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Human ACE2 And TMPRSS2 Interaction With SARS-Cov-2 In COVID-19 Patient: A Review On Male Reproductive Disorders

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Abstract

Since its inception in 2019 from China, the novel Coronavirus has caused an unprecedented havoc in the economic and public health sector. Many countries were forced to close their borders and cross-border interactions in order to limit the spread of the disease. Furthermore, many economic and commercial activities were adversely affected as many businesses had to close. The only ones that the pandemic spared were the ones providing essential services. By March 2020, many public healthcare facilities had already been overrun. Other governments devised alternative means of managing significant cases of COVID-19, such as introducing home-based care to give room for more critical cases to be taken care of in intensive care units. It is imperative to identify the disease's risk factors to mitigate the unexpected devastation caused by the SARS-CoV-2. Global epidemiological results indicate that men, especially the elderly, are more susceptible to Coronavirus infection. The number of reported Coronavirus cases varies by gender, and this disparity continues to grow in favor of male participants until they reach the age of 60. Other studies have also established that men more than women are susceptible to coronavirus infection. Further, male patients diagnosed with coronavirus infection were shown to have an elevated mortality rate. SARS-CoV-2 is the Covid-19 pathogen that is transmitted via respiratory globules, through indirect or direct interaction. Evaluation of the genome has revealed that SARS-CoV-2 is 79% similar to SARS-CoV-2; they employ ACE2 receptors to attack cells,

meanwhile it has been established that TMPRSS2 promotes ACE2, therefore causing more severe reactions in comparison to the other types of coronaviruses. Studies describe ACE2 as a gateway for viruses to enter cells. It is directly associated with the COVID-19 clinical symptoms. Research has shown that TMPRSS2 and ACE2 are expressed in the male reproductive system tract and testis and are controlled by testosterone. Thus, the male reproductive system has all the mechanism needed to bid SARs-CoV-2, and these possibilities raise the capability of ACE2 and TMPRSS2 as potential vectors of COVID-19. This review examines how the novel Coronavirus find its way into the human cells through known receptors such as ACE2, antibody Fcy R, etc. The examination is also done on the mechanisms of its spike proteins transition with the help of proteases such as cathepsins, Furin, and TMPRSS2. The study reviewed six articles selected based on PRISMA criteria.

Keywords ACE2, SARS-CoV-2, TMPRSS2, Male reproductive disorders

Introduction

In Rationale

Covid-19 is known as one of the most pathogenic infectious diseases that infect millions of people globally. According to the recent updates, approximately 198 million infection cases has been confirmed and over 400 million deaths were resulted from SARS-CoV-2 infections had been reported globally.(1) Besides the onset of the disease in Wuhan, China has seen countries worldwide take precautionary measures such as restricting cross-border and internal movements, washing hands with soaps or sanitizers, wearing masks, and maintaining social distance (WHO). The worrying trend of SARS-CoV-2 infections necessitated the World Health Organization to declare Coronavirus a pandemic in 2019. Furthermore, there have been cases of different variants associated with covid-19 globally that are more deadly and highly contagious.(2) SARS-CoV-2 infection is primarily spread by forming direct or indirect contact with infected respiratory droplets. Clinically, infected persons experience fatigue, fever, loss of smell, nasal congestion, and in most cases, dry coughs. SARS-CoV-2 has the ability to manifests asymptomatically or presents mild to moderate symptoms. SARS-CoV-2 infection has a variety of medical consequences, including severe viral pneumonia, moderate upper respiratory tract infection, asymptomatic infection, and even death.(3) Although uncommon, approximately 20% of reported cases were presented with severe symptomology. Due to the virus's rapid positivity rate, the undetected number of individuals with mild symptoms is likely to increase.

Females and males differ biologically, endocrinely, and immunologically, resulting in diverse disease processes and outcomes. Several studies have reported molecular-level differentiation and sex-specific differential gene expression that affects the kidney function, blood pressure, and cardiovascular health. Generally, females can activate more robust immune responses that protect them from most infectious disease processes; however, such advantage predisposes them to a range of autoimmune diseases. As countries around the world struggle with the new coronavirus epidemic, researchers, clinicians, and public health officials have recognized that the disease does not have the same impact on people. Markedly, males seem to be predisposed to covid-19 disease, and their rate of death is very high. Thus, SARS-CoV-2 infection incidences are prevalent in adult males between ages 34 to 59, implying that virulent actions of the virus are likely to affect the male population. Other risk factors associated with the virus include chronic comorbidities, such as diabetes, cardiovascular infections, and cerebrovascular infection.

