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ORIGINAL RESEARCH

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Time-reversibility of SARS-CoV-2 infection on basic semen parameters

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Abstract

SARS-CoV-2 infection may affect semen quality. However, there is limited evidence on whether this effect is reversible. Retrospective analysis was done on the same male patient's semen quality prior to SARS-CoV-2 infection, less than 74 days following recovery from SARS-CoV-2 infection, and more than 74 days following recovery in order to confirm. Consequently, the sperm concentration (44.10 (28.00, 75.20) *vs.* 66.00 (47.05, 135.30) $\times 10^6$/mL, $p < 0.001$), sperm motility ((43.22 $\pm$ 21.34)% *vs.* (51.65 $\pm$ 15.41)%, $p = 0.0105$), percentage of progressively motile sperm (PR) ((39.76 $\pm$ 20.58)% *vs.* (46.88 $\pm$ 15.26)%, $p = 0.0243$) and percentage of normal morphological sperm ((2.70 $\pm$ 1.82)% *vs.* (3.58 $\pm$ 2.00)%, $p = 0.0299$) of 45 male patients 30 to 71 days after recovery from SARS-CoV-2 infection were significantly lower than those before SARS-CoV-2 infection, while there was no significant difference in semen volume (3.20 (2.10, 4.65) mL *vs.* 3.20 (2.45, 4.55) mL, $p = 0.4819$) between them. The sperm concentration, motility and PR of 8 patients 77 to 125 days after recovery from SARS-CoV-2 infection (68.75 (31.50, 114.1) $\times 10^6$/mL, (44.23 $\pm$ 25.73)% and (39.76 $\pm$ 25.23)%) were higher than those 30 to 71 days after recovery from SARS-CoV-2 infection (20.05 (13.58, 30.10) $\times 10^6$/mL, (30.11 $\pm$ 22.05)% and (26.56 $\pm$ 21.55)%), and there were no significant differences from those before SARS-CoV-2 infection (50.05 (41.03, 90.35) $\times 10^6$/mL, $p = 0.8438$; (50.24 $\pm$ 13.62)%, $p = 0.5126$; and (45.76 $\pm$ 12.97)%, $p = 0.5251$). In conclusion, SARS-CoV-2 infection may affect one spermatogenesis cycle of a male patient for about 74 days, and the patient may return to the normal state in the next spermatogenesis cycle.

Keywords

SARS-CoV-2; Semen quality; Male; Recovery time; Spermatogenesis cycle

1. Introduction

On 31 January 2020, the World Health Organization (WHO) identified coronavirus disease 2019 (COVID-19) caused by novel coronavirus (SARS-CoV-2) infection as a public health emergency of international concern [1]. In addition to lung symptoms, COVID-19 also causes varying degrees of organ damage that occurs outside of the lung [2]. Since the outbreak of COVID-19, increasing number of evidences have proved that SARS-CoV-2 infection may affect male reproduction and sexual function [3–5]. In the clinics, male semen quality can best reflect a man's fertility. Previous studies [6–8] showed that SARS-CoV-2 infection might affect semen quality. However, there is limited evidence on whether this effect is reversible. A male's spermatogenic cycle takes approximately 74 days [9]. Therefore, this study intends to retrospectively analyze the semen quality of male patients before SARS-CoV-2 infection, lower than 74 days after recovery from SARS-CoV-2 infection and higher than 74 days after recovery from SARS-CoV-2 infection, to observe the impact of SARS-CoV-2 infection on male semen quality and whether this impact is reversible.

2. Materials and methods

2.1 Study population

Male patients who visited the male clinic of the Center for Reproductive Medicine, the Second Affiliated Hospital of Soochow University, between April 2022 and May 2023 were selected. The inclusion criteria were: (1) The patients were infected with SARS-CoV-2 between 13 December 2022, and 05 January 2023. The reason for choosing patients at this stage was that the Chinese government completely unsealed the prevention and control of COVID-19 on 08 December 2022. The pandemic fully emerged in the next 20 days, and on 22 December 2022, the number of people infected with COVID-19 reached its peak. (2) During 2–263 days before SARS-CoV-2 infection, the patients once had semen examinations in our hospital. They returned to our hospital after their SARS-CoV-2 infection was under control to recheck semen samples. (3) None of the patients took any other drugs or received any

other treatment during the study period except for symptomatic treatment after infection with SARS-CoV-2. The following were the exclusion criteria: (1) The patients with azoospermia and moderate to severe oligozoospermia; (2) The patients with male reproductive system diseases identified through medical history and physical examination; (3) The patients with genetic disorder.

