Pathological and molecular examination findings in postmortem testis biopsies reveal spermatogenesis damages in COVID-19 patients
10.21203/rs.3.rs-56526/v1
https://www.researchsquare.com/article/rs-56526/v1
Despite widespread interest in the pathophysiology of COVID-19 in respiratory and cardiovascular systems, little is known about the morphologic and molecular changes in the testis of patients with COVID-19 and the effects of SARS-CoV-2 infection on male fertility. We report here on the pathophysiology and molecular feature of testes obtained at autopsy from six men with COVID-19, as compared with those of testes from three men with age-matched, uninfected SARS-CoV-2. Our histopathological results showed that all COVID-19 patients had severe spermatogenesis damages compared with controls. Importantly, we detected the nuclear acid of the SARS-CoV-2 virus, viral particles, and SARS-CoV-2 spike S1 protein in COVID-19 patient testes, and we also found ACE2 and TMPRSS2 significantly elevated in the testes from COVID-19 patients. Furthermore, we observed a prominent leukocyte infiltration, including CD3+ T lymphocytes, CD20+ B lymphocytes, CD68+ macrophages, HLA-DR+ myeloid cells, and CD38+ plasma cells in the testes of COVID-19 patients. RNA-Seq analyses further revealed SARS-CoV-2 infection could lead to dysfunction of the genes that regulate the spermatogenesis and inflammation response-related pathways. Collectively, our pathological and molecular examination findings indicate that SARS-CoV-2 could directly attack testicular cells, thereby inducing the damage of testicular immune privilege and spermatogenesis defects.

Haghpanah A, Masjedi F, Alborzi S, et al. **Potential mechanisms of SARS-CoV-2 action on male gonadal function and fertility: Current status and future prospects** [published online ahead of print, 2020 Oct 27]. Andrologia. 2020;e13883. doi:10.1111/and.13883
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7645932/
The novel coronavirus was recognized in December 2019 and caught humanity off guard. The virus employs the angiotensin-converting enzyme 2 (ACE2) receptor for entry into human cells. ACE2 is expressed on different organs, which is raising concern as to whether these organs can be infected by the virus or not. The testis appears to be an organ enriched with levels of ACE2, while the possible mechanisms of involvement of the male reproductive system by SARS-CoV-2 are not fully elucidated. The major focus of the present studies is on the short-term complications of the coronavirus and gains importance on studying the long-term effects, including the possible effects of the virus on the male reproductive system. The aim of this review was to provide new insights into different possible mechanisms of involvement of male gonads with SARS-CoV-2 including investigating the ACE2 axis in testis, hormonal alterations in patients with COVID-19, possible formation of anti-sperm antibodies (ASA) and subsequently immunological infertility as a complication of SARS-CoV-2 infection. Finally, we suggest measuring the sperm DNA fragmentation index (DFI) as a determiner of male fertility impairment in patients with COVID-19 along with other options such as sex-related hormones and semen analysis. Invasion of SARS-CoV-2 to the spermatogonia, Leydig cells and Sertoli cells can lead to sex hormonal alteration and impaired gonadal function. Once infected, changes in ACE2 signalling pathways followed by oxidative stress and inflammation could cause spermatogenesis failure, abnormal sperm motility, DNA fragmentation and male infertility.

Anifandis G, Messini CI, Daponte A, Messinis IE. **COVID-19 and fertility: a virtual reality**. Reprod Biomed Online. 2020;41(2):157-159. doi:10.1016/j.rbmo.2020.05.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7206439/
The COVID-19 pandemic is an extraordinary global situation, and all countries have adopted their own strategies to diminish and eliminate the spread of the virus. All measures are in line with the recommendations provided by the World Health Organization. Scientific societies, such as the European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine, have

provided recommendations and guidance to overcome and flatten the growing curve of infection in patients who undergo IVF treatments. Although there is as yet no evidence that the virus causing COVID-19 might have negative effects on IVF outcomes, fertility treatments have been postponed in order to support healthcare systems by avoiding placing them under additional stress. The possibility of the virus affecting sperm function and egg performance cannot be excluded. In addition, an indirect effect of the virus on gametes and embryos during their manipulation cannot be ruled out. This commentary aims to provide some ideas on the possible effect of the virus on gametes and embryos, as well as how it could affect the normal functioning of the embryology laboratory.

Corona G, Baldi E, Isidori AM, et al. **SARS-CoV-2 infection, male fertility and sperm cryopreservation: a position statement of the Italian Society of Andrology and Sexual Medicine (SIAMS)** (Società Italiana di Andrologia e Medicina della Sessualità). J Endocrinol Invest. 2020;43(8):1153-1157. doi:10.1007/s40618-020-01290-w
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7252417/
Purpose
The recent pandemic of severe acute respiratory syndrome (SARS) due to coronavirus (CoV) 2 (SARS-CoV-2) has raised several concerns in reproductive medicine. The aim of this review is to summarize available evidence providing an official position statement of the Italian Society of Andrology and Sexual Medicine (SIAMS)
Methods
A comprehensive Pubmed, Web of Science, Embase, Medline and Cochrane library search was performed. Due to the limited evidence and the lack of studies, it was not possible to formulate recommendations according to the Oxford 2011 Levels of Evidence criteria.
Results
Several molecular characteristics of the SARS-CoV-2 can justify the presence of virus within the testis and possible alterations of spermatogenesis and endocrine function. Orchitis has been reported as a possible complication of SARS-CoV infection, but similar findings have not been reported for SARS-CoV-2. Alternatively, the orchitis could be the result of a vasculitis as COVID-19 has been associated with abnormalities in coagulation and the segmental vascularization of the testis could account for an orchitis-like syndrome. Finally, available data do not support the presence of SARS-CoV-2 in plasma seminal fluid of infected subjects.
Conclusion
Data derived from other SARS-CoV infections suggest that in patients recovered from COVID-19, especially for those in reproductive age, andrological consultation and evaluation of gonadal function including semen analysis should be suggested. Studies in larger cohorts of currently infected subjects are warranted to confirm (or exclude) the presence of risks for male gametes that are destined either for cryopreservation in liquid nitrogen or for assisted reproduction techniques.

Hsu AL, Finlinson A, Warncke K. **Mechanisms by Which SARS-CoV-2 May Impact Male Fertility** [published online ahead of print, 2020 Oct 6]. Reprod Sci. 2020;1-2. doi:10.1007/s43032-020-00304-5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7537772/
The COVID-19 pandemic is unlike anything we have experienced in over a century. In the USA, waves of COVID-19 have migrated from the Northeast to the Sun Belt to the Midwest over the past year. Compared with females, males are more susceptible to SARS-CoV-2 infection, have more severe COVID-19 disease, and have higher death rates. In many countries, men are consistently more likely to die by a factor of almost 2. This article describes some of the mechanisms by which COVID-19 may be associated with male infertility, as discussed by Dutta and Sengupta.

Cellular entry of SARS-CoV-2 is mediated by its spike glycoproteins (S proteins), which need priming by cellular proteases to facilitate viral fusion to cellular membranes. TMPRSS2 (transmembrane protease serine 2) is utilized for S protein priming and is found in prostate epithelial cells [3, 4]; androgen receptor activation is needed to trigger TMPRSS2 expression [1]. In addition, brain cells (neurons and glial cells) also express ACE2 receptors, raising the question of whether viral damage by SARS-CoV-2 may also disrupt the hypothalamic-pituitary-testicular axis and thereby disrupt normal male pubertal development and/or contribute to male infertility [2].

There is some evidence correlating high ACE2 expression with infertility, suggesting that "an overactivation of ACE2 might affect spermatogenesis." [2] Of note, the highest number of ACE2-positive cells was found in a 30-year-old man (compared with a 20-year-old and 60-year-old man) [1]. As the COVID-19 pandemic evolves to strike younger populations of reproductive age globally, it remains an outstanding question whether there will be a rise in male factor infertility (or overall infertility) in general, as a result of this pandemic.

Dutta and Sengupta discuss oxidative stress, inflammation, and the immunologic response to a high testicular viral load in the testes, as parts of a cascade that could lead to COVID-19-related male factor infertility [1]. Evidence of localized testicular damage also suggests the potential for adverse reproductive consequences at the anatomical, cellular, and molecular levels. With evidence that worldwide sperm counts have already declined 50–60% among men in North America, Europe, Australia, and New Zealand between 1973 and 2011 [5], the potential impact of COVID-19 on sperm and sperm function is an area that requires urgent further study.

Dutta S, Sengupta P. **SARS-CoV-2 and Male Infertility: Possible Multifaceted Pathology** [published online ahead of print, 2020 Jul 10]. Reprod Sci. 2020;1-4. doi:10.1007/s43032-020-00261-z
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7351544/

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19) has been declared a pandemic by the World Health Organization (WHO) on 11th March 2020. Bulk of research on this virus are carried out to unveil its multivariate pathology. Surprisingly, men are reportedly more vulnerable to COVID-19 even with higher fatality rate compared to women. Thus, it is crucial to determine whether SARS-CoV-2 infection can even affect male fertility as an immediate or long-term consequence of the disease. Among the discrete data available, an important finding is that angiotensin converting enzymes 2 (ACE2) receptor, that aids the SARS-CoV-2 entry into host cells, is profoundly expressed in testicular cells. In addition, the endogenous androgen milieu and its receptors are associated with ACE2 activation reflecting that enhanced testosterone levels may trigger the pathogenesis of COVID-19. In contrary, hypogonadism has also been reported in the acute phase of some COVID-19 cases. Moreover, SARS-CoV-2 infection-induced uncontrolled inflammatory responses may lead to systemic oxidative stress (OS), whose severe disruptive effects on testicular functions are well-documented. This article aims to precisely present the possible impact of COVID-19 on male reproductive functions, and to highlight the speculations that need in-depth research for the exact underlying mechanisms how COVID-19 is associated with men's health and fertility.

SAR-CoV2 can operate via multiple possible mechanisms which may lead to disruption of male reproductive functions. It is suggested that this virus activates oxidant-sensitive pathways via inflammatory responses, thereby inducing oxidative stress (OS), which presents a common pathological mechanism to disrupt several physiological functions via oxidative damage to host tissues. OS-mediated mechanisms of male infertility are widely documented, as OS can affect semen quality and disrupt sperm functions and morphology, intracellular oxidative damage to spermatozoa by lipid peroxidation of sperm membrane, sperm DNA damage, as well as inducing apoptotic pathways in spermatozoa [18, 19]. In SARS-CoV infections, the excessive production of reactive oxygen species (ROS) may trigger mainly the nuclear factor kappa-light-

chain-enhancer of activated B cells (NF-κB)-toll-like receptor (mainlyTLR-4) pathways [20]. This further stimulate release of cytokines causing exaggeration of the inflammatory responses [20]. As already discussed, this virus can potentially cause orchitis which also can lead to induction of OS. Moreover, SARS-CoV-2 infection causes psychological stress which is a major cause of systemic OS [21]. Besides the direct relation of SARS-CoV-2 infection and OS, treatment of COVID-19 include antiviral drugs like ribavirin which has been shown to be associated with induction of OS, reduced testosterone level, impaired spermatogenesis, and sperm abnormalities in animal studies [22, 23]. Moreover, ribavirin treatment showed reduced sperm count [24] and sperm DNA fragmentation (SDF) [25] up to 8 months following cessation of treatment [21, 26].

Khalili MA, Leisegang K, Majzoub A, et al**. Male Fertility and the COVID-19 Pandemic: Systematic Review of the Literature**. World J Mens Health. 2020;38(4):506-520. doi:10.5534/wjmh.200134
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7502312/

Purpose

Since its discovery in December 2019, the novel coronavirus SARS-CoV-2 has spread globally, causing the current COVID-19 (coronavirus disease-19) pandemic. As there is an increase of infections in the male population, concerns have emerged about the potential impact of COVID-19 on male reproductive organs and male fertility. Therefore, this study systematically investigates the current evidence of SARS-CoV-2 impact on male reproduction and pregnancy outcomes, discussing them in light of the evidence published on other coronaviruses.

Materials and Methods

Literature search was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A total of 24 original articles were included for the analysis, investigating the effects of the infection on semen parameters, male reproductive hormones, and pregnancy outcomes. Further, a Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis was conducted based on the available evidence linking the virus with male reproduction and conception.

Results

Although there is limited data, viral mRNA has been identified in semen of infected men, with some evidence of altered seminal parameters. Low testosterone and dihydrotestosterone with raised luteinizing hormone has been reported as well as preterm delivery in pregnant women; however, data regarding vertical transmission remains contradictory and inconclusive.

