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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

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Abstract

The study investigated whether there is a male reproductive system coronavirus disease-2019 (COVID-19) phenomenon. Thirty participants who met the inclusion criteria were enrolled in the study between April and May 2020. The participants were assigned in one of the three groups including COVID-19 patients before and after treatment, and controls. Presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) within the semen samples was investigated. Additionally, participant's demographics, semen parameters and serum sex hormone levels were compared between the groups. SARS-CoV-2 was not detected within the semen samples. Sperm morphology and serum sex hormone levels were significantly different between the groups. In the post hoc analysis, sperm morphology was significantly lower in the COVID-19 patients. Patients before treatment had significantly lower serum FSH, LH and T levels than controls. However, patients after treatment had similar serum FSH, LH and T levels with controls and patients before treatment. In our opinion, COVID-19 and its treatment had no specific deteriorative effect on male sexual health at a short-time period. In the patients before treatment, decreased serum of T, FSH and LH levels was consistent with acute patient stress due to COVID-19. Similarly, it seems that decreased sperm morphology was associated with the acute fever.

KEYWORDS
male, pilot study, SARS coronavirus, semen, sexual health

1 | INTRODUCTION

Since first reports of infection in the last quarter of 2019, a novel beta-coronavirus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected thousands of people and spread overseas (Jiang et al., 2020). Eventually on 11 March 2020, The World Health Organization (WHO) named the disease caused by the SARS-CoV-2 infection as 'COVID-19' and declared a pandemic status (Huang et al., 2020). Symptoms and signs of COVID-19 have a considerable similarity to influenza flu as both of the diseases mainly affect the respiratory system. As in influenza flu, the clinical presentation of COVID-19 can vary from an asymptomatic course to severe pneumonia (Kakodkar et al., 2020). At the pathophysiological basis of the SARS-CoV-2 infection, the angiotensin-converting enzyme 2 (ACE-2) receptor plays a central role. SARS-CoV-2 exploits the ACE-2 receptor and uses it to facilitate viral entry into the target cells (Zhang et al., 2020).

Being a respiratory system virus, primary target of SARS-CoV-2 is ACE-2 receptor expressing upper and lower respiratory tract (Li, Li, et al., 2020). ACE-2 expression, on the other hand, is not limited to the lungs and has also been demonstrated in many extra-pulmonary organs (Li, Li, et al., 2020; Zhang et al., 2020). For instance, testes are one of the highest ACE-2 expressing tissue (Li, Li, et al., 2020). In this context, the testes are potential target for SARS-CoV-2 infection. Considering the publications on COVID-19, most of the studies focus on epidemiological data and either the clinical presentation or outcome of the SARS-CoV-2 pneumonia (Yang et al., 2020). Although limited number of studies reported the effects of COVID-19 on the other organ systems (Behzad et al., 2020), characteristics of extra-pulmonary COVID-19 still remain unknown.

To our knowledge, there is no study evaluating whether the testes are potential target of COVID-19. In the present study, we primarily aimed to investigate whether COVID-19 has any deleterious effect on the male reproductive system (reproductive system COVID-19 phenomenon). The secondary aim of the study was to evaluate the effects of our COVID-19 treatment algorithm, consisting of hydroxychloroquine, azithromycin and low-molecular-weight heparin (LMWH), on semen parameters and male sex hormone profile.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

After approval by the Institutional Review Board (Approval No: 2020.05.1.08.040), a prospective, cross-sectional, analytical study was conducted in a tertiary care centre. The principles outlined in the Declaration of Helsinki were followed, and informed consent from all of the participants was obtained, as well. Approval of Turkish Ministry of Health also provided for the study. A total of 55 patients with a confirmed or probable diagnosis of COVID-19 disease and ten healthy volunteers as controls were prospectively evaluated between April and May 2020.

