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Effects of moderate COVID-19 infection on semen oxidative status and parameters 14 and 120 days after diagnosis

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Abstract. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus causing coronavirus disease 2019 (COVID-19). Because COVID-19 is a multisystem infection, there are some concerns regarding its possible effects on male fertility. This study aimed to investigate the effects of COVID-19 on semen oxidative status and parameters 14 and 120 days after diagnosis in patients presenting with moderate infection (defined as respiratory symptoms, with or without fever, with S_pO_2 <93% and >90% and lung involvement <50%). Semen samples were obtained from 20 participants at two time points: the first sample on Day 14 and the second on Day 120 after diagnosis. Semen parameters (sperm concentration, motility, morphology, and viability) were evaluated, as were levels of seminal reactive oxygen species (ROS), malondialdehyde (MDA), total antioxidant capacity (TAC) and sperm DNA fragmentation. Semen parameters, including sperm motility and DNA integrity, improved at 120 days after the COVID-19 diagnosis relative to values at 14 days. In addition, ROS and MDA levels were significantly reduced in patients 120 days after infection, and TAC increased at 120 days compared with 14 days (during the acute stage of infection). In conclusion, the present study shows that the detrimental effects of COVID-19 on sperm properties caused by oxidative stress decrease up to Day 120 after diagnosis.

Keywords: COVID-19, DNA, male fertility, oxidative stress, SARS-CoV-2, semen, spermatozoa, testis.

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Introduction

The new coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in December 2019 in Wuhan, China (Al-Qahtani 2020); thereafter, the resulting COVID-19 infection very quickly spread worldwide. There have been close to 132 million reported cases of COVID-19 globally, including approximately 3 million deaths as of 4 April 2021 (https://covid19.who.int/). Notably, this infection is characterised by a broad spectrum of clinical presentations and affects multiple body organs (Zaim *et al.* 2020). COVID-19 infections range from asymptomatic to life-threatening infections that require intensive care and special medicine (Azoulay *et al.* 2020).

SARS-CoV-2 enters host cells by binding to membrane angiotensin-converting enzyme II (ACE2) receptors (Bian and Li 2021). Therefore, organs containing a large number of cells with ACE2 receptors are susceptible to the infection caused by SARS-CoV-2 (Khalili *et al.* 2020). ACE2 receptor expression in

reproductive organs and the contribution of these receptors to sperm function and fertilisation have been reported previously (Khalili *et al.* 2020; Fan *et al.* 2021). Indeed, there are reports of enriched ACE2 receptor expression in testicular cells, including spermatogonia, Sertoli and Leydig cells and prostate cells (Wang and Xu 2020; Chen *et al.* 2020).

Although the shedding of some viruses through the seminal fluid has been reported previously (Dejucq and Jégou 2001; Qian *et al.* 2016), whether SARS-CoV-2 is shed through the seminal fluid remains unclear. In addition, it is unclear whether SARS-CoV-2 has the ability to affect testis tissue, the male urogenital tract and/or accessory glands. An association between SARS-CoV-1, another severe acute respiratory syndrome (SARS)-related coronavirus (SARSr-CoV), and orchitis was shown in a previous study (Guo *et al.* 2020). In addition, there is a report of testicular pathological changes, including germ cell damage, thickening of the basement membrane of the seminiferous tubules, infiltration of leucocytes and a reduction in the number of spermatozoa in the tubules, in the testes of patients who died because of SARS (Xu *et al.* 2006).

Some studies have investigated the semen characteristics of COVID-19 patients (Kayaaslan *et al.* 2020; Guo *et al.* 2021; Ma *et al.* 2021; Temiz *et al.* 2021). The results of these studies indicated possible detrimental effects of COVID-19 infection on semen parameters; however, controversy remains regarding the effects of SARS-CoV-2 on male fertility potential, because the available data are limited. These effects could be either a result of the systemic infection and the cytokine storms, which are a feature of COVID-19, or a direct effect of the viral infection on male reproductive organs. The systemic involvement of the body, in combination with fever and inflammation, may also dysregulate hormone production by the hypothalamic–pituitary–gonadal (HPG) axis; thus, sperm production may be impaired by this route. In addition, there is a reciprocal interaction between inflammation and oxidative stress, so one can cause the other (BaSalamah *et al.* 2018). The detrimental effects of oxidative stress on male fertility have been discussed previously (Agarwal *et al.* 2003; Sanocka and Kurpisz 2004; Tremellen 2008).

This study aimed to investigate the effects of COVID-19 on semen parameters and markers of oxidative stress in seminal fluid 14 and 120 days after a diagnosis of COVID-19 infection in patients hospitalised because of a moderate infection, defined according to the guidelines of the Iranian National Committee of COVID-19 (diagnosis criteria described in Maghbooli *et al.* 2020) as respiratory symptoms, with or without fever, with S_pO_2 <93% and >90% and lung involvement <50%.

Materials and methods

Patient selection

This prospective study was performed at Imam Khomeini University Hospital, Urmia, Iran, from July to August 2020. The study was approved by the internal ethics committee of the hospital. Written informed consent was obtained from all those who volunteered to take part in the study.

In all, 63 men aged between 20 and 50 years who were hospitalised due to a moderate COVID-19 infection were eligible for inclusion in the study. Diagnoses were made by infectious disease specialists according to the World Health Organization (WHO) guidelines (https://www.who.int/publications/i/item/9789241547789) and recommendations of the Iranian National Committee of COVID-19 (Maghbooli *et al.* 2020). All patients included in the study tested positive for SARS-CoV-2 infection following quantitative real-time polymerase chain reaction (PCR) analysis of pharyngeal–nose swabs. Thereafter, the infection was confirmed by chest computed tomography (CT). As noted above, moderate infections were classified according to the guidelines of the Iranian National Committee of COVID-19 as respiratory symptoms, with or without fever, an S_pO_2 <93% and >90% and <50% lung involvement. The fertility status of patients was determined on the basis of a questionnaire containing questions on age, occupation, fertility and medical history. Of the 63 participants eligible for inclusion in the study, 20 fertile men with proven fertility (whose wives gave birth to a healthy child within the previous 2 years) were selected for the study. The exclusion criteria were a history of varicocoele, cryptorchidism, congenital disorders, immunological and inflammatory diseases, hormone disruptions, diabetes, alcohol abuse and/or cigarette smoking, a history of any scrotal or inguinal surgery, malignant disease, the consumption of any drug affecting the male reproductive tract and a high-risk job environment (e.g. exposure to certain chemicals, pesticides, herbicides, organic solvents, painting materials, radiation, extreme heat etc.).

Semen sample collection and analysis

Semen samples were obtained at two time points: 14 and 120 days after the diagnosis of COVID-19 infection. Participants were trained to collect samples by masturbation, after 2–5 days of abstinence, into a sterile plastic container, which was then transferred to the laboratory within 30 min, avoiding cold or hot environments and any exposure to sunlight. After liquefaction at 37°C, the semen samples were immediately analysed according to the WHO guidelines (https://www.who.int/publications/i/item/9789241547789). After evaluation of the volume, colour, viscosity and pH of the semen sample, sperm parameters (i.e. sperm concentration, motility, morphology and viability) were determined under a light microscope (Nikon). Finally, the number of leucocytes in the semen sample was determined using the peroxidase-staining method.

Measurement of semen reactive oxygen species concentrations

Reactive oxygen species (ROS) concentrations in the neat semen sample were measured by the indirect luminol-dependent chemiluminescence assay, as described previously (Venkatesh *et al.* 2011). Briefly, luminol working solution (5 mM) was prepared using a stock solution (100 mM) of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma-Aldrich) and dimethyl sulfoxide (DMSO; Sigma-Aldrich). Thereafter, four tubes were prepared: (1) a blank tube containing 400 µL phosphate-buffered saline (PBS); (2) a negative control tube containing 400 µL PBS + 10 µL luminol working solution; (3) a test sample tube containing 400 µL semen sample + 10 µL luminol working solution; and (4) a positive control tube containing 400 µL PBS + 50 µL H_2O_2 + 10 µL luminol working

solution. The chemiluminescence of the samples was assessed using a luminometer (Berthold Detection Systems) in duplicate. Subsequently, the mean relative light unit (RLU) was calculated for the negative control, test samples and positive control. ROS levels in samples were calculated by subtracting the mean RLU of the negative control from that of the test samples. ROS levels were expressed as $\times 10^4$ RLU per minute and then normalised per 20×10^6 spermatozoa.

