

L-carnitine Acetylcarnitine

The Effect of L-carnitine and Acetylcarnitine on Sperm Parameters *in vitro*

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Objectives: To assess the scavenging effect of carnitine derivatives on oxidative damage to sperm during sperm processing, cryopreservation and thawing.

Materials and Methods: Fresh semen samples from 20 normal healthy volunteers were collected by masturbation after at least 48 hours abstinence. After liquefaction of semen samples at room temperature, the specimens were diluted with sperm wash media (Ham's F-10, Life technologies) to a uniform density of 20×10^6 /ml. L-carnitine or acetylcarnitine were added with various concentration of 0 μ M, 10 μ M, 30 μ M in semen sample or cryoprotectant. All specimens were cryopreserved at $-196 \text{ } ^\circ\text{C}$ LN₂ for 3 days. Sperm motility, vitality, fertilizing capacity, reactive oxygen species formation and the level of lipid peroxidation were analyzed by computer assisted semen analyzer, eosin-nigrosin stain, hypo-osmotic swelling test, chemiluminescence and thiobarbituric acid method, respectively, during sperm processing, cryopreservation and thawing.

Results: The sperm motility was only increased in proportion to the concentration of acetylcarnitine with no statistical significance ($p > 0.05$). The sperm vitality was also significantly improved in proportion to the concentration of acetylcarnitine with statistical significance ($p < 0.05$). The sperm fertilizing capacity was significantly increased in proportion to the concentration of L-carnitine and acetylcarnitine and reactive oxygen species generation and lipid peroxidation were significantly decreased with same fashion ($p < 0.05$). On comparison of effects between L-carnitine and acetylcarnitine, acetylcarnitine was superior to L-carnitine on the improvement of sperm motility and vitality as well as the suppression of reactive oxygen species generation and lipid peroxidation.

Conclusions: These results suggest that carnitine derivatives have a scavenging effect against oxidative damages during sperm processing, cryopreservation and thawing. Therefore, carnitine derivatives may be useful as an oral antioxidant in patients with male infertility due to increased ROS generation.

Key Words: L-carnitine, Acetylcarnitine, Cryopreservation, Sperm

가

가

1-4 sorbitol, kallikrein, carnitine, EDTA verapamil, pentoxifylline methylxantine, carnitine

5 Carnitine acyl

6,7 Sertoli cell Leydig cell 8

L-carnitine long chain acyl

, acetylcarnitine acetyl (structural lipid)

가

가 8-11 carnitine

가 12,13

carnitine L-carnitine acetylcarnitine 가

1. 가 20 5 가 20~30

가

L-carnitine 0 μ M, 10 μ M 30 μ M

가 A, I II , acetylcarnitine 0 μ M, 10 μ M 30 μ M 가 (Figure 1).

B, III IV I, II III, IV

2. 가 L-carnitine acetylcarnitine (Figure 1).

1) 2~3 27 20~30 Makler chamber (Fertility-Tech, USA) (SAIS, Medical Supply Co. Ltd., Korea), WHO 14

2 ml, 20 $\times 10^6$ /ml, 50%, 30% (Ham's F-10, Life technologies) 20 $\times 10^6$ /ml (1) (velocity distribution) static (grade 1), slow (grade 2), medium (grade 3), rapid (grade 4) grade 3 4 가 (%) (2) eosin-nigrosin (E-N) 가 20 $\times 10^6$ /ml E-N 50 μ l 1 ml eppendorf tube vortex mixer 50 μ l malinol xylene 2:1 mounting 1000 100 E-N