SARS-pathogenicity CoV-2's is extremely high. SARS-CoV-2 is a member of the Coronavirus family and belongs to the Betacoronavirus genus.(4) Other coronaviruses such as SARS-CoV and MERS-CoVs also belong to the same genus. These viruses gain entry into the mammalian body through various mechanisms. The nucleocapsid, small membrane protein, membrane, and spike are the four primary structural protein proteins in SARS-CoV-2. It uses its spike (S) protein to gain admission into the target's cells, where it links its spike protein to the target human cell's binding surface. S protein is a large multipurpose viral transmembrane protein that mediates coronavirus entrance into cells through interactions with receptors.

Even though most studies suggest that SARS-CoV-2 infects respiratory, cardiovascular, and gastrointestinal systems, recent research has shown that the virus can spread to other fundamental parts of the body[23]. Research indicates that SARS-CoV-2 can enter the male reproductive system using angiotensin-converting enzyme (ACE2), which is a SARS-CoV receptor. SARS-CoV's S protein attaches to ACE2 as a cell receptor. A significant number of studies indicate that SARS-CoV-2 share approximately 76% of the SARS-CoV amino acid and genome sequence, implying that SARS-CoV-2 can use the same receptors (ACE2) to infect terminal hosts. The coronavirus engages protease to enable membrane fusion. TMPRSS2 possesses the ability to sever S protein and ACE2, with possibility of eradicating the structural restraint S1 on S2 and freeing the internal membrane fusion peptide, thus promoting viral entrance. Complete ACE2 encompasses a C-terminal Collectrin-like domain and N-terminal Peptidase domain (PD). The ACE2-PD structure and that of complexed with RBD of the S-protein of SARS-CoV shows the molecular base of the relations between the RBD of S-protein and PD of ACE2.

ACE2 can be expressed in several organ systems, including testes, kidneys, enterocytes of the small intestine, and type I and type II alveolar. According to Wang and Xu, ACE2 is frequently expressed in the lung region, particularly in type II alveolar epithelial cells. Therefore, SARS-CoV-2 targets these receptors (ACE2) to bind its S proteins on the cell surface leading to lung infections such as pneumonia. SARS-CoV-2 requires the involvement of Transmembrane Serine Protease 2 (TMPRSS2) proteins to enter and spread into the rest of the body parts.(3) TMPRSS2 is expressed in various epithelial cells such as prostate cells. Moreover, ACE2 is expressed in testes (Leydig, germ cells, and Sertoli cells), indicating that SARS-CoV-2 can result in male reproductive disorders such as infertility.

Objective

This study intends to comprehensively review and identify the ACE2 and TMPRSS2 interaction with SARS-CoV-2 in male patients' reproductive system from interrelated texts. In addition, it will evaluate the potential transmission route of SARS-CoV-2, focusing on the challenges encountered by male reproductive health in this epidemic. To address these concerns, the study systematically analyzed available texts utilizing the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework.

Material and methods

Eligibility criteria

The articles included in the review were subjected to the main components of the PICO. PICO is an acronym for population, intervention, comparator group. In this case, reported cases of covid-19 patients

were included. Patients were confirmed using Rrt-PCR. The review focused on male patients that reported incidences of reproductive disorders or infections after an encounter with SARS-CoV-2 as the study population. However, Sample population within the research is inclusive of all ages The research focused particular attention on the incidence of ACE2 and TMPRSS2 interactions among covid-19 patients. Furthermore, reporting characteristics such as publication dates were considered. For instance, the study included articles published between 2019 -2021 on the topic of coronavirus. All the articles searched and included were peer-reviewed research publications. Cross-sectional studies reporting descriptive data or cases series were the primary study design. Letters, opinion articles, and review articles that do not present original data were excluded from the analysis. In addition, the language of publication was strictly limited to be English. The review also excluded covid-19 patients with chronic conditions such as prostate cancer or respiratory diseases because of predisposing factors such as ACE2.

Information sources

The review incorporated and consulted the following six online databases for source articles: PubMed, CINAHL, EMBASE, PsycINFO, SCOPUS, Gene Expression Omnibus. Systematic searches were conducted using these databases.

Search Strategy

The author completed a detailed systematic literature search of the six online databases: PubMed, CINAHL, EMBASE, PsycINFO, SCOPUS, Gene Expression Omnibus. The research utilized various keywords to search for relevant articles with topics that include Covid-19, SARS-CoV-2, ACE2, TMPRSS2, testicular cell, and male fertility.

Selection process

The selection of the materials included in the review is based on the screening method. The study achieved this by screening the title and abstract of the generated articles from the search databases. Studies that do not contain abstract written in English were excluded from the study during the screening stage. The researchers used a PRISMA flow diagram to summarize the total number of articles included in the research. Figure 1 below shows the PRISMA flow diagram used in the selection process.