Measurement of lipid peroxidation

Semen levels of malondialdehyde (MDA), as a marker of lipid peroxidation, were measured using the thiobarbituric acid-reactive substances (TBARS) assay as described by Sahnoun *et al.* (2017). Briefly, semen samples were centrifuged at 300*g* for 5 min at 25°C. The resulting pellet was then resuspended in distilled water and a suspension containing 10^7 sperm cells was subsequently prepared in 500 µL distilled water. The thiobarbituric acid (TBA) reagent (containing 0.8% TBA and 15% trichloroacetic acid in 0.25 M HCl) was added to the suspension, and the mixture was heated for 15 min at 95°C. The reaction was chilled to room temperature and the mixture was the centrifuged at 3000*g* for 10 min at 25°C. Finally, the MDA content of the supernatant was measured by colourimetric analysis using a spectrophotometer (BioTek). A standard curve was constructed using 1,1,3,3-tetraethoxypropane was as the standard. Results are then expressed as nanomoles of MDA per millilitre.

Total antioxidant capacity of seminal plasma

The total antioxidant capacity (TAC) of seminal plasma was measured using the ferric reducing ability of plasma (FRAP) method as described by Salimi *et al.* (2016). This method is based on the ability of the antioxidants in the seminal plasma to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}). For this purpose, the working FRAP reagent was prepared by mixing 10 volumes of acetate buffer (300 mM, pH 3.6), 1 volume of 2,4,6-tripyridyl-*S*-triazine (10 mM), 40 mM HCl and 1 volume of $FeCl_3 \cdot 6H_2O$ (20 mM). A 1.5-mL aliquot of this working solution was transferred to a glass tube and incubated for 5 min at 37°C. Then, either 50 µL seminal plasma, 50 µL distilled water or 50 µL $FeSO_4 \cdot 7H_2O$ solution (1000, 500, 250 or 125 µM; as the standard) was added to the 1.5 mL of working solution, and the mixture was incubated for 10 min at 37°C. Finally, absorbance was measured by a spectrophotometer (BioTek) at 593 nm. TAC results are expressed as millimoles per litre (mM).

Analysis of sperm DNA fragmentation

Sperm DNA fragmentation was assessed by the sperm chromatin dispersion (SCD) test, as described by Fernández *et al.* (2003) with some modification. In the SCD test, sperm cells with non-fragmented DNA appear with characteristic halos of dispersed DNA loops around the central core of the nucleus after both the denaturation and removal of nucleoproteins; in contrast, such halos do not appear around sperm cells with fragmented DNA. For the SCD test, a suspension of $5–10 \times 10^6$ spermatozoa mL^{-1} was first prepared in PBS. Thereafter, 50 µL sperm suspension was added to 50 µL low-melting-point aqueous agarose (1% in distilled water) at 37°C. After homogenisation of the mixture, a 50-µL droplet was placed on a glass slide precoated with 0.65% standard agarose. The droplet was covered with a coverslip and then left to solidify for 4 min at 4°C. The coverslip was then carefully removed and the initial acid treatment was performed using HCl solution (0.08 M) for 7 min at 22°C in the dark. After discarding the acid solution, lysis buffer was placed on the slide and the incubation was continued for 15 min at 22°C. Then, the slide was thoroughly washed in distilled water, dehydrated in sequential ethanol baths, air dried and finally stained with Giemsa stain. Slides were inspected under a light microscope (Nikon) to assess DNA fragmentation, as evidenced the by absence of halo or small halo or degraded nucleus. A sperm DNA fragmentation index (DFI), defined as the percentage of fragmented to total number of sperm cells counted, was also calculated for each sample.

Statistical analysis

GraphPad Prism (GraphPad Software) was used to analyse all data. Comparisons of mean values were made using paired Student's *t*-tests. Two-sided $P < 0.05$ was considered statistically significant. Data are presented as the mean ± s.d.

