Title: Impact of the SARS-CoV-2 Virus on Male Reproductive Health

Authors: Daniel E. Nassau, Jordan C. Best, Eliyahu Kresch, Daniel C Gonzalez, Kajal Khodamoradi and Ranjith Ramasamy*

Department of Urology, University of Miami

*Corresponding Author:

Ranjith Ramasamy, MD

1150 NW 14th Street, Ste 309

Miami, FL 33136

ramasamy@miami.edu

DR. DANIEL ELLIOT NASSAU (Orcid ID : 0000-0002-6884-4299)

Article type   : Review

Abstract

The Coronavirus 2019 (COVID-19) pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to more than 160 million infections and 3.5 million deaths globally. Men are disproportionately affected by COVID-19, having more severe disease with higher mortality rates than women. Androgens have been implicated as the underlying cause for more severe disease, as the androgen receptor has been noted to upregulate the cell surface receptors that mediate viral cell entry and infection. Unfortunately, despite testosterone's potential role in COVID-19 prognosis, androgen deprivation therapy is neither protective nor a treatment for COVID-19. Interestingly, the male reproductive organs have been found to be vulnerable in moderate to severe illness, leading to reports of erectile dysfunction and orchitis. COVID-19 viral particles have been identified in penile and testis tissue, both in live patients who recovered from COVID-19 and post-mortem men who succumbed to the disease. Although sexual transmission remains unlikely in recovered men, moderate to severe COVID-19 infection can lead to germ cell and Leydig cell depletion, leading to decreased spermatogenesis and male hypogonadism. The objective of this review is to describe the impact of SARS-CoV-2 on male reproductive health. There are still many unanswered questions as to the specific underlying mechanisms by which COVID-19 impacts male reproductive organs and the long-term sequalae of SARS-CoV-2 on male reproductive health.

Introduction

Coronavirus 2019 (COVID-19) is a novel viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported in Wuhan China in December 2019. Since then, COVID-19 has spread globally and has infected more than 166 million people and caused more than 3.5 million deaths around the world (1,2). Although there is no difference

in the percentage of men and women diagnosed with COVID-19, large-scale, multi-country statistical analyses have shown that men suffer from higher disease severity and mortality rate compared with women (3,4). While the underlying reason for worse prognosis of COVID-19 in men has not been fully elucidated, it does appear that male gender is a risk factor for severe infection worldwide.

Researchers have been attempting to discover the underlying mechanisms causing the disproportionate impact of COVID-19 on men. The underlying mechanisms for viral entry of SARS-CoV-2 could be influenced by androgens, which are well known to have higher serum concentrations in men (5). The potential role of androgens in COVID-19 severity has been investigated, and medical castration has even been explored as a potential treatment(6,7). The male reproductive organs have also been described as being vulnerable to infection believed to be due to direct viral entry. Erectile dysfunction has been reported after COVID-19 recovery and viral particles have even been documented in penile tissue up to 7 months after infection (8). Epididymoorchitis has been reported in almost a quarter of infected men, raising concerns about SARS-CoV-2's ability to enter the testis, break down the testis-blood barrier and effect spermatogenesis, and potentially be spread through sexual contact (9,10). In this review, we will briefly describe the underlying mechanisms for SARS-CoV-2's entry into human cells and describe how this may lead to worse infection in men, review the role of androgens in COVID-19 infection and implications for treatment, and discuss the virus' impact on male reproductive organs in order to comprehensively highlight the current evidence of SARS-CoV-2's impact on male reproductive health.

Viral Mechanism of Entry

SARS-CoV-2 is a single-stranded RNA virus around ~30kb in size, primarily spread through air by an infected person's cough or sneeze and less commonly by touching surfaces containing live virus droplets (11). Similar to other coronaviruses, SARS-CoV-2 contains four main structural proteins, including envelope (E), membrane (M), nucleocapsid (N) and spike (S) (12,13) The E protein has a crucial role in the permeability of the host cells' membrane (11). The M protein is the most abundant molecule on the virus surface and is the main organizer of the virion assembly

(14). The N protein forms a complex with the viral genome and enhances the efficiency of SARS-CoV-2 transcription and assembly in human cells (15). Finally, the S protein, located on the surface of SARS-CoV-2, is responsible for viral attachment and fusion between the virus and the host cell, leading to infection (13).

