New Microbes and New Infections 60–61 (2024) 101432

Contents lists available at ScienceDirect

New Microbes and New Infections

journal homepage: www.journals.elsevier.com/new-microbes-and-new-infections

Original Article

Investigating the effects of the Omicron variant of COVID-19 on sperm parameters and serum levels of male sexual hormones: Prospective observational study

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ARTICLE INFO

Handling Editor: Patricia Schlagenhauf

Keywords:
COVID-19
Sex hormones
Male fertility
Semen
Testis
Sexual health

ABSTRACT

Background: With the progress and prevalence of COVID-19, concerns have arisen regarding its impact on men's sexual health. Therefore, this study was conducted with the aim of examining the effects of COVID-19 on serum levels of sex hormones and semen.
Methods: Sixty participants who met the study inclusion criteria enrolled in this study between January and April 2022. The individuals were divided into three groups (n = 20): healthy, COVID-19 positive, and recovered from COVID-19. Blood and semen samples were collected from the participants. Serum levels of sex hormones and semen were evaluated both macroscopically and microscopically.
Results: Our study results showed that the most common symptoms observed in the COVID-19 group were cough (100 %), fever (100 %), fatigue (95 %), and runny nose (90 %). Serum levels of sex hormones (testosterone, FSH, and prolactin) in the COVID-19 group were significantly decreased compared to the healthy group. Microscopic examination of semen revealed significant differences in vitality, progressive, and motile parameters among the three groups, with a decrease observed in the COVID-19 group.
Conclusion: These results indicate that COVID-19 may have a negative impact on men's sexual health, potentially affecting hormone production and sperm quality. Further research is needed to determine the long-term effects of COVID-19 on male fertility and to explore potential treatment options.

1. Introduction

A novel type of pneumonia was defined by the World Health Organization (WHO) as coronavirus disease 2019 (COVID-19) in February 2020 [1]. This infection has infected thousands of people and led to the death of a large number of infected people [2].

The signs and symptoms of COVID-19 are remarkably similar to the flu as both diseases primarily affect the respiratory system [3]. As with influenza, the clinical presentation of COVID-19 can vary from an asymptomatic period to severe pneumonia [3,4]. So far, it has been established that the coronavirus is mainly transmitted through respiratory droplets [5]. In addition, viral RNA in various biological samples such as urine, feces, and blood has been observed [6].

Studies show that the male reproductive system, especially the process of spermatogenesis, is the target of the vulnerability of this virus [7] and increases the possibility that covid-19 may ultimately cause male infertility or sexual transmission of this virus, depending on the level of infection [7,8]. Angiotensin-converting enzyme receptor 2 (ACE-2) plays a major role in SARS-CoV-2 infection [9]. In such a way that SARS-CoV-2 exploits the ACE-2 receptor and uses it to facilitate the entry of the virus into target cells [10]. ACE-2 is expressed in several organs of human cells including the heart, kidney, and testes [11]. While the testes are immunologically privileged against viral infections, some viruses can breach the blood-testicular barrier and induce local inflammation [12].

The blood-testicular barrier (BTB) is a specialized structure that

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https://doi.org/10.1016/j.nmni.2024.101432
Received 8 October 2023; Received in revised form 20 April 2024; Accepted 14 May 2024
Available online 15 May 2024

separates the bloodstream from the seminiferous tubules within the testes. This barrier plays a crucial role in maintaining a specific microenvironment necessary for spermatogenesis [13]. The BTB restricts the passage of harmful substances and immune cells from the bloodstream into the testes, thereby protecting sperm cells from autoimmune reactions [14]. However, certain viruses and toxins can disrupt the BTB, potentially leading to testicular inflammation and impaired sperm production [15].

While fever is a common symptom of COVID-19 [16,17], it's not the only potential mechanism by which the disease may affect male fertility. The comment highlights other mechanisms to be explored: direct viral infection of testicular cells: studies are investigating the potential for SARS-CoV-2 to directly infect testicular cells, including spermatogonia stem cells, which are responsible for sperm production [7]. Inflammatory response: COVID-19 can induce an inflammatory response throughout the body. This inflammation could potentially reach the testes and disrupt spermatogenesis [18]. Hormonal imbalances: COVID-19 may affect the production of hormones essential for sperm production, such as testosterone [19].

Covid-19 disease typically manifests as viral pneumonia, with fever, dry cough, and fatigue being the most common symptoms. In some patients, it can also present with a runny nose, headache, and other symptoms. Diarrhea is less frequent [17]. Fever is a prominent symptom, observed in up to 80 % of COVID-19 patients [16,17].

