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REVIEW

Male Reproductive Health

Viral infections and implications for male reproductive health

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Viral infections have haunted humankind since times immemorial. Overpopulation, globalization, and extensive deforestation have created an ideal environment for a viral spread with unknown and multiple shedding routes. Many viruses can infect the male reproductive tract, with potential adverse consequences to male reproductive health, including infertility and cancer. Moreover, some genital tract viral infections can be sexually transmitted, potentially impacting the resulting offspring's health. We have summarized the evidence concerning the presence and adverse effects of the relevant viruses on the reproductive tract (mumps virus, human immunodeficiency virus, herpes virus, human papillomavirus, hepatitis B and C viruses, Ebola virus, Zika virus, influenza virus, and coronaviruses), their routes of infection, target organs and cells, prevalence and pattern of virus shedding in semen, as well as diagnosis/testing and treatment strategies. The pathophysiological understanding in the male genital tract is essential to assess its clinical impact on male reproductive health and guide future research.

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INTRODUCTION

Over the last 30 years, anthropogenic action on the environment has accelerated the incursion of potentially dangerous viral infections to humanity, sometimes taking on the character of a pandemic with devastating consequences.[1] The first cases of human immunodeficiency virus (HIV) infection in 1981 and the subsequent development of the acquired immunodeficiency syndrome (AIDS) pandemic have alerted the international scientific community and society about the real threat posed by sexual microorganism transmission.[2,3] Till date, 27 candidate viruses have been reported to exist in human semen. In most of them, the evidence concerning a possible sexual transmission is nonexistent or ambiguous.[4]

Since December 2019, a new viral disease has spread to 188 countries, infecting more than 35 million people and causing the death of over one million people worldwide over a 10-month period.[5–7] The current pandemic, called Coronavirus 2019 Disease (COVID-19), refers to the new coronavirus-2 associated with Severe Acute Respiratory Syndrome (SARS-CoV-2), its causative agent. Unprecedented efforts were launched by the international community, governments, private and public research organizations, and civil society worldwide to find a viable medical treatment option and a large-scale vaccine.[8]

Deaths from SARS-CoV-2 occur not solely because of respiratory tract complications but also from multiorgan involvement with an essential component of microthrombotic and other vascular affections.[9,10] Genitourinary organs such as the kidneys and the testis have molecular characteristics and express receptors in their cells that prioritize target organs for this new virus. That said, the understanding of the sexual transmission of the virus, testicular involvement with potential sperm function defects, and hormonal production imbalances in both acute and convalescent and recovered males warrant priority investigation.[11,12]

OVERVIEW OF VIRAL INFECTIONS AND THE MALE REPRODUCTIVE SYSTEM

Many families of viruses have a strong tropism for the male reproductive system, especially the testis, and about thirty different species can affect semen quality.[4,13] In addition, some of these viruses can impair fertility and be sexually transmitted.[14] Our knowledge concerning the routes of infection of several viruses in the male genital tract is notably limited. In general, viral infections reach the testis through the bloodstream, causing damage by direct harmful effects on target organs or indirectly through pro-inflammatory cytokines.[15,16] However, alternative routes to hematogenous spread exist, bypassing the epididymal immune capacity and contributing to

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viral dissemination in the reproductive system.[17–19] Viral translocation between the epididymis and testis has been documented from data obtained from studies involving simian immunodeficiency virus (SIV)-infected monkeys and micro-RNA-targeted viral Zika virus (ZIKV) clones in mice.[17,18]

In addition to the public health consequences of the high infectivity and spread of many viruses, there is growing concern about the potential contamination of male reproductive organs and spermatozoa. Such contaminations may lead, inter alia, to male sub-fertility or infertility resulting from signaling deficiencies in testicular cells that may be aggravated by pro-inflammatory mediators, leading to clinical or sub-clinical hypogonadism. In females, oocyte and embryonic infection may cause miscarriages and even fetal losses or fetal abnormalities. Moreover, there is a potential risk of transmission to subsequent generations by incorporation of the viral genome into the germ cell or embryonic genomes.[14,20] **Table 1** summarizes the main viruses known to be present in the male genital tract and their associated complications, and **Table 2** describes the main target organs of these viruses in the male reproductive tract.

Semen quality is considered an accurate biomarker of men's health.[21] However, several factors influence the presence of viruses in semen, including viremia levels, specific viral epitopes, immune replication mechanisms, viral structural stability, specific receptors of viral attachment to testis cells, and the presence of other sexually transmitted pathogens.[4] Additionally, different viruses target different cell types in the reproductive tract, and also the cellular response varies from species to species.[22] Thus, studies investigating the effect of specific infections on male reproductive health may be challenging owing to the high degree of specificity among the viruses and their hosts.[23,24]

Table 3 compiles the prevalence and pattern of virus shedding in human semen. In the next sections, we summarize the evidence concerning the presence and adverse effects of the most common viruses on the reproductive tract, their routes of infection, target organs and cells, prevalence and pattern of viral shedding in semen, and diagnosis/testing and treatment strategies.

MUMPS VIRUS

Summary evidence

Mumps virus (MuV) is an enveloped, nonsegmented, negative-sense RNA virus, a member of the Paramyxoviridae family, with a high tropism for the human testis.[25] Typically, 15%–30% of postpubertal men develop epididymo-orchitis as a complication of mumps. In most cases, the condition is unilateral, resulting in a minor transient change in the sperm parameter values (*e.g.*, decreased sperm count, morphology, and motility). However, 15%–30% of epididymo-orchitis is bilateral and causes testicular atrophy that progresses to infertility in 30%–87% of patients.[26–28]

Routes of infection and target organs and cells

Transient plasma MuV viremia leads to its hematogenous spread into different organs, including the testis.[26] The MuV has been isolated from testicular biopsy specimens of infected men,[29] but its testicular target cells and the complete mechanisms by which MuV infection damages the testis remain undefined. *In vitro* MuV replication is reported in human Leydig cells, causing impairment in testosterone production.[30] Data suggest that MuV-induced germ cell degeneration may be secondary to increased testicular temperature mediated by inflammatory mediators.[20] However, other mechanisms might also explain the observed cellular damage caused by MuV on the testis, including (I) modifications of the paracrine control of spermatogenesis exerted by testicular macrophages, Leydig, and Sertoli cells; (II) germ cell apoptosis induced by C-X-C motif chemokine ligand 10 (CXCL10) production by infected Sertoli cells;[31] and (III) congestion of the seminiferous tubules precipitated by the interstitial edema.[32]

