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RBMO

COMMENTARY

SARS-CoV-2: diagnostic and design conundrums in the context of male factor infertility

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ABSTRACT

The question of whether SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus-2 [SARS-CoV-2], leading to the COVID-19 infection) can be harboured in the testes and/or semen is currently unanswered. It is essential to understand the limitations of both antibody and real-time PCR tests in interpreting SARS-CoV-2 data in relation to analyses of semen and testicular tissue without appropriate controls. This article critically analyses the evidence so far on this, and the possible implications. The limitations of diagnostic tests in both sampling and testing methodologies, their validation and their relevance in interpreting data are also highlighted.

INTRODUCTION

With the mounting global death toll from the COVID-19 pandemic, caused by severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2), data from the UK Office for National Statistics indicate that the mortality rate for men was twice that for women in England and Wales in March 2002 (*ONS, 2020*).

The spread of SARS-CoV-2 led to an immediate shutdown of fertility clinics, primarily to reduce the footfall in hospitals and hence the complications arising from fertility treatment, but also to adhere to social distancing. The Society for Assisted Reproductive Technology has advised that prospective parents, patients undergoing assisted reproductive technology, gamete donors and gestational carriers who meet the diagnostic criteria for SARS-CoV-2 should avoid becoming pregnant or participate in any fertility programme (*ARCS & BFS, 2020*; *SART, ASRM, 2020*). To reinstate fertility services, clinics have received advice from the professional bodies regarding best practice in carrying out risk analyses, use of appropriate personal protective equipment (PPE) and social distancing measures before treatment can recommence (HFEA, ARCS & BFS, *ESHRE 2020*).

SARS-CoV-2 has been detected in respiratory fluids, saliva, gastrointestinal tract samples, blood, faeces and urine (*Wang et al., 2020*). In human reproduction, expression of the SARS-CoV-2 cellular entry receptor, angiotensin-converting enzyme 2 (ACE2), at the human maternal–fetal interface and in the main fetal organs, raises concerns of potential vertical transmission and placental dysfunction/abortion (*Li et al., 2020b*). Several cells in developing human embryos express the receptors for SARS-CoV-2 harbouring the necessary machinery for viral internalization and replication; this raises equal concerns in terms of embryo and fetal development (*Colaco et al., 2020*). In males, ACE2 receptor sites have been reported in testicular tissue that then have the capability to harbour SARS-CoV-2 virus, with eventual shedding into the semen, which has

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KEY WORDS

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implications for sexual transmission, early pregnancy or early in-utero embryonic development. This paper describes the available information on SARS-CoV-2 and male factors.

INFECTION AND THE EFFECTS ON THE MALE REPRODUCTIVE SYSTEM

As SARS-CoV-2 is a novel virus strain, there is little knowledge on the mechanism by which it seems to invade the respiratory system, although information has been drawn from the previous SARS and influenza studies. Therefore, SARS-CoV-2 viral RNA in a specimen cannot be directly interpreted as having a potential for disease transmission and infection. Furthermore, viral RNA can be detected long after the disappearance of the infectious virus. In addition, viral load data are absent from the most recent reports . Viral culture to evaluate viral virulence and activity has so far been absent in clinical practice because of the relatively long turnround time and the low sensitivity for virus detection. The virus can be neutralized by the immune system by lysis of its envelope or aggregation of virus particles, thereby preventing subsequent infection. However, the nucleic acid remains, being degraded over time. The presence of nucleic acids in PCR alone cannot be used to define viral shedding or infection potential as experienced with other viruses such as SARS-CoV, Middle East respiratory syndrome coronavirus, influenza virus, Ebola virus and Zika virus (*Atkinson, 2020*).

The main SARS-Cov-2 entry point into cells appears to be via the viral spike (S) protein attaching to the ACE2 receptor and employing the cellular transmembrane serine protease TMPRSS2 (*Hoffmann et al., 2020*). Both ACE2 and TMPRSS2 are also present in the testis, so testicular infection and subsequent sexual transmission is gaining interest among scientists. Studies analysing SARS-CoV-2 in seminal fluid or testicular biopsies have so far lacked appropriate controls, and the participants have suffered from predominantly mild infections and have been tested several weeks after the infection, thereby increasing the complexity of interpreting the results. In a study conducted in 34 men at a point 25–75 days after a mainly mild initial SARS-Cov-2 infection, RT-PCR showed that there was an absence of virus in the semen (*Pan et al., 2020*). This may be causally reassuring (*Eisenberg, 2020*), but the results cannot be generalized to men suffering severe infections and also do not account for the limitations of interpretation of PCR testing described earlier.

