SARS-COV-2 PRESENCE IN SEMINAL FLUID: MYTH OR REALITY

Donatella Paoli[1#], Francesco Pallotti[1#], Ombretta Turriziani[2], Laura Mazzuti[2], Guido Antonelli[2], Andrea Lenzi[1], Francesco Lombardo[1,*]

[#] **These Authors contributed equally**

[1] Laboratory of Seminology - Sperm Bank "Loredana Gandini", Department of Experimental Medicine, "Sapienza" University of Rome, Rome, Italy

[2] Laboratory of Virology, Department of Molecular Medicine, "Sapienza" University of Rome, Rome, Italy

Authors' email:

donatella.paoli@uniroma1.it

francesco.pallotti@uniroma1.it

ombretta.turriziani@uniroma1.it

laura.mazzuti@uniroma1.it

guido.antonelli@uniroma1.it

andrea.lenzi@uniroma1.it

francesco.lombardo@uniroma1.it

Short title: SARS-CoV-2 presence in seminal fluid

Keywords: SARS-CoV-2, COVID-19, spermatozoa, RT-PCR, seminal fluid

*** Corresponding Author:**

Francesco Lombardo

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/andr.12825

This article is protected by copyright. All rights reserved

Accepted Article

Laboratory of Seminology - Sperm Bank "*Loredana Gandini*", Department of Experimental Medicine, "Sapienza" University of Rome, Rome, Italy, Viale del Policlinico 155, 00161 Roma, Italy

Tel: +3390649970715

Fax: +3390649970717

Email: francesco.lombardo@uniroma1.it

ABSTRACT

Great concerns have been raised on SARS-CoV-2 impact on men's andrological well-being and one of the critically unanswered questions is whether it is present or not in the seminal fluid of infected subjects. The expression of ACE2 and TMPRSS2 in the testis and in the male genital tract allows speculations about a possible testicular involvement during the infection, possibly mediated by local and/or systemic inflammation that might allow a high viral load to overcome the haemato-testicular barrier. To date, few investigations have been carried out to ascertain the presence of SARS-CoV-2 in the seminal fluid with contrasting results. Furthermore, the cumulative number of subjects is far too low to answer the question unambiguously. Therefore, great caution is still needed when evaluating this data, otherwise we risk unleashing unmotivated concerns in the scientific world with troublesome consequences in reproductive medicine.

Introduction

The impact of coronavirus on men's andrological well-being, including its presence/absence in the seminal fluid is one of the many unanswered questions about this pandemic. SARS-CoV-2 cell entry is mediated by its spikes (S proteins), who give it a crown like appearance in electron microscopy. Furthermore, the spike protein needs priming by cellular proteases to facilitate viral and cellular membranes fusion. Angiotensin-converting enzyme 2 (ACE2) protein has been identified as the viral receptor and TMPRSS2 (transmembrane protease serine 2) is utilized for S protein priming[1,2]. Since ACE2 is present in the testis[3] and TMPRSS2 has been identified in the male genital tract[4], the possibility of a testicular involvement and, thus, viral contamination of the

This article is protected by copyright. All rights reserved

Accepted Article

seminal fluid have been hypothesized[5,6]. The isolation in the semen of men has been frequently reported for many viruses of different families, including replicating Zika, Ebola and Marburg viruses[7]. Some may also be particularly persistent, like the Zika virus which has been detected in the semen of asymptomatic men for up to 1 year after healing[8]. This wide range of viral families suggests that seminal contamination may not be fully dependent on specific viral characteristics (conserved epitopes, ability to replicate in male genital tract, capability to evade the immune system) but viral spread in the male reproductive tract may rather be associated to blood viral load. In fact the blood-testicular barrier may not constitute a perfect barrier to viruses, especially in the presence of systemic or local inflammation[7]. Several viruses that result in viremia can cause orchitis[7] as is the case of SARS-CoV[9]. Its high homology with the current SARS-CoV-2 strengthens the theory that the latter may also be detectable in semen. Nonetheless, we still do not know enough about the new COVID-19 to hypothesize its behavior towards the male reproductive system. Clarifying the presence of a viremia may be a critical step but, to date, few studies evaluated SARS-CoV-2 presence in blood samples with different results. Ling et al. reported the absence of viral RNA in serum samples from fourteen recovering subjects with positive pharyngeal swabs[10]. Likewise, Wang et al. revealed a minimal percentage of positive blood samples through RT-PCR amplification of viral RNA[11], whereas Zhang et al. detected the virus in 40% of blood samples[12]. Conversely, they detected viral presence in other body fluids (particularly in stool samples) opening the doors wide to the hypothesis that despite SARS-CoV-2 tropism for respiratory tissues[13], others extra-respiratory viral transmission routes cannot be excluded "a priori". However, this still does not provide evidence about the risk of contamination of human semen.