Prevalence and pattern of viral shedding in semen

In a case report, MuV was isolated from the semen using real-time-polymerase chain reaction (RT-PCR) 14 days after the onset of the disease, and MuV RNA persisted in semen for up to 40 days. The development of anti-sperm antibodies on day 84 may indicate testicular damage, although semen parameters recovered to average values.[33]

Diagnosis and testing

Mumps orchitis is a clinical diagnosis, rarely seen in children under 10 years old. It typically occurs 1 week to 2 weeks after parotitis.[34] Laboratory diagnosis relies on MuV culture, detection of viral RNA, or, more commonly, serological confirmation by measurement of immunoglobulin antibody levels.[26]

Treatment

There is no specific antiviral therapy for mumps, so treatment is primarily supportive (bed rest, scrotal support, and nonsteroidal anti-inflammatory agents).[26,34] Steroids should not be prescribed for mumps orchitis because they can suppress testosterone production, which could ease, rather than alleviate, testicular atrophy.[35] Despite that, a potential treatment for mumps orchitis is the subcutaneous administration of interferon alpha-2b during 2–4 days, with no evidence of testicular atrophy reported during the follow-up period. In a case series of four patients, three had oligoasthenozoospermia that returned to normal levels 2–4 months after interferon treatment.[36] In another study with 13 patients treated with interferon, four men persisted with oligoasthenozoospermia, even without testicular atrophy.[37]

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Summary evidence

HIV is a retrovirus capable of integrating its DNA into the host genome. This virus includes two main subtypes, HIV type 1 (HIV-1) and type 2 (HIV-2). HIV-1 presents with higher pathogenic potential and more prevalent than HIV-2.[38] AIDS patients infected with HIV-1 can develop chronic orchitis and, consequently, progressive hypergonadotropic hypogonadism, suggesting that testicular steroidogenesis is impaired.[39,40] The clinical scenario involving orchitis, hypogonadism, and leukocytospermia explains alterations in semen parameters, mostly oligozoospermia and teratozoospermia, which accompany HIV infections.[41] The most common testicular histopathology observed in men who have died of AIDS is the presence of "Sertoli-cell-only" syndrome (43%), followed by germ cell damage (27%) and peritubular fibrosis (15%). In only 3% of individuals, did the testis remain normal in appearance.[42]

Routes of infection and target organs and cells

Sexual intercourse is the principal route of HIV transmission, with about 2 million new HIV infections each year resulting from this single shedding route.[43] The primary viral target is $CD4^+$ T lymphocytes and some populations of macrophages and dendritic cell lineages expressing CD4 molecules. These molecules represent the central viral receptor, while CC-chemokine receptor 5 (CCR5) and CXC-chemokine receptor 4 (CXCR4) are the main HIV co-receptors.[44] Several clinical trials and epidemiological studies reported the importance of foreskin as an entry portal for HIV.[45–48] However, other penile sites such as glans

Table 1: Viruses: taxonomy, clinical presentation, and effects on male reproductive health

Virus	*Family*	*Genus*	*Genome*	*Clinical presentation*	*Effect on male reproductive health*	*Reference*
EBOV	Filoviridae	*Ebolavirus*	ssRNA (–)	Hemorrhagic fever	Testis as an anatomic reservoir for persistence	156–158
HBV	Hepadnaviridae	*Orthohepadnavirus*	dsDNA (RT)	Hepatitis, cirrhosis, and hepatocellular carcinoma	Sperm parameter alteration and infertility	97,120,128,130-132
HCV	Flaviviridae	*Hepacivirus*	ssRNA (+)	Hepatitis, cirrhosis, and hepatocellular carcinoma	Sperm parameter alteration and infertility	97,120,129,133–137
HSV-1	Herpesviridae	*Simplexvirus*	dsDNA	Herpes labialis and genital herpes	Prostatitis, epididymitis, infertility, and sperm parameter alteration	61, 63, 64, 66
HSV-2	Herpesviridae	*Simplexvirus*	dsDNA	Genital herpes	Prostatitis, epididymitis, infertility, and sperm parameter alteration	14,97,119–121
HIV	Retroviridae	*Lentivirus*	ssRNA (RT)	AIDS	Orchitis, "Sertoli-cell only" syndrome, and infertility	38–42
HPV	Papillomaviridae	*Alpha-, beta-, gamma-papillomavirus*	dsDNA	Warts and preneoplastic lesions related to oropharyngeal genital and anal cancers	Subfertility and infertility	13,94–98,101,102
Influenza virus	Orthomyxoviridae	*Influenzavirus*	ssRNA (–)	Systemic and respiratory symptoms	Sperm parameter alteration	164–166
MuV	Paramyxoviridae	*Rubulavirus*	ssRNA (–)	Swelling of the parotid glands, salivary glands, and other epithelial tissues	Epididymo-orchitis and infertility	25–28
SARS-CoV	Coronaviridae	*Betacoronavirus*	ssRNA (+)	Severe acute respiratory syndrome	Orchitis	8,171,194
SARS-CoV-2	Coronaviridae	*Betacoronavirus*	ssRNA (+)	Severe acute respiratory syndrome	Orchitis	5,6,8,10,171,195,196
ZIKV	Flaviviridae	*Flavivirus*	ssRNA (+)	Zika fever and congenital Zika	Orchitis, epididymo-orchitis, and infertility in mouse models. Sperm parameter alteration in men	1,72–75,85

EBOV: Ebola virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HSV-1: herpes simplex virus type 1; HSV-2: herpes simplex virus type 2; HIV: human immunodeficiency virus; HPV: human papillomavirus; MuV: mumps virus; SARS-CoV: severe acute respiratory syndrome-associated-coronavirus; SARS-CoV-2: severe acute respiratory syndrome-associated-coronavirus type 2; ZIKV: Zika virus; ssRNA (–): negative-sense, single-stranded RNA viruses; ssRNA (+): positive-sense, single-stranded RNA viruses; dsDNA: double-stranded DNA viruses; dsDNA (RT): double-stranded DNA reverse-transcribing viruses; ssRNA (RT): single-stranded RNA reverse-transcribing viruses

