#### **ORIGINAL ARTICLE**

# Examining changes on testicular structure and sperm analysis of COVID-19 patients

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#### Abstract

This study aimed to examine the testicular functions with sperm analysis of patients with COVID-19. The study was carried out with male patients aged between 18 and 50 years with positive RT-PCR test and SARS-CoV-2 virus between December 2020 and April 2021. A total of 103 participants were included in the study. The mean age was 31.24 ± 5.67 (19-45) years and the mean body mass index of the participants was 28.41 ± 4.68 kg/m<sup>2</sup>. The patients were divided into two groups, group-1 was patients who had COVID-19, group-2 was healthy men. A semen analysis of both groups was performed, and the serum total testosterone, FSH, LH, anti-mullerian hormone and Inhibin-B tests were analysed and recorded. The testicular dimensions and testicular densities were examined by ultrasound and elastography for both groups. Comparing the patient and control groups results, this study found that the sperm count per 1 cc (p = 0.01) and total motility (p = 0.01) in group-1 was lower than in the control group, the testicular dimensions decreased (for right testis group-2 was 15.39 ± 4.78 ml versus group-1 was 12.11 ± 4.62 cm<sup>3</sup> p < 0.01, for left testis group-2 was 16.01  $\pm$  5.12 versus group-1 was 11.92  $\pm$  4.78 cm<sup>3</sup>; p < 0.01), and the shear wave velocities were significantly higher in group-1 patients. In conclusion, sperm parameters deteriorate in men who have symptomatic disease with SARS-CoV-2 infection. The fact that the cause of this deterioration is characterized by changes at the cellular level in the testis raises doubts about the persistence of this condition.

KEYWORDS COVID-19, infertility, SARS-CoV-2, shear wave elastography, sperm

# 1 | INTRODUCTION

The 2020 coronavirus pandemic caused by SARS-CoV-2 (a virus belonging to the Coronaviridae family and causing COVID-19) spread globally. To counteract this major pandemic, mandatory medical protocols and procedures, as well as various health policies were put in place. These new changes were made to reduce the increasing number of infected people, the number of which posed an extraordinary challenge for health systems in almost every country. These

procedural changes and new information also affect a number of changes in andrology laboratories, especially in order to avoid possibly harmful results of assisted reproductive techniques (Banihani, 2022).

SARS-CoV-2 enters the host cell via the angiotensin-converting enzyme 2 (ACE2) receptors and causes infection (Bourgonje et al., 2020). At the same time, transmembrane serine protease 2 (-TMPRSS2), which allows SARS-CoV-2 to enter the host cell, also plays an important role. It has been shown that ACE2 and TMPRSS2 are highly expressed in spermatogonia and Sertoli and Leydig cells (Wang & Xu, 2020). SARS-CoV-2 enters the host cell and causes down-regulation of ACE2 expression, which causes excessive angiotensin production (Culha et al., 2021; Dijkman et al., 2012; Gurwitz, 2020). The excessive production of proinflammatory cytokines, autoimmune response and increasing leukocyte infiltration in testis may cause negative effects on the spermatogenesis pathway.

In the literature, the impact of COVID-19 on spermiograms has previously been evaluated (Hamarat et al., 2022; Moryousef et al., 2022). However, studies have not been done on sperm quality and sperm DNA fragmentation or damage after healthy individuals are infected with the SARS-CoV-2 virus.

This study aimed to evaluate the post-infection sperm quality of fertile men infected with the SARS-CoV-2 virus.

# 2 | METHOD

This study was carried out with 103 male patients aged between 18 and 50 years old with a positive RT-PCR test and SARS-CoV-2 virus at Van Yüzüncü Yıl University, Faculty of Medicine, Department of Urology between December 2020 and April 2021. Ethics committee approval was obtained for the study at 16 July 2020 number 07 in Van Yüzüncü Yıl University Faculty of Medicine Ethics Committee and written consent was obtained from each participant in the study. The study design was planned in accordance with the Declaration of Helsinki.

