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Effects of COVID-19 on sexual health in men with suspected infertility using semen analysis and serum hormone profile: A single-center experience

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ABSTRACT

Aim: To evaluate the effect of COVID-19 on sex hormone levels between men who have recovered from COVID-19 infection and men who have never been infected.

Method: This study included 80 men who applied to the Infertility Clinic with a diagnosis of primary or secondary infertility. Semen analysis was performed twice, before COVID-19 and after the treatment of COVID-19 disease. In addition, Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), testosterone (T), and 17β- estradiol (E2) levels were compared between the men after COVID-19 disease and uninfected men.

Results: There was a significant difference in progressive sperm motility and immobility before and after the COVID-19 disease. Progressive sperm motility was decreased after COVID-19 disease while immobility was increased after COVID-19. The serum T level was lower and the E2 level was higher in men after COVID-19 disease compared to uninfected men.

Conclusions: COVID-19 may adversely affect gonadal functions by causing to more deterioration of the hormone levels and semen parameters in infertile males. Therefore, gonadal function evaluation, including semen and sex-related hormones examination, is required to follow up the male COVID-19 patients with a reproductive plan.

Key words: COVID-19, male infertility, semen analysis, sex-related hormones.

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Introduction

COVID-19, caused by the SARS-CoV-2 virus, was announced by WHO as an international public health emergency [1]. The broad infection of SARS-CoV-2 clinical spectrum has ranged from mild respiratory diseases to severe pneumonia with multi-organ failure, respiratory failure, and death. The first study of 191 primary patients with the virus in Wuhan showed that old age and comorbidities such as hypertension, diabetes, and cardiovascular disease were among the primary diagnostic factors for the severity of the disease in patients [2]. In a study conducted in the United Kingdom, it was observed that characteristics such as male gender, old age, obesity, chronic heart diseases, asthma, and

diabetes are risk factors for COVID-19 deaths [3,4]. Although the male gender is seen as a risk factor for SARS-CoV-2 deaths, the factors causing it are not clear.

Some studies show that COVID-19 has an effect on the testicles [5,6]. The disease has devastating effects due to the inflammatory responses to the virus or the cytopathic effects of the virus on target cells [7]. Entry of the virus into the cell is based on viral spike (S) proteins that bind to cellular receptors via host cell proteases and S protein preparation. It is thought that receptors of angiotensin-converting enzyme-2 (ACE-2) are the primary passage of the virus to attack the target cells. It also activates transmembrane serine protease 2 (TMPRSS2) to prim the S protein, defining the S2 subunit, which allows cellular and viral membrane fusion [8,9]. Prostate tissue, Leydig cells, seminiferous tubules, spermatogonia, and Sertoli cells express receptors of ACE-2, showing the adverse effect of COVID-19 on the reproductive system of men [10,11]. It was found a significant reduction in semen volume, percentage of total motility, percentage of progressive motility, and normal sperm morphology was found after COVID-19 [12]. A different study observed that semen parameters such as DNA integrity and sperm motility improved 120 days after diagnosis of COVID-19 [13]. In line with this information, we aimed to evaluate the semen parameters of men with suspected infertility and COVID-19 disease. For this, we compared the sperm test results before and after COVID-19 treatment. We also evaluated sex hormone levels between men who recovered after COVID-19 disease and men who had never been infected, to assess the impact of COVID-19 on sex hormone levels.

Material and Methods

Our study was conducted with 80 men aged 20-50 years who were diagnosed with primary or secondary infertility and admitted to Andrology Laboratory between May and September 2021. Semen samples were examined before and after COVID-19 disease. All volunteers completed a questionnaire regarding marital status, type of infertility, age, Body Mass Index (BMI), smoking, medical history, and COVID-19 history. Those with varicocele and chronic diseases were not included in the study. Each patient gave written informed consent. The study was approved by Health Sciences University, Gazi Yasargil Education and Research Hospital Ethics Committee. (Date and number of approval: 2021/750). The principles of the Declaration of Helsinki were applied to conduct the study.

Semen sample collection and analysis

After 2-5 days of sexual abstinence, participants were trained to take samples through masturbation into a sterile plastic container. For the liquefaction of semen, it was incubated for 30-60 minutes in a CO_2 incubator at 37^{o}C. The liquefaction time, volume, and pH of samples were evaluated manually. All semen criteria based on the World Health Organization (WHO) guidelines [14], including sperm concentration, total motile sperm count **(**TMSC), leukocytes, and sperm motility were examined by an experienced embryologist. A light microscope (Olympus CX31) and Makler camera (counting chamber, Sefi Medical Instruments) were used to measure sperm count and motility after semen liquefaction.

The COVID-19 diagnosis

The COVID-19 diagnosis was made based on the detection of SARS-CoV-2 from the samples collected from the patient's oropharynx and the nasopharynx with a cotton swab. Samples were analyzed by reverse-transcription polymerase chain reaction (RT-PCR). RT-PCR test was

conducted in the COVID-19 Laboratory authorized by the Ministry of Health. All COVID-19 volunteers included in this study had been diagnosed as a moderate or mild COVID-19 infection, according to the guidelines provided by the Turkish National Committee on COVID-19 [15]. The patients received the treatment recommended by the Ministry of Health and were not hospitalized.