SARS-CoV-2 identification in seminal fluid: negative evidence

Recently several researchers focused their attention to the possible direct and indirect consequences of the COVID-19 pandemic in medicine of reproduction, with particular attention on testicular involvement, androgen production and sexuality[5,6]. Moreover, safety issues for patients and personnel in andrological services, medically assisted reproduction services and gamete cryopreservation have become subjects of lively comments[14–16]. However, the issue of SARS-CoV-2 in seminal fluid is not yet answered unequivocally. Recently, with currently available molecular methods we showed that a recovering 31-year-old Italian man affected by a relatively mild form of COVID-19 had no detectable virus in his ejaculate approximately one

This article is protected by copyright. All rights reserved

week after the last positive nasopharyngeal swab and fifteen days from the onset of the disease[17]. With all the limitations of a single case report, the absence of viral RNA amplification allowed us to speculate that either the virus had never been present or, if it was ever present at the peak of the infection, SARS-CoV-2 clearance kinetics in seminal fluid might coincide with the progressive clinical recovery. Nonetheless, it is still possible that a more severe disease and/or a semen sample collection in the acute phase (if possible) could have allowed viral detection. Other recent publications have produced comparable results. Song et al. tested a group of 12 Chinese COVID-19 patients in the recovery phase, defined as two consecutive negative quantitative real time polymerase chain reaction (qRT-PCR) tests or as a substantial improvement of symptoms and of chest computed tomography scans. None of the patients had detectable viral RNA in semen samples, although the authors do not disclose information on target genes. Noteworthy, the authors tested the testicular tissue from a COVID-19 deceased subject, also in this case without detecting the presence of viral RNA[18]. Similarly to our conclusions, the authors suggested that SARS-CoV-2 is unlikely to infect the testis and male genital tract, although a definitive answer would need more investigations. Pan et al. investigated a larger group of patients: a single ejaculated semen sample from 34 Chinese men was tested with qRT-PCR for viral RNA amplification, confirming again the absence of the virus in all samples[19]. Once again, the subjects were previously confirmed COVID-19 cases through positive qRT-PCR tests of pharyngeal swabs. The subjects were mostly affected by a mild disease and semen testing was performed on average one month after diagnosis. Furthermore, six subjects reported scrotal discomfort at the moment of COVID-19 confirmation. However, no testicular investigation was conducted in these patients to rule out this aspect and the possibility of a viral orchitis remains unclear. Nonetheless, it can be presumed that in these milder COVID-19 cases the seminal presence of SARS-CoV-2 seems unlikely, but the authors suggest that the investigation of severe acute cases with higher viral loads might bring different results. While overall this can be seen as reassuring, the cumulative number of subjects is still too low to consider conclusive this data. Furthermore, data from these caseloads are hard to generalize as definitions of recovered subjects is different among studies. Target genes differ in Paoli et al. and Pan et al., while Song et al. disclose no information on target genes. In fact, taken together these studies only allow us to infer that it is unlikely that recovering subjects may still harbor this coronavirus in their seminal fluids, leaving the uncertainty whether SARS-CoV-2 infection is capable of involving the testis and the seminal fluid in other circumstances.

Accepted Article

This article is protected by copyright. All rights reserved

SARS-CoV-2 identification in seminal fluid: positive evidence

In contrast with the previous works, Li et al. recently reported the detection of SARS-CoV-2 in 6 among 38 semen samples collected from both acute and recovering Chinese COVID-19 patients (4 and 2 positive cases, respectively)[21]. While this may seem in deep contrast with the previous investigations, the caseload presented in the paper is also quite different and, like all previous evidence, it needs to be cautiously interpreted. The first thing to be acknowledged is that it was conducted in the only designated hospital for the treatment of COVID-19 in Shangqiu and, while no deep description of the caseload was available, it was presumably composed of more severe cases of COVID-19 (the Authors cited 12 comatose or dying subjects). This may have influenced the results because, as we hypothesized, a more severe disease may correspond to a higher blood viral load and a higher chance to reach other organs and body fluids including the semen; moreover, this can induce a higher probability of pollution of the environment. In fact, semen collection is normally performed by masturbation, which can hardly be defined a sterile procedure. Indeed, there is a chance that, at least for some subjects, the authors registered false positive results due to contamination with respiratory droplets of the specimen containers.

CRITICAL ANALYSIS OF "Clinical Characteristics and Results of Semen Tests Among Men With Coronavirus Disease 2019"

Waiting for stronger evidence, we would like to discuss the conclusions of this study.

