Baseline levels of seminal reactive oxygen species predict improvements in sperm function following antioxidant therapy in men with infertility

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Abstract

Background: Poor sperm function is a major cause of infertility. There is no drug therapy to improve sperm function. Semen oxidative stress is a recently identified pathway for sperm damage. Commercial antioxidants such as L-carnitine and acetyl-L-carnitine (LAL) are commonly self-administered by infertile men. However, concerns have been raised whether inappropriate LAL therapy causes reductive stress-mediated sperm damage. It is imperative to investigate whether: (1) LAL improves sperm function by reducing reactive oxidative species (ROS); (2) LAL has differential effects on sperm function between men with normal and elevated ROS.

Methods: A prospective cohort study of routine clinical practice was performed in infertile men with abnormal sperm quality. Changes in sperm function and semen ROS levels following three months of oral LAL therapy were compared between participants with baseline seminal normal ROS (≤10RLU/SEC/10⁶sperm; n = 29) and High ROS (>10 RLU/SEC/10⁶sperm; n = 15) levels measured using an established colorimetric-luminol method.

Results: In normal ROS group, sperm function did not change following LAL therapy. In high ROS group, LAL therapy reduced semen ROS fivefold, increased sperm count by 50% (mean count in mill/ml: 21.5 + 7.2, baseline; 32.6 + 9.5, post-treatment, P = .0005), and total and progressive sperm motility each by 30% (mean total sperm motility in % 29.8 + 5.0, baseline: 30.0 + 5.5, post-treatment, P = .014 vs. baseline).

Conclusions: We report for the first time that LAL only improves sperm quality in infertile men who have baseline high-ROS levels prior to treatment. These data have important potential implications for couples with male infertility and their clinicians.
INTRODUCTION

Infertility affects 10% of couples, and nearly half of cases are due to sperm defects in the male partner. There are currently no available drug therapies to improve sperm function. Reactive oxygen species (ROS) are unstable products of metabolism causing cellular damage produced in the semen by leukocytes and the oxidative metabolism of spermatozoa. ROS generation is a recently identified mechanism for sperm damage and measurement of ROS is a potential tool of added value in the investigation of male infertility. Previous studies have suggested that elevated semen ROS levels are associated with reduced sperm function in men with idiopathic infertility and recurrent miscarriage. Furthermore, many exogenous factors such as genito-urinary infections, varicocele and adverse lifestyle choices have been observed to increase semen ROS. However, no current clinical guidelines support its use in routine practice. There is extensive research in the area with antioxidant therapy observed to improve semen parameters and live-birth rates in men with idiopathic infertility and varicocele-associated infertility. Accordingly, antioxidant therapy is a novel, potential therapy to improve sperm function in men with infertility.

Nutritional supplements containing the antioxidant, L-carnitine and acetyl-L-carnitine, are readily available over the counter for men to use as empirical therapies for improving sperm function. However, published data suggest that the effects of carnitines on sperm function are unclear. Some studies suggest that the orally administered amino acid-derived antioxidant L-carnitine and acetyl-L-carnitine (LAL) increase sperm concentration and motility parameters in men with infertility; however, other studies suggest that carnitines do not alter sperm function. Furthermore, some clinicians harbour concerns that infertile men without elevated semen ROS might suffer paradoxical impairment of sperm function following antioxidant therapy due to reductive stress. In summary, it is common practice that men with infertility often take antioxidant therapy, but the balance of potential benefit and/or harm from such therapy is not known.

In the absence of any pharmacological therapy for male infertility, it is important to identify which (if any) men with infertility could clinically benefit from self-administering the antioxidant carnitines to improve sperm function. We therefore conducted a single-centre prospective cohort study of routine clinical practice in men with infertility associated with reduced sperm function. Specifically, we compared changes in sperm function following carnitines therapy between participants with normal and elevated baseline semen ROS levels.

METHODS

Participants

Male participants under investigation for infertility were recruited from Reproductive Clinic at Hammersmith Hospital, London, UK. Participants were included in the study if they had male factor infertility, that is failure to conceive with a female partner after at least 12 months of regular unprotected sex, with at least one abnormal semen analysis parameter using WHO criteria. These included men with either oligozoosperma (sperm count < 15 million/mL), severe oligosperma (sperm count < 5 million/mL), asthenozoosperma (>40% total motility or <32% progressive motility), and/or teratozoosperma (>4% normal morphology).

