cryopreservation-induced oxidative injury.

The Vietnamese Ac chicken (*Gallus gallus domesticus* Brisson) is an indigenous breed of considerable economic and genetic value because of its high nutritional quality and medicinal importance. Preserving the genetic resources of this breed requires efficient semen cryopreservation protocols suitable for artificial insemination and long-term germplasm conservation. However, despite growing evidence supporting the protective effects of CoQ10 in other poultry breeds, information regarding the optimal concentration of CoQ10 for cryopreservation of Ac chicken semen remains unavailable. Therefore, the present study aimed to evaluate the effects of different concentrations of CoQ10 supplemented in a Beltsville freezing extender on the post-thaw quality of Ac chicken sperm by assessing sperm motility, viability, plasma membrane integrity, and lipid peroxidation.

II. LITERATURE REVIEW

Semen cryopreservation is an indispensable reproductive biotechnology in poultry breeding, enabling artificial insemination, genetic improvement, and long-term conservation of valuable genetic resources. However, the success of cryopreservation in chickens remains considerably lower than in many mammalian species because avian spermatozoa are highly susceptible to cryoinjury. Previous studies have demonstrated that freezing and thawing induce osmotic stress, intracellular ice crystal formation, mitochondrial dysfunction, membrane disruption, and excessive production of ROS, resulting in reduced sperm motility, viability, membrane integrity, and fertilizing ability [4, 16]. The high proportion of polyunsaturated fatty acids within the chicken

sperm plasma membrane further increases its susceptibility to lipid peroxidation and oxidative damage during cryopreservation [3, 16]. To overcome these limitations, the supplementation of antioxidants into semen extenders has been investigated. Conventional antioxidants such as vitamins C and E, catalase, dimethylacetamide, ginseng, sericin, and plant-derived extracts have shown varying degrees of success in improving post-thaw semen quality by reducing oxidative stress and preserving membrane integrity [4–9]. Particularly, Amini et al. [6] reported that vitamin E and vitamin C improved post-thaw rooster sperm quality when incorporated into a modified Beltsville extender, whereas Moghbeli et al. [7] demonstrated that the protective efficacy of catalase and vitamin E depended on sperm concentration. More recently, Mehaisen et al. [4] optimized dimethylacetamide concentration for rooster semen cryopreservation, while Ratchamak et al. [5] and Ratchamak et al. [8] showed that supplementation with ginseng or sericin enhanced antioxidant capacity, sperm freezability, and fertility. Although these antioxidants reduced cryopreservation-induced damage, their protective effects varied considerably among compounds and concentrations, suggesting that the search for more effective antioxidants remains necessary.

Among the available antioxidants, CoQ10 has emerged as a particularly promising candidate because of its dual biological functions. As an essential component of the mitochondrial electron transport chain, CoQ10 facilitates oxidative phosphorylation and ATP production, while its reduced form (ubiquinol) acts as a potent lipid-soluble antioxidant capable of scavenging free radicals and interrupting lipid peroxidation chain reactions [10]. In addition to protecting plasma membrane lipids, CoQ10 stabilizes mitochondrial membranes, reduces ROS generation, and maintains cellular bioenergetics, all of which are essential for normal sperm function [10, 11]. CoQ10 has been demonstrated to contribute to spermatogenesis, testicular function, and reproductive performance in several animal species

[11–13].

The application of CoQ10 in semen preservation has attracted increasing attention during the past decade. Masoudi et al. [14] demonstrated that supplementation of freezing extenders with CoQ10 significantly improved post-thaw motility, mitochondrial activity, plasma membrane integrity, antioxidant status, and fertility potential of rooster sperm. Similarly, Sharideh et al. [15] reported that CoQ10 enhanced rooster sperm quality during liquid storage by preserving mitochondrial function and reducing oxidative damage. Beneficial effects of CoQ10 have also been reported in goat bucks, where dietary supplementation improved seminal antioxidant capacity, sperm quality, and reproductive performance under heat stress [12]. Collectively, these studies indicate that CoQ10 effectively protects spermatozoa against oxidative damage by simultaneously improving mitochondrial bioenergetics and antioxidant defense mechanisms.

