Golestan University of Medical Sciences

Jorjani Biomedicine Journal

Online ISSN: 2645-3509

Protective effect of L-carnitine on sperm motility and membrane integrity in men with asthenoteratozoospermia: A Brief Report

Samira Naderi Noreini [1], Roya Ahmadi Niyatabesh [2], Mahdi Malmir [2*], Ali Asghar Ghafarizadeh [3]

1. Rastak Infertility Center, Arak, Iran
2. Department of Midwifery, Tuyserkan Branch, Islamic Azad University, Tuyserkan, Iran
3. Academic Center for Education, Culture and Research, Arak, Iran

* Correspondence: Ali Asghar Ghafarizadeh. Academic Center for Education, Culture and Research, Arak, Iran. Tel: +989189630563, Email: ghafarizade@acecr.ac.ir

Abstract

Background: Sperm preparation is a time-consuming process that contributes to oxidative stress induction in assisted reproductive techniques, which can be prevented by using antioxidants to an acceptable extent. This study aimed to evaluate the effect of L-carnitine on sperm parameters during semen preparation at different time intervals.

Methods: In this in vitro study, the semen samples of 50 asthenoteratozoospermic men were divided into control and L-carnitine groups (incubated with 0.5 mg/mL of L-carnitine; n=25 each). Total motility, progressive motility, sperm membrane integrity (by the hypo-osmotic swelling test), and lipid peroxidation (by the malondialdehyde level) were assessed in each group after 2, 4, and 6 hours. Afterward, the samples were analyzed by the World Health Organization (2010) criteria. The data were statistically analyzed via SPSS software.

Results: In the control group, total and progressive motility after 2 hr and the membrane integrity of the sperm were significantly decreased after 4 hr (P=0.028, P=0.019, and P=0.025, respectively), while malondialdehyde levels significantly increased after 4 hr (P=0.018). Sperm parameters in the L-carnitine group increased significantly after 2 hr, such as total motility (P=0.028), progressive motility (P<0.019), and sperm membrane integrity (P<0.025), while malondialdehyde levels significantly decreased compared to the control group in 6 hr (P<0.001 vs. P=0.045).

Conclusion: L-carnitine can conserve the motility and sperm membrane integrity of sperm from the hazardous impact of oxidative stress and lipid peroxidation during sperm preparation.

Article History

Received: 5 February 2023
Received in revised form: 21 May 2023
Accepted: 30 September 2023
Published online: 24 November 2023
DOI: 10.29252/jorjanibiomedj.11.2.19.

Keywords

Carnitine
Asthenoteratozoospermia
Membranes
Spermatozoa

Article Type: Brief Report

Highlights

What is current knowledge?

Antioxidants, including L-carnitine, can reduce oxidative stress and lipid peroxidation in human semen.

What is new here?

In the andrology laboratory, L-carnitine can be added as a supplement to the semen of asthenoteratozoospermic volunteers, increasing their chances of fertility.

Introduction

Lack of natural pregnancy is currently a problem for human society, which has caused considerable problems for couples (1-5). Based on universal circumstantial evidence, an average of 10-15% of young couples suffer from infertility (2, 4). Asthenospermia and teratozoospermia simultaneously or asthenoteratozoospermia (ATS) can be a factor in male sexual disorders (3, 4). Men with ATS have normal sperm motility of less than 40% and abnormal sperm morphology of more than 4% (3). Studies indicated that these men's sperm produce remarkable levels of reactive oxygen species (ROS) (4, 6, 7). Excess ROS cause mitochondrial dysfunction, reduce the levels of sperm antioxidant enzymes, and cause defective sperm production, which can emanate from the nicotinamide-adenine dinucleotide phosphate oxidase system (2, 4). Induction of lipid peroxidation by the same pathway can operate phospholipase-A2, which facilitates the extrication of polyunsaturated fatty acids (PUFA) in the phospholipid and increases the manufacture of ROS (5). An essential reason for oxidative induction in the membrane plasma of sperm is fatty acid generation (5, 6). Obviously, PUFA is an important factor in ROS risk (6). By damaging the mitochondria, the increase in ROS reduces the level of energy produced for sperm movement and causes the disruption of the integrity of the sperm membrane and sperm morphology (4, 7). Hence, mitochondria damage disrupts sperm motility (4). Assisted reproductive technology (ART) is a time-consuming process, and over time, ROS levels increase in the sperm. Moreover, as once noted, ROS levels are significantly high in ATS semen; it seems that these parameters lead to more violent oxidative stress, which contributes to considerable harm in sperm quality and quantity (3, 6, 7).

