


Does ergothioneine and thawing temperatures improve rooster semen post-thawed quality?

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ABSTRACT The present study focuses on the effect of different levels of ergothioneine and thawing temperature on rooster semen cryopreservation. Semen was diluted in Lake extender containing ergothioneine at 5, 10, 15, and 20 μM and cryopreserved. Two thawing temperatures (37°C for 30 s and 60°C for 5 s) were consequently examined. Sperm motility parameter, membrane integrity, abnormal morphology, viability, apoptotic status, mitochondria activity, and lipid peroxidation were determined after freeze-thaw process. Ergothioneine levels of 5 and 10 μM led to higher ($P < 0.05$) total motility (66.58 ± 1.44 and 72.11 ± 1.44 , respectively) and average path velocity (**VAP**) (34.54 ± 0.89 , 37.28 ± 0.89 , respectively). Higher ($P < 0.05$) significant membrane integrity and mitochondria activity after freeze-thawing were observed in the

groups supplemented with 10 μM ergothioneine (68.62 ± 1.24 and 69.12 ± 1.26 , respectively). Also, 5 and 10 μM of ergothioneine led to the lowest significant percentage of apoptotic and dead sperm. The total motility and progressive motility resulted in significantly ($P < 0.05$) higher amount when sperm were thawed with 60°C (60.58 ± 0.91 and 24.76 ± 0.53 , respectively) compared to thawed sperm in 37°C. The membrane integrity, viability and mitochondria activity led to significantly ($P < 0.05$) higher when sperm were thawed with 60°C (58.2 ± 0.78 , 63.21 ± 0.80 and 56.85 ± 0.79 , respectively). It could be concluded the addition of 5 and 10 μM ergothioneine in the semen extender and thawing temperature at 60°C in 5 s can be an efficient strategy to preserve rooster cryopreserved semen quality.

Key words: rooster, ergothioneine, thawing temperature, cryopreservation, sperm

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INTRODUCTION

The success of the poultry industry depends mainly on the reproductive status of the birds (Hanafy and Elnesr, 2021). Cryopreservation is known as an operative and effective method that can be used to preserve the genetic diversity of bird populations, especially vulnerable and important species. However, this method is not widely used in the poultry industry because the sperm quality (such as viability and fertility) of stored samples significantly decreases (Mehaisen et al., 2020).

In order to become successful in poultry production, sperm with high viability and standard motility are critical. Only motile sperm can pass through the vagina. There are many factors affecting sperm motility such as physiology, nutrition, and environment (Al-Qarawi, 2005;

Abdelnour et al., 2020). Reproductive performance is also affected by oxidative stress. The high fatty acids (**PUFA**) in the plasma membrane of rooster sperm, which are sensitive to oxidative stress, have negative correlation with sperm viability and motility (Najafi et al., 2020). However, when the production of active oxygen exceeds the amount of body antioxidant, oxidative stress occurs. Oxidative stress is defined as the presence of metabolic and radical substances or so-called reactive (oxygen, nitrogen, or chlorine) species (Elnesr et al., 2019; Elwan et al., 2019). Oxidative stress leads to the reduction of the number of gametes, sperm motility, and increasing the percentage of dead cells. A highly effective antioxidant system is needed to protect sperm membrane against oxidative damage (Mavi et al., 2020). Various studies have shown the protective effects of different types of antioxidants for conserving sperm motility and viability during cooling or freezing storage (Mehaisen et al., 2020).

Ergothioneine is a natural hydrophilic amino acid, a nutrient and antioxidant which has been shown to scavenge reactive oxygen species (Zullo et al., 2016). Compared with some popular antioxidants such as trolox, uric acid and glutathione (**GSH**) have shown the

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highest antioxidant activity and have reduced the most active free radicals (Franzoni et al., 2006). In some researches, ergothioneine protected sperm from chemicals derived from fructolysis, and enhanced the sperm motility and DNA integrity after freezing and thawing (Çoyan et al., 2012; Najafi et al., 2014).

Thawing rate is also a crucial factor for maintaining sperm quality and motility during cryopreservation. Various methods for thawing semen have been studied and suggested, but there is not any unanimous method. Traditionally, in most species, frozen semen has been thawed at 37°C (Nur et al., 2003). However, many studies have shown that a temperature of 60°C to 80°C could improve post-thaw motility (Nöthling and Shuttleworth, 2005). This is probably because the intracellular ice crystals raised during the freezing process will grow during the slow rewarming process and damage the sperm. Using higher temperatures seems to cause less cell damage; the extent of this effect is unclear (Malo et al., 2019).