Table 2: Viruses and target organs in the human male reproductive tract

Virus	*Testicle*	*Epididymis*	*Vas deferens*	*Seminal vesicle*	*Prostate*	*Penis*	*Reference(s)*
EBOV	Endothelial cells and seminiferous tubules	NA	NA	NA	NA	NA	157
HBV	Endothelial cells and fibroblasts	NA	NA	NA	NA	NA	141,142
HCV	NA	NA	NA	NA	NA	NA	133–136
HIV	Germ cells, macrophages, and T-lymphocytes	Viral RNA and antigens in leukocytes	NA	Viral RNA and antigens in leukocytes	Viral RNA and antigens in leukocytes	Glans, foreskin, and urethra	38,50,52,54,55
HPV	Sertoli and interstitial cells	Viral DNA	Viral DNA	Viral DNA	Viral DNA	Viral DNA in shaft, glans, foreskin, and urethra	102,105–107
HSV	Specific tropism not defined	NA	NA	HSV-2 isolation from biopsies	HSV-2 DNA	HSV-2 DNA in glans, shaft, and urethra and viral HSV-1 in foreskin	20,97
Influenza virus	NA	NA	NA	NA	NA	NA	168
MuV	Leydig cells	NA	NA	NA	NA	NA	29,30
SARS-CoV	Leydig and epithelial cells	NA	NA	NA	NA	NA	194
SARS-CoV-2	Spermatogonia, Leydig and Sertoli cells	NA	NA	NA	NA	NA	11,190
ZIKV	Immature germ cells	*Viral RNA	*Viral RNA	*Viral RNA	*Viral RNA	NA	74,79–84

*Experimental animal studies. EBOV: Ebola virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; HPV: human papillomavirus; HSV: herpes simplex virus; HSV-1: herpes simplex virus type 1; HSV-2: herpes simplex virus type 2; MuV: mumps virus; SARS-CoV: severe acute respiratory syndrome-associated-coronavirus; SARS-CoV-2: severe acute respiratory syndrome-associated-coronavirus type 2; ZIKV: Zika virus; NA: not available

meatus, urethra, and even explants of the inner foreskin are susceptible to HIV-1 infection.[49–51] HIV RNA and leukocyte antigens are detected in the testis,[52] epididymis,[53] prostate,[53,54] and seminal vesicles.[55] It is hypothesized that HIV-1 has a direct detrimental effect on Leydig cells, leading to interstitial tissue fibrosis and increased production of inflammatory cytokines that inhibit testosterone synthesis.[52,56] *In vitro* studies demonstrated that HIV attaches to spermatozoa through heparin sulfate proteoglycans,[57] but there is no evidence of the virus's

Table 3: Prevalence[a] and characteristics of virus shedding in human semen

Virus	*Acute stage of infection (%)*	*Chronic stage of infection (%)*	*Shedding pattern (%, continuous)[b]*	*Sexual transmission reported (Y/N)*	*Reference(s)*
EBOV	73–100		100	Y	158–160
HBV	NA	68	NA	Y	148–150
HCV	29–39	32–46	0–28	Y	151–155
HIV-1	61–100	81–100	Chronic: 56–61	Y	60–66
HPV	11.4		NA	Y	114
HSV	NA	0–10	50	Y	60–66
Influenza virus	NA	NA	NA	N	168
MuV	NA	NA	NA	N	33
SARS-CoV	NA	NA	NA	NA	NA
SARS-CoV-2	NA	NA	NA	NA	NA
ZIKV	50–68		100	N	86–90

[a]Prevalence of virus shedding in semen is calculated using the infected individuals and not the general population, [b]continuous shedding pattern in semen is estimated by longitudinal shedding rates determined by Kaplan–Meier survival analysis as reported by Gianella *et al.*[125] EBOV: Ebola virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV-1: human immunodeficiency virus type 1; HPV: human papillomavirus; HSV: herpes simplex virus; MuV: mumps virus; SARS-CoV: severe acute respiratory syndrome-associated-coronavirus; SARS-CoV-2: severe acute respiratory syndrome-associated-coronavirus type 2; ZIKV: Zika virus; NA: not available; Y: yes; N: no

vertical transmission (spermatozoon to the fetus). A meta-analytic study, including 3994 women with HIV-infected partners who had undergone a total of 11 585 assisted reproduction technology (ART) cycles, demonstrated that sperm-mediated HIV transmission never occurred following sperm processing.[58,59]

Prevalence and pattern of viral shedding in semen

HIV load in seminal fluid is usually lower than that in blood, but there are reports of infected patients with an unusually high semen viral load.[60,61] Various factors are associated with the persistence of HIV shedding in semen, including repeated sexual transmitted infections (STIs),[62,63] blood HIV load,[62] co-presence of seminal cytomegalovirus (CMV) or Epstein–Barr virus (EBV),[64–66] and seminal cytokine levels.[63,64] Even under efficient antiretroviral therapy and undetectable blood viral loads for several years, the persistence of HIV-1 DNA and RNA had been reported in some infected men.[67]

Diagnosis and testing

International guidelines usually recommend an initial screening test to be performed with a fourth-generation antigen–antibody assay. Positive results should be confirmed with an antibody assay to differentiate between HIV-1 and HIV-2 infections.[68,69] This assay measures immunoglobulin levels (IgM and IgG) against recombinant proteins or synthetic peptides and monoclonal antibodies against p24.[38]

Treatment

HIV treatment aims to suppress viral replication and maintain plasma HIV-1 below detection levels by antiretroviral therapy (ARTx). The World Health Organization (WHO) endorses a first-line treatment that consists of a combination of two nucleoside reverse-transcriptase inhibitors plus a nonnucleoside reverse-transcriptase inhibitor or an integrase inhibitor.[70] Of note, it is hard to dissociate the direct impact of HIV-1 on male fertility from the possible ARTx gonadotoxic effects. The latter can affect sperm parameter values and lead to decreased total sperm count, progressive motility, and an increase in sperm counts with abnormal morphology.[41]