Results

Severity of COVID-19 infection and medications

All patients ($n = 20$) included in this study had been diagnosed as having a moderate COVID-19 infection according to the guidelines provided by the Iranian National Committee of COVID-19 (diagnosis criteria described in Maghbooli *et al.* 2020). In addition, according to these guidelines, the medications used to treat these patients included dexamethasone (4–8 mg, b.i.d., for 10 days), enoxaparin (40 mg, q.d., as a preventive anticoagulant), bromhexine (7.5 mL, t.i.d., for 10 days), famotidine (20 mg, b.i.d., for 2 weeks), vitamin C (500 mg, q.d., for 2 weeks), and vitamin D (50 000 IU, once a week for 1 month).

Semen parameters

Table 1 shows the semen parameters of the 20 patients in this study on Days 14 and 120 after the COVID-19 diagnosis. Within individual patients, there were no significant differences in semen volume, pH, sperm concentration and viability between the two time points. In addition, all these parameters were within the normal range according to the WHO criteria (https://www.who.int/publications/i/item/9789241547789).

Although both sperm progressive motility and total motility were below the normal range at 14 days after the COVID-19 diagnosis, both parameters increased significantly at 120 days after the diagnosis and fell with the normal range according to the WHO criteria. In addition, sperm morphology improved significantly at 120 days, but the percentage of spermatozoa with normal morphology was still below the normal value according to the WHO criteria.

The number of peroxidase-positive leucocytes in semen samples was above normal according to the WHO criteria 14 days after the COVID-19 diagnosis, but the number of peroxidase-positive leucocytes decreased significantly at 120 days to within the normal range.

Semen ROS concentrations

ROS concentrations in patients' semen samples are shown in Fig. 1*a*. There was a significant decrease (by 55.52%) in semen

Table 1. Semen parameters of patients on Days 14 and 120 after the COVID-19 diagnosis
Unless indicated otherwise, data are given as the mean ± s.d. Normal WHO value, see https://www.who.int/publications/i/item/9789241547789

	Day 14	Day 120	Normal WHO value	*P*-value
Volume (mL)	3.8 ± 1.2	4.1 ± 1.3	≥1.5	0.4389
pH	7.4 ± 0.2	7.5 ± 0.2	≥7.2	0.1185
Sperm concentration ($\times 10^6$ mL^{-1})	47.6 ± 21.9	52.1 ± 24.3	≥15	0.5422
Progressive motility (%)	30.6 ± 8.2	44.1 ± 9.9	≥32	<0.0001
Total motility (%)	32.8 ± 8.9	47.5 ± 9.8	≥40	<0.0001
Normal morphology (%)	1.3 ± 1.1	3.2 ± 1.7	≥4	0.0002
Viability (%)	73.1 ± 11.6	80.3 ± 11.5	≥58	0.0569
Peroxidase-positive leucocytes ($\times 10^6$ mL^{-1})	1.5 ± 1.1	0.8 ± 0.6	<1	0.0124

