

The impact of SARS-CoV-2 and COVID-19 on male reproduction and men's health

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Many couples initially deferred attempts at pregnancy or delayed fertility care due to concerns about coronavirus disease 2019 (COVID-19). One significant fear during the COVID-19 pandemic was the possibility of sexual transmission. Many couples have since resumed fertility care while accepting the various uncertainties associated with severe acute respiratory syndrome coronavirus 2, including the evolving knowledge related to male reproductive health. Significant research has been conducted exploring viral shedding, tropism, sexual transmission, the impact of male reproductive hormones, and possible implications to semen quality. However, to date, limited definitive evidence exists regarding many of these aspects, creating a challenging landscape for both patients and physicians to obtain and provide the best clinical care. This review provides a comprehensive assessment of the evolving literature concerning COVID-19 and male sexual and reproductive health, and guidance for patient counseling. (Fertil Steril® 2021;115:813–23. ©2020 by American Society for Reproductive Medicine.)

Key Words: COVID-19, angiotensin-converting enzyme 2, coronavirus, semen, infertility, male

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In December 2019, the first case of a severe atypical pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was described in Wuhan, China. To date, nearly 45,700,000 cases of coronavirus disease 2019 (COVID-19) have been reported worldwide, with nearly 1.2 million deaths (<https://coronavirus.jhu.edu/>). The COVID-19 pandemic has had significant and profound long-lasting impacts on people's lives.

Many couples initially deferred attempts at pregnancy or delayed their fertility care. Currently, numerous couples have resumed fertility care and have accepted the associated remaining

uncertainties related to the ongoing pandemic. Nearly 70,000 peer-review publications and 20,000 preprint communications have been published in the short period since the discovery of SARS-CoV-2. However, for patients and providers delivering fertility care, there remain many unanswered questions about the impact of SARS-CoV-2 on reproduction, specifically male reproductive health. For infertile couples, the COVID-19 pandemic continues to have a significant impact for their fertility treatment and family planning.

Our understanding of other infectious viruses inform the possible impact

of systemic viral infection and inflammation on male reproduction (1). In addition, certain viruses are shed in human semen, permitting the possibility of sexual transmission of SARS-CoV-2. As the body of literature evaluating the impact of SARS-CoV-2 and COVID-19 on male reproduction develops, it becomes more challenging for patients and providers to draw appropriate conclusions and provide the best evidence-based clinical care. This review provides a comprehensive assessment of the evolving literature surrounding SARS-CoV-2, COVID-19, and men's health and reproduction.

VIRAL INFECTIONS OF THE MALE REPRODUCTIVE TRACT

Various microorganisms, including certain bacteria and viruses, may affect male reproductive function. As a result of direct testicular infection, men may have diminished sperm viability, reduced sperm counts, and impaired sperm motility, primarily through effects exerted on the testicles (2–4).

Received October 31, 2020; revised December 23, 2020; accepted December 29, 2020; published online January 1, 2021.

D.P.P. has nothing to disclose. N.P. has nothing to disclose. J.G. has nothing to disclose. J.P.A. has nothing to disclose. P.S.L. has nothing to disclose. J.M.H. reports a research and fellowship grant from Endo Pharmaceuticals and a fellowship grant from Boston Scientific, has a leadership position or is founder of Nanonc (a microfluidic sperm sorting company), StreamDx, Andro360, and Inherent Biosciences, and is an advisor for Turtle Health.

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Fertility and Sterility® Vol. 115, No. 4, April 2021 0015-0282/\$36.00

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Viruses typically reach the testicle through hematogenous spread. Under normal circumstances, testicular immune privilege protects the testicular germ cells from the host inflammatory response to a systemic infection. However, certain viruses may cross the blood-testis barrier and even invade testicular cells, eliciting an immune response within the testis (5). A basic understanding of viral infection physiology is essential to understanding both short- and long-term impact of SARS-CoV-2 on male reproductive function. Figure 1 provides a broader overview of previously reported viral infections of the male reproductive tract (2–4, 6). We specifically review the impact of mumps, human immunodeficiency virus (HIV), and Zika virus (viruses for which we have a basic understanding and known impact on male reproductive health) to frame the discussion regarding the impact of SARS-CoV-2 on male reproduction.

Mumps

The Mumps virus is part of the Paramyxoviridae family of single-stranded RNA viruses (4, 7). The hallmark of Mumps is painful swelling of the parotid glands. Nearly 20%–30% of postpubertal men with mumps develop unilateral epididymo-orchitis (7). Bilateral orchitis occurs in ~15% of cases and can lead to testicular atrophy, reduced sperm concentration and motility, and even azoospermia. Reproductive impairment due to mumps orchitis is thought to occur secondarily to the induced host inflammatory response and its subsequent impact on Leydig and Sertoli cell function, as shown in in vitro mouse models (8, 9). Infection of Leydig and Sertoli cells by the mumps virus activates the innate immune response with the release of proinflammatory cytokines such as interferon (IFN) α and tumor necrosis factor α (10). Under these inflammatory conditions, impaired testosterone

production and germ cell death can occur, although this mechanism is incompletely characterized (10). Although the mumps virus is primarily transmitted by direct contact or respiratory droplets, it has been isolated previously in both human urine and human semen (4).