According to the cases mentioned earlier, using antioxidant activity positively impacts men's fertility. L-carnitine is a natural antioxidant that is presented for the preparation process of sperm in ATS men. It is derived from lysine and methionine, which are commonly found in meat and dairy products (8). It is effective in amending sperm mobility by enhancing the available energy of sperm through beta-oxidation from long-chain fatty acids in mitochondria (8). This antioxidant can lower lipid peroxidation and malondialdehyde (MDA) levels and neutralize ROS (9). Epididymis has the highest concentration of carnitine in the body; however, this level is lower in infertile men (10). Moreover, carnitine can protect motility, DNA (deoxyribonucleic acid) integrity, fertilizing capacity, and membrane integrity of sperm (plasma, acrosome, and mitochondrial) against harms that are rooted in ROS production (10-13). Besides, a diet containing L-carnitine and L-acetyl carnitine can significantly increase sperm motility in oligoteratospermic men (8, 14). This in vitro research aimed to measure the impact of time-crossing and L-carnitine incubation on motility, membrane integrity, and MDA levels of sperm sampled from ATS men at different times during the preparation process.

Methods

Fifty infertile men aged 20-40 years (average age of 30 years) with simultaneous asthenospermia and teratozoospermia referred to the Omid Royan Infertility Center, Arak, Iran, from July to December 2016, participated in this in vitro study. All the participants had regular sexual intercourse twice a week without using any type of contraceptive. They lived in an industrial city. The ones who had taken any drugs such as vitamin supplements, alcohol, and tobacco or had a history of varicocele, azoospermia, or prostatitis were excluded. Then, semen samples were collected while 3 days had passed since their last ejaculation. Finally, the samples were analyzed by the 2010 World Health Organization (WHO) criteria (3).

Preparation of semen samples

The semen samples were placed in an incubator at 37 °C for 30 min according to the WHO 2010 guidelines to be liquefied (3). Next, the samples were randomly divided into control and L-carnitine groups, and each group had 25 samples. After removal of the upper layer by centrifugation (1000 g) for 10 min, the samples were added to Ham's F-10 medium (Merck, Germany) and stained twice. A dose of 0.5 mg/ml of L-carnitine (L-carnitine; Merck, Germany) was selected based on previous research (14). According to similar studies on the impact of various

antioxidants on sperm quality in vitro, after adding 0.5 mg/ml L-carnitine to the semen samples, they were incubated for 0, 2, 4, and 6 hours (h) at 37 °C.

Semen total antioxidant capacity (TAC) evaluation by ferric reducing antioxidant power assay (FRAP)

This evaluation was based on Benzie and Strain's method (15). This method is based on the ability of plasma to reduce Fe3+ ions and convert them to Fe2+ ions at acidic pH in the presence of 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) reagent (Sigma Aldrich, USA). The plasma's regenerative power is proportional to this complex's concentration. Then, a blue complex is formed in the presence of TPTZ at a low pH, which can be measured at 593 nm. Next, the standard solution of ferrous sulfate (Merck KGaA, Germany) was prepared with a concentration of 1 mM and different dilutions. Finally, the standard curve of iron sulfate was plotted in the FRAP test. The regression formula was derived from the standard curve ($R^2 = 0.9975$, $y = 0.6333x + 0.1013$), where Y equals survival (Figure 1). The regression formula from the standard curve was used to measure the FRAP of ATS men's seminal plasma (15).

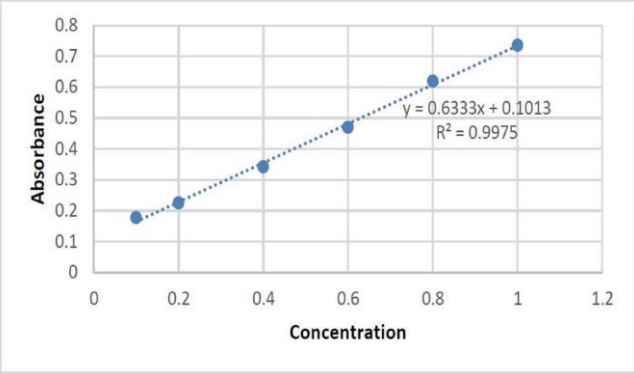

Figure 1. Standard curve and regression formula of iron sulfate in the ferric reducing antioxidant power assay (FRAP) method. (Y: Equal to conservation; R2: R-squared). FRAP measurement is a direct test to evaluate the total antioxidant power. Firstly, the standard curve of ferrous sulfate was drawn, and the regression formula obtained from the standard curve was used to measure FRAP