A total of 45 patients, aged (29.73 ± 4.44) years old were enrolled in this study. After SARS-CoV-2 infection, they had a fever of 38 ℃–41 ℃ lasting 1–7 days and a recovery time of 7–10 days. Their nucleic acid test results of SARS-CoV-2 were positive. The infection and recovery criteria for SARS-CoV-2 refer to the Diagnosis and Treatment Plan for Novel Coronavirus Infection (Tenth Edition for Trial Implementation) [10]. All patients were diagnosed with mild COVID-19. The semen quality of these patients had been detected 2–263 days (average of 100.47 ± 73.84 days) before SARS-CoV-2 infection. The mean interval between the reexamination of semen quality and recovery from SARS-CoV-2 infection was 45.36 ± 11.19 days, ranging from 30–71 days. All 45 patients underwent routine semen analysis, of which 22 performed the detection of sperm morphology. Among them, 8 patients underwent routine semen analysis again in 77–125 days (average of 100.5 ± 14.92 days) after recovery from SARS-CoV-2 infection.

2.2 Semen analysis

After abstinence for 2–7 days, semen samples were collected from each patient by masturbation. The weighing method was used to determine the semen volume [11]. The WT3002K electronic balance was purchased from WANT Balance Instrument Co., Ltd. (Changzhou, Jiangsu, China). After complete liquefaction, sperm concentration, motility and the percentage of progressively motile sperm (PR) were analyzed using the BEION S3-3 computer-assisted sperm analysis (CASA) system (Shanghai Beion Pharmaceutical Technology Co., Ltd., Shanghai, China). Sperm morphology was stained with the modified Papanicolaou staining solution (Nanjing Xindi Bio-Pharmaceutical Engineering Co., Ltd., Nanjing, Jiangsu, China) and observed under a phase contrast microscope (CX31, Olympus, Olympus (China) Co., Ltd., Shanghai Branch, Shanghai, China). Then, the percentage of normal morphological sperm was calculated. The above semen analysis was performed according to the WHO Laboratory Manual for Human Semen Examination and Treatment (6th edn.) [11]. The intra-laboratory quality control of sperm concentration was routinely carried out once a week. The latex beads solution (HARIOMED) used for sperm concentration quality control was supplied by Hua Yue Medical Technology Co., Ltd. (Guangzhou, China).

2.3 Statistical analysis

The data were analyzed using the statistical software SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The data were first performed by one-sample nonparametric tests (Kolmogorov-Smirnov test) to determine whether they were normally distributed. The data conforming to normal distribution were expressed as mean ± SD, and those conforming to non-normal distribution were expressed as median (P_{25}, P_{75}). If the data conformed to a normal distribution, a paired *t*-test was used; if the data conformed to a non-normal distribution, Wilcoson Signed Rank test was used. $p \leq 0.05$ was statistically significant.

3. Results

3.1 Comparisons of male semen quality before SARS-CoV-2 infection and 30 to 71 days after recovery from SARS-CoV-2 infection

Table 1 displays the data for 45 male patients' semen volume, sperm concentration, sperm motility, PR and percentage of normal morphological sperm before and 30 to 71 days after recovery from SARS-CoV-2 infection. The findings indicated that the sperm concentration, motility, PR and percentage of normal morphological sperm in 30 to 71 days following recovery from SARS-CoV-2 infection were significantly lower than those prior to SARS-CoV-2 infection ($p < 0.05$) and that there was no significant difference in semen volume between them.

3.2 Comparisons of semen quality in different periods after recovery from SARS-CoV-2 infection

The comparisons of semen quality of 8 patients before SARS-CoV-2 infection, 30 to 71 days after recovery from SARS-CoV-2 infection, and 77 to 125 days after recovery from SARS-CoV-2 infection were shown in Table 2. The results showed that compared with the sperm concentration, motility, and PR before SARS-CoV-2 infection, those in 30 to 71 days after recovery from SARS-CoV-2 infection were significantly reduced ($p < 0.05$), while those in 77 to 125 days after recovery from SARS-CoV-2 infection had no significant difference ($p > 0.05$).

4. Discussion

The COVID-19 pandemic caused by SARS-CoV-2 infection has had a certain impact on both male and female reproduction [12, 13]. The positive rate, hospitalization rate, mortality rate, and complications of male patients with COVID-19 are higher than those of female patients [14, 15].

Many studies [6–8, 16] reported that SARS-CoV-2 infection significantly reduced male sperm concentration, and that the number of sperm in men with fever was significantly lower than that without fever. In our study, all male patients had varying degrees of fever symptoms. The sperm concentration in 30 to 71 days after recovery from SARS-CoV-2 infection was significantly lower than that before SARS-CoV-2 infection, which was consistent with the literature.

