Peer Review File

Article Information: https://dx.doi.org/10.21037/tau-21-935

Reviewer A

Comment 1: The authors present a wonderful opportunity to learn more about this disease by following a recurrent semen donor through his infection period. My only caution in their telling of this story is to even further temper their hypothesis. They say that with the sperm count decline beginning in February and symptom onset not until end of March, that the donor must have been an asymptomatic carrier during this time. If at all possible, the authors need to provide a reference for this possibility of asymptomatic carrier status for a time period of 1 month. If no such reference exists, then state this clearly too.
Very important work.

Reply 1: *The referee makes an excellent point. We have modified the text to indicate that we cannot exclude the possibility that COVID-19 infection may have arrested spermatogenesis by attacking the late stages of this process, rather than its initiation. See Page 5, lines 89-112*

It is also possible that estimates of incubation time have been made on patients that go on to develop significant symptoms – in patients with low levels of infection, such as this case, the hiatus between infection and the appearance of symptoms may be significantly longer.

A reference has been included describing the normal incubation time associated with COVID-19 infection, as requested by the referee. See reference 12.

minor

Comment 2: last line of abstract mentions COVID still found by PCR. Please include if this was found in semen or in saliva.

Reply 2*: PCR positive via nasal swab.*

Comment 3: introduction: consider replacing "exploiting" with "exploring" or "closely following"

Reply 3*: replaced 'exploiting' with 'exploring' as requested. See Page 3 line 46.*

Reviewer B

Comment 4: It is stated multiple times that the patient began to have declining semen parameters 7 weeks prior to symptomatic COVID infection, and that this is a "sensitive indicator" for COVID infection. Symptomatic COVID has been shown to present 2-14 days after exposure, so what reference are you using which indicates that the virus can infect and impact a patient 7 weeks prior to symptoms? Semen parameters are quite variable, and to assume that the drop described from January to February is due to COVID is unsubstantiated.

Reply 4*: the referee makes a valid point. The argument a long incubation period prior to the appearance of azoospermia and symptoms of infection is based on the two assumptions.*

- *First, that the virus suppressed spermatogenesis by infecting the spermatogonia and preventing the initiation of a spermatogenic cycle.*

- *Secondly, azoospermia was preceded a significant fall in both motility and sperm count.*
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We fully acknowledge that the virus may have attacked the germ line at a much later stage of spermatogenesis and, that the fall in sperm motility and count observed some weeks earlier were unrelated to the COVID-19 infection. These alternative explanations are acknowledged in the revised text. See Page 5, lines 89-112

It is impossible with such a observational study to be certain about the chain of cause and effect; the one conclusion that we can draw with certainty however, is that male germ cell differentiation is exquisitely sensitive to COVI9 infection and that even a mild case of infection can result in transient azoospermia. See Page 5 lines 109-112.

Comment 5: Based on the pre-infection DFI values, the hypothesis that the infection caused an early increase in DNA fragmentation, as well as a lasting impact following recovery, does not seem to fit. It appears that the DFI was already 40% many months before any type of infection and levels actually dropped after the acute infection. Please explain.

Reply 5: *It is important to note that we are referencing DNA oxidation and not DNA fragmentation. Pre-COVID, 8OHGdG could be detected in an average 40.1 ± 3.6% of spermatozoa analyzed. Unfortunately, due to an insufficient number of spermatozoa, 8OHdG could not be determined for nearly 60 days post infection. However, when spermatozoa reappeared in the ejaculate towards the end of June, 56.7% of spermatozoa were 8OHdG positive, representing a 39.9% increase from the pre-COVID average. The total average 8OHdG of all readings within 6 months post-covid is 44.3% (still 9.53% higher that his pre-covid average). This point has been made in the revised text. See Page 5 lines 85-87.*

To date, no study reporting sperm DNA oxidation before and after COVID infection has been reported.

Comment 6: While the explanation appears to fit this individual scenario, this is not what we have seen clinically. There has been a wide variation in the impact of COVID on semen parameters, including extended periods of recovery outside of the expected 3-month cycle. While I agree that male fertility is often the "canary in the coal mine" for overall health, I am not certain that this single case report is applicable to the general population.

