INTRODUCTION

In December 2019, China reported a group of pneumonia cases of unknown etiology to the World Health Organization (WHO). A novel beta-coronavirus was detected in samples taken from the patients’ lower respiratory tract (Zhu et al., 2020). Due to the similarity of its genomic structure with severe acute respiratory syndrome coronavirus (SARS-CoV), it was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Gralinski & Menachery, 2020). The highly contagious coronavirus disease 2019 (COVID-19) spread rapidly, causing a pandemic.

SARS-CoV-2 infects host cells through angiotensin-converting enzyme 2 (ACE2) receptors (Bourgonje et al., 2020). In addition, transmembrane serine protease 2 (TMPRSS2) plays a major role in the entry of SARS-CoV-2 into the host cell. ACE2 and TMPRSS2 have been shown to be highly expressed in spermatogonia and Sertoli and Leydig cells (Wang & Xu, 2020). SARS-CoV-2 causes the downregulation of ACE2 expression after entering the host cell, resulting in excessive angiotensin production (Dijkman et al., 2012; Gurwitz, 2020). Overproduction of angiotensin leads to apoptosis by increasing reactive oxygen species (Aitken, 2021). In addition, excessive production of proinflammatory cytokines induced by SARS-CoV-2 infection may adversely affect spermatogenesis by increasing autoimmune response and leukocyte infiltration in the testis (Hedger & Meinhardt, 2003; Mahmudpour et al., 2020; Rival et al., 2006; Xu et al., 2006). All these findings suggest that SARS-CoV-2 infection...
may affect the testis and lead to possible harmful effects on spermatogenesis and testicular endocrine function (Kadihasanoglu et al., 2021).

To date, more than 27 viruses, including mumps, Zika and HIV, have been detected in semen, indicating that a virus has the potential to infect the organs of the male reproductive system (Gornet et al., 2016; Ternavasio-de la Vega et al., 2010; Zafer et al., 2016; Zea-Mazo et al., 2010). Moreover, besides the risk of transmission, the presence of some viruses, such as HIV, HBV and HCV, in semen has been reported to have detrimental effects on sperm DNA integrity and semen parameters (Garolla et al., 2013). However, there are limited data and conflicting results in the literature regarding the presence of SARS-CoV-2 in semen and its effects on semen parameters (Holtmann et al., 2020; Li et al., 2020; Pan et al., 2020; Paoli et al., 2020).

The aim of this study was to investigate the possible effects of SARS-CoV-2 infection on semen parameters in men recovering from COVID-19.

2 | PATIENTS AND METHODS

2.1 | Study design

This prospective cohort study was conducted in a tertiary academic centre between June 2020 and November 2020. Between these dates, a total of 24 male patients with a confirmed diagnosis of COVID-19 disease were prospectively evaluated. For all the participants, data on demographic and clinical characteristics, medications used for the treatment of COVID-19, and semen analyses were collected.

2.2 | Ethical approval

This study was approved by the Institutional Review Board (Approval number: 2020–2641) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each participant.

2.3 | Study population

Between January 2018 and June 2020, approximately 5,000 men presented to the urology outpatient clinic for a reproductive function evaluation. Of these patients, those aged 18–49 years whose semen analyses (including semen volume, sperm concentration, and motility) before COVID-19 were within the normal reference range according to the WHO 2010 fifth edition criteria were contacted by phone and invited to participate in the study. Among the cases that visited the department after this invitation, the availability of the results of a reverse transcription-polymerase chain reaction (RT-PCR) test of pharyngeal and nasal swabs performed due to suspected SARS-CoV-2 infection was checked. A total of 124 men were positive for SARS-CoV-2 in the RT-PCR test. The exclusion criteria were: (1) previous exposure to empirical treatments, such as anti-oestrogens, antioxidants, and gonadotropins; (2) a non-COVID-19 febrile illness in the last three months; (3) a previous urogenital tract infection history; and (4) testicular diseases, such as varicocele and undescended testis, which may be associated with deterioration in semen parameters over time. Individuals meeting the study criteria were asked to give a sample for semen analysis. The data of 24 men who agreed to participate in the study were included in the final analysis. Written informed consent for research purposes was obtained from each participant.

The severity of COVID-19 was determined according to the criteria in the COVID-19 guidelines (Xu et al., 2020). Accordingly, the mild disease was defined as having any of the symptoms of COVID-19 but not having lung involvement on chest imaging (X-ray and/or computed tomography). The presence of lung involvement in imaging and oxygen saturation (SpO$_2$ ≥ 94% in room air) was accepted as a moderate disease. Severe respiratory problems (SpO$_2$ < 94% and respiratory frequency >30 breaths/min) and more than 50% lung infiltration were accepted as severe disease.

