



Male Fertility and the COVID-19 Pandemic: Systematic Review of the Literature

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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.

Keywords: COVID-19; Infertility, male; Pregnancy; Semen; Severe acute respiratory syndrome coronavirus 2

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INTRODUCTION

Coronaviruses (CoVs) belong to the family of Coronaviridae, named based on the presence of antigens in their membrane which give them the characteristic “crown-like” appearance in ultramicrographs (from the Latin *corona* [crown]). Since the first identification in 1965 [1], several CoVs have been identified in animals and humans [2-5] and are currently classified into 46 different species [6]. Although some CoVs can cause minor flu-like symptomatology in humans (*i.e.*, human CoVs 229E and OC43) [7], others, such as the severe acute respiratory syndrome (SARS)-CoV-1 and Middle Eastern Respiratory syndrome (MERS)-CoV, have received significant attention due to their high rate of infections and mortality [8]. On the basis of data reported by the Centers for Disease Control and Prevention (CDC), both SARS-CoV-1 and MERS-CoV infections can cause fever and cough, and be associated with the development of other severe complications, such as diarrhea and pneumonia (<https://www.cdc.gov/sars/about/fs-sars.html>; <https://www.cdc.gov/coronavirus/mers/about/symptoms.html>).

A novel CoV emerged in December 2019, termed SARS-CoV-2 by the International Virus Taxonomy Committee (<https://talk.ictvonline.org/>) [9] due to its genetic similarities to the SARS-CoV-1 virus (79.5%) [10]. The infection has been termed “COVID-19” (coronavirus disease-19), as this is a CoV-related disease that was discovered originally in 2019. This virus has exceeded other CoVs in infection rate, and has spread rapidly from its origins in Wuhan, China [11,12]. SARS-CoV-2 has been identified as a member of the β -coronavirus subgroup, with a positive-sense single strand RNA, located within a nucleocapsid contained in an envelope [11,13,14]. The viral structure includes characteristic spike (S) proteins that are projected from the virion surface and assist viral cell entry, membrane (M) and envelope (E) proteins that assist in viral assembly, and the N protein which forms the nucleocapsid [11]. The S protein mediates viral transfer into host cells, and is composed of two unique subunits, S1 and S2. The S1 domain functions in virus binding to the host cell membrane, while the S2 domain is responsible for the fusion of virus to host cell membranes to facilitate the viral genome in entering the host cell. Viral S proteins undergo proteolytic priming by the transmembrane protease, serine 2 (TMPRSS2) [15]. Many receptors on

the human cell membrane are identified which are involved in S1 protein binding to host cells [11]. The SARS-CoV-2 may gain access to host cells through the angiotensin-converting enzyme 2 (ACE2) receptor, with a higher affinity than reported in SARS-CoV-1 [11,16] (Fig. 1). These receptors are widely expressed in the lungs (particularly type II pneumocytes and macrophages), cardiovascular system, gastrointestinal system, kidneys, neurological tissues, and the testes [10,17,18]. Here, the viruses replicate within the cells, releasing mature virions, which in turn infect new target cells [19].

Evidence strongly suggests human-to-human transmission through droplets spread [10,20]. The median incubation period is estimated to be 5 days following exposure, with a reported range of 2–14 days [21,22]. The typically described presentation includes pyrexia (88%), dry cough (67%), fatigue (38.1%), productive cough (33%), dyspnea (18%), pharyngitis (13%), and headache (13%) [12]. The more severe cases develop pneumonia [10,12]. Complications include acute respiratory distress syndrome, shock, liver dysfunction and secondary infection. Gastrointestinal symptoms, such as abdominal pain, poor appetite, nausea, vomiting, and diarrhoea have also been reported [10,23]. Laboratory results suggest leukopenia and lymphocytopenia [10]. Although the mortality rate is reported at 2% to 3%,

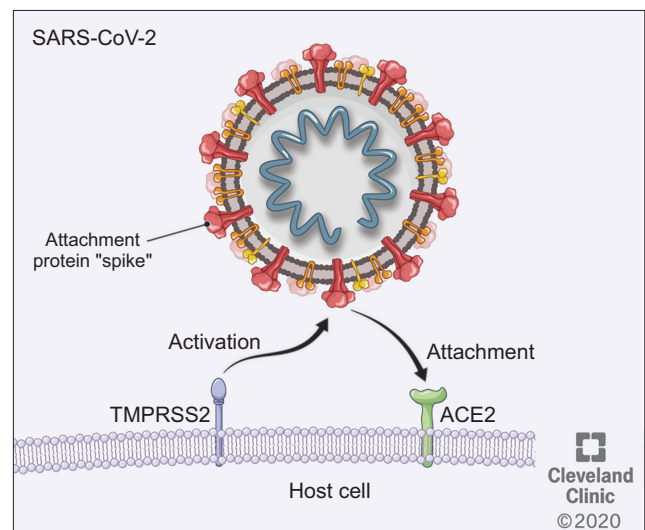


Fig. 1. Cellular entry mechanism. Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection is mediated by the binding between viral spike proteins and angiotensin I converting enzyme 2 (ACE2) cellular receptor, and the further proteolytic cleavage and activation of spike proteins by the transmembrane protease serine 2 (TMPRSS2).

which is significantly lower than MERS, SARS-CoV-2 is expected to result in many more deaths due to its widespread infections [12]. Currently, diagnosis is based on nucleic acid identification in oropharyngeal swabs through real-time polymerase chain reaction and next-generation sequencing [10]. Risk factors for hospitalization includes those who are age older than 60 years, male sex, obesity, smokers, and underlying conditions including hypertension, cardiovascular disease, diabetes, and chronic respiratory diseases [24].