Antioxidants added to freezing extenders have been tested on avian spermatozoa in several studies. Furthermore, by altering environmental conditions or using antioxidants during the freezing and thawing of sperm, many changes have been made to reduce the occurrence of oxidative stress in sperm. On the other hand, the role of ergothioneine and thawing temperature in the Lake extender has not yet been investigated. Our hypothesis for this experiment is that different levels of ergothioneine and thawing temperature will influence the quality of rooster sperm post-thawing, especially because high and low thawing temperatures moderate the critical stage of ice crystal formation.

MATERIALS AND METHODS

All materials were purchased from Merck (Darmstadt, Germany), unless indicated. This experiment was carried out following approval given by Animal Care Committee and Animal Research Ethics Board from Department of Animal Science, University of Tabriz, Iran.

Animals and Sample Preparation

The trial was carried out on 10 roosters (28 wk of age) were housed separately in cages (70 × 95 × 85 cm) held at 18°C to 20°C and subjected to 15 L:9 D photoperiod. Commercial feed of breeding flocks was selected for feeding the birds. Water was available ad libitum all the time.

For semen collection, the roosters were habituated to dorsoabdominal massage (Najafi et al., 2020). Therefore, dorsoabdominal massage technique was used to collect sperm twice a week. The ejaculates were immediately moved in a water bath (37°C) after semen selection. A total of 50 ejaculates (5 ejaculates/10 cocks) were prepared for semen analysis. Semen ejaculates with motility ≥80%, volume 0.2 to 0.6 mL, sperm concentration of

≥3 × 10⁹ sperm/mL, and abnormal morphology ≤10% were pooled and then divided into 6 aliquots for dilution in Lake extender comprised of D-fructose (8 g/L), sodium glutamate (19.2 g/L), polyvinylpyrrolidone (3 g/L), glycine (3.74 g/L), potassium citrate (5 g/L), magnesium acetate (0.7 g/L; pH 7.1 and osmolarity 310 mOsm/kg). Semen was extended at 37°C in Lake extender containing the different levels of ergothioneine (5 [E5], 10 [E10], 15 [E15], and 20 [E20] μM). Immediately following dilution, the prepared samples were drawn into 0.25 mL straws (IMV, L'Aigle, France) and polyvinyl alcohol was used for sealing, then equilibrated at 4°C for a period of 3 h (Mehdipour et al., 2021). Following equilibration, the straws were mounted on a rack 4 cm above the liquid nitrogen surface for 7 min, then the straws were plunged into liquid nitrogen. After a wk of leaving the straws in liquid nitrogen, they thawed individually in a water bath using 2 different conditions: at 1) 37 °C for 30 s, 2) at 60 °C for 5 s.

Motility Analysis

A sperm analyzer (CASA) system IVOS 12 (Hamilton-Thorne Biosciences, Beverly, MA) was applied for motility analysis. At first, the samples were thawed and diluted, then 3 μL of the prepared semen was placed onto a prewarmed chamber slide (37°C, Leja 4; 20 μm height; Leja Products, Luzernestraat B.V., Holland). Each sample had at least 200 cells analyzed. Total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, μm/s), velocity average path (VAP, μm/s), straight line velocity (VSL, μm/s), straightness (STR, %), and linearity (LIN, %) were assessed (Najafi et al., 2019a).

Plasma Membrane

For evaluating plasma membrane of rooster spermatozoa, 10 μL of frozen thawed sample was incubated with 100 μL hypo-osmotic swelling solution (sodium citrate 1.9 mM, fructose 5.0 mM in 100 mL of distilled water, osmolarity, 100 mOsmol/kg) for 30 min (Mehdipour et al., 2018). Under a phase contrast microscope (400× magnification), 200 spermatozoa were examined for intact (swelling in the tail region) and damaged plasma membranes (no swelling in tail region).

