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Testis and blood-testis barrier in Covid-19 infestation: role of angiotensin-converting enzyme 2 in male infertility

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) that causes COVID-19 infections penetrates body cells by binding to angiotensin-converting enzyme-2 (ACE2) receptors. Evidence shows that SARS-CoV-2 can also affect the urogenital tract. Hence, it should be given serious attention when treating COVID-19-infected male patients of reproductive age group. Other viruses like HIV, mumps, papilloma and Epstein–Barr can induce viral orchitis, germ cell apoptosis, inflammation and germ cell destruction with attending infertility and tumors. The blood-testis barrier (BTB) and blood-epididymis barrier (BEB) are essential physical barricades in the male reproductive tract located between the blood vessel and seminiferous tubules in the testes. Despite the significant role of these barriers in male reproductive function, studies have shown that a wide range of viruses can still penetrate the barriers and induce testicular dysfunctions. Therefore, this mini-review highlights the role of ACE2 receptors in promoting SARS-CoV-2-induced blood-testis/epididymal barrier infiltration and testicular dysfunction.

Keywords: angiotensin-converting Enzyme-2; blood-testis barrier; SARS-COV-2.

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Introduction

The male reproductive system

This system consists of the testes, epididymides and other accessory structures such as seminal vesicle, prostate, vas deferens, ejaculatory duct, glands of Littre, Tyson's gland, the ampullary and the erectile organ (penis). Their functions are sperm production, synthesis of hormones and coitus [1, 2].

The testes

The testes also referred to as the testicles are the male gonads concerned with the production of sperm cells where the haploid germ cell is generated and male reproductive hormone (testosterone). Each functional testis is about 15 mL or more in volume, but a size lower than 15 mL indicates injury to the seminiferous tubule, which occupies about 95% of the major volume of approximately 500 tubules per testis [3]. Each testicular lobule contains tightly packed seminiferous tubules and sparse interstitial connective tissue. The seminiferous tubules are lined by stratified germinal epithelium that consists of developing germ (spermatogenic) cells and supporting sustentacular or Sertoli cells. The process of spermatogenesis (including germinal cell division, maturation and transformation into mature spermatozoa) occurs in the seminiferous tubules. Surrounding each tubule are loose connective tissues, macrophages, blood capillaries, nerves as well as neurovascular bundles, lymphatic vessels, and between them are groups of steroid-secreting interstitial cells (Leydig) producing testosterone [4].

Spermatogenic cells of different stages are incorporated into the cytoplasm of Sertoli cells for growth (nurse cells). The cytoplasmic extensions (tight junctions) of the adjacent Sertoli cells formed the BTB which prevents spermatogenic cells against the body's immune system, thus preventing the formation of autoantibodies. The Sertoli cells produce a large number of proteins like transferrin, androgen-binding proteins, ceruloplasmin, cadherins, laminins, connexins, seminiferous growth factors, plus anti-mullerian hormone which are critical for transport, tight junction regulations and spermatogenesis control [5, 6]. Each seminiferous tubule is about 30–70 cm long, 200–300 μm in diameter, highly convoluted loop within the lobule to accommodate a long tubule in a very small lobule [7]. Sometimes very tight convolutions of the seminiferous tubule may result in their incomplete obstruction, thereby reducing intertubal space. As the tubule reaches the mediastinum, convolutions decrease and ultimately straightens out to form tubuli recti (straight ducts). The sperm cell produced move out of the tubular lumen beginning from the rete testes into the epididymis then into the vas deferens during ejaculation. Sperm cells in the vas deferens are joined by secretions from the accessory sex glands to form semen as they pass through the prostate via the ejaculatory ducts into the urethra [8].

Testes are mostly supplied by the long testicular arteries arising high up from the abdominal aorta just beneath the renal arteries and anastomoses with the artery of the ductus deferens. The venous drainage from the testis and epididymis form the network of veins known as pampiniform plexus with 8–20 veins surrounding the testicular artery in the spermatic cord. The heat conveyed by the testicular arteries is mostly absorbed back by the venous network in the pampiniform plexus. This structural arrangement, together with the cremasteric and dartos muscle, helps the testes to maintain a constant temperature [9].

