Received: 28 April 2023 | Accepted: 31 August 2023

DOI: 10.1002/jmv.29094

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Comparison of semen parameters in the same patients before and after diagnosis of COVID-19

Cihan Cakir[1] | Goktan Kuspinar[1] | Gizem Kurt[1] | Miray Berber[1] | Kiper Aslan[2] | Isil Kasapoglu[2] | Gurkan Uncu[2] | Berrin Avcı[1]

[1]Department of Histology and Embryology, Uludag University School of Medicine, ART Center, Bursa, Turkey

[2]Department of Obstetrics and Gynecology, Uludag University School of Medicine, ART Center, Bursa, Turkey

Correspondence
Cihan Cakir, Uludag, Department of Histology and Embryology, Uludag University School of Medicine, ART Center, Bursa, Turkey.
Email: cihancakir@uludag.edu.tr

Abstract

Clinical and histopathological evidence suggest that the male reproductive system may be negatively impacted in patients with coronavirus disease (COVID-19). The objective of this study is to investigate the effect of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on semen parameters by comparing semen analyses before and after COVID-19 diagnosis in the same patient. We retrospectively analyzed 342 semen analyses by reviewing medical records. The study included two groups of patients: (i) those who underwent two consecutive semen analyses within 6 months, one before ($n = 114$) and one after ($n = 114$) COVID-19 diagnosis, and (ii) a control group ($n = 114$) that was age-matched and did not receive a diagnosis of COVID-19. The study results indicated a significant decrease in semen volume, total sperm count per ejaculate, progressive motile sperm count, total motile sperm count, and normal sperm morphology after SARS-CoV-2 infection in comparison to their respective values before the infection. Subgroup analyses showed that the duration of COVID-19 diagnosis (short-term vs. long-term) did not impact the changes in semen parameters. However, fever during the COVID-19 process had a negative effect on semen parameters, particularly sperm concentration, unlike in patients without fever. In conclusion, our findings suggest that SARS-CoV-2 infection is associated with a decline in semen quality, which may potentially impact male fertility. Furthermore, it's important to note that the negative effects on semen parameters may persist in the long-term. Our results also indicate that fever during active infection could be a significant risk factor that negatively affects spermatogenesis.

KEYWORDS

COVID-19, male infertility, SARS-CoV-2, semen parameters, spermatogenesis

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel strain of coronavirus that causes coronavirus disease (COVID-19). It was first associated with severe acute respiratory syndrome in late 2019 and was declared a worldwide pandemic in March 2020.[1] Since its emergence, the COVID-19 pandemic has spread globally, infecting more than 600 million identifiable individuals and causing over 6.5 million deaths as of April, 2022.[2] This highlights the significant severity and virulence of SARS-CoV-2. The entry of SARS-CoV-2 into target cells is facilitated by the interaction between the virus's spike protein and the host cell's angiotensin-converting enzyme 2 (ACE2) receptor.[3] The spike protein is primed by transmembrane serine protease 2 (TMPRSS2) in the host cell, which enhances the ACE2 receptor-mediated entry of the virus.[4,5] Therefore, cells that possess both the specified receptors and enzymes are susceptible to infection by SARS-CoV-2. It is widely acknowledged that SARS-CoV-2 is not solely a respiratory virus and can affect multiple organs, making it a multisystemic virus. In a study conducted by Qi et al.[6] that utilized single-cell RNA sequencing data, the risk of viral infection was evaluated in 31 different organs, based on the expression levels of ACE2 receptor and TMPRSS2. The study's findings indicate that SARS-CoV-2 has the potential to affect multiple organ systems beyond the respiratory system, including the digestive, urogenital, nervous, and circulatory systems. Notably, the testis was identified as a high-risk organ for SARS-CoV-2 infection due to high expression levels of ACE2 receptor and TMPRSS2 expression in spermatogonia, peritubular myoid cells, testicular somatic cells, and spermatogonial stem cells.[6]

There is considerable evidence to suggest that SARS-CoV-2, the virus responsible for COVID-19, can have a detrimental effect on the male reproductive system, as evidenced by reports of testicular pain,[7] radiological imaging findings,[8,9] histopathological analyses,[10,11] decreased numbers of Leydig cells,[12] and decreased testosterone levels.[13,14] However, the existing literature on the impact of COVID-19 on semen parameters is conflicting, in terms of the extent, duration, and causes of these effects.[15–32] This inconsistency can be attributed to low-evidence level studies, which do not include preinfection semen parameters, have small sample sizes, short follow-up periods, and do not categorize patients according to disease severity.

The primary aim of our study is to determine the impact of SARS-CoV-2 on semen parameters by comparing the results of semen analyses conducted before and after the diagnosis of COVID-19 in the same patient. In addition, our secondary aim is to assess the influence of two potential factors on any observed changes in semen parameters: the duration of time elapsed since COVID-19 diagnosis, and the presence of fever during the infection period.

2 | MATERIALS AND METHODS

2.1 | Study design and population

This was a retrospective cohort study conducted at a single center, the Andrology Laboratory of the ART Centre located in Uludağ University Medical Faculty, which is a tertiary hospital. The study analyzed semen analyses that were performed between December 2019 and February 2023. We obtained a detailed medical history, including COVID-19 history, the date of a positive RT-PCR test result for nasopharyngeal SARS-CoV-2, the presence of fever and symptoms during the infection process, the need for hospitalization and medications used, tobacco and alcohol consumption, the presence of chronic systemic disease, other previous diseases, previous surgical interventions, and patients' demographic characteristics for all patients who underwent semen analysis at our centre since the beginning of the COVID-19 pandemic. We retrospectively reviewed a total of 1471 spermiogram analyses carried out between the specified dates, as well as the demographic and clinical characteristics of the patients.

The study population consisted of patients who underwent semen analysis at our center and were divided into two groups: the COVID-19 group and the control group. The COVID-19 group included patients whose semen analyses were performed before and after a positive RT-PCR test for SARS-CoV-2. Patients in the COVID-19 group had two consecutive semen analysis results within a maximum of 6 months before and after the diagnosis of COVID-19, and were not in the active phase of COVID-19 at the time of semen sampling. The control group was selected based on age-matching and similar demographic characteristics from patients who had undergone semen analysis during the same period. The preinfection semen parameters of the COVID-19 group were compared to those of the control group to assess their compatibility with the normal population. The study excluded individuals who did not meet the following criteria for both the COVID-19 patient group and the control group: (a) age outside the range of 18–45 years; (b) diagnosis of azoospermia; (c) presence of a fever-causing illness other than COVID-19 between semen analyses; (d) medical history or treatment that may have an impact on sperm production (such as a history of chemotherapy/radiotherapy, hypogonadism, use of hormonal medication, genital tract infection, mumps, chronic disease, or steroid therapy during COVID-19 treatment); and (e) a history of prior testicular or prostate surgery (including testicular tumor, cryptorchidism, varicocele, testicular torsion, or hydrocele).

The effects of COVID-19 on semen parameters were assessed by comparing spermiogram results before and after SARS-CoV-2 RT-PCR positivity in the same patient in the COVID-19 group. We also investigated the short-term (<75 days) and long-term (>75 days) effects of COVID-19 on male infertility by determining the time elapsed between the first semen analysis and the positive SARS-CoV-2 PCR test result date, and the time elapsed between PCR positivity and the second semen analysis in the COVID-19 group patients. Furthermore, the effect of fever during infection on the semen parameters of patients in the COVID-19 group was analyzed. The study included a total of 342 spermiogram results, with 228 from the COVID-19 group and 114 from the control group.

The study was conducted following the principles of the Declaration of Helsinki and was approved by the Republic of Türkiye Ministry of Health Ethics Committee (Approval No: 2021-04-13T15_40_41) and the

Istanbul Medipol University Institutional Clinical Research Ethics Committee (Approval No: E-10840098-772.02-2378).

2.2 | Semen analysis

All semen samples were analyzed in the same andrology laboratory and by an experienced embryologist, following the guidelines established by the World Health Organization (WHO).[33] Samples were obtained through masturbation into sterile, noncytotoxic containers after 2–5 days of sexual abstinence. Before semen analysis, the samples were incubated at 37°C for 30–60 min to promote liquefaction. The duration of liquefaction did not exceed 60 min in any of the semen samples analyzed. Sperm concentration and motility were manually analyzed using a counting chamber (Makler; Sefi Medical Instruments) under a phase contrast microscope (Olympus CX31) at ×20 magnification. The study evaluated various sperm parameters for each sample, including sperm concentration, total sperm count per ejaculate, percentage of progressive motility, percentage of total motility, progressive motile sperm count, and total motile sperm count. A detailed morphological evaluation was also performed by preparing a smear slide and staining it with a DIFF-3 rapid differential stain kit. Morphological evaluation of at least 200 spermatozoa was then performed at ×100 magnification under a light microscope, using Kruger's criteria.[34] The morphological examination assessed normal morphology as well as abnormalities in the head, midpiece, principal piece, and excess residual cytoplasm.

2.3 | Statistical analysis

The distribution characteristics of the variables were analyzed using the Shapiro–Wilk test. For continuous variables, mean (standard deviation) or median (25th–75th percentiles) were used based on the distribution characteristics. Categorical variables were presented as numbers and percentages. The Student *t* test was used to compare two sets of normally distributed data with continuous variables. If the continuous variables did not show normal distribution, the Mann–Whitney *U* test was used to compare two groups. The comparison of categorical variables was performed using either the Pearson's χ^2 test or Fisher's exact test. The Wilcoxon signed-rank test was used in dependent groups to compare semen parameters before and after COVID-19 in the same patients. All statistical analyses were performed using SPSS software (Statistical Package for Social Sciences, version 28.0, SPSS Inc). A $p < 0.05$ was considered statistically significant.