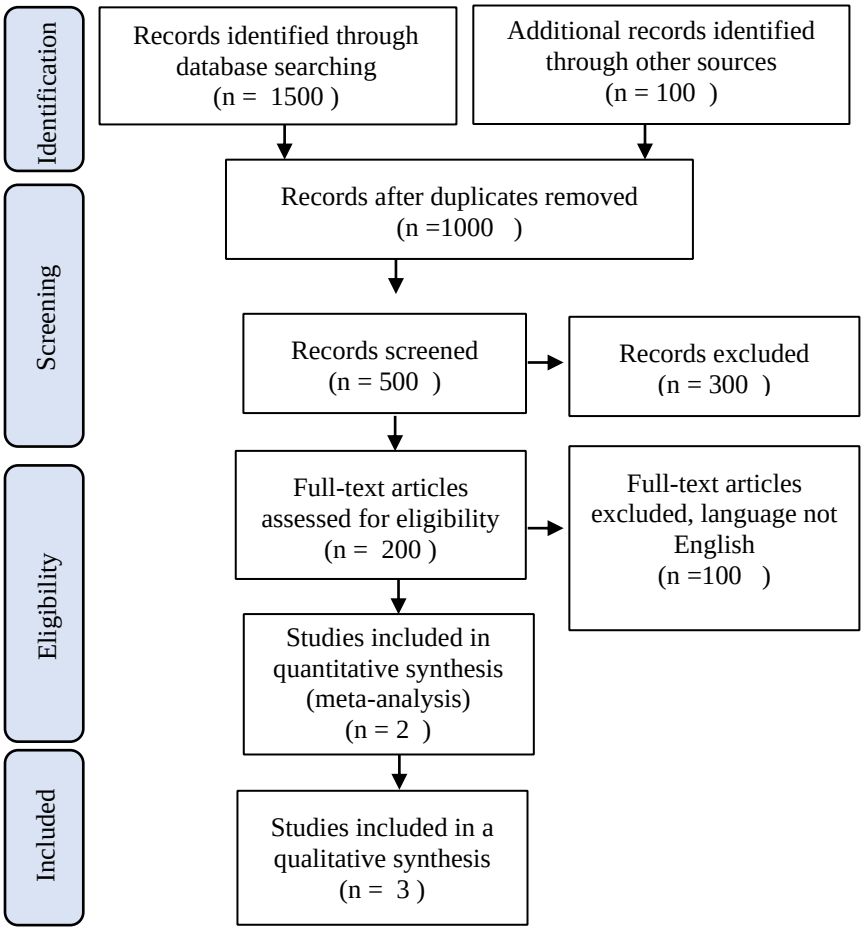


Figure 1: Works of literature finding Prisma flow diagram

Data collection process

The data collection process involved using extraction sheets in which the key areas of data extraction were divided amongst the researchers. Upon division of tasks, one of the reviewers used a pilot test to examine the mechanism of ACE2 entry into the testes and its impacts on the germ cells to examine the extraction process. According to the manufacturer's information, the automatic nucleic acid extraction system was used to extract the novel coronavirus nucleic acid. RT-PCR from Anda Gene Company was used to test and amplify reading frame lab and nucleus genes. The cycle threshold of less than 37 is positive, according to the Chinese national institute of viral diseases' recommendations. This cycle threshold is not a fixed value; it might vary depending on the kit used and the operational standards of the laboratory where the work is performed.

A full semen analysis was not always performed to avoid the virus's rapid transmission rate.[2] The prior examination was done of the sc RNA-seq data set of testicular cells, which were aimed to examine the level of manifestation of TMPRSS2 and ACE2. For example, three healthy young men's testicular cells were placed into the 10X genomics Chromium platform, which generated the libraries. 2 The Cell ranger was used to align and alter the resulting files. The genes were analyzed using a dimension reduction technique. Cells that were similar to one

other were clustered together. In addition, the researcher performed downstream analysis using a modified R script. The study also looked at the degree of expression of TMPRSS2 and ACE2 by projecting them onto t-SNE.

Data items

The targeted data were those of Covid-19 positive male patients who had experienced reproductive disorders after contraction of SARS-CoV-2. There was no intervention offered for the participants because the virus has no specific treatment and there were difficulties caused by the multiple mutations and SARS-CoV-2 variants. Governments and non-governmental organizations funded most of the studies. In other words, the projects were funded, and the author declared the fund sources.

Study risk of bias assessment

Each study was assessed using Cochrane review analysis. The author assessed the sources of bias to find incorrect or misleading studies. Some of the key areas assessed included the results and methodologies used in conducting the study. The conclusions of the studies were screened to evaluate if all judgments were derived based on scientific evidence. This was achieved by evaluating the quotes from external sources and reviewing the authors' explanations. Since the author utilized no intervention in the study, variability of the results from the rest of the articles was considered in the analysis. The reviewers worked independently and were guided by the evidence-based results from individual studies.

Effect measures

There were no treatment measures because the study adopted a mechanistic approach in which it examined the linkage between covid-19 patient and reproductive disorders.