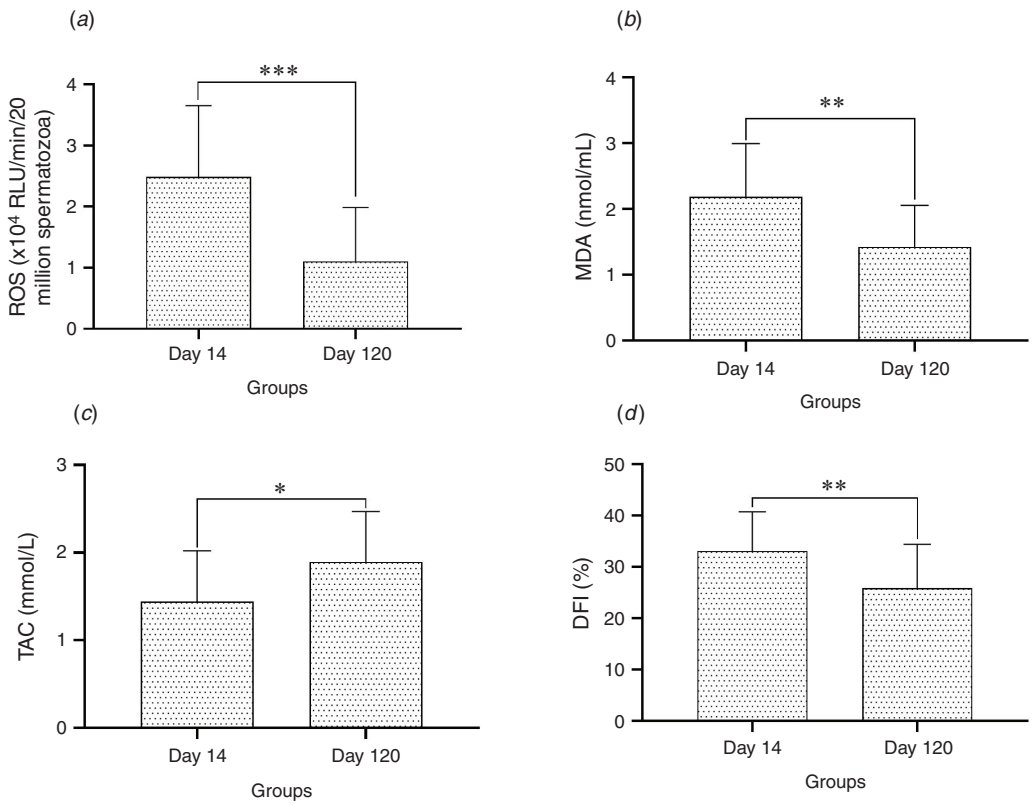

Fig. 1. Levels of (*a*) seminal reactive oxygen species (ROS), (*b*) malondialdehyde (MDA), (*c*) total antioxidant capacity (TAC) and (*d*) sperm DNA fragmentation index (DFI) in patients on Days 14 and 120 after COVID-19 diagnosis. Data are shown as the mean ± s.d. *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$.

ROS concentrations at 120 days after the COVID-19 diagnosis compared with Day 14.

Semen MDA concentrations

MDA concentrations, as a marker of lipid peroxidation, were measured in semen samples. There was a significant reduction (by 34.83%) in MDA concentrations in semen samples obtained 120 days after the COVID-19 diagnosis compared with Day 14 (Fig. 1*b*).

Semen TAC

Fig. 1*c* shows TAC in semen samples obtained from COVID-19 patients 14 and 120 days after diagnosis. There was a significant (by 31.52%) increase in TAC in semen samples 120 days after the COVID-19 diagnosis compared with Day 14.

Sperm DNA fragmentation

The DFI of spermatozoa in samples from the COVID-19 patients on Day 14 after diagnosis (33.10%; Fig. 1*d*) was above the

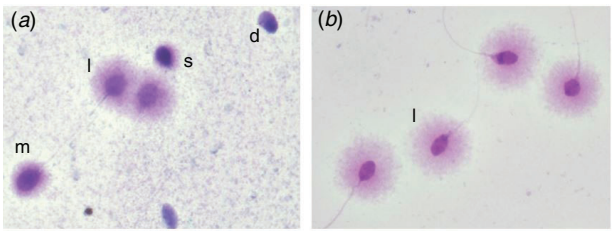

Fig. 2. Representative images of the sperm chromatin dispersion (SCD) test performed in an individual patient. (*a*) 14 and (*b*) 120 days after a COVID-19 diagnosis. l, spermatozoa with a large halo; m, spermatozoa with a medium halo; s, spermatozoa with a small halo; d, spermatozoa with a degraded nucleus.

defined value for optimal fertility; a DFI <30% is considered an essential prerequisite for optimal fertility (Pratap *et al.* 2017). Moreover, sperm DNA fragmentation decreased significantly (by 21.76%) at Day 120 after the diagnosis compared with Day 14, and fell within the normal range. Fig. 2 shows representative images of from the SCD test performed on semen samples of one of the patients at the two study time points.

Discussion

In this study we demonstrated that sperm parameters, including motility, morphology and the DFI, improved at 120 days after the COVID-19 diagnosis in patients hospitalised because of a moderate infection. Moreover, there was a significant reduction in semen ROS levels and lipid peroxidation at 120 days compared with 14 days after diagnosis of COVID-19. In addition, there was an increase in the TAC of the semen at 120 days compared with that during the acute phase.

