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Original Article

The effect of L-carnitine on ram epididymal sperm motility parameters during preservation in HTF medium with CASA

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ABSTRACT

L-carnitine, an endogenous amino acid, is highly effective in preventing programmed cell death. As an antioxidant, it also protects DNA structure against reactive oxygen species and increases the activity and amount of antioxidant enzymes. In this study, 20 pairs of mature ram testicles were collected immediately after Islamic slaughter from the Urmia industrial slaughterhouse. The samples were transferred to the faculty laboratory in a cool box. After cleaning and drying the testes from blood, a cut was made in areas without capillaries on the tail of the epididymis. The sperm were transferred to microtubes containing HTF (Human Tubal Fluid) culture medium with 10% bovine serum albumin. After preparing a dilution of 30 to 50 million sperm per ml of HTF, five levels of L-carnitine (0, 2, 4, 6, 8, 10 mM) were added to the medium. The samples were then kept in a refrigerator at 5 degrees Celsius for up to 36 hours. At 1, 12, 24, and 36 hours, the sperm motility pattern was evaluated with CASA. Statistical results showed that L-carnitine at levels of 6, 8, and 10 mM and at 24 and 36 hours caused a clear and significant increase in the motility indices of ram epididymal sperm compared to the control group ($p<0.05$).

تاثیر اِل -کارنیتین بر داده های حرکتی اسپرم اپیدیدیمی قوچ طی نگهداری در محیط کشت HTF با کاسا

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چکیده

اِل-کارنیتین در نقش اسید آمینه داخلی در پیش گیری از مرگ برنامه ریزی شده سلول بسیار موثر است. همچنین به عنوان آنتی اکسیدان، نقش حفاظتی از ساختار DNA را در برابر گونه های اکسیژن واکنشی بر عهده می گیرد. اِل کارنیتین فعالیت و میزان آنزیم های آنتی اکسیدان را نیز افزایش می دهد. در این تحقیق بلافاصله پس از ذبح اسلامی، ۲۰ جفت بیضه قوچ بالغ از کشتارگاه صنعتی ارومیه جمع آوری شد. نمونه ها در کول باکس به آزمایشگاه دانشکده منتقل گردید. پس از تمیز و خشک کردن بیضه ها از خونابه، در نواحی بدون مویرگ روی دم اپیدیدیم برش ایجاد شد. اسپرم های محل برش به میکروتیوب های حاوی محیط کشت HTF با ۱۰ درصد آلبومین سرم گاوی منتقل گردید. پس از تهیه رقت ۳۰ الی ۵۰ میلیون اسپرم در میلی لیتر محیط کشت، ۵ سطح اِل کارنیتین (۰- ۲-۴-۶-۸-۱۰ میلی مول) به محیط کشت اضافه شد. سپس نمونه ها در دمای ۵ درجه سلسیوس و تا ۳۶ ساعت در یخچال نگهداری شد. در زمان های ۱- ۱۲- ۲۴ و ۳۶ ساعت، الگوی حرکتی اسپرم با سیستم کاسا ارزیابی گردید. نتایج آماری نشان داد اسید آمینه اِل کارنیتین در سطح ۶-۸-۱۰ میلی مول و در زمان ۲۴ و ۳۶ ساعت، سبب افزایش واضح و معنی دار شاخص های حرکتی اسپرم اپیدیدیمی قوچ در مقایسه با شاهد گردید ($p<۰/۰۵$).

