

# The effect of N-acetyl cysteine consumption on men with abnormal sperm parameters due to positive history of COVID-19 in the last three months

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**Summary** Male infertility is an important factor accounting for 40-50% of infertility cases that may be due to disturbance in one of the parameters as concentration, motility and morphology observed in one or two semen analysis with an interval of 1 and 4 weeks. COVID-19 may affect male fertility through virus division, cytotoxic effects on testicular tissue and immunopathological effect. N-acetyl cysteine (NAC) improved sperm concentration and acrosome reaction while reducing reactive oxygen species (ROS) and oxidation of sperm DNA. This interventional study was conducted on 200 men who were referred to private infertility clinics for female factor (their previous semen analysis was normal) and got COVID-19 infection in the last 3 months showing an impairment of the latest semen analysis due to COVID. Men were placed in two groups of control (n = 100) and intervention (NAC consumption). Subjects who got COVID-19 infection had a significant impairment of sperm quality (sperm concentration, sperm motility, and normal sperm morphology) compared to their semen analysis evaluated before the COVID-19 infection. NAC consumption significantly improved sperm total motility, sperm morphology and sperm concentration. COVID-19 infection has a negative effect on sperm parameters. NAC supplementation may have positive effect on sperm parameters.

**KEY WORDS:** COVID-19; Sperm; Morphology; Infection.

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## INTRODUCTION

According to the *International Committee for Monitoring Assisted Reproductive Technology of World Health Organization* (WHO), infertility is a reproductive disorder that prevents clinical pregnancy after 12 months or more of unprotected intercourse (1). Studies have shown that nearly 72.4 million couples worldwide are involved in infertility problems (2). Male infertility is an important factor accounting for 40-50% of infertility cases that may be due to disturbance in one of the parameters of concentration, motility and morphology in one or two semen analysis with an interval of 1 and 4 weeks (3).

Normal sperm parameters are assessed based on WHO criteria (4). Semen analysis is still a powerful and essential tool with a sensitivity of 89.6% that can identify 9 out of 10 men with infertility problems (5). Normal semen volume is the total amount of fluid ejaculated that should

be  $\geq 1.5$  mL. The sperm concentration is reported as the number of sperm per mL of semen that should be  $\geq 15$  million per mL. Total sperm number (also known as 'total sperm count') is described as the total number of sperm in the ejaculate, calculated by multiplying the semen volume by the sperm that should be  $\geq 39$  million. Sperm progressive motility should be  $\geq 32\%$  motile within 60 minutes of ejaculation. Sperm vitality should be  $\geq 58\%$ . Sperm normal morphology is described as the percentage of the total number of sperms that should be  $\geq 4\%$  (4).

COVID-19 may affect male fertility through virus division, cytotoxic effects on testicular tissue and immunopathological effect (6). Coronavirus can directly affect testicular tissue as well as some sperm parameters by altering the expression of the *angiotensin-converting enzyme 2* gene pattern (ACE-2) (7) because seminiferous cells, spermatogonia, Leydig cells and Sertoli cells express this enzyme (8). Studies have shown that ACE-2 is present in the post-acrosomal region, neck and middle part of normal sperm (9). Sperm ACE binds to the *glycosyl-phosphatidyl-Inositol* (GPI) portion of the oocyte *Zona Pellucida* and is therefore involved in fertilization (10).

*N-acetylcysteine* (NAC), a derivative of amino acid L-cysteine, is used mainly as an antioxidant (11). NAC has free radical scavenging activity (12). In addition, daily treatment with NAC results in a significant improvement in sperm motility in comparison to placebo (13). NAC improved sperm concentration and acrosome reaction while reducing ROS and oxidation of sperm DNA (14).

This study investigates the effect of NAC on abnormal sperm parameters in men with COVID 19 infection.

## MATERIALS AND METHODS

### Design

This interventional study was conducted from March 2020 to July 2021. All couples whose infertility treatment cycle were canceled due to COVID-19 pandemic were included in this study. Male patients with abnormal sperm analysis before COVID-19 were excluded from the study. In total, 273 male patients were included in this study, 47 patients did not present to the infertility center to continue treatment during the time of this study and

26 patients presented to continue the treatment more than two months after positive PCR and were excluded from the study. Other exclusion criteria were diabetes, hypertension, mumps history, sexually transmitted diseases, varicocele, and chronic diseases history.

All the 200 eligible COVID-19 patients were willing to participate in the study and were randomly allocated in two groups of controls and treated subjects. All the cases gave a semen sample after completing written informed consent. Subjects having a positive nasopharyngeal swab test for COVID-19 (*ESwab collection kit, Copan diagnostics*) or positive *Immunoglobulin (Ig) M* and *IgG* antibodies were considered positive for COVID-19 (15).

### Intervention and assessment

The subjects took NAC 600 mg/day by oral route for 3 months (16). Variables including seminal parameters were measured before and after the intervention.

### Semen sampling method

Semen samples were collected once, at the beginning of study and at the end of the intervention. Sperm samples were taken by masturbation after 3-5 days of sexual abstinence and kept in a plastic container. Then the samples were incubated at 37°C for 30 minutes and analyzed after one hour. The sperms were counted by light microscope with a magnification of 400. Different characteristics of semen including appearance, volume, pH, color, viscosity, liquefaction time, sperm concentration and sperm motility were investigated.