Conclusions

The recent literature provides evidence that male gonads may be potentially vulnerable to SARS-CoV-2 infection, recommending caution to pregnant women and couples planning natural pregnancy or assisted reproduction.

Aitken RJ. **COVID-19 and human spermatozoa-Potential risks for infertility and sexual transmission?** [published online ahead of print, 2020 Jul 10]. Andrology. 2020;10.1111/andr.12859. doi:10.1111/andr.12859
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7404878/pdf/ANDR-9999-na.pdf

Cleavage of ACE2 following exposure to COVID-19 would be expected to induce a decrease in sperm viability and function, leading to a loss of fertility. Furthermore, the processing of ACE2 on the surface of human spermatozoa may effectively turn these cells into viral vectors, capable of sexually transmitting COVID-19 to other individuals in the community.

Actual fusion between the virus and human spermatozoa requires the presence of the above-mentioned protease, TMPRSS2, to cleave the viral spike proteins (S) at the S1/S2 boundary or within S2 subunit, thereby

removing the structural constraint of S1 on S2, and releasing the internal membrane fusion peptide. This protease is known to be present in prostasomes that are released into seminal fluid from the prostate gland at ejaculation. Since one of the major functions of these exosome-like structures is to transfer their contents, including proteins, to the spermatozoa following ejaculation, the incorporation of TMPRSS2 from this source seems probable. Furthermore, a close examination of the human sperm proteomic databases reveals the presence of related proteases TMPRSS11B and TMPRSS12 as well as FURIN, in these cells, all of which are thought to serve as activating proteases for viral infection including corona viruses. The presence of these activating proteases as well as ACE2 in the sperm plasma membrane would be expected to allow the COVID-19 virus to bind to the cell surface and ultimately fuse, either in the testes or during the prolonged sojourn of these cells in the epididymis. They also have a proven capacity to fuse with enveloped viruses and possess reverse transcriptase activity capable of generating proviral DNA.

Yang M, Chen S, Huang B, et al. **Pathological Findings in the Testes of COVID-19 Patients: Clinical Implications**. Eur Urol Focus. 2020;6(5):1124-1129. doi:10.1016/j.euf.2020.05.009
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7261470/

Background

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), involves multiple organs. Testicular involvement is largely unknown.

Objective

To determine the pathological changes and whether SARS-CoV-2 can be detected in the testes of deceased COVID-19 patients.

Design, setting, and participants

Postmortem examination of the testes from 12 COVID-19 patients was performed using light and electron microscopy, and immunohistochemistry for lymphocytic and histiocytic markers. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the virus in testicular tissue.

Outcome measurements and statistical analysis

Seminiferous tubular injury was assessed as none, mild, moderate, or severe according to the extent of tubular damage. Leydig cells in the interstitium were counted in ten 400× microscopy fields.

Results and limitations

Microscopically, Sertoli cells showed swelling, vacuolation and cytoplasmic rarefaction, detachment from tubular basement membranes, and loss and sloughing into lumens of the intratubular cell mass. Two, five, and four of 11 cases showed mild, moderate, and severe injury, respectively. The mean number of Leydig cells in COVID-19 testes was significantly lower than in the control group (2.2 vs 7.8, $p < 0.001$). In the interstitium there was edema and mild inflammatory infiltrates composed of T lymphocytes and histiocytes. Transmission EM did not identify viral particles in three cases. RT-PCR detected the virus in one of 12 cases.

Conclusions

Testes from COVID-19 patients exhibited significant seminiferous tubular injury, reduced Leydig cells, and mild lymphocytic inflammation. We found no evidence of SARS-CoV-2 virus in the testes in the majority (90%) of the cases by RT-PCR, and in none by electron microscopy. These findings can provide evidence-based guidance for sperm donation and inform management strategies to mitigate the risk of testicular injury during the COVID-19 disease course.

Patient summary

We examined the testes of deceased COVID-19 patients. We found significant damage to the testicular parenchyma. However, virus was not detected in testes in the majority of cases.

Segars J, Katler Q, McQueen DB, et al. **Prior and novel coronaviruses, Coronavirus Disease 2019 (COVID-19), and human reproduction: what is known?** Fertil Steril. 2020;113(6):1140-1149. doi:10.1016/j.fertnstert.2020.04.025
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7161522/

Objective

To summarize current understanding of the effects of novel and prior coronaviruses on human reproduction, specifically male and female gametes, and in pregnancy.

Design

Review of English publications in PubMed and Embase to April 6, 2020.

Method(s)

Articles were screened for reports including coronavirus, reproduction, pathophysiology, and pregnancy.

Intervention(s)

None.

Main Outcome Measure(s)

Reproductive outcomes, effects on gametes, pregnancy outcomes, and neonatal complications.

Result(s)

Seventy-nine reports formed the basis of the review. Coronavirus binding to cells involves the S1 domain of the spike protein to receptors present in reproductive tissues, including angiotensin-converting enzyme-2 (ACE2), CD26, Ezrin, and cyclophilins. Severe Acute Respiratory Syndrome Coronavirus 1 (SARS–CoV-1) may cause severe orchitis leading to germ cell destruction in males. Reports indicate decreased sperm concentration and motility for 72–90 days following Coronavirus Disease 2019 (COVID-19) infection. Gonadotropin-dependent expression of ACE2 was found in human ovaries, but it is unclear whether SARS–Coronavirus 2 (CoV-2) adversely affects female gametogenesis. Evidence suggests that COVID-19 infection has a lower maternal case fatality rate than SARS or Middle East respiratory syndrome (MERS), but anecdotal reports suggest that infected, asymptomatic women may develop respiratory symptoms postpartum. Coronavirus Disease 2019 infections in pregnancy are associated with preterm delivery. Postpartum neonatal transmission from mother to child has been reported.

Conclusion(s)

Coronavirus Disease 2019 infection may affect adversely some pregnant women and their offspring. Additional studies are needed to assess effects of SARS–CoV-2 infection on male and female fertility.

Perry MJ, Arrington S, Neumann LM, Carrell D, Mores CN. **It is currently unknown whether SARS-CoV-2 is viable in semen or whether COVID-19 damages spermatozoa** [published online ahead of print, 2020 May 29]. Andrology. 2020;10.1111/andr.12831. doi:10.1111/andr.12831
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7300609/

Research is needed to understand the presence of the SARS-CoV-2 virus in semen, sexual transmissibility, and impact on sperm quality. Several studies have examined men recovering from COVID-19, but large-scale community-based testing is needed to ascertain the effects on the male reproductive tract, and the potential for prolonged transmission.

The few studies on SARS-CoV-2 and semen thus far have shown conflicting results. Two studies were unable to detect the presence of SARS-CoV-2 in semen from men who had recovered from COVID-19. 9 , 10 These studies report differing conditions and varying disease severity of study participants. Pan et al (2020) analyzed samples collected from men one month after diagnosis exhibiting milder symptoms, while Song et al (2020) included symptomatic and asymptomatic men, and testis tissue specimens from a deceased patient. An additional study analyzed the semen of a patient with mild symptoms and did not detect the virus eight days after receiving a positive test. 11 However, a study of 38 male patients with COVID-19 found that 4 of 15 patients (26.7%) in the acute stage of infection and 2 of 23 patients (8.7%) in recovery had

detectable SARS-CoV-2 present in semen samples. 12 In addition to the semen studies, one study of 48 males and 20 females compared the viral clearance time of SARS-CoV-2 between sexes and observed that females achieved a negative PCR result two days earlier than males. 13

Groner MF, de Carvalho RC, Camillo J, Ferreira PRA, Fraietta R. **Effects of Covid-19 on male reproductive system**. Int Braz J Urol. 2021;47(1):185-190. doi:10.1590/S1677-5538.IBJU.2021.99.04
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7712710/
In view of the genetic similarity between the etiological agents of SARS and COVID-19, it is possible to infer the probable effects of SARS-CoV-2 on the male reproductive system based on previous studies on SARS-CoV. There are no reports on the presence of SARS-CoV in semen in patients with SARS, however there were descriptions of orchitis and deleterious effects on testicular tissue in autopsies (16, 17) with confirmation of the virus presence in the testicles (18).
Moreover, the mechanism of cellular infection of SARS-CoV-2 is similar to SARS-CoV, due to the link between the viral Spike (S) protein and the Angiotensin converting enzymes 2 (ACE2) cell receptor (19–21). Previous studies have shown the high concentration of these receptors in the germ and somatic cells of the testicular tissue (22). This fact may indicate the testicles tissue vulnerability to contamination by this new virus, reinforcing the importance of monitoring the reproductive function in infected patients.

Pascolo L, Zito G, Zupin L, et al. **Renin Angiotensin System, COVID-19 and Male Fertility: Any Risk for Conceiving?. Microorganisms**. 2020;8(10):1492. Published 2020 Sep 28. doi:10.3390/microorganisms8101492
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7601043/
The current knowledge concerning the connection between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the renin–angiotensin system (RAS) system in the male reproductive apparatus is still limited, so dedicated studies are urgently required. Concerns about the male fertility consequences of SARS-CoV-2 infection have started to emerge, since epidemiologic studies observed that this coronavirus affects male patients more frequently and with increased severity, possibly because of the hormone-regulated expression of angiotensin-converting enzyme 2 (ACE2) receptor. A disturbance in fertility is also expected based on studies of the previous SARS-CoV infection, which targets the same ACE2 receptor when entering the host cells. In addition, bioinformatics analyses reveal the abundant expression of ACE2 receptor in the male reproductive tissues, particularly in the testis. It has been proposed that pharmacological intervention favoring the angiotensin-(1–7)/ACE2/Mas receptor pathway and increasing ACE2 expression and activity could greatly prevent inflammatory lesions in this area. Finally, in laboratories performing assisted reproductive technologies it is recommended that more attention should be paid not only to sperm quality but also to safety aspects. Data about the potential infectivity of seminal fluid are in fact conflicting and do not exclude risks for both personnel and patients. The potential infectivity of SARS-CoV-2 in reproductive male tissues should be strongly considered and further investigated for the proper management of in vitro fertilization procedures.
Bioinformatic studies reported the abundant expression of the ACE2 receptor and TMPRSS2 in the male reproductive tissues and cells (spermatogonial stem cells, spermatids and spermatozoa), suggesting the particular vulnerability of the testis to the virus [34]. The testicular RAS seems to have an important role in puberty onset, spermatogenesis, endocrine function and sperm capacitation, as well having the ability to affect the fluid homeostasis of the testes [54,55,68]. Nevertheless, at present, we do not have clues on how the activation of AT1R vs. AT2R by Ang II contributes to the infective and inflammatory conditions, nor to other consequences triggered by these molecules [164].
Some recent studies were able to find viral RNA in the seminal fluid of affected patients [143] and orchitis was associated with COVID-19 [144]. It is still unclear whether the putative damage to the testes is immune-

mediated or caused by the viral entry, depending on the severity of symptoms and a temporary high viral load during the acute phase of the disease.
In addition, the therapeutic approaches adopted to fight the infection may have collateral toxicity that further suggests the need for a follow-up on testicular functionality in recovered patients. No data on fertility are available on Tocilizumab and Remdesivir, the most promising drugs for the fight against SARS-CoV-2, whereas the continued use of chloroquine might impair sperm quality [165]. Evidence is still insufficient to support conclusions on this topic, and follow-up studies examining the long-term effects are needed.

de Carvalho RC, Groner MF, Camillo J, Ferreira PRA, Fraietta R. **The interference of COVID-19 in the male reproductive system: Important questions and the future of assisted reproduction techniques**. Clinics (Sao Paulo). 2020;75:e2183. doi:10.6061/clinics/2020/e2183
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7442377/
Due to the pandemic caused by the novel coronavirus disease, researchers internationally have started to utilize their efforts in understanding its pathophysiology and method of action (1). Ongoing studies have reported that severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) is easily found in most human bodily fluids (2-4). In relation to semen, there is great interest in unraveling the possible interaction between this fluid sample and the SARS-Cov-2 microorganism, as well as the possible long-term consequences of this relationship (5). Perhaps the most significant dimension of this theme is related to our current knowledge of other viral infections, in which the causative agents are found in the seminal sample, which use this medium for their transmission while causing alterations in the fertile potential of their carriers (6-9).
In addition, the form of the SARS-Cov-2 infection that has been established in the literature, and its mechanism of action, are similar to that of the SARS-Cov virus (10). Using the cellular receptor angiotensin converting enzyme 2 (ACE2), this virus infiltrates the cells where it then starts its multiplication (11,12). Interestingly, these receptors are found in high concentrations within the germ and somatic cells of the human testicles (13); however, recent studies show that, for the cell infection process to be successful, the transmembrane serine protease 2 (TMPRSS2) protein that assists in the virus-cell fusion process needs to be present (14). However, the expression of this molecule is rarely found within testicular tissue (15). Thus, there are doubts as to whether the testis is an organ that is vulnerable to this new infection.

