The effect of treatment by L-carnitine for infertile men on semen parameters

Abdul Rahman Jihad Manssor1, Zaid Mohammed Mubarak Al–Mahdawi1, Azzawi Mustafa Hadi2
1 Department of biology, College of Science, University of Tikrit, Tikrit, Iraq
2 Department urology, College of Medicine, University of Tikrit, Tikrit, Iraq

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ABSTRACT

L-carnitine (LC) is highly concentrated in the epididymis and plays a crucial role in sperm metabolism and maturation. They are related to sperm motility and have antioxidant properties. The objective of this review is to summarize the multiple roles played by LC in male reproduction, and to highlight their limitations as well as their benefits in the treatment of male infertility. A variety of studies support the conclusion that LC at total daily amounts of at least 500mg per day can significantly improve both sperm concentration and total sperm counts among men with astheno- or oligoasthenozoospermia. Although many clinical trials have demonstrated the beneficial effects of LC in selected cases of male infertility. Additional, a well – designed study is necessary to further validate the use of carnitines in the treatment of patients with male infertility, specifically in men with poor semen quality.

Introduction

Infertility is defined as failure of the wife to achieve a successful pregnancy after one year or more of regular unprotected intercourse, [1]. Infertility is a common clinical problem affecting 13-15% of couples worldwide. The prevalence varies throughout developed and undeveloped countries, being higher in the latter in which limited resources for diagnosis and treatment exist. A male factor is solely responsible for infertility in approximately 20% and contributory in another 30-40% of couples; as such, a male factor is implicated in more than 50% of couples attempting to conceive [2]. About 1 in 7 couples have problems conceiving, with a similar incidence worldwide. Over 80% of couples who have regular sexual intercourse and do not use contraception will achieve a pregnancy within one year, and approximately 92% can achieve a pregnancy within 2 years [3,4].

Infertility is classically defined as the inability to conceive after at least one year of regular unprotected intercourse. It is a common medical condition affecting between 9 and 25% of couples worldwide [5,6].

Primary infertility is a term given when no prior conception is attained, while secondary infertility refers to delayed conception in a patient who was previously able to cause a conception. In a systematic analysis of demographic and reproductive health surveys, reported a 1.9 and 10.5% prevalence of primary and secondary infertility, respectively [7]. Most clinical guidelines agree that a fertility evaluation is generally advisable only after the time frame definition is met. However, couples may be evaluated earlier in the presence of male infertility risk factors such as a history of bilateral cryptorchidism, female infertility risk factors including advanced female age, or when the couple questions the male partner’s fertility potential (best practice statement).

In addition, researcher will perform an abbreviated evaluation (focused history and physical exam with semen analysis) in couples prior to 1 year if they express a desire for earlier assessment. As such, the patient in case (1) may be offered evaluation since he is seeking an initial assessment. Nonetheless, a discussion about the statistical chances of pregnancy can be initiated to raise patient awareness and possibly reduce his often experienced anxiety. In a healthy couple, there is about 20% chance of pregnancy with each cycle, provided that no obvious risks for infertility are present (a guide for patients. 2012). This chance is mainly affected by maternal age and drops to about 5% per cycle when the women...
Infertility is a major health problem [8]. Male infertility is defined as inability on the wife to conceive after six months of unprotected sex in the absence of female cause [9]. Subfertility and secondary infertility are developing states after an initial phase of fertility [10]. The incidence of infertility is difficult to be determined with precision because the control populations that lack social or artificial constraints on fertility are not easy to find [11]. The investigations were only performed when conception had not occurred after a year of unprotected intercourse [12].

Infertility is a significant problem in humans. According to WHO it is defined as the inability of a sexual active, non-contracepting couple to achieve pregnancy in one year [13]. Infertility affects fifteen percent of couples worldwide. Male and female factors coexist in about one third of cases, while one third of cases are secondary to male factors only [14]. Spermatozoa are non-motile and cannot fertilize an ovum after formation in the seminiferous tubules. Sperm develops the capability of motility and fertilization (post testicular maturation) only they pass through epididymis [15].