3 | RESULTS

The allocation diagram of our study is presented in Figure 1. We conducted a retrospective review of 1471 semen analyses performed within a specific time interval. From these records, we included

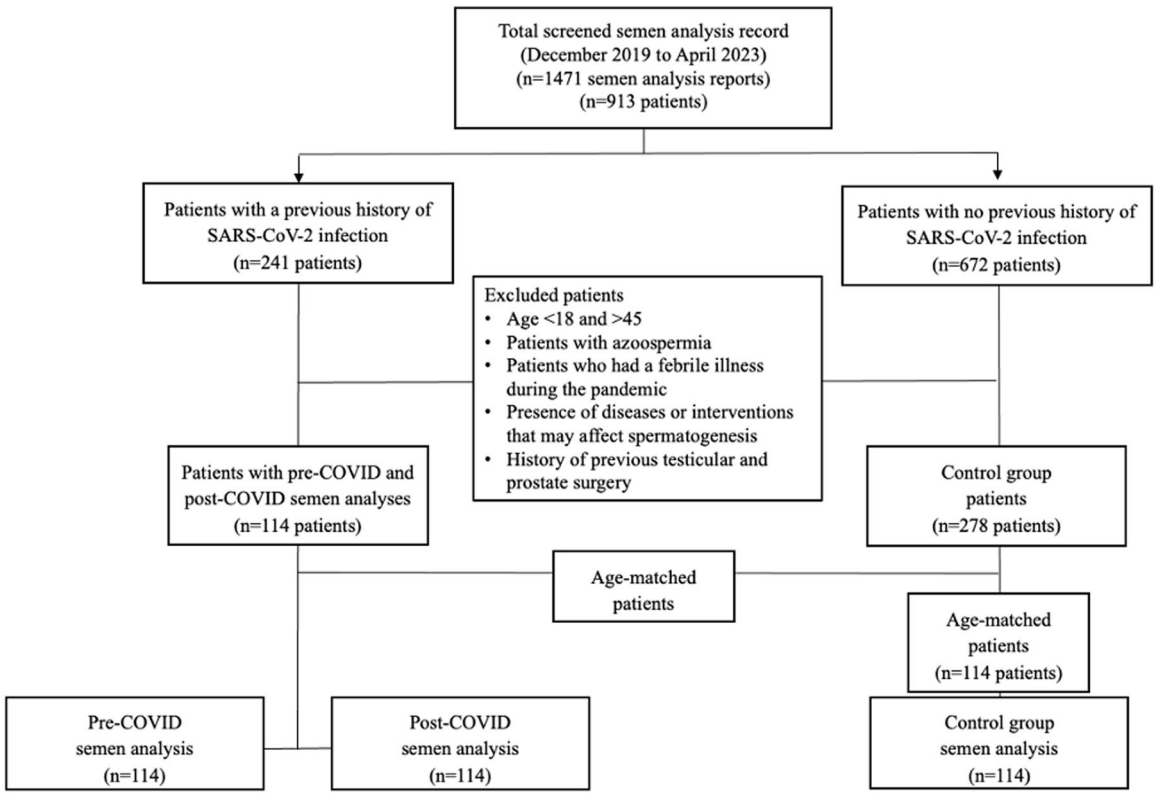

FIGURE 1 The allocation diagram of the study.

spermiogram results for 114 patients before and after they tested positive for SARS-CoV-2 via RT-PCR, and an age-matched control group ($n = 114$) meeting inclusion criteria. We evaluated a total of 342 semen analysis results.

The demographic and clinical characteristics of the study population are presented in Table 1. Parameters such as body mass index (BMI) ($p = 0.695$), smoking ($p = 0.085$), and alcohol consumption ($p = 0.347$), as well as comorbidity rates such as hypertension ($p = 0.722$), asthma ($p = 1.000$), and diabetes ($p = 1.000$), which are known to be important factors in the prognosis of COVID-19, were found to be statistically similar between patients in the COVID-19 group and the control group. Furthermore, there was no significant difference in the number of patients diagnosed with varicocele who did not undergo surgery between the groups ($p = 0.477$). In patients in the COVID-19 group, the median time interval between the first semen analysis and the confirmation of a positive PCR test was 41.50 days (*interquartile range [IQR]: 31.75–68*). The median time interval between the positive PCR test and the second semen analysis was 82 days (*IQR: 42.75–120*). It was observed that 53.5% of the patients with COVID-19 had a fever of >38°C for at least 3 days, and 12.3% were hospitalized. None of the patients required intensive care or endotracheal intubation. Among the patients, 51.8% received hydroxychloroquine and 42.1% received favipiravir. The results of the semen analysis for the control group, pre-COVID-19 group, and post-COVID-19 group are presented in Table 1. There was no significant difference observed between the groups in terms of the duration of sexual abstinence. The semen parameters of the pre-COVID-19 group were similar to those of the control group. When comparing the pre- and post-COVID-19 semen parameters of the patients, there was no statistically significant difference in terms of sperm concentration ($p = 0.083$), progressive motility ($p = 0.170$), total motility ($p = 0.428$), or percentage of sperm with normal morphology ($p = 0.106$). However, there was a significant decrease in semen volume ($p < 0.001$), and based on semen volume, the total number of sperm per ejaculate ($p < 0.001$), progressive motile sperm count ($p = 0.005$), total motile sperm count ($p = 0.003$), and number of sperm with normal morphology ($p < 0.001$) following SARS-CoV-2 infection compared to preinfection values. In addition, a statistically significant difference was observed in similar semen parameter between the post-COVID-19 group and the control group; semen volume ($p < 0.001$), the total number of sperm per ejaculate ($p = 0.035$), progressive motile sperm count ($p = 0.045$), total motile sperm count ($p = 0.022$), and number of sperm with normal morphology ($p = 0.032$) (Table 1).

In addition, a statistical difference was found in similar parameters when the post-covid-19 group was compared with the control group.

The effect of the time elapsed between the positive PCR test result for COVID-19 and the second semen analysis on semen parameters was investigated in two groups of patients, namely the short-term group ($n = 46$) with an interval of <75 days, and the long-term group ($n = 68$) with an interval of >75 days. The pre-COVID-19 and post-COVID-19 semen parameters of the patients in each group were compared (Table 2). The two patient groups with different intervals were found to be similar in terms of demographic, prognostic and clinical characteristics. However, a comparison of the pre-COVID-19 semen parameters between two groups revealed statistically significant differences in several aspects. These included the total sperm count per ejaculate ($p = 0.009$), progressive motile sperm count ($p = 0.008$), total motile sperm count ($p = 0.009$), and the number of sperm with normal morphology ($p = 0.002$). The time elapsed between COVID-19 diagnosis and the second semen analysis was 40 (30.75–60) days in the short-term group and 96.5 (86–123) days in the long-term group ($p < 0.001$). There was no statistically significant difference in sperm concentration, progressive motility, total motility, and percentage of sperm with normal morphology between the patients in both groups before and after COVID-19. In patients in the short-term group, as well as in the overall COVID-19 group, a statistically significant decrease was observed in post-COVID-19 semen volume ($p < 0.001$), total number of sperm per ejaculate ($p = 0.006$), progressive motile sperm count ($p = 0.010$), total motile sperm count ($p = 0.006$) and number of sperm with normal morphology ($p = 0.005$) when compared to their pre-COVID-19 values. On the other hand, in patients from the long-term group, it was found that only semen volume ($p < 0.001$), total sperm count per ejaculate ($p = 0.007$), and number of sperm with normal morphology ($p = 0.003$) showed statistically significant decrease following COVID-19, while progressive motile sperm count ($p = 0.216$) and total motile sperm count ($p = 0.145$) were similar to pre-COVID-19 values.

Another subgroup analysis was conducted in the COVID-19 group by creating two groups based on the presence or absence of fever during active infection: fever-positive group ($n = 61$) and fever-negative group ($n = 53$) (Table 3). The two groups had similar demographic and clinical characteristics, except for the treatment received for COVID-19. The fever-positive group had a higher percentage of patients treated with hydroxychloroquine (*60.7% vs. 41.5%*, $p = 0.041$) and favipiravir (*50.8% vs. 32.1%*, $p = 0.043$) compared to the fever-negative group. Moreover, the time elapsed between the positive PCR test result and the second semen analysis was similar in both groups ($p = 0.759$). Additionally, no statistically significant differences were observed in the pre-COVID-19 semen parameters between the two groups. In the fever-positive group, as well as in the entire COVID-19 group, a statistically significant decrease was observed in post-COVID-19 semen volume ($p < 0.001$), total sperm count per ejaculation ($p < 0.001$), progressive motile sperm count ($p = 0.003$), total motile sperm count ($p = 0.002$), and total number of sperm with normal morphology ($p < 0.001$) compared to pre-COVID-19 values within the same patients. Notably, sperm concentration also significantly decreased after infection in the fever-positive group ($p = 0.005$). On the other hand, in patients in the fever-negative group, it was observed that only semen volume decreased significantly ($p < 0.001$) following SARS-CoV-2 infection, and all other semen parameters remained similar before and after the disease.

To assess the duration of fever's impact on semen parameters, two subgroups were established: Fever-positive short-term group and fever-positive long-term group (Table 4). Despite similarities in

TABLE 1 Demographic characteristics, clinical features, and semen analysis results of patients.

	COVID-19 Group	Control group	*p* Value
No. of patients (*n*)	114	114	
Age (years)	33 (31–38)	33 (31–38)	1.000[a]
BMI (kg/m^2)	26.44 (24.18–29.39)	26.03 (24.51–29.39)	0.695[a]
Smoking (*n*) (%)	60/114 (52.6%)	47/114 (41.2%)	0.085[b]
Alcohol consumption (%)	14/114 (12.3%)	19/114 (16.7%)	0.347[b]
Clinical varicocele presence (%)	17/114 (14.9%)	21/114 (18.4%)	0.477[b]
Comorbidity			
Hypertension (*n*) (%)	5/114 (4.4%)	3/114 (2.6%)	0.722[c]
Asthma (*n*) (%)	2/114 (1.8%)	3/114 (2.6%)	1.000[c]
Diabetes mellitus (*n*) (%)	3/114 (2.6%)	2/114 (1.8%)	1.000[c]
Time from the first semen analysis to the diagnosis of COVID-19 (days)	41.50 (31.75–68)	-	-
Time from the diagnosis of COVID-19 to the second semen analysis (days)	82 (42.75–120)	-	-
<75 days (*n*) (%)	68/114 (59.6%)	-	-
>75 days (*n*) (%)	46/114 (40.4%)	-	-
Treatment		-	-
Hydroxychloroquine % (*n*/total)	59/114 (51.8%)	-	-
Favipiravir (*n*) (%)	48/114 (42.1%)	-	-
Fever (>38°C) (*n*) (%)	61/114 (53.5%)	-	-
Hospitalization (*n*) (%)	14/114 (12.3%)	-	-

Semen parameters	COVID-19 group pre-COVID-19 semen analysis	COVID-19 group post-COVID-19 semen analysis	Pre-COVID-19 vs. post-COVID-19 *p* Value	Control group semen analysis	Control group vs. pre-COVID-19 *p* Value	Control group vs. post-COVID-19 *p* Value
Sexual abstinence (days)	4 (3–4)	4 (3–4)	1.000[d]	3.5 (3–4)	0.304[a]	0.304[a]
Semen volume (mL)	3 (2–4.5)	2.5 (2–3)	**<0.001**[d]	3 (2–4)	0.401[a]	**<0.001**[a]
Sperm concentration (×10^6/mL)	38 (17–77.5)	33.5 (17–69)	0.083[d]	43 (18–66.75)	0.695[a]	0.773[a]
Total number of sperm per ejaculate (×10^6)	127.75 (52–239.63)	86 (37.13–166.75)	**<0.001**[d]	109.25 (51.75–196.88)	0.414[a]	**0.035**[a]
Progressive motility (PR, %)	59 (44.75–71.25)	63 (49.5–71)	0.170[d]	59 (46.75–70)	0.969[a]	0.313[a]
Total motility (PR + NP, %)	64 (48–75.25)	65 (51.75–74)	0.428[d]	62 (49.75–72.25)	0.842[a]	0.441[a]
Progressively motile sperm count (×10^6)	61.68 (18.51–177.98)	51.36 (19.02–111.7)	**0.005**[d]	66.74 (29.36– 142.53)	0.729[a]	**0.045**[a]
Total motile sperm count (×10^6)	68.49 (22.23–187.07)	55.8 (19.37–115.2)	**0.003**[d]	69.35 (31.20–151.20)	0.623[a]	**0.022**[a]
Sperm morphology (normal forms, %)	3 (1.75–4)	3 (1–4)	0.106[d]	3 (1–4)	0.789[a]	0.865[a]
Total number of normal forms (×10^6)	2.63 (0.99–6.34)	1.89 (0.78–4.11)	**<0.001**[d]	2.25 (0.95–4.11)	0.218[a]	**0.032**[a]
Abnormal head (%)	95 (93–97)	95 (93–96)	0.945[d]	94 (92–96)	0.216[a]	0.302[a]

(Continues)

TABLE 1 (Continued)

Semen parameters	COVID-19 group pre-COVID-19 semen analysis	COVID-19 group post-COVID-19 semen analysis	Pre-COVID-19 vs. post-COVID-19 *p* Value	Control group semen analysis	Control group vs. pre-COVID-19 *p* Value	Control group vs. post-COVID-19 *p* Value
Abnormal midpiece (%)	1 (0–2)	1 (1–2)	0.146[d]	1 (0–2)	0.867[a]	0.055[a]
Abnormal principal piece (%)	0 (0–2)	1 (0–2)	0.074[d]	1 (0–2)	0.118[a]	0.839[a]
Excess residual cytoplasm (%)	0 (0–0.25)	0	0.093[d]	0 (0–1)	0.973[a]	0.169[a]

Note: Data presented as number (percentage of total) or median (IQR). Bold values denote statistical significance at the $p < 0.05$ level.