The epididymis

The epididymis is another important structure in the male reproductive system concerned with sperm maturation. Sperm leaving the testis becomes motile and acquires functional competence during their transit in the epididymis [10]. Epididymal secretion plays a vital role in sperm mitochondrial metabolism, antioxidant defense, and sperm-zona pellucida interaction. These secretions contain L-carnitine, Myo-inositol, glycerophosphocholine alpha-glucosidase, glutamate, sialic acid, taurine, electrolytes and lactate are also present [11]. Many proteins are also secreted, such as transferin, albumin, immobilin, metalloproteins, clusterin (SGP - 2), and proenkephalin which are involved in the regulation of epididymal functions. Spermatozoa undergo physiological changes during their transit time (estimated to be between 2 and 6 days) in the epididymis, which is facilitated by the fluid microenvironment within the epididymis [12]. Seminal fluid analysis is a valuable test to examine male fertility status. The factors usually analyzed include volume (2 mL), motility (50% within the first 60 min after ejaculation), counts (40 million/ml), morphology (14%), liquefaction (within 30 min of ejaculation at room temperature), pH slightly alkaline (7.2), fructose level (13 μmol/mL), smell, leucocytes levels (majorly neutrophils) 1×10^6/mL, and a specific gravity of 1.028 according to WHO [13].

Accessory sex glands

The secretions from the seminal vesicles are viscid, yellowish-white alkaline fluid and forms the bulk of semen (46–80%) which is rich in fructose, prostaglandin, electrolytes, fibrinogen, flavins, vitamin C, phosphorylcholine, ergothioneine, semenogelin I and II, a coagulating enzyme (vesculase). The activity of the mucous-secreting epithelial membrane containing many goblet cells of the seminal vesicles is regulated by the secretion of the androgen and fructose serve as an energy source for sperm. The seminal vesicles contain about 40 million times higher concentration of prostaglandins than the blood. This has been demonstrated to enhance sperm motility in the female reproductive system upon reacting with cervical mucus and stimulating smooth muscle contraction in both sexes [14].

The prostatic secretion contributes to about 13–33% of semen volume and is slightly acidic, biochemically active with a large number of enzymes (e.g. proteases, peptidases, fibrinogenase and hyaluronidase) and other constituents (buffers, zinc, spermine, phospholipids and electrolytes). This secretion plays a vital role in semen clotting, liquefaction and breakdown of clot. The acid phosphatase, prostate-specific antigen and citrate which are classical markers of prostatic functions are also present. The bulbourethral glands (Cowper's gland) are mucus-secreting two pea-size glands situated in the deep perineal pouch surrounded by sphincter urethrae muscle. The volume of secretion is small about 2–5% but rich in mucoproteins, which serves as a lubricant for the urethrae to facilitate ejaculations of the seminal fluid. The gland also produces the secretory immunoprotein-immunoglobulin G (IgG) [15].

Blood-testis barrier

Blood-testis barrier (BTB), otherwise known as Sertoli cell or seminiferous epithelium barrier, functions as a physiological, anatomical, and immunological barrier and segregates the seminiferous epithelium into two compartments—basal and adluminal compartments. This barrier is considered as one of the closely-fitted blood–tissue barriers in the mammalian body compared to other types of blood tissue barriers [16]. Unlike other barriers (e.g. blood–brain barrier and the blood–retina barrier), which are primarily formed by the tight junctions between endothelial cells of the small capillaries and supported by other cell types; the BTB is more elaborate and extensive owing to the presence of several components including desmosome, gap junction (GP), tight junction (TJ) and basal ectoplasmic specialization (basal ES) that forms this barrier [17, 18].

Structural barrier

BTB serves as a structural barrier with the presence of tight junction formed by some structural protein which includes occludin, tricellulin and scaffolding proteins, basal ES, desmosome, and gap junction [19]. BTB is solely formed by adjacent Sertoli cells in proximity of the basement membrane and devoid of penetration by neurovascular bundles [20, 21]. Occludin mediates the control and the localization of polarity proteins for cell polarity, thereby modulating the movement germ cell across the BTB and assisting Ca^{2+}-independent adhesion [18]. Claudin expression in the Sertoli cells is controlled by androgens, follicle stimulating hormone, cytokines such as TNFα and (TGFβ), and even the presence of germ cells [18, 22]. The discontinuities of microfilament strands in TJs are prevented by Tricellulin, which could increase paracellular electrical resistance as well as reduction in the movement of charged particles and solutes across the TJs. Thereby prevents the passage of macromolecules by forming a seal in the epithelium. Tricellulin promotes junction formation by regulating cell division cycle 42 (CDC42) via actin polymerization and subsequent binding to CD42 guanine nucleotide exchange factor that stimulates junction formation in the epithelial [23].