DNA Fragmentation of the sperm chromatin assay

The sperm chromatin dispersion (SCD) test was used to investigate the fragmentation of sperm DNA. First, 70 µl of samples (5-10 million/mL) from control and L-carnitine groups were blended with 1% agarose (Merck, Germany) at different times at 37 °C. The microscope slides were then covered with 65% agarose, and the suspension was placed on them. Then, a lamella was laid on the slide and stored at 4 °C for 4 min. Subsequently, with the removal of the lamella, each slide was placed horizontally in the ordinary 0.08% chloridic acid solution (Merck, Germany) for 7 min, in absolute darkness and room temperature, and was then immersed in a lysis solution for 25 min. After being washed with distilled water for 5 min, they were flooded in alcohol (70, 90, and 100%) for 2 min. After drying, the slides were stained for 10 min with wright and phosphate-buffered saline (PBS). Finally, after being washed with water, they were examined under a light microscope. In this technique, the range of DNA fragmentation can be classified into 5 types of cells according to the presence of a halo around the nucleus and by examining its size. The percentage of fragmented spermatozoa in the form of nuclear DNA with a small halo, no halo, and destroyed cells, as well as unfragmented spermatozoa in the form of nuclear DNA with a large and medium halo, was reported (16).

Sperm motility assay

Sperm motility parameters were evaluated considering the WHO criteria (2010) (3). First, 10 mL of the suspension containing the medium and sperm was placed on the slide to measure sperm motility under a light microscope (40X). Then, for each slide, 5 microscopic fields containing at least 250 sperm were observed. Finally, the sperm motility percentage was calculated by reporting progressive, nonprogressive, and nonmotile sperm.

Hypo-osmotic swelling (HOS) test for the sperm membrane integrity (SMI) assay

The HOS was done based on Check's method (17). Sperm plasma membrane integrity was assessed via its osmotic roles (Figure 2). Spermatozoa swell when exposed to hypo-osmotic fluid through fluid permeation with a normal plasma membrane. First, 30 µL of the seminal fluid suspension was incubated simultaneously, and then, 300 µL of the hypo-osmotic solution 150 mOsm/Kg (Merck, Germany) was added. Next, they were placed on the slide for 1 hour (37 °C), and then, 200 sperm were counted in approximately 5 fields of sight via phase contrast microscopy. Finally, the percentage of swollen (normal) tailed spermatozoa was identified and recorded as a response to the hypo-osmotic solution (Sperm with a normal plasma membrane showed a bent tail, and sperm with a defective plasma membrane showed no tail swelling) (17).

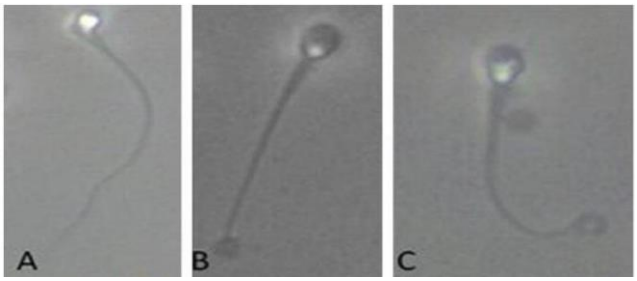

Figure 2. Sperm membrane integrity by hypo-osmotic swelling (HOS) staining, showed different degrees of tail swelling in the human sperm (A-C). Sperm with a normal plasma membrane and bent tail (B & C), Sperm with a defective plasma membrane by no tail swelling (A). (×1000 magnification)

Malondialdehyde assay

Based on the method of Buege and Aust (1), malondialdehyde reacts with thiobarbituric acid (Sigma, USA), generating an orange mixture to indicate rays by a wavelength of about 532-535 nm. First, trichloroacetic acid (15% w/v)-thiobarbituric acid (0.375% w/v)-hydrochloric acid (0.25N HCl) (Sigma, USA) reagent was mixed (2:1) with the semen suspension. Then, the samples were placed in a boiling bath (15 min) and centrifuged for 10 min. After removing the supernatant, their absorbance was read spectrophotometrically in the presence of a blank. Finally, the concentration of MDA was calculated with the extraction coefficient (1.56×105 M-1 cm-1) (18, 19).

Ethical consideration

The present study was conducted in compliance with ethical principles with the permission (IRANKMU.REC.1394.37) of the Medical Ethics Committee of Arak University of Medical Sciences, Arak, Iran. Verbal informed consent was obtained from all the participants.

Statistical analysis

The data were statistically analyzed via SPSS v. 20 (Statistical Package for Social Sciences; Chicago, IL, USA).

Repeated-measures analysis of variance (ANOVA) was used to compare the time intervals, and independent *t*-tests were run to compare the control and L-carnitine groups. All data are presented as means ± standard error (SE), and $P<0.05$ was considered statistically significant.

Results

Spermatogram of ATS men

Table 1 demonstrates the mean of sperm parameters in 50 volunteers, analyzed by the WHO guideline (2010) before incubation. As expected, the semen samples illustrated remarkable disorders in motility, viability, morphology, and DNA fragmentation of sperm. The spermatozoa of all the participants had abnormal motility and tail morphology. According to the WHO, all the volunteers had teratozoospermia and asthenospermia, and their sperm had a high level of DNA fragmentation (Table 1; $P<0.05$).