[a]Mann–Whitney *U* test.

[b]Pearson's χ^2.

[c]Fisher's exact test.

[d]Wilcoxon signed-rank test.

demographic, prognostic, and clinical characteristics between the two patient groups with different time intervals, statistically significant differences were observed in sperm concentration *(p = 0.025)*, total number of sperm per ejaculate *(p = 0.007)*, progressive motile sperm count *(p = 0.007)*, total motile sperm count *(p = 0.008)*, and the number of sperm with normal morphology *(p = 0.001)* parameters before COVID-19 infection. Both the short-term and long-term analyses of patients with fever during the illness revealed statistically significant reductions in semen volume *(p = 0.002 and p = 0.002, respectively)*, sperm concentration *(p = 0.037 and p = 0.043, respectively)*, total number of sperm per ejaculate *(p = 0.006 and p = 0.012, respectively)*, and the total number of normal forms *(p = 0.011 and p = 0.003, respectively)* when compared to pre-COVID-19 infection. Furthermore, it was determined that while the parameters of progressive motile sperm count and total motile sperm count decreased in the short term after infection *(p = 0.009 and p = 0.007, respectively)*, they remained similar in the long-term groups before and after the disease.

We observed that 51.8% of the patients included in the study were administered hydroxychloroquine, while 42.1% received favipiravir treatment. Our analysis focused on patients who did not experience fever during the active infection of COVID-19, dividing them into two distinct subgroups based on drug usage: fever-negative drug-positive group and fever-negative drug-negative group (Table 5). Comparative analysis revealed a significant decrease ($p = 0.002$) in semen volume alone after SARS-CoV-2 infection, when compared to pre-infection values, within fever-negative drug-negative group. However, all other semen parameters remained comparable before and after the disease. Similarly, no statistically significant differences were observed in the semen parameters before and after COVID-19 within the fever-negative drug-positive group.

We have also presented the parameters exhibiting significant differences using categorical scatter plot graphs, wherein each value was visually represented as a dot. This graphical representation aims to enhance the ease of observing and interpreting the obtained results (Figures 2–6).

4 | DISCUSSION

The main objective of our study was to investigate the potential detrimental effects of COVID-19 on semen parameters. Additionally, we aimed to assess the impact of time elapsed after infection and the presence of fever during the active infection period on changes in semen parameters. To achieve these aims, we conducted a comparative analysis of consecutive pre- and postinfection semen samples obtained within 6 months from 114 patients who had recovered from COVID-19. Our findings revealed that semen volume decreased significantly following COVID-19 infection, regardless of the presence of fever or the duration of infection. However, there was no significant impact on sperm concentration, percentage of motile sperm, or percentage of sperm with normal morphology. Additionally, we found that the impact on semen parameters remained largely unchanged over both short-term (<75 days) and long-term (>75 days) periods. Notably, we observed that the presence of fever during the active infection period had a negative effect on semen parameters, with sperm concentration being the most significantly affected parameter.

Numerous studies have been conducted to evaluate the impact of SARS-CoV-2 on male fertility at the hormonal level. Upon reviewing the literature, there are conflicting findings regarding the effect of COVID-19 on serum luteinizing hormone (LH) levels. Some studies have reported a significant increase in LH levels after COVID-19,[13,14,35] while others have found no change in LH levels.[36] Similarly, there are contradictory findings regarding the effect of COVID-19 on serum testosterone levels. Some studies have suggested a decrease in testosterone levels after COVID-19,[14,35] while others have found no postillness changes in testosterone levels.[13,37] Histopathological changes in testicular tissue following COVID-19 have been proven with many autopsy samples. These studies have shown degeneration in testicular tissue and germ cells,[11] damage to seminiferous tubule cells, lymphocytic inflammation, decrease in Leydig cells,[12,38] increase in the number of apoptotic cells, macrophage infiltration,[29,39] decreased spermatogenesis, and

TABLE 2 Stratified analysis of recovered COVID-19 patients with less and greater than 75 days' follow-up since recovery.

	COVID-19 short-term group <75 days	COVID-19 Long-term group >75 days	*p* Value
No. of patients (*n*)	46	68	
Age (years) median (IQR)	34 (31–38.25)	32 (30–38)	0.407[a]
BMI (kg/m^2)	26 (23–29.55)	26.54 (25.35–29.39)	0.424[b]
Smoking (*n*) (%)	27/46 (58.7%)	33/68 (48.5%)	0.286[c]
Alcohol consumption (%)	7/46 (15.2%)	7/68 (10.3%)	0.432[c]
Clinical varicocele presence (%)	8/46 (17.4%)	9/68 (13.2%)	0.541[c]
Comorbidity			
Hypertension (*n*) (%)	3/46 (6.5%)	2/68 (2.9%)	0.391[d]
Asthma (*n*) (%)	1/46 (2.2%)	1/68 (1.5%)	1.000[d]
Diabetes mellitus (*n*) (%)	1/46 (2.2%)	2/68 (2.9%)	1.000[d]
Time from the first semen analysis to the diagnosis of COVID-19 (days)	47.50 (34.55–87)	40.5 (30–67.5)	0.286[a]
Time from the diagnosis of COVID-19 to the second semen analysis (days)	40 (30.75–60)	96.5 (86–123)	**<0.001**[a]
Treatment			
Hydroxychloroquine % (*n*/total)	22/46 (47.8%)	37/68 (54.4%)	0.490[c]
Favipiravir (*n*) (%)	20/46 (43.5%)	28/68 (41.2%)	0.807[c]
Fever (>38°C) (*n*) (%)	24/46 (52.2%)	37/68 (54.4%)	0.807[c]
Hospitalization (*n*) (%)	4/46 (8.7%)	10/68 (14.7%)	0.337[c]

	Short-term group pre-COVID-19 <75 days (*n*) (%)	Short-term group post-COVID-19 <75 days (*n*) (%)	*p* Value	Long-term group pre-COVID-19 >75 days (*n*) (%)	Long-term group post-COVID-19 >75 days (*n*) (%)	*p* Value	Short-term group pre-COVID-19 vs. long-term group pre-COVID-19 *p* Value
Sexual abstinence (days)	3.5 (3–4)	4 (3–4)	0.414[e]	4 (3–4)	3 (3–4)	0.480[e]	0.872[a]
Semen volume (mL)	3.5 (3–5)	2.5 (2–3.5)	**<0.001**[e]	3 (2–4.38)	2.5 (1.63–3.00)	**<0.001**[e]	0.056[a]
Sperm concentration (×10^6/mL)	45.5 (21.25–85.75)	44.5 (26.75–75.25)	0.236[e]	29 (14–73.75)	28.5 (13.25–63.25)	0.233[e]	0.062[a]
Total number of sperm per ejaculate (×10^6)	161 (80.88–280.13)	105 (62.75–184.5)	**0.006**[e]	86 (39.38–213.75)	75.25 (22.88–163)	**0.007**[e]	**0.009**[a]
Progressive motility (PR, %)	61 (47.75–76.25)	64 (52–71.25)	0.750[f]	58 (38.25–69.75)	61.5 (45–71)	0.098[e]	0.070[a]
Total motility (PR + NP, %)	65 (57–78)	65.5 (58.75–76)	0.972[f]	60.5 (44.25–72)	62 (50–73.5)	0.218[e]	0.054[a]
Progressively motile sperm count (×10^6)	91.65 (42.25–192.99)	58.31 (33.91–129.26)	**0.010**[e]	48.5 (13.68–145.59)	35.85 (10.91–93.95)	0.216[e]	**0.008**[a]
Total motile sperm count (×10^6)	94.04 (44.94–198.98)	59.27 (33.91–134.12)	**0.006**[e]	53.72 (18.20–146.06)	42.18 (12.04–99.50)	0.145[e]	**0.009**[a]
Sperm morphology (normal forms, %)	3 (2–4)	3 (2–4)	1.000[e]	3 (1–4)	3 (1–4)	0.050[e]	0.158[a]

(Continues)

TABLE 2 (Continued)

	Short-term group pre-COVID-19 <75 days (*n*) (%)	Short-term group post-COVID-19 <75 days (*n*) (%)	*p* Value	Long-term group pre-COVID-19 >75 days (*n*) (%)	Long-term group post-COVID-19 >75 days (*n*) (%)	*p* Value	Short-term group pre-COVID-19 vs. long-term group pre-COVID-19 *p* Value
Total number of normal forms (×10^6)	4.22 (1.95–10.24)	3.05 (1.47–5.53)	**0.005**[e]	1.94 (0.72–5.38)	1.32 (0.44–2.64)	**0.003**[e]	**0.002**[a]
Abnormal head (%)	95.5 (93–97)	95 (93–97)	0.377[f]	95 (92–97)	95 (93–96)	0.552[e]	0.625[a]
Abnormal midpiece (%)	1 (0–2)	1 (1–2)	0.051[e]	1 (0–2.75)	1 (1–2)	0.787[e]	0.197[a]
Abnormal principal piece (%)	0 (0–1)	1 (0–1)	0.260[e]	1 (0–2)	1 (0–2)	0.163[e]	0.111[a]
Excess residual cytoplasm (%)	0 (0–0)	0 (0–0)	0.322[e]	0 (0–1)	0 (0–0)	0.152[e]	0.415[a]

Note: Data presented as number (percentage of total) or median (IQR). Bold values denote statistical significance at the $p < 0.05$ level.

[a]Mann–Whitney *U* test.

[b]Independent-samples *T* test.

[c]Pearson's χ^2.

[d]Fisher's exact test.

[e]Wilcoxon signed-rank test.

[f]Paired-samples *T* Test.

vascular changes.[10] The consistent findings across these studies indicate that SARS-CoV-2 infection can significantly impair spermatogenesis due to increased immune response in the testicular tissue.

