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REVIEW

Unlocking the potential of antioxidant supplementation with N-acetylcysteine to improve seminal parameters and analysis of its safety: A systematic review and meta-analysis of randomized controlled trials

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Summary ***Introduction and objectives: N-acetyl-cysteine (NAC) is one of the oldest and most powerful antioxidants used to treat various diseases. It plays an important role in protecting cells against oxidative damage and has the potential to improve seminal parameters in male with infertility. This systematic review and meta-analysis aim to comprehensively evaluate the efficacy and safety profile of antioxidant supplementation with NAC in male with infertility or impaired semen parameters.***

Materials and methods: This systematic review and meta-analysis adhered to Cochrane Handbook guidelines. A literature search across PubMed, ScienceDirect, Cochrane Library and Scopus on February 21, 2024 of studies evaluating NAC supplementation for male infertility or impaired semen parameters was conducted. Study quality was assessed using Revised Cochrane's risk of bias (RoB 2.0) and RevMan 5.4 was used for meta-analysis.

Results: Search yielded 1.106 articles and 5 studies were included in this meta-analysis. Our study showed that patients who received NAC had statistically significant results in improving sperm volume [MD: 0.69 (0.26-1.12), P = 0.002], sperm concentration [MD: 4.43 1.50-7.36), P = 0.003], sperm total motility [MD: 9.69 (6.61-12.77), P < 0.00001], and normal sperm morphology [MD: 1.36 (0.70-2.03), P < 0.0001] compared to control. Additionally, patients given NAC had no reported side effects based on our included studies.

Conclusions: We found NAC supplementation significantly improves seminal parameters and has a favorable safety profile. These findings highlight the potential role of NAC as a safe supplementation for male with infertility or in male with impaired semen parameters.

KEY WORDS: *Antioxidant; Sperm parameters; Safety; Meta-analysis; N-acetylcysteine.*

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INTRODUCTION

The male component significantly accounts for around 50% of all infertility cases (1). Male fertility is primarily influenced by spermatogenesis, the process of spermatozoa formation from spermatogonia within the testes. Male infertility is caused by defective sperm parameters (spermatogenic failure), including total absence (azoospermia), low count (oligozoospermia), abnormal morphology (teratozoospermia), and/or impaired motility (asthenozoospermia). The failure in dealing with male infertility is mostly because of the multifaceted etiology that arises from the interaction of genetics, lifestyle choices, environmental influences, and concomitant conditions (2).

Reactive oxygen species (ROS) are normal products of cellular metabolism, mainly produced in the mitochondria during oxidative phosphorylation. Free radicals form during oxygen reduction for energy production. When oxidants increase, the balance shifts toward oxidative stress, which is linked to over 100 disorders, including infertility. ROS can induce protein degradation, lipid peroxidation, DNA damage and apoptosis. Evidence indicates that ROS-mediated damage to sperm significantly contributes to 30%-80% of all cases (3, 4).

N-acetyl-cysteine (NAC) is a dietary supplement and mucolytic drug utilized in the treatment of acetaminophen and paracetamol overdoses. As a thiol-based derivative of the amino acid L-cysteine and a precursor to glutathione peroxidase, it has potent anti-inflammatory, mucolytic, and antioxidant properties. Numerous studies indicate that NAC supplementation can elevate sperm counts, improve motility, diminish abnormal morphology, minimize DNA fragmentation, promote acrosomal activity, and function as a potent semen antioxidant (5).

This review investigates the potential of NAC supplementation in the latest studies on seminal parameters and its safety in male with infertility or impaired semen parameters.

METHOD

Search strategy

Two authors independently searched for and reviewed all randomized controlled trial describing the efficacy and safety of NAC in male with infertility or impaired semen parameters from inception until February 21, 2024 from several databases comprised of *PubMed, ScienceDirect,*

Scopus and *Cochrane Library*. The subsequent keywords were generated by integrating several terms, including "*N-acetyl-cysteine*" AND "*Semen quality*" OR "*seminal parameters*" OR "*Semen*". This study applied no restrictions regarding country or publication year. The protocol of this meta-analysis was registered in PROSPERO (CRD42024516001). This study also followed the guideline of PRISMA 2020 (6).

