Effect of L-carnitine on *in vitro* sperm activation of infertile men

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Abstract:

Background

The effect of L-carnitine (LC) on the male infertility have been previously reviewed. However there are few studies about the effect of LC on sperm function parameters *in vitro* .

Objectives

The objective of this study was to improve certain sperm function parameters *in vitro* of asthenozoospermic infertile men using LC as a motility stimulant substance which may enhance the medium used for assisted reproductive technologies (such as artificial insemination) in future.

Methods:

Semen was collected from 50 infertile men involved in the current study. Each semen sample was divided into two portions. One part was considered as a control and *in vitro* activated by using culture medium only. The other portion was considered as treated group and *in vitro* activated by adding LC (0.5mg) to the culture media. Certain sperm function parameters were examined before and following *in vitro* activation using simple layer technique.

Results

The results revealed highly significant increment ($P<0.01$) in the percentage of total sperm motility grades (A+B+C) and progressive sperm motility grade (A) with a significant improvement ($P<0.05$) in the percentage of progressive sperm motility grade (B) and morphologically normal sperm when using LC medium in comparison with control medium after 30 minutes incubation .

Conclusion

It is concluded from the results of the present study that adding 20% LC to the culture medium of the *in vitro* sperm activation leads to an improvement in certain sperm function parameters.

Key words: L-carnitine, *in vitro* sperm activation, sperm function parameters

Introduction

Infertility is the inability of a sexually active non-contraception couple to achieve pregnancy in one year (1). Infertility is either primary, when no pregnancy has ever occurred, or secondary, where there has been a pregnancy, regardless of the outcome. About 67-71% and 29-33% of patients have primary and secondary infertility, respectively (2). Asthenozoospermia is one of the major causes of infertility or reduced fertility in men. *In vivo* and *in vitro* treatment of this factor depends mainly on the sperm motility stimulants such as Ca^{++}, glutamine, pyruvate ,glucose and so many others(3) .L-Carnitine is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine (4). Is a natural vitamin like substance that acts in the cells as a receptor molecule for activated fatty acids. A shortage of this substance results primarilyin impaired energy metabolism and membrane function (5). In the human body, the primary function of L-carnitine is to carry fatty acids into the mitochondria where they can be broken down with the ultimate production of energy (6). In the epididymis, the sperm use fatty acids as their source of metabolic energy and scientists believe that one of the functions of LC in sperm is to carry fatty acids into the sperm

mitochondria, thereby assisting in the production of energy. Secondly, the conversion of some of the LC to acetyl-L-carnitine(ALC) in the mature sperm facilitates the continuation of energy production within the sperm and the newly formed ALC serves as a readily available source of acetyl groups, i.e. energy, for the sperm (7). Men with infertility issues frequently have a problem with the number, shape or motility of their sperms. In fact, it is estimated that 40 percent of human infertility is entirely or partly due to deficiencies in sperm quality (8). At the beginning of the 1990s, a pilot multicenter uncontrolled study was begun to test the efficacy of LC in selected cases of idiopathic male infertility (9). Clinical research over the last few decades has reported that LC and its metabolite, ALC are found in high concentrations in sperm, play a part in sperm energy metabolism and most importantly, may support sperm quality (10). In another study (11) LC and ALC are found in high concentrations in the epididymis, where they also act as antioxidants protecting spermatozoa against damage caused by reactive oxygen species and play a crucial role in sperm metabolism and nutrition. They are related to sperm motility. Indeed, the initiation of sperm motility occurs in parallel with the increase in concentration of free LC in the epididymal lumen (7).Therefore the aim of the present study was to use LC as a motility stimulant

Materials and Methods

Semen samples were obtained from 25 infertile men during their attendance to Institute of Embryo Research and Infertility treatment, Al-Nahrain University. The mean ages of patient's was 39 ± 0.96 years old with a range of 25 to 59 years. The samples of seminal fluid were collected after 3 to 5 days of abstinence directly into a clean, dry and sterile disposable Petri-dishes by masturbation in a room near the laboratory (12). After liquefaction time, analysis of semen samples was done before and after swim up preparation *in vitro* to determine the sperm count and sperm motility percentage. Atleast 200 spermatozoa in five different microscopic fields were evaluated from each sample. The specimens were examined in details by macroscopic and microscopic examinations using standardization of WHO (12).