Despite these advances, several important knowledge gaps remain. Most previous studies have evaluated CoQ10 in commercial rooster breeds or under liquid storage conditions, whereas little information is available regarding its application in indigenous chicken breeds subjected to cryopreservation [12, 14, 15]. The effects of different CoQ10 concentrations on the cryopreservation of Vietnamese Ac (Silkie) chicken semen using a Beltsville extender and a conventional liquid nitrogen vapor freezing protocol remain insufficiently characterized. Furthermore, previous studies have generally focused on a limited number of semen quality parameters, whereas comprehensive evaluations integrating sperm motility, viability, plasma membrane integrity, and lipid peroxidation remain scarce [12, 14, 15]. The study builds upon previous cryopreservation protocols established for rooster semen by adopting the Beltsville extender, glycerol-based cryoprotection, conventional freezing procedures, and standardized methods for evaluating sperm quality, including computer-assisted sperm analysis (CASA), Eosin–Nigrosin staining, the hypo-osmotic swelling test, and malondialdehyde

(MDA) determination [6, 17–21]. Building on these established methodologies, a comprehensive evaluation of different concentrations of CoQ10 in the cryopreservation of Vietnamese Ac chicken semen was carried out. The findings provide new evidence regarding the optimal concentration of CoQ10 for improving post-thaw sperm quality and contribute to the development of more efficient cryopreservation protocols for poultry genetic resource conservation and artificial insemination programs.

III. RESEARCH METHODS

A. Study period and location

The research was carried out between January and May 2026 at the Experimental Center and the Animal Science and Veterinary Medicine Experimental Farm, School of Agriculture and Aquaculture, Tra Vinh University, Vietnam.

B. Animals

Semen was obtained from ten clinically healthy Ac roosters aged between six and eight months, with body weights ranging from 1.2 to 1.5 kg. Prior to inclusion in the experiment, all birds were assessed and confirmed to have satisfactory reproductive performance. The roosters were housed under standardized environmental conditions and provided with individual feeding and watering systems to facilitate precise dietary management. Feed and drinking water were available ad libitum throughout the study period. The chickens received routine vaccinations against infectious diseases and regular antiparasitic treatments. The experiment was conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Tra Vinh University, Vietnam.

C. Semen collection and initial evaluation of semen quality

Semen samples were collected twice per week. Before the experiment, all roosters were acclimated to the semen collection procedure and trained using the abdominal massage method

described by Burrows et al. [17]. Only semen samples meeting the following quality criteria were selected: sperm motility > 75%, sperm concentration > 400×10^6 spermatozoa/mL, abnormal spermatozoa < 5%, and sperm viability > 90%.

Sperm motility was assessed using a CASA system. The percentages of abnormal and viable spermatozoa were determined using the Eosin–Nigrosin staining technique as described by Tamuli et al. [18]. Before evaluation, the semen samples were diluted with Beltsville extender and equilibrated at 37°C in a thermostatic water bath.

D. Experimental design and semen cryopreservation procedures

A completely randomized design comprising six treatments with different concentrations of CoQ10 was employed. The CoQ10 concentrations used in this study were selected based on a preliminary experiment conducted to identify a suitable dose range for cryopreserved Ac chicken semen. Accordingly, CoQ10 was evaluated at the concentrations of 0, 5, 10, 20, 40, and 80 µg/mL in the extender. Each treatment was replicated four times, with each replicate consisting of an independently prepared pooled sample of 10 ejaculates. The Beltsville extender described by Amini et al. [6] served as the basal diluent, and its composition is shown in Table 1. Glycerol was incorporated as a cryoprotective agent to obtain a final concentration of 11% in the semen extenders. All chemicals used were purchased from Sigma-Aldrich. Coenzyme Q10 (catalogue No. C9538; purity $\geq 98\%$) was dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solution. The same final DMSO concentration (0.8%, v/v) was maintained in all treatments, including the control.

