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SCAN ME

Review Paper

DOES COVID-19 HAVE ANY IMPECT ON MALE REPRODUCTIVE SYSTEM?

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Abstract

COVID-19 since its emergence in 2019 has become a global health challenge to deal with. Apart from other affected organs, the studies reporting presence of SARS-CoV-2 virus in male reproductive tract of Covid-19 patients has instigated a concern regarding its impact on male fertility. The high expression of Angiotensin-converting enzyme 2 (ACE2) receptor protein and TMPRSS2 in testis and other male accessory organs which facilitate the entry of virus in the human cells has increased the probability of viral invasion and its adversity in these organs. In this study we have systematical reviewed the current information available on different online portals to explore the possibility of direct and indirect effects if any of SARS-CoV-2 virus on male fertility organs following its clinical interpretations and consequences on male fertility outcome. The available literature showed disrupted hypothalamus-pituitary-testicular axis (hypogonadism), testicular orchitis and altered sperm parameters as major unavoidable risk of SARS-CoV-2 infection in male patients. However, with the limited clinical data, it is currently not appropriate to reach any conclusion and more follow-up studies on COVID-19 male survivors as well as in-depth studies for better understanding of molecular mechanism of SARS-CoV-2 virus inducing pathogenesis in male reproductive system affecting male reproductive health and fertility outcome are need to be conducted.

Key words: SARS-CoV-2, ACE2, Male fertility, Testis, hypogonadism, Orchitis.

INTRODUCTION

Since its first emergence in December 2019 in Wuhan city of China, the COVID-19 infection, caused by the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) commonly known as corona virus is creating havoc across the world

[1]. SARS-CoV-2 disease symptoms resemble severe acute respiratory syndrome coronavirus 1 SARS-CoV-1) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Due to rapid human-to-human transmission and intercontinental spread it was declared as pandemic by WHO in March 2020. Until Jan 2022, over 409 million confirmed cases and over 5.8 million deaths were caused globally by this virus [2]. Till date WHO have identified several variants of SARS-CoV-2 virus and the latest one is omicron. **The principal mode of infection of COVID-19 is mainly through exposure to respiratory fluids and common** symptoms includes fever, sore throat, dry cough and fatigue. However, severe cases may result in respiratory distress syndrome and pneumonia. The traces of SARS-CoV-2 virus has been detected in feces, blood and urine specimens of some patients suggesting its transmission through other bodily fluids too [3-5].

Although the source of SARS-CoV-2 is still unknown, however, it has been found to have approximately 80% genetic similarity with SARS-CoV-1 virus [6,7]. Thus both the viruses use transmembrane serine protease 2 (TMPRSS2) and receptor angiotensin converting enzyme 2 (ACE2), a type I integral membrane protein in renin-angiotensin system (RAS) to infect the human cell [8]. However, SARS-CoV-2 virus shows a higher affinity for ACE2 receptor than SARS-CoV-1 [9].

SARS-CoV-2 virus has been categorized into four structural proteins namely, spike (S), envelop (E), membrane (M) and nucleocapsid (N) proteins. The ability of virus to invade the host cell is through binding of its spike (S) protein to the ACE2 receptors present on host cell membrane where it replicates, releases copies and induce cytotoxicity [10]. Expression of ACE2 is cell-type specific and therefore cells with higher ACE2 expression may augment viral infection. In addition to being abundantly expressed in pulmonary epithelium, ACE2 has also been expressed significantly high in other organs such as small intestine, kidney, liver, brain and heart suggesting multi organ involvement of SARS-CoV-2 virus [11,12]. The human reproductive organs also show widespread expression of ACE2 receptor protein hence arousing the probability of SARS-CoV-2 virus invasion in these organs and exposing them to its adversities. Though the virus has shown to induce its adversities on both male and female reproductive system, however, gender differences in incidences and outcome of COVID-19 infection has been

observed with high percentage of severity and mortality recorded among males than to their female counterparts [13-15].