There has been a significant rise in publications and pre-publications on SARS-CoV-2 and COVID-19 with more than 6 million cases worldwide until June 2020. Male patients have a higher risk for infection and symptoms compared to women in both SARS and COVID-19 [14,25,26]. However, any adverse effects on the male reproductive system have been generally under-investigated. This is relevant, as SARS-CoV-1 virus has reportedly been able to impact the testicles and cause viral orchitis [27]. On February 20, 2020, the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) recommended for couples to avoid or postpone pregnancy and assisted reproductive techniques (ART) in cases of suspected subclinical or confirmed COVID-19 diagnosis (<https://www.sart.org/news-and-publications/news-and-research/press-releases-and-bulletins/sart-and-asrmissue-advice-for-infertility-patients-concerning-the-novel-coronavirus-covid-19/>). In the case of other infectious diseases, such as human immunodeficiency virus (HIV), sperm processing techniques including washing steps were reported to significantly reduce seminal viral loads and the probability of infection [28]. However, there is a lack of evidence regarding the possible adverse effect of SARS-CoV-2 on the reproductive system [29].

Therefore, the aim of this study is to systematically review the emerging data on SARS-CoV-2 in order to understand its known and potential impact on the male reproductive system and male fertility.

MATERIALS AND METHODS

1. Review criteria: search strategy

The literature search was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The following keyword string was used to retrieve publications from

the PubMed and Google Scholar databases: (“severe acute respiratory syndrome–coronavirus 2” OR “severe acute respiratory syndrome coronavirus 2” OR “COVID-19” OR “SARS-CoV-2” OR “2019-nCoV” OR “SARS CoV2” OR “SARS CoV 2”) AND (“semen” OR “sperm*” OR “seminal” OR “testes” OR “testicular” OR “male fertil*” OR “male infertil*” OR “epididymis” OR “prostate” OR “testosterone” OR “LH” OR “FSH” OR “pregnancy” OR “ART” OR “assisted reproduc*” OR “IVF” OR “in vitro fertilization” OR “ICSI” OR “intracytoplasmic sperm injection” OR “cryopreservation”).

The literature search was conducted independently by two authors (RF and KL) *via* manual screening of the titles and abstracts of retrieved articles. Original studies conducted in humans evaluating the impact of SARS-CoV-2 infection on semen quality, reproductive hormones and pregnancy outcomes were included in this systematic review without any restriction on publication language. Reviews, meta-analysis and other type of publications not reporting original clinical data were excluded. Also, studies conducted *in vitro* or in animal models and studies investigating the impact of other CoVs on reproductive system were excluded. After the preliminary screening, the full text of the selected articles was checked for eligibility.

RESULTS

1. Search strategy results

A total number of 1,171 articles were identified through the application of the keyword search strategy. Duplicates (n=579) and not relevant articles (n=459) were excluded by screening the title and abstract (Fig. 2). Additionally, 109 articles were excluded after the full-text was screened for eligibility. After screening, 24 articles were considered eligible for inclusion in this study, including studies investigating the impact of SARS-CoV-2 infections on semen parameters (n=6), male reproductive hormones (n=3), and pregnancy outcome (n=15) (Table 1).

2. Severe acute respiratory syndrome-coronaviruse-2 in semen

Six studies investigated semen samples from infected patients [30-34]. Five of the 6 studies involving a total of 82 patients with active or resolving infection failed to detect the presence of SARS-CoV-2 viral RNA in any of the semen samples [30-34]. On the other hand,