واژه های کلیدی: قوچ، اسپرم، اِل کارنیتین، HTF، کاسا

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INTRODUCTION

The mammalian sperm membrane is rich in unsaturated fatty acids (PUFA). The composition of these fatty acids plays a significant role in sperm function. Any manipulation of sperm in an environment outside the body, such as lab conditions, can lead to the production of free radicals and reactive oxygen species (ROS), causing peroxidation of sperm membrane lipids. This oxidative damage reduces the quality and fertility of sperm cells [1]. As an endogenous amino acid, L-carnitine plays a role in mitochondrial function and the oxidation of long-chain fatty acids. It is effective in preventing cell apoptosis and, as an antioxidant compound, it preserves the DNA structure of cells against damage from free radicals and ROS. L-carnitine can also increase the levels and activity of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase, thus potentially strengthening sperm resistance to oxidative damage [1]. Carnitine is a vitamin-like amino acid, a quaternary ammonium compound with many functions in reproduction [2]. In the epididymal ducts, there is a high concentration of L-carnitine, which is necessary for energy balance and sperm maturation. L-carnitine increases sperm fertility indicators. It plays a role in the metabolism of abdominal fat in geese and ducks and subcutaneous fat in pigs. Detailed studies have been conducted on the effect of L-carnitine on the metabolism of Sertoli cells before the maturity of rats. There are also reports of the beneficial effects of L-carnitine after damage caused by gamma radiation [2]. Carnitine is involved in energy metabolism in most mammals, plants, and microorganisms, playing a major role in fatty acid metabolism by facilitating the passage of fatty acids through the mitochondrial membrane. In the inner and outer membrane of mitochondria, two transporters, palmitoyl transferase 1 and 2, carry carnitine in its long-chain form called acetyl carnitine, allowing fatty acids to pass through the mitochondrial membrane to complete the beta-oxidation pathway [3]. Carnitine is mainly produced in the liver and is present in high amounts in the sperm and epididymis of mammals. The epididymal epithelium and the sperm obtain their energy from epididymal fluids through carnitine. Increasing the concentration of carnitine in epididymal fluids increases sperm motility. Additionally, carnitine acts as an antioxidant by transporting fatty acids into the mitochondria and producing ATP, reducing the fat available for peroxidation. Carnitine plays a critical role in mitochondrial respiration by supporting the activity of the pyruvate dehydrogenase enzyme. In various studies, a diluent containing L-carnitine has been effective in increasing the sperm quality of species such as stallions, roosters, rabbits, and bulls [3]. During the freezing and thawing process, sperm cells suffer structural damage, reducing sperm motility and fertility. Sperm are exposed to the harmful effects of reactive oxygen and nitrogen species, impairing mitochondrial function due to lipid peroxidation in the mitochondrial membrane. Free radicals or ROS cause sperm DNA fragmentation, hindering gene expression and embryo development. L-carnitine reduces the destructive effects of these oxidants on sperm cells by neutralizing free ferrous ions, radicals, nitrogen, and competing with superoxide ions [4]. This study aimed to determine the effect of L-carnitine on ram epididymal sperm motility parameters during preservation in HTF medium with CASA.

MATERIALS AND METHODS

Sperm sampling

This study was conducted in a specialized veterinary clinic. For this purpose, 20 pairs of mature ram testicles were collected from the industrial slaughterhouse after routine slaughter. All of the samples were obtained immediately after the routine slaughter of animals according to Islamic Sharia standards in the industrial slaughterhouse of Urmia. The samples were transferred to the laboratory inside a Styrofoam box containing a cold pack. After transferring the samples to the laboratory of the veterinary faculty of Islamic Azad University of Urmia, the testicles were first removed from the scrotum. After cutting the white covering of the testis, tunica albuginea, the tail of the epididymis was fixed between two fingers. In testicle samples where the tail of the epididymis was prominent and relatively large, the tubules of the area were full of sperm and contained many blood capillaries. To avoid staining the blood with sperm during epididymal extraction, the blood vessels were slowly extracted, and the blood in the area was cleaned and dried with a tampon. Then, by cutting in the dry area without blood secretions of the tail of the epididymis, the sperm were collected and transferred to four ml microtubes containing two ml of HTF (Human Tubal Fluid) culture medium with 10% bovine serum albumin (BSA). BSA was added to the culture medium to protect the sperm membrane and prevent sperm heads from sticking together. Sperm samples from several testicles were transferred to microtubes. 950 µl of HTF with 10% BSA was transferred to each microtube, then 50 µl of the medium containing the sperm sample was added so that the final dilution between 30 and 50 million sperm per ml of microtube was obtained. Using an autoclavable micropipette of 1 to 10 µl of Socorex brand (Acura manual 825, Swiss made), levels of 0, 2, 4, 6, 8, and 10 mM L-carnitine were added to one ml microtubes containing HTF and sperm cells. The microtubes were stored in a refrigerator at 5 degrees Celsius for up to 36 hours [5-8].