In total, forty-six men were assessed, and forty-four completed the study. Patients were excluded from the study if they received previous antioxidant therapy, or had history of genital or urinary tract infection, varicocele or other intrascrotal pathology.

Ethics and protocol

An NHS Service Evaluation of clinical practice was conducted in accordance with the National Health Service (NHS) Health Research Authority/ Medical Research Council Decision Tool. Institutional ethics committee approval was not required for this NHS service evaluation.

All participants underwent assessment of semen analysis and semen ROS testing at study commencement. Then, LAL was orally self-administered by all participants on a daily basis for 3 months, immediately after which semen analysis and semen ROS testing was repeated. All semen samples were produced on site to minimize variability in analysis times. The protocol is summarized in Figure 1.

Sample analyses and ROS measurement

All semen analyses were performed according to WHO 2010 methodology in the UK Accreditation Service (UKAS) certified laboratory at Hammersmith Hospital, London, UK following 2-7 days of sexual abstinence. ROS testing was performed on site within 20 minutes of semen production using an established in-house luminol-based colorimetric assay as detailed: A 100mmol/L luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma-Aldrich) stock solution was prepared in advance in dimethylsulphoxide (DMSO) and stored at room temperature in the dark in a foil-covered polystyrene Falcon tube. A luminol working solution (5mmol/L luminol prepared in
DMSO) was freshly prepared from the stock solution each day, covered in foil, and stored in the dark, and discarded at the end of each day. Negative control samples contained 400µL phosphate-buffered saline solution (PBS) with 10 µL 5 mmol/L luminol working solution. Positive control samples contained 395µL PBS, 5µL 30% hydrogen peroxide, and 10 µL 5 mmol/L luminol working solution. The solutions were mixed gently with care to avoid any bubbles and placed in the luminometer. For measuring chemiluminescence in the semen samples, liquefied whole semen was gently mixed with a plastic pipette, and 400 µL of semen was aliquoted into a 1.5 mL microfuge tube and 10 µL 5 mmol/L luminol working solution was added and mixed gently avoiding any bubbles before reading in the luminometer. Furthermore, because the reagent is light sensitive, the assay was performed with electrical lights switched off to reduce light exposure. The assay was carried out at temperatures of 20-25 degrees Celsius. Measurements were generated at 1-minute intervals over the course of ten minutes for each control and test sample. ROS levels generated were reported in Relative Light Units (RLU)/×10⁶/mL and results were assessed against reference values.

ROS analysis was carried out using a Turner Biosystems single cuvette Modulus as outlined in previous literature. The LAL-containing supplement provided was marketed under the name of Proxeed Plus supplied by Sigma-Tau HealthScience, Utrecht, The Netherlands. The supplement formulation consisted of 1000 mg of L-carnitine, 725 mg of fumarate, 500 mg of acetyl-L-carnitine, 1000 mg of fructose, 50 mg of citric acid, 50 µg of selenium, 20 mg of coenzyme Q10, 90 mg of vitamin C, 10 mg of zinc, 200 µg of folic acid and 1.5 µg of vitamin B12.

### 2.5 Statistical methods

All analysis was performed with GraphPad Prism v.8. Quantitative data were assessed for normality with the D’Agostino & Pearson test, followed by paired t-testing or Wilcoxon rank-sum test. All hypothesis testing was two-tailed; \( P < .05 \) was considered statistically significant. All data are presented as standard error of mean (SEM) unless stated otherwise. Proportions were compared using Fisher’s exact test.

### 3 RESULTS

#### 3.1 Baseline characteristics

Forty-four infertile men with reduced sperm function were included in the study. The mean age of participants was 40 ± 1 years. Baseline semen parameters are summarized in Table 1. Fifteen of the
44 patients had abnormally elevated semen ROS levels (>10 RLU/SEC/10⁶ sperm). No significant differences in baseline characteristics were observed between men with elevated ROS levels (‘High ROS’ group) and normal ROS levels (‘Normal ROS’ group).

3.2 | Effects of antioxidant treatment on sperm function in men with infertility

We investigated whether sperm function, measured by the WHO 2010 criteria, changed following a 3-month course of daily LAL administration in infertile men with at least one abnormal semen analysis parameter with either normal or elevated baseline semen ROS levels.