2.4 | Semen analysis

Semen samples were obtained from the participants by masturbation after a three-day abstinence period during the recovery phase of COVID-19 which had been detected on the nasopharyngeal swab sample at least three months earlier. All semen analyses were carried out by the same andrology laboratory according to the WHO 2010 guidelines (Lu et al., 2010). Semen samples were placed in non-cytotoxic sterile containers and liquefied at 37°C degrees for 30 min. Semen analysis was performed within one hour of ejaculation. The gravimetric method was used for semen volume measurement. Sperm concentration was assessed using an improved Neubauer chamber. The motility of each spermatozoon was measured manually and graded as progressive motile, non-progressive motile, and immotile. Sperm morphology was not assessed due to safety concerns, similar to the study by Holtmann et al. (Holtmann et al., 2020). To investigate the impact of SARS-CoV-2 infection on semen parameters (semen volume, sperm concentration, motility, and total motile sperm count), the participants’ pre-COVID-19 and post-COVID-19 semen analyses were compared.

2.5 | Outcomes

The primary outcome of the study was the effect of SARS-CoV-2 infection on semen parameters. The secondary outcome was the effects on semen parameters in cases with and without symptoms of fever during active SARS-CoV-2 infection.
2.6 | Statistical analysis

Sample size determination was based on changes in sperm count in semen analysis. Type I error \( \alpha \) was 0.05, type II error \( \beta \) was 0.05, two-tailed \( p \)-value was less than 0.05, the difference of standard error in the pre- and post-COVID-19 semen counts was 2.7, loss to follow-up rate was 10\%, and at least 24 patients were required for the final analysis. The suitability of the data for normal distribution was examined using the Shapiro-Wilk test. Non-normally distributed data were expressed as mean or median (interquartile range) values and percentages for continuous and categorical variables, respectively. The paired \( t \)-test was used to compare the pre- and post-infection semen analyses. For statistical analyses, STATA 14 software (Stata Corp.) was used. \( p \) values less than 0.05 were accepted as statistically significant.

3 | RESULTS

The study cohort consisted of 24 men who had recovered from COVID-19. The mean age and mean body mass index of the participants were 34.7 ± 6.4 (range 21–48) years and 27.4 ± 3.7 kg/m\(^2\), respectively. Two (8.3\%) participants reported pre-existing chronic diseases, including hypertension and type II diabetes. Twelve (50\%) participants were smokers, and one (4.2\%) participant reported alcohol consumption. The demographic and clinical characteristics of the study cohort are given in Table 1.

All the participants with COVID-19 were diagnosed with mild disease and described symptoms during active infection, with the most common being myalgia (66.7\%), cough (62.5\%), and fever (50\%). Eleven (45.8\%) participants had headache, nine (37.5\%) had anosmia, and seven (29.2\%) had sore throat symptoms. The remaining symptoms are detailed in Table 1. Of the participants, 91.7\% received medical treatment due to COVID-19. Hydroxychloroquine, favipiravir, and both were used in 70.8\% (17/24), 41.7\% (10/24), and 20.8\% (5/24) of the patients, respectively.

The median interval between the positive nasopharyngeal swab test and obtaining semen samples was 111.5 (158) days. The results of the pre-COVID-19 and post-COVID-19 semen analyses of the participants are presented in Table 2. In the overall cohort, total motility (\( p = .01 \)) and total motile sperm count (\( p = .02 \)) decreased significantly after SARS-CoV-2 infection compared to the pre-infection values. There was no significant difference in semen parameters before and after COVID-19 in terms of semen volume (\( p = .56 \)), sperm concentration (\( p = .06 \)), and progressive motility (\( p = .14 \)).

In addition, the cohort was divided into two subgroups as those with and without symptoms of fever during active infection to compare the changes in semen parameters in these subgroups. In the group with fever, no statistically significant difference was found between the pre- and post-infection semen parameters (semen volume (\( p = .59 \)), sperm concentration (\( p = .12 \)), progressive motility (\( p = .88 \)), total motility (\( p = .14 \)), and total motile sperm count (\( p = .11 \)). In the group without fever, total motility (\( p = .03 \)) significantly decreased after SARS-CoV-2 infection compared to the pre-infection values; however, there was no significant change in semen volume (\( p = .78 \)), sperm concentration (\( p = .28 \)), progressive motility (\( p = .07 \)), and total motile sperm count (\( p = .07 \)). The pre- and post-COVID-19 semen parameters of the participants with and without fever during infection are presented in Table 3.

4 | DISCUSSION

The semen parameters of the men with a history of mild COVID-19 were negatively affected by SARS-CoV-2 infection. The post-COVID-19 sperm total motility and total motile sperm count significantly decreased compared to the pre-COVID-19 values (\( p = .01 \) and \( p = .02 \), respectively). In addition, although not statistically significant, the decrease in sperm concentration was notable (\( p = .06 \)). Moreover, total motility significantly decreased after SARS-CoV-2 infection among the participants without fever symptoms during COVID-19 (\( p = .03 \)).