While the definitive evidence for direct testicular damage by the coronavirus is still emerging, elevated scrotal temperatures due to fever can negatively impact sperm production [20]. This emphasizes the need to investigate the broader effects of COVID-19 on male fertility beyond just the presence of the virus in the testes.

In November 2021, the WHO designated a new variant of the coronavirus as "Omicron." By January 6, 2022, this variant had been confirmed in 149 countries, triggering a widespread global surge in cases [21]. This underlines the importance of ongoing research into the effects of COVID-19 variants on male health. The current study aimed to investigate the impact of the Omicron variant on male sexual health by analyzing sperm parameters and sex hormone levels.

2. Materials and methods

2.1. Study design and setting

After approval by the ethics committee of Birjand University of Medical Sciences (ethics No: IR.BUMS.REC.1401.164), a prospective analytical study was conducted in a medical center located in the city of Birjand. During the study, the principles stated in the Helsinki Declaration were observed and followed, and informed consent was obtained from all participants. In total, 40 patients with confirmed diagnosis of COVID-19 by PCR test and 20 healthy individuals were evaluated as the control group between January and April 2022.

2.2. Participants

Patients with COVID-19 were selected from the treatment center. The control group were initially examined based on self-reporting and without any symptoms and then entered the study. Inclusion criteria included men aged 18 to 50 and patients confirmed to have COVID-19 by PCR testing. Exclusion criteria included individuals with a history of infertility, hard occupations, heavy exercise, palpable testicular atrophy and/or varicocele, history of testicular trauma or surgery, acute epididymitis or orchitis, history of varicocelectomy, any known malignant disease or abnormality, or the use of any medication that affects the male reproductive system. In addition, individuals who could not abstain from ejaculation for two to five days prior to sampling or were unable to ejaculate were excluded from the study.

Respiratory samples were collected using nasal and oropharyngeal swabs to diagnose the Omicron variant of COVID-19, and were evaluated using real-time reverse transcription-polymerase chain reaction (RT-PCR) assay. The RT-PCR measurement was performed according to the manufacturer's Pishtazteb kit (Tehran, Iran) instructions. Finally, participants were divided into three groups (n = 20): Group 1: Healthy (without any symptoms), Group 2: COVID-19 (patients confirmed positive for COVID-19 Omicron strain by RT-PCR test), and Group 3: Recovered (individuals who have recovered from Omicron COVID-19 strain and at least two weeks have passed since their complete recovery).

2.3. Sample collection

The study was conducted on the serum and seminal fluid of the participants. Eight hours after fasting, blood was taken from the participants, and after clotting, the samples were centrifuged for 10 min at 4000 g, and the serum was obtained. Serum samples were used to measure male sex hormones, including testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL). The serum levels of sex hormones were measured using the DiaSorin kit (Saluggia, Italy) and the chemiluminescence technique.

In order to collect semen samples, participants were asked to abstain from intercourse for two to five days in order to collect semen samples. The semen samples were collected in a wide-mouth sterile graduated container. The analysis of the semen was performed using the laboratory manual of the World Health Organization for the examination and processing of human semen (5th edition) [22]. We evaluated the main parameters of semen in terms of microscopic and macroscopic parameters, including volume, sperm count, pH, motility, percentage of normal morphology, and semen leukocytes.

Demographic information, including age, marital status, occupation, Body Mass Index (BMI) ($BMI = \frac{\text{Body Weight } (kg)}{\text{Height } (m)}$), smoking history, comorbidities, and clinical symptoms, were collected from individuals using a designed questionnaire.

2.4. Statistical analysis

Based on a similar study by Nora et al. [23] and considering α of 0.05 and β of 0.1, and $x_1 = 89.5$ and $\sigma_1 = 69.6$ for the healthy group, and $x_2 = 32$ and $\sigma_2 = 22.6$ for the sick group, a sample size of 17 individuals was determined for each group. Taking into account a 0.2 dropout rate in the samples, the final sample size for each group was set to be 20 individuals.

The collected data were analyzed using SPSS version 16 software. Descriptive statistical methods such as mean, standard deviation, frequency, and percentage will be used to describe the data, and inferential statistics will be used for data analysis. The Kolmogorov-Smirnov test will be used to examine the normality assumption, and if the normality assumption is met, one-sided analysis of variance (ANOVA) and Tukey's post hoc test will be used for group comparison. If the normality assumption is not met, the nonparametric equivalent test, Kruskal-Wallis test, will be used. In all tests, a significance level of less than 0.05 will be considered.