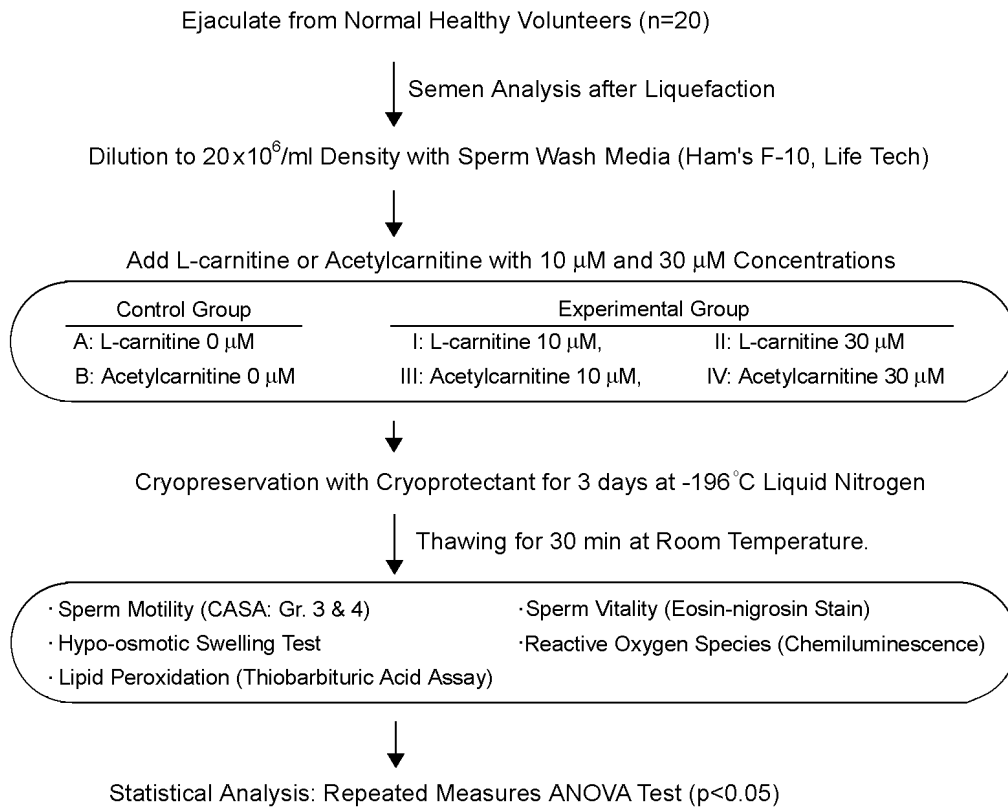


Figure 1. Study design and experimental flow diagram

2) 5 20×10^6 /ml 100 μ l 30 cover glass

0.5 ml 400 swelling rate

(9971, Irvine Scientific Co., Santa Ana, USA) 1:1 52%

가 cryomatic cellevator tray (L.A.O. Enterprises, U.S.A) (liquid-nitrogen gas tank, Harsco Co., Hamilton, U.S.A.) 4 (2) chemiluminescence

-196 1 Hepes balanced salt solution/bovine serum albumin (BSA) buffer (130 mM NaCl, 4 mM KCl, 1 mM CaCl_2 , 14 mM fructose, 10 mM Hepes, pH 8.0 and 1 mg/ml BSA) 500 μ l

(1) Hypo-osmotic swelling test (HOS) 4 mM luminol (5-amino-2,3-dihydro-1, 4-phthalazinedione, Wako, Japan) 25 μ l 가 lumiphotometer (TD4000, Laboscience, Tokyo, Japan)

가 Fructose (M.W = 180.16) 150 mM sodium citrate \cdot H_2O_2 (M.W = 294.11) 50 mM 가 luminol

