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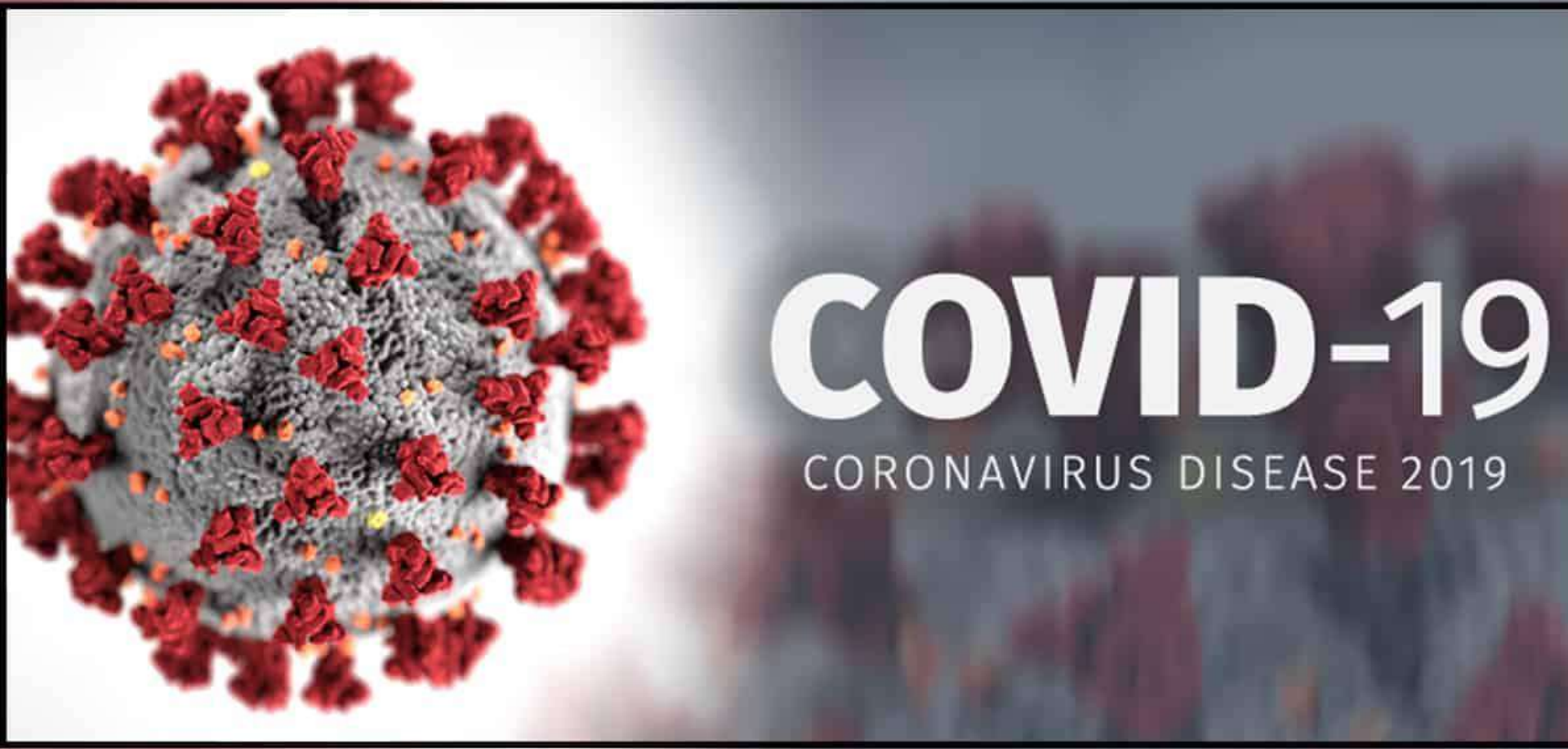
Will COVID-19 affect male fertility?



By: Zahra Rashidi Ph.D Reproductive Biology
Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences (KUMS)
May 2020

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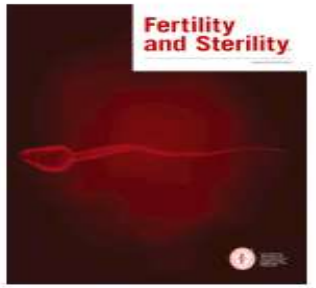

Prior and Novel Coronaviruses, COVID-19, and Human Reproduction: What Is Known?

James Segars, MD, Quinton Katler, MD, MS, Dana B. McQueen, MD, Alexander Kotlyar, MD, Tanya Glenn, MD, Zac Knight, PhD, Eve C. Feinberg, Hugh S.

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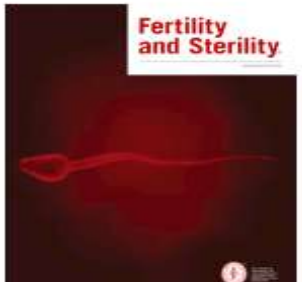


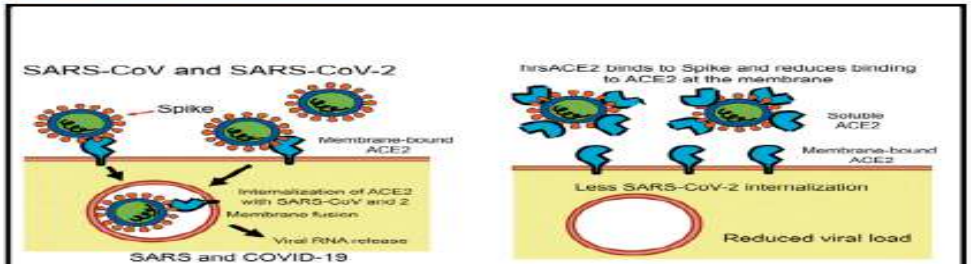
Prior and Novel Coronaviruses, COVID-19, and Human Reproduction: What Is Known?

James Segars, MD, Quinton Katler, MD, MS, Dana B. McQueen, MD, Alexander

scRNA-seq Profiling of Human Testes Reveals the Presence of ACE2

Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2

Graphical Abstract



Authors

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In Brief

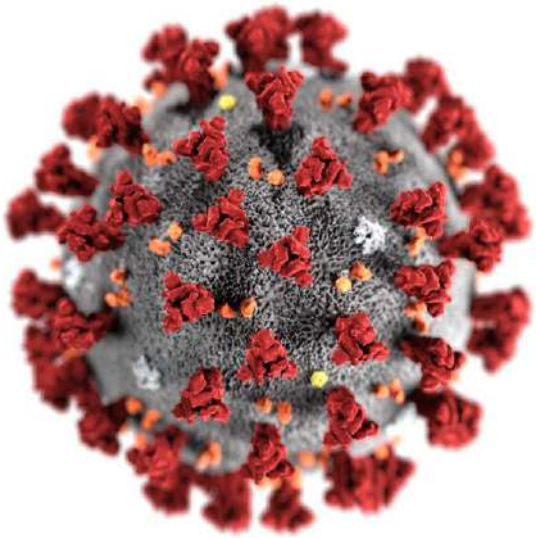
Clinical-grade recombinant human ACE2 can reduce SARS-CoV-2 infection in cells

Coronavirus

On December 31, 2019, the Health Commission of Hubei Province, People's Republic of China, announced a cluster of unexplained cases of pneumonia.

The virus was isolated, its genome was sequenced, and it was identified as the 2019 novel coronavirus (2019-nCoV).

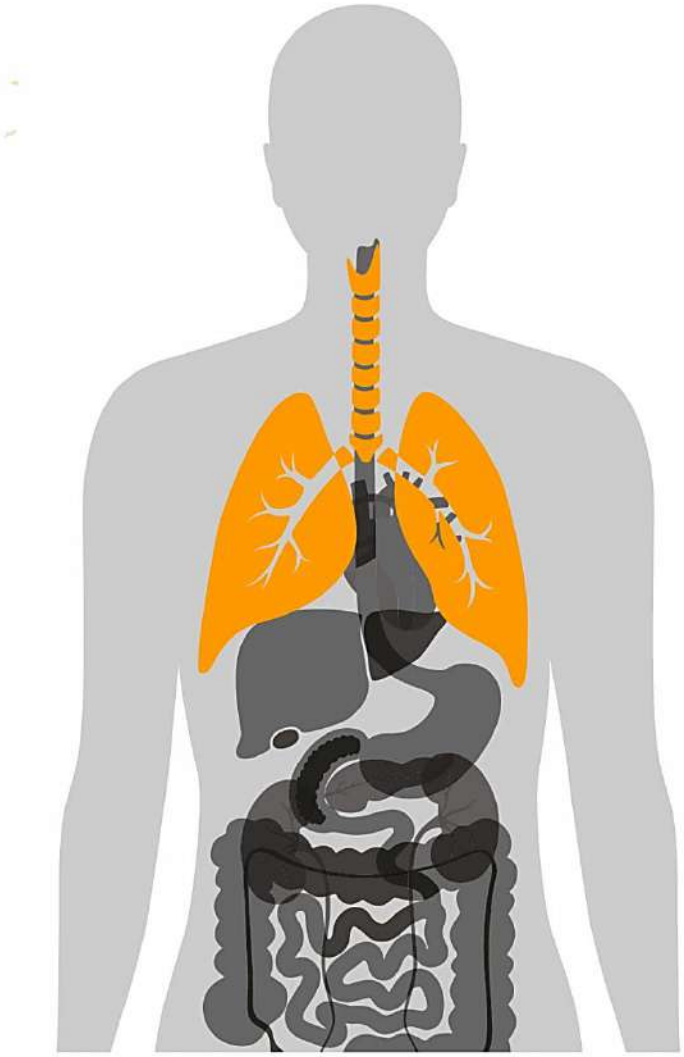
On February 11, 2020, the International Committee on Taxonomy of Viruses defined the virus as ''acute severe respiratory syndrome coronavirus 2'' (SARS-CoV-2) with the associated respiratory disease COVID-19 (CO-rona VI-rus D-isease 2019).



