Original Article

Detection of SARS-CoV2 virus using the real-time reverse transcriptase polymerase chain reaction in semen and seminal plasma from men with active COVID-19 infection – A pilot study

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ABSTRACT

Introduction: SARS-CoV-2 has been detected in various body fluids. Its presence in semen has been tested with contradictory results. This study aimed to detect the presence of SARS-CoV-2 virus using the real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) in semen and seminal plasma from men with active COVID-19 infection.

Methods: In a cross-sectional study at a COVID facility, men aged 20–45 years with active COVID-19 infection provided semen samples within 7 days of symptom onset or 5 days of nasopharyngeal rRT-PCR test positivity in asymptomatic men. Testing of SARS-CoV-2 was performed using rRT-PCR and semen analysis was done for sperm counts and motility as per the WHO (2010) standards.

Results: A total of 37 men with a mean age of 32.2 ± 5.6 years were tested. SARS CoV-2 virus could not be isolated in any of the samples. Further, microscopic analysis done on 17 samples showed normal semen parameters during the active phase of disease.

Conclusion: Men with mild COVID-19 disease or asymptomatic individuals do not shed virus in their semen, ruling out sexual contact as a mode of transmission in this subset of population.

INTRODUCTION

Reported first from Wuhan, China, the novel coronavirus (SARS-CoV-2) pandemic has swept the globe, creating a public health emergency.[1] The RNA virus attaches to the angiotensin-converting enzyme (ACE-2) receptors expressed predominantly within type II alveolar cells of the lung, besides aerodigestive tract and kidneys.[2] Other body tissues also express these receptors and apart from nasopharyngeal secretions, the virus has been isolated in other body fluids including feces, urine, and blood.[3]

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The ACE-2 receptor has been isolated in the testis and male reproductive tract, and it is theoretically possible that the virus may be present in semen.[4] However, the data on the presence of the virus in semen are conflicting. While preliminary data suggest the presence of virus in the seminal plasma of men who tested positive on real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) on nasopharyngeal swabs,[5] others have refuted this.[6-12]

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Most of this data comes from case reports or studies with testing done during the recovery phase of disease. Since viral shedding from the nasopharynx is highest during the acute phase of infection, it is possible that these studies do not provide complete data on the viral presence in semen.

Determining the presence of the virus in semen is important for multiple reasons. The majority of patients infected with the virus are asymptomatic.[13] Men outnumber women among asymptomatic infections, and many of them are in the reproductive age group.[13] Viral infection of the semen may impact fertility through affecting either the sperm or seminal plasma. In addition, it is possible that asymptomatic, infective men with presence of the virus in their semen could potentially transmit it to the female partner or impact a pregnancy conceived when they were infected. Hence, the presence of the virus in the semen during the acute phase of infection needs to be assessed. Further, viral presence may not be the same during all phases of the disease and determining trends of viral shedding during both the active and recovery phases would be useful.

Apart from the potential impact on fertility, the presence of the virus in semen could have an impact on best practices for handling semen samples and the potential need for screening for COVID-19 in men before semen handling for analysis or sperm preparation techniques.

Our institution is a dedicated, high-volume, COVID-19 hospital and we conducted this study to determine the presence of SARS-CoV-2 virus in the semen of men with mild symptoms in the active phase of disease and to ascertain the clearance of virus with recovery of illness after 2 weeks. We also evaluated the semen parameters of these men to assess the impact of infection on them.

METHODS

Patient selection

This cross-sectional study was conducted between October and December 2020 at the National Cancer Institute, All India Institute of Medical Sciences, New Delhi, India, which was a designated tertiary COVID care facility. As a policy, symptomatic patients with mild disease (defined as the presence of upper respiratory tract symptoms or fever without shortness of breath) or moderate disease (defined as respiratory rate >24/min and O2 saturation <94% on room air) and asymptomatic patients without home isolation facilities were admitted at this site. The study was approved by the Institutional Ethics Committee vide its approval number (IEC-799/07.08.2020). All patients provided written, informed consent for inclusion in the study and the authors confirm the availability of all original data.