3.2.1 | Normal ROS group

LAL administration had no significant effect on semen volume, sperm count, total and progressive sperm motility or sperm morphology in men with reduced sperm function and normal baseline ROS levels (Figure 2A-E). Semen ROS levels increased nearly twofold higher post-treatment when compared to pretreatment, but this increase was nonsignificant (mean semen ROS in RLU/SEC/10⁶ sperm: 1.4 ± 0.3, baseline: 2.6 ± 1.0, post-treatment, \( P = .22 \) vs. baseline) (Figure 2F).

3.2.2 | High ROS group

Semen volume and sperm morphology did not change significantly following LAL treatment when compared with baseline in men with reduced sperm function and High ROS (Figure 3A and E). However, mean levels of sperm count increased by approximately 50% following 3 months of LAL treatment in men with reduced sperm function and High ROS (mean count in million/ml: 21.5 ± 7.2, baseline: 32.6 ± 9.5, post-treatment, \( P = .0005 \) vs. baseline) (Figure 3B). Mean total and progressive sperm motility also increased significantly following 3 months of LAL treatment in men with reduced sperm function and High ROS (mean total sperm motility in %: 29.8 ± 5.0, baseline: 39.4 ± 6.2, post-treatment, \( P = .004 \) vs. baseline; mean progressive sperm motility in %: 31.9 ± 4.6, baseline: 30.0 ± 5.5, post-treatment, \( P = .014 \) vs. baseline) (Figure 3C&D). Furthermore, mean semen ROS levels reduced significantly by approximately fivefold, after 3 months of LAL therapy in men with reduced sperm function and High ROS (mean semen ROS in RLU/SEC/10⁶ sperm 55.2 ± 14.3, baseline: 10.6 ± 2.5, post-treatment, \( P = .0001 \) vs baseline) (Figure 3F).

3.3 | Comparing how semen ROS levels influence the response of men with reduced sperm function to antioxidant treatment

Sperm count increased in 13/15 (86.7%) men in the high ROS group which was significantly higher when compared with the normal ROS group (14/29 (48.3%), \( P < .05 \) (Figure 4B). Total sperm motility increased in 12/14 (85.77%) men in the high ROS group which was significantly higher when compared with the normal ROS group (12/29 (41.4%), \( P < .01 \) (Figure 4C). Progressive sperm motility increased in 12/15 (80%) men in the high ROS group which was significantly higher when compared with the Normal ROS group (11/29 (37.9%), \( P < .05 \) (Figure 4D). Furthermore, semen ROS levels reduced in all (14/14; 100%) men in the high ROS group which was significantly lower when compared with the Normal ROS group (11/29 (42.3%), \( P < .001 \) (Figure 4E). No significant difference was noted in sperm volume (Figure 3A) or sperm morphology (Figure 4E).

4 | DISCUSSION

Semen ROS is a novel potential marker of sperm function with increasing evidence of its aetiological role in male infertility. Oxidative stress may negatively affect fertility by adversely affecting sperm membrane lipid peroxidation, sperm motility, the acrosome reaction, chromatin maturation and subsequent sperm DNA fragmentation, resulting in defective paternal DNA passage to the offspring. With nearly 30%-80% of infertile men with high ROS, high oxidative stress is associated with reduced sperm function in

### TABLE 1 Baseline characteristics of patients with male factor infertility

| Characteristics          | All patients (n = 44) | Normal ROS (n = 29) | High ROS (n = 15) | \( P \)-value |
|--------------------------|-----------------------|---------------------|-------------------|--------------|
| Age (years)              | 40 ± 1                | 40 ± 1              | 38 ± 2            | .41          |
| Semen volume (mL)        | 3.4 ± 0.2             | 3.4 ± 0.3           | 3.5 ± 0.4         | .86          |
| Sperm count (million/ml) | 28.6 ± 4.2            | 32.2 ± 5.2          | 21.5 ± 7.2        | .14          |
| Total sperm motility (%) | 34.8 ± 3.4            | 37.3 ± 4.4          | 29.8 ± 5.0        | .19          |
| Progressive sperm motility (%) | 28.2 ± 2.8          | 30.9 ± 3.5          | 23.1 ± 4.6        | .19          |
| Sperm morphology (%)     | 2 ± 0.3               | 2.4 ± 0.4           | 1.5 ± 0.4         | .09          |

Note: The Normal reactive oxygen species (ROS) group had semen ROS ≤ 10 RLU/SEC/10⁶ sperm at baseline. The High ROS group had semen ROS > 10 RLU/SEC/10⁶ sperm at baseline. All data are mean ± standard error of the mean (SEM).
men with idiopathic infertility and in cases of recurrent miscarriage. Subsequently, ‘male oxidative stress infertility’ or MOSI is a new proposed term by Agarwal et al to describe men with idiopathic infertility who have raised semen OS. However currently, there is no consensus on which patients to select to test for ROS and lack of standardized method of ROS assessment and which patients would benefit from treatment. Furthermore, although useful, these tests are expensive, time-consuming and require technical training.