CORONAVIRUS PANDEMIC

COVID-19

COVID-19 is an infectious disease caused by SARS-CoV-2, a new type of coronavirus detected in China in late 2019.



Data shows the disease is mild in 80 percent of patients, severe in 13 percent, and critical in 6 percent.

Most common symptoms:

- Fever
- Fatigue
- Dry cough

Some patients may also have:

- Aches and pains
- Runny nose
- Sore throat
- Shortness of breath
- Diarrhoea

In critical cases, COVID-19 can cause severe pneumonia or a multiple-organ failure and can lead to death.

Approximately **80%** of infections are **mild** with flu-like symptoms,
15-20% are **severe**, requiring hospitalization and supplemental oxygen,
and **5%** are **critical** and require mechanical ventilation.

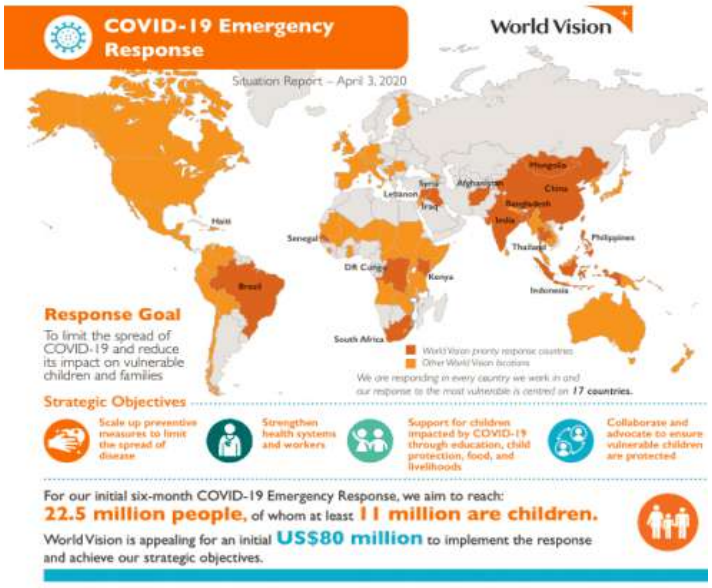
Risk factors for severe illness include age and underlying medical comorbidities such as cardiovascular disease, diabetes, chronic respiratory disease, hypertension and cancer.

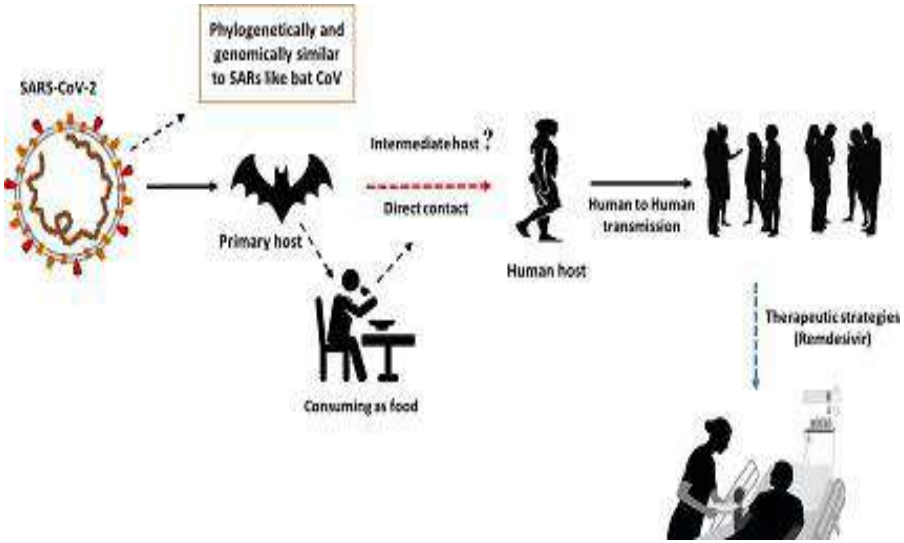
Death may occur in up to 3% of infections.

Death from SARS-CoV-2 is more common in individuals over age 60 or with underlying medical issues but can occur in younger persons, perhaps related to the inoculum.

Primary transmission is believed to occur through respiratory droplets from coughing and sneezing and contagion requires close proximity (less than 6 feet distance) between individuals.

The incubation period for COVID-19 is 3 to 7 days but can be as long as 2 weeks from infection to symptoms .





The COVID-19 pandemic brought unique challenges to the global healthcare community, with rapid escalation of the number of affected individuals and associated mortality over a handful of weeks.

Shortly **before puberty**, the sex cords acquire a lumen and become the **seminiferous tubules.** At about the same time, PGCs give rise to **spermatogonial stem cells**.

