

POSSIBLE PROTECTIVE MECHANISMS OF COENZYME Q10 ACTION ON SPERMATOZOA DURING CRYOPRESERVATION OR COOLED-STORED CONDITION

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Abstract

Artificial insemination (AI) with frozen or cooled-stored semen plays a key role in the widespread distribution of germplasm of elite livestock resources and the protection of endangered species. Cryopreservation provides long-term preservation of sperm and also encourages a greater exchange of genetic material between distant populations. However, freezing has some detrimental effects on sperm, including premature induction of acrosome response, reduced sperm motility, reduced viability, and impaired sperm DNA integrity and fertility. The transition of the membrane phase occurs when the sperm cools down, and lipid accumulation damages the micro-domain, thereby impairing membrane functions, leaving a gap between the gel and the liquid membrane region. Coenzyme Q10 (CoQ10) is a vital lipophilic molecule found in all respiratory eukaryotic cells, including spermatozoa. When such a lipophilic antioxidant is added to the sperm, it can directly diffuse into the polyunsaturated lipid chain present in the plasma membrane, thereby affecting the structure and function of the sperm by generating energy and preventing reactive oxygen. Coenzyme Q10 treatment of sperm from various species improves sperm quality during cryopreservation and cooled-stored condition. It is, however, unclear how this antioxidant affects sperm to improve survival during freezing or cooled-stored condition. Thus, this review highlights the potential protective mechanisms of coenzyme Q10 action during the sperm freezing process.

Keywords: coenzyme Q10; cryopreservation; cooled-stored; mechanism; spermatozoa

Abbreviations: TM, total motility; ATP, adenosine triphosphate; LPO, lipid peroxidation; ROS, reactive oxygen species; OXPHOS, oxidative phosphorylation; mPTP, mitochondrial permeability transition pore; AI, artificial insemination; CoQ10, coenzyme Q10; ICM, inner cell mass; IVF, in vitro fertilization.

INTRODUCTION

Artificial insemination (AI) is a method of rapidly spreading exclusive livestock genetic resources. For the detection of genes in a population, AI is a more economical, simpler and a more successful technique than embryo transfer technology or natural mating (53). Cryopreservation of sperm plays an important role in protecting endangered species and livestock AI. This freezing process preserves semen for long periods of time and promotes the exchange of genetic material between distant populations. In the course of semen processing procedures for cryopreservation, sperm is subjected to various stresses, destabilization of the plasma membrane with impaired motility and function (1). During cryopreservation, sperm damage occurs due to intracellular ice crystals formation (32), phase shifts caused by membrane changes due to cooling (52, 78), and osmotic stresses associated with changes in cryoprotectants from freezing media (59). In addition, oxidants (reactive oxygen species and lipid peroxidation) are increased (8, 9, 43) and seminal antioxidants are decreased (40). Consequently, the integrity of sperm DNA during cryopreservation is impaired (44), resulting in a decrease in sperm quality. During the freezing process, only about half of the sperm survive and the surviving sperm has reduced fertilization capacity (26). The membrane phase transition occurs when the sperm cools and lipid accumulation causes damage to the micro-domains, thereby disrupting the function of the membrane, and a gap forms between the gel and the area of the liquid membrane (6).

Coenzyme Q (CoQ) is a naturally occurring molecule positioned in the hydrophobic domain of the phospholipid bilayer of all biological membranes. Shortly after its discovery, it was considered to be the necessary electron transport chain component in mitochondria, with mitochondria being particularly abundant. Since then, more cellular physiological effects have been reported, including as an antioxidant, in signaling, and in death prevention. This antioxidant Q_{10} (1,4-benzoquinone) or ubiquinone, in most cases abbreviated as CoQ10, is also a fat-soluble biomolecule ubiquitous in mitochondria of almost all bacteria and animals (67). Because of its fat-like nature, it releases phospholipids into the cell membrane and

protects the sperm plasma membrane from these transformations [76]. The letter Q represents the