2.2 | Participants

All of the patients were selected from our department. The controls were also selected from healthcare workers in our department. Inclusion criteria were being 18- to 60-year-old males, laboratory-confirmed COVID-19 patients immediately after their treatment, highly suspected COVID-19 patients with specific computerised tomography (CT) imaging findings (Ai et al., 2020) before treatment and healthy controls without any clinical or laboratory COVID-19 findings. History of infertility, presence of palpable varicocele and/or testicular atrophy, previous history of scrotal or inguinal surgery or scrotal trauma, presence of acute or history of previous epididymitis or orchitis, history of mumps during the childhood period, presence of any known malignant disease and usage of any drug affecting male reproductive tract were the exclusion criteria. Patients who could not meet two to five days ejaculatory abstinence period before semen sampling and could not ejaculate semen were also not included in the study.

Diagnosis of the COVID-19 was confirmed by identification of SARS-CoV-2 RNA extracted from upper respiratory specimens including nasopharyngeal and oropharyngeal swabs with real-time reverse-transcription polymerase chain reaction (RT-PCR) assay in our genomic laboratory. The RT-PCR assay was performed according to manufacturer's instructions (Coyote Bioscience Co., Ltd., Beijing, China). Patients were diagnosed as probable COVID-19 cases based on the criteria listed on the latest updated version of National COVID-19 Guidelines (Turkish COVID-19 science committee, 2020). Cases with specific chest CT findings consistent with the disease but do not have positive RT-PCR result were termed as highly suspected COVID-19 cases. We again used latest updated version of our National COVID-19 Guidelines to determine the treatment algorithms, as well. The treatment of the patients who were highly suspected for COVID-19 was initiated upon admission to the hospital even before the RT-PCR results were obtained to prevent any complications due to the delay in the treatment. For uncomplicated cases, the treatment protocol was defined as oral hydroxychloroquine 400 mg once a day and oral azithromycin 500 mg twice daily for the first day as a loading dose and 250 mg per day for 4 days thereafter. For the patients with mild pneumonia, oral hydroxychloroquine treatment was started as 400 mg twice daily for the first day and was continued as 400 mg once daily for the next four days with oral administration of azithromycin 500 mg twice daily for the first day as a loading dose and 250 mg per day for 4 days thereafter. Low-molecular-weight heparin (LMWH) was also injected subcutaneously with a dosage of 0.75mg kg day^{-1} as a single dose.

Initially, we stratified the participants into the three groups as follows: Group 1: highly suspected COVID-19 cases before treatment, Group 2: confirmed COVID-19 patients after treatment and Group 3: controls. As the treatment was initiated before the RT-PCR results, we selected only highly suspected COVID-19 cases to minimise the inclusion of negative cases. Confirmed COVID-19 cases with positive RT-PCR tests for swab samples and completed the 5-day treatment protocol included in the Group 2. The blood and semen samples were obtained immediately after the end of the treatment, in this patients. Blood and semen samples were collected from the patients of Group1 and 3, as well.

2.3 | Outcomes

Outcomes of interest were as follows: transmission of the SARS-CoV-2 into the semen, effects of SARS-CoV-2 on semen parameters and serum male sexual hormone levels, and adverse effects of the treatment protocol for COVID-19 on male sexual and reproductive health.

2.4 | Data source/measurement and variables

Venous blood samples were collected at 8:30 to 10:30 a.m. after an eight hours of starving to measure serum male sex hormone levels including testosterone (T), prolactin (PRL), luteinising hormone (LH) and follicle-stimulating hormone (FSH). After centrifugation of the blood samples for 10 min at 4,000 *g*, serum was obtained and serum levels of the parameters detected by electro-chemiluminescent immunoassays using UniCel™ DxI 800 Access Immunoassay System (Beckman Coulter Inc., Brea, CA, USA). Semen samples were obtained by masturbation, and all of the ejaculate was collected into a sterile wide-mouthed calibrated container after ansexual abstinence period of two to five days. Semen analysis was performed according to World Health Organization Laboratory Manual for the Examination and Processing of Human Semen (Fifth Edition). (Cooper et al., 2010). We evaluated the main sperm parameters: volume, pH, sperm counts, motility, percentage of normal morphology and seminal leukocytes. After completion of semen analysis, remaining semen samples were analysed for the detection of SARS-CoV-2 using RT-PCR.