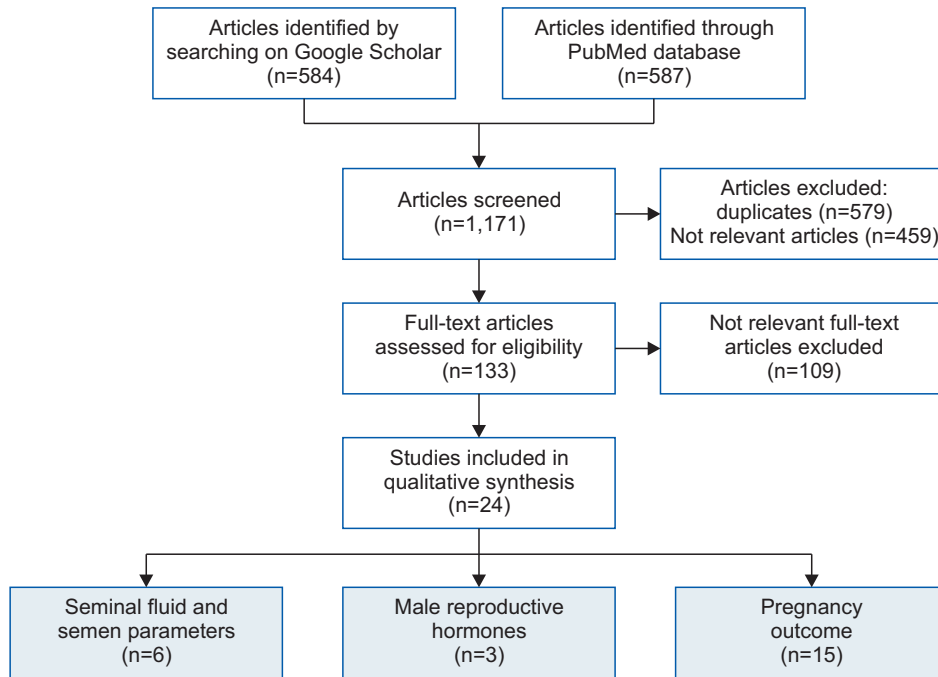


Fig. 2. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) workflow reporting the literature search strategy.
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Li et al [35] tested semen samples from 38 patients and identified SARS-CoV-2 RNA in the semen of 6 patients (15.8%), 4 (66.7%) of whom were in the acute stage of infection and 2 (33.3%) were recovering. Furthermore, the authors did not find any significant difference in clinical characteristics (age, urogenital disease history) or disease progression (days since onset, days since hospitalization, or days since clinical recovery) between patients with negative and positive SARS-CoV-2 infection [33]. Therefore, the data available on a combined number of 120 patients in these 6 studies reveals that the virus is identified in the semen of 6 patients (5%) with COVID-19 infection [30-34].

A single study investigated the impact of SARS-CoV-2 viral infection on semen analysis results [32]. The authors compared conventional semen parameters between 20 COVID-19 patients (18 recovered, 2 active) and 14 healthy volunteers. Patients were sub-classified into mild and moderate groups based on whether hospitalization was required during active infection. Patients with moderate infection had significantly lower sperm concentration ($16.2 \pm 22.4 \times 10^6/\text{mL}$), total number of sperm per ejaculate ($11.9 \pm 13.4 \times 10^6$), total number of motile sperm ($4.7 \pm 5.5 \times 10^6$), and total number of progressively motile sperm ($2.4 \pm 2.7 \times 10^6$) compared to patients with mild infection (sperm concentration: $95.9 \pm 50.5 \times 10^6/\text{mL}$; total number of sperm per ejaculate: $243.7 \pm 140.4 \times 10^6$; total number of motile sperm:

$157.1 \pm 120.8 \times 10^6$; total number of progressively motile sperm: $125.3 \pm 96.4 \times 10^6$) or the control group (sperm concentration: $89.5 \pm 69.6 \times 10^6/\text{mL}$; total number of sperm per ejaculate: $233.1 \pm 234.4 \times 10^6$; total number of motile sperm: $124.0 \pm 124.9 \times 10^6$; total number of progressively motile sperm: $102.1 \pm 102.3 \times 10^6$) ($p < 0.05$ for all).

3. COVID-19 and male reproductive hormones

The impact of SARS-CoV-2 on male reproductive hormones was investigated by three studies. In the first study, Ma et al [36] reported the first evidence linking SARS-CoV-2 infection with disturbances in male sex hormones in a non-peer reviewed study. The authors compared sex-related hormones between 81 reproductive-aged men with SARS-CoV-2 infection and 100 age-matched healthy men. Although serum testosterone was not statistically different between both groups, significantly increased serum luteinizing hormone (LH) levels ($p < 0.0001$) and decreased testosterone:LH ($p < 0.0001$) and follicle stimulating hormone:LH ($p < 0.0001$) ratios were observed in recovered patients compared to the healthy counterparts [36]. Linear regression analysis showed that the testosterone:LH ratio in COVID-19 patients was negatively associated with disease severity ($p = 0.0236$), aspartate transaminase concentration ($p = 0.0287$), and c-reactive protein (CRP) levels ($p < 0.0001$), while a positive association was reported with serum AMH level ($p = 0.0128$) [36].