The pathological effects of viral infections on testicular tissue can be direct, indirect or both. In addition, it has been demonstrated that some viruses, such as mumps and hepatitis B, can enter the male reproductive tract and directly cause orchitis and impair fertility (Dejucq and Jégou 2001; Qian *et al.* 2016). ACE2 receptors are required for SARS-CoV-2 entry into host cells. These receptors have so far been found in spermatogonia and Sertoli and Leydig cells. Transmembrane serine protease 2 (TMPRSS2) is another critical enzyme, the presence of which is mandatory for facilitating SARS-CoV-2 entry into cells after binding to the ACE2 receptor (Hallak *et al.* 2021). Recently, it was reported that <0.1% of cells in the testes coexpress ACE2 receptor and TMPRSS2 (Pan *et al.* 2020). Consequently, this may decrease the feasibility of SARS-CoV-2 entering these cells, thereby reducing the likelihood of direct pathological effects on testicular tissue. Thus, SARS-CoV-2 infection probably interferes with testicular function indirectly, similar to other systemic viral infections (e.g. influenza; Fijak *et al.* 2018). During viral infections, some conditions, such as fever and inflammation, may result in dysregulation of the HPG axis, subsequently impairing sex hormone production and disrupting spermatogenesis (Carlsen 2003; Liu *et al.* 2018).

Postmortem examination of testis samples during the past SARS epidemic revealed that the infection caused by SARS-CoV can be accompanied by some degree of orchitis (Xu *et al.* 2006). Because of the high similarity between the genomes of SARS-CoV and SARS-CoV-2 (Malik 2020), the new virus (SARS-CoV-2) may cause similar complications in testicular tissue (Madjunkov *et al.* 2020). In a previous study, Carneiro *et al.* (2021) evaluated testicular involvement in 26 patients with mild-to-moderate COVID-19 infection and reported that none of those patients had scrotal complaints and/or orchitis. However, a significant percentage of those individuals (42.3%) showed signs of epididymal inflammatory changes upon radiological examination. Although scrotal examination was not performed in the present study, none of the patients had scrotal discomfort during the time of their infection or recovery period, as reported in the questionnaire. Other postmortem examinations of the testes from COVID-19 patients reported orchitis with fibrin microthrombi, mild lymphocytic inflammation, a decreased Leydig cell population and significant lesions in the seminiferous tubules (Duarte-Neto *et al.* 2020; Yang *et al.* 2020). Therefore, the pathogenesis of COVID-19 in testicular tissue remains contentious.

Currently, *in situ* hybridisation has failed to detect SARS-CoV-2 in testicular tissue (Yang *et al.* 2020; Teixeira *et al.* 2021), although, because of the presence of SARS-CoV-2 RNA in semen samples reported in previous studies (Li *et al.* 2020; Pan *et al.* 2020; Song *et al.* 2020; Holtmann *et al.* 2020), these results are controversial. Of note, different studies, conducted in 82 patients during the active or recovery phases of the infection, failed to detect SARS-CoV-2 RNA in individuals' semen samples (Holtmann *et al.* 2020; Pan *et al.* 2020; Song *et al.* 2020; Guo *et al.* 2021). However, Li *et al.* (2020) detected SARS-CoV-2 RNA in semen samples from six of 38 infected individuals (15.8%). In addition, of these six patients, four were in the acute phase of the infection and the remaining two were in the recovery phase.

The results of the present study demonstrate that semen parameters, including both progressive motility and total motility, increased significantly at 120 days after the COVID-19 diagnosis and fell within the normal WHO ranges (https://www.who.int/publications/i/item/9789241547789). In addition, although sperm morphology improved 120 days after the diagnosis, the percentage of normal spermatozoa did not fall into the normal WHO range. Few studies have investigated sperm parameters in COVID-19 patients with mild to moderate levels of infection (Guo *et al.* 2021; Ma *et al.* 2021). In their study, Ma *et al.* (2021) assessed semen samples obtained from 12 reproductive-aged men on Days 56–109 from the onset of COVID-19. Sperm parameters were normal in most of the study patients (66.7%), with reduced sperm motility being recorded in only one patient after infection compared with the results of a semen analysis report before the infection (Ma *et al.* 2021). Furthermore, Holtmann *et al.* (2020) demonstrated that sperm concentration and motility decreased significantly in patients who had recovered from a moderate type of COVID-19 infection compared with control subjects. In that study, 18 semen samples were collected from recovered men at 8–54 days after their symptoms had disappeared. In the study of Guo *et al.* (2021), sperm parameters were reportedly normal in all samples ($n = 23$), regardless of the severity of the infection in the patients from whom the samples were collected. Moreover, in that study, the interval between COVID-19 diagnosis and semen sample collection was nearly 32 days. One possible explanation for the heterogeneous results reported by these studies could be the