After obtaining the RT-PCR tests results of respiratory samples, participants were divided into three final groups as 'COVID-19 patients before treatment', 'COVID-19 patients after treatment' and 'controls'.

The parameters age, body mass index (BMI) (calculated by dividing the weight in kilograms to the height in metres squared), smoking history, clinical quantification of cigarette smoking (pack years of smoking), comorbidities (including diabetes mellitus, hypertension, coronary heart disease or any chronic illnesses requiring long-term medicine/pills usage or well-known effects on sperm production), semen volume, semen pH, sperm counts, percentages of sperm motility, percentage of normal sperm morphology, seminal leukocytes and serum FSH, LH, T and PRL levels were statistically compared between the groups. Serum T/LH, FSH/LH and PRL/T ratios were also compared (for calculating the T/LH ratio, the unit of T was converted from ng/mL to IU/L). We preferred to additional use of PRL/T ratio because of the fact that the PRL/T ratio is superior to PRL or T alone in identifying potential pituitary pathology in hypogonadal men (Naelitz et al., 2020).

2.5 | Data analysis

We determined the minimum number of participants required using G*power version 3.0.10 data analysis software (Department of Cognitive and Industrial Psychology, Heinrich Heine Universität, Düsseldorf, Germany). The alpha level (the probability of detecting a significant difference) and power were considered as 0.05 and 0.75, respectively, in determining the sample size. In the beginning, the effect size was investigated considering the one-way ANOVA test for 20 sample in each group for all parameters. The minimum effect size found as 0.56578. Later, the effect size: 0.56, alpha level:0.05 and power: 0.75 values and number of groups: 3 were inserted to G*power calculator as input parameters with the settings of *F* test as test family, one-way ANOVA statistic test, and a priori power analysis type. The output revealed that total sample size as 33 and actual power as 0.7872. As a result, we decided to sample size as 30 participants and 10 for each group.

Statistical analysis was performed using IBM SPSS Statistics version 22.0 statistic software package (IBM SPSS Inc., Chicago, IL). Data distributions and test of normality were evaluated with Shapiro–Wilk test. Descriptive statistic methods (mean ± standard deviation and median ± Interquartile range) were used to evaluate data. We compared the normally distributed and not normally distributed parametric data between the three groups, using one-way ANOVA test, and Kruskal–Wallis test respectively. The Tukey's test was used for post hoc analysis of one-way ANOVA test. Post hoc analysis of Kruskal–Wallis test was performed using independent *t* test and Mann–Whitney *U* test for normally distributed and not normally distributed parameters respectively. To determine the effects of given treatment on the parameters, independent *t* test and Mann–Whitney U test were used for normally distributed and not normally distributed parametric data respectively. Chi-square test was also used in the comparison of the nonparametric categorical variables. Differences were considered significant at $p < .05$ and 95% confidence interval.

3 | RESULTS

Among the 55 participants, 30 of them who met the inclusion criteria were enrolled. In the initial 'highly suspected COVID-19 cases before treatment' group, for seven patients, RT-PCR confirmed the disease after 2–3 days. However, due to the high probability based on the clinical findings including signs and symptoms related to COVID-19 history of close contact to a person with COVID-19 and specific chest CT findings, we assumed remaining patients as confirmed COVID-19 cases even if their RT-PCR results were reported as negative. In this regard, the group was named as COVID-19 patients before treatment group (Group 1).

After completion of the treatment protocol, all patients enrolled to the study were discharged to home without any complications. All patients within the Group 1 had fever with a median body temperature of 38.7°C (range: 38.5–39.2°C). On the other hand, body temperatures of patients in Group 2 and Group 3 were within the normal range (36.1–37.2°C).