Semen was collected from ten Ac roosters and subjected to an initial quality assessment. Samples meeting the selection criteria were pooled and equally distributed into six sterile tubes. Each tube contained 200 µL of pooled semen and 3 mL of Beltsville extender supplemented with

Table 1: Composition of the six semen extenders used for chicken semen dilution

Extender components	Semen extenders					
	Control	CQ-5	CQ-10	CQ-20	CQ-40	CQ-80
Potassium citrate tribasic monohydrate (g)	0.64	0.64	0.64	0.64	0.64	0.64
Sodium-L- glutamate (g)	8.67	8.67	8.67	8.67	8.67	8.67
Magnesium chloride (g)	0.34	0.34	0.34	0.34	0.34	0.34
D-(-)-Fructose (g)	5.00	5.00	5.00	5.00	5.00	5.00
Dipotassium phosphate trihydrate (g)	7.59	7.59	7.59	7.59	7.59	7.59
Potassium phosphate monobasic (g)	0.70	0.70	0.70	0.70	0.70	0.70
N-[Tris(hydroxymethyl) methyl]-2-aminoethanesulfonic acid (g)	2.70	2.70	2.70	2.70	2.70	2.70
Sodium acetate trihydrate (g)	3.10	3.10	3.10	3.10	3.10	3.10
Penicillin (1,000,000 UT)-Streptomycin (1 g) (g)	5.00	5.00	5.00	5.00	5.00	5.00
Glycerol (ml)	110.00	110.00	110.00	110.00	110.00	110.00
Coenzyme Q10 (mg)	0.00	5.00	10.00	20.00	40.00	80.00
DMSO (mL)	8.00	8.00	8.00	8.00	8.00	8.00
Distilled water (ml)	To 1000	To 1000	To 1000	To 1000	To 1000	To 1000

Note: CQ-5, CQ-10, CQ-20, CQ-40, and CQ-80 correspond to 5, 10, 20, 40, and 80 µg/ml of CoQ10, respectively

11% glycerol and CoQ10 at 0, 5, 10, 20, 40, or 80 µg/mL. The semen-to-extender ratio was 1:15, resulting in a final sperm concentration of approximately 40 × 106 spermatozoa/mL. Each treatment received a total of approximately 120 × 106 spermatozoa.

Following dilution, the semen samples were placed in a Styrofoam container containing water maintained at approximately 25°C and gradually cooled to 5°C at an estimated rate of 0.3°C/min through the progressive addition of ice over a 1-h period. The samples were then equilibrated at 5°C for an additional hour. Subsequently, the diluted semen was packaged into 0.5-mL French straws and sealed using a heat-sealing device.

For cryopreservation, the straws were exposed to liquid nitrogen vapor in a two-step freezing process. Initially, they were positioned 11 cm above the liquid nitrogen surface for 15 minutes and then transferred to a position 3 cm above the liquid nitrogen surface for a further five minutes. Thereafter, the straws were immersed directly into liquid nitrogen and stored at -196°C in liquid nitrogen tanks according to the procedure described by Ratchamak et al. [8]. Frozen semen samples were maintained under storage conditions for seven days.

Before post-thaw evaluation, the straws were

thawed in a 4°C water bath for three minutes following the protocol of Lin et al. [19]. For each replicate, semen quality was evaluated after thawing six straws by assessing progressive motility, sperm viability, plasma membrane integrity, and lipid peroxidation.

E. Sperm motility evaluation

Sperm motility was assessed using a CASA system (ROTO Vision II, China). Briefly, 5 µL of each thawed semen sample was loaded into a 2X-CEL counting chamber and equilibrated on a MiniTherm heated stage maintained at 38°C. For each sample, a minimum of 200 spermatozoa were analyzed across five randomly selected microscopic fields. The CASA system was used to determine total motility (TM, %), progressive motility (PM, %), average path velocity (VAP), straight-line velocity (VSL), and curvilinear velocity (VCL).