Blumenfeld Z. **Possible impact of COVID-19 on fertility and assisted reproductive technologies**. Fertil Steril. 2020;114(1):56-57. doi:10.1016/j.fertnstert.2020.05.023
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7245206/
Whereas the blood-testicular barrier is not perfect, SARS-CoV-2 may inoculate the male reproductive tract, especially in the presence of inflammation ([4]). To date, 27 viruses have been detected in human semen in association with viremia ([4]). It has been speculated that the presence of viruses in semen may be more common than appreciated, and that traditional non–sexually transmitted viruses may be present in the genital secretions ([4]). Indeed, Li et al. recently identified SARS-CoV-2 in six out of 38 positive patients (15.8%), including four of 15 patients (26.7%) in the acute stage of infection ([4]). Furthermore, two of the 23 recovering patients (8.7%) also tested positive for SARS-CoV-2 in their semen, with no difference in days since clinical recovery, suggesting that semen may be contagious for the virus not only in the acute stage of illness but even later on. Because there was no difference between the positive and negative results, it is unknown yet for how long the semen may be contagious, which is definitely alarming.

Eisenberg ML. **Coronavirus disease 2019 and men's reproductive health**. Fertil Steril. 2020;113(6):1154. doi:10.1016/j.fertnstert.2020.04.039
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7174172/

This is reassuring regarding possible viral transmission or lack thereof. However, it is important to note that the men studied were often several weeks removed from acute infections, and many had had only mild symptoms, so it is conceivable that earlier time points or higher viral loads could lead to different results. But given the normal time course of semen turnover, that appears less likely.
Given the known mechanisms of SARS-Cov-2 entry into cells and the requirement for dual expression of ACE and TMPRSS2 proteins, the authors used their existing single-cell RNA seq cellular data to show that only 4 of 6,490 (<0.1%) testicular cells contain RNA for both proteins. Thus, it appears unlikely that SARS-Cov-2 can enter into any cells in the testis (e.g., germ cells, Leydig cells, or Sertoli cells) as has been hypothesized.
Next, the authors reported another interesting and novel clinical observation: 6 (17.6%) of 34 men reported scrotal discomfort at the time of COVID-19 infection.

Bendayan M, Robin G, Hamdi S, Mieusset R, Boitrelle F. **COVID-19 in men: With or without virus in semen, spermatogenesis may be impaired** [published online ahead of print, 2020 Oct 30]. Andrologia. 2020;e13878. doi:10.1111/and.13878
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7645883/
We would like to congratulate you for the review recently published in Andrologia entitled 'Could SARS-CoV-2 affect male fertility?' (Vishvkarma R, & Rajender S, 2020). In just a few months, several million humans have been infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This brutal pandemic, called coronavirus-19 disease (COVID-19), disproportionately affects men and may have lasting impacts relating to aspects such as fertility. Some of these impacts are described in the literature, and others may be published in the coming months. Today, the literature focuses on whether the virus is present in semen, a possibility that is causing significant panic in the media and the public because the presence of the virus in the ejaculate would make COVID-19 an sexually transmitted infection (STI). However, beyond the answer to this question, there are symptoms of COVID-19 (e.g. fever) with the known andrological impacts that should be highlighted.
SARS-CoV-2 can have negative impacts on spermatogenesis and male fertility. Even in cure patients, the presence of the virus in semen is not impossible, and apart from the presence of virus in semen, spermatogenesis can be impaired by COVID-19-related fever. Therefore, as a precautionary measure, clinical, hormonal and semen parameter evaluations of patients diagnosed with COVID-19 are recommended at the time of infection and during follow-up appointments (3 and 6 months), especially in severe forms. For infertile men, a postponement of ART activities to three months post-infection is advisable.

Holtmann N, Edimiris P, Andree M, et al. **Assessment of SARS-CoV-2 in human semen-a cohort study**. Fertil Steril. 2020;114(2):233-238. doi:10.1016/j.fertnstert.2020.05.028
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7256599/
Objective
To investigate the presence of viral RNA in human semen of patients with severe acute-respiratory syndrome coronavirus 2 (SARS-CoV-2) and to evaluate its presence and relevance in semen parameters.
Design
Pilot cohort study.
Setting
University hospital.
Patient(s)
Thirty-four men were distributed as: 1) patients in convalescence (patients with confirmed SARS-CoV-2 infection in pharyngeal swab according to reverse-transcription polymerase chain reaction [RT-PCR] or antibodies); 2) negative control group (no antibodies); and 3) patients with an acute infection (detection of SARS-CoV-2 in pharyngeal swab).

Intervention
Semen and a blood sample were collected from each individual.
Main Outcome Measure(s)
Analysis of semen quality according to the World Health Organization standards. Detection of SARS-CoV-2 by RT-PCR in the native semen sample and after density gradient preparation. Confirmation of immunoglobulin (Ig) A und IgG antibodies in the blood.
Result(s)
Eighteen semen samples from recovered men were obtained 8–54 days after absence of symptoms, 14 from control subjects, and 2 from patients with an active COVID-19 infection. No RNA was detected by means of RT-PCR in the semen, including semen samples from two patients with an acute COVID-19 infection. Subjects with a moderate infection showed an impairment of sperm quality.
Conclusion(s)
A mild COVID-19 infection is not likely to affect testis and epididymis function, whereas semen parameters did seem impaired after a moderate infection. SARS-CoV-2 RNA could not be detected in semen of recovered and acute COVID-19–positive men. This suggests no viral transmission during sexual contact and assisted reproductive techniques, although further data need to be obtained.

Payne K, Kenny P, Scovell JM, Khodamoradi K, Ramasamy R. **Twenty-First Century Viral Pandemics: A Literature Review of Sexual Transmission and Fertility Implications in Men**. Sex Med Rev. 2020;8(4):518-530. doi:10.1016/j.sxmr.2020.06.003
https://www.sciencedirect.com/science/article/pii/S2050052120300676?via%3Dihub
Introduction
The 21st century has seen a series of viral pandemics that have collectively infected millions of individuals. To understand factors that may contribute to viral spread and address long-term health sequelae for survivors, it is important to review evidence regarding viral presence in semen, sexual transmission potential, and possible effects on fertility.
Aim
To review the current literature regarding the sexual transmissibility of recent viral pandemics and their effects on semen parameters and fertility. We review evidence for the following viruses: Ebola, Zika, West Nile, pandemic influenza, severe acute respiratory syndrome (SARS), and SARS-corona virus-2 (SARS-CoV-2).
Methods
A literature search was conducted to identify relevant studies. Titles and abstracts were reviewed for relevance. References from identified articles were searched and included, if appropriate.
Main Outcome Measures
The main outcome measure of this study was reviewing of peer-reviewed literature.
Results
Both the Ebola virus and Zika virus are present in semen, but only the Zika virus shows consistent evidence of sexual transmission. Current evidence does not support the presence of the West Nile virus, pandemic influenza, SARS, and SARS-CoV-2 in semen. The Zika virus appears to alter semen parameters in a way that diminishes fertility, but the effect is likely time limited. The West Nile virus and SARS have been associated with orchitis in a small number of case reports. Viruses that cause febrile illness, such as pandemic influenza, SARS, and SARS-CoV-2, are associated with decreased sperm count and motility and abnormal morphology. SARS and SARS-CoV-2 may interact with angiotensin-converting enzyme 2 receptors present in the testes, which could impact spermatogenesis.
Conclusions

We have reported the presence in semen, sexual transmission potential, and fertility side effects of recent viral pandemics. Overall, semen studies and fertility effects are highly understudied in viral pandemics, and rigorous study on these topics should be undertaken as novel pandemics emerge.

Fraietta R, Pasqualotto FF, Roque M, Taitson PF. **SARS-COV-2 and Male Reproductive Health** [published online ahead of print, 2020 Jul 14]. JBRA Assist Reprod. 2020;24(3):347-350. doi:10.5935/1518-0557.20200047
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7365526/
Critical challenges for the public and private health, research, and medical communities have been posed by the COVID-19 outbreak. Some of these challenges are related to the possible adverse effects of SARS-CoV-2 on male reproductive health, and whether other potential modes of transmission may occur, such as sexual transmission. Moreover, concerns have been raised in terms of whether the COVID-19 outbreak may have an impact on fertility worldwide. In this study, we will discuss the origins of SARS-CoV-2. We will further describe its mechanism of action, diagnosis, symptoms, and potential effects on the male reproductive system.
There is the theoretical possibility that testicular damage and subsequent infertility may result following COVID-19 infection, and also the possibility of sexual transmission, as SARS-CoV-2 has been identified in the semen of infected patients. However, the available data and study findings are recent, based on small sample sizes, and present conflicting information.

Sharun K, Tiwari R, Dhama K. **SARS-CoV-2 in semen: Potential for sexual transmission in COVID-19** [published online ahead of print, 2020 Nov 13]. Int J Surg. 2020;84:156-158. doi:10.1016/j.ijsu.2020.11.011
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7664476/
Direct contact with respiratory droplets serves as a major mechanism for efficient human-to-human transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections on a pandemic scale. Although the transmission of SARS-CoV-2 occurs predominantly through respiratory droplets, the virus has also been isolated from blood and fecal samples of patients with coronavirus disease of 2019 (COVID-19) indicating that the infection may at times be systemic [1]. This has raised concerns over the shedding of SARS-CoV-2 through other body fluids, including semen, thereby acting as an alternative mode of transmission. Li et al. (2020) have reported that the semen collected from patients with COVID-19 had oligozoospermia and increased levels of leucocytes [2]. Moreover, there was also an increase in the seminal levels of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). Further, the impairment of spermatogenesis can be attributed to the elevated immune responses in patients with COVID-19. The occurrence of autoimmune orchitis in some patients with COVID-19, confirms the same [2].

Massarotti C, Garolla A, Maccarini E, et al. **SARS-CoV-2 in the semen: Where does it come from?** [published online ahead of print, 2020 Jun 13]. Andrology. 2020;10.1111/andr.12839. doi:10.1111/andr.12839
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323151/pdf/ANDR-9999-na.pdf
We have no data on the presence of SARS-CoV-2 in the prostate, but we know that TMPRSS2 is highly expressed by the epithelium of the human prostate and is androgen-responsive [13]. Moreover, even if ACE2 expression in the prostate is reported as “very low”, the two receptors are co-expressed in at least a small percentage of prostate hillock cells [5, 14], therefore a prostate infection by SARS-CoV-2 cannot be excluded. If this hypothesis would be confirmed, it could provide an explanation for the virus presence in the seminal fluid and could justify the persistence of viral RNA in recovering patients. The third site of interest is the urinary tract. Clinical data from patients with SARS-CoV and MERS-CoV showed evidence of tubular damage [15]. Moreover, both ACE2 and TMPRSS2 are highly expressed by renal tubular cells [5, 16]

and SARS-CoV-2 was detected in urines [17]. Since the distal urinary and reproductive tract are overlapping in males, the RNA detected in semen could have been just a residual of urinary shedding.