Eligibility criteria

This systematic review and meta-analysis examined studies of randomized controlled trials that met the specified criteria. The studies were included into this meta-analysis if they fulfilled the following criteria. (1) Evaluated NAC supplementation for male with infertility or impaired semen parameters; (2) Each article provided precise data, mostly involving the subject count and indicator outcomes of: semen volume, sperm concentration, total motility, normal sperm morphology and adverse event (AE) reported; and (3) Full-text article in English language and related data can be obtained. Studies presented as abstracts, review articles, and case reports were removed.

Selection process

Duplicate studies were identified and excluded after the initial search. The titles and abstracts of the remaining literature were screened by at two independent reviewers to determine eligibility. Studies meeting the criteria were included, while those not meeting requirements were excluded. Conflicts in study classification were resolved through group discussion. The results of the literature screening adhere to the Preferred Reporting Items for Systematic Review and Meta-Analyses (*PRISMA guidelines*).

Data extraction

The following data were collected for each study by different reviewers: (1) First author's name; (2) Published time; (3) The type of study design; (4) Patients description; (5) Patient's received therapy, dosage and treatment period; (6) Number of patients in each group; (7) age, and (8) Data on semen volume (mL), sperm concentration (10^6/mL), total motility (%), normal sperm morphology (%) and AE reported.

Quality assessment

Two authors independently assessed all identified inclusion studies, and any disagreements that emerged were addressed with the input of a third reviewer. Cochrane *Risk of Bias* (ROB) Tools 2 was used to assess the quality of each study. ROB 2 instrument was utilized to assess the risk of bias in *randomized controlled trials* (RCTs), focusing on five domains (7). Overall risk-of-bias judgement of these instruments was classified into 3 groups which low risk of bias (if the study is judged to be at low risk of bias for all domains for this result), some concerns (if there is some concern at least in one domain) and high risk of bias (if the study is judged to be at high risk of bias in at least one domain for this result). *Risk-of-bias Visualization* (ROBVIS) was used for the visualization of risk of bias graph (8).

Statistical analysis

The acquired data was analyzed with Review Manager 5.4. (*Cochrane Collaboration, UK*). Variations between baseline (study entrance) and study completion (end-point measure) were utilized to indicate changes in the results. *Mean difference* (MD) was used to explain continuous data and *odds ratio* (OR) for dichotomous results with the corresponding 95% *confidence interval* (CI). I^2 value refers to statistical analysis of heterogeneity. The fixed-effects model is used if $I^2 < 50\%$, while the random-effects model is used if $I^2 \geq 50\%$. The results will be presented in a forest plot, and the overall effect is considered significant if $p < 0.05$. Asymmetry tests, including Egger's test for assessing potential publication bias via funnel plots, will not be performed if the meta-analysis comprises fewer than 10 studies due to their restricted reliability (9). Furthermore, subgroup analysis and sensitivity analysis will also not be conducted if there is a small number of studies (10).

Results

Literature search, screening results and characteristic of studies

From various databases, 1.106 studies were initially identified using keywords. After excluding 82 duplicates, two reviewers independently screened the remaining 1.024 study titles and abstracts, which excluded 1.015 articles according to the inclusion and exclusion criteria. After reviewing only nine full texts, we excluded four studies because they lacked sufficient data or did not meet our study criteria.

Finally, 5 RCT studies (11-15) were included in our analysis with a total of 666 patients. Full details of the search and selection process are presented in the PRISMA flow diagram (Figure 1) and the characteristics of these studies are stated in Table 1.

Quality assessment result

Three studies (11-13) raised concerns regarding the randomization process (domain 1), while one study (11) indicated issues related to the lack of personnel and patient blinding, which pertains to deviations from intended interventions (domain 2) and bias in outcome measurement (domain 4). Additionally, one study (13) expressed concerns about missing outcome data due to a significant number of patient withdrawals, resulting in an imbalance in sample sizes between groups (domain 3). Four studies (11, 12, 14, 15) raised concerns about bias in the selection of reported results due to the absence of a published protocol (domain 5). A detailed assessment of the risk of bias is presented in Figure 2.

