Archives of Razi Institute, Vol. 76, No. 6 (2021) 1797-1802

DOI: 10.22092/ari.2021.356398.1836

Original Article

Conservation of Buck's Spermatozoa during Cooling Storage Period through Cooling Medium Supplementation with L-Carnitine

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Received 3 October 2021; Accepted 26 October 2021
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Abstract

This research examined the influence of the addition of L-carnitine (LC) to cooling medium on buck's semen quality during cooling storage periods at 4ºC. Semen samples were collected, diluted, assigned into four groups, and received LC (0, 1, 5, and 10 mM LC). The samples were then chilled to 4ºC and stores for 48 h. Sperm total motility, progressive motility, viability, lipid peroxidation, membrane integrity, and mitochondrial activity were examined at 0, 24, and 48 h of cooling storage. At time 0 of cooling storage, different treatments showed no impact on the quality of sperm samples ($P>0.05$). During 24 and 48 h of chilling periods, the supplementation of cooling medium with 5 mM LC presented greater motility, viability, membrane integrity, and mitochondrial activity ($P\leq0.05$), compared to the other groups. Moreover, the treatment of 5 mM LC caused lower lipid peroxidation ($P\leq0.05$) than the other treatments at 24 and 48 h storage times. In conclusion, the supplementation of buck's cooling storage medium with 5 mM LC is a suitable way to protect buck spermatozoa during 24 and 48 h storage against cold-induced structural and functional damages.

Keywords: Buck, Cooling, L-carnitine, Quality evaluation, Spermatozoa

1. Introduction

Although cold storage of semen reduces sperm metabolism, it preserves sperm viability and fertility potential. Researchers have performed several studies to improve the efficiency of semen storage for more than 24 h (1). The cooling process causes a reduction in fertility ability because of the unique physiological features of sperm in small ruminants (1).

Buck's sperm may be exposed to cooling-related injuries that cause membrane damage and consequent reduction of fertility potential; therefore, it is necessary to enhance the efficacy of the protection method of buck's sperm storage periods. The tris-based extender is a common medium for buck's sperm preservation, which is composed of Tris, fructose, and citric acid (2). On the other hand, the addition of an auxiliary additive to this medium can be helpful to improve the protection rate of buck sperm against the cooling situation. The cooling process can result in reactive oxygen species production that distracts sperm structure (1).

Metabolic and antioxidant roles of L-carnitine (LC) encouraged us to perform this study. The LC plays important roles in the generation of metabolic energy for sperm cells via facilitating the transportation of fatty acids into the mitochondrion (3). Its antioxidant characteristics are associated with stabilizing the membrane of mitochondria and protecting the DNA structure against reactive oxygen species (ROS) (4),

which is related to the ability of LC to reduce lipids availability for peroxidation by fatty acids transportation into the mitochondria for β-oxidation (5). According to the literature, dietary LC enhanced the activity of the antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase in spermatozoa (6), which are effective in ROS scavenging in cooled sperm cells. The helpful effects of LC were observed in sperm motility in boar (7) and rooster (4).

To the best of our knowledge, no study has yet reported the useful influence of cooling medium supplementation with LC on buck's semen quality during cooling storage; therefore, the current study evaluated the motility, viability, mitochondrial activity, lipid peroxidation, and membrane functionality of cooled buck's spermatozoa.

2. Materials and Methods

2.1. Semen Samples Processing

Semen samples were collected from five Saanen goats (3-4 years old) by an artificial vagina. Samples with the following characteristics were selected for cooling storage: abnormal morphology of ≤ 15%, sperm concentration of ≥ 3×10^9 spermatozoa/ml, motility of ≥ 70%, and volume of 1-2 ml. Semen samples were pooled for elimination of individual differences and diluted in the extender [citric acid (1.64 g/100ml), fructose (1.26 g/100ml), Tris (3.07 g/100ml), soybean lecithin (1.5% w/v), glycerol (5% v/v), osmolarity (425 mOsm), pH (6.8)]. Subsequently, the samples were assigned into four aliquots, namely extender without LC (LC0), extender with 1 mM (LC1), 5 mM (LC5), and 10 mM (LC10) LC. The final concentration was 400×10^6 sperm/ml, and then, the samples were placed in a rack and cooled from 37ºC to 4ºC for 30 min, in a cold cabinet, and maintained at 4ºC. Motility, viability, mitochondrial activity, lipid peroxidation, and membrane functionality were examined at 0, 24, and 48 h of storage.

2.2. Semen Quality Evaluation

Sperm class analysis software (Version 5.1; Microptic, Barcelona, Spain) was used to analyze motility parameters. An amount of 5 µl of diluted semen was placed into a prewarmed chamber slide (38°C, Leja 4; 20 mm height; Leja Products, Luzernestraat B.V., Holland). Afterward, six fields that contained a minimum of 400 sperm were evaluated for each sample at a 5-sec average time to read each sample. Total motility (%) and progressive motility (%) were recorded (8).

