L-carnitine and assisted reproduction

Hussein Abdelrazik[1], Ashok Agarwal[2]

[1]Department of Andrology, Suez Canal University, Ismailia, Egypt
[2]Center for Reproductive Medicine, Cleveland Clinic, Cleveland, Ohio, USA

Submitted: 2 October 2008
Accepted: 30 November 2008

Arch Med Sci 2009; 5, 1A: S43–S47

Corresponding author:
Prof. Ashok Agarwal
Center for Reproductive Medicine
Glickman Urological and Kidney Institute
and OB-GYN
and Women's Health Institute
Cleveland Clinic
9500 Euclid Avenue
Desk A19.1, Cleveland
Ohio 44195 USA
Phone: 216-444-9485
Fax: 216-445-6049
E-mail: agarwaa@ccf.org

Abstract

L-carnitine is required during the oxidation of lipids for the transport of fatty acids from the cytosol into the mitochondria for generation of energy. L-carnitine exerts a substantial antioxidant, anti-cytokines and anti apoptotic actions providing multi-mechanisms protective effects for the cell. The gametes *in vitro* may be exposed to damaging effects from oxidative stress (OS) due to either the high oxygen tension compared to the in vivo condition or the preparation producers preceding the *in vitro* fertilization (e.g. sperm preparation procedures preceding the *in vitro* fertilization). Moreover OS can affect the gametes *in vivo* under certain conditions as in endometriosis or leukocytospermia. Oxidative stress and cell apoptosis may adversely impact the fertilization process and the subsequent embryo development and pregnancy outcome. This review discusses the protective effects of L-carnitine for gametes and embryos and how it may help improve the embryogenesis and the *in vitro* fertilization outcome.

Key words: free radicals, antioxidant, ICSI, DNA damage.

Introduction

L-carnitine (LC) is a small water-soluble molecule that plays an important role in fat metabolism. It is essential for the normal mitochondrial oxidation of fatty acids and excretion of acyl-coenzyme A (acyl-CoA) esters and affects adenosine triphosphate (ATP) levels [1]. Free carnitine was first isolated from bovine muscle in 1905, and only the L-isomer was found to be bioactive [2]. L-carnitine protects the cell membrane and DNA against damage induced by free oxygen radicals and has a pivotal role in mitochondrial oxidation of long-chain fatty acids that increase energy supply to the cell [2].

Mitochondrial dysfunction may lead to incomplete detoxification of free radicals, which may lead to oxidative damage to macromolecules such as lipids, proteins and DNA. L-carnitine has free radical-scavenging activity and the ability to scavenge superoxide anion and inhibit lipid peroxidation, thereby conferring protection against damage induced by hydrogen peroxide (H_2O_2) [3].

L-carnitine levels also may affect ATP levels. L-carnitine has a role in intramitochondrial fatty acid oxidation. Acyl-CoA esters cannot directly cross the mitochondrial inner membrane, and their entry into the mitochondrion is a major point for control and regulation of the β-oxidation of fatty acids [4].

Expression of fatty acid oxidation enzymes such as carnitine palmitoyl transferase 1 and medium-chain acyl-CoA dehydrogenase has been demon-

strated in embryonic and fetal tissues and human placenta [5]. In addition to producing glucose, fatty acid oxidation may be important for generation of ATP necessary to meet the energy requirement of developing embryos [6, 7].

L-carnitine has antioxidant activity that combines both free radical-scavenging and metal-chelating properties. L-carnitine was found to be effective in reversing age-related trends and improved the mitochondrial function during the aging process in rat skeletal muscle mitochondria [3].

The anti-apoptotic effect of LC also has been demonstrated in human lymphoma cells treated with apoptosis-inducing agents [8]. Higher concentrations of tumor necrosis factor alpha (TNF-α) have been reported in the serum and follicular fluids of infertile women with polycystic ovary syndrome [9]. In various tumors and inflammatory diseases, elevated serum level of TNF-α decreased after treatment with LC [10].