ZIKA VIRUS

Summary evidence

Zika infection, transmitted by mosquitoes of the genus *Aedes*, is caused by an RNA virus belonging to the Flaviviridae family. This infection has recently emerged partly owing to the spread of arthropod vectors into new deforested geographical areas, particularly in the Amazon and Atlantic Rainforest regions of Brazil.[1] ZIKV can be sexually transmitted, with worrisome public health implications, not only for people living or transiting in endemic areas but also for their sexual partners in nonendemic places.[71] ZIKV can cause orchitis, epididymo-orchitis, and testicular atrophy, resulting in infertility and hypogonadism in animal models.[72–74] Alterations in semen, including decreased total sperm count and an increase in sperm abnormalities up to 90 days after the onset of acute ZIKV infection, can cause infertility and might be explained by direct damage to the testis and epididymis.[75] Testosterone concentration remains unchanged during the acute-phase infection.[75]

Routes of infection and target organs and cells

Hematogenous spread probably corresponds to the ZIKV leading entry portal into the male genital tract.[76] *In vitro* and animal studies show that testicular and prostate cells are susceptible to ZIKV infection. However, the testes are the organs of choice for viral replication, capable of sustaining high viral loads for extended periods.[74,77–82] ZIKV replicates in human germ cells *in vivo* and within testis explants, basically composed of macrophages and Leydig, Sertoli, and peritubular cells.[79] Viral RNA has also been detected in the epididymis,[74,80,83,84] vas deferens,[83] and seminal vesicles,[74,81,82,84] in different animal models. In about 30% of affected spermatozoa, ZIKV was present primarily at the mid-piece of mature cells. The Tyro3 receptor is the viral binding and entry site in this cell.[78] As ZIKV is detected in motile spermatozoa, vertical transmission via spermatozoa is a real possibility that deserves further investigation.[75] Another possible route of vertical transmission is the exposure of the pregnant women to ZIKV-infected semen that can favor hematogenous spread and transplacental infection; a high viral load in the mother might be a significant risk factor for vertical transmission.[85]

Prevalence and pattern of viral shedding in semen

The prevalence of ZIKV shedding in human semen samples collected up to 30 days after symptom onset varies from 50% to 68%.[86–89] Viral RNA remains positive in the seminal fluid until day 370 after the onset of systemic symptoms.[86] For ZIKV, the mean clearance time from semen is 25 days to 83 days compared with 5–15 days in the bloodstream.[86–88,90] This prolonged virus shedding is influenced by host characteristics such as age, anejaculation, joint pain, and conjunctivitis.[87]

Diagnosis and testing

As clinical presentation of ZIKV infection is often nonspecific, diagnosis relies on nucleic acid detection by RT-PCR or serological

testing.[91] RT-PCR for ZIKV-nucleic acids should be performed during the acute phase of the infection, with ZIKV DNA by RT-PCR detecting up to 14 days of symptoms onset in saliva and urinary samples, and up to 60 days in blood.[92] Currently, ZIKV serological diagnosis is debated, mainly owing to the cross-reactivity of ZIKV to sera of other flaviviruses (*e.g.*, vaccination for Yellow-fever virus) and previous infection by other species within the Flavivirus family.[93]

Treatment

Clinical management of acute ZIKV infection is supportive care. Antiviral therapy and vaccine are under evaluation in clinical trials.[93] Prevention of sexual transmission is based on sexual abstinence or protected coitus in individuals with a suspected infection. Protected sexual intercourse for 2 months in females and 3 months in males is highly recommended.[92]

HUMAN PAPILLOMAVIRUS (HPV)

Summary evidence

HPV is a nonenveloped, double-stranded circular DNA virus, a member of the Papillomaviridae family.[94] It is the most common sexually transmitted virus in humans, with over 80% of the sexually active adults being infected by one HPV type at least once in their lifetime.[95,96] HPV infections are clinically expressed by genital warts and are usually cleared by immune cells within a few months of acquisition. However, subclinical infections represent the majority of nonsuspected infections and are likely to spread the virus silently.[94,97] Nonetheless, a small percentage of diseases of specific HPV subtypes can persist and progress to cancer, including penile cancer. Thus, the classification of HPV encompasses those at high risk (*e.g.*, HPV-16, 18, 45) or low risk for cancer onset and development (*e.g.*, HPV-6, 11), based on their oncogenic potential.[94] Among high-risk HPVs, HPV-16, HPV-18, and HPV-45 are involved in the pathogenesis of penile squamous cell carcinoma.[98] Few studies suggest that HPV can be a risk factor for prostate cancer aggressiveness.[99,100] HPV is also responsible for specific alterations in semen quality, eventually leading to infertility.[101] The proposed mechanisms for male infertility associated with HPV infection include (I) direct modification of semen quality, (II) damage to sperm DNA integrity, and (III) production of anti-sperm antibodies that can interfere with sperm motility and sperm–oocyte binding.[13,95,102] The hypothesis of a direct viral effect on sperm is substantiated by the findings of high sperm DNA fragmentation rates and decreased progressive sperm motility in infertile men with asymptomatic chronic high-risk HPV infection.[103,104]

Routes of infection and target organs and cells

HPV infects the stratified penile epithelium's basal cells and may take months or even years before squamous intraepithelial lesions can be clinically detectable.[105] HPV infection is mostly asymptomatic in men rather than in women. Still, the virus is easily found in almost all parts of the male reproductive system, including the urethra, vas deferens, epididymis, testicles, prostate, and seminal fluid.[14,102,106,107] Vertical transmission through the spermatozoon is possible. Therefore, HPV can potentially be transferred to the embryo upon fertilization, thus impairing embryo development and invading trophoblast cells, potentially increasing miscarriage rates.[108–110] Conventional sperm-washing protocols rarely eliminate HPV, so it may be prudent to include HPV in the semen-screening panel prior to ART procedures.[111,112]

Prevalence and pattern of viral shedding in semen

A meta-analysis of 31 studies comprising more than 5000 men, a part of the general population (HPV prevalence of 11.4%) and a part attending fertility clinics (HPV prevalence of 20.4%), reported that HPV in semen was associated with a two-fold increased risk of infertility. High-risk HPV-16 and 56 were the most common types in semen found in both populations.[113] Another meta-analysis also demonstrated high HPV prevalence in semen (16% in men with subfertility *vs* 10% in the general population).[106] In an analysis of HPV-infected men, the median duration of persistence of any HPV type was 15.3 months in semen samples.[114]