HOS 1 ml 37 10 10

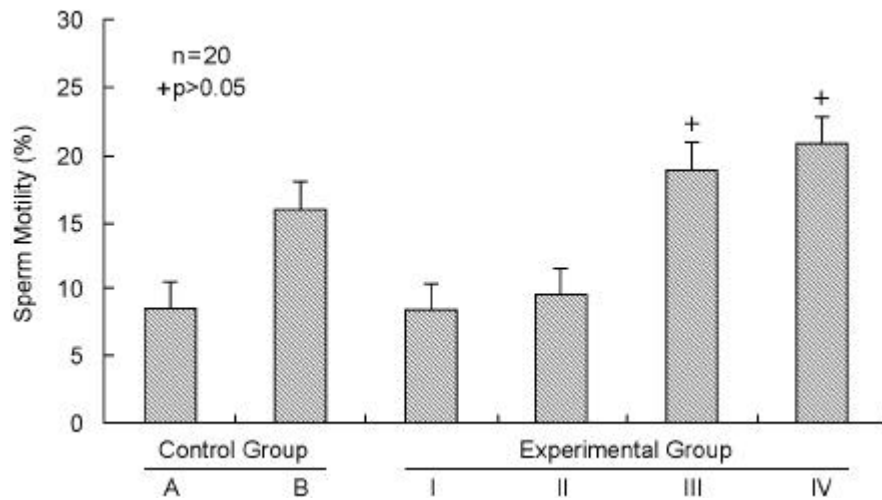


Figure 2. Effect of L-carnitine and acetylcarnitine on sperm motility after cryopreservation and thawing

10 1000 가 p-value 0.05
 unit (AU) arbitrary
 (3)
 malondialde-
 hyde thiobarbituric acid (TBA) 1.
 2000 rpm 10 IV 8.4 ±3.4%, 9.5 ±5.0%, 19.0 ±11.0%
 sperm pellet 1% phosphoric acid (Kanto 20.8 ±10.0% A B 8.5 ±4.0%,
 Chemicals Co., INC., Tokyo, Japan) 750 µl, 0.6% 2- 16.0 ±10.5% 가
 tribarbituric acid (Sigma, St. Louis, MO., U.S.A.) 250 µl (p>0.05) (Figure 2).
 가 vortex mixer (Scientific industry, U.S.A.) 2.
 . 100 (,)
 60 가 vortex mixer , 4 I, II
 30 n-butanol (Junsei 33.4 ±7.5%, 36.6 ±10.3% A
 Chemical CO., Ltd., Tokyo, Japan) 1 ml 가 34.2 ±7.6% 가 (p>0.05).
 vortex mixer , 3000 rpm III, IV 56.8 ±
 25 diode 11.9%, 64.0 ±14.0% B 52.1 ±14.0%
 array spectrophotometer (Hewlett Packard, U.S.A.) 가 (p<0.05) (Figure 3).
 510 nm 534 nm ,
 nmole/mg protein 3.
 3. I, II, III
 IV 60.6 ±6.8%, 62.2 ±5.9%, 66.6 ±7.3%
 SPSS+ version 70 package 69.4 ±6.6% A B 55.8 ±6.2%, 63.7 ±
 Repeated measures ANOVA test , 6.9%

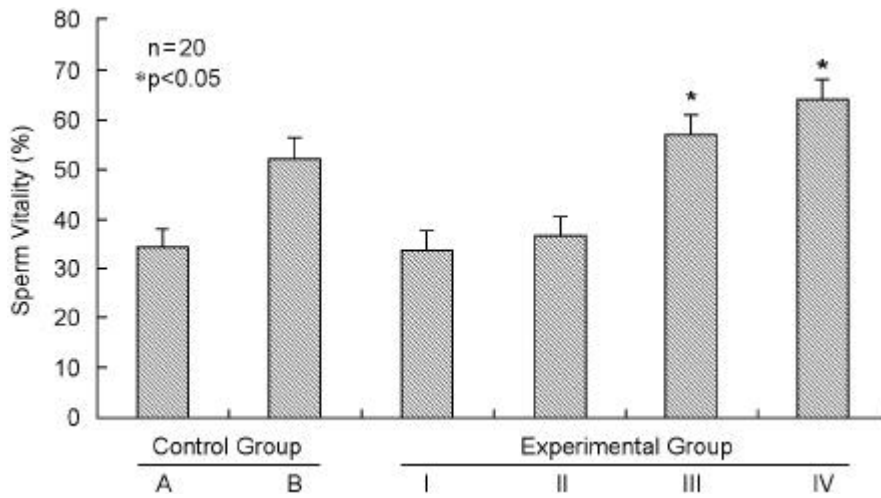


Figure 3. Effect of L-carnitine and acetylcarnitine on sperm vitality after cryopreservation and thawing