The epididymis is a highly coiled tube measuring 5-6 meters if unwound fully. It connects the tubes of the testes to the vas deferens and plays an important role in maintaining a physiological milieu in the epididymal canal suitable for sperm maturation.

To diagnose male factor infertility, the male partner is studied by his medical history and physical examination, including semen analysis according to standards set by the WHO [16]. The most common cause for male infertility is varicocele, affecting around 20% of men in general population and up to 40% of infertile men [17]. It is a condition that involves dilatation of scrotal veins. Links between varicocele and testicular dysfunction have remained obscure, with venous reflux and testicular temperature elevation as one of the possible culprits (The Practice Committee of American Society for Reproductive Medicine, 2008). However, increase in seminal levels of proinflammatory cytokines and oxidative stress as well as reduced total antioxidant capacity have been suspected as much [18, 19]. Besides, in the pathophysiology of varicocele impaired spermatogenesis because of autoimmunity has been proposed [20, 21].

Infertility in the male due to immunological causes is mostly associated with antisperm antibodies (ASA), which can develop as a result of testicular damage, infection or inflammation. As a consequence, sperm antigens are able to pass through blood-testis barrier and may activate corresponding antigen-specific Tand B lymphocytes. ASA can affect sperm quality and fertilization capacity by causing sperm agglutination, inhibiting sperm mobility and impairing sperm capacitation and acrosome reaction. In the female, ASA can additionally disrupt sperm-oocyte fusion, act embryotoxic or hamper embryo implantation by 18 binding to the hatching embryo [22, 23, 24]. Male infertility can additionally be caused by congenital genetic factors, for example anomalies at the chromosomal or DNA level.

L- carnitine (America Medic & Science), It is biologically active amino acid that was first isolated from beef muscle in 1905 [15]. Meat and milk are the most significant dietary sources of exogenous carnitine for humans [25]. Approximately 75% of the body stores of L- carnitine are derived from the diet, whereas only 25% are synthesized de novo from lysine and methionine [26]. It has long been assumed that carnitine is not an essential component of diet as humans have the ability to synthesize this compound. However, when groups of strict vegetarians were studied, the results showed that their average plasma concentration of carnitine was significantly lower than those of the respective omnivorous controls, which may be attributed to the much less carnitine that strict vegetarians consumed per day [27]. L-carnitine is concentrated in high energy demanding tissues such as skeletal and cardiac muscles and in a transferring long –chain fatty acids into the mitochondria for oxidation, producing energy. In addition, modulation of acyl-CoA/CoA ratio, storage of energy as acylcarnitine, and the modulation of toxic effects of poorly metabolized acyl groups by excreting them as carnitine esters are the functions of L- carnitine [28]. In 1973, Casillas [29] demonstrated that spermatozoa accumulate carnitine in mammalian epididymis, which is closely related with the development of fertilizing capacity by spermatozoa. The concentration of L-carnitine in epididymal plasma and spermatozoa varies from 2 to 100 mmole, which is nearly 2000 fold greater than circulating levels (10-50 mole). In epididymis, free L-carnitine is taken up from the blood plasma and is transported into the epididymal fluid. It is then passively diffused into the spermatozoa, where it accumulates as both free and acetylated L-carnitine. The initiation of sperm motility occurs in parallel with the increase in concentration of free L-carnitine in the epididymal lumen [25].

Another potential use of seminal free- L-carnitine is in the diagnosis of the etiology of azoospermia. Men with obstructive azoospermia whose level of obstruction is post epididymal, such as those with agenesis of vas deference, have extremely low concentration of carnitine. On the other hand, men with pre-epididymal obstruction like at the level of rate testis have normal concentration of carnitine in the seminal fluid. In 1986, Tomamichel and bandhaur [30] demonstrated that free- L-carnitine concentration in human semen correlates with the level of epididymal obstruction. Lower the carnitine levels, the more distal the occlusion is likely to be located and better the prognosis is after surgery.
Evaluation of seminal free L-carnitine will not only diagnosis the level of obstruction but also helps in postoperative prognosis regarding fertility. Therefore, the present study was done to test the hypothesis (HI) that free L-carnitine helps in maintaining normal fertility.