Reporting bias assessment

We looked for asymmetries in the covariance of the trial mean variations to see whether there was any likelihood of selection bias, which can occur when small studies with unfavorable outcomes are not published. We used a normalized rank correlation analysis and multivariate asymmetries test as formalized statistical measures for publication bias considering graphical judgment can be discretionary. Other factors, such as variations in experiment quality or real study variability, could cause asymmetries in funnel plots, which we recognize.

Certainty assessment

We analyze bias by outlining the techniques for assessing bias risk in specific research (including whether this was done at the study, outcome, or both levels), as well as how this information will be used in any data synthesis. The authors conducted blind literature reviews, which were carried out by more than one individual and had to be completed separately. As a result, the claim that study variables such as TMPRSS2 and ACE2 can be employed in diagnosing SARS-CoV-2 when clinically applied is maintained with high assurance.

Results

Study selection

For the review, a total of 5 studies involving SARS-CoV-2 and male reproductive problems were identified. A total of 1500 citations were found after searching PubMed, CINAHL, EMBASE, PsycINFO, SCOPUS, and Gene Expression

Omnibus. 1000 remained after correcting for duplication. After analyzing the abstracts, the author eliminated 500 studies since they did not fulfill the requirements. Because the entire text of the study was not available or the publication could not be translated into English, ten further studies were excluded. The author reviewed the remaining 470 citations' full text in depth.

Study characteristics

The six studies were reviewed to identify representative objectives. Each of the studies were unique and is published in English. All of these studies were non-randomized control except one.

Risk of bias in studies

The author reviewed the articles for bias using the Cochrane review approach. In this approach, the study identified errors that may arise due to incorrect studies. Further, the reviewer looked for asymmetries in the covariance of the experiment mean variations to see whether there was any likelihood of selection bias when small studies with unfavorable outcomes were not published. The author adopted an amended rank correlational analysis and multivariate asymmetries test as formalized statistical analyses for publishing bias considering visual assessment can be discretionary. Anomalies in funnel plots were caused by various variables, which include discrepancies in trial performance or real study variability.

Result of individual studies

Testis was identified as a critical reproductive organ responsible for producing reproductive cells, sperms. These studies have shown an abundance of the ACE2 cells expressed in the testis cells, such as Sertoli cells, Leydig, and spermatogonia,(3) the primary function in the Leydig cells is associated with the manufacture of sex steroid hormones and testosterone. Besides, Angiotensin 1-7 acquired because of ACE2 could regulate testosterone secretion. Virus entry into the human body is through body fluids such as saliva. Due to the current studies, there is potential transmission through sexual intercourse because some studies have shown that semen can also transmit the novel Coronavirus. Furthermore, researchers hypothesized that when the SARS-CoV is attached to the ACE2, it increases the expression of ACE2 in the body, leading to inflammatory response, hence induces the malfunctioning of the Sertoli Leydig cells. These studies have tried to use single-cell RNA sequencing approach to delineate cells and help uncover heterogeneity which can help the current studies to determine the expression of the ACE2 (Table 1).

Significant damage to the testicular parenchyma was discovered in the testes of deceased COVID-19 individuals. In the majority of instances, however, the virus was not found in the testes. COVID-19 individuals had substantial seminiferous tubular damage, less Leydig cells, and mild lymphocytic inflammation, according to tests. By RT-PCR, we discovered no evidence of SARS-CoV-2 virus in the testes in 90% of the cases (90%) and none by electron microscopy. These data can be used to provide evidence-based sperm donation advice as well as management methods to reduce the risk of testicular injury during the COVID-19 disease course. Although, the findings and limitations of this study can be addressed, Sertoli cells displayed enlargement, vacuolation, and cytoplasmic rarefaction, separation from tubular basement membranes, and loss and sloughing into lumens of the intratubular cell mass on microscopy. Edema and mild inflammatory infiltrates constituted of T lymphocytes and histiocytes were found in the interstitium. In three cases, transmission EM failed to detect virus particles. In one of the 12 cases, the virus was discovered using RT-PCR.(5)

Most sex-related hormones (T, FSH, and LH levels) in males infected with SARS-CoV-2 stay within normal reference ranges after recovery from COVID-19, and no significant relationships were found between T level and disease duration or severity. Although there is currently insufficient evidence to suggest that SARS-CoV-2 causes hypogonadism and sterility, the risk should not be ignored.(6)

Table 1: Summary of the findings

S/No	Source	The goal of the study	Methodology	Findings
1	(7,8)	To investigate the SARS-CoV-2 viral transmission to the target cells using ACE2 and whether TMPRSS2 can be inhibited.	Meta-analysis of covid-19 patients	The results showed that SARS-CoV-2 gained entry into the target cells with the help of ACE2 receptors. Further, the authors found that TMPRSS2 acts as a priming protein that can clinically be blocked to prevent the virus from spreading to other parts of the body, such as testes.
2	(9,10)	To investigate the presence of SAR-CoV-2 in the testes of Covid-19 patient	RT-PCR analysis of testes during postmortem examination	The result showed swelling of Sertoli cells and severe injuries of the testes. The number of Leydig cells reduced compared to normal levels. Further, 90% of the cases reported no evidence of SARS-CoV-2 in the patient's testes.
3	(11,12)	To examine the patterns of ACE2 expression in adult testes	Sc RNA analysis of covid-19 patient's testes	High levels of ACE2 were expressed in the testes, particularly Sertoli cells, spermatogonia, and Leydig cells. The finding suggests that SAR-CoV-2 can penetrate the human testes.

