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Evaluation of SARS-CoV-2 in Human Semen and Effect on Total Sperm Number: A Prospective Observational Study

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Purpose: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has created a surge of research to help better understand the breadth of possible sequelae. However, little is known regarding the impact on semen parameters and fertility potential. We sought to investigate for presence of viral RNA in semen of men with SARS-CoV-2 infection and to evaluate its effect on semen parameters in ejaculate.

Materials and Methods: We prospectively recruited thirty men diagnosed with acute SARS-CoV-2 infection using real-time reverse transcriptase polymerase chain reaction (RT-PCR) of pharyngeal swab specimens. Semen samples were collected from each individual using mailed kits. Follow-up semen samples were done with mailed kits or in-person in office setting. Semen analysis and PCR was performed after samples were received.

Results: Thirty semen samples from recovered men were obtained 11–64 days after testing positive for SAR-CoV-2 infection. The median duration between positive SAR-CoV-2 test and semen collection was 37 days (interquartile range [IQR]=23). The median total sperm number (TSN) in ejaculate was 12.5 million (IQR=52.1). When compared with age-matched SARS-CoV-2(-) men, TSN was lower among SARS-CoV-2(+) men (p=0.0024). Five men completed a follow-up sperm analysis (median 3 months) and had a median TSN of 18 million (IQR=21.6). No RNA was detected by means of RT-PCR in the semen in 16 samples tested.

Conclusions: SARS-CoV-2 infection, though not detected in semen of recovered men, can affect TSN in ejaculate in the acute setting. Whether SARS-CoV-2 can affect spermatogenic function long-term remains to be evaluated.

Keywords: COVID-19; Infertility, male; SARS-CoV-2; Semen; Sperm count

INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); it has spread worldwide since being identified in December 2019 in Wuhan, China [1]. The World Health Organization (WHO) has recorded more than 20,162,474 cases resulting in over 737,417

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deaths as of August 12, 2020 [2]. It is estimated that up to 70% of cases are asymptomatic or with very mild symptoms [3]. Early epidemiological data has suggested that the primary mode of transmission is through respiratory droplets, but the presence of SARS-CoV-2 has been identified in other bodily fluids such as feces, urine, and semen [4-6]. Due to these findings, questions have been raised about the possibility of viral shedding and transmission through semen in addition to its conceivable impact on male fertility.

Steps have been taken to identify all sources of viral transmission and complete our understanding of the effects of SARS-CoV-2. It has been established that SARS-CoV-2 utilizes angiotensin converting enzyme 2 (ACE2) for cell entry [6]. Among other organs, ACE2 receptor expression has been identified in genitourinary organs and the testis [7-10]. Li et al [6] detected SARS-CoV-2 in semen samples, making possible that the virus might be seeded in the reproductive system and affect the function of the testis. Furthermore, it was previously shown in 2005 that SARS-CoV viral particles were still present in urine and feces after men cleared virus from nasopharyngeal secretions [11]. The potential presence of SARS-CoV-2 in other bodily fluids is especially concerning for men who show little or no symptoms who may act as a potential reservoir of the virus and a long-term source for transmission [12]. Taken together, these findings have raised concern that SARS-CoV-2 can serve as a vector for transmission through semen or have an impact on sperm parameters.

Our study sought to evaluate the impact of SARS-CoV-2 on male reproduction among American men. We collected semen samples from men who recovered from SARS-CoV-2 infection to evaluate for the presence of SARS-CoV-2. We also assessed the impact of SARS-CoV-2 infection on total sperm number (TSN) in ejaculate both during the acute phase after testing positive and a smaller cohort of men at follow-up.

MATERIALS AND METHODS

1. Study design and participants

Men aged 18 to 70 who were diagnosed with SARS-CoV-2 infection were enrolled in this study. Subjects were identified using an electronic medical record search for men age 18–70 years old who had tested positive for SARS-CoV-2 infection. Using the interim guidance from the WHO and previous literature, a laboratory confirmation of SARS-CoV-2 infection was determined using real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay of nasal or pharyngeal swabs upon hospital presentation (WHO 2020) [13]. Exclusion criteria included prisoners, subjects unable to consent, critically ill, history of mumps, ejaculatory disfunctions, vasectomy, prior testosterone replacement therapy, history of sexual transmitted infections, history of male factor infertility, or if they were unwilling to donate semen.

