Minireview

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Perspectives into the possible effects of the B.1.1.7 variant of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on spermatogenesis

Abstract: B.1.1.7 is a recently discovered variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) associated with increased transmissibility. Recent findings indicate that this variant has a propensity to infect adolescents and children at higher rates than adults. The virus gains entry into various body cells utilizing angiotensin-converting enzyme 2 (ACE-2) and basigin (CD147) as receptors. The virus mainly affects type II pneumocytes of lungs, endothelial cells, enterocytes, and renal tubular cells. It is reported to affect testes, causing testicular pain, and producing histopathological changes, as observed in some autopsies. The B.1.1.7 variant can also affect various cells in the testes. This raises a major concern regarding the long-term effects of the viral infection on spermatogenesis and highlights the pressing need for a robust database of serum samples from infected male children.

Keywords: B.1.1.7; COVID-19; infertility; SARS-CoV-2; spermatogenesis; subfertility; testes; variant.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019 in Wuhan, Hubei Province, China, in the respiratory tracts of pneumonia patients. After identifying the pathology in several countries, the World Health Organization declared the coronavirus disease 2019, or COVID-19, a pandemic on March 11, 2020, which has since resulted in over 94.5 million people across the world with confirmed positive cases [1, 2].

Patients with confirmed cases of COVID-19 experience a wide array of symptoms including, but not limited to, fever or chills, cough, dyspnea, muscle aches, anosmia, pharyngitis, congestion, nausea or emesis, and diarrhea [3]. Patients also complained of testicular pain [4]. Histopathology of testes in patients of COVID-19 demonstrated the tubular detachment of Sertoli cells and reduced spermatogenesis [5].

On December 14, 2020, a new, potentially more transmissible variant of SARS-CoV-2, B.1.1.7, also known as variant of concern 202012/01, was discovered and spreading quickly through the United Kingdom and the rest of the world [6]. Unfortunately, this B.1.1.7 variant has a propensity to infect children and adolescents [7].

Our aim is to discuss the potential implications of the B.1.1.7 variant of the SARS-CoV-2 on testes of children and adolescent males and the possibility of subfertility or infertility in the years to come.

Viral structure and entry into host cells

SARS-CoV-2 is an enveloped, single-stranded RNA virus with a characteristic crownlike appearance on the outer surface. This coronavirus has four main structural proteins: (1) the spike (S) glycoprotein, (2) the small envelope (E) glycoprotein, (3) the membrane (M) glycoprotein, and (4) the nucleocapsid (N) protein [8]. The spike protein, a transmembrane protein, allows the virus to bind to host
cells via interactions with angiotensin-converting enzyme 2 (ACE2). This binding permits for viral entry, aided by the widespread expression of ACE2 in various tissues [9]. The ACE2 expression is found in multiple cells in the body, including but not limited to the type II pneumocytes of the lung, alveolar epithelial cells, enterocytes of the small intestine, endothelial cells of blood vessels, sustentacular cells, Leydig cells, and spermatogonia of the testes [10, 11]. In 2020, Wang and colleagues discovered a different pathway of viral entry; this pathway uses basigin (CD147) as the host cell receptor that interacts with the SARS-CoV-2 spike protein [12]. CD147 is a transmembrane protein activated by cyclophilins A and B (CypA and CypB, respectively) [13]. Cells with higher expression of specific membrane proteins, such as ACE2, transmembrane serine protease 2 (TMPRSS2), and CD147, may be more susceptible to a SARS-CoV-2 infection [9, 12–14]. TMPRSS2 cleaving of ACE2 also augments SARS-CoV-2 viral entry [14].

**SARS-CoV-2 effect on spermatogenesis**

Sertoli and Leydig cells are involved in spermatogenesis and express ACE2, TMPRSS2, and CD147 [15, 16]. Leydig cells produce cytokines, growth factors, and hormones—such as testosterone, which play a crucial role in spermatogenesis [17]. TMPRSS2, which can be coexpressed with ACE2, is predominantly present in spermatids and spermatogonial stem cells [18].