Diagnosis and testing

Genital warts are often diagnosed based on their clinical appearance, so the molecular tests for the presence of HPV are not usually necessary. However, one must keep in mind that up to 80% of male HPV infections are subclinical and can only be detected if inspection using magnification and 5% acetic acid are employed.[115] Histologic examination of biopsy specimens is recommended to rule out squamous cell carcinomas, mainly when the lesions are atypical and when the disease does not respond to or worsens during standard therapy, as in immunocompromised patients. In these situations, HPV-DNA testing should be performed to categorize the virus.[116]

Treatment

The recommended treatments for HPV genital warts consist of patient-applied regimens (*e.g.*, imiquimod cream, podofilox solution or gel, and sinecatechins ointment) and physician-applied regimens (*e.g.*, bichloracetic or trichloroacetic acid, cryotherapy, and surgical excision).[116] CO_2 laser with magnification is one of the most successful and efficient methods to eliminate clinical and subclinical HPV lesions in the external genitalia.[117] Besides the evident advantage in terms of primary female disease prevention, HPV vaccines in men might help to prevent high-risk HPV infection diffusion, decreasing the risk of developing anal, cervical, and penile cancers.[115] In addition, it is suggested that HPV vaccination in infertile men has a protective role because it has been associated with lower detection of HPV DNA in semen, improved semen quality, increased pregnancy rates, and lower miscarriage rates.[118]

HERPES SIMPLEX VIRUS (HSV)

Summary evidence

HSV, a DNA virus, is one of the most common infections in humans. Genital HSV has two subtypes, namely, HSV-1, which causes recurrent ulcers in the mouth and sometimes in the genital area, and HSV-2, which has a tropism for the genital area.[119] HSV is detected in the semen and is likely to be associated with male infertility and abnormal semen parameters (*e.g.*, low sperm count and motility).[14,97,120]

Routes of infection and target organs and cells

HSV is a sexually transmitted virus that infects the penile mucosa and induces a lifelong infection in humans.[121] HSV first replicates in keratinocytes and then infects nerve cells, where it rests latent. When reactivated, HSV returns to the epithelial surface to create vesicular lesions due to virus-induced cell death.[122] HSV-2 can infect almost all organs and tissues of the male genital tract, except the seminiferous tubules, owing to the protection conferred by the blood–testicular barrier.[20,97] Sources of seminal HSV DNA have yet to be clarified, but it is currently accepted that HSV-2 can be internalized into healthy, motile spermatozoa, and are likely to cause direct sperm damage.[119] Therefore, HSV can be vertically transmitted through spermatozoa during ART, with a consequent increased miscarriage risk, and potentially adverse effects to the fetus and the newborn.[123]

Prevalence and pattern of virus shedding in semen

In chronic infection stages, the prevalence of HSV shedding in semen

ranges from 0 to 10%, in studies performed in healthy men and co-infected by HIV.[64,66,124–126] In a study on HIV-infected men, half of the HSV-co-infected samples showed a continuous pattern of seminal shedding.[124]

Diagnosis and testing

Clinical diagnosis consists of identifying single or clustered recurrent vesicles that ulcerate before resolving in genitalia and adjacent areas, sometimes accompanied by fever, malaise, and local adenopathy in primary infections.[121] In the presence of active lesions, PCR assay is the method of choice for differentiating type-specific HSV. In contrast, serologic testing is useful when the medical history is suggestive. Still, there is no lesion, or the PCR assay is negative, or when the patient's partner is infected.[127]

Treatment

Treatment aims to reduce the severity, duration, and recurrence of the disease, and prevent transmission to uninfected partners.[121] Regimens are the same for both HSVs and are based on nucleoside analogs (acyclovir, famciclovir, and valacyclovir), whose mechanism of action is the inhibition of viral DNA.[127]

HEPATITIS B (HBV) AND HEPATITIS C (HCV) VIRUSES

Summary evidence

HBV and HCV viruses are present in the human semen and are active sexually transmitted.[97,120] HBV is an enveloped DNA virus, a member of the Hepadnaviridae family,[128] whereas HCV is an RNA virus that belongs to the Flaviviridae family.[129] HBV is known to induce the production of reactive oxygen species (ROS), which can cause lipid peroxidation (LPO) and subsequent sperm DNA fragmentation and increase the rate of chromosome mutations.[130] Patients chronically infected with HBV generally have reduced sperm motility, a higher proportion of necrotic spermatozoa, and abnormal sperm morphology.[131] Current data show an increased incidence and risk of infertility in men infected with HBV.[132] HCV infection is also associated with alterations in semen quality, including decreased sperm count, reduced motility, and abnormal sperm morphology.[133,134]

In several studies comparing uninfected versus chronically HCV-infected men, hormonal imbalances, such as decreased serum levels of testosterone and inhibin B, as well as altered semen parameters, such as abnormal morphology and lower sperm motility, have been reported.[131,133–137] It is noteworthy that treatment with ribavirin and interferon further worsened semen quality in HCV-infected patients.[133,137] In chronically HCV-infected men, viral replication is associated with worsening of all sperm functional tests, including high levels of seminal ROS and high sperm DNA fragmentation.[135]

Routes of infection and target organs and cells

In areas of a low or intermediate prevalence of HBV, sexual intercourse is the most common way of dissemination.[138] The mechanism of entry into the genital mucosa has not yet been fully explained, mainly owing to the lack of an adequate viral cell culture system.[139] The target cells for HBV are the hepatocytes, lymphoid cells,[140] and specifically in the testis, the endothelial cells and fibroblasts.[141,142] Spermatozoa can probably act as a vector for the transmission of HBV because HBV DNA has been detected inside them, directly damaging its structure.[143] A study has revealed that the frequency of sperm chromosomal aberration in an HBV-infected group was significantly higher (14.8%) than that in an uninfected control group (4.3%).[144] HBV can be vertically transmitted, and spermatozoa transfected with HBV are susceptible to apoptosis and have reduced fertilization capacity.[145]

By contrast, HCV-infected patients have a reduced viral load in semen, so the risk of transmission through sexual intercourse is low, accounting for about 5% of transmissibility, according to a few studies.[133,146] Semen HCV is not associated with altered semen quality, suggesting that the virus does not have a significant direct deleterious effect on spermatozoa.[147] Vertical transmission of HCV has not been reported so far.[146]