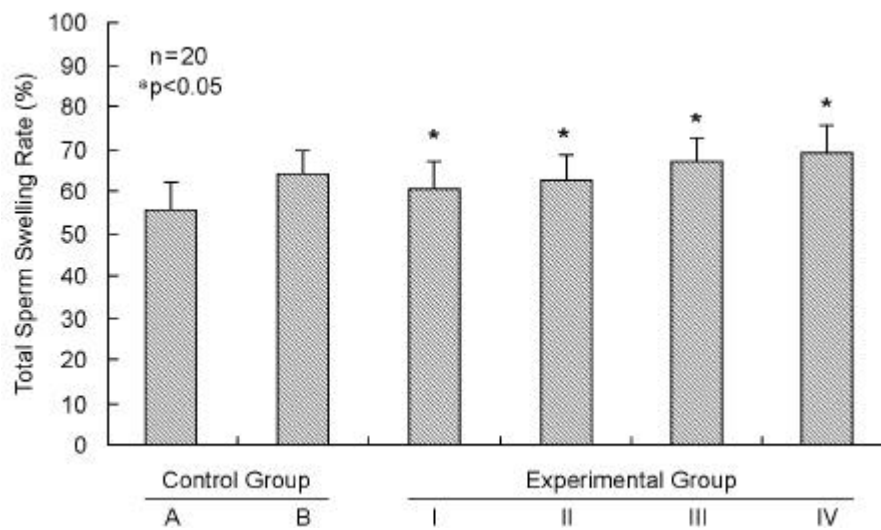


Figure 4. Effect of L-carnitine and acetylcarnitine on total sperm swelling rate after cryopreservation and thawing

(p<0.05) (Figure 4).

4.

III IV 12.2 ± 2.3 × 10³ AU, 10.2 ± 1.7 × 10³ AU, 11.7 ± 2.0 × 10³ AU, 10.5 ± 2.0 × 10³ AU
 A B 12.6 ± 2.4 × 10³ AU, 13.1 ± 2.3 × 10³ AU
 AU

(p<0.05) (Figure 5).

5.

I, II, III IV 91.3 ± 37.7 nM/mg protein, 68.2 ± 32.0 nM/mg protein, 75.3 ± 33.4 nM/mg protein, 54.8 ± 30.4 nM/mg protein
 A B 121.2 ± 50.8 nM/mg protein, 109.9 ± 27.2 nM/mg protein

(p<0.05) (Figure 6).

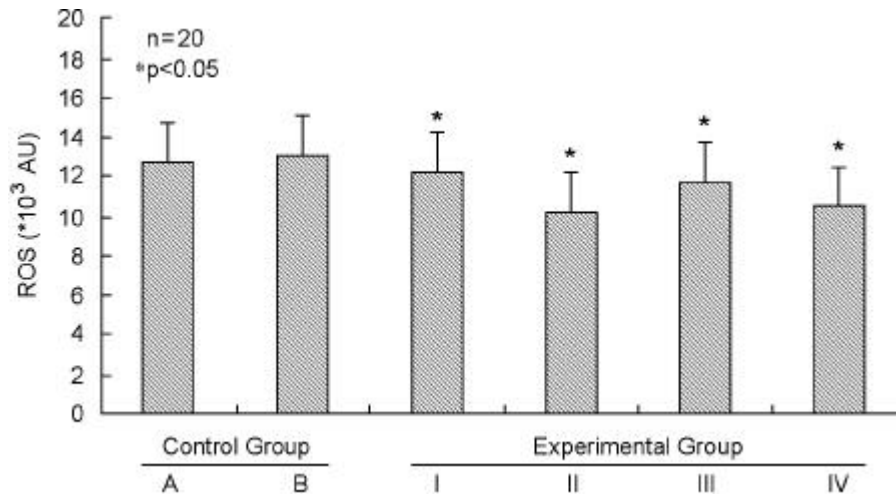


Figure 5. Effect of L-carnitine and acetylcarnitine on level of reactive oxygen species after cryopreservation and thawing