The study included men aged between 20 and 45 years, diagnosed with mild or moderate COVID-19 infection, presenting within 7 days of symptom onset. For asymptomatic patients, semen was collected within 3–5 days of being tested positive for COVID-19 infection. All infections were diagnosed based on positivity on SARS-CoV-2 rRT-PCR from nasopharyngeal swabs, as per the manufacturer's recommendation for determining positivity. All patients with symptom onset of >7 days, active pneumonia, need for ventilatory support, and presence of comorbidities such as diabetes mellitus, immunocompromised status or on immunosuppressants, or with underlying lung disease were excluded.

Men meeting the criteria were asked to provide a semen sample by masturbation as per the WHO criteria of semen collection.[14] Samples were collected in the sterile universal container labeled with patient details and allowed to undergo liquefaction. Semen analysis was done using disposable counting slides (ISASD2C10G, Proiser, Spain) according to the WHO guidelines 2010.[14] The samples were transported in an ice box to the Virology laboratory for the determination of viral presence. Adequate personal protective equipment (PPE) was provided to those handling and transporting the semen samples.

Patients with the presence of virus in the acute phase of infection were to be followed up 2 weeks after recovery for a repeat test.

Laboratory procedure

All semen samples were processed in a class II, type A2 biological safety cabinet using biosafety level-2 work practices. The samples were kept at the room temperature for 10–15 min and then transferred to cryovials. Each sample was centrifuged at 3000 rpm for 10 min. The supernatant and sediment from the cryovials were aliquoted separately in storage vials and stored at –80°C until testing.

RNA extraction and real-time reverse transcriptase-polymerase chain reaction assay

RNA was extracted from the semen samples using the QIAamp Viral RNA Mini Kit (Qiagen, Cat. No. 52906). RNA was extracted from 200 µl of sample and eluted in 30 µl of elution buffer provided with the RNA extraction kit. It was reverse transcribed and amplified by using an FDA-EUA approved, Indian Council of Medical Research recommended National Institute of Virology (NIV) multiplex, single tube SARS-CoV-2 RT-PCR assay in Agilent AriaMx real-time PCR system. The results were interpreted as per the kit manufacturer's instruction.

Statistical analysis

The sample size of 118 was calculated based on a study by Li *et al.* as reference,[5] assuming a seminal viral prevalence of 26.7% among men with active COVID-19 infection, and an absolute precision of 8% for 95% confidence intervals. Assuming a nonresponse rate of 20%, the final sample size

came out to be 140. However, considering this study in a pilot mode we recruited 50 subjects for the study.

Categorical variables were reported in proportions, whereas continuous variables reported in mean (standard deviation) or median (interquartile range) depending on the distribution. All tests were performed using Stata version 15.0 (Statacorp, College Station, Texas, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Among the 50 samples, 13 were inadequate to perform testing (volume insufficient for RNA extraction) and the remaining 37 were analyzed. The demographic details of patients are given in Table 1. Twenty-four (64.9%) participants had mild symptoms, whereas the remaining 13 (35.1%) were asymptomatic. Of these 37 patients, 31 patients were sexually active. A total of four female partners of these patients also tested positive on their nasopharyngeal swabs.

On seminal rRT-PCR, SARS CoV-2 RNA was not isolated in any of the 37 semen samples. Seventeen semen samples were subjected to routine semen analysis, and all samples showed normal semen parameters [Table 2]. Since none of the patients had the presence of virus in the acute phase of infection, no repeat testing was performed at 2 weeks.

DISCUSSION

This was first of its kind from India to evaluate the presence of SARS-CoV2 virus in the semen of COVID-19 positive men during the acute phase of infection. The absence of the virus in the semen sample of all of these 37 men who were in their active phase of disease (including symptomatic and asymptomatic individuals) provides evidence of absence of risk of shedding of this virus in seminal plasma. This further indicates the absence of risk of transmission of SARS CoV-2 from men to women through sexual contact.