differences in when semen samples were collected after the COVID-19 diagnosis. In addition, these studies compared results from COVID-19-patients with those of non-infected men as a control group, and this type of comparison may not be suitable because of the large variability in sperm parameters among individuals. In this regard, in the present study we collected samples at two time points, once during the acute phase of COVID-19 and once after the recovery period, to compare results in the same affected individual. In addition, the 120-day interval between sample collection was selected based on the duration of the spermatogenesis cycle to avoid misleading results that may be caused by affected cycles during the acute phase of the infection.

It is well documented that normal sperm parameters, including sperm count, motility and morphology, do not guarantee fertility because many other factors that are not evaluated during routine semen analysis could affect fertility potential (Alahmar *et al.* 2021). For example, high levels of ROS and lipid peroxidation in the semen have been found to be linked to different forms of infertility (Tremellen 2008). Furthermore, it has been demonstrated that excessive ROS have detrimental effects on the proteins and lipids of the sperm plasma membrane, and can also induce the breakdown of sperm DNA (Agarwal *et al.* 2003; Sanocka and Kurpisz 2004). Of note, any damage to the plasma membrane consequently disturbs its fluidity, resulting in impaired motility and dysregulated membrane fusion events during the acrosome reaction and fertilisation (Sanocka and Kurpisz 2004). ROS-induced DNA damage can occur in both the nuclear and mitochondrial genomes of spermatozoa (Gharagozloo and Aitken 2011). DNA fragmentation is caused by ROS attacking the DNA, particularly the phosphodiester backbone or guanine bases (Gharagozloo and Aitken 2011). The results of the present study reveal significant decreases in semen ROS levels and MDA content, as well as a significant increase in semen TAC, on Day 120 compared with Day 14 after the COVID-19 diagnosis. In addition, there was a significant decrease in the DFI on Day 120 after the diagnosis compared with Day 14. This decrease in DFI may possibly be due to changes in ROS concentrations in the seminal fluid of infected individuals during the recovery period.

Major sources of ROS in the seminal fluid are abnormal sperm cells (Sakkas *et al.* 2003) and leucocytes, and mostly neutrophils and macrophages (Gharagozloo and Aitken 2011). It is of note that increased levels of leucocytes in the seminal fluid (leucocytospermia) are seen in systemic infections; however, the reason for this increase has not yet been clarified (Sandoval *et al.* 2013). The systemic involvement during COVID-19 infection (Laforge *et al.* 2020) may be one of the sources for the high peroxidase-positive leucocyte count and increased ROS concentrations in the seminal fluid. In line with the results reported by Temiz *et al.* (2021), the results of the present study showed a mild increase statistically in the leucocyte count in semen samples obtained from the COVID-19 patients on Day 14 after diagnosis; however, this increase had declined on Day 120 after the diagnosis. Extensive studies are needed to investigate leucocytospermia and its detrimental effects on both sperm structure and function in COVID-19 patients.

One major limitation of the present study was its small sample size. Furthermore, all the patients in this study had a moderate infection, and we were not able to include severely ill patients. In addition, we did not investigate sperm function in this study to unveil the possible detrimental effects of COVID-19 infection on sperm physiology. Notably, due to the variable nature of semen parameters, large-scale studies with long-term patient follow-up are needed to investigate the effects of COVID-19 on male reproduction. It should be mentioned that medication use during the infection can have some adverse effects on semen parameters, and ROS levels in particular. Because all the patients in this study were hospitalised in the same place and received the same medications, any possible detrimental effects of pharmaceuticals are common to all patients. More studies are required to distinguish the effects of medication use during COVID-19 infection from those of SARS-CoV-2.

Conflicts of interest

The authors declare no conflicts of interest.

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