F. Sperm viability evaluation

Sperm viability was determined using the Eosin–Nigrosin staining method according to Tamuli et al. [18]. A 5-µL aliquot of diluted semen was mixed with an equal volume of Eosin–Nigrosin stain on a clean glass slide. After

gentle mixing for 30 seconds, the mixture was smeared evenly across the slide and allowed to air-dry. The preparations were examined under a light microscope (400× magnification). Spermatozoa with damaged plasma membranes absorbed the stain and appeared pink, whereas viable spermatozoa excluded the dye and remained unstained. Four replicate assessments were performed for each treatment.

G. Sperm plasma membrane integrity evaluation

Plasma membrane integrity was assessed using the hypo-osmotic swelling test as previously described by Shahverdi et al. [20]. Briefly, 30 μL of thawed semen (20×10^6 spermatozoa/mL) was mixed with 300 μL of a hypo-osmotic solution (100 mOsm/kg; containing 9 g/L fructose and 4.9 g/L sodium citrate) and incubated at 37°C for 30 minutes. Following incubation, 5 μL of the suspension was transferred onto a clean glass slide, covered with a coverslip, and examined under a light microscope using a 40× objective lens. A minimum of 200 spermatozoa were evaluated per sample and classified according to the presence or absence of tail swelling. Spermatozoa with functionally intact plasma membranes exhibited characteristic hypo-osmotic swelling responses, including curled tails, swelling of the tail tip or midpiece, and shortened, thickened tails. Conversely, spermatozoa with damaged plasma membranes showed no swelling response. Each treatment was analyzed in four independent replicates.

H. Sperm lipid peroxidation evaluation

Lipid peroxidation was evaluated by measuring MDA levels using thiobarbituric acid reactive substances (TBARS) assay as described by Maia et al. [21]. Lipid peroxidation was induced by incubating the sperm samples with 0.24 mM FeSO_4 at 37°C for 15 minutes. Subsequently, 1 mL of TBARS reagent (thiobarbituric acid 0.375% (w/v), trichloroacetic acid 15% (w/v) in hydrochloric acid 0.25N) was added to 0.5 mL of each sperm sample, and the mixture was incubated in a water bath for 15 minutes. After cooling to room temperature, the mixture was

centrifuged at $1,000 \times g$ for 10 minutes. The supernatant was collected, transferred to a 96-well microplate, and its absorbance was measured at 535 nm using a microplate spectrophotometer. MDA concentrations were calculated by comparing the absorbance values of the samples with an MDA standard curve and expressed as nmol MDA per 20×10^6 spermatozoa.

I. Statistical analysis

Data were analyzed using IBM SPSS Statistics version 22. Differences among treatments supplemented with different concentrations of CoQ10 in the semen extender were evaluated by one-way ANOVA, and mean comparisons were performed using Tukey's multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

IV. RESULTS AND DISCUSSION

A. Results

Sperm motility characteristics Coenzyme Q10 supplementation significantly affected the motility characteristics of frozen-thawed chicken sperm ($P < 0.05$; Table 2). Total motility increased progressively with increasing CoQ10 concentration from 5 to 40 $\mu\text{g/mL}$, reaching the highest value in the CQ-40 group ($47.12 \pm 0.24\%$), which was significantly greater than all other treatments. Compared with the control ($26.82 \pm 0.63\%$), total motility was enhanced by approximately 76% in the CQ-40 group. However, supplementation with 80 $\mu\text{g/mL}$ CoQ10 resulted in a marked reduction in total motility ($28.37 \pm 0.35\%$), although it remained slightly higher than the control.

A similar pattern was observed for progressive motility. The CQ-40 extender produced the highest progressive motility ($44.87 \pm 1.37\%$), followed by the CQ-20 ($33.84 \pm 0.87\%$), CQ-10 ($31.17 \pm 0.72\%$), and CQ-5 ($28.16 \pm 0.92\%$) groups. The control group exhibited the lowest progressive motility ($24.11 \pm 0.36\%$), while the CQ-80 treatment ($27.26 \pm 0.46\%$) showed a significant decline compared with CQ-20 and CQ-40.