Sperm Morphological Abnormalities

Ten μL of semen sample was placed to eppendorf tubes containing 1 mL of Hancock solution for assessing sperm morphological abnormalities. Two hundred sperm per experimental treatment per replicate were evaluated with a phase contrast microscope and sperm total abnormalities were recorded (Najafi et al., 2019b)

Externalization of Phosphatidylserine (Annexin VIPI)

The phosphatidylserine detection kit (IQ Products, Groningen, the Netherlands) was applied for detecting the PS flip-flop motion across the membrane. At first, samples were washed in 100 μL calcium buffer and the cell concentration was readjusted to 1×10^6 sperm/mL. Next, 10 μL Annexin V FITC was added and it was then incubated for 20 min at 24°C. Ten μL of PI was then added to the cell suspension from the previous step and they were all incubated for 10 min at 24°C. Flow cytometry was used to evaluate the stained sperm right away, measuring fluorescence emission at 530/30 nm (FL1 canal) and 585/42 (FL3 canal). The viable sperm was designated as (An-/PI-), early apoptotic sperm as (An+/PI-), apoptotic sperm as (An+/PI+), and necrotic sperm as (An-/PI+) (Najafi et al., 2018).

Mitochondrial Activity

The Rhodamine-123 (RH-123) assay was applied for evaluating sperm mitochondrial activity. Semen samples were thawed, diluted and 250 μL of this sample were mixed with 5 μL of R123 solution (0.01 mg/mL stock) and PI solution (1 mg/mL stock). Before flow cytometry, the processed samples were incubated at 37°C for 15 min in a dark place. For determining the mitochondrial activity, the percentage of sperm with R123 high fluorescence and no PI fluorescence was recorded (Mehdipour et al., 2020).

Malondialdehyde (MDA) Concentrations

For defining MDA concentrations, thiobarbituric acid reaction was selected as an indicator for lipid peroxidation. As explained by Mehdipour et al. (2016), for obtaining protein precipitate, 1 mL of cold trichloroacetic acid (20% [wt/vol]) was added to 1 mL of diluted semen (250×10^6 spermatozoa/mL). They were then pelleted by centrifuging ($963 \times g$ for 15 min), and 1 mL of the obtained supernatant was eliminated and incubated in a boiling water bath at 100°C for 10 min with 1 mL of thiobarbituric acid (0.67% [wt/vol]). They were then cooled and finally the absorbance was detected by a spectrophotometer (T80 UV/VIS PG Instruments Ltd, Lutterworth, UK) at 532 nm.

Statistical Analysis

The normal distribution was assessed using the UNIVARIATE protocol and the Shapiro-Wilk test. The MIXED procedure of SAS software (SAS Institute V 9.1, 2002, Cary, NC) was selected for analyzing the data. The statistical model included the fixed effects of ergothioneine levels and thawing temperature, and their interaction. Since the model did not result in any significant interaction between thawing temperature and ergothioneine, we considered each factor as a main effect. The results were stated as Lsmmeans \pm SEM with Tukey's test to define the significant differences. It should be noted that the differences with P values < 0.05 were regarded significant.

RESULTS

The effect of the ergothioneine on the motility parameters of frozen sperm is depicted in Table 1. Ergothioneine levels of 5 and 10 μM resulted in significantly ($P < 0.05$) higher total motility (66.58 ± 1.44 and 72.11 ± 1.44 , respectively) and VAP (34.54 ± 0.89 , 37.28 ± 0.89 , respectively) compared to the control group. Furthermore, total motility (49.82 ± 1.44) and progressive motility (18.99 ± 0.83) were significantly ($P < 0.05$) lower in level 20 of antioxidant compared to the control and other treatments. For Amplitude of Lateral head displacement (ALH), the curvilinear velocity (VCL), and Beat cross frequency (BCF), no significant difference was detected between control, 5 and 15 (μM). For STR no significant difference was detected among groups.

Effects of different levels of ergothioneine on sperm membrane integrity, mitochondria activity, total abnormality, sperm viability, and MDA level are shown in Table 2. Higher ($P < 0.05$) significant of membrane integrity and mitochondria activity after freeze-thawing were observed in groups containing 10 μM ergothioneine (68.62 ± 1.24 and 69.12 ± 1.26 , respectively) compared to the control (50.09 ± 1.2 and 46.90 ± 1.26 , respectively), and 20 μM ergothioneine (48.01 ± 1.24 , and 44.83 ± 1.26 , respectively).

The obtained results of Annexin V/PI test after sperm freeze-thawing under different levels of ergothioneine are shown in Table 3. The viability amounts were significantly ($P < 0.05$) higher in 5 and 10 μM of ergothioneine (61.16 ± 1.78 , 65.75 ± 1.78 , respectively) compared to the control. Furthermore, 5 and 10 μM of ergothioneine

Table 1. Effect of different levels of ergothioneine on motility parameters of rooster sperm.

Antioxidant (μM)	TM(%)	PM(%)	VAP ($\mu\text{m/s}$)	VSL ($\mu\text{m/s}$)	VCL ($\mu\text{m/s}$)	LIN(%)	STR (%)	ALH (μm)	BCF (Hz)
0	51.72 ^{bc}	18.15 ^d	30.87 ^c	17.06 ^c	52.74 ^{ab}	32.35 ^b	55.59	4.22 ^{ab}	15.55 ^{ab}
5	66.58 ^a	27.50 ^b	34.54 ^{ab}	20.23 ^b	54.81 ^{ab}	37.00 ^{ab}	58.95	4.59 ^{ab}	17.68 ^{ab}
10	72.11 ^a	31.80 ^a	37.28 ^a	23.06 ^a	55.94 ^a	41.20 ^a	62.17	4.82 ^a	18.30 ^a
15	56.03 ^b	22.61 ^c	32.48 ^{bc}	18.70 ^{bc}	52.65 ^{ab}	35.80 ^{ab}	57.81	4.47 ^{ab}	16.40 ^{ab}
20	49.82 ^c	18.99 ^d	31.38 ^{bc}	16.89 ^c	51.01 ^b	33.32 ^b	54.41	4.18 ^b	15.08 ^b
SEM	1.44	0.83	0.89	0.66	1.16	1.36	2.63	0.15	0.78

^{a-d}Different superscripts within the same column indicate differences among groups ($P < 0.05$).

Abbreviations: ALH, mean amplitude of the lateral head displacement; BCF, mean of the beat cross frequency; LIN, linearity; PM, progressive motility; STR, straightness; TM, total motility; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

Table 2. Effect of different levels of ergothioneine on membrane integrity, mitochondria activity, total abnormality, viability, and MDA of rooster sperm.

Antioxidant (μM)	Membrane integrity (%)	Total abnormality (%)	Mitochondria activity (%)	Viability (%)	MDA (nmol/mL)
0	50.09 ^c	18.30 ^a	46.90 ^d	54.33 ^{cd}	4.14 ^a
5	63.22 ^b	15.23 ^{ab}	63.50 ^b	69.58 ^b	1.85 ^c
10	68.62 ^a	14.13 ^b	69.12 ^a	74.92 ^a	1.68 ^c
15	51.94 ^c	16.79 ^{ab}	52.25 ^c	58.87 ^c	3.58 ^b
20	48.01 ^c	18.78 ^a	44.83 ^d	52.35 ^d	4.19 ^a
SEM	1.24	0.89	1.26	1.27	0.11

^{a-d}Different letters within the same column show significant differences among the groups ($P < 0.05$).

Table 3. Effect of different levels of ergothioneine on live and apoptosis status of rooster sperm after freeze-thawing.

Antioxidant (μM)	Live sperm (%)	Apoptotic sperm (%)	Dead sperm (%)
0	43.31 ^{bc}	24.73 ^a	31.95 ^a
5	61.16 ^a	15.92 ^b	22.91 ^b
10	65.75 ^a	13.17 ^b	21.07 ^b
15	49.11 ^b	21.82 ^a	29.05 ^{ab}
20	40.61 ^c	25.79 ^a	33.59 ^a
SEM	1.78	1.14	2.17

^{a-c}Different letters within the same columns show significant differences among the groups ($P < 0.05$).

led to the lowest significant percentage of apoptotic and dead sperm compared to the control group.

Effects of thawing temperature on motility parameters of rooster sperm are displayed in [Table 4](#). The percentages of total motility and progressive motility resulted in significantly ($P < 0.05$) higher when sperm were thawed at 60°C (60.58 ± 0.91 and 24.76 ± 0.53 , respectively) compared to thawed sperm at 37°C. For other motility parameters (VAP, VSL, VCL, LIN STR, BCF, ALH), no significant difference was detected between thawed temperatures.

Effect of temperature degree on the percentage of membrane integrity, total abnormality, mitochondria activity, and MDA levels of thawed rooster sperm are displayed in [Table 5](#). The MDA level with 60°C (2.93 ± 0.07) was significantly the lowest compared with 37°C (3.25 ± 0.07). Likewise, no significant differences were detected between experimental groups for the

Table 4. Effect of 2 different thawing temperatures on motile parameters of rooster sperm.

Motility parameters	Thawing temperature		SEM
	37°C	60°C	
TM (%)	57.92 ^b	60.58 ^a	0.91
PM (%)	22.86 ^b	24.76 ^a	0.53
VAP ($\mu\text{m/s}$)	32.62	34.00	0.56
VSL ($\mu\text{m/s}$)	18.66	19.71	0.42
VCL ($\mu\text{m/s}$)	53.32	53.54	0.73
LIN (%)	35.04	36.82	0.86
STR (%)	57.20	58.37	1.66
ALH (μm)	4.41	4.51	0.09
BCF (Hz)	16.38	16.82	0.49

^{a-b}Different superscripts within the same row indicate differences among groups ($P < 0.05$).

Abbreviations: ALH, mean amplitude of the lateral head displacement; BCF, mean of the beat cross frequency; LIN, linearity; PM, progressive motility; STR, straightness; TM, total motility; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

percentage of total abnormality. The percentages of membrane integrity, viability and mitochondria activity were significantly ($P < 0.05$) higher when sperm were thawed with 60°C (58.2 ± 0.78 , 63.21 ± 0.80 and 56.85 ± 0.79 , respectively) compared to thawed sperm in 37°C. The lowest amount of thawed sperm was observed in MDA with 60°C (2.93 ± 0.07) compared with 37°C.

Effect of temperature degree on viability, apoptotic, and dead sperm of thawed rooster sperm are displayed in [Table 6](#). No significant differences were noticed among treatments for the percentage of viability, apoptotic, and dead sperm.

DISCUSSION

The high amount of polyunsaturated fatty acids (PUFAs) in the sperm membrane maintain the stability of the membrane during fertilization, but will increase the sensitivity of sperm to lipid peroxidation produced by ROS during storage ([Long and Kramer, 2003](#); [Min et al., 2016](#); [Mehdipour et al., 2017](#)). It is known that lipid peroxidation has a negative impact on the morphology, motility, and fertilization ability of bird sperm. This study was conducted to evaluate whether ergothioneine as an antioxidant can be used to increase the quality of post-thawing sperm. To achieve this goal, we tested different concentrations of ergothioneine in the extenders used for cryopreservation. In addition, we tested the 2 different methods for thawing straws. The results obtained from the flow cytometric analysis in the current research showed beneficial effects of ergothioneine on cryopreserved sperm cell quality and structure. The mitochondrial activity in the sperm after thawing was higher in groups containing 5 and 10 μM ergothioneine. These results are consistent with previously reported results ([Najafi et al., 2020](#)), which represents that antioxidants can stabilize mitochondria and plasma membranes from oxidative stress in rooster semen during cryopreservation. The energy from intracellular ATP storages responsible for sperm motility is produced by mitochondria, which covers axosome and related dense fibers of the midpiece of sperm cells ([Bucak et al., 2015](#)). In the process of sperm cryopreservation, the reactive oxygen species produced by free radicals, are neutralized due to the inhibitory effect of ergothioneine, thereby protecting cell organs including mitochondria. In the present study ergothioneine supplementation in cryopreservation medium resulted in a significant increase in the post-thaw sperm motility and

Table 5. Effect of 2 different thawing temperatures on plasma membrane integrity, mitochondria activity, and total abnormalities of rooster sperm.

	Thawing temperature		SEM
	37°C	60°C	
Membrane integrity (%)	54.55 ^b	58.2 ^a	0.78
Total abnormality (%)	17.11	16.19	0.56
Viability (%)	60.80 ^b	63.21 ^a	0.80
Mitochondria activity (%)	53.79 ^b	56.85 ^a	0.79
MDA (nmol/mL)	3.25 ^a	2.93 ^b	0.07

^{a-b}Different letters within the same row show significant differences among the groups ($P < 0.05$).