In contrast, in another study of 38 men providing semen samples (*Li et al., 2020*) 23 (60.5%) had clinically recovered, while 15 (39.5%) were in the acute stage of the infection. Semen was SARS-CoV-2 positive in six patients (15.8%), including four of 15 patients (26.7%) who were in the acute stage of the infection. However, the other two men came from the 23 men in the group who were recovering (accounting for 8.7% of this group), which is particularly noteworthy (*Li et al., 2020*). Other viruses such as Zika, human immunodeficiency virus (HIV) and cytomegalovirus are found in semen, and it is possible for viral shedding to occur if viral loads are high because of the severity of the infection (*Salam et al., 2017*). This is especially true if the blood–testes/deferens/epididymis barriers have been damaged by systemic local inflammation. It is unlikely that SARS-CoV-2 could replicate in the male reproductive tract (*Li et al., 2020*)

Considering SARS-CoV-2-infected male patients, is there a possibility that a virus with a size of 70–90 nm could breach the blood–testis barrier? Furthermore, is the mechanism of filtering out SARS-CoV-2 so complex and efficient that ACE2 might be absorbing the entire SARS-CoV-2 virus, in which case should alterations of the ACE-2 receptor site be analysed instead? This particularly applies to the testes, where immune privilege may protect immunogenic germ cells from some systemic viral infections. However, a number of viruses, for example mumps, HIV, human herpesvirus-8, Ebola and Zika viruses, are confirmed as being able to traverse the blood–testis barrier to elicit an immune response within the testicle (*Salam et al., 2017*; *Zhao et al., 2014*). In the case of mumps, men can develop orchitis that is associated with testicular atrophy and azoospermia (*Philips, 2006*). There is also a report relating to SARS infection and orchitis (*Xu et al., 2006*). However, the mere presence of viruses in a semen sample does not mean that the disease is sexually transmitted (*Feldmann, 2018*).

CRYOPRESERVATION AND SPERM DONATION IN THE SARS-COV-2 ERA

On a practical level, this may have an impact for cryopreserving semen for oncology patients and highlights the need to resume fertility services. For men with older female partners, age-dependent subfertility may become irreversible if clinics remain closed except for emergency oncology patients. For this reason, a measured approach is suggested to resume fertility services (*HFEA, 2020*; *Tesarik, 2020*). Likewise, if ovarian stimulation and fertilization were to occur, freezing the resulting embryos and delaying embryo transfer might be another strategy. The use of highly secure devices and segregated cryovessels is suggested, while acknowledging the risks associated with transporting cryopreserved samples between clinics (*Yakass and Woodward, 2020*).

The general problem of recruiting sperm donors in light of the incubation period for SAR-Cov-2 also raises uncertainties. The 2-week suspension from the end of symptoms currently suggested for donors showing respiratory symptoms or those who have returned from high-risk areas seems not to be based on any particular follow-up studies or latency period for SARS-Cov-2 (*La Marca et al., 2020*). No data exist on semen quality following SARS-CoV-2 infection but it is likely that the stress period will increase cortisol concentrations and depress sperm quality, similar to the effects of febrile systemic illnesses (*Chan et al., 2020*). It is important to establish in controlled experiments whether the testis could harbour SARS-Cov-2, and if so to identify its dormancy period, before drafting policies on reproductive health (*Cardona Maya et al., 2020*), while also managing risk for patients and staff (*Anifandis et al., 2020*).

IMPLICATION OF NON-PEER REVIEWED REPORTS ON SARS-COV-2 AND MALE FACTOR INFERTILITY

Against this backdrop are the numerous publications concerning SARS-Cov-2 and reproductive health on the non-peer reviewed MedRxiv platform (https://www.medrxiv.org/), which cautions readers about its use to guide clinical practice or health-related behaviours, while urging

news media to refrain from reporting these.

The non-peer-reviewed papers suggest that there is no clinical evidence on whether SARS-CoV-2 infection can affect male gonadal function. In a study of 81 reproductive-aged men with SARS-CoV-2, serum LH concentration was significantly increased, but the ratios of testosterone to LH and of FSH to LH were dramatically decreased (*Ma et al., 2020*). No semen parameters were reported, and stress and corticosteroid therapy could also have separately affected the hypothalamic–pituitary–gonadal axis. The question of whether SARS-CoV-2 can directly infect the testes or the male genital tract and be sexually transmitted from mildly infected males has also been considered (*Song, 2020*). In this study, the RT-PCR result was negative in 11 semen samples and one testicular biopsy specimen from a deceased 67-year-old man. Although the authors concluded that there was no evidence of sexual transmission of SARS-CoV-2 from males, there were no data to support this.

