Original Article

Preservation of Buck Semen Quality During Chilling Storage Using Coenzyme Q10

Mokhtar Mohajer[1], Nader Asadzadeh[1*], Hoda Javaheri Barfourooshi[1], Navid Dadashpour Davachi[2], Reza Masoudi[1]

1. Animal Science Research Institute of Iran, Agricultural Research Education and Extension Organization, Karaj, Iran.
2. Department of Research, Breeding and Production of Laboratory Animals, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran.

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ABSTRACT

Background: Storing spermatozoa in a cold environment decreases spermatozoa metabolism while maintaining sperm fertility. Because of their unique physiological characteristics, sperm cells' reproductive ability in small ruminants is reduced by cooling.

Objectives: This study aimed to assess the impact of supplementing chilling medium with coenzyme Q10 (CoQ10) on the quality parameters of buck's semen after chilling at 4°C.

Methods: Semen was collected, diluted, and divided into five groups with different CoQ10 supplementation concentrations (0, 1, 2, 5, and 10 µM CoQ10). Collected semen were stored at 4°C for 50 h. The motility, mitochondrial activity, membrane integrity, viability, and lipid peroxidation were measured after 0, 25, and 50 h of chilling.

Results: According to the findings, different concentrations of CoQ10 had no impact (P>0.05) on semen at time 0 of storage. After 25 and 50 h of storage, supplementing the chilling medium with 5 µM CoQ10 increased (P≤0.05) progressive motility (PM), total motility (TM), viability, mitochondrial activity, and membrane integrity compared to other groups. Furthermore, 5 µM CoQ10 produced less lipid peroxidation (P≤0.05) after 25 and 50 h than the other groups.

Conclusion: Adding 5 µM CoQ10 to the buck's semen cooling medium is an effective procedure for protecting the quality of buck spermatozoa during cooling storage.

Keywords: Cooling, CoQ10, Goat, Quality, Semen

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*** Corresponding Author:**
Nader Asadzadeh, Associate Professor.
Address: *Animal Science Research Institute of Iran, Agricultural Research Education and Extension Organization, Karaj, Iran.*
Phone: *+98 (21) 46029772*
E-mail: *nader.asadzadeh4@gmail.com*

Introduction

Preserving spermatozoa in a cold situation decreases its metabolism while protecting reproductive functioning. Scientists have attempted to improve the output of sperm preservation after 24 h (Zarei et al., 2021; Javaheri et al., 2023). The cooling procedure reduces fertility in small ruminants due to the unique physiological characteristics of their spermatozoa (Zarei et al., 2021). A standard medium for sperm preservation in small ruminants is a tris-based extender composed of tris, citric acid, and fructose (Masoudi et al., 2021). Adding exogenous antioxidants to sperm extenders could help preserve sperm quality in farm animals during storage (Sharafi et al., 2015).

Coenzyme Q10 (CoQ10) comprises a benzoquinone ring and a lipophilic isoprenoid side chain in mitochondrial inner membranes. It plays an effective role in the electron transport chain used in ATP synthesis and acts as a liposoluble chain-breaking antioxidant (Masoudi et al., 2018). The testis contains high concentrations of CoQ10, demonstrating its cell-protective impact against free radicals. CoQ10 also influences sperm function by facilitating the production of sperm energy (Yousefian et al., 2018), and in human sperm, it works as a motility enhancer (Telavi et al., 2013). Other animal studies show that CoQ10 improves sperm cryo-resistance in horses (Yousefian et al., 2014), roosters (Masoudi et al., 2019), and goats (Yousefian et al., 2018). Because no research has been conducted to assess the impact of chilling medium supplementation with CoQ10 on the buck sperm quality during chilling storage, this study was conducted to determine the effects of CoQ10 on progressive motility (PM), total motility (TM), mitochondrial activity, viability, membrane integrity, and lipid peroxidation of buck cooled semen.

Materials and Methods

Processing of semen

The samples of semen were collected from 5 Saanen goats via an artificial vagina. The samples were selected if their abnormal morphology, sperm concentration, motility, and volume were ≤15%, ≥3×10^9 spermatozoa/mL, ≥70%, and 1-2 mL, respectively. The samples were pooled and diluted in the extender (fructose, 1.26 g/100 mL; citric acid, 1.64 g/100 mL; glycerol, 5% v/v; soybean lecithin,1.5% w/v; Tris, 3.07 g/100 mL; osmolarity,425 mOsm; pH, 6.8). Then, the samples were divided into 4 aliquots as follows: Medium without CoQ10 (Q0), medium with 1 µM (Q1), 2 µM (Q2), 5 µM (Q5) and 10 µM (Q10) CoQ10. The final concentration was 400×10^6 sperm/mL.

