Review Article

SARS-CoV-2 infection and male fertility problems

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Abstract

In 2019, the coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), has killed more than 6.6 millions of people around the world as of end of 2022. The long-term impact of COVID-19 is persisted, including its impact on male reproduction. SARS-CoV-2 enters into host cells using the angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) receptors. Both of these receptors are expressed more in men, and therefore men are more susceptible to SARS-CoV-2. COVID-19 potentially cause infertility by damaging testicular tissues and interfering with the process of spermatogenesis. A decrease in serum levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) as well as a decrease in sperm quality in men with COVID-19 compared to healthy men of the same age has been reported in several studies. Utilizing existing research data, this study aims to explore in detail of how SARS-CoV-2 tends to affect male fertility.

Keywords: SARS-CoV-2, male fertility, testis, sperm analysis, hormones

Introduction

Coronavirus disease 2019 initially emerged in Wuhan, China in December 2019 and has spread to many nations all over the globe (World Health Organization, 2020; Zhou et al., 2020). The virus, severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), is a member of the Coronaviridae family. The virus has many similarities with severe acute respiratory syndrome corona virus (SARS-CoV) (Zhou et al., 2020) that caused SARS-CoV epidemic in 2003 and Middle-East respiratory syndrome coronavirus (MERS-CoV) that cause epidemic in 2012 (Mascio et al., 2020). Causing the global pandemic, COVID-19 has killed millions of people internationally. The long-term impact of COVID-19 is persisted even two years after the pandemic started, including its impact on reproduction. COVID-19 could affect human fertility (Bechmann et al., 2022). Research in 2020 revealed that most male patients who had been infected with COVID-19 experienced decreased fertility even though they had fully recovered (Guan et al., 2020). In this review, the authors would like to explore how SARS-CoV-2 infection could affect male fertility.

SARS-CoV-2

Coronavirus is a large, single-stranded and enveloped RNA virus of about 32 kilobases with a genome contained in the nucleocapsid (Ortega et al., 2020; Shantanam, 2018). The nucleocapsid is contained in the viral envelope and the envelope consists of three proteins: membrane protein, envelope protein and spike proteins which mediates the entry of the virus into the host cell (Shantanam, 2018). The spike protein also plays a role in determining the specificity of host tissue, a marker of the exposed surface that is recognized by the virus and induces a host immune response (Hulswit et al., 2019; Tortoricia & Veesler, 2020; Vlasak et al., 1988). The spike protein of the coronavirus consists of two subunits, domain S1 and
domain S2. The S1 domain consists of a spike protein that plays a role in the binding and attachment of the virus to the host cell membrane. These features also true for SARS-CoV-2. Angiotensin-converting enzyme-2 (ACE2), CD26, Ezrin, and cyclophilins are some of the receptors known to be involved in S1 subunit binding in humans on the attachment and penetration stage of the SARS-CoV-2 into the host cells. Complex interactions between the virus and the host are involved in this process so that the virus can replicate rapidly in the target cells. However, the role of SARS-CoV-2 infestation in the reproductive system is not completely understood. Some coronavirus variants, particularly SARS-CoV, can provide information about the virus’s tissue-specific physiopathology. A cohort study of immunohistochemical tests and in situ hybridization on ovarian and uterine tissue of women who died from infection with SARS-CoV showed that the female reproductive system can avoid infection with the SARS-CoV virus, even though there is an ACE2 receptor (Ding et al., 2004). The ACE2 protein is the main receptor for the entry of the coronavirus. Because Leydig cells (testicular cells) actively express this receptor, coronavirus infection is thought to affect the male reproductive tract (Douglas et al., 2004).

ACE2 in testicular cells

ACE is expressed almost all over the body, while ACE2 is initially found in the heart, kidney, and testes (Donoghue et al., 2000). Immunohistochemical tests showed the presence of ACE2 in adult human testes in both Sertoli cells and Leydig cells, whereas ACE2 in adult rat testes was only found in Leydig cells (Douglas et al., 2004). A study was conducted to see the expression of ACE2 in rats. The researchers found that the expression of ACE2 in the rat testes increased progressively on the 7th and 19th day postpartum when progenitor cells mature into steroidogenic Leydig cells (Douglas et al., 2004). Researchers also discovered that using testosterone therapy to suppress LH and testosterone levels had no effect on the ACE2 activity expression in the testes, indicating that the ACE2 enzyme is not hormonally regulated (Douglas et al., 2004). Because SARS-CoV-2 uses the same signalling pathway as SARS, the testes are a potential target for SARS-CoV-2 infestation. Researchers discovered ACE2 expression and transcripts in human spermatozoa in a recent genomic study, which were then validated for their expression in germ cells and testicular cells (Jan et al., 2017). Transcriptomic analysis from a pool of 500 laser microdissected cells of human testicular germ cell subtypes captured individually by RNA-Seq found ACE2 transcripts in human spermatogonia, spermatocytes, and spermatids, as well as ACE2 transcripts in the mouse testes (Jan et al., 2017).