Measurement of sex- related hormone levels

Peripheral blood samples were taken from the men participating in the study to compare their sex-related hormone levels. Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), 17β-estradiol (E2), and testosterone (T) levels were evaluated between the groups. For hormone analysis, after centrifugation of peripheral blood at 4000 rpm for 15 minutes, serum was obtained. Sex-related hormone levels were measured using Cobas 6000 (Roche, Germany) auto analyzer device using commercial kits (Roche, Germany).

Statistical analysis

Statistical evaluation was performed using SPSS 21 for Windows (IBM SPSS Inc., Armonk, NY, USA). The normality of data distribution was tested with the Kolmogorov- Smirnov test. Student T-test was used for pairwise comparison of normally distributed parametric data. When the data did not fit the normal distribution, Mann Whitney U tests were used for pairwise comparison. Chi-square test was used for the comparison of categorical data. Data were defined as mean, standard deviation and min-max. It was tested to see whether there was a significant difference between the two groups in terms of means of pre- and post-COVİD-19 data. $P \leq 0.05$ was considered significant.

Results

Demographic and clinical characteristics of the men with suspected infertility are shown in Table 1.

Table 1. Demographic features and clinical characteristics of the men.

Demographic characteristics	**Men with suspected infertility (n=80)**
Age (years), mean±SD	33.7± 3.82
BMI (kg/m^2), mean±SD	25.88 ± 2.88
Smoking status (yes/no), n (%)	44/36 (55/45)
Infertility type	
Primary, n (%)	54 (67.5)
Secondary, n (%)	26 (32.5)

***SD**: Standard deviation, **BMI**: Body Mass Index.*

Semen parameters

Semen samples were collected from 80 men before and after COVID-19 disease. There was no significant difference among the volunteers in semen volume, sperm concentration, total sperm count, non-progressive sperm motility, and total progressive sperm motility before and after COVID-19 diagnosis (*p>0.05*). There was a significant difference in terms of progressive sperm motility and immotility. Progressive sperm motility was decreased after COVID-19 infection while immotility was increased after COVID-19 infection (*p=0.009, p=0.021*, respectively). Table 2 shows the semen parameters of 80 men before and after COVID-19 disease.

Sex-related hormone levels

The sex hormone levels were assessed between men who have recovered from the COVID-19 disease and men who have never been infected. The serum T level was lower in the men with post- COVID-19 treatment compared to the non-infected men (*p=0.000*). Serum E2 level was higher in the COVID-19 group compared to the non-infected group (*p=0.0001*). Serum FSH and LH did not differ significantly between the men with post- COVID-19 and the non-infected group. Sex-related hormone levels are shown in Table 3.

Table 2. Semen analysis results of the 80 men before and after the COVID-19.

Semen parameters	**Before COVID-19**	**After COVID-19**	**p**
Abstinence time (days) (Mean ± SD),	3.11±0.43	3.08±0.44	0.11
Semen volume (ml) (Mean ± SD),	2.90 ±1.24	3.10 ±1.46	0.17
Sperm concentration ($x10^6$/ ml) (Mean ± SD),	50.42 ±45.89	43.71± 32.64	0.13
Total sperm count ($x10^6$) (Mean ± SD),	136.4 ±168.04	124.17 ±102.29	0.52
Progressive sperm motility (%) (Mean ± SD),	52.30 ±19.26	45.85 ±18.73	**0.009**
Non-progressive sperm motility (Mean ± SD)	10.03 ±8.48	8.42 ±5.11	0.43
Immotility (%) (Mean ± SD)	39.37±18.19	44.17± 17.55	**0.021**
Total Progressive motile sperm count ($x10^6$) (Mean ± SD)	72.33±108.02	69.21 ±65.04	0.66

Table 3. Hormone levels between men after COVID-19 and non-infected men.

Hormone levels	**COVID-19 post-treatment group (n: 80)**	**Uninfected healthy group (n:80)**	***p***
FSH (IU/L)(Mean ± SD)	4.62 ±3.69	4.65 ±2.02	*0.08*
LH (IU/L)(Mean ± SD)	6.49± 2.02	6.16 ± 2.01	*0.12*
T (ng/mL)(Mean ± SD)	3.75± 1.47	5.00± 2.18	***0.00***
E2 (pg/mL)(Mean ± SD)	33.18± 8.36	23.50± 7.97	***0.00***

COVID-19, Coronavirus Disease 2019; FSH: Follicle-Stimulating Hormone LH: Luteinizing Hormone; T: Testosterone; E2: Estradiol; SD, Standard Deviation. The results statistically significant was shown in bold.