Ruan Y, Hu B, Liu Z, Liu K, Jiang H, Li H, Li R, Luan Y, Liu X, Yu G, Xu S, Yuan X, Wang S, Yang W, Ye Z, Liu J, Wang T. **No detection of SARS-CoV-2 from urine, expressed prostatic secretions, and semen in 74 recovered COVID-19 male patients: A perspective and urogenital evaluation**. Andrology. 2020 Nov 4. doi: 10.1111/andr.12939. Epub ahead of print. PMID: 33150723.
https://onlinelibrary.wiley.com/doi/10.1111/andr.12939

Background
The coronavirus disease 2019 (COVID-19) has been spreading all over the world since December 2019. However, medical information regarding the urogenital involvement in recovered COVID-19 patients is limited or unknown.
Objectives
To comprehensively evaluate urogenital involvement in recovered COVID-19 patients.
Materials and methods
Men aged between 20 years and 50 years who were diagnosed with SARS-CoV-2 infection and recovered when the study was conducted were enrolled in our study. Demographic and clinical characteristics, and history of hospitalization were collected and analyzed. Urine, expressed prostatic secretions (EPSs), and semen samples were collected for SARS-CoV-2 RNA detection. Semen quality and hormonal profiles were analyzed.
Results
Among 74 male recovered COVID-19 patients, 11 (14.9%) were asymptomatic, classified into mild type, and 31 (41.9%) were classified into moderate type. The remaining patients (32/74, 43.2%) had severe pneumonia. No critically ill recovered COVID-19 patient was recruited in our cohort. The median interval between last positive pharyngeal swab RT-PCR test and semen samples collection was 80 days (IQR, 64-93). The median age was 31 years (IQR, 27-36; range, 21-49), and the median body mass index (BMI) was 24.40 (IQR, 22.55-27.30). Forty-five (61.6%) men were married, and 28 (38.4%) were unmarried. Fifty-three (72.6%) patients denied cigarette smoking, 18 (24.7%) were active smokers, and 2 of them were past smokers. The majority of our participants (53/74, 72.6%) did not consume alcohol. Fever occurred in most of the patients (75.3%), and 63 of them had abnormal chest CT images. Only one patient complained of scrotal discomfort during the course of COVID-19, which was ruled out orchitis by MRI (data not shown). A total of 205 samples were collected for SARS-CoV-2 detection (74 urine samples, 70 semen samples, and 61 EPS samples). However, viral nucleic acid was not detected in body fluids from the urogenital system. In terms of hormonal profiles, the levels of FSH, LH, testosterone, and estradiol were 5.20 [4.23] mIU/mL, 3.95 [1.63] mIU/mL, 3.65 [1.19] ng/mL, and 39.48 [12.51] pg/mL, respectively. And these values were within the normal limits. The overall semen quality of recovered COVID-19 patients was above the lower reference limit released by the WHO. While compared with healthy control, sperm concentration, total sperm count, and total motility were significantly declined. In addition, different clinical types of COVID-19 have no significant difference in semen parameters, but total sperm count showed a descending trend. Interestingly, subjects with a longer recovery time showed worse data for sperm quality. Small sample size and lacking semen parameters before the infection are the major limitations of our study.
Discussion and conclusions
To the best of our knowledge, it is the largest cohort study with longest follow-up for urogenital evaluation comprehensively so far. Direct urogenital involvement was not found in the recovered COVID-19 male patients. SARS-CoV-2 RNA was undetectable in the urogenital secretions, and semen quality declined slightly, while hormonal profiles remained normal. Moreover, patients with a long time (≥90 days) since

recovery had lower total sperm count. Great attention and further study should be conducted and follow-up on the reproductive function in the following months.

Cardona Maya WD, Du Plessis SS, Velilla PA. **SARS-CoV-2 and the testis: similarity with other viruses and routes of infection.** Reprod Biomed Online. 2020 Jun;40(6):763-764. doi: 10.1016/j.rbmo.2020.04.009. Epub 2020 Apr 17. PMID: 32362571; PMCID: PMC7162782.
https://www.rbmojournal.com/article/S1472-6483(20)30188-7/fulltext
Since the start of the latest coronavirus (SARS-CoV-2) outbreak, the number of infected individuals and cases of coronavirus disease (COVID-19) has been increasing exponentially worldwide. Of interest is existing evidence that orchitis can develop due coronavirus infection. It is therefore not unreasonable to believe that SARS-CoV-2 could be transmitted by semen. Consequently, it is of paramount importance that individuals who could potentially be infected take all possible care to mitigate the likely risk of passing on the infection through sexual intercourse.

Sheikhzadeh Hesari F, Hosseinzadeh SS, Asl Monadi Sardroud MA. **Review of COVID-19 and male genital tract**. Andrologia. 2020 Nov 24:e13914. doi: 10.1111/and.13914. Epub ahead of print. PMID: 33236375.
https://onlinelibrary.wiley.com/doi/10.1111/and.13914
COVID-19 pandemic leads to health challenges globally, and its diverse aspects need to be uncovered. Multi-organ injuries have been reported by describing potential SARS-CoV-2 entrance routes: ACE2 and TMPRSS2. Since this cell surface receptors' expression has been disclosed within the male reproductive system, its susceptibility to being infected by SARS-CoV-2 has been summarized through this literature review. Expression of ACE2 and TMPRSS2 at RNA or protein level has been reported across various investigations indicates that the male genitalia potentially is vulnerable to SARS-CoV-2 infection. Presence of SARS-CoV-2 within semen samples and following direct viral damage, secondary inflammatory response causing orchitis or testicular discomfort and finally the amount of viral load leading testicular damage and immune response activation are among probable underlying mechanisms. Therefore, genital examination and laboratory tests should be considered to address the male reproductive tract complications and fertility issues.
We speculated that SARS-CoV-2 could be able to infect the male genital tract according to the abovementioned process. Afterwards, we discussed underlying mechanisms:
1) Direct damage has proposed at first place via virus-receptor binding; however, this damage is also dependent on its replication and stabilization capabilities inside the target cells (Salam & Horby, 2017). Xu and colleagues reported no positive staining testis by SARS-CoV infected patients with orchitis in 2006 (Xu et al., 2006). Recently, Song et al. collected 12 semen samples of SARS-CoV-2 infected patients during the recovery period to diagnose COVID-19 by polymerase chain reaction (PCR) test, resulting in no positive sample (Song, Wang, et al., 2020). Interestingly, one recent cohort study in China has reported positive semen samples of COVID-19 patients: four positive for SARS-CoV-2 samples during the acute phase of the disease, and two positive ones during the recovery period among total 38 semen samples of infected patients (Li, Jin, et al., 2020).
2) Secondary inflammatory response by inflammatory cytokines has suggested as another underlying mechanism; like most infectious diseases, fever has accused of testicular damage due to the destruction of germ cells in constant high temperature, and leucocyte infiltration by destroying Leydig cells and following a decline in testosterone (T) level (Xu et al., 2006). Similarly, in one recent study in China, SARS-CoV-2 infected males were compared to healthy men in reproductive age to assess the changes in sex hormone levels. Consequently, the possibility of hypogonadism and Leydig cell destruction were hypothesized due to decline in T: LH ratio. It is worth noting that the potential effect of various therapies, corticosteroids as an example, on hypothalamic–pituitary–gonadal axis must need to be taken into consideration (Ma et al., 2020).

Additionally, a couple of investigations have suggested the role of sex steroid abnormalities in the severity of symptoms and poor prognosis among COVID-19 patients (Giagulli et al., 2020; Stopsack et al., 2020).
3) Additionally, viral load has recommended as an influential factor in following complications like testicular damage, on one hand (Hikmet et al., 2020), and immune response activation, adaptive and innate, to fight against the virus on the other hand (Li, Li, et al., 2020).
Further studies are required to address this issue by more patients' evaluation and examination, more semen samples collecting during different phases of the disease, additional hormonal function analysis and more studies in both RNA and protein expression levels.
Conclusively, we suggest physicians gain more attention to male genital examinations besides pulmonary, cardiovascular and other involved organs. These examinations should be done in the course of the acute and recovery phase to recognise any genital discomfort complaints, orchitis, epididymitis or infertility problems.

Patel DP, Guo J, Hotaling JM. **The jury is still out: COVID-19 and male reproduction**. Fertil Steril. 2020;114(2):257-258. doi:10.1016/j.fertnstert.2020.06.013
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7287438/
The current report further questions whether SARS-CoV-2 can be transmitted in the semen. Additionally, it is one of the first reports about SARS-CoV-2 and effects on semen parameters, although there are several limitations. Larger-scale community-based testing for SARS-CoV-2 and semen analyses in both symptomatic men with a wide range of COVID-19 severity and asymptomatic men are needed before we can better understand whether sexual transmission can occur and the effects of SARS-CoV-2 on semen parameters.

Cavalcante MB, Sarno M, da Silva ACB, Barini R. **Letter: COVID-19 and human reproduction: hypothesis needs to be investigated**. Mol Hum Reprod. 2020;26(7):549-550. doi:10.1093/molehr/gaaa041
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7313839/pdf/gaaa041.pdf
the hypothesis of Fan *et al*. (2020) started to be investigated. Li *et al*. (2020) demonstrated the presence of SARS-CoV-2 in 06 semen samples from a total of 38 men (06/38, 15.8%) diagnosed with COVID-19. Among men with a positive semen sample, the time since onset of symptoms varied from 6 to 16 days. The authors did not describe results of sperm quality and concluded that further studies are required with respect to the detailed information about virus shedding, survival time, and concentration in semen (Li *et al*., 2020). Ma *et al*. (2020) observed elevated luteinizing hormone (LH) levels in men infected with SARS-CoV-2, warning about the relationship between COVID-19 and gonadal dysfunction (Ma *et al*., 2020). Studies that assess the long-term impact of infection are also needed.
Fei C, Jing Y, Run-Qian L, Ya-Bin L, Hao-Ran W. **Reply: COVID-19 and human reproduction: hypothesis needs to be investigated.** Mol Hum Reprod. 2020 Jul 1;26(7):551-552. doi: 10.1093/molehr/gaaa042. PMID: 32502239; PMCID: PMC7313789.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7313789/pdf/gaaa042.pdf

José FG, González JGÁ, Molina JMC, et al. **Infección por SARS-CoV-2: implicaciones para la salud sexual y reproductiva. Una declaración de posición de la Asociación Espan˜ola de Andrología, Medicina Sexual y Reproductiva (ASESA)** Rev Int Androl . 2020; 18 (3): 117-123. doi: 10.1016 / j.androl.2020.06.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7351068/pdf/main.pdf
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7351068/

Omolaoye TS, Adeniji AA, Maya WDC, du Plessis SS. **SARS-COV-2 (Covid-19) and Male Fertility: where are we?** [published online ahead of print, 2020 Nov 26]. Reprod Toxicol. 2020;99:65-70. doi:10.1016/j.reprotox.2020.11.012
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7689309/

Severe acute respiratory syndrome coronavirus 2 (SARS−COV-2), a single-stranded RNA virus, was found to be the causal agent of the disease called coronavirus disease. During December 2019, China informed the World Health Organization (WHO) of an outbreak of cases of pneumonia of unknown etiology, which caused severe-acute respiratory distress. The disease was termed coronavirus disease 2019 (Covid-19). Due to alarming levels of spread and severity, on the 11th of March 2020, the WHO declared the outbreak as a global pandemic. As of September 14, 2020, more than 29 million cases have been reported, with over 900,000 deaths globally. Since the outbreak, although not conclusive, discoveries have been made regarding the understanding of the epidemiology, etiology, clinical features, clinical treatment, and prevention of the disease. SARS−COV-2 has been detected in saliva, respiratory fluids, blood, urine, and faeces. Findings are however controversial regarding its presence in the semen or the testis. Hence, this review aimed to further analyze the literature concerning (i) the effects of previously identified human coronaviruses on male fertility (ii) the impact of Covid-19 on male fertility and (iii) the implication for general health in terms of infection and transmission.

Bringing all the findings together, although most of the studies reported the absence of SARS−COV-2 in the semen and prostatic secretions, as well as testicular tissues, which may reduce the possibility of transmission through this route, it is evident that (i) there is testicular injury and inflammatory infiltration (ii) viral orchitis may occur, as patients experienced scrotal discomfort (iii) there is altered semen parameters and (iv) the number of spermatozoa with DNA fragmentation is increased. These results collectively suggest that infection with SARS−COV-2 may lead to potential fertility issues.

Xia J. **Does immune privilege result in recovered patients testing positive for COVID-19 again?** Biosci Trends. 2020 Jul 17;14(3):209-211. doi: 10.5582/bst.2020.03154. Epub 2020 May 16. PMID: 32418947.
https://www.jstage.jst.go.jp/article/bst/14/3/14_2020.03154/_pdf/-char/en

Recently, an increasing number of reports have indicated that a few patients who were believed to have recovered from COVID-19 initially tested negative but later tested positive. Several hospitals in different countries have detected SARS-CoV-2 RNA in the semen and cerebrospinal fluid of patients with severe COVID-19. Given the fact that the testes and central nervous system are both immune privilege sites and the fact that Ebola virus and Zika virus can avoid immune clearance and continue proliferating and spreading by hiding in those sites, the question of whether SARS-CoV-2 is present in immune privilege sites, it attacks those sites, and it spreads again after proliferating in those sites needs to be investigated.

Certain sites in the human body have immune privilege, meaning that they are able to tolerate the introduction of antigens without eliciting an inflammatory immune response. Immunologically privileged sites include: the eyes, the placenta and fetus, the testicles, the central nervous system (CNS), and the anagen hair follicles (7) (Figure 1). Immune privilege is thought to be an evolutionary adaptation to protect vital structures from the potentially damaging effects of an inflammatory immune response. Inflammation in the brain or eye can lead to loss of organ function, while immune responses directed against a fetus can lead to a miscarriage. Thus, these are niches where viruses may be protected from the host immune response.