Due to the wide spread presence of ACE2 receptor protein in male reproductive system, SARS-CoV-2 virus is considered to be a threat to male reproductive organs and its related adverse effects on male fertility outcome. Hence it become important to investigate the mode of action of SARS-CoV-2 virus in the male reproductive tract and its possible adversities on male fertility outcome, especially in present time when the cases of infertility involving male factors are on increase globally. Therefore, in the present article we have systemically reviewed the information available on various online portals about the current information of mode of action of SARS-CoV-2 virus invasion in male reproductive organs, its effects on male reproductive physiology causing alterations in fertility parameters in male patients survived with COVID-19 severity.

METHODOLOGY:

To fulfill the objectives of the current article a comprehensive and systematic reviewing of available literatures was undertaken. An extensive search for available literature on severe acute respiratory syndrome coronavirus-2, the possible mode of action of COVID 19 and its influence on male reproductive system and consequential alterations in male fertility outcome in Covid-19 male survivors using published articles/peer reviewed articles, internet and web based published materials was conducted. Literature search were done under the keywords such as Covid 19, Angiotensin-converting enzyme 2 receptor, transmembrane serine protease 2, male infertility, testis, spermatogenesis, steroidogenesis, hypothalamus-pituitary-testicular axis, orchitis, semen analysis, oxidative stress, sperm DNA fragmentation and anti-sperm antibodies. The search strategy involved step by step processes including the Electronic databases like PubMed, Scopus and Google Scholar identifying relevant literature published and the use of cross references from the selected articles and some related summary papers and forward citation queries were also scanned by hand.

RESULTS:

3.1. Localization and Expression of ACE2 in Male Reproductive System:

ACE2 enzyme belongs to the dipeptidyl carboxydipeptidases family which is homologous to human angiotensin-1-converting enzyme and is part of renin-angiotensin-system (RAS) [16]. The high levels of catalytic activity of ACE2 is mostly present in all organs along with male reproductive organs [17-19], however, human testes are recently being reported as one of the most ACE2 enriched organ in the body [20]. Spermatogonial cells, Sertoli cells and Leydig cells have been observed significantly rich in ACE2 expression whereas low expression levels were reported in epithelial and somatic cells, different stages of spematids and spermatocytes [20-22]. Its activity has been found 13-fold higher in sperm plasma membrane as compare to seminal plasma [23]. The widespread expression of ACE2 protein in Leydig cells proves its substantial role in regulation of testosterone production [24,25]. The localization of ACE2 in other male accessary reproductive organs such as in glandular cells of seminal vesicles, furin domain of epididymis and epithelial cells of prostate lumen suggest the risk of involvement of SARS-CoV-2 virus in these organs too [22,26,27]. Its expression is also found to be varied with age as men in 30's had highest testicular expression of ACE2 than the younger aged males [28,29], raising the chances of more damage to male reproductive physiology at younger age.

3.2. Role of Renin-Angiotensin System (RAS) and Functional Significance of ACE2 in Male Reproductive System:

Renin-Angiotensin System **(**RAS) which is a complex enzyme-protein cascade plays important role in cardiovascular and renal regulation [30]. The presence of RAS enzymatic cascade in male genitals draws attention towards its potential involvement in functioning of male reproductive tract [31]. The identified key components of RAS in testes and other male reproductive organs are ACE1, ACE2, MAS receptor which plays important regulatory roles in spermatogenesis and steroidogenesis [11,32-35]. Traditionally ACE1 (angiotensin-converting enzyme 1), converts angiotensin I (AngI) into angiotensin II (AngII) which participates in ACE2, a homolog of ACE1. ACE2 which plays important role in the regulation of male reproductive performances is mediated by angiotensin-(1–7) [Ang-(1–7)] and functions through its receptor Mas [36]. Regulatory role of testicular ACE2 in spermatogenesis and steriodogenesis was confirmed when Reis et al observed severe spermatogenesis impairment in human

