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SARS-CoV-2, Zika viruses and mycoplasma: Structure, pathogenesis and some treatment options in these emerging viral and bacterial infectious diseases

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ABSTRACT

The molecular evolution of life on earth along with changing environmental, conditions has rendered mankind susceptible to endemic and pandemic emerging infectious diseases. The effects of certain systemic viral and bacterial infections on morbidity and mortality are considered as examples of recent emerging infections. Here we will focus on three examples of infections that are important in pregnancy and early childhood: SARS-CoV-2 virus, Zika virus, and *Mycoplasma* species. The basic structural characteristics of these infectious agents will be examined, along with their general pathogenic mechanisms. Coronavirus infections, such as caused by the SARS-CoV-2 virus, likely evolved from zoonotic bat viruses to infect humans and cause a pandemic that has been the biggest challenge for humanity since the Spanish Flu pandemic of the early 20th century. In contrast, Zika Virus infections represent an expanding infectious threat in the context of global climate change. The relationship of these infections to pregnancy, the vertical transmission and neurological sequels make these viruses highly relevant to the topics of this special issue. Finally, mycoplasmal infections have been present before mankind evolved, but they were rarely identified as human pathogens until recently, and they are now recognized as important coinfections that are able to modify the course and prognosis of various infectious diseases and other chronic illnesses. The infectious processes caused by these intracellular microorganisms are examined as well as some general aspects of their pathogeneses, clinical presentations, and diagnoses. We will finally consider examples of treatments that have been used to reduce morbidity and mortality of these infections and discuss briefly the current status of vaccines, in particular, against the SARS-CoV-2 virus. It is important to understand some of the basic features of these emerging infectious diseases and the pathogens involved in order to better

Abbreviations: ACE2, Angiotensin-converting enzyme 2 receptors; ALA, Alpha-lipoic acid; ARDS, Acute respiratory distress syndrome; COVID-19, Coronavirus disease 2019; CoVs, Coronaviruses; CQ, Chloroquine; DMVs, Double-membrane vesicles; E, Envelope; ENac, Epithelial sodium channel; ERS, Endoplasmic reticulum; γCoV, Gammacoronavirus; HCQ, Hydroxychloroquine; HGT, Horizontal gene transfer; HI, Herd immunity; IL, Interleukins; J-H, Jarisch-Herxheimer reactions; M, Membrane; MHC, Major histocompatibility complex; N, Nucleocapsid; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; nsp, Nonstructural proteins; ORF, Open reading frames; pp1a, Polyproteins 1a; pp1b, Polyproteins 1b; RdRp, RNA-dependent RNA polymerase; RTC, Replicase-transcriptase complex; S, Spike; TMPRSS2, Type II transmembrane serine protease; TNF-α, Tumor necrosis factor-α; WHO, World Health Organization; ZIKV, Zika Virus; αCoV, Alphacoronavirus; βCoV, Betacoronavirus; δCoV, Deltacoronavirus.

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appreciate the contributions of this special issue on how infectious diseases can affect human pregnancy, fetuses and neonates.

1. Introduction

According to the U.S. National Institute of Allergy and Infectious Diseases, emerging infectious diseases are commonly defined as outbreaks of previously unknown diseases, in contrast to known persistent diseases that are uncontrolled and rapidly increasing in incidence or geographic range [1]. Emerging infectious diseases should not be confused with reemerging infectious diseases, which are diseases that reappear after they have been in significant decline due to several reasons. Emerging and reemerging infectious diseases remain among the leading causes of death and disability worldwide. All of these infectious pathogens are subject to changes in their genomes, epigenomes, and their interactions with particular hosts, modifying the susceptibility of populations to new diseases [2]. All emerging and reemerging infectious diseases have to be evaluated in the context of human reproduction [3]. Reproduction is an essential step in the conservation of a species. There can be effects before and after conception. After conception there are many physiological changes related to reproduction that take place in higher organisms, such as in a mammalian mother and its offspring during pregnancy, resulting in adaptations of the individuals within the milieu of mother/placenta-fetus/neonate interactions [4]. Some changes are related to the presence of new individuals, such as changes in maternal breathing, circulation and immunity patterns, and other changes are associated with the generation of a new organ, the placenta, dedicated to the support and relationship between offspring and mother. As a result of the physiological changes that occur during pregnancy, there are also different susceptibilities to infections of the mother, the placenta, and the embryo/fetus at different stages of pregnancy [5]. Because of this, emerging and reemerging infectious diseases should be evaluated in their effects after conception at the level of the offspring and several stages of development (embryo, fetus, newborn), the placenta and the mother (during pregnancy and after-pregnancy).

Recently added to the list of emerging diseases, COVID-19 is the result of infection by the SARS-CoV-2 coronavirus. The recent COVID-19 pandemic serves as an interesting model for studying the relationship between infection and pregnancy related to a novel emergent pathogen. Studies so far suggest that there may be an increased risk of adverse pregnancy outcomes, such as preterm birth, among pregnant women with COVID-19 disease [6]. A recent Morbidity and Mortality Weekly Report from the Centers for Disease Control and Prevention (CDC) suggests that pregnant women with COVID-19 disease are more likely to be hospitalized and are at increased risk for intensive care unit (ICU) admission and receipt of mechanical ventilation than nonpregnant women. Interestingly, the risk of death is similar for both groups, but much remains unknown about this aspect of COVID-19 disease [7]. Another example of an older but recent emergent disease is Zika virus. The most peculiar finding about this viral infection and offspring is microcephalia. Regarding the reemerging infectious diseases, mycoplasma infections due to overuse of antibiotics has led to its reemergence in some parts of the world.

In this review we will focus on these two recent emerging viral diseases, SARS-CoV-2 and Zika viruses, while considering a reemerging infectious disease by intracellular bacterial pathogens, such as mycoplasmas. We will discuss mostly the structure to prevention of these pathogens, summarizing their effects on humans after conception. This chapter is to understand why these pathogens may cause diseases with special peculiarities in human reproduction after conception and what to expect in the following years.

2. General considerations of COVID-19 disease

Coronavirus infectious disease-2019 (COVID-19) was first identified in 2019 and was internationally recognized in January 2020 as a new emergent disease that originated in Hubei, China. It is a disease caused by a newly identified coronavirus, SARS-CoV-2, discovered in Wuhan City, Hubei Province, China in December 2019. It was initially viewed as primarily a respiratory disease, in some cases progressing to viral pneumonia. It is now recognized as a complex, potentially lethal systemic disease that affects many organ systems, and it often progresses, especially in older males, to a severe course that requires ICU support. Among those admitted to an ICU, up to one-half may not survive [8]. Patients with COVID-19 that had progressed to lethality generally were older and had other underlying health conditions, such as hypertension, cardiovascular disease, chronic obstructive pulmonary disease, diabetes, chronic kidney disease, malignancy, or other conditions [9,10,11]. The severe complications associated with non-survival in patients with COVID-19 were primarily acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, coagulation dysfunction and multiple organ failure (primarily lung, heart, and/or kidney) [12,13].

COVID-19 disease can be initiated when the SARS-CoV-2 virus is transmitted from one human to another, primarily via inhalation, oral ingestion or mucous membrane contact with virus-containing aerosol droplets. The virus likely enters epithelial cells in the nasal or oral cavity or pulmonary system using the SARS-CoV-2 viral spike protein that

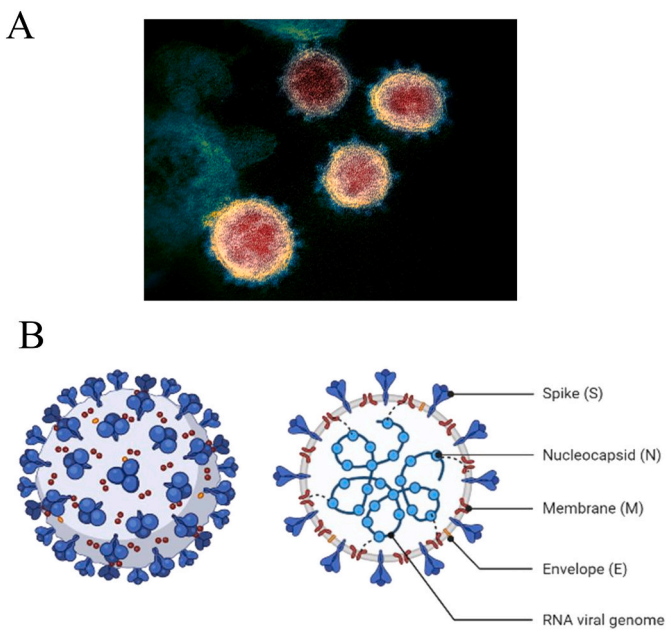

Fig. 1. Structure of SARS-CoV-2. (A) Image and structural scheme of SARS-CoV-2 coronavirus. Spikes and an envelope surrounding the genetic material from viral particles observed using an electron microscope. (B) Structural scheme of SARS-CoV-2 with the main components summarized to the right. S is the characteristic Spike protein, E, the envelope protein, M, the membrane protein and N the nucleocapsid protein, attached to the single stranded RNA genome. The dots indicate that these particles contact the M-proteins in some regions. Images from NIH, page from NIH with freely available SARS-CoV-2 pictures, https://www.niaid.nih.gov/news-events/novel-coronavirus-sarscov2-images, and inside https://www.flickr.com/photos/niaid/49534865371/in/album-72157712914621487/, Scheme made with Biorender https://biorender.com/.

binds to the angiotensin-converting enzyme 2 (ACE2) receptors expressed on mucosal and pulmonary epithelial cells (Fig. 1). Virus–receptor binding subsequently results in endocytosis and fusion of the viral envelope membrane with the epithelial plasma membrane and the entry of the viral nucleocapsid components into the cell, enabling the viral RNA to be replicated. The SARS-CoV-2 virus uses host cell ribosomes to translate its viral genome, thereby leading to the generation of excessive amounts of viral RNA and viral proteins. Ultimately the viral components assemble into new intact viruses that bud from the surface of infected cells or are contained within vacuoles that are released from cells [13].

Initially, local tissue propagation of the SARS-CoV-2 virus can initiate a limited innate immune response, and at this stage, infected individuals can be infectious to others. Viral load appears to be highest around the time of symptom onset, but it generally decreases over the following 5–7 days, with viable virus no longer cultivable beyond 10 days from symptom onset [14]. These features likely account, in part, for the high transmissibility of the virus. Physiologically the SARS-CoV-2 virus propagates and travels down the respiratory tract, where a more robust innate immune response is generally triggered. This process is also characterized by the production of systemic pro-inflammatory cytokines and activated immune cells. By then, COVID-19 disease may be clinically manifest with, in most cases, self-limiting mild-to-moderate symptoms of an upper respiratory tract infection along with other nonspecific symptoms, such as myalgia and fatigue. In about 20% of patients, the virus will infect alveolar cells, once again entering cells via binding to the ACE2 receptor.

In the most severely symptomatic patients, an exaggerated immune response can occur as a cytokine 'storm.' This is characterized by extremely high concentrations of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and various interleukins (ILs), granulocyte colony-stimulating factor, and several chemokines [15]. This pattern can mimic secondary haemophagocytic lymphohistiocytosis, an under-recognized hyperinflammatory syndrome characterized by fulminant hypercytokinaemia and in extreme cases multi-organ failure. It also resembles the cytokine release syndrome that is seen as a complication of chimeric antigen receptor T-cell therapy for lymphoproliferative malignancies and other forms of cancer [16].

2.1. Structure of SARS-CoV-2 virus

Coronaviruses (CoVs) are enveloped positive sense, single-stranded RNA viruses that belong to the subfamily *Coronavirinae*, family *Coronavirdiae*, order *Nidovirales*. Four genera of CoVs, namely alphacoronavirus (αCoV), betacoronavirus (βCoV), deltacoronavirus (δCoV), and gammacoronavirus (ɣCoV) have been isolated. This group of viruses is of zoonotic origin with αCoV and βCoV found in bats and rodents, whereas δCoV and ɣCoV are found in avian species [17]. SARS-CoV, MERS-CoV, and SARS-CoV-2 belong to the family of *Coronaviridae* and the genus *Betacoronavirus*. All coronaviruses are enveloped, positive-sense, single-stranded RNA viruses that feature the largest known RNA virus genomes, ranging in size from approximately 26 to 32 kb [18]. Their diameters are about 65–125 nm, and each viron contains a single strand of RNA. The outer viral surfaces of CoVs are adorned with crown-like viral protein spikes. The SARS-CoV-2 virus is a novel coronavirus of zoonotic origin. It is of spherical shape, but it can also exist as larger pleomorphic particles, measuring between 80 and 160 nm in length.

The unique SARS-CoV-2 virus was named after the previously identified SARS-CoV and MERS-CoV viruses. These latter viruses cause pulmonary failure and potentially fatal respiratory tract infections and were identified in outbreaks in Guandong, China, and Saudi Arabia, respectively. In the case of the SARS-CoV-2 virus, several studies have found that bats were suspected as the key zoonotic reservoir of the virus. As much as 96.2% of the genomes of SARS-CoV-2 and bat CoV RaTG13 viruses are identical [19]. Similar to all CoVs, the SARS-CoV-2 virus contains four structural proteins: envelope (E), spike (S), membrane (M), and nucleocapsid (N) proteins. The S, M, and E proteins together form the envelope of the virus and are embedded in a phospholipid membrane bilayer that surrounds the N protein and associated virus RNA. The M protein is the most abundant viral protein and is mostly responsible for the shape of the envelope. The E protein is the smallest structural protein. The S and M proteins are transmembrane proteins that are involved in virus assembly during replication and virus binding to cell surfaces. The N proteins remain associated with the RNA and form a nucleocapsid structure inside the membrane envelope. Although the N protein is largely involved in processes relating to the viral genome, it is also involved in other aspects of the coronavirus replication cycle, such as virus assembly and budding and the host cellular response to viral infection. Polymers of S proteins remain embedded in a phospholipid bilayer facing outward from the viral membrane envelope, giving the virus its crown-like appearance under electron microscopic observation, and thus the name *coronavirus* [20]. A general scheme showing the main structural components of the novel SARS-CoV-2 coronavirus is shown in Fig. 1.

2.2. Components of the SARS-CoV-2 virus

2.2.1. E protein

The CoV E-protein or envelope protein is a trans-membrane protein common to CoVs and many other viruses [21]. It has a basic structure of homopentamers that form cation-selective ion channels (Fig. 2A). The structure of the E protein has been examined using NMR, and its PDB-IB for the analogous 2003 SARS protein is 5×29 [22]. This protein appears to be implicated in several important processes related to viral infectivity, such as membrane curvature necessary for membrane fusion of the virus with the epithelial cell plasma membrane. It may also be involved in the disruption by virus of host cell membrane pores [23]. The CoV E protein has a well-established role in the assembly of virions, where it induces membrane curvature or aids in membrane scission. Recent studies have expanded the role of CoV E protein beyond viral assembly. Thus the CoV E protein also appears to be critical for the efficient trafficking of virions through the secretory pathway from the Golgi apparatus, a function that may be related to its ion channel-like structure [24]. In addition, the CoV E protein has recently been shown to inhibit host cell stress responses, implicating it in viral pathogenesis [21]. It has been shown to activate the host NLRP3 inflammasome, leading to IL-1β overproduction [25,26]. It is unclear how the regulation of ion fluxes mediated by these proteins can affect virus viability. In many viruses the ability to replicate is impaired by blocking these proteins. For example, in influenza viruses there is an analogous ion channel protein, the M2 protein. In fact, an early antiviral drug, amantadine, had previously been shown to be an influenza virus M2 channel blocker [27]. In summary, although the E protein is a minor component of the virus membrane, it is deemed to be important for several stages of virus infection and replication [28–30].