Type A spermatogonia

↓ **Mitotic Division**

Type B spermatogonia

↓ **Mitotic Division**

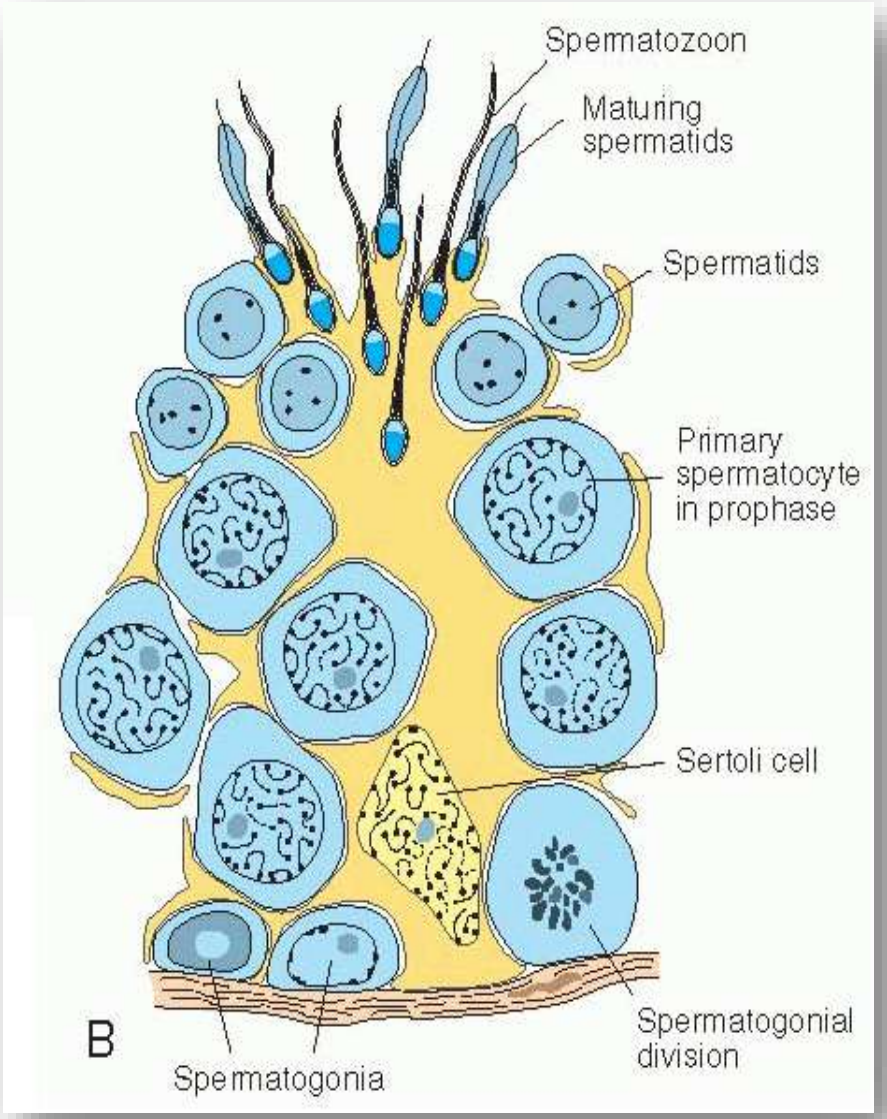
Primary spermatocytes

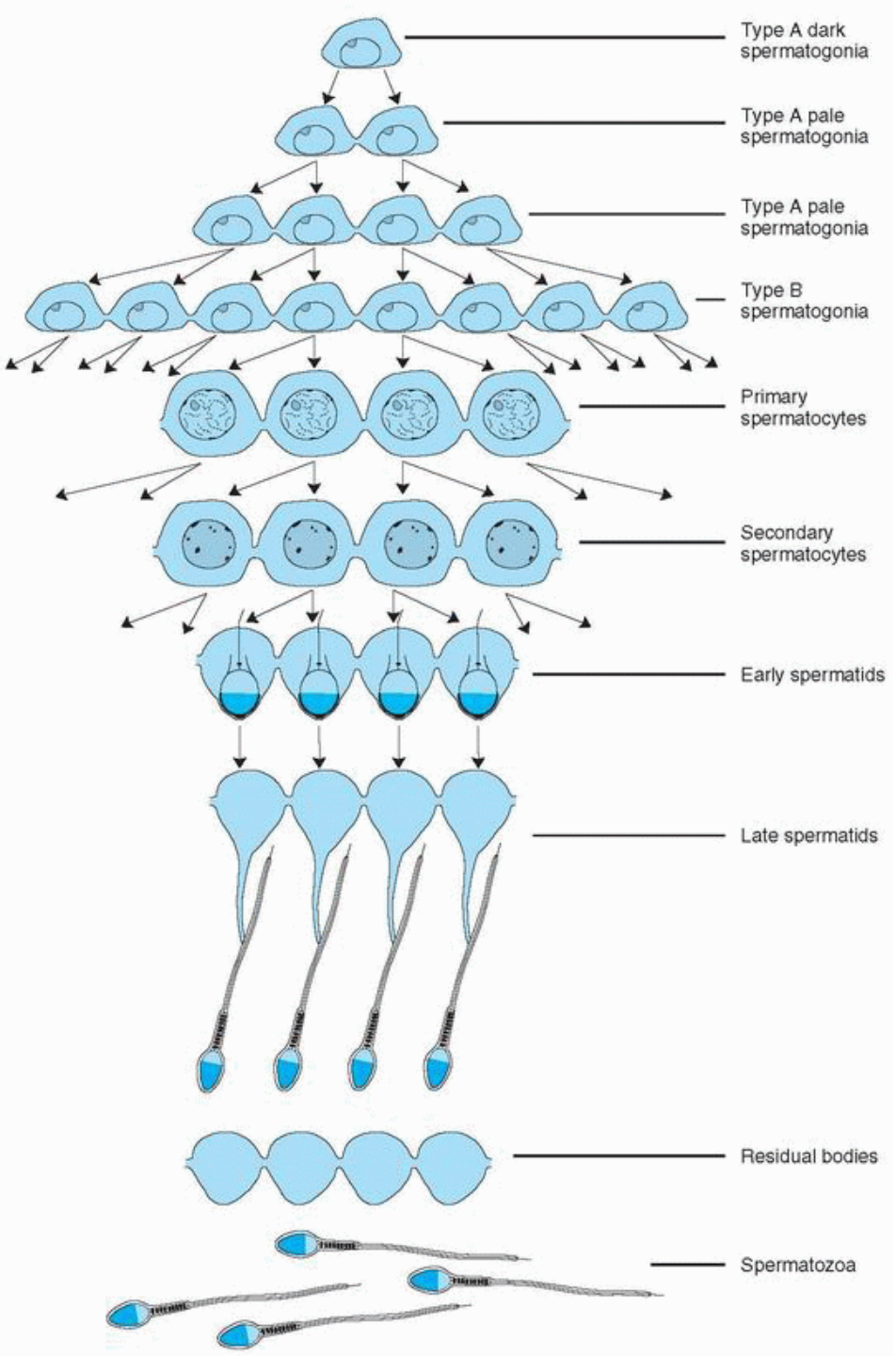
↓ **1st Meiotic Division**

Secondary spermatocytes

↓ **2nd Meiotic Division**

Spermatids





Mitotic Division

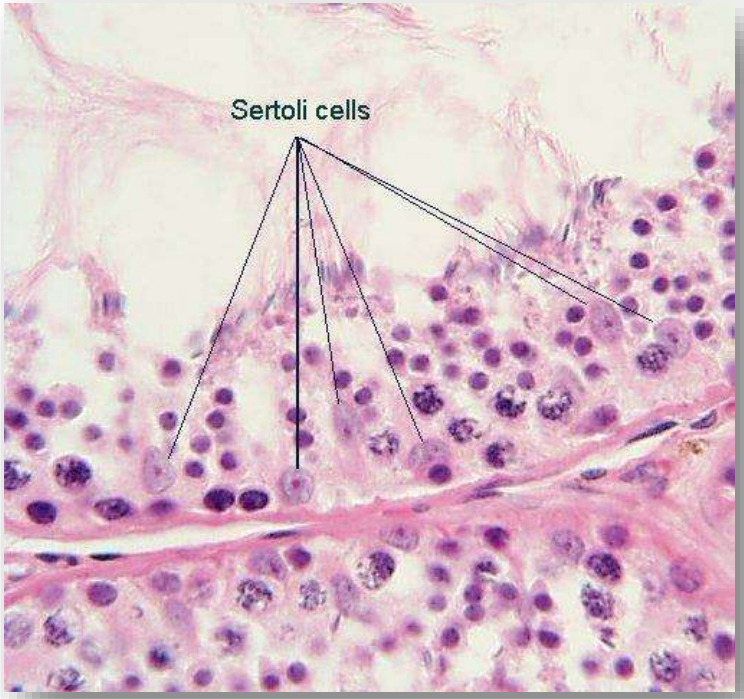
Meiotic Division

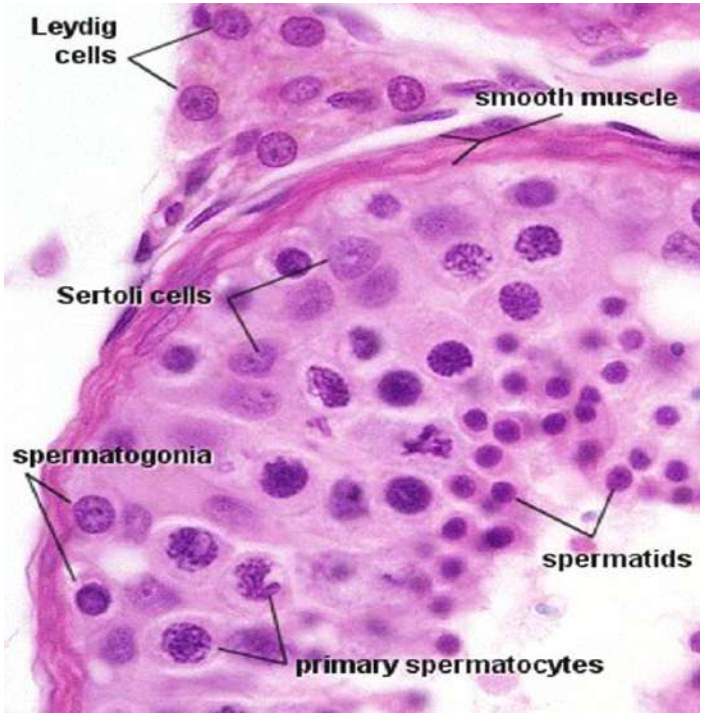
Furthermore, spermatogonia and spermatids remain embedded in deep recesses of Sertoli cells throughout their development .

Sertoli cells:

- ❖support and protect the germ cells
- ❖participate in their nutrition
- ❖assist in the release of mature spermatozoa

Leydig cells, also known as **interstitial cells of Leydig**, are found adjacent to the seminiferous tubules in the testicle. They produce **testosterone** in the presence of **luteinizing hormone (LH).**





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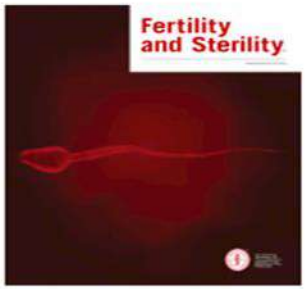


Prior and Novel Coronaviruses, COVID-19, and Human Reproduction: What Is Known?

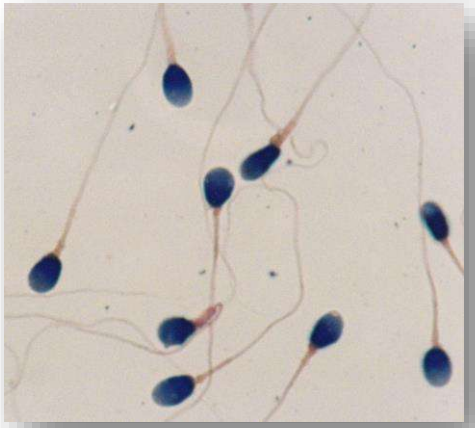
James Segars, MD, Quinton Katler, MD, MS, Dana B. McQueen, MD, Alexander Kotlyar, MD, Tanya Glenn, MD, Zac Knight, PhD, Eve C. Feinberg, Hugh S. Taylor, MD, James P. Toner, MD, PhD, Jennifer F. Kawwass, MD, for the ASRM Coronavirus/COVID-19 Task Force

PII: S0015-0282(20)30385-X
DOI: https://doi.org/10.1016/j.fertnstert.2020.04.025
Reference: FNS 32441

There are **limited data** regarding the impact of SARS-CoV-2 on human reproduction as the virus is novel and has only recently infected humans.

To date, there have been **no reports** of the virus in the female reproductive tract, in vaginal secretions, in amniotic fluid or in peritoneal fluid.

Although there is nothing to suggest that female or male gametes would be impacted directly by infection with SARS-CoV-2 or other coronaviruses, there is evidence that fever can impact **spermatogenesis.**



Therefore, male fertility may be diminished for 72-90 days following COVID- 19 due to **decreased sperm concentration** and **motility.**

The SARS-CoV-2 virus utilizes **ACE2 receptors** to gain entry into the human cells. The male reproductive system expresses ACE2 within adult **Leydig cells** in the testis and there are data to suggest that **ACE2 plays a role in spermatogenesis.**

The presence of ACE2 receptors is **much more prominent in the male reproductive** system than the female reproductive system.

These precautions are not currently recommended for SARS-CoV-2, given the lack of evidence for transmission through blood or sexual contact.

Similarly, there is no current recommendation for screening oocyte or sperm donors for SARS-CoV-2.