Table 1. Studies included in this systematic review

Number	Study	Reported outcome	Grade of evidence ^a
Seminal fluid and semen parameters			
1	Li et al (2020) [35]	15.8% of cohort tested positive for seminal SARS-CoV-2; 66.7% acute infectious patients tested positive; 33.3% recovering patients tested positive.	Level 4
2	Ning et al (2020) [34]	All samples were negative for the N gene and ORF1ab gene.	Level 3
3	Holtmann et al (2020) [32]	No detection of virus RNA in all semen samples; Moderate infections showed impaired semen parameters.	Level 3
4	Pan et al (2020) [30]	No detection of the virus in any semen sample; Clinical evidence of orchitis at time of COVID-19 diagnosis in 19% of patients; Single-cell scRNA-seq demonstrates sparse expression of ACE2 and TMPRSS2.	Level 3
5	Paoli et al (2020) [33]	No detection of virus RNA in urine or semen.	Level 4
6	Song et al (2020) [31]	No detection of the virus in any semen sample.	Level 4
Male reproductive hormones			
7	Rastrelli et al (2020) [37]	Low total testosterone levels were observed in the most severe COVID-19 cases.	Level 2
8	Ma et al (2020) [36]	Significantly increased serum LH levels and decreased testosterone:LH and FSH:LH ratios were observed in recovered patients compared to the healthy counterparts.	Level 2
9	Schroeder et al (2020) [38]	Most of the men with COVID-19 had low testosterone and dihydrotestosterone levels, associated with high levels of inflammatory cytokines, such as IFN- γ and IL-2.	Level 3
Pregnancy outcomes			
10	Zhang et al (2020) [50]	No significant intraoperative complications in COVID-19 patients; no difference in fetal birth weight between COVID-19 patients and controls. No detection of COVID-19 infection in newborn infants and development of neonatal complications.	Level 3
11	Li et al (2020) [53]	Significantly higher preterm delivery due to maternal complications in COVID-19 patients. COVID-19 infection was not detected in newborn infants and none developed neonatal complications.	Level 3
12	Liao et al (2020) [51]	No difference in maternal (gestational age, postpartum hemorrhage, and perineal resection rates) and neonatal outcomes (birth weight and neonatal asphyxia rates) reported for COVID-19 patients.	Level 2
13	Futterman et al (2020) [40]	1 patient (22 weeks of gestation) underwent intrauterine fetal demise. 1 patient (29 weeks of gestation) underwent emergency cesarean delivery for non-reassuring fetal status.	Level 4
14	Sentilhes et al (2020) [39]	3 out of 21 pregnant women underwent preterm deliveries due to maternal respiratory complications.	Level 3
15	Knight et al (2020) [41]	5% of the infants were positive for COVID-19 infection.	Level 2
16	Cooke et al (2020) [42]	No detection of COVID-19 infection in newborn infants.	Level 4
17	Govind et al (2020) [43]	1 out of 9 newborns was positive for SARS-CoV-2 RNA.	Level 4
18	González Romero et al (2020) [52]	No detection of COVID-19 infection in newborn infant.	Level 4
19	Yang et al (2020) [46]	No differences were reported in maternal status in the prenatal and postpartum period. No detection of COVID-19 infection in newborn infants.	Level 3
20	Khan et al (2020) [47]	No detection of COVID-19 infection in newborn infants.	Level 4
21	Khan et al (2020) [48]	5 out of 17 pregnant women showed complications. All of them required caesarean section. 2 out of 17 babies were positive to the SARS-CoV-2 RNA, while 5 out of 17 showed neonatal pneumonia.	Level 4
22	Chen et al (2020) [49]	No detection of COVID-19 infection in newborn infants.	Level 3
23	Liu et al (2020) [44]	Successful delivery reported for all pregnant patients, with no neonatal complications.	Level 4
24	Liu et al (2020) [45]	5 out of 13 patients required caesarean section due to complications. No detection of COVID-19 infection in newborn infants.	Level 4

SARS-CoV-2: severe acute respiratory syndrome-coronaviruse-2, ACE2: angiotensin-converting enzyme 2, TMPRSS2: transmembrane protease, serine 2, LH: luteinizing hormone, FSH: follicle stimulating hormone, IFN: interferon, IL: interleukin.

^aGrade of evidence is evaluated based on Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence (<https://www.cebm.net/wp-content/uploads/2014/06/CEBM-Levels-of-Evidence-2.1.pdf>). For more details: <https://www.cebm.net/2011/06/explanation-2011-occebm-levels-evidence/>.

In the second study, Rastrelli et al [37] reported that, in COVID-19 recovered patients, total testosterone levels were negatively related to CRP levels, with low total testosterone levels observed in the most severe cases. In the third study, a non-peer reviewed study from Germany reported that the majority of men with COVID-19 had low testosterone and dihydrotestosterone levels [38]. These results suggest that hypogonadism may be considered as a risk factor for COVID-19, leading to higher morbidity and mortality.

4. COVID-19 and pregnancy outcomes

The coronavirus epidemic also has implications on pregnancy and natural conception. Our literature search identified 15 studies including 598 confirmed cases of SARS-CoV-2 investigating maternal and neonatal consequences of the disease [39-53]. Symptoms of COVID-19 were reported by 98.3% of pregnant women, with fever (64.2%), cough (56.2%), and dyspnea (34.05%) being the most common. Intensive care unit admission was required in 8.5% of pregnant women, and maternal mortality was identified in 0.8% of the cases. Preterm delivery occurred in 33.2% of women, and caesarean section was the most common method of delivery, occurring in 88% of cases. Perinatal mortality was 1.5%, with 2 intrauterine fetal deaths, 4 still births, and 2 neonatal deaths reported out of 528 cases. Low birth weight was reported in 7.6% of neonates, and 30% required neonatal intensive care unit (NICU) admission. Vertical transmission was reported in 2.3% of cases.