3. Results

3.1. Demographic information

In this study, 60 samples participated in three groups of healthy individuals, COVID-19 patients, and COVID-19 recovered individuals. The mean age of the samples in the healthy group was 29.90 ± 6.585 years, in the COVID-19 patients' group was 28.75 ± 5.571 years, and in the COVID-19 recovered group was 27.25 ± 4.983 years. Statistical analysis showed no significant difference in age between the three groups ($p <$ 0.351). Also, no significant difference was observed between the three groups in terms of education, marital status, living environment, and

tobacco use ($p > 0.05$). Table 1 shows the frequency and percentage of demographic indicators of the samples under study according to the study groups.

3.2. Factors exposure to covid 2019

The results showed that in the study of factors exposure to Covid 2019, three groups did not differ significantly in terms of mask use ($p = 0.338$), exposure to a person with COVID-19 ($p = 0.122$), and attendance at crowded gatherings in enclosed spaces ($p = 0.754$) (Table 2).

3.3. Clinical symptoms

The results of the clinical symptoms examination in the study sample showed that the most common symptoms in the COVID-19 patients group were Cough (100 %), Fever (100 %), Fatigue (95 %), and Runny nose (90 %), while the most rare symptoms were Nausea (5 %), Chest pain (10 %), and Diarrhea (15 %). Additionally, in the recovered group, the most common clinical symptoms reported were Cough (80 %), Fatigue (45 %), and Sore throat (35 %). Results indicated that, except for Nausea ($p = 0.3$) and Diarrhea ($p = 0.07$), there was a significant decrease in other clinical symptoms in the recovered group compared to the COVID-19 patients ($p < 0.05$). Table 3 shows the frequency of clinical symptoms in the studied groups.

3.4. Vaccination of participants

The results of the study showed that all samples in the three study groups had received their first vaccine dose, with the highest vaccine received in the healthy group being AstraZeneca, COVIran Barekat, and Sinopharm (30 %), in the COVID-19 group being COVIran Barekat (40 %), and in the recovered group being Sinopharm (45 %). There was no significant statistical difference between the three groups regarding the type of vaccine received for the first dose ($P = 0.626$). Except for one person (5 %) in the healthy group, all other samples received their second vaccine dose. The highest vaccine received in the healthy group for the second dose was Sinopharm (40 %), in the patient group being Sinopharm and COVIran Barekat (35 %), and in the recovered group being Sinopharm (35 %). There was no significant statistical difference between the three groups regarding the type of vaccine received for the second dose ($P = 0.344$). Additionally, among the study samples, 26.7 % had not received their third vaccine dose, with the highest percentage in the healthy group. The highest vaccine received for the third dose in the healthy group was Sinopharm and COVIran Barekat (25 %), and in the patient and recovered groups was AstraZeneca (35 %). There was no significant statistical difference between the three groups regarding the type of vaccine received for the third dose ($P = 0.451$) (Table 4).

Table 1
Frequency and percentage of demographic characteristics of the samples under study by the studied groups.

			group			Total
			Healthy	COVID-19	Recovered	
marital status	single	N	7	8	8	23
		%	30.4 %	34.8 %	34.8 %	38.3 %
	married	N	13	12	12	37
		%	35.1 %	32.4 %	32.4 %	61.7 %
place of residence	city	N	15	14	17	46
		%	32.6 %	30.4 %	37.0 %	76.7 %
	Village	N	5	6	3	14
		%	35.7 %	42.9 %	21.4 %	23.3 %
Education	Middle school	N	4	1	2	7
		%	57.1 %	14.3 %	28.6 %	11.7 %
	Diploma	N	7	8	4	19
		%	36.8 %	42.1 %	21.1 %	31.7 %
	Bsc	N	7	11	10	28
		%	25.0 %	39.3 %	35.7 %	46.6 %
	Msc	N	2	0	4	6
		%	33.3 %	0.0 %	66.7 %	10 %
Smoking	No	N	13	16	12	41
		%	31.7 %	39.0 %	29.3 %	68.3 %
	2	N	2	1	2	5
		%	40.0 %	20.0 %	40.0 %	8.3 %
	3	N	3	2	5	10
		%	30.0 %	20.0 %	50.0 %	16.7 %
	4	N	2	0	1	3
		%	66.7 %	0.0 %	33.3 %	5 %
	5	N	0	1	0	1
		%	0.0 %	100.0 %	0.0 %	1.7 %

Table 2
Shows the frequency and percentage of exposure factors by study groups.