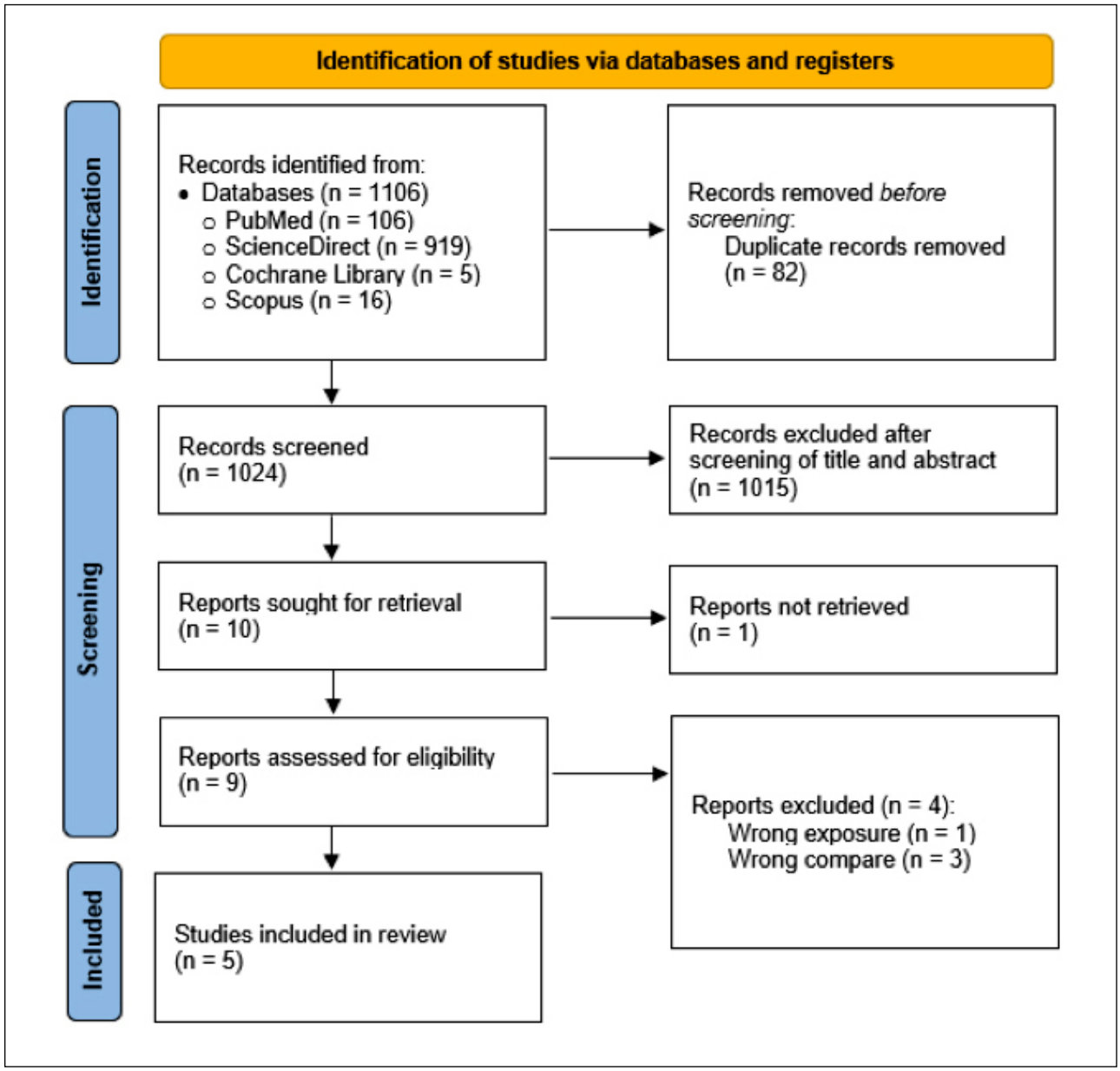
Sperm volume

Sperm volume from two studies assessing the efficacy of NAC showed a marked improvement of sperm volume (MD: 0.69; 95% CI: 0.26-1.12; P = 0.002), NAC demonstrated a markedly larger enhancement in sperm volume in relation to the control group (Figure 3A).