The study included 103 men of reproductive age, 53 of which had been diagnosed with COVID-19 within 3 months in the patient group, and 50 healthy men who had not been previously diagnosed with COVID-19 in the control group. The COVID-19 patients were under favipiravir treatment, in accordance with the protocol of the Ministry of Health of the Republic of Turkiye. There were no vaccinated patients included in the study. Criteria for exclusion from the study included prior exposure to empirical treatments, such as antiestrogens, antioxidants; have a history of febrile illness other than COVID-19 in the past 3 months; patients with a previous diagnosis of urogenital system infection (including orchitis) and testicular abnormalities. The demographic and clinical characteristics of all participants, as well as their semen analyzes, serum total testosterone, FSH, LH, anti-mullerian hormone (AMH), Inhibin-B levels, were collected and recorded. In addition, scrotal ultrasound and shear wave elastography were performed and recorded for each participant.

For blood hormone analysis of the groups, 5 cc of blood was drawn from the right arm median cubital vein, between 8 AM and 11 AM. After at least 3 days of sexual abstinence, the participants were asked to provide a semen sample by masturbation. All semen analyzes were performed in accordance with WHO 2010 guidelines (Lu et al., 2010). Semen samples were taken sterile and liquefied for 30 min at 37°C. The semen analysis was done 1 h after ejaculation. The semen volume was measured using the gravimetric method. The sperm motility measurement was done manually and graded as motile, nonprogressive motile and nonmotile.

A scrotal US and elastography examination were performed (Garra, 2007). All elastographic evaluations were carried out by the same practitioner. A USG and elastography examination were performed with a Siemens ACUSON S2000<sup>™</sup> (Siemens Healthcare, Erlangen, Germany) brand device. An Acoustic Radiation Force Impulse (ARFI) elastography examination was performed using the 4–9 MHz 9L4 linear probe with the Virtual Touch IQ option.

In the examinations, testicular echogenicity and homogeneity were examined with grey scale USG during normal breathing while the participants were lying in the supine position. Bilateral testicular volumes were calculated automatically with software in the ultrasound device after measuring the longest three axis diameters of each testis.

During the elastography measurement, the patient was told not to take a deep breath, cough, strain or move. The radiologist made contact with the scrotal skin so that the probe would not exert external pressure on the testicular tissue and affect the measurement and accounted for the gel thickness between the probe and the skin. In the elastography examination, each testis was evaluated as three parts, the upper, middle and lower 1/3. The examiner took three separate measurements in each 1/3 part. In the colour-coded ROI (Region of Interest) established in the area. six small boxes were placed giving the shear wave velocity in m/sec, and a total of 18 velocity values were obtained for each segment. While doing the calculations, the 18 values were ordered from smallest to largest. then the three largest and the three smallest values were removed, and the average of the 12 remaining values was taken. Then, the average shear wave velocity of a single testis was found by taking the mean of the average SW velocity values found for the three separate 1/3 parts.

To investigate the effect of the SARS-CoV-2 infection on testicular function, the data of the COVID-19(+) patients in group-1 and the healthy control participants in group-2 were compared.

# 2.1 | Statistical analysis

In this prospective study, primary characteristic was considered Semen Volume (ml). According to previous studies (Pazir et al., 2021); the standard deviation (s) for Semen Volume (ml) was considered as 0.5 ml. In the study, sample size was determined by considering %80 power ( $\alpha = 0.05$  and  $\beta = 0.20$ ) value, 0.15 effect size (d), and 1.96 Z value. Thus minimum sample size was found 43 by using the " $n = Z2 s^2/d2$  "equation for sample size calculation. Considering the possible losses that may occur during the data collection process, 50 patients were included in the study. Descriptive statistics for the continuous variables (characteristics) were presented as mean and standard deviation while count and percent for the categorical variables. Normality assumption of the continuous variables was tested with Kolmogov-Simirnov test. After normality test, Student t test was used for the comparison of means in normally distributed characteristics while Mann-Whitney U test for non-normal distributed characteristics. Statistical significance level was considered as 5% and IBM

SPSS Statistics 25.0 (IBM) statistical program was used for all statistical computations.

# 3 | RESULTS

A total of 103 male participants were included in the study. Of these, 53 were patients with a diagnosed COVID-19 infection were assigned to group-1, and 50 healthy men were assigned as the control participants in group-2. The mean age of the participants was  $36.07 \pm 10.09 (19-56)$  years and the mean body mass index of the participants was  $28.41 \pm 4.68 \text{ kg/m}^2$ . Eight participants reported preexisting chronic diseases, including hypertension (n = 5) and type II diabetes (n = 3). Fifty-one of the participants were smokers (Table 1).