Table 1. Descriptive data of sperm parameter averages in 50 asthenoteratospermic men aged 20-40 years (average age of 30 years) before incubation, such as volume, pH, concentration, total motility, progressive motility, viability, normal morphology, total antioxidant capacity, and sperm DNA fragmentation

Parameters	Mean ± Standard deviation	Normal range
Volume	3.35 ± 1.78	≥ 1.5 (mL)
pH	7.91 ± 0.28	≥ 7.2
Concentration	74.4 ± 26.6	≥ 15 (106 per mL)
Total motility	34.2 ± 5.7 L	≥ 40%
Progressive motility	23.7 ± 10.57 L	≥ 32%
Viability	57.6 ± 18.5 L	≥ 58%
Normal morphology	1.6 ± 0.70 L	≥ 4%
Total antioxidant capacity	1339.2 ± 150.3	
Sperm DNA fragmentation	22.53 ± 6.74 H	15% ≤

Total sperm motility

There were significant differences between the L-carnitine group at different incubation times and controls in terms of total sperm motility ($P=0.028$). Besides, the total sperm motility significantly declined in the control group after 2 hr ($P<0.001$) (Figure 3; $P<0.05$).

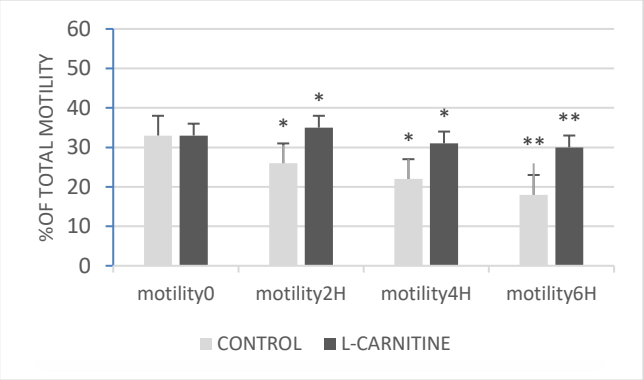

Figure 3. The effects of L-carnitine (0.5 mg mL^{-1}) on total sperm motility after 2 hr of incubation. Mean ± standard deviation and P<0.05. *P<0.05, **P<0.01 significant difference versus the control group. ●P<0.01, ●●P<0.001 significant difference versus the control group before incubation.

Progressive motility of sperm

There were significant differences between the L-carnitine group at different incubation times and controls in terms of sperm progressive motility (P<0.019). Furthermore, sperm progressive motility significantly decreased in the control group after 2 hr (P<0.001) (Figure 4; P<0.05).

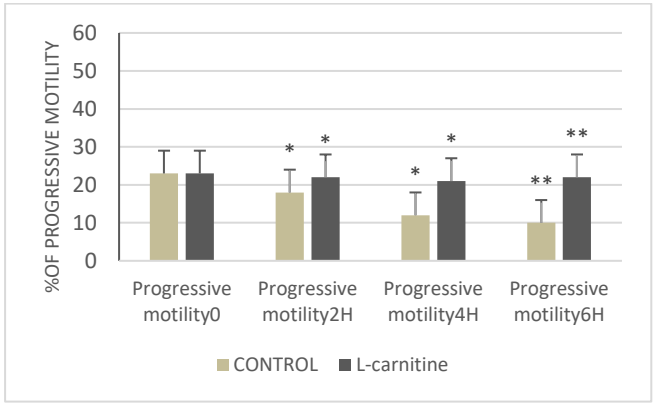

Figure 4. The effects of L-carnitine (0.5 mg mL^{-1}) on the progressive sperm motility after 2 hr of incubation. Mean ± standard deviation, and P<0.05. *P<0.03, ** P<0.01 significant difference versus the control group. ●P<0.01, ●●P<0.01 significant difference versus the control group before incubation.

Sperm membrane integrity

There were significant differences between the L-carnitine group at different incubation times and controls in terms of SMI (P<0.025). Moreover, SMI significantly decreased in the control group after 2 hr (P<0.018) (Figure 5; P<0.05).