There are scaffolding proteins (Zona occludens proteins), also known as TJ protein, at the TJ uniting structural proteins with the cytoskeleton [24]. Maintenance of TJs and cell polarity is facilitated by three different types of protein complexes: the apical Crumbs (CRB)-PALS-PATJ complex; the apicolateral partitioning-defective (PAR) 3-PAR6 -atypical protein kinase C (aPKC) complex; and the basolateral scribble (SCRIB)-discs-large (DLG)-lethal giant larvae (LGL) [25]. Ectoplasmic specialization-mediated adhesion could be apical or basal in location but the basal form is the one involved in BTB. It is a cadherin-catenin multifunctional complex, closely-packed with actin-filament bundles that lie close to the plasma membrane. It is found between the endoplasmic reticulum and cell membrane of two connecting Sertoli cells [26–28]. Another junctional component found in BTB is gap junction (GJ), which serves as a channel for solute including transport of therapeutic drugs between two cells and extracellular space. The GJ channels, in this case, enhance homeostasis between Sertoli cells in BTB and form the basic building blocks of connexins [29]. Connexins are structural proteins with a short half-life of approximately 1–6 h in cells which are translated and oligomerize in the rough endoplasmic reticulum to form connexions. A combination of connexions from two opposite cells creates GJ [30]. The opening of connexions is graded to regulate the movement of molecules which is controlled by cellular Ca^{2+} concentration, mechanical stress, voltage, intracellular H^+ ions, redox potential, and level of phosphorylation of connexins [29]. There are several members of the connexin family with each member appearing to have unique functions like maintenance of fertility and viability of offspring. These functions become evident by impairment in gametogenesis in both male and female $Cx43^{-/-}$ mice (a member of the connexin family) [31]. Myoid peritubular cells (MPCs) and Sertoli cells (SCs), produce substances that constitute the basal lamina surrounding the seminiferous epithelium, this is further reinforced by the BTB to prevent the movement of substances into the adluminal compartment especially in rodents but the efficiency of this barrier is compromised in human [32]. The endothelial TJ in the testicular interstitium also gives some degree of restriction thereby assisting BTB [33, 34]. It has been reported that the structural integrity of the BTB is maintained by crosstalk between actin microfilaments, intermediate filaments, microtubules and Cx43 connexin [18]. Also, testosterone was reported to be a key player in the maintenance of the BTB dynamic ultrastructure. It supports of Sertoli-germ cell junction assembly and disassembly, and its deficiency will result in detachment of advanced germs cell from the Sertoli cells [35].

The immunological barrier

Some germ cell-specific antigens (proto-oncogenes and oncogenes) expressed during meiosis and spermiogenesis, can elicit an immunological reaction, seen in some pathological state (autoimmunity) which could cause infertility

[36]. Hence, BTB serves not only as a physical barrier by separating the events of spermatogenesis but rather allow the cellular events to take place in an immune-privileged apical compartment [16]. The immunological barrier provides microenvironment devoid of an attack on germ cells that have crossed from the basal compartment to the adluminal region. This occurs by mediating immune cells and other possibly toxic components in the lymphatic system and blood that may be bathing the basal aspect of the seminiferous epithelium [37]. Sertoli cells have been suggested to secrete immunosuppressive molecules such as TG-β, IDO, galectin-1, activin A that block immune response [16, 38]. The immunosuppressive factors inhibit the proliferation of lymphocytes and the secretion of interleukin-2 [39]. Male germ cells have also been found to secrete cytokines (e.g. IL-1α and TNF-α) and expression Fas ligand (FasL), which induces apoptosis in Fas-bearing lymphocytes, by so contributing to the provision of immune privilege microenvironment [40]. Germ cells in the basal compartment of BTB are saved by nonspecific immunosuppressive, lymphocyte trafficking, and suppressor T cells, antigen presentation, as well as hormones like testosterone and guiding of BTB by a selected population of lymphocyte as suggested by some studies [41, 42]. The combination of the physical structure of testicular cells, cytokines, and controlled immune responses in the testis, give the testicular immune privilege compartment. The testis locally produces a reliable innate immune system to counter infections which may lead to orchitis [34]. The immunological barrier is not lost despite transient passage of preleptotene spermatocytes from basal compartment to adluminal compartment [43]

