Abstract | Although many studies have focused on SARS-CoV-2 infection in the lungs, comparatively little is known about the potential effects of the virus on male fertility. SARS-CoV-2 infection of target cells requires the presence of furin, angiotensin-converting enzyme 2 (ACE2) receptors, and transmembrane protease serine 2 (TMPRSS2). Thus, cells in the body that express these proteins might be highly susceptible to viral entry and downstream effects. Currently, reports regarding the expression of the viral entry proteins in the testes are conflicting; however, other members of the SARS-CoV family of viruses — such as SARS-CoV — have been suspected to cause testicular dysfunction and/or orchitis. SARS-CoV-2, which displays many similarities to SARS-CoV, could potentially cause similar adverse effects. Commonalities between SARS family members, taken in combination with sparse reports of testicular discomfort and altered hormone levels in patients with SARS-CoV-2, might indicate possible testicular dysfunction. Thus, SARS-CoV-2 infection has the potential for effects on testis somatic and germline cells and experimental approaches might be required to help identify potential short-term and long-term effects of SARS-CoV-2 on male fertility.

In late December 2019, SARS-CoV-2 was discovered in Wuhan, China. The resulting disease was termed coronavirus disease 2019 (COVID-19). The virus causes similar clinical characteristics to viral pneumonia and went on to kill >70,000 people in the first 50 days of the epidemic. The novel coronavirus quickly spread: at the time of writing, 250 million people have been infected, and 5 million people have died worldwide. Many studies have focused on the effects of SARS-CoV-2 infection in the lungs, and comparatively little is known about the effects of COVID-19 on male fertility.

This Perspectives article will cover the potential risks of infection on somatic and germline cells of the testis, as well as considering other male organs that are susceptible to SARS-CoV-2 infection and that could affect semen parameters as well as considering the models and methods that could be used to examine the effects of SARS-CoV-2 on male fertility.

Characteristics of SARS-CoV-2 and COVID-19 infection

Major risk factors for SARS-CoV-2 infection include being male and aged over 65 years. Comorbidities such as hypertension, obesity, hypercholesterolaemia, diabetes, cardiovascular disease and respiratory diseases seem to significantly worsen the prognosis of COVID-19. COVID-19 is primarily characterized by fever, cough, fatigue and myalgia, which follow a standard course of four main stages: first, upper respiratory tract infection, followed by onset of dyspnoea and pneumonia, then, third, cytokine storm and hyperinflammatory state and, finally, death or recovery. However, a range of symptoms have been reported across multiple organ systems. Gastrointestinal symptoms are reported in 40% of patients and include anorexia, nausea, vomiting and diarrhoea. Neurological manifestations affect 36% of patients and 45% of those with severe infection; these include symptoms of dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, seizures, olfactory disorders and nerve pain. The incidence of cardiovascular manifestations is high and diverse; early studies indicate that 7–20% of COVID-19 patients experience acute myocardial injury, 24% had heart failure and 17% displayed cardiac arrhythmia, which is probably due to a systemic inflammatory response and immune system inhibition during disease progression.

This wide array of symptoms indicates that SARS-CoV-2 is able to access several organ systems through indirect and direct mechanisms, possibly gaining entry to multiple organ systems that express viral entry proteins. The broad tropism of SARS-CoV-2 is also indicated by the detection of the virus in the pharynx, trachea, lungs, blood, heart, vessels, intestines, liver, male genitals, brain and kidneys. Furthermore, many COVID-19 symptoms persist after acute infection (long COVID) suggesting possible tissue damage.

Coronaviruses are large, enveloped, positive single-stranded RNA viruses ranging from 27 to 32 kb and are known to infect humans, other mammals and birds, causing multi-organ system disease. They are split into four types: alpha-, beta-, delta- and gammacoronaviruses, according to their antigenic activity. Seven coronaviruses have been shown to cause human disease characterized by respiratory infection; two alphacoronaviruses — H-CoV-NL63 and H-CoV-229E — and two betacoronaviruses — H-CoV-OC43 and H-CoV-HKU1 — cause mild respiratory infection, whereas three betacoronaviruses — SARS-CoV, MERS-CoV and SARS-CoV-2 — are highly pathogenic. SARS-CoV-2 is genomically similar to SARS-CoV, with which it shares 79.5% of its sequence, and to Middle East respiratory syndrome coronavirus (MERS-CoV), sharing 50% genomic homology. However, SARS-CoV-2 has high transmissibility and infectivity and a low mortality rate compared with both SARS-CoV and MERS-CoV. Two studies have shown that the basic reproduction number of SARS-CoV-2 falls between 2 and 3, whereas the reproduction number of SARS-CoV is between 2.2 and 3.
3.6 (REF.34) and that of MERS-CoV is 2.0–6.7 (REF.35), indicating the higher transmissibility of SARS-CoV-2 (REF.36).

All coronaviruses encode proteins for viral replication and nucleocapsid and envelope formation. The viral envelope is composed of three structural proteins: the matrix and envelope proteins, which are involved in virus assembly, and the spike surface glycoprotein, which mediates viral entry36. These spike proteins form large protrusions on the surface, giving the appearance of a crown.