The median time between a positive nasopharyngeal swab test and semen sample collection was 138 (95–162) days in the COVID-19 infected group-1. In the evaluation of semen analyzes between the groups, the sperm count per 1 cc (p = 0.03) and total motility (p = 0.01) in men with SARS-CoV-2 infection were found to be significantly lower. There was no significant difference found between the groups in relation to age, co-morbidity and smoking status. There was no significant difference in semen parameters between groups in terms of semen volume, sperm concentration, advanced motility, and

#### **TABLE 1** Demographic characteristics

<i>n</i> = 103	Mean ± SD	Min-max
Age (years)	31.24 ± 5.67	19-45
Body mass index	28.41 ± 4.68	21.12-36.78
Comorbidity (%)	8 (7.77)	
Smoking (%)	51 (49.51)	
Covid-19 symptoms	n = 53	
Fever	30 (56.60)	
Cough	33 (62.26)	
Headache	19 (35.85)	
Myalgia	36 (67.92)	
Other symptoms	43 (81.13)	

 TABLE 2
 Comparison of sperm

 parameters serum hormone levels
 between COVID-19 patients and control

 groups
 groups

morphology. Total testosterone levels of the patients in group-1 were lower than the control participants in group-2 (p = 0.03). There was no significant difference found in FSH, LH, AMH and Inhibin-B levels (p > 0.05) (Table 2).

When the testicular volumes and elastography values of the patients in group-1 were compared with the control participants in group-2, the testicular dimensions decreased (for right testis in group-2 was  $14.70 \pm 2.43$  cm<sup>3</sup> versus in group-1 was  $12.60 \pm 2.11$  cm<sup>3</sup> p < 0.01, and for left testis in group-2 was  $14.80 \pm 2.67$  versus in group-1 was  $11.92 \pm 2.77$  cm<sup>3</sup>; p < 0.01). The shear wave velocity in the right testis was 2.60 m/s in the patient group-1, while it was 2.09 m/s in the healthy control group-2. The shear wave velocity in the left testis was 2.68 m/s in the patient group-1 and 2.12 m/ sec in the healthy control group-2. The shear wave velocities were significantly higher in COVID-19 infected patients. (Table 3).

# 4 | DISCUSSION

As a result of this prospective observational study, a decrease in sperm count and motility was observed in patients with the COVID-19 disease compared to healthy men. In addition, a reduction in testicular size and deterioration in testicular elasticity was also observed in men who had COVID-19.

Viral diseases (such as Zika, HBV and HCV) have a negative effect on spermatogenesis (Garolla et al., 2013; Joguet et al., 2017). Significant reductions in concentration, motility and morphology were detected in sperm samples taken from HBV and HCV patients (Lorusso et al., 2010). As a result of the research that Joguet et al. did on the effects the Zika virus had on semen parameters, he reported that there was a 50% decrease in total sperm count and total motile sperm count on the 60th day compared to the 7th day after symptom onset (Joguet et al., 2017). In our study, we found that the mean total motile sperm count, the sperm count per 1 cc, and the sperm total motility were significantly reduced in COVID-19 patients.

In recent studies, SARS-CoV-2 RNA has been isolated in the sperm of patients with SARS-CoV-2 infection (Li et al., 2020). In other studies, the presence of SARS-CoV-2 viral RNA was not detected in

	Control group ( $n = 50$ )	COVID-19 group (n = 53)	p value
Semen volume (ml)	3.8 ± 0.4	3.8 ± 0.5	0.88
Sperm concentration (million/ml)	28.4 ± 10.9	23.4 ± 12.3	0.03
Progressive sperm motility (%)	28.4 ± 7.5	27.4 ± 7.3	0.55
Total sperm motility (%)	41.2 ± 5.7	31.0 ± 8.9	<0.01
Sperm Morphology (%)	3.9 ± 0.6	$3.8 \pm 1.4$	0.55
Total testosterone	4.3 ± 1.6	3.7 ± 1.2	0.03
FSH	7.76 ± 2.54	8.13 ± 4.38	0.09
LH	5.48 ± 2.21	4.12 ± 2.45	0.03
AMH (ng/ml)	3.10 ± 1.57	3.07 ± 1.76	0.54
Inbibin-B (ng/L)	225.69 ± 112.51	231.58 ± 107.62	0.11

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	Control group (n = 50)	COVID-19 group (n = 53)	p value
Testis volume(ml) (right)	14.70 ± 2.43	12.60 ± 2.11	<0.01
Testis volume (ml) (left)	14.80 ± 2.67	11.92 ± 2.77	<0.01
Shear wave velocity (right) (m/s)	2.09 ± 0.68	2.60 ± 0.53	<0.01
Shear wave velocity (left) (m/s)	2.12 ± 0.75	2.68 ± 0.65	<0.01

**TABLE 3**Comparison of ultrasoundand elastograhpy results betweenCOVID-19 patients and control groups

the semen of patients with active infections or those who had recovered (Holtmann et al., 2020; Pan et al., 2020).