Studies have shown that a complex interdependent and well-coordinated process maintains BTB integrity when preleptotene spermatocytes move from basal to the adluminal region. When this occurrs, another BTB is formed behind the preleptotene spermatocytes leaving the initial barrier to disintegrate after its formation. This process occurs seamlessly with the help of cytokines that increase adhesion protein complexes (such as occludin-ZO-1, N-cadherin-β-catenin, claudin-5-ZO-1), androgen, actin regulatory proteins (like., Eps8, Arp2/3 complex), endocytic proteins (like dynamin-2, clathrin, caveolin), non-receptor protein kinases (like., focal adhesion kinase, c-Src, c-Yes) and polarity proteins (like, PAR6, Cdc42, 14-3-3) [44].

Efflux pump barrier

The BTB restricts the entry of many drugs and other molecules into the seminiferous tubules with the help of an efflux pump but this could sometimes prevent the distribution of some therapeutic drug meant for testis; for this, the efflux pump modulation is strongly considered. Efflux pumps are integral membrane proteins (as shown in Figure 1) that selectively influence both entry and exit of substrates such as drugs and xenobiotics, including nonhormonal contraceptives across the BTB thereby protecting spermatogenesis [45]. The pumps (transporters) are also involved in the modulation of inflammation, proliferation of the immune cell, detoxification, movement of lipids and hormones as well as development and retention of stem cells [46]. Active transporter of substrates can be classified into primary, secondary and tertiary.

The primary transporter uses ATP to bring about substrate movement; therefore belongs to ATP-binding cassette (ABC) superfamily, also known as efflux transporter, while secondary and tertiary transporter functions without using ATP directly and belong to solute carrier family (SLC). Knowledge of efflux pump distribution is essential in understanding the role they play in BTB which could have conferred best protection of spermatogenesis [47]. There are about 50 members of ABC but important ones are P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP) [48]. ABC transporters have a common molecular configuration and two distinct domains which are: the transmembrane domain, consists of six α-helices and the cytoplasmic nucleotide-binding domain, containing an active site for ATP [45].

The distribution and localization of efflux pump vary in testis cells. Leydig cells expressed P-gp (P-glycoprotein) and MRP1, myoid cells and endothelial cells strongly expressed P-gp and BCRP. In contrast, the Sertoli cells expressed MRP1; however, their marked expression of MRP2 (multidrug-resistance associated proteins 2) in the BTB and other testicular cells, except the myoid cells [47]. The efflux pumps are not restricted to BTB but also found in other blood tissue barriers (like blood–brain barrier, blood-placenta barrier) and organ involve in excretory functions (like uterus, liver, kidney and small intestine) [49]. There is variation in the expression of MRP (MRP1, MRP4, MRP5 and MRP8) across the species, testicular cell and BTB compartment. While MRP1 and MRP4 are basolaterally located in the membrane of Sertoli cells, MRP5 is confined to Leydig cells, and MRP8 is present in round spermatids. MRP1 and MRP4 are considered to limit drug movement into the apical compartment of BTB while MRP5 and MRP8 are not involved [50]. Mdr1, encoding p-gp in humans, function primarily by extruding hydrophobic substrates with molecular mass ranging from 300 to 4000Da from the intracellular aspect of the cell membrane to the exterior via active transportation [51]. P-gp

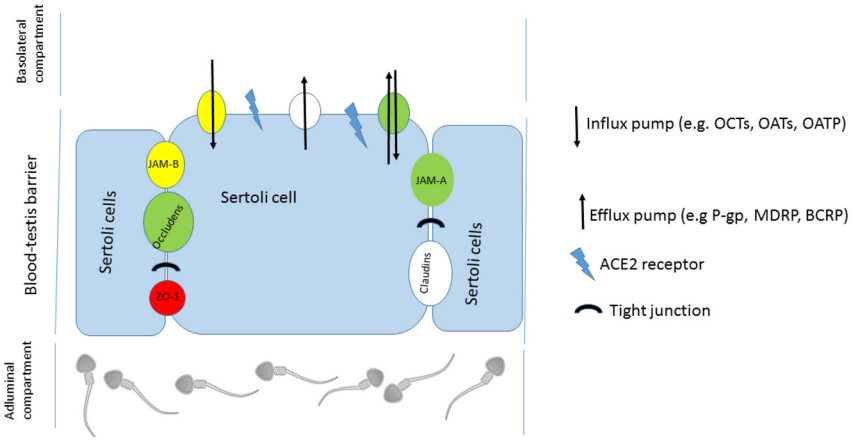


Figure 1: Hypothetical diagram showing the relative distribution of various transporters, including influx and efflux pumps localized on the Sertoli cells that form the BTB. The transporters and ACE2 receptors are associated with the integral proteins found on the basolateral membrane of Sertoli cells and mediate the transport of substances between basal and adluminal compartment.

prevents the penetration of BTB by xenobiotic and other chemicals to protect germ cell development [52]. The functionality of P-pg has been considered as the yardstick for measuring BTB integrity [45].