**Aims of the Study:**
This study aimed to search the evaluation physiological variations in men seving of infertile conduits to determinate the following steps.

1- To examine the effect of L-Carnitine (500mg) per day supplement on semen quality of male infertility.
2- General analysis of semen fluid of patient & control.
3-L-carnitine helps in maintaining normal fertility.

**Patients and Methods**

**Semen collection:**
This study has been conducted at General Salahauddin Hospital and Private Clinics and laboratories in Tikrit city. The treatment lasted four months starting from 1st of March to 1st of July. Infertile men were treated with (L-Carnitine 500mg) once daily. At least 2-3 semen analyses were done during 4 months of treatment for each patient before making a final conclusion regarding the base line sperm parameters. Semen specimens were collected from all patient and control subjects after at least 3 days of sexual abstinence in sterile containers. Samples were obtained by masturbation in room beside the laboratory. Containers were closed and labeled (name, age, time of ejaculates and duration of abstinence). The ejaculates were allowed to liquefy in an incubator at 37°C for 30 minutes. Within ½-1Hr., semen parameters were analyzed according to World Health Organization (WHO) guidelines (WHO Lab. Manual, 1999).

**Table (1): WHO (2010),Guidelines for Normal Seminal Fluid Analysis**

| Parameter          | Accepted Range                  |
|--------------------|---------------------------------|
| Semen Volume       | ≥ 1.5 ml                        |
| Sperm Count        | ≥20 million/ml                  |
| Sperm Motility     | ≥ 50% (Progressive motility + Sluggish progressive motility) |
| Sperm Vitality     | > 30% vital                     |
| Sperm Normal Morphology | ≥ 15% normal forms           |

**Liquefaction time:**
Every semen sample was mixed gently immediately after delivery, then introduced into the incubator at 37°C. After that the sample was examined every 5 minutes for evidence of liquefaction. When the sample was completely

**Sperm motility:**
For the examining of sperm motility the time of between the collection of semen and the examination was recorded. One hundred sperms were counted within 5 – 10 different microscopic fields, and then the following were recorded approximate percentage of what are activity motile or called forward progression motility which includes rapid linear progressive motility. Slow or moderate linear progressive (straight) and rapid nonlinear progressive motility (zigzag). In normal semen specimen, sperm with forward progressive motility are ≥ 50 %.

**Spermatozoa Concentration and Sperm Count:**
Sperm concentration is expressed in millions of spermatozoa per ml of semen, while sperm count is the total number of spermatozoa in the ejaculation. The normal range of sperm concentration is ≥20x10⁶/ml, [31]. The sperm count is performed in ordinary laboratory method of performing blood cell count. Immobilization of the sperm cell is accomplished by using a diluting fluid composed of a 4% solution of sodium bicarbonate and 1% phenol. (This may be prepared by mixing 16 g of sodium bicarbonate and 4 g of phenol in 400 ml distilled water). A white blood cell pipette (haemocytometer) is used in combination with the red blood cell field of the standard Neubauer counting chamber.

The semen specimen is thoroughly mixed and part is drawn up into the white blood cell pipette. If numerous sperm cells (more than 50/Hpf) have been observed when the drop of the ejaculate was examined directly, then a 1:20 dilution is made, with the semen being drawn up to the 0.5 mark half way up the stem of the pipette. The pipette is then filled to the mark at the top of its bubble-chamber with the bicarbonate-phenol solution, and thoroughly shaken. If, on the other hand, only a relatively small number of sperm were observed in the direct examination of the drop of semen, a 1:10 dilution is made by drawing the semen all the way up to the 1 mark at the top of the stem, just below the bubble-chamber of the pipette. The pipette chamber is then filled with the diluents fluid as before. After the mixture in the pipette chamber has been thoroughly shaken, a few drops of the fluid from the stem of the pipette are discarded, and both sides of the Neubauer counting chamber with its cover slip are carefully filled with the pipette mixture.