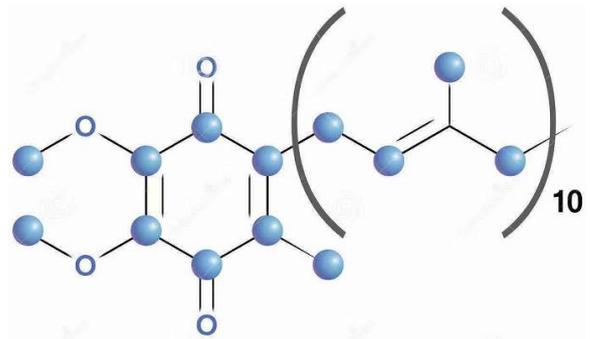


Figure 1. Structure of coenzyme Q10.

chemical group of quinone, and the number 10 represents the isoprenyl repeat of the tail of the chemical structure $(CH_2=C(CH_3)-CH=CH-)$ as illustrated in Figure 1. The repeat number of the isoprenyl varies from species to species (from 6 to 10). For example, in humans, the predominant form is coenzyme Q10 whereas in rats the principal form is coenzyme Q9 (16, 25). Such changes occurring in the number of isoprenyl repeats affects the chemical properties of the molecule, such as motility and membrane protein interaction, thereby influencing the stability in the mitochondrial membrane (77).

Coenzyme Q10 also has a protective effect on cells from free radicals and can also affect sperm function, thereby contributing to the production of energy for the spermatozoa [49], with a concomitant increase in human sperm motility (72). There are several reports showing an improvement in cryo-resistance and cryo-survival of CoQ10 treated sperm of goats (80), horses (81), pigs (60), rooster (51), cattle and buffalo (62) and sheep (27) during the freezing process. In addition, several studies have also been conducted on various types of sperm in vitro, such as bull (14), ram (80) and horse (23, 57). This evidence indicates that using coenzyme Q10 as an additive to sperm can also improve its quality in cooled-stored condition.

In addition to these important roles of coenzyme Q10 in sperm freezing, other reports have suggested that this lipophilic molecule is biologically active in other systems, tissues and ovaries. For example, in reproductive biology, coenzyme Q10 has been shown to play a crucial role in the reproduction of both male and female systems (12). We have also reported reduced

levels of CoQ10 and increased oxidation in the seminal plasma and sperm cells of infertile men with idiopathic asthenozoospermia (10). Coenzyme Q10 administration in these patients caused an upsurge the content of CoQ and improved its oxidation status and semen kinetics features (11, 74). In the female reproductive system, early reports of CoQ10 content in various rat tissues after exogenous supplementation suggested that the adrenal glands and ovaries take up large amounts of CoQ10. The total coenzyme Q10 content of these organs more than doubled, while other organs did show only modest increases (19).

Research by Stoikovic et al (71) studied the role of coenzyme Q10 as a supplement for bovine embryos cultured in vitro, and found a significantly higher cleavage rate of early embryo, blastocyst formation rate, hatching rate, percentage of blastocyst expansion and larger size of the inner cell mass (ICM). In the same paper, the authors also observed an upsurge in ATP content in the group of embryos cultured with CoQ10. All those parameters propose enriched embryo quality. Based on this observation, Ben-Meir et al recently displayed that coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging (17), emphasizing the key role of coenzyme Q10 in reproductive aging. This is depicted using animal models of insufficient CoQ10 synthesis and showing a reduction in ovarian reserve that can be saved by supplementation with CoQ10. In human clinical studies, we evaluated that coenzyme Q10 levels in follicular fluid are associated with oocyte fertilization and embryo grading (75). Compared with dysmorphic oocytes, mature coenzyme Q10 levels are significantly higher. Likewise, coenzyme Q10 levels resulted in significant enhancement of grades I-II as against III-IV embryos.