These are areas in which further investigation is necessary in order to assure the safety of stored gametes and the safety of patients undergoing assisted reproduction.

Comments (4)

Effect of SARS-CoV-2 infection upon male gonadal function:A single center-based study

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doi: https://doi.org/10.1101/2020.03.21.20037267

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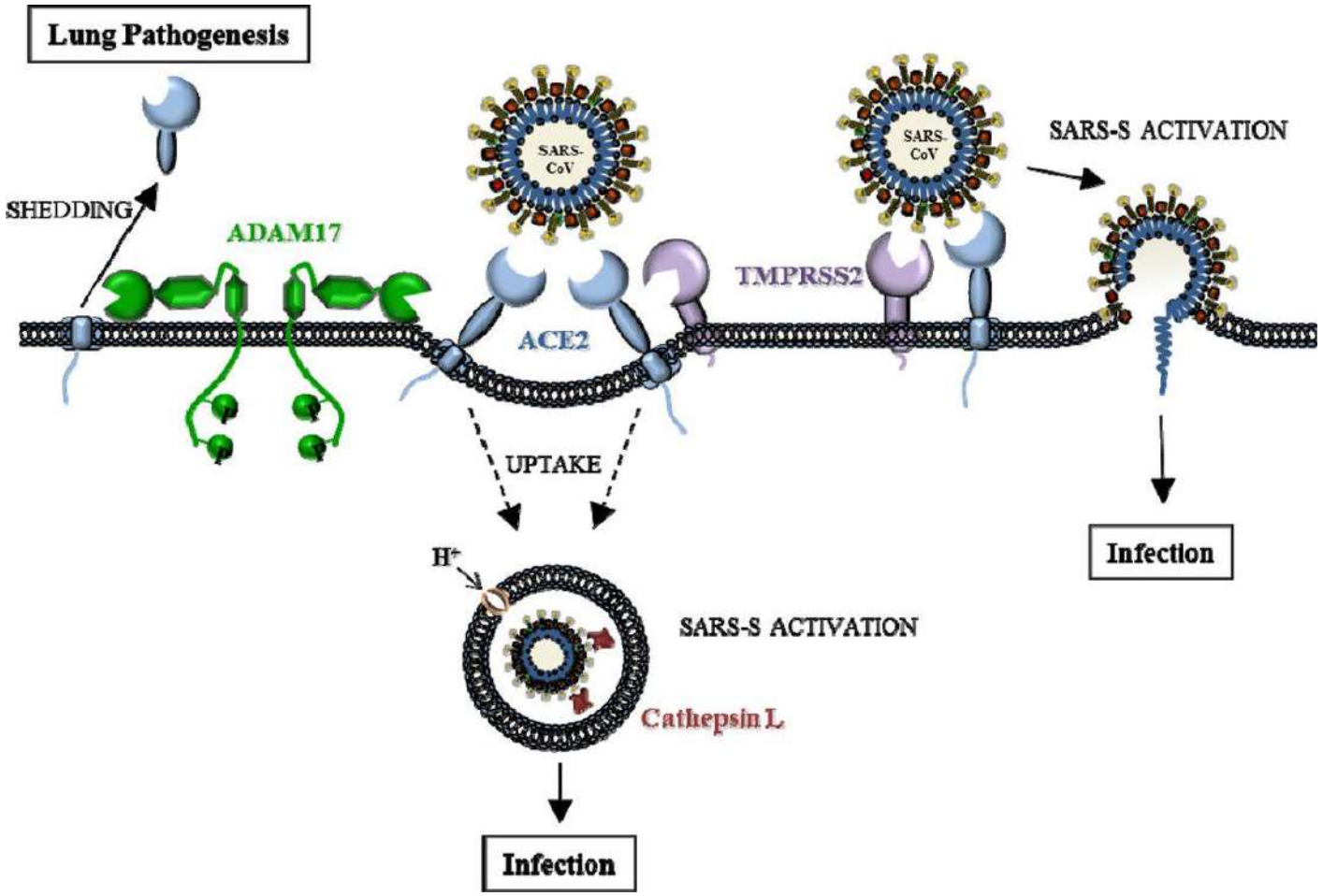
Except for the respiratory symptoms such as cough, fever and even acute respiratory failure, evidences of SARS-CoV-2 attack to multiple organs such as digestive, cardiovascular, urinary systems have been reported.

Angiotensin converting enzyme 2 (ACE2) is considered as the receptor for binding and entry into host cells by SARS-CoV-2.



According to the online database ***The Human Protein Atlas portal***, **testes** shows the **highest** expression level of **ACE2 protein and mRNA in** the body [8].

Based on scRNA-seq profiling of human testes, Wang ZP et al. also reported that ACE2 is predominantly enriched in **spermatogonia, Leydig and Sertoli cells** [9]. All the findings suggest the potential risk of male gonad to be vulnerable to SARS-CoV-2 attack.

In this study, we compared the sex-related hormones between

reproductive-aged men (100 age-matched healthy men)

SARS-CoV-2 infection and age-matched healthy men, **(81 male patients)**

Wuhan Leishenshan Hospital from Mar 5 to Mar 18, 2020.

All patients aged from **20~54 years** (with a median of 38 yrs).

In the study group:
serum testosterone **(T),**
estradiol **(E2),**
progesterone **(P),**
prolactin (**PRL),**
luteinizing hormone **(LH),**
follicle stimulating hormone **(FSH),**
antimullerian hormone **(AMH)**

were detected by **electrochemiluminescent immunoassays**

serum luteinizing hormone **(LH)**
testosterone (T) to LH **(T/LH)**
testosterone (T) to estradiol **(T/E2),**
the ratio of follicle stimulating hormone (FSH) to LH **(FSH/LH)**

Since the major roles of testes are spermatogenesis and androgens secretion, the sex-related steroids can be used to evaluate the status of male gonad.

Serum T levels did not statistically change in the COVID-19 group
a significant increase in serum LH level
a dramatic decrease in serum T: LH.

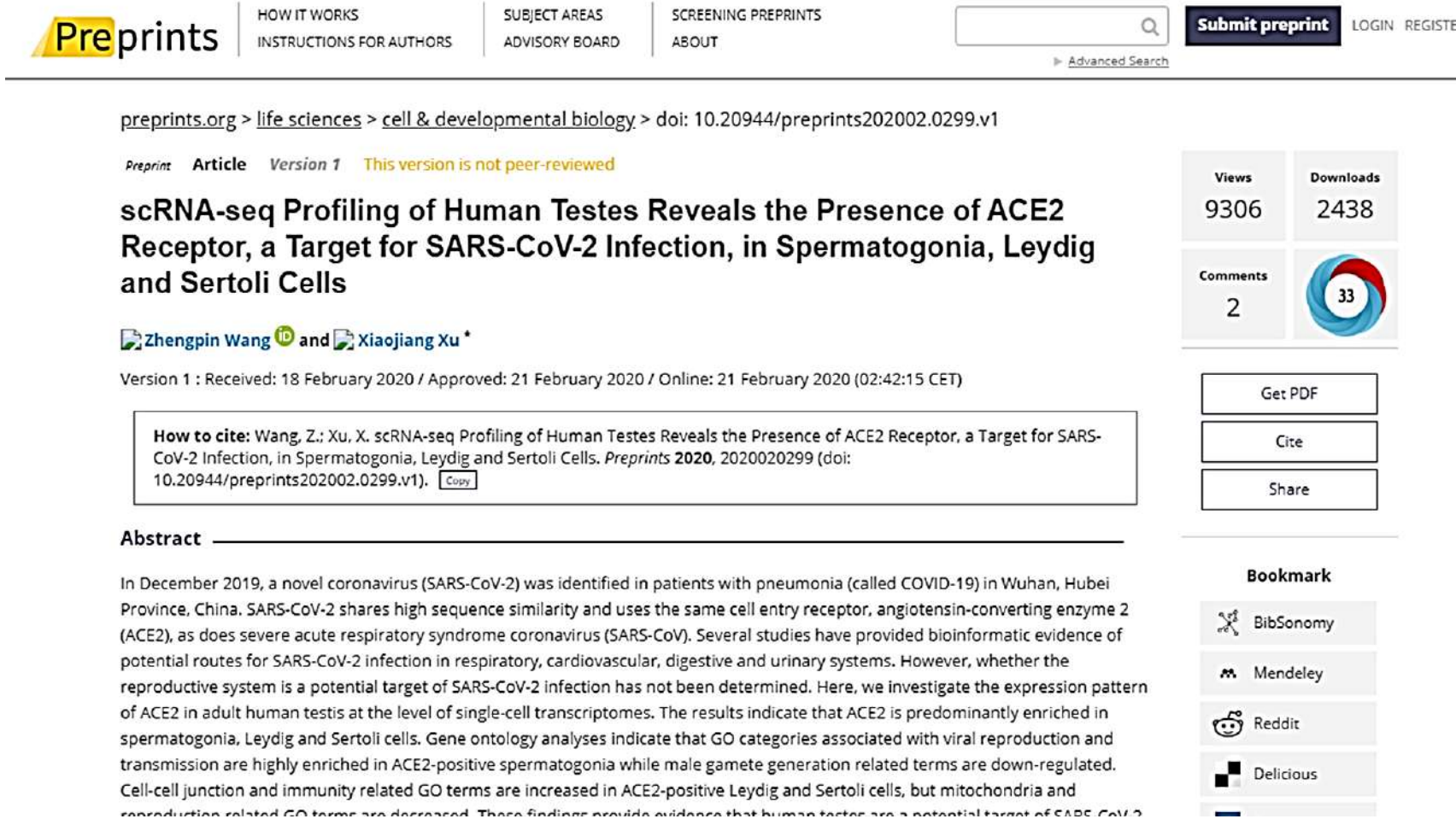
As known, there is a subtle negative feedback between T in testes and LH in pituitary. In the early stage of hypogonadism, impaired T production may stimulate the release of LH which can maintain T level temporarily.