The immobilized sperm cell within the red blood cell field of the counting chamber is examined, and a count is made of all sperm cell lying within 5 blocks of 16 small squares each, or one-fifth of the entire red blood cell field. The total number of sperm cells within five blocks (or 80 squares) is obtained. The clicker-counter in securing the total. The sperm cell count in millions /ml is then computed as follows:

For the 1:20 dilution, six zeros are added to the figure obtained for the total count within the five blocks. For the 1:10 dilution, the total number of cells within the five-block area is divided by two, and six zeros are added.

If, with the 1:10 dilution, there are very few cells found in the counting chamber, then all the sperm cells present in the entire red blood cell field rather than just in the 5 blocks are counted. The number of sperm cells found in all 25 blocks is totaled and six zeros are added to the figure obtained.
Following the laboratory examination the semen was immediately centrifuged at a speed of 1800 rotations per minute at room temperature for the period of 10 minutes in order to separate plasma from sperms.

**Result and Discussion**

The study excluded those men whose wives are infertile and whose wives exceeded 50. Men who suffer from some chronic diseases as diabetic have been excluded as well. The study included 30 normal men as a control group. After four months of treatments with : L-Carnitine (500mg) once daily and, it has been found a significant increase in the number, movement, and activity of sperms and the decrease of dead sperms and abnormal cells thorough comparing those parameters before and after the treatment as compared with the control group.

**Sperm Count.**

There is significant increase in sperm count in infertile men treated with (L-carnitine, and the mean and standard deviation of increment in sperm count after four months of treatment (51 ±4.78), (table 1). And the percentage of increment in treated 51 %, here was a highly significant different (p<0.01) regarding sperm count, motility, viability and normal morphology between infertile men and control group.

There was a significant different (p<0.01) regarding ejaculate volume between infertile men and control group table (2).

These results were agreed with the findings of Marbut, et al (2006), who found that sperm count, sperm motility& morphology sperm in seminal in infertile men a low than healthy control subjects [32].The present study, show a decrease in sperm motility in infertile men as compared with control. The lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa, and it was associated with loss of motility and impairment of spermatogenesis (Sharma and Agarwal,1996).

In the present study, there was a low of sperm count in infertile men compare with control group. Patients with a low sperm count have a reduced chance of initiating a pregnancy. The lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa, and it was associated with loss of motility and impairment of spermatogenesis[33], poor motility poor morphology & a low sperm count [34].

**Table (2) : The mean & standard deviation of semen analysis of infertile men before & after treatment with L – Carnitine.**

| Parameters               | Mean ± S.D. before | Mean ± S.D. after | P value |
|--------------------------|--------------------|-------------------|---------|
| COUNT Total              | 27±2.2886          | 51±4.7871         | 0.01    |
| Active                   | 23±4.787           | 52±6.412          | 0.05    |
| Sluggish                 | 47±6.466           | 18±4.7            | 0.01    |
| Non Motile               | 52±6.432           | 22±2.761          | 0.05    |
| Normal                   | 22±5.761           | 52±6.123          | 0.01    |
| Abnormal                 | 55±4.087           | 22±3.210          | 0.05    |
| PH                       | 7.3±0.101          | 7.1±0.107         | Ns      |
| Viability                | 33.88±14.31        | 75.7±6.2          | 0.01    |
| Ejaculate Volume ml      | 2.00±0.55          | 3.12±0.82         | 0.05    |

**L-Carnitine**

L-carnitine [35].Carnitine is a zwitterionic amino acid (3-Hydroxy-4-trimethylamino-buticyic acid). It is found in different food items and derived endogenously from lysine and methionine [36].L-carnitine is an essential cofactor that could accelerate lipid metabolism and has a pivotal role in mitochondrial β-oxidation of long-chain fatty acids for cellular energy production [38]. L-carnitine and L-acetylcarnitine are highly concentrated in the epididymis and play a crucial role in sperm metabolism and maturation [15,37]. The redox system in the spermatozoa regulates the processes that are crucial for fertilization [39], but increased reactive oxygen species (ROS) observed in semen of infertile men might cause cellular damage and this have brought about the widespread use of antioxidants [34]. Many vitamins such as vitamin C, vitamin E, vitamin B12, and many other antioxidants were used to improve sperm quality for the treatment of idiopathic oligoasthenozoospermia [40]. In addition, sperm concentration was increased in a number of studies on subfertile men after treatment with zinc and folic acid [41,42].