Although there is controversy regarding the histopathological and hormonal changes associated with SARS-CoV-2 infection, existing literature suggests that semen parameters may be adversely affected following infection. However, when examining studies that have evaluated semen parameters after COVID-19, inconsistent findings have been reported.[13,14,22,26–29,32,36] Many studies have reported statistically significant reductions in sperm concentration, total sperm count, and total motile sperm count.[13,26–28,32,40] However, Temiz et al.[14] conducted a study comparing semen parameters before and after COVID-19 treatment, and found no significant difference between the groups. In our study, contrary to previous findings, we did not observe significant differences in sperm concentration, progressive motility, total motility, and percentage of sperm with normal morphology before and after COVID-19. However, we observed a significant decrease in semen volume and semen volume-related parameters (total number of sperm per ejaculate, progressive motile sperm count, total motile sperm count, and total number of sperm with normal morphology) following COVID-19.

The long-term effect of SARS-CoV-2 on fertility is still a matter of debate. Considering that the process of spermatogenesis takes approximately 74 days from the initiation of spermatocyte differentiation to the formation of motile sperm,[41] it has been suggested that the effects of COVID-19 may persist for a duration of 74 days. Several studies have supported this notion, indicating that SARS-CoV-2 infection has adverse effects on semen parameters in the short term, but that these values tend to normalize or improve over the long term.[21,28] For instance, Guo et al.[28] found a significant increase in sperm concentration, motility, and total sperm count in semen samples collected on average on the 84th day post-COVID-19 compared to the 56th day. Similarly, Falahieh et al.[42] showed that sperm concentration, motility, morphology, and viability parameters improved in semen analyses performed on the 120th day after COVID-19 compared to the 14th day. Another study that analyzed semen parameters of patients who had recovered from COVID-19 and a healthy control group every 10 days for 2 months found that semen volume, sperm concentration, and morphology were lower in the patient group than in the control group for 60 days, but progressive sperm motility increased significantly after the 30th day.[21] On the other hand, a different study comparing two patient groups based on the time elapsed since recovery from SARS-CoV-2 infection (<90 and >90 days) did not observe any improvement in sperm parameters in the long term.[27] However, limitations in these studies included the lack of pre-COVID-19 spermiogram results and comparison of postinfection semen parameters with a control group. Hamarat and colleagues conducted a comparable investigation involving two groups categorized according to the duration between SARS-CoV-2 infection and semen analysis: one group within <70 days and the other beyond 70 days, similar to our present study. Unlike our findings, their study did not reveal any statistically

TABLE 3 Stratified analysis of recovered COVID-19 patients based on the presence or absence of fever during infection.

	COVID-19 fever-positive group	COVID-19 fever-negative group	*p* Value
No. of patients (*n*)	61	53	
Age (years) median (IQR)	32 (30–38.5)	33 (32–38)	0.229[a]
BMI (kg/m^2)	26.54 (24.28–29.98)	26.24 (24.06–29.34)	0.459[b]
Smoking (*n*) (%)	34/61 (55.7%)	26/53 (49.1%)	0.476[c]
Alcohol consumption (%)	9/61 (14.8%)	5/53 (9.4%)	0.388[c]
Clinical varicocele presence (%)	12/61 (19.7%)	5/53 (9.4%)	0.126[c]
Comorbidity			
Hypertension (*n*) (%)	3/61 (4.9%)	2/53 (3.8%)	1.000[d]
Asthma (*n*) (%)	2/61 (3.3%)	0/53 (0%)	0.498[d]
Diabetes mellitus (*n*) (%)	2/61 (3.3%)	1/53 (1.9%)	1.000[d]
Time from the first semen analysis to the diagnosis of COVID-19 (days)	42 (32–74)	40 (30–68)	0.579[a]
Time from the diagnosis of COVID-19 to the second semen analysis (days)	82 (47–120)	82 (41.5–120)	0.759[a]
<75 days (*n*) (%)	24/61 (39.3%)	22/53 (41.5%)	0.814[c]
>75 days (*n*) (%)	37/61 (60.7%)	31/53 (58.5%)	0.814[c]
Treatment			
Hydroxychloroquine % (*n*/total)	37/61 (60.7%)	22/53 (41.5%)	**0.041**[c]
Favipiravir (*n*) (%)	31/61 (50.8%)	17/53 (32.1%)	**0.043**[c]
Hospitalization (*n*) (%)	9/61 (14.8%)	5/53 (9.4%)	0.388[c]

	Fever-positive group pre-COVID-19	Fever-positive group post-COVID-19	*p* Value	Fever-negative group pre-COVID-19	Fever-negative group post-COVID-19	*p* Value	Fever-positive group pre-COVID-19 vs. fever-negative group pre-COVID-19
Sexual abstinence (days)	3 (3–4)	3 (3–4)	0.739[e]	4 (3–4)	4 (3–4)	0.655[e]	0.845[a]
Semen volume (mL)	3 (2–4)	2.5 (1.75–3)	**<0.001**[e]	3.5 (2–5)	2.5 (2–3.75)	**<0.001**[e]	0.201[a]
Sperm concentration (×10^6/mL)	44 (16–91)	33 (17–65)	**0.005**[e]	34 (17–59.5)	34 (17.5–73)	0.425[e]	0.153[a]
Total number of sperm per ejaculate (×10^6)	111 (46.5–258)	81 (30–151.5)	**<0.001**[e]	129 (66.5–219.75)	101.5 (41–187)	0.123[e]	0.781[a]
Progressive motility (PR, %)	59 (37.5–73.5)	62 (45–71)	0.438[e]	59 (47.5–70.5)	65 (50–70.5)	0.175[f]	0.977[a]
Total motility (PR + NP, %)	64 (44.5–76)	63 (50–74)	0.669[e]	61 (50.5–75.5)	65 (55.5–73.5)	0.365[f]	0.937[a]
Progressively motile sperm count (×10^6)	61.48 (17.74–189.35)	45.89 (14.45–93.38)	**0.003**[e]	61.88 (24.30–151.02)	60.68 (21.11–125.4)	0.465[e]	0.807[a]
Total motile sperm count (×10^6)	68.16 (19.46–197.14)	48.84 (14.85–99.28)	**0.002**[e]	69.16 (28.5–151.02)	60.68 (22.10–128.6)	0.282[e]	0.856[a]

(Continues)

TABLE 3 (Continued)

	Fever-positive group pre-COVID-19	Fever-positive group post-COVID-19	*p* Value	Fever-negative group pre-COVID-19	Fever-negative group post-COVID-19	*p* Value	Fever-positive group pre-COVID-19 vs. fever-negative group pre-COVID-19
Sperm morphology (normal forms, %)	2 (1–4)	2 (1–4)	0.251[e]	3 (2–4)	3 (2–4)	0.248[e]	0.119[a]
Total number of normal forms ($\times 10^6$)	2.32 (0.9–6.34)	1.62 (0.67–3.16)	**<0.001**[e]	2.84 (1.31–6.35)	2.04 (1–5.98)	0.067[e]	0.674[a]
Abnormal head (%)	95 (93–97.5)	95 (93–96.5)	0.587[e]	95 (92–96.5)	95 (93–96)	0.705[e]	0.487[a]
Abnormal midpiece (%)	1 (0–2)	1 (1–2)	0.237[e]	1 (0–2)	1 (1–2)	0.407[e]	0.808[a]
Abnormal principal piece (%)	0 (0–2)	1 (0–2)	**0.032**[e]	0 (0–2)	1 (0–1)	0.752[e]	0.793[a]
Excess residual cytoplasm (%)	0 (0–0)	0 (0–0)	0.254[e]	0 (0–1)	0 (0–0)	0.222[e]	0.614[a]

Note: Data presented as number (percentage of total) or median (IQR). Bold values denote statistical significance at the $p < 0.05$ level.

[a]Mann–Whitney *U* test.

[b]Independent-samples *T* test.

[c]Pearson χ^2.

[d]Fisher's exact test.

[e]Wilcoxon signed-rank test.

[f]Paired-samples *T* Test.

significant difference in semen volume and motility parameters between the short-term and long-term intervals. However, both the short and long-term intervals showed a significant decrease in mean sperm concentration and total sperm count.[23] In contrast to previous findings, our study determined that both short-term group (<75 days) and long-term group (>75 days) patients exhibited similar sperm concentration, progressive motility, total motility, and sperm with normal morphology between pre- and post-COVID-19 disease. However, in both groups, there was a statistically significant decrease in semen volume, total number of sperm per ejaculate, and total number of sperm with normal morphology compared to pre-COVID-19 values in the same patients. Interestingly, in the long-term patient group, progressive motile sperm count and total motile sperm count were similar to pre-COVID-19 values, unlike the short-term patient group. These variations between the studies could potentially be attributed to differences in the chosen time intervals for assessing postinfection semen characteristics. An important limitation of our study is the lack of a statistically normal distribution in the pre-COVID-19 semen parameters of the patients included. In comparison to the short-term group, the long-term group exhibited statistically lower values for various parameters, including total sperm count per ejaculate, progressive motile sperm count, total motile sperm count, and number of sperm with normal morphology before COVID-19 infection. Therefore, to validate our findings on the progressive motile sperm count and total motile sperm count in the long term following COVID-19 infection, it is crucial to conduct additional studies with larger sample sizes.

The effects of SARS-CoV-2 infection on testicular tissue may vary depending on the viral load and disease severity. In addition, the impact of SARS-CoV-2 infection on testicular tissue may vary depending on viral load and disease severity. Holtmann et al.[32] observed that semen parameters were not significantly affected in patients with mild symptoms, but moderate COVID-19 infection resulted in significant reductions in sperm concentration, total number of sperm per ejaculate, and total motility. Similarly, Erbay et al.[30] demonstrated that the severity of COVID-19 symptoms correlated with varying effects on semen parameters. Additionally, Gacci et al.[22] reported that the likelihood of oligozoospermia and cryptozoospermia in post-COVID-19 patients was associated with disease severity. Given that fever is a common symptom that can impair testicular function, the presence of fever during COVID-19 may have negative effects on spermatogenesis. Several studies have reported that increased body temperature can impact semen parameters for a period of 1–3 months.[43,44] Holtmann et al.[32] conducted a study which revealed that patients with COVID-19 infection and fever had lower semen volume, sperm concentration, and motility compared to those without fever. Similarly, another study showed that the decrease in the percentage of normal sperm morphology after COVID-19 infection was attributable to fever observed in all patients.[14] In contrast to these studies, Pazir et al.[31] did not detect a significant difference in any semen parameters of patients with fever before and after COVID-19. In our study, we determined that the presence of fever during active infection was an

TABLE 4 Changes in sperm parameters of patients with fever during COVID-19 infection: Long-term and short-term effects.