In addition, there are serious limitations of interpretation, as mentioned above. For example, these studies should have included PCR results from respiratory tissues or ocular fluids in optimally infected cases most likely to show a viral presence. In addition, no SARS-CoV-2 was detected in expressed prostatic secretions of 18 confirmed COVID-19-infected patients and five men strongly suspected to have the infection, but semen analyses were absent. Another Quans paper commented on the safety of sexual intercourse and virus transmission remaining unknown, without providing supporting controlled data (*Quan et al., 2020*). Furthermore, one paper took a leap into the unknown by analysing vaginal fluids from 35 infected female patients for SARS-CoV-2, as a potential mode of sexual transmission; 42.9% of partners were subsequently infected, completely disregarding infections from the lack of social distancing measures (*Cui et al., 2020*). All these studies lack adequate controls, and apparently infected males were analysed well after the infectious period. We have further highlighted the limitation of being able to make interpretations from the diagnostic tests. Despite the warnings issued on using these data, it seems impossible to correct their appearance in the media.

DIAGNOSTIC TESTING AND ITS IMPLICATIONS FOR FERTILITY PATIENTS

Standard testing kits for the public have so far used non-validated procedures for identifying antibodies that may carry up to a 50% false-negative rate (*Blanchard, 2020*). In hospital and research establishments, testing of bodily fluids and tissues is expected to be more sensitive, but these methods have also not been validated for diagnosis of SARS-CoV-2 infection. Caution therefore needs to be exercised in accepting the validity of a SARS-CoV-2 infection test (*FDA1. FOOD AND DRUG ADMINISTRATION 2020*), while the predictive value of a positive (or negative) test result is dependent on the prevalence of antibody-positive individuals in a given population as well as the sensitivity and specificity of the test. In other words, the predictive value of a serological test may be higher where there is widespread infection. To minimize false-positive results, the serological tests must specifically be able to identify antibodies against SARS-CoV-2 and should not 'cross-react' with other antigens or those from similar respiratory viruses (*FDA2. FOOD AND DRUG ADMINISTRATION 2020*). Different types of clinical specimens from SARS-CoV-2-infected individuals tested by RT-PCR have shown a high degree of respiratory tissue positivity for broncho-alveolar lavage fluid (93%), sputum (72%), nasal swabs (63%), pharyngeal swabs (32%), faeces (29%), blood (1%) and urine (0%) (*Wang et al., 2020*). Control data on the specificity and sensitivity of tissue-specific tests with 'spiking' are absent. This limited report provides a glimmer of hope for the blood transfusion sector but, equally, raises questions about offering diagnostic SARS-CoV-2 testing in blood samples versus swab tests.

Overall, little confidence exists in the clinical application of serological antibody tests showing a heterogeneous assay performance. In SARS-CoV-2 RT-PCR-positive individuals, seropositivity showed time dependence and peaked at 81.8–100.0% in samples taken more than 20 days after the onset of symptoms. Test specificity ranged from 84.3% to 100.0% in pre-COVID-19 specimens, and immunoglobulin (Ig) M detection was more variable than that of IgG, with optimal detection when IgM and IgG results were combined. Agreement between two enzyme-linked immunosorbent assays and 10 lateral flow assays ranged from 75.8% to 94.8%. Improving serological tests will require evaluation of the full spectrum of SARS-CoV-2 infections, from asymptomatic or mild infection to severe disease, and later convalescence (*Whitman et al., 2020*).

In contrast, RT-PCR, which is used extensively in diagnostic virology and is expected to give a precise result, is lacking in several key aspects. Such testing for SARS-CoV-2 infection has been validated against previous 2002/2003 SARS viral strains and aided by the use of synthetic nucleic acid technology (*Corman et al., 2020*). Other SARS-CoV RNA has been used as a positive control. Although the sensitivity of the molecular assay for SARS-coronavirus (SARS-CoV) is too low in the early stages of the infection, it improves with severity of infection (*Pan et al., 2020*). These types of tests normally take months rather than weeks to validate. Although work is in progress to improve the diagnostic tests, the limits of using the tests, along with the sample type – fluid or tissue – sampling technique, severity of infection, and above all use of appropriate controls, need to be factored into the results. Positive results from RT-PCR swab kits do not necessarily mean that infection exists as the virus is not known to be alive and active. Sensitivity of RT-PCR in symptomatic patients is approximately 60–95% (*He et al., 2020*), while sample collection, storage and its transport may contribute adversely to the quality of results, increasing false negative results.