The aim of the present study is to investigate and compare the efficacy of L-carnitine, multivitamins and their combination therapies on semen characteristics (seminal fluid volume, sperm concentration, sperm count, sperm morphology, sperm motility, progressive motile sperm count and round cells count) in idiopathic male infertility. Table (2) and figure (1) show the semen parameters of infertile men before & after use supplement (L-Carnitine). The mean & SD for sperm count in infertile men after use L- Carnitine group is 51±4.7871) million /ml (m/ml), while the mean & SD for sperm count in infertile men before use L-Carnitin is ( 27±2.2886) (m/ml). There is a significant different regarded sperm count between infertile men before & after use L- carnitin in regard to sperm count (P<0.01). In the present study, semen parameters were measured in infertile men before & after use supplement L- Carnitin. As expected, the semen parameters of infertile ment before treatment (sperm count, motility, viability & morphology) was...
significantly lower in infertile patient before use supplement than that of after use supplement fertile subjects (table 1).

The mean & SD values of motility of sperm in after use treatment & infertile patients before use treatment are( 52±6.123 & 22±5.76) (table 2), significant different regarded sperm motility between infertile men before and after use L-Carnitin (P<0.05).The mean & SD for sperm viability in infertile men after use treatment group are (75.7±6.2), while the mean & SD for sperm viability in infertile men before use treatment is (33.88±14.31) (table 2).There is a significant different regarded sperm viability between infertile men before & after use treatment (P<0.05). The mean &SD values of normal morphology of sperm in infertile men after use supplement & infertile patients before use supplement are (52±6.123 & 22±5.76) (table 2). There are significant different regarded normal morphology of sperm between infertile men before and after use supplement group regarding ratio of morphology of sperm (P<0.05).

The mean & SD for sperm viability in infertile men after use treatment group are (75.7±6.2), while the mean & SD for sperm viability in infertile men after use treatment is (33.88±14.31) (table 2). There is a significant different regarded ejaculate volume

Conclusions

1- L- Carnitine has potential antioxidant effects of causing significant improvement in most semen parameters of most male infertility.
2- There was a positive relationship between L- Carnitine level and sperm function tests (count, motility & normal morphology).

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تأثير الكارنتين في علاج الرجال العقيمين

عبد الرحمن جهاد منصور 1، زيد محمد مبارك 1، عزاوي مصطفى هادي 2

1 قسم علم الحياة، كلية العلوم، جامعة تكريت، تكريت، العراق
2 قسم جراحة المسالك البولية، كلية الطب، جامعة تكريت، تكريت، العراق

المملخص
(الكارنتين) يتركز بشدة في البربخ ويمعب دورا حاسما في استقلاب الحيوانات المنوية والنضج.

ترتبط بحركة الحيوانات المنوية وله خصائص مضادة للأكسدة. الهدف من هذه المراجعة هو تلخيص الأدوار المتعددة التي تلعبها الكارنتين في إعادة إنتاج الذكور، وإبراز دورها وكذلك فوائدها في علاج العقم عند الذكور. تدعم مجموعة متنوعة من الدراسات الاستنتاج القائل بأن الكارنتين الكميات اليومية التي تستخدمها الشخص والتي لا تقل عن 500 ملغ في اليوم يمكن أن يحسن بشكل كبير كل من تركيز الحيوانات المنوية وعدد الحيوانات المنوية الكلية لدى الرجال العقيمين، أو قلة في عدد الحيوانات. على الرغم من أن العديد من التجارب السريرية قد أثبتت الأثار المفيدة في الكارنتين في حالات معينة ومختارة من العقم عند الذكور، إضافية، دراسة مصممة بشكل جيد ضروري لمزيد من التحقق من صحة استخدام الكارنتين في علاج المرضى الذين يعانون من العقم عند الذكور، وخاصة في الرجال الذين يعانون من نوعية السائل المنوي الفقيرة (قليلة في العدد والنشاط).