testis with lower expression of ACE2, Ang-(1-7) and Mas receptor [32]. Hence, a correct balance between ACE1/AngII/AT1R (angiotensin type 1 receptor) and ACE2/Ang-1-7)/Mas receptor pathway is found to be important for male reproductive health. This pathway was also found to be involved in epididymal contractility, sperm maturation and functioning [11,26,37-39]. Contrary to these, ACE2 knockout animal studies showed no adverse effect on male fertility which was supposed to be due to the involvement of some rescue mechanisms in the RAS pathway which was compensating ACE2 loss in testis however more confirmatory studies are need to be conducted [26,39,40]. In view of these findings, it is of clinical importance to study how SARS-CoV-2 virus acts in the male reproductive tract through RAS pathway since downregulated expression of ACE2 and increased Ang II levels have been observed in COVID-19 positive male patients.

3.3. Potential mechanism of Action of SARS-CoV-2 in Male Reproductive System: Interaction between SARS-CoV-2 and ACE2:

The entry of SARS-CoV-2 virus into human cells depends on the recognition and binding of its Spike (S) protein to its receptor ACE2 (Angiotensin Converting Enzyme 2) present on host cells and TMPRSS2 [41]. TMPRSS2 is recruited by the virus to cleave ACE2 and S protein and eliminate the structural constraint of S1 on S2 and finally release the internal membrane fusion peptide, thereby enhancing viral entry in the host cell to complete its intracellular replication, release of copies and induce cytotoxicity [42]. It is a member of type II transmembrane serine proteases family, one of the largest families of proteolytic enzymes, involved in many physiological processes, such as development, digestion, coagulation, inflammation, fertility and immunity [43].

As per the available literature there are different views on its mode of action in male reproductive system. First, the virus may attack the testis directly and affect the seminiferous epithelium containing Sertoli cells and Leydig cells and abruptly stop spermatogenesis causing a marked reduction in sperm population [21]. Second, it may target the spermatognial cells provoking immune and inflammatory reactions that result in cytokine-mediated bursts inside the tubules and damage the testicular epithelium [44]. Third, the virus which entered the Sertoli cells indirectly impaired the functional dynamics of developing spermatozoa by disrupting the blood-testis barrier (BTB) and stimulating the immunological and inflammatory responses [45]. As evident

with previous viruses such as Zika [46], Ebola [47] and Marburg [48] viruses, BTB may not constitute a perfect barrier to viruses since an active viral replication found in human semen during these infections.

3.4. Clinical Implications of Covid-19 on Male Reproductive Tract:

High levels of ACE2 expression in germ cells and somatic cells of male reproductive tract drew the attention of researchers to study the potential damages caused by COVID-19 virus on male genital tract affecting the male fertility outcome [49]. The studies reporting the presence of viral particles in the testicular biopsies and semen samples of COVID-19 male patients are very limited, however, evidences of testicular dystrophy, abnormal hormonal levels, orchitis has been observed in number of COVID-19 positive cases [50-53]. On the basis of available information on direct and indirect role of COVID-19 virus in causing deleterious effects on male reproductive system and its possible long term clinical implications on male fertility, we have categorized these impacts in three categories i.e. Pre-testicular, Testicular and Post-testicular effects.

3.4.1. Pre-Testicular Effects: Impact of SARS-CoV-2 on Hypothalamus-Pituitary-Testicular Axis

Studies related to the deteriorated impacts of SARS-CoV-2 on Hypothalamic region of brain suggest that the virus may have the capability to cross the Blood-Brain–Barrier (BBB) and infect the ACE2 rich cells thereby causing inflammation and pathogenesis in hypothalamic region which controls various physiological functions of the body including hormonal balance [54-55]. Male reproductive system which is highly under control of hypothalamus-pituitary-testicular (HPT) axis may get affected since changes in gonadotropic hormones (LH and FSH) release have been observed in COVID-19 positive male patients [56]. The reversible hypositis or direct hypothalamic damage that could have led to transient hypothalamic-pituitary dysfunction leading to adverse effect on release of gonadotropins were also been reported in studies on SARS-1 and SARS-2 viruses infected patients [57,58].