P-gp suppresses caveolin endocytic function, also seclude drugs to the intracellular vesicles and subsequently eliminate them by exocytosis [53, 54]. Another way P-gp was found to regulate substrate movement is by interacting (although less significant) with numerous tight junction proteins, including occludin, claudin-11 and junctional adhesion molecule-A (JAM-A). In the presence of a substrate that can be harmful to the germ cells, it causes this protein-to-protein interaction to be stronger ,thereby preventing further entry of such toxin. Thus it is a part of the junctional complex and possibly controls the distribution of tight junction proteins in BTB [55]. About eight different multidrug resistance-associated proteins (MRPs) have been identified in various body organs like the brain, kidney, placenta and testis [47]. MRP1 is the most studied of the MRPs that transports anti-cancer and antiviral drugs, toxicants, antibiotics, androgens, cyclic nucleotides, glutathione, glucuronate or sulphate conjugates – the end-products of detoxification reactions and heavy metal oxyanions [56, 57]. Absence or reduction in MRP1 expression could affect testosterone levels in the testis thereby causing loss of seminiferous epithelium and detachment of germ cells from the Sertoli cell [58]. Transcription factors, recognized as nuclear hormone receptors (e.g., pregnane-x), also help to upregulate some transporters (e.g. MRP and P-gp) at blood-tissue barriers [59].

Angiotensin-converting enzyme 2 (ACE2) and blood-testis barrier

ACE2 or angiotensin-converting enzyme homologue (ACEH) is an important enzyme that mediates multiple physiological functions in the body resulting from its ability to regulate the Renin-angiotensin system (RAS), transport amino acid and function as a potent receptor for coronaviruses [60–62]. Thus, the abnormal activities of this enzyme have been implicated in various pathologies (including pulmonary abnormalities, cardiovascular and renal dysfunctions). At the same time, its structure and multiple functions increase its susceptibility as a receptor for SARS-CoV2 [63, 64]. The analysis of the total length of human ACE2 cDNA revealed that it comprises of endothelium-bound carboxypeptidase/metallopeptidase having a similar identity with the *N*-terminal catalytic domain of ACE1.It is made up of two regions: an amino-acid terminal and a carboxylic terminal [65]. Studies have shown that ACE2 has poor substrate specificity and it acts by removing an amino acid from the carboxylic terminal of the peptides, thereby converting angiotensin I to angiotensin-(1–9) [66]. ACE2 enzymatically produce angiotensin-(1–7) from angiotensin II, and less efficiently, angiotensin-(1–9) from angiotensin I, by the hydrolyzing reaction of angiotensin I. Angiotensin-converting enzyme can then convert angiotensin-(1–9) to angiotensin-(1–7) [67].

The ACE2 receptor mRNA and protein expression have been localized in various organs, including oral mucosa, lungs, stomach, intestine, spleen, brain and kidney, mostly in cell membranes bound state and rarely in a soluble form [68, 69]. Recent studies showed mapping of ACE2 in human testicular tissue using single-cell transcriptome resolution with ACE2 protein expression localized in spermatogonia, Leydig, and Sertoli cells [70, 71], implying testicular pathogenesis in Covid-19 infection. The testis has been found to have the highest level of ACE2 expression compared to other tissue and all have the same isoform [71]. Douglas et al. [72], in his study, revealed that the increase in Leydig cell count is directly related to ACE2 expression during development and also demonstrated that adult-type Leydig cell contains the primary source of testicular ACE2.