4	(13)	To investigate the ACE2 expression in the mRNA of germ and somatic cells	Single-cell RNA-sequence analysis	The somatic and germ cells expressed ACE2. There was a higher rate of infertility among covid-19 patients compared to control groups.
5	(14)	To investigate the relationship between SARS-CoV-2 and Male reproductive disorders.	Bioinformatic analysis Leydig, germ and spermatogenic cells	Reproductive dysfunction was evident in the testicular functions. The reproductive cells, including Leydig, germ, and spermatogenic cells, expressed ACE2, suggesting the pathogenicity of SARS-CoV-2 in male reproduction.

Result of synthesis

We can conclude that human ACE2 and TMPRSS2 facilitate the entry of SARS-CoV-2 into the male reproductive system among covid-19 male patients, based on the goal of this study, which was to review and identify the ACE2 and TMPRSS2 interaction with SARS-CoV-2 in male patients' reproductive system from interrelated texts. The following are the main points' outcomes:

1. The results showed that SARS-CoV-2 entered the target cells with the help of ACE2 receptors. Further, the authors found that TMPRSS2 acts as a priming protein that can clinically be blocked to prevent the virus from spreading to other parts of the body, such as testes.
2. The result showed swelling of Sertoli cells and severe injuries of the testes. The number of Leydig cells reduced compared to normal levels. Further, 90% of the cases reported no evidence of SARS-CoV-2 in the patient’s testes.
3. High levels of ACE2 were expressed in the testes, particularly Sertoli cells, spermatogonia, and Leydig cells. The finding suggests that SAR-CoV-2 can penetrate the human testes.
4. The somatic and germ cells expressed ACE2. There was a higher rate of infertility among covid-19 patients compared to control groups.
5. Reproductive dysfunction was evident in the testicular functions. The reproductive cells, including Leydig, germ, and spermatogenic cells, expressed ACE2, suggesting the pathogenicity of SARS-CoV-2 in male reproduction.

These findings were derived from a variety of studies utilizing a variety of methods, ranging from meta-analysis to laboratory techniques such as RT PCR, sc RNA analysis, single cell RNA-sequence analysis, and bioinformatic analysis.

Discussion

The general interpretation of the result

Considering that the SARS-Cov-2 infections are a universal pandemic, it is important to recognize all methods of viral transmission. While adapting to their new human hosts, SARS-CoV-2, like other RNA viruses, is susceptible to

genetic evolution with the formation of mutations over time, resulting in mutant variations with different features than their ancestral strains. Several SARS-CoV-2 variations have been identified during the pandemic, however only a few are classified variants of concern (VOCs) by the WHO due to their worldwide public health impact. Five SARS-CoV-2 VOCs have been detected since the beginning of the pandemic, according to a recent WHO epidemiological update, as of December 11, 2021:

1. Alpha (B.1.1.7): in late December 2020, the United Kingdom (UK) reported the first version of concern.
2. Beta (B.1.351): first reported in December 2020 in South Africa.
3. Gamma (P.1): first detected in early January 2021 in Brazil.
4. Delta (B.1.617.2) was discovered for the first time in India in December 2020.
5. Omicron (B.1.1.529) was originally discovered in November 2021 in South Africa.(15)