Reply 6: *We contend that this case report is highly valuable in that other studies have not been able to achieve such acute monitoring of individuals pre and post infection. The results shown here should warrant further larger controlled studies to better understand the true impact of COVID-19 infection on male fertility.*

Reviewer C

Comment 7: This case report presents interesting anecdotal facts regarding a known complication of COVID19 infection. It is, on the basis of the topic, a very current and relevant report. However, the described phenomenon has been recently vastly described on the infertility and reproduction literature on larger sample populations, thus it does not bring innovative evidence. It has however value on the methods, namely providing follow up and the hypothesized timeline between the infection, the mechanism of insult to spermatogenesis, and the recovery; as well as objective evidence on lingering DNA damage. If this hypothesized pathophysiological process can be further backed with other cases - even if only one or two more patients -, I would suggest its acceptance to this journal. As a single case,

unfortunately the evidence becomes anecdotal over a previously observed phenomenon, and would perhaps be better suited for a journal with bigger focus on case reports.

Reply 7: *As mentioned above, there are no studies that been able to attain such close monitoring of the relationship between COVID-19 infection and semen quality as we have achieved in this case study. The value of this case report is that it provided an insight into the dynamic changes in semen quality in a patient on whom a significant volume of pre-infection data had been acquired, This is a unique situation – it is clearly not possible to design prospective human studies where semen quality is monitored before, during and after an infectious episode. The only way that such data can be acquired is retrospectively and in such cases the gaps between semen analysis and the onset of the disease (up to one year) and between recovery and the post infection analysis (at least 3 months) are considerable. This patient represents a unique opportunity to collect semen quality data around the time of infection.*

Reviewer D

This case report is very interesting and with some edits, could be an important contribution to to the COVID literature.

Comment 8: Main issue: The CDC reports that COVID symptoms appear 2-14 days after virus exposure. Therefore the timeline suggested in the case report is well out of this range. The authors state that the patient was likely exposed in early February but did not show symptoms until the end of March (the authors state this could be 7 weeks. This is way outside of the reported exposure-to-symptom time frame for COVID. Therefore many questions arise: Did the patient have another viral infection before COVID and that yielded the decline in sperm count in early February?
Was this patient every vaccinated? This answer should be included.

Reply 8: *The referee makes a valid point. The long incubation time was inferred from the changes in semen quality observed in the lead up to azoospermia and the assumption that spermatogenesis was suppressed by preventing the entry of spermatogonia into spermatogenesis. The possibility that the virus might have induced spermatogenic arrest by targeting a much later spermatogenic stage is acknowledge in the revised version of this case report. See Page 5 , lines 89-112*

Reviewer E

Comment 9: I enjoyed your article and I believe it can be improved with a paragraph adding a that the covid can itself being found the semen per see, only to articles described it and I believe your article would be enriched if you add them:

1- Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical Characteristics and Results of Semen Tests Among Men With Coronavirus Disease 2019. JAMA Netw Open. 2020;3(5):e208292. doi:10.1001/jamanetworkopen.2020.8292

2- Machado B, Barcelos Barra G, Scherzer N, Massey J, Dos Santos Luz H, Henrique Jacomo R, Herinques Santa Rita T, Davis R. Presence of SARS-CoV-2 RNA in Semen-Cohort Study in the United States COVID-19 Positive Patients. Infect Dis Rep. 2021 Feb 4;13(1):96-101. DOI: 10.3390/idr13010012. PMID: 33557147; PMCID: PMC7930957.

our article had 14 references and TAU accepts up to 20

Reply 9*: these references have been inserted into the revised text, as requested – references 4 and 5.*

As for COVID virus in semen, The patient was never tested for levels of Covid-19 in the semen, only via PCR nasal swab test, but this would have been interesting to know.

Comment 10: Another interesting piece of information would be if you can let us know if after the semen had recovered to the normal parameters the sperm was able to fertilize an egg and be implanted.

***Reply 10**: This patient was also only an external clinical research volunteer, and not a patient pursuing ART, so no ART outcomes are available.*