The **serum PRL level** also significantly elevated in COVID-19 patients.
high PRL level may lead to pituitary suppression and decreased gonadotropins, while serum LH was increased in this study.

Taken together, we infer that the elevated LH and decreased T:LH ratio are more likely to be caused by testes dysfunction, such as the possible damage of Leydig cells.

This study has several strengths and limitations.

- The study provides the **first evidence** about the alteration of sex-related hormones under COVID-19.
- First, neither semen parameters nor existence of SARS-CoV-2 in semen was detected, which are more straightforward evidence for testes injury caused by SARS-CoV-2.
- The condition of COVID-19, some other factors such as stress and corticosteroid therapy may also influence hypothalamic-pituitary-gonadal axis.
- However, it should be mentioned that corticosteroids are usually believed to impair LH release instead of promoting it as seen in this study.
- In the end, repeated detection with appropriate time interval (such as 3 months or 6 months later) is necessary.



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scRNA-seq Profiling of Human Testes Reveals the Presence of ACE2 Receptor, a Target for SARS-CoV-2 Infection, in Spermatogonia, Leydig and Sertoli Cells

Zhengpin Wang and Xiaojiang Xu *

Version 1 : Received: 18 February 2020 / Approved: 21 February 2020 / Online: 21 February 2020 (02:42:15 CET)

How to cite: Wang, Z.; Xu, X. scRNA-seq Profiling of Human Testes Reveals the Presence of ACE2 Receptor, a Target for SARS-CoV-2 Infection, in Spermatogonia, Leydig and Sertoli Cells. *Preprints* **2020**, 2020020299 (doi: 10.20944/preprints202002.0299.v1). Copy

Abstract

In December 2019, a novel coronavirus (SARS-CoV-2) was identified in patients with pneumonia (called COVID-19) in Wuhan, Hubei Province, China. SARS-CoV-2 shares high sequence similarity and uses the same cell entry receptor, angiotensin-converting enzyme 2 (ACE2), as does severe acute respiratory syndrome coronavirus (SARS-CoV). Several studies have provided bioinformatic evidence of potential routes for SARS-CoV-2 infection in respiratory, cardiovascular, digestive and urinary systems. However, whether the reproductive system is a potential target of SARS-CoV-2 infection has not been determined. Here, we investigate the expression pattern of ACE2 in adult human testis at the level of single-cell transcriptomes. The results indicate that ACE2 is predominantly enriched in spermatogonia, Leydig and Sertoli cells. Gene ontology analyses indicate that GO categories associated with viral reproduction and transmission are highly enriched in ACE2-positive spermatogonia while male gamete generation related terms are down-regulated. Cell-cell junction and immunity related GO terms are increased in ACE2-positive Leydig and Sertoli cells, but mitochondria and

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Integrative Bioinformatics, NIEHS, National Institutes of Health, Research Triangle Park, NC, 27709, USA.

In this study, we investigate the **RNA expression** profiles of **ACE2** in adult human testis at single-cell resolution.

Our study documents that ACE2 is predominantly enriched in **spermatogonia, Leydig** and **Sertoli** cells.

A recent epidemiologic study indicates that some patients infected with SARS-CoV-2 have signs of severe liver damage (1).
Moreover, recent studies has explored the composition and proportion of ACE2-expressing cells

- Respiratory system
- the digestive system (10)
- cardiovascular systems (11).
- urinary systems (11).

have been reported as potential organ targets of SARS-CoV-2 infection.

However, whether **the reproductive system** is susceptible to SARS-CoV-2 infection has not been determined.

In this study, we investigate the RNA expression profiles of ACE2 in adult human testis at single-cell resolution.

Our study documents that ACE2 is predominantly enriched in **spermatogonia, Leydig** and **Sertoli** cells.

ACE2-positive cells possess higher abundance of transcripts associated with viral reproduction and transmission and lower abundance of transcripts related with male gametogenesis.

Taken together, ACE2 expression in human testis suggests that SARS-CoV-2 could infect the male gonad and risk male reproductive dysfunction.

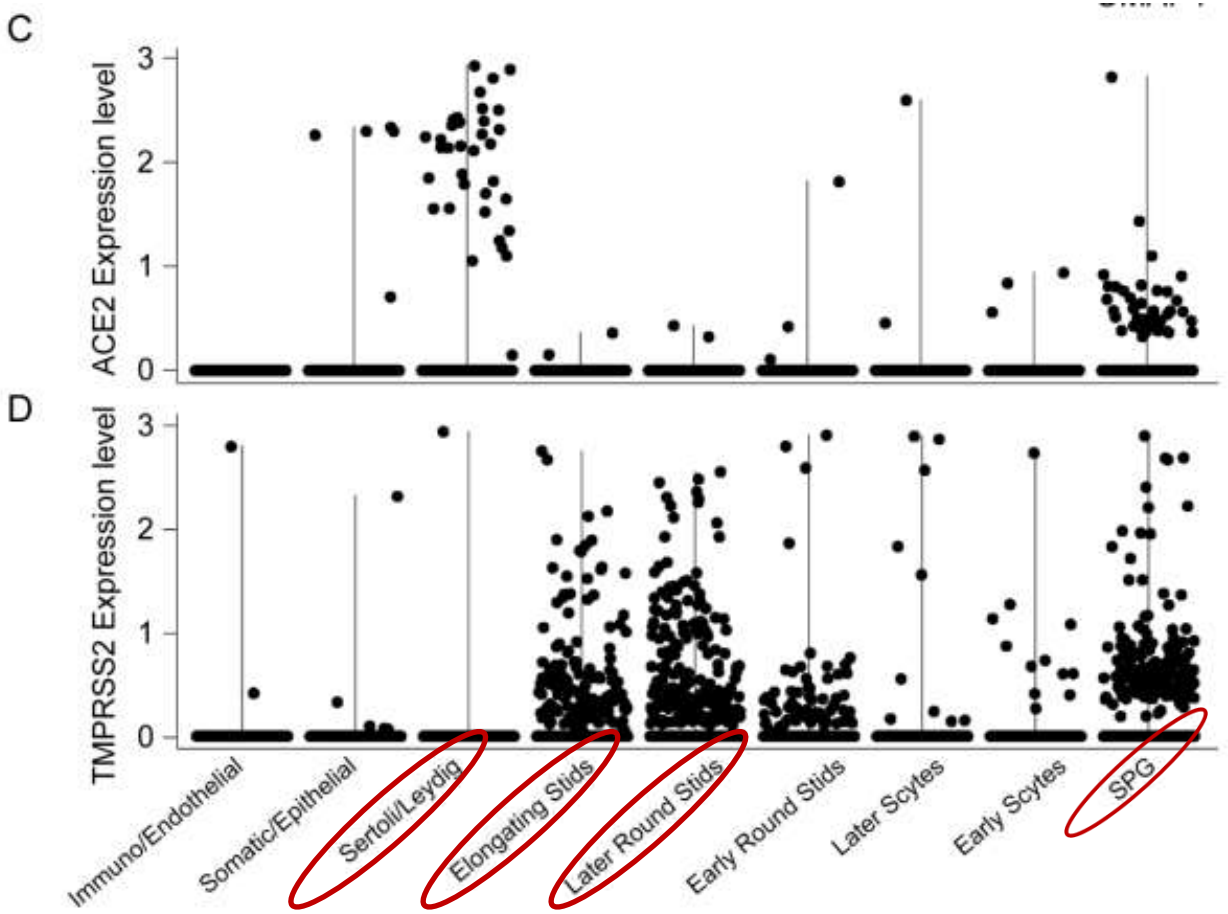


Figure 2. ACE2 expression pattern in adult human testis. (A) Per-cell expression level of ACE2 of human testicular cells visualized on the UMPA plot. (B) UMAP plot of TMPRSS2 expression across all cell clusters. (C) Violin plots of ACE2 expression in all identified cell types. (D) Violin plots for TMPRSS2 expression across all cell types.