The sperm velocity parameters were also significantly improved by CoQ10 supplementation. The highest VAP, VSL, and VCL were recorded in the CQ-40 group, with values of $45.79 \pm 0.45 \mu\text{m/s}$, $33.68 \pm 0.81 \mu\text{m/s}$, and $74.01 \pm 0.29 \mu\text{m/s}$, respectively ($P < 0.05$). These values were significantly higher than those observed in the control group (29.58 ± 0.67 , 18.54 ± 0.29 , and $52.90 \pm 0.30 \mu\text{m/s}$, respectively). Although supplementation with 5–20 $\mu\text{g/mL}$ CoQ10 significantly improved all velocity parameters compared with the control, the enhancement was concentration-dependent and reached its maximum at 40 $\mu\text{g/mL}$. In contrast, the CQ-80 treatment significantly reduced VAP, VSL, and VCL relative to the CQ-20 and CQ-40 groups, indicating that excessive CoQ10 supplementation diminished the beneficial effects on sperm motility after cryopreservation.

Overall, supplementation of the semen extender with 40 $\mu\text{g/mL}$ CoQ10 yielded the greatest improvement in post-thaw sperm motility and kinematic parameters, whereas increasing the concentration to 80 $\mu\text{g/mL}$ adversely affected sperm movement, although values remained generally higher than those of the untreated control.

Sperm viability and plasma membrane integrity

Coenzyme Q10 supplementation significantly improved the viability and plasma membrane integrity of frozen–thawed chicken sperm compared with the control ($P < 0.05$; Table 3). Both parameters exhibited a concentration-dependent increase from 5 to 40 $\mu\text{g/mL}$ CoQ10, followed by a decline at the highest supplementation level (80 $\mu\text{g/mL}$).

Sperm viability was lowest in the control group ($41.43 \pm 0.29\%$) and increased significantly with CoQ10 supplementation to $47.82 \pm 0.21\%$, $51.63 \pm 0.52\%$, and $53.77 \pm 0.31\%$ in the CQ-5, CQ-10, and CQ-20 groups, respectively. The highest viability was observed in the CQ-40 group ($59.09 \pm 0.16\%$), representing an approximately 43% increase compared with the control. However, increasing the CoQ10 concentration to 80 $\mu\text{g/mL}$

significantly reduced sperm viability to $46.20 \pm 0.23\%$, although this value remained significantly higher than that of the control group.

A similar trend was observed for plasma membrane integrity. The percentage of sperm with intact plasma membranes increased significantly from $41.72 \pm 0.28\%$ in the control group to $48.04 \pm 0.38\%$, $51.76 \pm 0.12\%$, and $54.41 \pm 0.38\%$ in the CQ-5, CQ-10, and CQ-20 groups, respectively. The CQ-40 treatment produced the highest plasma membrane integrity ($59.51 \pm 0.34\%$), which was significantly greater than all other treatments ($P < 0.05$). In contrast, supplementation with 80 $\mu\text{g/mL}$ CoQ10 significantly decreased plasma membrane integrity to $45.94 \pm 0.03\%$, although it remained higher than the control. Overall, supplementation of the semen extender with 40 $\mu\text{g/mL}$ CoQ10 was the most effective concentration for preserving sperm viability and plasma membrane integrity following cryopreservation, whereas increasing the concentration to 80 $\mu\text{g/mL}$ diminished these protective effects.

Sperm lipid peroxidation

As shown in Table 4, CoQ10 supplementation significantly reduced lipid peroxidation in frozen–thawed chicken sperm, as evidenced by decreased MDA concentrations. MDA levels progressively declined from the control group to the treatment supplemented with 40 $\mu\text{g/mL}$ CoQ10, followed by a slight increase at the highest supplementation level (80 $\mu\text{g/mL}$). The control group exhibited the highest MDA concentration ($42.92 \pm 0.39 \text{ nmol}/20 \times 10^6 \text{ spermatozoa}$), whereas supplementation with 5 $\mu\text{g/mL}$ ($36.03 \pm 0.08 \text{ nmol}/20 \times 10^6 \text{ spermatozoa}$) and 10 $\mu\text{g/mL}$ ($34.63 \pm 0.02 \text{ nmol}/20 \times 10^6 \text{ spermatozoa}$) significantly reduced MDA production compared with the control ($P < 0.05$). However, no significant difference was observed between these two treatments ($P > 0.05$). Further reductions in MDA concentration were observed in the 20 $\mu\text{g/mL}$ treatment ($34.31 \pm 0.05 \text{ nmol}/20 \times 10^6 \text{ spermatozoa}$), while the lowest MDA level was recorded in sperm supplemented with 40 $\mu\text{g/mL}$ CoQ10 ($25.38 \pm 1.16 \text{ nmol}/20 \times 10^6$