			group			Total
			Healthy	COVID-19	Recovered	
Mask	Yes	N	10	6	10	26
		%	38.5 %	23.1 %	38.5 %	43.3 %
	No	N	10	14	10	34
		%	29.4 %	41.2 %	29.4 %	56.7 %
COVID-19 Exposure	Yes	N	16	14	10	40
		%	40.0 %	35.0 %	25.0 %	66.7 %
	No	N	4	6	10	20
		%	20.0 %	30.0 %	50.0 %	33.3 %
Party	Yes	N	11	13	13	37
		%	29.7 %	35.1 %	35.1 %	61.7 %
	No	N	9	7	7	23
		%	39.1 %	30.4 %	30.4 %	38.3 %

3.5. Effect of COVID-19 on the profile of sex hormones

The results of the study on the levels of sexual hormones testosterone, LH, FSH, and prolactin among the study participants showed a significant decrease in the other hormones, except LH, in the group with COVID-19 compared to the healthy group ($p < 0.05$). However, this difference was only present in prolactin hormone between the recovered group compared to the control group ($p < 0.05$) (Fig. 1).

3.6. Effect of COVID-19 on macroscopic and microscopic indexes of sperm

The examination of the indices related to macroscopic parameters of the spermogram showed that the mean Liquefaction time in the healthy, COVID-19, and recovery groups was 32.3 ± 4.28, 31.4 ± 2.89, and 30.25 ± 3.43 min, respectively. Also, the mean volume of sperm samples in the healthy, COVID-19, and recovery groups was 2.2 ± 0.44, 2.46 ± 0.63, and 2.45 ± 0.66 mL, respectively. Statistical analysis indicated no significant difference in terms of Liquefaction and Volume among the three groups of healthy, disease, and recovery ($p > 0.05$). (Fig. 2).

In the examinations of the indices related to the microscopic parameters of spermogram, the results showed that the mean number of spermatozoa in the samples per milliliter ($p = 0.28$) and their percentage of natural morphology ($p = 0.3$) did not have a significant difference between the healthy, COVID-19 infected, and recovered groups. However, a significant difference was observed between the three groups in terms of the percentage of vitality, progressive, and motile ($p < 0.05$). Specifically, significant differences were observed in the percentage of vitality between the control group and the COVID-19 infected ($p =$

Table 3
Shows the frequency of clinical symptoms in the study samples by groups.

			group			Total
			Healthy	COVID-19	Recovered	
Cough	No	N	20	0	4	24
		%	100.0 %	0.0 %	20.0 %	40.0 %
	Yes	N	0	20	16	36
		%	0.0 %	100.0 %	80.0 %	60.0 %
Fever	No	N	20	1	20	41
		%	100.0 %	5.0 %	100.0 %	68.3 %
	Yes	N	0	19	0	19
		%	0.0 %	95.0 %	0.0 %	31.7 %
Ague	No	N	20	6	20	46
		%	100.0 %	30.0 %	100.0 %	76.7 %
	Yes	N	0	14	0	14
		%	0.0 %	70.0 %	0.0 %	23.3 %
Fatigue	No	N	20	1	11	32
		%	100.0 %	5.0 %	55.0 %	53.3 %
	Yes	N	0	19	9	28
		%	0.0 %	95.0 %	45.0 %	46.7 %
Anosmia and Ageusia	No	N	20	13	20	53
		%	100.0 %	65.0 %	100.0 %	88.3 %
	Yes	N	0	7	0	7
		%	0.0 %	35.0 %	0.0 %	11.7 %
Sore throat	No	N	20	5	13	38
		%	100.0 %	25.0 %	65.0 %	63.3 %
	Yes	N	0	15	7	22
		%	0.0 %	75.0 %	35.0 %	36.7 %
Headache	No	N	20	5	13	38
		%	100.0 %	25.0 %	65.0 %	63.3 %
	Yes	N	0	15	7	22
		%	0.0 %	75.0 %	35.0 %	36.7 %
Body pain	No	N	20	10	18	48
		%	100.0 %	50.0 %	90.0 %	80.0 %
	Yes	N	0	10	2	12
		%	0.0 %	50.0 %	10.0 %	20.0 %
Stomach	No	N	20	15	20	55
		%	100.0 %	75.0 %	100.0 %	91.7 %
	Yes	N	0	5	0	5
		%	0.0 %	25.0 %	0.0 %	8.3 %
Chest pain	No	N	20	18	20	58
		%	100.0 %	90.0 %	100.0 %	96.7 %
	Yes	N	0	2	0	2
		%	0.0 %	10.0 %	0.0 %	3.3 %
Runny nose	No	N	20	2	17	39
		%	100.0 %	10.0 %	85.0 %	65.0 %
	Yes	N	0	18	3	21
		%	0.0 %	90.0 %	15.0 %	35.0 %
eye redness	No	N	20	4	18	42
		%	100.0 %	20.0 %	90.0 %	70.0 %
	Yes	N	0	16	2	18
		%	0.0 %	80.0 %	10.0 %	30.0 %
Nausea	No	N	20	19	20	59
		%	100.0 %	95.0 %	100.0 %	98.3 %
	Yes	N	0	1	0	1
		%	0.0 %	5.0 %	0.0 %	1.7 %
Diarrhea	No	N	20	17	20	57
		%	100.0 %	85.0 %	100.0 %	95.0 %
	Yes	N	0	3	0	3
		%	0.0 %	15.0 %	0.0 %	5.0 %
Decreased libido	No	N	20	10	16	46
		%	100.0 %	50.0 %	80.0 %	76.7 %
	Yes	N	0	10	4	14
		%	0.0 %	50.0 %	20.0 %	23.3 %

