Effect of SARS-CoV-2 infection on semen parameters

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Abstract

Introduction: There are not enough studies showing the degree to which the SARS-CoV-2 virus affects the semen parameters after coronavirus infection in comparison to before they were infected. In this study, we aimed to assess the effect of SARS-CoV-2 infection on semen parameters.
Methods: Patients were screened if they had had semen analysis performed from October 1, 2019, to December 1, 2020, in the assisted reproduction unit and later had positive polymerase chain reaction (PCR) test for SARS-CoV-2 infection. The patients' semen parameters were recorded before and after SARS-CoV-2 infection, along with degrees of SARS-CoV-2 infection, dates of SARS-CoV-2 infection, durations between the treatment for SARS-CoV-2 infection and the second semen analysis, time of symptom onset, duration of their symptoms, ages, comorbidities, and any medications patients were taking.
Results: Forty-one patients were included in the study. The mean age of the patients was 31.29±5.95 years. The mean duration from first semen analysis to the PCR test was 7.74±3.03 months. The mean duration between the PCR test and later semen analysis was 2.35±1.35 months. The median sperm concentration for the patients before and after SARS-CoV-2 infection were 24 mil/ml and 13 mil/ml, respectively ($p<0.001$). The normal morphology percentage before infection was 3.16±0.92, while it was 2.44±1.04 after infection ($p=0.011$). In 26 patients, the period from the time of infection to the second semen analysis was over 70 days, while this period was less than 70 days in the other 15 patients; however, in both patient groups, a significant decrease was detected in the sperm concentrations and total sperm count.
Conclusions: In the semen samples we assessed, we observed a significant decrease in the mean sperm concentration, total sperm count, and mean percentage of samples with normal morphology after SARS-CoV-2 infection.

Introduction

The first case of a novel coronavirus disease was observed in Wuhan City, China, in 2019, and following this it spread around the world.[1] It has been proposed that the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes cell injury to the transmembrane serine protease 2 (TMPRSS2) enzyme, which binds to the angiotensin-converting enzyme 2 (ACE2) receptor that is expressed in many organs, including the testis.[2] The virus binding to ACE-2 through "the spike protein" it contains. Following entry, it starts a proinflammatory process and cause cell damage. Primarily target tissues are respiratory epithelia which high expressed ACE-2.[3] In addition, the testis which expresses high amounts of ACE2 mRNA in seminiferous tubule cells, spermatogonia, Leydig cells and Sertoli cells, may be an important target. [4,5] ACE improve Leydig and Sertoli cells and a significant role for the regulation of spermatogenesis. In addition, it is recognized as a regulator of the sperm cell function and epididymal contractility.[6] Thus a number of studies have stated that the increased inflammatory response and linked oxidative stress that develops in SARS-CoV-2 infection may cause loss of function in the testis.[7,8]

Although many studies have been performed on the effect of SARS-CoV-2 on reproductive health in the short term, there are not enough studies showing the degree to which the virus affects the semen parameters after coronavirus infection in patients in comparison to before they were infected. In this study, we aimed to assess the effect of SARS-CoV-2 infection on semen parameters by investigating sperm parameters in male patients who had experienced this infection.

Methods

Ethics committee approval was received for this study from the Ethics Committee of The Konya Chamber of Commerce Karatay University Karatay Faculty of Medicine no: E-41901325-050.99-693. Patients were screened if they had had semen analysis performed from 1 October, 2019, to 1 December, 2020, in the assisted reproduction unit and had later had positive polymerase chain reaction (PCR) tests for SARS-CoV-2 infection. . These patients consisted of those who had previously been examined in the urology outpatient clinic for infertility or varicocele. Patients were contacted by telephone and given appointments with the assisted reproduction unit to provide semen samples. Semen analysis was performed on the patients after three days of sexual abstinence. Patients were excluded from the study if they were under 18 years of age, had azoospermia, had had any urogenital operations in this period, had undergone assisted reproduction techniques, were using steroids for infection treatment or were using medications that could affect semen parameters (testosterone, supplements, antibiotics, etc.). The patients' semen parameters were recorded before and after SARS-CoV-2 infection, along with degrees of SARS-CoV-2 infection, dates of SARS-CoV-2 infection, durations between the treatment for SARS-CoV-2 infection and the second semen analysis, times of the onset of their symptoms, durations of their symptoms, their ages, comorbidities, and any medications they were taking. Patients who were treated at home were recorded as having mild infection, those admitted to the hospital with oxygen requirements

were recorded as having moderate infection, and patients treated in the intensive care unit were recorded as having severe infection.[9]