2.2.2. M protein

The M protein, also known as E1 membrane glycoprotein or matrix protein, is one of three major membrane proteins of the coronavirus together with the S and the E proteins [31]. The M protein is the most structure-conforming protein of the coronavirus membrane, and it plays an important role in determining the shape of the virus envelope. Its structure resembles that of sugar transporters, though it is unknown if it works as such in the virus [32]. The M protein can bind to all of the other coronavirus structural proteins. Binding with M protein helps to stabilize the virus N protein-RNA complex. The M protein promotes the completion of viral assembly by stabilizing the N-protein-RNA complex inside the virus [28]. It has been postulated that the M protein is related to viral infectivity through its binding to the viral S protein and to particular host surface receptor(s), promoting membrane fusion of the virus membrane with the host cell membrane [33]. The M protein also appears to be involved in virus antigenicity, as demonstrated by virus-

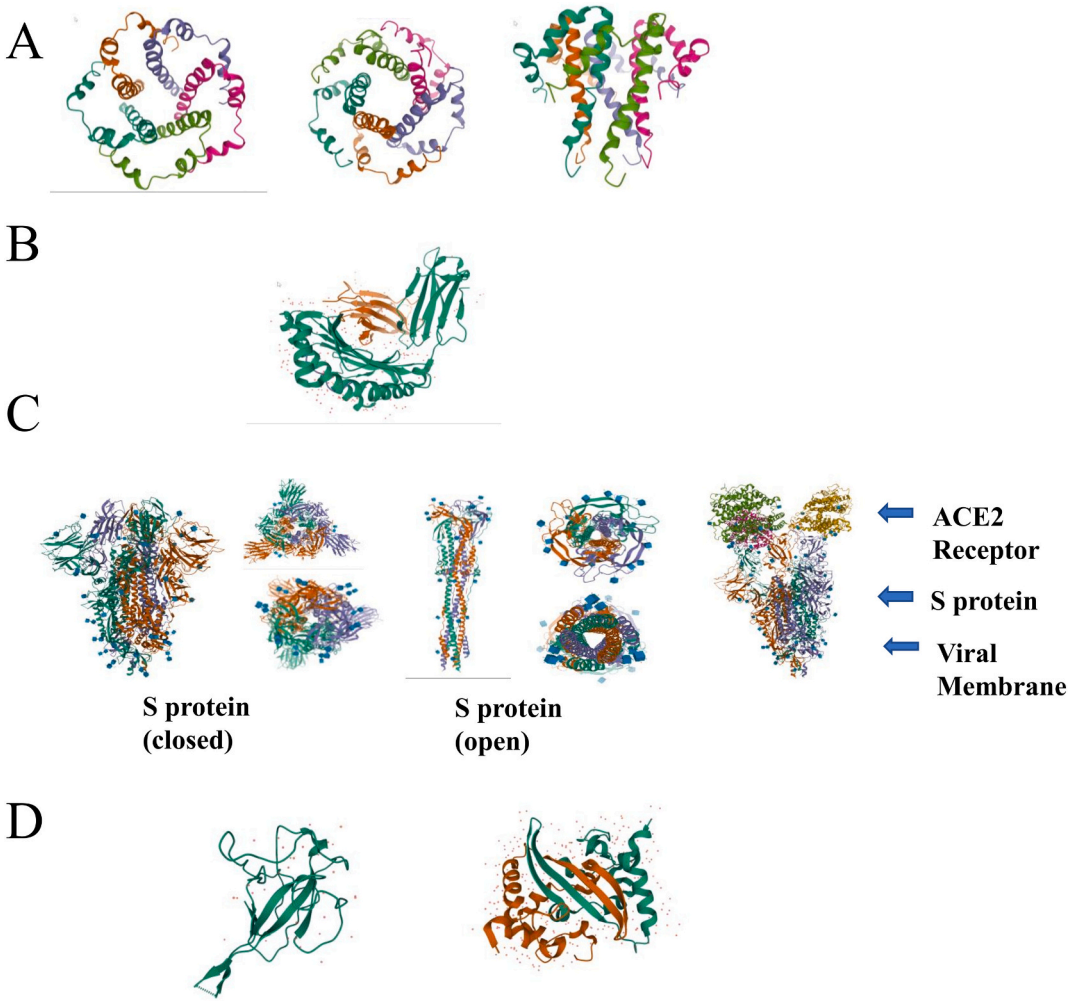

Fig. 2. Main structural proteins and host receptor proteins for SARS-CoV2, A, (A) Structure of the Envelope protein obtained from the Protein Data Bank (PDB) site from NIH https://www.rcsb.org/ligand/IB. View from outside the virus (*left*), inside (*middle*), lateral, the pore structure can be observed (*right*). (B) The M protein in contact with the histocompatibility complex HLA-A blood type. Peptide antigenic chains from HLA are in green. (C) Structure of the unbound Spike protein (*left*) and the ACE2-receptor bound Spike protein (*right*). (D) Structure of the N-protein attached to the M protein and viral RNA genome. All structures were obtained from PDB sequences from NIH.

induced immune responses of the host [34]. As the most abundant protein in the SARS-CoV-2 coronavirus, the M protein may also be one of the key components in viral assembly and morphogenesis, such as in the regulation of replication and the packaging of the virus RNA into viral particles [35]. It is quite interesting that there are several points of contact between the M protein and N protein with its bound RNA (Fig. 1) [36]. The M protein also inhibits NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation [37]. This is important because NF-κB is a protein complex found in almost all animal cell types that controls, in part, transcription of DNA, and indirectly it regulates cytokine production and cell survival. NF-κB is involved in several cellular responses to many stimuli, such as bacterial or viral antigens [38]. It can also induce apoptosis through activation of the Akt survival pathway [39]. Of particular interest, the M protein can bind to the human leukocyte antigen serotype within the HLA-A serotype group, which is a particular class I major histocompatibility complex (MHC) allele group at the HLA-A locus. It is not known if this could explain the more severe COVID-19 cases observed in patients with blood group type A. This interaction has been structurally determined as PDB ID 3I6K and G for SARS-CoV viruses (Fig. 2B) [40].

2.2.3. *S protein*

The S or spike protein is approximately 150 KDa in size and is the most extensively studied membrane protein of the SARS-CoV-2 virus. This heavily glycosylated transmembrane protein forms a homotrimeric structure with high conformational flexibility, protruding outward from the viral lipid bilayer [41,42]. The S-protein trimers facilitate the binding of the SARS-CoV-2 virus to host cells by its attachment to the ACE2 proteins expressed on lower respiratory tract epithelial cells, especially alveolar type II cells. ACE2 is a membrane-bound enzyme (carboxypeptidase) that contributes to the inactivation of angiotensin II, and therefore, it physiologically counters the effects of angiotensin II [43]. ACE2 receptors are also found in the upper esophagus on stratified epithelial and other cells, such as absorptive enterocytes in the ileum and colon, cholangiocytes, myocardial cells, kidney proximal tubule cells, and bladder urothelial cells. The amino acid-binding domain of the S protein of the SARS-CoV-2 virus are present at residues 331 to 524, and this amino acid sequence binds strongly to human and bat ACE2 [44].

After binding to host cells, the S glycoprotein is cleaved by a host cell enzyme, a furin-like protease that is found in abundant quantities in the lungs, into two subunits (S1 and S2). The cleavage takes place within a highly conserved sequence of the S protein (between amino acids R and S in the highly conserved sequence RRARSVAS). This amino acid sequence is also present in the epithelial sodium channel (ENac), which is highly expressed in the lungs and intestine and is essential in controlling the fluid-air interphase in order to avoid edema. It has been

found consistently that the expression of ENac is decreased by SARS coronavirus infections [45]. It is believed that the same enzymes (prohormone convertases or PKCs) that modulate ENac function in transepithelial transport could be the enzymes responsible for the cleavage of SARS-CoV-2 S proteins [46]. After cleavage, part S1 of the S protein appears to be responsible for determining the host-virus range and cellular tropism, whereas S2 functions to help mediate virus fusion with host cell membranes. The S protein cleavage products S1 and S2 are two distinct functional domains of the S protein, both of which are necessary for a coronavirus to successfully enter a human cell. A sequence of events occurs after coronavirus S protein sequence S1 attaches to a host epithelial cell membrane via the ACE2 receptor, and eventually the virus enters host cells where it replicates. Thus, the cleavage of the S glycoprotein into S1 and S2 fragments is important in the endocytosis of the coronavirus and entry into the endosomes of the host cell where it eventually fuses with cellular membranes [47–51].

The successful endocytosis of SARS-CoV viruses also requires a type II transmembrane serine protease, an enzyme that in humans is encoded by the gene TMPRSS2 (TMPRSS2). This process is known as priming of the S protein in human lung cells, which is an essential step for viral entry into cells [48]. Hence, the SARS-CoV-2 virions have to be activated for membrane fusion by TMPRSS2 and can thus be inhibited by TMPRSS2 inhibitors [48]. Similar to other SARS-CoV viruses, the SARS-CoV-2 virus uses the ACE2 receptor for entry and the serine protease TMPRSS2 for S protein priming to complete viral endocytosis. After virus fusion occurs, the TMPRSS2 located in the surface of host cells will degrade the ACE2 receptors and activate the receptor-attached spike-like S proteins [52,53]. This priming process of the S protein unmasks the fusion peptide S2 and activates a further membrane fusion within the endosomes [54,55]. Thus, S2 acts as a viral fusion peptide that is unmasked upon S protein cleavage by TMPRS22 and other proteases during endocytosis. The S protein can be further altered by proteolysis and activation by cathepsin CTSL inside endosomes. While inside host endosomes the S protein exhibits an anti-BST2 activity. BST2 is a protein related to innate immunity against viral activity, and thus CoVs can inhibit the antiviral activity of innate immunity [56]. The structure of the soluble, overexpressed S protein and its distribution in situ on the virion surface have been determined with high-resolution cryo-electron microscopy [13,48–50,57,58]. The structure of the S protein in its closed conformation (prefusion) is shown in Fig. 2C (PDB 6VXX) [50]. Upon binding of the S protein receptor-binding domain (RBD) to the cell ACE2 receptor, the S protein elongates, facilitating the contact between the virion and the host cell. These results in a post-fusion conformational change are shown in Fig. 2C (PDB 6M3W) [59]. The structure of the RBD bound to the ACE2 receptor has also been determined, and this is shown in Fig. 2C, (PDB 6M17, 7KNI) [60,61]. Because of its importance in viral-cell binding, the S protein has been used as an important target for vaccines and antiviral therapeutics [62].

2.2.4. N protein

The N protein or coronavirus nucleocapsid protein is a structural and multifunctional protein that forms complexes with genomic viral RNA. It interacts with the other viral membrane proteins during virus assembly. The N protein also plays a critical role in enhancing the efficiency of virus transcription and assembly. It helps package the positive strand viral genome RNA into a helical ribonucleocapsid. In addition, it plays a fundamental role during virus assembly through its interactions with coronavirus membrane protein M. The N protein also plays an important role in enhancing the efficiency of subgenomic viral RNA transcription as well as viral replication [63]. Amino acid sequence comparisons have shown that SARS-CoV N proteins have three distinct and highly conserved domains, including two structural and independently folded structural regions, namely the N terminal domain (NTD/domain 1) and the C-terminal domain (CTD/domain 3). These two domains are separated by an intrinsically disordered central region (RNA-binding domain/domain 2). All three N domains have been shown in different SARS-CoVs to bind with viral RNA [64].

The use of static light scattering, size exclusive chromatography, and small-angle X-ray scattering have shown that the purified N proteins are largely present as dimers in solution. The SARS-CoV N protein has a high percentage of disorder at room temperature, whereas it is more structured at 55 °C. Fluorescence polarization has shown that it has non-specific nucleic acid-binding capability, which raised a concern if it was to be chosen as a diagnostic marker [65]. Because the N protein is bound to RNA, this protein is involved in processes related to the viral genome, the viral replication cycle, and the cellular response of host cells to viral infections [28,44]. The N protein is also heavily phosphorylated, and this has been proposed to result in structural changes that enhance the affinity of N protein to viral RNA [66,67]. Finally the N protein may modulate transforming growth factor-β signaling by binding the host receptor SMAD3, thereby blocking the apoptosis of infected cells [68]. The crystal structure of the N-terminal region of the N protein has been obtained (PDB 6M3M, green, Fig. 2D, *left panel*) [69] as well as the crystal structure of the N protein P1 dimerization domain (PDB 6WZO, Fig. 2D, *right panel*).

2.2.5. Viral RNA

The RNAs of CoVs are enveloped, single-stranded, positive-sense RNAs with genome sizes ranging between 26.2 and 31.7 Kb. Coronavirus genomes are among the largest genomes of RNA viruses [70]. The large, capped and polyadenylated genomes of coronaviruses contain seven common genes in the following conserved order: 5′-ORF1a-ORF1b-S-ORF3-E-M-N-3′ [71]. The open reading frames (ORF) 1a/b encompasses two-thirds of the coronavirus genomes and collectively produce a genome-length mRNA (mRNA1) that encodes two overlapping viral replicase proteins called polyproteins 1a (pp1a) and 1b (pp1b) [72]. The S, M, N and E genes encode the major structural proteins discussed in Sections 2.2.1. to 2.2.4, above. The polyproteins pp1a and pp1b are read with a ribosomal frame shift that results in a complex pseudoknot RNA structure [73]. This RNA structure is translated and then proteolytically processed by virally encoded proteases into mature nonstructural proteins (nsp1 to nsp16), which in turn assemble to form a membrane-associated viral replicase-transcriptase complex (RTC) [72,74,75]. The remaining one-third of the coronavirus genome yields subgenomic (sg) mRNAs that encode the four structural proteins discussed above (S, E, M, and N proteins), as well as a number of accessory proteins [76,77]. A scheme of the SARS-CoV-2 RNA reference sequences with the different regions specified above, according to NIH, is shown in Fig. 3.

2.3. The life cycle of SARS-CoV-2

Inside host cells the duplication of the SARS-CoV-2 virus involves non-structural proteins (nsps). Once the virus enters a host cell, it releases its genomic material into the cytoplasm to begin its replication cycle, as summarized in Fig. 4. The coronavirus RNA is then transferred to the nucleus for duplication, and the viral mRNA ready for translation is bound by the cell's ribosomes and translated into viral proteins. The genomic material released by coronaviruses contain 14 ORF, each of which encodes a variety of structural and nsps proteins that play roles in cell binding and entry, replication, survival and virulence. The gene segments that encode the nonstructural SARS-CoV-2 polyproteins are translated first into ORF1*a* and ORF1*b* to produce two large overlapping polyproteins, pp1a and pp1ab [70].

The SARS-CoV-2 polyproteins require processing by viral proteases, namely a papain-like protease (PLpro) and a serine-type chymotrypsin-like protease (3CLpro) that are encoded by nsp3 and nsp5, respectively. Subsequently, pp1a and pp1ab are cleaved into nsps proteins 1–11 and 1–16, respectively. The nsps play important roles in several host cell and viral processes, such as the inhibition of the host immune responses that promote both cellular degradation and inhibition of RNA translation (nsp1 and 3 and nsp 16), promotion of cytokine expression, cleavage of viral polyprotein (nsp 3 and 5), processing of RNA polymerase (complex

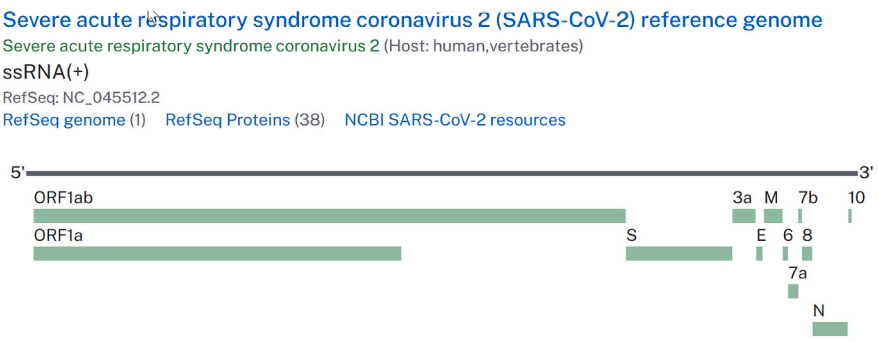

Fig. 3. The genome of SARS-CoV-2 with its structural and non-structural codifying proteins and their corresponding open reading frames. The open reading frames (ORF) encoded proteins are critical for viral replication. Data obtained from analysis of NIH public repositories https://www.ncbi.nlm.nih.gov/nuccore/1798174254. The RefSeq NC_045512.2 is the first original genome reported from SARS-CoV-2 [422]. It is a single-stranded RNA (+). Near the 5the Open Reading Frames 1ab and 1a are located. ORF1ab is the largest gene. It encodes polyproteins named PP1ab and PP1a. When cleaved, they become 16 different non-structural proteins. The regions encoding proteins S, M, E and N are shown. ORF3a encodes viroporin3a, important for inflammasome NLRP3 activation [423]. The rest of the RNA ORF indicated in the figure, encode proteins whose functions are being characterized.