**Viral entry into cells**
SARS-CoV-2 has the typical coronavirus structure22,36, sharing a high degree of spike homology with SARS-CoV12,23. The spike protein of SARS-CoV-2 mediates viral entry into host cells and is the determinant factor of viral infectivity, host range, tissue tropism and immune response induction34. The SARS-CoV-2 spike protein consists of the S1 subunit, a clover-shaped trimer, and S2 subunits, trimeric stalks linked to the S1 trimer23,36. The receptor-binding domain (RBD) lies on the tip of the S1 head2 and is only loosely connected, enabling the infection of multiple host cells27 [FIG. 1]. The RBDs of SARS-CoV, MERS-CoV and SARS-CoV-2 recognize exopeptidases on the host cell35. Angiotensin-converting enzyme 2 (ACE2) is the critical receptor for cellular entry of H-CoV-NL63, SARS-CoV and SARS-CoV-2 (REF.37), but not MERS-CoV35,39,40,41. The SARS-CoV RBD on the S1 unit binds to the outer surface of the host ACE2, away from its zinc-chelating enzymatic site42–44. Upon exopeptidase binding, entry into the cell depends on cellular proteases; transmembrane protease serine 2 (TMPRSS2) splits the spike protein enabling further penetration of SARS-CoV-2 (REFS35,36).

The entry mechanisms of SARS-CoV-2 have been directly demonstrated in a study showing that the virus uses ACE2 as a cellular entry receptor in multiple species, including humans, and does not use other typical coronavirus receptors such as aminopeptidase N and dipeptidyl peptidase 4 (REFS34,45–47). ACE2 binding is facilitated by furin, a poly-substrate convertase48, which cleaves the SARS-CoV-2 polybasic cleavage site on the viral spike28 causing the RBD to swing out and become available for ACE2 binding. The ubiquitous expression of furin means it has great potential for viral infiltration, and it is commonly hijacked by viruses such as Ebola, herpes and retrovirus. Additionally, furin is transactivated by cytokine stimulation49, possibly allowing increased infectivity during an immune response. SARS-CoV-2 is the only coronavirus to display furin cleavage2, which helps to explain its infectivity.

RNA viruses have a high mutation rate30, which results in changes to protein surface amino acids that can alter viral function and possibly lead to increased infectivity, transmissibility and mortality50. Mutations in the spike protein are of particular interest, as they can enhance viral entry and protein binding, for instance, the D614G mutation increases entrance efficiency through enhanced ACE2 binding affinity54. From September 2020 to June 2021, a total of 71 unique SARS-CoV-2 lineages were identified in England that contributed to SARS-CoV-2 infectivity56. Mutation sites have been identified, distributed across the spike protein sequence of SARS-CoV-2, with the maximum mutation density near the protease cleavage site. Additionally, the viral RBD is associated with 44 known mutation sites57.

The importance of furin, ACE2 and TMPRSS2 in viral entry and infection suggests that environmental factors regulating these proteins could affect SARS-CoV-2 infection and severity. Specific comorbidities — such as hormone dysregulation58–60, increased age61, obesity2, diabetes62 and high blood pressure63 — can substantially increase SARS-CoV-2 risk by altering expression of ACE2, TMPRSS2 or furin64,65. Furthermore, other factors, such as alcohol intake, cholesterol levels61, smoking55 and hormone-altering chemicals66, can also affect COVID-19 severity by potentially dysregulating and aggregating viral entry proteins. Furin is ubiquitous among all human cell types64, thus, infection is primarily driven by ACE2 and TMPRSS2 co-expression35. ACE2 and TMPRSS2 display a broad distribution in various human organs, being co-expressed in the lung, nose, gall bladder, small intestine, large intestine, oesophagus, brain, fallopian tube, testis, heart and kidney. However, SARS-CoV and SARS-CoV-2 have also been shown to enter cells through promiscuous proteases such as cathepsin B/L27–29. Furin is expressed in extracellular vesicles, it might be able to reach tissues distant from where it is expressed, possibly contributing

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**Fig. 1 | Mechanisms of SARS-CoV-2 cellular entry.** a | The transmembrane convertase furin35 cleaves and activates the S1 subunit of the SARS-CoV-2 spike glycoprotein, opening the viral receptor-binding domain (RBD). b | When the RBD is in an open conformation, it can attach to angiotensin-converting enzyme 2 (ACE2)35,37,38. c | Transmembrane protease serine 2 (TMPRSS2) is recruited to cleave the S1 subunit of the SARS-CoV-2 spike protein28, enabling entry. d | After ACE2 binding and protease cleavage, viral entry can occur.
to SARS-CoV-2 infection\(^6\). Although the necessity of TMPRSS2 for SARS-CoV-2 infection is still uncertain, ACE2 expression alongside a suitable protease clearly drives COVID-19 pathology.