	Fever-positive short-term Group	Fever-positive long-term group	*p* Value
No. of patients (*n*)	24	37	
Age (years) median (IQR)	33 (31.5–38)	31 (29.5–37)	0.073[a]
BMI (kg/m^2)	27.28 (24.25–30.06)	26.51 (24.17–29.87)	0.970[b]
Smoking (*n*) (%)	12/24 (50%)	22/37 (59.5%)	0.467[c]
Alcohol consumption (%)	4/24 (16.7%)	5/37 (13.5%)	0.729[d]
Clinical varicocele presence (%)	5/24 (20.8%)	7/37 (18.9%)	1.000[d]
Comorbidity			
Hypertension (*n*) (%)	1/24 (4.2%)	2/37 (5.4%)	1.000[d]
Asthma (*n*) (%)	1/24 (4.2%)	1/37 (2.7%)	1.000[d]
Diabetes mellitus (*n*) (%)	0/24 (0%)	2/37 (5.4%)	0.515[d]
Time from the first semen analysis to the diagnosis of COVID-19 (days)	55 (35–90)	41(30.5–63)	0.209[a]
Time from the diagnosis of COVID-19 to the second semen analysis (days)	40 (30.25–60)	96 (84–125.5)	**<0.001**[a]
Treatment			
Hydroxychloroquine % (n/total)	13/24 (54.2%)	18/37 (48.7%)	0.674[c]
Favipiravir (*n*) (%)	12/24 (50%)	19/37 (51.4%)	0.918[c]
Hospitalization (*n*) (%)	2/24 (4.2%)	7/37 (18.9%)	0.462[d]

	Fever-positive short-term group pre-COVID-19 (*n*) (%)	Fever-positive short-term group post-COVID-19 (*n*) (%)	*p* Value	Fever-positive long-term group pre-COVID-19 (*n*) (%)	Fever-positive long-term group post-COVID-19 (*n*) (%)	*p* Value	Fever-positive short-term group pre-COVID-19 vs. fever-positive long-term group pre-COVID-19 *p* Value
Sexual abstinence (days)	4 (3–4)	4 (3–4)	0.564[e]	3 (3–4)	3 (3–4)	0.414[e]	0.694[a]
Semen volume (mL)	3.5 (3–4.5)	2 (2–3)	**0.002**[f]	3 (2–4)	2.5 (1.5–3)	**0.002**[e]	0.179[a]
Sperm concentration (×10^6/mL)	65.5 (38.25–131.50)	50 (27–88.50)	**0.037**[e]	29 (13.5–81.5)	24 (9–52.5)	**0.043**[e]	**0.025**[a]
Total number of sperm per ejaculate (×10^6)	219.75 (86.63–419.50)	99 (69.13–159.50)	**0.006**[e]	68 (36.5–216)	66 (15.75–132)	**0.012**[e]	**0.007**[a]
Progressive motility (PR, %)	69 (49–79.25)	63 (52–71.75)	0.551[f]	57 (26.5–68.5)	57 (44–71)	0.115[e]	0.115[a]

(Continues)

TABLE 4 (Continued)

	Fever-positive short-term group pre-COVID-19 (*n*) (%)	Fever-positive short-term group post-COVID-19 (*n*) (%)	*p* Value	Fever-positive long-term group pre-COVID-19 (*n*) (%)	Fever-positive long-term group post-COVID-19 (*n*) (%)	*p* Value	Fever-positive short-term group pre-COVID-19 vs. fever-positive long-term group pre-COVID-19 *p* Value
Total motility (PR + NP, %)	72 (61–81.75)	65 (53.75–74.75)	0.543[f]	60 (30.5–72.5)	62 (46–74)	0.236[e]	0.121[a]
Progressively motile sperm count (×10^6)	143 (56.16–320.13)	57.15 (35.24–109.06)	**0.009**[e]	39.1 (14.44–144.18)	30.24 (9.47–90)	0.143[e]	**0.007**[a]
Total motile sperm count (×10^6)	153.74 (62.84–337.47)	57.15 (38.54–115.68)	**0.007**[e]	39.1 (17.74–145.11)	30.24 (10.25–91.20)	0.131[e]	**0.008**[a]
Sperm morphology (normal forms, %)	3 (2–4)	3 (2–4)	0.705[e]	2 (1–3.5)	1 (1–3)	0.083[e]	0.126[a]
Total number of normal forms (×10^6)	4.74 (2.28–13.73)	3.05 (1.77–4.46)	**0.011**[e]	1.36 (0.68–5.03)	0.92 (0.28–1.80)	**0.003**[e]	**0.001**[a]
Abnormal head (%)	94.5 (93–96.75)	94.5 (93–96)	0.618[f]	95 (92.5–98)	95 (93.5–97)	0.829[e]	0.557[a]
Abnormal midpiece (%)	1 (0–2)	1 (1–2)	0.378[e]	1 (0–3)	1 (1–2)	0.405[e]	0.497[a]
Abnormal principal piece (%)	0.50 (0–1)	1 (0–1.75)	0.197[e]	0 (0–2)	1 (0.5–2)	0.086[e]	0.448[a]
Excess residual cytoplasm (%)	0 (0–0)	0 (0–0)	0.477[e]	0 (0–0.5)	0 (0–0)	0.379[e]	0.944[a]

Note: Data presented as number (percentage of total) or median (IQR). Bold values denote statistical significance at the $p < 0.05$ level.

[a]Mann–Whitney *U* test.

[b]Independent-Samples *T* test.

[c]Pearson's χ^2.

[d]Fisher's exact test.

[e]Wilcoxon signed-rank test.

[f]Paired-aamples *T* Test.

TABLE 5 Alterations in sperm parameters in patients' exposed to drug use during COVID-19 infection.

	Fever-negative drug-positive group	Fever-negative drug-negative group	*p* Value
No. of patients (*n*)	22	*31*	
Age (years) median (IQR)	33 (31–37)	33 (32–38)	0.429[a]
BMI (kg/m^2)	25.37 (23.30–29.39)	26.54 (25.60–29.30)	0.234[b]
Smoking (*n*) (%)	10/22 (68.2%)	16/*31* (35.5%)	0.654[c]
Alcohol consumption (%)	3/22 (13.6%)	2/*31* (6.5%)	0.638[d]
Clinical varicocele presence (%)	3/22 (13.6%)	2/*31* (6.5%)	0.638[d]
Comorbidity			
Hypertension (*n*) (%)	2/22 (9.1%)	0/*31* (0%)	0.168[d]
Asthma (*n*) (%)	0/22 (0%)	1/*31* (0%)	1.000[d]
Diabetes mellitus (*n*) (%)	1/22 (4.5%)	0/*31* (0%)	0.415[d]
Time from the first semen analysis to the diagnosis of COVID-19 (days)	41.5 (31.5–68.75)	40 (30–68)	0.800[a]
Time from the diagnosis of COVID-19 to the second semen analysis (days)	82 (41.5–120)	81.5 (38–122)	0.651[a]
<75 days (*n*) (%)	8/22 (36.4%)	14/*31* (45.2%)	0.522[c]
>75 days (*n*) (%)	14/22 (63.6%)	17/*31* (54.8%)	0.522[c]
Hospitalization (*n*) (%)	2/22 (9.1%)	3/31 (9.7%)	1.000[d]

	Fever-negative drug-positive group pre-COVID-19 (*n*) (%)	Fever-negative drug-positive group post-COVID-19 (*n*) (%)	*p* Value	Fever-negative drug-negative group pre-COVID-19 (*n*) (%)	Fever-negative drug-negative group post-COVID-19 (*n*) (%)	*p* Value	Fever-negative drug-positive group pre-COVID-19 vs. fever-negative drug-negative group pre-COVID-19 *p* Value
Sexual abstinence(days)	3 (3–4)	4 (3–4)	0.564[e]	4 (3–4)	4 (3–4)	1.000[e]	0.840[a]
Semen volume (mL)	3.75 (3–5.5)	3 (2–4)	0.058[e]	3 (2–4)	2.5 (2–3.5)	**0.002**[e]	0.167[a]
Sperm concentration (×10^6/mL)	38.5 (16.25–60.5)	33.5 (24.75–74.5)	0.434[e]	29 (17–60)	35 (16–72)	0.681[e]	0.607[a]
Total number of sperm per ejaculate (×10^6)	132 (67.63–239.25)	119 (46.5–260.25)	0.322[e]	94 (65.5–214.5)	100.5 (32–176)	0.213[e]	0.367[a]

(Continues)

TABLE 5 (Continued)

	Fever-negative drug-positive group pre-COVID-19 (*n*) (%)	Fever-negative drug-positive group post-COVID-19 (*n*) (%)	*p* Value	Fever-negative drug-negative group pre-COVID-19 (*n*) (%)	Fever-negative drug-negative group post-COVID-19 (*n*) (%)	*p* Value	Fever-negative drug-positive group pre-COVID-19 vs. fever-negative drug-negative group pre-COVID-19 *p* Value
Progressive motility (PR, %)	58.5 (46.25–74.25)	65 (56–70)	0.219[f]	60 (49–70)	62 (50–71)	0.416[f]	0.825[b]
Total motility (PR + NP,%)	60 (55.75–76.5)	66.5 (59.5–76.25)	0.446[f]	64 (50–72)	62 (53–72)	0.520[f]	0.712[b]
Progressively motile sperm count ($\times 10^6$)	64.68 (30.36–163.73)	66.47 (25.53–182.99)	0.485[e]	51 (12.87–147.6)	60.68 (14.95–123.2)	0.814[e]	0.417[a]
Total motile sperm count ($\times 10^6$)	76.19 (30.36–167.42)	68.79 (27.83–184.31)	0.372[e]	63.25 (18.36–147.6)	60.68 (17.55–126.72)	0.610[e]	0.427[a]
Sperm morphology (normal forms, %)	3 (2.75–3.25)	3 (2–3)	0.564[e]	3 (2–4)	3 (2–4)	0.317[e]	0.712[a]
Total number of normal forms ($\times 10^6$)	3.64 (1.65–7.81)	3.48 (0.98–8.04)	0.173[e]	2.53 (0.74–5.4)	1.94 (1.01–4.44)	0.229[e]	0.339[a]
Abnormal head (%)	96 (93.75–97)	95 (93–96)	0.470[f]	94 (92–96)	95 (93–96)	0.318[e]	0.163[a]
Abnormal midpiece (%)	1 (0–1)	1 (1–2)	0.059[e]	1 (0–2)	1 (1–2)	0.491[e]	0.215[a]
Abnormal principal piece (%)	0 (0–1.25)	0.5 (0–1)	0.782[e]	1 (0–2)	1 (0–2)	0.837[e]	0.129[a]
Excess residual cytoplasm (%)	0 (0–0)	0 (0–0)	0.496[e]	0 (0–1)	0 (0–0)	0.255[e]	0.273[a]

Note: Data presented as number (percentage of total) or median (IQR). Bold value denotes statistical significance at the $p < 0.05$ level.

[a]Mann–Whitney *U* test.

[b]Independent-samples *T* test.

[c]Pearson's χ^2.

[d]Fisher's exact test.

[e]Wilcoxon signed-rank test.

[f]Paired-samples *T* Test.