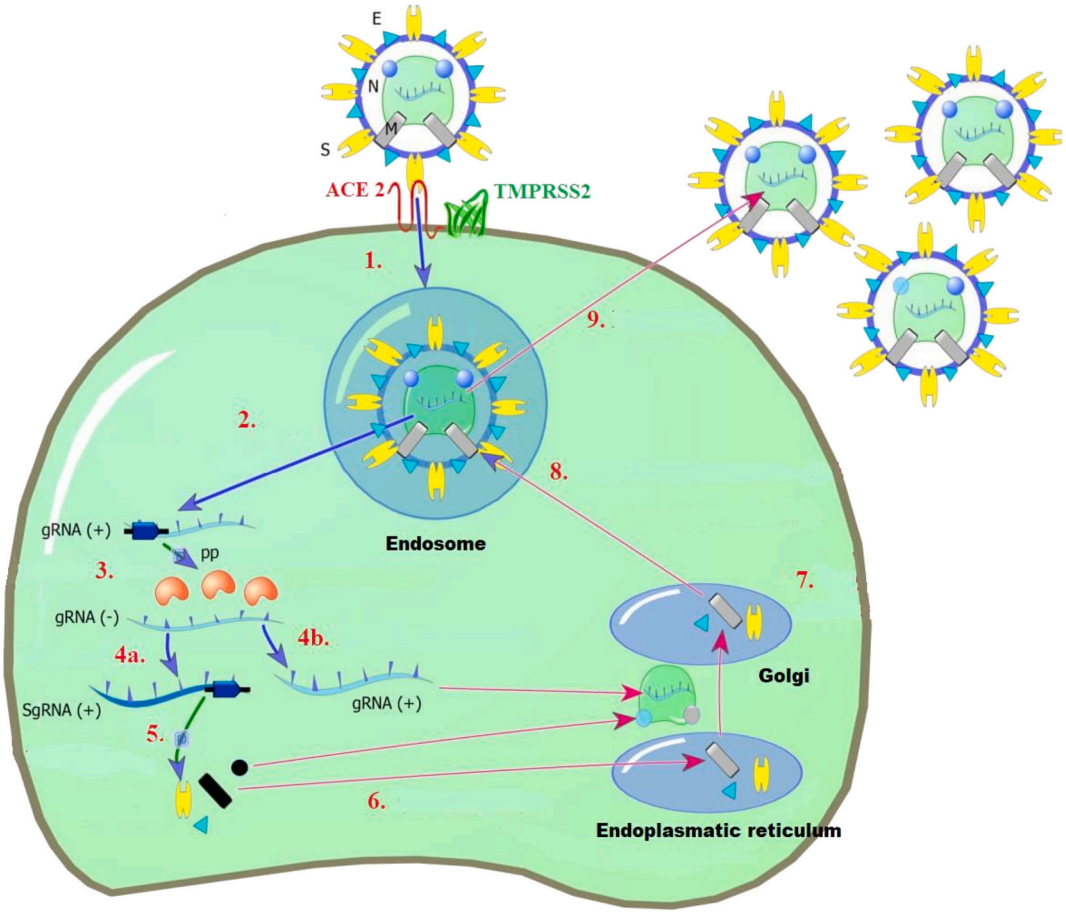

Fig. 4. The invasion and replication of SARS-CoV-2 in host cells. Invasion and replication of SARS-CoV-2 require several steps: (*1*) Viral Entry. The ACE2 (red) and TMPRSS2 (green) membrane receptors from the host cells, are critical for binding of the cleaved S protein of the virus (in yellow). (*2*) Release of viral genome. Once the virus is inside the endosomes from host cells, the RNA is released to the cytoplasm. (*3*) Translation of polyproteins and polymerases from the virus to yield a replication complex. (*4*) Transcription and replication of the viral RNA. Once the replication complex is formed, the viral genome can replicate. (*5*) Translation. Proteins from the viral genome are continuously being translated treating a positive feedback mechanism to increase the proto-viral load of the host cells. (*6*) Translocation. The main viral proteins like S, M and E proteins are translocated inside the ER. (*7*) Ensemble of new viral particles. The replicated nucleocapsid-RNA complexes are then surrounded by Golgi and endoplasmic reticulum membrane with the translocated proteins to ensemble new viral particles. (*8*) Mature virion particles are formed and ready to be secreted outside the invaded host cell by exocytosis. (*9*) Exocytosis. The mature viral particles are finally expelled from the host cells to repeat this cycle in other cells. Figure made by Axel Santander, Tinkercell.

of nsp7/8), binding of protein phosphatases (nsp 9) to RNA, stimulation of RNA-dependent RNA polymerase for viral RNA replication, RNA helicase (nsp12 and 13), proofreading of the transcript viral genome (nsp 14), and finally viral endoribonuclease and protease activation (nsp 15) [76,78,79].

Many of the nsps proteins subsequently form RTCs that are moved inside double-membrane vesicles (DMVs). These DMV vesicles are mainly used for viral complex assembly by RNA-dependent RNA polymerase (RdRp)- and helicase-containing subunits, such as the canonical RdRp domain of CoV nsp 12 and nsp 9. This complex transcribes an

endogenous genome template of viral negative-sense genes of both the progeny genome and subgenomic RNA, as intermediate products, followed by transcription to positive-sense mRNAs that are mainly mediated by RdRp [78,80,81]. Finally, the structural proteins (S, M, E and N) and some accessory proteins are subsequently translated and sequestered in the endoplasmic reticulum where they can be moved to the endoplasmic reticulum-Golgi intermediate compartment. Simultaneously, the previously replicated RNA viral genome joins with the N protein to form the nucleocapsids, which also move into the endoplasmic-reticulum-Golgi intermediate compartment. In this intermediate compartment, the nucleocapsids interact with other viral structural proteins to build the so-called small-wallet-vesicles, which will be then be transported outside of the host cells through exocytosis [76,80] (see Fig. 4). It is interesting that ORF3a encodes for a calcium-selective channel or viro-porin that could play a role in membrane exocytosis, thus promoting the release of virus from host cells [82]. All the protein components of the SARS-CoV-2 virus, and especially those components involved in its binding to host cells, are potential targets for vaccine development or antiviral treatments [83].

2.4. Mutations and genomic variants of SARS-CoV-2

During the replication of viruses inside host cells, errors in the viral genome can induce changes in the encoding regions for the virion proteins. The repair mechanisms for RNA replication have more intrinsic errors than the DNA repair mechanisms inside the nucleus, so mutations in RNA viruses can be a million times more frequent than found in their hosts [84–86]. A typical SARS-CoV-2 virus inside humans accumulates only two single-letter mutations per month in its genome—a rate of change about one-half that of influenza and one-quarter that of HIV-1 [87]. SARS-CoV-2 has an RNA proofreading mechanism that keeps the mutation rate low in comparison with other viruses. Since its appearance at the end of 2019, many unique strains of SARS-Cov-2 have been detected in different countries [88,89]. There are also some hot-spot locations that show increased rates of mutations [86,90]. Sometimes these changes have resulted in an increase in the adaptation of the virus to the host that could facilitate the infectivity of the different viral strains. This turns out to be very important in locating different virion targets for vaccines and antivirals [90].

All of the different proteins from SARS-CoV-2 can experience mutations, and differing rates of mutations for different strains can vary in different locations in the world [86]. As of Dec 2020, the GISAID (Global Science Initiative and Primary Source providing open access to genomic data of pandemic pathogens [91]) has identified seven clades (O, S, L V, G, GH, GR) or different evolutionary groups of the human SARS-CoV-2 virus where mutations have accumulated one after another. Another open-source site for sequences of SARS-CoV-2 genomes, NEXTSTRAIN, has identified at least five clades with slightly different criteria (19A, 19B, 20A, 20B, and 20C). Regarding the main mutations that can result in a new clade, Guan et al. [91] identified five clades representing those mutations (G_{614}, S_{84}, V_{251}, I_{378}, and D_{392}) [89,91–93] (Fig. 5). Those mutations that affect the S protein are particularly relevant for virus infectivity, prevention, and therapeutics and have been found mostly in Europe [86,94,95]. As early as May 2020, a mutation in the cleavage site between S protein domains named D614G was identified in SARS-CoV-2, and this mutation has been associated with higher infectivities of the resulting mutated viruses, although the picture remains incomplete [87,96,97]. It is estimated that since the end of May 2020, most of the circulating SARS-CoV-2 virions within Europe had changed from D614 to G614 [97]. The UK public health services in Great Britain have

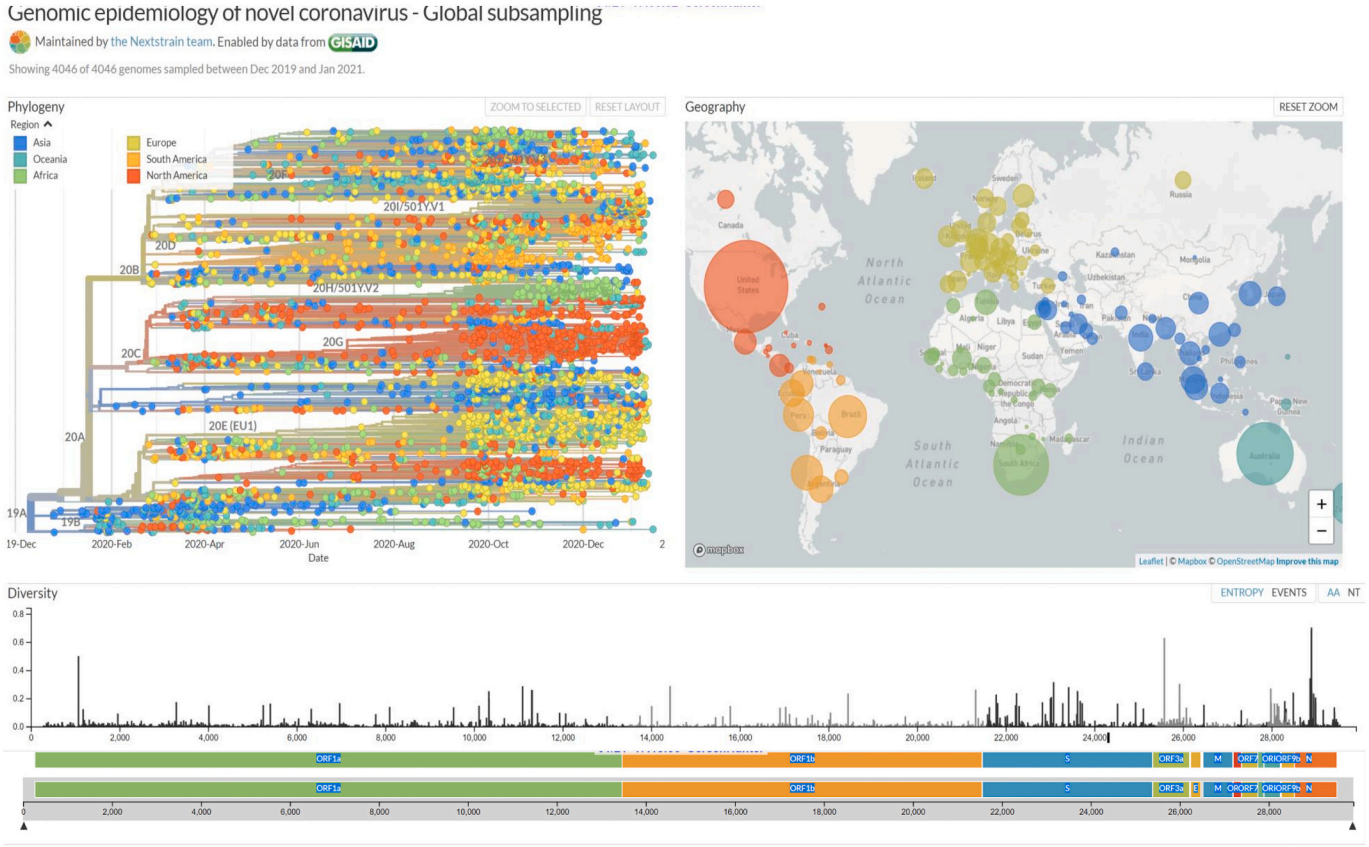

Fig. 5. Different strains of SARS-CoV-2. The image was obtained by the GISAID initiative and NEXTSTRAIN site (https://nextstrain.org). Each color in the *left panel* represents the strains predominantly found in each region of the world as shown in the *right panel*. The diversity found in different spots along the SARS-CoV-2 genome is shown below in the plot of diversity versus base position of SARS-CoV-2. The corresponding proteins of the virus are shown below. Note the intense variability of nucleotides in the region corresponding to the S protein.

initiated a new identification system of mutations, taking into account variants of SARS-CoV-2 of concern and also those that require further investigation [98]. These new variants are of concern because of their possible increased infectivity, lethality, and also their potential evasion of immunization strategies.

An important new strain of SARS CoV-2 was identified in December 2020 in Great Britain [99]. This emergent lineage has been termed B.1.1.7 or 202012/01 (Public Health England Report) [100]. What makes this variant especially intriguing and worrisome is that from the 17 mutations that it carries, at least 8 are in critical regions of the S protein [101]. British scientists estimate this new variant is 50% to 74% more transmissible, and it is already replacing most of the strains of SARS-CoV-2 that are in circulation in the UK [101]. This new strain could potentially interfere with the vaccination programs already under development in several countries. The patients with more severe illness usually have a higher SARS-CoV-2 mutation rate than those with mild disease, and it is thus thought that this lineage may have evolved from a patient with a severe illness [101]. This strain is among the variants of concern that have mutations located in the S protein. Other important mutant strains accumulating multiple mutations are P681H and N501Y, with mutations located in the receptor-binding-domain, and the deletion 69/70, among others, located in the S protein-encoding region [94,101,102].

Another example is mutation N501Y, which occurs in one of the few contact residues within the receptor-binding domain (RBD). This mutation increases the binding affinity of S protein to human ACE2 receptors. The mutation P681H is quite close to the furin cleavage site. Finally, the spike protein deletion 69-70del occurs in a region critical to evading human immune responses [101]. Another complication has been the finding of increasing rates of mutations per month–increasing from 2 to 12 per month [103,104]. This suggests that a new lineage has evolved that includes one or more mutations at replication sites in the viral genome, promoting additional errors during the life cycle of the virus. Though more information is needed, most of the variants reported so far remain sensitive to most of the available vaccines, because they were designed with several epitope regions in mind, mostly in the S protein, though some regions that might escape antibody recognition have also been detected [105,106]. Mutations in SARS-CoV-2 are an important issue that can develop rather quickly genomically and geographically [89,104,107]. Thus, new variants that are currently under study have emerged in Brazil (gamma, P.1 or B.1.1.248 and P2, descendants from B.1.1.28, with notable mutations N501Y, E484K) and South Africa (B.1.351, with notable mutations the same as Brazil strains but adding K417N) in January 2021. The more recent variants of concern that could affect the effectivity of the vaccines and/or increase the rate of infection are the so-called delta variant from India (B1.617.2), with mutations in the spike protein like D614G, T478K, P681R and L452R [108], and the lambda variant from southern Brazil and Peru (C.37), with mutations in the ORF1a gene: Δ3675–3677; Spike gene: Δ246–252, G75V, T76I, L452Q, F490S, D614G, and T859N [109]. It is inevitable that many other mutants will appear in the future as this virus continues to adapt and evolve. These new variants have the potential to reinfect a patient with COVID-19 disease, even when they were infected previously with another viral strain. It is not just about developing new vaccines and antivirals but also about molecular epidemiology, surveillance and viral evolution. Until we know more about this emergent virus and the disease that it causes, we will be trying to keep pace with an increasing number of new mutant strains [95].

2.5. Clinical symptoms, diagnostic tests, treatment, and vaccines related to SARS-CoV-2 in adults

2.5.1. Symptoms of COVID-19

COVID-19 disease is a multisystemic disease, and as such its symptoms are mostly nonspecific and comprise several organs, tissues and functions of the body. The disease can vary from asymptomatic to severe presentations leading to death. In the COVID-19 presentations that are apparent, conditions can range from mild, self-limiting flu-like illnesses to more moderate illnesses with fever, cough and other symptoms, to life-threatening diseases characterized by severe symptoms of pneumonia with ARDS, multi-organ failure and a high mortality rate. Early prodromal symptoms can include hyposmia, anosmia and dysguesia as well as fever, sore throat, cough, chest tightness, shortness of breath, headaches, myalgias, gastrointestinal (nausea, vomiting, pain and diarrhea) and other symptoms. Highly progressed patients that have severe, life-threatening disease usually suffer ARDS, excessive inflammation, coagulation and cytokine problems, metabolic acidosis, sepsis and septic shock, and/or multi-organ failure [11,12,110].

The overall mortality rate of COVID-19 is around 3% [111]. The rate of ICU admission is about 5%, and the rate of hospital admission is about 20% [112]. These values can vary among different populations, geographical regions and the types of healthcare systems. The severity of the disease is related to the age of patients and the presence or absence of comorbidities. It is more severe in men than in women. Blood type has also been found to be relevant. Blood type A seems to have the worst prognosis, and O the best prognosis, between the A, B, O types [113,114]. The lethality of COVID-19 increases dramatically with the age of affected individuals.