Eight studies have been published on this issue with all, barring one, showing no virus in semen.[5-12] However, most of these studies have been done during the recovery phase. Kayaaslan *et al.* included patients within 7 days of illness and they too did not find any viral shedding in semen.[12] However, this study was limited due to its small sample size of 16 men. Only one study by Li *et al.* where 6 out of 38 patients tested positive for SARS-CoV-2 virus included patients from day 6 to 16 of diagnosis.[5] The authors also hypothesized possibility of delayed shedding of virus due to privileged immunity of testis. However, this study does not mention the method of semen collection and the possibility of contamination by respiratory droplets cannot be ruled out. Song *et al.* apart from testing SARS CoV-2 virus in semen, also biopsied the testis from a deceased man which tested negative.[7]

Table 1: Baseline characteristics of enrolled participants (*n*=37)

Variable	Value
Age (years)#	32.2±5.6
BMI (kg/m^2)#	25.8±1.8
Hindu$	86.4
Muslim	5.4
Sikh	8.2
Christian	0
Employed$	86.5
Unemployed	13.5
Married$	83.7
Unmarried	16.2
Rural$	32.5
Urban	67.5
Smoking$	18.9
Alcohol$	18.9
Duration of symptoms (days)*	2 (1-3)
Symptomatic$	64.9
Time from symptom onset to semen collection (days)#	4.5±0.5
Time from testing to semen collection (days)*	3 (2-4)

#Mean±SD, *Median (IQR), $Proportion. SD=Standard deviation, IQR=Interquartile range

Table 2: Baseline laboratory investigations of study participants and semen parameters

Laboratory investigation (*n*=37)	Value (normal range)
Hemoglobin (g/dl)#	14.1±1.2
Total leukocyte count (cells/μl)*	5110±1197
Platelet count (lakh cells/μl)*	1.86±0.69
LDH (IU/L)#	241±68 (120-246)
CRP (mg/dl)#	0.48±0.62 (0-0.5)
Ferritin (ng/ml)#	243±293 (22-322)
Creatine phosphokinase (U/L)#	115±72 (32-294)
Fibrinogen (mg/dl)#	304±69 (180-350)
Procalcitonin (ng/ml)#	0.02±0.02 (<0.1)
Interleukin 6 (pg/ml)#s	3.8±3.9 (0-4.4)
Prothrombin time (s)#	12.1±0.8 (10.2-13.2)
Activated partial thromboplastin time (sec)#	33.7±4.4 (25.4-38.4)
D dimer (ng/ml)#	124±105 (<500)
Semen parameters (*n*=17)	
Abstinence (days)	7±2
Semen volume (ml)#	2.8±0.7
Sperm concentration (million/ml)#	45.9±22.2
Sperm motility (PR + NPR %)#	62.9±15.2

#Mean±SD, *Median (IQR), $Proportion. PR=Progressive motility, NPR=Nonprogressive motility, SD=Standard deviation, IQR=Interquartile range, CRP=C-reactive protein, LDH=Lactate dehydrogenase

Along with testing the presence of virus in semen in the present study, semen analysis was also performed from 17 men positive for COVID-19 infection. We found all semen parameters within normal range among these men. Our findings corroborate the findings of Guo *et al.* who also found normal sperm parameters.[10] Holtmann *et al.* analyzed the semen parameters after categorizing patients with and without fever and found lower counts and motility in patients with fever as compared to those without fever at the time of semen collection.[11] This finding can be an effect of fever and inflammatory cytokines that spike during pyrexia *per se* on semen parameters and not specific to SARS-CoV-2 as the parameters though reduced but were

still within the limits of normal values. The morphological evaluation of semen samples could not be done due to technical constraints; however, the mild form of disease is unlikely to alter the semen parameters as in our series.

We had planned to repeat semen testing at 2 weeks among individuals demonstrating the presence of virus in initial semen sample, to determine the duration of viral shedding. It could have suggested the time period required for abstinence. As we did not isolate virus from any initial sample, we believe that there is no need of abstinence after recovery from this infection. This also allays anxiety of all laboratory personnel involved in semen handling as they hesitated performing semen analysis during COVID-19 pandemic fearing infection from sample of an asymptomatic individual providing sample. The American Society of Reproductive Medicine guidelines recommend the use of universal precautions only while handling semen samples of COVID-19 patients due to inconclusive evidence on seminal transmission.[15] However, national guidelines demand testing for COVID-19 infection before getting a semen analysis.[16] As assisted reproductive techniques have slowly returned to normal across the globe, RT-PCR testing before semen analysis and use of PPE for the laboratory personnel puts extra burden on the system and increases the overall cost of infertility treatment. Based on our study results, we recommend against routine COVID-19 testing and use of PPE before semen analysis.

