L-carnitine and assisted reproduction

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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 (H₂O₂) [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-
implantation development of the embryo [14]. That have a deleterious effect on pre- and post-

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 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].

H₂O₂ 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 H₂O₂ 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 H₂O₂. 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 H₂O₂ 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 embryonic 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 H2O2. Exposure of the oocytes to both TNF-α alone and in combination with H2O2 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 epididymal 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].

Afkanian 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