Reagents

All media, chemicals, and reagents used in this study were procured from Sigma-Aldrich Chemical Co. Ltd through Barnard Urmia Company (L-carnitine hydrochloride/c9500-25g/lot# 022P4058/Sigma-Aldrich. HTF (Human Tubal Fluid), EmbryoMax® HTF/MR070/Catalogue number: 637428).

Sperm evaluation

To assess sperm motility, at 1, 12, 24, and 36 hours after storage of ram epididymal sperm at 5 degrees Celsius, 5 microliters of the sample from each microtube was placed on a Makler chamber of CASA (HFTCASA Computer Aided Sperm Analysis, system Version 7.00, Hooshmand Fanavar Co. Limited) at 36 degrees Celsius. After two minutes, the motility pattern of at least 1000 sperms was evaluated with CASA hardware and software [4], [9-10].

Statistical analysis

In this research, after repeating the experiment three times, Statistical Package for Social Sciences (SPSS version 26) software was used for statistical analysis. After the data were examined for the significance of the difference in variances, multi-domain tests were performed. When assuming the equality of the variances of the groups, Tukey's test was used, and when assuming the inequality of the variances of the groups, the Tamaneh test was used at a 95% confidence level (Statistical significance was set as $p \leq 0.05$). The results were recorded as mean ± standard error of the mean in Tables 1-7.

RESULTS

The results of the effect of L-carnitine on the epididymal sperm of ram with CASA showed that the average percentage of rapid-progressive motility in terms of numerical values at 1 and 12 hours after storage in liquid form was higher than the control at all levels of L-carnitine. Although the difference in means was not significant, at 24 and 36 hours after sperm storage, the difference in means was often significant ($p<0.05$). At 24 hours, the difference between the levels of 6, 8, and 10 mmol L-carnitine was significant with the control, and the average values of the L-carnitine group were higher than the control. At 36 hours, the average level of 6 mmol L-carnitine was significantly higher than the control ($p<0.05$) (Table 1). The results of the effect of L-carnitine on the motile sperm variable with CASA showed that the mean percentage of this data was higher than the control at 1 and 12 hours after storage in most treatment levels, but the difference between the means was not significant. However, at 24 hours, all treatment levels were higher than the control, and the difference with the control was significant at the levels of 6, 8, and 10 mmol ($p<0.05$). At 36 hours, the difference between the treatment and the control was significant only at the levels of 6 and 10 mmol, and the mean of L-carnitine was higher than the control ($p<0.05$) (Table 2). Regarding the effect of L-carnitine on the curvilinear velocity of ram epididymal sperm, the average numerical values of this data, in terms of micrometers per second, at 1 hour, in all levels of L-carnitine were higher than the control, but the difference between the means was not significant. At 12 hours, all L-carnitine levels, except for 2 mM, had a numerical average higher than the control, but again the differences were not significant. At 24 hours, all L-carnitine levels were higher than the control, and the differences were significant at 2, 6, 8, and 10 mM levels ($p<0.05$). At 36 hours, the means of all L-carnitine levels were higher than the control, and the difference between the means at the levels of 6 and 10 mM with the control was significant ($p<0.05$) (Table 3). Regarding the straight-line velocity data, the value of this index at 24 hours was higher than the control at all levels of L-carnitine, and the difference between the averages at the levels of 6 and 8 mmol with the control was significant. At 36 hours, the means of 6 mM L-carnitine was significantly higher than the control ($p<0.05$) (Table 4). The last speed index evaluated by CASA is the average path velocity of the sperm. The results showed that up to 24 hours, all L-carnitine levels were faster than the control, and the difference between the means and the control was significant at the levels of 2, 6, and 8 mM L-carnitine ($p<0.05$). At 36 hours, the difference between the means was significant only at the level of 6 mmol L-carnitine with the control ($p<0.05$) (Table 5). Regarding the amplitude lateral head variable, the amount of this data at 24 hours at the levels of 2, 6, 8, and 10 mM L-carnitine was higher than the control ($p<0.05$). At the 36th hour, the value of this parameter at the levels of 6 and 10 mM L-carnitine was higher than the control, and the difference between the means was significant ($p<0.05$) (Table 6). The results of the effect of different levels of L-carnitine on the linearity index of the sperm motility path showed that no significant difference was observed between the averages until the first 12 hours. At 24 and 36 hours, this data was significantly higher than the control only at the level of 6 mM L-carnitine ($p<0.05$) (Table 7).