The median hospital stay was 4.5 (4–5) days for patients in Group 1 and Group 2. One patient in Group 1 had type 2 diabetes mellitus, and one patient in Group 2 had a psoriasis. Of the patients within Group 3, one had type 2 diabetes mellitus and one had hypertension.

The mean age, smoking rate, serum PRL level and serum FSH/LH ratio of the whole study group were 37.21 ± 8.59 years, 20.70 ± 7.40 package-year, 7.15 ± 4.09 ng/ml and 0.92 ± 0.38 respectively. The median BMI, semen volume, semen pH, sperm count, total sperm count, progressive motility, non-progressive motility, total motile sperm count and sperm morphology of the participants were 26.30

(1.85) kg/m^2, 1.75 (1.13) ml, 8.0 (1.0), 42.0 (49.0) × 10^6/ml, 66.0 (55.5) × 10^6, 35.5 (9.0) %, 8.5 (4.0) %, 26.74 (22.0) × 10^6 and 1.5 (2.0) % respectively. The median serum FSH, LH and T levels and serum T/LH ratio of the participants were 3.10 (2.14) IU/L, 3.45 (2.95) IU/L and 2.30 (1.89) ng/ml and 395.91 (281.369) respectively.

The semen RT-PCR test results revealed that none of the patients with COVID-19 had SARS-CoV-2 in their semen samples. All the semen parameters except semen morphology were normal limits in patients and controls. The patient demographics and mean and median levels of the semen parameters were provided in the Table 1 for patients and controls. The normal morphology was %3 in controls, whereas it was %1.5 and %1 in patients before and after treatment groups ($p = .006$). All the hormone levels were at normal limits in both patients and controls. However, the levels of the parameters exhibited some significant differences, even if they were normal limits, between patients and controls. The median serum FSH, LH and T levels showed significant difference, whereas the mean serum PRL level and the median serum T/LH and mean FSH/LH ratios were similar (Table 1). According to the post hoc analysis, the difference was associated with lower per cent of normal sperm morphology in the COVID-19 patients (Table 2). Post hoc analysis also revealed that the source of significant differences in serum FSH, LH and T levels was decreased serum levels in Group 1 (Table 2).

All the parameters were similar between patients in Group 1 and Group 2 (Table 3).

4 | DISCUSSION

Despite the de-escalation of COVID-19 pandemic crisis, it has already strained the health systems worldwide. Healthcare workers and healthcare systems are facing a multitude of challenges at all stages of the pandemic (Tanne et al., 2020). With the accumulation of data and experience, articles about COVID-19 have been published successfully all around the World (Yang et al., 2020). As expected, most of the initial studies were case reports, case series or epidemiological investigations. Studies on extra-pulmonary COVID-19, especially on genitourinary involvement, with appropriate methodology are still lacking.