1. The results necessitate further confirmations in order to highlight the possibility of a SARS-CoV-2 sexual transmission. Declaring that "*If it could be proved that SARS-CoV-2 can be transmitted sexually in future studies, sexual transmission might be a critical part of the prevention of transmission, especially considering the fact that SARS-CoV-2 was detected in the semen of recovering patients*" might cause unreasonable panic, considering the small caseload. In particular, this would require epidemiological demonstration of viral transmission from male recovered subjects to previously unaffected sexual partners which, as far as we know, it has not yet been reported. Moreover, the authors' concerns regarding a possible viral reservoir constituted by semen may be true for viruses like Zika, which has a remarkably different pathophysiology, but it is still quite unclear for SARS-CoV-2.

This article is protected by copyright. All rights reserved

2. An arguable point in this paper is that the methodology for the detection of SARS-CoV-2 in semen is not specified. In fact, the Authors state they used RT-PCR to detect viral RNA of nasal and pharyngeal swabs; what about semen? We can only suppose that used RT-PCR also for semen. How did the authors extract viral RNA? What was the limit of detection of their molecular method? A description of methods used to detect viral RNA would be useful for different reasons. Since this study is the only one to detect SARS-CoV-2 in seminal fluid, it is important to understand if their method of extraction and/or amplification is somehow better than those used in other studies. The most important point is the lack of information about limit of detection (LoD), gene targets and cycle threshold (Ct) values for positive samples. A real time PCR with a high sensitivity (low LoD), able to detect very low amount of virus, could explain the positive results found. Usually, the gene targets of SARS-CoV-2 are E, S, N1, N2, and RpRd. Recently some authors observed that N2 gene may be prone to false positive results[20]. Particularly high Ct value (>40) has been detected in nasopharyngeal swab using N2 gene as RT PCR target, suggesting either "very low" viral load or "false positive" results. To date the clinical relevance of this "very low" amount of virus is unknown. For this reason, it should be important to know the Ct values detected in seminal fluid in order to clarify the viral presence in semen.
3. Finally, we do not know anything about the collection modality of semen samples (masturbation, electrovibrator or other). This is a critical point because the collection of seminal fluid is completely different from the collection of other biological media (such as blood) and can be easily subject to contamination, especially in a COVID Unit. In fact, the obsession of these days is to wash our hands because the virus could be present on the epidermis. Moreover, a "positive" PCR result reflects only the detection of viral RNA and does not necessarily indicate presence of viable virus[22], and the doubt remains as to whether the detected RNA or RNA fragments are from contamination.

IS IT POSSIBLE TO DRAW FINAL CONCLUSIONS FROM CURRENT LITERATURE?

In our opinion, in this pandemic period, the hectic activity of the researchers, in order to investigate and try to understand the spread of the virus, risks to cause an insufficient critical evaluation of the data produced. We must have great caution right now otherwise we risk

This article is protected by copyright. All rights reserved

Accepted Article

unleashing fear and unmotivated concern in lay and scientific world. Although most studies indicate a low risk of seminal infection, the great variability of severity of the clinical manifestations induced by the virus, make a greater and more in-depth studies mandatory. This may have critical implications for sperm cryopreservation, since many concerns have been raised in the possible collection, shipping and utilization of these samples for medically assisted reproduction[16]. In fact, viruses stored in liquid nitrogen could also maintain their pathogenic properties[23] and sperm cryopreservation might allow preservation of viral species that potentially contaminate the semen sample.

Acknowledgements

We are grateful to the Post Graduate Students of our Department who made a great work during the Covid emergency. A special thank to Dr Stefano Colangelo, Dr Giulia Nigro, Dr Claudio Lecis, Dr Alessandra Caputi, Dr Francesca De Giorgi, Dr Carmen Mignogna.

This is a tribute to all Medical Doctors, Biologist and Healthcare Personnel who have contracted COVID-19 while on duty and suffered or lost their lives because of it.

Author Contributions

FL and DP conceived and designed the manuscript. FP and DP wrote the manuscript. OT, LM and GA gave their expertise on the virological issues. FL and AL revised critically the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Declaration of interests

The Authors declare no conflict of interest.

Funding

None

References

1. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol*. 2020;5(4):562-569.