ACE2 on Sertoli cells and TMPRSS2 on testicular germ cells will interact during contact between cell types, enabling SARS-CoV-2 viral entry into testicular tissue. Similarly, CD147 is also highly expressed in testes, specifically in Sertoli cells, Leydig cells, spermatocytes, and spermatids [19, 20]. In a study by Bi and colleagues, the CD147 knockout mice showed arrested development at an early-round spermatid stage and increased apoptosis of germ cells. Damage to cells expressing CD147 will compromise the blood–testis barrier (BTB) integrity and result in apoptosis of the germ cells. Thus, CD147’s role is essential for developing germ cells into spermatids [21].

Serum interleukin (IL)-6 levels are elevated in patients with severe SARS-CoV-2 disease resulting in shock, organ failure, and respiratory distress [22]. The dramatic increase in IL-6 production results in hyperinflammation leading to spermatooza damage by producing reactive oxidative species and decreased testosterone production [23, 24]. This oxidative stress can damage the sperm membrane and induce apoptosis overall, affecting sperm quality, sperm function, and motility. Furthermore, an increase in testicular temperature from a fever can increase the sperm DNA fragmentation index, reflecting the amount of DNA damage in the sperm [24]. SARS-CoV-2 is reported to be present in the semen samples of male patients who test positive for the virus. However, after the resolution of SARS-CoV-2 clinical symptomatology, the semen samples tested negative [25, 26].

High fever and inflammatory cytokine response negatively impact spermatogenesis during an active SARS-CoV-2 infection that continues for 72–90 days [27]. Elevation of IL-6 in SARS-CoV-2 patients raises core body temperature and simultaneously decreases the stability of the BTB [28]. Damage to the BTB increased viral entry and subsequently compromised spermatogenesis. The evaluation of testes during the autopsy of SARS-CoV-2-positive individuals revealed decreased sperm concentration, interstitial edema, congestion, and red blood cell exudation compared to the control group [29]. If spermatogenesis is affected, then the cascade’s downstream outcome could result in nonfunctional sperm and negatively affect male reproductive health [30]. A study done by Verma and colleagues in 2020 showed an overall increase in luteinizing hormone (LH) levels and a decrease in testosterone (T) to LH (T/LH) and follicle-stimulating hormone (FSH) to LH (FSH/LH) ratios in SARS-CoV-2-positive male patients, indicating hypogonadism [31].

**SARS-CoV-2 B.1.1.7 variant**

By 2021, two new variants of SARS-CoV-2 were reported by the Centers for Disease Control and Prevention—B.1.1.28 or 20J/501V.V3 (emerged in Brazil) and B.1.351 or 20H/501V.V2 (emerged in South Africa) [6, 32]. The B.1.351 variant has additional mutations (E484K and K417N) relative to the B.1.1.7 variant and includes the N501Y mutation that potentially increases the variant’s transmissibility [33, 34]. However, it is unclear whether these variants, unlike the B.1.1.7 variant, have a propensity to infect children and adolescents.

Despite the variants undergoing frequent mutations, most mutations have no significant effect on the virus’ virulence or structure. The B.1.1.7 variant consists of 14 mutations; the most functionally altering mutation, N501Y, affects one of the six key amino acid residues in the binding domain on the viral spike protein [35]. This missense mutation allows the virus to bind to ACE2 more effectively, as N501Y increases affinity for ACE2 [34, 36]. Thus, this variant of the virus is up to 71% more transmissible than previous variants [7]. This B.1.1.7 variant has a propensity to infect
children and adolescents. The explanation for why this viral variant infects children more readily than adults remains unclear. However, this increased susceptibility of the B.1.1.7 variant toward children may be due to the difference in expression of the ACE2 in children compared to adults [7].

