

Peer Review File

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Reviewer A

The authors are seeking to evaluate potential impacts of mild COVID-19 infection on healthy males' semen parameters, sex-hormones, urinary symptoms, and erectile function. Specifically, regarding semen samples, they were collected from 22 men recovered from COVID-19 at approximately 3 months after infection, comparing this to semen samples from 36 men who are health age-matched controls. The authors present useful data that can shed additional light on a disease process that we are still learning more about.

Comment 1: A major point that will need revising is the author's description of "low sperm concentration" in the recovered male cohort. Their sperm concentrations averaging 41.5 million / mL are statistically lower, but still technically well within normal regardless of WHO edition. One could also come to an alternative conclusion that males recovered from COVID-19 have normal sperm concentration. The authors will need to revise their language regarding this finding and temper their conclusions to simply state that recovered males had lower concentrations and volumes that were still within the normal range. Possibly this study can reassure men who only have mild symptoms that the data doesn't suggest they'd be rendered subfertile. The authors do acknowledge the limitation that there were no baseline values for comparison.

Reply 1: Thank you for your comments. We revised the language regarding our findings, and the description of "low sperm concentration" in the recovered male cohort was removed from the manuscript to temper our conclusions, as suggested by the reviewer. Also, some texts referring to "low sperm concentration" were also removed or modified to temper our conclusions.

Changes in the text: We have modified our text as advised.

See Pages 3-4, lines 76-79: "*Levels of semen volume (control: 2.5 mL vs COVID-19: 1.9 mL; $p < 0.05$) and sperm concentration (control: 59 x106/mL vs COVID-19: 41.5 x106/mL; $p < 0.005$) were significantly lower in males recovered from COVID-19, but still technically well within normal regardless of WHO edition.*"

See Page 4, lines 88-93: "*Reproductive-age males recovered from COVID-19 have normal sperm concentration. Sperm concentration did not correlate with other semen parameters, sex-*

related hormones, IIEF-5, and IPSS. Further studies should be performed to evaluate whether the lower sperm concentration and semen volume that were still within the normal range are a transient or prolonged downregulation resulting from the COVID-19 attack.”

See Page 10, lines 219-221: *“This difference was associated with lower semen volume (Figure 1A) and sperm concentration (Figure 1B) that were still within the normal range in the recovered COVID-19 group.”*

See Page 12, lines 271-273: *“The logistic regression analysis in this study revealed that sperm concentration, but not semen volume, was associated with the men who recovered from COVID-19.”*

See Page 15, lines 351-352: *“Hence, the findings suggest that men who recovered from mild COVID-19 could have a normal sperm concentration.”*

See Page 16, lines 354-359: *“In summary, these findings suggested that the levels of sperm concentration were significantly lower but still technically well within normal regardless of WHO edition and associated with reproductive-age males recovering from COVID-19. Further studies should be performed to evaluate whether it is a transient or prolonged downregulation resulting from the COVID-19 attack. Moreover, the mechanism of a long-term effect must be clarified.”*

Comment 2: Title should be revised to be more descriptive of the authors' conclusions/message they want to convey. Major revision needed - they should not use "low sperm concentrations" anywhere to describe the recovered cohort given their numbers falling well within reference ranges.

Reply 2: Due to the sperm concentration number or count falling well within the reference range, we decided not to use and to remove the words "low sperm concentrations" and some texts referring to "low sperm concentration", as mentioned above. In this sense, we also revised the title of the manuscript and decided to change it from “Correlation between sperm concentration and semen parameters and hormone profile in males recovered from COVID-19” to “Sperm concentrations do not correlate with semen parameters and hormone profiles in males recovered from COVID-19”, as suggested by the reviewer.

Changes in the text: We have modified our text as advised.

See Page 1, lines 1-2: *“Normal sperm concentrations do not correlate with semen parameters and hormone profiles in males recovered from COVID-19”*

Reviewer B

Comment 1: I read with interest this study about correlation between sperm concentration and semen parameters in males recovered from COVID-19. The paper is well written and the authors explain the problems related with the treatment of this “difficult” disease. The references are lacking, tables and figures are sufficiently updated. The mechanism of SARS-COV2 infection is that the virus binds to the angiotensin 2 converting enzyme (ACE2) through glycoproteins membrane S. The ACE2 enzyme is strongly expressed in lung, kidney, cardiac, gastrointestinal, bladder and testicular cells. In the testis it is found in both in the cells of the seminiferous ducts, in particular spermatogonia, and in the cells of Leydig and Sertoli. Hence the hypothesis that the testicle may be a reservoir of the disease SARS-COV2 could, through various pathogenic pathways, increase oxidative stress, increase DNA methylation and fragmentation and decrease male fertility. Also, direct cellular damage is possible through the ace enzyme on Leidig cells and spermatocytes

Can the authors add this reference and considerations to the Discussion section? (Delle Fave et all. Arch Ital Urol Androl. 2021 Mar 22;93(1):115-119. doi: 10.4081/aiua.2021.1.115)

Reply 1: In this new submission, we included the lacking references (including the reference suggested by the reviewer: Delle Fave et all. Arch Ital Urol Androl. 2021) on the mechanism of SARS-CoV-2 affecting spermatogenesis.

Changes in the text: We have modified our text as advised.

See Pages 13-14, lines 397-308: “Donders et al. suggested that a reduction in sperm concentration was correlated with the anti-S1 SARS-CoV-2 IgG antibodies and time lapse after COVID-19.²⁵ Semen proteomics of COVID-19-recovered males identified pathways involved in reproductive functions such as extracellular matrix, cell motility regulation, and sperm-oocyte recognition.²⁰ Furthermore, Ghosh et al. revealed significant downregulation of prosaposin and semenogelin 1, two proteins related to male fertility.²⁰ Therefore, to discern fertility-related biological processes triggered by SARS-CoV-2 infection, a protracted assessment of disease impact in men recovered from COVID-19 is required. In this sense, ACE-2 receptor is present and highly expressed on seminiferous tubules, Sertoli cells and the germ cells from the testes, making them a potential site for SARS-CoV-2 infection which could impact spermatogenesis.^{26,27”}