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COVID-19 and Male Reproduction: A Thorny Problem

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Abstract

With the global epidemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the increasing number of infections, little is known about how SARS-CoV-2 affects the male reproductive system during infection or after recovery. Based on the existing research data, we reviewed the effects of SARS-CoV-2 on the male reproductive system and discussed its possible mechanism of action. SARS-CoV-2 enters host cells through the angiotensin-converting enzyme 2 (ACE2)/transmembrane serine protease 2 (TMPRSS2) pathway, and males are more susceptible than females. After infection, immunopathological damage is noticed in the testicles, and the semen index is significantly reduced. Second, abnormalities of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) levels were also observed, suggesting that there may be dysfunction of the hypothalamic–pituitary–gonadal (HPG) axis. Even after recovery, the effect of SARS-CoV-2 on the male reproductive system can last for at least a period. There are still many unresolved questions about the effect of SARS-CoV-2 infection on the male reproductive tract. Other receptors involved during the invasion of human cells by SARS-CoV-2 remain to be identified. Will the mutation of SARS-CoV-2 increase the diversity of receptors? How does SARS-CoV-2 affect the HPG axis? The long-term effects of SARS-CoV-2 on the male reproductive system remain to be evaluated. SARS-CoV-2 infection can affect male reproductive function. Standard treatment strategies should be developed in time to protect the fertility of infected patients. For recovered patients with fertility requirements, fertility assessments should be performed and professional fertility guidance should be provided at the same time.

Keywords

COVID-19, SARS-CoV-2, male infertility, testis, hypothalamus–pituitary–gonad

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Introduction

Although many countries are combating coronavirus disease 2019 (COVID-19), more than 150 million people have been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and over 120 million people have recovered from the infection. As the elimination of this virus in the near foreseeable future is uncertain, humankind will have to live with SARS-CoV-2 at least for a few years. Although SARS-CoV-2 infection is a respiratory disease, a large number of infections due to this virus has compelled us to pay attention to its adverse effects on other organs. SARS-CoV-2 enters host cells by binding to the cell surface receptors of angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) (Hoffmann, Kleine-Weber, Krüger et al., 2020). Apart from the lungs, the ACE2

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receptor is present in different cell types in the testis (Wang & Xu, 2020). A previous study on the clinical characteristics of COVID-19 revealed that male patients of reproductive age accounted for a significant proportion of the study patients and their reproductive system may still be affected by SARS-CoV-2 until recovery (Guan et al., 2020). Therefore, the potential risk of SARS-CoV-2 infection on the male reproductive system requires further study.

Herein we review features of SARS-CoV-2 infections that may impact male fertility whether the patient is previously infected or newly diagnosed. We suggest that we should pay more attention to the disease history of SARS-CoV-2 infection in infertile cases.

Invasion Mechanism of SARS-CoV-2 and Sex-Based Susceptibility

Several studies have elucidated the process of SARS-CoV-2 invasion: virus–host cell fusion is mediated by SARS-CoV-2 surface spike glycoprotein and its receptors of ACE2 and TMPRSS2 on the host cell surface. The former is responsible for SARS-CoV-2 entry and the latter is related to S-protein priming (Hoffmann, Kleine-Weber, Krüger et al., 2020; Letko et al., 2020; W. Li et al., 2003; Zhou et al., 2020). Specifically, TMPRSS2, located on the host cell surface, cleaves the S protein into two subunits (S1 and S2 subunits) when the S protein binds to its receptor ACE2, thereby promoting the endocytosis, translation, and replication of the virus (Hoffmann, Kleine-Weber, Krüger et al., 2020; Letko et al., 2020).