L-carnitine was reported to down-regulate cytokines such as IL-1, IL-6 and TNF-α and/or increase clearance of these cytokines in rats implanted subcutaneously with sarcoma tumor. A statistically significant reduction in the concentrations of the cytokines (IL-1β: 423±33 vs. 221±60, IL-6: 222±18 vs. 139±38, TNF-α: 617±69 vs. 280±77 pg/ml, P<0.05) was seen in the sarcomas groups that were untreated vs. those treated with LC [11].

In vitro culture media supplementation with L-carnitine

In vitro fertilization culture media may be an exogenous site of reactive oxygen species (ROS) generation affecting the oocytes and the pre-implantation embryo. Higher day 1 ROS levels in culture media were associated with delayed embryonic development, high fragmentation and development of morphologically abnormal blastocysts after prolonged culture. A significant correlation was reported between increased ROS levels in day 1 culture media and lower fertilization rates in patients undergoing intracytoplasmic sperm injection (ICSI) [12].

Females exhibit an age-related decline in the number and quality of follicles and oocytes. Reactive oxygen species may damage the oocytes [13]. Aging of the oocytes affects many biochemical pathways that have a deleterious effect on pre- and post-implantation development of the embryo [14].

H_2O_2 at a concentration of 50 mmol/l is reported to significantly decrease the blastocyst development rate (%BDR) in two-cell mouse embryos [15]. Incubation of mouse embryos in 500 mmol/l H_2O_2 at 10 times the toxic concentration significantly decreased the %BDR and increased the level of apoptosis (P<0.001 and P<0.01) compared with the control group. L-carnitine has a very potent antioxidant effect in the mouse embryo culture medium because it is able to neutralize the embryotoxic effects of exogenous induction of oxidative stress by H_2O_2. L-carnitine at 0.3 and 0.6 mg/ml significantly improved the %BDR (P<0.001 and P<0.001, respectively) and decreased the level of apoptosis (P<0.006 and P<0.007, respectively) compared with the group treated with H_2O_2 alone (500 μmol/ml) [16].

Studies have suggested that blastomere fragmentation in mouse and human pre-implantation embryos may be indicative of apoptosis [17]. Human embryos generated from IVF also exhibit varying degrees of cytoplasmic fragmentation [18]. L-carnitine is able to stabilize mitochondrial membranes and increase the supply of energy to the organelle and protect the cell from apoptotic death [19].

Reduction of apoptosis through the mitochondrial pathway by the administration of LC to mouse fibroblasts in culture medium has been demonstrated [19]. Treatment of the embryos with actinomycin-D at 0.005 mg/ml for 4 h significantly decreases %BDR and increases the level of apoptosis (P<0.001 and P<0.001, respectively), compared with the control group. L-carnitine (0.3 mg/ml) significantly improved the %BDR (P<0.002) compared with the groups treated with actinomycin-D (0.005 mg/ml) alone. A significant decrease in the level of apoptosis induced by actinomycin-D was also seen in embryos treated with both 0.3 and 0.6 mg/ml of LC (P<0.001 and P<0.001, respectively) [16].

Significantly higher concentration of basal levels of TNF-α from the granulosa cells have been reported from women with endometriosis [20]. Granulosa, cumulus and sperm cells are potential sources of cytokine production, especially during the first 24 h [21]. TNF-α can inhibit cell proliferation and glucose consumption in the rat blastocyst [22].

Elevated TNF-α has been reported to restrict inner cell mass and trophectoderm proliferation in the mouse blastocyst. This leads to retardation of embryo development, reduced viability of embryos and even embryonic death [23, 24]. Exposure of mouse embryos to TNF-α (50 ng/ml) affects their protein synthesis both quantitatively and qualitatively in the morula and blastocyst stage [23, 24].