Table 2: Effects of coenzyme Q10 supplementation in the semen extender on sperm motility characteristics of frozen–thawed chicken sperm

Parameters	Control	CQ-5	CQ-10	CQ-20	CQ-40	CQ-80	P-value
Total motility (%)	26.82±0.63 ^f	30.12±1.14 ^d	33.43±0.23 ^c	35.16±0.81 ^b	47.12±0.24 ^a	28.37±0.35 ^e	< 0.001
Progressive motility (%)	24.11±0.36 ^e	28.16±0.92 ^d	31.17±0.72 ^c	33.84±0.87 ^b	44.87±1.37 ^a	27.26±0.46 ^d	< 0.001
VAP (µm/s)	29.58±0.67 ^e	34.21±0.30 ^c	35.19±0.54 ^c	41.12±0.36 ^b	45.79±0.45 ^a	32.98±0.45 ^d	< 0.001
VSL (µm/s)	18.54±0.29 ^d	23.37±0.44 ^c	23.47±0.98 ^c	28.60±0.71 ^b	33.68±0.81 ^a	22.84±0.47 ^c	< 0.001
VCL (µm/s)	52.90±0.30 ^e	58.62±0.47 ^d	59.80±0.10 ^c	66.57±0.49 ^b	74.01±0.29 ^a	57.91±0.52 ^d	< 0.001

Note: CQ-5, CQ-10, CQ-20, CQ-40, and CQ-80 represent semen extenders supplemented with 5, 10, 20, 40 and 80 µg/ml of CoQ10, respectively; VAP: average pathway velocity, VSL: straight line velocity, and VCL: curvilinear velocity; data are presented as the mean ± standard deviation of four independent replicates, with each replicate consisting of a pooled sample of ten ejaculates; within the same row, values bearing different superscript letters (a–f) differ significantly among treatments ($P < 0.05$).

Table 3: Effects of coenzyme Q10 supplementation in the semen extender on the plasma membrane integrity and viability of frozen–thawed chicken sperm

Parameters	Control	CQ-5	CQ-10	CQ-20	CQ-40	CQ-80	P-value
Viability (%)	41.43±0.29 ^f	47.82±0.21 ^d	51.63±0.52 ^c	53.77±0.31 ^b	59.09±0.16 ^a	46.20±0.23 ^e	< 0.001
Plasma membrane integrity (%)	41.72±0.28 ^f	48.04±0.38 ^d	51.76±0.12 ^c	54.41±0.38 ^b	59.51±0.34 ^a	45.94±0.03 ^e	< 0.001

Note: CQ-5, CQ-10, CQ-20, CQ-40, and CQ-80 represent semen extenders supplemented with 5, 10, 20, 40, and 80 µg/ml of CoQ10, respectively; data are presented as the mean ± standard deviation of four independent replicates, with each replicate four independent replicates, each consisting of a pooled sample of ten ejaculates; within the same row, values bearing different superscript letters (a–f) differ significantly among treatments ($P < 0.05$).

spermatozoa), which differed significantly from all other treatments ($P < 0.05$). Increasing the CoQ10 concentration to 80 µg/mL resulted in a significant increase in MDA production ($32.75 \pm 1.23 \text{ nmol}/20 \times 10^6$ spermatozoa) compared with the 40 µg/mL treatment, although the value remained significantly lower than that of the control group ($P < 0.05$).