Eosin-nigrosine staining examined sperm viability via counting 200 sperm cells using a phase-contrast microscope (×400). The live cells showed unstained heads, and dead cells were stained/partial stained heads (4).

Hypo Osmotic Swelling Test examined sperm cells membrane functionality (9). The samples were observed via a phase-contrast microscope (×400) after incubation (for 30 min). About 300 sperm cells were counted. Sperm with swollen tails were recorded as a functional membrane.

Mitochondrial activity was evaluated by Rhodamine 123 (R123; Invitrogen TM, Eugene, OR, USA) and propidium iodide during a flow cytometry study using the FACSCalibur flow cytometer (Becton Dickinson, San Khosoz, CA, USA) (10). About 10,000 events were assessed for each assay at a 100 cells/s flow rate. The data were analyzed using FlowJo software (Treestar, Inc., San Carlos, CA). The procedure was performed according to the method was described by Masoudi, Sharafi (10).

Lipid peroxidation was evaluated by measuring Malondialdehyde (MDA) concentrations via the reaction of thiobarbituric acid at 532 nm by a spectrophotometer set (UV-1200, Japan) (11), and the concentrations were recorded as nmol/ml. The procedure was conducted according to the method described by Esterbauer and Cheeseman (11).

2.3. Statistical Analysis

The data in the current study (six replicates) were analyzed by the GLM procedure of SAS 9.1 software. Tukey's test was used to determine the statistical differences between groups. The p values of ≤0.05 were considered significant.

3. Results

Table 1 presents the impact of LC on the total motility and progressive motility of buck's sperm. No significant difference was observed among groups at the storage time of 0. At 24 h and 48 h cooling storage, LC5 showed significantly greater total motility ($P \leq 0.05$) and progressive motility than the other groups. However, there was no significant difference among the other groups ($P > 0.05$).

Table 2 summarizes the effects of LC on sperm viability and lipid peroxidation during cooling storage. No difference was observed between groups at time 0 of storage for sperm viability and lipid peroxidation. Greater viability rates were recorded in LC5 at 24 h and 48 h cooling storage times ($P \leq 0.05$), compared to the other groups. No difference was recorded among LC0, LC1, and LC10 ($P > 0.05$). In the case of MDA concentration at 24 h and 48 h storage, LC5 showed a lower lipid peroxidation in contrast to the other groups ($P \leq 0.05$), and there was no difference among the other groups in this regard ($P > 0.05$).

Table 3 presents the impact of LC on mitochondrial activity and membrane integrity of cooled semen. Differences in mitochondrial activity and membrane integrity were not significant among groups at time 0 of storage. At times of 24 h and 48 h cooling storage, a higher significant mitochondrial activity and membrane functionality were observed in LC5 ($P \leq 0.05$), compared to the other groups. No significant difference was found among the rest groups in cases of sperm mitochondrial activity and membrane functionality ($P > 0.05$).

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

LC	TM (%)			PM (%)		
concentrations	0 h	24 h	48 h	0 h	24 h	48 h
LC0	85.5±1.1	55.5±1.5^{b}	20.4±1.3^{b}	65.0±1.0	24.6±1.4^{b}	10.5±1.7^{b}
LC1	86.2±1.1	56.3±1.5^{b}	20.5±1.3^{b}	66.1±1.0	25.2±1.4^{b}	11.5±1.7^{b}
LC5	85.0±1.1	59.8±1.5^{a}	24.0±1.3^{a}	66.3±1.0	30.1±1.4^{a}	15.5±1.7^{a}
LC10	96.5±1.1	56.0±1.5^{b}	19.6±1.3^{b}	65.5±1.0	24.6±1.4^{b}	10.0±1.7^{b}

LC: L-carnitine; TM: Total motility; PM: Progressive motility
Different letters within the same column show significant differences among the groups ($P \leq 0.05$).

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

LC	Viability (%)			MDA concentration (nmol/ml)		
concentrations	0 h	24 h	48 h	0 h	24 h	48 h
LC0	87.6±1.3	56.0±1.7^{b}	21.5±1.8^{b}	2.75±0.30	4.20±0.25^{b}	8.15±0.20^{b}
LC1	88.2±1.3	57.2±1.7^{b}	21.0±1.8^{b}	2.85±0.30	4.15±0.25^{b}	8.00±0.20^{b}
LC5	88.0±1.3	62.2±1.7^{a}	25.5±1.8^{a}	2.80±0.30	3.55±0.25^{a}	7.57±0.20^{a}
LC10	88.5±1.3	55.9±1.7^{b}	21.0±1.8^{b}	2.70±0.30	4.10±0.25^{b}	8.05±0.20^{b}

LC: L-carnitine; MDA: Malondialdehyde
Different letters within the same column show significant differences among the groups ($P \leq 0.05$).