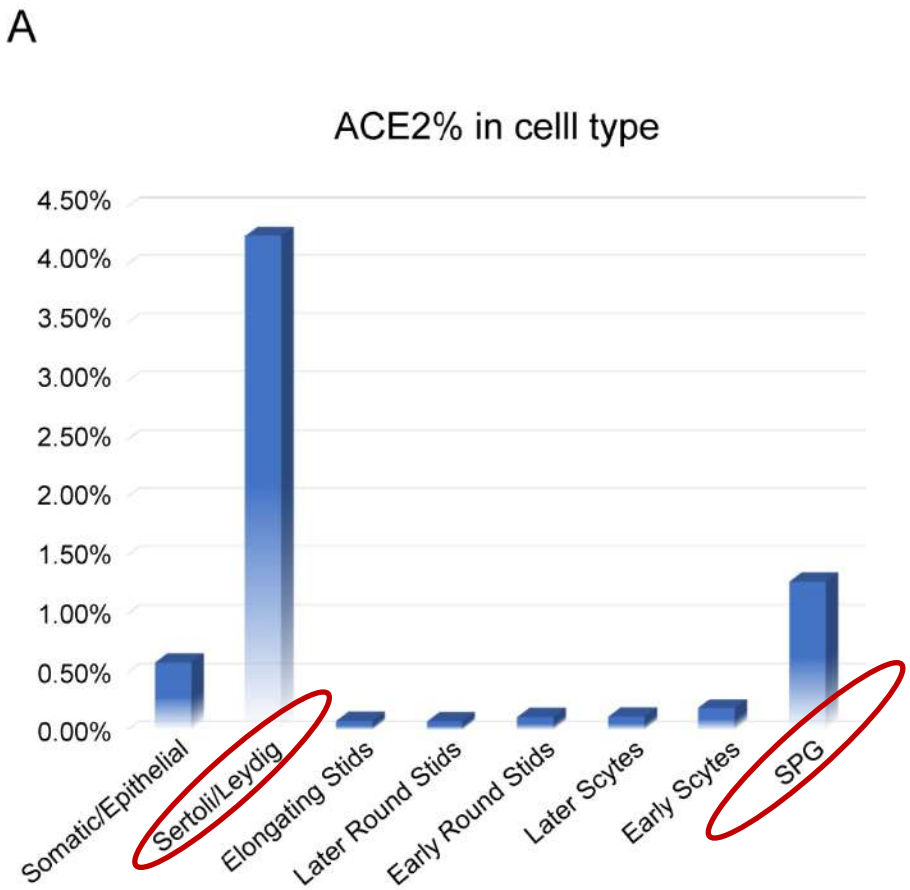


Figure 3. Composition of ACE2-positive cells and pseudotime analysis of human testicular cells. (A) ACE2-expression cells in each identified cell type.

By analyzing the expression pattern of ACE2 in adult human testis at single-cell transcriptome resolution, we find that ACE2 is primarily expressed in spermatogonia, Leydig and Sertoli cells in the human testis.

ACE2-positive spermatogonia express a higher number of genes associated with viral reproduction and transmission and a lower number of genes related to spermatogenesis compared to ACE2-negative spermatogonia.

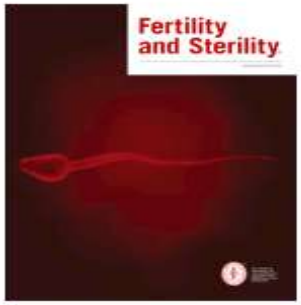
ACE2-positive Leydig and Sertoli cells express higher genes involved in cell-cell junction and immunity and lower genes associated with mitochondria and reproduction.

These findings suggest that the testis is **a high-risk organ** vulnerable to SARS-CoV-2 infection that may result in spermatogenic failure.

These investigations may provide potential clues for further investigation and may have translational implications for treatment of reproductive defects caused by SARS-CoV-2 infection.

Journal Pre-proof

Impact factor: 5.4



Prior and Novel Coronaviruses, COVID-19, and Human Reproduction: What Is Known?

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PII: S0015-0282(20)30385-X

DOI: https://doi.org/10.1016/j.fertnstert.2020.04.025

Reference: FNS 32441

We identified adult Chinese male patients (range 18-57 years) diagnosed with COVID-19 in Wuhan, China between Jan 26th, 2020 and March 2nd, 2020.

A total of **34 adult male patients** were recruited for this study.

Patients were initially diagnosed with COVID-19 based on clinical symptoms, **confirmed with qRT-PCR** of pharyngeal swab samples.

1) detection of SARS-CoV-2 in **the semen of patients** recovering from COVID-19
2) determine the expression profile of **ACE2** and **TMPRSS2** within the human testicle.

Table 1: Individual characteristics for adult males with confirmed COVID-19 providing semen sample for SARS-CoV-2 testing.

Patient	Age (yrs)	BMI (kg/m2)	Time between COVID-19 diagnosis and semen sample obtained (days)	SARS-CoV-2 Semen Result
1	31	26	33	Negative
2	55	23.5	28	Negative
3	31	29.4	32	Negative
4	20	31.2	31	Negative
5	28	24.6	33	Negative
6	50	25.1	29	Negative
7	35	27	27	Negative
8	54	18.1	25	Negative
9	46	24.9	30	Negative
10	30	24.6	40	Negative
11	30	21.7	40	Negative
12	37	29.2	31	Negative
13	49	24.3	31	Negative
14	50	22.2	30	Negative
15	49	26.4	33	Negative
16	55	26.2	37	Negative
17	33	25.1	27	Negative
18	50	23.1	37	Negative
19	37	22	15	Negative
20	27	29.9	31	Negative
21	47	26.6	30	Negative
22	36	22.8	36	Negative
23	36	24.1	33	Negative
24	32	30.9	14	Negative
25	44	26.6	31	Negative
26	32	28.7	33	Negative
27	26	24.1	9	Negative
28	32	32	36	Negative
29	43	22.2	36	Negative
30	39	35.5	8	Negative
31	54	26	35	Negative
32	18	23	29	Negative
33	39	24.3	56	Negative
34	55	22.9	75	Negative

Abbreviations: COVID-19 - coronavirus disease 2019; SARS-CoV-2- severe acute respiratory syndrome coronavirus 2; BMI – body mass index;

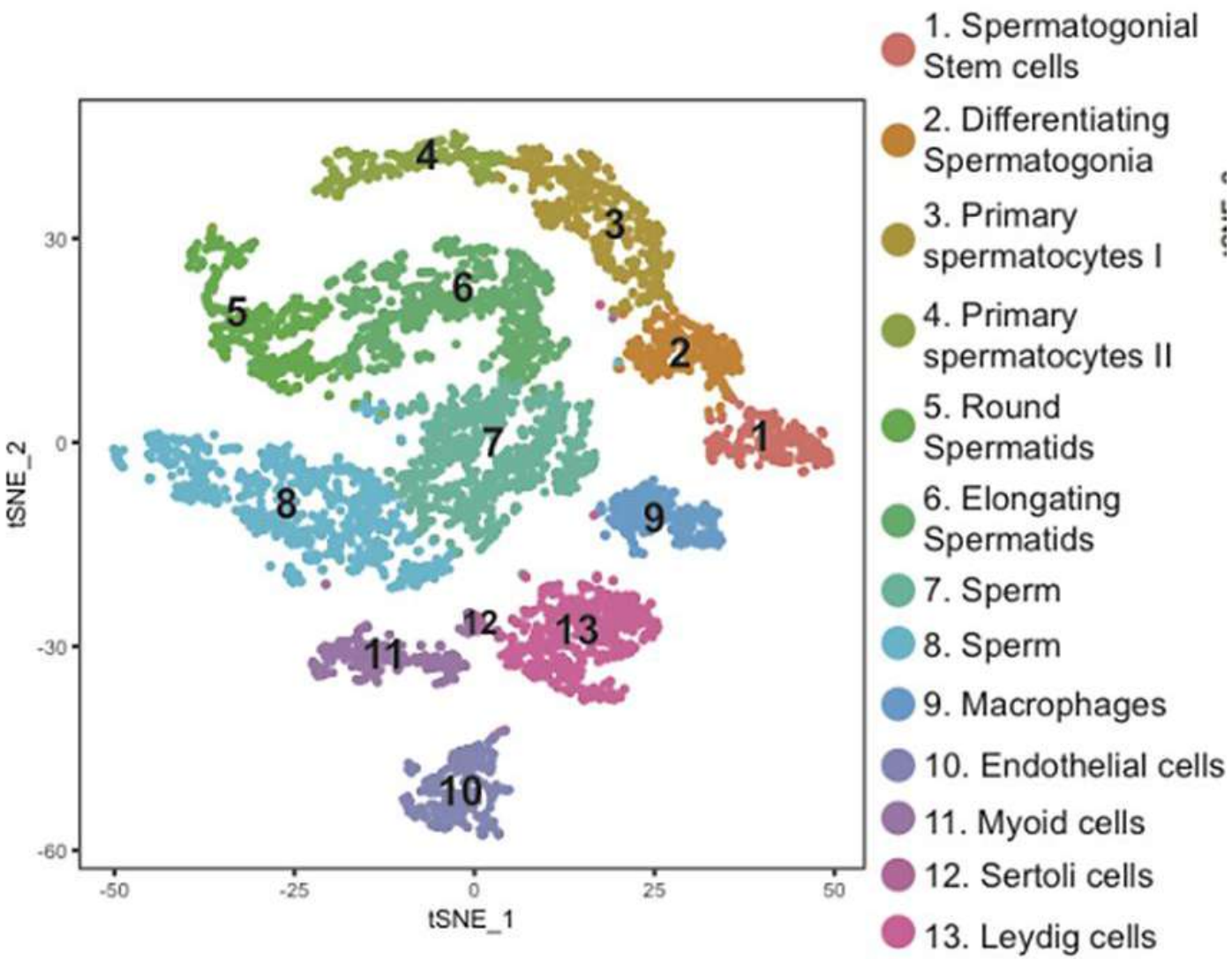

Figure 1: Expression of ACE2 and TMPRSS2 in human testicular cells using single-cell RNA-seq dataset A. Dimension reduction (t-SNE) analysis of single-cell transcriptome data from human testes (n= 6490). Each dot represents a single cell and is colored according to its cluster identity, as indicated on the figure key.

Conclusion:

We did not detect SARS-CoV-2 within semen of adult Chinese males recovering from COVID-19, approximately one month after the initial COVID-19 confirmation.

Unfortunately, we cannot definitively rule out the presence of SARS-CoV-2 in the seminal fluid during an acute infection with severe COVID-19 symptoms.