Men taking antioxidants have an associated significant increase in sperm parameters and in live-birth rates. Cavallini et al assessed the effect of antioxidant therapy on idiopathic and varicocele-associated oligoasthenospermia patients with improvement in sperm parameters and pregnancy rate compared to placebo. Furthermore, a recent Cochrane review indicated that there may be an increase in live-birth rate for those couples with the male partners taking antioxidants; however, the overall quality of evidence was low from only seven small randomized controlled trials.

Antioxidants may represent a popular empirical therapy for male infertility; however, previous evidence underpinning their efficacy has been controversial. Consequently, many primary care and specialist clinicians remain sceptical about the proposed benefits of antioxidant therapy for infertile men. Our study suggests for the first time that a commercially available LAL preparation improves sperm count, total and progressive motility while dramatically reducing semen ROS levels in men with infertility. However, we report that LAL therapy is only effective for infertile men with reduced sperm function in whom semen ROS levels are abnormally elevated at baseline.

To date, twelve randomized, double-blinded placebo-controlled studies have reported that carnitine administration improves at least one aspect of sperm function in men with infertility: one, three and ten of these studies reported increases in semen volume, sperm concentration, total sperm motility and progressive sperm motility respectively. Lenzi et al (2003) reported that L-carnitine therapy improved sperm motility and concentration in men with infertility, but only after the exclusion of five outlier patients with spontaneous increase of sperm motility during the second treatment period or spontaneous decrease of sperm motility during the washout period. However, other studies failed to observe any improvements in sperm function following L-carnitine administration to men with infertility. Sigman et al (2006) failed to show any significant improvement in sperm motility or total motile sperm count following

![Figure 2](https://wileyonlinelibrary.com)
daily treatment with L-carnitine (1000 mg) and L-acetyl-carnitine (500 mg) of men with idiopathic asthenospermia. Conversely, we observed that 3 months of LAL supplementation increased sperm concentration significantly by over 50%, and total and progressive motility both increased by approximately 30% when compared with baseline in the elevated ROS group. Furthermore, supplementation with carnitines significantly reduced semen ROS levels by fivefold in the elevated ROS group. However, LAL had no significant effect on sperm function or semen ROS levels in infertile men with normal semen ROS levels ≤ 10 RLU/SEC/10^6. Results of our study may partially explain the inconsistency in the previously observed effects of carnitine therapy on male reproductive function when not stratified by baseline semen ROS levels. We did not observe any significant impairment in sperm volume, count, total or progressive motility men with infertility and normal baseline semen ROS levels. Our data therefore provide important preliminary safety data by suggesting that men without elevated semen ROS could take carnitines therapy without impairment of sperm function.

Compared with empirical hormonal therapies such as aromatase inhibitors and selective oestrogen receptor modulators, antioxidant therapy is relatively safe, inexpensive and widely available. None of the patients in this study had any observed side effects from the antioxidant therapy. However, clinical guidelines are needed to avoid indiscriminate overuse of antioxidants.

Hypothesizing that carnitines are likely to affect sperm function by reducing semen ROS levels, we explored the relationship between changes in ROS and changes within individual markers of sperm function in men with infertility. Our data therefore provide important preliminary safety data by suggesting that men without elevated semen ROS could take carnitines therapy without impairment of sperm function.

Figure 3 Sperm characteristics of patients with elevated baseline ROS (ROS > 10). Bar graphs compare semen volume (A), sperm count (B), total sperm motility (C), progressive sperm motility (D), sperm morphology (E) and semen ROS (F) at baseline versus after 3 months of L-carnitine and acetyl-L-carnitine (LAL) therapy. Data are mean ± SEM. ROS: Reactive oxygen species. **P < .01; ***P < .001; ****P < .0001 [Colour figure can be viewed at wileyonlinelibrary.com]
significantly associated with increase in sperm count, total and progressive motility. These data provide biological plausibility to the hypothesis\textsuperscript{23,29} that novel drugs targeted to reduce semen ROS could be used to treat men with infertility.