Preparation of LC for *in vitro* sperm activation

The concentration of LC working solution was prepared by adding 0.5mg of LC powder to 10 ml of phosphate buffer solution in plastic test tube contained broad spectrum antibiotic (Ampicillin 0.004 gm) to prevent bacterial growth. The media used for activation contained 20% of LC working solution by adding 2 ml of LC working solution to 8ml of FertiCultTM flushing medium (FertiCult Company,Belgium).The solution was filtered using Millipore 0.45 µM and it's pH was adjusted to be 7.4-7.8 at 25C°.

Experimental design

Each semen sample was divided into two equal parts. One part was added to equal volume of FertiCultTM flushing medium, which was considered as a control, while the other part of semen was added to equal volume of LC media. Sperm activation was performed using simple layer technique .One ml of semen sample was layered beneath one ml of the FertiCultTM flushing medium in culture tube. Then incubated at 37C° for 30 minute(13). A drop (10µL) taken from the top layer was examined under 40X-objective. The effect of prepared media on certain sperm parameters was studied.

Statistical Analysis:

The data were expressed as mean ± SE. Analysis of variance (ANOVA) test and least significant (LST) were used to detect the significance between the variables(14). Analyses were conducted with Microsoft Excel/ Microsoft Office XP 1985- 2001 and Statistica/ version 6.0 (USA) statistical package(14).

Results

Table 1 shows the effect of LC on *in vitro* activation of human sperm of infertile men. There was a highly significant (P<0.01) decrease in sperm concentration compared to the result before activation. Total sperm motility percentages (A+B+C) was highly increased (P<0.01) in both control and treated semen samples compared to the results before activation. There was a highly significant (P<0.01) improvement in total motility percentage in treated samples when compared to control samples after 30 minute of incubation. Concerning the percentage of progressive sperm motility grade (A) there was highly significant (P<0.01) increment in both control and treated group following 30 minutes of incubation compared with the results obtained before activation. There was highly significant (P<0.01) elevation in the percentage of progressive sperm motility grade (A) in treated group compared to control group after *in vitro* activation. A significant (P<0.05) increase was noticed in the percentage of progressive sperm motility grade (B) after activation for 30 minutes in both control and treated group compared to before activation. There was a significant (P<0.05) improvement in the percentage of progressive sperm motility grade (B) in treated group compared to control group after incubation for 30 minutes. There was a significant (P<0.05) decrease in the percentage of sperm motility grade (C) in the samples treated with LC compared to before activation. While no significant (P>0.05) difference was observed in control sperm sample when compared to results before activation. No significant (P>0.05) difference in the percentage of sperm motility grade (C) was noticed between control and treated group. Activation of human sperm caused a highly significant (P<0.01) elevation in the percentage of normal sperm morphology in both control and treated group following incubation for 30 minutes compared to the results before activation. While there was a highly significant (P<0.01) increment in the percentage of morphologically normal sperm of treated groups compared to control group (Table.1). A highly significant (P<0.01) decrease in the mean of round cells was observed in both control and treated group compared to the results before activation

Discussion

The reduction in the concentration of spermatozoa following *in vitro* activation may be due to the inability of the dead and abnormal sperm with poor motility spermatozoa to swim up and migrate from sperm pellet to the upper layer of culture medium (15,16). The increase in the percentage of total sperm motility, progressive sperm motility grade (A) and (B) after 30 minutes incubation is in agreement with other studies that reported a significant increase in the sperm motility when LC was added to ejaculated human spermatozoa (17,18). The contribution of LC to enhance sperm motility may be explained by its role in sperm energy metabolism in vivo and most importantly, in supporting sperm quality (10). In addition the initiation of sperm motility occurs in parallel with the increase in concentration of free LC in the epididymal lumen (12). Other studies (11,19) demonstrated a significant correlation between seminal LC concentration and sperm concentration, sperm motility, rapid forward progression, live sperm count, membrane function, capacity for cervical mucus penetration, linearity of spermatic movement, amplitude of lateral sperm head movement and nuclear DNA integrity.

Table 1. Effect of LC on certain sperm characters of infertile men following *in vitro* activation for 30 minutes.