Our study has certain limitations, the most important being the small sample size due to technical constraints and strict inclusion criteria. Another limitation was the noninclusion of antibody testing in the study cohort which could have identified individuals who were earlier exposed to virus and developed antibodies. This would have resulted in mild illness and absence of virus in semen. Inability of individuals with moderate-to-severe disease to provide semen sample prevents us from studying shedding in various stages to make a strong recommendation. However, for asymptomatic and mild individuals, it is safe to conclude that sexual contact does not serve as a mode to transmit this disease.

CONCLUSION

SARS CoV-2 virus was not found in the semen of any of the men with active COVID-19 infection. This finding suggests that laboratory handling semen samples of such men, peno-vaginal intercourse, or the use of semen for artificial reproductive techniques may not be associated with risk of transmission of COVID-19.

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REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
2. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, *et al*. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020;382:1708-20.
3. Mohseni AH, Taghinezhad-S S, Xu Z, Fu X. Body fluids may contribute to human-to-human transmission of severe acute respiratory syndrome coronavirus 2: Evidence and practical experience. Chin Med 2020;15:58.
4. Wang ZP, Xu XJ. Sc RNA-seq profiling of human testes reveals the presence of ACE2 receptor, a target for SARS-CoV-2 infection in spermatogonia, leydig and sertoli cells. Cells 2020;9:920.
5. 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:e208292.
6. Paoli D, Pallotti F, Colangelo S, Basilico F, Mazzuti L, Turriziani O, *et al.* Study of SARS-CoV-2 in semen and urine samples of a volunteer with positive naso-pharyngeal swab. J Endocrinol Invest 2020;43:1819-22.
7. Song C, Wang Y, Li W, Hu B, Chen G, Xia P, *et al*. Absence of 2019 novel coronavirus in semen and testes of COVID-19 patients. Biol Reprod 2020;103:4-6.
8. Pavone C, Giammanco GM, Baiamonte D, Pinelli M, Bonura C, Montalbano M, *et al.* Italian males recovering from mild COVID-19 show no evidence of SARS-CoV-2 in semen despite prolonged nasopharyngeal swab positivity. Int J Impot Res 2020;32:560-2.
9. Pan F, Xiao X, Guo J, Song Y, Li H, Patel DP, *et al.* No evidence of severe acute respiratory syndrome-coronavirus 2 in semen of males recovering from coronavirus disease 2019. Fertil Steril 2020;113:1135-9.
10. Guo L, Zhao S, Li W, Wang Y, Li L, Jiang S, *et al.* Absence of SARS-CoV-2 in semen of a COVID-19 patient cohort. Andrology 2021;9:42-7.
11. Holtmann N, Edimiris P, Andree M, Doehmen C, Baston-Buest D, Adams O, *et al.* Assessment of SARS-CoV-2 in human semen-a cohort study. Fertil Steril 2020;114:233-8.
12. Kayaaslan B, Korukluoglu G, Hasanoglu I, Kalem AK, Eser F, Akinci E, *et al.* Investigation of SARS-CoV-2 in semen of patients in the acute stage of COVID-19 infection. Urol Int 2020;104:678-83.
13. Falahi S, Kenarkoohi A. Sex and gender differences in the outcome of patients with COVID-19. J Med Virol 2021;93:151-2.
14. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. WHO press, Geneva, Switzerland; 2010.
15. Patient Management and Clinical Recommendations during the Coronavirus (covid-19) Pandemic- American Society for Reproductive Medicine. Available from: http://www.asrm.org. [Last accessed on 2020 Mar 17].
16. Prasad S, Trivedi P, Malhotra N, Patil M, Swaminathan D, Shukla S, *et al.* Joint IFS-ISAR-ACE Recommendations on Resuming/Opening Up Assisted Reproductive Technology Services. J Hum Reprod Sci 2020;13:82-8.

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