Semen analysis was performed by liquidizing ejaculate, obtained through masturbation after three days of sexual abstinence in a sterile container, for 30–60 minutes at room temperature and then investigating this under a microscope. Semen analysis was performed by the same embryologist according to the World Health Organization (WHO) and Kruger's criteria.[10] This includes sperm concentration, total sperm count, semen volume, sperm motility (progressive motility, nonprogressive motility and immotility percentages), morphological features of the sperm (determined as normal morphology percentage in addition to head, neck and tail anomaly percentages). Leukocytes and round cells were counted manually and microscopically by the embryologist, not performed peroxidase test.

Statistical analyses

SPSS (Version 23) was used for statistical analysis. With this software the mean age and body mass index of the patients are given as means ± standard deviations. Data adhering to normal distribution and parametric data are expressed as means and standard deviations. Data that do not meet parametric distribution criteria are given as median and interquartile ranges. The paired t test or Wilcoxon test was used for comparative analysis of sperm parameters before and after SARS-CoV-2 infection in the patients. Values with $p<0.05$ were accepted as statistically significant.

Results

A total of 79 patients who had had semen analysis in our assisted reproduction center and had later had positive SARS-CoV-2 PCR tests were identified. These patients were contacted by telephone, and 46 patients who had had no treatment for infertility attended our center to provide sperm samples. The data for 46 patients who had had semen parameters investigated before SARS-CoV-2 were recorded. Of these patients, five were excluded from the study: two had azoospermia and three had undergone genital operations. Therefore 41 patients were included in the study. Semen analysis was performed in seven of these patients for varicocele, 25 for primary infertility, and nine for secondary infertility. The mean age of the patients was 31.29±5.95 years. One patient had hypertension, one had asthma and no others had comorbid diseases. Only two patients had moderate infection, while 39 had mild infection. The onset of symptoms was an average of 4.03±1.48 (2–7) days before the PCR test, and the symptoms lasted an average of 7.22±1.96 (5–12) days. All the patients stated that they had had fever at least once, and no patient reported a fever above 38.5 degrees, except for two hospitalized patients. These two patients had fevers of up to 39.5 degrees. After their diagnoses, all patients received 40 tablets of favipiravir (200mg favicovir in one tablet) for five days, along with vitamin C and parol treatment if necessary. The mean duration from the first semen analysis to the PCR test was 7.74±3.03 months. The mean duration between the PCR test and later semen analysis was 2.35±1.35 months. Table 1 shows the demographic characteristics of the patients.

The median sperm concentrations for the patients before and after SARS-CoV-2 infection were 24 mil/ml and 13 mil/ml, respectively ($p<0.001$). The normal morphology

percentage before SARS-CoV-2 infection was 3.16±0.92, while it was 2.44±1.04 after infection (p:0.011). Table 2 provides the semen parameters for patients before and after SARS-CoV-2 infection, with 13 patients having oligospermia before SARS-CoV-2 infection and 18 having oligospermia after infection. In 26 patients, the period from the time of infection to the second semen analysis was over 70 days, while this period was less than 70 days in the other 15 patients. The patients were also separated into groups of 26 and 15 and the changes in the semen parameters are shown in Table 2. In the semen analysis performed after infection in both patient groups, a significant decrease was detected in the sperm concentrations and total sperm counts. In addition, a significant decrease was found in the normal morphology percentages in the group for whom the time of infection to that of the second semen analysis was less than 70 days.

The semen parameters of three patients had round cells above five million before they had SARS-CoV-2, while this number was six after infection. In addition, the leukocytes were above one million on the semen parameters of three patients before SARS-CoV-2, and these three patients had leukocytes above one million after infection. Agglutination was not observed on the semen parameters of any patient before SARS-CoV-2 infection, while one patient had grade 4 agglutination after SARS-CoV-2 infection.