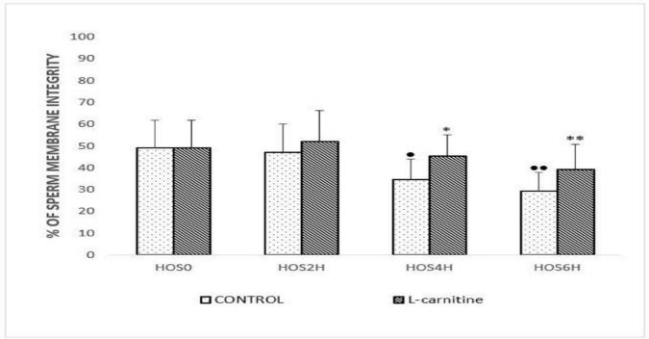

Figure 5. The effects of L-carnitine (0.5 mg mL^{-1}) on sperm membrane integrity (SMI) by the hypo-osmotic swelling (HOS) assay after 2 hr of incubation. Mean ± standard deviation, and P<0.05. *P<0.04, ** P<0.018 significant difference versus the control group. ●P<0.04, ●●P<0.025 significant difference versus the control group before incubation.

Lipid peroxidation by MDA

There were significant differences between the L-carnitine groups at different incubation times and controls in terms of MDA levels (P<0.017). Moreover, MDA levels significantly increased in the control group after 2 hr (P<0.001) (Figure 6; P<0.05).

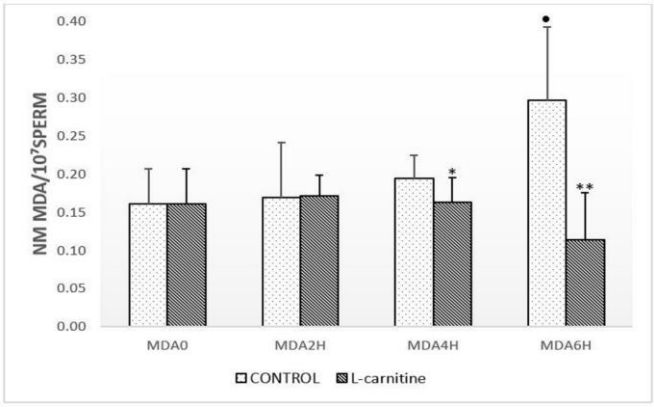

Figure 6. The effects of L-carnitine (0.5 mg mL^{-1}) on the malondialdehyde (MDA) levels after 2 hr of incubation. Mean ± standard deviation and P<0.05. *P<0.03, **P<0.017 significant difference versus the control group. ●P<0.001 significant difference versus the control group before incubation.

Discussion

The findings showed that as time passed, by increasing lipid peroxidation in the semen of ATS men, the quality of sperm parameters significantly decreased, and L-carnitine significantly compensated for this damage and upregulated sperm parameters. In our study, the mean of semen parameters in 50 ATS men before incubation showed significant abnormalities in viability, morphology, motility, and sperm DNA fragmentation. Moreover, in the control group, sperm parameters declined significantly, except for the MDA level, which significantly increased. Hence, the results of the present study confirm previous andrological studies (4, 20-22). In agreement with our results, Ghafarizadeh et al. (2018) and Fanaei et al. (2014) found a decline in motility and viability, as well as an increase in the MDA of sperm 0.5 to 6 hr after incubation (4, 23). Human semen has low antioxidant enzyme capacity and, therefore, fails to completely deal with oxidative stress (23). Besides, ROS produced by sperm and leukocytes can decrease adenosine triphosphate (ATP) levels, which have a destructive effect on sperm motility (23). The sperm membrane has a lot of polyunsaturated fatty acids, which are attacked by free radicals (20). Furthermore, ROS can directly damage chromatin by penetrating through the membrane nucleus (24). Kobayashi et al. (2001) showed a relationship between low sperm viability and high ROS levels (25). High MDA levels also destroy sperm membrane integrity so that ionic transfer, chemical messengers, and the ion concentration gradient are disturbed on both sides of the membrane (26, 27). According to our results and those of similar studies (4, 20-22), it can be concluded that oxidative stress and lipid peroxidation increase in sperm as time passes, which in turn damages sperm motility and membrane integrity. A common method today to prevent oxidative stress in sperm during ART is the use of antioxidants. Hence, according to this study, L-carnitine was applied as a natural antioxidant to upregulate sperm parameters.