Sperm concentration

Five studies evaluating the efficacy of NAC exhibited that the NAC group had a significant improvement (MD: 4.43; 95% CI: 1.50-7.36; P = 0.003) (Figure 3B), it

Figure 1.
Flow of literature search and selection based on Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA).



demonstrated that NAC is more effective in increasing sperm concentration compared to the control (Figure 3B).

Total sperm motility

Four studies revealed that patients who received NAC intervention had a significant improvement in total sperm motility (MD: 9.69; 95% CI: 6.61-12.77; P < 0.00001) (Figure 4A), which shown that NAC was more effective in enhancing sperm motility than the control (Figure 4A).

Normal sperm morphology

There were four studies that assessed normal sperm morphology. The random effects model showed that there was significant increase in normal sperm morphology in the NAC group (MD: 1.36; 95% CI: 0.70-2.03; P < 0.0001), which demonstrated that NAC resulted in a much higher enhancement in sperm normal morphology relative to the control (Figure 4B).

Safety profile

The included studies reported that patient receiving NAC supplementation at a dosage of 600 mg/day for twelve weeks or 26 weeks, resulted in improved sperm quality without any reported side effects.

Discussion

The results of this meta-analysis showed that NAC supplementation significantly increased sperm volume, sperm concentration, total sperm motility and normal sperm mor-

Table 1.
Characteristics data of included studies.

No	Study (Author)	Study design	Population	Mean Age (years)		Sample size		Treatment Type		Treatment Cycle	Outcome Assesments				
				NAC	Control	NAC	Control	NAC	Control		Semen volume (mL)	Sperm Concentration (10^6/mL)	Total Motility (%)	Normal Sperm Morphology (%)	Adverse Events Reported
1	*Rafiee et al. (2021)*	RCT	Men with impaired semen parameters of sperm concentration, motility and normal morphology due to a history of COVID-19 infection within the last three months.	36.1 ± 4.1		100	100	Oral NAC 600 mg/day	Placebo	12 Weeks	-	✓	✓	✓	NAC supplementation resulted in no adverse events.
2	*Jannatifar et al. (2019)*	RCT	Infertile men diagnosed with asthenoteratozoospermia with no previous report of pregnancy in the couple and normal female partner.	34.7 ± 4.17		50	50	Oral NAC 600 mg/day	Placebo	12 Weeks	✓	✓	✓	✓	-
3	*Barekat et al. (2014)*	RCT	Men with primary infertility due to left-sided varicocele (grades II, III) diagnosed by palpation and Doppler duplex ultrasound.	30.73 ± 1.4	29.64 ± 0.74	15	20	Oral NAC 600 mg/day	No Medication	12 Weeks	-	✓	✓	✓	NAC did not lead to any negative side effects.
4	*Ciftci et al. (2009)*	RCT	Men diagnosed with idiopathic infertility according to medical history and physical and seminal examination findings.	3.1 ± 4.5	32.8 ± 3.7	60	60	Oral NAC 600 mg/day	Placebo	12 Weeks	✓	✓	✓	✓	None of the patients in the study reported any side effects.
5	*Safarinejad et al. (2009)*	RCT	Men with oligo-asthenoteratospermia with more than 2 years of failed attempts at conception, a sperm count of greater than 5 ± 10^6/ml and no female factors.	32 ± 10	31 ± 9	105	106	Oral NAC 600 mg/day	Placebo	26 Weeks	✓	✓	✓	✓	NAC consumption did not lead to any negative side effects.

RCT: Randomized controlled trial; NAC: N-acetylcystein.