Incubation of two-cell mouse embryos with 500 ng/ml TNF-α significantly decreased the %BDR (P<0.001) but did not increase the level of apoptosis compared with the control group (P=0.59) [16]. The lack of any significant effect on the level of apoptosis suggests that TNF-α may have an antiproliferative effect on the developing mouse embryos, which may not necessarily be through the induction of apoptosis. This finding coincides

with the reports of Pampfer et al. who showed that incubation of mouse embryos with 50 ng/ml TNF-α did not increase the incidence of apoptosis compared with the control group, although it decreased the number of nuclei in the developing blastocyst [22]. Lalitkumar et al. showed that exposure of mouse embryos to TNF-α (50 ng/ml) affected their protein synthesis both quantitatively and qualitatively in the morula and blastocyst stage [25]. L-carnitine at 0.3 and 0.6 mg/ml was able to neutralize this antiproliferative effect and improve the %BDR (P<0.001 and P<0.004, respectively) compared with the group treated with TNF-α alone [16].

Endometriosis is characterized by changes in the intrafollicular and peritoneal fluid environment and increased levels of certain cytokines, such as TNF-α, and ROS, which affect oocytes and embryo development. Significant alterations in the oocyte microtubule chromosomal alignment were reported after exposure to high concentration of H_2O_2. Exposure of the oocytes to both TNF-α alone and in combination with H_2O_2 resulted in a concentration- and time-dependent increase in spindle damage compared with controls [26].

Incubation of oocytes with peritoneal fluid from patients with endometriosis significantly increased the microtubule and chromosome scores compared with the oocyte group incubated in peritoneal fluid from patients with tubal ligation (P<0.001 and P<0.006, respectively). This may be attributed to the high concentration of ROS [27] and TNF-α or other cytokines that have been reported to be higher in patients with endometriosis [28].

Statistically significant improvement in the microtubule score was seen when 0.6 mg/ml of LC was added to the endometriosis peritoneal fluid (P<0.001). Oocytes incubated in peritoneal fluid from patients with endometriosis and LC demonstrated an improvement in chromosomal alignment (P=0.06). The improvement in microtubule and chromosome alignment after addition of LC may be due to the strong antioxidant properties of LC. Additionally, it may be the result of down-regulation of the cytokines that are known to be present in the peritoneal fluid of endometriosis patients [29].

The embryotoxicity of peritoneal fluid from patients with and without endometriosis has been studied before, but the results have been conflicting. Although some investigators have demonstrated that peritoneal fluid from individuals with endometriosis is not embryotoxic when studied in an *in vitro* mouse embryo model [30, 31], others have shown that embryotoxicity is increased in women with endometriosis [32]. These conflicting results may be due to differences in the peritoneal fluid concentration, severity of the disease, incubation time or type of culture media [33].

Incubation of the pre-implantation mouse embryos with peritoneal fluid from patients with endometriosis significantly increased the level of apoptosis compared with the controls (P<0.001). Statistically significant improvement in the apoptosis level was seen after adding 0.6 mg/ml of LC to the peritoneal fluid from endometriosis patients (P<0.001). Again, the improvement in embryo apoptosis level after addition of LC may be due to the strong antioxidant properties of LC and its down-regulation of the cytokines that are present in the peritoneal fluid of endometriosis patients [29].

Another study evaluated 617 oocytes obtained from 38 infertile couples (12 with male factor infertility, 11 female factor infertility and 15 combined male and female factor infertility) incubated in two groups with or without LC 0.3 mg/ml immediately after injection and through blastocyst development. Comparison of the percentage of grade 1 embryos at Day 3 and %BDR at Day 5 between the two groups showed that LC at a concentration of 0.3 mg/ml significantly improved embryo quality embryos at Day 3 and the %BDR at Day 5 compared with the untreated group (P<0.001 and P<0.001, respectively). Supplementation of LC in the culture media resulted in a significant improvement in embryo quality and %BDR, which may provide a novel approach to improving ICSI outcomes in infertile couples [34].

L-carnitine and semen parameters

L-carnitines are important conditionally essential nutrients in the organism with extensive physiological functions and highly concentrated in the epididymis and sperm. Carnitines play an important role not only in initiating sperm motility, promoting sperm maturation and enhancing sperm fertilizing, but also in regulating Sertoli cell function, protecting sperm against oxidative damage, reducing apoptosis of spermatogenic cells and inhibiting sperm aggregation [35].