Table 3. Effects of LC on buck's cooled sperm mitochondrial activity and membrane functionality

LC	Mitochondrial activity (%)			Membrane functionality (%)		
Concentrations	0 h	24 h	48 h	0 h	24 h	48 h
LC0	88.0±1.0	58.0±1.5^{b}	26.7±1.2^{b}	88.8±1.2	61.0±1.4^{b}	25.0±1.5^{b}
LC1	89.0±1.0	58.4±1.5^{b}	27.5±1.2^{b}	89.3±1.2	60.2±1.4^{b}	24.4±1.5^{b}
LC5	88.5±1.0	63.5±1.5^{a}	30.2±1.2^{a}	88.4±1.2	65.5±1.4^{a}	29.3±1.5^{a}
LC10	89.5±1.0	59.5±1.5^{b}	26.4±1.2^{a}	88.0±1.2	60.7±1.4^{b}	23.7±1.5^{b}

LC: L-carnitine
Different letters within the same column show significant differences among the groups ($P \leq 0.05$).

4. Discussion

Sperm cooling storage is an applied method to preserve spermatozoa for reproductive goals in ruminants (12); in this regard, semen samples must be diluted with a suitable extender enriched with enough protective additives (13). In this research, the helpful effects of LC in buck's sperm cooling medium were evaluated at 4°C for 48 h. According to the results, a time-dependent reduction was observed in cooled sperm motility, mitochondrial activity, viability, and membrane functionality; nevertheless, this reduction was less in the LC5 group than in other groups. The addition of 5 mM LC to the buck's semen cooling medium resulted in higher total motility, progressive motility, viability, mitochondrial activity, and membrane integrity as well as lower MDA concentration than the other LC concentrations during 24 and 48 h of cooling storage.

According to the pieces of literature, in rooster, dietary LC improved semen quality (6), and sperm extender supplementation with LC was also useful (5); however, while there is no study investigating the case of buck. In the current study, improvement in sperm viability, motility, and membrane functionality might be related to the metabolic roles of LC (14). Facilitation in fatty acids transportation across the inner mitochondrial membrane via LC leads to an improvement in the adenosine triphosphate (ATP) production via β-oxidation (15), which provides a better supply of energy for the motility of spermatozoa. Moreover, LC could be effective in scavenging accumulated ROS and causing lower damages during sperm storage (5). On the other hand, higher LC concentrations were found in semen plasma than in the blood plasma (16), presenting the important role of LC in energy production through the pyruvate cycle (12).

As an antioxidant, LC scavenges free radicals, inhibits xanthine oxidase activity, and destroys metal chelation and hydrogen peroxide (17). Moreover, lipid peroxidation reduction is presented in researches and is normally shown in clinical attempts (18). The results about lipid peroxidation in the current study verified the data obtained for sperm viability, motility, and membrane integrity because lipid peroxidation was lower in LC5, which was in line with the previous findings regarding the useful impacts of LC on different cells (19) and sperm cells (14).

There is also a logical relationship between mitochondrial activity and motility. Sperm motility is relatively dependent on mitochondrial activity (20). It could be due to the osmolyte role of LC in the extender. Extender supplementation with LC leads to the partial removal of Na^+ from diluent to maintain isotonicity (21). The Na^+ increases the ATP depletion by activating Na-ATPase pumps (21); consequently, the beneficial effects of LC may be due to the Na^+ removal (12). The Na^+ reduction in the medium decreases the spermatozoon energy demands resulting in a slower rate of ATP depletion, which effectively maintains and improves sperm mitochondrial activity and viability for extended periods of storage (12).

5. Conclusion

L-carnitine-supplemented buck's sperm cooling medium protects cooled semen quality via reducing lipid peroxidation and preserving mitochondrial activity. Therefore, the supplementation of buck cooling medium with 5 mM LC is a practical way by which buck's semen could be transported to far-away farms for reproductive goals and the insemination of commercial flocks, without serious quality losses.

Authors' Contribution

Study concept and design: R. M.
Acquisition of data: M. H.
Analysis and interpretation of data: B. G. and Gh. M.
Drafting of the manuscript: R. M.
Critical revision of the manuscript for important intellectual content: B. G. and Gh. M.
Statistical analysis: M. H.
Administrative, technical, and material support: R. M.

Ethics

The present study was approved by the Ethics Committee of the University of Tabriz, Tabriz, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

This study was carried out based on a PhD thesis and supported by the University of Tabriz, Tabriz, Iran.

Acknowledgment

The authors would like to thank the Animal Science Research Institute of Iran for supporting the current study.

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