The study is not without its limitations. The normal (<10) and high (>10) ROS levels in our study were arbitrary cut-offs in men who had abnormal baseline semen parameters. There is lack of standardization and inconsistencies in establishing a reference range. Furthermore, the established reference ranges attempt to define physiological levels of seminal ROS as opposed to high and low ROS levels within men with abnormal baseline semen parameters or infertility. In addition, sexual abstinence duration may influence ROS levels. A recent study showed that a group of men with > 4 days of abstinence duration had higher seminal oxidative stress compared with men with sexual abstinence of 4 days or less, albeit using different ROS assays.\textsuperscript{35} All semen samples in our study were collected between 2 to 7 days of sexual abstinence as per the WHO 2010 guidelines\textsuperscript{14}; therefore, this variation in abstinence duration may have influenced the ROS levels. In addition, it was a small sample size, requiring future larger studies to confirm these findings. Furthermore, a placebo group could not be included in this study of routine clinical practice; however, we consider this would have provided limited additional benefit to the study given that the included control group (infertile men with normal ROS) did not respond to LAL therapy. Published studies suggest that mean sperm counts would not change significantly during serial measurements in the absence of L-carnitine therapy.\textsuperscript{33,36} We also acknowledge that the antioxidant supplement contains other ingredients other than LAL; therefore, the effects observed cannot be attributed only to the LAL ingredient. In addition, we did not measure reproductive hormones or paternity outcomes in this cohort.

In summary, we have performed a prospective study reflecting real-world practice with important implications for the use of antioxidants to treat men with infertility. Three months of over the counter LAL therapy improved sperm function in some but not all men with infertility. Critically, our data suggest LAL therapy only improves sperm function in men with elevated semen ROS. Our data therefore suggest that LAL administration has clinical utility to improve sperm function in men with infertility, but only benefits a subset of patients. Furthermore, LAL did not impair sperm function.
in infertile men without elevated semen ROS levels. Future studies are warranted to investigate if LAL therapy can improve pregnancy and live-birth outcomes in couples affected by male infertility.