L-carnitine is a chemical compound with strong antioxidant properties. It is produced in the brain, kidneys, and liver, helps the body to convert fat into energy, and increases sperm motility in semen (8, 20). Our results showed that the total motility, progressive motility, and membrane integrity of sperm were significantly higher in the L-carnitine group compared with the control group after 2 hr of incubation. However, MDA levels were significantly lower in the L-carnitine group compared to the control group at 6 hr of incubation. Studies by other researchers on sperm parameters confirm the results of the present results (14, 22, 28, 33-35). Banihani et al. (2012), in an in vitro study, evaluated the effect of different concentrations of L-carnitine (0.1, 0.5, and 1 mg/mL) on human sperm at 2 and 4 hr of incubation and, then, after 20 min centrifuging; they found an increase in sperm motility (14). In the study by Al-Dujaily et al. (2014), the impact of L-carnitine (0.5 mg/mL) on men with asthenospermia disorder was investigated during sperm preparation by the concentration gradient. Eventually, the progressive motility of sperm significantly increased compared to the control group (28). Besides, Zhang (2020) reported an increase in total and progressive sperm motility by using L-carnitine in idiopathic oligoasthenoteratozoospermia patients (35). L-carnitine and L-acetyl carnitine act as a buffering system to regulate the concentration of acetyl CoA, which is essential for the tricarboxylic acid cycle and energy production and can increase sperm motility (20). Besides, L-carnitine facilitates the transport of long-chain fatty acids along the inner membrane of the mitochondria, and these fatty acids are broken down by beta-oxidation to produce ATP, which can provide the energy needed by sperm (21, 34). Besides, the property of L-carnitine, as a ROS-destroyer, can improve sperm motility and viability (10, 32, 35, 36). Oral administration of L-carnitine and acetyl-L-carnitine showed an increase in the activity of total antioxidants and sperm motility in individuals with idiopathic asthenospermia (14). Furthermore, L-carnitine can protect sperm membranes against the harmful effects of ROS by its antioxidant activity (22, 32, 34). It seems that L-carnitine helps facilitate the transfer of fatty acids to the

mitochondrial matrix and lipid peroxidation decreasing, and it takes advantage of this way to improve the sperm membrane integrity and mitochondrial membrane potential (22, 34). Recently, researchers have reported that selenium (4), melatonin (20), and L-carnitine (22) can have a positive effect on DNA fragmentation in the sperm of ATS patients. Recent studies have also reported the effect of reducing MDA levels and increasing membrane integrity in human sperm by L-carnitine treatment (34, 36). Besides, it can improve the total and progressive motility of sperm in them (21). Such studies and the present research show that antioxidant therapy would remarkably impact the sperm of ATS men.

Conclusion

ART is a time-consuming process, and over time, sperm quality decreases; nevertheless, the use of an antioxidant in this process can prevent this damage. This point is more important in AST patients in whom sperm quality is inherently low. The present study showed that L-carnitine as an antioxidant can improve the progressive and total motility and sperm membrane integrity and can also reduce lipid peroxidation as time passes. Therefore, it can maintain sperm parameters during ART and enhance the fertility rate in ATS patients.

Since half of the participants did not return, it was not possible to evaluate the percentage of fertility in them. It is suggested that in future studies, the fertility percentage of the participants is also assessed because it can provide more information about the effect of L-carnitine. Finally, the repeatability of using L-carnitine in different doses and treatment times can reveal the hidden dimensions of this antioxidant in improving male fertility.

Acknowledgement

The authors appreciate the efforts of Tayebeh Faragi.

Funding sources

This experimental study was carried out by self-funding.

Ethical statement

This research was conducted in agreement with ethical principles by the license (IRANKMU.REC.1394.37) of the Medical Ethics Committee of Arak University of Medical Sciences, Arak, Iran. All the participants provided oral informed consent.

Conflicts of interest

There is no conflict of interest.

Author contributions

Nader Noreini conceived and planned the experiments. Ahmadi Niyatabesh carried out the experiments. Malmir contributed to the interpretation of the results. Ghafarizadeh supervised the study and took the lead in writing the manuscript. All the authors provided critical feedback and helped shape the research, analysis, and manuscript.