Prevalence and pattern of viral shedding in semen

The prevalence of virus shedding in semen is around 68% in a population of chronically HBV-infected men.[148] In two case reports, HBV-DNA was still detected in semen 120 days after it was no longer detected in the serum.[149,150] The prevalence of HCV in semen varies according to the stage of infection, ranging from 29% to 39% in early stages,[151,152] and from 32% to 46% in chronic stages.[151–155] Studies with HIV–HCV-coinfected men reported a continuous seminal shedding pattern of HCV in 0–28% of samples.[153,154]

Diagnosis and testing

Diagnosis of hepatitis B is based on a clinical presentation of acute hepatitis or cirrhosis, confirmed with a serological assay. The key serological marker of hepatitis B is the detection of hepatitis B surface antigen (HBsAg) in the patient's serum. The combination of different detected specific antigens–antibodies establishes the diagnostic state of acute/chronic disease, while the detection of HBV DNA indicates the standpoints for therapy and treatment monitoring.[128] Acute HCV infection usually occurs asymptomatically or less commonly as acute hepatitis. In around 80% of cases, the disease progress to a chronic infection that can culminate in cirrhosis and hepatocarcinoma.[129] Different virological tests are used to diagnose HCV infection, such as enzyme-linked immunosorbent assays to detect anti-HCV serum/plasma antibodies and detection of HCV RNA to confirm active infection and evaluate the response to ART.[129]

Treatment

Medications used in HBV treatment are classified into two broad categories, the immunomodulatory and antiviral agents. The former comprises interferon α-2b and PEGylated interferon α-2a. The latter includes nucleoside or nucleotide analogs, such as lamivudine, adefovir dipivoxil, entecavir, telbivudine, tenofovir disoproxil fumarate, and tenofovir alafenamide.[128] Only patients with detectable HCV RNA in the serum should receive ARTx. The main HCV treatment relates to direct-acting antiviral agents, mainly NS3/4A protease inhibitors, NS5B polymerase inhibitors, and NS5A replication complex inhibitors.[129]

EBOLA VIRUS

Summary evidence

Ebola virus (EBOV) is a linear, nonsegmented, single negative-stranded RNA virus from the Filoviridae family.[156] It causes a severe zoonotic spillover whose potential for the persistence of the virus in the semen of male survivors raises concern regarding the possibility of sexual transmission in nonepidemic geographical areas.

Routes of infection and target organs and cells

EBOV entries into the male genital tract via the hematogenous route primarily. EBOV antigens are found in testicular endothelial cells and seminiferous tubules.[157] Therefore, the testis is very likely to be an anatomic reservoir for EBOV persistence in humans.

Prevalence and pattern of viral shedding in semen

EBOV RNA can be encountered in the semen of survivors for up to 1178 days (median 158 days after the onset of the disease),[158] and sexual

transmission from male survivors to female partners was identified in up to 470 days after the illness offset.[159]

Diagnosis and testing

Initial symptoms are nonspecific but progressive to hemorrhagic fever, and thus suspicion depends intensely on the epidemiologic surveillance data. Two different diagnostic tests are available, namely rapid diagnostic tests for detecting a viral protein and the RT-PCR to identify EBOV genomic material in various body fluids, including semen.[156] In semen during convalescence, higher viral loads proved by the detection of positive RNA RT-PCR suggest persistent active replication of EBOV within the male reproductive system.[160]

Treatment

During recent outbreaks, aggressive supportive care and ARTx improved outcomes. Favipiravir deployed a weak antiviral response against EBOV when there is a low viral load.[156] Male survivors should have their semen tested by RT-PCR 90 days after the onset of disease; those with positive results must use condoms or abstain from sex until the monthly semen PCR test is negative on two occasions.[161]

INFLUENZA VIRUS

Summary evidence

Influenza A virus is one of the most common infectious agents in the world. It was responsible for the 1918 pandemic that infected 500 million people and killed 50 million.[162] Over the past 100 years, four influenza pandemics have spread and ravaged humanity: Spanish H1N1 in 1918, Asian H2N2 in 1957, Hong Kong H3N2 in 1968, and H1N1 swine Influenza in 2009.[163] Influenza A virus circulates in the reservoir of wild birds and can crossbreed with several different species, sometimes requiring adaptive mutation, possibly using intermediate hosts, making influenza A a true paradigm for an emerging viral threat.[24] Acute infection with influenza virus has systemic implications and can alter semen quality. Decreased sperm motility and sperm count, as well as altered sperm morphology, have been reported and occur up to 4–11 weeks after febrile episodes.[164–166] There is also evidence that sperm DNA integrity may be compromised by influenza.[166,167]

Routes of infection and target organs and cells

Influenza has never been detected in the male genital tract, and currently no studies have examined the presence of the viral receptor in the human genital system.[168] However, two nonmutual mechanisms have been postulated to affect testicular function negatively during systemic influenza infection: (I) fever-induced increase in testicular temperature, which is deleterious to the germ cell line, and (II) the orchitis that may result in systemic illness, which may impair exocrine and endocrine functions of the testis.[166]

Prevalence and pattern of viral shedding in semen

Neither the presence of the influenza virus in semen nor sexual transmission has been reported.[168]

Diagnosis and testing

Seasonal influenza ranges from asymptomatic presentation to fulminant illness, depending on the host and viral determinants.[24,163] The most common symptoms include systemic features such as fever, myalgia, headache, chills, anorexia, and malaise, along with respiratory symptoms, including runny nose and nonproductive cough.[163] Clinical diagnosis is difficult to make and laboratory tests may be recommended, whenever possible, including specific culture, DNA testing, and antigen detection.[163,169]

Treatment

Only four classes of antiviral drugs are approved for the treatment of influenza, namely adamantanes, membrane fusion inhibitors, neuraminidase inhibitors, and RNA-dependent RNA polymerase inhibitors.[169,170] Seasonal vaccination is undoubtedly the most effective method of disease prevention and control.[169]

CORONAVIRUSES (SARS-COV AND SARS-COV-2)