All of the semen samples from recovering patients that were found to have viral RNA were collected 2 or 3 days after recovery. Thus, different findings from the earlier studies and the more recent one is probably the result of differences in disease severity and the timing of sample collection

The presence of viral RNA in immunologically privileged sites in patients does not necessarily mean that an infectious virus is present. Thus, what must be determined is whether infectious viruses can also be isolated from immunologically privileged sites in COVID-19 patients and survivors. Isolation of SARS- CoV-2 in those sites would lead to 3 questions: first, will the virus attack eyes, CNS, testes, or a fetus; second, will SARS-CoV-2 spread through sexual transmission or pregnancy; and third, can detection of SARS-CoV-2 in immunologically privileged sites serve as a prognostic marker or is it a necessary indicator for recovered patients, and especially those who with severe disease. In the future, more studies need to investigate

whether immunologically privileged sites play an important role in SARS-CoV-2infection and the recurrence of COVID-19. In the meantime, the sensible move would be for patients recovering from COVID-19 to remain in self-isolation until further research determines how long the virus remains in immunologically privileged sites.

Hallak J, Teixeira TA, Bernardes FS, Carneiro F, Duarte SAS, Pariz JR, Esteves SC, Kallas E, Saldiva PHN. **SARS-CoV-2 and its relationship with the genitourinary tract: Implications for male reproductive health in the context of COVID-19 pandemic**. Andrology. 2020 Sep 1. doi: 10.1111/andr.12896. Epub ahead of print. PMID: 32869939.
https://onlinelibrary.wiley.com/doi/10.1111/andr.12896
Background
The current outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, named coronavirus disease 19 (COVID-19), is not the first well-known spillover of an animal originated virus to infect humans. However, one of the few to make such a fast jump in a powerful evolutionary shortcut. The incredible pattern of aggressiveness worldwide since the beginning of the outbreak is that up to 20% of those infected need hospitalizations and 5% evolve to critical conditions, not limited to respiratory-related issues, but rather to systemic involvement.
Objective
This study aims to summarize the current knowledge about the effects of SARS-CoV-2 infection on the male genitourinary tract.
Materials and methods
A narrative review was carried out to identify articles on the SARS-CoV-2 infection on the male genitourinary system.
Results
Considerations were made about the molecular characteristics of SARS-CoV-2 and immune response to coronavirus. We discussed the influence of the virus on the urinary system, potential mechanisms of COVID-19- related acute kidney injury (AKI), and the role of cytokine release syndrome on the renal pathophysiology of the disease. In the male reproductive tract, it was discussed the testis' vulnerability to SARS-CoV-2 invasion and the possible adverse effects on its function and the seminal findings of COVID-19.
Discussion and conclusion
During the COVID-19 pandemic, an international coordinated scientific effort must arise to understand the role of the urogenital system in the SARS-CoV-2 infection in the clinical setting.
SARS-CoV-2 infection probably interferes indirectly with testicular function, like other systemic viral infections (eg, influenza).50 High inflammatory response with fever, immune cell activation, and inflammatory mediators such as interferons and cytokines, which could inhibit steroidogenesis and spermatogenesis, could affect the testis induced by SARS-CoV-2 infection.51, 52 COVID-19-related fever could impair spermatogenesis, so semen parameters such as sperm concentration and motility might be reduced for 72 to 90 days following the infection.53, 54
The influence of SARS-CoV-2 infection on testicular hormonal function was evaluated in a study comparing hormone levels of 119 infected men with 273 age-matched control subjects. The authors found a significant increase in luteinizing hormone (LH) levels and a concomitant decrease in serum total testosterone/luteinizing hormone (T/LH) ratio in the infected group. The T/LH ratio was negatively associated with C-reactive protein and white blood cell count, possibly by injury to Leydig cells, meaning an early transient stage of hypogonadism.55
Up to now, there are no reports of sexual transmission, and evidence involving the presence of SARS-CoV-2 in semen remains scarce. In an analysis of 38 semen samples, SARS-CoV-2 was detected by RT-PCR in six patients, two of whom were in the convalescent period.62 However, two key points must be emphasized

despite these startling results: (a) absence of data regarding the viral load prevents a thorough analysis of its significance for sexual transmission and (b) lack of a precise description of RT-PCR technique and semen collection methods do not allow verification of the hypothesis by independent scientific groups.

Li H, Xiao X, Zhang J, et al. **Impaired spermatogenesis in COVID-19** patients. EClinicalMedicine. 2020;28:100604. doi:10.1016/j.eclinm.2020.100604
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7584442/
https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(20)30348-5/fulltext

Background

The current study aimed to determine the impact of SARS-CoV-2 infection on male fertility.

Methods

This is a single-center, hospital-based observational study that included autopsied testicular and epididymal specimens of deceased COVID-19 male patients (n=6) and recruited recovering COVID-19 inpatients (n=23) with an equal number of age-matched controls, respectively. We performed histopathological examinations on testicular and epididymal specimens, and also performed TUNEL assay and immunohistochemistry. Whereas, we investigated the semen specimen for sperm parameters and immune factors.

Findings

Autopsied testicular and epididymal specimens of COVID-19 showed the presence of interstitial edema, congestion, red blood cell exudation in testes, and epididymides. Thinning of seminiferous tubules was observed. The number of apoptotic cells within seminiferous tubules was significantly higher in COVID-19 compared to control cases. It also showed an increased concentration of CD3+ and CD68+ in the interstitial cells of testicular tissue and the presence of IgG within seminiferous tubules. Semen from COVID-19 inpatients showed that 39.1% (n=9) of them have oligozoospermia, and 60.9% (n=14) showed a significant increase in leucocytes in semen. Decreased sperm concentration, and increased seminal levels of IL-6, TNF-α, and MCP-1 compared to control males were observed.

Interpretation

Impairment of spermatogenesis was observed in COVID-19 patients, which could be partially explained as a result of an elevated immune response in testis. Additionally, autoimmune orchitis occurred in some COVID-19 patients. Further research on the reversibility of impairment and developing treatment are warranted.

Bendayan M, Boitrelle F. **Covid-19 and impairment of spermatogenesis: What if fever was the only cause?** EClinicalMedicine. 2020;29:100670. doi:10.1016/j.eclinm.2020.100670
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7700723/

We would like to congratulate you for the original research recently published in EclinicalMedicine entitled 'Impaired spermatogenesis in COVID-19 patients' [1]. The authors found an increased concentration of CD3+ and CD68+ cells and apoptotic cells in the testicles/epidymes of men who died from Covid-19. In some patients cured of COVID-19, they noted oligozoospermia, leukocytospermia and increased seminal levels of IL-6 and MCP-1. All of these signs would be, for the authors, due to an autoimmune orchitis.

First, the presence of CD3+ and CD68+ cells is physiological in epididymides [2]; they play a role in physiological sperm phagocytosis. Fever is a symptom observed in more than 80% of patients infected with COVID-19. This fever, even of limited duration, can, on its own, induce oligozoospermia and the appearance of apoptotic cells [3]. Thus, COVID-19-induced fever can alter semen parameters even in the absence of testicular immune response. The return to basal state of semen parameters may take up to three months [4].

In addition, leukocytospermia (also called pyospermia) is defined by the WHO as the presence of >1.106 granulocytes (not lymphocytes) /ml. This leukocytospermia is traditionally accompanied by an increase in IL-6 and MCP-1 [5]. Leukocytospermia can be a sign of a bacterial or viral infection, systemic

inflammation or simply an infrequent ejaculation. Leukocytospermia is not evidence of inflammation of the testicles.
In conclusion, the question of SARS-CoV-2 tropism for the testicles remains unresolved. Moreover, the ability of this novel coronavirus to induce an autoimmune orchitis is not proven to date.

Wang Z, Xu X. **scRNA-seq Profiling of Human Testes Reveals the Presence of the ACE2 Receptor, A Target for SARS-CoV-2 Infection in Spermatogonia, Leydig and Sertoli Cells**. Cells. 2020;9(4):920. Published 2020 Apr 9. doi:10.3390/cells9040920
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7226809/
In December 2019, a novel coronavirus (SARS-CoV-2) was identified in COVID-19 patients in Wuhan, Hubei Province, China. SARS-CoV-2 shares both high sequence similarity and the use of the same cell entry receptor, angiotensin-converting enzyme 2 (ACE2), with severe acute respiratory syndrome coronavirus (SARS-CoV). Several studies have provided bioinformatic evidence of potential routes of SARS-CoV-2 infection in respiratory, cardiovascular, digestive and urinary systems. However, whether the reproductive system is a potential target of SARS-CoV-2 infection has not yet been determined. Here, we investigate the expression pattern of ACE2 in adult human testes at the level of single-cell transcriptomes. The results indicate that ACE2 is predominantly enriched in spermatogonia and Leydig and Sertoli cells. Gene Set Enrichment Analysis (GSEA) indicates that Gene Ontology (GO) categories associated with viral reproduction and transmission are highly enriched in ACE2-positive spermatogonia, while male gamete generation related terms are downregulated. Cell–cell junction and immunity-related GO terms are increased in ACE2-positive Leydig and Sertoli cells, but mitochondria and reproduction-related GO terms are decreased. These findings provide evidence that the human testis is a potential target of SARS-CoV-2 infection, which may have significant impact on our understanding of the pathophysiology of this rapidly spreading disease.

Huang HH, Wang PH, Yang YP, et al. **A review of severe acute respiratory syndrome coronavirus 2 infection in the reproductive system.** J Chin Med Assoc. 2020;83(10):895-897. doi:10.1097/JCMA.0000000000000388
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7526564/
An outbreak of pneumonia associated with coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) occurred in Wuhan, China, in December 2019, and has been spread worldwide rapidly now. Over 5.3-million confirmed cases and 340,000 disease-associated deaths have been found till May 25, 2020. The potential pathophysiology for SARS-CoV-2 to affect the target is via the receptor, angiotensin-converting enzyme 2 (ACE2). ACE2 can be found in the respiratory, cardiovascular, gastrointestinal tract, urinary tract, and reproductive organs such as human ovaries and Leydig cells in the testis. This receptor plays a dominant role in the fertility function. Considering the crucial roles of testicular cells of the male reproductive system, increasing numbers of studies focus on the effects of SARS-CoV-2 on the testis. In this literature, we reviewed several studies to evaluate the relevance between SARS-CoV-2, ACE receptor, and female and male reproductive system and found that the risk of being attacked by SARS-CoV-2 is higher in males than in females. Since men infected with SARS-CoV-2 virus may have the risk of impaired reproductive performance, such as the orchitis and an elevated of luteinizing hormone (LH), and additionally, SARS-CoV-2 virus may be found in semen, although the latter is still debated, all suggest that we should pay much attention to sexual transmitted disease and male fertility after recovering from COVID-19.

Sun J. **The hypothesis that SARS-CoV-2 affects male reproductive ability by regulating autophagy**. Med Hypotheses. 2020;143:110083. doi:10.1016/j.mehy.2020.110083
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7347466/

The outbreak of CoronaVirus Disease19 (COVID19) in December 2019 posed a serious threat to public safety, and its rapid spread caused a global health emergency. Clinical data show that in addition to respiratory system damage, some male patients with COVID-19 are also accompanied by abnormal renal function and even renal damage. As the main receptor of syndrome coronavirus 2 (SARS-CoV-2), angiotensin converting enzyme 2 (ACE2) is also found to be highly expressed not only in respiratory mucosa and alveolar epithelial cells, but also in renal tubule cells, testicular Leydig cells and seminiferous tubule cells. This suggests that SARS-CoV-2 has the possibility of infecting the male reproductive system, and the recent detection of SARS-CoV-2 in the patient's semen further confirms this theory. In previous studies, it has been found that ACE2 has the ability to regulate autophagy. Not only that, recent studies have also found that SARS-CoV-2 infection can also lead to a reduction in autophagy. All of these associate SARS-CoV-2 with autophagy. Furthermore, autophagy has been shown to have an effect on male reproduction in many studies. Based on these, we propose the hypothesis that SARS-CoV-2 affects male reproductive function by regulating autophagy. This hypothesis may provide a new idea for future treatment of COVID-19 male patients with reproductive function injury, and it can also prompt medical staff and patients to consciously check their reproductive function.

Autophagy is a degradation system in eukaryotic cells, which can not only degrade intracellular aging-damaged organelles and unneeded metabolites to provide cell energy and nutrients, but also play an important role in the elimination of intracellular pathogenic microorganisms. Moreover, more and more studies have proved that autophagy is involved in a wide range of cellular events in the male reproductive system, affecting male reproductive ability. In the process of spermatogenesis, autophagy is very important to ensure the formation of specific structures and the degradation of some components in spermatogenesis [26].