Human Immunodeficiency Virus

HIV belongs to the Lentivirus family and includes enveloped single-stranded RNA viruses (11). Acute HIV infection can cause symptoms of mild systemic viral illness followed by a period of clinical latency (11). HIV has been detected in the semen after infection, and sexual transmission is the primary mode of transmission (12). Large randomized trials have suggested that circumcision may reduce HIV transmission among heterosexual couples (13). Leukocytes are the main vectors of HIV in the semen (12). Progression to acquired immunodeficiency syndrome (AIDS) is characterized by opportunistic infections and increased cancer risk due to viral immunosuppression (11). Male patients with AIDS can develop chronic orchitis and hypogonadism (12). HIV has also been found in testicular germ cells, however, the mechanism of viral entry remains poorly understood because the primary HIV receptor, CD4, is not found on testicular germ cells (12, 14). The inflammatory response, impaired testosterone production by Leydig cells, and HIV infection of testicular germ cells can affect male reproductive and endocrine function (12, 14).

Zika

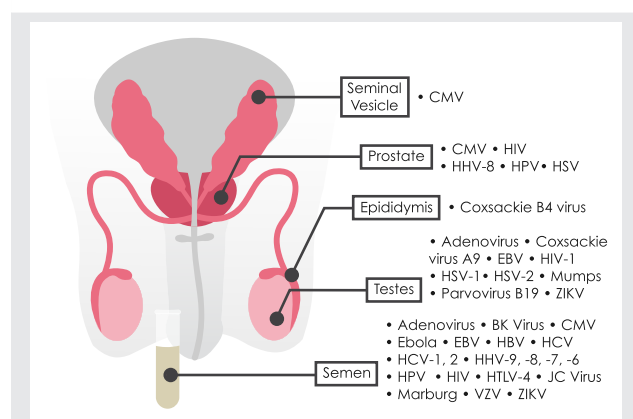
The Zika virus is a single-stranded RNA virus that is part of the Flaviviridae family. The most severe clinical manifestations include Guillain-Barré syndrome and congenital microcephaly (15). Zika has been isolated in the semen and can be sexually transmitted (15, 16). Interestingly, in men infected with Zika, viral levels are usually much higher in the semen than in the serum and may persist for more than 188 days in the semen whereas the virus is cleared from the serum after initial viral symptoms subside (16). Given the relatively recent emergence of the Zika virus, data are limited on its long-term impact of on human male reproductive and endocrine function. In mouse models, Zika infection has been shown to cause significant epididymo-orchitis leading to decreased sperm counts and diminished sperm motility (17). In vitro models suggest that Zika predominantly infects Sertoli cells but also germ cells (18).

Mumps, HIV, and Zika virus can lead to orchitis and have been detected in the semen. This poses many questions about the impact of SARS-CoV-2, also a single-stranded RNA virus on male reproductive health and detection in the semen.

SARS-CoV-2 TAXONOMY

SARS-CoV-2 is a single-stranded RNA virus of the coronavirus subfamily. There are seven different coronaviruses that can infect humans (19). The first four, 229E, NL63, OC43, and HKU1, cause mild viral symptoms (19). The other three, SARS-CoV-1, MERS-CoV, and SARS-CoV-2, can cause more severe respiratory symptoms (19). The SARS-CoV-2

FIGURE 1



Viruses found in the male reproductive tract. CMV = cytomegalovirus; EBV = Epstein-Barr virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HHV = human herpesvirus; HIV = human immunodeficiency virus; HPV = human papillomavirus; HSV = herpes simplex virus; HTLV = human T-lymphotropic virus; VZV = varicella zoster virus; ZIKV = Zika virus.

Patel. Impact of COVID-19 on men's health. *Fertil Steril* 2020.

envelope has 20-nm spikes that resemble a crown under electron microscopy, whence the name coronavirus (20). SARS-CoV-2 has the largest genome among known RNA viruses (19). Similarly to other viruses, a nucleoprotein surrounds the RNA genome to form a helical structure, which is surrounded by the viral envelope. The matrix protein is embedded in the viral envelope, and the spike (S) proteins are anchored to the viral envelope. The S proteins are important for receptor recognition, cell attachment, and fusion during viral infection (21). The S proteins are a trimeric glycoprotein found in all human coronaviruses and other viruses, including HIV, influenza, and Ebola (21).

MECHANISM OF SARS-CoV-2 VIRAL ENTRY

Viral entry into host cells is mediated by the viral S proteins and the host cell receptor angiotensin-converting enzyme 2 (ACE2) (21–24). When the S protein binds to the ACE2 receptor, transmembrane protease serine 2 (TMPRSS2), found on the host cell surface, primes the S protein as well as other cellular protease to cleave the S protein into S1 and S2 subunits (Fig. 2) (22). This critical step promotes viral entry into the host, as both ACE2 and TMPRSS2 are needed for viral entry. Once viral entry occurs, the viral RNA is released, and the viral genome's replication and transcription begins.