The S protein includes two subunits: S1 mediates the attachment process; S2 is involved in membrane fusion. The receptor-binding domain (RBD) on S1 binds to angiotensin-converting enzyme 2 (ACE2). ACE2 is a cell entry receptor and is found in a variety of human tissue, in particular lung cells, but also male organs including the testis, notably in the Leydig and Sertoli Cells (16–20). After binding to ACE2 through RBD, the virus uses the host's cellular serine protease type II transmembrane serine protease (TMPRSS2) and is proteolytically activated (21). TMPRSS2 is expressed in many cell types, including lung, intestine, colon, salivary gland, stomach, and prostate (5,22). Furthermore, expression of TMPRSS2 is reported in all cell clusters within the testis, such as spermatogonia and spermatids (23). Under the influence of a disintegrin and metalloproteinase 17 (ADAM17), ACE2 can become soluble and is believed to be protective against severe disease (Figure 1); however, expression of ADAM17 is not well understood (24). The presence of target proteins for the SARS-CoV-2 may help explain the unique consequences of COVID-19 infection in males.

COVID-19 Infection differences in Men and Women

While the susceptibility to SARS-CoV-2 infection is similar between men and women, several studies have shown that the severity of the disease and the rate of death is higher in men (3,4). Importantly, no difference has been reported in the number of infected patients with COVID-19 between the men and women, suggesting that either differences in lifestyle or an underlying physiologic reason could account for the increased disease severity in men (16–20,25,26). Unhealthy lifestyles, which are known to be prominent in men, such as smoking, physical inactivity and obesity were associated with hospitalization for COVID-19 in a large population based study (27). On the contrary, there is growing evidence that ACE2 and TMPRSS2 expression mediates the sex disparity during the mechanism of viral entry (5). In women, estrogen has been shown to not only regulate the expression of ACE2 in differentiated airway epithelial cells, but also increase the immune response (28,29). Expression and activity of

TMPRSS2 are higher in alveolar type 1 and 2 epithelial cells of men in contrast to women with higher severity of COVID-19 (5). TMPRSS2 is expressed in various male reproductive parts, such as the testes and the genital tract, as well as in the prostate epithelial cells which implies its presence the seminal fluid (22,23,30). Since the effect of SARS-CoV-2 infection is increased by TMPRSS2 proteolysis, there is likely a positive association between TMPRSS2 expression and disease severity. Furthermore, androgen receptors (ARs) modulate the expression of TMPRSS2, and due to significantly higher expression of ARs in men compared to women, this likely causes men's increased risk for more severe COVID-19 (4).

Androgens and SARS-CoV-2

The relatively high incidence of complications from severe COVID-19 in males led investigators to study the relationship between androgens and infection early on in the pandemic. While there have been competing theories about whether testosterone improves or worsens the severity of COVID-19, it is likely that testosterone is a key factor in infection and progression of the illness. (25,31–34). Testosterone is a co-regulator of expression of both ACE2 and TMPRSS2, which likely aides in the internalization of SARS-CoV-2. Interestingly, in contrast, both obesity and advanced age, both of which commonly result in low levels of serum testosterone, have also been associated with the development of complications from COVID-19 (6,33).

Testosterone's influence on severity of COVID-19 may be illuminated by an association between milder cases of infection and androgen deprivation therapy (ADT). ADT, the mainstay treatment for metastatic prostate cancer, lowers serum testosterone to castrate levels. Two studies have evaluated COVID-19 in patients receiving ADT: one found no relationship (35), whereas the other found ADT to be partially protective (36). While both of these conflicting studies included large cohorts (>4000), Klein et al. evaluated an even larger number of patients on ADT and found no association between ADT and COVID-19 severity.

ADT was also investigated as a treatment for COVID-19 to limit the severity of symptoms (37). Although the study was limited to a small sample size, there were significantly lower rates of hospitalization, infection and supplemental oxygen utilization in the ADT group compared to those not on ADT. There were also trends towards decreased intubation and increased overall