Four case-control studies on pregnant women have been published thus far. Zhang et al [50] retrospectively compared sixteen COVID-19-positive with forty-five COVID-19-negative women. None of the cases progressed to severe pneumonia and both infected and controls delivered by caesarean section with no significant difference in the gestational age between both groups [50]. Additionally, no significant intraoperative complications were encountered, and the fetal birth weight was not significantly different between both groups. Li et al [53] compared sixteen COVID-19 confirmed patients with pneumonia to eighteen suspected COVID-19 pneumonia patients who were admitted to labour. Preterm delivery due to maternal complications was reported in 3 patients (18.8%), and was significantly higher than controls (16.7%) [53]. In both studies, COVID-19 infection was not detected in newborn infants and none developed neonatal complications. Liao et al

[51] retrospectively analysed the records of 10 pregnant women diagnosed with COVID-19 and compared their outcomes with 53 pregnant controls. No significant differences in maternal outcomes (gestational age, postpartum haemorrhage, and perineal resection rates) or neonatal outcomes (birth weight and neonatal asphyxia rates) were reported between the two groups [51]. Finally, Yang et al [46] retrospectively reviewed the charts of 55 pregnant women who were suspected to have COVID-19 (13 confirmed cases and 42 controls). No significant differences were reported in peripartum complications, mode of delivery, or neonatal outcomes between the two groups [46].

DISCUSSION

1. Coronaviruses infection and male reproduction

Although there is significant individual variation, some viruses have the potential for ongoing viral shedding though seminal fluid. Ebola viral RNA may be detected in seminal fluid for more than 13 months following infection [54]. Animal models also suggest prolonged shedding of the Zika virus in seminal fluid [55]. Based on the reported evidence, it is still unclear if the SARS-CoV-2 virus originates from testis, epididymis or accessory sex glands or even the renal system.

Other CoVs, such as SARS-1-CoV, have been associated with the development of orchitis. Xu et al [27] reported pathological testicular changes including germ cell damage, thickened basement membrane, and leukocyte infiltration, with few or no spermatozoa in the seminiferous tubules of patients who died for SARS. Viral orchitis may occur as a complication of a viral infection and is typically due to haematogenous dissemination through viremia. Mumps is the most common viral pathology associated with orchitis, presenting as testicular pain, swelling, warmth and erythema of the testes in the acute setting [56]. Additional viruses that are known to causes orchitis include hepatitis B and C viruses, human papilloma virus, herpes simplex virus, influenza virus, Epstein–Barr virus, Coxsackie viruses, HIV, Zika virus, Ebola virus, Arbovirus, and Marburg virus.

Both spermatogenesis and testosterone production may be affected by viral orchitis. Viral infection of testicular tissue is associated with perivascular lymphocytic infiltration and interstitial edema, which can

induce hyalinisation of the seminiferous tubules and fibrosis and atrophy of the testes [57]. In the acute phase of infection, scrotal discomfort and orchidoptosis prevail with variable intensity [56]. Fever, a common symptom in viral illnesses, is well known to cause transient impairment of spermatogenesis [58]. Nonetheless, the persistent reduction in semen parameters that has been reported following post-pubertal mumps orchitis is believed to be due to viral induced damage of the seminiferous tubules [59].

Testosterone has a sex specific protective effect on inflammation and vascular aging [60]. Low testosterone levels in COVID-19 patients are associated with high levels of inflammatory cytokines, such as interferon- γ and interleukin-2 [38]. Since hypogonadism is a common finding in systemic illnesses, it is unknown whether low testosterone levels observed in COVID-19 patients are the cause or the result of severe infection [61,62]. Moreover, testosterone has anti-inflammatory and immunomodulatory properties as it can regulate the differentiation of T-lymphocytes [63]. However, contrary to the potentially protective nature of testosterone, others have suggested that COVID-19 infection may be mediated by testosterone [64]. This is based on the fact that TMPRSS2 is activated by androgen receptors and is believed to be an essential protease for viral spread and transmission [61]. TMPRSS2 can also cleave both ACE2 and viral S protein to facilitate viral entry into the cells [65]. Testosterone mediated modulation of TMPRSS2 expression is believed to be one of the reasons for male predominance of COVID-19 infections [66]. These facts advocate the need for further research investigating the relationship between testosterone levels and COVID-19 infection.

2. Coronaviruses infection and pregnancy outcomes

While the viruses responsible for SARS and MERS had a more limited spread than SARS-CoV-2, their impact on overall human health including natural conception was more severe. In SARS, pregnant women were three times more likely to require mechanical ventilation and had a higher incidence of mortality than non-pregnant women [67]. Miscarriage was observed in more than half of pregnant women who contracted SARS during their first trimester, and 80% of women contracting the disease in the third trimester delivered prematurely [68,69]. While there was no evi-

dence of vertical transmission, infants born to SARS-positive women either developed respiratory distress syndrome requiring surfactant or had fetal growth restriction [68,69].