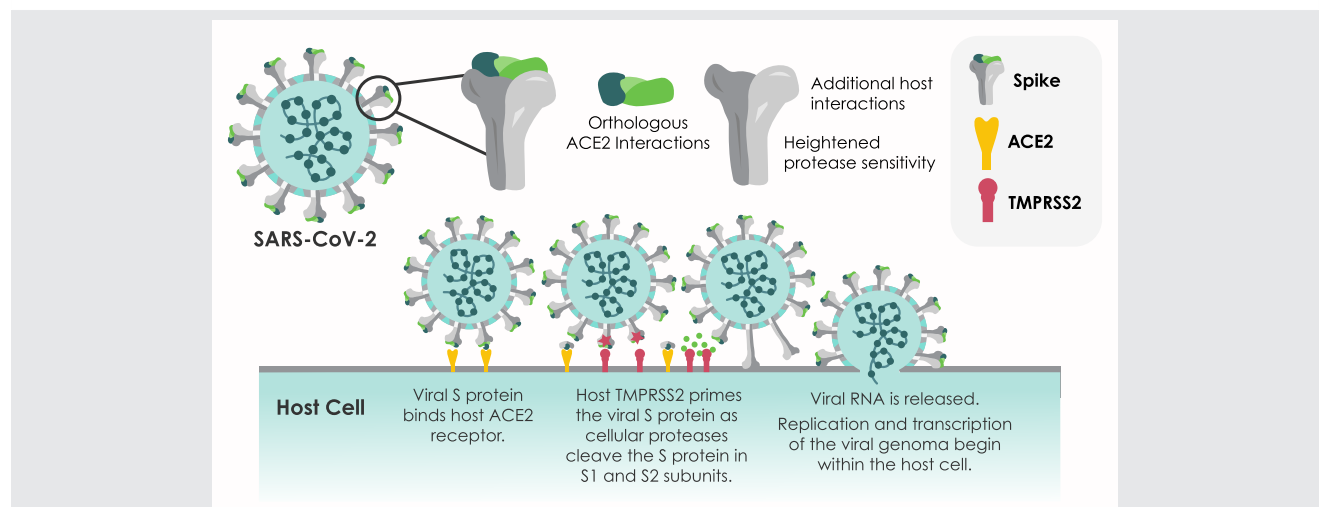
IMMUNOPATHOGENESIS OF COVID-19

SARS-CoV-2 is primarily transmitted through respiratory droplets from infected individuals (25, 26). The incubation period for COVID-19 ranges from 1 to 14 days after initial exposure, although most infected patients show symptoms of COVID-19 by day 5 after initial exposure (27, 28). Once the virus is transmitted to an individual, SARS-CoV-2 begins

replication within the airway epithelial cells. This is mediated primarily by the interaction between host ACE2 and TMPRSS2, as discussed above (22). Infection of the airway epithelium can typically cause fevers, myalgia, sore throat, and shortness of breath (29). In more severe cases, COVID-19 is characterized by severe pneumonia, acute respiratory distress syndrome, sepsis, septic shock, and death (29). Interestingly, the down-regulation of ACE2 expression is associated with acute lung injury based on our understanding of SARS, but this may contribute to a sex susceptibility to COVID-19, as discussed below (30). In addition, alternative host cells with higher ACE2 expression include enterocytes, which can lead to the gastrointestinal symptoms of COVID-19 such as diarrhea (29).

One of the challenges in understanding the impact of SARS-CoV-2 infection on male reproductive health is the variability in COVID-19 severity and the immune response to SARS-CoV-2. It has been hypothesized that higher viral loads in the blood lead to hematogenous spread to the male reproductive tract and severe viral illness causing heightening host immune response within the testicle (4). Based on our knowledge of other viral illnesses (e.g., mumps), SARS-CoV-2 may affect male reproduction. Older age (>65 years), male sex, African-American or Asian race, diabetes, and hypertension are among many well established risk factors for more severe symptoms and death from COVID-19 (31–33). However, 5% of all cases of severe COVID-19 are in younger healthy adults (32). Regardless of the known risk factors, men are more likely to have more severe disease and clinical courses (33). Some hypotheses, which are discussed later, relate to differential expression of ACE2/TMPRSS2 (which have higher expression levels in the male-specific organs), an androgen-dependent relationship with higher levels possibly conferring worse

FIGURE 2



SARS-CoV-2 viral entry in host cell. ACE2 = angiotensin-converting enzyme 2; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TMPRSS2 = transmembrane protease serine 2.

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disease, and innate immune differences in women that portend less significant disease (34, 35).

Our understanding of the viral dynamics and the immune response is evolving but is relatively limited. The innate immune response requires recognition of SARS-CoV-2 as a pathogen. Host macrophages identify the pathogen-associated molecular patterns and viral RNA that activates innate immune response through IFN-1 (36). SARS-CoV-2 may exhibit immune evasion through inhibition of IFN signaling pathways, which may contribute to the variability in incubation periods (37). Subsequent activation of natural killer cells contributes to the innate immune response through a major histocompatibility complex-independent mechanism. Adaptive immunity against SARS-CoV-2 is initially mediated by cellular immunity through helper T cells, which release IFN- γ , tumor necrosis factor α , and interleukin-2 in response to antigen presentation by antigen presentation cells (36). The cytokine release by helper T cells activates cytotoxic T cells that attack and destroy virus-infected host cells (36). Humoral immunity is another component of adaptive immunity that is mediated by B cells (36). B-Cell activation leads to the production of IgM and IgG SARS-CoV-2 S-protein antibodies. There is variability in the timing of serum antibody production after initial infection, but an increase in levels of IgM and IgG SARS-CoV-2 S-protein antibodies is usually noted after 10 days (38). An improved understanding of the immune response to SARS-CoV-2 will help elucidate the sex susceptibility to COVID-19 and identify novel therapeutics.