Table 1. Effect of different L-Carnitine levels on Mean ± SEM rapid progressive motility (Class A, %)

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	63.78 ± 1.64	48.14 ± 1.84	11.87 ± 0.33^{b}	5.31 ± 0.32^{b}
L-Carnitine 2	65.27 ± 2.6	48.49 ± 4.43	16.58 ± 3^{ab}	1.17 ± 0.19^{c}
L-Carnitine 4	72.8 ± 4.9	52.84 ± 1.53	13.19 ± 1.36^{b}	3.02 ± 0.46^{bc}
L-Carnitine 6	69.11 ± 1.4	48.11 ± 3.39	20.91 ± 2.55^{a}	12.25 ± 1.37^{a}
L-Carnitine 8	68.93 ± 2.95	56.39 ± 1.2	19.6 ± 3.16^{a}	5.64 ± 0.53^{b}
L-Carnitine 10	66.77 ± 3.59	58.57 ± 2	16.01 ± 2.3^{ab}	6.8 ± 1.75^{b}

In each column values with different superscripts are significantly different ($p< 0.05$).

Table 2. Effect of different L-Carnitine levels on Mean ± SEM motile sperm (Class A+B+C, %)

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	82.84 ± 1.29	77.16 ± 1.08	30.92 ± 1^{C}	17.98 ± 0.86^{cd}
L-Carnitine 2	86.10 ± 3.52	76.45 ± 4.29	42.95 ± 4.31^{bc}	14.79 ± 1.12^{d}
L-Carnitine 4	93.47 ± 1.51	84.7 ± 0.08	41.14 ± 2.16^{bc}	23.04 ± 4.89^{bcd}
L-Carnitine 6	92.61 ± 0.71	79.24 ± 4.52	53.17 ± 6.55^{ab}	36.55 ± 0.32^{a}
L-Carnitine 8	88.77 ± 2.51	85.82 ± 1.54	59.59 ± 5.59^{a}	27.88 ± 1.92^{abc}
L-Carnitine 10	91.8 ± 2.29	85.3 ± 1.71	55.30 ± 4.88^{ab}	31.32 ± 7.5^{ab}

In each column values with different superscripts are significantly different ($p < 0.05$).

Table 3. Effect of different L-Carnitine levels on Mean ± SEM curvilinear velocity VCL **(**μm s^{-1})

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	94.9 ± 2.38	73.15 ± 2.7	17.48 ± 0.52^{d}	8.91 ± 0.51^{cd}
L-Carnitine 2	97.33 ± 6.97	67.82 ± 5.37	27.68 ± 5.54^{bc}	6.39 ± 0.48^{d}
L-Carnitine 4	109.44 ± 5	77.64 ± 2.24	25.04 ± 1.3^{cd}	10.42 ± 2.31^{bcd}
L-Carnitine 6	99.30 ± 0.56	69.90 ± 6.28	35.72 ± 4.61^{ab}	21.70 ± 0.71^{a}
L-Carnitine 8	94.90 ± 3.61	82.72 ± 3.6	37.54 ± 4.1^{a}	14.14 ± 1.01^{bc}
L-Carnitine 10	105.17 ± 7	80.88 ±2	31.63 ± 2.12^{abc}	15.85 ± 3.13^{ab}

In each column values with different superscripts are significantly different ($p< 0.05$).

Table 4. Effect of different L-Carnitine levels on Mean ± SEM straight-line velocity VSL (μm s^{-1})

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	73.59 ± 1.98	50.81 ± 2.57	9.28 ± 0.3^{c}	3.92 ± 0.27^{bc}
L-Carnitine 2	72 ± 5.8	45.93 ± 4.66	14.57 ± 4.2^{abc}	1.61 ± 0.16^{c}
L-Carnitine 4	83.44 ± 7.16	53.11 ± 2.19	11.05 ± 0.58^{bc}	3.04 ± 0.54^{bc}
L-Carnitine 6	72.68 ± 3.05	47.12 ± 3.86	17.64 ± 2.8^{a}	10.09 ± 0.97^{a}
L-Carnitine 8	72.49 ± 3.28	58.79 ± 2.61	16.85 ± 2.08^{ab}	5.24 ± 0.54^{b}
L-Carnitine 10	75.5 ± 5.07	57.23 ± 1.24	12.82 ± 1.2^{abc}	5.69 ± 1.21^{b}

In each column values with different superscripts are significantly different ($p< 0.05$).