**The importance of ACE2 and the RAAS**

ACE2 has a vital role in regulating the renin–angiotensin–aldosterone system (RAAS)\(^7\) (FIG. 2). The RAAS is activated in response to low blood pressure, low fluid volume and specific agonists, such as salt loading\(^7\) and macrophage differentiation\(^8\), resulting in activation of angiotensin receptor 1 (ATR1) to cause vasoconstriction, cell proliferation, inflammation and fibrosis.\(^7\) This process occurs via the angiotensin-converting enzyme–angiotensin I (ANG I)–angiotensin II (ANG II)–ATR1 axis. ACE2 is activated to mitigate these effects\(^9\) through its effector protein angiotensin-(1–7) (ANG-(1–7)) and major receptor mitochondrial assembly 1 (MAS1)\(^10,11\). The ACE2–ANG-(1–7)–MAS1 axis has a protective effect, causing vasodilation, anti-inflammation, antiproliferation and antifibrosis, which mitigate the effect of ATR1 (REF.\(^9\)).

Common COVID-19 comorbidities such as hypertension, diabetes and heat exhaustion are associated with RAAS overactivation or ACE2 deficiency\(^12\). During RAAS overactivation, ACE2 can be upregulated, providing additional entry points for SARS-CoV and SARS-CoV-2, which enter cells via ACE2 and inhibit its protective response in ACE2-expressing tissues\(^9\). Furthermore, in patients with deficient ACE2 expression, infection could further dysregulate the ACE–ANG I–ANG II–ATR1 and ACE2–ANG-(1–7)–MAS1 axes\(^9\). Several studies\(^7,9,13\) suggest that these viruses became highly lethal because they deregulate a lung-protective pathway. Decreasing ACE2 activity through SARS-CoV-2 binding increases the acute effects of angiotensin\(^14\), which are in line with many COVID-19 symptoms\(^15\).

The RAAS is activated more in men than in women, characterized by higher levels of ACE\(^16\). However, men display lower levels of ACE2 (REF.\(^7\)). This discrepancy might account for the worse COVID-19 outcomes observed in men than in women. The substantial involvement of ACE2 and the RAAS in vascular inflammation and remodelling illustrate the sensitivity of the vascular system to changes in blood pressure and inflammation\(^17\). Organs such as the brain, kidneys and testes are at an increased risk of viral-mediated damage\(^11\). In the brain, which displays similar immune-privilege, gene expression\(^9\) and decreased inflammatory response to foreign antigens to that seen in the testis\(^18\), RAAS overactivation through ACE2 inhibition causes cognitive impairment\(^18\). Alongside the neuro-invasive potential of SARS-CoV-2 (REFS.\(^19,20\)), ACE2 inhibition seems to be integral to understanding SARS-CoV-2-associated neuropathology.

**The RAAS and male fertility.**

The RAAS might also be relevant to male fertility—that RAAS functions in the testes is well established and various aspects of the RAAS, including renin, ACE, MAS and ANG II, are expressed in Leydig cells, germ cells, and sperm\(^21\)–\(^24\). These components are also found in the epididymis, vesicular glands, prostate, seminal vesicles, vas deferens and seminal plasma\(^25\). Additionally, infertile men with impaired spermatogonia displayed lower levels of ACE2, ANG–(1–7) and MAS\(^26\). MAS is also found at the mRNA and protein level of matured sperm in the acrosomal and tail regions and incubation of MAS with asthenozoospermic sperm improves sperm mobility\(^27\). Furthermore, MAS-null rodents exhibit a reduction in testis weight and sperm production\(^25\).

However, the functions of local tissue-specific RAAS might operate independently of the systemic RAAS\(^28\). Notably, the brain, heart and testis display a local RAAS and the testes express a testicular ACE isoform that might have important roles in male fertility\(^29\). The involvement of the RAAS in the testis means that SARS-CoV-2 infiltration and interference with testicular ACE2 could lead to infection, loss of protective effects during inflammatory...
states, and coagulation and inflammation, which can disrupt spermatogenesis and testicular function\(^{10,11}\). Clinical evidence of an aetiologial role of the RAAS in male infertility is currently lacking; however, many SARS-CoV-2-associated symptoms and comorbidities can affect male fertility, such as fever, hypoxia, inflammation, immune-system inhibition, obesity and cholesterol dyshomeostasis\(^{12}\). These interactions further complicate the question of whether SARS-CoV-2 can directly affect the testis via the RAAS or whether effects might be indirect through various immune responses and pre-existing pathological conditions.

**Does SARS-CoV-2 affect testicular function?**

The human testis is a complex multicellular system with the primary function of producing spermatozoa and androgens, the inhibition of which by viral pathogens could have severe effects on testicular function\(^{13}\).

**Testicular cell types**

The testis comprises three major cell types: Leydig, Sertoli and germ cells. The interaction between these cells drives spermatogenesis. Sertoli and germ cells are the primary components of the seminiferous epithelium\(^{14}\). Spermatogonial stem cells (SSCs) are localized in the basal epithelium and are adjacent to the Sertoli cells, which produce various factors, such as glial-derived neurotrophic factor and CYP26B1 to maintain SSC homeostasis\(^{15}\). Notably, Sertoli cells form junctions to create the blood–testis barrier (BTB), which divides the seminiferous epithelium into the basal and adluminal compartments. This division is crucial, as it helps to keep circulating toxicants, systemic immune components, and pathogens away from differentiating germ cells\(^{16}\).