Discussion

Studies have shown that many viruses such as human papilloma virus (HPV), herpes simplex virus (HSV), mumps virus, human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, and Coxsackie virus, negatively affect the reproductive system and semen quality.[11,12] Although the mechanism by which viruses damage the testis is not clearly known, different hypotheses have been proposed. Viruses may directly enter the testis and cause testicular damage. Moreover, the immune response directed against viruses and the resulting inflammation and increase in temperature may damage the testicular tissue.[13] In 2006, Xu et al. showed that SARS-CoV-1 could cause testicular damage. Subsequently, the number of studies on the effects of SARS-CoV-2 on the genital system and semen quality has rapidly increased.[14] Pan et al. did not detect SARS-CoV-2 in the semen samples of patients collected nearly 1 month after recovery from SARS-CoV-2 infection.[15] However, Li et al. detected SARS-CoV-2 in the semen samples.[16] Theoretically, SARS-CoV-2 may affect all cells that have ACE2 receptors and express TMPRSS2. The ACE2 mRNA and protein expression levels in the testis are higher than those in many other tissues; this increases the probability of damage by the virus.[4] One of the most important functions of the testis is to produce sperm and infections may deteriorate the sperm quality. In the present study, we investigated the effect of SARS-CoV-2 on semen parameters by comparing the semen parameters of patients before and after infection and observed significant changes in the sperm count and morphology.

Yang et al. examined the testes of 12 patients who died of SARS-CoV-2 infection. They observed normal spermatogenesis in three samples, but noted spermatogenesis defects in the rest.[17] Li et al. assessed the semen parameters of 23 patients who had recovered from SARS-CoV2 infection and found that their mean sperm count was 11.9 million; this count was 40.9 million in healthy controls belonging to a similar age group. In their study, in the

SARS-CoV-2 patient group, nine patients were found to have oligospermia.[18] To date, studies have compared the semen parameters of patients after SARS-CoV-2 infection with those of a demographically similar control group; however, no study has compared the semen parameters of patients before and after infection. In the present study, semen analyses performed after a mean duration of 2.35±1.35 months after SARS-CoV-2 infection revealed that the median sperm concentration was 24 (2–93) mil/ml before infection and 13 (0.1–63) mil/ml after infection ($p<0.001$). In addition, oligospermia was identified in 13 patients before SARS-CoV-2 infection, and in 18 patients after infection. Moreover, the mean percentages of samples with normal morphology and tail anomaly were significantly lower after infection than before infection. On the other hand the percentage of samples with head anomalies significantly increased after infection. Although the mean percentage of total motile sperm decreased after infection, the difference was not statistically significant (Table 2).

It is proposed that inflammation-mediated testicular damage may cause infertility[19]. Xu et al. identified orchitis during the testis autopsies of six patients who died of infection due to SARS-CoV-1, which is 85% similar to SARS-CoV-2. Moreover, they noted leukocyte infiltration that may damage the Leydig cells, blood–testis barrier, and seminiferous tubules in the testes. Although these findings were noted on autopsy, the patients did not have symptoms of orchitis during their clinical follow-up when they were alive.[14] However, Özveri et al. reported normal semen parameters on analyzing the semen of a SARS-CoV-2 patient who visited the hospital only with symptoms of orchitis.[20] In the present study, no significant change was noted in the leukocyte counts in the semen samples before and after infection; only three patients (7.3%) stated that they experienced testicular pain during SARS-CoV-2 infection.

It has been suggested that while focal edema, diffuse alveolar damage and pneumonia develop in the lungs in the early period due to the SARS-Cov-2 infection, the virus may cause permanent damage by causing the development of fibrosis in the lungs in the late period.[21] Virus-related fever, inflammation and antiviral drugs used in the treatment may cause spermatogenesis defects in the early period by damaging the germ cells. In addition, the SARS-Cov-2 virus can cause permanent damage to the testes, similar to the pathophysiology suggested in the lung. Inflammatory cytokines, which increase due to the immune response to the virus, may cause deterioration of the blood-testicular barrier and damage to the testes. Increased expression of ACE-2 in Sertoli, Leydig and germ cells, especially in men of reproductive age, may make these cells more vulnerable to the virus.[22] The patients were separated into two groups in order to understand whether the deterioration in semen parameters that we observed in the present study was caused by an early temporary spermatogenesis defect or a late period spermatogenesis defect. In 26 patients, the period from the time of infection to the second semen analysis was over 70 days, while this period was less than 70 days in the other 15 patients (Table 2). In the semen analysis performed after infection in both patient groups, a significant decrease was detected in the sperm concentrations and total sperm counts. In addition, a significant decrease was found in the normal morphology percentages in the group for whom the time of infection to that of the second semen analysis was less than 70 days. Continuing deterioration in semen parameters in the late period after SARS-Cov-2 infection may be caused by permanent damage due to the

virus. In the present study the number of patients evaluated at least six months after infection was two. Future studies to include longitudinal studies on post-infection men are needed to show the long-term effect of infection.