Recently, high prevalence of low total testosterone (TT) levels, higher LH levels and significantly low TT: LH ratio and impaired fertility potential in many post-hospitalized COVID-19 survivors were found to be associated to the testicular pathophysiology induced by inhibition of HPT axis [59]. However, this condition was assumed due to the

reduced levels of angiotensin (1-7) which negatively affect the regulation of steroidogenesis at testicular levels rather than the inhibitory effect of COVID-19 virus on hypothalamus [44], since earlier it was observed that certain infections can lead to hypogonadism via primary aetiology, rather than causing gonadotropic hypogonadism [60]. As per an another speculation the suppressive impact on the hypothalamus-pituitary-gonadal axis in COVID-19 patients was due to the use of corticosteroids as a treatment protocol [44], since Corticosteroids are supposed to restrain the release of gonadotropin-releasing hormones at hypothalamus level and could affect the pituitary-gonadotropin action at testicular level [61].

Though the presently available information on the direct or indirect impact of SARS-CoV-2 virus mediated adversity in HPT axis is not conclusive, however, the data from limited studies reporting the abnormal circulatory levels of gonadotropins and sex steroid as well as inflammation in hypothalamic region suggest that the COVID-19 infection may have negative impact on HPT axis which augment the probabilities of testicular dis-functioning, altered circulatory testosterone levels and finally abnormal semen parameters. Hence more clinical investigations to find direct or indirect the long term adversities of SARS-CoV-2 virus on male fertility via hypothalamic region in post hospitalized Covid-19 male patients are needed.

3.4.2. Testicular Effect: Deleterious effects of SARS-CoV-2 on human testis

The significant presence of ACE2, Ang (1-7) and Mas receptor protein in various testicular cells (Spermatogonia, spermatids, Leydig cells and Sertoli cells) and evidences from previous SARS-CoV-1 virus raised the concern of testis being one of the target organ for viral attack. The potential risk of direct damage of testicular functioning following SARS-CoV-2 exposure was considered due to the reduced levels of Ang (1-7) in progressive inflammatory phase of infection [3][3] when impaired spermatogenesis was observed in COVID-19 positive hospitalized male patients [62]. The common clinical manifestations observed in such patients were development of testicular orchtis and reproductive tract inflammation (acute or chronic) which are one of the evident marker of male infertility [33,50,51,63,64]. Furthermore, testicular failure due to high destruction or inactivation of Leydig cells may impede testosterone production causing an acute stage hypogonadism in man which may finally trigger a cascade of events

[61,65,66]. This situation not only disrupt the process of sperm production and maturation, absence of testosterone may also exaggerate inflammatory responses ensuing in an increase of unrestrictive release of inflammatory cytokines in testis [67,68]. Although, inflammatory cytokines such as TNF-α, IL-1β, IL-6 etc have significant role in male reproduction, on the other hand, their increased levels along with inflammation can induce the down-regulation of junctional proteins of the blood-testis barrier (BTB) which can disrupt BTB consequently stimulate an immune response resulting in disrupted spermatogenesis [69]. SARS-CoV-2 induced immunogenic factors may also play crucial role in causing in-direct testicular damage since high immunoglobulin G precipitation was detected in seminiferous epithelium, suggesting an immune-mediated testicular damage through the release of cytokines in response to infiltrated activated immune cells [70].

SARS-CoV-2 induced testicular orchitis is also associated with generation of reactive oxygen species (ROS) and stimulation of Oxidative stress (OS) through innate immune pathways [71] which may indirectly affect testicular functioning [72]. High ROS levels may lead to activation of the nuclear factors that trigger the release of pro-inflammatory mediators and cytokines thereby exaggerating the inflammatory responses [73] which can reportedly further lead to testicular orchitis and OS [50]. The reduced circulatory testosterone levels in COVID-19 positive males has also been related to testicular inflammation, generation of OS and impaired spermatogenesis resulting in abnormal fertility parameters [55]. Other co-morbid conditions such hypertension, diabetes mellitus, etc were also found to contribute majorly in induction of OS in SARS-CoV-2 infected male patients which further have adverse effect on testicular functioning indirectly [74].