SSCs are undifferentiated, whereas outside the BTB, up to the preleptotene stage\(^{17}\), they remain spermatogonial, but as they progress inwards, SSCs mature towards primary and secondary spermatocytes before becoming spermatids and undergoing spermiogenesis, to ultimately become spermatozoa\(^{18}\). Leydig cells are interstitial and produce testosterone to maintain spermatogenesis\(^{14}\). Testicular macrophages are the most heterogeneous and abundant testicular immune cells; they are phagocytic towards cell debris, foreign particles, and invading pathogens and help to maintain the immunoprivileged state within the testis, increasing in number during inflammation. Other testicular cell types include endothelial cells\(^{19}\), which aid in SSC renewal through the expression of glial-derived neurotrophic factor, fibroblast growth factor 2, stromal cell-derived factor 1, macrophage inflammatory protein 2 and insulin-like growth factor binding protein 2 \(\text{[Ref.}17\text{]}\); and myoid cells\(^{20}\), which provide ultrastructure and express leukaemia inhibitory factor to help maintain SSC homeostasis\(^{12}\). Although all stages of spermatogenesis can be indirectly affected by viral interaction with testicular cells that provide support during proliferation, differentiation and maturation, spermatogonium — which sit outside the BTB — might be at an increased risk of direct viral interaction.

**Viral interaction with the testis**

Viral infections and their induced immune response are poorly understood aetiologial factors that can functionally impair male reproduction, via effects on the testis and epididymis\(^{12}\). Viruses can directly affect spermatozoa, resulting in sperm death, reduction in sperm counts and decreased motility, or can act indirectly by inducing inflammatory cytokine production, inhibiting hormone production and altering the function of the genital organs\(^{22}\).

**Viral entry.** Viral infection of any of the main testicular cell types can negatively affect sperm output and quality (FIG. 3), via mechanisms including BTB breakdown and immune entry, hormone alteration, or a direct effect on spermatogonial\(^{19}\).

Viral entry into the testis normally occurs haematogenously. The BTB provides protection and immune privilege for germ cells; however, some viruses, such as Zika and HIV\(^{127,128}\), can pass through the BTB, eliciting an immune response\(^{19}\). The BTB is markedly different from other cell barriers, such as the blood–brain barrier and the pulmonary epithelial barrier: the BTB is much tighter than most barriers and is made up of multiple types of co-functioning junctions, unlike other barriers that are exclusively comprised of tight junctions\(^{128}\).
Table 1 | Expression of ACE2 and TMPRSS2 in male reproductive cells

| Cell type                  | ACE2 | TMPRSS2 |
|----------------------------|------|---------|
| Non-testicular             | ✓    | ✓       |
| Testis                    | ✓    | ✓       |
| Leydig                    | ✓    | X       |
| Sertoli                   | ✓    | X       |
| Myoid                     | ✓    | X       |
| Stromal                   | ✓    | ✓       |
| Spermatogonia             | ✓    | ✓       |
| Spermatocyte              | X    | ✓       |
| Spermatids                | X    | ✓       |

ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2.

However, the BTB also has some similarities to the epithelial barriers found in the lungs and brain. The tight junction, although not the only barrier in the BTB, is still a mainstay of the barrier; furthermore, the BTB is much more dynamic in nature than barriers found elsewhere. Additionally, the BTB can be modulated by viruses, notably Zika virus and SARS-CoV-2. SARS-CoV-2 has been shown to disrupt and pass through both epithelial and endothelial barriers, such as those found in the lungs and brain, demonstrating a potential means by which SARS-CoV-2 can disrupt normal BTB function, potentially resulting in immune cell infiltration into the seminiferous tubules.

**ACE2 and TMPRSS2 expression.** For SARS-CoV-2 viral entry to occur, ACE2 and TMPRSS2 must be co-expressed in cells. Both are primarily expressed in bronchial transient cells, especially alveolar type 2 cells; thus, the respiratory tract is often the first organ system to be affected and usually incurs the most damage. While in the lungs, SARS-CoV and SARS-CoV-2 show viral targeting of ACE2 expressing cells such as alveolar epithelial cells, vascular endothelial cells and macrophages. Although the full mechanism for COVID-19 pathology is not understood, the destruction of infected cells, breakdown of barriers and binding of ACE2 are fundamental.

The data are conflicting regarding the exact expression of ACE2 and TMPRSS2 in the testis (Table 1) despite both being present. One study has suggested limited expression of ACE2 and TMPRSS2 in testis tissue and no overlapping expression of these genes in testicular cells, indicating that viral entry into testicular cells is unlikely to occur. However, other studies have shown that ACE2 is expressed in the myoid cells, Leydig cells, Sertoli cells and spermatogonia, indicating that these cell types could be highly susceptible to SARS-CoV-2 (Table 1). TMPRSS2 is shown to be expressed in SSC, spermatogonia, spermatocytes and spermatid cells. ACE2 and TMPRSS2 are both expressed in many secondary sex organs, including the prostate, seminal vesicles and adrenal glands. Notably, only one study has failed to show co-expression of ACE2 and TMPRSS2 in any male reproductive tissues (Table 1). Additionally, substantial inconsistencies are seen between mRNA and protein presence of ACE2 and TMPRSS2. In addition, the presence of promiscuous proteases and extracellular vesicular TMPRSS2 means that infection might not solely depend on ACE2 and TMPRSS2 co-expression, requiring only ACE2. Furthermore, RAAS involvement in the testis means that direct infection of testicular cells might not be necessary for the disease to decrease testicular function.