Summary evidence

Coronaviruses are a family of enveloped, strand-positive RNA viruses that infect vertebrates such as humans, bats, birds, cats, and livestock since times immemorial.[8] Within this family, there are three known viruses, one that causes in 2012 the Middle East Respiratory Syndrome (still ongoing), which was named the Middle East Respiratory Syndrome Related Coronavirus (MERS-CoV), and two others that cause Severe Acute Respiratory Syndrome (SARS). These include the SARS-CoV, discovered in 2003, and the new SARS-CoV-2, responsible for the current and still uncontrolled outbreak of the COVID-19 pandemic.[8,171] Almost all human coronaviruses are serologically related to one of the two primary strains, 229E and OC43, related to lower respiratory tract diseases. In a study of older adult patients hospitalized because of pulmonary illness and who tested negative for influenza and respiratory syncytial viruses, approximately 8% were positive for either human coronavirus OC43 or human coronavirus 229E.[172]

Genetic analysis of SARS-CoV-2 reveals close similarities with bat coronaviruses, particularly the BAT CoVRaTG13 found in South China's Yunnan caves (96.2% similarity). However, the intermediate host remains uncertain.[173–176] The SARS-CoV-2 genome has only 380 amino acid differences from its SARS-CoV equivalent, whereas 27 new mutations have been identified for the spike (S) protein responsible for cell–cell interaction and invasion. This mutation rate could explain the higher pathogenicity of SARS-CoV-2 than SARS-CoV, including its genitourinary tract effect, but further studies are warranted and urgently needed.[177,178]

Routes of infection and target organs and cells

All coronaviruses bind their protein S to ectopeptidases for entry into cells. While MERS-CoV binds to dipeptidyl-peptidase 4 (DPP4), angiotensin-converting enzyme 2 (ACE2) is described as the host cell receptor for SARS-CoV and the novel SARS-CoV-2.[179] These two receptors are critical elements in the pathogenesis of the respective diseases, owing to their distinct distribution in different tissues and organs, thus governing potential lesions and their clinical manifestations.[180,181] MERS-CoV has not been found in the human male reproductive tract and, therefore, it is not within the scope of this review.

The ACE2 link is only the first step of a regulated cell entry process for SARS-CoV-2. Indeed, the transmembrane serine protease 2 (TMPRSS2) is crucial for priming the S protein to increase the virus's entry after binding to ACE2.[182] Therefore, it plays a critical role in the pathogenesis of both SARS and MERS coronaviruses, the Asian H7N9 influenza virus, and several H1N1 subtype influenza A viruses.[183]

ACE2 receptors are expressed in alveolar lung cells, proximal kidney tubular cells and podocytes, and, in other organs, including the testes.[184–186] According to data obtained from the Genotype-Tissue Expression (GTEx) project, the testicles have the highest expression of ACE2, while the highest expression of TMPRSS2 is found in the prostate.[187]

Theoretically, the testis should be highly susceptible to systemic SARS-CoV-2 infection owing to its high ACE2 levels.[11,188,189] ACE2 receptors are also present in Leydig cells and cells within the seminiferous tubules, including Sertoli cells and spermatogonia.[190] ACE2 is a physiological regulator of the male reproductive system, modulating steroidogenesis affecting germ cell development.[11,185,191] The prostate and bladder have a lower risk of local infection because, despite a high TMPRSS2 level, ACE2 expression is low.[187]

On the basis of the mass RNA expression data (from the Human Proteome Map) and the Protein Platform dataset (from the Atlas of Human Proteins), the testes exhibit moderate expression of ACE2 with little or no TMPRSS2 receptors.[192] Similarly, a descriptive analysis of single-cell RNA sequencing data failed to demonstrate the co-expression of ACE2/TMPRSS2 in testicular cells, including spermatozoa, although Sertoli cells were underrepresented in the sample.[192]

Therefore, for the current COVID-19 pandemic, two questions remain to be answered, namely, (I) does SARS-CoV-2 replicate in the genitourinary organs, and (II) how does the high expression of ACE2 in genitourinary tissues contribute to the infectivity of SARS-CoV-2.[186,193]

It is known that since the first global SARS outbreak linked to a coronavirus in 2003, testicular involvement was demonstrated.[194] In a small case series of six men who died of SARS-CoV infection in China, the testicular analysis confirmed that orchitis was a complication of the systemic disease, with germ cell destruction, few or no spermatozoa in the seminiferous tubules, basement membrane thickening, peritubular fibrosis, leukocyte infiltration, and vascular congestion in the interstitium.[194]

Testicular involvement has already been described during the SARS-CoV-2 pandemic. In a single study presenting the results of first autopsies of COVID-19 in the metropolitan area of São Paulo, Brazil, orchitis with fibrin microthrombi was a common feature in postmortem testicular samples.[10] In another study, the examination of testes from 12 deceased COVID-19 patients demonstrated reduced Leydig cell count, significant seminiferous cellular injury, and mild lymphocytic inflammation. In contrast, direct evidence of the presence of SARS-CoV-2 occurred in just 10% of the testes studied.[195] In a series of patients with mild-to-moderate symptoms of COVID-19, six out of 34 (17.65%) men complained of some scrotal discomfort at diagnosis. However, orchitis was not confirmed by physical examination or Doppler ultrasound (US).[196] Besides, the presence of SARS-CoV and SARS-CoV-2 has not been established in testicular biopsy samples.[184,194,197,198]

Therefore, from these preliminary results, two main nonmutually exclusive hypotheses are proposed to explain the pathophysiology of testicular injury caused by SARS-CoV-2. First, the virus reaches the testis hematogenously, and it affects the Leydig and Sertoli cells, creating alterations in the steroidogenic pathway and recruiting immune cells that might potentiate the pathogenic presentation of orchitis.[199] Other SARS-CoV-2 infection might indirectly interfere with testicular function, inducing a systemic "cytokine storm" as it is the case in other viral infections, such as the influenza virus, avian H5N1, and SARS-CoV.[186,200,201]

Regarding the effects of SARS-CoV-2 infection on testicular endocrine function, a study comparing hormone levels of 119 reproductive-aged infected men with 273 age-matched subjects in a control group reported a significant increase in luteinizing hormone (LH) and a decrease in serum testosterone/LH ratio in the COVID-19 group. The testosterone/LH ratio was negatively associated with white blood cell counts and C-reactive protein, probably reflecting an immunological injury to Leydig cells, provoking an early stage of transient hypogonadism.[12]