Table 4
Frequency and percentage of vaccination factors in the first, second, and third doses by study groups.

			group			Total
			Healthy	COVID-19	Recovered	
Vac1	AstraZeneca	N	6	3	5	14
		%	30.0 %	15.0 %	25.0 %	23.3 %
	COVIran Barekat	N	6	8	4	18
		%	30.0 %	40.0 %	20.0 %	30.0 %
	Cov Pars	N	1	0	0	1
		%	5.0 %	0.0 %	0.0 %	1.7 %
	Sinopharm	N	6	6	9	21
		%	30.0 %	30.0 %	45.0 %	35.0 %
	Sputnik v	N	1	3	2	6
		%	5.0 %	15.0 %	10.0 %	10.0 %
Vac2	Unvaccinated	N	1	0	0	1
		%	5.0 %	0.0 %	0.0 %	1.7 %
	AstraZeneca	N	4	3	7	14
		%	20.0 %	15.0 %	35.0 %	23.3 %
	COVIran Barekat	N	6	7	3	16
		%	30.0 %	35.0 %	15.0 %	26.7 %
	Cov Pars	N	0	2	0	2
		%	0.0 %	10.0 %	0.0 %	3.3 %
	Sinopharm	N	8	7	7	22
		%	40.0 %	35.0 %	35.0 %	36.7 %
	Sputnik v	N	1	1	3	5
		%	5.0 %	5.0 %	15.0 %	8.3 %
Vac3	Unvaccinated	N	7	5	4	16
		%	35.0 %	25.0 %	20.0 %	26.7 %
	AstraZeneca	N	3	7	7	17
		%	15.0 %	35.0 %	35.0 %	28.3 %
	COVIran Barekat	N	5	6	3	14
		%	25.0 %	30.0 %	15.0 %	23.3 %
	Sinopharm	N	5	2	6	13
		%	25.0 %	10.0 %	30.0 %	21.7 %

0.003) and recovered groups ($p = 0.001$). Similarly, significant differences were observed in terms of progressive and motile between the control group and the COVID-19 infected and recovered groups ($p < 0.05$). Additionally, a significant difference was observed in the pH level between the control and recovered groups ($p = 0.046$). (Fig. 2).

4. Discussion

4.1. Main findings

There are various evidences and documents indicating the vulnerability of male reproductive systems to viral infections [24]. Bacterial infections usually attack the accessory glands and epididymis, but viruses can attack the testes through blood circulation. Numerous studies