References

1. Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. Indian J Med Res. 2009;129(4):357-67. [View at Publisher] [Google Scholar] [PMID]
2. Fanaei H, Khayat S, Halvaei I, Ramezani V, Azizi Y, Kasaeian A. Effects of ascorbic acid on sperm motility, viability, acrosome reaction and DNA integrity in teratozoospermic samples. Iran J Reprod Med. 2014;12(2):103-10. [View at Publisher] [Google Scholar] [PMID]
3. Lu JC, Huang YF, Lu NQ. [WHO laboratory manual for the examination and processing of human semen. its applicability to andrology laboratories in China]. Zhonghua Nan Ke Xue. 2010;16(10):867-71. [View at Publisher] [Google Scholar] [PMID]
4. Ghafarizadeh AA, Vaezi G, Shariatzadeh MA, Malekirad AA. Effect of in vitro selenium supplementation on sperm quality in asthenoteratozoospermic men. Andrologia. 2018;*50*(2):e12869. [View at Publisher] [Google Scholar] [DOI] [PMID]
5. Safarinejad MR, Hosseini SY, Dadkhah F, Asgari MA. Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: a comparison between fertile and infertile men. Clin Nutr. 2010;29(1):100-5. [View at Publisher] [Google Scholar] [DOI] [PMID]
6. Nabi A, Khalili MA, Halvaei I, Roodbari F. Prolonged incubation of processed human spermatozoa will increase DNA fragmentation. Andrologia. 2014;46(4):374-9. [View at Publisher] [Google Scholar] [DOI] [PMID]
7. Yun JI, Gong SP, Song YH, Lee ST. Effects of combined antioxidant supplementation on human sperm motility and morphology during sperm manipulation in vitro. Fertil Steril. 2013;100(2):373-8. [View at Publisher] [Google Scholar] [DOI] [PMID]
8. Lenzi A, Sgro P, Salacone P, Paoli D, Gilio B, Lombardo F, et al. A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and l-acetyL-carnitine treatment in men with asthenozoospermia. Fertil Steril. 2004;81(6):1578-84. [View at Publisher] [Google Scholar] [DOI] [PMID]
9. Lee YJ, Lee SH, Kim YJ, Hwangbo Y, Lee S, Cheong HT, et al. Effects of L-carnitine and Nicotinic Acid on Sperm Characteristics in Miniature Pigs. Reprod Devel Biol. 2016;40(1):1-5. [View at Publisher] [Google Scholar] [DOI]
10. De Rosa M, Boggia B, Amalfi B, Zarrilli S, Vita A, Colao A, et al. Correlation between seminal carnitine and functional spermatozoal characteristics in men with semen dysfunction of various origins. Drugs R D. 2005;6(1):1-9. [View at Publisher] [Google Scholar] [DOI] [PMID]
11. Galarza DA, López-Sebastián A, Santiago-Moreno J. Supplementing a skimmed milk–egg yolk-based extender with L-carnitine helps maintain the motility, membrane integrity and fertilizing capacity of chilled ram sperm. Reprod Domest Anim. 2020:55(7):805-13. [View at Publisher] [Google Scholar] [DOI] [PMID]
12. Nery Igor HAV, Araújo Silva RAJ, Souza HM, Arruda LCP, Monteiro MM, Seal DCM, et al. Effects of l-carnitine on equine semen quality during liquid storage. Biopreserv Biobank. 2020;18(5):403-8. [View at Publisher] [Google Scholar] [DOI] [PMID]
13. Jiang XH, Jiang C, Yu L, Li XL, Zuo T, Gu PF, et al. Supplementation with L-carnitine rescues sperm epigenetic changes in asthenospermic male semen with altered Acetyl-L-carnitine levels. Reprod Dev Med. 2020;4(3):146-58. [View at Publisher][Google Scholar][DOI] [PMID]
14. Banihani S, Sharma R, Bayachou M, Sabanegh E, Agarwal A. Human sperm DNA oxidation, motility and viability in the presence of l-carnitine during in vitro incubation and centrifugation. Andrologia. 2012;44(S1):505-12. [View at Publisher][Google Scholar][DOI] [PMID]
15. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005:53(6):1841-56. [View at Publisher][Google Scholar][DOI] [PMID]
16. Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril. 2005;84(4):833-42. [View at Publisher][Google Scholar][DOI] [PMID]
17. Check ML, Kiefer D, Check JH, Hourani W, Long R. Treatment of sperm with subnormal host scores with chymotrypsin/viable pregnancy after IUI. Arch Androl. 2002;48(2):155-8. [View at Publisher][Google Scholar][DOI] [PMID]
18. Czinner E, Hagymasi K, Blazovics A, A Kery, É Szőke, Lemberkovics E. The in vitro effect of Helichrysi flos on microsomal lipid peroxidation. J Ethnopharmacol. 2001:77(1):31-5. [View at Publisher][Google Scholar][DOI] [PMID]
19. Mayorga-Torres BJM, Cardona-Maya W, Cadavid A, Camargo M. Evaluation of sperm functional parameters in normozoospermic infertile individuals. Actas Urol Esp. 2013;37(4):221-7. [View at Publisher][Google Scholar][DOI] [PMID]
20. Malmir M, Naderi Noreini S, Ghafarizadeh A, Faraji T, Asali Z. Ameliorative effect of melatonin on apoptosis, DNA fragmentation, membrane integrity and lipid peroxidation of spermatozoa in the idiopathic asthenoteratospermic men: In vitro. Andrologia. 2021;53(2):e13944. [View at Publisher][Google Scholar][DOI] [PMID]
21. Ghafarizadeh AA, Malmir M, Naderi Noreini S, Faraji T, Ebrahimi Z. The effect of vitamin E on sperm motility and viability in asthenoteratozoospermic men: In vitro study. Andrologia. 2020;53(1):e13891. [View at Publisher][Google Scholar][DOI] [PMID]
22. Naderi Noreini S, Malmir M, Ghafarizadeh A, Faraji T, Bayat R. Protective effect of L-carnitine on apoptosis, DNA fragmentation, membrane integrity and Lipid peroxidation of spermatozoa in the asthenoteratospermic men. Andrologia. 2021;53(2):e13932. [View at Publisher][Google Scholar][DOI] [PMID]
23. Fanaei H, Khayat S, Halvaei I, Ramezani V, Azizi Y, Kasaeian A, et al. Effects of ascorbic acid on sperm motility, viability, acrosome reaction and DNA integrity in teratozoospermic samples. Iran J Reprod Med. 2014:12(2):103-10. [View at Publisher][Google Scholar][DOI] [PMID]
24. Aitken RJ, Bronson R, Smith TB, De Iuliis GN. The source and significance of DNA damage in human spermatozoa; a commentary on diagnostic strategies and straw man fallacies. Mol Hum Reprod. 2013;19(8):475-85. [View at Publisher][Google Scholar][DOI] [PMID]
25. Kobayashi H, Gil-Guzman E, Mahran AM, Rakesh, Nelson DR, Thomas Jr AJ, et al. Quality control of reactive oxygen species measurement by luminol-dependent chemiluminescence assay. J Androl. 2001;22(4):568-74. [View at Publisher][Google Scholar][DOI] [PMID]
26. Bravo A, Quilaqueo N, Jofré I, Villegas JV. Overtime expression of plasma membrane and mitochondrial function markers associated with cell death