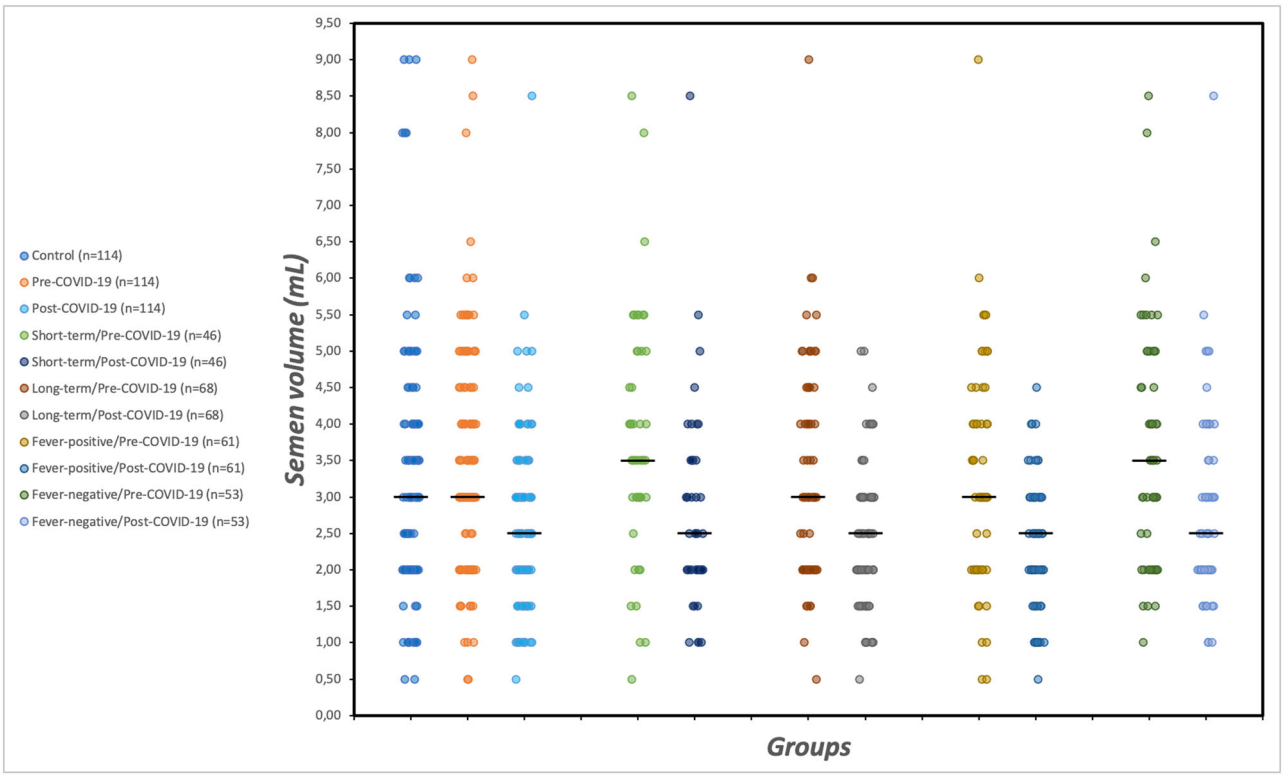

FIGURE 2 The sperm volume values of individuals in each group. Each distinct dot on the graph represents the sperm volume value of each individual. Each solid line on the graph represents the median semen volume value for the respective group.

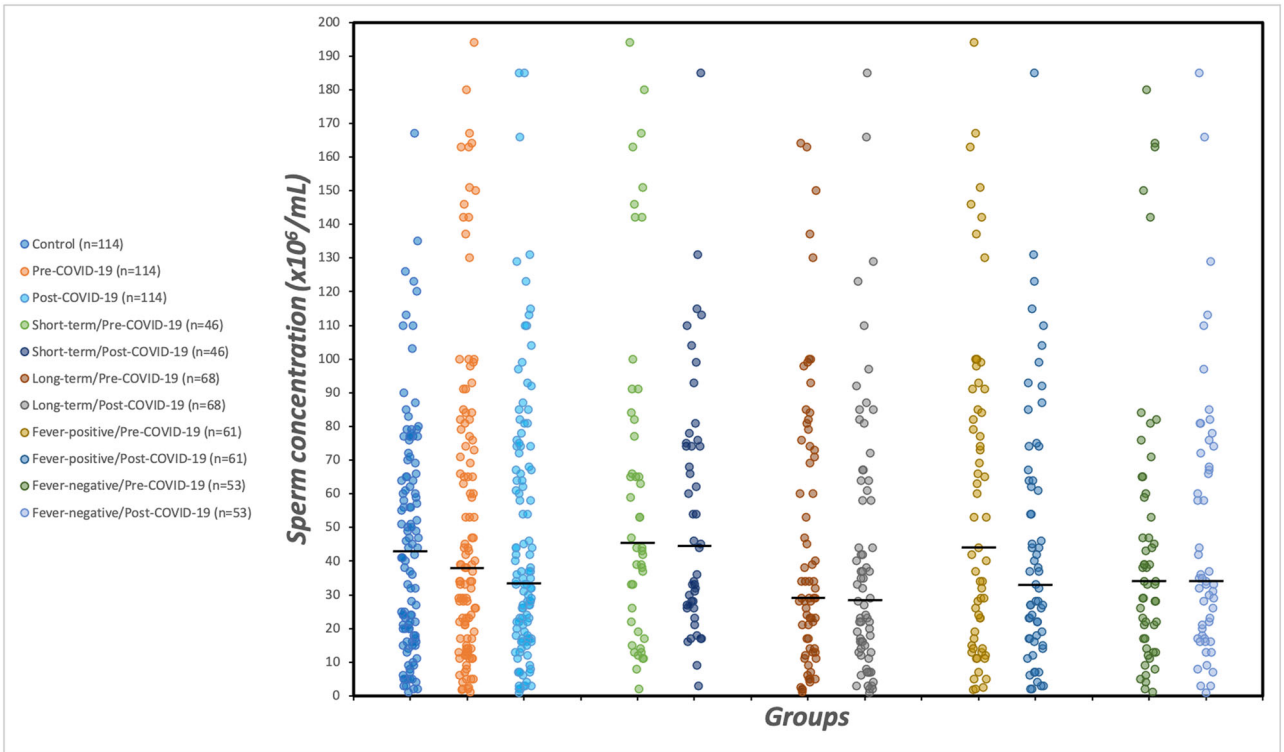

FIGURE 3 The sperm concentration values of the individuals in each group. Each distinct dot on the graph represents the sperm concentration value of each individual. Each solid line on the graph represents the median sperm concentration value for the respective group.

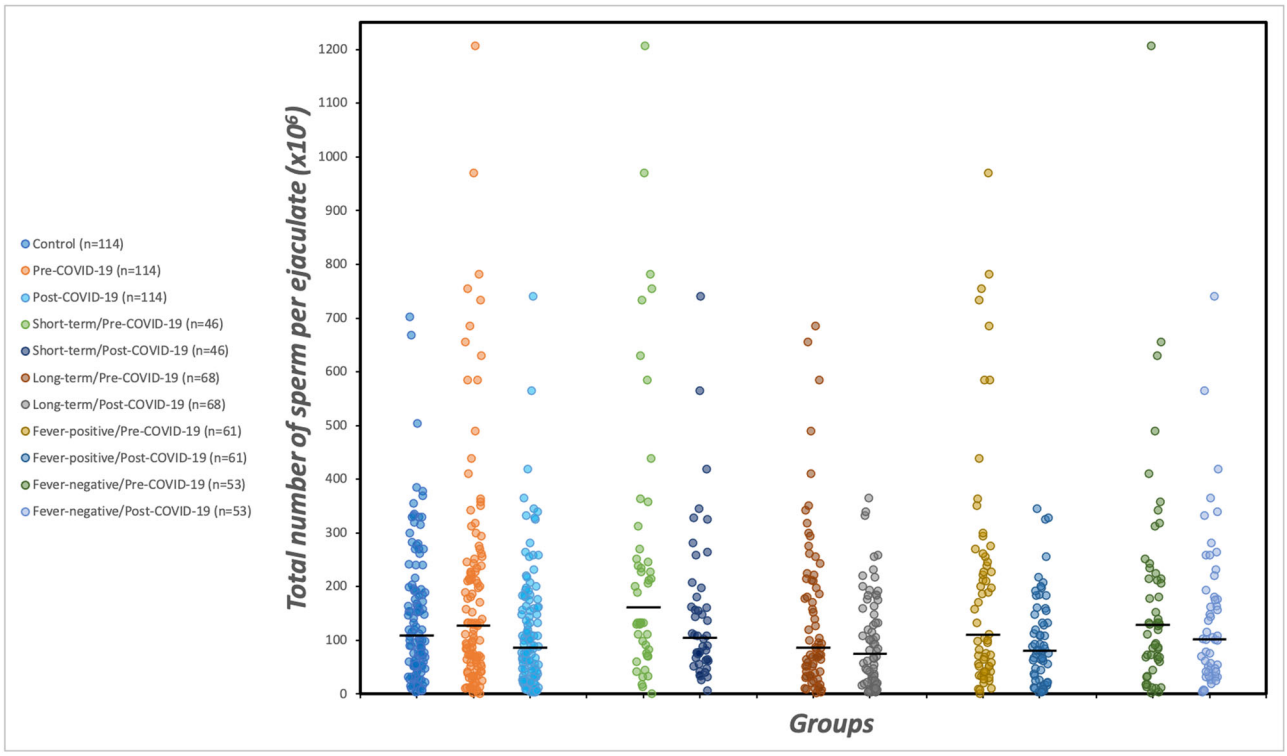

FIGURE 4 The total number of sperm per ejaculate values of the individuals in each group. Each distinct dot on the graph represents the total number of sperm per ejaculate value of each individual. Each solid line on the graph represents the median total number of sperm per ejaculate value for the respective group.

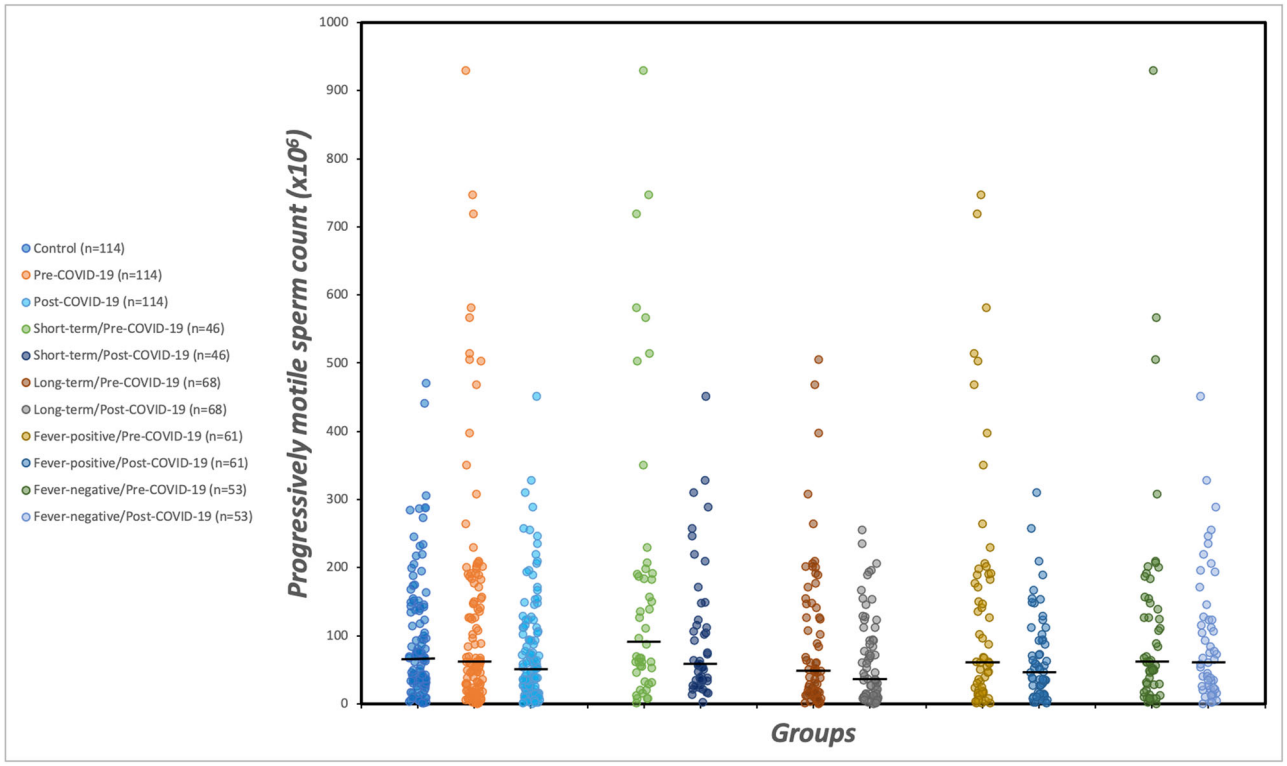

FIGURE 5 The progressively motile sperm count values of the individuals in each group. Each distinct dot on the graph represents the progressively motile sperm count value of each individual. Each solid line on the graph represents the median progressively motile sperm count value for the respective group.