COVID-19 symptoms usually appear 2 to 3 days after exposure to an infected individual. The most common mode of transmission is through air droplets, and this implies the utility of some common measures that the WHO has recommended for preventing the spread of this disease, such as physical distance among people, use of protective masks, especially in closed spaces, washing hands thoroughly, and disinfection of places occupied by COVID-19-positive patients [115]. The disease course usually lasts about 14 days, and patients can infect other uninfected individuals, even in pre-symptomatic stages. The more severe the presentation of the disease, the longer it can take to recover, and patients can thus infect more people. The measurement of the infectivity (Ro) of SARS-CoV-2 varies between 2.4 and 3.1 or more, if no prevention abatement procedures are undertaken [116]. In the acute phase of the disease, patients can have different chronological appearances of symptoms. First to appear, as the virus invades components of the upper respiratory tract are anosmia and dysguesia, or a loss of sense of smell and taste. The loss of smell seems to involve non-neuronal components for the sense of olfaction [117]. Neurons do not have ACE2 receptors, but this receptor is expressed on cells that are essential for support of the olfactory neurons, and also stem cells from blood vessels [117]. These early symptoms have been reported in as much as 90% of SARS-CoV-2-infected, COVID-19-positive patients [110,118]. These symptoms seem to be more common in mild or nearly asymptomatic cases [119].

Markov models have been applied to thousands of patients to predict the order of appearance of the following symptoms: fever, cough, sore throat, headache, myalgia with nausea and vomiting [120]. Other sets of common nonspecific symptoms that may appear in any order and can be superimposed on other symptoms, such as fatigue, breathlessness, joint and chest pain, vision problems, lack of appetite, among others [121]. It is interesting that several of the later symptoms can persist for longer periods after the acute phase of the infection and last several weeks or even months after onset of the illness [121].

2.5.2. Testing for COVID-19

Testing and tracing are an invaluable tool in the SARS-CoV-2 pandemic, although several considerations have to be taken into account when interpreting test results [122–124]. First, validation or verification should ensure that the testing platforms operate well and that tests are promptly conducted. Laboratories must define assay detection limits, and their staff should recognize how disease prevalence can alter the predictive results. Rapid communication of test results following national reporting procedures is important for planning and design of public health and outbreak control interventions. The interaction between public health experts, clinicians and local laboratory

experts to discuss strategies, potential problems and solutions, is an essential part of an adequate COVID-19 response.

Up to December 2020 the virological assays for detecting SARS-CoV-2 routinely used oral or nasal samples to detect either the SARS-CoV-2 nucleic acids through a nucleic acid amplification technique, such as the Polymerase Chain Reaction (PCR), or the viral antigens using specific immunological reagents. Infected individuals may test positive for viral nucleic acids or proteins without expressing symptoms (asymptomatic), before symptom onset (pre-symptomatic), and after symptoms have occurred (symptomatic) and patients are experiencing the disease [125–131]. As for any virus, SARS-CoV-2 will continue to acquire genetic changes/mutations over time, and this requires constant updating of testing materials and procedures which can reduce the risk of false-negative results [132–135]. The sensitivity of different antigen tests performed using immunological reagents compared to more sensitive nucleic acid detection using RT-PCR in specimens from nasopharyngeal swabs appears to be highly variable [136–138]. More false positives can occur with the viral antigen detection assays, because the antibodies used sometimes recognize other viral antigens from different CoVs than from the SARS-CoV-2.

Antibody tests can also have different uses. For example, after infection with SARS-CoV-2 there is usually an increase in specific antibodies against the virus that can last for several months and is thought to be an important potential source of anti-viral immunity. Vaccines take advantage of this response, and they elevate the number of specific antibodies against the virus [139–141]. The results obtained from serologic assays vary widely in patients with mild versus moderate-to-severe disease and in young versus older patients, and other variables, such as the timing of testing and the target viral protein.

Finally, co-infections of SARS-CoV-2 with other pathogens have been reported; therefore, a positive test for another pathogen does not rule out COVID-19 and vice versa [142,143]. Recent investigations have suggested the presence of coinfections in patients with SARS and MERS, including co-pathogens such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Chlamydia pneumonia*, among others [144]. Co-infections together with possible activation of latent bacterial and viral infections might be a strong determinant of a fatal disease course. For example, *Mycoplasma* species have been found in COVID disease, and the severity of signs and symptoms could be related to this additional pathogen and/or other infections, increasing the probability of cytokine storm, hemodynamic dysfunction, autoimmune activation, and other major clinical problems seen in cases of COVID-19 [143].

2.5.3. Treatment of COVID-19

Since the appearance of COVID-19, there have been several procedures that have been employed to treat the virus directly as well as some to treat the symptoms and complications that accompany the virus. In addition, there have been efforts to reduce the severity of symptoms and provide adequate patient support. This is especially important in patients that progress to express very severe symptoms requiring ICU support [145,146]. Finally, procedures have been developed to treat possible co-infections that together with SARS-CoV-2 make the condition more severe and life-threatening [143]. Most of the patients that progress to a severe form of the disease require ICU support, and these are usually older patients that also have underlying health conditions, such as hypertension, diabetes, obesity, cardiovascular and respiratory disease (asthma, emphysema), hemorrhagic or ischemic strokes, immunosuppression, cancer, chronic kidney or liver disease and secondary infections [110,146,147].

In the treatment of SARS-CoV-2 virus infections a variety of anti-inflammatory and anti-microbial agents have been investigated to determine if they have therapeutic potential. Among the first examined were hydroxychloroquine (HCQ) and chloroquine (CQ) (often in combination with azithromycin), and this was followed by the antivirals Remdesivir, Lopinavir, and other anti-proliferative agents. HCQ and CQ are more well-known as anti-malarial agents, but they have been used against viral infections, and previously they were found to inhibit the proliferation of SARS viruses in cell cultures [148,149].

Pilot studies revealed that CQ and HCQ could, in fact, inhibit viral infections in patients [150,151]. The action of CQ and HCQ during SARS infections may revolve around their abilities to increase endosome internal pH and prevent Toll-like receptor activation [152] as well as their interference with the glycosylation of ACE2 receptors, causing reductions in binding efficiency between the SARS virus S protein and ACE2 receptors [153]. By increasing the pH inside endosomes and lysosomes where SARS viruses enter cells by the process of endocytosis, the fusion of the virus membrane with the endosome membrane could be prevented or reduced, and this potentially blocks subsequent entry of viral RNA into the cell cytoplasm where replication of the virus occurs [153]. Since CQ and HCQ also have some immunomodulatory properties, such as preventing Toll-like receptor activation and antigen presentation, these drugs may also interfere with host immune activation [154]. The immunomodulatory properties of CQ and HCQ are thought to be important in reducing the over-activity of host responses that seem to worsen COVID-19 symptoms, such as excess production and release of inflammatory cytokines [155]. These immunomodulatory properties were considered important in the treatment of rheumatic diseases with CQ and HCQ [156]. In COVID-19 patient studies in France the use of HCQ suggested there was some benefit. For example, treatment with HCQ reduced viral carriage in nasopharyngeal swabs on the 6th day after starting treatment [157]. In this preliminary study on 20 patients who were treated with HCQ, 14 had negative swabs at day 6 compared to 2 of 16 controls. An analysis of the effects of CQ and HCQ in patients in 3 of 4 randomized clinical trials reported favorable treatment effects, such as duration of cough and fever, viral shedding, recovery, death [158]. However, follow-on studies in 25 different hospitals using 1438 COVID-19 patients indicated that there were no significant differences in mortality for patients taking HCQ (±azithromycin) compared to azithromycin alone [145]. In a subsequent systematic review and analysis of 37 clinical studies involving 45,913 patients there was no significant difference in treatment efficacy between HCQ with azithromycin and control groups [159]. It is interesting to note that the use of azithromycin, an antibiotic, has no role in the treatment of viral infections, but it could be important in suppressing co-infections, such as mycoplasmas, that have been found in COVID-19 patients with severe symptomatology [143]. In addition to CQ and HCQ, colchicine, another drug with anti-inflammatory properties has been used to treat COVID-19, [160], especially in those patients where COVID-19 affects the heart [161] or in those where prevention of COVID-19 complications are needed [162]. The results, however, remain controversial and are under further study [163].

Certain antivirals have proved useful in treating COVID-19 patients and reducing viral load. One of the approved antivirals is Remdesivir, which selectively inhibits viral RNA-dependent RNA polymerase and has broad-spectrum antiviral activity against several viruses [145]. This antiviral drug was found to be a potent inhibitor of SARS-CoV-2 replication in human nasal and bronchial airway epithelial cells [164]. In a randomized, double-blinded, placebo-controlled, multi-centered trial Remdesivir did not significantly improve the time to clinical improvement or time to clearance of SARS-CoV-2 in severe cases of COVID-19 compared to placebo, but it did cause reductions in viral load in bronchoalveolar lavage and pulmonary infiltrates [145]. Other trials did, however, find some benefits over placebo and faster recoveries without seeing significant differences in mortality [145]. In one randomized, placebo-controlled trial containing 237 patients in China, patients with symptom durations of 10 days or less that received Remdesivir showed faster times to clinical improvement than those receiving placebo, although these differences did not reach statistical significance [165]. In another trial 584 patients with moderate COVID-19 were randomized to a 5-day course of Remdesivir and had statistically significant better outcomes compared with standard care, but those randomized to a 10-day course of treatment were not statistically significantly different

from controls [166]. Another example was the double-blind, randomized, placebo-controlled trial using 1062 patients who received a 200 mg loading dose of Remdesivir on day 1, followed by 100 mg daily for 9 additional days or placebo [167]. Patients who received Remdesivir were found to be more likely than those who received placebo to show clinical improvement at day 15 (odds ratio = 1.5). Estimates of mortality were 6.7% with Remdesivir and 11.9% with placebo by day 15, and 11.4% with Remdesivir and 15.2% with placebo by day 29 [167]. Other antivirals, such as Favipiravir, Lopinavir, Ribavirin and Arbidol, have been proposed as possible new antivirals to treat COVID-19 but have not been fully evaluated [168].

Another drug that has been under consideration for the treatment of COVID-19 is ivermectin [169]. Although this drug has been used as a broad-spectrum anti-parasitic agent with antiviral activities, its clinical use in COVID-19 patients has been limited due to formulation challenges [169]. Ivermectin has been shown to have efficacy against SARS-CoV-2 virus in vitro, but standard doses used clinically did not show differences between drug and control COVID-19 patients [15,170].

Specific monoclonal antibodies against the SARS-CoV-2 S protein have been approved for use in treatment of COVID-19. One such therapeutic, Bamlanivimab, is a neutralizing monoclonal antibody (LY-CoV555) that targets the receptor-binding domain of the SARS-CoV-2 S protein. A Phase 2, randomized, placebo-controlled, multi-centered trial of Bamlanivimab suggested a potential benefit for outpatients with mild to moderate COVID-19; however, the study had a relatively small number of participants in each dosage arm (3 different dose levels were used) of the study. The authors reported more than a 3-log decrease in viral RNA at day 11 after receiving a single dose of the monoclonal antibody and slightly lower symptom severity scores, and the infusions lowered the subsequent hospitalization rates from 6.3% to 1.6% [171]. Another approach has been to use a cocktail of neutralizing monoclonal antibodies, such as REGN-CoV2 [172]. This mix of monoclonal antibodies reduced viral load, with a greater effect in patients whose immune response had not yet been initiated against SARS-CoV-2 or who had a high viral load at baseline [172].

Non-specific therapeutic approaches have also been used in SARS-CoV-2 infections. In SARS infections where no specific therapies were available, convalescent plasma has been used, and COVID-19 is no different [168]. Patients who develop humoral immunity to the SARS-CoV-2 virus and recover from COVID-19 are potential plasma donors for other COVID-19 patients. The protective and therapeutic benefits of convalescent plasma against COVID-19 are being evaluated, and this methodology is being used on an emergency basis for patients who have a severe case of COVID-19 with rapid progression [173].

In severe cases of COVID-19 supportive care and suppression of secondary events, such as inflammation and coagulatory processes, are essential for improving survival [174]. Among the suggested supportive care approaches are oxygen (with ventilation), hydration and dielectric balance, anti-inflammatory treatments and controlling cytokine storms, anti-coagulation, nutritional interventions, along with other supportive care approaches, are collectively important [145,146]. Since COVID-19 is a systemic multi-organ viral disease that is frequently complicated by severe host reactions, a combination of interventions is often necessary, especially in the severe cases [175].

As described above, the severity of ARDS and other COVID-19 pathologies are usually linked to excessive inflammation and oxidative stress, so important treatments that are directed at these processes are especially critical in severe ICU COVID-19 cases [176]. Indeed, the severity of COVID-19 appears not to be directly related to viral load, but it is instead related to inflammatory processes associated with cytokine storm and an increase in pro-inflammatory cytokines and chemokines [15,147]. This can cause a hyper-inflammatory syndrome in COVID-19 patients that is characterized by a fulminant and fatal hypercytokinemia, ARDS, and progression to mutli-organ failure (kidney, liver, heart) [15]. Effective treatment of severe COVID-19 cases usually requires reduction of inflammation and control of thrombosis [147]. For example, dexamethasone has been used in hospitalized COVID-19 patients to reduce inflammation. In a randomized, open label trial 2104 patients received oral or IV dexamethasone for 10 days, and mortality was determined at day 28. Fewer patients died in the dexamethasone group (22.9% compared to 25.7% in the control group) [177], although there was no benefit for patients who were not receiving respiratory support and could be harmful in some patients [177,178].

A common finding in patients who die from COVID-19 is widespread thrombosis with severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes [178,179]. Histologic analyses of pulmonary vessels in these patients revealed extensive alveolar capillary fibrin microthrombi and evidence of angiogenesis as well as diffuse intravascular coagulation and large vessel thrombosis, which are often linked to multi-system organ failure [178]. For example, the use of heparin to control coagulation appeared to be useful for severe COVID-19 presentations [180]. Thus, anti-coagulation treatments have been used to improve survival of COVID-19 patients [146,181]. Several alternative support supplements have also been proposed for COVID-19 patients [146,181], including vitamins C and D, glutathione, alpha-lipoic acid, phospholipids and a variety of plant extracts [146,181–186].

2.5.4. Prevention of COVID-19

The most widespread method of infectious disease prevention is mass vaccination in order to quickly acquire herd immunity (HI) in the vaccinated population. Vaccines against COVID-19 have been developed at a pace never done before in the history of mankind. Once SARS-CoV-2 was identified and sequenced in January 2020, the first mass vaccinations of a susceptible population were achieved under a national health plan starting in January 2021 [187]. The alternative to mass vaccination depends on HI, which is the resistance to the spread of an infectious disease within a population based on a high proportion of individuals having pre-existing immunity achieved as a result of previous natural infections (or previous vaccination campaigns). The HI against SARS-CoV-2 via allowing natural infections has not proved successful based on rates of hospitalization and mortality, for example in Sweden [188]. Hence, HI is most likely obtained using vaccines. The percentage of individuals in a population needed to obtain HI varies with the reproduction number, Ro, that indicates the average number of people who will contract a contagious disease from one person with that disease. The Ro for SARS-Cov-2 is estimated at 2 to 3 [189]. The efficacy of a vaccine to immunize a population, the speed of a vaccination campaign and other factors also influence the population percentage needed for HI. The percentage calculated with these variables usually ranges between 60 and 90% of the population, and the time to achieve HI can vary from 1 to 2 years, depending on the size of the population and vaccination rate [190]. The different vaccines that have been used against SARS-CoV-2 make use of the injection of mRNA to produce antigenic viral proteins, viral vectors carrying non-infectious viruses with CoV-2 antigens, viral subunits, inactivated virus and also a nasal spray of live attenuated viruses [190]. Several of these vaccines have proved effective in preventing or limiting the disease, reducing hospitalizations and preventing mortalities and are currently being introduced into various populations around the world. At the time that this contribution was being prepared, it was still too soon to conclude which approach to vaccination against SARS-Cov-2 was the most successful.

2.6. Effects of SARS-CoV-2 in human reproduction after conception

2.6.1. Offspring

ACE2 receptors and TMPRSS2 proteases are found in human oocytes, zygotes and embryos, showing the potential from early stages in conception to be infected by SARS-CoV-2 [191]. In cells collected from biopsies from human embryos in the blastocysts-stage, it has been reported the SARS-CoV-2 infection of cultured embryos by detection of the expression of the spike glycoprotein fused with green-fluorescent-protein the embryos collected [192,193]. Those receptors needed for

viral infection into these cells are present in the female reproductive tract, the embryo since early stages, and the maternal-fetal interface [194]. However, though the potential entry exists, a systematic review of pregnant women in early stages concludes that the levels of expression may be too low at the interphase to account for vertical transmission of SARS-CoV-2 at these stages [195]. Neither there is evidence of vertical transmission at advanced stages of pregnancy, except for some reports showing transplacental passage of SARS-CoV-2 and infection of the offspring showing neurological symptoms [196,197]. Though further research is needed, to date there is no evidence that SARS-CoV-2 could impact negatively human reproduction in its early and advanced stages [198,199]. Nevertheless, although the molecular evolution and mutation rate of SARS-CoV-2 seems to be less than for influenza or SARS-CoV-1, special attention is needed as in other emergent diseases such as Zika Virus, a single mutation was enough to increase the Zika Virus neurotropism and increase the incidence of microcephaly in the Brazil outbreaks in 2015 [200,201]. Though there is no evidence to support frequent vertical transmission and teratogenic effects in the unborn child from infected patients, due to the increased risk of respiratory viral infections during pregnancy [202,203], the continuous molecular evolution of variants of SARS-CoV-2 [204], and the adverse outcomes of pregnancy such as preterm birth, preeclampsia, intrauterine growth restriction [205–207], there is a consensus in the scientific community that SARS-CoV-2 infection during pregnancy is worthy of special attention and further studies [208–210].