In the present study, we investigated the effects of COVID-19 on male sexual and reproductive health with a pilot study design. First report about the detection of SARS-CoV-2 in specimens obtained other than respiratory tract was published by Wang et al. (2020) on 11 March 2020. The authors investigated the presence of SARS-CoV-2 in different types of clinical specimens and revealed that the virus may be found in blood and faeces in addition to respiratory specimens, but not in urine. Following this initial report, possibility of the existence of SARS-CoV-2 within the semen has started to attract attention. First report about this topic was published by Song Ci et al. (2020) on 10 April 2020. In this report, the authors could not demonstrate the presence of the virus in the semen or testicular biopsy specimens by using RT-PCR. They concluded that there is no evidence to support the sexual transmission of SARS-CoV-2 from males. A few days later, similarly, Paoli et al. (2020) reported the negative semen investigation results of a 31-year-old man diagnosed with COVID-19. Contrary to these initial reports, Li et al. (2020b) demonstrated SARS-Cov-2 positivity in semen samples of six patients out of 38 COVID-19 patients. Within a couple of weeks, the first cohort study on the topic was reported by Nora et al. (2020), which included the semen results of 34 COVID-19 patients. This was also the first report investigating the standard semen analysis of the patients. They reported that a mild COVID-19 infection did not likely to affect testicular and epididymal function, whereas semen parameters began to impairment after a moderate infection. SARS-CoV-2 RNA could not be detected in semen of recovered and acute COVID-19 positive males by the authors. The latest report by Pan et al. (2020) revealed that SARS-CoV-2 was not detected in the semen of 34 patients recovering from COVID-19 one month after COVID-19 diagnosis. In the present study, we also revealed that SARS-CoV-2 did not pass to the semen. Unlike findings of the current study as well as the several previous ones, Li et al. (2020a) identified the virus within the semen samples. They reported the findings of the patients diagnosed between 26 January 2020 and 16 February 2020. During that period, no standard treatment protocol had been established yet and various trials were ongoing to define the best treatment method for COVID-19. Therefore, it is possible that some of those treatment agents might have affected their results. Similarly, Song Ci et al. (2020), Nora et al. (2020) and Pan et al. (2020) also did not describe their treatment protocols. Only Paoli et al. (2020) provided the information of paracetamol treatment for COVID-19 patient who gave semen sample. Some studies showed that harmful environmental toxicants, xenobiotics and/or therapeutic substances (e.g. chemotherapeutic agents) induce blood-testis barrier (BTB) and testis damage (Pereira & Garcia e Costa, 2007; Su et al., 2011).

In the present study, we described our treatment algorithm clearly and obtained some of the semen samples from the patients before treatment period. This allowed us to see and comment the possible effects of the given treatment. Our findings revealed that all the parameters were similar between the COVID-19 patients before and after treatment groups (Table 4). In this regard, our treatment protocol consisting azithromycin and hydroxychloroquine was safe in terms of male sexual and reproductive health. Azithromycin is a well-known drug commonly used for urogenital infections. To date, only one report has underlined the deleterious effects on male genital system when used doubled dosage of Azithromycin more than six days (El-Sayed et al., 2017). We used a much lower dosage in the treatment of COVID-19 patients enrolled to the study. Similarly, chloroquine has no known potential for damage to the testes in the literature, even more it increases the transepithelial resistance of immature Sertoli cells (Okanlawon & Dym, 1996).

In our cohort, we described significant decrease in the percentage of normal morphology on the semen analysis of the COVID-19 patients. We attributed this finding to the fever seen all COVID-19 patients, which was described and supported previously in the literature (Andrade-Rocha, 2013; Carlsen et al., 2003). For instance, Carlsen et al. investigated the effect of fever on semen parameters