MERS had a similar impact on natural conception as a high incidence of NICU admissions (64%) and overall mortality (23%) were observed in infected pregnant women [70-74]. A total of 9 cases were reported in the literature to have contracted MERS after their first trimester, showing substantial complications. Complications were reported in 6 patients: One patient had a miscarriage at 20-weeks' gestation [75], one developed pre-eclampsia and had an intrauterine fetal demise, one with pre-existing respiratory issues died from the disease, and three delivered prematurely [71].

3. Angiotensin-converting enzyme 2 in male reproductive tissues

There is limited evidence regarding the effects of SARS-CoV-2 on male reproduction [76]. However, bioinformatic analysis suggests that this virus may potentially infect reproductive tissues. The Human Protein Atlas database (<https://www.ebi.ac.uk/gxa/home>) reports the expression of ACE2 receptors in both male and female reproductive organs [77-79]. This receptor has a significant role in sperm function [80] and in oocyte fertilization [81]. Increased ACE2 receptor expression is reported in testicular tissues, particularly in spermatogonia, Sertoli and Leydig cells, as well as in prostate epithelial cell clusters, fibroblasts and pericytes (Fig. 3). There is a lower expression of ACE2 in

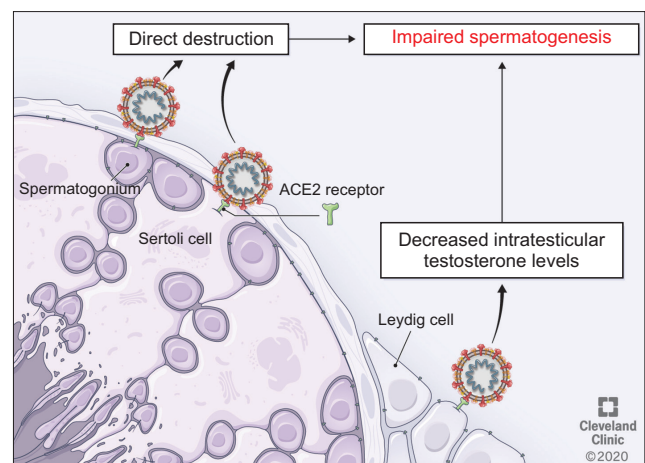


Fig. 3. The putative mechanisms by which severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) might affect spermatogenesis are here summarized. ACE2: angiotensin-converting enzyme 2.

spermatocytes, late spermatocytes, spermatids and other somatic cells [82,83]. Furthermore, a high expression of the protease TMPRSS2 is also reported in germ and somatic cells. Androgens are the only identified transcriptional regulator for TMPRSS2 gene expression [84], due to the presence of a 15 base pair (bp) androgen response element in the TMPRSS2 gene. *In vitro* and *in vivo* studies confirm that castration leads to decreased expression of both ACE2 and TMPRSS2, while androgen supplementation therapy can reverse this decrease [83]. However, TMPRSS2 and ACE2 proteins show a different expression profile, with the former highly expressed in Sertoli cells and the latter in spermatogonial stem cells. This seems to exclude a possible viral infection of testicular tissue, however an interplay between somatic and spermatogenic cells might provide the virus the necessary molecular components to infect the cells.

Wang and Xu [85] reported an enrichment of Gene Ontology (GO) terms related to viral reproduction and transmission in ACE2-positive cells and reduced GO terms related to male reproduction, suggesting a possible disruption of spermatogenesis in case of infection. Moreover, the enrichment of GO terms in ACE2-positive cells related to the organization of cell junctions suggest a viral transmission through the cellular junctions while the reduced GO terms related to mitochondria and reproduction suggest a reduced capability of

Sertoli and Leydig cells to support spermatogenesis [83].

4. Strengths-weaknesses-opportunities-threats (SWOT) analysis

A SWOT analysis has been conducted to assess the available evidence linking the virus with male reproduction and conception (Fig. 4).

1) Strengths

The available evidence demonstrates an impact of COVID19 infection on male and female reproduction (Table 1). Histopathologic studies identifying the expression of ACE2 in testicular tissues have revealed that the SARS-CoV-2 virus can gain access to reproductive tissues. Viral shedding in the seminal fluid has been reported in men with active disease or those recovering from infection. Others have linked SARS-CoV-2 infection with disturbances in male sex hormones [37], or have even identified hypogonadism as a risk factor for worse COVID-19 outcomes [38]. With regards to the impact of SARS-CoV-2 on pregnancy outcomes, the available evidence reveals less severe consequences compared to those witnessed with SARS and MERS.