A transcriptome analysis of >62,000 individual germ cells from adult mice using single-cell RNA-Seq uncovered the presence of ACE2 transcripts in spermatogonia and spermatids (Hermann et al., 2018). Wang, et al conducted an analytical study of ACE2 expression in adult human testes by single-cell transcriptome sequencing and found that there was ACE2 expression in Leydig and Sertoli cells in human testes (Wang & Xu, 2020). Gene Enrichment Analysis revealed that ACE2-positive spermatogonia had a greater amount of genes related to virus replication and transmission than ACE2-negative spermatogonia. ACE2-positive Leydig and Sertoli cells have a higher number of immunity-related genes than mitochondria- and reproduction-related genes. It can be concluded that the testes are highly vulnerable to infection of SARS-CoV-2, which can result in spermatogenic malfunction (Wang & Xu, 2020). Sertoli cells, Leydig cells, and spermatogenic stem cells all express ACE2. Positive ACE2 in infertile men is higher than fertile men because ACE2 that is too active can affect spermatogenesis and is highest in men aged 30 years (Shastri et al., 2020). The incidence and severity of SARS-CoV-2 in men are higher than in women. A research of 68 people (48 men, with a median age of 37 years old), found that women were able to eradicate the virus more quickly than men. They also
observed three families based on gender and found that female family members achieved clearance of SARS-CoV-2 infection earlier than men. They also examined ACE2 expression using 3 independent RNA expression databases in several tissues and found that ACE2 expression was present in testicular tissue, but very low in ovarian tissue (Shastri et al., 2020).

**SARS-CoV-2 infection and invasion mechanism**

The SARS-CoV-2 virus's entry process begins when the spike protein binds to the ACE2 enzyme on the cellular membrane by the S1 domain and fused into the target cell membrane by the S2 domain is mediated by Transmembrane Serine Protease 2 (TMPRSS2) on the outside of the host cell membrane which is utilized for priming protein S so that the virus can enter (Glowacka et al., 2011; Hoffmann et al., 2020; Wang & Xu, 2020). Some studies mention that TMPRSS2 causes a conformational change in the S protein into two, S1 and S2 domains (Hoffmann et al., 2020; Jiang et al., 2020). Once the virus successfully diffuses into the target cell membrane, it sheds its genetic material, replicates its RNA using host cell organelles, and releases fully developed virions to attack another cell (Figure 1) (Boopathi et al., 2020; Jiang et al., 2020). Because ACE2 levels in men are greater than in women, recent investigations have found that men are more likely to contract COVID-19 and experience more severe symptoms (Guan et al., 2020; Letko et al., 2020). ACE2 and TMPRSS2 expression in men is higher in certain organs.

ACE2 was discovered to be extensively expressed in spermatogonia, Leydig cells, and Sertoli cells in a single-cell RNA sequencing (scRNA-seq) analysis of human testis cells (wang z & xu x, 2020). TMPRSS2 is an important host factor for virus attachment and initiation for replication. TMPRSS2 is overexpressed in the prostate which is usually associated with prostate oncogenesis (H. Li et al., 2020). Androgen levels in male circulation are directly proportional to TMPRSS2 levels in cells (Ko et al., 2015). The researchers also investigate the expression of ACE2 and TMPRSS2 in female ovarian cells using scRNA-seq technology, discovering that ACE2 expression in the ovarian cortex cells was insignificant, and no other ovarian cells expressed TMPRSS2 (Chen et al., 2010). A study on the analysis of virus clearance rates conducted in Mumbai (India), from 68 COVID-19 patients, 48 male and 20 female, found that the rate of viral clearance in women was considerably faster than men (4 vs 6 days) (Shastri et al., 2020). This disparity could be attributed to male inherent immunity, which generates less antiviral interferon (Trypsteen et al., 2020).

**SARS-CoV-2 influence on male reproduction system**

**Male reproductive hormones**

Several studies were conducted to investigate the effect of SARS-CoV-2 infestation on male reproductive hormones, as SARS-CoV-2-mediated disturbances in male reproduction are also linked to androgen synthesis. The study findings indicate that infection with SARS-CoV-2 in males results in acute-stage hypogonadism associated with increased levels of pro-inflammatory cytokines, especially IL-1β, IL-6, and TNF-α. This inflammatory process can affect the regulation of steroidogenesis (Ma et al., 2020; Sengupta et al., 2020). Hormone levels in males fluctuate greatly, especially under conditions of acute illness or physiological stress (Simmons & Roney, 2009). Ma et al. evaluated by comparing reproductive hormone levels in 119 men of reproductive age with SARS-CoV-2 infection to 273 healthy men as controls in their study. The study revealed higher levels of prolactin and LH in patients with COVID-19 compared to healthy men, but no statistical difference in serum testosterone and Estradiol levels (Ma et al., 2021). Rastrelli et al. (2021) discovered that men with severe disease or death caused by SARS-CoV-2 pneumonia had reduced testosterone levels.
levels (5 nmol/L) than men with low disease and clinical recovery. Another study found in COVID-19 patients an upsurge in circulating LH levels and a reduction in the testosterone: LH ratio, suggesting a detrimental effect on Leydig cell function (Ma et al., 2021). The study by Koç and Keseroglu proved a decrease in post-infection circulating testosterone levels, while the study by Cinislioglu et al. suggested that this reduction is associated with disease severity (Cinislioglu et al., 2022; Koç & Keseroglu, 2021).