SEX SUSCEPTIBILITY TO SARS-CoV-2 INFECTION AND COVID-19

Early epidemiologic studies from China suggested a significant male gender susceptibility for the rate of severe COVID-19 symptoms and mortality (32). Similar trends have been seen in other countries as well (39). Initially, this susceptibility was thought to be confounded by worse overall health status, chronic disease, and other lifestyle factors, such as smoking (26). However, two other theories have been proposed to explain the observed sex differences in COVID-19 outcomes. First, ACE2 is located on the short arm of the X chromosome, and therefore females have two copies. In normal development, one of the two X chromosomes is silenced in the late blastocyst stage of development, causing condensation of the X chromosome into a Barr body (40). However, some genes escape this inactivation, which is more likely to occur on the short arm of the X chromosome where the ACE2 gene is located (40). This may explain differences in ACE2 expression between different sexes, although this finding has not been consistent in the literature (41). Furthermore, ACE2 is a regulatory component of the renin-angiotensin system, protecting against vascular compromise and severe organ damage. It is hypothesized that increased ACE2 expression in women is protective against more severe COVID-19 symptoms because rapid viral saturation of ACE2 is less likely to occur (41). The second theory to explain the sex differences in COVID-19 symptoms and outcomes is the association between TMPRSS2 and androgen sensitivity.

The androgen response element is a transcriptional promoter for TMPRSS2, which was initially described in the context of the TMPRSS2-ERG fusion gene and prostate oncogenesis (42). It is thought that lower levels of circulating androgens in women lead to lower cellular expression levels of TMPRSS2 and down-regulation of this host receptor. 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.

Severe COVID-19 and mortality have been documented in healthy young adults as well. This has led to investigations into genetic variants that may confer a more severe COVID-19 course due to a subclinical primary immunologic defect. Van der Made et al. reported a study of four young healthy men (pairs of brothers) with severe COVID-19 admitted to the intensive care unit in the Netherlands (43). Whole-genome sequencing identified loss-of-function variants of the *TLR7* gene on the X chromosome. Functional testing in primary immune cells of these brothers suggested the down-regulation of type I and type II IFN signaling. As discussed above, the IFN pathway is important in the innate immune response and subsequent activation of natural killer cells. These novel findings suggest that genetic variants may also affect the severity of COVID-19 and male sex susceptibility, although this warrants further investigation.

IMPACT OF SARS-COV-2 ON MALE SEXUAL FUNCTION

COVID-19 affects sexual function and sexual activity. Early in the pandemic it was reported in a study from China that sexual activity had decreased in 37% of those surveyed, and 44% reported a decrease in the number of sexual partners (44). Interestingly a study from Bangladesh, India, and Nepal suggested minimal change in sexual activity and perhaps even an increase in frequency in a small subset (45). Sexual practices during COVID-19 may be affected through social isolation leading to changes in mood as well as fear of transmission. It has also been suggested that cardiovascular disease from COVID-19 and subsequent treatment may lead to erectile dysfunction, and neurologic manifestations from cerebrovascular disease or hemorrhage may affect sexual desire as well as erectile and ejaculatory function (46). These and other sexual practice-related issues continue to be explored, including self-stimulation practices and pornography use during the pandemic (44).

IMPACT OF SARS-COV-2 INFECTION ON MALE REPRODUCTIVE HORMONES

Several studies have also explored the impact of SARS-CoV-2 infection on male reproductive hormones. Unfortunately, male hormones vary tremendously at baseline with acute illness or physiologic stress, so these early results should be interpreted with caution (47). A further limitation of these data is the absence of long-term hormone data as well as pre- and post-COVID-19 infection hormone levels to reinforce an association between SARS-CoV-2 infection and male reproductive hormone changes.

Ma et al. compared male reproductive hormone levels in 119 reproductive-age men with SARS-CoV-2 infection and 273 age-matched control men (48). They found that patients with COVID-19 had higher serum LH levels and decreased T:LH ratio compared with control men, but there was no statistical difference in serum T levels (3.97 ng/mL in case subjects vs. 4.79 ng/mL in control subjects). No differences were seen also for E₂, but there were significant increases in PRL levels. In addition, on multivariable analyses, serum T:LH ratio among case subjects was negatively associated with serum white blood cell count and C-reactive protein levels. A study by Rastrelli et al. described the association between T levels and clinical outcomes after severe COVID-19 (49). They reported on 31 male patients with SARS-CoV-2 pneumonia in a respiratory care unit where men were categorized into different cohorts based on increased versus decreased levels of care and mortality. Men who required a higher level of care or died had lower total serum T levels than those who recovered clinically. In addition, an increase in mortality and intensive care unit transfer was seen in men with total T <5 nmol/L. These data have not been reproduced in large-scale prospective trials.

Additional studies have explored this androgen hypothesis for COVID-19. In a report on 41 men from Spain, 71% of those admitted to hospital with COVID-19 had androgenetic alopecia (male-pattern hair loss), which is a known androgen-mediated phenomenon (50). Because the androgen receptor is also composed of trinucleotide repeats, it has been hypothesized that this may be implicated in some of the variable disease courses for COVID-19 (51). To further corroborate an androgen hypothesis, data from a large Italian series of 4,532 men demonstrated that men with prostate cancer who received androgen-deprivation therapy (ADT) had significantly lower risks of disease than those without ADT, and this difference was more significant when compared with any other malignancies (52). Although this study was conducted in a specific subset of men, it suggests a protective effect of ADT in COVID-19 outcomes and prompted some to consider clinical trials of ADT.