2) Weaknesses

This pandemic has significantly affected the health care systems of various countries around the globe. To

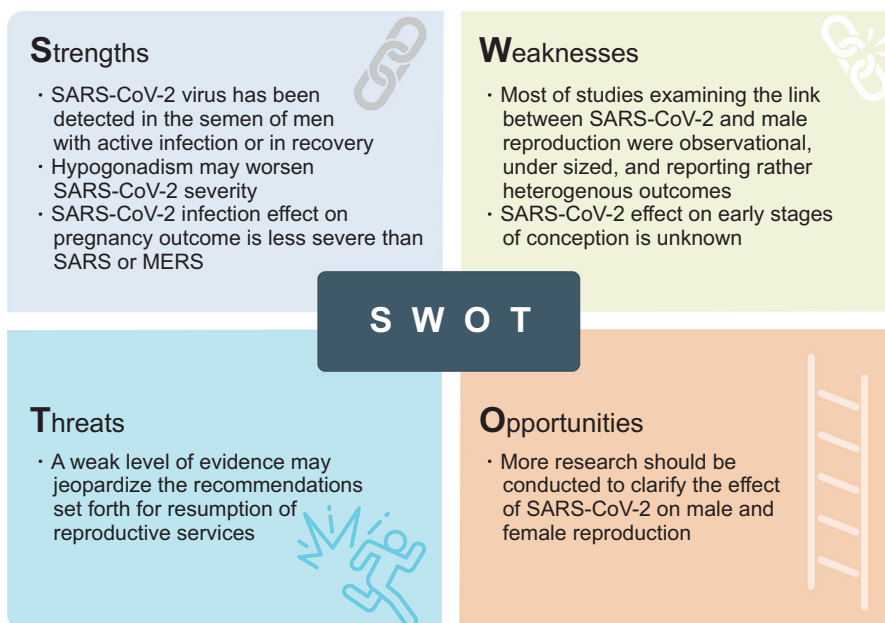


Fig. 4. Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis has been conducted to define if there is an impact of the virus in the male reproduction. SARS-CoV-2: severe acute respiratory syndrome-coronaviruse-2, MERS: Middle Eastern Respiratory syndrome.

facilitate the dissemination of findings on multiple aspects of this novel infection, almost all scientific journals implemented fast tracking for COVID-19 related publications. While such action is needed, the less diligent peer-review process may certainly affect the quality of the published literature. Moreover, few number of studies included are not peer-reviewed.

Most of the published studies examining the link between SARS-CoV-2 and male reproduction were observational, under-sized, and reporting rather heterogeneous outcomes. This may certainly affect the accuracy of the available evidence and, to a variable extent, might influence the creation of optimum recommendations and guidelines. Furthermore, conception studies were mostly conducted in the third trimester or in women at the time of delivery and little is known on the consequences of this infection on the earlier stages of pregnancy at this time.

3) Opportunities

Reproduction is an essential field of medicine and many couples are eagerly waiting to resume treatment. Further research is needed to accurately assess the effect of SARS-CoV-2 on the testis, semen parameters, reproductive hormones, and pregnancy outcomes to help determine when and how couples should begin reproductive treatments again.

4) Threats

Taking into consideration that this pandemic will most likely persist for several years, efforts have been made to propose new standards to healthcare practices. Establishing recommendations to resume healthcare services (and reproductive services in particular) on the basis of feeble evidence could be considered a potential threat, as this may place both patients and healthcare workers at risk of infection or unwanted complications.

5. Assisted reproductive technique practice in COVID-19 pandemic

No research has been conducted to study the impact of any of the CoVs on the outcome of ART. The limited research examining the effect of COVID-19 infection on pregnancy outcomes coupled with previous experiences with other CoVs, led international societies to publish recommendations regarding the practice of ART during this pandemic. On March 17th, 2020, the ASRM published their recommendations for fertility

related therapies during the COVID-19 pandemic [86]. The ASRM advised physicians to “1) Suspend initiation of new treatment cycles, including ovulation induction, intrauterine inseminations (IUIs), *in vitro* fertilization (IVF) including retrievals and frozen embryo transfers, as well as non-urgent gamete cryopreservation. 2) Strongly consider cancellation of all embryo transfers whether fresh or frozen. 3) Continue to care for patients who are currently “in-cycle” or who require urgent stimulation and cryopreservation. 4) Suspend elective surgeries and non-urgent diagnostic procedures. 5) Minimize in-person interactions and increase utilization of telehealth”.

These recommendations have been updated five times since the initial statement and the most recent update was published on June 8, 2020 [87]. In their third update, the ASRM recognized that the COVID-19 pandemic has become a reality that needs to be managed for a prolonged period of time [88]. Since fertility care is an essential health service, the committee proposed measures to resume patient care. After appropriate risk assessment and mitigation, fertility related therapies can be resumed provided that the following measures are applied: social distancing, continued use of telehealth to minimize the number of in-person consultations and to conduct patient counselling and consenting, minimization of the number of in-cycle monitoring visits, performing serologic testing for SARS CoV2 infection on all patients opting for surgery and the application of enhanced or expanded precautions for infection control.

