

A comparative study of preimplantation embryos development of young and aged mice treated with L-carnitine

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Received 13/04/2023, Revised 18/06/2023, Accepted 20/06/2023, Published Online First 20/11/2023, Published 1/6/2024



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Abstract

Female aging is one of the main factors influencing reproductive fertility. The existing experimental study was planned to investigate the L-carnitine (LC) treatment effect on the body weight of adult young 8–10 weeks old and aged 26–28 weeks old female mice and the preimplantation embryonic development of their embryos. This study involved 40 mature young (n=20) and aged (n=20) old female mice. The animals were weighed and divided into 4 groups according to their age (n = 10). The control groups of young and aged mice groups were orally administered distilled water, while the young and aged mice that were treated orally with 10 mg/kg LC daily for 2-3 estrous cycles were considered as treated groups. Then, all female mice were mated with adult males. The weight of pregnant mice on 1-day post coitum was recorded and then euthanized to harvest early cleavage embryos. The embryo development was examined and evaluated their grading according to A, B, C and D. The results showed that the body weight of mice in both young LC and aged LC groups reduced significantly ($P \leq 0.01$). The grade A embryo in 1-day post coitum in young and aged LC groups improved significantly ($P \leq 0.01$). However, the development of embryos grade A in the young LC group was higher than that of the aged LC group. It was concluded from these findings that the oral supplementation of LC can reduce body weight and improve the preimplantation embryonic development proportions.

Keywords: Aged mice, Body weight, L-carnitine, Preimplantation embryonic development, Young mice.

Introduction

One of the most critical factors affecting female fertility is aging. Physiologically, female fertility decreased with aging. It has been noticed that fertility declines slowly and steadily in women aged 30–35 years, but it accelerates beyond 35 due to a decline in ovarian reserve and the quality of oocytes ¹. An oocyte's ability to restart meiotic division, be fertilized, implant in the uterus, and eventually grow

into an intact embryo and subsequent progeny is determined by its developmental potential ². Therefore, female infertility is caused by poor oocyte quality ³. It has been found that one of the causes that resulted in poor oocyte quality is reactive oxygen species (ROS) generation. Through the respiratory chain, β -oxidation of fatty acids generates ROS such as peroxide, hydroxyl, and superoxide radicals ⁴.

Excessive ROS production causes inactivation of the enzyme, peroxidation of lipids, and covalent DNA and protein modification, all of which can affect the oocytes and embryos⁵.

Many nutrients and pharmaceutical medications, including proteins, hormones, growth factors, and antioxidants, are recommended to enhance oocyte quality and reduce fertility problems⁶. L-carnitine (LC) can carry activated fatty acids into the matrix of the mitochondria, promoting the utilization of fatty acid and energy, while also simultaneously raising ATP concentration⁷. Thus, LC is important in lipid metabolism because it regulates the transport of fatty acids to the matrix of mitochondria for β -oxidation⁸. Furthermore, LC functions as an antioxidant, reducing cell stress and suppressing apoptosis, a

response to mitochondrial dysfunction^{3,9}. L-carnitine also regulates oxidative and metabolic states in the reproductive system of female and is essential for mammalian oocyte in vitro maturation (IVM) and embryo culture¹⁰. It is discovered that adding LC to the IVM medium improved β -oxidation as well as the rates of nuclear maturation, fertilization, and blastocyst development in mice¹¹. Also, LC increases levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E2) in the blood. Furthermore, it increases certain sperm function parameters in vitro, e.g., sperm motility¹². Consequently, the aim of this research is to study the effects of LC on the body weight and quality of early cleavage embryos of young and aged mice.

Materials and Methods

Animals' management:

This study complies with current Iraqi law, was permitted for animal research by the Research Ethics Committee, College of Science, University of Baghdad and was approved under protocol number CSEC/1020/0053. Forty adult female albino Swiss mice were weighed and divided into four groups based on their ages and treatment with LC (n=10 per group): (young control and young LC groups with age range 8-10 weeks old), and (aged control and aged LC groups with age range 26-28 weeks old). The animals utilized in this investigation were purchased from the Al-Razi Center for Research and Medical Diagnostic Kit Production/Ministry of Industry and Minerals.

The mice were housed separately in plastic cages, fed ad libitum by conventional laboratory pellets, and kept on a 12:12 hour light-dark cycle with a temperature of 22°C and a relative humidity of 30-40%. Both young and aged control groups were given distilled water orally, while both young and aged LC groups received 10 mg/kg LC (AMS, U.S.A.) orally daily for 2-3 estrous cycles, then mated with adult male albino Swiss mice. The presence of vaginal plague (an indication of mating) was discovered the next morning. The animals were weighed and euthanized after 24 hours post-mating with carbon dioxide (CO₂) gas inhalation to harvest early cleavage embryos (1-day post coitum)¹³.

Detection of female estrous cycle:

Under a light microscope, a vaginal smear was utilized to assess the stage of the estrous cycle in each female mouse. Every day between the hours of 8:00 a.m. and 1:00 p.m., the smear was conducted. The four stages of the estrous cycle in female mice were detected using the vaginal smear method as described by Rafiee and her colleagues¹⁴. The slides were examined under a light microscope to determine the estrous cycle phase.

Mating procedure and timing of pregnancy:

After the adaptation of female mice, LC oral supplementation and, detection of the estrous cycle in them, mating was carried out by putting two females within the estrus stage in the breeding cage of one male overnight^{15,16}, following which the females were checked for indications of a vaginal copulatory plug. The presence of a vaginal copulatory plug the next morning established the starting of gestation, and the day was identified as day 'zero' of pregnancy¹⁷.

Retrieval and grading of embryos:

Following animal CO₂ gas inhalation, each animal's uterine horns and oviducts were isolated from the body and removed using sterile surgical tools, and the specimens were placed directly in a Petri plate loaded with phosphate-buffered saline (PBS with pH 7.3). The horns of the uterus were dissected under a stereomicroscope (Zeiss, Germany). The "flushing and dissection" technique was used to retrieve

embryos from uterine horns or oviducts^{18, 19}. An inverted microscope (TECHNO, Japan) was used to examine the morphology of all retrieved early-stage (i.e., 2-cell) embryos. The early embryos (1-day post coitum) were graded into four categories: A, B, C, and D according to Khalili and Anvari²⁰.

Statically analysis:

IBM SPSS for Windows, version 28 (SPSS Inc. Chicago, Illinois, United States) was used for data

Results and discussion

L-carnitine and body weight gaining:

According to the findings of Table 1, the weight of young mice after LC treatment showed a significant ($P \leq 0.01$) reduction in comparison with their weight before the LC treatment, while there was no significant difference in the weight of female mice of the young control group during the experiment period. On the other hand, the weight of aged mice after LC administration decreased significantly ($P \leq 0.01$) compared to their weight prior to the administration of LC as illustrated in Table 2. The present investigation established that the oral administration of LC to both young and aged mice for 2-3 estrous cycles resulted in body weight

analysis. Frequency and proportions were used to represent categorical data. The proportions were compared by using the Chi-square test. In order to detect statistical differences between means, one-way ANOVA was employed. Prior to the investigation, $P \leq 0.01$ was selected to represent significance.

reduction. Several published studies demonstrated that LC supplementation can reduce body weight in human and experimental animals^{21, 22}. This observation may result from the mechanism of LC that leads to a reduction in fat mass^{22, 23}. It has been found that the action of LC in the transport of long-chain saturated fatty acids into the mitochondria and later β -oxidation resulted in a decrease in body weight^{24, 25}. In addition, the relationship between LC diet and weight loss involves multiple factors. While LC diets can lead to a decrease in plasma leptin levels and an increase in fat metabolism, these effects may contribute to limiting the proliferation of adipocytes (fat cells). This can ultimately result in reduced fat mass and weight loss²⁶.

Table 1. The results of young female mice weight after LC administration.

Groups of 8-10 weeks young mice (n=10)	Wt. Before LC-treatment (g)	Wt. After LC-treatment (g)	P value
Control	26.50 \pm 0.30 ^a	26.70 \pm 0.3 ^a	0.98
LC	27.00 \pm 0.9 ^a	23.66 \pm 0.4 ^b	0.003

Different small superscripts represent significant differences at $P \leq 0.01$ between weights in the columns (before and after LC administration).

The mean and standard error mean are used to represent the data (SEM). LC: L-carnitine group.

Table 2. The results of aged female mice weight after LC administration.

Groups of 26-28 aged mice (n=10)	Wt. Before LC-treatment (g)	Wt. After LC -treatment (g)	P value
Control	31.64 \pm 0.55 ^a	31.48 \pm 0.38 ^a	0.86
LC	32.10 \pm 0.55 ^a	28.20 \pm 0.80 ^b	0.001

Different small superscripts represent significant differences at $P \leq 0.01$ between weights in the columns (before and after LC administration).

The mean and standard error mean are used to represent the data (SEM). LC: L-carnitine group.

L-carnitine improves preimplantation embryos development:

Based on the results illustrated in Table 3, a significant raise ($P \leq 0.01$) in the mean number of 2-

cell embryos (1-day post coitum) grade A from young mice received LC was assessed in comparison with untreated mice of the same age group. On the other hand, the number of 2-cell embryos grade A from aged mice treated with LC improved

significantly ($P \leq 0.01$) in comparison with the aged control group but declined considerably ($P \leq 0.01$) when compared to young mice in the LC group.

In the young LC group, the mean number of 2-cell embryos grade B revealed a significant ($P \leq 0.01$) reduction compared to the young control group. The proportions of the same grade embryos in the control and LC groups of aged mice revealed a non-significant ($P > 0.01$) difference. Furthermore, the mean number of 2-cell embryo grade B in the young and aged LC groups showed non-significant ($P > 0.01$) variations.

On the other hand, the results proved in the same table showed that the mean number of 2-cell of grade C embryos in LC and control groups from young female mice had no significant ($P > 0.01$) difference between them. In the aged LC group, there was a significant ($P \leq 0.01$) reduction when compared to the aged control group. Whereas there was no considerable change in the mean number of 2-cell of embryos grade C between the young and aged LC groups. At the same time, the proportion of grade D embryos in the young LC group decreased significantly ($P \leq 0.01$) when compared to the young control group, and there were no significant ($P > 0.01$) differences between the young LC and aged LC groups, as observed in Table 3.

The grades of the early embryo (A, B, C and D) are shown in Fig. 1. The current study confirmed that the treatment of mice with LC improved the mean of

embryonic development in the early embryos and the quality of embryos after 24 hrs. of fertilization in both young and aged mice and decreased the mean of embryonic development of the 2-cell embryo of grade D, that characterized by unequal size blastomeres and severe fragmentation. It has been demonstrated that LC increases the preimplantation and developmental competence of embryos in experimental animals, due to the improvement of cytoplasmic and nuclear maturation of oocytes that play an initial role in the consequent development of fertilized oocyte to embryo, and the reduction of ROS levels²⁷, which may be due to its vital role in increasing glutathione (GSH) levels inside the cells that can prevent free radical damage to embryos²⁸.

On the other hand, it has been reported that LC tends to induce nuclear maturity and increase the number of early embryos in camels²⁹. While, LC when added to the diet of pigs, rates of oocyte maturation, cleavage of early embryos, and formation of early embryos significantly improved³⁰. In mice, the inclusion of LC has been demonstrated to improve the rate of oocyte maturation and fertilization, and early embryonic development¹¹. It seemed that LC also increases the cleavage of preimplantation embryo of mice, which is likely due to the intracellular lipid storage utilization. L-carnitine has been shown in several studies to have good effects on early embryonic development in canines³¹, cattle^{32, 33}, camels²⁹, and mice³⁴.

Table 3. Percentage of embryos grading in young and aged female mice treated with LC.

Embryo Grade	Groups (n=10)	8-10 weeks mice		26-28 weeks mice		P value
		Young Control	Young LC	Aged Control	Aged LC	
		%	%	%	%	
A		70 ^c	89.473 ^a	60 ^d	88.235 ^b	0.006
B		20 ^a	7.894 ^b	16 ^b	8.823 ^b	0.002
C		0 ^a	0 ^a	4 ^b	0 ^a	0.01
D		10 ^b	2.631 ^c	20 ^a	2.941 ^a	0.004

(Chi-square test; $P \leq 0.01$): $P \leq 0.01$ indicates that values with distinct small superscript (s) are significantly different. Different small superscripts represent significant differences at $P \leq 0.01$ between columns (groups).

LC: L-carnitine group.