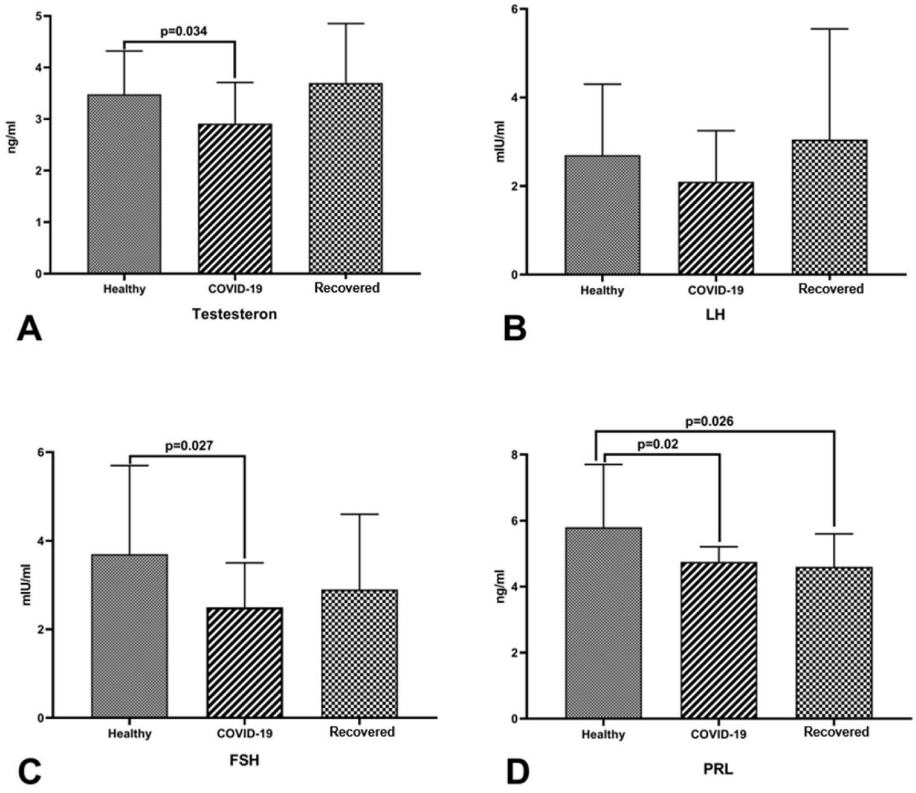

Fig. 1. Mean levels of testosterone (A), LH (B), FSH (C), and prolactin (D) according to the study groups.

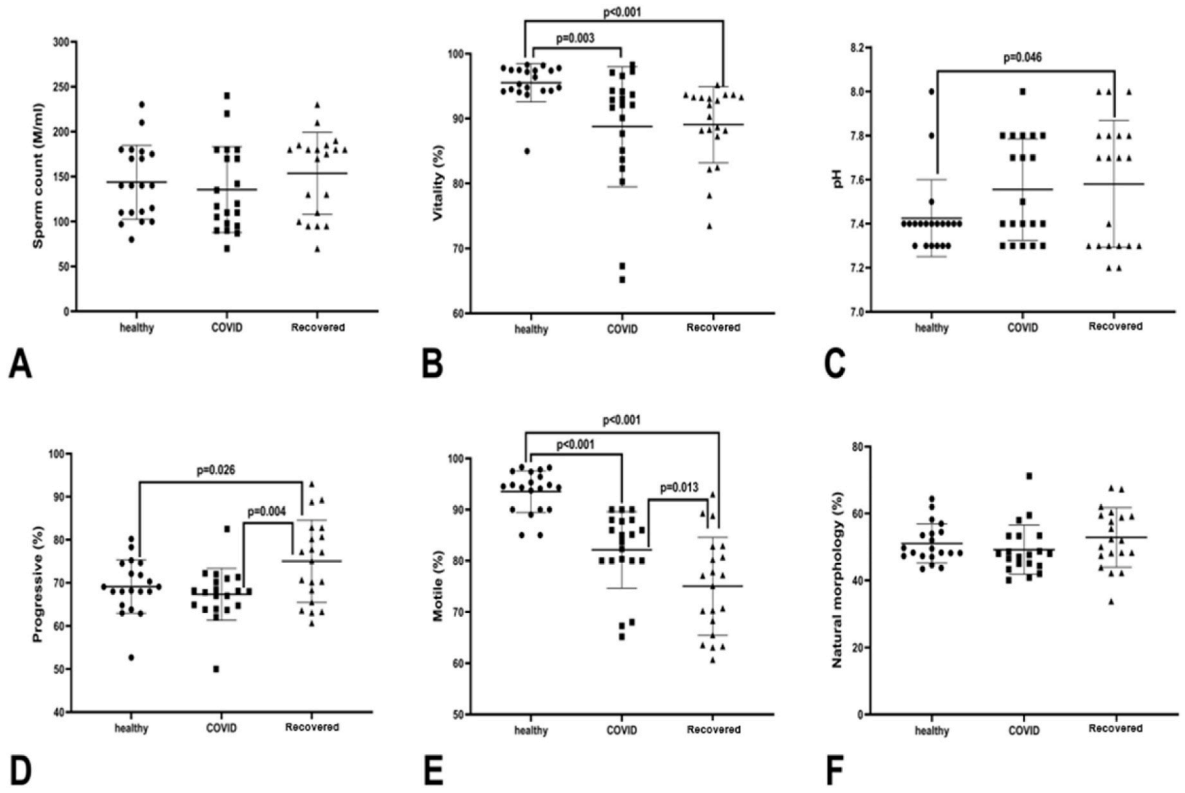

Fig. 2. Mean of sperm count (A), Vitality (B), pH (C), progressive(D), Motile (E), and Natural morphology (F) according to the study groups.