Early epidemiological studies in China have reported that men are more likely to be infected with COVID-19 and develop more severe symptoms (Guan et al., 2020). Punjani et al. (2020) have reported similar observations. ACE2 levels in males are higher than those in females, at least in the lungs (Li et al., 2020). Higher levels of ACE2/TMPRSS2 expressed in specific organs in men may explain this sex-based sensitivity. Single-cell RNA sequencing (scRNA-seq) analysis of human testicular cells identified that ACE2 was highly expressed in spermatogonia, Leydig cells, and Sertoli cells (Wang & Xu, 2020). TMPRSS2, identified as a critical host factor for viral entrance and activation, has been reported to be overexpressed in the prostate (Ko et al., 2015). The androgen response element, a transcriptional promoter for TMPRSS2, was originally described in the context of the TMPRSS2-ERG fusion gene and prostate oncogenesis (see Figure 1). Correlation analysis identified that circulating androgen levels in men are positively associated with cellular levels of TMPRSS2 (Chen et al., 2010). On the contrary, researchers used scRNA-seq technology to analyze the expression of ACE2 and TMPRSS2 in the ovarian cells of women and reported that the expression of ACE2 was very low in stromal cells and perivascular cells of the ovarian cortex. Surprisingly, TMPRSS2 was not expressed in different types of ovarian cells (Goad et al., 2020). The second explanation is related to the removal of the virus. An analysis of the virus clearance rate in 68 (48 males and 20 females) COVID-19 patients from Mumbai (India) identified that the speed to clear the virus in female patients was significantly higher than that in male patients, that is, an average of 4 days for females and 6 days for males (Shastri et al., 2020). This difference may be related to the innate immunity of men, who produce fewer antiviral interferons (van der Made et al., 2020).

Effect of SARS-CoV-2 Infection on Semen Parameters

Whether SARS-CoV-2 is present in the testis and semen samples of COVID-19 patients remains debatable (Holtmann et al., 2020; Ma et al., 2021). A previous study reported that reverse transcription polymerase chain reaction (RT-PCR) is a feasible molecular diagnostic method for detecting SARS-CoV-2 RNA in semen samples (Paoli et al., 2021), and SARS-CoV-2 has been actually detected in the semen samples of patients infected with SARS-CoV-2. However, most reports in the current literature have indicated that SARS-CoV-2 is undetectable in the semen samples of male COVID-19 patients. For instance, Gacci et al. collected multiple types of samples from 43 convalescent patients with SARS-CoV-2 infection, including saliva, pre-ejaculatory urine, semen, and post-ejaculatory urine. Among the 43 patients, three patients were positive for SARS-CoV-2, but they were diagnosed as negative after resampling. As the collection of semen and urine samples is not completely sterile, they were possibly contaminated during the process of sample collection (Gacci et al., 2021). However, the patients' semen parameters significantly decreased after SARS-CoV-2 infection (Patel et al., 2021). There have been many reports regarding the detection of SARS-CoV-2 in the semen of patients previously diagnosed with COVID-19. The majority of reports in the current literature, however, suggest that SARS-CoV-2 is not detected in the semen.

In a prospective cohort research center, researchers consecutively observed 84 male patients diagnosed with COVID-19 and 105 healthy controls, and identified that patients with COVID-19 showed higher ACE2 enzyme activity and inflammatory factor levels in the semen samples. In addition, significantly lower semen volumes, progressive motility, sperm morphology, sperm concentration, and the number of spermatozoa were observed in the COVID-19 group compared with the control group (Hajizadeh Maleki & Tartibian, 2021).

