Investigation of the effect of COVID-19 on sperm count, motility, and morphology

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus that causes many diseases such as respiratory diseases, cardiovascular diseases, and gastrointestinal diseases. Although it has been shown that the angiotensin-converting enzyme 2 receptor, which has a high affinity for the SARS-CoV-2 is mostly expressed in the lungs, it is also expressed especially in the cells of the testicular tissue. Although there are studies showing the effect of SARS-CoV-2 on spermatogenesis, the effects of COVID-19 on sperm count, motility, and morphology are still unclear. The aim of this study is to investigate changes in sperm quality in men who had recovered and never had COVID-19, therefore semen samples were analyzed from all individuals in the patient and control groups aged 20–50 years who agreed to participate in the study and voluntary in SBU Ministry of Health Adana City Training and Research Hospital. (Toros University Ethics Committee Decision Number: 1433, Date: April 15, 2021) (Adana Provincial Health Directorate Ethics Commission Decision dated May 27, 2021/5). Two groups were selected (100 men had and recovered from COVID-19, and 100 men never had COVID-19) spermiograms from both groups were analyzed in accordance with the World Health Organization standards. The sperm concentration of the COVID-19 negative group was significantly higher than those in the COVID-19 positive group. No statistically significant difference was detected between the groups for sperm motility and morphology. It was observed that men with COVID-19 had decreased sperm concentrations suggesting that COVID-19 may have a negative effect on male fertility. However, in the long term, more comprehensive studies with a large sample size are needed to understand better the changes in sperm concentration.

Keywords
fertility, SARS-CoV-2, semen, sperm parameters
1 | INTRODUCTION

Coronaviridae are single-stranded RNA viruses belonging to the Coronaviridae family. There are many coronaviruses that affect humans. The first coronavirus that infects humans was characterized in the 1960s.\textsuperscript{1,2} At the end of December 2019, a new strain of coronavirus emerged in Wuhan, China, with a resemblance to severe acute respiratory syndrome coronavirus (SARS-CoV). This virus, called SARS-CoV-2, spread rapidly all over the world and caused a pandemic.\textsuperscript{3} Although current information shows that the clinical findings of COVID-19 are fever, cough, and nasal congestion, in some cases, the clinical picture may be more severe and even show serious symptoms, such as cardiovascular diseases, gastrointestinal diseases, and pneumonia.\textsuperscript{4} Although it has been shown that the angiotensin-converting enzyme 2 (ACE-2) receptor, which has a high affinity for COVID-19 virus, is mostly expressed in the lungs, it is known that the ACE-2 receptor is also widely expressed, especially in spermatagonia, Leydig and Sertoli cells.\textsuperscript{5} In some studies, it is thought that in addition to the cytopathic effects of COVID-19 on cells, gonadal functions may be impaired as a result of the inflammatory response to the virus.\textsuperscript{6} In this context, testicles are considered to be a potential target for COVID-19. As a result, male reproductive health may be impaired. In research supporting this, it was suggested that the immune response developed in the testicular tissue negatively affects spermatogenesis.\textsuperscript{7,9} Although various studies have shown the effects of COVID-19 on the male reproductive system, more studies are needed to show the effect of infection on sperm parameters. As a result of postmortem studies, the researchers found orchitis in the testicles of six men who died from SARS. They observed that there were very few sperm cells in the seminiferous tubules, but increased inflammatory response in the tissue. In addition, swelling in Sertoli cells and a decrease in the number of Leydig cells were noted as one of the remarkable findings.\textsuperscript{10}

Although there are many studies showing the effects of COVID-19 on the male reproductive system, there is a need for more comparative studies showing the effect of the disease on sperm parameters in our country. This study was to compare the sperm parameters of individuals with and without COVID-19 and to investigate the effect of COVID-19 on sperm parameters.

2 | METHODS

2.1 | Study design

The study was started after the approval of the Scientific Research and Publication Ethics Committee of Toros University (Decision number: 1433, Date: April 15, 2021). Informed consent was obtained from all patients. A total of 200 men coming to the Ministry of Health Adana City Training and Research Hospital in vitro fertilization (IVF) unit andrology laboratory were included. The patients were selected among men aged 20–50 years, who were sent to the andrology laboratory for examination from the urology unit with the suspicion of preconceptional screening. The anamnesis of these people was taken. Most of them were married. None of them were smokers and had no previous history of cryptorchidism or varicocele operation, chronic disease, and using medication regularly. And all of them had hormone levels within limits. One hundred men did not have COVID-19 (we named them the COVID-19-negative group), and 100 of them had COVID-19 infection (we named them the COVID-19-positive group) proven by a positive polymerase chain reaction test. All of the patients in the COVID-19 group were exposed to COVID-19 infection from 4 months to 1 year ago. Those who had COVID-19 in the last 3 months were not included in the study. In addition, we did not have information about whether any of the people in the COVID-19 positive and COVID-19 negative groups had a sperm analysis before and the results. Of 100 people, 58 survived the infection with moderate symptoms and 42 with mild symptoms but none of them were sent to a hospital owing to infection. Sperm samples were not taken from people who had a severe infection and were hospitalized because it was thought to affect sperm results. Appointments were made after taking their anamnesis. The duration of sexual abstinence was randomly selected between 2 and 7 days in both the patient group and the control group based on the World Health Organization (WHO) data. Individuals with a semen volume of less than 1.5 ml were not included in the study, since sperm samples below 1.5 ml were not considered normal according to WHO criteria.\textsuperscript{11}