SARS-CoV-2 TROPISM FOR THE MALE REPRODUCTIVE TRACT

Host cell coexpression of ACE2 and TMPRSS2 for S-protein priming is a critical component of viral entry. ACE2 converts angiotensin II to angiotensin I and is predominantly found in the lung, intestine, heart, and kidney (23, 24). TMPRSS2 is found predominantly in the gastrointestinal tract, genitourinary tract, and the prostate (42). It is still uncertain whether the male reproductive system is susceptible to SARS-CoV-2 infection, primarily because coexpression of both ACE2 and TMPRSS2 is needed by an individual host cell to facilitate viral entry and it is unclear how frequently these are coexpressed (22, 53).

To evaluate the immediate and long-term impact of COVID-19 on testis and male reproduction, pathologic examination of the testes tissues from COVID-19 patients during and after illness will be very useful. Traditional approaches using histologic examination can provide a general under-

standing of how SARS-CoV-19 infection affects the testis physiology and subsequent reproductive health. However, more systematic approaches are also needed to explore the impact on human testis to bring in molecular and mechanistic insights. Single-cell RNA sequencing (scRNA-seq) can serve as a very useful tool for such purposes. Particularly, scRNA-seq allows obtaining unbiased transcriptional profiles of all cell types in the human testis at single-cell resolution. Therefore, if researchers can use scRNA-seq to generate the single-cell transcriptomes of testis biopsies from COVID-19 patients and make comparisons with those from healthy men who are fertile and uninfected, we could gain a systematic understanding of whether and how each testicular cell type may alter its gene expression program in response to COVID-19 infection. This knowledge, combined with physiologic examination, can provide researchers and physicians in-depth understanding of the impact of COVID-19 on male reproduction as well as underlying mechanisms and may lead to personalized treatment options.

Testes

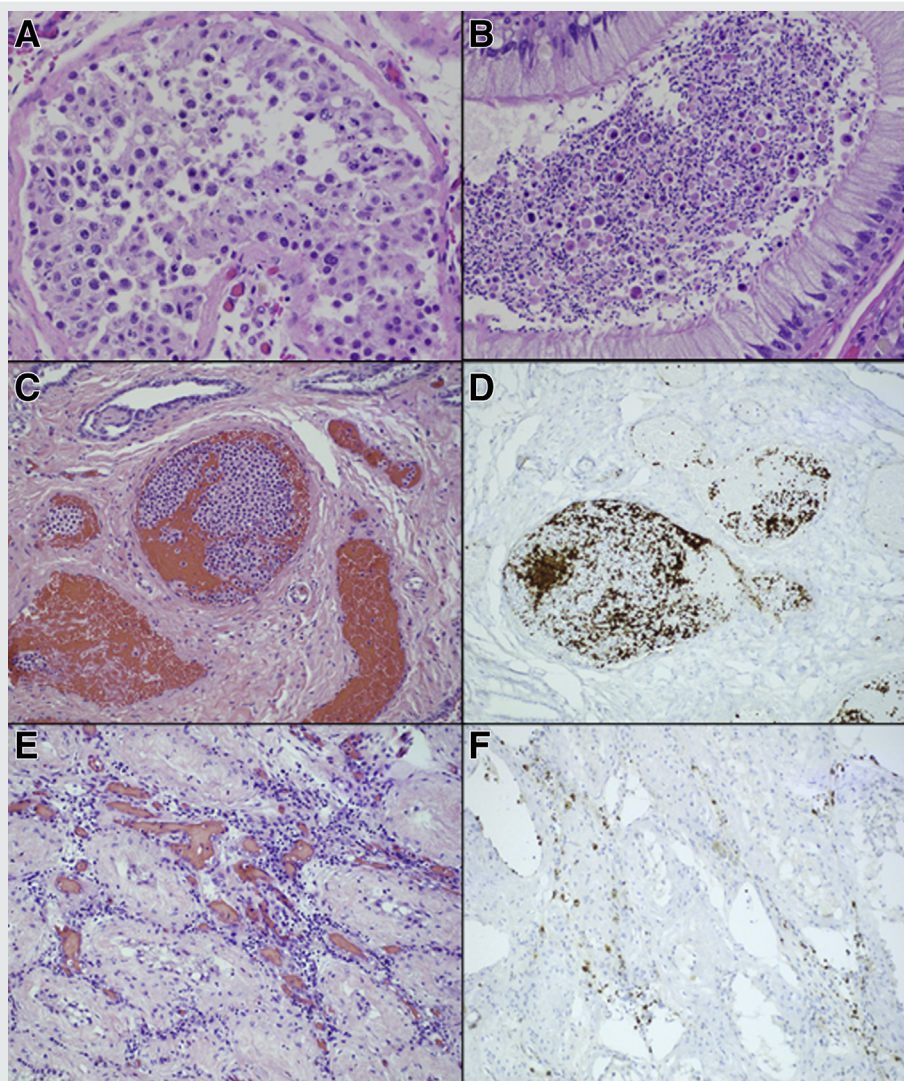
Pan et al. found that expression levels of ACE2 and TMPRSS2 occurred at relatively low and sparse levels within different cells of the testes, with almost no overlapping of gene expression (54). This analysis was performed with the use of dimension-reduction (t-distributed stochastic neighbor embedding) analysis of single-cell transcriptome data from the testes of three healthy young men. Stanley et al. performed a similar analysis of scRNA-seq data from testicular cells and found that coexpression of both ACE2 and TMPRSS2 occurred less than 0.05% of the time (55). Wang et al. performed a similar analysis using scRNA-seq datasets from the Gene Expression Omnibus and Sequence Read Archive with three adult human testicular samples (56). Uniform manifold approximation and projection clustering of adult testicular cells and marker gene analyses showed the expression of ACE2 in two clusters: spermatogonia and Leydig/Sertoli cells. Similar analyses have demonstrated that the expression of TMPRSS2 was concentrated in spermatogonia and spermatids (56). Liu et al. presented their analyses of scRNA-seq data from adult testes, including seven men with obstructive azoospermia and two healthy donors (57). That group found that TMPRSS2 is expressed at high levels and ACE2 at low levels in spermatogonial stem cells. They also reported that Sertoli cells have higher ACE2 expression level and lower TMPRSS2 expression level. To date, most studies have focused on examining the gene expression levels of ACE2 and TMPRSS2 to infer the likelihood of SARS-CoV-2 invading human testes; further studies are needed to evaluate the protein expression patterns of ACE2 and TMPRSS2 in human testis to better reflect the potential of SARS-CoV-2 infection.