Regarding neonates from infected mothers, most of them are not infected with SARS-CoV-2 if precautions such as avoiding aspirations from the birth canal during delivery are taken [211]. In Sweden it has been found a mildly higher rate of neonatal respiratory dysfunction (3% versus 2%), but not mortality rates (0.3–0.12%) [212]. Mothers having COVID-19 does not necessarily require cesarean surgery for delivery [213]. It is even recommended the management of the neonate that is not infected, to be next to the mother with breastfeeding, though sharing the bed is not recommended [213]. In those newborns that are infected, and test positive for a PCR reaction diagnostic test, when they are symptomatic it is common to observe lymphopenia, anemia (low hemoglobin levels) and low albumin levels, accompanied by liver and renal dysfunction [214,215]. In these cases, or even if they present suspected symptoms, it is recommended to transport them in a dedicated isolated incubator to quarantine in the neonatal intensive care unit (NICU), for at least 14 days taking care of isolating their infectiveness through contention of their droplets [214]. At the NICU they need to be hemodynamically and respiratory monitored and stabilized. The only drugs that are being studied for using in those cases where the disease get severe are corticosteroids and tocilizumab to prevent cytokine storms [214]. If the neonates test positive for COVID-19, but are asymptomatic, they can spend their quarantine time at home [214]. Psychological support of the families and healthcare workers is recommended in these situations [214].

2.6.2. Placenta

It has been reported that the placenta from symptomatic COVID-19 positive mothers gets infected, especially the syncytiotrophoblast cells at the materno-fetal interface of the placenta [216]. This infection can lead to severe preeclampsia and placental abruption though there is no evidence of vasculopathy, finding instead a dense macrophages infiltration [216]. The infection of the human placenta may lead to alterations in the local renin-angiotensin system, explaining the preeclampsia like outcomes during pregnancy [217]. This finding shows the potential for severe complications of pregnancy promoted by infection by SARS-CoV-2. This is quite important as it has been shown that due to severe infections of the placenta by SARS-CoV-2, there can be important impacts in neonatal outcomes, even in the absence of vertical transmission [218]. However, as it happens with vertical transmission, severe infection of the placenta in COVID-19 infected pregnant women, is not frequently observed [218]. This is because usually there is not a high level of expression of ACE2 receptors and TMPRSS2, both critical for viral entry into the host cells, in the placenta [219]. The findings reported previously are for the second and third trimester of pregnancy. The infection of the placenta and possible transmission to the embryo during the first trimester has been controversial, though there are reports of miscarriages of COVID-19 symptomatic pregnant women with severe infection of their placentas and that the lungs and kidneys from the early stages of offspring development can be affected by SARS-CoV-2 [220].

In pregnant women infected with SARS-CoV-2 and mild to moderate symptoms alterations of the placenta observed at gestational weeks 40 or 41 (after birth), some showed signs of villitis, frequent maternal malperfusion, though fetal malperfusion was less frequent [221]. About 60% of these women who had mild COVID-19 during pregnancy, showed negative tests for SARS-CoV-2 and inflammation of the placenta was not found [221]. However, these findings are controversial, because in a recent cohort study with careful observation of placental alterations in COVID-19 positive pregnant women, maternal malperfusion and villitis were not the main findings [222]. Instead the significant differences reported in this study between infected pregnant women and control cases, were fetal malperfusion (21% versus 4%), with decidual arteriopathy and inflammation, perivillous fibrin deposition (30–40% versus 1–3%), and finally fetal vessel thrombi (22% versus 1%) [222]. Another recent study, showed that in about 5% of SARS-CoV-2 positive pregnant women (9 in 198 cases), a strong correlation between trophoblast damage and placental SARS-CoV-2 infection was observed [223]. This finding might explain why there is an increment of fetal death associated with COVID-19 disease in pregnant women [223], though some reports argue that placental infection it is a rather rare finding in SARS-CoV-2 positive women [224]. The receptors for host cell entry by SARS-CoV-2 (ACE2 and TMPRSS2) have been found in throphoblasts from placentas in the 3rd trimester of gestation [225]. A relevant finding important for vaccination strategies, is that antibodies can be transferred from the mother to the offspring and traverse the placenta, since the 2nd trimester of gestation [226].

2.6.3. Mother

In mothers, most of the reported cases have occurred between the 2nd and 3rd trimester of gestation, developing a severe disease only 1% of the infected mothers [214]. In a study performed in the United Kingdom, from 1148 hospitalized pregnant women that were COVID-19 positive, 63% had symptoms [227]. The symptomatic cases had a higher risk of severe disease and admission to Intensive Care Unit. They were mostly overweight or obese as well [227]. It was estimated an incidence of hospitalizations of symptomatic COVID-19 pregnancies of 2 in 1000 maternities and an incidence of 1.2 in 1000 maternities for asymptomatic COVID-19 pregnancies [227]. The risk factors predicting illness severity in a study performed in the United States were age (30–39), obesity, healthcare occupation, black and non-Hispanic ethnicity, and preexisting diseases such as cardiovascular diseases, diabetes, hypertension and chronic lung diseases [228]. Pregnant women should be vaccinated as soon as they can because of their higher risk and concerns for potential future COVID-19 infections [229]. Any person can conceive after being vaccinated waiting not longer than one or two days. The main issue about vaccination in pregnant women is to protect the mother from being infected by SARS-CoV-2. Regarding conferring immunity to offspring, transfer will occur especially since the 2nd trimester of gestation because of the maternal-fetal barrier, though the main purpose of vaccination during pregnancy is the mother and not the offspring [230,231]. It has been shown that the breast milk of vaccinated mothers contains SARS-CoV-2 specific antibodies. Vaccination during breastfeeding is also encouraged [232]. The effects of SARS-CoV-2 infection in human reproduction after conception are summarized in Table 1.

Table 1
Reported observations and recommendations of SARS-CoV-2 infections in offspring, placenta, and mother.

Offspring	Placenta	Mothers
Before delivery		
- Rare or no vertical transmission (ACE2 receptors and TMPRS22 expressed) [191].	- Rare [218]	- Symptomatic cases of infection (higher risk of severe disease with the presence of comorbidities) [228]
- Increase in fetal death [223]		- Pre-eclampsia like complications [216]
After delivery		
Neonates	- If present (villitis and malperfusion) [221]	Recommended actions
- Usually not infected [212]		- Vaccination (any stage during pregnancy) [229]
- If infected with symptoms (translate to NICU) [214]		- Breastfeeding (it can transfer antibodies) [232]

ACE2: Angiotensin-converting enzyme 2, TMPRS22: Transmembrane serine protease 2, NICU: Neonatal Intensive Care Unit.

3. General considerations of the Zika virus disease

The Zika virus (ZIKV) is an RNA flavivirus that belongs to the *Arbovirus* genre, and the family *Flaviviridae* [233,234]. It is phylogenetically quite close to dengue virus, yellow fever virus, West Nile fever virus, and the Japanese encephalitis virus [233]. It is a single-stranded RNA virus with two different evolutionary lineages: the Asian lineage and the African lineage [235–237]. The ZIKV was first identified in the Zika forests in Uganda in 1947 in Rhesus monkeys [235–237]. ZIKV causes a zoonotic emergent infectious disease that was first reported in sporadic cases in Africa and Southeast Asia. These cases were considered rare and sporadic until 2007, when it is believed that a mutation in the ZIKV genome in Micronesia allowed the virus to be better adapted to infect humans [235–237]. The virus is transmitted mainly through female mosquito bites, especially from the species *Aedes aegypti* and *Aedes albopictus*. Other methods of Zika disease transmission that are less frequent are: mother to neonate, sexual, transfusions, transplants, and by rare laboratory accidents [81,238–240].

The ZIKV was not considered as a widespread pathogen capable of causing considerable outbreaks and sequels until a recent large outbreak in Brazil in 2015. In Brazil the ZIKV was also associated with microcephaly and Guillain-Barré syndrome. Two distinct manifestations of ZIKV infections are sexual and trans-placental transmissions associated with neurological sequels such as the Guillain-Barré syndrome and microcephaly [241]. Similar with most arboviruses, the ZIKV participates in a complex cycle of transmission between insects and vertebrates. After the flaviviruses are transmitted via female mosquito bites, they initially replicate in the immune dendritic cells next to the skin, and later they inhabit the lymphatic ganglia. Finally, they spread systemically throughout the whole body through the blood [242].

Since its appearance in Micronesia, ZIKV has spread around the world, and it is attributed to large outbreaks in the Asian Pacific and the Americas. As mosquito vectors are increasing in their worldwide distribution along with global warming, so is the ZIKV. According to the World Health Organization (WHO), in July 2019 a total of 87 countries and territories documented the existence of autochthonous transmission of ZIKV through mosquito bites [243]. There are now 61 countries and territories where a mosquito vector (*Aedes aegypti*) has been found. This indicates that the spread of the Zika virus around the planet is far from over, and the potential risk of spread to uninfected territories remains a real possibility. Thus, ZIKV has to be added to the risk of reemergence or reintroduction of the virus in places where infectious outbreaks of the disease are now under control [243].

3.1. Structure of Zika virus

The ZIKV, like other flaviviruses (i.e. dengue), has an icosahedral envelope and a nucleocapsid structure. The virus particles are spherical and small in size (approximately 50 nm in diameter) with an electron-dense core of approximately 30 nm in diameter with an external and an internal surface. The virion outer surface contains the three most important structural proteins, envelope protein dimers, the membrane/pre membrane proteins (M) proteins, and on the internal surface the capsid protein [244–246]. The ZIKV and its genome, a 10.7 Kb, positive, single-stranded RNA, are shown in [247] Fig. 6.

ZIKV is highly homologous to dengue virus (60% homology) [248]. The ZIKV genome encodes three structural proteins, the capsid (C), envelope (E), and membrane (prM) proteins. The genome also encodes for seven non-structural proteins (nsps proteins), which are important for virus replication, protein processing, and countering host antiviral immune responses. Following cell binding and endocytosis, the Zika viral RNA is uncoated, released, and translated into a single polyprotein in the cytoplasm [249]. This polyprotein is cleaved into mature viral proteins by nsps and cellular proteases. These events occur in association with the endoplasmic reticulum (ER), where the viral components are packaged by the C protein, while they acquire their lipid bilayer envelope from the ER. There is subsequent processing, consisting of glycosylation and cleavage of prM by a host furin protease, which appears to be necessary to release the mature virions from the infected cells. The E-protein seems to be vital for the ZIKV life-cycle, and it is critical for viral entry into host cells, membrane fusion, and virus assembly [250]. Some of the epitopes designed for detection antibodies and also putative antiviral compounds have been directed towards the E protein and some of the structural proteins and nsps proteins [241].

3.2. Zika virus clinical symptoms, diagnostic tests, treatment, and vaccines

Zika virus infections are usually acute and self-limited, and the transmission of ZIKV can be vector or non-vector borne. The life cycle in vector-borne transmissions (the most frequently observed) starts with virus acquisition by a mosquito during a blood meal. The virus then replicates inside the mosquito until it reaches its salivary gland, where it can be eventually part of the injected fluid that flows into a new host during a blood meal [249]. The human skin in the region of the bite is the first site of viral replication in the new host. There is a preference for replication in fibroblasts, keratinocytes and immature dendritic cells. From the inoculation site, ZIKV can travel through lymphatic vessels to lymphatic ganglia, and finally it can be spread as a blood-borne infection throughout the entire host organism where it can produce viremia [251]. The ZIKV incubation period can vary between 3 and 12 days, and this is followed by a consolidated illness state where the presentation can differ between asymptomatic (80% of the cases) to a moderate dengue-like fever presentation (20% of the cases). The virus can be found in the blood of the patients during this period [252].

The symptoms of most ZIKV infections are fever, macular or papular rash, conjunctivitis (red eyes), arthralgia, headache, and myalgia, sometimes with retro-orbital pain [252]. These symptoms usually last between 4 and 7 days [238,253]. Sometimes it is difficult to differentiate the symptoms of ZIKV from other infectious diseases caused by other arboviruses, especially dengue and chikungunya. The presence of exanthema (pruriginous maculopapular rash) starting in the face and/or trunk suggests a ZIKV infection [254,255].

During infections the Zika virus can be found in urine, saliva, and other body secretions weeks after the initiation of the infection, indicating that the ZIKV infections are systemic [240]. It is epidemiologically relevant that in men the virus is attracted to testicular tissue (positive tropism) and can be excreted in semen for months after the infection was initiated, making ZIKV infections also sexually transmitted diseases [256]. Moreover, ZIKV can have complications with sequelae

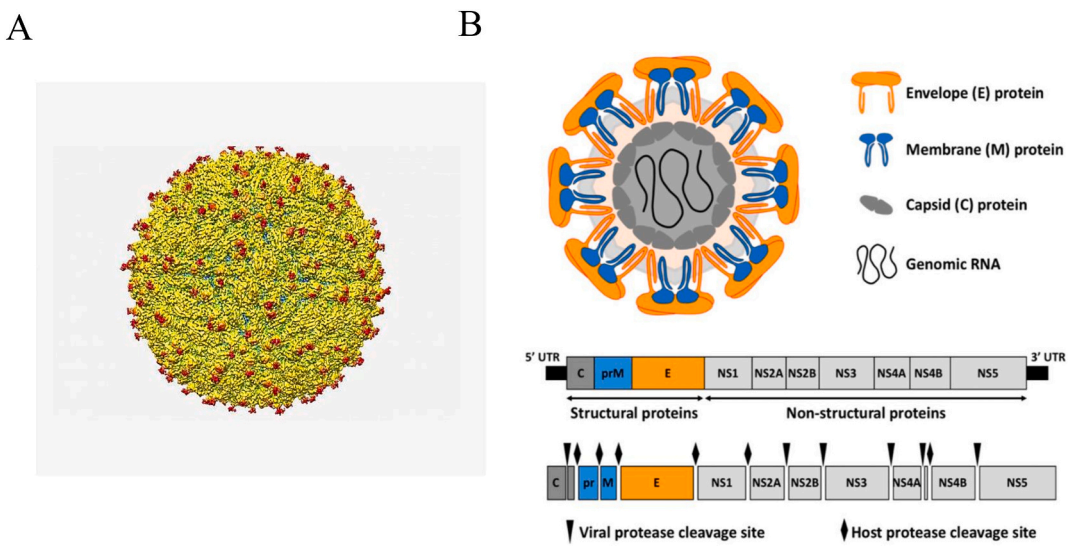

Fig. 6. The Zika Virus. (A) A scheme showing the structure of a Zika virus from its exterior where its membrane and rough surface due to membrane proteins are shown. (B) The main structural components and the genome of the Zika virus are shown in this scheme. The image was obtained by cryoelectron microscopy from public repositories at NIH (Rossman and Kuhn team [245,246]).

like the Guillain-Barré syndrome and hemorrhagic symptoms [241]. Guillain-Barré syndrome is an autoimmune disease of the nerves that starts with weakness and tingling to later cause a flaccid paralysis of the body. The temporal coincidence of the incidence between the Guillain-Barré syndrome with Zika virus outbreaks has been essential in linking these diseases. Most of the ZIKV-Guillain-Barré syndrome patients also had elevated levels of IgM antibodies against Zika virus and experienced rapid progression of the neurological symptoms [257]. The hemorrhagic complications of ZIKV are usually mild, though in some cases they can be severe [258].

The outbreaks reported in French Polynesia between 2013 and 2014 and in Brazil in 2015 were important in establishing the association between Zika virus infection, Guillain-Barré Syndrome, and microcephaly. Other less frequent complications that can happen, usually in <1% of ZIKV patients, and can include: transverse myelitis, meningoencephalitis, myocarditis, and thrombocytopenic purpura [254,259,260].

