THE GEORGE WASHINGTON UNIVERSITY
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# SARS-CoV-2 Viability in Seminal Fluid

## Introduction

- Over 27 viruses found in human semen and sexual transmission confirmed in non-STIs, including Zika, Ebola, Chikungunya, and Dengue[1].
- Viruses can pass the blood-testis barrier in the presence of systemic/local inflammation and may persist in reproductive tract as a reservoir due to immunological privilege required for sperm survival[2].
- Previous studies have found that SARS-CoV-2 is likely capable of infecting the testes due to high expression of ACE2 and TMPRSS2, proteins necessary for viral attachment and entrance, in sperm cells, Leydig cells, and Sertoli cells[3].
- Li, Jin, & Bao (2020) found evidence of SARS-CoV-2 in semen of six (15.7%) patients out of 38 total, with 4 patients in the acute stage and 2 already recovered[4].

## Methodology

Part I

- Uninfected seminal fluid (SF) from biobank samples collected from men prior to the COVID-19 pandemic were pooled and spiked with SARS-CoV-2 stock virus (1.7 x $10^6$ viral particles/mL) for 1 hour at 34°C.
- The baseline Ct of the virus was measured via RT-qPCR.
- After the initial incubation with SF, the virus/SF mixture was tested via RT-qPCR, plated on Vero E6 cells in triplicate and a plaque assay was conducted.
- The mixture was added to an additional CPE plate, allowed to incubate for an hour, and then removed and washed thoroughly before new media was added.
- Media from the CPE plate was collected for RT-qPCR 3 dpi and photographed for cytotoxicity.
- The CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel was used to test for presence the viral genetic markers Nucleoprotein 1 and 2, indicating current infection.

Part II

- A cryopreserved, whole semen sample obtained from a sperm bank was infected with stock virus at an MOI of 0.5 before incubating for 1 hour at 34°C.
- After incubation, the mixture was washed twice to remove the virus and resuspended in sterile media before testing via RT-qPCR and plating on Vero E6 cells.
- The Ct value was measured after 72 hours and cytotoxicity was photographed.

## Results

- Compared to the baseline Ct values for the stock virus, we found that the N1 and N2 values did change significantly72 hours following the addition of and incubation with seminal fluid for 1 hour at 34°C (Table 1).
- The Ct values for N1 and N2 do not appear to be substantially lower after mixing with sperm for 1 hour at 34°C but were reduced after 72 hours of incubation (Table 1)
- For the sample control, seminal fluid alone appeared to cause no cytotoxicity (Figure 1B).
- Infectious virus (plaques and CPE) found after incubation with seminal fluid (Figure 1 and Figure 2).

| Sample Type | Gene target | Ct Value |
|---|---|---|
| Stock virus baseline | N1 | 17.42 |
| | N2 | 16.16 |
| Seminal fluid 72 hours | N1 | 9.32 |
| | N2 | 8.64 |
| Sperm 1 hour | N1 | 16.29 |
| | N2 | 15.68 |
| Sperm 72 hours | N1 | 12.26 |
| | N2 | 11.18 |

**Table 1:** RT-qPCR results showing quantitative measurement of genetic markers for CoV-2 at different timepoints with seminal fluid and sperm. N1= Nucleoprotein 1; N2= Nucleoprotein 2.

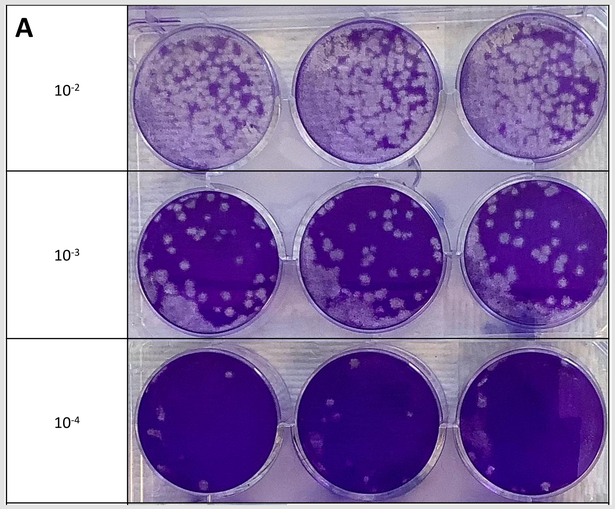



**Figure 1:** A) The plaques (small white circles) are visible in dilutions of a plaque assay on Vero E6 cells conducted after SARS-CoV-2 viral stock was mixed and incubated with SF at at 34°C. SF: Seminal fluid.

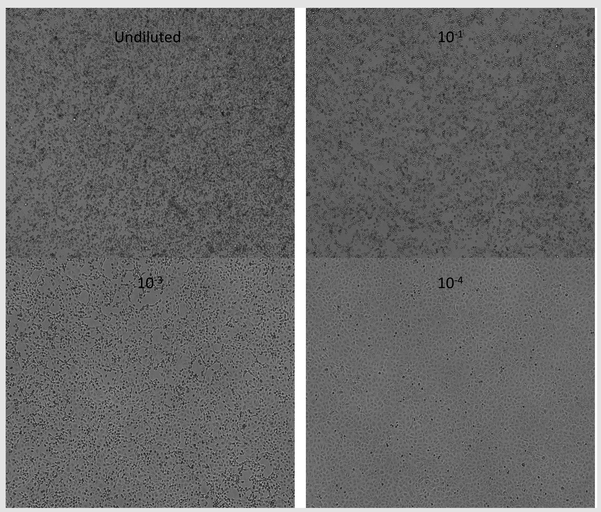


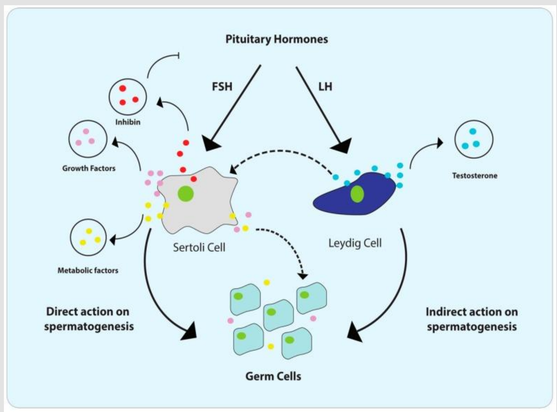

**Figure 2:** Cytotoxicity dilution on Vero E6 cells after stock virus was mixed with SF, incubated for 1 hour at at 34°C, washed and incubated for 72 hours at 37°C.

## Conclusion

- Detection of infectious virus after mixing suggests that SF is a suitable environment for SARS-CoV-2 to survive.
- Sperm appears to be capable of infection by SARS-CoV-2.

## Recommendations

- Is SARS-CoV-2 present in the semen of men with active infections?
- Is virus present in seminal fluid, sperm, or both?
- Is presence influenced by infection severity, including fever?
- What are the impacts of SARS-CoV-2 infection on sperm parameters?
- Does infection impact spermatogenesis by affecting the function of Sertoli and Leydig cells?




**Source:** Almeida et al. (2020) "The Cellular Impact of the ZIKA Virus on Male Reproductive Tract Immunology and Physiology"

## References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