Recently, Akarsu et al (4) have highlighted the role of coenzyme Q10 in protecting follicular lipoproteins from oxidation and maintaining their functionality. In addition, different studies indicate that coenzyme Q10 supplementation (600 mg per day for 60 consecutive days) has the ability to improve ovarian response in women with reduced ovarian reserve (33, 79). Such treatment is required to improve responsiveness in terms of reduced gonadotropin requirements during IVF (in vitro fertilization), thereby promoting pre-stimulation of follicles. It is worth noting that in recent

human studies, the authors have verified the bioavailability of coenzyme Q10 to the follicular environment and its biological effects. This study aimed at verifying whether coenzyme Q10 provided as an oral supplement at a dosage considered as a dietary supplement, which according to the European Union is 200 mg/day for 30 days, could cause an increase in coenzyme Q10 follicular fluid content. Finally, it was found that coenzyme Q10 can improve the oxidative metabolism of follicular fluid and oocyte quality, especially in women over 35 years old. A summary of the research studies to date that demonstrate a direct effect of coenzyme Q10 on sperm, testosterone, tissues and various systems and organs is presented in Table 1.

However, so far, little research has been conducted on the possible protective mechanisms of coenzyme Q10 action on sperm during cryopreservation or cooled-stored condition. Hence, this review discusses the current progress in understanding of the possible protective mechanisms of coenzyme Q10 action on spermatozoa during cryopreservation or storage under cooled conditions.

Possible protective mechanisms of co-enzyme Q10 action on spermatozoa during freezing or cooled-stored condition

Co-Q10 is part of the mitochondrial respiratory chain and plays two important roles, firstly, in metabolism, and secondly, as a fat-soluble antioxidant to protect sperm membranes and related lipoproteins(28). This lipid-soluble antioxidant concentrates in the mitochondria of another cell, such as sperm, and plays a crucial role in energy metabolism (22). Coenzyme Q10 also acts as a fat-soluble chain cleavage antioxidant and participates in the regeneration of endogenous antioxidants, such as superoxide dismutase, which in turn inhibits oxidative stress (56). In vivo studies carried out in humans and rats have confirmed that the addition of coenzyme Q10 to the diet improves overall antioxidant capacity and sperm quality (34, 54), while the exogenous administration of this fat-soluble antioxidant improved sperm motility in men (11). There is a positive relationship between the concentration of coenzyme Q10 and overall sperm motility (48). Sperm motility and viability are positively correlated with mitochondrial function, and also indicates a significant relationship between ATP

concentration with motility and energy index (57). For example, in avian species, energy production for sperm motility is provided by oxidative phosphorylation in mitochondria, rather than glycolysis (45). Thus, when supplying molecular oxygen during in vitro storage of sperm, more ATP production is needed. In addition, molecular oxygen is involved in lipid degradation by peroxidation (46). High peroxide index phospholipids are the

main components of avian sperm (45). Although CoQ10 protects sperm lipids from peroxide damage, promotes membrane stability, removes superoxide anion and peroxides, and also plays a central role in the maturation of the sperm and its development (3), these membrane-bound phospholipids are lost when storing spermatozoa in vitro, which, in turn, leads to a decrease in the viability and integrity of the plasma membrane (46).

Table 1. A summary of the main research studies conducted on coenzyme Q10 and its reported effects on sperm, testosterone, various organs, tissues and systems