In autophagy-deficientt mouse testis, due to the accumulation of a negative cytoskeleton organization regulator, PDLIM1, the cytoskeleton structure is disordered, the assembly of extracellular specialized (ES) is destroyed, and finally lead to sperm head deformity [26]. Besides, autophagy has also been shown to synthesize testosterone by promoting cholesterol uptake and degradation of intracellular low density lipoprotein, which may be involved in the metabolism and elimination of testosterone, as well as the production of other cholesterol-based hormones [8]. It can be seen that autophagy plays an active role in male spermatogenesis and endocrine process.

We know that SARS-CoV-2 may infect host cells by binding to ACE2 on the surface of reproduction-related cells such as Leydig cells. SARS-CoV-2 itself or ACE2 can cause cell dysfunction by regulating the level of autophagy, and some viral proteins can also directly induce or inhibit the autophagy pathway to achieve virus survival [27]. For example, the dual action of HIV and autophagy pathway increases virus production by using the early stage of autophagy and inhibiting the late stage of autophagy [28], [29]. This information links SARS-CoV-2, autophagy and male fertility, leading us to speculate whether SARS-CoV-2 may eventually cause male reproductive disorders by regulating the level of autophagy in male germ cells.

Illiano E, Trama F, Costantini E. **Could COVID-19 have an impact on male fertility?** Andrologia. 2020;52(6):e13654. doi:10.1111/and.13654

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267130/

The pandemic caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to several hypotheses of functional alteration of different organs. The direct influence of this virus on the male urogenital organs is still to be evaluated. However some hypotheses can already be made, especially in the andrological field, for the biological similarity of the SARS-CoV and SARS-CoV2. As well as SARS-CoV, SARS CoV-2 uses the 'Angiotensin Converting Enzyme-2' (ACE2) as a receptor to enter human cells. It was found that ACE2, Angiotensin (1-7) and its MAS receptors are present, over in the lung, also in the testicles, in particular in Leydig and Sertoli cells. A first hypothesis is that the virus could enter the testicle and lead to

alterations in testicular functionality. A second hypothesis is that the binding of the virus to the ACE2 receptor, could cause an excess of ACE2 and give rise to a typical inflammatory response. The inflammatory cells could interfere with the function of Leydig and Sertoli cells. Both hypotheses should be evaluated and confirmed, in order to possibly monitor fertility in patients COVID-19+.

Vishvkarma R, Rajender S. **Could SARS-CoV-2 affect male fertility?** Andrologia. 2020;52(9):e13712. doi:10.1111/and.13712
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7361071/
We performed this systematic review to evaluate the possibility of an impact of SARS-CoV-2 infection on male fertility. SARS-CoV-2 enters the cells with the help of ACE2; therefore, testicular expression of ACE2 was analysed from transcriptome sequencing studies and our unpublished data. Literature suggested that SARS-CoV-1 (2002-2004 SARS) had a significant adverse impact on testicular architecture, suggesting a high possibility of the impact of SARS-CoV-2 as well. Out of two studies on semen samples from COVID-19 affected patients, one reported the presence of SARS-CoV-2 in the semen samples while the other denied it, raising conflict about its presence in the semen samples and the possibility of sexual transmission. Our transcriptome sequencing studies on rat testicular germ cells showed ACE expression in rat testicular germ cells. We also found ACE2 expression in transcriptome sequencing data for human spermatozoa, corroborating its presence in the testicular germ cells. Transcriptome sequencing data from literature search revealed ACE2 expression in the germ, Sertoli and Leydig cells. The presence of ACE2 on almost all testicular cells and the report of a significant impact of previous SARS coronavirus on testes suggest that SARS-CoV-2 is highly likely to affect testicular tissue, semen parameters and male fertility.

Sengupta P, Dutta S. **Does SARS-CoV-2 infection cause sperm DNA fragmentation? Possible link with oxidative stress.** Eur J Contracept Reprod Health Care. 2020 Oct;25(5):405-406. doi: 10.1080/13625187.2020.1787376. Epub 2020 Jul 9. PMID: 32643968.
https://pubmed.ncbi.nlm.nih.gov/32643968/
http://www.okilab.es/sars-cov-2-infection-vs-sperm-dna-fragmentation/
following the limited literature correlating SARS-CoV2 infection and male reproductive disruption, it can be hypothesised that OS is the central player of this infection induced male reproductive changes, resulting in sperm DNA damage and subsequent infertility. In addition, as discussed above, some anti-SARS-CoV-2 drugs, such as ribavirin treatment can also cause SDF even following cessation of treatment.
Thus, it can be concluded that SARS-CoV-2 infection may possibly affect sperm DNA and cause SDF through induction of OS following various direct oxidant-sensitive pathways, altering reproductive endocrinological milieu, or through the course of COVID-19 treatment using certain broad-spectrum anti-viral drugs. These reflections suggest that SARS-CoV-2-mediated SDF is surprisingly an unexplored area awaiting immediate research attention.
Renu K, Subramaniam MD, Chakraborty R, et al. **The role of Interleukin-4 in COVID-19 associated male infertility - A hypothesis** [published online ahead of print, 2020 Sep 30]. J Reprod Immunol. 2020;142:103213. doi:10.1016/j.jri.2020.103213
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7526609/
COVID-19 is a present-day complex pandemic infection with unpredictable levels of morbidity and mortality in various global populations. COVID-19 is associated with the different comorbidities with its change in biological function such as causing heart dysfunction via deregulating ACE-2 receptor, gastrointestinal risk via causing vomiting, diarrhea, and abdominal pain, chronic kidney disease via proteinuria and hematuria, diabetes mellitus, liver injury via increasing ALT, AST and bilirubin level, lung injury, CNS risk, ocular risk, and cancer risk. In this, we are focused on the COVID-19 connected with male infertility. Some of the studies show that the patients of COVID-19 are associated with impaired spermatogenesis. Impaired

spermatogenesis via COVID-19 decreases the level of testosterone by disturbing cytokines such as TNF-α, IL-4, IL-6, and IL-12 and further, attenuates the sperm count. COVID-19 is causing inflammation via TNF-α and interferons. IL-4 plays an eminent role in the activation of the JAK-STAT pathway and leads to the disturbing pro-inflammatory cytokine as well as further cause's male infertility. Th2 activates the IL-4 through IgG and IgE and mediates apoptosis with the triggering of STAT signaling. The activated STAT signaling augments Batf/Irf4, and the Bach2/Batf pathway. On the other hand, SARS-CoV-2 is activating the level of Th2 cells. So, we hypothesized that the augmented Th2 cells would disturb the level of IL-4, JAK-STAT signaling, Batf/Irf4, and Bach2/Batf pathway. The disturbed IL-4 decreases the level of the ACE-2 with the inflammation. This further leads to male infertility in COVID-19 patients. So, in this hypothesis, we focused on the role of IL-4 in COVID-19 patients associated with male infertility via Th2 cells and JAK-STAT signaling.

Younis JS, Abassi Z, Skorecki K**. Is there an impact of the COVID-19 pandemic on male fertility? The ACE2 connection.** Am J Physiol Endocrinol Metab. 2020;318(6):E878-E880. doi:10.1152/ajpendo.00183.2020
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7276979/
emerging evidence indicates that a subgroup of patients with severe COVID-19 might have a secondary cytokine storm syndrome (hemophagocytic lymphohistiocytosis) (16). This is an underrecognized, hyperinflammatory syndrome characterized by sustained fever, with fulminant and fatal hypercytokinemia with multiorgan failure. These patients have a particular serum blood cytokine profile with cytopenia and hyperferritinemia. These findings also suggest that immunomodulatory therapy (IL-6 antagonist) may improve mortality rate considerably in these patients (16, 24).
As cytokines contribute to testicular function and maintenance of male reproductive health, and to the pathologies associated with their abnormal activity in this organ, COVID-19-induced changes in cytokines profile may have further implications to male fertility (13). In addition, immunomodulatory therapies may provoke potential long-term effects on male fertility and are a matter of concern. Furthermore, cytokine microenvironment deviations within the testis may have tumorigenic adverse effects on the cellular level, leading eventually to testicular cancer, a second long-term matter of concern (13).

Sansone A, Mollaioli D, Ciocca G, et al. **Addressing male sexual and reproductive health in the wake of COVID-19 outbreak** [published online ahead of print, 2020 Jul 13]. J Endocrinol Invest. 2020;1-9. doi:10.1007/s40618-020-01350-1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7355084/
The viral pandemic of the coronavirus disease 2019 (COVID-19), generated by a novel mutated severe acute respiratory syndrome coronavirus (SARS-CoV-2), has become a serious worldwide public health emergency, evolving exponentially. While the main organ targeted in this disease is the lungs, other vital organs, such as the heart and kidney, may be implicated. The main host receptor of the SARS-CoV-2 is angiotensin converting enzyme 2 (ACE2), a major component of the renin-angiotensin-aldosterone system (RAAS). The ACE2 is also involved in testicular male regulation of steroidogenesis and spermatogenesis. As the SARS-CoV-2 may have the potential to infect the testis via ACE2 and adversely affect male reproductive system, it is essential to commence with targeted studies to learn from the current pandemic, with the possibility of preemptive intervention, depending on the findings and time course of the continuing pandemic.
Erectile function, as a surrogate marker of cardiovascular/pulmonary health, could also become extremely valuable as a quick and inexpensive first-line assessment of the pulmonary and cardiovascular complications for COVID-19 survivors. In this regard, evidence coming from diagnostic procedures, such as penile color-doppler ultrasound [43] and hypothalamic-pituitary–testicular axis evaluation [100], will be necessary to assess the extent to which COVID-19 has been able to impair erectile, and finally vascular, function, the former being an efficient predictor of complete restitutio ad integrum. *Additionally, tailored psychological*

interventions would be necessary to adequately support patients who develop sexual dysfunction consequently to the containment measures.

Zupin L, Pascolo L, Zito G, Ricci G, Crovella S. **SARS-CoV-2 and the next generations: which impact on reproductive tissues?.** J Assist Reprod Genet. 2020;37(10):2399-2403. doi:10.1007/s10815-020-01917-0
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7419027/
Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) is a severe global pandemic, affecting mostly the respiratory system. Understandably, attention is also being directed towards the urogenital tract. In this work, expression patterns of various host molecules possibly involved in viral entry and replication were investigated in human female and male reproductive systems by inquiring online repositories, including the Human Protein Atlas, GTEx, FANTOM5. Our findings highlight that male reproductive tissues could be targeted by SARS-CoV-2, particularly the testis since it co-expresses the receptor (ACE2) and the protease (TMPRSS) needed for viral entry. We hypothesized that SARS-CoV-2 infection could have repercussions on the fertility status of male individuals Potential infectivity of SARS-CoV-2 in reproductive tissues should be considered in reproductive medicine and management of in vitro fertilization in present and future generations.

Olaniyan OT, Dare A, Okotie GE, Adetunji CO, Ibitoye BO, Bamidele OJ, Eweoya OO. **Testis and blood-testis barrier in Covid-19 infestation: role of angiotensin-converting enzyme 2 in male infertility.** J Basic Clin Physiol Pharmacol. 2020 Oct 5;31(6):/j/jbcpp.2020.31.issue-6/jbcpp-2020-0156/jbcpp-2020-0156.xml. doi: 10.1515/jbcpp-2020-0156. PMID: 33006953.
https://www.degruyter.com/view/journals/jbcpp/31/6/article-20200156.xml
Available pieces of evidence have now shown that SARS-COV-2 is not usually restricted to the respiratory system alone but may also attack other vital tissues in the body. This could explain in part its passage into the testicular microcirculation where reduced blood flow and presence of its receptor (ACE2) could enhance testicular infection and adversely affect male fertility. However, coronaviruses will rarely get into the testis, —hence, with the alarming global cases of COVID-19 infections, there is a potential risk of significant reproductive disturbance that could occur in severe cases. This can result from the similar characteristics exhibited by SARS-COV-2 with other viruses (such as ZIKA, MERS-COV, HEC67 N) attacking the testis, ability to remain in the testicular genome for a longer period and the possibility of reactivation in the future during immune-suppression and cellular stress. Therefore, it is highly recommended to enhance the continuous prevention of SARS-COV-2 transmission and identification of people who are or have been infected to have their fertility tested. Since the virus may hide from been identified by the immune system, thereby hindering its complete clearance from the body system even when the patient recovers from illness. Also, the health care providers should take cognizance of fertility during care especially in male patients of reproductive age.