survival. It still is not clear if medical castration could be used to treat severe COVID-19. A sub-analysis of Montopoli's data calculated the number needed to treat with ADT to prevent one SARS-CoV-2 infection was 434, and considering the adverse effects associated with ADT, O'Callaghan and colleagues concluded it would not be a good treatment or prevention option. Interestingly, even though ADT was essentially found to be neither detrimental nor protective in COVID-19, lower testosterone levels are also associated with severe COVID-19. In an elegant study comparing hormonal levels between hospitalized patients with COVID-19, patients with non-COVID respiratory disease, and age-matched controls, Kadihasanoglu and colleagues found significantly lower testosterone levels in COVID-19 patients compared to the other groups (38). Almost 75% of COVID-19 patients met the criteria for testosterone deficiency and lower testosterone levels were correlated with an increased hospitalization time. Similarly, testosterone levels were significantly decreased in severe disease compared to those with milder cases of COVID-19 (39). Low testosterone levels were also significantly associated with higher levels of inflammatory markers, including interleukin-6, C-reactive protein, interleukin 1 receptor antagonist, hepatocyte growth factor, and interferon γ-inducible protein 10. Testosterone can modulate immune response, so higher inflammatory markers could partly explain why severe disease has been associated with lower testosterone levels (7).

Unfortunately, as pre-infection testosterone levels are not available in the previously mentioned studies, it remains unclear if lower testosterone levels are a risk factor for more severe disease or if severe SARS-CoV-2 infection leads to hypogonadism. Interestingly, in Kadihasanoglu's study, serum luteinizing hormone levels were elevated, suggesting a primary hypogonadism and testicular failure, instead of a secondary cause more likely to be influenced by a severe inflammatory state (38). Testicular compromise could be the underlying etiology of hypogonadism in severe COVID-19. Leydig cells, which produce testosterone, have high expressions of ACE2, although co-expression of TMPRSS2 is unknown (40). It is possible that direct SARS-CoV-2 invasion with inactivation or destruction of Leydig cells leads to testicular hypofunction in severe disease. In fact, decreased Leydig cell populations have been described in post-mortem pathological evaluation of the testis in men who died from COVID-19 (41). Taken together, the association of lower testosterone levels in severe COVID-19 appears to be due to the sequala of SARS-CoV-2 infection, and not because men were hypogonadal before infection.

Given this information, some experts have even postulated about the potential use of testosterone replacement therapy (TRT) in severe infection (6). Unfortunately, given the high rates of venous thromboembolic events (VTE) in severe SARS-CoV-2 infection, concerns have been raised that TRT could accentuate VTE. Fortunately, when investigating COVID-19 patients already on TRT compared to those who were not, Rambhatla et al. did not find an increased risk of VTE (42). Furthermore, there was no relationship found between men taking TRT and more severe COVID-19. Taken together, although testosterone can modulate ACE2 and TMPRSS2 expression, there is currently not enough clinical evidence to suggest a direct relationship between T levels and COVID-19 outcomes.

Despite the well described role of androgens in SARS-CoV-2 cell entry, the effects of baseline testosterone levels on disease severity and any therapeutic roles remain unknown. Since the onset of the pandemic there has been conflicting data about whether castrate, low or high testosterone levels are protective or detrimental in COVID-19, which has led to investigations into potential treatment using both ADT and TRT. Unfortunately, use of both TRT and ADT is limited by adverse effects, especially in those with severe COVID-19. Nevertheless, larger data sets and long-term effects of SARS-CoV-2 are needed to elucidate the true relationship between androgens and COVID-19 severity.

COVID-19's Impact on Male Reproductive Organs Tract

The presence of ACE2 and TMPRSS2 throughout the male genitourinary tract make the male reproductive organs vulnerable to damage from SARS-CoV-2 infection. Furthermore, the influence of testosterone on these organs and the presence of the androgen receptor which promotes the TMPRSS2 gene would further explain viral uptake (23,43). One of the hallmarks of COVID-19 infection has been the variability in symptom severity and immune response (32). The current theory is that hematogenous viral spread, in men with more severe disease, is responsible for direct effects of the virus on the male reproductive organs (22).

Penis and Erectile Dysfunction

Endothelial dysfunction is a cornerstone of severe COVID-19 infection and is likely the underlying pathophysiology behind the finding of erectile dysfunction (ED) after recovery. ACE2 and TMPRSS2 are ubiquitous in endothelial cells, the target for SARS-CoV-2 uptake. TMPRSS2 cleaves the S protein into S1 and S2 domains, which produces an active fusion fragment. TMPRSS2 also promotes the cleavage of ACE2, creating an increase in viral uptake via the endosomal cysteine proteases cathepsin B and L pathway. In this process, the endosome releases the virus to the cytoplasm of the cells for replication and cell damage (44,45). This can create a vasculopathy which leads to microvascular damage, a hallmark of vasculogenic erectile dysfunction (46).