TABLE 1 Comparison of the parameters between the groups

	Controls ($n = 10$)	COVID−19 patients before treatment ($n = 10$)	COVID−19 patients after treatment ($n = 10$)	p
Age (years) (Mean ± *SD*)	36.64 ± 9.63	38.00 ± 8.28	37.00 ± 8.69	.93[a]
BMI (Median ± IQR)	26.57 ± 2.71	25.55 ± 2.08	26.55 ± 1.14	.07[b]
Smoking status (*n*, %)				.63[c]
Yes	4, 13.3	6, 20.0	7, 23.3	
No	6, 20.0	4, 13.3	3, 10.0	
Smoking rate (pock-year) (Mean ± *SD*)	17.50 ± 8.66	21.00 ± 9.03	22.28 ± 5.55	.61[a]
Comorbidity (*n*, %)				.38[c]
None	8, 26.7	8, 26.7	9, 30.0	
Diabetes mellitus	1, 3.3	1, 3.3	0, 0.0	
Hypertension	1, 3.3	0, 0.0	0, 0.0	
Others	0, 0.0	1, 3.3	1, 3.3	
SARS-CoV−2 positive semen sample (*n*)	0	0	0	
Semen volume (ml) (Median ± IQR)	2.00 ± 1.63	1.25 ± 1.13	1.60 ± 1.63	.14[b]
Semen pH (Median ± IQR)	8.00 ± 0.10	8.00 ± 1.00	9.00 ± 1.00	.09[b]
Sperm count ($x10^6$/ml) (Mean ± *SD*)	41.00 ± 36.30	57.00 ± 36.62	45.10 ± 36.90	.56[a]
Total sperm count ($x10^6$) (Mean ± *SD*)	48 ± 147.25	67.40 ± 29.61	69.90 ± 57.01	.93[a]
Progressive sperm motility (%) (Median ± IQR)	35.00 ± 9.75	39.50 ± 15.50	33.00 ± 14.75	.18[b]
Non-progressive sperm motility (%) (Median ± IQR)	9.50 ± 3.25	8.00 ± 5.50	9.50 ± 4.25	.48[b]
Total motile sperm count ($x10^6$) (Mean ± *SD*)	18.68 ± 79.06	27.97 ± 14.88	23.54 ± 18.53	.69[a]
Normal sperm morphology (%) (Median ± IQR)	3.00 ± 3.00	1.50 ± 2.00	1.00 ± 1.00	.006[b]
Leukocytes detected in semen (*n*, %)				.08[c]
Yes	0, 0.0	4, 13.3	3, 10.0	
No	10, 33.3	6, 20.0	7, 23.3	
Serum FSH (IU/L) (Median ± IQR)	3.92 ± 2.35	2.04 ± 1.36	3.15 ± 2.70	.01[b]
Serum LH (IU/L) (Median ± IQR)	4.46 ± 2.06	2.98 ± 1.65	3.22 ± 3.83	.04[b]
Serum PRL (ng/ml) (Mean ± *SD*)	5.71 ± 1.44	5.44 ± 2.54	8.31 ± 4.65	.09[a]
Serum T (ng/ml) (Median ± IQR)	2.90 ± 1.87	1.13 ± 1.28	2.26 ± 1.86	.02[b]
Serum T/LH ratio (Median ± IQR)	0.60 ± 0.25	0.42 ± 0.36	0.41 ± 0.48	.18[b]

(Continues)

TABLE 1 (Continued)

	Controls ($n = 10$)	COVID–19 patients before treatment ($n = 10$)	COVID–19 patients after treatment ($n = 10$)	p
Serum FSH/LH ratio (Mean ± *SD*)	0.97 ± 0.35	0.87 ± 0.42	0.93 ± 0.39	.85[a]
Serum PRL/T ratio (Median ± IQR)	2.14 ± 3.24	3.96 ± 3.15	4.40 ± 3.92	.03[b]

Abbreviations: COVID-19, coronavirus disease-2019, BMI, body mass index, FSH, follicle-stimulating hormone, IQR, interquantile range; LH, luteinising hormone, PRL, prolactin, *SD*, standard deviation, T, testosterone.

[a]One-way ANOVA test

[b]Kruskal–Wallis test

[c]Chi-square test.

TABLE 2 Post hoc analysis of the Kruskal–Wallis test

	Controls versus COVID–19 patients before treatment	Controls versus COVID–19 patients after treatment	COVID–19 patients before versus after treatment
Normal sperm morphology (%)(Median ± IQR)	3.0 ± 3.0 versus 1.5 ± 2.0 $p = .07$[a]	3.0 ± 3.0 versus 1.0 ± 1.0 $p < .002$[a]	1.0 ± 1.0 versus 1.0 ± 1.0 $p = .16$[a]
Serum FSH (IU/L) (Median ± IQR)	3.92 ± 2.35 versus 2.04 ± 1.36 $p < .004$[a]	3.92 ± 2.35 versus 3.15 ± 2.70 $p = .19$[a]	2.04 ± 1.36 versus 3.25 ± 2.70 $p = .16$[a]
Serum LH (IU/L) (Median ± IQR)	4.46 ± 2.06 versus 2.98 ± 1.65 $p < .007$[a]	4.46 ± 2.06 versus 3.22 ± 3.83 $p = .39$[a]	2.981±0.65 versus 3.223 ± 3.83 $p = .21$[a]
Serum T (ng/mL) (Median ± IQR)	2.90 ± 1.87 versus 1.13 ± 1.28 $p < .009$[a]	2.90 (1.87) versus 2.26 ± 1.86 $p = .12$[a]	1.13 ± 1.28 versus 2.26 ± 1.86 $p = .16$[a]

