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REVIEW

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# SARS-CoV-2 and the possible connection to ERs, ACE2, and RAGE: Focus on susceptibility factors

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**Abstract**

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has provoked major stresses on the health-care systems of several countries, and caused the death of more than a quarter of a million people globally, mainly in the elderly population with preexisting pathologies. Previous studies with coronavirus (SARS-CoV) point to gender differences in infection and disease progression with increased susceptibility in male patients, indicating that estrogens may be associated with physiological protection against the coronavirus. Therefore, the objectives of this work are threefold. First, we aim to summarize the SARS-CoV-2 infection pathway and the roles both the virus and patient play in COVID-19 (Coronavirus disease 2019) progression, clinical symptomatology, and mortality. Second, we detail the effect estrogen has on viral infection and host infection response, including its role in both the regulation of key viral receptor expression and the mediation of inflammatory activity. Finally, we describe how ERs (estrogen receptors) and RAGE (receptor for advanced glycation end-products) play a critical role in metabolic pathways, which we

**Abbreviations:** AC, adenylyl cyclase 1; ACE1, angiotensin-converting enzyme 1; ACE2, angiotensin-converting enzyme 2; AECC, American-European Consensus Conference; AGE, advanced glycation end-products; AGT, angiotensin; AngA, angiotensin A; AP1, activator protein I; ARDS, acute respiratory distress syndrome; AT2, alveolar type 2 pneumocytes; ATR1, angiotensin II receptor type 1; CFR, case fatality rate; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; eNOS, endothelial nitric oxide synthase; ERE, estrogennonbreakingspaceresponse element; ERK 1/2, extracellular signal-regulated kinase 1/2; ERs, estrogen receptors; ERα, estrogen receptors α; ESR1, estrogen receptor 1; ESR2, estrogen receptor 1; G-CSF, granulocyte colony-stimulating factor); GPER1, G protein-coupled estrogen receptor 1; HCV, hepatitis C virus; HMGB1, high mobility group box 1 protein; IL-10, interleukin-10; IL-2, interleukin-2; IL-7, interleukin-7; IP10, inducible protein 10; JNK, c-Jun N-terminalnonbreakingspacekinase; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated proteinnonbreakingspacekinase; MCP1, monocyte chemoattractant protein-1; MERS-CoV, Middle East respiratory Syndrome Coronavirus; MIP1a, macrophage inflammatory protein 1 alpha; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor Kappa B; NO, nitric oxide; PAMP, pathogen-associated molecular patterns; PI3K-AKT, phosphatidylinositol 3-kinase-proteinnonbreakingspacekinasenonbreakingspaceB; PKC, protein kinase C; PLC, phospholipase C; PMN, polymorphonuclear cells; RAGE, receptor for advanced glycation end-products; RNS, reactive nitrogen species; ROS, reactive oxygen species; Rp3 NP, Rp3nonbreakingspacenucleocapsid protein; S100A4/Mts1, S100 calcium-binding protein A4; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SERMs, selective estrogen receptor modulators; SRC, proto-oncogene non-receptor tyrosine kinase; TMPRSS2, type II serine protease; TNFα, tumor necrosis factor-α; WHO, World Health Organization.

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envisage could maintain a close interplay with SARS-CoV and COVID-19 mortality rates, despite a current lack of research directly determining how. Taken together, we present the current state of the field regarding SARS-CoV-2 research and illuminate where research is needed to better define the role both estrogen and metabolic comorbidities have in the COVID-19 disease state, which can be key in screening potential therapeutic options as the search for effective treatments continue.

**KEYWORDS**
ACE2, COVID-19, estrogen, RAGE

# 1 | INTRODUCTION

## 1.1 | SARS-CoV-2 and COVID-19

Coronavirus disease 2019 (COVID-19) is a disease caused by the new coronavirus called SARS-CoV-2 (SARS-coronavirus 2). In December 2019, the first case of COVID-19 was diagnosed in the city of Wuhan, China.[1] The virus disseminated rapidly and the World Health Organization (WHO) declared SARS-CoV-2 as a pandemic on March 11th 2020, given that it had already spread to more than 188 countries on five continents. Updated epidemiological data from the Johns Hopkins University indicator shows that there are more than 20 million cases and over 730 thousand deaths due to COVID-19 by the beginning of August 2020 (https://coronavirus.jhu.edu/map.html—accessed on August 11, 2020 14:00 GMT). The Case Fatality Rate (CFR) for COVID-19 increases exponentially with age.[2,3] For example, for patients aged between 65 and 74 years old, the CFR is 3%-5%, 4%-11% CFR for 75 and 84 years old and 10%-27% CFR for the patients above 85 years old.[2]

Obesity, diabetes, and hypertension are comorbidities associated with increased risk for developing the severe form of COVID-19. Data attributed to the Centers for Disease Control and Prevention (https://www.cdc.gov/coronavirus/2019-ncov/need-extraprecautions/groups-at-higher-risk.html) shows that diabetes is one of the major risk factors for fatal outcomes from COVID-19. Considering that diabetic patients usually have hyperglycemia, impaired immune function, and several comorbidities such as hypertension, dyslipidemia, and cardiovascular disease, this group is more susceptible to be severely infected by SARS-CoV-2.[4] Another important risk for fatal COVID-19 is obesity, particularly in males.[5,6] Moreover, COVID-19 also affects more severely individuals with metabolic syndrome, probably because these patients have a pro-inflammatory condition that may contribute to enhance the COVID-19-mediated host immune dysregulation.[7] In addition, data from Italy show the CFR is over a third higher for men as compared to women.[3] Interestingly, the authors pointed that although the bias is observed at all ages apparently there is a reduction in the relative risk in older man.[3] To date, many recent publications are arising to reinforce the role of sex bias in COVID-19,[8,9] which suggests that it might be potentially considered in future public health policies.