Angiotensin-converting enzyme 2 activity affects both the basal and Luteinizing hormone-stimulated testosterone biosynthesis, and the ablation of ACE2 has been found to affect fertility in males [73]. Secondly, ACE2 could have been involved in the local regulation of testicular microvasculature in terms of fluid movement and permeability as evidenced by the reduction in interstitial fluid and blood flow when Leydig cell was ablated; however, this effect could be insignificant in the presence of testosterone [74]. The angiotensin-(1–7) receptor, Mas (G protein-coupled receptor) are majorly localized in the interstitial compartment and cytoplasm of the Leydig cells. They are essential in the regulation of spermatogenesis [75]. Thus, ACE2 regulates spermatogenesis by producing angiotensin-(1–7) [76, 77]. Upregulation of ACE2 was noticed at puberty in rodents with some of the Leydig cells ablated prior puberty, and its expression seems not to be controlled by the pituitary–testicular-hormonal axis [72]. Angiotensin II type 2 receptor has been found in semen that plays a vital role in sperm motility [78]; this suggests ACE2 may help in making spermatogonia and newly formed sperm cells quiescent till it gets to a stage of maturity.

Endocrine control of the blood-testis/epididymal barrier

The increase in serum gonadotropin that occurs at puberty has been reported to facilitate BTB formation [79]. Also, a reduced or delayed formation of TJ protein has been observed in idiopathic hypogonadotropic hypogonadism, a condition in which the gonads release a low amount of sex hormone due to abnormalities in the pituitary gland or hypothalamus [80]. Thus, reproductive hormones (most importantly, follicle-stimulating hormones (FSH), luteinizing hormone (LH) and testosterone) perform a significant function in the formation and conservation of BTB [81] with considerable "cross-talk" between endothelial cells of capillaries, dendritic cells, mast cells, peritubular cells and interstitial endocrine cells [82]. However, androgen (testosterone) is the principal hormone that upregulates mRNA expression, increases the production of structural protein and mobilization of tight junction proteins into the Sertoli cell junction as well as maintenance of the BTB integrity [83, 84]. While FSH can directly act on Sertoli cell androgen receptors to restore and localize tight junction protein, LH stimulates the synthesis of testosterone that upregulates the structural proteins in the tight junctions [85, 86]. Experimental studies have shown that FSH or testosterone, alone or in combination, increases the transepithelial electrical resistance of the tight junction, thereby enabling the barrier to effectively regulate the movement of substances across the seminiferous interstitium [87]. Testosterone upregulates tight junction integral protein (claudin-11 and claudin-3) mRNA expression as well as enhances localization of structural proteins at tight junctions [88, 89]. On the contrary, Sertoli cell androgen receptor (AR) knock-out showed a significant reduction in structural protein (especially claudin-11 and occludin) with the reduced formation and incomplete function of the BTB [90, 91].

SARS-COV-2-induced blood-testis/epididymal barrier inflammation, the role of ACE2

Although the BTB and blood-epididymis barrier (BEB) are tight barriers in the male reproductive tract that helps to inhibit immune response and testicular inflammation [16, 92]. They undergo restructuring to facilitate the transition of spermatocytes in the seminiferous tubules during spermatogenesis [93]. Studies have shown that a wide range of viruses (e.g. Mumps virus, Zika virus, HIV) can still penetrate the barriers and seen in semen [94, 95] where they induce orchitis, inflammation, apoptosis and oxidative damage to the testis thereby affecting male fertility [96, 97]. Thus, increased blood viral load (as seen in SARS-COV infection) can increase the vulnerability and risk of male gonads to viral infection and predominantly affect testicular functions [98–100]. The testicular aberration could occur via ACE2 present in the testes, which can be utilized as a potent receptor by the virus to penetrate the testes [71, 72], as well as reduced efficiency of the BTB to prevent the testis against viral entry [37]. The rapid replication of the virus after entry may promote pyroptosis of the immune cells and apoptosis of the endothelial cells in the testes with subsequent release of inflammatory biomolecules including cytokines, chemokines and adhesion molecules. Also, inflammatory cytokines (TNF-α, IL-1β) may promote oxidative stress in the Sertoli cells and compromise BTB integrity [101, 102]. This results from the significant role of the cytokines to alter the integral proteins at the BTB, promoting the destruction of tight Junction fibrils, desmosome and gap junctions in Sertoli cells [103, 104]. A significant increase in cellular inflammation has been well-correlated with cell death and multiple organ damage during viral infection including Covid-19 infection [105–108]. At the molecular level, increased cytokines enhance oxidative stress which may invariably activate either focal

adhesion kinase (FAK) or phosphoinositide 3 kinase-based (P13K-c-Src-PAR) signaling pathway. These pathways impair the phosphorylation of the adhesion protein complex at the BTB with the increased polarity of the Sertoli cells. Ultimately, these pathways render the cell adhesion molecules unstable and disrupt the BTB and promote testicular dysfunction [109, 110].