Figure 2.
Risk of bias assessment using the revised Cochrane risk-of-bias tool algorithm for randomized trials (RoB 2.0).

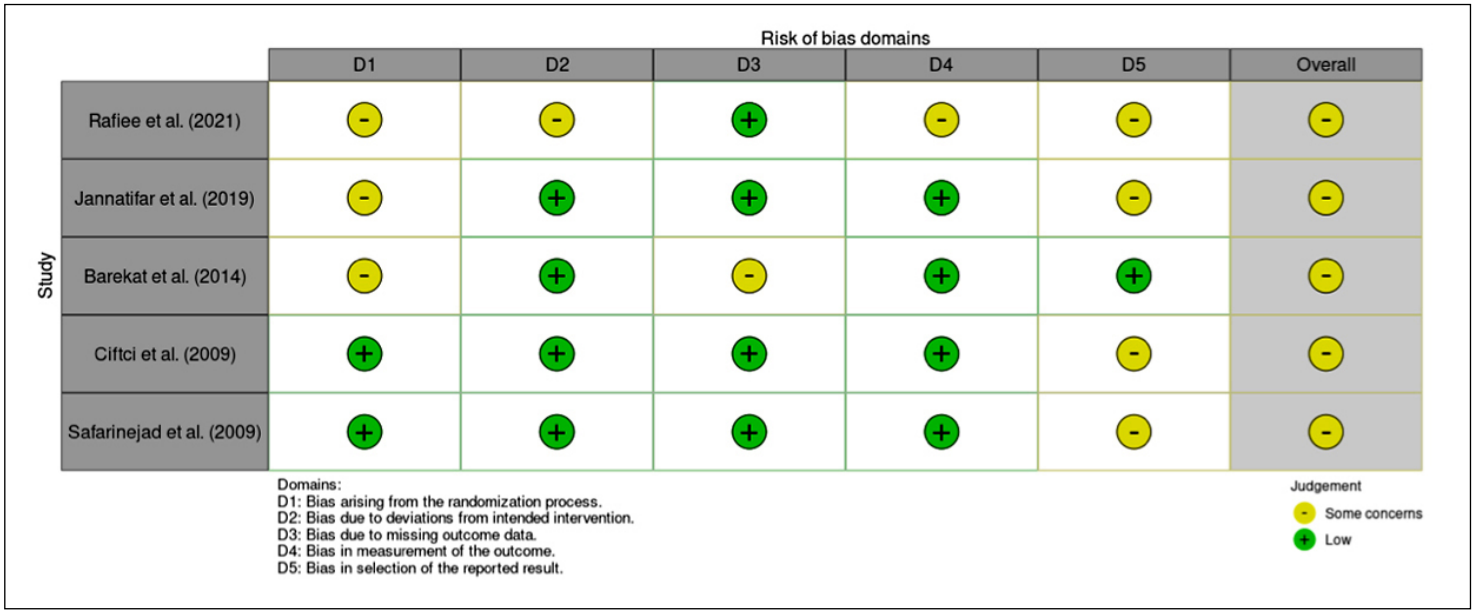
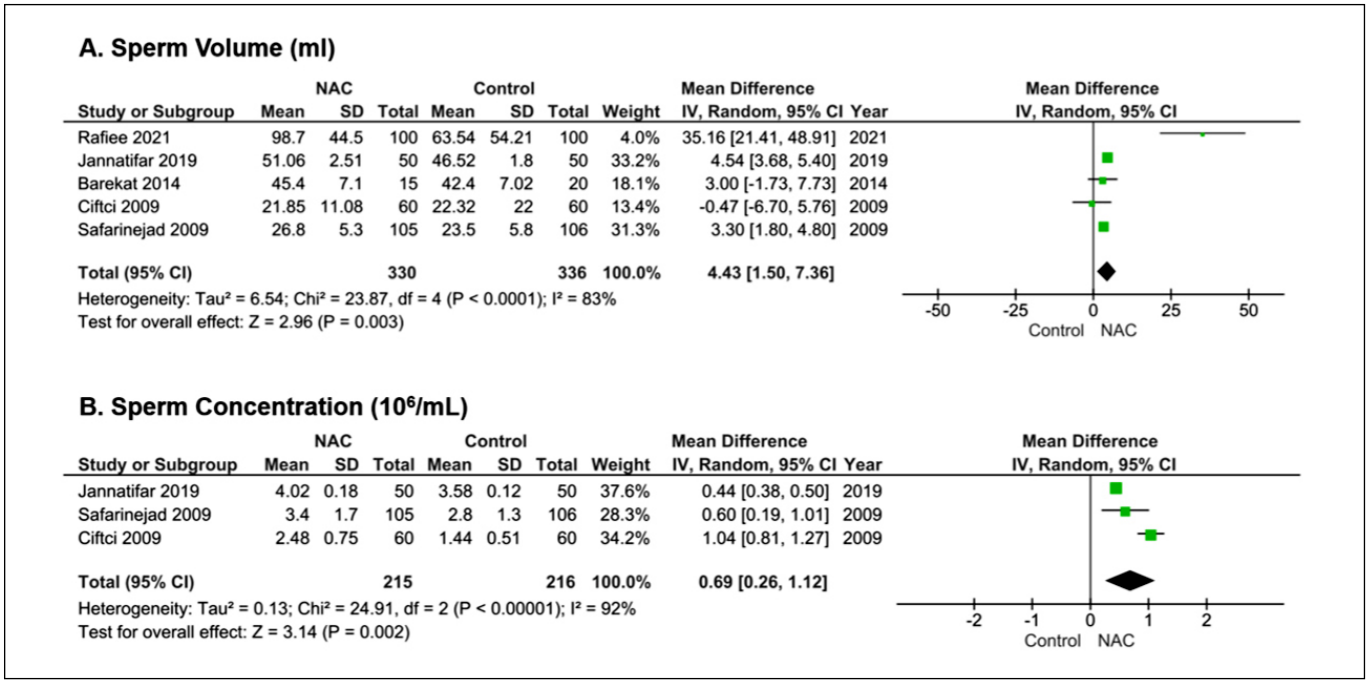
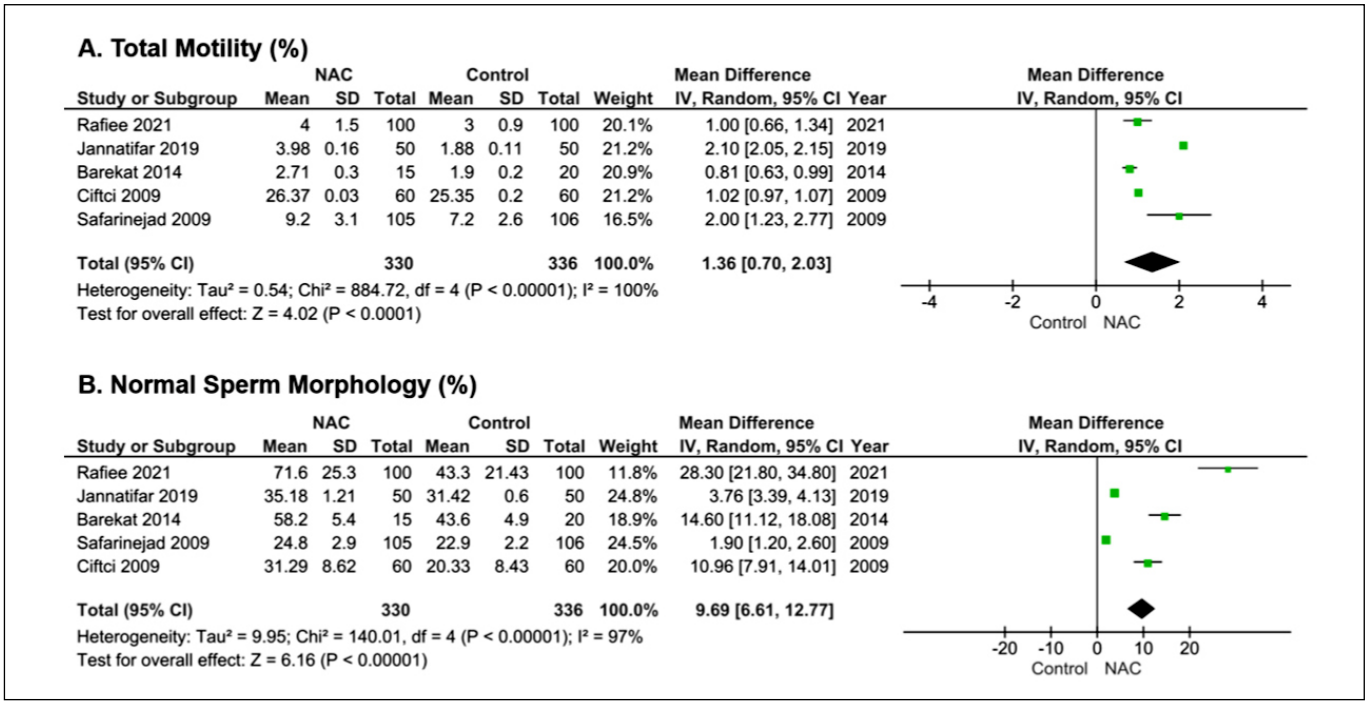