Male sexual dysfunction in the form of ED was explored early in the pandemic. Survey studies out of China and Italy evaluated how COVID-19 impacted sexual health and found that the prevalence of ED was higher among previously infected men (47,48). Aside from endothelial dysfunction, other underlying causes associated with illness recovery could impact erectile function, including subclinical hypogonadism, psychological distress, and impaired pulmonary hemodynamics (49). While ED pathophysiology is often multi-factorial, a recent report of SARS-CoV-2 within the corpora cavernosa (Image 1) of two previously infected men strongly suggests that direct penile damage, likely from endothelial dysfunction, plays a major role in ED after severe COVID-19 (8). The presence of the virus within the penile tissue up to 7 months after infection illustrates how SARS-CoV-2 can directly damage cavernosal endothelium, leading to male sexual dysfunction. Although the majority of these studies are based on small sample sizes, it will be interesting to learn if COVID-19 severity is indeed a risk factor for erectile dysfunction as longer-term survivor data becomes available.

Prostate and Seminal Vesicles

Despite expression of both ACE2 and TMPRSS2, the prostate and seminal vesicles are the rare male reproductive organs that have not been commonly described to have been impacted in COVID-19. This is possibly due to very low ACE2 expression, as shown from bioinformatic analysis from the Human Protein Atlas database (50). Conversely, TMPRSS2 expression is noted to be at low to medium levels in the prostate. Colocalization was identified by scRNA-seq in <1% of prostate epithelial cells, while 0.3% expressed ACE2 and 18.6% expressed TMPRSS2

(51). Not surprisingly, three studies investigated if SARS-CoV-2 was present in prostatic secretions, and across these studies, all 89 samples were negative for the virus (52–54).

Testis and Epididymis

Unlike the prostate, despite low co-expression of ACE2 and TMPRSS2, the testis has been vulnerable SARS-CoV-2, as there are reports of both orchitis and decreased sperm production during infection. Interestingly, scRNA-seq data suggests that co-expression of both ACE2 and TMPRSS2 occurs less than 0.05% of the time in testicular tissue (55–57). ACE2 expression was found mostly in Leydig and Sertoli cells, while TMPRSS2 was concentrated in spermatogonia and spermatids (23). Since current models suggest that both receptors are needed for SARS-CoV-2 entry, there may be another mechanism by which the virus infects testicular tissue.

It has been estimated that 10-22% of men with acute COVID-19 infection develop orchitis or epididymo-orchitis, likely due to direct testicular infection (55,58–60). In two studies evaluating the presence of SARS-CoV-2 in seminal fluid, 18% of men reported scrotal discomfort (55,58). Similarly, when evaluating for inflammation such as tunica albuginea thickening, enhanced echogenicity of the testis or epididymis via scrotal ultrasound, 22% of men with acute COVID-19 were found to have acute orchitis with or without epididymitis (59). It is important to recognize that these small studies were conducted primarily in hospitalized patients; therefore, the high rates of testicular pain and orchitis is likely more common in moderate-severe COVID-19 compared to asymptomatic or mild disease.

Testicular inflammation from COVID-19 could be due to direct viral invasion, even though SARS-CoV-2 receptors are infrequently expressed. In post-mortem testicular biopsies, multiple studies using transmission electron microscopy and RT-PCR found virus in 17% of samples, similar to the clinical rate of epididymo-orchitis (41,61,62). Although RT-PCR was positive for SARS-CoV-2 in the testis, it is possible that the samples, which were predominantly composed of fibrovascular tissue, could have contained a contaminant from blood instead of testicular tissue (41). Interestingly, Ma and colleagues also analyzed transcriptome changes within the testes with SARS-CoV-2, demonstrating dysfunction of the genes regulating spermatogenesis and inflammation-related changes (62). Additionally, four of five samples demonstrated

extensive germ cell loss, histologically similar to patients with Sertoli cell-only syndrome, suggesting that SARS-CoV-2 can not only damage the testis-blood barrier, but also negatively impact male fertility.