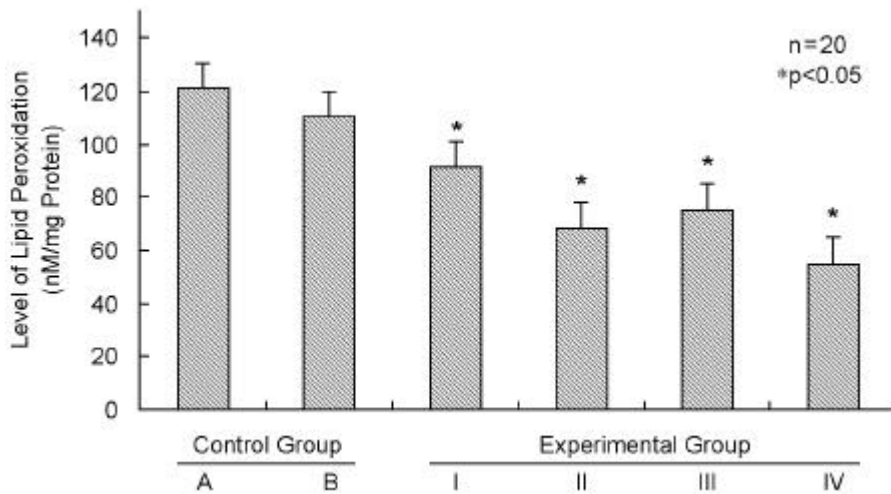


Figure 6. Effect of L-carnitine and acetylcarnitine on level of lipid peroxidation after cryopreservation and thawing

6. L - carnitine acetylcarnitine

(Table).

IV 가
 acetylcarnitine L-carnitine , ,
 , hypo-osmotic swelling lysine methionine
 II 가 L-carnitine .¹⁶
 L-carnitine
 II IV ,
 가 L-carnitine acetylcarnitine . 98%가 ,

Table. Percent changes of sperm parameters according to the concentration of L-carnitine and acetylcarnitine after cryopreservation and thawing

Experimental group	Sperm motility	Sperm vitality	HOS	ROS	LPO
I	-1.2	-2.3	+8.6	-3.9	-24.7
II	+11.8	+7.0	+11.5	-19.7	-43.7
III	+18.8	+9.0	+4.6	-10.7	-31.5
IV	+30.0	+22.8	+8.9	-19.8	-50.1

*HOS; Hypo-osmotic swelling test, ROS; Reactive oxygen species, LPO; Lipid peroxidation

1~6%, 0~6%, acetylcarnitine 가
 carnitine 10~50 μmol/L, L- Duru 22 가
 nm/mL 2000 acetylcarnitine
 carnitine L-carnitine, acetyl- cryosurvival
 carnitine acyl-L-carnitine 가
 L-carnitine 4 amine L-carnitine
 가 3-hydroxyaminobutyrate
 , acetylcarnitine carnitine , acetylcarnitine
 , carnitine 가
 L-carnitine, acetylcarnitine propionyl-L-carnitine L-carnitine acetylcarnitine
 , L-carnitine acetylcarnitine carnitine
 , propionyl-L-car- 가
 nitine 19-21,23
 17-20 L-carnitine acetylcarnitine carnitine
 carnitine 가 Carnitine
 , L-carnitine
 , Campaniello 18 propionyl-L-carnitine
 (asthenozoospermia) , DNA
 L-carnitine , 25% , Vitali 13 Vanella 13 L-carnitine
 40% 가 , Vitali 13 Vanella 13 L-carnitine
 19 20% 가 , Costa 20 17% xide supero-
 38% 가 , Acetylcarnitine nitine 가 L-carnitine acetylcarn-
 가 ,
 Moncada 21 (oligozoospermia)
 acetylcarnitine
 , L-
 7 carnitine acetylcarnitine

가 carnitine

가 carnitine

가 carnitine

가 carnitine

carnitine

가 L-carnitine

carnitine acetylcarnitine

1. acetylcarnitine (p>0.05).

2. L-carnitine acetylcarnitine (p<0.05).

3. L-carnitine acetylcarnitine acetylcarnitine

가 L-carnitine

carnitine

L-carnitine acetylcarnitine

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