2.2 | Semen analysis

Internal and external quality controls and calibrations of the devices in Adana City Hospital Andrology Laboratory were completed. The laboratory supervisor was a doctor who was in charge of the Embryology Laboratory. All analyzes in the laboratory were performed in accordance with WHO criteria.\textsuperscript{11} Received samples were kept in an incubator (Thermo Fisher Scientific\textsuperscript{®}) at 37°C for 30 min for liquefaction. Liquefied samples were taken into a laminar flow which is a sterile cabinet. After examining the macroscopic properties of the samples, their volumes were measured by the pipetting method, and their viscosity was noted. Viscosity was measured by the comparison method as less fluid, fluid, or very fluid. After liquefaction, the viscosity of the sample can be estimated by observing the filament that forms when semen is gently aspirated through a large bore (approximately 1.5 mm diameter) disposable plastic pipette and allowed to drip by gravity. Normal semen falls out of the pipette in the form of distinct small drops. If the viscosity is abnormal, the drop forms a strand longer than 2 cm. Semen samples were taken from each sample with a pipette and placed in the Counting Chamber Makler. Sperm count was performed under a phase contrast microscope (Olympus CX\textsuperscript{2}) at ×200 (×20 objective, ×10 ocular) magnification. Sperm counting was performed in a 100-square area. Sperm analysis was analyzed in accordance with WHO (WHO, 2010) criteria.\textsuperscript{11} Spermatozoa in each of the rows were assessed in accordance with WHO criteria as rapid progressively
motile (Type a), slow progressively motile (Type b), motile in situ (Type c), and immobile (Type d). The total number was converted to concentration from the volume assessed. The percentage of a, b, c, and d type sperm cells was calculated, and total motility was determined (a% + b% + c%). The total sperm count in semen was calculated by multiplying the sperm concentration by its volume. Samples with concentrations above $5 \times 10^9$/ml were stained with a staining kit (Spermac Stain; FertiPro®) and evaluated according to Kruger morphology criteria. In the morphological examination, 200 spermatozoa were assessed for the percentage with normal morphology, head anomaly, neck anomaly, and tail anomaly. Staining was not applied to those with concentrations below $5 \times 10^9$/ml.

2.3 Statistical analysis

Analyses were performed on SPSS (IBM SPSS Statistics 24). The degree of statistical significance between the groups was accepted as $p < 0.05$. Parametric methods were used for measurement values suitable for normal distribution and the “Independent Sample” test (t-table value) method was used to compare the measurement of two independent groups. Nonparametric methods were used for the measurement values that did not conform to the normal distribution and the “Mann–Whitney U” test (Z table value) method was used to compare the measurements of two independent groups. χ²-cross tables (Fisher Exact, Pearson) were used to examine the relationships between two qualitative variables.

3 RESULTS

The ages, periods of sexual abstinence, semen volumes, and sperm concentrations of men with and without COVID-19 were compared. The sperm concentration of the COVID-19 negative men was significantly higher than that of the men in the COVID-19 group. However, sperm motility and morphology of COVID-19 negative individuals were not significantly higher than the sperm motility and morphology of infected men. The duration of sexual abstinence (days) and concentration values in the COVID-19 negative group were significantly higher than those in the COVID-19 positive group. Since the selection was made randomly from patients with a sexual abstinence period between 2 and 7, the significant difference is not important in terms of evaluating the results. (Table 1). In addition, it was observed that no one in the COVID-19 negative group was azoospermic, while four men in the COVID-19 positive group were azoospermic (Table 2).

4 DISCUSSION

Some studies argue that SARS-CoV-2 may have negative effects on spermatogenesis and the male reproductive system. However, spermatogenesis may be impaired when COVID-19 causes fever. In our study, when the spermogram results were evaluated, 4 out of 100 men in the COVID-19-positive group were observed to be azoospermia. In contrast, azoospermia was not observed in any of the men in the COVID-19 negative control group. It was noticed that the sperm concentrations of people with COVID-19 were significantly reduced compared to the control group. However, since the previous sperm histories of azoospermic individuals in the COVID-19 group are not known, it is not possible to make a clear comment about the effect of COVID-19. The study by Holtman et al. concluded that COVID-19 significantly reduces sperm concentration. When these groups were compared in terms of sperm count and total progressive motility, it was stated that the sperm count of men reporting fever was significantly lower than those without fever. In our study, it was noticed that the sperm counts of the patients with moderate signs of infection were lower than those who survived a mild infection. However, there was no statistically significant difference between the two groups in terms of volume, motility, and morphology.