B. Discussion

Cryopreservation is well known to impair sperm function through a combination of intracellular ice crystal formation, osmotic stress, oxidative damage, and membrane disruption. These injuries reduce mitochondrial activity, destabilize the plasma and acrosomal membranes, and impair DNA integrity, ultimately compromising post-thaw sperm survival and fertilizing ability [1, 22]. In the research, supplementation

of the freezing extender with CoQ10 significantly improved post-thaw sperm motility, viability, plasma membrane integrity, and reduced lipid peroxidation. Among the tested concentrations, 40 µg/mL consistently produced the greatest improvement in all evaluated sperm quality parameters, whereas increasing the concentration to 80 µg/mL reduced these beneficial effects.

The improvement in sperm motility observed after CoQ10 supplementation is consistent with previous reports in rooster semen [14, 15]. Total motility, progressive motility, and sperm velocity parameters (VAP, VSL, and VCL) all increased in a concentration-dependent manner up to 40 µg/mL CoQ10. Since sperm motility depends primarily on ATP generated by mitochondria located in the sperm midpiece, preservation of mitochondrial function during cryopreservation is essential for maintaining flagellar movement

Table 4: Effects of coenzyme Q10 supplementation in the semen extender on malondialdehyde levels (nmol/20 × 10⁶ spermatozoa) in frozen–thawed chicken sperm

Parameters	Control	CQ-5	CQ-10	CQ-20	CQ-40	CQ-80	P-value
MDA	42.92±0.39 ^a	36.03±0.08 ^b	34.63±0.02 ^{bc}	34.31±0.05 ^{cd}	25.38±1.16 ^e	32.75±1.23 ^d	< 0.001

Note: CQ-5, CQ-10, CQ-20, CQ-40, and CQ-80 represent semen extenders supplemented with 5, 10, 20, 40, and 80 µg/mL CoQ10, respectively; data are presented as the mean ± standard deviation of four independent replicates, with each replicate consisting of a pooled sample of ten ejaculates; within the same row, values bearing different superscript letters (a–e) differ significantly among treatments (P < 0.05).

[13]. Coenzyme Q10 functions as a lipid-soluble electron carrier within the mitochondrial electron transport chain, facilitating oxidative phosphorylation and ATP synthesis while simultaneously limiting electron leakage and ROS production [10]. Consequently, improved mitochondrial bioenergetics likely contributed to the enhanced motility observed in the present research. Similar mechanisms have been proposed in rooster sperm cryopreservation, where CoQ10 supplementation maintained mitochondrial membrane potential and improved post-thaw fertility potential [14]. Furthermore, Sharideh et al. [15] reported that CoQ10 supplementation during liquid storage also enhanced rooster sperm motility by preserving mitochondrial function and antioxidant capacity. Significant improvements in sperm viability and plasma membrane integrity following CoQ10 supplementation were demonstrated. Cryopreservation exposes spermatozoa to severe osmotic fluctuations and oxidative stress, causing structural damage to plasma membranes, mitochondrial membranes, and the acrosome [1]. Chicken sperm are particularly susceptible to oxidative injury because their plasma membranes contain exceptionally high proportions of polyunsaturated fatty acids, which are readily oxidized by ROS [3]. Oxidative degradation of membrane lipids decreases membrane fluidity and permeability, ultimately reducing sperm survival after thawing [16]. In the study, supplementation with 40 µg/mL CoQ10 significantly preserved membrane integrity and increased sperm viability, indicating effective protection against cryo-induced membrane damage. These findings agree with those reported by Masoudi et al.

[14], who observed improved plasma membrane integrity, mitochondrial activity, and fertility following CoQ10 supplementation in frozen rooster semen. Comparable beneficial effects of CoQ10 on sperm quality have also been reported in goat bucks under heat stress, where CoQ10 enhanced seminal antioxidant capacity, sperm viability, and reproductive performance [12].