have shown that different viruses, including Orin, Zika, influenza, Coxsackie, and the human immunodeficiency virus (HIV), may cause orchitis and, consequently, male infertility [25]. In addition, HIV, hepatitis B/C, Zika, and Ebola provide sexual transmission through entry into seminal fluid [26]. The adverse effects of viruses on male infertility can include abnormal secretion of sexual hormones and direct

damage to spermatogenesis. Puggioni et al. [27] reported that Bluetongue virus, by entering the testes and affecting Leydig cells, leads to a decrease in testosterone biosynthesis as well as destruction of Sertoli cells. With the prevalence and progress of the coronavirus, concerns about its effects on male sexual health exist. Therefore, a study was conducted to investigate the effects of coronavirus-2019 Omicron strain on male sexual health using semen and sexual hormones analysis.

Our study results showed that the most common symptoms observed in COVID-19 patients included cough, fever, fatigue, and runny nose, while the least common clinical symptoms were nausea, chest pain, and diarrhea. Additionally, our results indicated a significant decrease in the levels of sexual hormones (FSH, PRL, and testosterone) in COVID-19 patients compared to healthy and recovered individuals. Moreover, the analysis of semen fluid revealed that the macroscopic and microscopic indices were reduced in COVID-19 patients compared to healthy and recovered individuals, although it was not statistically significant.

There is little evidence and documentation regarding the impact of the coronavirus on the male reproductive system. However, bioinformatic analyses indicate that this virus may potentially infect reproductive tissues [28]. The virus uses the angiotensin-converting enzyme 2 (ACE2) receptor to enter human cells [29]. ACE2 has been expressed on various organs and tissues, including the testes, which are enriched with ACE2 surface levels [30]. This concern has arisen as to whether these organs can be infected by the virus or not.

New studies have provided a better understanding of the function of ACE2 and its effects on the male reproductive system. This enzyme leads to a decrease in blood pressure by breaking down angiotensin II (Ang-II), and also regulates sperm motility and function through the PI3K/AKT signaling pathway [31,32]. Given the role of ACE2 in reducing Ang-II levels, it appears to modulate spermatogenesis and function [33]. Therefore, potential male infertility cannot be denied. However, it remains to be determined if this could lead to testicular involvement during a systemic infection or potential male infertility. In any case, ACE2 expression patterns in various tissues enable the virus to enter different parts of the body. The possibility of testicular involvement for SARS-CoV has been investigated, but the evidence is limited and conflicting, requiring further research.

Our results showed that fever was one of the most common symptoms observed in COVID-19 patients. Fever, a common sign in viral diseases, is well-known to cause transient disruption of spermatogenesis [34]. Under normal conditions, the temperature of the testes in the scrotum is 1–2 °C lower than the internal body temperature, and an increase in testicular temperature can affect the process of spermatogenesis [35]. In our study, macroscopic and microscopic parameters of semen in COVID-19 patients decreased, and we attributed this finding to fever, which is seen in all COVID-19 patients, as previously described and supported in the literature, for example by Carlsen et al. [36] They investigated the effect of fever on semen parameters and showed that following fever, semen parameters were affected in such a way that spermatogenesis decreased by 32.6 %. Based on the results of our study, we announce that the parameters of semen in men who have experienced fever are affected. Another noteworthy point is that it has been previously described in the literature that viral infections have a negative effect on semen parameters such as volume, sperm count, and motility [23]. In this study, we were able to demonstrate that sperm parameters in terms of Vitality, progressive, and Motile decrease significantly in the COVID-19 group, which may be due to the severity of the disease or higher viral load. It seems that this could be a temporary effect as we observed a normalization of the parameters in the improved group. Moreover, as mentioned in various studies, mature sperm require DNA integrity to maintain their fertilization capacity [37]. DNA fragmentation is mainly the result of apoptosis and reactive oxygen species (ROS), which have various etiologies such as smoking, drugs, oxidative stress from infection, and inflammation caused by infection, which may lead to increased infertility [33].