**Hormonal implications.** Alongside the risk of direct infection of Leydig cells, the hypothalamic–pituitary–gonadal (HPG) axis could also be dysregulated by SARS-CoV-2 infection, resulting in altered levels of FSH, LH and testosterone, which can potentially lead to spermatogenic impairment, hypogonadism and infertility (Fig. 4). Many endocrine organs, including the pituitary, thyroid, hypothalamus, pancreas, adrenal glands, pancreas, ovaries and testis, express ACE2 and TMPRSS2. Although hypothalamic SARS-CoV and SARS-CoV-2 infection could theoretically result in decreased GnRH, affecting gonadal hormone levels, this hypothesis has yet to be observed. FSH and LH levels increase with COVID severity, possibly indicating inflammatory activation of gonadotropin-producing cells. The hypothalamic–pituitary–adrenal (HPA) and HPG axes have a reciprocal relationship, whereby they function in a tandem, flexible, and bidirectional manner and any insult to one will impact the other. Additionally, the ACE2-expressive adrenal gland, which has a major role in the RAAS and HPA axis, is negatively affected by both SARS-CoV-2 and SARS-CoV-2 infections, further implicating hormone disruption, possibly through hypothalamic and pituitary interactions.

**Clinical studies in patients with COVID-19**

Many viruses such as HIV, hepatitis B and C, mumps, Epstein–Barr, papilloma and, notably, SARS-CoV, have been shown to cause testicular dysfunction or inflammation (orchitis). SARS-CoV-2 displays similar pathology to SARS-CoV and could cause similar clinical effects, such as germ cell death, orchitis and immune intrusion. Several case reports and studies have noted testicular pain and orchitis associated with SARS-CoV-2 infection. Ediz et al. noted that 10.9% of 91 male patients with COVID-19 reported testicular pain, and Pan and colleagues reported that 19% of 34 male patients with COVID-19 displayed testicular discomfort suggestive of orchitis. In addition, Kim et al. presented a clinical report of a patient with testicular and abdominal pain associated with COVID-19, whilst Bridwell and colleagues presented clinical cases of bilateral orchitis and orcho-epididymitis, respectively. A report by Chen and colleagues noted that 22.5% of 142 male patients had acute orchitis, epididymitis, or epididymo-orchitis associated with COVID-19, and also observed that scrotal infection risk increased with age, with 53.3% of patients over 80 years experiencing testicular symptoms. They also noted that the risk of epididymo-orchitis increased with severity of infection (P = 0.037). Furthermore, a study by Falahieh and co-workers, which observed 20 male patients with COVID-19 at day 14 and day 120 after infection, noted a 34.83% reduction in malondialdehyde and a 31.52% increase in total antioxidant capacity 120 days after infection. After 14 days a 33.1% increase in sperm DNA fragmentation, an increase in leukocytes, and a 31.52% increase in sperm DNA capacity 120 days after infection. After 14 days a 33.1% increase in sperm DNA fragmentation, an increase in leukocytes, and progressive and total sperm motility below the WHO criteria were noted, all of which had returned to normal levels 120 days after infection. However, the majority of men with COVID-19 do not report testicular pain.

Testicular and epididymal autopsy sections from six patients with COVID-19 showed signs of orchitis characterized by interstitial oedema, congestion, red blood cell exudation, increased concentration of CD3/CD68, IgG within the seminiferous tubule, thinning of the seminiferous tubules and a significant increase in apoptotic cells within the seminiferous tubule (P = 0.018). Semen collected from 23 patients with mild or ordinary cases of COVID-19 showed that 39.1% had oligozoospermia and 60.9% had increased seminal leukocytes. The patients also had decreased sperm concentration and increased II-6, TNF and MCP1 compared with men in the control group. Furthermore, assessment of 81 men with moderate-to-severe SARS-CoV-2 infection...
showed significantly elevated serum LH levels (P < 0.0001) and decreased ratios of serum testosterone:LH (P < 0.0001) and FSH:LH (P < 0.0001)\(^\text{180}\). Saliccia et al. and Rastrelli et al. both observed reduced total testosterone in patients with severe COVID-19 and showed a negative correlation with biochemical risk factors such as neutrophil count, lactate dehydrogenase, C-reactive protein, IL-6, D-dimer, pH and procalcitonin\(^\text{186,177}\). Similar effects have been brought on by SARS-CoV\(^\text{178}\). However, although hormonal changes were observed, none of these studies assessed the long-term effects on sperm production in the affected patients.