Orchitis-induced male infertility

Local or systemic infection (predominantly during viral infection) can cause orchitis (an inflammatory lesion of the testicles) due to the hematogenous propagation of the pathogens [111–113]. Orchitis has been reported to be a risk factor for male infertility reported in 15% of infected or recovered patients undergoing fertility tests [114]. This pathological condition is characterized by the infiltration of leucocytes into the seminiferous tubules with resultant tubular and cellular damage, and sometimes associated with mild pain and swelling in the scrotum [115, 116]. The infiltration of leucocytes into the Sertoli cells could disrupt the immune-privileged environment behind the BTB necessary for sperm maturation as well as the production of anti-sperm antibodies that alter sperm concentration and quality [117, 118]. Also, Leydig cell dysfunction, secondary to infiltration of activated leucocytes, could impair the production and release of reproductive hormones (majorly testosterone), resulting in hypogonadism [114]. Furthermore, inflammation may spread to the epididymis, especially when effective therapy is delayed, to alter the development of spermatozoa as well storage of mature sperm cells [119].

The pro-inflammatory stimulus (interleukin-6) present in the epididymis during infection can freely permeate the BTB via protein inhibition or activating extracellular-signal-regulated kinase (ERK) signaling pathway in the Sertoli cells to affect the seminiferous tubules [120]. These inflammatory molecules can promote germ cells apoptosis and alter spermatogenesis by inhibiting ZFp637 (Zinc-finger protein-637) protein that downregulates SOX2 (Sex-determining region Y-Box-2) expression, a transcription factor necessary for cell proliferation [121, 122]. Thus, orchitis can induce male infertility via leucocyte infiltration into the seminiferous tubules, thereby acting on Sertoli cells and Leydig cells to impair spermatogenesis and hypogonadism respectively. Also, chronic inflammation resulting from delayed therapy can promote the infiltration of activated leucocytes into the epididymis to negatively impact the epididymal physiology.

Blood–testis barrier and resistance to antiviral drugs

Numerous drug transporters are universally expressed in the adult testes and help to ensure that circulating xenobiotic and drugs do not cross the BTB into the developing germ cells in the apical membrane [123]. These drug transporters exist as integral proteins that mediate active transport of substances across the cell membrane as well as distribution, toxicity and clearance of these drugs from the cell under physiological and pathological conditions. These integral proteins could either serve as an influx pump (such organic transporters (OCT)) transporting drugs into the cells or an efflux pump (such as P-gp, MRPs etc.) that facilitate the removal of drugs out of the cells and restricting unwanted substances from entering the cell [47]. Thus, they play a significant role in preserving testicular functions during infection and therapy by resisting drugs and xenobiotic across the BTB. Drug transporters are localized on the basolateral membrane of Sertoli cell that constitutes BTB and germ cells found outside the BTB and are structurally associated with tight junction protein complexes (e.g. occluding, JAM-A, ZO-1, and claudins) at the BTB [50].

Exposure of the testes to toxicants (including drugs and viral infections) causes upregulation of drug resistance protein such as P-glycoprotein and enhances its association with tight junction proteins. This process of protein–protein interaction between the integral proteins in the basolateral membrane (P-gp) and tight junction proteins (occluding, claudins, JAM-A) that constitute the BTB has been shown to intensify closure of the tight junctions resulting from increases in the efficacy of the BTB [124]. Therefore, drug transporters (influx and efflux) in concert with tight junction proteins regulate the number of drugs and toxins in the apical compartment of where spermiogenesis and spermiation occur [33, 125].

Pathophysiology of SARS-COV2 on the reproductive system

Statistics have shown the increased vulnerability of men to Covid-19 infection compared to women due to the upregulation of ACE2 expression in the testis compared to ovarian tissue as well as the activity of androgen receptor to promote TMPRSS2 gene transcription [126]. Also, the immune response to pathogens is more potent in females than males resulting from hormonal differences (estrogen, progesterone) and genetic composition of sex chromosomes, both of