The European Society of Human Reproduction and Embryology (ESHRE) has recognized that since only few cases of COVID-19 infection during pregnancy have been reported, caution is advised with regards to the interpretation of its potential effects, especially since no information is available during the initial stages of pregnancy [89]. A joint statement by the ESHRE, ASRM, and the International Federation of Fertility Societies was published on May 29th, 2020 urging fertility centers to monitor local conditions, including governmental regulations and availability of resources [90]. Prioritized care strategy may be offered provided that risk assessment and mitigation strategies have been implemented to maximize the safety of patients and staff.

The detection of the SARS-CoV-2 virus in semen samples of infected men, even while recovering [33],

Sexual transmission	Natural pregnancy	Assisted reproduction	Cryopreservation
Detection of SARS-CoV-2 virus in semen samples from 5% of men with active disease or in convalescence may indicate the possibility of sexual transmission, though this assumption warrants further investigation.	The impact of SARS-CoV-2 on pregnancy appears to be less severe than other coronaviruses. Preterm delivery and low fetal birth weight are among the most common implications of SARS-CoV-2.	ART during the SARS-CoV-2 pandemic may be conducted after incorporating risk assessment and mitigation strategies and when measures to maximize the safety of patients and staff have been employed.	The SARS-CoV-2 may present a major risk of cross-contamination during cryopreservation



Fig. 5. Hypothesis and evidence regarding the possible severe acute respiratory syndrome-coronaviruse-2 (SARS-CoV-2) sexual transmission, as well as its impact on natural pregnancy, assisted reproduction and cryopreservation are summarized. ART: assisted reproductive technique.

represents a major risk for cryopreservation. It is well known that most viruses can withstand ultra-low temperatures especially when stored in proteinaceous media [91]. In one study, the influenza virus remained infectious after 40 years of cryopreservation [92]. These facts necessitate not only the testing for SARS-CoV-2 virus in semen samples of patients opting for cryopreservation, but also the strict implementation of laboratory safety procedures such as using closed system vitrification with sterile liquid nitrogen or washing and storage in separate cryo-containers [93]. In addition, a recent recommendation from fertility specialists identified patients who might have a transitory “fertility window” and who should be prioritized for cryopreservation [94]. This list includes patients with advanced paternal age, those planning to receive gonadotoxic therapies (cancer, inflammatory or autoimmune systemic disorders), azoospermic men who responded to medical treatment and patients with cryptozoospermia.

6. Future directions and recommendations

In the reproductive field, treatments such as cryopreservation or ART procedures may be considered essential services as well as time-sensitive in case of specific clinical conditions or advanced female age. As the main goals are to guarantee the medical services and, at the same time, the safety of patients and staff, ART centers have been recommended to reconsider their procedures during the early months of the COVID-19 outbreak. While the first reaction to the pandemic has been to interrupt or postpone any treatment, a cautious reopening has progressively begun. Nevertheless, further research regarding the mechanisms of the viral infection is highly warranted. The above evidence as well as the previously described reports of ACE2 expression in the male genital tract suggest that extra

precautions should be taken for COVID-19 patients in order to prevent viral transmission during sexual activity and for handling their semen specimens during cryopreservation and ART procedures. Moreover, the impact of a SARS-CoV-2 infection on reproductive outcomes must be further investigated (Fig. 5). Accordingly, larger studies need to be conducted by including SARS-CoV-2 positive or recovered male subjects of reproductive age. Finally, as the number of pediatric patients might be underestimated, longitudinal studies are required to assess the long-term impact of SARS-CoV-2 infection on testicular function and spermatogenesis in these patients, who may experience potential testicular damage and infertility at a later stage.

CONCLUSIONS

The evidence regarding a putative impact of SARS-CoV-2 infection on male reproduction, as well as the potential of SARS-CoV-2 viral transmission through seminal fluids, remains inconclusive. However, studies conducted on other CoVs suggest potential testicular damage and subsequent infertility mediated via direct viral invasion or secondary immunological or inflammatory response that may negatively affect fertility in adults and future reproductive outcomes in pediatric patients. Currently, extra precautions are strongly recommended for natural or ART-related conception, as clear evidence regarding the impact of the SARS-CoV-2 and the possible complications of COVID-19 on reproductive outcomes require needs additional investigation.

Key points

- 1) Male patients are reportedly slightly more affected than women by both SARS-CoV-1 and SARS-CoV-2 infections.

2) Although the mechanism by which SARS-CoV-2 can gain entry in the cell has been explained, there remains a lack of evidence to support a significant susceptibility of the male reproductive tract.

3) In 5 out of 6 studies, viral mRNA was not detected in seminal fluids of COVID-19 patients, so the potential sexual transmission of SARS-CoV-2 is likely low but requires further investigation.

4) Studies reporting the influence of COVID-19 infection on testosterone are contradictory, although this hormone is generally reported to be lower in COVID-19 patients.

5) Preliminary studies reported that COVID-19 may increase the risk of preterm delivery in pregnant women while reports about vertical transmission are still contradictory.

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Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA, MAK. Writing – original draft: all the authors. Writing – review & editing: all the authors.

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