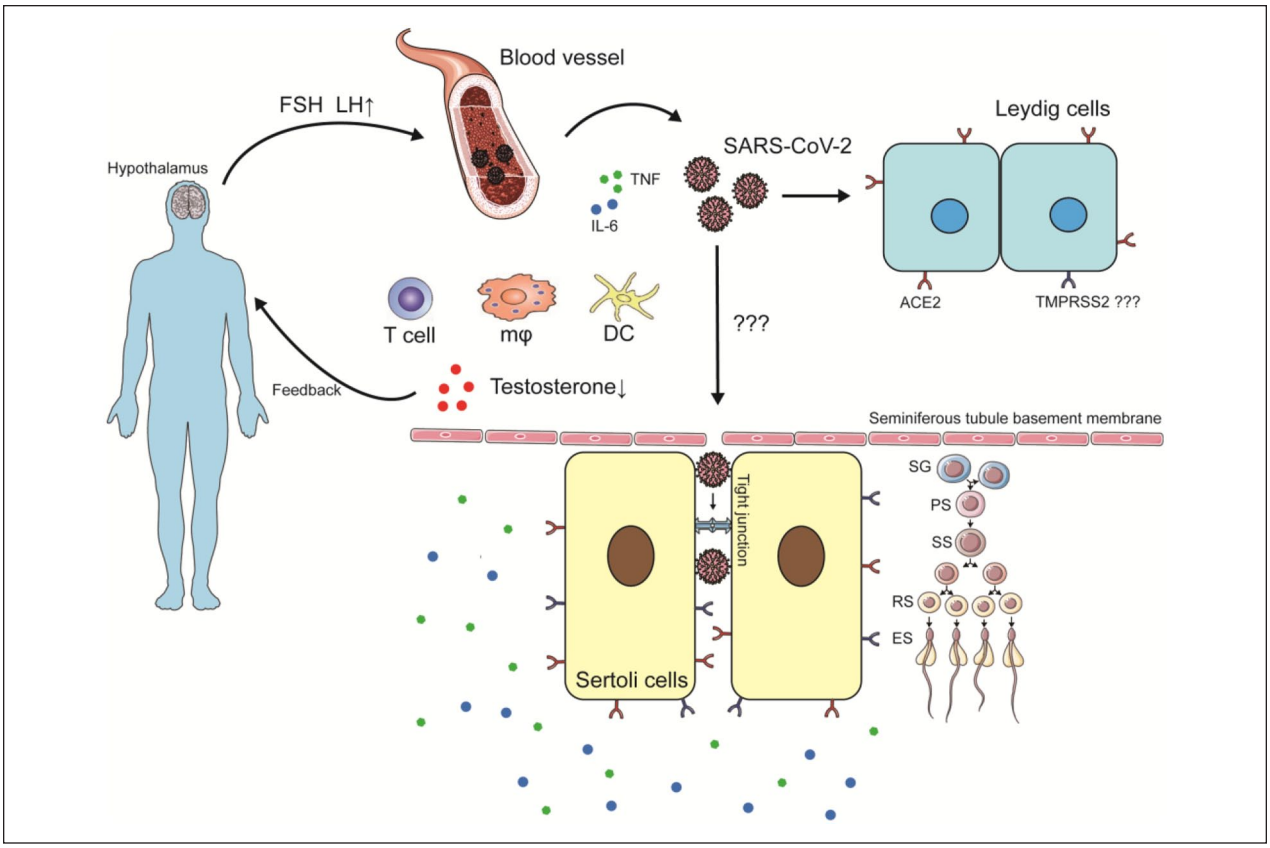

Figure 1. The Possible Mechanism of SARS-CoV-2-Mediated Testicular Injury.
Note. The SARS-CoV-2 reaches the testis through the circulatory system and induces a local inflammatory reaction. Immune cells, such as $CD3^+$ T-cells, $CD68^+$ macrophages, and dendritic cells, infiltrate and increase the levels of IL-6 and TNF. Hyperpyrexia and persistent inflammation damage the tight junctions between Sertoli cells, leading to the destruction of the blood–testis barrier, ultimately resulting in the invasion of the SARS-CoV-2 virus and testicular damage. The decreased testosterone level in the serum induces GnRH synthesis in the hypothalamus and promotes FSH and LH secretion in the pituitary gland in turn. FSH = follicle-stimulating hormone; LH = luteinizing hormone; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TNF = tumor necrosis factor; IL-6 = interleukin 6; ACE2 = angiotensin-converting enzyme 2; TMPRSS2 = transmembrane serine protease 2; mφ = macrophage; DC = dendritic cell; SG = spermatogonia; PS = primary spermatocyte; SS = secondary spermatocyte; RS = round spermatid; ES = elongating spermatid; GnRH = gonadotropin-releasing hormone.

Another study performed by Li et al. (2020) reported that among the semen samples provided by 23 hospitalized patients (age range: 27–55 years, average age: 40.8 years), more than one third of them had sperm density lower than the World Health Organization (WHO) standard (15×10^6/mL) and nearly two thirds of hospitalized patients contained no less than 1×10^6 white blood cells in the semen. Moreover, the levels of seminal inflammatory factors, such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant protein–1 (MCP-1), were significantly higher in SARS-CoV-2 infected patients than in males in the control group. Leukocytes and cytokines could affect spermatogenesis and interfere with fertility. The increased concentration of seminal leukocytes may cause sperm abnormalities by activating reactive oxygen species. It has been noted that cytokines could affect Sertoli cell functions and their altered level could impair spermatogenesis (Aziz et al., 2004; Fraczek & Kurpisz, 2015). An up-to-date review about human semen quality as affected by SARS-CoV-2 infection showed that seven of the research studies have tested the effect of SARS-CoV-2 infection on sperm parameters, three of which were conducted in China (Banihani, 2021). Also in this up-to-date review, six studies revealed the negative effects of SARS-CoV-2 infection on sperm parameters. It reminds us that SARS-CoV-2 has a very large impact on the reproductive system.

Does SARS-CoV-2 Affect the Testis?