in human spermatozoa exposed to nonphysiological levels of reactive oxygen species. Andrologia. 2021:53(2):e13907. [View at Publisher][Google Scholar][DOI] [PMID]
27. Mahfouz RZ, du Plessis SS, Aziz N, Sharma R, Sabanegh E, Agarwal A. Sperm viability, apoptosis, and intracellular reactive oxygen species levels in human spermatozoa before and after induction of oxidative stress. Fertil Steril. 2010;93(3):814-21. [View at Publisher][Google Scholar][DOI] [PMID]
28. Al-Dujaily S, Al-Sultani YK, Alddin NNS. DNA normality following in vitro sperm preparation with pentoxifylline and L-carnitine for asthenozoospermic infertile men. Global Journal of Medical Research 2014;13(K7). [View at Publisher][Google Scholar]
29. Casillas ER, Chaipayungpan S. The distribution of carnitine and acetylcarnitine in the rabbit epididymis and the carnitine content of rabbit spermatozoa during maturation. J Reprod Fertil 1979;56(2):439-44. [View at Publisher][Google Scholar][DOI] [PMID]
30. Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med. 2004;25(5-6):455-73. [View at Publisher][Google Scholar][DOI] [PMID]
31. Gulcin İ. Antioxidant and antiradical activities of L-carnitine. Life Sci. 2006;78(8):803-11. [View at Publisher][Google Scholar][DOI] [PMID]
32. Nateghian Z, Nasr-Esfahani MH, Talaei-Khozani T, Tavalaee M, Aliabadi E. L-carnitine and pentoxifylline supplementation improves sperm viability and motility at low temperature. Int J Fertil Steril. 2023;17(1):61-6. [View at Publisher][Google Scholar][DOI] [PMID]
33. Chang D, Kong F, Jiang W, Li F, Zhang C, Ding H, et al. Effects of l-carnitine Administration on Sperm and Sex Hormone Levels in a Male Wistar Rat Reproductive System Injury Model in a High-Altitude Hypobaric Hypoxic Environment. Reprod Sci. 2023;30(7):2231-47. [View at Publisher][Google Scholar][DOI] [PMID]
34. Ghorbani F, Nasiri Z, Koohestanidehaghi Y, Lorian K. The antioxidant roles of L-carnitine and Nacetyl cysteine against oxidative stress on human sperm functional parameters during vitrification. Clin Exp Reprod Med. 2021;48(4):316-21. [View at Publisher][Google Scholar][DOI] [PMID]
35. Zhang X, Cui Y, Dong L, Sun M, Zhang Y. The efficacy of combined l-carnitine and l-acetyl carnitine in men with idiopathic oligoasthenoteratozoospermia: a systematic review and meta-analysis. Andrologia. 2020;52(2):e13470. [View at Publisher][Google Scholar][DOI] [PMID]
36. Altyeb YH, Absy G, Medan MS, Hassan ST, Elsayed DH. The effects of cysteine and L-carnitine on the DNA integrity of post-thaw sperm of frozen buck semen. Iran J Vet Res. 2022;23(3):196-203. [View at Publisher][Google Scholar][DOI] [PMID]

How to Cite:
Naderi Noreini S, Ahmadi Nia Tabesh R, Malmir M, Ghafarizade AA. Protective effect of L-carnitine on sperm motility and membrane integrity in men with asthenoteratozoospermia: A Brief Report. *Jorjani Biomedicine Journal.* 2023;11(2):19-23.