Abbreviations: COVID-19, coronavirus disease-2019; FSH, follicle-stimulating hormone; IQR, interquantile range; LH, luteinising hormone; PRL, prolactin; *SD*, standard deviation; T, testosterone.

[a]Mann–Whitney *U* test.

TABLE 3 Post hoc analysis of the one-way ANOVA test

	Controls versus COVID–19 patients before treatment	Controls versus COVID–19 patients after treatment	COVID–19 patients before versus after treatment
Serum PRL (ng/mL) (Mean ± *SD*)	5.71 ± 1.44 versus 5.44 ± 2.54	5.71 ± 1.44 versus 10.31 ± 5.36	5.44 ± 2.54 versus 10.31 ± 5.36
p	.98[a]	.01[a]	.01[a]

Abbreviations: COVID-19, coronavirus disease-2019; PRL, prolactin.

[a]Tukey's test.

and they showed that sperm concentration significantly decreased by 32.6% following fever during meiosis and by 35.0% following fever during the post-meiotic period of spermatogenesis (spermiogenesis). The percentage of morphologically normal spermatozoa decreased by 7.4%, and the percentage of immotile spermatozoa increased by 20.4% (6.0; 36.8) by fever during spermiogenesis, as well. According to their findings, the authors concluded that the number of days the men experienced fever significantly affected their semen parameters (Carlsen et al., 2003).

In our opinion, COVID-19 may cause decrease in semen morphology due to the fever instead of the direct effect of SARS-CoV-2 on testes. The primary support of this assumption was that significantly decreased serum testosterone, FSH and LH levels with similar PRL levels in our COVID-19 patients when compared to our normal controls. Increased LH and FSH levels would have been accompanied decreased testosterone levels if the COVID-19 had caused testicular damage in our patients. Some studies showed that stress reduces gonadotropin secretion with gonadotropin-inhibitory hormone independently of PRL (Breen & Mellon, 2014; Clarke et al., 2016). In our patients, after the disappearing of COVID-19 associated stress at the end of the treatment, serum T, FSH and LH levels showed an increase at the least. Our findings revealed a similar T/LH and FSH/LH levels between the groups, as well. Because of T/LH ratio is a prognostic parameter of infertility and Leydig cell function (Giagulli & Vermeulen, 1988), similar T/LH level of the groups again demonstrated that COVID-19 had no direct effect on testes.

Our study has some limitations. The major one was the relatively small sample size. However, we had a prospective pilot