The entry of the ZIKV into host cells is not fully understood, but phosphatidyl receptors (TIM-1, TIM-4, AXL, and Tyro-3) seem to be quite important [251]. AXL (Tyrosine-protein kinase receptor UFO anexelecto), seems to be the receptor linked to ZIKV neurotropism, and TIM-1 (cell surface phosphatidylserine receptor T cell immunoglobulin mucin domain 1) is the receptor related to placental infection [261,262]. The host immune response promoted by interferons-I and -II may slow down the viral replication and infection of ZIKV [251]. The small-membrane-associated-interferon-induced transmembrane proteins (IFITMs) that inhibit the replication of many viruses are also quite effective against ZIKV replication [263]. To defend against the host immune system ZIKV has developed mechanisms that inhibit host responses, such as methylation of the viral genome and inactivation of interferon signaling through the NS viral proteins [249]. Recent studies have identified some of the host proteins and enzymes related to the ER that are needed for efficient viral replication in host cells [264].

The techniques used for the diagnosis of ZIKV infections received a boost in 2016, when the USA declared a public health emergency related to an outbreak of ZIKV in that country [265,266]. Several molecular assays and serological assays were developed during the following two years [267]. The recommended diagnostic techniques, molecular or serological, vary during the course of the infection [267]. The diagnosis of infection by ZIKV can be difficult because of the overlapping symptomatology with other flaviviruses and also because there is immunological cross-reactivity with other flavivirus infections [241]. A diagnosis of ZIKV infection requires confirmation by at least one of the following laboratory testing criteria: found in the specimen samples: (*a*) detection of Zika virus antigens (serological tests) or RNA (usually RT-PCR); (*b*) detection of the intact virus; (*c*) an increase in host IgM antibodies against Zika virus while ruling out cross-reactivity with other viruses; and (*d*) seroconversion of a four-fold increase in antibodies against Zika virus in paired clinical samples.

During the first few days of ZIKV infection (acute or viremic phase) it is recommended that RT-PCR be used for the detection of the viral RNA. After this viremic phase, it appears to be more useful to do serial serologic tests through the combined detection of anti-ZIKV IgM and IgG levels, searching for an increase in their usual serum concentrations by comparing paired samples. IgM antibodies usually appear on day 7 after the infection and are usually increased within 2–3 months, whereas IgG antibodies appear 3 days after IgM antibodies and remain increased for many years [268]. There is also a possibility of cross-reaction with other *Flaviviridae* viruses [269]. Because of this, this later assay should be combined with other diagnostic tests to distinguish between other viral infections, such as dengue, and also bacterial infections.

There is no specific treatment for ZIKV infections, and specific antiviral drugs are not available. The recommendations for therapy are usually symptomatic and support treatments [270]. Prevention and control and avoiding mosquito bites are the main protective measures to avoid ZIKV infection (use of mosquito repellent, adequate clothes, mosquito nets, etc.). Vector control, blood and tissue bank tests, and prevention of sexual and vertical transmission are also useful for reinforcement of protective policies. In those countries where there is a possibility of vertical transmission from mother to fetus, community measures to control mosquitoes and eliminate mosquito reservoirs are needed [238]. There is no vaccine against the ZIKV to prevent the disease, though some vaccines are currently under development and being tested [270]. Some advances in antiviral therapeutics and vaccines are underway, but they are still in early phases of development. Due to the similarity with Dengue virus, there have been proposals of designing a unified ZIKV and Dengue virus vaccine, or to share resources to get both vaccines [248].

3.3. Effects of Zika virus in human reproduction after conception

3.3.1. Offspring

The infection with ZIKV during any stage of pregnancy can cause birth defects, though the adverse outcomes in the offspring have a higher risk at ZIKV infections during the early stages of pregnancy. The estimated rate of mother-offspring transmission can vary between 7 and 26% depending on the methodology of study [271]. Between the adverse outcomes of pregnancy, 1–4% are fetal loss, and 4–9% can develop congenital Zika syndrome (CZS). The CZS is characterized by multisystemic birth defects such as microcephaly (33–64%), ventriculomegaly (63–92%), widespread calcifications (71–92%), and neurological symptoms in the newborn such as abnormal motor development and movements (77–100%) and epilepsy (9–54%), hearing loss and other neurological impairments [271,272]. The neurological symptoms are very important in CZS and it has been established that there are five symptoms and signs that are characteristic of this condition, viz, 1) severe microcephaly and collapsed skull, 2) thin cerebral cortices with subcortical calcifications, 3) macular marks with pigmentary blotchy retina, 4) congenital contractures, and 5) hypertonia and signs of extrapyramidal syndrome involvement [272]. Another set of observations that helps to explain these symptoms are anomalies of the corpus callosum (71–100%) and of the posterior fossa (21–82%). Extra-neurological signs observed were intrauterine growth restriction (14%), placentomegaly, hepatitis and anemia [271]. The high neurological in the offspring is due to the positive neurotropism of the ZIKV, once it goes through the placental barrier [273]. The neurotropism is also present in ZIKV infected adults, that can develop a Guillain-Barre syndrome [274]. The molecular basis of this neurotropism has been related to the expression of the Axl gene, which is a Tyrosin Protein-Kinase receptor that has been found in glial cells [275]. Glial activation and proliferation through Toll-like receptors (essential for the innate immune responses), is activated resulting in apoptosis, cell death and inflammation in the nervous system [276]. As a result there is cell-cycle arrest and the developing neurons die [277]. This is important to know, as a good therapeutic strategy with pregnant women infected with ZIKV to diminish the risks of fetal development is to inhibit the interaction of ZIKV with its host cell receptors (besides prevention through vaccination and diminishing *Aedes Agypti* contaminated populations) [278].

Regarding newborns, those that acquire ZIKV infection perinatally or after delivery can have symptoms like maculopapular rash, conjunctivitis, arthralgia (joint pain) and fever, but CZS is rare [279,280]. ZIKV can be found in breast milk in ZIKV infected women [281]. Even though there is controversial evidence of newborns infections with ZIKV through breast milk [282], the advantages of breastfeeding surpasses the risk of acquiring ZIKV disease by the babies and breastfeeding by infected mothers is encouraged [283]. Most babies that acquire ZIKV infection seem to be asymptomatic or experience a mild disease like it is observed in adults. However, the information regarding long term outcomes of babies infected with ZIKV is still limited and further research is needed.

3.3.2. Placenta

Villous immaturity is the main histopathological finding among ZIKV infected women, although placentas without any abnormalities have been also frequently observed [284]. Infections during the 3rd trimester are characterized by syncytial and fetal macrophages hyperplasia (Hofbauer cells) [284]. ZIKV from infected mothers, have to cross a series of barriers in order to reach the brain cells from the offspring. The maternal-fetal barrier has a series of multicellular layers between the fetus and the mother that results mostly from the differentiation of cytotrophoblasts being composed of endothelial fetal vessels, mesenchymal interior of the villus branch, layer of cytotrophoblast cells of the villus branch and a syncytio-trophoblast layer of the villus branch, surrounding all the previous structures [285]. There are many junctional structures among cells that can be disrupted during inflammation [286]. The syncytiotrophoblast layer of the villus branch seems to be the most resistant layer of the barrier [287]. Once it penetrates this layer, ZIKV is able to infect the rest of the cells, especially macrophages (like Hofbauer cells) and trophoblast cells, disrupting their junctions [288,289]. It has been shown that ZIKV can pass through the placental barrier, reducing the expression of occludine and ZO-1 (tight junction proteins), facilitating a paracellular passage through human placenta trophoblast cells [290]. The blood-brain barrier from the offspring, can be infected by ZIKV, and a transcytosis passage of ZIKV has been proposed [290].

3.3.3. Mother

Regarding mothers and ZIKV infection, the symptoms in adults have already been considered. Prevention of ZIKV infection besides protection against mosquito bites and vaccines (on trial) [291], considers waiting for at least two months in ZIKV infected women and up to six months in infected men for clearance of the ZIKV from the body, before having sexual relationships [292]. Once the virus is cleared-up from the body, there is no evidence that future pregnancies can be compromised. As ZIKV infection confers long lasting immunity this is some sort of natural vaccination by getting the disease though it has been shown there is a decline of immunity through the years, especially in adults [293]. There is evidence that having ZIKV infection increases the risk of having dengue disease [294]. There are cross-reaction effects of infections by similar viruses like dengue. These effects and consequences are currently under study [295]. As it has been mentioned before, ZIKV can be found in breast milk, though there is a broad consensus that breastfeeding by infected mothers should continue because the benefits are higher than the risks [283]. The effects of ZIKV infection in human reproduction after conception are summarized in Table 2.

4. Pathogenic mycoplasma

4.1. Mycoplasma structure and general considerations

Mycoplasmas are members of the *Mollicutes* class of prokaryotes, the

Table 2
Reported observations and recommendations in ZIKA virus infections in offspring, placenta, and mother.

Offspring	Placenta	Mothers
Before delivery		
- Vertical transmission (CZS with microcephaly, because of positive viral neurotropism) [272], and multisystemic symptoms that might not configure a CZS [271]	- Villous immaturity - Macrophages hyperplasia [284]	- Symptomatic cases of infection (treatment like the rest of the adult population, though intensive studies should be performed in the offspring due to the likely vertical transmission) [270].
- Increase in intrauterine growth restriction, hepatitis < anemia [271]		- Diagnostic tests (RT-PCR, seroconversion, others) [267,268].
After delivery		
Neonates infection - Maculopapular rash, conjunctivitis, joint pain, fever [279,280]	- Placentomegaly [271]	Recommended actions - Prevention (avoid mosquito bites, sexual intercourse in ZIKV infected adults, 2 months for females and up to 6 months for men) [292] - Breastfeeding (though ZIKV is found in milk breastfeeding should not stop) [283]

CZS: Congenital Zika Syndrome, ZIKV: Zika Virus Infection.

Mycoplasmataceae family, and are known as the smallest forms of autonomous self-replicative life in terms of microorganism dimensions and genome size. With their limited genomes mycoplasmas have been used as a simple live model for the identification of the minimal gene set required for the survival and growth of a free-living organism [296,297]. For example, the small genomes of *Mycoplasma genitalium* (*M. genitalium*) and *Mycoplasma pneumoniae* (*M. pneumoniae*) encode approximately 400–600 proteins, compared to about 4000 encoded by the genome of *E. coli* [296]. Mycoplasma genomes can be composed of single-stranded RNA or double-stranded DNA. As intracellular infections, they have an osmotrophic form of nutrition, and they use a replication disk for their cell division [296]. They are extremely common in all forms of life, living as symbiotic or infectious intracellular forms. Importantly, mycoplasmas still maintain all of the essential genes for their replication, transcription, and translation as well as the minimal number of energy metabolism genes needed for their intracellular, parasitic modes of life. Thus, they can survive, grow and replicate with a core number of slightly <400 essential genes [298].

Mollicutes comprise over 200 species, and members are characterized by the absence of an external cell wall and what appears in electron microscopy to be a pseudo-trilayered membrane structure (Fig. 6) [299]. Mycoplasmas and Ureaplasmas are the most frequently identified species found as intracellular bacterial infections in humans. This includes *M. pneumoniae*, *M. genitalium*, *Mycoplasma hominis* (*M. hominis*), *Mycoplasma fermentans* (*M. fermentans*), *Ureaplasma parvum*, *Ureaplasma urealyticum* and other species. Their pathogenic role in humans has remained controversial, because they have also been found as part of the normal flora of healthy individuals located at superficial sites in the urinary, genital and respiratory tracts. They are rarely found in the blood and the internal organs and tissues in normal, non-symptomatic humans [300,301].

Recently specific mycoplasmas have been identified as important pathogens in almost every variety of species: humans, animals, plants and insects, among others [301–304]. There is evidence in humans that pathogenic mycoplasmas are associated with certain chronic diseases where they could function as causative agents, cofactors or opportunistic infections that cause morbidity [301,304–306]. For example, pathogenic mycoplasmas in humans are often associated with respiratory infections, urogenital infections, fatiguing illnesses, autoimmune diseases, neurodegenerative and neurobehavioral diseases and cardiac infections, oral infections, periodontal diseases, sexually transmitted diseases, complications affecting the central nervous system, and systemic infections found in various solid cancers and leukemias and immunosuppressive diseases, such as HIV-AIDS [300,304,307,308] (see Fig. 7).

Essentially all mycoplasmas can live as parasites or commensals in various species of animals and plants, where they are usually found attached to or inside host cells [300,301,303]. Thus, a significant number of mycoplasma genes are devoted to encoding cell adhesion and attachment structures to allow entry into cells as well as variable membrane surface antigens to maintain parasitism and evade host immune and non-immune surveillance systems [296,300,301,303,308]. The adherence of mycoplasmas to specific tissue and cell surfaces is a crucial step in the establishment of pathogenic infections, and pathogenic mycoplasmas possess specialized structures that permit targeted cell attachment to specific host cells and host cell receptors. For example, *M. pneumoniae*, which is commonly found in cases of atypical childhood and adult pneumonia, requires a network of interactive adhesion molecules and accessory proteins for its adherence to host lung epithelial cells [301,303]. The adhesion molecules must cluster cytoadherence-related accessory proteins where specific mycoplasma organelles make cell contact. These adherence proteins appear to function together and comprise a primitive mycoplasma membrane adhesion structure [2,300–302,309].

Mycoplasmas are known to be able to adapt quickly to new microenvironments, especially intracellular environments [303,310]. This

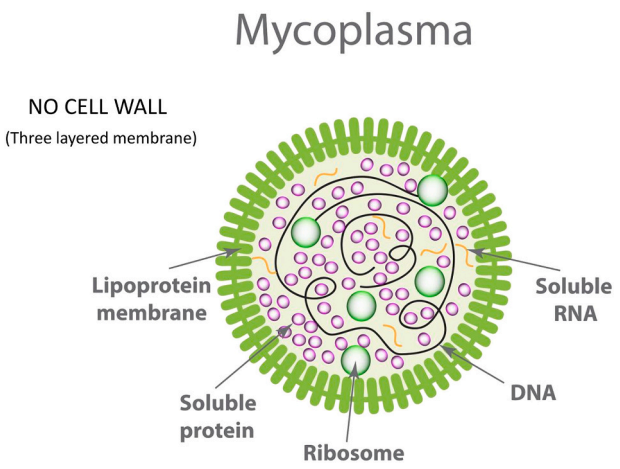

Fig. 7. Mycoplasmas. A structural scheme that shows the absence of a bacterial cell wall and some remarkable aspects, such as a multi-layered cellular membrane and internal contents of nucleic acids, proteins and ribosomes without a nuclear envelope. The scheme resembles a primitive bacterium. Free image, https://s3.amazonaws.com/commercio/goldbio-2018/pages/Mycoplasma%20structure%20b.png

adaptation is an important element in mycoplasma pathogenicity, and it can be attributed to their varying genomic structures and abilities to undergo rapid change [301,302,310]. When mycoplasmas evolved and adapted to parasitic intracellular modes of life, their transformation was likely made possible by devoting many of their genes to parasitic functions. Thus the genetic evolutions of mycoplasmas have ensured rapid alterations in cell membrane characteristics, such as membrane lipid phase variations and variable regulations of distinct membrane surface proteins involved in cell adherence, intracellular colonization and host immune system avoidance. Some examples include the size and sequence variations in the structural domains encoding surface proteins, changes in epitope masking and demasking, and alterations in numbers and surface presentations of surface cytoadherence proteins [300,301,310].

The ability to variably express structurally heterogeneous cell surface antigens and adhesion molecules make mycoplasmas very adaptable [301,310]. For example, variations in the genes encoding cell surface adherence molecules, such as the variable adherence-associated (Vaa) antigen, have revealed distinct patterns of mutations capable of generating multiple changes in mycoplasma cell surface antigen molecules. These deviations also generate changes in antigenic size and structural diversity [310,311]. In addition, mycoplasmas can scavenge host structures, such as the glycans from host glycolipids and proteoglycans, for their own surface glyco-molecules in order to avoid detection by host surveillance systems [301,310].

Displaying variable surface antigenic structures and rapidly changing of their expression are both thought to be important in the pathogenesis of mycoplasma infections. By rapidly providing altered epitope structures this enables mycoplasmas to escape from host immune responses and changes in cell host adhesion structures [310,311]. The result is that the changes in mycoplasma surface components can influence cell and tissue colonization and penetration of mucosal barriers, which are, in turn, directly related to mycoplasma pathogenicity [300,311].

As mentioned, mycoplasmas have small and unique genomes that contain repetitive and other elements that contribute to the variability in surface antigenic structures [310,311]. For example, the genome of *M. genitalium* was recently sequenced and found to encode a number of identifiable membrane proteins as well as membrane glycolipoproteins whose sequences do not resemble previously sequenced genes [312]. One recently discovered example in *M. genitalium* is the repeat fragments of a gene encoding a 140 kDa adhesion lipoprotein (MgPa), and

interestingly, this lipoprotein was subsequently localized to the tips of mycoplasma protrusions where it facilitates cell attachment and penetration [313].