Additional age-matched samples were collected during the same time period on men who had a negative SARS-CoV-2 test and presented for semen analysis (SA). To minimize bias, only men who presented for fertility checks or sperm cryopreservation prior to vasectomy were included as controls; those who presented with male-factor infertility, history of testosterone replacement, or a history of sexual transmitted infections were excluded. These age-matched SARS-CoV-2 negative subjects were included to compare semen analyses to the SARS-CoV-2 positive cohort.

2. Specimen collection and processing

Subjects enrolled in the study received at-home testing kits with sterile cup. They were instructed to produce semen samples into the sterile cup following a minimum of 48-hours and maximum of 7 days of sexual abstinence. All biohazard safety protocols were followed with regards to shipping Biological Substance Category B (UN3373) materials. All samples were collected in sterile containers which were placed in a biohazard bag, followed by putting into a rigid container with absorbent material inside and then a plastic overlay with proper biohazard labeling and shipment information. For subjects who were quarantining, or those who did not feel comfortable leaving the house, a dedicated team-member assisted with scheduling the package to be securely picked up from their house. This team-member also assisted with any questions regarding the consent, collection or mailing protocols. All participants then returned the samples by overnight mail at room temperature to our laboratory using the included pre-paid package inside and following all safety protocols.

Participants who initially provided samples and consented to the study were also asked to provide a follow-up semen sample *via* this same process 90 days after

their initial sample using the same mailing kit and overnight return mail. SA, including semen volume, concentration, pH, and motility, was performed according to the WHO laboratory manual for the examination and processing of human semen (5th edition) [14]. For counting "total sperm number", 6 μL of semen was placed on a Microcell chamber (vitrolife, CA, USA) and counted using a light microscope with a phase-contrast stage at 400× magnification. Quality control is performed each day of testing using QC-Beads (Bioscreen, NY, USA).

3. Detection of SARS-CoV-2 in semen

The whole semen aliquots were centrifuged at 4℃ for 7 minutes at 10,000 rpm and supernatant was removed without disturbing the sperm pellet. The pellet was washed 2 times with Phosphate-Buffered Saline (Gibco, Carlsbad, CA, USA). The total RNA was isolated from purified sperm samples using the TRIzol (ambion catalog #15596018; Invitrogen, Carlsbad, CA, USA) method (with brief modifications). The modifications include-adding 1 mL of TRIzol containing β-Mercaptoethanol (Sigma catalog #M3148; Sigma-Aldrich, St. Louis, MO, USA) (10 μL/mL) to the sperm pellet and homogenized by passing through a 26-G needle attached to a 5-mL syringe 20 to 25 times. Then the samples were vortexed for 1 minute and incubated for 20 minutes on ice for complete dissociation of the sperm membrane. This is followed by the standard RNA isolation protocol. Post isolation, RNA was reverse transcribed to complementary DNA using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The quantitative RT-PCR for indicated genes was performed in SYBR Universal PCR Master Mix (BIO-RAD, Hercules, CA, USA). Quantitation of mRNAs was performed using BIORAD Gene Expression Assays according to the manufacturer's protocol. Samples were analyzed using the BIORAD sequence detection system. All PCRs were performed in triplicate, and the specificity of the reaction was determined by melting curve analysis at the dissociation stage. The relative quantitative method was used for the quantitative analysis. The calibrator was the average ΔCt from the untreated cells. The 2019-Novel Coronavirus Real-time RT-PCR Primers sequences were referred from Centers for Disease Control and Prevention website (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html).

4. Data collection

Subjects medical history, clinical course, and demographic data were documented upon presentation to the hospital. Additional information including specific symptoms, duration of symptoms, prior fertility, history of vasectomy, *etc.*; were collected during the phone interview with the subjects during initial recruitment.

5. Statistical analysis

Subject demographics are represented with descriptive statistics. Continuous variables are described using median and interquartile ranges (IQRs) or means and standard deviations and analyzed with Mann-Whitney U-test or t-test, as required according to the data distribution. Data was analyzed using SPSS version 25.0 software for Mac (IBM Corp., Armonk, NY, USA). A p-value <0.05 was considered statistically significant.