Discussion

Steroidogenesis and spermatogenesis were the major functions of the testes. Hepatitis C, B viruses, human papillomavirus, flu virus, herpes simplex virus, Epstein-Barr virus, Zika virus, Ebola virus, and the SARS-CoV-2 virus cause orchitis [16]. A complication of a viral infection leads to viral orchitis typically caused by hematogenous dissemination with viremia. Viral orchitis may affect the production of both testosterone and spermatogenesis. There is an association between testicular tissue viral

infection and interstitial edema, and perivascular lymphocytic infiltration inducing hyalinisation of the seminiferous tubule atrophy and fibrosis of the testes [17]. For this reason, the sexual transmission possibility and the status of spermatogenesis and reproductive endocrine should be examined to better show the COVID-19 impact on the male reproductive system.

Xu et al. [18] in the autopsy series, reported that SARS-CoV-2 caused SARS disease, associated with orchitis development. In histopathological examinations, seminiferous tubes were observed with inflammatory infiltrates. Although viral genomic materials were not isolated in testicular tissue in situ hybridization samples, it was interpreted as orchitis due to virus-related immunological and inflammatory reactions. Some autopsy series showed comparable damages in the histopathological analysis of the reproductive system among men infected with SARS-CoV-2. Bian and his team reported that SARS-CoV-2 was isolated from testes in the autopsy series. In addition, different degrees of damage to testicular cells and a decrease in the number of spermatogenic cells were reported [7]. Achua et al. [5] also showed positivity of SARS-CoV-2 in the testes, detecting spermatogenesis impairment.

In a study comparing semen analysis results of 18 COVID-19 patients with 14 healthy men, sperm motility and concentration were found to be higher in the control group [19]. A study of 41 male patients of reproductive age who recovered from COVID-19 analyzed serum sex hormones and semen parameters at a median time of 56 days after hospital discharge [20]. When COVID-19 patients and non-infected controls were compared for semen parameter values, patients had a significantly lower rate of progressive motile spermatozoa, motile spermatozoa, and a higher rate of asthenozoospermia than controls. An analysis of many studies on this topic found a statistically significant decrease in sperm quality and T levels in COVID-19 patients [21]. In a retrospective study, Schroeder et al. reported that male patients with severe COVID-19 had significantly lower T levels than control groups [22]. However, sex hormone levels showed no statistically significant differences in severe female COVID-19 patients. These results showed that severely male patients with COVID-19 may affect sex hormone metabolism. According to Ma et al. comparing the data of 273 healthy men and 119 COVID-19 patients, there was no significant difference in FSH and T levels, while higher serum LH and lower T/LH ratio were observed in the COVID-19 group [23]. Salonia et al. found significantly lower LH and T levels in COVID-19 patients compared to healthy controls [24]. A study in the USA showed an association between disease severity and low T concentrations in male patients with COVID-19 [25]. But there was no difference in the E2 level where severely ill males with COVID-19 compared to healthy controls. A study conducted in Italy reported that men with COVID-19 had decreased T level and increased E2 level than the control group who did not have COVID-19 [27]. According to the data, whether the COVID-19 interrupts the operation of the hypothalamic-pituitary-gonadal axis is unclear [19]. The effect of the COVID-19 virus through ACE-2 receptors and on Leydig cells could reduce T levels and negative affect sperm production and parameters [28].

Our study investigated whether the COVID-19 disease affects sperm quality and sex-related hormones in suspected infertile men. As far as we know, our study is the most extensive single-center study on this subject in Turkey. Depending on the exposure of the testicles to any infection or trauma, the change in sperm parameters can occur after an average of 3 months, but the spermatozoa life cycle is

approximately 74 days [26]. Therefore, the effects of COVID-19 can occur after the end of the life cycle of spermatozoa. Men who had a semen analysis for an average of 83 (65-134) days after COVID-19 diagnosis were included in this study. This period was thought to be appropriate for examining the changes in sperm parameters during the infection period. When sperm parameters were examined, we found that progressive sperm motility was lower in after the COVID-19 treatment compared to before the COVID-19, while immotility increased after the COVID-19 compared to before the COVID-19 (Table 2). Our results show that COVID-19 negatively affects sperm motility. Sperm motility is the concept that refers to the ability of the sperm to move properly through the woman's reproductive system to reach and fertilize the woman's egg. For fertilization, fast and forward-moving sperm are needed. The present study is in line with the previous studies regarding the significant reduction of sperm motility after COVID-19.

In this study, we compared the hormone levels of men with suspected infertility after COVID-19 treatment with those of uninfected men. We found that the serum T level was significantly lower after COVID-19 than in uninfected men. Also, serum E2 level was significantly higher in the men after COVID-19 than in the uninfected men's group. (Table 3). A decrease in T level can harm the sperm production process. According to the data obtained in this study, it was thought that low T concentration and increased E2/ T ratio could be used as diagnostic parameters, especially for men who had COVID-19. There was no significant difference between the FSH and LH values between after the treatment of COVID-19 and uninfected men. These results were in line with the previous studies.

This study has certain limitations. There were relatively few COVID-19 patients. However, our series was the most studied series in Turkey as far as we know. Another limitation was that those with severe disease were not studied.

Conclusions

COVID-19 may cause deterioration of the semen parameters and hormone levels in infertile men and may adversely affect gonadal functions. Therefore, gonadal function evaluation, including semen and sex-related hormones examination, should be performed in the reproductive plan follow-up men with COVID-19.

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Open Access Statement

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