Although early epidemiologic studies have suggested that men are at higher risk of more severe COVID-19 and mortality, causal mechanisms remain unknown and require further investigation. Host cell co-expression of ACE2 and TMPRSS2 for S-protein priming is a critical component of viral entry. While ACE expression is mostly ubiquitous, ACE2 transcripts are found only in the heart,

kidney, and testis (Donoghue et al., 2000). TMPRSS2 is found predominantly in the gastrointestinal tract, genitourinary tract, and prostate. It is still uncertain whether the male reproductive system is susceptible to SARS-CoV-2 infection, primarily because co-expression of both ACE2 and TMPRSS2 is needed by an individual host cell to facilitate viral entry (Hoffmann, Kleine-Weber, Schroeder et al., 2020; Sungnak et al., 2020). Stanley et al. (2020) performed an analysis of scRNA-seq data from testicular cells and found that co-expression of both ACE2 and TMPRSS2 occurred less than 0.05% of the time. As mentioned above, the male reproductive organs significantly express ACE2, while TMPRSS2 is rarely co-expressed in testicular cells and sperm. TMPRSS2 is required for SARS-CoV-2 cell entry, indicating that SARS-CoV-2 is unlikely to enter testicular cells by the mechanism mediated by the ACE2/TMPRSS2 pathway (Hoffmann, Kleine-Weber, Schroeder et al., 2020; Pan et al., 2020; Stanley et al., 2020). However, Kim et al. (2020) reported that most COVID-19 patients experienced testicular pain regardless of the common respiratory symptoms. Several studies have reported that SARS-CoV-2 infection can induce the circulation of pro-inflammatory cytokines, thus promoting the pathogenic progress of COVID-19 (Shi et al., 2020; Tay et al., 2020). Similarly, male patients showed an increased level of cytokines in the plasma than female patients (Takahashi et al., 2020). Professor Li Honggang's team analyzed the pathological changes in the testis and epididymal tissues of patients with new coronavirus pneumonia and identified that these tissues had hyperemia and edema. Observation of $CD3^+$ and $CD68^+$ cell infiltration in testicular tissues indicated an increased concentration of T-lymphocytes and macrophages in the interstitial space of testicular tissues of COVID-19 patients compared with the testis of healthy patients. There is an obvious infiltration of T-lymphocytes around the blood vessels of the testes and epididymal tissues, and the occasional presence of T-lymphocytes around the epididymal duct of COVID-19 patients. Some patients had red blood cell exudation, wherein the number of seminiferous epithelial cells decreased while the apoptosis of spermatogenic cells increased. Moreover, IgG was detected in the seminiferous tubules of some patients (Li et al., 2020). Li et al. used the reliable semen RNA extraction method, combined with reverse transcription quantitative polymerase chain reaction (RT-PCR) and the more sensitive MNGS (metagenomic next-generation sequencing), to detect SARS-CoV-2 in semen samples. They also performed an ultrastructural pathological diagnosis of testicular tissues; however, SARS-CoV-2 was not detected in semen and testicular tissue samples (Li et al., 2020). Pathological edema and hyperemia were seen in the testis and epididymis of infected patients, a non-specific inflammatory response to COVID-19. The blood–testis barrier (BTB) confers an isolated immune-privileged microenvironment for sperm in the testis. However, serious inflammation induced by infection can disrupt the BTB resulting in orchitis which adversely affects testicular function and male fertility. However, whether the virus can be present in the testis does not entirely depend on the viral infection. There are other conditions where the virus might enter and disrupt testicular tissue, including high blood viral load, local inflammation, hyperpyrexia, and imperfect BTB. Thus, the possibility of SARS-CoV-2 directly disrupting testis cannot be ruled out. Professor Li Honggang's paper analyzed the pathological changes in the testis and epididymal tissues of patients with new coronavirus pneumonia and identified that these tissues had hyperemia and edema. Observation of $CD3^+$ and $CD68^+$ cell infiltration in testicular tissues indicated an increased concentration of T-lymphocytes and macrophages in the interstitial space of testicular tissues of COVID-19 patients compared with the testis of healthy patients. The study is a case series in which autopsy specimens of testis and epididymis were obtained from patients who died of COVID-19 ($n = 6$), and an equal number of surgical specimens from male patients other than COVID-19 were taken as controls ($n = 6$); the specimens included in the investigation for the control group were of the patients with no other history of medical illness (as per the documented medical history records). The experiment has ruled out the possibility of other virus infections. This pathological feature is the manifestation of COVID-19, although it is not specific. It reminds us that SARS-CoV-2 not only affects the respiratory system but also has a very large impact on the reproductive system, which should be paid attention to.