6. Ethics statement

Once identified, subjects were contacted about their participation in the study at which time details regarding the study were discussed and they were counseled regarding their voluntary participation in the study. For those who were willing to participate, the informed consent and HIPPA forms were explained in detail and the subjects had an opportunity to ask any questions. After this initial consent was given, men were mailed a securely packaged testing kit through express mail to provide semen samples. Additionally, all informed consent documents and HIPPA forms were mailed to obtain a wet signature for full consent in the study. All participants gave written informed consent before they were included. This study was approved by the Institutional Review Board of the University of Miami (IRB No: #20200401).

RESULTS

A total of 183 men between 18–70 years old who tested positive for SARS-CoV-2 were contacted regarding participation in this study. Of those, 83 agreed to participate in the study and packages including the collection containers and all required consent paperwork were mailed to their homes. Currently, 30 subjects have returned all required consent documents and samples that were analyzed.

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Subject demographics and clinical characteristics of the 30 enrolled subjects are included in Table 1. Overall, the median age was 40 (IQR=24.75) and median body mass index was 26.25 (IQR=3.57). Previous smoking history was present in 6.9%. A total of 17 (56.7%) of men had proven history of fertility, which was assessed by either having a child or having gotten a partner pregnant in the past. Of the 30 men, 1 was asymptomatic and the remaining 29 had symptomatic infection with a mean duration of 12 days (±8.97). Only one (3.4%) subject described bilateral testis pain (suggestive of orchitis) as one of the symptoms experienced and only 8 (27.6%) men required hospitalization. Only 6 subjects received any treatments during their infective period, of which, treatments included Azithromycin (n=2), convalescent plasma (n=3), dexamethasone (n=3) and Hydroxychloroquine (n=2).

A SA was performed on all 30 initial semen specimens collected, as well as the 5 follow-up specimens collected. For the 30 initial semen samples, the semen parameters including volume, sperm concentration, pH are included in Table 2. The median duration between positive SAR-CoV-2 test and semen collection was 37 days (IQR=23) and median duration between semen collection and SA was 1 day (IQR=1.75). The median semen volume was 2.1 mL (IQR=1.23), median sperm concentration was 11.5 million/mL (IQR=26.8), median pH was 7.2 (IQR=0.8), and median TSN in ejaculate was 12.5 million (IQR=52.1). Sperm motility could not

Table 1. Patient demographics and clinical characteristics

Variable	Value
Median age (y)	40 (IQR=24.75)
BMI (kg/m^2)	26.25 (IQR=3.57)
Smoking status	
Never	93.1
Former	6.9
Symptoms	
Abdominal pain	6 (20.7)
Anosmia	13 (44.8)
Chills	18 (62.1)
Cough	19 (65.5)
Diarrhea	11 (37.9)
Dyspnea	8 (27.6)
Fever	21 (72.4)
Headache	13 (44.8)
Myalgia	19 (65.5)
Nasal congestion	6 (20.7)
Orchitis	1 (3.4)
Pharyngitis	7 (24.1)
Rhinorrhea	7 (24.1)
Vomiting	1 (3.4)
Median duration of symptoms (d)	10.5 (IQR=9.5)
Requiring hospitalization	
Yes	8 (27.6)
No	21 (72.4)
Median interval between SARS-CoV-2 positive and semen collection (d)	37 (IQR=23)

Values are presented as median (IQR), percentage only, or number (%).
BMI: body mass index, IQR: interquartile range, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

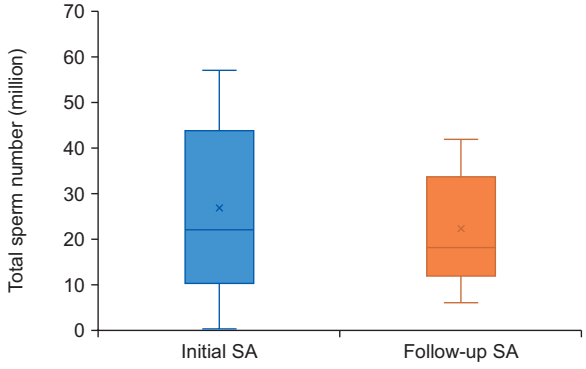

Fig. 1. Comparison of total sperm number on initial semen analysis (SA) *vs*. follow-up SA for the 5 subjects who agreed for a follow-up analysis.