Evaluation of the semen quality

Sperm motility parameters were assessed via the sperm class analysis program. About 400 spermatozoa in each sample were evaluated, and the PM (%) and the TM (%) were reported (Khodaei-Motlagh et al., 2022). Sperm viability was assessed using eosin-nigrosin staining, which colors the head of dead sperm cells and not the live ones (Heidari et al., 2022). The hypo-osmotic swelling test looked into how the membrane worked (Masoudi et al., 2022). A total of 300 spermatozoa were counted. As a functional membrane, swollen tails sperm cells were recorded. Mitochondria activity was measured using flow cytometry (Zarei et al., 2022). At a flow rate of 100 cells/s, each assay analyzed approximately 10000 events. The FlowJo program was used to process the data. The peroxidation of lipids was assessed via the concentration of malondialdehyde (MDA) using a spectrophotometer set (UV-1200, Japan) at 532 nm (Sharafi et al., 2015).

Statistical analysis

The current study's data (6 replicates) were analyzed using SAS software, version 9.1 GLM procedure. Tukey's test was used to determine statistical differences between groups. The differences were statistically significant if the $P<0.05$.

Results

Motility parameters

Table 1 presents the impact of CoQ10 on the TM and PM of a buck's semen. At storage time 0, there were no significant differences between treatments. Q5 had significantly higher ($P\leq0.05$) PM and TM than other treatments after 25 and 50 h of storage.

Viable spermatozoa and MDA concentration

The impacts of CoQ10 on viability and MDA content within low-temperature storage are shown in Table 2. Time 0 showed no significant difference among groups for viability and MDA content. After 25 and 50 h of chilling, the Q5 showed a greater ($P\leq0.05$) viability than the others. No statistically significant differences ($P>0.05$) were found between Q0, Q1, Q2, and Q10. Lipid peroxidation was lower ($P\leq0.05$) in Q5 than in the other treatments after 25 and 50 h of storage.

Table 1. Effects of CoQ10 on buck's cooled sperm TM and PM

Treatment	(%)					
	TM			PM		
	0 h	25 h	50 h	0 h	25 h	50 h
Q0	82.2±0.7	52.0±1.0^{b}	20.0±1.1^{b}	64.0±1.0	25.8±1.5^{b}	12.5±1.1^{b}
Q1	83.1±0.7	52.2±1.0^{b}	20.2±1.1^{b}	62.8±1.0	27.6±1.5^{b}	13.3±1.1^{b}
Q2	82.5±0.7	53.5±1.0^{b}	21.4±1.1^{b}	63.2±1.0	28.4±1.5^{b}	14.2±1.1^{b}
Q5	83.0±0.7	55.6±1.0^{a}	25.3±1.1^{a}	62.5±1.0	32.5±1.5^{a}	16.7±1.1^{a}
Q10	84.1±0.7	52.2±1.0^{b}	19.8±1.1^{b}	63.9±1.0	26.2±1.5^{b}	12.0±1.1^{b}

$^{a, b}$Significant differences among the groups (P≤0.05).

Table 2. Effects of CoQ10 on buck's cooled sperm viability and lipid peroxidation

Treatment	Viability (%)			MDA Concentration (nmol/mL)		
	0 h	25 h	50 h	0 h	25 h	50 h
Q0	87.0±0.6	54.2±1.2^{b}	22.5±1.0^{b}	2.55±0.10	4.25±0.20^{b}	8.25±.0.25^{b}
Q1	87.5±0.6	55.0±1.2^{b}	23.0±1.0^{b}	2.65±0.10	4.10±0.20^{b}	8.05±0.25^{b}
Q2	88.0±0.6	55.4±1.2^{b}	23.4±1.0^{b}	2.60±0.10	4.00±0.20^{b}	7.85±0.25^{b}
Q5	87.1±0.6	58.9±1.2^{a}	27.8±1.0^{a}	2.65±0.10	3.00±0.20^{a}	7.15±0.25^{a}
Q10	87.0±0.6	53.5±1.2^{b}	22.0±1.0^{b}	2.60±0.10	4.30±0.20b	8.35±0.25^{b}

$^{a, b}$Significant differences among the groups (P≤0.05).