In the literature, various viral diseases, such as Zika, HBV and HCV, have been reported to have deleterious effects on spermatogenesis (Garolla et al., 2013; Joguet et al., 2017). Lorusso et al. found

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**TABLE 1** Demographic and clinical parameters of the study cohort (\( n = 24 \))

| Parameter                        | Value |
|----------------------------------|-------|
| Age (years)                      | 34.7 ± 6.4 |
| Body mass index (kg/m\(^2\))     | 27.4 ± 3.7 |
| Comorbidity                      | 2 (8.3\%) |
| Diabetes                         | 1 (4.2\%) |
| Hypertension                     | 1 (4.2\%) |
| Smoking                          | 12 (50\%) |
| Alcohol consumption              | 1 (4.2\%) |
| COVID-19-related symptoms        |       |
| Myalgia                          | 16 (66.7\%) |
| Cough                            | 15 (62.5\%) |
| Fever                            | 12 (50\%) |
| Headache                         | 11 (45.8\%) |
| Anosmia                          | 9 (37.5\%) |
| Sore throat                      | 7 (29.2\%) |
| Dyspnea                          | 4 (16.7\%) |
| Fatigue                          | 4 (16.7\%) |
| Taste loss                       | 2 (8.3\%) |
| Treatment                        | 22 (91.7\%) |
| Hydroxychloroquine               | 17 (70.8\%) |
| Favipiravir                       | 10 (41.7\%) |
| Both hydroxychloroquine and favipiravir | 5 (20.8\%) |
| Interval between nasopharyngeal swab test and semen collection (days) (median, IQR) | 111.5 (158) |

Note: Data are presented as n (\%) and mean ± standard deviation unless specified otherwise.

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that sperm concentration, motility, morphology and viability were significantly impaired in HBV- and HCV-seropositive patients compared to the control group (Lorusso et al., 2010). Joguet et al. investigated the effects of Zika virus on semen parameters and reported an approximately 50% reduction in total sperm count and total motile sperm count on day 60 compared to day 7 after symptom onset (Joguet et al., 2017). In the current study, the mean total motile sperm count and sperm total motility were significantly decreased in patients with COVID-19.

Various studies have investigated the presence of SARS-CoV-2 in semen and reported conflicting results. Li et al. identified SARS-CoV-2 RNA in the semen samples of patients with acute infection and those that had recovered from the disease (Li et al., 2020). However, in other studies on this topic, the presence of SARS-CoV-2 viral RNA was not detected in any of the semen samples of active infection or recovery groups (Holtmann et al., 2020; Pan et al., 2020; Paoli et al., 2020; Song et al., 2020).

The presence of SARS-CoV-2 in semen has motivated researchers to focus on the impact of COVID-19 on semen parameters. In a prospective cohort study by Holtmann et al., the semen analysis of 18 patients who had recovered from COVID-19 with mild or moderate symptoms and 14 healthy volunteers were evaluated (Holtmann et al., 2020). The authors found that patients exhibiting moderate symptoms had a significant deterioration in semen parameters (sperm concentration, total sperm count, total progressive motile sperm count, and total motile sperm count) compared with those who had recovered from mild COVID-19 and the control group. In another study, Guo et al. collected semen samples from 18 patients with mild infection and five with moderate infection during the acute and recovery periods of SARS-CoV-2 infection (Guo et al., 2021). They reported that semen parameters were within the normal range after COVID-19. However, the major limitation of these two studies is that patients’ pre-COVID-19 semen parameters were not available. In our study, the study cohort consisted of participants with mild symptomatic disease, and unlike the previous two studies, we demonstrated that sperm total motility and total motile sperm count significantly decreased after mild COVID-19. Additionally, although not statistically significant, the decrease in sperm concentration was notable. The differences and strengths of our study compared to previous research include the higher number of cases, the presence of a healthy study population with normal semen parameters before COVID-19, and the comparison of sperm parameters before and after COVID-19. We consider that this provided more accurate information about the effect of COVID-19 on semen parameters.