There are also mycoplasma repetitive sequence elements that are variably transcribed but do not appear to encode surface-expressed mycoplasma proteins. Recombination of these repetitive elements with other genes and their ultimate expression in different mycoplasma strains may generate unique structures that explain the appearance of the polymorphisms within the genes and their encoded surface proteins. Thus the repetitive elements found in the *M. genitalium* genome may provide a reservoir of available protein sequences that could combine and contribute to the variability of antigenic structures expressed by mycoplasmas. By changing the adhesive properties found in pathogenic mycoplasmas this could affect the ability of mycoplasmas to penetrate into specific tissues and organs and cause various symptoms and illnesses [314].

4.2. Mycoplasma host responses and pathogenicity

Pathogenic mycoplasmas have a complex relationship with host response systems, especially in animals and humans [300,314]. These pathogens can either activate or suppress host response systems, and they use these and other strategies to evade host immune surveillance [300,315]. As an example, pathogenic mycoplasmas can act as immune cell suppressors or activators and inhibit, or under different conditions they can stimulate, the proliferation of various lymphocyte subsets involved in memory, suppression, responses and other possible outcomes. Pathogenic mycoplasmas can induce B-cell differentiation, and they can prompt cells to secrete pro-inflammatory cytokines, including various interleukins (IL). The induced molecules include IL-1ß, IL-2, IL-6, IL-8, among other ILs, tumor necrosis factor-α (TNFα), various interferons and granulocyte macrophage-colony stimulating factor [316,317]. This is an important event in vivo in patients with pathogenic mycoplasmal infections, because it can cause morbidity, and it is certainly involved in the prediction of the most severe mycoplasma cases [300,316]. In fact, the release of inflammatory cytokines is predictive of refractory mycoplasmal infections in children [316]. In terms of cell-mediated immune responses against mycoplasmas, it is known that mycoplasma-derived lipopeptides can directly stimulate host responsive cells, such as those mediated by macrophages. Certain mycoplasma lipoproteins have been found to be highly effective at immune stimulation, even as effective as endotoxins derived from other pathogenic bacteria [318]. Stimulation of macrophages can be measured, for example, by the release of nitric oxide, and this has been shown to be an indicator of immune stimulation by a *M. fermentans*-derived lipopeptide [318]. In another study from the same laboratory, *M. fermentans*-derived lipoproteins were shown to interfere with the interferon gamma-dependent (IFN-ɣ-dependent) expression of MHC class II molecules expressed on macrophage surfaces, another indication of how mycoplasmas can use different mechanisms to affect host responses [317].

Mycoplasmas are also able to secret soluble factors that can have duel activities, for example, in different lymphocyte subsets. An illustration of this is the activation and stimulation of proliferation or the inhibition of the growth and differentiation of particular immune competent cells. An example of this is the *M. penetrans* induction of significant proliferative responses in peripheral blood mononuclear cells, and this induction of proliferation was found to also be associated with the expression of specific surface markers of lymphocyte activation. This was seen in lymphocytes (both CD4+ and CD8+ T lymphocytes) from healthy human donors as well as in lymphocytes from HIV-1-infected subjects at different stages of AIDS progression [319]. This example indicates that pathogenic mycoplasmas have evolved with the ability to rapidly modulate and interfere with host responses in different ways.

Another property of pathogenic mycoplasma interference in host responses is the ability of some species to modify the secretion of immune-modulating substances. The secretion of immune-modulating substances stimulated by pathogenic *Mycoplasma* species is an important facet of mycoplasma immune modulation [315]. In the case of the mycoplasma human pathogen *M. fermentans*, a secreted lipoprotein was found that stimulated the induction of several monocyte cytokines and chemokines [320]. Another example of a secreted mycoplasma immunomodulatory lipoprotein is spiralin, which can stimulate the in vitro proliferation of human peripheral blood mononuclear cells and murine splenocytes. Eventually this results in the secretion of several proinflammatory cytokines, such as TNFα, IL-1 and IL-6. In addition, spiralin can also induce the maturation of murine B-cells. The spiralin-mediated stimulation of cytokine secretion may be similar to other immune-modulating lipoproteins secreted by other pathogenic bacteria [321]. The stimulation of various cytokines by pathogenic mycoplasmas is an important property that can contribute significantly to patient morbidity [316].

Mycoplasmas also evade immune recognition and destruction due to their ability to undergo rapid surface antigenic variations [315]. Mycoplasmas have slow intracellular growth rates compared to other bacteria, allowing them to be targeted by host immune surveillance systems. However, they can escape host surveillance by rapidly altering their cell surface antigenic structures. This along with the ability to alter host immune responses allows pathogenic mycoplasmas to evade host surveillance [315]. Mycoplasmas, especially intracellular mycoplasmas, grow slowly compared to extracellular bacteria that use the speed of their division to outpace and evade immune responses by overwhelming host defensive abilities, so mycoplasmas have developed other mechanisms to evade host surveillance. Their slow intracellular growth rates may also help explain the chronic nature of mycoplasmal infections. Slow growth and other properties of mycoplasmas have forced them to evolve with properties other than rapid growth to allow them to hide and evade host surveillance systems [300,302].

The abilities of pathogenic mycoplasmas to adapt to unique host tissue and cellular microenvironments are usually accompanied by changes in surface adhesion receptors for cell-binding and entry as well as structural protein changes that mimic host antigenic structures. This latter property allows mycoplasmas to 'hide' from host surveillance. One of the ways that this occurs is by the property of antigen 'mimicry,' seen during chronic pathogenic mycoplasma infections, and driven by quickly changing the size, structural diversity and expression of cell adhesion antigens. For example, the divergence of variable surface antigens mentioned previously can affect the adherence properties of mycoplasmas and enhance their abilities to evade foreign protein recognition by host immune systems. This adaptive ability helps mycoplasmas to survive undetected in their human hosts, but this is only one example of the abilities of pathogenic mycoplasmas to achieve survival and at the same time change their pathogenic properties [300,301,303,310,315].

There are various methods that pathogenic mycoplasmas use to overcome host mechanisms that attempt to limit their ability to colonize cells and tissues. Moreover, one strategy appears to rely on changing structural proteins and glycoproteins while separately weakening host anti-microbial mechanisms. This can result in changes that cause systemic results that come at the detriment of the host. For example, the stimulation of host inflammatory responses in order to kill or incapacitate a microbial invader can result in the release of inflammatory cytokines that also cause severe host symptoms. In the case of pathogenic mycoplasma infections, the severity of host symptoms closely parallels the elevated expression of inflammatory cytokines [310,315,316].

Other possible virulence mechanisms are implicated in the pathogenesis of mycoplasmas [301,322]. One example is the intracellular competition for nutrients and metabolites. This competition between mycoplasmas and their host cells can deplete biosynthetic precursors and disrupt metabolic and synthetic pathways that weakens the host and its ability to respond the invader mycoplasmas. Although mycoplasmas can stimulate host enzymes and other proteins, most mycoplasmas can

synthesize and secrete many of their own enzymes, such as lipases, proteases, nucleases and other enzymes. These mycoplasma enzymes can disrupt and interfere with host substrates, products and metabolic cycles [301,310]. Mycoplasmas also have the capacity to stimulate the generation of cellular-damaging molecules, such as hydrogen peroxide and superoxide radicals, that can damage host cellular membranes and other structures [322]. There are other possible ways that pathogenic mycoplasmas can be involved in damaging host cellular structures and normal cellular processes, such as direct membrane-membrane interactions found in cell adhesion, membrane fusion, vacuolization, and release of toxins or cytopathic molecules from cells [300,301,322]. The goal remains for pathogenic mycoplasmas to hide and survive, not to kill their host. However, in the process host cells can die. For example, one property of pathogenic mycoplasmas is the ability to initiate programmed cell death or apoptosis. This can be useful, for example, if the host cells are involved in immune responses [322]. Some mycoplasmas can induce or enhance apoptosis of peripheral mononuclear cells. In this case, the human pathogen *Mycoplasma penetrans* can program for an endonuclease (p40) that has been identified as a pathogenic determinant [323]. Pathogenic mycoplasma-released nucleases may also be involved in secondary necrosis, and this has been documented in some progressive mycoplasmal infections [324]. Other pathogenic properties of mycoplasmas are mediated by unknown or partially known mycoplasma-encoded molecules [325].

In addition to stimulating cell and organ death, pathogenic mycoplasmas can also release growth inhibitory molecules into their surroundings. For example, the enzyme arginine deaminase is an example of a growth-inhibitory enzyme derived from certain mycoplasmas that inhibits the growth of human T-cells and T-lymphoblastoid cell lines. Arginine deaminase suppresses IL-2 production and receptor expression in T-cells stimulated by non-specific mitogens, while also inducing the morphologic features of dying cells, including the type of DNA fragmentation pattern seen during apoptosis [326,327]. This enzyme has been followed in patients with community-acquired pneumonia as a possible marker for *M. pneumoniae* infections [328].

Pathogenic mycoplasmas can also release toxins that directly damage cells or activate innate host response systems [329,330]. For example, Becker et al. [331] have isolated a *M. pneumoniae*-released toxic factor, called the community-acquired respiratory distress syndrome toxin (CARDS), which is an ADP-ribosylating and vacuolating cytotoxin. This pathogenic mycoplasma toxin activates the NLRP3 inflammasome complex and causes subsequent release of IL-1, among other inflammatory cytokines, and causes hyper-inflammation that can result in tissue damage and other pathologies. This mycoplasma toxin appears to cause pulmonary inflammation, cytokine release, and significant airway dysfunction and may be responsible, in part, for respiratory failure and fatal outcomes found in acute *M. pneumoniae* infections [332].

Pathogenic mycoplasmas can cause life-threatening conditions in some patients. For example, cardiovascular and pulmonary manifestations caused by mycoplasma infection can result in extreme patient morbidity [329,330,333]. There are several examples of this in the literature, and they have been reported as caused by vascular occlusion due to thrombosis triggered by stimulation of autoimmunity and the formation of vascular immune complexes. In severe mycoplasma infections vascular occlusion has been reported for heart, lung, kidney, brain and other organs, but this is usually only seen in the most pathogenic mycoplasma infections [329,330,333–335].

4.3. Diseases associated with Mycoplasma species infections

Although mycoplasmas in humans have been described in the literature since the 1930s, it has been only recently that they have been shown to play a pathogenic role in many illnesses and diseases, from pneumonia to sexually transmitted diseases [305,308] as well as in several chronic illnesses of unknown origin [304]. The evidence that *Mycoplasma* species are involved in the pathogenesis of certain diseases and chronic illnesses has been based mainly on the positive responses of most patients to therapies directed specifically at intracellular mycoplasmas in patients who are mycoplasma-positive [300,304,307]. Using anti-microbial and integrative treatments pathogenic *Mycoplasma* species infections have been suppressed slowly, resulting in gradual reductions in morbidity, but this is not seen in every patient [300]. Even if mycoplasmas are not currently considered a cause for specific illnesses, they do appear to be important in the inception, progression, morbidity and relapse of several chronic illnesses [300,301,304,307]. Often this can also be due to the important part they can play as co-infections [300,336,337].

Evidence gathered over the last 30 years demonstrated the presence of pathogenic mycoplasma species in the body fluids and tissues from patients with a variety of chronic clinical conditions, such as atypical pneumonia, asthma and other respiratory conditions; oral cavity infections; urogenital conditions; neurodegenerative and neurobehavioral diseases; autoimmune diseases; immunosuppressive diseases; inflammatory diseases; and illnesses and syndromes of unknown origin (for example, fatiguing illnesses) [300,301,304,307]. In these illnesses and diseases mycoplasmas might be present initially as superficial flora, such as in the oral and urogenital cavities; however, they are not thought to be pathogenic at these superficial sites. When they penetrate into body fluids, blood and tissues, they are capable of intracellular colonization of internal tissues and organs, and it is at this point that they can slowly exert their full pathogenic properties [300,304,336]. In some cases this might not be fully apparent until an appropriate mixture of co-infections, including mycoplasmas, is fully expressed [143,300,337].

4.4. Testing for mycoplasmas and prevention

Testing for the presence of *Mycoplasma* species in patients has generally been lacking until the recent advances in PCR testing of clinical samples. Mycoplasmas and Ureaplasmas are fastidious microorganisms and generally grow very poorly in culture, so this method of bacterial identification and detection is not useful for most clinical samples [300,338]. Although seriological testing for some *Mycoplasma* species is commercially available, for example for *M. penumoniae*, most *Mycoplasma* species do not have adequate, routine antibody tests that are sensitive and reliable [300]. Thus, more recent studies have used nucleic acid detection techniques, such as various methods of PCR for mycoplasma detection [300,338] Problems remain in the use of PCR due to specimen limitations, availability of clinical samples that contain pathogenic mycoplasmas, rapid sample degradation, the presence of inhibitors or interfering factors and other limitations [300]. Non-amplified DNA hybridization methods have also been used successfully in a few studies, for example for *M. fermentans* sequences [339], but in general these test formats are not in routine use due to the complex nature of the tests, their reliability and the requirement for sufficient numbers of microorganisms for a positive result [339].

Prevention of *Mycoplasma* species infections has been difficult to achieve. Most mycoplasma prevention approaches have depended on health maintenance rather than more specific approaches, such as vaccinations [340]. Mycoplasma vaccines have been under development for a few *Mycoplasma* species, the most common being *M. pneumoniae* [341]. In a small study a vaccine against inactivated *M. pneumoniae* was partially successful against challenge with viable *M. pneumoniae*. A majority of participants developed growth-inhibiting antibodies and only one of the volunteers who responded to the vaccine became ill; whereas most of the participants that did not respond to the vaccine became ill with respiratory tract disease [341]. Various studies have shown that patients can develop cellular responses to mycoplasma antigens [342]. Indeed, cellular responses against mycoplasma antigens were shown to be durable in patients with *M. pneumoniae* infections [343]. Cellular immunity to mycoplasmas can develop during pulmonary mycoplasmal infections, and this is thought to reduce the severity

of lung inflammation and course of the disease [344]. However, overall the development of mycoplasma vaccines compared to other bacterial infections has proved to be disappointing. Future developments will likely focus on the development of vaccines that reduce morbidity and secondary complications [345].

4.5. Effects of Mycoplasma in human reproduction after conception

4.5.1. Offspring

The most dangerous species for the offspring are *Mycoplasma hominis* (MH), *Ureaplama ureatylicum* (UU), and *Ureaplasma parvum* (UP). Ureaplasma species are being increasingly recognized as pathogens raising prenatal, perinatal and postnatal morbidity [346]. UU is part of the lower urogenital tract flora, but in occasions ascends and cause bacterial vaginosis, chorioamnionitis increasing the adverse outcomes of pregnancy such as preterm birth [346]. Several reports agree that UU leads to infection of the chorioamnion which associates with premature spontaneous labor and delivery [347–349]. It plays an important role in perinatal and postnatal morbidities increasing bronchopulmonary dysplasia, neonatal sepsis and meningitis [346]. Though reports in the pathogenesis of bronchopulmonary dysplasia in the offspring were initially controversial, most of the evidence is consistent with a role of UU in the pathogenesis of this disease [350–352]. This can lead to prolonged mechanical ventilation and certain chronic lung diseases in neonates with premature birth [353–355]. Systemic neonatal infection as sepsis has also been reported for this pathogen [356]. It has been debated if UU infection can lead to central nervous system infections in neonates showing as meningitis [357]. All this can lead to an increment in morbidity and long-term hospitalization in low-weight birth newborns [358]. The diagnostic and treatment of UU infections can be challenging because it can be normally found in the absence of pathologies and there is a lack of diagnostic tests [346,359]. Everything reported for UU has also been reported for MH, though the rate of spontaneous abortions seems to be higher with MH [360,361]. Though these reports show that UU and MH can have adverse outcomes by themselves, UU and MH infections are also frequently associated [362,363]. They can lead together to most of the pathologies with UU or MH alone and exacerbate them [364]. Coinfection with UU and MH can lead to funisitis (inflammation of the umbilical stump) [365]. They also impact in the neonates and they have been found in neonatal blood, cerebrospinal fluid or tissues such as lungs. It also faces the challenge of appropriate diagnostic and treatment [366]. When and how should be the treatment to reduce the incidence of adverse outcomes in pregnancy and neonates, remains unclear [366]. It is being under study the higher risk to get these pathogens during in-vitro fertilization procedures [367]. In neonates and newborns, *Mycoplasma genitalium* (MG) is also a cause of usually milder diseases in the neonate, from the same sort as those found with UU and MH [368]. MG seems to be more important as a possible cause of infertility promoting endometritis, cervicitis and salpingitis in infected women [368]. It is being under study its potential to enhance HIV transmission [368]. UP has also been associated with preterm birth and an increment of mortality in newborns [369], though most of the studies have been centered in UU and MH.