The COVID-19 virus adversely affects the testosterone levels of patients. Testicular dysfunction is seen due to the affinity of ACE receptors in Leydig cells. In the study conducted by Kadıhasanoğlu et al. (Kadihasanoglu et al., 2021), the testosterone levels of the 265 patients with COVID-19 were lower and the serum LH levels were higher than the control groups. Likewise, in our study, it was determined that serum total testosterone levels were lower and serum LH levels were higher in COVID-19 patients when compared to the control group.

After detecting the presence of SARS-CoV-2 in semen, studies on semen parameters were planned. In a prospective study, the semen analysis of 18 patients and 14 healthy volunteers who recovered from COVID-19 was evaluated (Holtmann et al., 2020). A significant deterioration in semen parameters (sperm concentration, total sperm count, total progressively motile sperm count, and total motile sperm count) was observed in those who had mild symptoms of COVID-19. In another study conducted by Guo et al., they collected semen samples from 18 mildly infected and five moderately infected SARS-CoV-2 patients during the acute and convalescent periods of the infection (Guo et al., 2021). The fact that the sperm parameters of patients were not known before contracting COVID-19 stands out in Guo's study. We also see a lack of studies in the literature.

Pazir et al. demonstrated that, when the sperm parameters of 24 patients, before and after COVID-19, were compared, it was shown that the sperm total motility and the total motile sperm count significantly decreased after COVID-19 (Pazir et al., 2021). In our study, a decrease in total sperm motility was found.

The basic principle of ultrasound elastography techniques is to measure the response of the tissue by applying an external force and the changes in the surrounding tissue after the displacement and deformation of the tissue caused by the force (Bamber et al., 2013). In the SW Elastography technique, acoustic waves are sent, focused on the tissue at the target depth and the diffusion speed of the shear waves that are formed in the tissue are measured. The velocities of the shear waves are expressed in m/s, and the elasticity of the tissue in kilo Pascals (kPa) (Ferraioli et al., 2012).

Elastography studies have been carried out on many organs. Aigner et al., with real-time sonoelastography, reported that stiffness increased in neoplastic testicular lesions and softened in orchitis and partial infarction (Aigner et al., 2012). In their study, Anastasi et al. found a significant relationship between low volume and shear wave velocity using an ARFI technique on 23 volunteers without testicular pathology. In the same study, a positive correlation was found between advanced age and shear wave velocity, and increased tissue stiffness was considered as the cause (D'Anastasi et al., 2011). In our study, the increase in wave velocity caused by the stiffness of the tissue was found to be significant in patients with COVID-19. In addition, a decrease was observed in sperm values and total testosterone levels in the group that had the disease. We determined that this change was the testicular damaging effect of COVID-19. It can be argued that the decrease in testicular size and loss of elasticity may affect the testicular tissue, and this will consequently negatively affect sperm parameters.

The study has some limitations. The first is the relatively small sample size for which only a single semen analysis was performed. In addition, the seminal oxidative stressor effect of the COVID-19 infection has not been evaluated. Another limitation is the absence of testicular histology.

# 5 | CONCLUSION

In conclusion, sperm parameters deteriorate in men who have symptomatic disease with SARS-CoV-2 infection. The fact that the cause of this deterioration is characterized by changes at the cellular level in the testis raises doubts about the persistence of this condition. Additional large-scale, multicenter, and long-term studies are needed to evaluate the effects of COVID-19 on the male reproductive system.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

Research data are not shared.

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How to cite this article: Ertaş, K., Eryilmaz, R., Yokuş, A., Körpe, K., Gedük, N., Özkan, M., & Aslan, R. (2022). Examining changes on testicular structure and sperm analysis of COVID-19 patients. *Andrologia*, e14609. <u>https://doi.org/10.</u> 1111/and.14609