Dose (mode of treatment)	Duration	Study population	Effects	Reference
200 mg day ⁻¹ (orally)	30 days	Infertile couples	Improvement of follicular fluid content and oocyte quality	(35)
600 mg day ⁻¹ (orally)	60 days	Female	Increased ovarian reserve in women	(33), (79)
22 mg kg ⁻¹ (injected subcutaneously)	12 weeks	Aged mice	Increased oocyte mitochondrial function, ovulation and pregnancy rates	(17)
30 µM (exogenous administration)	24 hours	Cattle and buffalo semen	Prevents LPO and DFI	(62)
200 mg day ⁻¹ (orally)	21 weeks	Patients with hormonally untreated carcinoma of the prostate	No effects	(37)
900 mg day ⁻¹ (orally)	12 months	Infertile men with idiopathic oligoasthenoteratozoospermia	No effects	(64)
10 mg kg ⁻¹ day ⁻¹ (orally)	20 days	Male rats with isoproterenol-induced reproductive toxicity	Increased	(34)
10 mg kg ⁻¹ day ⁻¹ (intraperitoneally)	5 days	Male rats with sodium arsenite-induced reproductive toxicity	Increased	(31)
200 mg day ⁻¹ (orally)	8 weeks	Patients with polycystic ovary syndrome	Decreased	(39)
300 mg day ⁻¹ (orally)	26 weeks	Infertile men	No effects	(63)
125, 250, and 500 mg kg ⁻¹ day ⁻¹ (orally)	96 days	Bilateral orchidectomized male mice	No effects	(68)

However, in studies conducted on rooster (55), cattle (14), and man (72), it was found that the inclusion of coenzyme Q10 to sperm improved the integrity of the plasma membrane at post-thaw. Therefore, since coenzyme Q10 acts as a fat-soluble antioxidant, it is postulated that it can diffuse directly into the polyunsaturated lipid chain present in the plasma membrane, thereby protecting the plasma membrane of the spermatozoa.

In addition to the mechanisms that protect the integrity of the plasma membrane of these species, CoQ10 is also able to remove free radicals that cause damage to DNA proteins and lipids (2). Previous studies have also depicted that coenzyme Q10 can maintain sperm quality, such as weak sperm in humans (41) and normal sperm (72), as well as rooster sperm (55) and horse sperm (57). Besides, a study by Nath and colleagues (55) also demonstrated *in vitro* studies by incubating pooled semen from Rhode Island Red roosters with 0.3 mg/mL of coenzyme Q10 at 4°C for 36 h, which significantly improved sperm motility, viability, and integrity of their plasma membranes. Coenzyme Q10 is a stronger antioxidant than other fat-soluble antioxidants (such as vitamin E), and can also oxidize vitamin E into strong antioxidants (24). It appears to shield sperm from the adverse effects of oxidative stress on plasma membrane integrity and functionality during the process of freezing. Despite the ability of CoQ10 to enhance several sperm quality parameters, its addition to sperm extender improved the motility, functionality, and integrity of the rooster spermatozoa acrosome (for example, in the cooled-stored condition), which subsequently increased fertility (66). However, its antioxidative effect on other sperm parameters (namely mitochondrial activity, the activity of antioxidant enzymes and lipid peroxidation) have not been investigated. Therefore, it is recommended that future studies explore the effect of CoQ10 on these parameters, most importantly on mitochondrial activity, which is a source of energy for the spermatozoa when stored in the chilled state.

In addition, the efficacy of coenzyme Q10 has been demonstrated in the function of sperm membranes of horses and goats (80, 81). When the antioxidant properties of CoQ10 directly neutralize the ability of lipid peroxide radicals, its antioxidant properties also increase the action potential of mitochondria (48). Besides,

coenzyme Q10 can regenerate α -tocopherol from free radicals anions of α -tocopheroxyl (76), and can also remove free radicals (18), preventing the accumulation of cytotoxic aldehydes. It acts as an energy carrier that facilitates the synthesis of adenosine triphosphate (ATP) (42) and regulates (mPTP) mitochondrial transition pore permeability (76). Mitochondria are vital and distinctive organelles located in the middle of sperm. These organelles are included in the main part of the flagellum. In addition to ATP production, mitochondria are also involved in various cellular functions such as calcium homeostasis, ROS production, and its core apoptotic pathway (7). In fact, changes in mitochondrial integrity and function are associated with decreased sperm quality parameters (especially parameters related to decreased motility, viability, and DNA integrity) (7). A recent study by Yousefian and colleagues (80) showed that the protective effect of coenzyme Q10 at 1 μ M on mitochondrial activity of a buck sperm may be associated with decreased MDA content and subsequent maintenance of mitochondrial membrane integrity. This finding supports the hypothesis of other researchers (7) that the sperm motility and viability may be relatively dependent on mitochondrial activity.

Furthermore, the administration of CoQ10 improved sperm variables values in men with sperm pathology (11, 63), in stallions (81), in bulls (36), cocks (50, 51) and billy goats (80) following cryopreservation. There was also a higher proportion of mitochondrial activity, along with a lower percentage of apoptotic-like changes in the semen of rams following the freeze-thaw process (50), which is likely attributable to CoQ10 functional protective mechanisms. Similarly, the storage of spermatozoa in the cooled-stored condition revealed that the addition of 5 μ M CoQ10 to cockerel sperm extender preserved the quality of the cooled-stored sperm by preserving mitochondrial activity and reducing lipid peroxidation (50). Besides, the optimal dose of CoQ10 in lake solution maintained sperm fertility during the cooled-stored process. Although storage of the diluted sperm with 5 μ M CoQ10 supplementation showed good efficacy within 24 h, it was not as pronounced as compared within 48 h of preservation. This suggests that it can be a useful strategy for transporting chilled stored semen to different locations without compromising semen quality.

This lipid-soluble antioxidant functions to maintain the quality of sperm by mediating the modulation of mitochondrial permeability in transition pores, and can also deactivate potential depolarization of mitochondrial membranes, reduce ATP levels, and decrease caspase-9 activity (76). Moreover, there was a decrease in TM, membrane integrity, mitochondrial active potential, and acrosome integrity, according to some previous studies while the percentage of LPO and dead sperm was higher in groups receiving 5 and 10 μM CoQ10. This indicates that a relatively higher concentration of antioxidants at higher concentrations than specific threshold concentrations can be toxic and also cause oxidative effects (21, 58). It can be assumed that higher concentrations of CoQ10 may turn into its toxic redox form, semiquinone, which can have a noticeably harmful effect on spermatozoa (29).

Nonetheless, coenzyme Q10 was most effective when added to a centrifuge extender rather than a freezing extender. Although centrifugation during the process of freezing may be harmful and may result in mechanical damage to the plasma membrane (15), the inclusion of coenzyme Q10 prior to centrifugation appears to offset the lack of antioxidants in stallions with poor sperm freezing capability. At centrifugation, sperm reach the apex of their energy metabolism, thereby facilitating material uptake and ion exchange (69). Antioxidant supplementation associated with seminal plasma extraction, and thus byproducts of sperm metabolism (e.g., ROS), increases sperm viability during the process of cryopreservation (15). Coenzyme Q10 can stimulate greater sperm cell stability in the plasma membrane of stallions classified as poorly frozen compared to controls, indicating the potential for inhibition by this antioxidant to early capacitation. Besides, this efficacy of coenzyme Q10 during the process of cryopreservation, it can also improve overall motility and reduce lipid peroxidation after cooling equine semen to 5°C for 72 h (57). However, further research is needed to determine concentrations and protocols more effectively, especially with regard to individual sensitivities and variability in ejaculation. Conversely, the inclusion of an antioxidant directly to the semen at a concentration of 1 mM (such as CoQ10) does not improve the parameters related to the motility of frozen or

cooled semen of equine; but according to observations, it can impair sperm quality eventually. This effect, combined with data from other authors, lead us to suggest that species-specific influences may depend not only on the type of antioxidant added but also on its concentration. The ameliorative ability of coenzyme Q10 can also be related to its role in testicular tissue, which improves spermatogenesis rather than in spermatozoa directly, emphasizing the significance of increasing its presence in feed rather than in sperm extender. However, some studies have also shown that lipid peroxidation is not significant during semen preservation. For example, stallion sperm appears to be resilient to induced membrane peroxidation. Therefore, further studies are needed to elucidate the role of exogenous antioxidants' influence in the sperm of stallion and/or to choose the optimal concentration of antioxidants, the route of administration and their possible effects at the level of spermatogenesis. Moreover, the results should be improved by increasing the number of stallions in order to obtain more detailed conclusions.

The supplementation of CoQ10 to the sperm diluent as an antioxidant significantly improved the overall motility of buffalo and cattle sperm compared to their control group, especially after thawing (62). This antioxidant action is evident through its protective mechanism to reduce the strong effect of ROS on the motility of sperm and damage to the acrosome, especially in semen after thawing (72). Besides, in studies performed *in vitro* on bovine (36) and human (72), it was depicted that supplementation CoQ10 to semen extender prevented chromatin fragmentation at ambient temperature for 5 to 6 h of incubation. CoQ10 is able to remove free radicals that can damage DNA proteins and lipids, degradation of the DNA base and DNA fragmentation (36, 65), which is important as spermatozoa with impaired DNA lose their capability to fertilize an egg and have a low conception ratio (3). For instance, in a study of 32 infertile men, CoQ10 inhibited the production of H_2O_2 in seminal plasma and seminal fluid (5). Similarly, in an *in vitro* study of 22 sperm samples from spermatozoa of attenuated men, incubation with 50 mM CoQ10 significantly improved their sperm motility after the cryopreservation process. Besides, the addition of 60 mg of CoQ10 to 17 infertile men increased their rate of fertilization

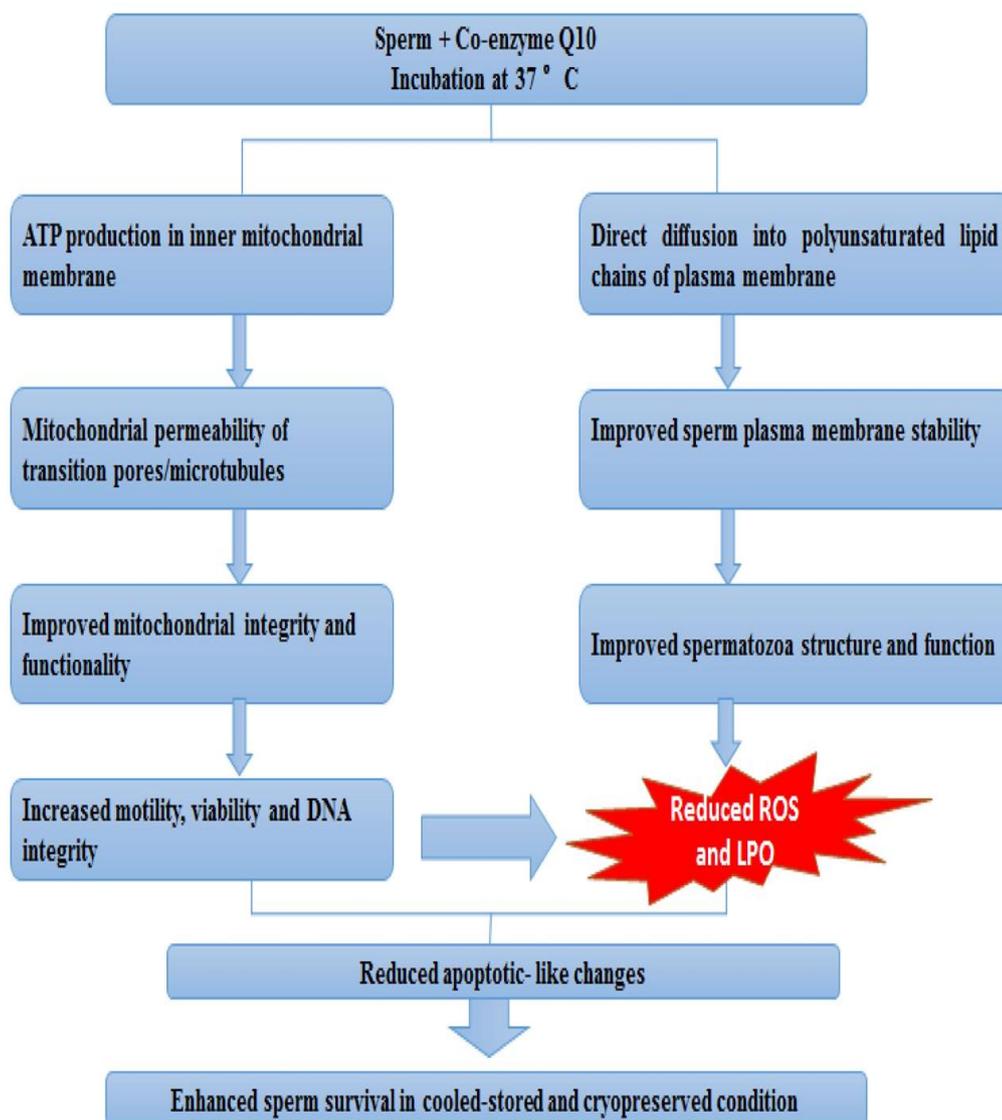


Figure 2. Possible mechanisms for improved cryopreservation and cooled storage of co-enzyme Q10 treated sperm.

without altering sperm parameters (41). These results show that the effects of various concentrations of coenzyme Q10 on sperm motility are beneficial and improve sperm motility, making it an ideal antioxidant for incubating sperm based on the shelf life prior to assisted reproductive techniques (38).

Regarding the frozen semen of dogs, this fat-soluble antioxidant has the ability to improve the motility progression after thawing (70). However, in this case, the effect is affected by a single factor that may be associated with membrane integrity, mitochondrial potential, and lipid peroxidation. On the other hand, the

storage of boar semen also had an affirmative influence on sperm survival and acrosome integrity in the presence of different concentrations of coenzyme Q10. Although little is known about the mechanism by which CoQ10 affects sperm cell viability, it can be said to be an important antioxidant previously used for the preservation of sperm prior to AI. However, in an experiment, it was specifically observed on days 3 and 7, that only the highest dose of CoQ10 (8 μ M and 1 mM) caused a statistically significant increase in cell viability and percent acrosome integrity. However, there is a great interest in the extent to which spermatozoa are

affected if the storage time is extended to more than 7 days and the concentration of CoQ10 exceeds 1 mM. This can, however, be determined through further research. On the other hand, decreased sperm motility may be associated with mitochondrial damage. CoQ10 is the only lipid-stabilized electron carrier in the mitochondrial electron transport system. The energy provided is in the form of ATP, which can be synthesized by glycolysis in the cytoplasm [30], or by oxidative phosphorylation (OXPHOS) in mitochondria (47). The transfer of ATP produced in the mitochondrial membrane by OXPHOS into the microtubules stimulates motility. Stress factors that can inhibit oxidation and phosphorylation in mitochondria can be considered as effectors that contribute to the depletion of electrons and the formation of superoxide radicals responsible for the loss of sperm membrane integrity, motility, vitality, and fertility. CoQ10 is an integral part of the redox and proton host components of the mitochondrial respiratory chain and plays a key role in energy metabolism and also has powerful antioxidative properties that protect the integrity of sperm cellular membranes (29) during cryopreservation or cooled-stored condition. This protective mechanism of CoQ10 action on sperm cell membrane integrity may reasonably suggest that this lipophilic molecule can diffuse into the cell membrane bilayer phospholipids. In that location it can aid in the maintenance/recycling of vitamin E, one of the major cell membrane antioxidants (73) which is also present naturally in the semen (20). These possible mechanisms for improved cryopreservation and cooling storage of coenzyme Q10 treated sperm are illustrated in Figure 2.

Furthermore, sperm viability, fertilization, and storage efficiency are extremely reliant on the expression of the effective antioxidant ability in these cells and the surrounding seminal plasma. It has been revealed that exogenous lipids from a diluent are closely related to the plasma membrane of sperm, preventing membrane damage during cooling/freezing. This can then successfully preserve sperm viability, motility, fertility (61). There were also experiments aimed at studying the mechanisms by which antioxidants, especially coenzyme Q10, played a protective role in the spermatozoa during cryopreservation or cooled-stored condition. It is well known that CoQ10 is a highly lipophilic molecule and as such, it leads

us to assume that when this antioxidant is transported from peripheral blood to human testis and accessory glands (10), it has the capability to diffuse through the phospholipid bilayer of the cell membrane. However, it is not clear whether this transmission is passive or active and this warrants further investigation. Nevertheless, the distribution of CoQ10 between intracellular and extracellular compartments appears to be an active process. Besides, there is a positive correlation between the level of coenzyme Q10 in seminal plasma and sperm motility (13). Consequently, it can be argued that in some cases, an increase in stress due to oxidation in spermatozoa can somehow suppress levels of CoQ10, which can negatively affect the bioenergetic effects on spermatogenesis.

However, a deeper understanding of the molecular mechanism of coenzyme Q10 may lead to a greater understanding of infertility (13). In contrast, Littaru and colleagues (42) observed that the addition of coenzyme Q10 increased the level of ubiquinol-10 in circulating lipoproteins, while low-density lipoproteins in humans also increased resistance to lipid peroxidation, thereby reducing oxidative stress. The contents of the reduced form and the oxidized form (ubiquinol / ubiquinone) of CoQ10 were measured in 32 infertile patients, and the concentration of organic peroxide in seminal plasma and semen was also measured (5). There was a significant positive correlation between the content of ubiquinol, sperm count and motility. There was also an inverse correlation between the content of ubiquinol and the concentration of organic peroxide. These results indicate that ubiquinol-10 hinders organic peroxide formation in seminal plasma and semen, thereby reducing the oxidative stress that may cause damage to the sperm eventually during the cryopreservation or cooled-stored condition.

CONCLUSIONS AND FUTURE CONSIDERATIONS

The evidence suggests that the diffusion rate of coenzyme Q10 through the phospholipid bilayer (lipid chains) of the sperm plasma membrane or in the hydrophobic domain of the phospholipid bilayer of the inner membrane system of the mitochondria, coupled with the production of ATP in the mitochondrial inner membrane or the degree of mitochondrial permeability of the transitional pore, may

regulate sperm resistance, making it undergo phase transformation during several cooling processes. After incubation of spermatozoa with CoQ10, increased spermatozoa membrane stability together with increased integrity and functionality of the mitochondrial membrane leads to a decrease in apoptosis-like changes, which can cause an increase in sperm survival. Increased osmotic tolerance of spermatozoa can be associated with increased integrity of the plasma membrane as a result of the use of coenzyme Q10 for cryopreservation of sperm or cooling during storage. A decrease in ROS and LPO in spermatozoa treated with coenzyme Q10 can also lead to a decrease in apoptosis-like changes, thereby improving sperm quality during cold-storage or cryopreservation process. Hence, it is recommended that future studies should pivot on the selection of more potent alternative antioxidants with properties to influence the diffusion rate into polyunsaturated lipid chains or to regulate the permeability of mitochondrial transition pore into the phospholipids bilayer of the sperm plasma membrane. Such insights will help increase the survival of the spermatozoa and also influence fertility rate after the AI process following cryopreservation or after cooled storage. Furthermore, since CoQ10 has proved effective in the field of spermatozoa cryopreservation for a wide variety of animal species, it is recommended that future studies focus on oocyte and ovarian tissue cryopreservation using CoQ10 as, presently, there is less information in this sphere.

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Abbreviations: TM, total motility; ATP, adenosine triphosphate; LPO, lipid peroxidation;

ROS, reactive oxygen species; OXPHOS, oxidative phosphorylation; mPTP, mitochondrial permeability transition pore; AI, artificial insemination; CoQ10, coenzyme Q10; ICM, inner cell mass; IVF, in vitro fertilization.

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