**Male reproductive organs**

Oxidative stress occurs when the production of pro-oxidants in the cells is more than antioxidants (Bisht et al., 2017). One of the causes of oxidative stress is inflammation induced by SARS-CoV. The incoming SARS-CoV virus evokes an innate immune response and triggers inflammation and excessive production of reactive oxygen species (ROS), resulting in oxidative stress (Delgado-Roche & Mesta, 2020). The systemic cytokine storm elicits a severe inflammatory response and causes severe tissue injury (Huang et al., 2020). Severe systemic inflammation through the spread of blood-borne pathogens or through secondary inflammation, can adversely affect male reproductive function (Dutta et al., 2020; Schuppe & Meinhardt, 2005). Testicular cells with high ACE-2 expression may have undergone direct viral intrusion (Figure 1) (Fan et al., 2021; X. Li et al., 2022). Testicular function may be impacted by secondary SARS-CoV-2 inflammation. Intracellular inflammatory mediators, particularly interleukin (IL)-1A, IL-1B, type 1 and type 2 interferons, nitric oxide, tumour necrosis factor α (TNF-α), and transforming growth factor B3 (TGF B3), are sensitive to Sertoli cells (Schuppe & Meinhardt, 2005).

![Figure 1. Mechanism of testicular tissue damage mediated by ACE2 and TMPRSS2](Li et al., 2022).

In COVID-19 patients, pathological examination of testicular tissue can be done both during and after infection to gauge the short- and long-term effects of the disease on the testicles and male reproduction. Single-cell RNA sequencing (scRNA-seq) is a viable molecular technique that can be done to generate independent and objective transcriptional profiles of all cell types in the human testis at single-cell resolution. To better understand how each type of testicular cell can alter its gene expression
program when infected with COVID-19, single-cell transcriptomes from testicular biopsies from COVID-19 patients are compared to those from healthy, fertile men. Both tests will give researchers and doctors a thorough understanding of how COVID-19 affects male reproduction and how to treat each patient individually (Chen et al., 2010; Van Der Made et al., 2020).

Sperm analysis
Decreased quality of spermatozoa occurs due to increased damage to sperm DNA due to oxidative stress and inflammation acquired from SARS-CoV-2 infection (Maleki & Tartibian, 2021). The impact of SARS-CoV-2 infection on sperm analysis has been documented for the first time in 14 men with mild symptoms (35 days after recovering from COVID-19 symptoms), four men with moderate symptoms (25 days after recovering from COVID-19 symptoms) and 14 healthy men as controls were included in the study (Holtmann et al., 2020). The study found a significant decrease in the concentration, number, and progressive motility of spermatozoa in men with SARS-CoV-2 infection compared to control men (Holtmann et al., 2020). A cohort in 12 men (one with mild and 11 men with moderate symptoms) an average of 78.5 days after COVID-19 manifestation, they discovered that the volume, concentration, morphology, motility, and sperm DNA fragmentation index of eight men were all within normal ranges (Ma et al., 2021). Four men had low sperm motility and two of them had a lot of abnormal sperm cells (Ma et al., 2021). A study found a decrease in sperm morphology after SARS-CoV-2 infection (Temiz et al., 2021). Another study found that the severity of the decline in spermatozoa's quality was significantly correlated with the severity of the disease in 25% of men who recovered from COVID-19, with the majority becoming azoospermia (Gacci et al., 2021). According to Li et al study, 39% of recovered COVID patients had oligozoospermia, and sperm analysis revealed a higher level of leukocyte infiltration (Li et al., 2020).

Conclusion
The attachment of SARS-CoV-2 to host cells is mediated by ACE2 and TMPRSS2. By affecting semen parameters, the reproductive tract, hormones, and sexual function, SARS-CoV-2 infection can harm and lessen the reproductive function of adult men. Many studies have discussed the impact of COVID-19 on the male reproductive organs, but the long-term effects of COVID-19 on male fertility cannot be fully assessed to date. Researchers are still continuing to carry out long-term studies with a larger number of samples and molecular examination parameters to understand the possible effects of COVID-19 on male reproduction and to obtain more potential therapeutic approaches.

Authors’ contributions
Conceptualization: IIB and ZZ; Data Curation: IIB, ZZ and GG; Formal Analysis: DS and FH; Resources: WY and MA; Supervision: ZZ, GG and DS; Validation: ZZ, GG, FH, DS, WY and MA; Writing – Original Draft Preparation: IIB; Writing – Review & Editing: IIB.

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