Additionally, we did not find evidence of *ACE2* and*TMPRSS2* co-expression, SARS-CoV-2 would likely not be able to gain entry to testicular cells through an *ACE2/TMPRSS2*-mediated mechanism.

Further research is needed to understand the long-term impact of SARS-CoV-2 on male reproductive function including fertility and testicular endocrine function.

Article

Impact factor: 36.216 (2018)

Cell

Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2

Vanessa Monteil,[1] Hyesoo Kwon,[2] Patricia Prado,[3] Astrid Hagelkrüys,[4] Reiner A. Wimmer,[4] Martin Stahl,[5] Alexandra Leopoldi,[4] Elena Garreta,[3] Carmen Hurtado del Pozo,[3] Felipe Prosper,[6] Juan Pablo Romero,[6] Gerald Wirnsberger,[7] Haibo Zhang,[8] Arthur S. Slutsky,[8] Ryan Conder,[5] Nuria Montserrat,[3,9,10,*] Ali Mirazimi,[1,2,*] and Josef M. Penninger[4,11,12,*]

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We have previously provided the first genetic evidence that angiotensin converting enzyme 2 (ACE2) is the critical receptor for severe acute respiratory syndrome coronavirus (SARS-CoV), and ACE2 protects the lung from injury, providing a molecular explanation for the severe lung failure and death due to SARS-CoV infections.

ACE2 has now also been identified as **a key receptor for SARS-CoV-2 infections**, and it has been proposed that inhibiting this interaction might be used in treating patients with COVID-19.

In a normal adult human lung, ACE2 is expressed primarily in alveolar epithelial type II cells, which can serve as a viral reservoir (Zhao et al., 2020).

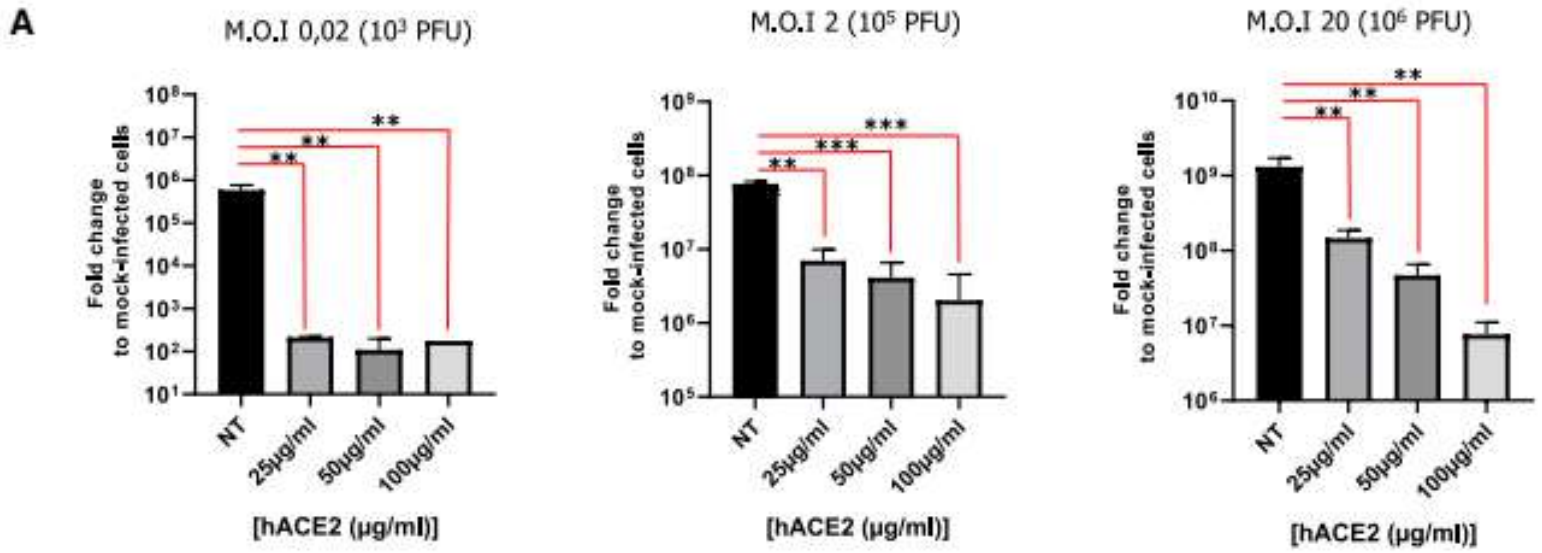
These cells produce **surfactant** that reduces surface tension, thus preventing alveoli from collapsing, and hence are critical to the gas exchange function of the lung (Dobbs, 1989).

Injury to these cells could explain the severe lung injury observed in COVID-19 patients.

hrsACE-2 Can Inhibit SARS-CoV-2 Infection in a Dose Dependent Manner

These data demonstrate that hrsACE2 inhibits the attachment of the virus to the cells.

Importantly, as expected from a neutralizing agent, this inhibition was dependent on the initial quantity of the virus in the inoculum and the dose of hrsACE2



hrsACE-2 Inhibits SARS-CoV-2 Infections of Human Capillary Organoids

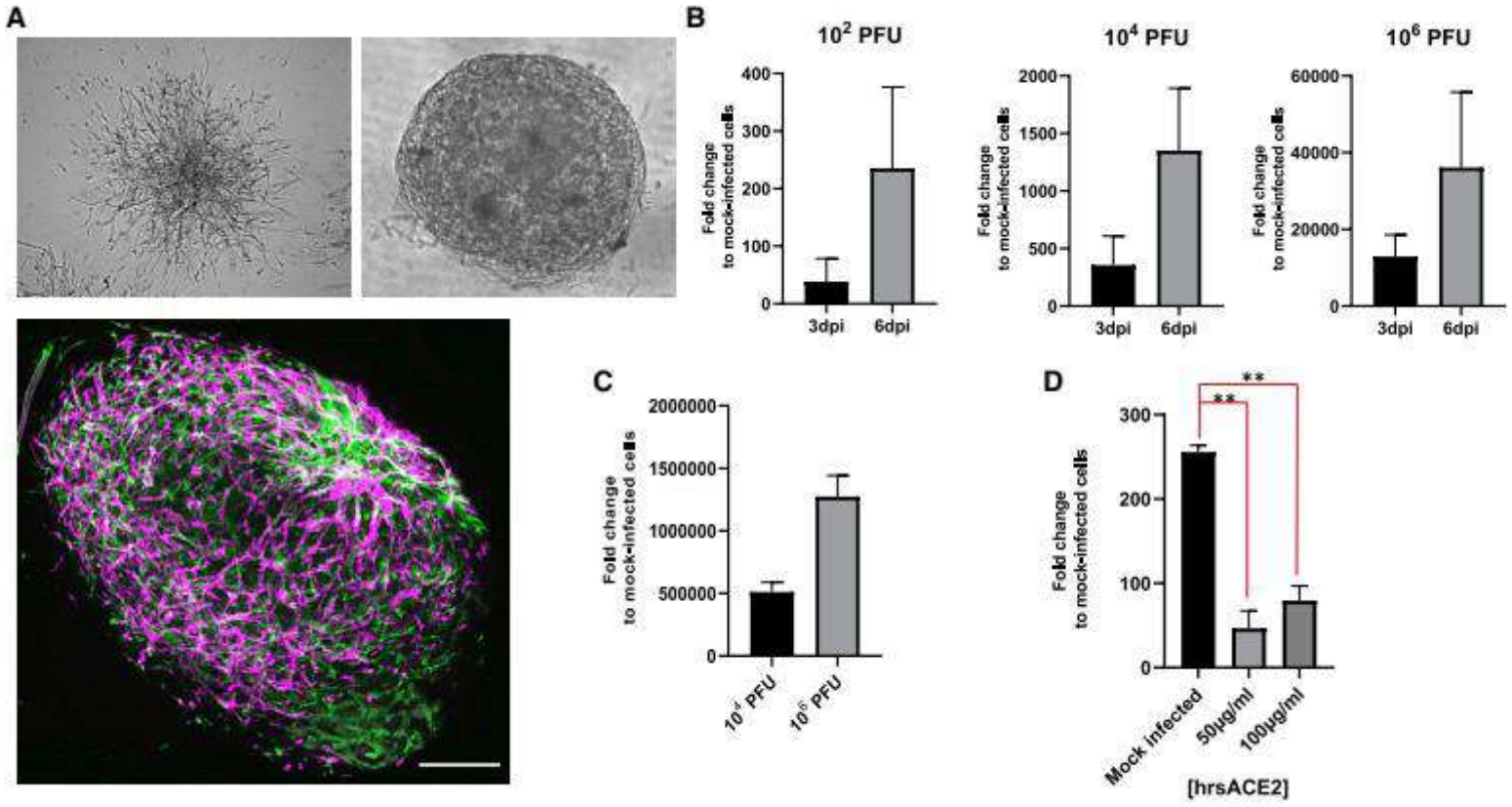
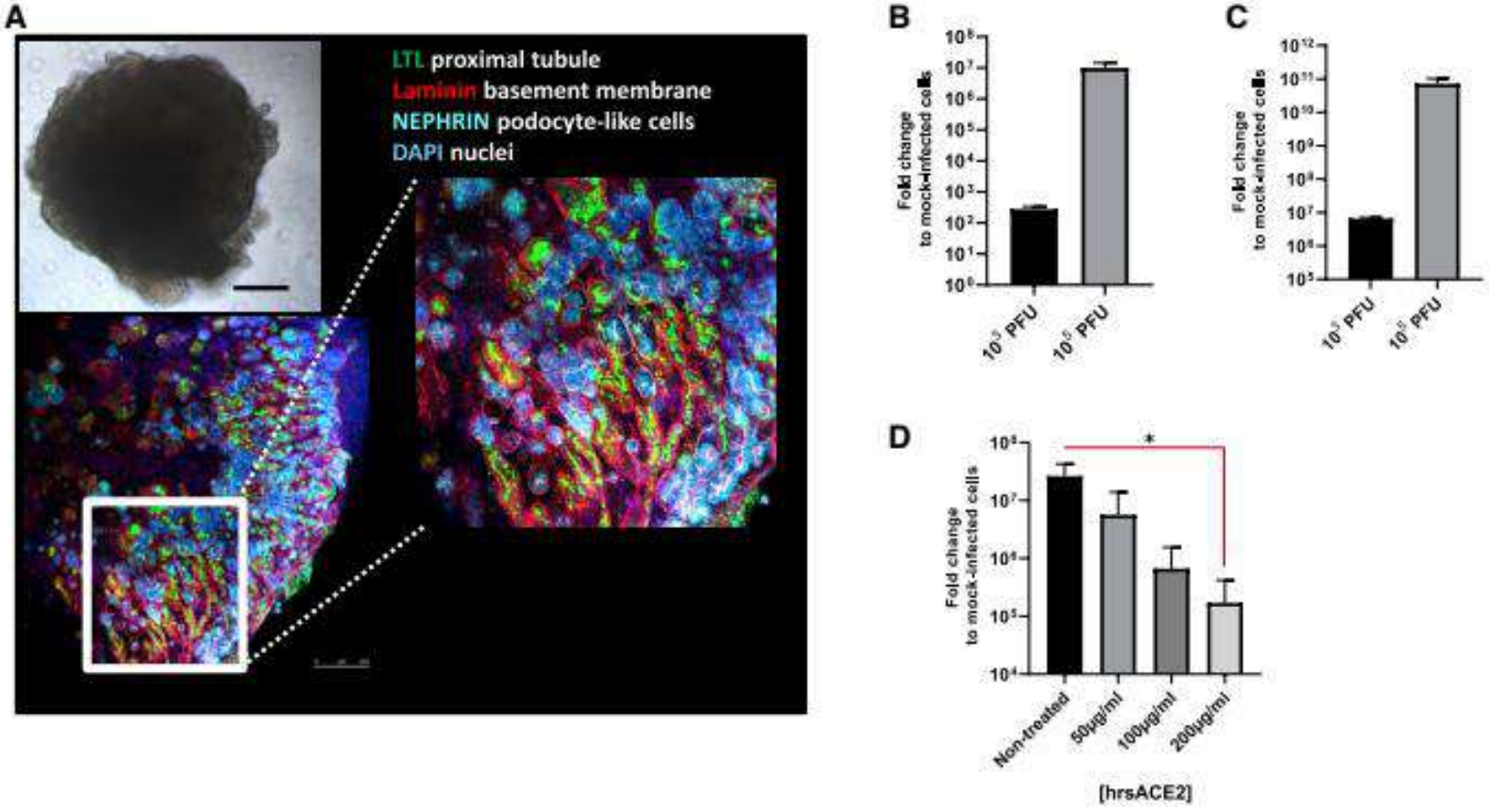