Reports of testicular discomfort and altered hormone levels in patients with COVID-19 indicate that SARS-CoV-2 might be able to infect testicular cells to directly affect testicular function. Viral presence in semen is a potential indicator of viral entry into the testis through the BTB, although whether SARS-CoV-2 is able to penetrate the BTB remains unclear\(^\text{179}\). Although presence of the SARS-CoV-2 virus in semen is debated, two groups have detected viral RNA in semen\(^\text{103,104,167}\). SARS-CoV-2 presence in the semen would suggest the possibility of sexual transmission of the virus and, importantly, a direct effect on the sperm itself, which are known to express and house ACE2 and TMPRSS2 (REF\(^\text{188}\)).

Testicular origin — rather than penetration of the BTB — is one explanation for viral presence in semen. In this scenario, viral entry might originate from other sources, such as the epididymis, seminal vesicles, prostate and urethra\(^\text{184}\), particularly as expression of TMPRSS2 (and other proteases) has been shown in the seminal vesicles and prostate, as well as the kidneys\(^\text{185,190}\).

Notably, although viral presence in semen is concerning, it does not necessarily indicate a negative effect on fertility. Even so, semen testing should be performed in future studies to ensure that SARS-CoV-2 is not present in the semen and, at the time of writing, many reproductive clinicians have suspended fertility treatments for infertile couples until more evidence is obtained\(^\text{187}\).

Clinical evidence suggesting widespread infection throughout several organ systems related to reproductive function, alongside the reports of testicular pain, orchitis and hormonal changes, justify concerns of a possible effect of SARS-CoV-2 on male fertility\(^\text{157,160,173,175,180}\). Moreover, although the exact testicular expression of ACE2 and TMPRSS2 is debated, their testicular presence has been observed for 20 years\(^\text{186,189}\). The viral modulation of ACE2 and TMPRSS2 through comorbidities and environmental factors\(^\text{186}\) in specific cell types, both within and outside the testis and the BTB, leads to various pathological conditions, such as changes in hormone levels, BTB breakdown, immune response, or direct effects on the germ line, all of which have the potential to affect male fertility.

**Models of potential testicular effects of SARS-CoV-2**

The conflicting reports regarding SARS-CoV-2 entry into the testes cells mean that various informative models need to be considered for future studies in order to rigorously examine the potential effects of SARS-CoV-2 infection on male fertility. A comprehensive approach is essential as evidence of possible long-term effects of SARS-CoV-2 on many organ systems is increasing. The primary need must be to elucidate viral entry into the testis and, if this is confirmed, by what mechanism. A combination of animal, in vitro and cadaver models, guided by data from cohort and clinical studies, will be advantageous (FIG. 5). Animal models can be used to cheaply and efficiently pinpoint at-risk organ systems, whereas in vitro models can be used to investigate how specific testicular cell types might be affected by SARS-CoV-2 infection.

**Cohort studies**

For the clinical data to be useful, patients must be segregated by their age and disease status, most notably asymptomatic and symptomatic. As more information arises regarding the long-term health consequences of COVID-19, the formation of clinical cohorts will become even more important. Sperm and blood serum samples will need to be taken throughout the course of the infection and through several sperm production cycles after viral clearance. These time points will be key to observing any changes in sperm parameters and morphology, hormone levels and immune responses throughout infection, and to determine whether any of these changes persist after viral clearance.

One pilot cohort study of 69 male patients with COVID-19 noted a significant change in sperm parameters in patients with mild and moderate COVID-19: semen volume (P < 0.001), sperm concentration (P = 0.008), total sperm number (P = 0.001), progressive motility (P < 0.001), total motility (P < 0.001) and vitality (P = 0.001)\(^\text{187}\). The conclusions drawn from this study could have been improved through the addition of more outputs such as systemic hormone

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**Fig. 4 | Effects of SARS-CoV-2 on hormone production.** Through angiotensin-converting enzyme 2 (ACE2) inhibition, SARS-CoV-2 infection might alter hormone production. SARS-CoV-2 ACE2 inhibition can cause renin–angiotensin–aldosterone system-mediated overactivation of the hypothalamic–pituitary–adrenal axis, which can interfere with the hypothalamic–pituitary–gonadal axis. SARS-CoV-2 can also directly affect the hypothalamic–pituitary–gonadal axis.
levels, blood pressure, morphological analysis and characterization of immune response; however, it provides a promising springboard for further studies.

Unfortunately, these cohort studies cannot provide any mechanistic data or solid evidence of viral presence in the testis, as tissue samples, in the form of testicular biopsy samples, would be required to confirm SARS-CoV-2 in testicular tissue, but cohort data will be vital for establishing the long-term implications of SARS-CoV-2 infection for male fertility.

**Post-mortem studies**

Post-mortem studies have a long tradition. Specifically in coronaviruses, autopsy reports for SARS and MERS were essential for elucidating their pathology and will be necessary to fully understand the reproductive pathology of SARS-CoV-2 (REFS [196,197]). Only a small number of SARS-CoV-2 patient autopsy studies have been published to date, only ten have examined testicular tissue [198–201]. One of these performed post-mortem examinations on the testis of 12 men who had died with COVID-19. Of these 12 men, 1 displayed normal histology, 2 mild testicular injury, 5 moderate injury and 4 had severe injury. In the 11 patients with abnormal histology, Sertoli cells showed swelling, vacuolation and cytoplasmic rarefaction, as well as detachment from tubular basement membranes, and loss and sloughing into lumens of the intratubular cell mass. The study also illustrated substantial seminiferous tubular injury, reduced Leydig cells and mild lymphocytic inflammation. However, SARS-CoV-2 virus was not found to be present in the testes [199].