Table 2. Comparison of semen parameters in COVID (+) men and age-matched controls

Variable	COVID (+) cohort (n=30)	COVID (-) cohort (n= 30)	p-value
Age (y)	40 (IQR=24.75)	42 (IQR=9.8)	0.8732
Volume (mL)	2.1 (IQR=1.23)	2.2 (IQR=2.15)	0.3841
pH	7.2 (IQR=0.8)	7.2 (IQR=0.4)	0.2304
Concentration (million/mL)	11.5 (IQR=26.8)	21.5 (IQR=21.5)	0.0048
Total sperm number (million)	12.5 (IQR=52.1)	59.2 (IQR=70.5)	0.0024

Values are presented as median (IQR).
COVID: coronavirus disease, IQR: interquartile range.
$p<0.05$ was considered significant.

be assessed because samples were shipped, and we discouraged SARS-CoV-2 (+) men from leaving their residence during quarantine period. Comparing the SARS-CoV-2 (+) men to age-matched SARS-CoV-2 (-) men who obtained SA during the same time period, the demographics for the control group are included on Supplement Table 1. The concentration and TSN for SARS-CoV-2 (+) men (median 11.5, IQR=26.8; median 12.5, IQR=52.1, respectively) was significantly lower than SARS-CoV-2 (-) men (median 21.5, IQR=21.5; median 58.2, IQR=70.5, respectively) (p=0.0048; p=0.0024, respectively) (Table 2).

A total of 5 subjects completed a follow-up SA after a median of 91 days (IQR=61) after the initial SA. The full data for initial and follow-up SA for these 5 subjects are included on Supplement Table 2. For these 5 men, the initial SA showed a median TSN of 22 million (IQR=34) and their follow-up SA had a median TSN of 18 million (IQR=21.6). The initial and follow-up SA for these subjects are illustrated on Fig. 1. No SARS-CoV-2 RNA was detected in the 16 samples that were able to be tested *via* RT-PCR. The remaining 14 samples were unable to be analyzed for viral presence due to the decreased sperm counts and quality of specimens.

DISCUSSION

The SARS-CoV-2 is a novel pandemic which has changed the world as we once knew it. While many of preventive measures being advised by the WHO are focused in precautions against respiratory droplets, solid data about sexual transmission is lacking [15]. Additionally, SARS-CoV-2 impact on semen parameters is not clear and could affect several men in reproductive age seeking to conceive in the future. We hypothesized that SARS-CoV-2 would not be present in semen and that SARS-CoV-2 would impair semen parameters. In our study, we evaluated semen parameters after acute SARS-CoV-2 infection and for the presence of SARS-CoV-2 virus in the semen. The median TSN of men previously infected with SARS-CoV-2 was 12.5 million (IQR=52.1), which is less than the 5th percentile (20 million) of TSN according to WHO guidelines for the general population of men who may have not been screened for fertility [14]. Additionally, 83.3% (25/30) of our cohort had a TSN less that the 25th percentile and 50% (15/30) had a TSN less than the 2.5th percentile [14]. The 5 subjects who completed a follow-up SA, whose demographics were similar to the remainder of the cohort, showed a similar TSN (median 18 million, IQR=21.6) compared to the initial analysis (median 22 million, IQR=34). While studies have demonstrated that we can expect semen parameters to be impacted during an acute illness with fever, this TSN is still considered low when comparing the results to other studies which evaluated parameters following acute illness [16]. Therefore, we believe men should be counseled regarding the necessity of close monitoring of semen parameters, as well as offering the possibility of cryopreservation of sperm for men with oligozoospermia. Also, there is not enough data at this time to provide a safety statement regarding sexual transmission of SARS-CoV-2 during the acute phase of infection.

Since the beginning of the SARS-CoV-2 pandemic, numerous studies have focused on evaluating the impact of SARS-CoV-2 on the reproductive axis and the potential for sexual transmission. Ma et al [17] suggested that SARS-CoV-2 infection can result in abnormal sexual hormone secretion and lead to dysfunction in reproduction function. Holtmann et al [18] proposed that infection can affect spermatogenesis and found that moderate infections have a statistically significant impairment of sperm quality when compared to controls. Eight studies have investigated semen specimens for the presence of SARS-CoV-2. Only one study, by Li et al [6], identified the presence of viral RNA in six (15.8%) semen specimens, with four of them being present in acutely infected men. On the other hand, seven other studies that evaluated a combined 134 men did not detect SARS-CoV-2 in semen in men who were in the acute or recovery phase [17-23]. Thus, studies so far have demonstrated that SARS-CoV-2 can impact spermatogenesis while the majority has suggested that the virus cannot be transmitted sexually.