Summary and outlook

Compared to elderly patients and immunocompromised individuals, children and young adults tend to present with milder symptomatology when infected with SARS-CoV-2. Infection may present asymptomatically, and consequently, long-term effects—such as spermatogenesis impairment—may go undetected for several years.

A prospective longitudinal study is required to determine the long-term effects of SARS-CoV-2 on spermatogenesis and its role in male reproductive health, with specific emphasis on the B.1.1.7 variant due to its prevalence among young adults. The semen analysis from patients of COVID-19 demonstrated reduced sperm count that recovered after the symptoms subsided. This finding in adults may not reflect the way in which the virus might affect testes of prepubertal subjects.

Our major concern is that male children affected with SARS-CoV-2 might present with subfertility or infertility a decade or more after being infected with undetected subclinical or mild symptoms of COVID-19. At that time, the serum antibody titer against SARS-CoV-2 might be undetectable, providing no substantial etiological basis for subfertility or infertility.

A repository with a robust database of serum samples from a large number of male children might prove indispensable for providing insight into the potential long-term effects of SARS-CoV-2 on male fertility.

SARS-CoV-2 is directly implicated in spermatogenesis through a multifactorial process partly facilitated by elevated expression of ACE2, TMPRSS2, and CD147 in the testes [9, 13, 14]. The recently discovered B.1.1.7 variant has high transmissibility rates due to a missense mutation on its receptor-binding domain that alters its affinity. In addition to initial reports of its affinity for children and adolescents, this increase in transmissibility has significant implications for future fertility in males infected during prepubertal age. A prospective, longitudinal study on a large sample size is needed to confirm these implications.