The concentration of free LC (P<0.01) and the activity of α-glucosidase (P<0.05) were significantly reduced in the infertile group as compared with the control. A statistically significant positive correlation was found between seminal plasma free LC level and α-glucosidase activity (r=0.504, P<0.001). The determination of free LC level in seminal plasma is a useful test in the evaluation of epi-didymal function, which may serve as a guide for the clinical treatment of male infertility as well as for the study of the mechanisms of male reproduction [36].

The etiologic cause in nearly one-third of male factor infertility is unknown. The percentage of men with idiopathic infertility who have been

successfully treated by empirical therapeutic modalities is not high [37]. A lack of adequate sperm motility has been associated with low fertility or infertility [38]. This correlation may be mediated by centrosome destruction, which affects both sperm motility and oocyte fertilization [39], thus increasing sperm motility plays an important role in improving outcomes of assisted reproductive technology. In a study investigating the effect of combined treatment by LC and acetyl-LC on 30 patients with idiopathic asthenospermia 3 months after failure to respond to the integrated therapy of empirical medicine, combined LC and acetyl-LC statistically improved sperm vitality, grade a + b sperm motility and the total sperm count per ejaculate, with a significant difference from pre-treatment ($P<0.05$). Combined LC and acetyl-LC has a supplementary effect in the treatment of idiopathic asthenospermia and improves sperm motility [40].

Afsaneh and colleagues investigated the effect of LC on sperm parameters in patients who underwent ICSI as infertility treatment. A total of 65 men presenting with primary infertility due to idiopathic oligoasthenoteratozoospermia were treated with LC 1 g orally every 8 h for 3 months. Semen analysis was performed at baseline and after the conclusion of the LC treatment. The proportion of patients who had motile sperm rose significantly after treatment, and the percent of sperm with abnormal morphology decreased significantly after treatment. Improving sperm motility potentially will improve ICSI outcomes [37].

Conclusion

Oxygen-controlled incubators have been introduced recently in clinical embryology procedures, and these may help reduce the harmful effects of free radicals and thereby improve blastocyst rates. However, LC works by multiple mechanisms; one of them is by antagonizing ROS formation through its antioxidant effect. In addition, LC also can decrease the level of apoptosis induced by an apoptotic inducer and decrease the anti-proliferative effect induced by presence of an interleukin such as TNF-α.

In conclusion, success rates of ART are still unsatisfactory. Relatively few *in vitro* cultured embryos reach the blastocyst stage. Mouse embryos are an excellent research model, because pre-implantation development is fairly similar to that of human embryos. Various studies have shown that tumor TNF-α, oxidative stress and apoptosis significantly affect embryogenesis. Improvement in embryo developmental competence may be accomplished by LC supplementation through its potent antioxidant effect, its ability to reduce DNA damage, and by protecting the cells from the harmful effect of TNF-α. Improvement in the %BDR and reduction in the level of DNA damage at low LC concentrations (0.3 and 0.6 mg/ml) is a novel approach that has a combination of beneficial effects on embryos and may result in a higher yield of good quality embryos. Moreover, LC protects the oocyte spindle structure and, subsequently, improvement in embryo quality. Selected patients with asthenozoospermia can reveal the effects of carnitine therapy. This approach may have significant clinical applications in the ART setting, and its use may improve fertility outcomes and prove to be cost-effective.