Postmortem studies have provided further insight into the impact of COVID-19 on the male reproductive tract. Previously, postmortem examination of the testes of six men who died of SARS suggested that SARS-CoV infection led to orchitis (58). The authors found that the testes contained few spermatozoa within the seminiferous tubules, significant germ cell

death, and considerable inflammatory infiltrate. This was one of the only studies assessing the impact of SARS-CoV on the male testis. Concerning SARS-CoV-2, an early autopsy study of two men with COVID-19 showed testicular atrophy in one of the men (59). Two other studies on the postmortem examination of the testes from men infected with SARS-CoV-2 have provided additional insight. Flaifel et al. presented their findings from ten autopsies who had previously tested positive for SARS-CoV-2 according to nasopharyngeal swab at the time of hospital admission (60). SARS-CoV-2 was found in all of the subjects' respiratory tracts, but it was not found in the testes

(60). Microscopic findings from the testes showed sloughing of the spermatocytes, elongation of spermatids, and swelling and vacuolization of Sertoli cells suggestive of an acute testicular injury (Fig. 3). In two cases, they found multifocal microthrombi in the testicular vasculature, which has also been shown in lung tissue after SARS-CoV-2 infection. This may suggest that hypoxic injury may also contribute to possible testicular damage from SARS-CoV-2. A similar study by Yang et al. examined the testes from 12 patients who had died of complications from COVID-19 (61). Reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2

FIGURE 3



Pathologic changes of the testes associated with severe acute respiratory syndrome coronavirus 2 infection. (A) Seminiferous tubule injury including chromatin condensation, acidophilic alteration of the cytoplasm of spermatocytes, and swelling of the Sertoli cells (hematoxylin and eosin stain [H&E], $\times 400$ magnification). (B) Accumulation of sperm and immature spermatocytes in the epididymis (H&E, $\times 400$ magnification). (C) Multifocal platelet aggregation and microthrombi (H&E, $\times 200$ magnification). (D) CD61 immunostain highlights platelet clusters within testicular vessels ($\times 200$ magnification). (E) Mononuclear inflammatory infiltrate in the testicular interstitium and atrophic seminiferous tubules consistent with orchitis (H&E, $\times 200$ magnification). (F) Immunohistochemical studies reveal a predominant CD8-positive T-cell infiltrate (CD8, $\times 200$ magnification). Reproduced from Flaifel et al. (60).

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found one case that was positive for virus in the testis, but the testicular tissue sample was predominantly fibrovascular tissue with very few seminiferous tubules, suggesting that viral detection was a contaminant from the blood and not from within testicular cells. In that same individual, there was also significant damage to the seminiferous tubules. They found significant interstitial edema and inflammatory infiltration with T cells. Sloughing of the spermatocytes was also noted along with swelling of the Sertoli cells and reduced number of Leydig cells. These pathologic findings correlate with clinical findings that have been reported in previous case reports and series. The same group examined testicular tissue from three cases with the use of electron microscopy and did not detect SARS-CoV-2 viral particles.

Very early in the pandemic, a patient presented with testicular and abdominal pain, highlighting one of the first reports of testicular discomfort (62). Since then, there have been two further reports of testicular pain as the predominant presenting symptom of SARS-CoV-2 infection in young men (63, 64). In addition, Pan et al. and Holtmann et al. found that 6 out of 34 (7%) and 1 out of 14 (19%) patients, respectively, reported scrotal discomfort at the time of diagnosis of COVID-19 (54, 65).

Epididymis, Seminal Vesicles, and Prostate

There is relatively limited evidence regarding SARS-CoV-2 tropism for the epididymis, seminal vesicles, and prostate. Bioinformatics analysis from the Human Protein Atlas database (<https://www.proteinatlas.org/ENSG00000130234-ACE2/tis->

[sue](#)) suggests that ACE2 expression occurs at low levels within the seminal vesicles and at very low levels within the epididymis and prostate. Conversely, TMPRSS2 expression has been noted at low to medium rates in the epididymis, seminal vesicles, and prostate within this database. Gene fusions of TMPRSS2 with ETS/ERG transcription factors have been associated with prostate cancer in an androgen-sensitive manner (66). TMPRSS2 expression also is androgen sensitive within the prostate. Song et al. analyzed TMPRSS2 and ACE2 coexpression with the use of scRNA-seq from an existing normal human prostate dataset (67). They found that 0.32% and 18.65% of prostate epithelial cells expressed ACE2 and TMPRSS2, respectively, and colocalization was identified in <1% of cells (67). Pathologic evaluation of prostate tissue in men recovering from COVID-19 has not been explored.