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References

1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus – infected pneumonia. N Engl J Med 2020;382:1199–207.
2. Coronavirus (COVID-19) cases. https://ourworldindata.org/covid-cases [Accessed 18 Jan 2021].
3. CDC. Symptoms of Coronavirus. Published January 4, 2021 https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html [Accessed 18 Jan 2021].
4. La Marca A, Busani S, Donno V, Guaraldi G, Ligabue G, Girardis M. Testicular pain as an unusual presentation of COVID-19: a brief review of SARS-CoV-2 and the testis. Reprod Biomed Online 2020;41:903–6.
5. Yang M, Chen S, Huang B, Zhong JM, Su H, Chen YJ, et al. Pathological findings in the testes of COVID-19 patients: clinical implications. Eur Urol Focus 2020;6:1124–9.
6. CDC. Emerging SARS-CoV-2 variants. Published January 29, 2021 https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html [Accessed 23 Feb 2021].
7. Mahase E, Covid-19: what have we learnt about the new variant in the UK? BMJ 2020;371:m4944.
8. Ysrafi A, Al B, Al K, Al S, Al Z, Al M, Al M, Al G, Al L. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): an overview of viral structure and host response. Diabetes Metab Syndr 2020;14:407–12.
9. Younis JS, Abassi Z, Skorecki K. Is there an impact of the COVID-19 pandemic on male fertility? The ACE2 connection. Am J Physiol Endocrinol Metab 2020;318:E878–80.
10. Hikmet F, Mérar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. Mol Syst Biol 2020;16:e9610.
11. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. J Autoimmun 2020;112:102463.
12. Wand K, Chen W, Zhang Z, Deng Y, Lian JQ, Du P, et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal Transduct Target Ther 2020;5:283.
13. Radzikowska U, Ding M, Tan G, Zhakparov D, Peng Y, Wawrzyniak P, et al. Distribution of ACE2, CD147, CD26, and other SARS-CoV-2 associated molecules in tissues and immune cells in health and in asthma, COPD, obesity, hypertension, and COVID-19 risk factors. Allergy 2020;75:2829–45.
14. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281–92.e6.
15. Wang Z, Xu X. scRNA-seq profiling of human testes reveals the presence of the ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, Leydig and Sertoli cells. Cells 2020;9:920.
16. Fok KL, Chen H, Ruan YC, Chan HC. Novel regulators of spermatogenesis. Semin Cell Dev Biol 2014;29:31–42.
17. Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, et al. The roles and mechanisms of Leydig cells and Myoid cells in regulating spermatogenesis. Cell Mol Life Sci 2019;76:2681–95.
18. Navarra A, Albani E, Castellano S, Arruzzolo L, Levi-Setti PE. Coronavirus Disease-19 infection: implications on male fertility and reproduction. Front Physiol 2020;11:574761.
19. Igakura T, Kadomatsu K, Kaname T, Muramatsu H, Fan QW, Miyauchi T, et al. A null mutation in Basigin, an immunoglobulin superfamily member, indicates its important roles in peri-implantation development and spermatogenesis. Dev Biol 1998;194:152–65.
20. Mahdian S, Shahhoseini M, Moin A. COVID-19 mediated by Basigin can affect male and female fertility. Int J Fertil Steril 2020;14:262–3.
21. Bli J, Li Y, Sun F, Saalbach A, Klein C, Miller DJ, et al. Basigin null mutant male mice are sterile and exhibit impaired interactions between germ cells and Sertoli cells. Dev Biol 2013;380:145–56.
22. Huang L, Zhao X, Qi Y, Li H, Ye G, Liu Y, et al. Sepsis-associated severe interleukin-6 storm in critical coronavirus disease 2019. Cell Mol Immunol 2020;17:1092–4.
23. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020;8:420–2.
24. Evenson DP, Wixon R. Clinical aspects of sperm DNA fragmentation detection and male infertility. Theriogenology 2006;65:979–91.
25. Ruan Y, Hu B, Liu Z, Liu K, Jiang H, Li H, et al. No detection of SARS-CoV-2 from urine, expressed prostatic secretions, and semen in 74 recovered COVID-19 male patients: a perspective and urogenital evaluation. Andrology 2020;9:99–106.
26. Li R, Yin T, Fang F, Li Q, Chen J, Wang Y, et al. Potential risks of SARS-CoV-2 infection on reproductive health. Reprod Biomed Online 2020;41:89–95.
27. Segars J, Katler Q, McQueen DB, Kotlyar A, Glenn T, Knight Z, et al. Prior and novel coronaviruses, Coronavirus Disease 2019 (COVID-19), and human reproduction: what is known? Fertil Steril 2020;113:1140–9.
28. Huang C, Ji X, Zhou W, Huang Z, Peng X, Fan L, et al. Coronavirus: a possible cause of reduced male fertility. Andrology 2021;9:80–7.
29. Li H, Xiao X, Zhang J, Zafar MI, Wu C, Long Y, et al. Impaired spermatogenesis in COVID-19 patients. EClinicalMedicine 2020;28:100604.
30. Yan W. Male infertility caused by spermiogenic defects: lessons from gene knockouts. Mol Cell Endocrinol 2009;306:24–32.
31. Verma S, Saksena S, Sadri-Ardekani H. ACE2 receptor expression in testes: implications in coronavirus disease 2019 pathogenesis. Biol Reprod 2020;103:449–51.
32. Galloway SE, Paul P, MacCannell DR, et al. Emergence of SARS-CoV-2 B.1.1.7 lineage—United States, December 29, 2020–January 12, 2021. MMWR Morb Mortal Wkly Rep 2021;70:95–9.
33. Rondinone V, Pace L, Fasanella A, Manzulli V, Parisi A, Capobianchi MR, et al. VOC 202012/01 Variant Is effectively neutralized by antibodies produced by patients infected before its diffusion in Italy. Viruses 2021;13:276.
34. Le Page M. Threats from new variants. New Sci 2021;249:8–9.
35. WHO. SARS-CoV-2 variant – United Kingdom of Great Britain and Northern Ireland. Published online December 21, 2020 https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON304 [Accessed 11 Mar 2021].
36. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KH, Dingens AS, et al. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell 2020;182:1295–310.e20.