Potential for Sexual Transmission and Effect on Male Fertility

The presence of SARS-CoV-2 within the testis, potential for breakdown of the testis-blood barrier, and depletion of germ cells has raised the question of whether COVID-19 could be spread sexually and what the effects could be on male reproduction. Early in the pandemic, a study by Li et al. identified SARS-CoV-2 viral particles in 6 out of 38 men who submitted a semen sample (63). This finding raised the question about whether the virus had the potential to be present in high enough concentrations to be transmitted through sexual contact. A recent systematic review evaluated the available data on SARS-CoV-2 presence in semen, using 19 articles that met the inclusion criteria. All studies investigated for the presence of viral presence in the semen; however, Li and colleagues were the only group to detect seminal COVID-19 (10). This is likely because Li et al. was the only study that evaluated viral presence during acute infection, notably among many severely infected men. Given the low rates of SARS-CoV-2 within seminal fluid, andrology technicians are not likely to be at risk to contract COVID-19 while performing semenanalysis; however, universal precuations should still be used. Similarly, although there has not been any reported evidence of strictly sexual transmission, it is unlikely, given that seminal presence of SARS-CoV-2 is only during severe, acute infection and likely resolves with resolution of the illness. Current data suggests that the risk of sexual transmission from men who have recovered from infection is negligible.

While SARS-CoV-2 concentrations within the testis are unlikely to be high enough for sexual transmission, the virus is able to impact male fertility through germ cell depletion (62). An early COVID-19 study evaluated semen parameters of 2 acutely ill and 18 recovered men. Men with moderate infection had a statistically significant impairment in semen parameters compared to those with mild infection or within the control group, even though the values were within the normal WHO ranges (58). A prospective cross-sectional analysis of 43 men recovered from SARS-CoV-2 infection found 11 men to have semen impairment; of these, 8 had azoospermia and 3 oligospermia (sperm concentration <15million/ml) (64). A more recent study evaluated 30

men who recovered from SARS-CoV-2 infection and included a follow-up semen analysis from 5 men (9). Median total sperm number in the ejaculate was 12.5 million which was significantly lower than an age-matched control group of healthy, non-SARS-CoV-2 infected men (Table 1). At a median of 3 months, the 5 men with follow-up semen analyses had an increase of median total sperm number to 18 million. Given these findings, it appears that COVID-19 infection can negatively impact spermatogenesis, at least temporarily. Although the long-term effects of SARS-CoV-2 infection on semen quality are not yet known, after infection and normalization of semen parameters, which may take up to 3 months for spermatogenesis recovery, the sperm is likely safe enough for cryopreservation and/or use for assisted reproductive techniques such as in-vitro fertilization. Since long-term sperm quality is not yet known in men who have recovered from COVID-19, those who wish to conceive should consider undergoing a fertility evaluation to assess sperm quality.

Conclusion

There are still many questions as to why the COVID-19 pandemic has disproportionately affected men, leading to more severe disease and worse mortality rates than women. In addition to men generally having more comorbid conditions than women, higher androgen levels have been identified as a potential mechanism for viral cell entry. The ability for the AR to upregulate ACE2 and TMPRSS2 gene could be an underlying reason for worse disease severity; however, the data is mixed on whether clinical differences exist amongst men on TRT and ADT. Furthermore, despite extremely low levels detected of both ACE2 and TMPRSS2 in reproductive organs such as the testis, there have been higher than expected rates of orchitis and spermatogenesis impairment amongst men with moderate to severe COVID-19, suggesting that there may be another mechanism by which the virus impacts male reproductive health. As the long-term effects of infection in males are not yet available, it is important for men who have overcome COVID-19 with concerns about potential consequences of the disease to seek medical care. Future research is needed to elucidate the sequalae of infection, develop methods to decrease SARS-CoV-2's impact on male reproductive health, and address the long-term consequences of severe disease.

Disclosures of interest

Ranjith Ramasamy

Acerus Pharmaceuticals	Consultant, Grant Recipient
Boston Scientific	Consultant, Grant Recipient
Coloplast	Consultant, Grant Recipient
Endo Pharmaceuticals	Consultant, Grant Recipient
Empower Pharmacy	Grant Recipient
Nestle Health	Consultant
Olympus	Grant Recipient

No other interests to disclose.