The reduction in MDA concentration further supports the antioxidant role of CoQ10 during cryopreservation. MDA is a major end-product of lipid peroxidation and is widely used as an indicator of oxidative damage in spermatozoa [23]. MDA concentrations decreased progressively with increasing CoQ10 concentration up to 40 µg/mL, indicating effective suppression of lipid peroxidation. Lower MDA levels coincided with higher sperm motility, viability, and plasma membrane integrity, suggesting that reduction of oxidative stress directly contributed to improved sperm quality. Coenzyme Q10 possesses a benzoquinone ring and a long isoprenoid side chain, allowing it to localize within lipid bilayers of plasma and mitochondrial membranes where it scavenges free radicals and interrupts lipid peroxidation chain reactions [10]. Similar reductions in MDA following CoQ10 supplementation have been reported in cryopreserved rooster semen by Masoudi et al. [14] and during liquid preservation by Sharideh et al. [15], supporting the present findings. Moreover, previous studies have demonstrated that antioxidant supplementation during avian semen preservation effectively reduces oxidative damage and enhances post-thaw sperm function [2, 5].

Interestingly, increasing the CoQ10 concentra-

tion to 80 $\mu\text{g/mL}$ significantly reduced sperm motility, viability, and plasma membrane integrity compared with the 40 $\mu\text{g/mL}$ treatment, which produced the best results among the tested concentrations. Although MDA remained lower at 80 $\mu\text{g/mL}$ than in the control, it was higher than at 40 $\mu\text{g/mL}$, indicating a non-linear dose response. Other antioxidants used in semen preservation have shown similar responses, suggesting that excessive antioxidant supplementation may have detrimental effects [2]. Physiological ROS levels contribute to sperm functions such as capacitation, hyperactivation, and the acrosome reaction; therefore, excessive ROS scavenging has been proposed to disrupt essential redox signaling [24]. High concentrations of lipophilic antioxidants may also alter membrane properties, interfere with mitochondrial electron transport, or exert direct cytotoxic effects, potentially impairing sperm function. Comparable inhibitory effects at excessive antioxidant concentrations have been reported in canine semen preserved with plant-derived antioxidants [25]. However, these explanations remain hypothetical because this study did not directly evaluate intracellular ROS, mitochondrial function, ATP production, or CoQ10 cytotoxicity. Further mechanistic and cytotoxicity assessments are therefore required to explain the reduced sperm quality observed at 80 $\mu\text{g/mL}$ and to define an appropriate CoQ10 concentration for semen cryopreservation.

Among the tested concentrations, 40 $\mu\text{g/mL}$ CoQ10 (approximately 46.3 μM) produced the best post-thaw sperm quality. This concentration falls within the range of effective CoQ10 concentrations reported in previous studies [14, 15], supporting the potential protective role of CoQ10 during rooster semen cryopreservation. Nevertheless, direct comparisons should be made cautiously because the previous studies used different chicken breeds, semen extenders, and freezing–thawing protocols. In contrast, this work evaluated Vietnamese Ac chickens using a Beltsville-based freezing extender. These biological and methodological differences may influence sperm responses to CoQ10 and explain

variations in the most effective concentrations reported across studies. Therefore, 40 $\mu\text{g/mL}$ should be considered the best concentration under the present experimental conditions rather than a universally optimal dose for avian semen cryopreservation.

Overall, the findings indicate that supplementation of the Beltsville freezing extender with 40 $\mu\text{g/mL}$ CoQ10 was associated with the best post-thaw laboratory outcomes among the tested concentrations, including higher sperm motility, viability, and plasma membrane integrity and lower MDA levels. These results are consistent with previous findings in cryopreserved rooster semen and provide breed-specific evidence for Ac chickens under the protocol.

V. CONCLUSION AND RECOMMENDATIONS

Supplementation of the Beltsville freezing extender with CoQ10 produced a non-linear dose response in the post-thaw quality of Ac chicken sperm. Among the tested concentrations, 40 $\mu\text{g/mL}$ resulted in the highest sperm motility, viability, and plasma membrane integrity and the lowest MDA level, whereas these beneficial effects declined at 80 $\mu\text{g/mL}$. These findings indicate that supplementation with 40 $\mu\text{g/mL}$ CoQ10 was associated with improved post-thaw sperm quality and reduced lipid peroxidation under the present experimental conditions. However, its effects on fertility, hatchability, and artificial-insemination outcomes require validation through *in vivo* studies. Further research should also evaluate mitochondrial activity, DNA integrity, and antioxidant enzyme activity to clarify the mechanisms underlying the observed effects.

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