mitochondrial activity. The usage of possibly suboptimal antioxidant amounts and the interaction of the antioxidants with the egg yolk are 2 factors that help to explain these findings. This indicates that ergothioneine ions can protect fragile mitochondrial DNA and other mitochondrial components from damage by ROS caused by the electron transport chain. The results of Paul and Snyder (2010) support this theory, suggesting that when cells are exposed to H₂O₂, the damage of mitochondrial DNA increases in response to OCTN1 decline. In addition, the authors observed that cells depleted of ergothioneine also lead to increased protein carbonyls, lipid peroxidation, and increased sensitivity to H₂O₂-induced cell death (Paul and Snyder, 2010). The results of this study revealed that compared with the control group, ergothioneine treatment markedly decreased the percentage of apoptotic spermatozoa. Notably, it was stated that ergothioneine contains an organic cation transporter-1 (OCTN1) in the neuronal cells that can enter the central nervous system through the blood-brain barrier while preserving normal membrane fluidity leading to neuronal cell apoptosis decrease (Gründemann et al., 2005).

Cryopreservation causes chemical and physical stresses on the sperm membrane, thereby affecting the motility, viability, and membrane integrity (Baghshahi et al., 2014). The current study proved that concentrations of 5 and 10 μM ergothioneine during freezing–thawing processes can effectively protect rooster spermatozoa. Moreover, it is demonstrated that sperm plasma membrane have an influential role in viability, motility, and conjugating capability of spermatozoa (Gandeshmin et al., 2020).

In the current study, different concentrations of ergothioneine did not influence sperm morphology, which is consistent with the study of Najafi et al. (2014), which pointed out that in the sperm cryopreservation, ergothioneine could not increase the percentage of ram

Table 6. Effect of 2 different thawing temperatures on viable, apoptotic, and dead of thawed rooster sperm.

	Thawing temperature		SEM
	37°C	60°C	
Viable (%)	50.43	53.55	1.12
Apoptotic (%)	20.95	19.62	0.72
Dead (%)	28.61	26.82	1.37

sperm with normal morphology. Similarly, our results corroborate the previous studies in which antioxidant in cryopreservation did not influence the sperm morphology (Mehdipour et al., 2018). Studies have shown that in vitro manipulation and processing have no effect on sperm abnormalities (Chenoweth, 2005).

A critical factor in maintaining sperm quality and viability is thawing which few researchers have focused on (Yavas and Bozkurt, 2011). In general, thawing rate should be high to prevent recrystallization (Lahnsteiner, 2000). According to the results of the current study higher temperatures have beneficial effects to restore sperm metabolism and membrane stability. It has also been shown that through this thawing procedure, the recrystallization and formation of ice crystals during thawing process was reduced or the reactivation of enzyme activity improved (Lahnsteiner, 2000). Another remarkable reason is exposing sperm to the concentrated solute and glycerol for a shorter time, and also faster restoration of the extracellular and intracellular equilibrium compared with slow thawing (Andrabi, 2009). Thawing from -196°C to 4°C is known as a critical phase owing to potential recrystallization (Purdy, 2006). Concerning the thawing rates, higher temperature (60°C for 5 s) compared to 37°C for 30 s led to better sperm quality. Total and progressive sperm motility were consistently and significantly better for the fast thawing temperature (60°C for 5 s). In line with our result, Malo et al. (2019) reported that total and progressive motility at 0 h and 1 h ($P < 0.001$) was higher for 60°C thawing temperature comparing 37°C. Moreover, our findings are in line with those of Iaffaldano et al. (2016); that thawing turkey sperm at 50°C/10 s were more proficient than 4°C/5 min. The positive correlation between sperm motility and thawing rate observed in the present study corroborates Ahmad (1984), who stated that more rapid thawing rates lead to better sperm motility and acrosomal integrity.

The other fundamental result of these experiments was the obvious evidence of the effect of the thawing procedure on rooster spermatozoa freezability. Thus, our results demonstrated that mitochondria activity and membrane integrity were significantly affected by the thawing procedure. When sperm was thawed at 60°C for 5 s, the results of mitochondrial activity and membrane integrity were significantly higher than the results of thawing at 37°C for 30 s.

CONCLUSION

Rooster medium supplementation with 5 and 10 μM ergothioneine in the semen extender improves post-thawing quality. Moreover, total and progressive sperm motility, mitochondria activity and membrane integrity were significantly increased by thawing at 60°C in 5 s. It could be concluded; addition of 5 and 10 μM ergothioneine in the semen extender and thawing temperature at 60°C in 5 s can be an efficient strategy to preserve rooster cryopreserved semen quality.

DISCLOSURES

The authors declare no conflicts of interest.

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