Our findings are in accordance with the majority of studies which are available in the literature to this point [17-23]; we did not detect SARS-CoV-2 RNA in any semen samples tested. Therefore, based on our results, males that have recovered from SARS-CoV-2 acute phase are unlikely to be a source for sexual transmission of SARS-CoV-2. However, until more studies are published with long-term data, we recommend that men with positive history of SARS-CoV-2 who are interested in fertility should be evaluated by a fertility specialist. Since most men were tested 37 days (IQR=23) after the initial diagnosis, we cannot af-

firm with certainty whether or not recently diagnosed males can sexually transmit the virus. Furthermore, *in vitro* fertilization (IVF) clinics should consider assessing SARS-CoV-2 RNA in the semen and sperm banking facilities should consider excluding men with positive history of SARS-CoV-2.

It is still unclear what SARS-CoV-2's long-term impact on male fertility will be. While it's evident that SARS-CoV-2 infection has implications in the short-term, long-term follow-up of SARS-CoV-2 positive males and data on testicular histology will clarify whether the variation of semen parameters is related to the acute febrile illness or not [16]. Furthermore, the evident decrease in semen parameters could be a sequelae of SARS-CoV-2 infection and the resulting systemic inflammatory state and immune response against the seminiferous epithelium [24]; or caused by a breach on the blood-testis barrier [25]. Although the 5 subjects in our study had a median TSN that was similar to the initial analysis (20 million to 18 million), the sample size is small and larger numbers of follow-up data is still needed before we can assess the effect-period. A direct effect on the testes by SARS-CoV-2 cannot be concluded due to the lack of RNA detected in the semen specimens. We can only speculate that temporary variations on semen quality may be attributed to acute febrile illness, while irreversible findings should be related to immune response and/or disruption of the blood-testis barrier.

Our study has strengths and limitations. Strength of our study is that this is the first study that provides insight into both the presence of SARS-CoV-2 in semen and its effect on semen parameters in the US population. However, our study is also not without limitations. First, most of the semen samples came from non-severe men of whom were in the recovery stage and lacked symptoms. Secondly, men were excluded from the study if they had a known male infertility factor, however not all men were examined prior to enrollment and it is possible that these subjects could have an undiagnosed male infertility factor. Additionally, our sample size was limited due to minimized in-person patient communication and we only collected one SA without evaluation of sperm motility at each time point, which we understand is likely to have variations; and endocrinology data was unable to be collected in a safe manner, which could have provided additional insight. Also, follow-up SA was not collected from the control group. Furthermore, to minimize contact with positive subjects, overnight mail-in SA kits were used during the acute phase of infection – which prevented us from evaluating motility in these samples. Lastly, we did not have SA information from men prior to their SARS-CoV-2 infection, so comparison with their baseline was not possible; and without a known timeline for sperm production following an acute illness – the timing of our follow-up SA was based off of the spermatogenesis timeline and the specific recovery time is still unknown for SARS-CoV-2 infection. Future studies will evaluate long-term impacts of SARS-CoV-2 diagnosis on sperm parameters and the hypothalamic-pituitary-gonadal axis axis of men.

CONCLUSIONS

The sperm concentration and TSN of men diagnosed with SARS-CoV-2 was lower than men who tested negative. Nevertheless, the 5 men who completed follow-up analysis had similar TSN compared to the initial analysis. SARS-CoV-2 RNA could not be detected in any semen samples tested. The immediate impact on TSN in recovered men from SARS-CoV-2 infection is concerning, nevertheless long-term follow-up of these men is critical to determine whether the sperm counts will recover with time or show further decline.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: RR, JCB, MK. Data curation: JCB, MK, KK, TFNL, FSF, JA, OR, BM, HA, EI, RR. Formal analysis: JCB, MK. Investigation: RR, JCB, MK. Methodology: RR, JCB, MK, HA, EI. Project administration: JCB, MK, TFNL, JA, FSF, OR, BM. Resources: RR. Supervision: RR, EI, HA. Validation: RR, KK, EI, HA. Visualization: RR, JCB, MK. Writing – original draft: RR, JCB, MK, TFNL. Writing – review & editing: RR, JCB, MK, TFNL.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.200192.

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