### Statistical analysis

For statistical analysis was used the Statistical Package for Social Science (*SPSS Inc, Chicago, Illinois, USA*) version 16.0. P value significance was set at 0.05 and confidence interval was at 95%. Paired t-test was used to compare the results before and after interventions. Independent t test was used to compare between control and intervention group.

## RESULTS

The average time between initial normal sperm analysis and COVID-19 infection was 2 months (range 1-5 months). Average time from COVID-19 infection and sperm analysis re-evaluation was 6 weeks (range 3-8 weeks). Patients who presented more than 2 months after COVID-19 infection were excluded.

Subjects suffering from COVID-19 infection had a statistically significant impairment of sperm quality (sperm concentration, sperm motility, and normal sperm morphology) compared to their semen analysis before the COVID-19 infection (Table 1).

In Table 2, results of sperm analysis after NAC treatment were not significantly different from initial results of sperm analysis (before COVID).

In Table 3 the results of sperm analysis during the follow-up of controls are described.

Results of the initial sperm analysis (before COVID) and the last sperm analysis at 3 month follow-up were significant different for sperm motility ( $p = 0.04$ ) and sperm concentration ( $p = 0.03$ ).

**Table 1.**

Age, body mass index and semen parameters for individuals before and after COVID-19 infection.

	Before COVID	After COVID	P value
Age	36.1 ± 4.1	36.1 ± 4.1	0.96
BMI (kg/m <sup>2</sup> )	23.12 ± 2.5	21 ± 3.1	0.04*
Volume (ml)	3.5 ± 0.9	2.9 ± 0.6	0.05*
Sperm concentration (10 <sup>6</sup> )	115.1 ± 35.1	68.7 ± 53.6	0.01*
Total motility (%)	69.9 ± 32.7	30.1 ± 29.6	0.01*
Morphology	4 ± 1.2	2 ± 0.9	0.03*

**Table 2.**

Comparison of semen parameters before and after COVID and after 3 months treatment with NAC. NAC consumption significantly improved sperm total motility, sperm morphology and sperm concentration.

Sperm parameters	Before COVID	After COVID before NAC	After NAC	P value before vs after NAC
Volume (ml)	3.10 ± 0.56	2.3 ± 0.6	4.02 ± 0.18	0.03*
Total motility (%)	81.1 ± 23.2	35.1 ± 29.6	71.6 ± 25.3	0.01*
Sperm concentration (10 <sup>6</sup> /mL)	115 ± 32.5	61.7 ± 53.6	98.7 ± 44.5	0.04*
Morphology (%)	4 ± 0.6	2 ± 0.9	4 ± 1.5	0.03*

**Table 3.**

Comparison of semen parameters before and immediately after COVID infection and after 3 months of follow-up in the control group.

Sperm parameters	Before COVID	After COVID	After 3 months of follow-up	P value after COVID vs 3 months follow-up
Volume (ml)	4.13 ± 0.61	2.5 ± 0.3	4.44 ± 1.32	0.04*
Total motility (%)	65.10 ± 19.63	37.5 ± 25.1	43.3 ± 21.43	0.06
Sperm concentration (10 <sup>6</sup> /mL)	110 ± 21.42	58.8 ± 25.1	63.54 ± 54.21	0.071
Morphology (%)	3 ± 0.71	2 ± 2.1	3 ± 0.9	0.06

## DISCUSSION

*Coronavirus disease 2019* (COVID-19) is a highly transmissible infectious disease caused by the *Severe Acute Respiratory Syndrome Coronavirus* (SARS-CoV-2), a single-strand enveloped RNA virus belonging to the family of *coronaviridae* (17).

This virus enters host cells mainly through ACE-2 and *transmembrane protease serine 2* (TMPRSS2) (18). Recent advances suggest a possible infection of the endocrine system in COVID-19 patients (19-21).

In the present study, we evaluated semen parameters after acute SARS-CoV-2 infection. Subjects suffering from COVID-19 infection had a statistically significant impairment of sperm quality (semen volume, sperm concentration, sperm motility, and normal sperm morphology) compared to their semen analysis before the covid infection. Our findings are in accordance with other studies which are available in the literature (22-24). It is recommended that men with positive history of SARS-CoV-2 who are interested in fertility should be evaluated by a fertility specialist.

*N-acetylcysteine* (NAC) is a thiol-based antioxidant that plays an important role in the protection of cellular con-

stituents against oxidative damage. The hypothetical action of NAC comes from the ability to stimulate and sustain intracellular levels of reduced glutathione and to detoxify ROS. NAC is one of the oldest and most powerful antioxidants that treat various diseases, including respiratory disorders, heart disease, heavy metal poisoning, overdose with acetaminophen and epilepsy (25). Safarinejad *et al.* also reported significant improvements in all semen parameters in subjects receiving selenium or NAC (13). The results of this study showed that sperm parameters (volume, concentration, motility and normal morphology) significantly improved after NAC supplementation. NAC also improved sperm concentration and morphology.

## CONCLUSIONS

COVID-19 infection has a negative influence on sperm parameters. NAC oral supplementation may improve sperm parameters.

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