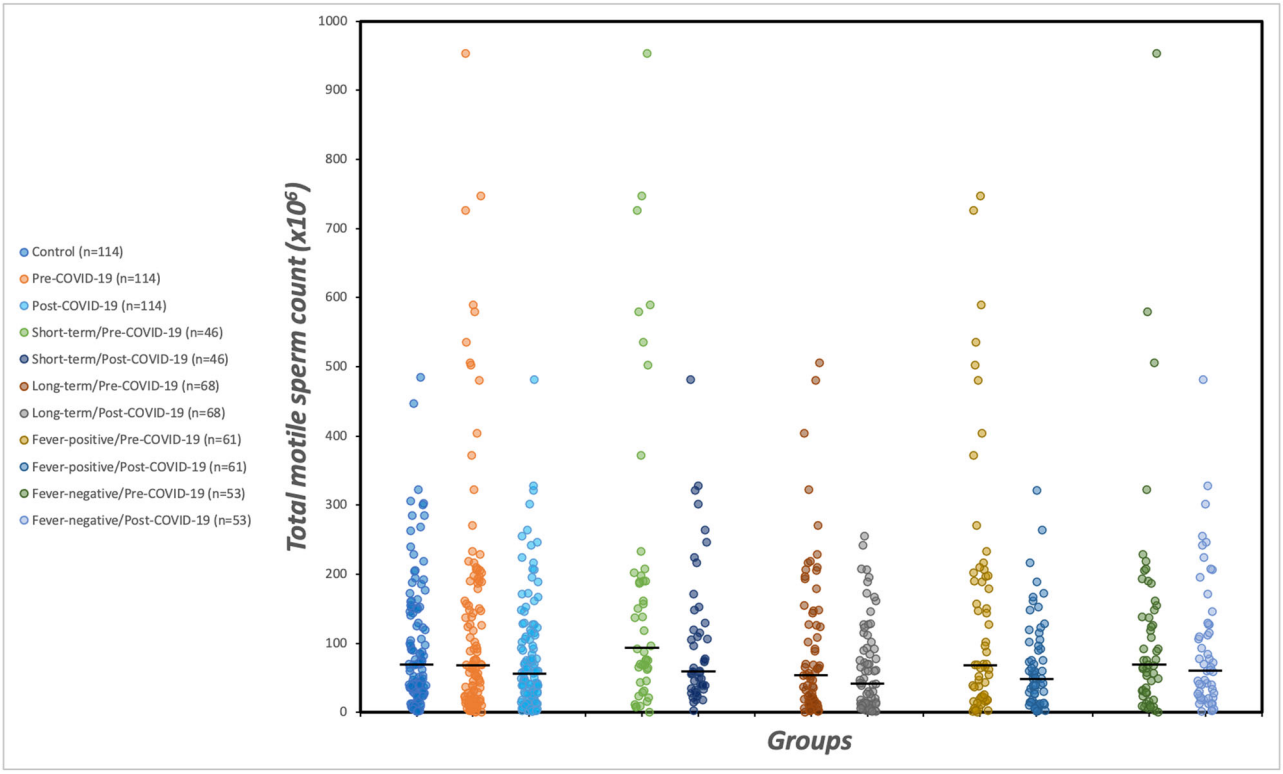

FIGURE 6 The total motile sperm count values of the individuals in each group. Each distinct dot on the graph represents the total motile sperm count value of each individual. Each solid line on the graph represents the median total motile sperm count value for the respective group.

important marker for semen parameters. We also determined that only the semen volume of patients without fever decreased significantly after infection compared to the previous values, and that there was no change in all other parameters. In contrast, patients who experienced fever during the COVID-19 period showed a significant decrease in semen volume, sperm concentration, total number of sperm per ejaculate, progressive motile sperm count, total motile sperm count, and total number of sperm with normal morphology after COVID-19. Both short-term and long-term analyses of patients experiencing fever during illness demonstrated significant reductions in semen volume, sperm concentration, total sperm count per ejaculation, and the total number of normal sperm compared to pre-COVID-19 infection. Additionally, it was observed that while the parameters of progressive motile sperm count and total motile sperm count decreased in the short-term following infection, they remained comparable in the long-term groups before and after the disease. It is important to note that there is currently a lack of standardized definitions for fever in COVID-19 patients, which should be taken into consideration when interpreting the findings of previous studies and our current study.

We would like to highlight an important aspect regarding the patient population included in our study, namely, a significant proportion of individuals received treatment with hydroxychloroquine and favipiravir during their course of treatment. This raises the possibility that these medications might impact the process of spermatogenesis. To address this question, we aimed to assess patients who received and did not receive these drugs separately, during the active phase of COVID-19 infection, within two distinct groups. However, we recognized that the higher usage rates of hydroxychloroquine (60.7% vs. 41.5%, $p = 0.041$) and favipiravir (50.8% vs. 32.1%, $p = 0.043$) in the fever positive group compared to the fever negative group could introduce potential bias and lead to misleading findings. Consequently, we decided to solely examine the impact of drug usage on semen parameters within the subgroup of patients without fever. Despite the limited sample size in this subgroup analysis, we found no detrimental effects on semen parameters associated with the use of hydroxychloroquine or favipiravir. Our findings are supported by the absence of evidence in previous studies indicating any negative impact of hydroxychloroquine or favipiravir on male fertility.[45,46] A safety study that evaluated semen parameters showed that favipiravir did not affect sperm concentration or motility.[47] Moreover, medications such as steroids or ribavirin, which are known to negatively affect semen parameters, were not used in the patient group included in the study.

The impact of COVID-19 on semen volume has been a subject of controversy in previous studies. While some studies have reported a decrease in semen volume following COVID-19 infection,[24,30,32] other studies have found no effect on semen volume.[14–16,18,19,23,24,26–28,31] In our study, we compared semen volume in COVID-19 patients before and after infection, as well as in subgroups with different time intervals and the presence or

absence of fever. Our findings showed a significant decrease in semen volume after COVID-19 infection. This finding may be explained by the potential impact of COVID-19 on prostate cells and seminal vesicles, which are major contributors to the production of seminal fluid. The prostate and seminal vesicles have been shown to express high levels of ACE2 and TMPRSS2, which are the receptors that COVID-19 uses to enter host cells.[6,48,49]

Although numerous studies have evaluated the effects of COVID-19 on semen parameters, our knowledge about the subject remains limited due to several limitations in previous research. Notably, a critical limitation of many studies was the lack of pre-COVID-19 semen parameter data for the included patients. When reviewing the existing literature, several studies have compared semen analysis results before and after the diagnosis of COVID-19 within the same patients, similar to our study.[23,24,30,31] However, these previous studies have certain limitations. Many of these studies suffered from small sample sizes, analysis of a single patient sample, short follow-up periods, retrospective data analysis, and the inclusion of patients with varying disease severity and viral loads. For instance, in the study conducted by Pazir and colleagues, the comparison of semen analysis results before and after COVID-19 diagnosis was limited by a small sample size ($n = 24$). Furthermore, the study did not assess the short-term or long-term effects of COVID-19 on male fertility.[31] Similarly, the study conducted by Erbay et al.[30] had a relatively small sample size ($n = 69$) and employed a multicenter design, potentially leading to inadequate standardization in laboratory analysis procedures. The studies conducted by Koç et al.[24] and Hamarat et al.[23] which examined pre- and post-COVID-19 semen analysis results, did not include subgroup analyses based on disease severity or symptom analysis. These studies, like others, were also limited by small sample sizes ($n = 21$ and $n = 41$, respectively). In comparison, our current study, conducted at a single center, exhibits notable strengths in terms of a relatively large sample size and comprehensive assessment of both fever presence/absence and short-term/long-term effects of COVID-19.

Our study has several limitations, including its retrospective design, lack of biochemical or histopathological data beyond semen parameters, inability to perform a power analysis, and reliance on a single semen sample before and after COVID-19. Additionally, the severity of the disease could not be determined with certainty due to the absence of detailed symptom analysis in our patient cohort. On the other hand, the current study has several strengths. It compared pre-COVID-19 and post-COVID-19 sperm parameters within the same patient and was performed by the same embryologist in a single center. The study also included results about the short-term and long-term effects of COVID-19, as well as the presence or absence of fever. Notably, to the best of our knowledge, our study had the largest sample size among studies investigating the effect of SARS-CoV-2 infection on semen parameters.

In conclusion, our study indicates that SARS-CoV-2 infection has a negative impact on semen parameters, particularly in terms of semen volume, total sperm count, and total motile sperm count. Moreover, the presence of fever during active infection is a significant risk factor affecting spermatogenesis. It is important to note that the decline in semen parameters may not improve in the long term. There is a need for prospective studies that include larger sample sizes to confirm or update our findings on the effect of SARS-CoV-2 on semen parameters. Future studies should aim to prospectively analyze a series of semen samples from the same patient, monitor both clinical outcomes and offspring results, and evaluate testicular and accessory genital organs with multiple parameters. Further investigation is necessary to understand the mechanism by which COVID-19 affects the male reproductive system and to develop potential therapeutic interventions. This will ensure that patients who have recovered from COVID-19 can be properly advised regarding the appropriate monitoring of reproductive functions and preservation of fertility.

AUTHOR CONTRİBUTİONS

Berrin Avci and Cihan Cakir contributed to conception and design of the study. Goktan Kuspinar, Gizem Kurt, and Miray Berber contributed to the laboratory analysis of semen. Cihan Cakir and Kiper Aslan contributed to the data collection and processing. Gurkan Uncu, Isil Kasapoglu, and Kiper Aslan provided the clinical data. Cihan Cakir was responsible for the manuscript drafting. All authors interpreted data, provided critical review, and approved the final version of the manuscript. All authors contributed to the study.

CONFLICT OF INTEREST STATEMENT

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.