Although population testing targets preoccupy the political–public arena, little attention is paid to critical appraisal of the underlying inaccurate diagnostic science, highly commercialized without complete validation. This false reassurance constitutes a major risk factor for the public through free movement of virus-releasing spreaders. Therefore, the best policy is to presume that all patients are infected and to use the best available infection prevention methods and PPE during this pandemic. The limitations of tests also cast doubt on the many publications coming through describing how to interpret a negative test result. To understand the tests, the public needs to understand the underlying science.

The design and validation of SARS-CoV-2 RT-PCR testing has been further improved by the availability of the SARS-CoV-2 genome sequence and the

incorporation of variant viruses from an animal reservoir to theoretically ensure broad sensitivity (*Corman et al., 2020*). Sampling problems contribute to false-negative results, and multiple site sampling may improve test sensitivity (*Zhang et al., 2020*). The rapid introduction of new diagnostic tests and controls during this evaluation study may add to the uncertainty of measurements. In non-pregnant patients, false-negative results ranged from 17% to 63% for SARS-CoV-2 RT-PCT (*Kelly et al., 2020*); there was no clear gold standard test available. Diagnostic test characteristics include sensitivity and specificity, while positive and negative predictive values of SARS-CoV-2 RT-PCR assays are difficult to determine. False-negative RT-PCR testing for SARS-CoV-2 has major clinical implications, especially in pregnant women, in men with suspicion of severe/critical SARS-CoV-2 infection and for the research reports emerging. Two individuals with a history of exposure to SARS-CoV-2-infected patients showed positive results on RT-PCR a day before onset of symptoms (*Pan et al., 2020*), suggesting that infected individuals can be infectious before they become symptomatic.

Finally, the timing of sampling needs to be understood to ensure that optimal virus detection is possible. Unlike the earlier SARS the positivity of the results peaks, the SARS-CoV-2 peaks at around 5–6 days after symptom onset. Sputum samples generally show higher viral loads than throat swab samples, while in the two patients described above, viral RNA was not detected in urine or stool samples (*Pan et al., 2020*); this raises the question of whether severity of illness alters detection at other sites and whether adequate viral spiking controls were performed to rule out sensitivity of testing at these sites. Clearly, as many as 3–5 repeat SARS-CoV2 RT-PCR tests may be required to obtain a positive result (*Pan et al., 2020*).

The World Health Organization recommends two negative SARS-CoV-2 RNA PCR tests, at least 24 h apart, before discharging the patient, although this is not widely practised (*WHO, 2020*). A prolonged presence of SARS-CoV-2 RNA may suggest the potential for viral replication to occur. As the incubation time for SARS-CoV-2 also remains unclear, it is difficult to provide guidance to patients and clinics, especially when fertility services are to resume.

Due to limitations in the accuracy of the diagnostic methods, every man with infertility who is having a semen test and all women should be treated as potentially virus-positive and infective even when test results are negative. SARS-CoV-2 has brought unique challenges to the global reproductive healthcare community, requiring new ways to risk-manage patients (*Anifandis et al., 2020*)

FUTURE OUTLOOK

Unravelling the mechanism of SARS-CoV-2 entry into the semen and testes would help in assessing the early impact on male reproductive function. Equally, the impact of SARS-CoV-2-related stress and fever manifesting in depressed semen quality cannot be underestimated, and possible intergenerational effects should also be taken into account (*Chan et al., 2020*). The limited and poorly reported small studies on seminal plasma and testes lack the adequate controls needed to establish evidence-based guidelines for the public and are unable to produce guidance on sexual practices or reproduction, given that the lack of social distancing appears to be a major risk in performing such studies. The main weaknesses of reports relate to poorly validated diagnostic procedures, lack of controls, mildly infected males tested several weeks after infection, the fact that vital respiratory tissue most likely to harbour viral RNA has never been reported alongside these data, and the lack of control analyses.

CONCLUSION

Proof of SARS-CoV-2 shedding into semen or being present in the testes is still not established and there is a lack of good quality evidence. Therefore, no firm conclusions can be drawn on sexual transmission of SARS-CoV-2 but it makes sense to use barrier protection to minimize sexually transmitted disease in general. The degree of uncertainty of SARS-CoV-2 detection seems to have evaded public scrutiny, while media reporting from mainly non-peer-reviewed reports is unacceptable. Further studies are required at all levels to help improve the quality of evidence in order to understand the effects of SARS-CoV-2 on male fertility and the health of the testes.

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