References

1. Vanella A, Russo A, Acquaviva R, et al. L-propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector. Cell Biol Toxicol 2000; 16: 99-104.
2. Zhou X, Liu F, Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. Asia Pac J Clin Nutr 2007; 16 Suppl 1: 383-90.
3. Panneerselvam KS, Kumaran S. REMOVED: l-Carnitine and alpha-lipoic acid improves mitochondrial function during ageing process. Clin Nutr 2006 Epub ahead of print.
4. Eaton S. Control of mitochondrial beta-oxidation flux. Prog Lipid Res 2002; 41: 197-239.
5. Oey NA, Ruiter JP, Attié-Bitach T, Ijlst L, Wanders RJ, Wijburg FA. Fatty acid oxidation in the human fetus: implications for fetal and adult disease. J Inherit Metab Dis 2006; 29: 71-5.
6. Berger PS, Wood PA. Disrupted blastocoele formation reveals a critical developmental role for long-chain acyl-CoA dehydrogenase. Mol Genet Metab 2004; 82: 266-72.
7. Oey NA, Ijlst L, van Roermund CW, Wijburg FA, Wanders RJ. dif-1 and colt, both implicated in early embryonic development, encode carnitine acylcarnitine translocase. Mol Genet Metab 2005; 85: 121-4.
8. Qi SN, Zhang ZF, Wang ZY, Yoshida A, Ueda T. L-carnitine inhibits apoptotic DNA fragmentation induced by a new spin-labeled derivative of podophyllotoxin via caspase-3 in Raji cells. Oncol Rep 2006; 15: 119-22.
9. Amato G, Conte M, Mazziotti G, et al. Serum and follicular fluid cytokines in polycystic ovary syndrome during stimulated cycles. Obstet Gynecol 2003; 101: 1177-82.
10. Sener G, Ekşioğlu-Demiralp E, Cetiner M, et al. L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell Biol Toxicol 2006; 22: 47-60.
11. Winter BK, Fiskum G, Gallo LL. Effects of L-carnitine on serum triglyceride and cytokine levels in rat models of cachexia and septic shock. Br J Cancer 1995; 72: 1173-9.
12. Bedaiwy MA, Falcone T, Mohamed MS, et al. Differential growth of human embryos in vitro: role of reactive oxygen species. Fertil Steril 2004; 82: 593-600.
13. Tarin JJ. Potential effects of age-associated oxidative stress on mammalian oocytes/embryos. Mol Hum Reprod 1996; 2: 717-24.
14. Tarín JJ, Pérez-Albalá S, Cano A. Consequences on offspring of abnormal function in ageing gametes. Hum Reprod Update 2000; 6: 532-49.
15. Zhang X, Sharma RK, Agarwal A, Falcone T. Effect of pentoxifylline in reducing oxidative stress-induced embryotoxicity. J Assist Reprod Genet 2005; 22: 415-7.