Figure 3. SARS-CoV-2 Infections of Blood Vessels Organoids

The 'Vero' lineage was isolated from **kidney epithelial cells** extracted from an African green monkey

anti-CD31 to detect endothelial cells and anti-**PDGFRb** ('platelet derived growth factor receptor alpha') to detect pericytes. **DAPI** (blue) was used to visualize nuclei.

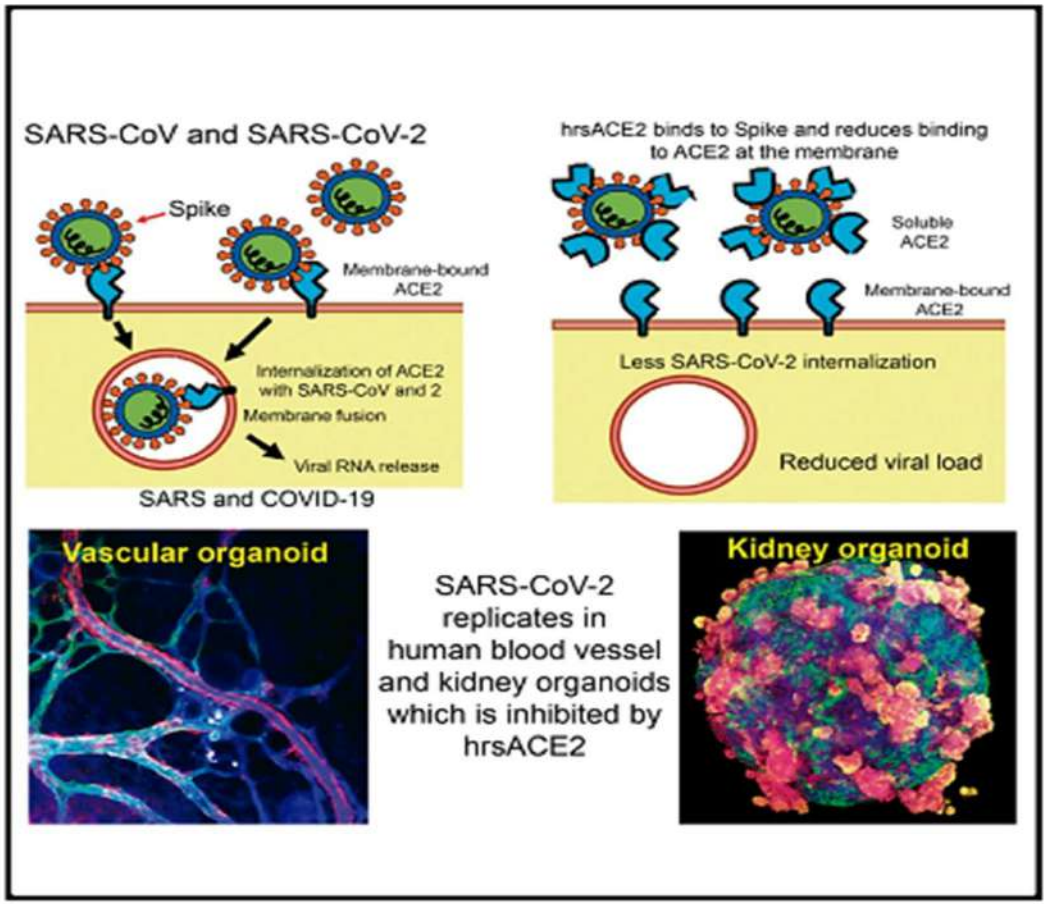
hrsACE-2 Can Inhibit SARS-CoV-2 Infections of Human Kidney Organoids.



it is not known whether human recombinant soluble ACE2 (hrsACE2) blocks growth of SARS-CoV-2.

We also show that SARSCoV-2 can directly infect engineered human blood vessel organoids and human kidney organoids, which can be inhibited by hrsACE2.

These data demonstrate that hrsACE2 can significantly block **early stages of SARS-CoV-2 infections.**



Andrade-Rocha reported that adverse effects of an acute fever on semen variables, particularly in the sperm concentration and morphology. The recovery of the semen parameters occurred 4 to 5 weeks after the fever. *(Fernando Tadeu Andrade-Rocha et al., 2013).*

Considering that one of the primary symptoms of COVID-19 is a high fever, it's reasonable to assume that men infected with coronavirus will also experience reduced fertility.

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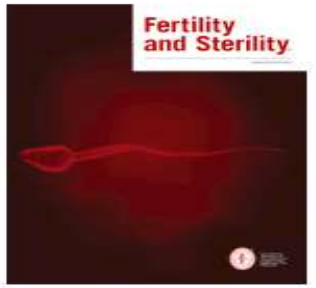

Prior and Novel Coronaviruses, COVID-19, and Human Reproduction: What Is Known?

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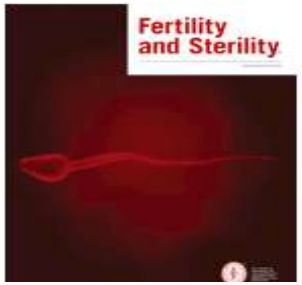

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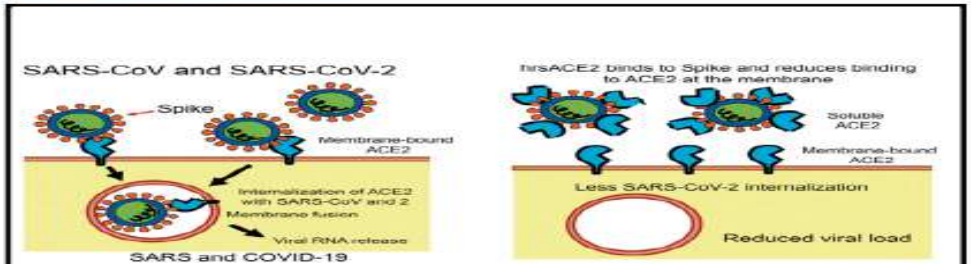
scRNA-seq Profiling of Human Testes Reveals the Presence of ACE2

Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2

Graphical Abstract



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In Brief

Clinical-grade recombinant human ACE2 can reduce SARS-CoV-2 infection in cells


THANKS