DETECTION OF SARS-CoV-2 IN THE SEMEN

Recently, 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 (48, 54, 65, 68–72). These findings are summarized in Table 1. Li et al. are the only group to detect SARS-CoV-2 by means of RT-PCR in 6 of 38 semen samples (16%) from men with acute symptoms of or recovering from COVID-19 (73). However, details regarding semen collection and protocol for that study are not comprehensive. Compared with other series, semen testing from the onset of clinical symptoms of COVID-19 was shorter (median 10.5 days). A

TABLE 1

SARS-CoV-2 transmission in the semen.

Study	Country	Sample size	Median time from clinical diagnosis of COVID-19 to semen testing, days	Cohort description	Key findings
Li et al. (73)	China	38	10.5 (range 6–16)	Acutely infected and recovered men	In total, 6/38 (15.8%) men recovering from COVID-19 had SARS-CoV-2 detected in the semen: 4/15 (26.7%) men with acute symptoms of COVID-19 and 2/23 (8.7%) men recovering from COVID-19
Pan et al. (54)	China	34	31 (range 8–75)	Symptomatically recovered men	No viral detection in semen samples
Holtmann et al. (65)	Germany	34	~45 (IQR ~39.5–50.5)	18 symptomatically recovered men, 2 acutely infected men, and 14 control men	No viral detection in semen samples
Paoli et al. (69)	Italy	1	8	Symptomatically recovered man	No viral detection in semen samples
Song et al. (68)	China	12	N/A	Recovered men	No viral detection in semen samples
Guo et al. (70)	China	23	32 (IQR 27.5–33)	11 recovered men and 12 acutely infected men	No viral detection in semen samples
Pavone et al. (71)	Italy	9	7 (range 4–13)	7 recovered men and 2 men with mild residual COVID-19 symptoms	No viral detection in semen samples
Ma et al. (48)	China	12	78.5 (range 56–109)	Recovered men	No viral detection in semen samples
Kayaaslan et al. (72)	Turkey	16	1 (range 0–7)	Acutely infected hospitalized men with COVID-19	No viral detection in semen samples. Four semen samples were provided on the same day as a positive SARS-CoV-2 nasopharyngeal swab and six samples ~1 day after a positive SARS-CoV-2 nasopharyngeal swab

Note: COVID-19 = coronavirus disease 2019; IQR = interquartile range; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

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more recent case series was unable to detect SARS-CoV-2 in the semen of 16 men acutely infected with COVID-19, with a median time between positive nasopharyngeal swab and semen evaluation of 1 day (72). Although the virus may be cleared from the semen in the acute phase of the disease, larger-scale community-based testing for SARS-CoV-2 of the semen in symptomatic men with a broader range of COVID-19 severity as well as asymptomatic men is needed before we can determine whether sexual transmission of SARS-CoV-2 can occur.

In an alternate research approach, Zhang et al. evaluated for the presence of SARS-CoV-2 in extraprostatic secretions (EPSs) of men with recent COVID-19 (74). EPSs are a large component of semen. They performed a two-glass test of EPSs in ten men recovering from COVID-19. They did not detect presence of SARS-CoV-2 in either pre-prostate massage urine or post-prostate massage EPS.

IMPACT OF SARS-CoV-2 INFECTION ON SEMEN PARAMETERS

Holtmann et al. were the first to report the impact of SARS-CoV-2 infection on semen parameters (65). They described 14 men with mild symptoms of COVID-19, 4 with moderate symptoms of COVID-19, and 14 healthy control men. For those with COVID-19, the median times from the resolution of COVID-19 symptoms to semen collection were 35 days for those with mild symptoms and 25 days for moderate symptoms. The authors found a significant negative impact of SARS-CoV-2 infection on sperm concentration, total sperm count, and total progressive motility compared with control men. Two men with moderate COVID-19 symptoms were excluded from the analysis owing to cryptozoospermia. For men with COVID-19 who reported fever, only total motile sperm count was significantly lower than those without a fever. Owing to the small sample size, lack of semen analysis before SARS-CoV-2 infection, and single semen analysis after SARS-CoV-2 infection, only limited conclusions may be drawn from that study regarding the impact of SARS-CoV-2 on semen parameters. Furthermore, the authors reported that the findings may be confounded by certain medications used to treat symptoms of COVID-19 as well as from the febrile illness alone.

Guo et al. described a cohort of 23 men who underwent semen analysis following an abstinence period of 3–6 days at a median 32 days after diagnosis of pharyngeal-swab-positive SARS-CoV-2 infection (70). Eighteen men (78%) had mild symptoms of COVID-19 and the remainder had moderate symptoms ($n = 5$; 22%). There were no patients with severe symptoms of COVID-19. Two patients were excluded because of low ejaculate volume. The authors concluded that there was no impact of recent SARS-CoV-2 infection on sperm concentration, motility, and morphology.

