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P-073 SARS-CoV2 infection in human testis and sperm: in vivo and in vitro studies

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Study question: Can severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) enter somatic and germinal cells of human testis or ejaculated sperm, thus affecting male reproductive function?

Summary answer: This research provides a biological background of the potential route for infection of SARS-CoV-2 and may enable rapid deciphering of COVID-19-induced male-related reproductive disorders.

What is known already: Epidemiologic studies suggest a significant male sex susceptibility for severe COVID19 symptoms. SARS-CoV-2 is known to affect certain cell types based on their expression of angiotensin-converting enzyme 2 (ACE2) and Transmembrane serine protease2 (TMPRSS2). ACE2 makes available the binding site for the Spike protein of SARS-CoV-2. TMPRSS2 facilitates virus entry by cleaving the S antigen into S1 (the active binding site). Several studies reported the presence of ACE2 in Leydig and Sertoli cells as well as in germ cells from spermatogonia to spermatozoa. These data suggest that the human testis and gametes are a target for SARS-CoV-2.

Study design, size, duration: To address this question, we examined the gene expression profile of SARS-CoV-2-associated receptors and proteases (ACE2-TMPRSS2) as well as their protein expression and localization in testicular tissue of males undergoing diagnostic surgery and in sperm of healthy, normozoospermic donors referring to the Unit of Assisted Reproduction, Siena University Hospital, from April 2020 to January 2022.

Participants/materials, setting, methods: Assays were performed on tissue biopsies (n = 3) or on freshly ejaculated sperm of men (n = 6) undergoing routine semen analysis after granting informed consent. To this end, tissue biopsies and ejaculated sperm have been co-cultivated for 5-12h with infected VERO E6 cells. Immunoelectron microscopy, in situ hybridization, and highly sensitive digital droplet PCR analysis, have been used to assess if particles containing SARS-CoV- 2 antigens can be detected in testicular tissue or spermatozoa *in vitro*.

Main results and the role of chance: We carefully investigated the presence of the SARS-CoV-2 virus and the possible effect on male fertility, both at the molecular and ultrastructural levels. To elucidate the mechanisms underlying virus infection in germ cells and male gametes, we analyzed the expression of both ACE2 and TMPRSS2. Both genes are expressed in testes and ejaculated sperm, confirming the possibility that SARS-CoV2 can enter these cells. We also provided evidence of the expression of the corresponding proteins in both testis and sperm by immunofluorescence assays. The immunofluorescence staining of paraffin-embedded slices of testicular tissue with anti-ACE2 antibody revealed a strong signal in Leydig cells. However, ACE2 staining was also present in human Sertoli cells and was concentrated in the adluminal half of the cell, i.e. surrounding spermatocytes and spermatids.

The analysis of TRPMSS2 showed similar results. Indeed, this protease is localized mainly in the interstitium, at the level of Leydig cells. This is an intriguing datum, since, while Leydig cells may be considered as a high-risk cells because of the co-expression of TMPRSS2 and ACE2, germ cells may not be at increased risk of ACE2and TMPRSS2-mediated viral entry and spread, given the lack of co-expression in these testicular cell type.

Limitations, reasons for caution: The low number of analyzed samples may limit the statistical power of this study. Whether the SARS-CoV-2 infection change the gene expression profile of other SARS-CoV-2-associated proteases is under investigation

Wider implications of the findings: This validated RT-PCR assay allows reliable screening of SARS-CoV-2 in sperm, useful for investigating the presence of the virus in patients undergoing ART, as well as for explaining the molecular and cellular mechanisms related to the gender specificity of the more severe infection-related symptoms.

Trial registration number: Not applicable