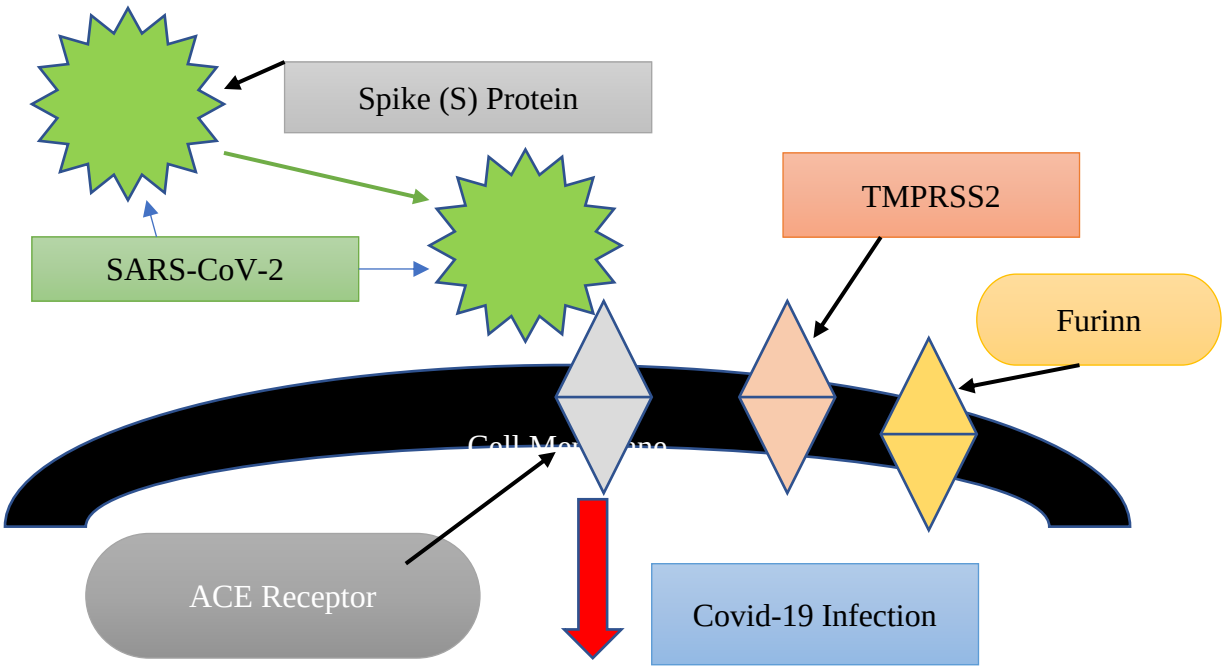
Although there is uncertainty about covid-19 transmission through seminal fluid, more studies should be done since over 25 viruses have been detected in people's semen. SARS-CoV-2 can be found in the sperm of COVID-19 patients, and it can also be found in the sperm of recovered patients. SARS-CoV-2 could be seeded to the male reproductive tract due to the ineffective blood-testes/deferens/epididymis barriers, especially in the context of systemic local inflammation. Even if the virus is unable to multiply in the male reproductive system, it may remain due to testes' special immunity. Researchers have discovered 27 viruses linked to viremia in human sperm so far. However, viruses in sperm may be more frequent than previously thought, and non–sexually transmitted viruses should not be presumed to be completely absent from genital secretions.(16) Pan study established that 19% of men infected with SARS-CoV-2 experienced scrotal discomfort like males diagnosed with orchitis. Nonetheless, the prsecence of SARS-CoV-2 in sperms and semen should be reevaluated since several studies have shown contentious findings. Pan and Song studies were unable to detect the virus presence in the semen. In this systematic review, the author addressed how ACE2 and TMPRSS2 interact with SARS-CoV-2 in COVID-19 patients' male reproductive systems. Five research studies were selected and undergone a meta-analysis on SARS-CoV-2 interactions with ACE2 and TMPRSS2 receptors in semen.

Most of the studies discussed entry mechanisms of the novel coronavirus and associated body cells to aid in formulation of therapeutic design measures. There are ways in which viruses enter the body. These mechanisms include receptor-mediated endocytosis, receptor-mediated plans membrane fusion, and antibody-dependent viral entry. The surfaces of the host cells play a crucial role because they have receptor proteins that aid the attachment of the virus into the host cells. The association between then-novel Coronavirus and the receptor cells has provided a significant basis by which researchers can find safe and effective clinical interventions.(17) SARS-CoV-2 appears to enter cells and cause pathogenic effects on respiratory tract cells. As a result, local TMPRSS2 and ACE2 expression is linked to further spread in the host, including testes. According to the findings, ACE2 is mostly found in human testes, spermatogonia, and Sertoli and Leydig cells. As a result, guys with severely impaired spermatogenesis have lower levels of ACE2. ACE2 plays a crucial role in testosterone production variation. TMPRSS2 is also found in spermatogonia, prostate epithelial cells, and prostasomes, where it is discharged into the semen. (18)

The ACE2, part of the renin-angiotensin-aldosterone system (RAAS), has been recognized as a potential functional receptor cell for the virus. Studies have suggested that elevated ACE2 levels may lead to increased vulnerability towards covid-19 infection. (18)Even though the ACE2 receptor is the most recognized host factor for Covid-19, research on TMPRSS2 has also presented its importance towards virus infection rate. Membrane fusion and receptor r5ecognition happens via SARS-CoV-2 spike (S) protein (Figure 1). The entry of the virus needs S

protein priming by TMPRSS2 that encompasses S protein cleavage at S1 AND S2 subunits. This is followed by the viral release of the S1 subunit for post-fusion confirmation.(18) Consequently, the S1 binds to ACE2, while membrane fusion occurs through the S2, a critical mechanism for viral infection.