Membrane integrity and mitochondria activity

Table 3 presents the effect of CoQ10 on membrane integrity and mitochondria activity. Time 0 showed no significant difference among groups. Q5 demonstrated higher (P≤0.05) membrane integrity and mitochondria activity than the others after 25 and 50 hours of chilling storage. No significant difference (P>0.05) was found between the other groups.

Discussion

The beneficial effect of adding CoQ10 to the buck's sperm chilling extender for storage at 4°C for 50 hours was investigated in this study. The quality parameters

Table 3. Effects of CoQ10 on buck's cooled sperm mitochondrial activity and membrane integrity

Treatment	(%)					
	Mitochondrial Activity			Membrane Functionality		
	0 h	25 h	50 h	0 h	25 h	50 h
Q0	91.0±1.0	62.1±1.2^{b}	28.8±1.6^{b}	87.6±1.0	54.5±1.0^{b}	24.5±1.3^{b}
Q1	90.0±1.0	63.5±1.2^{b}	30.2±1.6^{b}	88.5±1.0	55.0±1.0^{b}	25.8±1.3^{b}
Q2	89.8±1.0	64.0±1.2^{b}	31.5±1.6^{b}	89.0±1.0	55.5±1.0^{b}	26.3±1.3^{b}
Q5	90.5±1.0	66.8±1.2^{a}	35.5±1.6^{a}	89.7±1.0	60.0±1.0^{a}	31.0±1.3^{a}
Q10	91.5±1.0	62.0±1.2^{b}	28.5±1.6^{a}	89.4±1.0	54.0±1.0^{b}	24.2±1.3^{b}

$^{a, b}$Significant differences among the groups (P≤0.05).

in chilled sperm showed a time-dependent decrease across treatment groups. Meanwhile, the Q5 group experienced less sperm motility reduction than the other groups. Buck's chilling extender supplemented with 5 µM CoQ10 demonstrated higher TM, PM, membrane integrity, viability, mitochondrial activity, and lower lipid peroxidation (LPO) during storage periods than other groups. Because the generation of reactive oxygen species (ROS) during cooling storage reduces the quality of chilled-stored sperm and damages the plasma membrane of spermatozoa, the presence of antioxidants is important for preserving sperm viability and activity (Balercia et al., 2009). The antioxidant properties of CoQ10 may account for the higher quality of stored sperm in extenders supplemented with CoQ10. The increased percentage of TM, PM, and viability of stored sperm cells under the effects of CoQ10 are consistent with the findings of previous studies that demonstrated the effective role of CoQ10 in frozen and chilled stored spermatozoa (Yousefian et al., 2018; Masoudi et al., 2019). Using 5 µM CoQ10 resulted in a higher viability rate because a suitable concentration of CoQ10 reduces apoptosis through plasma membrane stabilization and ROS scavenging. CoQ10 has been shown to reduce the apoptosis rate in cattle blastocysts (Gualtieri et al., 2014). The CoQ10 treatments did not affect the rate of abnormal morphology in sperm cells. Because sperm abnormality occurs during the spermatogenesis step, laboratory manipulation does not affect it (Zarei et al., 2022). The sperm morphology results were consistent with previous studies that found in vitro manipulation does not affect the morphology of rooster spermatozoa (Masoudi et al., 2019). Membrane integrity in spermatozoa can be maintained by reducing polyunsaturated fatty acid peroxidation, so LPO inhibition by CoQ10 protects sperm membrane integrity (Littarru & Tiano, 2007). The improvement in membrane integrity observed in this study is consistent with previous research that found CoQ10 to be beneficial to stallion sperm membrane integrity (Yousefian et al., 2018) CoQ10's antioxidant properties increased mitochondrial activity by neutralizing lipid peroxyl radicals (Mancini et al., 1998). CoQ10 also regenerates tocopherol from tocopheroxyl radicals (Turunen et al., 2004). It scavenges free radicals by preventing cytotoxic aldehyde accumulation. As an energy carrier, CoQ10 aids in the synthesis of ATP (Littarru & Tiano, 2007) and regulates the permeability of transition pores in the mitochondria (Turunen et al., 2004). Higher doses of CoQ10, on the other hand, were not as beneficial as the optimum concentration for sperm cells because higher concentrations of additives could be toxic for cooled sperm cells, indicating that the antioxidant concentration must be carefully fine-tuned to achieve the best performance (Yousefian et al., 2018). Exogenous additives such as antioxidants may unintentionally increase ROS concentrations, resulting in a decrease in the rate of functional sperm cells (Asadzadeh et al., 2021; Mohajer et al., 2024).