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CONFLICT OF INTEREST

The views expressed are those of the authors and not necessarily those of the above-mentioned funders, the NHS, the NIHR, or the Department of Health. Proxeed Plus was provided free of charge through an investigator-led grant (JWR) by Sigma-Tau HealthScience (Utrecht, Netherlands). The authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. *J Hum Reprod Sci*. 2015;8(4):191-196.
2. Darbandi M, Darbandi S, Agarwal A, et al. Reactive oxygen species and male reproductive hormones. *Reprod Biol Endocrinol*. 2018;16(1):87.
3. Agarwal A, Mulgund A, Alshahranı S, et al. Reactive oxygen species and sperm DNA damage in infertile men presenting with low level leukocytospermia. *Reprod Biol Endocrinol*. 2014;12(1):126.
4. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. *Am J Reprod Immunol*. 2008;59(1):2-11.
5. Jayasena CN, Radia UK, Figueiredo M, et al. Reduced testicular steroidogenesis and increased semen oxidative stress in male partners as novel markers of recurrent miscarriage. *Clin Chem*. 2019;65(1):161-169.
6. Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cell. Mol. Life Sci*. 2020;77(1):93-113.
7. Imamovic Kumatic S, Pinter B. Review of clinical trials on effects of oral antioxidants on basic semen and other parameters in idiopathic oligoasthenoteratozoospermia. *Biomed Res Int*. 2014;2014:426951.
8. Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G, Vitali G. Cinnomic and L-carnitine/acyetyl-L-carnitine treatment for idiopathic and varicoceles-associated oligoasthenospermia. *J Androl*. 2004;25(5):761-772.
9. Balercia G, Regolì F, Armenì T, Koverech A, Mantero F, Boscaro M. Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. *Fertil Steril*. 2005;84(3):662-671.
10. Lenzi A, Sgrò P, Salacone P, et al. A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acytethyl-carnitine treatment in men with asthenozoospermia. *Fertil Steril*. 2004;81(6):1578-1584.
11. Raigani M, Yaghmaei B, Amirjannti N, et al. The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. *Andrologia*. 2014;46(9):956-962.
12. Sigman M, Glass S, Jyor J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. *Fertil Steril*. 2006;85(5):1409-1414.
13. Henkel R, Sandhu IS, Agarwal A. The excessive use of antioxidant therapy: A possible cause of male infertility? *Andrologia*. 2019;51(1):e13162.
14. World Health Organization. *Laboratory manual for the examination and processing of human semen*, 5th edn. Cambridge: Cambridge University Press; 2010.
15. Vessey W, Perez-Miranda A, Macfarquhar R, Agarwal A, Homa S. Reactive oxygen species in human semen: validation and qualification of a chemiluminescence assay. *Fertil Steril*. 2014;102(6):1576-1583.e4.
16. Homa ST, Vessey W, Perez-Miranda A, Riyait T, Agarwal A. Reactive Oxygen Species (ROS) in human semen: determination of a reference range. *J Assist Reprod Genet*. 2015;32(5):757-764.
17. Agarwal A, Sharma RK, Nallella KP, Thomas AJ JR, Alvarez JG, Sikka SC. Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril*. 2006;86(4):878-885.
18. Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: A systematic review on evaluation and management. *Arab J Urol*. 2019;17(2):87-97.
19. Bui AD, Sharma R, Henkel R, Agarwal A. Reactive oxygen species impact on sperm DNA and its role in male infertility. *Andrologia*. 2018;50(8):e13012.
20. Agarwal A, Parekh N, Panner Selvam MK, et al. Male Oxidative Stress Intensity (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. *World J Mens Health*. 2019;37(3):296-312.
21. Wagner H, Cheng JW, Ko EY. Role of reactive oxygen species in male infertility: An updated review of literature. *Arab J Urol*. 2016;16(1):35-43.
22. Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. *Cochrane Database Syst Rev*. 2019;3(3):CD007411.
23. Micic S, Nalic D, Djordjevic D, et al. Double-blind, randomised, placebo-controlled trial on the effect of L-carnitine and L-acetylcarnitine on sperm parameters in men with idiopathic oligoasthenozoospermia. *Andrologia*. 2019;51(6):e13267.
24. Wu ZM, Lu X, Wang YW, et al. Short-term medication of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine treatment in men with asthenozoospermia. *Fertil Steril*. 2004;81(6):1578-1584.
25. Lenzi A, Lombardo F, Sgrò P, et al. Use of carnitine therapy in idiopathic asthenozoospermia. *Andrologia*. 2012;18(3):253-256.
26. Wu ZM, Lu X, Wang YW, et al. Short-term medication of L-carnitine before intracytoplasmic sperm injection for infertile men with oligoasthenozoospermia. *Zhonghua Nan Ke Xue* 2012;18(3):253-256.
27. Costa M, Canale D, Filicori M, D’Iddio S, Lenzi A. L-carnitine in idiopathic asthenozoospermia: a multicenter study. Italian Study Group on Carnitine and Male Infertility. *Andrologia*. 1994;26(3):155-159.

28. Moncada ML, Vicari E, Cimino C, Calogero AE, Mongioi A, D’Agata R. Effect of acetylcarnitine treatment in oligoasthenospermic patients. *Acta Eur Fertil*. 1992;23(5):221-224.

29. Busetto GM, Agarwal A, Virmani A, et al. Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-asthenoteratozoospermia, with and without varicocele: A double-blind placebo-controlled study. *Andrologia*. 2018;50(3):e12927.

30. Vicari E, Calogero AE. Effects of treatment with carnitines in infertile patients with prostatovesiculo-epididymitis. *Hum Reprod*. 2001;16(11):2338-2342.

31. Vicari E, Rubino C, De Palma A, et al. Antioxidant therapeutic efficiency after the use of carnitine in infertile patients with bacterial or non bacterial prostatovesiculo-epididymitis. *Arch Ital Urol Androl*. 2001;73(1):15-25.

32. Vicari E, La Vignera S, Calogero AE. Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculooepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertil Steril*. 2002;78(6):1203-1208.

33. Mongioi L, Calogero AE, Vicari E, et al. The role of carnitine in male infertility. *Andrology*. 2016;4(5):800-807.

34. Buhling KJ, Chan P, Kathrins M, Showell M, Vij SC, Sigman M. Should empiric therapies be used for male factor infertility? *Fertil Steril*. 2020;113(6):1121-1130.

35. Degirmenci Y, Demirdag E, Guler I, Yildiz S, Erdem M, Erdem A. Impact of the sexual abstinence period on the production of seminal reactive oxygen species in patients undergoing intrauterine insemination: A randomized trial. *J Obstet Gynaecol Res*. 2020;46(7):1133-1139.

36. 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(1):383-390.

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