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Preservation of Buck Semen Quality During Chilling Storage Using Coenzyme Q10

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Abstract

BACKGROUND: Storing spermatozoa in a cold environment decreases spermatozoa metabolism while maintaining sperm fertility potential. Because of their unique physiological characteristics, cooling reduces the reproductive ability of sperm cells in small ruminants.

OBJECTIVES: The purpose of this study was 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 assigned into five groups with different CoQ10 supplementation concentrations (0, 1, 2, 5, and 10 µM CoQ10). Collected semen were stored at 4 degrees Celsius during 50 h. The motility, mitochondrial activity, membrane integrity, viability, and lipid peroxidation were measured after 0, 25, and 50 h of chilling.

RESULTS: Different concentrations of CoQ10 had no impact ($P>0.05$) on semen at time 0 of storage, according to the findings. After 25 and 50 hours of storage, supplementing chilling medium with 5 µM CoQ10 increased ($P\leq0.05$) progressive motility, total motility, viability, mitochondrial activity, and membrane integrity than other groups. Furthermore, 5 µM CoQ10 produced less lipid peroxidation ($P\leq0.05$) compared to the other groups after 25 and 50 h.

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CONCLUSIONS: Adding 5 M 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

Introduction

Preservation of spermatozoa at a cold situation decreases its metabolism while protecting reproductive performance. Scientists have attempted to improve the output of sperm preservation for 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 common 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 extender could be a useful way for preserving sperm quality in farm animals during storage (Sharafi *et al.*, 2015).

CoQ10 is made up of a benzoquinone ring and a lipophilic isoprenoid side chain found in mitochondrial inner membranes. It plays an effective role in the electron transport chain used in ATP synthesis, as well as acting 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 production of sperm energy (Yousefian *et al.*, 2018), and in human sperm, it works as a motility enhancer

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(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 assess the impact of CoQ10 on progressive motility, total motility, 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 abnormal morphology ≤15%, sperm concentration of ≥3×10^9 spermatozoa/ml, motility ≥70% and volume: 1-2 mL. 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 10µM (Q10) CoQ10. The final concentration was 400×10^6 sperm/mL.

Evaluation of the quality of semen

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Sperm motility parameters were assessed via sperm class analysis program. The samples were observed About 400 spermatozoa in each sample were evaluated and the PM (%) and the TM (%) were reported (Masoudi *et al.*, 2020a).

Sperm viability was evaluated using Eosin-nigrosine staining. The Live cells did not show stained head, whereas the heads in dead cells were stained (Masoudi *et al.*, 2020b).

The HOST 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 10,000 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 (six replicates) were analyzed using SAS 9.1 software's GLM procedure. To determine statistical differences between groups, Tukey's test was used. If the P values were less than 0.05 ($P \leq 0.05$), the differences were statistically significant.

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Results

Motility parameters

Table 1 shows the impact of CoQ10 on the TM and PM of a buck's semen. At storage time zero, there were no significant differences between treatments. Q5 had significantly higher ($P \leq 0.05$) PM and TM than other treatments after 25 and 50 hours 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 hours of chilling, the Q5 showed a greater ($P \leq 0.05$) viability than the others. No statistically significant differences ($P > 0.05$) was found between Q0, Q1, Q2, and Q10. Lipid peroxidation was lower ($P \leq 0.05$) in Q5 than in the other treatments after 25 and 50 hours of storage.

Membrane integrity and mitochondria activity

The findings (Table 3) revealed the effect of CoQ10 on the membrane integrity and mitochondria activity. Time 0 showed no significant difference among groups. Q5 demonstrated

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higher ($P \leq 0.05$) 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 rest 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 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 5M CoQ10 demonstrated higher TM, PM, membrane integrity, viability, mitochondrial activity, and lower LPO during storage periods than other groups. Because the generation of 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). The use of 5 M 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

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the rate of apoptosis in cattle blastocysts (Gualtieri *et al.*, 2014). The CoQ10 treatments had no effect on the rate of abnormal morphology in sperm cells. Because sperm abnormality occurs during the spermatogenesis step, laboratory manipulation has no effect on it (Zarei *et al.*, 2022). The sperm morphology results were consistent with previous studies that found in vitro manipulation has no effect on 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 and 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 adenosine triphosphate (ATP) (Littarru and 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

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decrease in the rate of functional sperm cells (Asadzadeh *et al.,* 2021; Mohajer and Davachi, 2023).

Conclusion

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 transportation of buck's spermatozoa during reproductive programs without a significant reduction in quality and fertility potential.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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

Treatments	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]

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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}

Different letters within the same column show significant differences among the groups (P≤0.05).

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

Treatments	**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 ± 0.20^{b}	8.35 ± 0.25^{b}

Different letters within the same column show significant differences among the groups (P≤0.05).

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

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Treatments	Mitochondrial activity (%)			Membrane functionality (%)		
	0 h	25 h	50 h	0 h	25 h	50h
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}

Different letters within the same column show significant differences among the groups (P≤0.05).

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

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چکیده

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

هدف: هدف از این مطالعه بررسی اثر افزودن کوانزیم کیوتن به محیط سردسازی بر کیفیت اسپرم بز طی فرایند ذخیره سرمایی در دمای 4 درجه سانتیگراد بوده است.

روش کار: نمونه های اسپرم پس جمع آوری و رقیق سازی به پنج قسمت تقسیم شده و مقادیر 0، 1، 2، 5 و 10 میکرومولار کوانزیم کیوتن را دریافت نمودند. سپس نمونه ها در دمای 4 درجه سانتیگراد سرد شده و طی 48 ساعت ذخیره شدند. جنبایی کل و پیشرونده، زنده مانی، سلامت غشا، فعالیت میتوکندری و پراکسیداسیون لیپیدهای غشایی در زمان های 0، 25 و 50 ساعت ذخیره سرمایی مورد ارزیابی قرار گرفتند.

نتایج: در زمان 0، تیمارها تاثیری بر کیفیت نمونه های اسپرم نداشتند ($P>0.05$). در زمان های 25 و 50 ساعت از ذخیره سرمایی، تیمار 5 میکرومولار کوانزیم کیوتن مقادیر بالاتر ($P\leq0.05$) جنبایی کل و پیشرونده، زنده مانی، سلامت غشا و فعالیت میتوکندری را نسبت به سایر گروه ها نشان داد. همچنین تیمار 5 میکرومولار کوانزیم کیوتن موجب پراکسیداسیون لیپیدی کمتر ($P\leq0.05$)در زمان های 25 و 50 ساعت از ذخیره سرمایی نسبت به سایر گروه ها شد.

نتیجه‌گیری نهایی: در نتیجه، استفاده از 5 میکرومولار کوانزیم کیوتندر محیط ذخیره سرمایی اسپرم بز می تواند راهی مناسب برای محافظت از اسپرم بز در هنگام 25 و 50 ساعت سردسازی در مقابل آسیب های ساختاری و عملکردی طی ذخیره سرمایی باشد.

کلمات کلیدی: سردسازی، کوانزیم کیوتن، بز، کیفیت، منی.

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