This article is protected by copyright. All rights reserved

Accepted Article

doi:10.1038/s41564-020-0688-y

2. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181(2):271-280.e8. doi:10.1016/j.cell.2020.02.052

3. Douglas GC, O'Bryan MK, Hedger MP, et al. The novel angiotensin-converting enzyme (ACE) homolog, ACE2, is selectively expressed by adult Leydig cells of the testis. *Endocrinology*. 2004;145(10):4703-4711. doi:10.1210/en.2004-0443

4. Chen Y-W, Lee M-S, Lucht A, et al. TMPRSS2, a serine protease expressed in the prostate on the apical surface of luminal epithelial cells and released into semen in prostasomes, is misregulated in prostate cancer cells. *Am J Pathol*. 2010;176(6):2986-2996. doi:10.2353/ajpath.2010.090665

5. Simoni M, Hofmann M-C. The COVID-19 pandemics: shall we expect andrological consequences? A call for contributions to ANDROLOGY. *Andrology*. April 2020. doi:10.1111/andr.12804

6. Corona G, Baldi E, Isidori AM, et al. "SARS-CoV-2 infection, male fertility and sperm cryopreservation: a position statement of the Italian Society of Andrology and Sexual Medicine (SIAMS) (Società Italiana di Andrologia e Medicina della Sessualità)." *J Endocrinol Invest*. 2020. doi:10.1007/s40618-020-01290-w

7. Salam AP, Horby PW. The Breadth of Viruses in Human Semen. *Emerg Infect Dis*. 2017;23(11):1922-1924. doi:10.3201/eid2311.171049

8. Kurscheidt FA, Mesquita CSS, Damke GMZF, et al. Persistence and clinical relevance of Zika virus in the male genital tract. *Nat Rev Urol*. 2019;16(4):211-230. doi:10.1038/s41585-019-0149-7

9. Xu J, Qi L, Chi X, et al. Orchitis: a complication of severe acute respiratory syndrome (SARS). *Biol Reprod*. 2006;74(2):410-416. doi:10.1095/biolreprod.105.044776

10. Ling Y, Xu S-B, Lin Y-X, et al. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin Med J (Engl)*. February 2020. doi:10.1097/CM9.0000000000000774

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

11. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA*. March 2020. doi:10.1001/jama.2020.3786

12. Zhang W, Du R-H, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect*. 2020;9(1):386-389. doi:10.1080/22221751.2020.1729071

13. Chu H, Chan JF-W, Wang Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clin Infect Dis*. April 2020. doi:10.1093/cid/ciaa410

14. Esteves SC, Lombardo F, Garrido N, et al. SARS-CoV-2 pandemic and repercussions for male infertility patients: a proposal for the individualized provision of andrological services. *Andrology*. May 2020. doi:10.1111/andr.12809

15. De Santis L, Anastasi A, Cimadomo D, et al. COVID-19: the perspective of Italian embryologists managing the IVF laboratory in pandemic emergency. *Hum Reprod*. April 2020. doi:10.1093/humrep/deaa074

16. Yakass MB, Woodward B. COVID-19: should we continue to cryopreserve sperm during the pandemic? *Reprod Biomed Online*. April 2020. doi:10.1016/j.rbmo.2020.04.004

17. Paoli D, Pallotti F, Colangelo S, et al. Study of SARS-CoV-2 in semen and urine samples of a volunteer with positive naso-pharyngeal swab. *J Endocrinol Invest*. 2020:1-4. doi:10.1007/s40618-020-01261-1

18. Song C, Wang Y, Li W, et al. Absence of 2019 Novel Coronavirus in Semen and Testes of COVID-19 Patients. *Biol Reprod*. April 2020. doi:10.1093/biolre/ioaa050

19. Pan F, Xiao X, Guo J, et al. No evidence of SARS-CoV-2 in semen of males recovering from COVID-19. *Fertil Steril*. 2020. doi:10.1016/j.fertnstert.2020.04.024

20. Pujadas E, Ibeh N, Hernandez MM, et al. Comparison of SARS-CoV-2 Detection from Nasopharyngeal Swab Samples by the Roche cobas(R) 6800 SARS-CoV-2 Test and a Laboratory-Developed Real-Time RT-PCR test. *J Med Virol*. May 2020. doi:10.1002/jmv.25988

21. Li D, Jin M, Bao P, Zhao W, Zhang S. Clinical Characteristics and Results of Semen Tests

This article is protected by copyright. All rights reserved

Among Men With Coronavirus Disease 2019. *JAMA Netw open*. 2020;3(5):e208292. doi:10.1001/jamanetworkopen.2020.8292

22. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. *Jama*. 2020;2019:2019-2021. doi:10.1001/jama.2020.8259

23. De Paoli P. Bio-banking in microbiology: from sample collection to epidemiology, diagnosis and research. *FEMS Microbiol Rev*. 2005;29(5):897-910. doi:10.1016/j.femsre.2005.01.005

Accepted Article

This article is protected by copyright. All rights reserved