Gülsemin *et al.* [17] found that a man's PR was 59% prior to contracting SARS-CoV-2, and it dropped to 0% a month after he recovered. Mannur *et al.* [18] reported that a 36-year-old male patient with normal semen parameters showed oligoasthenospermia in the semen sample on the 43rd day after recovery from SARS-CoV-2 infection. When the semen sample of the 135th day after recovery from SARS-CoV-2

TABLE 1. Comparisons of semen quality of 45 patients before SARS-CoV-2 infection and 30 to 71 days after recovery from SARS-CoV-2 infection.

Groups	Abstinence time (d) (n = 45)	Semen volume (mL) (n = 45)	Sperm concentration ($\times 10^6$/mL) (n = 45)	Sperm motility (%) (n = 45)	Sperm PR (%) (n = 45)	Normal morphological sperm (%) (n = 22)
Before SARS-CoV-2 infection	3 (3, 4)	3.20 (2.45, 4.55)	66.00 (47.05, 135.30)	51.65 ± 15.41	46.88 ± 15.26	3.58 ± 2.00
30 to 71 days after recovery from SARS-CoV-2 infection	3 (3, 4)	3.20 (2.10, 4.65)	44.10 (28.00, 75.20)	43.22 ± 21.34	39.76 ± 20.58	2.70 ± 1.82
p	0.6636	0.4819	<0.001	0.0105	0.0243	0.0299

PR: Percentage of progressively motile sperm. The semen quality of 45 male patients was determined in 2 to 263 days before SARS-CoV-2 infection and 30 to 71 days after recovery from SARS-CoV-2 infection, respectively. The weighing method was used to determine the semen volume. An automated system for computer-assisted sperm analysis (CASA) was used to analyze sperm concentration, motility, and PR. Sperm morphology was stained with the modified Papanicolaou staining and observed under a phase contrast microscope. The data of abstinence time, semen volume, and sperm concentration conformed to non-normal distribution and were expressed as median (P_{25}, P_{75}). The data of sperm motility, PR and percentage of normal morphological sperm conformed to a normal distribution, and were expressed as mean ± SD. The sperm concentration, motility, PR and percentage of normal morphological sperm in 30 to 71 days after recovery from SARS-CoV-2 infection were significantly lower than those before SARS-CoV-2 infection ($p < 0.05$), and there was no significant difference in semen volume between them.

TABLE 2. Comparisons of semen quality before SARS-CoV-2 infection, 30 to 71 days after recovery from SARS-CoV-2 infection and 77 to 125 days after recovery from SARS-CoV-2 infection (n = 8).

Groups	Abstinence time (d)	Semen volume (mL)	Sperm concentration ($\times 10^6$/mL)	Sperm motility (%)	Sperm PR (%)
Before SARS-CoV-2 infection	4.00 ± 1.07	3.93 ± 1.59	50.05 (41.03, 90.35)	50.24 ± 13.62	45.76 ± 12.97
30 to 71 days after recovery from SARS-CoV-2 infection	3.50 ± 0.76	3.35 ± 1.31	20.05 (13.58, 30.10)	30.11 ± 22.05	26.56 ± 21.55
77 to 125 days after recovery from SARS-CoV-2 infection	3.38 ± 0.92	3.59 ± 1.47	68.75 (31.50, 114.10)	44.23 ± 25.73	39.76 ± 25.23
*p*1	0.3506	0.1754	0.0078	0.0225	0.0372
*p*2	0.1395	0.4315	0.8438	0.5126	0.5251

PR: Percentage of progressively motile sperm. p1: Comparisons between 30 to 71 days after recovery from SARS-CoV-2 infection and before SARS-CoV-2 infection; p2: Comparisons between 77 to 125 days after recovery from SARS-CoV-2 infection and before SARS-CoV-2 infection. The semen quality of 8 male patients was determined in 2 to 228 days before SARS-CoV-2 infection, 30 to 71 days after recovery from SARS-CoV-2 infection, and 77 to 125 days after recovery from SARS-CoV-2 infection, respectively. Semen analysis methods were the same as in Table 1. The data of sperm concentration conformed to non-normal distribution and were expressed as median (P_{25}, P_{75}). The data of abstinence time, semen volume, sperm motility and PR conformed to a normal distribution and were expressed as mean ± SD. Compared with the sperm concentration, motility and PR before SARS-CoV-2 infection, those in 30 to 71 days after recovery from SARS-CoV-2 infection were significantly reduced ($p < 0.05$), while those in 77 to 125 days after recovery from SARS-CoV-2 infection had no significant difference ($p > 0.05$).