3.4.3. Post Testicular Effect: Adverse effects of SARS-CoV-2 on other male accessory reproductive organs

Although evidences of expression of ACE2 and TMPRSS in other male reproductive tract organs such as epididymis, seminal vesicle and prostate are very limited [74], still, they have been found to be vulnerable in moderate to severe illness in COVID-19 positive male patients since erectile dysfunction and orchitis conditions have been reported in some of the studies [75]. As per Li et al [76] the incidences of SARS-CoV-2 virus in these

organs may be due to the damaged blood-testes/deferens/epididymis barriers, especially in the presence of systemic local inflammation. He suggests that persistent of corona virus in the male reproductive system may possible, however, it still cannot replicate possibly due to the privileged immunity of testes [76]. Another study reports Epididymo-orchitis condition in 10-12% of men with acute covid-19 infection which was considered to be through direct testicular infection [77]. Though there are speculations that testicular orchitis and depleted testosterone levels may cause severity and hypo functioning in these testosterone dependent organs, however, the present data is not sufficient to confirm the direct or indirect involvement of SARS-CoV-2 virus and its resulting adversities in accessory sex organs and further more investigations are need to be conducted.

3.4.4. Presence of SARS-CoV-2 in human semen and its impact on semen parameters:

The data on presence of SARS-CoV-2 RNA in human semen samples are very limited and majority of the studies carried out did not find any traces of SARS-CoV-2 in semen samples of recovering male patients [70,78,79]. The adverse effects of SARS-CoV-2 virus on quality of semen was first reported by Li et al in many COVID-19 positive male patients [76]. Later, Best et al published that the virus did not have any direct effects but it may affect total sperm number in ejaculate which was thought to be due the resulting systemic inflammatory state and immune response against the seminiferous epithelium or caused by a breach on the blood-testis barrier [80]. Later Delaroche et al reports that SARS-CoV-2 is rarely present in semen specimens during acute phase of the disease and if present it is due to oral or manual contamination during semen collection [81]. The prospects of sexually transmission of SARS-CoV-2 virus also remains unlikely since no viral transmission has been observed till date during assisted reproductive technologies [82]. However, importance of accessory organs being a major contributors of seminal fluid (testis and epididymis contribute less than 10% to semen, whereas, seminal vesicles 65–75% and the prostate contributes 25–30% of the total ejaculate volume) as potential viral reservoirs for sexual transmission on longer run which should be evaluated in future studies especially when such conditions has been experienced with previous viruses [83].

CONCLUSION:

The abundant presence of ACE2 receptor protein on membrane of different testicular cells facilitating the entry of the SARS-CoV-2 virus, its replication and cytotoxicity in the cells raised the concern among scientists globally about its potential adversities on male fertility organs. The deleterious effects of SARS-CoV-2 virus in testis majorly explained as altered hormone levels, orchitis, disrupted spermatogenesis, oxidative stress, and an increased level of antisperm antibodies generation which impaired blood-testis barrier and further generate immune responses. However, the reported investigations are majorly done on acute phase infection when the COVID-19 positive male patients were hospitalized and further follow-up studies on reproductive parameters of these patients post hospitalization are missing, hence, at present with limited information, it is not justifiable to reach any conclusion. Also, in time when rise in infertility cases due to male factors observed worldwide, consequential adversities in male reproductive parameters due to the COVID-19 pandemic, might further multiply the complications. Therefore, more long term follow-up studies on male survivors with both severe and moderate COVID-19 infections as well as in depth scientific studies are requisite to bridge the knowledge gaps in understanding the mechanism of SARS-CoV-2 virus induced direct or indirect pathogenesis in male reproductive tract causing male fertility impairment.

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