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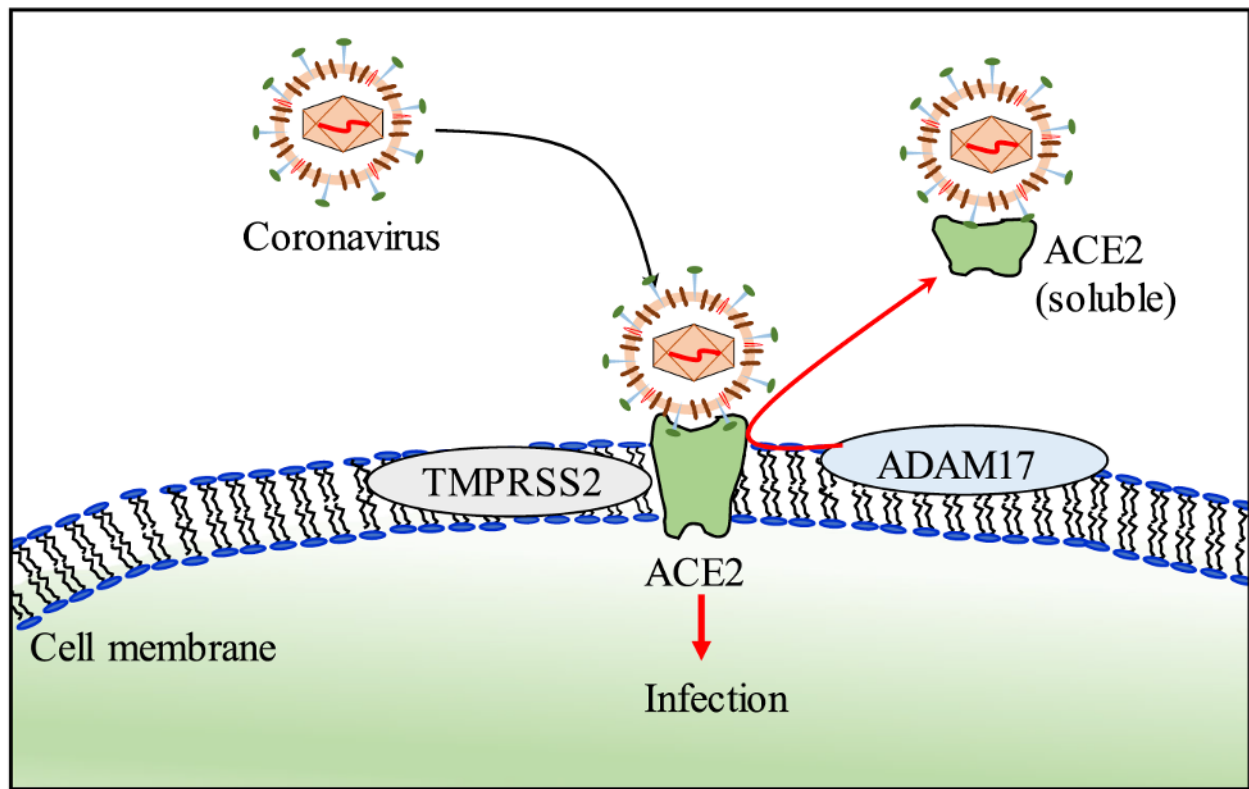
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Figure 1. Entry of SARS-CoV-2 into the cell. The spike protein binds to ACE2 and is then cleaved by TMPRSS2, leading to cell membrane fusion and viral entry. ADAM17 can induce a soluble form of ACE2, which may be protective against severe infection (24).

Image 1. Transmission electron microscopy image of SARS-CoV-2 within cavernosal tissue of a previously severe COVID-19 infected patient undergoing penile prosthesis surgery 7 months after infection. Viral particle (red circle) exhibiting prominent spikes and nucleocapsid (electron dens material).

Table 1. Comparison of semen parameters in men with COVID-19 infection and age-matched controls. Concentration and total sperm number were significantly lower in COVID(+) men. Values are presented as median (IQR). COVID: Coronavirus disease, IQR: interquartile range p<0.05 was considered significant (9).

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Variable	COVID (+) cohort (n=30)	COVID (-) cohort (n=30)	p-value
Age (y)	40 (IQR=24.75)	42 (IQR=9.8)	0.8732
Volume (mL)	2.1 (IQR=1.23)	2.2 (IQR=2.15)	0.3841
pH	7.2 (IQR=0.8)	7.2 (IQR=0.4)	0.2304
Concentration (million/mL)	11.5 (IQR=26.8)	21.5 (IQR=21.5)	**0.0048**
Total Sperm Number (million)	12.5 (IQR=52.1)	59.2 (IQR=70.5)	**0.0024**