Conclusion

The addition of CoQ10 to the cooling medium of buck sperm preserves the quality of spermatozoa via reducing the peroxidation of lipids and protecting mitochondria active potential, so buck's cooling media supplementation with 5 µM CoQ10 could be an applied technique for the transportation of buck's spermatozoa during reproductive programs without a significant reduction in quality and fertility potential.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Animal Sciences Research Institute of Iran (Code: IR.ASRI.REC..2022.0898).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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مطالعه پژوهشی

محافظت از کیفیت اسپرم بز در هنگام ذخیره سرمایی با استفاده از کوانزیم کیوتن

مختار مهاجر[1]، *نادر اسدزاده[1]، هدی جواهری بار فروشی[1]، نوید داداشپور دواچی[2]، رضا مسعودی[1]

۱. مؤسسه تحقیقات علوم دامی کشور، سازمان تحقیقات آموزش و ترویج کشاورزی،کرج، ایران.
۲. گروه تحقیق، پرورش و تولید حیوانات آزمایشگاهی، مؤسسه تحقیقات واکسن و سرم‌سازی رازی، سازمان تحقیقات آموزش و ترویج کشاورزی، کرج، ایران.

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چکیده

زمینه مطالعه: ذخیره سرمایی اسپرم متابولیسم اسپرم را کاهش می‌دهد، درحالی‌که قابلیت باروری و زنده‌مانی اسپرم حفظ می‌شود. به‌علت ویژگی‌های خاص اسپرم نشخوارکنندگان کوچک، فرایندسردسازی توانایی باروری اسپرم را در این گونه‌ها کاهش می‌دهد.

هدف: این مطالعه با هدف بررسی اثر افزودن کوانزیم کیوتن به محیط سردسازی بر کیفیت اسپرم بز طی فرایند ذخیره سرمایی در دمای ۴ درجه سانتی‌گراد انجام شد.

روش کار: نمونه‌های اسپرم پس از جمع‌آوری و رقیق‌سازی به ۵ قسمت تقسیم شدند و مقادیر ۰، ۱، ۲، ۵ و ۱۰ میکرومولار کوانزیم کیوتن را دریافت کردند. سپس نمونه‌ها در دمای ۴ درجه سانتی‌گراد سرد و طی ۴۸ ساعت ذخیره شدند. جنبایی کل و پیشرونده، زنده‌مانی، سلامت غشا، فعالیت میتوکندری و پراکسیداسیون لیپیدهای غشایی در زمان‌های ۰، ۲۵ و ۵۰ ساعت ذخیره سرمایی مورد ارزیابی قرار گرفتند.

نتایج: در زمان صفر، تیمارها تأثیری بر کیفیت نمونه‌های اسپرم نداشتند ($P>0/05$). در زمان‌های ۲۵ و ۵۰ ساعت از ذخیره سرمایی، تیمار ۵ میکرومولار کوانزیم کیوتن مقادیر بالاتر ($P\leq0/05$)، جنبایی کل و پیش‌رونده، زنده‌مانی، سلامت غشا و فعالیت میتوکندری را نسبت به سایر گروه‌ها نشان داد. همچنین تیمار ۵ میکرومولار کوانزیم کیوتن موجب پراکسیداسیون لیپیدی کمتر ($P\leq0/05$) در زمان‌های ۲۵ و ۵۰ ساعت از ذخیره سرمایی نسبت به سایر گروه‌ها شد.

نتیجه‌گیری نهایی: درنتیجه استفاده از ۵ میکرومولار کوانزیم کیوتندر محیط ذخیره سرمایی اسپرم بز می‌تواند راهی مناسب برای محافظت از اسپرم بز در هنگام ۲۵ و ۵۰ ساعت سردسازی در مقابل آسیب‌های ساختاری و عملکردی طی ذخیره سرمایی باشد.

کلیدواژه‌ها: سردسازی، کوانزیم کیوتن، بز، کیفیت، منی

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* **نویسنده مسئول:**
دکتر نادر اسدزاده
نشانی: کرج، سازمان تحقیقات آموزش و ترویج کشاورزی، مؤسسه تحقیقات علوم دامی کشور.
تلفن: ۴۶۰۲۹۷۷۲ (۲۱) ۹۸+
رایانامه: *nader.asadzadeh4@gmail.com*