Youssef K, Abdelhak K. **Male genital damage in COVID-19 patients: Are available data relevant?** [published online ahead of print, 2020 Jun 21]. Asian J Urol. 2020;10.1016/j.ajur.2020.06.005. doi:10.1016/j.ajur.2020.06.005
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7306201/
Over the past few weeks, we have observed increasing concern about the possible impact of coronavirus disease 2019 (COVID-19) which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus) on male fertility. Precise mechanisms of male reproductive damages are still unclear, but it seems that high temperature resulting from persistent fever and triggering a secondary autoimmune response leading to an autoimmune orchitis are the most likely involved mechanisms. Also, angiotensin conversion enzyme 2 (ACE2) plays a highly important role in cellular entry for SARS-CoV-2 and male genital system presents high ACE2 expression. All these preliminary findings suggest that COVID-19 could impact men's reproductive

health. Thus, we examined available data including published and unpublished articles to assess the potential risk of COVID-19 in particular on the male reproductive system.

Li R, Yin T, Fang F, et al**. Potential risks of SARS-CoV-2 infection on reproductive health**. Reprod Biomed Online. 2020;41(1):89-95. doi:10.1016/j.rbmo.2020.04.018
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7192111/
The outbreak of 2019 novel coronavirus disease (COVID-19) has become a major pandemic threat worldwide. Such a public health emergency can greatly impact various aspects of people's health and lives. This paper focuses on its potential risks for reproductive health, including the reproductive system and its functioning, as well as gamete and embryo development, which could be affected by the virus itself, drug treatments, chemical disinfectants and psychological effects related to panic during the COVID-19 outbreak.

Achua JK, Chu KY, Ibrahim E, Khodamoradi K, Delma KS, Iakymenko OA, Kryvenko ON, Arora H, Ramasamy R. **Histopathology and Ultrastructural Findings of Fatal COVID-19 Infections on Testis**. World J Mens Health. 2020 Nov 3. doi: 10.5534/wjmh.200170. Epub ahead of print. PMID: 33151050.
https://wjmh.org/Synapse/Data/PDFData/2074WJMH/wjmh-38-e56.pdf
Purpose: To evaluate the presence and analyze the pathological changes within the testes of patients who died or recovered from severe acute respiratory syndrome coronavirus 2 (COVID-19) complications. *Materials and Methods*: Testis tissue was collected from autopsies of COVID-19 positive (n=6) and negative men (n=3). Formalin-fixed paraffin-embedded tissues were stained with hematoxylin and eosin (H&E) and subjected to immunofluorescence for angiotensin-converting enzyme 2 (ACE-2) expression. Fluorescent-labeled tissue slides were imaged on a quantitative pathology scope with various zoom levels allowing for qualitative and quantitative interpretation. Tissue from four CO- VID-19 positive autopsy cases and a live seroconverted patient was imaged with transmission electron microscopy (TEM).
Results: H&E histomorphology showed three of the six COVID-19 biopsies had normal spermatogenesis while the remain- ing three had impaired spermatogenesis. TEM showed the COVID-19 virus in testis tissue of one COVID-19 positive autopsy case and the live biopsy, H&E stain on the same autopsy case demonstrated interstitial macrophage and leukocyte infiltration. Immunofluorescent stained slides from six COVID-19 positive men demonstrated a direct association between increased quantitative ACE-2 levels and impairment of spermatogenesis.
Conclusions: The novel COVID-19 has an affinity for ACE-2 receptors. Since ACE-2 receptor expression is high in the testes, we hypothesized that COVID-19 is prevalent in testes tissue of infected patients. This study suggests the male reproductive tract, specifically the testes, may be targets of COVID-19 infection. We found an inverse association between ACE-2 receptor levels and spermatogenesis, suggesting a possible mechanism of how COVID-19 can cause infertility.

Pacchiarotti A, Frati G, Saccucci P. A **surprising link with unexplained infertility: a possible Covid-19 paradox?**. J Assist Reprod Genet. 2020;37(11):2661-2662. doi:10.1007/s10815-020-01911-6
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7447604/
lockdown and smart working during the pandemic have increased the frequency of sexual intercourse and could have unmasked the real cause of infertility or unexplained subfertility in these couples. It seems unlikely that a reduction of psychological stress would have contributed to the success to the natural conception of a baby in these seven couples, because of the extremely high stress levels felt in Italy during these months, in which more than 25,000 deaths due to COVID-19 were reported.
Our observation seems to suggest that insufficient sexual activity should always be ruled out in couples referring to reproductive centers for fertility issues. It is interesting to note that couples confined to home, naturally increase the amount of sexual intercourse. Clinicians probably should resist to the temptation to

begin an active treatment and not immediately propose medical treatments in these couples. The real impact of insufficient sexual activity on unexplained infertility warrants further clarification in larger studies.

Verma S, Saksena S, Sadri-Ardekani H. **ACE2 receptor expression in testes: implications in coronavirus disease 2019 pathogenesis.** Biol Reprod. 2020;103(3):449-451. doi:10.1093/biolre/ioaa080
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7314215/
Expression of angiotensin-converting enzyme 2, receptor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is high in the testes, therefore SARS-CoV-2 infection and its association with male reproductive health should be investigated in male coronavirus disease 2019 patients.
Although SARS-CoV-2 is not detected in the semen of recovered men, it does not rule out the possibility of testicular infection during the early and symptomatic stage of disease when other organs including heart and lung are infected. At least three studies have reported the presence of SARS-CoV, another pathogenic coronavirus, in the testes [9]. In the study by Xu et al. [9], testes from all six SARS-CoV patients displayed signs of orchitis and testicular damage including germ cell depletion and apoptotic death, and leukocyte infiltration in the interstitium. These findings set a precedent of coronavirus-induced orchitis and testicular dysfunctions.
Since ACE2 expression is high in testis, we speculate that during peak viremia, SARS-CoV-2 reaches testicular interstitium via blood route and LC might be one of the first target in the testes (Figure 1B). Virus infection in the LC may cause alterations in the steroidogenic pathways that may also explain the LC dysfunction and decreased serum T/LH ratio as reported by Ma and colleagues [8]. In addition, virus may infect SC, another ACE2 expressing cell type in the seminiferous tubules. Cytokines and chemokines induced by SARS-CoV-2 entry into the LC and SC may recruit peripheral immune cells including macrophages and virus-specific T cells that may further potentiate inflammation and orchitis in accordance with reported symptoms from 19% of patients in study by Pan et al. [6] but may also facilitate virus clearance. Since orchitis is not a common symptom reported in COVID-19 males, it is likely that the immunosuppressive properties of SC and testicular macrophages may play a critical role in suppressing inflammation and limiting virus-associated testicular damage unlike other organs. However, SARS-CoV-2-associated inflammation might transiently affect blood–testis barrier (BTB) integrity that may affect spermatogenesis.

Singh B, Gornet M, Sims H, Kisanga E, Knight Z, Segars J. **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and its effect on gametogenesis and early pregnancy**. Am J Reprod Immunol. 2020;84(5):e13351. doi:10.1111/aji.13351
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7537037/pdf/AJI-9999-e13351.pdf
SARS-CoV-2 infection and pregnancy has been the topic of hundreds of publications over the last several months, however, few studies have focused on the implications of infection in early pregnancy and reproductive tissues. Here we analyzed available evidence pertaining to SARS-CoV-2 infection, early pregnancy, and reproductive tissues. We searched PubMed and Embase databases in accordance with guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for publications from inception to June 4, 2020. Four reviewers screened titles and abstracts, and obtained full text articles for analysis. 62 studies were included in the review. Biological plausibility for infection with SARS-CoV-2 exists in testis, ovaries, and placenta as they express ACE2 receptor activity. In males, SARS-CoV-2 infection could lead to functional abnormalities leading to spermatogenic failure and male infertility. In females, an alteration of the ACE2 cascade via SARS-CoV-2 infection could lead to impairment in important follicular and luteal processes. There is also evidence of significant placental pathology in SARS-CoV-2 infection, but it is unclear what effects there may be for early pregnancy, though available data suggest less severe effects compared to other respiratory virus outbreaks. Further investigation is needed regarding SARS-CoV- 2 in reproductive function and early pregnancy.

Paoli D, Pallotti F, Turriziani O, et al. **SARS-CoV-2 presence in seminal fluid: Myth or reality** [published online ahead of print, 2020 May 26]. Andrology. 2020;10.1111/andr.12825. doi:10.1111/andr.12825
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7283802/pdf/ANDR-9999-na.pdf
Great concerns have been raised on SARS-CoV-2 impact on men's andrological well-being and one of the critically unanswered questions is whether it is present or not in the seminal fluid of infected subjects. The expression of ACE2 and TMPRSS2 in the testis and in the male genital tract allows speculations about a possible testicular involvement during the infection, possibly mediated by local and/or systemic inflammation that might allow a high viral load to overcome the haemato-testicular barrier. To date, few investigations have been carried out to ascertain the presence of SARS-CoV-2 in the seminal fluid with contrasting results. Furthermore, the cumulative number of subjects is far too low to answer the question unambiguously. Therefore, great caution is still needed when evaluating this data, otherwise we risk unleashing unmotivated concerns in the scientific world with troublesome consequences in reproductive medicine.

Li D, Jin M, Bao P, Zhao W, Zhang S. **Clinical Characteristics and Results of Semen Tests Among Men With Coronavirus Disease 2019** [published correction appears in JAMA Netw Open. 2020 Jun 1;3(6):e2010845]. JAMA Netw Open. 2020;3(5):e208292. Published 2020 May 1. doi:10.1001/jamanetworkopen.2020.8292
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7206502/
This cohort study examines the clinical characteristics of men with coronavirus disease 2019 whose semen tested positive for severe acute respiratory syndrome coronavirus 2.
Introduction
In December 2019, an outbreak of pneumonia associated with coronavirus disease 2019 (COVID-19) occurred in Wuhan, China, and rapidly spread to other parts of China and overseas.1 It has been confirmed that COVID-19 has the characteristic of human-to-human transmission, mainly through respiratory droplets and contact. Other routes require further verification. The virus responsible for COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been detected in stool, gastrointestinal tract, saliva, and urine samples.2 However, little is known about SARS-CoV-2 in semen.
Methods
This cohort study was performed after patients gave written informed consent for research purposes, and in compliance with the Helsinki Declaration3 with the approval of the ethics committee of Shangqiu Municipal Hospital, Shangqiu, China. This study is reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.
We identified all male patients with laboratory-confirmed COVID-19 aged 15 years and older between January 26, 2020, and February 16, 2020, in Shangqiu Municipal Hospital, which is the only designated hospital for the treatment of COVID-19 in Shangqiu, in the east of Henan province. Following guidance from the World Health Organization,4 laboratory confirmation for COVID-19 was defined as positive result for SARS-CoV-2 in real-time reverse transcriptase–polymerase chain reaction assay of nasal and pharyngeal swabs.1 Enrolled patients were asked to provide a semen sample for SARS-CoV-2 testing.
Groups were compared using the t test, $\chi 2$ test, or Mann-Whitney or Kruskal-Wallis test. All statistical analyses were performed using SPSS statistical software version 19 (IBM). P values were 2-tailed, and $P < .05$ was considered to indicate significant differences.
Results
Among 50 patients identified, 12 patients were unable to provide a semen specimen because of erectile dysfunction, being in a comatose state, or dying prior to recruitment; therefore, a total of 38 patients were enrolled for semen testing. Of these 38 participants who provided a semen specimen, 23 participants

(60.5%) had achieved clinical recovery and 15 participants (39.5%) were at the acute stage of infection. Results of semen testing found that 6 patients (15.8%) had results positive for SARS-CoV-2, including 4 of 15 patients (26.7%) who were at the acute stage of infection and 2 of 23 patients (8.7%) who were recovering, which is particularly noteworthy. But there was no significant difference between negative and positive test results for patients by age, urogenital disease history, days since onset, days since hospitalization, or days since clinical recovery.