The greatest limitation of this study was that the effect on semen parameters of medications used in our country for SARS-CoV-2 infection were not clearly known. Additionally, not having peroxidase test in semen analysis, the relatively low number of patients included in the study and nearly all having mild SARS-CoV-2 infection were other elements limiting the study.

Conclusions

We observed a significant decrease in the mean sperm concentration, total sperm count and mean percentage of samples with normal morphology after SARS-CoV-2 infection in the semen samples. Male patients attending infertility clinics should be questioned about whether they had SARS-CoV-2 infection and patients should be informed about this topic. There is a need for more studies showing the long-term effects of SARS-CoV-2 infection.

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Figures and Tables

Table 1. Demographic characteristics of the patients	
Number of patients	41
Age (years), mean ± SD (min-max)	31.29±5.95 (22-46)
Body mass index, mean ± SD (min-max)	23.21±2.05(18.8-26.2)
Comorbidity, n Hypertension Asthma	 1 1
Mild infection, n Moderate infection, n Severe infection, n	39 2 -
Time from the first semen analysis to the PCR test (months), mean ± SD (min-max)	7.74±3.03 (1-11)
Time from PCR test to second semen analysis (moths) (mean ± SD) (min-max)	2.35±1.35 (1-8)
Time from PCR test to second semen analysis, n <70 days >70 days	 15 26

PCR: polymerase chain reaction; SD: standard deviation;

Table 2. Comparison of semen parameters before and after SARS-CoV-2 infection									
	Before SARS-CoV-2 infection	**After SARS-CoV-2 infection**		**Before SARS-CoV-2 infection**	**After SARS-CoV-2 infection**		**Before SARS-CoV-2 infection**	**After SARS-CoV-2 infection**	
	<70 days	**<70 days**	**p**	**>70 days**	**>70 days**	**p**	**Total**	**Total**	**p**
Patients (n)	15	15		26	26		41	41	
Volume (ml), mean ± SD	2.67±0.79	2.51±0.81	0.347*	2.58±1.55	2.26±1.16	0.128*	2.61±1.25	2.35±1.03	0.069*
Sperm concentration (mil/ml), median (min–max)	20 (4–73)	9 (1–18)	**0.008****	42 (2–93)	30 (0.1–63)	**0.010****	24 (2–93)	13 (0.1–63)	**<0.001****
Total sperm number (million, median (min–max)	53.5 (5–219)	20.75 (2.5–72)	**0.013****	67.2 (3–318)	40.3 (0.4–168)	**<0.001****	63 (3–318)	28.8 (0.4–168)	**<0.001****
Progressive motility	29.05±8.44	27,35±12.21	0.660*	36.23±15.53	32.70±13.78	0.251*	33.66±13.59	30.74±14.28	0.230*
Total motility (%)	37.01±9.68	35.63±16.70	0.761*	44.88±17.74	40.47±16.39	0.194*	42±15.52	38.70±16.35	0.215*
Immotility (%)	62.98±9.68	64.34±16.70	0.761*	55±17.67	59.47±16.48	0.189*	57.92±15.48	61.25±16.41	0.211*
Normal morphology (%)	2.99±1.15	1.85±1.06	**0.030***	3.27±0.78	2.81±0.87	0.176*	3.16±0.92	2.44±1.04	**0.011***

Head anomaly (%)	85.48±2.76	88.21±4.37	0.170*	83±1.73	84.54±3.11	0.161*	83.94±2.43	85.94±3.96	**0.040***
Neck anomaly (%)	2.85±0.89	2.52±0.78	0.457*	3.09±0.83	3.36±0.67	0.465*	3.00±0.84	3.05±0.80	0.830*
Tail anomaly (%)	10.51±3.10	8.18±4.88	0.199*	13±2.86	11.36±3.10	0.127*	12.05±3.11	10.16±4.06	**0.037***

*Paired t-test. ** Wilcoxon signed-rank test. SD: standard deviation.