TABLE 4 Changes in the parameters after treatment for COVID-19 patients

	COVID−19 patients before treatment ($n = 10$)	COVID−19 patients after treatment ($n = 10$)	p
Age (years) (Mean ± *SD*)	38.00 ± 8.28	37.00 ± 8.69	.79[a]
BMI (Median ± IQR)	25.55 ± 2.08	26.55 ± 1.14	.29[b]
Smoking status (*n*, %)			
Yes	6, 30.0	4, 20.0	.63[c]
No	7, 35.0	3, 15.0	
Smoking rate (pocket/year) (Mean ± *SD*)	21.00 ± 9.03	22.28 ± 5.55	.75[a]
Comorbidity (*n*, %)			.58[c]
None	8, 40.0	9, 45.0	
Diabetes mellitus	1, 5.0	0, 0.0	
Hypertension	0, 0.0	0, 0.0	
Others	1, 5.0	1, 5.0	
SARS-CoV−2 positive semen sample (*n*)	0	0	
Semen volume (ml) (Median ± IQR)	1.25 ± 1.13	1.60 ± 1.63	.63[b]
Semen pH (Median ± IQR)	8.00 ± 1.00	9.00 ± 1.00	.31[b]
Sperm count (x10^6/ml) (Median ± IQR)	57.00 ± 36.62	45.10 ± 36.90	.52[a]
Total sperm count (x10^6) (Mean ± *SD*)	67.40 ± 29.61	69.90 ± 57.01	.90[b]
Progressive sperm motility (%)(Median ± IQR)	39.50 ± 15.50	33.00 ± 14.75	.89[b]
Non-progressive sperm motility (%)(Median ± IQR)	8.00 ± 5.50	9.50 ± 4.25	.43[b]
Total motile sperm count (x10^6) (Mean ± *SD*)	27.97 ± 14.88	23.54 ± 18.53	.56[b]
Normal morphology (%)(Median ± IQR)	1.50 ± 2.00	1.00 ± 1.00	.16[b]
Leukocytes detected in semen (*n*, %)			.63[c]
Yes	4, 20.0	3, 15.0	
No	6, 30.0	7, 35.0	
Serum FSH (IU/L) (Median ± IQR)	2.04 ± 1.36	3.15 ± 2.70	.16[b]
Serum LH (IU/L) (Median ± IQR)	2.98 ± 1.65	3.22 ± 3.83	.21[b]
Serum PRL (ng/mL) (Mean ± *SD*)	5.44 ± 2.54	8.31 ± 4.65	.06[b]
Serum T (ng/mL) (Median ± IQR)	1.13 ± 1.28	2.26 ± 1.86	.16[b]
Serum T/LH ratio (Median ± IQR)	347.64 ± 299.72	342.96 ± 399.33	.97[b]
Serum FSH/LH ratio (Mean ± *SD*)	0.87 ± 0.42	0.93 ± 0.39	.71[a]
Serum PRL/T ratio (Median ± IQR)	3.96 ± 3.15	4.40 ± 3.92	.91[b]

Abbreviations: BMI, body mass index; COVID-19, coronavirus disease-2019; FSH, follicle-stimulating hormone; IQR, interquantile range; LH, luteinising hormone; PRL, prolactin; *SD*, standard deviation; T, testosterone.

[a]Independent *t* test

[b]Mann–Whitney u test

[c]Chi-square test.

design making up the shortages of previous related reports such as lack of the patient selection criteria and group homogenisation. Indeed, our groups were similar in terms of age, smoking rate, comorbidity and BMI. The second major limitation was the lack of the investigation of testicular histology. The other limitation was the lack of the long-term results. However, we intended to perform a pilot study with optimal methodology and we will make an effort to maintain the protocol. Finally, three patients with negative RT-PCR were considered as confirmed COVID-19. Even if it seems as other limitation, sometimes a negative RT-PCR may not exclude COVID-19, especially if clinical suspicion is high with specific CT findings. The major strength of the present study was the inclusion of COVID-19 patients before treatment and the exclusion of the patients with scrotal surgery, previous genital tract infection history, previous scrotal trauma history and patients using any drug affecting male reproductive tract, because all those criteria could have affected the results.

In conclusion, our findings primarily revealed that COVID-19 has no specific deteriorative effect on male sexual and reproductive health at a short-time period. Decreased serum T, FSH and LH levels was associated with acute patient stress due to the COVID-19 and its uncertainties. Diminished sperm morphology was also associated

with fever. Our secondary conclusion is that treatment of COVID-19 with hydroxychloroquine and azithromycin was safe and effective.

However, these are preliminary findings and studies with tissue confirmation and long-term follow-up are needed to complete our final report.

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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