Figure 3.
Forest plots of seminal parameters of (A) Semen volume. (B) Sperm concentration.



CI: Confidence interval; IV: Inverse variance; SD: standard deviation.

Figure 4.
Forest plots of seminal parameters of (A) Total motility. (B) Normal sperm morphology.



CI: Confidence interval; IV: Inverse variance; SD: standard deviation.

phology in male with infertility or impaired semen parameters. *Wei et al.* previously carried out a meta-analysis assessing the effectiveness of *L-carnitine/L-acetyl-carnitine* (LC/LAC) and NAC in males with idiopathic asthenozoospermia. The findings indicated that supplementation with LC/LAC and NAC significantly enhanced sperm motility, normal morphology, sperm concentration, and ejaculate volume (16). Different from that study, this meta-analysis included 5 RCT studies evaluating NAC supplementation and providing new insights into the effective-

ness of NAC for improving semen parameters. Several previous studies have shown that around 30-80% of male infertile patients have increased ROS in their semen (17). An increase in ROS that surpasses the antioxidant capacity in semen leads to an imbalance between pro-oxidants and antioxidants, resulting in *oxidative stress* (OS) conditions that damage the spermatozoa plasma membrane and sperm DNA. This explains why increased ROS can have a negative impact on sperm parameters and cause infertility in men (18). One way to prevent OS is to increase the antioxidant capacity of semen by providing antioxidant supplements.

NAC is a derivative of the natural amino acid L-cysteine, which has an important role in cellular protection against oxidative damage (19). NAC is one of the oldest and most powerful antioxidants in treating several diseases, including its application in decreasing the viscosity and flexibility of mucus due to its capacity to break disulfide bonds. In addition, NAC has the potential to interact directly with oxidants and with some thiols, which are excellent hydroxyl radical scavengers (20). The results of this meta-analysis show that NAC administration can improve the quality of sperm parameters. This is thought to be related to the main role of NAC as a stem-form antioxidant, which has the ability to increase intracellular glutathione concentration, the most crucial biothiol responsible for cellular redox imbalance (21). NAC administration can also increase the total antioxidant capacity (TAC) in semen fluid (20). Increased TAC will prevent OS due to overproduction of ROS. This is proven by the study of *Barekat et al.*, who reported that patients given NAC reduced the percentage of ROS in semen (13). Preventing OS in semen can prevent damage to spermatozoa and enhance the integrity of sperm DNA, leading to a significant increase in the number of sperm in the semen (19). Several studies have reported that the antioxidant effect in semen can also increase the mitochondrial function of spermatozoa, thereby providing more energy and increasing their motility (22). *Jannatifar et al.* indicated that following NAC administration, the levels of seminal *malondialdehyde* (MDA) drastically reduced. MDA served as a particular indicator of lipid peroxidation, whereas TAC exhibited a considerable rise. NAC mitigates the intensity of oxidative stress, therefore diminishing lipid peroxidation (12). The theoretical basis and mechanisms explain the results of this meta-analysis, which show that NAC is effective and safe in improving sperm parameters. In addition, NAC is relatively safe and well tolerated, even at high doses (21). Of all the included studies, none reported any significant side effects of NAC administration on male with infertility or impaired semen parameters.