A study of five patients aged 51–83 years who had died from COVID-19 showed germ cell sloughing into the lumen, seminiferous tubules characterized in a similar manner to Sertoli-cell-only syndrome, increased apoptotic cells, lymphocyte and macrophage infiltration, and — strikingly — SARS-CoV-2 presence in testicular cells using S1 antibody staining and the identification of coronavirus-like particles in the interstitial space of the testis providing direct evidence of testicular entry and attack by SARS-CoV-2 (REF. 202). Similarly, post-mortem examinations on 6 men who died with COVID-19 showed that 3 of them had impaired spermatogenesis [203]. In one patient, transmission electron microscopy (TEM) showed the presence of SARS-CoV-2 in the testes and haematoxylin and eosin (H&E) staining demonstrated macrophage and leukocyte infiltrations in the same patient. Immunofluorescence studies in all six patients revealed a direct association between increased ACE2 levels ($P < 0.05$) and spermatogenic impairment [204].

Further studies are needed to determine whether SARS-CoV-2 has direct effects on testicular dysfunction or whether the virus acts indirectly through secondary mechanisms such as elevated blood pressure, fever or inflammation triggered by SARS-CoV-2 infection [205]. However, post-mortem studies are associated with several limitations. For example, only patients who had severe infections are studied, which might not reflect accurate pathology in the majority of patients. Furthermore, many of these patients are likely to have other underlying comorbidities and risk factors that have predisposed them to severe infection as well as independent effects on fertility. Finally, death and organ collection generally occurs days to weeks after acute infection and viral clearance could have occurred by the time of organ collection.

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**Fig. 5 | Proposed flow chart for evaluating effects of SARS-CoV-2 on male fertility.** A systematic approach is essential to guide research. To understand possible effects on male fertility, the first step is to determine viral presence in the testes or semen, using human cohort and post-mortem studies alongside in vivo animal models. In clinical studies, patient cohorts should be divided primarily by their age and disease status. If viral entry is observed, a mechanism for viral entry and possible effects on male fertility and testicular function can then be investigated. If viral entry is not observed, this does not rule out an effect on testicular function, which will still require investigation, owing to concerns raised by clinical data and viral involvement in the renin–angiotensin–aldosterone system pathway, which could have indirect effects on fertility.
**In vivo models**

Alongside clinical and cadaver data, animal models are essential for researching disease progression, pathogenesis and immunological response. An optimal animal model of viral effects on male fertility has similar host–pathogen interactions and disease progression to humans and should enable the measurement of parameters such as clinical symptoms, viral growth and spread, and immune response. Thus, various animal models are required to expand pathophysiological relevance and for specific research stages. The animal models that are currently being used to research SARS-CoV-2 were vital for studying SARS and MERS biology; the best-characterized models in use are mice, ferrets, hamsters and non-human primates (NHPs). However, many of these animals display clinical symptoms of SARS-CoV-2 that are much milder and shorter than humans.

Mice were used for studying both SARS-CoV and MERS; human ACE2 (hACE2) transgenic mice have been shown to better model SARS and MERS infection than wild-type mice. SARS-CoV-2 infection in hACE2 transgenic mice was shown to cause weight loss, viral replication in the lungs, interstitial pneumonia, macrophage infiltration and accumulation, interstitial lymphocyte infiltration and viral antigen presence in bronchial epithelial cells, macrophages and alveolar epithelia. None of these pathological conditions was observed in wild-type mice infected with SARS-CoV-2. The hACE2 transgenic model is likely to be valuable for evaluating routes of infection, barrier breakdown, therapies and vaccines of COVID-19. Additionally, the hACE2 transgenic mouse model provides a means of quickly observing the effect of ACE2 inhibition on fertility, the possible degradation of the BTB and viral impact on testicular somatic cells. Ferrets and Syrian golden hamsters are often used to study airborne viruses, particularly SARS, as they display human-like pulmonary characteristics. One study has found viral RNA in the testes of intranasally infected hamsters up to 1 month after infection. However, despite their consistent use in modelling pulmonary viral infections, including SARS-CoV-2, their use in studies of male fertility is minimal. Owing to kinetic and biological differences in spermatogenesis between rodents, mustelids and humans, these models cannot be recommended for standalone use in the study of SARS-CoV-2 and spermatogenesis. Furthermore, animal models are burdened with ethical dilemmas as well as money and time constraints. However, as animals are able to model organs and organ systems better than in vitro models, they remain a suitable model for viral entry, BTB damage and effects on testicular somatic cells.