Viral diseases can directly affect spermatogenesis and testicular endocrine function through viral invasion, secondary inflammatory, or immunological response (Hedger, 2011; Satie et al., 2011). In addition, high fever, one of the most common symptoms of COVID-19, can have an indirect negative effect on spermatogenesis by causing degeneration of germ cells (Xu et al., 2000). Considering the duration of spermatogenesis, semen parameters, such as sperm concentration and motility, may continue to decrease for 72–90 days after febrile infection (Carlsen et al., 2003; Jung & Schuppe, 2007). Since semen parameters can take up to three months to return to the basal state, it is desirable to perform their analysis after at least three months in order to more accurately assess the effects of viral diseases. In the current study, the median interval between the positive nasopharyngeal swab and semen collection was approximately four months, and according to our findings, sperm total motility and total motile sperm count in the overall cohort were significantly lower compared to their pre-COVID-19 values. Interestingly, unlike the overall cohort, there

### TABLE 2 Pre-COVID-19 and post-COVID-19 semen parameters of the study cohort (n = 24)

| Semen parameter          | Pre-COVID-19 | Post-COVID-19 | p  |
|--------------------------|--------------|---------------|----|
| Volume (ml)              | 3.6 ± 1.6    | 3.5 ± 1.5     | .56|
| Sperm concentration (million/ml) | 42.6 ± 18.0 | 35.3 ± 20.2   | .06|
| Progressive motility (%) | 34.5 ± 1.5   | 28.9 ± 9.1    | .14|
| Total motility (%)       | 45.8 ± 5.0   | 40.4 ± 10.9   | .01|
| Total motile sperm count (million) | 77.6 ± 72.4 | 55.7 ± 47.7   | .02|

Note: Data are presented as mean ± standard deviation.

### TABLE 3 Pre-COVID-19 and post-COVID-19 semen parameters of the participants with and without fever during infection

| Semen parameter          | Fever positive (n = 12) | Fever negative (n = 12) |
|--------------------------|-------------------------|-------------------------|
|                         | Pre-COVID-19 | Post-COVID-19 | p  | Pre-COVID-19 | Post-COVID-19 | p  |
| Volume (ml)              | 3.6 ± 1.4    | 3.5 ± 1.5     | .59| 3.6 ± 1.4    | 3.5 ± 1.6     | .78|
| Sperm concentration (million/ml) | 44.9 ± 20.9  | 36.6 ± 18.8   | .12| 40.3 ± 15.2  | 33.9 ± 22.1   | .28|
| Progressive motility (%) | 34.2 ± 1.7   | 30.3 ± 11.1   | .88| 34.7 ± 1.4   | 27.5 ± 6.9    | .07|
| Total motility (%)       | 46.7 ± 4.9   | 41.6 ± 12.1   | .14| 45.0 ± 5.2   | 39.2 ± 9.9    | .03|
| Total motile sperm count (million) | 89.5 ± 74.2  | 63.7 ± 57.7   | .11| 65.7 ± 41.8  | 46.4 ± 34.1   | .07|

Note: Data are presented as mean ± standard deviation.
was no significant difference between semen parameters before and after COVID-19 among the participants with fever during active infection. Additionally, in the group of participants without fever, only total motility significantly decreased after COVID-19.

Various medications, such as monoclonal antibodies, corticosteroids, anti-viral, anti-rheumatic and anti-malarial agents are used in the treatment of COVID-19. More than 90% of the cases in our cohort were given hydroxychloroquine and/or favipiravir in the treatment of COVID-19, which is in line with the recommendations of guidelines (Xu et al., 2020). This brings to mind the possibility that these medications may also have an effect on semen parameters. However, there is no evidence that hydroxychloroquine or azithromycin has a negative effect on male fertility and semen parameters (Bermas, 2020; Drobnis & Nangia, 2017; Temiz et al., 2021). In a safety study evaluating the effect of favipiravir on testicular function, no harmful effects on sperm count and motility were detected (Pilkington et al., 2020). It has been reported that other agents used in the treatment of COVID-19, such as steroids and ribavirin, may have negative effects on semen parameters and cause testicular damage (Gur et al., 2005; Lorusso et al., 2010). However, in our cohort, none of the patients used steroids or ribavirin. In light of these findings, the COVID-19 treatment protocol including hydroxychloroquine and favipiravir does not seem to have a detrimental effect on semen parameters.

This study has both strengths and limitations. The strengths of the study include the availability of pre-COVID-19 normal semen parameters of the same cohort according to the WHO 2010 criteria and all semen analyses being carried out by the same andrology laboratory. In addition, we excluded pathologies other than COVID-19 that may cause negative effects on semen parameters. The limitations of the study are the relatively small sample consisting of only participants that had recovered from mild COVID-19 and only a single semen analysis being performed after COVID-19. In addition, the effects of COVID-19 infection on other sperm quality parameters, such as seminal oxidative stress and DNA fragmentation, were not evaluated in our study.

5 | CONCLUSIONS

This study demonstrated that sperm motility and total motile sperm count were the semen parameters which showed a significant reduction in cases with a history of mild COVID-19. Considering that the majority of patients recovering from COVID-19 are of reproductive age, periodic monitoring of these patients for reproductive health might be beneficial.

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DATA AVAILABILITY STATEMENT

All data underlying the results are available as part of the article and no additional source data are required.
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