The primary goal of infertility therapy is to achieve pregnancy. In a study conducted by *Barekat et al.*, NAC was administered to men following varicocelectomy, leading to significant improvements in semen quality and a higher reported clinical pregnancy rate in the NAC group compared to the control group. This indicates a notable enhancement in fertility outcomes associated with NAC supplementation (13). Unfortunately, our analysis could not assess pregnancy rates because the studies we included did not provide sufficient data for further evaluation.The dosage and duration of NAC supplementation have been extensively evaluated in studies related to male fertility. Most studies have administered NAC at a dosage of 600 mg each day for three months, which has consistently resulted in significant improvements in sperm quality and oxidative stress markers. *Jannatifar et al.* reported enhanced sperm parameters following this regimen (12), while *Rafiee et al.* observed similar benefits in men with impaired semen parameters (11). In a larger trial by *Safarinejad et al.*, participants received 600 mg of NAC daily for 26 weeks, leading to significant improvements in multiple semen parameters (15). *Comhaire et al.* also noted improvements in sperm concentration and acrosome reaction at similar dosages (23). These studies suggest that NAC supplementation at dose of 600 mg each day for durations of three to six months should be preferred for enhance seminal parameters.

Several studies have reported on the safety profile of NAC supplementation. *Erkkilä et al.* found no cytotoxic effects of NAC on human male germ cells in vitro, supporting its safety for reproductive health applications (24). A systematic review by *Zafarullah et al.* highlighted that NAC is also safe in various clinical applications, being generally well-tolerated even at higher doses used for treating conditions such as chronic obstructive pulmonary disease and acetaminophen overdose (25). This study suggests that NAC is a safe and effective antioxidant supplement for enhancing male fertility parameters without a significant risk of adverse events.

Declarations

Ethical approval: *This study did not need ethical approval.*

Availability of data and material: *All data and materials from this research are available to the researcher and we will provide it upon request if the researcher needs it.*

Competing interests: *The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.*

Funding: *All funding for this research comes from researchers without receiving reimbursement of research costs or research grants from third parties.*

Authors' contributions: *Contribution Details (to be ticked marked as applicable):*

	SS	MAG	ATF
Concepts	√	√	√
Design	√	√	√
Definition of intellectual content	√		
Literature search	√	√	
Data acquisition	√	√	√
Data analysis	√	√	√
Statistical analysis	√	√	√
Manuscript preparation	√	√	√
Manuscript editing	√		
Manuscript review	√	√	√
Guarantor	√		

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We need to acknowledge some limitations of this study. First, there are only a few trials included in our study. However, all studies used in this research are randomized controlled trials, hence augmenting the robustness of the findings. In addition, bias due to deviations from intended interventions may affect the final results of this study. Secondly, this meta-analysis is limited by the limited number of RCT studies. Based on these limitations, it is important to interpret the findings of this review cautiously, as they may warrant further studies. Additionally, future studies should analyze and compare the combination of NAC and other types of antioxidant supplements to improve sperm parameters. Based on our analysis, we recommend NAC supplementation for males experiencing impairment of semen parameters, either at a dose of 600 mg or at a dose of 200 mg NAC three times a day for a minimum of 12 weeks, and up to 26 weeks.

Conclusions

A significantly improvement of seminal parameter was found in male with infertility or impaired semen parameters who received NAC supplementation. NAC supplementation has also been proven to be safe. These findings highlight the potential role of NAC as a safe supplementation for males with infertility or impaired semen parameters.

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