Coronaviruses are single-stranded and enveloped RNA viruses that belong to the Coronaviridae family.[10] Several members of this family circulate in the human population

and usually lead to mild respiratory diseases. However, two coronavirus subtypes, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), cause the severe respiratory diseases SARS and MERS, respectively.[11,12] Coronaviruses have four structural proteins—spike proteins (S, spike), envelope (E), membrane (M), and nucleocapsid (N).[13] Protein S is important for virus binding, fusion and entry into host cell, and therefore, is a potential target for drugs or vaccines. Once in the respiratory system, coronavirus entry into the host cells appears to depend on the interaction between S protein on the surface of the virus and angiotensin-converting enzyme 2 (ACE2) molecules on the outer side of lung epithelial cells,[14] and uses the type II serine protease (TMPRSS2) for spike protein priming.[15] When viral S protein binds to ACE2, it is cleaved by TMPRSS2 into S1 and S2 subunits. S1 is bound to ACE2, and S2 is sequentially cleaved into S2' which has the role of promoting the fusion of the viral envelope with the cell membrane.[16,17] Besides lung localization, ACE2 and TMPRSS2 are also expressed in intestine, kidney epithelial cells, and endothelial cells.[18] The respiratory and intestinal epithelial cells are the target cells for the replication of SARS-CoV-2, which leads to cytological changes and clinical symptoms.[19] Therefore, the ACE2 enzyme acts as the main receptor, which mediates SARS-CoV and SARS-CoV-2 entry into human gut and lung cells, and both ACE2 and TMPRSS2 are possible therapeutic targets for COVID-19. An important factor is that SARS-CoV-2 appears to have a higher affinity for ACE2 than SARS-CoV,[20] which could explain the significantly larger number of both infected patients. However, the presence of ACE2 alone does not seem to be sufficient to make cells susceptible to infection. SARS-CoV has failed to infect some cell types, such as endothelial and intestinal cells, that have high ACE2 expression.[21] In contrast, some cells that have a low amount of these receptors, such as hepatocytes, could be infected,[22] which reinforces the importance of looking for other mechanisms.

## 1.2 | ACE2 in lung and differences in age and gender

ACE2 is a surface membrane protein that is implicated in heart function, diabetes, hypertension, and several viral infections in the pulmonary system, such as the SARS-CoV and SARS-CoV-2.[23-25] ACE2 is prominent in pulmonary tissue—including alveolar and bronchial epithelium, the lung parenchyma, and pulmonary vascular structures.[17] ACE2 appears to be increased in the lungs of patients with lung diseases such as chronic obstructive pulmonary disease (COPD),[26] and when this protein is overexpressed it confers a protective effect in pulmonary hypertension, and subsequent cardiac remodeling.[27-29]

Jia et al determined several features regarding ACE2 distribution in pulmonary cells and the impact it has on coronavirus infection susceptibility. Localization of ACE2 on the surface of pulmonary cells is highly polarized in human airway epithelia. As a result, coronavirus had significantly higher transduction efficiency in the highly ACE2 populated, apical cell surface in cultured primary human pulmonary epithelial cells, compared to basolateral exposed groups.[25] Pulmonary cell differentiation state is also highly associated with cell susceptibility to coronaviruses. These authors also demonstrated that while fully differentiated pulmonary epithelial cells highly express *ACE2* mRNA and have a high density of ACE2 protein in their membranes, undifferentiated epithelial cell membranes have little to no ACE2 expression on both the mRNA and protein level and, as expected, show strong resistance to transduction in comparison to their fully differentiated counterparts.[25]

Besides the cellular factors of membrane polarization and differentiation state that play important roles in ACE2 localization and expression in pulmonary epithelial cells, organism-scale factors such as age and sex also impact ACE2 presence in the pulmonary system. ACE2 maps to the X sex chromosome and has been considered to potentially have a sex-dependent expression profile in both gene and protein form.[30] On a fetal and neonatal level, both mRNA and protein expression of renal ACE2 increased from birth through the first year of life with no sex difference in ACE2 expression present at this early stage of development in a sheep model.[31] This absence of sex differences in ACE expression in early life stages was observed in other species as well. For example, the ACE2 expression in lung epithelium of young adult and middle-aged rats demonstrated no sex differences, although both male and female groups showed decline in ACE2 protein levels with age.[30] It is only in the old rat group that a significant sex difference in ACE2 expression is seen in this study—with old male rats demonstrating a significantly lower level of ACE2 when compared to the corresponding female group.[30] