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An Impact of Intravenous Injection of L-Carnitine on the Sexual Characteristics of Aged Sheep

DHEYAB D. RADHI^{1*}, RASHEED H.A. AL-AIDI², ALI AHMED KHALAF³¹Department of Animal Production Technologies, Shatra Technical Institute, Southern Technical University, Iraq;²Ministry of Agriculture, Wasit Agriculture Directorate, Iraq; ³Department of Agricultural Biotechnology, Kut-Tech Technical Institute, Middle Technical University Iraq.

Abstract | The study aimed to improve the semen quality of aged Awassi rams (local Iraqi sheep) by injecting L-carnitine directly into the venous blood. For this purpose, twelve rams with ages ranged between 5 and 6 years were used. The animals were distributed into three groups, each group consisted of four rams. The first group was given an intravenous injection of 5 ml of distilled water as a control group. The second group was given an intravenous injection of L-carnitine at a rate of 25 mg/kg of body weight. The third group was given an intravenous injection of L-carnitine at 50 mg/kg body weight. The results of the study showed that giving an intravenous injection of L-carnitine for 70 days, led to improvement into the quality of semen in aged Awassi rams, the most important of which was a significant increase ($p < 0.05$) in sperm concentration, which reached $3.41 (10^6/\text{ml})$ at a concentration of 50 mg/kg body weight from L-carnitine, compared to the control treatment ($2.89, 10^6/\text{ml}$). The results also showed a significant decrease ($P < 0.05$) in the percentage of abnormal sperm, which amounted to 17.12% at a concentration of 50 mg/kg body weight from L-carnitine, compared to the control treatment (25.32%). In addition, an improvement in the biochemical indicators including LH, testosterone, ALT, AST, and glucose in blood serum was also observed.

Keywords | Iraqi sheep, L-carnitine, Sperm quality, Sexual ability

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***Correspondence** | Dheyab D. Radhi, Department of Animal Production Technologies, Shatra Technical Institute, Southern Technical University, Iraq; Email: dheyab.radhi@stu.edu.iq**Citation** | Radhi DD, Al-Aidi RHA, Khalaf AA (2024). An impact of intravenous injection of L-carnitine on the sexual characteristics of aged sheep. J. Anim. Health Prod. 12(s1): 258-262.DOI | <https://dx.doi.org/10.17582/journal.jahp/2024/12.s1.258.262>

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INTRODUCTION

Sheep are valued as a food source because they provide Meat, dairy, and other items (Holcomb, 1994). The ram is the most significant and frequently overlooked member of the flock. Ram makes up half of the flock's genetic makeup, and his breeding prowess will greatly increase the likelihood of successful lamb production (Swelum *et al.*, 2014). Any breed's semen quality is influenced by a variety of factors, the most significant of which are

dietary factors and environmental factors like temperature, humidity, daylight hours, and age, the latter of which is one of the most significant factors affecting ram's sheep's sexual ability and semen quality (Malejane *et al.*, 2014). Research on production performance has received a lot of attention up to this point, but little has been done on reproductive performance, particularly the quality of sheep sperm (Akhatayeva *et al.*, 2023). By evaluating sperm quality, it is possible to identify guys with high and low reproductive performance. As a foundation for subsequent

reproductive management, data on cattle reproductive performance is required. Performance and productivity in livestock are tightly correlated. In order to make attempts to increase reproductive efficiency in livestock, it is required to investigate information regarding reproductive performance since it is an expression of reproductive features (Zaher *et al.*, 2020). The aim of this study was to evaluate the sperm quality of aged Iraqi sheep following L-carnitine injections.

MATERIAL AND METHODS

LOCATION OF STUDY

The study was conducted at the livestock research station affiliated with the Shatra Technical Institute, Southern Technical University, Dhi Qar Governorate, Iraq. (Operation with laboratories in Southern Technical University Ethical Approval No. Us030101).

DESIGN OF THE EXPERIMENTS AND DIETARY

The study included the use of twelve Iraqi Awassi sheep. Their ages ranged between 5 and 6 years, and they had similar body weights. The animals were divided into three groups. The first group was given an intravenous injection of 5 ml of distilled water as a control group. The second group was given an intravenous injection of L-carnitine at 25 mg/kg body weight. The third group was given an intravenous injection of L-carnitine at 50 mg/kg body weight. The experiment lasted 70 days, a 10-day acclimation period. The animals were taught to collect semen using an artificial vagina. For 60 days, the animals were given intravenous injections every day. During the experimental period, the animals were fed a similar diet for all groups as in Table 1, in addition to being given free wheat straw and water. Semen was collected from animals every two weeks in the early morning using an artificial vagina. The volume of ejaculate(ml), sperm concentration (10^6 /ml), motility (%), livability (%) and abnormal (%) sperm were calculated according to what was stated in Swelum *et al.* (2018a, b).

Table 1: The various concentrated ration ingredients.

Nutrients	Percentage of feed materials included in experimental diets %
Barley	50
Wheat bran	20
Corn	20
Soy bean meal	7.5
Mineral premix 1	2.5

Blood samples were collected from the animals at the end of the experiment at the eighth week via a jugular vein using a sterile medical syringe with a capacity of 10 ml. It was placed in a test tube devoid of any anticoagulant to

allow it to coagulate and facilitate the process of obtaining the serum. Then the process of separating the serum was carried out using a centrifuge at a speed of 3000 rpm, and for 15 minutes after that, it was stored in a test tube until the biochemical and hormonal tests were performed.

The ELISA reader (MCL-202) was used at 450 nm to estimate FSH, LH and Testosterone in the blood serum of ram's sheep, using the ready-made kit manufactured by the German company GmbH. The same steps were followed in conducting tests for each hormone according to the enzymatic facilities.

The concentration of AST and ALT in blood serum was measured using a ready-made kit manufactured by GmbH, Germany. A method is summarized that uses the colorimetric determine the activity of the aspartate amino transferase enzyme. Oxalo-acetate hydrazone is formed with 2,4-dinitrophenyl hydrazine. To estimate the concentration of pyruvic-glutamic transaminase in blood serum by observing the concentration of pyruvate hydrazone formed with dinitrophenyl hydrazine-2,4, the samples are read with a spectrophotometer (DR3900).

The level of glucose in the blood serum was measured using a ready-made test kit produced by the Spanish company Spinreact and according to the Kopper method. The measurement method is summarized as follows: We take 10 microliters of blood serum and put it in a tube, then clean it with 1 ml of glucose reagent, shake the tubes well, and incubate. For 10 minutes at a temperature of 37 °C, the samples are read with a spectrophotometer (DR3900) at a wavelength of 505 nm.

STATISTICAL ANALYSES

The data analysis employed a totally random design. The effects of treatments on the qualities under inquiry were determined using a one-way ANOVA technique (SPSS, 2018). The statistical software used to find the significant differences between treatment means employed the least significant test.

RESULTS AND DISCUSSION

SEMEN QUALITY

The results outlined in Table 2 show that there was a significant improvement ($p < 0.05$) in ejaculate volume when L-carnitine was injected at concentrations of 50 and 25 mg/kg body weight. At the eighth week, it produced 2.12 and 1.19 ml, respectively, compared to the control group, which amounted to 1.38 ml.

Injection of 50 and 25 mg/kg body weight of L-carnitine led to a significant increase ($p = 0.0001$) in sperm concentration

at the eighth week, which amounted to 3.41 and 3.27 10⁶/ml, respectively, compared with the control group, which amounted to 2.89 10⁶/ml. This is shown in Table 3.

Table 2: The effect of L-carnitine on sperm ejaculate volume (ml) (mean ± SE).

Treatment	Week 2	Week 4	Week 6	Week 8
Control	1.26 ± 0.05 ^a	1.28 ± 0.02 ^a	1.31 ± 0.27 ^a	1.38 ± 0.39 ^a
T1	1.27 ± 0.02 ^a	1.32 ± 0.14 ^b	1.56 ± 0.07 ^b	1.91 ± 0.57 ^b
T2	1.30 ± 0.01 ^a	1.37 ± 0.19 ^b	1.81 ± 0.03 ^c	2.12 ± 0.61 ^c
P-value	0.2370	0.0364	0.0002	0.0001

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

Table 3: The effect of L-carnitine on sperm concentration in semen (10⁶/ ml) (mean ± SE).

Treatment	Week 2	Week 4	Week 6	Week 8
Control	2.77±0.03 ^a	2.79±0.71 ^a	2.80±0.06 ^a	2.89±0.25 ^a
T1	3.15±0.68 ^b	3.19±0.01 ^b	3.21 ± 0.09 ^b	3.27±0.52 ^b
T2	3.26±0.57 ^c	3.28±0.22 ^b	3.30 ± 0.45 ^c	3.41±0.36 ^b
P-value	0.0004	0.0003	0.0001	0.0001

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

Table 4: The effect of L-carnitine on sperm motility (%) (mean ± SE).

Treat-ment	Week 2	Week 4	Week 6	Week 8
Control	55.23±2.67 ^a	56.34±4.07 ^a	57.68±2.61 ^a	58.12±3.15 ^a
T1	59.24±1.02 ^b	59.49±3.19 ^b	61.55±3.29 ^b	61.69±1.93 ^b
T2	60.01±3.11 ^b	60.17±1.88 ^b	61.81±6.01 ^b	62.86±4.22 ^c
P-value	0.0001	0.0002	0.0010	0.0011

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

There was a significant difference ($p < 0.05$) in sperm motility when injecting 50 and 25 mg/kg body weight of L-carnitine in the eighth week, as in Table 4. We note that sperm motility amounted to 62.86 and 61.69%, respectively, compared to the control group, which amounted to 58.12%.

The data in Table 5 show that there was a significant improvement ($P < 0.05$) in the percentage of sperm livability for all weeks when injected with 50 mg/kg body weight L-carnitine, which amounted to 67.48, 69.47, 71.80, and 72.92% for weeks 2, 4, 6, and 8, respectively, compared to the group. The control amounted to 62.11, 62.53, 65.03, and 65.49% for weeks 2, 4, 6, and 8, respectively.

The results show a significant decrease ($P = 0.0001$) in

the percentage of abnormal sperm when injected with L-creatine at levels of 50 and 25 mg/kg body weight at the eighth week, as in Table 6. We notice in the Table 6 that the T2 and T1 treatments amounted to 17.12 and 18.48%, respectively. Compared to the control group, which amounted to 25.32%.

Table 5: the effect of L-carnitine on sperm livability (%) (mean ± SE).

Treat-ment	Week 2	Week 4	Week 6	Week 8
Control	62.11±1.37 ^a	62.53±3.12 ^a	65.03±1.52 ^a	65.49±4.31 ^a
T1	67.35±1.44 ^b	68.82±1.71 ^b	69.59 1.90 ^b	70.16±3.27 ^b
T2	67.48±1.83 ^b	69.47±2.66 ^b	71.80±2.78 ^b	72.92±3.14 ^b
P-value	0.0070	0.0001	0.0002	0.0001

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

Table 6: The effect of L-carnitine on sperm abnormal (%) (mean ± SE).

Treat-ment	Week 2	Week 4	Week 6	Week 8
Control	25.26±0.65 ^a	25.29±0.92 ^a	24.33±0.25 ^a	25.32±0.89 ^a
T1	23.27±2.72 ^{ab}	22.52±1.18 ^b	20.56±0.77 ^b	18.45±0.51 ^b
T2	22.30±0.71 ^b	20.37±0.49 ^b	19.79±0.23 ^b	17.12±0.68 ^b
P-value	0.0060	0.0070	0.0002	0.0001

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

Table 7: The effect of L-carnitine on FSH, LH and Testosterone in the serum of Awassi sheep rams at week eight (mean ± SE).

Treatment	FSH (ng/ml)	LH (ng/ml)	Testosterone (ng/ml)
Control	0.28± 0.01 ^a	0.25 ± 0.09 ^a	0.88 ± 0.02 ^a
T1	0.62 ± 0.01 ^b	0.65 ± 0.01 ^b	1.51 ± 0.07 ^b
T2	0.69 ± 0.02 ^b	0.67 ± 0.04 ^b	1.55 ± 0.02 ^b
P-value	0.0411	0.0322	0.0112

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. FSH: Follicle-stimulating hormone. LH: Luteinizing Hormone. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

PHYSIOLOGICAL TRAITS

The results in Table 7 showed the effect of L-carnitine on FSH, LH, and testosterone in the serum of Awassi sheep rams to a significant increase ($p \leq 0.05$), as the concentration of FSH, LH, and testosterone amounted to 0.69, 0.67, and 1.55 ng/ml, respectively, when injecting 50 mg/kg body weight of L-carnitine. The table also shows that after injecting 25 mg/kg body weight of L-carnitine, no significant difference appeared with the third treatment

compared with the control treatment, which showed a significant decrease in hormone concentrations of FSH, LH, and testosterone, which amounted to 0.28, 0.25, and 0.88 ng/ml, respectively.

Table 8: The effect of L-carnitine on AST, ALT and Glucose in the serum of Awassi sheep rams at week eight. (mean ± SE).

Treatment	AST (IU/L)	ALT (IU/L)	Glucose (mg/100 ml)
Control	85.16±2.55 ^a	42.39±1.92 ^a	61.30±2.25 ^a
T1	120.27±3.02 ^b	64.12±1.18 ^b	68.53±4.76 ^b
T2	122.30±1.31 ^b	67.40±2.49 ^b	68.84±1.03 ^b
P-value	0.0031	0.0055	0.0004

T1: L-Carnitine injection 25mg/kg body weight. T2: L-Carnitine injection 25mg/kg body weight. AST: Aspartate transaminase. ALT: Alanine transaminase. SE: standard error. P-value: significantly differed ($p \leq 0.05$).

The results in Table 8 showed the effect of L-carnitine on AST, ALT, and glucose in the serum of Awassi sheep rams to a significant increase ($p \leq 0.05$), as the concentrations of AST, ALT, and glucose amounted to 122.30, 67.40 IU/L, and 68.84 mg/100 ml, respectively, when injecting 50 mg/kg body weight of L-carnitine. The Table 8 also shows that after injecting 25 mg/kg body weight of L-carnitine, no significant difference appeared with the third treatment compared with the control treatment, which showed a significant decrease in hormone concentrations of AST, ALT, and glucose, which amounted to 85.16, 42.39 IU/L, and 61.30 mg/100 ml, respectively.

The data indicated that there are not sufficient numbers of studies examining the effects of L-carnitine on improving the reproductive characteristics of aged Iraqi Awassi sheep. L-carnitine is a type of amino acid that the body can naturally manufacture or that can also be acquired from specific meals and supplements. The body can benefit from L-carnitine in many ways. L-carnitine plays a crucial role in the production of energy, specifically by transporting fatty acids into the mitochondria of the body's cells. The body can then use the energy that mitochondria produce by burning fat as a source of fuel. L-carnitine is also beneficial for a variety of bodily functions, including heart and brain function, as well as muscle mobility. According to Chatzifotis *et al.* (1996), the addition of L-carnitine to feed has the potential to positively affect development and fat catabolism. L-carnitine contributes to the metabolic intermediates needed for the mitochondria to oxidize long-chain fatty acids and generate metabolic energy (Owen *et al.*, 2001). In addition, CoA/CoA-SH ratio regulation, which is crucial for both carbohydrate and fat metabolism, is regulated by L-carnitine (Chatzifotis *et al.*, 1996; Vaz *et al.*, 2002). According to numerous studies, using L-carnitine supplements on a regular basis can

enhance sperm quality, boost sperm count, and maximize sperm motility (Elokil *et al.*, 2019). Injecting damask goats with carnitine at a concentration of 50 mg/kg of live body weight significantly improved semen quality ($P < 0.01$). Ejaculation volume (ml), wave motility, sperm motility (%), sperm concentration (106/ml), total sperm production ($\times 10^6$), and abnormal sperm (%) were increased in male semen. In addition to a significant improvement ($P < 0.01$) in AST and testosterone levels compared to the control group (Abd-Allah *et al.*, 2021), It is possible to sustain boar sperm quality measures, including sperm motility and membrane integrity, by supplementing with 50 mM L-carnitine (Yang *et al.*, 2020). Plasma membrane function, motility, and other related dairy goat semen parameters are markedly enhanced when semen freezing extenders containing 50 mM L-carnitine are added (Zhao *et al.*, 2023). Significant increase ($P \leq 0.05$) in testosterone in the blood, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) compared to the control group, as well as improved semen properties (ejaculation volume/ml, sperm concentration (10^6 /mL). live sperm % and sperm abnormalities %) When male rabbits are dosed with L-carnitine at a dose of 140 mg/kg body weight (Abdel-Hamed *et al.*, 2014), L-carnitine injection of 20 mg/kg live body weight in Aleppo goats (Damascus) during the last month of subcutaneous pregnancy once a week improves glucose concentration compared to the control group.

CONCLUSIONS AND RECOMMENDATIONS

The results showed that the intravenous injections (carnitine 50 and 25 mg/kg body weight) given to rams from aged Iraqi Awassi sheep worked to improve the qualitative characteristics of semen and sexual ability. Thus, this study can be used to benefit from good, aged rams for the longest possible period in disseminating their genetic makeup within the herd.

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NOVELTY STATEMENT

Ethical approval, Animal care was permitted according to local ethics A committee at the Technical Institute, Shatra, Southern Technical University

AUTHOR'S CONTRIBUTION

All authors had supervision contributions. The first to the

author had a role in collecting and organizing the data. The second author contributed to the laboratory work and discussion of the results, while the third author was responsible for the statistical analysis of the data and reviewing the writing of the research.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Abd-Allah M, El-Kelawy H, Ibrahim H, El-Gaafary N, El-Ela A (2021). Effect of treatment with L-carnitine, co-enzyme Q10 and zinc-methionine as stimulants on semen quality and some blood parameters of damascus goat bucks during summer season of Egypt. J. Prod. Dev., 26(4): 671-688. <https://doi.org/10.21608/jpd.2021.203272>
- Abdel-Hamed OA, El-Sayed AI, Iraqi MM, Saad AM, Radwan AA (2014). Comparative evaluation of different herbal formula and L-Carnitine on reproductive performance of male Moshtohor rabbits. Anim. Biotechnol. (Rabbits and Rats), 11(17): 11.
- Akhataeva Z, Bi Y, He Y, Khan R, Li J, Li H, Lan X (2023). Survey of the relationship between polymorphisms within the BMPRI1B gene and sheep reproductive traits. Anim. Biotechnol., 34(3): 718-727. <https://doi.org/10.1080/10495398.2021.1979023>
- Chatzifotis S, Takeuchi T, Seikai T (1996). The effect of carnitine supplementation on growth of red sea Bream (*Pagrus major*) fingerlings at two levels of dietary lysine. J. Aquicult., 147: 235-248. [https://doi.org/10.1016/S0044-8486\(96\)01403-2](https://doi.org/10.1016/S0044-8486(96)01403-2)
- Elokil AA, Bhuiyan AA, Liu HZ, Hussein MN, Ahmed HI, Azmal SA, Yang L, Li S (2019). The capability of L-carnitine-mediated antioxidant on cock during aging: Evidence for the improved semen quality and enhanced testicular expressions of GnRH1, GnRHR, and melatonin receptors MT ½. Poult. Sci., 98(9): 4172-4181. <https://doi.org/10.3382/ps/pez201>
- Holcomb GB (1994). A small-scale agriculture alternative: Dairy and meat goats. USDA, office for small-scale Agriculture.
- Kaşar C, Zonturlu AK, Karapehlivan M, Ari UÇ, Ögün M, Cital M (2010). The effects of L-carnitine administration on energy metabolism in pregnant Halep (Damascus) goats. Turk. J. Vet. Anim. Sci., 34(2): 163-171. <https://doi.org/10.3906/vet-0805-11>
- Malejane CM, Greyling JPC, Raito MB (2014). Seasonal variation in semen quality of Dorper rams using different collection techniques. South Afr. J. Anim. Sci., 44(1): 26-32. <https://doi.org/10.4314/sajas.v44i1.4>
- Owen KQ, Maxwell CV, Nelseen JL, Goodb RD, Tokach MD, Tremblay GC (2001). Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristic of swine. J. Anim. Sci., 79: 3104-3112. <https://doi.org/10.2527/2001.79123104x>
- Statistical Packages of Social Sciences (SPSS) (2018). Statistical packages of social sciences. Version 24 for windows. SPSS. Inc.
- Swelum AAA, Saadeldin IM, Moumen AF, Ali MA, Alowaimier AN (2018a). Efficacy of controlled internal drug release (CIDR) treatment durations on the reproductive performance, hormone profiles, and economic profit of Awassi ewes. Small Rumin. Res., 166: 47-52. <https://doi.org/10.1016/j.smallrumres.2018.07.018>
- Swelum AAA, Saadeldin IM, Moumen AF, Ali MA, Ba- Awadh H, Alowaimier AN (2018b). Efficacy of using previously used controlled internal drug release (CIDR) insert on the reproductive performance, hormone profiles and economic measures of sheep. Reprod. Domest. Anim., 53(5): 1114-1122. <https://doi.org/10.1111/rda.13212>
- Swelum AA, Al-Owaimier AN, Abouheif MA (2014). Effects of breed and number of embryos transferred on the efficacy of MOET in sheep. J. Anim. Vet. Adv., 13: 98-103.
- Vaz FM, Melegh B, Bene F, Cuebas D, Gace DA, Bootsama A, Vreken P, Gennip AHV, Biebir LL, Wanders RJA (2002). Analysis of carnitine biosynthesis metabolites in urine by HPLC-electrospray tandem mass spectrometry. Clin. Chem., 48(6): 826-834. <https://doi.org/10.1093/clinchem/48.6.826>
- Yang K, Wang N, Guo HT, Wang JR, Sun HH, Sun LZ, Zhou JB (2020). Effect of L-carnitine on sperm quality during liquid storage of boar semen. Asian Austral. J. Anim. Sci., 33(11): 1763. <https://doi.org/10.5713/ajas.19.0455>
- Zaher HA, Alawaash SA, Swelum AA (2020). Effects of season and breed on the reproductive performance of sheep. J. Anim. Reprod. Biotechnol., 35(2): 149-154. <https://doi.org/10.12750/JARB.35.2.149>
- Zhao J, Meng P, Jin M, Ma X, Ma H, Yang H, Liu J (2023). Combined addition of L-carnitine and L-proline improves cryopreservation of dairy goat semen. Anim. Reprod. Sci., 257: 107325. <https://doi.org/10.1016/j.anireprosci.2023.107325>