Certain sperm characters	Before Activation	After activation Control	After activation Treated
Sperm concentration (X10^{6} sperm /ml)	44.6 ± 4.3^{a}	12.7 ± 2.3^{b}	18.1± 1.7^{b}
Total sperm motility (%) (A+B+C)	44.1 ±5.2^{B}	81.0 ± 2.4^{A}	89.0 ± 2.4^{A}
Sperm motility grade(C) %	9.2 ± 0.89^{a}	36.0 ± 4.9^{a}	53.3 ± 6.0^{a}
Sperm motility grade (B) %	25.1 ± 2.6^{A}	31.5 ± 5.5^{A}	37.4± 4.5^{A}
Sperm motility grade (A) %	18.0 ± 2.5^{A}	14.4 ± 1.7^{B}	15.2 ± 2.5^{B}
Morphologically normal sperm (%)	40.5 ±2.0^{B}	78.0 ± 3.2^{A}	83.3 ± 1.4^{A}
Round cells(cell/ HPF)	12.2 ± 3.2^{A}	2.6 ± 2.2^{B}	2.6 ± 1.4^{B}

Values express as mean± SEM
Different small letters mean significant at P<0.01.
Different capital letters mean significant at P<0.05.
Number Samples=25

The other positive effect of addition of LC to the medium is its function to carry fatty acids into the sperm mitochondria, thereby assisting the production of energy. Moreover, the conversion of some of the LC to ALC in the mature sperm facilitates the continuation of energy production within the sperm and the newly formed ALC serves as a readily available source of acetyl groups, i.e. energy for the sperm (7).

Activation of human sperm with LC caused a significant increment in morphologically normal sperm (MNS) of treated parts of semen which were incubated for 10, 30 and 60 minutes . Abd El-baset *et al.* (20) recorded that LC treatment has ameliorative impact on the quality of spermatozoa in the infertile men, resulting in a decrease in morphologically abnormal spermatozoa. Yeste et al. (21) concluded that the addition of LC to the diet of males may maintain the level of normal sperm morphology in boars.Furthermore, morphological defect rates of spermatozoa in the samples supplemented with LC were lower than that in control . Thus the observation of improvement of MNS following LC addition *in vitro* may be explained by the fact that LC has a strong antioxidant effect on sperm antioxidant defense enzymes in the cauda epididymis such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (22). Consequently LC can protects sperm membranes against pathological effect of ROS. *In vitro* addition of LC may reduce the pathway of lipid peroxidation by transporting fatty acids into the mitochondria for β-oxidation to generate adenosine triphosphate (ATP) energy. Thus LC can protects the cell membrane and DNA against damage induced by ROS (23) . It has been recorded that LC can neutralize the sperm toxic effects of oxidative stress induced by hydrogen peroxide (24). It seems that LC confers antioxidant activity that reduce the testicular levels of nitric oxide (NO), malondialdehyde (MDA), in addition to the healthy sperm and histology (25).

It has been documented that LC may be responsible for

removing excess intracellular toxic acetyl-COA, that is responsible for mitochondrial ROS production (26,27). On the other hand, ALC inhibits arachidonic acid incorporation into phospholipids. Arachidonic acid plays an important role in the formation of oxygen free radicals. In addition, it represents an important pool for accumulated lysophospholipids. This role is critical as a repair mechanism following insult from ROS (28). It has been reported that the inclusion of ALC in culture media during sperm processing can yield a higher fraction of motile spermatozoa with better velocity parameters (29). ALC is thought to maintain sperm plasma membrane stability through its involvement in acetylation of membrane phospholipids and exerts an antioxidant activity(27).Reactive oxygen species in particular hydrogen peroxide produced either by spermatozoa or leukocytes in the seminal plasma have been described to affect different sperm functions including motility (30). Their effects seem to be dual depending on the concentration of ROS: low levels can induce the cyclic adenosine monophosphate (cAMP) signaling leading to an increase in sperm motility and tyrosine phosphorylation of proteins stimulating capacitation, while high levels exert an inhibitory role (31). In vitro supplements used during sperm preparation and assisted reproductive technique also help to protect spermatozoa against ROS. Moreover, adding antioxidants to the culture media neutralizes ROS produced by the leukocytes and immature spermatozoa and improves sperm-oocyte fusion (32). The absence of statistically significant differences between mean of round cells in treated groups compared to control groups after human hyperactivation indicated that LC have no detrimental effect on round cells.

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