infection was analyzed again, although the number of sperm and sperm motility were improved, it still showed the characteristics of teratospermia. Paoli *et al.* [19] pointed out that the indirect damage of SARS-CoV-2 to testicular tissues seemed to be temporary, suggesting that infertile couples delay the plan of having children for three months. These studies indicated that SARS-CoV-2 infection could affect semen quality, and that semen quality might be recovered with the extension of recovery time after SARS-CoV-2 infection. In our study, the comparisons of semen quality of 8 patients before SARS-CoV-2 infection, 30 to 71 days after recovery from SARS-CoV-2 infection and 77 to 125 days after recovery from SARS-CoV-2 infection showed that compared with the sperm concentration, motility and PR before SARS-CoV-2 infection, those in 30 to 71 days after recovery from SARS-CoV-2 infection were significantly reduced ($p < 0.05$), while those in 77 to 125 days after recovery from SARS-CoV-2 infection had no significant difference ($p > 0.05$). It was indicated that the impact of SARS-CoV-2 infection on semen quality was reversible and that semen quality could basically recover to the state before

SARS-CoV-2 infection in 3 to 4 months after recovery from SARS-CoV-2 infection.

According to recent research, the mechanisms by which SARS-CoV-2 infection affects male reproductive functions may include the following. First, angiotensin-converting enzyme 2 (ACE2) is highly expressed in spermatogonia, Leydig cells and Sertoli cells of male testes [20]. SARS-CoV-2 may enter the male reproductive system by the ACE2 and transmembrane serine protease 2 (TMPRSS2) pathway, thus affecting spermatogenesis [13]. Second, the increase of inflammatory cytokines and oxidative stress caused by SARS-CoV-2 infection may affect the ability of Leydig cells to produce testosterone and destroy spermatogenic cells to prevent spermatogenesis [21, 22]. Third, the hypothalamic pathology caused by neuroinflammation related to SARS-CoV-2 infection may change testicular function by affecting the secretion of gonadotropins [23]. Fourth, the cytokine storm caused by SARS-CoV-2 infection may destroy the blood-testis barrier, thus promoting the occurrence of orchitis and affecting spermatogenesis [24, 25]. In addition, Lauritsen *et al.* [26] reported that serum insulin-like growth factor 3 (INSL3) levels were reduced and testicular function was impaired in men with mild SARS-CoV-2 infection. The risk of SARS-CoV-2 RNA transmission through semen appears to be low. Fever attacks may affect testicular function, but the direct impact of SARS-CoV-2 cannot be ruled out.

Some researchers [27] put forward that the fever caused by SARS-CoV-2 infection may change semen parameters, which may take up to 3 months to recover. It is indicated that the effect of SARS-CoV-2 infection on spermatogenesis may cover the whole spermatogenesis cycle [28–30]. Our study confirms this viewpoint, that is, SARS-CoV-2 infection may affect one spermatogenesis cycle of a male patient for about 74 days, and the patient may return to the normal state in the next spermatogenesis cycle.

This study contains certain flaws as well. Firstly, there are comparatively few patients included. It is associated with the prevention and control of COVID-19 because, during the epidemic, a significant decline in primary care visits occurred. We extended the period before SARS-CoV-2 infection to 9 months in an attempt to maximize sample size, which could introduce more confounding variables that skew the results. The data will be more compelling if they only include patients who were infected with SARS-CoV-2 three months prior to the infection. Future research with a larger sample size is expected to confirm this conclusion. Second, we did not pay attention to whether the COVID-19 patients had symptoms or signs like scrotal tenderness. If such symptoms or signs exist, it will provide better support for our conclusion. Third, the patients with COVID-19 in this study are all mild. If they are serious COVID-19 patients, whether the recovery time of their influence on semen quality is longer still needs further research. Last, this study does not consider the impact of specific lifestyle factors such as sleep, smoking, frequency of alcohol consumption, and other factors such as mental stress on semen parameters, which may have certain limitations.

5. Conclusions

SARS-CoV-2 infection has a negative impact on male reproduction and semen parameters, which may lead to the reduction of sperm concentration, motility, PR, and percentage of normal morphological sperm. Fortunately, this impact is reversible. Generally, the sperm quality of male patients can recover to the preinfection level 3–4 months after recovery from SARS-CoV-2 infection.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

CLL and JCL—designed the study, wrote the paper. CLL, KF, FL and MWC—collected and analyzed the data. HZ—directed the research. All authors approved final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Reproductive Medicine Ethics Committee of the Second Affiliated Hospital of Soochow University (No. JD-HG-2023-50), and all patients signed the informed consent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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