Table 5. Effect of different L-Carnitine levels on Mean ± SEM average path velocity VAP (μm s^{-1})

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	80.12 ± 2.07	58.36 ± 2.56	12.10 ± 0.34^{c}	5.65 ± 0.34^{bc}
L-Carnitine 2	79.61 ± 5.82	53.84 ± 5.1	19.4 ± 4.71^{ab}	3.24 ± 0.3^{c}
L-Carnitine 4	91.77 ± 6.77	61.54 ± 2.3	15.93 ± 0.48^{bc}	5.54 ± 1.11^{bc}
L-Carnitine 6	81.44 ± 2.33	55.16 ± 4.31	23.71 ± 3.58^{a}	14.19 ± 1.03^{a}
L-Carnitine 8	80.10 ± 3.37	66.35 ± 2.48	23.26 ± 2.69^{a}	8.01 ± 0.76^{b}
L-Carnitine 10	84.36 ± 5.35	65.41 ± 1.64	18.55 ± 1.59^{abc}	8.82 ± 1.88^{b}

In each column values with different superscripts are significantly different ($p< 0.05$).

Table 6. Effect of different L-Carnitine levels on Mean ± SEM amplitude lateral head displacement (ALH,µm)

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	2.95 ± 0.63	2.62 ± 0.56	0.85 ± 0.26^{d}	0.49 ± 0.03^{cd}
L-Carnitine 2	3.23 ± 0.18	2.58 ± 0.16	1.28 ± 0.16^{bc}	0.37 ± 0.27^{d}
L-Carnitine 4	3.42 ± 0.35	2.91 ± 0.63	1.25 ± 0.7^{cd}	0.63 ± 0.15^{bcd}
L-Carnitine 6	3.38 ± 0.12	2.69 ± 0.24	1.07 ± 0.22^{ab}	1.07 ± 0.003^{a}
L-Carnitine 8	3.13 ± 0.11	2.97 ± 1	1.87 ± 0.18^{a}	0.78 ± 0.05^{abc}
L-Carnitine 10	3.53 ± 0.22	2.94 ± 0.47	1.69 ± 0.13^{ab}	0.89 ± 0.19^{ab}

In each column values with different superscripts are significantly different ($p< 0.05$).

Table 7. Effect of different L-Carnitine levels on Mean ± SEM epididymal sperm linearity (LIN, %)

Treatment	Time (hrs)			
	1	12	24	36
L-Carnitine 0	57.92 ± 1.25	45.23 ± 1.42	13.46 ± 0.48^{c}	6.78 ± 0.4^{bc}
L-Carnitine 2	57.96 ± 3.07	44.64 ± 3.76	18.28 ± 3.22^{abc}	3.47 ± 0.23^{c}
L-Carnitine 4	65.14 ± 4.29	49.66 ± 1.1	15.24 ± 0.78^{bc}	5.73 ± 1.17^{bc}
L-Carnitine 6	61.48 ± 1.95	45.59 ± 2.68	21.62 ± 3.18^{ab}	13.51 ± 0.78^{a}
L-Carnitine 8	61.71 ± 2.59	53.07 ± 1.3	22.4 ± 2.85^{a}	8.37 ± 0.74^{abc}
L-Carnitine 10	59.86 ± 2.47	52.91 ± 1	19.39 ± 1.5^{abc}	10.41 ± 2.84^{ab}

In each column values with different superscripts are significantly different ($p< 0.05$).