NHPs have a close phylogenetic and physiological relationship with humans; they are remarkably similar in their susceptibility to infectious disease, viral replication and immunological response and are, therefore, used to model viral pathogenesis, male fertility and their intersection. Various NHP models were successfully used to uncover SARS and MERS pathogenesis and will be useful for elucidating SARS-CoV-2 infection. Although SARS-CoV-2 infection presents with less severe symptoms in NHPs than in humans, a more relevant pathology can be replicated by repeated infections. Additionally, similarities between human and NHP SARS-CoV-2 pathology have been reported. Rhesus macaques and African green monkeys were both shown to display a COVID-19-like disease, which will be useful for elucidating human pathology. Additionally, historical use of NHPs to model viruses that are known to affect male fertility, such as Zika and mumps, suggest that NHPs will be a valuable candidate system for studying SARS-CoV-2 and male fertility.

**In vitro models**

Although animal models are extremely useful, they do not fully recapitulate human processes such as human spermatogenesis; a human-derived in vitro model is necessary to fill these gaps. Two-dimensional culture systems have enabled insights into germ cell, somatic cell, and extracellular matrix interactions and the effects of growth factors, hormones and viruses; however, such monolayer systems often display irrelevant physiology and cell communications that fail to mimic human biological responses. Organoids derived from human primary or stem cells represent a more biologically relevant system than most in vivo and 2D culture systems and are useful for modelling viral infections. Several human testicular organoids have been developed over the past few years. In 2019, the generation of human testicular organoids using a microwell culture system was reported. These organoids display testis-specific morphology and tight junction protein expression, and response to retinoic acid was similar to that observed in the testis in vivo. Germ cells displayed reduced levels of autophagy compared with those seen in 2D models, indicating reduced cellular stress. Overall, this model seems promising for the study of the effect of SARS-CoV-2 on male fertility. Although organoids have been used to model SARS-CoV-2 infection in the brain, they have yet to be used for testicular cells. However, many of these organoids cannot provide an accurate view of viral pathogenicity as they fail to reproduce the complex organization of the testis and recapitulate spermatogenesis.

Testicular explants have been used to show Zika infection of testicular tissue and germ cells, but not widely in SARS-CoV-2. One study has shown viral replication in hamster testes using an ex vivo infection model. However, ex vivo models have been used to model SARS-CoV-2 infection in the lungs. This type of model enables study of an organism’s physiological and pathological environment, including tissue architecture, cell–cell interactions and local immune response. However, these models are best suited for short-term studies and rely on organ donors.

If SARS-CoV-2 is shown to pass through or degrade the BTB or is demonstrated to be present in patient semen, determining the effects of interactions between developing sperm cells and the virus will be essential. However, most of these spermatogenic models discussed above fail to recapitulate human in vivo effects such as germ cell death and cannot sustain chronic viral exposure, which is necessary for studying the effects of viral intrusion. An unbiased high-throughput format has been reported, which mirrored aspects of human spermatogenesis and mimicked the effects of environmental exposures in vivo.

Although this model has so far been used primarily for studying environmental toxicants, such as perfluoroalkyl and polyfluoroalkyl substances and polybrominated biphenyls, it is also well suited to observing the effects of SARS-CoV-2 and other viruses on spermatogenesis, particularly SSCs and early spermatogenic stages. However, as this model represents only spermatogenesis and lacks the somatic cell niche, its utility is limited to studying only the direct effects of SARS-CoV-2 on spermatogenic cells. Additionally, the model is limited by low haploid generation and lacks the capability to produce fully differentiated sperm.

**Conclusions**

The focus of SARS-CoV-2 research has so far been on the lungs and immune response, as these two systems are highly engaged during infection. Although little research has
investigated effects on male fertility, several studies have reported SARS-CoV-2-induced orchitis, altered hormone levels, testicular damage and the possible presence of the virus in sperm. As increasing data indicate the long-term effects of COVID on other organ systems, long-term effects on the tests will also need to be examined. Whether these symptoms arise from the direct effects of SARS-CoV-2 on the tests or whether they are mediated by other mechanisms, such as immune response and inflammation, remains to be determined; alternatively, underlying risk factors for SARS-CoV-2 could even be the ultimate cause. It is essential to remember that any disease state or environmental factor that disregulates furin, ACE2 or TMPRSS2 can affect SARS-CoV-2 infection and its intersection with male fertility. The primary proteins involved in SARS-CoV-2 pathology, particularly ACE2 and TMPRSS2, are expressed throughout the body, including in the tests. Thus, their infection or negative inhibition by SARS-CoV-2 could have severe consequences for male fertility. Although the RAAS is involved in male fertility, the extent to which ACE2 and TMPRSS2 are expressed in the tests is uncertain and requires more research. Several in vivo and in vitro models show promise for studying the effects of SARS-CoV-2 on male fertility. Tandem use of a variety of models along with clinical data will be necessary to fully elucidate the effects of SARS-CoV-2 infection on male fertility. As long-term sequelae of SARS-CoV-2 begin to be examined, viral effects on sperm production and male fertility should be considered to guide research and to modify clinical advice for patients. Multiple justifications exist for a potentially detrimental effect of SARS-CoV-2 on male fertility; however, a multifaceted approach is required for validation.

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