In general, free radicals mediated by pro-inflammatory cytokines and oxidative stress can cause the degradation of testicular cell components (Asadi et al., 2017). Decreased testosterone (T) levels and impaired sperm production observed in COVID-19 patients are also associated with persistent fever and immune-related pathological reactions in the testis (Youssef & Abdelhak, 2020). Therefore, we concluded that the SARS-CoV-2 virus can affect the testis by causing inflammation in the body; however, to determine whether the virus can enter the cells in the testis, a large number of samples are required for verification as the results of RT-PCR and electron microscopy are still inconsistent (Dittmayer et al., 2020; Goldsmith et al., 2020). However, whether SARS-CoV-2 could be present in the testis does not merely depend on the presence or absence of viral infection. In some other cases, SARS-CoV-2 may also enter and damage parts of the testicular tissue in cases with high viral load, local inflammatory response, high fever, and a broken BTB. The present studies have not provided any evidence suggesting that the semen of the female

reproductive tract can transmit SARS-CoV-2 (Pan et al., 2020; Quan et al., 2020; Ruan et al., 2021; Zhang et al., 2020).

COVID-19 and Dysfunction of the Hypothalamic–Pituitary–Gonadal (HPG) Axis

The hypothalamus regulates visceral and endocrine activities. It integrates and regulates various physiological processes, including water and salt metabolism, body temperature, food intake, sleep, reproduction, visceral activities, and emotions (Guijarro et al., 2006; Lechan &Toni, 2016; Saper et al., 2005). Generally, the HPG axis is regulated by hypothalamic gonadotropin-releasing hormone (GnRH) in response to changes in circulating sex steroid hormones (Selvaraj et al., 2021). Hypothalamus-derived GnRH can stimulate the anterior pituitary to synthesize and secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Kandasamy et al., 2019). In the testis, FSH targets Sertoli cells and LH acts on Leydig cells to jointly promote the synthesis of T (Ramaswamy & Weinbauer, 2014). The circulating concentration of T acts on the hypothalamus to affect the release of GnRH, thereby controlling the synthesis of FSH and LH in the pituitary and forming an HPG axis feedback loop (Clavijo & Hsiao, 2018; Pozzilli & Lenzi, 2020). The concentration of T in the testis is closely related to spermatogenesis. The dysfunction of the HPG axis can disrupt sperm production in the testes, leading to infertility (Selvaraj et al., 2020).

Ma et al. (2021) reported that serum LH levels of SARS-CoV-2 patients (119 men) were higher, and both the ratios of FSH:LH and T:LH were abnormal; however, the serum T levels of these men were not statistically different when compared with the levels of 273 age-matched control men. Another study performed by Çayan et al. (2020) identified that the concentration of LH and FSH increased with the severity of SARS-CoV-2 infection. The study in patients with severe SARS-CoV-2 infection performed by Rastrelli et al. (2021) observed that men with severe COVID-19 infection or dead patients had lower serum T levels than men who had recovered clinically. In addition, Rastrelli et al. identified that the mortality and severe illness rates of men with a total T concentration of <5 nmol/L have also increased. The existing results cannot rule out whether a low T level will lead to susceptibility to SARS-CoV-2. However, the above studies strongly suggest hypogonadism in severe SARS-CoV-2-infected patients. The phenomenon of low levels of T and dihydrotestosterone in COVID-19 patients has been reported by Schroeder et al. (2020). Another study also identified a negative relationship between T levels and inflammatory factors, suggesting that the inflammatory reaction caused by SARS-CoV-2 infection may be the direct predisposition of a low T level (Salciccia et al., 2020). Temiz et al compared the serum levels of FSH, LH, and T in SARS-CoV-2 infected patients before and after treatment, and found that patients after treatment had similar serum FSH, LH, and T levels with patients before treatment (Temiz et al., 2021).

Damaged Leydig cells after SARS-CoV-2 infection can reduce androgen secretion, which may eventually lead to a decrease in T levels and an increase in LH and FSH levels through pituitary feedback. A neuroimaging study performed by Pascual-Goñi et al. (2020) reported that a SARS-CoV-2-positive female patient had hypothalamic neuropathy accompanied by an enlarged pituitary gland. Pituitary hypertrophy found in this SARS-CoV-2-infected patient may be caused by a transient increase in GnRH.

In summary, the above evidence suggests that SARS-CoV-2 infection has posed an unexpected threat to the HPG axis, leading to its dysfunction. There are multiple elements involved in this progress. It is uncertain whether this disorder is caused by SARS-CoV-2 itself and whether it affects the pituitary or testis; however, inflammation induced by virus–host interaction seems to play a crucial role. To date, scientific data on this issue are limited; nevertheless, an increasing number of studies have reported that the interruption of gonadotropin regulation might be a consequence of testicular lesions caused by SARS-CoV-2. The findings of systemic cytokines, increased FSH, LH, and low T may simply derive from the comorbidities demonstrated in those men that lead to severe disease. The biggest limitation of these studies is the lack of long-term follow-up survey data on sex-related hormone levels before and after SARS-CoV-2 infection, which may strengthen the relationship between SARS-CoV-2 infection and male reproductive hormone changes. In addition, there is a need to discover the long-term effects of sex hormone changes on male fertility.

H1N1/Influenza and SARS-CoV-2

Some complications of SARS-CoV-2 infection (such as fever) can also harm the male reproductive system. To eliminate the factors of common complications, we compared the different effects of H1N1/influenza and SARS-CoV-2 on the male reproductive system. The influenza virus is among the most common infectious illnesses worldwide. It is worth noting that in the 2009 H1N1 influenza pandemic, young people are particularly at risk, and one third of people over the age of 60 have antibodies, which may come from historical infection or influenza vaccination (Payne et al., 2020). There is no report of the influenza virus in semen. There is also a lack of evidence

on the sexual transmission of influenza, which may be due to other more likely routes of infection. There is evidence that although direct contact with infection is possible, it is unlikely to be an important mode of transmission compared with the transmission through aerosols and respiratory droplets (Killingley & Nguyen-Van-Tam, 2013).

As a pyrogenic virus, influenza affects sperm quality. MacLeod noted that patients with febrile influenza have decreased sperm count, motility, and morphological parameters. The morphology and vitality recovered at 4 weeks, as did sperm count at 8 weeks (MacLeod, 1951). The mouse model shows that influenza virus particles are also destructive. Sharma and Polasa (1978) injected mice intraperitoneally with influenza virus for the first time and recorded a significant increase in numerical and structural alteration of meiotic chromosomes. Other mouse studies have also found that both inactivated and purified influenza can cause similar DNA damage, indicating that virus particles may have direct cytotoxicity (Pathki & Polasa, 1988). Therefore, despite the evidence that there is a local inflammatory response (Le Tortorec et al., 2020), the observed transient sperm changes after influenza virus infection may be due to the combination of systemic fever and direct DNA damage, leading to cell apoptosis and transient decline in fertility. Compared with SARS-CoV-2, influenza virus infection has a shorter duration of influence on the male reproductive system and milder symptoms.

The Possible Effects of COVID-19 on Recovered Male Patients

Two studies have confirmed that patients with moderate SARS-CoV-2 infections have the risk of impaired spermatogenesis (Holtmann et al., 2020; Ma et al., 2021); however, the duration and severity of these abnormalities and their possible threats on the offspring are still unclear. Therefore, a large-scale prospective, long-term research is required to further clarify the adverse effects of SARS-CoV-2 on the male reproductive system. A regional follow-up study reported that within 60 days of recovery from SARS-CoV-2, increased levels of inflammatory cytokines and reactive oxygen species, and caspase activity can still be detected in seminal plasma samples. These changes tend to exist over a long period and are associated with significant reductions in semen volume, sperm motility, sperm morphology, sperm concentration, and sperm count (Hajizadeh Maleki & Tartibian, 2021). These findings indicate that we should accurately track and evaluate the reproductive function of male patients who have recovered from this disease. Clinical examination and consultation are recommended in patients in the recovery period before they plan parenthood. For the recovery of fertility after exposure to SARS-CoV-2, zinc supplementation seems to have a positive effect (Sethuram et al., 2021).

Conclusion

ACE2 and TMPRSS2 mediate the fusion between SARS-CoV-2 and host cells. SARS-CoV-2 infection can damage the male reproductive function by affecting semen parameters, orchitis, and HPG. For patients who recovered and have fertility requirements, they should undergo fertility assessment and receive professional fertility guidance at the same time.

Declaration of Conflicting Interests

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