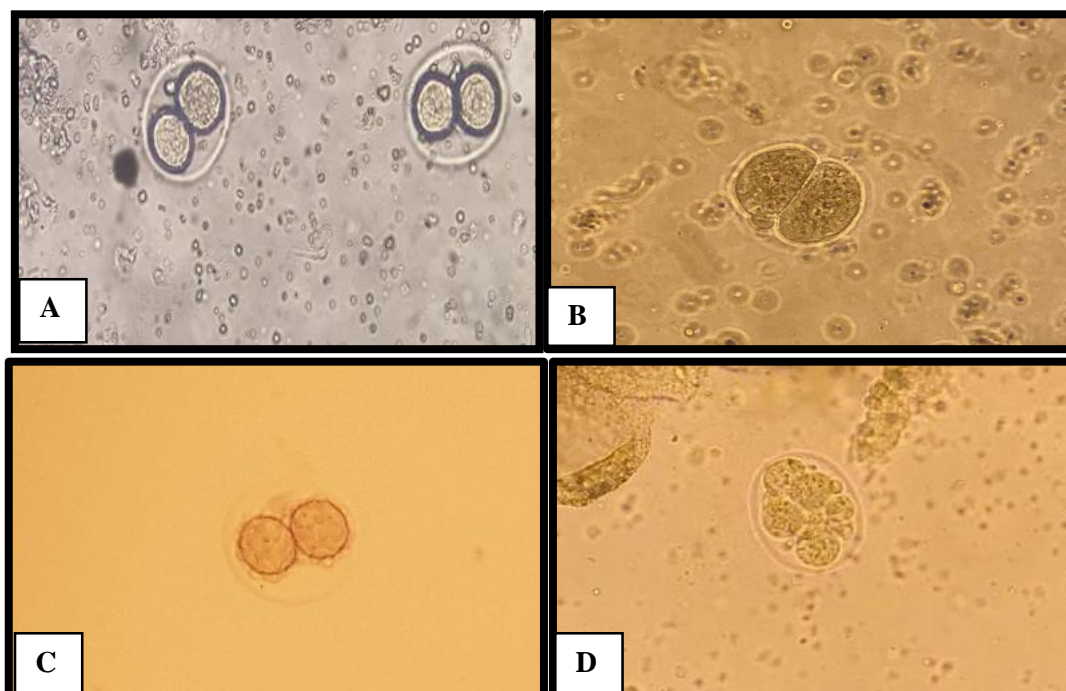


Figure 1. Shows 2-cell embryos; grade A good quality embryo with equal size blastomeres (Young LC group), grade B embryo of slightly unequal size blastomeres (Aged LC group), grade C embryo of moderately unequal size blastomeres (Aged control group) and grade D embryo with unequal size blastomeres and sever fragmentation (Young control group), by inverted microscope. Magnification x400.

Conclusion

In conclusion, the study found an important benefit in LC oral administration to decrease the body weight of treated female mice and enhance the quality and count of preimplantation embryonic development. A significant decrease in the grading

of abnormal embryos (i.e., Grade D) was noticed after treatment with LC. These results can be utilized for other mammals to increase the pregnancy outcome in young and aged females.

Acknowledgement

The authors appreciate the consultant office at the Forensic DNA Center for Research and Training at

Al-Nahrain University for providing the facilities necessary for us to complete this work.

Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given permission for re-publication attached with the manuscripts.

- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Author's Contribution

A. T. Al., S. S. Al. and L.A. S. contributed to the design and implementation of the research, to the

analysis of the results and to the writing of the manuscript.

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دراسة مقارنة لتكوين الأجنة قبل الانغراس في الفئران اليافعة والمتقدمة في السن المعاملة بالكارنتين اليساري

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الخلاصة

يعد التقدم في السن لدى الإناث أحد العوامل الرئيسية التي تؤثر على الخصوبة والتكاثر. تهدف الدراسة الحالية لدراسة تأثير العلاج بالكارنتين اليساري على وزن الفئران البالغة اليافعة والمتقدمة في السن وتكوين الأجنة قبل الانغراس. أشتملت هذه الدراسة على أربعين فأراً أنثى بالغة 8-10 أسابيع و 26-28 أسبوعاً. تم وزن الفئران الأناث وقسمت إلى أربع مجموعات حسب عمرها (كل مجموعة تحوي عشريناً) وهي مجموعتي السيطرة اليافعة والمتقدمة في السن ومجموعتي المعاملة بالكارنتين اليساري اليافعة والمتقدمة في السن. جرعت مجموعتي السيطرة فمويًا بالماء المقطر، بينما جرعت مجموعتي المعاملة فمويًا بالكارنتين اليساري بـ 10 مجم / كجم من الكارنتين اليساري يوميًا لمدة دورتين إلى ثلاث دورات شبق. ثم تزوجت جميع الإناث مع ذكور بالغين. وزنت الفئران الحوامل بعد يوم واحد من الجماع ثم شرحت الأمهات لجمع الأجنة في المراحل الأولى قبل الانغراس. تم فحص وتصنيف الأجنة وتقييم تكوينها وفقاً للدرجات A و B و C و D. أظهرت النتائج أن التجريب الفموي للكارنتين اليساري يقلل من وزن الجسم لدى الفئران في كل من مجموعتي المعاملة بالكارنتين اليساري اليافعة والمتقدمة في السن معنوياً ($P \leq 0.01$) وكما عزز من التكوين الجنيني للأجنة من الدرجة A في مجموعتي المعاملة بالكارنتين اليساري اليافعة والمتقدمة في السن بشكل معنوي ($P \leq 0.01$). ومع ذلك، فإن نسبة تكوين الأجنة من الدرجة A في المجموعة اليافعة المعاملة بالكارنتين اليساري أظهرت زيادة معنوية ($P \leq 0.01$) بالمقارنة مع مجموعة المعاملة بالكارنتين اليساري المتقدمة في السن. استنتج من هذه الدراسة أن التجريب الفموي للكارنتين اليساري بتركيز 10 مجم / كجم يوميًا لمدة 2-3 دورات شبق يمكن أن يقلل من وزن الجسم ويزيد نسبة التكوين الجنيني المبكر قبل الانغراس.

الكلمات المفتاحية: الفئران المتقدمة في السن، وزن الجسم، الكارنتين اليساري، التكوين الجنيني قبل الانغراس، الفئران اليافعة.