4.5.2. Placenta

Mollicutes is a class including simplest bacteria that cause infection in pregnant women. The most usual findings by Mollicute infections in the placenta are chorioamnionitis, with the presence of the pathogens in fetal membranes and fluids (chorion, amnios, and amniotic fluid) [370]. Chorioamnionitis is an acute inflammation of the placenta membranes and chorion, most commonly due to ascending polymicrobial infection from the lower urogenital tract, partially characterized by a neutrophil infiltration in the amnios and subchorion of the infected placentas that are observed in preterm delivery [371]. It is important to distinguish it because not all chorioamnionitis are clinically symptomatic and it might be quite difficult to obtain a successful culture of the infecting Mollicutes. In several cases it can be extended to the umbilical cord as funisitis with leukocyte infiltration of the umbilical vessel or the Wharton's jelly [372]. Chorioamnionitis by Mollicutes can occur without rupture of the placenta [349]. It has been established that 65% of them are polymicrobial and from those, almost half are because of UU, MH and Mollicutes infections [373,374]. The starting point is usually the flora present in the lower urogenital tract that ascends infecting the uterus, placenta and fetus. This condition happens in up to 2% of births in the USA, being an important cause of premature labor and premature rupture of membranes [370]. Ureaplasmas are the most frequent agents causing chorioamnionitis and the adverse outcomes of pregnancy reported above. The molecular basis of its pathogenesis and why some can go up from the lower urogenital tract is currently being unraveled. Most of the evidence so far, indicates that the Major Band Antigen (MBA, a surface- exposed lipoprotein), is a major player in the pathogenesis of Ureaplasma species for causing chorioamnionitis, in fact is being considered a predicted virulence factor of the Ureaplasma species [375]. The molecular reason behind the pathogenesis, seems to be that certain antigenic variation at the level of MBA makes Ureaplasmas able to avoid immune recognition in the host, causing chronic infections and colonization of the upper urogenital tract [375]. Variations in the size of the MBA lipoprotein, modulates the host immune response [376]. MBAs from different ureaplasma species are different and respond to different antibodies as well [377]. In HeLa cells in culture, it has been observed that Ureaplasmas enter the cells through clathrin-mediated endocytosis after binding of the pathogen by sulfoglycolipids membrane receptors [378]. Later it remains in the perinuclear region being secreted through exosomes. It is thought that this mechanism could help them to avoid the immune responses [379].

The fetal complications of chorioamnionitis are fetal death, neonatal sepsis and fetal inflammatory response syndrome with funisitis and chorionic vasculitis (FIRS, fetal counterpart of the systemic inflammatory response syndrome or SIRS) [380]. During FIRS, which is the fetal immune response to infection there is release of several pro-inflammatory mediators such as cytokines and chemokines like interleukin 6, TNF-α, C-reactive protein, and matrix metalloproteinases [381]. FIRS-associated fetal immune response leads to responses that vary through preterm labor to perinatal death, being associated with multi-systemic failure in newborns (especially those preterm). The multi-systemic failure includes chronic lung disease, periventricular leukomalacia and cerebral palsy [382,383].

The neonatal complications of chorioamnionitis are seen at or shortly after birth. These are perinatal death, asphyxia, sepsis, and neurological complications such as intraventricular hemorrhage, white matter damage in the encephalus and cerebral palsy [384,385]. These outcomes are worst in preterm newborns [386].

4.5.3. Mother

The clinical findings in the mother that suggest chorioamnionitis are fever, sensitivity to pain in the fundus of the uterus, maternal and fetal tachycardia (>100 bpm and 160 bpm respectively), and purulent or abnormal amniotic fluid [387,388]. Mollicutes can be isolated from the amniotic fluid in pregnancies with preterm birth, even without signs of clinical chorioamnionitis, but with histological findings [348]. From the clinical symptoms, though unspecific, maternal fever is the most important sign being present in 95–100% of the cases [389]. In women with prolonged labor and epidural anesthesia, the so-called epidural fever is often found [390]. Maternal fetal tachycardia is the second common clinical finding occurring up to 80–70% of the cases. The combination of fever plus tachycardia strongly suggests intrauterine infections such as chorioamnionitis [389]. Changes in pain sensitivity in the uterus and amniotic fluid odor are both subjective and can be distinguished well only in up to 25% of the cases [389]. Subclinical chorioamnionitis can also be found and have their manifestation through preterm labor or premature rupture of the membranes [389]. Clinical diagnosis of chorioamnionitis solely, should be considered when

accessing to the amniotic fluid or placenta is not possible, as they are invasive studies [391]. As mentioned above, fever, tachycardia and a weaker 3rd sign (uterine pain, abnormal amniotic fluid), are needed to have a very strong prediction of chorioamnionitis based solely on clinics [389]. When the clinical diagnosis is not clear, laboratory tests should be done if they can be performed with the patient. Maternal leukocytosis can be found in 70–90% of chorioamnionitis but it has to be accompanied with other of the reported signs of chorioamnionitis [389]. Other blood tests are being under discussion. The amniotic fluid testing obtained by amniocenteses is quite reliable but it can last up to 3 days to confirm the diagnosis. It is also limited because it is an invasive method [389,391].

Not all chorioamnionitis are produced by Mollicutes, there are other pathogens that might produce it, even with other ways of access to the chorioamnios like the blood [388]. However, addressing Mollicutes actions regards with the fact of their high frequency, their association with reemergent disease in some parts of the world, and their difficulty in diagnosis and treatment [389]. The maternal complications related to chorioamnionitis are: increased risk for cesarean delivery, endomyometritis, wound infection, pelvic abscess, bacteriemia and hemorrhage after delivery [392]. When there is premature rupture of membranes, chorioamnionitis has to be suspected because it is major cause of that adverse outcome of pregnancy [372].

The management of chorioamnionitis from offspring, interphase and mother include antibiotics and supportive measures. The antibiotic therapy should be done with broad spectrum antibiotics, and initiated as soon as possible (intrapartum), in order to prevent maternal, interphase and fetal complications [387]. Of special interest is their administration when there is premature rupture of the membranes as this adverse outcome of pregnancy is usually indicative of chorioamnionitis [372]. Antibiotics also reduce the degree of chorioamnionitis, neonatal sepsis, being able to prolong the gestational time and expected time-of-delivery in those women who are not in labor [393]. There is no consensus regarding the best antibiotic therapy because it will depend on the main pathogens found. The most used have been intravenous ampicillin and clindamycin or macrolides (especially erythromycin) for anaerobic coverage, usually lasting 7–10 days [394]. This treatment should continue with one intravenous dose more after delivery [389]. Oral routes after delivery can be given as well [395]. Steps to develop a universal treatment of chorioamnionitis after knowing its cause, are being currently undertaken [396]. The use of antipyretics is encouraged, especially during the intrapartum period, as the high fever in the mother can yield to fetal acidosis or neonatal encephalopathies [397]. It might also reduce fetal tachycardia [389]. If there is premature rupture of membranes, induction of labor and delivery it is recommended after 34–37 gestational weeks to avoid more severe adverse outcomes and complications [398]. The effects of Mollicutes infection in human reproduction after conception are summarized in Table 3.

Table 3
Reported observations and recommendations of Mollicute infections in offspring, placenta, and mother.

Offspring	Placenta	Mothers
Before delivery - Bronchopulmonary dysplasia [350–352] - Sepsis [356] - Meningitis [357] - Cerebral palsy [382,383] - FIRS [380] - Fetal death [380] - Preterm birth [346] (main mollicutes alone or combined with UU, MH, UP)	- Chorioamnionitis [370] (Mollicutes alone or polymicrobial, MBA is a major determinant and predicted virulence factor) [375]. Other pathogens may be the determinant ones) [388]	- Symptomatic infection [387,388] - Fever - Maternal and fetal tachycardia (>100 and >160 bpm, respectively) - Pain sensitivity in uterus - Abnormal amniotic fluid - Subclinical infection - Histological [348] - Maternal complications [392] - Cesarean delivery - Endomyometritis - Wound infection - Pelvic abscess - Bacteriemia - Hemorrhage after delivery
After delivery Neonates - Perinatal death [384,385] - Asphyxia [384,385] - Sepsis [384,385] - Cerebral palsy [384,385] - More important in preterm neonates [366] (same Mollicutes alone or combined as in before delivery adding MG) [368]	- Premature rupture of membranes [372]	Recommended actions - Antibiotics [387] (broad spectrum, early beginning intrapartum, intravenous ampicillin, clindamycin, macrolides, erythromycin) - Supportive measures (Antipyretics) [397] - Induction of labor (after 34–37 gestational weeks) [398]

FIRS: Fetal Immune Response Syndrome, UU: *Ureaplasma Ureatylicum*, MH: *Mycoplasma Hominis*, UP: *Ureaplasma Parvum*, MBA: Multiple Banded Antigen, MG: *Mycoplasma Genitalium*.

4.6. Treatment of mycoplasmal infections

Conventional antimicrobial treatments effective against pathogenic mycoplasmal infections mostly include systemic antibiotic therapy, but the choice of antibiotic(s) for a given *Mycoplasma* species is quite important. Mycoplasmas do not have a cell wall like most bacteria; thus antibiotics that act on cell wall synthesis are ineffective against mycoplasmas [301,303,304,307,339,345,399,400]. Therefore, mycoplasmas are usually treated with anti-microbials that attack their replication, synthesis of structural components, metabolism, or other bacterial targets. Most mycoplasmas and ureaplasmas are generally sensitive to tetracyclines, such as doxycycline, minocycline, among others, and these are often considered for frontline antibiotic treatment. Alternatively, quinolones (ciprofloxacin, sparfloxacin, levofloxacin, ofloxacin, among others) have been effectively used for mycoplasma treatments [306,400,401]. Some *Mycoplasma* species, such as *M. pneumoniae* or *M. genitalium*, are quite sensitive to macrolides (azithromycin, clarithromycin, erythromycin, among others), whereas *M. hominis* strains are usually more resistant [402,403]. Ureaplasmas are moderately susceptible to macrolides [403,404]. Some *Mycoplasma* species, such as *M. hominis* and *Ureaplasma urealyticum*, are generally more resistant to tetracyclines [405,406], and some *M. hominis* strains have been shown to be quinolone-resistant [407].

Treatment of pathogenic mycoplasma infections with antibiotics generally involves daily or pulsed treatment. For example, every-other-day administration at the maximum dose recommended for a particular antibiotic has been successfully used to suppress mycoplasma infections [340,408]. The rationale of pulsed treatment is that mycoplasmal proliferation is often cyclic, and thus some organizations recommend every-other-day antibiotic regimens [300]. In either case long-term treatment is often required, even as much as 6–12 months, due to the slow growth rates of mycoplasmas and their relative insensitivities to various treatments [300].

Another consideration is the age of the patient. For example, macrolides might be considered as first line therapy for young children with pathogenic mycoplasmal infections due to the potential side effects of tetracyclines and quinolones in young children [409]. Some adverse effects, such as staining of developing teeth in children under the age of eight with tetracyclines, have not been a problem with antibiotics like doxycycline, and in some cases low-dose doxycycline has been used to

treat children with bacterial infections without incident [410].

Antibiotic resistance can occur during treatment of pathogenic mycoplasmas [411]. This is exemplified by the shifting minimum inhibitory dose concentrations required to treat certain mycoplasma infections with antibiotics; for example, the treatment of *M. genitalium* infections with oral tetracyclines [412]. To overcome this problem, increasing antibiotic dose levels or shifting to a different antibiotic regimen has been utilized [300]. Another problem has been the appearance of Jarisch-Herxheimer reactions (J-H reactions) [300,413]. These are observed as temporary increases in the severity of signs and symptoms, and J-H reactions generally involve fevers, chills, muscle aches, fatigue, skin rashes, pain and other signs and symptoms related to cytokine release [413]. There are some rather simple methods to reduce the severity of some J-H reactions, and their appearance with antimicrobial treatments is thought to be due to the release of mycoplasma particles and fragments and subsequent host response and cytokine release [300]. Some feel that strong J-H reactions suggests efficacy of treatment, but this is not necessarily an indication of treatment failure or success [300]. Conventional use of antibiotics suggests using only limited treatment times, but limited treatment times fail to resolve mycoplasma infections in many chronic illnesses [307,408,409]. Since most mycoplasmas are slow-growing, cyclic, and fastidious, they are less sensitive, in general, to antibiotics compared to extracellular bacteria [300,414].

Host immune responses may also be essential in killing survivor mycoplasmas that resist antimicrobial treatments. Variant microorganisms can resist host surveillance by alteration or suppression of host responses [300,301,310]. Alternatively, mycoplasmas could go into non-growth phases where they are insensitive to therapies like antibiotics that target bacterial metabolism [415]. There are a variety of other possibilities that could explain why lengthy treatments of antibiotics are required in most chronically ill patients to achieve significant mycoplasma suppression that would allow patient recovery [300,307,415].

A major clinical problem seen during mycoplasma infections is caused by inflammation. This can be especially problematic in pathogenic mycoplasmal infections, and it is most troublesome when multiple infections are involved [416]. The most obvious example of this is that inflammatory cytokines are often produced and released into the circulation during pathogenic mycoplasmal infections [316,320,322]. Importantly, the blood levels of inflammatory cytokines have been correlated to patient morbidity [289]. In patients with severe mycoplasma infections inflammation can be serious, and anti-inflammatory treatments are often necessary [415]. In children who have severe *M. pneumoniae* infections corticosteroid treatment was associated with clinical and radiographic improvements. Such anti-inflammatory therapy was considered important in reducing patient signs and symptoms [417]. In mycoplasma cases with severe inflammation patients have been treated with steroids or other immunosuppressive drugs with or without intravenous administration of immunoglobulins [418].

Various natural supplements have been used to reduce inflammation during mycoplasma infections. Some natural cytokine inhibitors to reduce inflammation, such as alpha-lipoic acid (ALA), have been added as dietary supplements. ALA has also been shown to be a good inhibitor of inflammatory cytokines in rheumatoid arthritis patients [419]. Other herbs and vegetables, such as curcumin, broccoli seed extracts, cordyceps, Chinese skullcap, Isatis and Houttuynia extracts, have also been used to specifically reduce inflammation during mycoplasma infections (review [300]). In addition to ALA, other supplements, such as L-carnitine, ALA, CoQ10, and other components, especially membrane glycerophospholipids [420], have been used to support mitochondrial function [421].

After antibiotic therapies for mycoplasma infections are discontinued, patients often require additional anti-microbial treatments to prevent relapse [300]. Thus, some herbal supplements have been added to treatment strategies during and after antibiotics have been stopped. Most of these herbs and natural remedies have not been tested in controlled clinical trials with mycoplasma-infected patients, but there is some indication that they can be useful (reviewed in [300]).

5. Final comment

Human beings are exposed to infectious diseases from microorganisms adapted to the evolution of mankind and some other unexpected ones appearing as emergent or reemergent diseases. These latter microorganisms affect public health and produce unexpected outbreaks. We have considered here some examples of microorganisms that are mostly intracellular pathogens, such as viruses and mycoplasma. As they invade host cells intracellularly, they are more difficult to treat, diagnose and prevent, and they can easily spread among the population causing outbreaks of the diseases they promote with significant morbidity and sometimes, mortality and sequelae in human populations. We have briefly reviewed the current knowledge of the main biological, pathogenic, diagnostic, treatment, and prevention issues regarding SARS-CoV-2, Zika virus and mycoplasmas, as this special issue deals, in particular, with such pathogens.

Great effort has been made by many disciplines trying to understand why these pathogens promote various diseases and how we can stop these threats. The new techniques in biology that have matured during the last few years has allowed great advances in the topics covered in this review and will continue to evolve to include a more comprehensive data at the molecular and pathophysiological level of the diseases they promote worldwide. Understanding these will require not only new techniques, but a molecular explanation of the basis of these diseases as well as coordination to increase the accessibility of these advances to avoid the spread of emergent diseases.

The continuity of the human species requires knowing how these before-mentioned pathogens can affect pregnancy, fetuses and neonates. Though various emerging viruses and bacteria have been implicated in causing clinical morbidity and mortality, the examples chosen for this special issue (SARS-CoV-2, Zika viruses and pathogenic species of mycoplasma) may not be among the most deadly pathogens identified in human diseases, but they are very important in particular circumstances, such as in pregnancy and in the health of fetuses, neonates and children. In another chapter of this special issue dedicated to these pathogens, their pathological implications, susceptibility of pregnant women and forms of presentation during pregnancy, fetuses and neonates will be discussed.

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Declaration of competing interest

The authors confirm that there is no conflict of interest.

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