which contribute to decreased susceptibility to infection as well as increased viral clearance during infection [99, 127]. At this phase, in the pathogenesis of this novel infection, little is known about the effect of SARS-COV-2 infection on male reproductive functions. However, due to the significant properties shared by this virus with the other viruses (e.g. Hepatitis, HIV, papilloma, etc.), it is much likely to exert similar effects on reproductive function, especially in patients of reproductive age [128–130]. Existing literature has shown that viral infections (including mumps, HIV) adversely affect testicular functions, especially spermatogenesis and production of male reproductive hormones [131]. In theory, there is an increased risk of SARS-C0V-2 infections in most cells expressing ACE2 due to the high affinity of ACE2 for the outer domain of Covid-19 virus (SARS-COV-2) spike protein than previous SARS-COV spike protein [132–134]. This may result from the presence of spike glycoprotein and viral spike (S1) protein that appears as homologs [135, 136]. Therefore, once within the testicular milieu, the SARS-COV-2 can integrate into the testicular cell membrane by using the viral spike (S1) protein to bind with the testicular ACE2 and utilizing the cellular serine protease (TMPRSS2) to prime the viral spike [137]. After proper integration, the virus initiates a cascade of viral responses with subsequent testicular dysfunction.

Testicular autopsy of patients who died of SARS-COV infection (a virus sharing similar pathogenicity with Covid-19 disease) revealed that there was inflammation of one or both testes resulting from the infection with substantial destruction and death of spermatogenic cells. Also, there is a significant reduction in sperm cells present in the germinal epithelium, macrophages infiltration into the tubular lumen and thickness of the basement membrane [138]. Also, a pathological examination of the testis of Covid-19 infected patients has been reported by [139]. The report of this study showed that there was testicular inflammation with significant tubular damage which could affect spermatogenesis as well as reduced Leydig cells that produce testosterone. Furthermore, there have been suggestions and reports that Covid-19 infection might reduce male fertility [140, 141] via alteration in gonadal function, injury to the seminiferous tubule and testicular inflammation [142]. This observation was further examined in a study consisting of 181 men conducted in China. The outcome of this study shows that Covid-19 infected male patients have an increase in serum luteinizing hormone (LH) and prolactin with no changes in testosterone (T) or follicle stimulating hormone (FSH). However, a drastic reduction in T/LH ratio and FSH/LH ratio was recorded when compared with noninfected healthy men [143]. While reports were supporting the testis as a viral reservoir for SARS-COV-2 and its presence in the semen of infected male patients [144, 145], other studies reported that there were no traces of SAR-CoV-2 virus in the semen of male patients that recovered from the Covid-19 infection [146].

Therefore, till date, there is no convincing proof to validate the exact impact of Covid-19 infection on male reproductive function and the exact mechanism by which SARS-CoV2 induce testicular impairment. However, some mechanisms have been proposed and these include: direct testicular damage caused by viral entry into the testicular tissue via ACE2 [137], increased inflammation activated by the virus and loss of immune-privilege environment following an injury to the Sertoli cell [36] as well as adverse effect of increased scrotal temperature (considering high fever as a symptom during Covid-19 infection) on spermatogenesis [147]. Detailed and comprehensive studies need to be conducted to evaluate the status of important biomarkers such as sperm count, motility, semen quality, hormones and neurochemicals that mediate fertility as well as male sexual behavior in patients who recovered from this infection. Also, healthcare providers should pay attention to male fertility when caring for infected patients interested in reproduction as the testis could be a haven for the virus to spread further through sexual intercourse.

Conclusion and future perspectives

Available pieces of evidence have now shown that SARS-COV-2 is not usually restricted to the respiratory system alone but may also attack other vital tissues in the body. This could explain in part its passage into the testicular microcirculation where reduced blood flow and presence of its receptor (ACE2) could enhance testicular infection and adversely affect male fertility. However, coronaviruses will rarely get into the testis, —hence, with the alarming global cases of COVID-19 infections, there is a potential risk of significant reproductive disturbance that could occur in severe cases. This can result from the similar characteristics exhibited by SARS-COV-2 with other viruses (such as ZIKA, MERS-COV, HEC67 N) attacking the testis, ability to remain in the testicular genome for a longer period and the possibility of reactivation in the future during immune-suppression and cellular stress. Therefore, it is highly recommended to enhance the continuous prevention of SARS-COV-2 transmission and identification of people who are or have been infected to have their fertility tested. Since the virus may hide from been identified by the immune system, thereby hindering its complete clearance from the body system even when the patient recovers from illness. Also, the health care providers should take

cognizance of fertility during care especially in male patients of reproductive age.

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