In a study by Ma et al., semen samples were obtained from 12 men, 1 with mild and 11 with moderate COVID-19 symptoms. They evaluated eight men within reference ranges for semen quality such as semen volume, concentration, morphology, and total motility. Of the men, four reported low sperm motility, and two reported abnormal morphology. In our study, when we compared the sperm motility and morphology of the group with and without COVID-19, we could not find a significant difference between the two groups. In some studies, it has been shown that sperm concentrations are low in semen samples taken from men who have had COVID-19, and that sperm concentrations improve over time. For example, in a study by Mannur et al., the sperm sample taken from a 36-year-old male patient to the IVF center was evaluated as normozoospermia, and it was later learned that the coronavirus test of this patient was positive. After being evaluated as COVID-19 negative, semen analysis was performed again on the 43rd day, and the sperm sample taken this time was reported as oligoasthenoteratozoospermia. It has also been stated that sperm cells show DNA damage. In the sperm analysis performed on the semen sample taken from the same patient on the 135th day, it was reported that although sperm count and motility improved, its morphology continued with the features of teratozoospermia. The findings of this study formed the view that COVID-19 and its effects may disrupt sperm morphology.

In a study by Guo et al., semen samples were taken from 38 COVID-19 patients, but no virus was found in the semen samples of any of them. In addition, spermogram results were evaluated in the samples collected from these patients but no significant differences were found in sperm concentration, motility, and morphology. It was thought that the reason why COVID-19 changed sperm parameters was due to temporary tissue damage of fever and inflammatory response. In the comparative study reported here, we observed that the sperm concentrations of the patients who had COVID-19 were significantly lower than those in the control group. Researchers have stated that fever caused by COVID-19 can change semen parameters and it may take up to 3 months for these parameters to return to their original state.
There is a need for studies that evaluate sperm parameters in people who have had COVID-19, during the disease, and in the early and late periods after the disease has passed. In a similar study in terms of motility, Çiçek et al. examined the sperm motility of a male patient who had previously applied to an IVF center and evaluated whether COVID-19 could cause a loss of sperm motility. Progressive motile sperm motility was found to be 59% in the individual. Later, when the semen sample was taken again, they found that the motility of progressively motile sperm was 0%. When they examined the patient's anamnesis, they learned that he had a COVID-19 infection 1 month ago. This result made the researchers think that COVID-19 may have a negative effect on sperm motility.

Guo et al. analyzed semen samples from 41 male patients who had recovered from COVID-19 approximately 56 days later and compared them with normal controls. It was observed that the total sperm count, sperm concentration, and percentage of advanced motile sperm were lower in patients who recovered from COVID-19 than in patients in the

**TABLE 1** Comparison of some parameters according to groups

| Variable                  | Covid negative (n = 100) |          | Covid positive (n = 100) |          | Statistical analysis* |
|---------------------------|-------------------------|----------|--------------------------|----------|-----------------------|
|                           | X ± SD | Median [min–max] | X ± SD | Median [min–max] | Possibility |
| Age (year)                | 32.18 ± 7.24 | 30.0 [17.0–64.0] | 31.08 ± 6.05 | 30.5 [21.0–51.0] | Z = 0.832 |
|                           |         | p = 0.405      |         |                |           |
| Sexual abstinence (day)   | 3.98 ± 0.85 | 4.0 [2.0–7.0] | 3.44 ± 0.57 | 3.0 [3.0–5.0] | Z = −5.275 |
|                           |         | p = 0.001      |         |                |           |
| Volume (ml)               | 3.42 ± 1.62 | 3.0 [0.5–7.5] | 3.50 ± 1.42 | 3.2 [1.0–9.0] | Z = −0.479 |
|                           |         | p = 0.632      |         |                |           |
| Concentration             | 48.19 ± 36.24 | 38.0 [1.9–189.0] | 31.78 ± 32.09 | 18.0 [0.6–167.0] | Z = −3.964 |
|                           |         | p = 0.001      |         |                |           |
| Motility %                | 49.09 ± 17.46 | 52.0 [8.0–82.0] | 46.93 ± 1.83 | 48.0 [2.0–84.0] | t = 0.841 |
|                           |         | p = 0.402      |         |                |           |
| Normal morphology %       | 1.73 ± 1.48 | 1.0 [1.0–9.0] | 1.76 ± 1.62 | 1.0 [1.0–7.0] | Z = −0.485 |
|                           |         | p = 0.628      |         |                |           |
| Head anomaly %            | 43.38 ± 11.28 | 43.0 [19.0–68.0] | 41.44 ± 10.46 | 41.0 [21.0–67.0] | t = 1.124 |
|                           |         | p = 0.263      |         |                |           |
| Neck anomaly %            | 27.31 ± 6.88 | 28.0 [4.0–47.0] | 27.73 ± 7.49 | 28.0 [13.0–43.0] | t = −0.376 |
|                           |         | p = 0.707      |         |                |           |
| Tail anomaly %            | 27.59 ± 12.31 | 25.0 [8.0–76.0] | 29.20 ± 10.26 | 29.0 [8.0–60.0] | t = −0.890 |
|                           |         | p = 0.375      |         |                |           |