Figure 1, Human ACE2 AND TMPRSS2 Interaction with SARS-CoV-2 in Spermatozoa, Spermatogonia, Leydig and Sertoli Cells



The ACE2 cell moves to the cell's surface after the transcription along with the N terminal signal peptide. The anchoring depends on the C-terminal Transmembrane domain. The ACE2 protein has two lobes where the novel coronavirus spike attaches during transmission. There are many other receptors that viruses can use to enter the body. Some of these include aminopeptidase, dipeptidyl peptidase, etc. Different coronaviruses use different receptors to enter the body.

The primary receptor for SARS-CoV-2 has been identified as ACE2. Because other human coronaviruses are known to require co-receptors for viral cell entrance, early molecular docking modeling studies have revealed that DPP4 (CD26) could be a potential new binding target or co-receptor. Recent biophysical studies, however, have revealed that this connection is quite weak.(19)

Viruses can also infiltrate the body when antibodies are used. Many highly contagious viruses, including ZIKA and Dengue, have been found to utilize this type of mediated entry. Antibody-dependent enhancement refers to the mechanism by which viruses infiltrate a human cell via antibodies (ADE). The Fab region of the antibody molecule is used to interact with the virus. The antibody's Fc region interacts with the host cell's Fc receptor. Because the anti-S antibody aids ACE2 internalization, it's critical to understand the significance of coronavirus entry via antibodies.(20)

During the early phases of its emergence, the new Coronavirus is highly transmissible, having a negative effect. Before producing a safe and efficient vaccination, many nations were obliged to come up with containment methods. (2) Despite numerous studies, the underlying molecular mechanism that causes the new Coronavirus to be contagious remains unknown.

The novel coronavirus requires the protease to facilitate the entry into the body cells. (4)The spike of the Coronavirus is constantly subjected to proteolysis during the time of viral infection. Once the virus has attached itself to the receptor cells, plasma membrane fusion or endocytosis starts, leading to proteolysis with the aid of other protease activators. During binding to host receptors, the SARS-CoV-2 S protein undergoes a conformational transition. The exposed fusion peptide enables endosomal or cytoplasmic membrane fusions, leading to virus entry into the body cell. Research has shown that other host proteases such as Furi, trypsin, elastase, factor Xa, and thermolysin are also involved in the virus entry into the body; they reinforced the cleaving of SARS-CoV-1-S. Furin belongs to the family of endopeptidase that is serine-dependent. It is expressed in various organs cells such as gastrointestinal, lung, and reproductive tissues. The most recent protease that had been used in recent research is the TMPRSS2. It has an essential function in the proteolytic processing of novel Coronavirus S protein. Thus, TMPRSS2 is a vital host cell factor for SARs-Cov-2.

Unlike Furin, TMPRSS2 is a type II membrane protease. The TMPRSS2 is visible in the basal prostate cells and other tissues such as ovaries, alveoli, etc. The determination of the normal physiological function of the TMPRSS2 has been made impossible by the lack of phenotype. Furthermore, TMPRSS2 cleaves the protein of both the novel coronavirus and SARS-Cov-2. Most of the TMPRSS2 is found on the surface of the lung cells. Novel coronavirus entry into the body needs both TMPRSS2 and Furin to help in the S protein activation. Furin-mediated cleavage separates the S1 and S2 subunits of the novel coronavirus during the CAE2 binding, exposing the fusion peptidase.

Study results revealed the presence of the novel Coronavirus in all the reviewed articles except for one that discussing the examination of ejaculated semen of recovering patients.(17) However, 19% of the participants presented scrotal discomfort around the time the Coronavirus was confirmed. However, it remains unclear how significant the discomfort presented in the study. Also, TMPRSS2 and ACE2 were sparse in the Testicular cells, and the expressions were almost overlapped. Since its discovery, ACE2 has been used as the primary receptor in clinical trials of most viruses, which includes the novel Coronavirus.(2) Human ACE2 moves to the cell's surface after the transcription with the N-terminal peptide and anchors itself successfully via the C-terminal membrane. When the Coronavirus encounters the host cells, the spike RBD binds the tips of the lobe of the ACE2 to initiate the viral entry. Other places that the ACE2 receptor is located include lungs, liver intestines, etc. ACE2 coverts Angiotensin I into angiotensin II. The ACE2 functions as a receptor for the spikes Glico proteins. The Coronavirus depends on the host receptors such as ACE2 and the host proteases such as TMPRSS2 to enter the human cells. They play a crucial role in allowing the novel Coronavirus to enter the body. Hence, it can also play a key role in determining the therapeutic measures devised to help combat the novella coronavirus infections.