DISCUSSION

Motility is one of the important indicators in the evaluation of sperm quality, providing important information about the energy status of mammalian sperm. The evaluation of sperm motility is done either visually, resulting in many differences and variations, or by a computer using CASA (Computer Aided Sperm Analysis), which provides an accurate evaluation of sperm motility and various sperm motility parameters [11]. In the current research to investigate L-carnitine on the motility patterns of ram epididymal sperm, CASA was used. In the present study, L-carnitine had a significant and useful effect on the movement kinetics recorded by CASA, and this effect was dose-dependent. L-carnitine is a type of amino acid with a vitamin-like structure and the ability to dissolve in water, naturally existing in microorganisms, plants, and animals. Its amount is diverse in different animals according to species, type of tissue, and nutritional status. L-carnitine is an important and necessary amino acid involved as the main cofactor in the metabolism of fats, facilitating the transfer of fatty acids into the mitochondria and playing an important role in energy metabolism. L-carnitine is present in high dilutions in mammalian epididymis and sperm. Epididymal tissue and sperm supply their energy from L-carnitine in epididymal fluid [12]. Sariozkan et al. have reported that L-carnitine at 12 and 24 hours, and in doses of 0.5, 1, and 2 mM, caused a significant increase in rabbit sperm motility during liquid storage [12]. In our study, the effect of L-carnitine in increasing sperm motility indices at 24 and 36 hours, especially at the levels of 6 and 8 mmol, was significantly higher than the control. Epididymal carnitine is significantly consumed by epididymal sperm and increases mitochondrial oxygen consumption in rat, rabbit, and bull sperm. Therefore, it is believed that carnitine plays a role in maintaining energy metabolism and increasing sperm quality. Additionally, the start of sperm motility increases in parallel with the increase in carnitine concentration in the epididymal ducts [12]. The findings of the researchers indicate a relationship between carnitine and sperm motility. The sperm

removed from the epididymis are not motile because they have not yet been affected by carnitine, and the addition of L-carnitine increases their motility [13]. In the study by Agarwal et al., in a clinical trial, the concentration of sperm and the percentage of linearity and progressive motility of sperm in men who received L-carnitine were significantly higher than the control [13]. This confirms the results of our research. L-carnitine protects the genetic structure of the cell by stabilizing the mitochondrial membrane, and because it reduces the amount of fat available, the amount of fat peroxidation also decreases. On the other hand, L-carnitine plays a role in the activation of antioxidant enzymes such as superoxide dismutase and sperm glutathione peroxidase. These enzymes play an important role in cleaning ROS in chilled sperm. The beneficial effects of L-carnitine on sperm motility of human, pig, stallion, quail, bull, rainbow trout, and rooster have been determined. In our research, the stimulating effect of carnitine in increasing the motility indices of ram epididymal sperm was determined [14]. In medicine, L-carnitine is also involved in improving the quality and function of human sperm and causes an increase in the decarboxylation of sperm mitochondria. By improving the movement structures of sperm, it plays a role in treating infertility cases such as oligoasthenozoospermia (decreased sperm motility) [15]. L-carnitine reduces the harmful effects of cold shock during the cooling and freezing stages of pig sperm, increasing sperm motility kinetics after freezing and thawing [16]. In Heidari et al.'s study on the effect of L-carnitine on goat sperm freezing, the results showed that the addition of L-carnitine in the diluent increases the overall motility and progressive motility of goat sperm, with the best result obtained at a dose of 5 Mm [17]. Contrary to the research conducted in different species of mammals, which indicates the beneficial effects of L-carnitine on quality parameters and especially sperm motility, Souza et al.'s study on the effect of L-carnitine in two types of Tris-egg yolk extender and commercial IMV extender (optiXcell) on ram sperm motility parameters stated that although L-carnitine improves some sperm motility parameters such as VAP, LIN, VSL, WOB, and STR, especially in the optiXcell commercial extender, and although L-carnitine improves sperm motility in humans and rabbits, the effect of L-carnitine is probably different depending on the dose and species of mammal, and more studies and further investigation are needed in this field [9]. Contrary to Souza's study, and in agreement with the research mentioned above, in our study, although it was conducted in HTF culture medium and in liquid storage mode at 5 degrees Celsius, L-carnitine in dilutions of 6, 8, and 10 mmol, especially at 24 hours after storage, is effective on the motility indices of ram epididymal sperm in HTF culture medium, and its use is recommended to increase the quality of ram epididymal sperm. It is better to study the effect of L-carnitine on the motility parameters of ram epididymal sperm in other types of culture media and types of diluents with or without the presence of egg yolk or quail and ostrich egg yolk in the state of sperm storage in liquid form or after freeze and thawing.

CONCLUSION

Altogether, our results showed that L-carnitine at levels of 6, 8, and 10 mM and at 24 and 36 hours caused a clear and significant increase in the motility indices of ram epididymal sperm compared to the control group ($p<0.05$).

ETHICS

Approved.

CONFLICT OF INTEREST

None.

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