Dutta S, Sengupta P. **SARS-CoV-2 infection, oxidative stress and male reproductive hormones: can testicular-adrenal crosstalk be ruled-out?** J Basic Clin Physiol Pharmacol. 2020 Sep 7;31(6):/j/jbcpp.2020.31.issue-6/jbcpp-2020-0205/jbcpp-2020-0205.xml. doi: 10.1515/jbcpp-2020-0205. PMID: 32889794.
https://www.degruyter.com/view/journals/jbcpp/31/6/article-20200205.xml
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus, reportedly first identified in December 2019 in the Wuhan city in China [1]. It causes coronavirus disease 2019 (COVID-19), which has been declared a global pandemic by the World Health Organization (WHO) on 11th March 2020 [1]. Surprisingly, men are more vulnerable to this disease compared to women and the underlying causatives for this phenomenon remain elusive [2].
Angiotensin converting enzyme-2 (ACE2) receptor aids the entry of SARS-CoV-2 into the host cells and thus, is a key role player in COVID-19 pathogenesis. ACE2 expressions have been found in various organs including the endocrine glands and gonads [3]. Nevertheless, cells expressing higher levels of ACE2 are rendered more susceptible to COVID-19 [3]. The transmembrane protease, serine-2 (TMPRSS2) is a major protease mediating the priming of the spike proteins of this virus with the target host cell receptor, and mainly cleaving the ACE2 receptor [3]. Moreover, the striking observation of testes being among the body tissues with the highest ACE2 expressions, indicate associations of SARS-CoV-2 infections with male reproductive dysfunctions [3]. ACE2 mRNA and protein are profoundly expressed in the seminiferous duct cells, spermatogonia, Leydig cells and Sertoli cells [4]. Moreover, distinctly high ACE2 expression in testicular cells, while comparatively low expression levels of ACE2 in ovarian cells [2], may also support higher vulnerability of male gonadal functions. Despite the high possibilities of substantial endocrine impacts of SARS-CoV-2-infection owing to ACE2 expressions in endocrine glands and testicular cells, clinical/pre-clinical data in support of the hypotheses are still lacking. This article aims to precisely present whether SARS-CoV-2 infection operates via the primary endocrine-reproductive axes, the hypothalamic-pituitary-testicular (HPT) and hypothalamic-pituitary-adrenal (HPA) axes, their crosstalk, or the virus directly affects testicular cells to subsequently disrupt male reproductive functions.

Wambier CG, Goren A. **Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is likely to be androgen mediated.** J Am Acad Dermatol. 2020;83(1):308-309. doi:10.1016/j.jaad.2020.04.032
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7151476/
Coronavirus disease 2019 (COVID-19) pandemic fatalities are rare before adrenarche/puberty (<10 years of age), and the vulnerability of males to severe disease1 has been constantly reported over the past months of pandemic.
The first biologic step required for potential infectivity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the priming of the spike proteins by transmembrane protease, serine 2 (TMPRSS2). Although other proteases have been described to activate the spikes in vitro, only TMPRSS2 activity is regarded as essential for viral spread and pathogenesis in the infected hosts.2 TMPRSS2 may also cleave angiotensin converting enzyme 2 (ACE2) for augmented viral entry.3
Androgen receptor activity has been considered a requirement for the transcription of the TMPRSS2 gene because no other known TMPRSS2 gene promoter has been described in humans to date.4 , 5

Male vulnerability may be further enhanced by X-linked inheritance of genetic polymorphisms (androgen receptor and ACE2 genes loci are in chromosome X). Obvious dermatologic signs of hyperactivation of androgen receptors are pattern reduction of density of scalp hair, increased density of facial and chest hair, acne, and oily skin. Theoretically, the hyperandrogenic phenotype might correlate with COVID-19 increased viral load, increased viral dissemination, and severity of lung involvement
Studies are still required for epidemiologic conclusions. Acknowledging the importance of androgens during the COVID-19 pandemic may offer another targeted therapy for trials, with androgen suppression to reduce host vulnerability when infection risk is high.

He W, Liu X, Feng L, Xiong S, Li Y, Chen L, Li Y, Wang G, Li D and Fu B (2020) **Impact of SARS-CoV-2 on Male Reproductive Health: A Review of the Literature on Male Reproductive Involvement in COVID-19.** Front. Med. 7:594364. doi: 10.3389/fmed.2020.594364
https://www.frontiersin.org/articles/10.3389/fmed.2020.594364/full
Coronavirus Disease 2019 (COVID-19) has created a global pandemic. Global epidemiological results show that elderly men are susceptible to infection of COVID-19. The difference in the number of cases reported by gender increases progressively in favor of male subjects up to the age group ≥60–69 (66.6%) and ≥70–79 (66.1%). Through literature search and analysis, we also found that men are more susceptible to SARS-CoV-2 infection than women. In addition, men with COVID-19 have a higher mortality rate than women. Male represents 73% of deaths in China, 59% in South Korea, and 61.8% in the United States. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the pathogen of COVID-19, which is transmitted through respiratory droplets, direct and indirect contact. Genomic analysis has shown that SARS-CoV-2 is 79% identical to SARS-CoV, and both use angiotensin-converting enzyme 2 (ACE2) as the receptor for invading cells. In addition, Transmembrane serine protease 2 (TMPRSS2) can enhance ACE2-mediated virus entry. However, SARS-CoV-2 has a high affinity with human ACE2, and its consequences are more serious than other coronaviruses. ACE2 acts as a "gate" for viruses to invade cells and is closely related to the clinical manifestations of COVID-19. Studies have found that ACE2 and TMPRSS2 are expressed in the testis and male reproductive tract and are regulated by testosterone. Mature spermatozoon even has all the machinery required to bind SARS-CoV-2, and these considerations raise the possibility that spermatozoa could act as potential vectors of this highly infectious disease. This review summarizes the gender differences in the pathogenesis and clinical manifestations of COVID-19 and proposes the possible mechanism of orchitis caused by SARS-CoV-2 and the potential transmission route of the virus. In the context of the pandemic, these data will improve the understanding of the poor clinical outcomes in male patients with COVID-19 and the design of new strategies to prevent and treat SARS-CoV-2 infection

Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. **SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor**. Cell. 2020 Apr 16;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052. Epub 2020 Mar 5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102627/
The recent emergence of the novel, pathogenic SARS-coronavirus 2 (SARS-CoV-2) in China and its rapid national and international spread pose a global health emergency. Cell entry of coronaviruses depends on binding of the viral spike (S) proteins to cellular receptors and on S protein priming by host cell proteases. Unravelling which cellular factors are used by SARS-CoV-2 for entry might provide insights into viral transmission and reveal therapeutic targets. Here, we demonstrate that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming. A TMPRSS2 inhibitor approved for clinical use blocked entry and might constitute a treatment option. Finally, we show that the sera from convalescent SARS patients cross-neutralized SARS-2-S-driven entry. Our results reveal important

commonalities between SARS-CoV-2 and SARS-CoV infection and identify a potential target for antiviral intervention.

Effects of 2019 novel coronavirus on male reproductive system: a retrospective study
doi:10.20944/preprints202004.0280.v1
https://www.preprints.org/manuscript/202004.0280/v1/download
A novel and highly pathogenic coronavirus (2019-nCoV)-induced pneumonia spread worldwide in a short time. However, studies on the effects of 2019-nCoV on the male reproductive system are limited. The aim of this study is to describe the clinical characteristics of the male reproductive system of COVID-19 patients and to explore the presence of 2019-nCoV in semen.
Retrospective, single-center case series of 112 male patients with confirmed COVID-19 who were admitted to Renmin Hospital of Wuhan University from January 2 to March 7, 2020. Demographic data, symptoms and signs related to the male reproductive system, throat swabs and semen samples were collected and analyzed. 2019-nCoV RNA measured in throat swab and semen samples. The organ distribution of ACE2 mRNA and protein in human tissue on The Human Protein Atlas portal and investigated immunohistochemistry (IHC) images of the testis. The HPA dataset revealed relatively high levels of ACE2 protein and RNA expression in the testis. A total of 3 severe COVID-19 patients (2.7%) presented with orchidoptosis, while no patients experienced other symptoms or signs related to the male reproductive system. The analysis of 2019-nCoV RNA in semen included 17 patients with fertility needs. Among these patients, 9 (52.9%) remained positive for 2019-nCoV according to throat swab analysis, and 8 (47.1%) became negative. In the semen 2019-nCoV analysis, all 17 patients were negative for the N gene and ORF1ab gene. In view of the potential impairment, long-term follow-up for male COVID-19 patients with fertility needs is of great significance.

La Marca A, Busani S, Donno V, Guaraldi G, Ligabue G, Girardis M. **Testicular pain as an unusual presentation of COVID-19: a brief review of SARS-CoV-2 and the testis**. Reprod Biomed Online. 2020;41(5):903-906. doi:10.1016/j.rbmo.2020.07.017
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7377719/
Research question
Can the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus induce testis damage and dysfunction?
Design
This is the description of the case of a young man presenting with heavy testicular pain as the first symptom of COVID-19 infection. A review of the literature is also presented.
Results
SARS-CoV-2 may enter into the host cell by binding to angiotensin-converting enzyme 2. This receptor seems to be widely expressed in different testicular cell types, making possible the occurrence of orchitis in male patients with COVID-19 infection. From a review of the literature, it seems that there is currently no evidence of sexual transmission of SARS-CoV-2; however, the possibility of virus-induced testis damage and dysfunction cannot be excluded.
Conclusions
Further studies are necessary on the pathological effect of SARS-CoV-2 in the male reproductive system and to ensure a proper andrological follow-up for male patients.

Shoar S, Khavandi S, Tabibzadeh E, et al. **A Late COVID-19 Complication: Male Sexual Dysfunction**. Prehosp Disaster Med. 2020;35(6):688-689. doi:10.1017/S1049023X20001223
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7545238/

Since the beginning of the coronavirus infectious disease 2019 (COVID-19) pandemic, an exponentially large amount of data has been published to describe the pathology, clinical presentations, and outcomes in patients infected with the severe acute respiratory syndrome novel coronavirus 2 (SARS-CoV-2). Although COVID-19 has been shown to cause a systemic inflammation predisposing the involvement of multiple organs, its mechanism affecting the urogenital system has not been well-documented. This case report presents the clinical course of two male patients with COVID-19 who developed sexual dysfunction, as anorgasmia, following recovery from the infection. Although no evidence of viral replication or inflammatory involvement could be identified in these cases' urogenital organs, a lack of other known risk factors for anorgasmia points to the role of COVID-19 as the contributing factor.

Youssef K, Abdelhak K. **Male genital damage in COVID-19 patients: Are available data relevant?** [published online ahead of print, 2020 Jun 21]. Asian J Urol. 2020;10.1016/j.ajur.2020.06.005. doi:10.1016/j.ajur.2020.06.005
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7306201/
Over the past few weeks, we have observed increasing concern about the possible impact of coronavirus disease 2019 (COVID-19) which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 virus) on male fertility. Precise mechanisms of male reproductive damages are still unclear, but it seems that high temperature resulting from persistent fever and triggering a secondary autoimmune response leading to an autoimmune orchitis are the most likely involved mechanisms. Also, angiotensin conversion enzyme 2 (ACE2) plays a highly important role in cellular entry for SARS-CoV-2 and male genital system presents high ACE2 expression. All these preliminary findings suggest that COVID-19 could impact men's reproductive health. Thus, we examined available data including published and unpublished articles to assess the potential risk of COVID-19 in particular on the male reproductive system.

Akhigbe RE, Hamed MA. **Possible links between COVID19 and male fertility**. Asian Pac J Reprod 2020; 9(5): 211-214.
http://www.apjr.net/temp/AsianPacJReprod95211-1191939_001951.pdf
The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may have a ripple effect that puts men at a risk of infertility. This article reviews the possible link between SARS-CoV-2 infection and male reproduction following speculations that the single stranded RNA viruses could directly invade the testes. SARS-CoV-2 enters the human lung cells via angiotensin converting enzyme 2 (ACE2). ACEs, its products, angiotensin-(1-7), and its receptor, MAS receptor, are expressed in the testes. Although the binding of SAR-CoV-2 to ACE2 could lead to excess angiotensin II with possible enhanced inflammation, angiotensin II could also promote sperm motility. In addition, the pathophysiology of SAR-CoV-2, especially in relation to male fertility, is yet to be fully understood; the suppression of androgen observed in COVID-19 infected men calls for the need for andrological assessment in infected male.

Napolitano L, Barone B, Crocetto F, Capece M, La Rocca R. T**he COVID-19 Pandemic: Is It A Wolf Consuming Fertility?**. Int J Fertil Steril. 2020;14(2):159-160. doi:10.22074/ijfs.2020.6302
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7382680/

The reported different spillover episodes, the well-established reproductive problems related to coronaviruses in mammals and birds and finally the evidence regarding the presence of ACE-2 receptors in human genital tract does not let us excluding potential reproductive issues in humans. Particular attention should be given to asymptomatic patients who are often the major carriers of the Covid-19 infection (8). It is also necessary to identify all potential clinical presentations and the possible, long-term consequences of

Covid-19 -infection. According to the literature data, a possible reproductive system localization and, particularly spermatozoa localization with possible implications for male fertility, cannot be excluded. Further studies are needed to better define the physiopathology and clinical implications of respiratory virus infections on male fertility.