ORCID

Cihan Cakir https://orcid.org/0000-0002-8332-7353
Goktan Kuspinar https://orcid.org/0000-0002-0338-8368
Gizem Kurt https://orcid.org/0000-0001-5828-610X
Miray Berber https://orcid.org/0000-0003-3017-7352
Kiper Aslan https://orcid.org/0000-0002-9277-7735
Isil Kasapoglu https://orcid.org/0000-0002-1953-2475
Gurkan Uncu https://orcid.org/0000-0001-7660-8344
Berrin Avcı https://orcid.org/0000-0001-8135-5468

REFERENCES

1. WHO. *WHO Director-General's Opening Remarks at the Media Briefing on COVID-19-11 March 2020-World Health Organization*. World Health Organization; 2020. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020
2. Johns H. *COVID-19 Map-Johns Hopkins Coronavirus Resource Center*. Johns Hopkins Coronavirus Resource Center; 2022. https://coronavirus.jhu.edu/map.html
3. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*. 2020;5(4):562-569. doi:10.1038/s41564-020-0688-y

4. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181(2):271-280. doi:10.1016/j.cell.2020.02.052
5. Matsuyama S, Nao N, Shirato K, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2- expressing cells. *Proc Natl Acad Sci*. 2020;117(13):7001-7003. doi:10.1073/pnas.2002589117
6. Qi J, Zhou Y, Hua J, et al. The scRNA-seq expression profiling of the receptor ACE2 and the cellular protease TMPRSS2 reveals human organs susceptible to SARS-CoV-2 infection. *Int J Environ Res Public Health*. 2021;18(1):284. doi:10.3390/ijerph18010284
7. Kim J, Thomsen T, Sell N, Goldsmith AJ. Abdominal and testicular pain: an atypical presentation of COVID-19. *Am J Emerg Med*. 2020;38(7):1542.e1-1542.e3. doi:10.1016/j.ajem.2020.03.052
8. Chen L, Huang X, Yi Z, et al. Ultrasound imaging findings of acute testicular infection in patients with coronavirus disease 2019: a single-center–based study in Wuhan, China. *J Ultrasound Med*. 2021;40(9):1787-1794. doi:10.1002/jum.15558
9. Carneiro F, Teixeira TA, Bernardes FS, et al. Radiological patterns of incidental epididymitis in mild-to-moderate COVID-19 patients revealed by colour Doppler ultrasound. *Andrologia*. 2021;53(4):e13973. doi:10.1111/and.13973
10. Duarte-Neto AN, Teixeira TA, Caldini EG, et al. Testicular pathology in fatal COVID-19: a descriptive autopsy study. *Andrology*. 2022;10(1):13-23. doi:10.1111/andr.13073
11. Ma X, Guan C, Chen R, et al. Pathological and molecular examinations of postmortem testis biopsies reveal SARS-CoV-2 infection in the testis and spermatogenesis damage in COVID-19 patients. *Cell Mol Immunol*. 2021;18(2):487-489. doi:10.1038/s41423-020-00604-5
12. Yang M, Chen S, Huang B, et al. Pathological findings in the testes of COVID-19 patients: clinical implications. *Eur Urol Focus*. 2020;6(5):1124-1129. doi:10.1016/j.euf.2020.05.009
13. Ma L, Xie W, Li D, et al. Evaluation of sex-related hormones and semen characteristics in reproductive-aged male COVID-19 patients. *J Med Virol*. 2021;93(1):456-462. doi:10.1002/jmv.26259
14. Temiz MZ, Dincer MM, Hacibey I, et al. Investigation of SARS-CoV-2 in semen samples and the effects of COVID-19 on male sexual health by using semen analysis and serum male hormone profile: a cross-sectional, pilot study. *Andrologia*. 2021;53(2):e13912. doi:10.1111/and.13912
15. Hu B, Liu K, Ruan Y, et al. Evaluation of mid- and long-term impact of COVID-19 on male fertility through evaluating semen parameters. *Transl Androl Urol*. 2022;11(2):159-167. doi:10.21037/tau-21-922
16. Aksak T, Satar DA, Bağci R, Gültekin EO, Coşkun A, Demirdelen U. Investigation of the effect of COVID-19 on sperm count, motility, and morphology. *J Med Virol*. 2022;94:5201-5205. doi:10.1002/jmv.27971
17. Paoli D, Pallotti F, Anzuini A, et al. Male reproductive health after 3 months from SARS-CoV-2 infection: a multicentric study. *J Endocrinol Invest*. 2022;46:89-101. doi:10.1007/s40618-022-01887-3
18. Enikeev D, Taratkin M, Morozov A, et al. Prospective two-arm study of the testicular function in patients with COVID-19. *Andrology*. 2022;10(6):1047-1056. doi:10.1111/andr.13159
19. Wang M, Hu J, Huang B, et al. Investigating the impact of SARS-CoV-2 infection on basic semen parameters and in vitro fertilization/intracytoplasmic sperm injection outcomes: a retrospective cohort study. *Reprod Biol Endocrinol*. 2022;20(1):46. doi:10.1186/s12958-022-00918-1
20. Donders GGG, Bosmans E, Reumers J, et al. Sperm quality and absence of SARS-CoV-2 RNA in semen after COVID-19 infection: a prospective, observational study and validation of the SpermCOVID test. *Fertil Steril*. 2022;117(2):287-296. doi:10.1016/j.fertnstert.2021.10.022
21. Hajizadeh Maleki B, Tartibian B. COVID-19 and male reproductive function: a prospective, longitudinal cohort study. *Reproduction*. 2021;161(3):319-331. doi:10.1530/REP-20-0382
22. Gacci M, Coppi M, Baldi E, et al. Semen impairment and occurrence of SARS-CoV-2 virus in semen after recovery from COVID-19. *Hum Reprod*. 2021;36(6):1520-1529. doi:10.1093/humrep/deab026
23. Hamarat MB, Ozkent MS, Yılmaz B, Aksanyar SY, Karabacak K. Effect of sars-cov-2 infection on semen parameters. *Can Urol Assoc J*. 2021;16(3):E173-E177. doi:10.5489/cuaj.7292
24. Koç E, Keseroğlu BB. Does COVID-19 worsen the semen parameters? Early results of a tertiary healthcare center. *Urol Int*. 2021;105(9-10):743-748. doi:10.1159/000517276
25. Ali BR, Atiyah SA, Yser HT, Khelewe AM, Hameed HN. The influence of SARS-CoV -2 on semen parameters of infected infertile male in comparison with those that noninfected. *J Clin Lab Anal*. 2022;36(8):e24568. doi:10.1002/jcla.24568
26. Best JC, Kuchakulla M, Khodamoradi K, et al. Evaluation of SARS-CoV-2 in human semen and effect on total sperm number: a prospective observational study. *World J Men's Health*. 2021;39(3):489. doi:10.5534/WJMH.200192
27. Ruan Y, Hu B, Liu Z, et al. No detection of SARS-CoV-2 from urine, expressed prostatic secretions, and semen in 74 recovered COVID-19 male patients: a perspective and urogenital evaluation. *Andrology*. 2021;9(1):99-106. doi:10.1111/andr.12939
28. Guo TH, Sang MY, Bai S, et al. Semen parameters in men recovered from COVID-19. *Asian J Androl*. 2021;23(5):479-483. doi:10.4103/aja.aja_31_21
29. Li H, Xiao X, Zhang J, et al. Impaired spermatogenesis in COVID-19 patients. *EClinicalMedicine*. 2020;28:100604. doi:10.1016/j.eclinm.2020.100604
30. Erbay G, Sanli A, Turel H, et al. Short-term effects of COVID-19 on semen parameters: a multicenter study of 69 cases. *Andrology*. 2021;9(4):1060-1065. doi:10.1111/andr.13019
31. Pazir Y, Eroglu T, Kose A, Bulut TB, Genc C, Kadihasanoglu M. Impaired semen parameters in patients with confirmed SARS-CoV-2 infection: a prospective cohort study. *Andrologia*. 2021;53(9):e14157. doi:10.1111/and.14157
32. Holtmann N, Edimiris P, Andree M, et al. Assessment of SARS-CoV-2 in human semen—a cohort study. *Fertil Steril*. 2020;114(2):233-238. doi:10.1016/j.fertnstert.2020.05.028
33. W.H.O. *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 6th ed. W.H.O; 2021.
34. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril*. 1988;49(1):112-117. doi:10.1016/S0015-0282(16)59660-5
35. Kadihasanoglu M, Aktas S, Yardimci E, Aral H, Kadioglu A. SARS-CoV-2 pneumonia affects male reproductive hormone levels: a prospective, cohort study. *J Sex Med*. 2021;18(2):256-264. doi:10.1016/j.jsxm.2020.11.007
36. Guo L, Zhao S, Li W, et al. Absence of SARS-CoV-2 in semen of a COVID-19 patient cohort. *Andrology*. 2021;9(1):42-47. doi:10.1111/andr.12848
37. Li F, Zhang H, Shi W, et al. Controlled ovarian hyperstimulation protocol in infertile patients during the COVID-19 pandemic. *Front Physiol*. 2021;12:12. doi:10.3389/fphys.2021.732709
38. Flaifel A, Guzzetta M, Occidental M, et al. Testicular changes associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Arch Pathol Lab Med*. 2021;145(1):8-9. doi:10.5858/arpa.2020-0487-LE
39. Achua JK, Chu KY, Ibrahim E, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections on testis. *World J Men's Health*. 2021;39(1):65-74. doi:10.5534/wjmh.200170
40. Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical characteristics and results of semen tests among men with coronavirus disease 2019.

JAMA Netw Open. 2020;3(5):e208292. doi:10.1001/jamanetworkopen.2020.8292

41. Heller CG, Clermont Y. Spermatogenesis in man: an estimate of its duration. *Science (80-)*. 1963;140(3563):184-186. doi:10.1126/science.140.3563.184.
42. Falahieh FM, Zarabadipour M, Mirani M, et al. Effects of moderate COVID-19 infection on semen oxidative status and parameters 14 and 120 days after diagnosis. *Reprod Fertil Dev*. 2021;33(12):683-690. doi:10.1071/RD21153
43. Sergerie M, Mieusset R, Croute F, Daudin M, Bujan L. High risk of temporary alteration of semen parameters after recent acute febrile illness. *Fertil Steril*. 2007;88(4):970.e1-970.e7. doi:10.1016/j.fertnstert.2006.12.045
44. Carlsen E. History of febrile illness and variation in semen quality. *Hum Reprod*. 2003;18(10):2089-2092. doi:10.1093/humrep/deg412
45. Drobnis EZ, Nangia AK. Antivirals and male reproduction. *Adv Exp Med Biol*. 2017;1034:163-178. doi:10.1007/978-3-319-69535-8_11
46. Bermas BL. Paternal safety of anti-rheumatic medications. *Best Pract Res Clin Obstet Gynaecol*. 2020;64:77-84. doi:10.1016/j.bpobgyn.2019.09.004
47. Pilkington V, Pepperrell T, Hill A. A review of the safety of favipiravir—a potential treatment in the COVID-19 pandemic? *J Virus Erad*. 2020;6(2):45-51. doi:10.1016/S2055-6640(20)30016-9
48. Fan C, Lu W, Li K, Ding Y, Wang J. ACE2 expression in kidney and testis may cause kidney and testis infection in COVID-19 patients. *Front Med*. 2021;7:563893. doi:10.3389/fmed.2020.563893
49. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. *Infect Dis Poverty*. 2020;9(1):45. doi:10.1186/s40249-020-00662-x

How to cite this article: Cakir C, Kuspinar G, Kurt G, et al. Comparison of semen parameters in the same patients before and after diagnosis of COVID-19. *J Med Virol*. 2023;95:e29094. doi:10.1002/jmv.29094