Note: Bold values indicate a statistically significant difference was found between the groups in terms of sexual abstinence duration (days) and concentration (p < 0.05). The duration of sexual abstinence (days) and concentration values in the Covid-19 negative group were significantly higher than those in the Covid-19 positive group.

Abbreviation: SD, standard deviation.

*Independent sample t* test (*t*-table value) statistics were used to compare the measurement values of two independent groups with normally distributed data. The Mann–Whitney U’ test (Z-table value) statistics were used to compare the measurement values of two independent groups in the data that did not have a normal distribution.

**TABLE 2** Examining the relationships between the group and marital status and concentration class

| Variable                  | Covid negative (n = 100) |          | Covid positive (n = 100) |          | Statistical analysis* |
|---------------------------|-------------------------|----------|--------------------------|----------|-----------------------|
|                           | n   | %  | n   | %  | Possibility |
| Marital status            |     |    |     |    |            |
| Married                   | 84  | 84.0 | 88  | 88.0 | χ² = 0.374 |
|                           |     | p = 0.514      |     |                |           |
| Single                    | 16  | 16.0 | 12  | 12.0 | p = 0.514 |
| Concentration             |     |    |     |    |            |
| Azoospermia               | –   | –   | 4   | 14.3 | p = 1.000 |

Note: There was no statistically significant relationship between the group and marital status and concentration classes (p > 0.05). The groups are independent and homogeneous in terms of the specified characteristics.

*Fisher-exact* and *Pearson*-χ² crosstabs were used to examine the relationships between two qualitative variables.

There is a need for studies that evaluate sperm parameters in people who have had COVID-19, during the disease, and in the early and late periods after the disease has passed. In a similar study in terms of motility, Çiçek et al. examined the sperm motility of a male patient who had previously applied to an IVF center and evaluated whether COVID-19 could cause a loss of sperm motility. Progressive motile sperm motility was found to be 59% in the individual. Later, when the semen sample was taken again, they found that the motility of progressively motile sperm was 0%. When they examined the patient’s anamnesis, they learned that he had a COVID-19 infection 1 month ago. This result made the researchers think that COVID-19 may have a negative effect on sperm motility. Guo et al. analyzed semen samples from 41 male patients who had recovered from COVID-19 approximately 56 days later and compared them with normal controls. It was observed that the total sperm count, sperm concentration, and percentage of advanced motile sperm were lower in patients who recovered from COVID-19 than in patients in the

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control group. However, no significant changes in sperm morphology were reported. It was observed that sperm concentration and motility increased in the COVID-19 positive group, which was examined again after about 29 days by taking a semen sample for the second time. These findings suggest that COVID-19 may have adverse effects on sperm quality, but these effects are reversible.21-23

In our study, we examined four sperm parameters in a total of 200 male semen samples. We observed that the sperm concentrations of COVID-19 patients decreased significantly compared to the control group, and 4 out of 100 were azoospermic. The large sample size of our study reveals the difference between groups in sperm concentration was significant and the negative impact of COVID-19 on sperm concentration in men. As a matter of fact, there are many studies supporting our study. However, studies with sperm samples to be taken both before, after, and in the long term, will be very important in terms of understanding how COVID-19 affects male infertility.

5 CONCLUSION

Although there are studies showing that COVID-19 negatively affects male reproduction, no study with a large sample size has been found. In our study, we observed that the sperm concentrations of men with COVID-19 were low, and as a result, COVID-19 adversely affected male fertility. Long-term studies with larger samples (comparative before and after COVID-19) are needed to better understand the changes in sperm concentration values.

AUTHOR CONTRIBUTIONS

Collecting and evaluating data and writing manuscripts: Tińce Aksak. Collecting data and testing: Deniz A. Satar. Collecting data and testing: Ridvan Bağcı. Statistical testing: Efdal O. Gültekin. Article scanning process: Arzu Coşkun. Collecting data and testing: Umut Demirdelen.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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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