16. Abdelrazik H, Sharma R, Mahfouz R, Agarwal A. L-Carnitine decreases DNA damage and improves the in vitro blastocyst development rate in mouse embryos. Fertil Steril 2008 Epub ahead of print.
17. Jurisicova A, Latham KE, Casper RF, Casper RF, Varmuza SL. Expression and regulation of genes associated with cell death during murine preimplantation embryo development. Mol Reprod Dev 1998; 51: 243-53.
18. Jurisicova A, Antenos M, Varmuza S, Tilly JL, Casper RF. Expression of apoptosis-related genes during human preimplantation embryo development: potential roles for the Harakiri gene product and Caspase-3 in blastomere fragmentation. Mol Hum Reprod 2003; 9: 133-41.
19. Pillich RT, Scarsella G, Risuleo G. Reduction of apoptosis through the mitochondrial pathway by the administration of acetyl-L-carnitine to mouse fibroblasts in culture. Exp Cell Res 2005; 306: 1-8.
20. Carlberg M, Nejaty J, Fröysa B, Guan Y, Söder O, Bergqvist A. Elevated expression of tumour necrosis factor alpha in cultured granulosa cells from women with endometriosis. Hum Reprod 2000; 15: 1250-5.
21. Austgulen R, Arntzen KJ, Vatten LJ, Kahn J, Sunde A. Detection of cytokines (interleukin-1, interleukin-6, transforming growth factor-beta) and soluble tumour necrosis factor receptors in embryo culture fluids during in-vitro fertilization. Hum Reprod 1995; 10: 171-6.
22. Pampfer S, Moulaert B, Vanderheyden I, Wuu YD, De Hertogh R. Effect of tumour necrosis factor alpha on rat blastocyst growth and glucose metabolism. J Reprod Fertil 1994; 101: 199-206.
23. Głabowski W, Kurzawa R, Wiszniewska B, Baczkowski T, Marchlewicz M, Brelik P. Growth factors effects on preimplantation development of mouse embryos exposed to tumor necrosis factor alpha. Reprod Biol 2005; 5: 83-99.
24. Whiteside EJ, Boucaut KJ, Teh A, Garcia-Aragon J, Harvey MB, Herington AC. Elevated concentration of TNF-alpha induces trophoblast differentiation in mouse blastocyst outgrowths. Cell Tissue Res 2003; 314: 275-80.
25. Lalitkumar PG, Sengupta J, Ghosh D. Effect of tumour necrosis factor-alpha (TNF-alpha) on protein synthesis by mouse preimplantation stage embryos in vitro. Indian J Physiol Pharmacol 2005; 49: 139-47.
26. Choi WJ, Banerjee J, Falcone T, Bena J, Agarwal A, Sharma RK. Oxidative stress and tumor necrosis factor-alpha-induced alterations in metaphase II mouse oocyte spindle structure. Fertil Steril 2007; 88: 1220-31.
27. Harada T, Iwabe T, Terakawa N. Oxidative damage and mitochondrial DNA mutations with endometriosis. Ann N Y Acad Sci 2005; 1042: 186-94.
28. Iwabe T, Harada T, Terakawa N. Role of cytokines in endometriosis-associated infertility. Gynecol Obstet Invest 2002; 53 Suppl 1: 19-25.
29. Mansour G, et al. L-carnitine supplementation reduces oocyte cytoskeleton damage and embryo apoptosis induced by incubation in peritoneal fluid from patients with endometriosis. Fertil Steril 2008 Epub ahead of print.
30. Awadalla SG, Friedman CI, Haq AU, Roh SI, Chin NW, Kim MH. Local peritoneal factors: their role in infertility associated with endometriosis. Am J Obstet Gynecol 1987; 157: 1207-14.
31. Wu MY, Chen SU, Chao KH, Chen CD, Yang YS, Ho HN. Mouse embryo toxicity of IL-6 in peritoneal fluids from women with or without endometriosis. Acta Obstet Gynecol Scand 2001; 80: 7-11.
32. Gómez-Torres MJ, Acién P, Campos A, Velasco I. Embryotoxicity of peritoneal fluid in women with endometriosis. Its relation with cytokines and lymphocyte populations. Hum Reprod 2002; 17: 777-81.
33. Morcos RN, Gibbons WE, Findley WE. Effect of peritoneal fluid on in vitro cleavage of 2-cell mouse embryos: possible role in infertility associated with endometriosis. Fertil Steril 1985; 44: 678-83.
34. Abdelrazik H, El-Damen A, Badrawy H, Sharma R, Agarwal A. L-Carnitine improves embryo quality and increases blastocyst development rate in couples undergoing ICSI. 64th Annual Meeting of the American Society of Reproductive Medicine, San Francisco, CA, Nov 8-12, 2008.
35. Shang XJ, Wang XL, Huang YF. Carnitines and male reproduction [Chinese]. Zhonghua Nan Ke Xue 2006; 12: 726-9.
36. Li K, Li W, Huang YF, Shang XJ. Correlation of free L-carnitine level with accessory gland markers and its clinical significance [Chinese]. Zhonghua Nan Ke Xue 2007; 13: 507-10.
37. Afsaneh K, et al. The effect of L-carnitine on sperm parameters in patients candidated for intracytoplasmic sperm injection. Iranian J Reprod Med 2004; 2: 65-9.
38. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002; 23: 25-43.
39. Tesarik J, Mendoza C, Greco E. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. Hum Reprod 2002; 17: 184-9.
40. Cheng HJ, Chen T. Clinical efficacy of combined L-carnitine and acetyl-L-carnitine on idiopathic asthenospermia [Chinese]. Zhonghua Nan Ke Xue 2008; 14: 149-51.