In our opinion, Covid-19 may affect the testes and disrupt semen parameters due to fever. The main support for this hypothesis is that in our study the serum levels of FSH, PRL, testosterone (significantly) and LH (non-significantly) in COVID-19 patients have decreased compared to other groups. Some studies have shown that stress caused by infection reduces gonadotropin secretion [38,39]. In the improved group of COVID-19 patients, the serum levels of testosterone, FSH, and LH showed at least an increase. One of the most important hormones that affects inflammatory responses is testosterone, which has a special protective effect on sexual responses [40]. Low levels of testosterone in COVID-19 patients are accompanied by high levels of inflammatory factors such as interleukin-2 and interferon-γ [41]. It has been shown that hypogonadism is a common finding in systemic diseases [42], but it is unclear whether the low levels of testosterone in COVID-19 patients are due to severe infection or not. In addition, due to its regulation of T lymphocyte differentiation, testosterone has anti-inflammatory and immune properties [43]. However, contrary to the potential protective nature of testosterone, it has been suggested that COVID-19 infection may be mediated by testosterone [44]. These facts support the need for further research on the relationship between testosterone levels and COVID-19 infection.

The testes are generally composed of two types of cells: Sertoli cells that produce sperm (spermatogonia) and Leydig cells that are responsible for producing testosterone. It has been reported that SARS-CoV-2 uses not only ACE2 but also the serine protease TMPRSS2 to enter host cells [45]. According to the analysis by Wang ZP et al. [46], TMPRSS2 is predominantly present in spermatogonia and spermatids, while ACE2 is more widely expressed in spermatogonia, Leydig, and Sertoli cells. Therefore, it can be said that SARS-CoV-2 exerts its greatest impact on the testes through ACE2. It has been shown that Ang-II reduces the synthesis of baseline testosterone and LH by Leydig cells [47], and in addition, ACE2 in Leydig cells can also change local microvascular flow and permeability in favor of inflammation [48]. Consequently, we believe that Leydig cells are more affected by COVID-19 than Sertoli cells. As a result, the testes may be at risk of damage and dysregulation under COVID-19.

In this study, we observed a significant decrease in serum PRL levels in COVID-19 patients compared to other groups. Various factors such as medications, diet, stress, and so on can affect serum PRL levels [49], so the decrease in PRL in infected patients was not surprising. We hypothesize that SARS-CoV-2 may indirectly act on the hypothalamic-pituitary axis. Since the hormonal profile reflects male sexual health, these findings should be interpreted with caution until more data is available. It can be assumed that SARS-CoV-2 may disrupt the sexual gland function in males unless proven otherwise.

4.2. Strengths and limitations

This study has several strengths. 1) According to our knowledge, it was the first study to simultaneously investigate three groups including healthy, COVID-19 infected, and COVID-19 recovered individuals. 2) We had a prospective study that addressed the limitations of previous studies such as non-standardized patient selection criteria and group homogeneity. Our groups were similar in terms of age, BMI, smoking status, and other demographic information. 3) In addition, a comprehensive evaluation of the sexual hormone profile and semen analysis was performed. Furthermore, 4) it is noteworthy that all participants were of reproductive age, with 61.7 % being married and 38.3 % being single.

Also, this study has some limitations, including: 1) relatively small sample size, 2) lack of testicular histology examination, 3) not investigating oxidative stress parameters and inflammatory factors, 4) lack of long-term outcomes. However, we aimed to conduct a preliminary study with an optimized method and will strive to maintain the protocol.

5. Conclusion

Since the main role of the testes is secretion of androgens and spermatogenesis, analyzing the fluid and hormone profile can reveal the testicular function. Our study results showed that COVID-19 patients experienced more fever, cough, nausea, and runny nose, and the serum levels of sexual hormones and microscopic indices of semen in infected patients were decreased compared to other groups, although these reductions were within the normal range, and these results should be interpreted with caution. We believe that these changes may be due to infection, and depending on the severity of the infection, the extent of the changes may vary, although they appear to be transient and temporary. And because in the recovered group, the clinical symptoms, levels of sexual hormones, and semen parameters approached those of the healthy group, we suggest that COVID-19 may not have a significant impact on men's sexual health. However, further studies are needed to confirm this hypothesis.

Ethical considerations

The study was approved by the Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1401.164).

Consent for publication

The authors consent to publish in this journal.

Availability of data and materials

All Data and Materials are available.

Funding source

This study was performed with the financial support of Birjand University of Medical Sciences (Grant No.: 5894).

CRediT authorship contribution statement

Yaser Mohammadi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Javad Ranjbaran:** Data curation, Methodology, Writing – original draft. **Morteza Mamashli:** Data curation, Writing – original draft. **Hadi Zare Marzuni:** Formal analysis, Methodology. **Ali Dashtgard:** Conceptualization, Investigation, Methodology. **Seyed Mostafa Mohsenizadeh:** Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

We are extremely grateful for the cooperation and assistance of the staff of the health center located in Minudasht and all the patients.

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