Immune privilege shields the germ cells from host responses. Anaya et al. define autoimmune ecology as "the study of the immune response to environmental agents in general, as well as microbiota, cigarette smoking, alcohol and coffee use, socioeconomic status (SES), gender and sex hormones, vitamin D, chemical solvents, and vaccinations in particular.". (21)According to research on other viruses such as HIV, ZIKA mumps, etc., the virus could be detected in the human semen, leading to orchitis. (4) Moreover, the viruses can negatively impact spermatogenesis and the normal functioning of the male reproductive system. The Leydig cells produce hormone

testosterone hormone. The testis is an immune-privileged organ. Immunological privilege has two features in mammals: some tissues generate tolerance following transplantation to an allogenic recipient, and some tissues readily accept foreign cells without triggering immune rejection. Both features of immune privilege are present in the testis. During spermatogenesis, a vast number of new proteins are expressed in developing germ cells. Because sperms are unique to the organism and arise long after immunological competence has established, sperm production poses a challenge to the immune system. The testis, on the other hand, tolerates these unique antigens. When autoantigens are administered elsewhere in the body, they cause significant autoimmune responses, therefore the testis provides protection. Furthermore, researchers also think that because the Sertoli cells and Leydig are found in the blood vessels, the virus, including the novel Coronavirus, can lead to infertility. (22)

Furthermore, previous studies have also shown that the viruses can spontaneously replicate in the prostate.[3] Before this study was done, little was known about the novel coronaviruses' impact on the reproductive system. However, Xu et al. reported pathological assessment of the testicular cells after the autopsy was done on the six patients that had died from Coronavirus. Furthermore, those authors also found that covid-19 destroyed the germ and sperm cells. They also theorized that SARS-CoV-2 caused reproductive system impairment and caused orchitis. The receptor for viral entry was localized in Leydig and Sertoli cells. The data from the single cell showed that the ACE2 RNA occurred at low levels. Consequently, the coronavirus entry mediated by ACE2 of the novel Coronavirus into the targets cells of the host is unlikely to appear in the testicles.

In conclusion, the coronavirus epidemic has produced many concerns on male reproduction and fertility because of SARS-CoV.2. In this study, there is strong evidence in the literature reviewed that both TMPRR2 and ACE2 are expressed in testicular cells, which implies that they may negatively impact fertility. Besides, the studies have shown that semen may be a viral method of spreading the virus. Studies have proposed that TMPRRSS2 can promote ACE2-mediated viral entry through the release of some prostatic cells into seminal fluid.

Limitation of evidence

The study on ACE2 and TMPRSS2 mediated SARS CoV-2 entry into testicular cells has some limitations, like other studies previously done on Coronavirus. There is a limitation of the methodology utilized when conducting these studies. One of the limitations is that the sample size was considerably small and biased, as some Covid infected men may have mild COVID-19 symptoms. The past studies done on coronaviruses suggest that greater viral loads can be associated with severe symptoms. In addition, a comprehensive semen analysis was not done due to the high transmissibility of the Novel Coronavirus. Only a single semen sample was used in this sample. The small sample size restricts the researchers' capacity to provide the data for semen viral shedding.

The fourth limitations were that the researchers could not assess the hormone profile in patients. This includes luteinizing hormone, total testosterone, etc. The hormone profiles could be used to provide the assessment of the testicular functions. The study's findings and the data obtained cannot determine the lasting impact of the pandemic. Moreover, the appearance of TMPRSS2 and ACE2 was only tested using RNA.

Limitation of review process used.

Further or future studies should be focused on a large sample and the long-term impacts of Coronavirus in the reproductive system of human beings. According to the survey, traces of the novel Coronavirus was not detected from the semen sample one month after the confirmation of the diseases. Due to ethical and technical issues,

severe acute respiratory syndrome caused by coronavirus two cannot be ruled out in the seminal fluid if the symptoms of Covid 19 are severe. Evidence of ACE2 traces and protease expression in the RNA dataset were not apparent, which further confirms that the Coronavirus may not have gained entrance into the testicular cells TMPRSS2/ACE2 mechanism. Finally, further research was needed to determine the lasting impacts of novel Coronavirus on the reproductive system. Due to the limited knowledge on the novel coronavirus ability to affect the reproductive system, the World Health Organization and other agencies have not been able to tell people to abstain from sex once they are infected. This is because limited knowledge is available on the ability of the virus to be transmitted through bodily reproductive fluids such as the virginal fluids and the semen.(17) The novelty of this study is that RNA datasets were used to determine the presence of novel Coronavirus in the seminal fluid. Other than the functions of ACE2 and TPMRSS2 in the novel coronavirus entering the body, which other studies have tackled, therefore presenting more room for further studies. The long-term impact of the novel Coronavirus should also be emphasized by future studies. Furthermore, this study was also limited because the comprehensive analysis was not done due to the virus's transmissibility, leading to restrictions on its impact.

The implication of the results for practice policy and future research

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Conflict of Interest

The study was conducted without any financial or commercial ties that could be perceived as a potential conflict of interest, according to the author. As a result, the author revealed that no conflict of interest existed.

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