Ma et al. described a cohort of 12 men (1 with a history of mild symptoms related to COVID-19 and 11 with moderate symptoms) who had semen analysis at a median 78.5 days from the start of COVID-19 symptoms. Eight (66.7%) of the men were within the reference ranges for various semen parameters including volume, sperm concentration, total motile

sperm count, motility, morphology, and sperm DNA fragmentation index (assessed by means of sperm chromatin dispersion testing). Four of the men had low motility, and two had poor morphology. Three of the 12 men had an initial semen analysis before SARS-CoV-2 infection, which permitted direct comparison in those individuals. Although there was considerable variability between repeated semen analyses, even for the same individual, one patient showed a decrease in sperm motility in the semen analysis after SARS-CoV-2 infection compared with before. Further prospective longitudinal studies are needed to study the viral impacts on semen quality.

IMPLICATIONS FOR CHILDREN BORN TO MEN RECOVERED FROM COVID-19

At this time, data are limited on the implications for children born to men who were infected with COVID-19. Current evidence from the Society for Maternal-Fetal Medicine suggests minimal risk of vertical transmission from mother to newborn, but there is concern for respiratory transmission to a new fetus (75). In the few cases of vertical transmission, discussion has occurred regarding the nature of transmission, i.e., transplacentally or transcervically (76). Longitudinal studies are needed to further assess the long-term impacts of children born to parents with histories of COVID-19 infection.

ADDITIONAL GUIDANCE FOR DELIVERY OF FERTILITY CARE

Many reproductive care centers have resumed fertility care delivery after both risk assessment and mitigation, consideration for resource availability, and careful counseling. Beginning in March 2020, the American Society of Reproductive Medicine has released monthly updates on patient management and clinical recommendations during the COVID-19 pandemic (77), and the Society for Assisted Reproductive Technology, Society for Reproductive Biologists and Technologists, and College of Reproductive Biology have issued a joint statement on laboratory guidance during COVID-19 pandemic (78). We recommend referring to these guidelines and guidance from the Centers for Disease Control (CDC), about the delivery of fertility care during the COVID-19 pandemic. Furthermore, owing to restrictions during the pandemic, many clinicians treating patients with infertility adopted telemedicine to continue to provide care to these patients. This has been supported by the Society for Male Reproductive Medicine and Urology (79). These guidelines do, however, reinforce the importance of physical examination for patient assessment and the use of adjunctive tests when required.

One of the most significant considerations regarding SARS-CoV-2 and male reproductive health is collection, handling, and preservation of semen samples. There is currently limited evidence suggesting that SARS-CoV-2 can be isolated from the semen of a recovered male or asymptomatic male with SARS-CoV-2 infection. Efforts should be made to screen patients for possible signs and symptoms of COVID-19 or recent exposures to someone diagnosed with COVID-19 before on-site semen sample collection. In previously symptomatic patients who recovered from COVID-19, the CDC

recommends discontinuation of isolation after at least 10 days have passed since symptom onset, at least 24 hours have passed since the resolution of fever, and other COVID-19 symptoms are improved (80). In patients infected with SARS-CoV-2 who did not demonstrate any COVID-19 symptoms, isolation may be discontinued 10 days after their first positive RT-PCR test. Every effort should be made to protect the health of all patients, staff members, and health care providers by strict adherence to infection prevention and control measures such as hand hygiene, social distancing, environmental infection control, and appropriate use of personal protective equipment. Semen collection laboratories may consider off-site semen collection if feasible, including at-home collection following standard protocols.

Furthermore, appropriate risk assessment and mitigation strategies should be used by the reproductive care center to handle semen samples from patients. Currently, the CDC recommends using eye protection or face shield, medical-grade gloves, and medical-grade mask in the handling of all body fluids, including semen, by staff members (81). Although the primary potential risk to staff would be through semen samples splashing, every effort should be made to prevent aerosol formation during the procedure, such as during pipetting, centrifugation, and mixing. If possible, a physically separate space and dedicated instruments and equipment should be used for semen samples from patients recovered from COVID-19. Another consideration includes the cryopreservation of semen or testicular samples from recovered COVID-19 patients and use for assisted reproductive technologies (ART). Currently, there is no indication for routine testing of semen samples for SARS-CoV-2 by means of RT-PCR before cryopreservation or ART. There is no evidence for any specific microbicide or processing protocol to protect against possible viral transmission from a recovered patient. However, published protocols for handling semen samples and performing ART from individuals infected with HIV or hepatitis B or C virus may be used as an additional precaution, but this practice may vary between laboratories (82). Although viral cross-contamination between different preserved samples has never been reported, use of high-security straws and segregated cryovessels should be considered, if possible, for semen samples or testicular tissue from recovered COVID-19 patients.

CONCLUSION

Many questions remain unanswered regarding the short- and long-term impacts of SARS-CoV-2 on male reproductive health. Based on the current evidence, the likelihood of SARS-CoV-2 transmission through the seminal fluid is very low. Although there have been many reports regarding viral tropism for the male reproductive tract, an important consideration for viral entry is coexpression of both ACE2 and TMPRSS2 at sufficient levels. There are very limited data to characterize the impact of SARS-CoV-2 infection on male reproductive hormones and semen parameters. Larger-scale community-based evaluation for SARS-CoV-2 in semen samples and longitudinally collected hormone profiles and semen analyses from recovered men are needed before we can better

understand the impact of SARS-CoV-2 on male reproduction. As many reproductive care centers resume fertility care delivery globally, men and their partners must be appropriately counseled regarding what is known and remains unknown about SARS-CoV-2 and male reproductive health.

Acknowledgments: The authors thank Brenna Kelly for graphic design assistance. NP is supported by the Frederick J. and Theresa Dow Wallace Fund of the New York Community Trust.

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