Prevalence and pattern of viral shedding in semen

Like SARS-CoV, there is no evidence of sexual transmission of SARS-CoV-2 infection, but data concerning the presence of viruses in the human semen are minimal. A Chinese case series of 12 COVID-19 men reported no SARS-CoV-2 virus in semen samples with a median time of 78.5 days between semen collection and the symptoms' onset.[12] Another small case series of 34 men revealed that the presence of SARS-CoV-2 in semen was not reported in recovered patients. In this study, semen specimens were examined using RT-PCR 30 days or longer after the confirmation of COVID-19.[196] The authors hypothesized that the likelihood of testicular viral infection is small because <0.1% of all different testicular cells (including spermatozoa, spermatogonia, myoid, endothelial, macrophages, and Leydig and Sertoli cells) express both ACE2 and TMPRSS2 receptors.[196] However, it is worth mentioning that all participants of both studies were evaluated several days after the acute phase of COVID-19, which may have influenced the chances of finding the virus. In a study on 12 infected men (aged 22–38 years old), almost all with mild disease, SARS-CoV-2 was absent in the semen in both acute and recovery phases of COVID-19.[198] In a German cohort study, 16 semen samples were recovered between 8 and 54 days after the absence of symptoms, two from active SARS-CoV-2 infection, and 14 from control healthy individuals. RT-PCR did not reveal an RNA virus in any samples (mean recovery time of 32.7 days). It is noteworthy to mention that men with moderate infection had impaired semen parameters compared with recovered and healthy men.[202] By contrast, in another small cohort study, SARS-CoV-2 was found in the semen of a subset of COVID-19 patients. The authors reported the virus's presence in 4 of 15 patients (26.7%) suffering from COVID-19, and in 2 of 23 convalescent patients (8.7%).[203] Despite these alarming results, an accurate description of the RT-PCR and semen collection methods utilized had not been provided.

In addition, the lack of information about viral load prevents a thorough analysis of its implications for sexual transmission. For example, the location of the virus has not been provided, and it is therefore speculative whether it could exist free in the seminal fluid, bound to the surface of the spermatozoa, or even internalized.[204] The virus is not known to be present in the seminal fluid. Thus, the possibility of eliminating the virus through washing procedures remains equivocal.[205–207] It is suggested that although SARS-CoV-2 might be found in the semen, possibly taking advantage of the privileged testicular immune environment, it would seem unlikely to replicate in the reproductive tract and be sexually transmitted.[208]

For MERS-CoV, the viral shedding in the seminal plasma of dromedary camels has been described.[209] Nonetheless, there is no evidence for the same phenomena in human semen.

Diagnosis and testing

The most common symptoms for both SARS and COVID-19 are fever, dyspnea, and dry cough. Common complications among hospitalized men include acute respiratory distress syndrome (ARDS), pneumonia, acute kidney injury, and cardiac injury.[5,6,210,211]

Laboratory diagnostic tests for SARS are based on RT-PCR assay for nasopharyngeal aspirate during the first 3 days. Retrospective diagnosis is confirmed with seroconversion on a virus immunoassay (enzyme-linked immunosorbent or immunofluorescence assay).[211] The diagnosis of the initial phase of COVID-19 is possible using RT-PCR collected by the nasal swab. In contrast, probable diagnosis is often based on the clinical scenario, serology testing, laboratory findings, and computed tomographic imaging.[210]

Treatment

Owing to the limited knowledge about these newly emerged viruses, many drugs are currently under evaluation and development to minimize the effects or combat SARS-CoV-2 and COVID-19. Public health measures such as contact tracing, social distancing, proper hand washing, and face masks are imperative until the development of effective vaccines, chiefly for SARS-CoV-2 infection.[210,211]

GAPS IN KNOWLEDGE AND FUTURE PERSPECTIVES

A growing range of virus families has been recently discovered in the male reproductive system, with potentially dramatic consequences for both the individual and public health. Understanding the pathophysiology of these viruses in the reproductive system is of utmost importance to prevent sexual transmission and protect male reproductive health. Preventative measures must be outlined for the current and future outbreaks, although many questions are still unresolved. Remarkably, the following issues seem to be the leading directions for future studies in this area.

The mechanisms underlying the harmful consequences of chronic viral infections on semen parameter values and male fertility statistics are broadly unknown, principally discriminating systemic from a more direct viral effect on the reproductive system. A better understanding of the infection routes and the male reproductive tract's target cells is critical for developing adequate strategies for treatment and prevention.

The semen as a vector for sexual and vertical viral transmission must also be studied further; nevertheless, the mere presence of viral nucleic acids in semen does not indicate infection. Further research should be aimed at detecting accurate viable viral loads and particles in semen capable of causing disease. Studies in this area should investigate potential and likely possible risks with the use of medically assisted reproduction and also anticipate future epidemics when the virus presents with efficient persistence mechanisms in the semen as ZIKV and EBOV.[86,158] The immunosuppressive environment that protects spermatozoa from the autoimmune response might constitute an "invisible mantle" for some viruses, a hypothesis that warrants investigation.[208]

Lastly, it is indispensable to reveal some viruses' complex role in the etiology and the progression of such male genital cancers, such as penile, prostatic, and testicular. The presence of a human virome in the male genital system can elicit discoveries of viruses or a combination of them in some once-unsuspected asymptomatic men that could hide unhealthy conditions.[212]

Further studies of these viruses may provide useful information for a better understanding of the pathogenesis, routes of infection, and target tissues. Moreover, relevant research can elucidate the mechanisms of virus persistence in semen and confirm sexual and vertical transmission mechanisms. Thus, adequate and specific treatments can be developed either to cure or prevent possible sequelae in the male reproductive system, the partner, the offspring, and beyond benefiting future generations.

AUTHOR CONTRIBUTIONS

TAT and JH contributed to the design and data collection, conceived the manuscript, and wrote the first draft. YCO and FSB added to data collection. SCE contributed to the design and wrote sections of the document. EGK, AND-N and JRD wrote parts of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

TAT, YCO, FSB, EGK, AND-N, JRD, and JH state that the research was conducted in the absence of any commercial or financial relationship that could constitute a potential conflict of interest. SCE declares the receipt of unrestricted research grants from Merck and lecture fees from Merck.

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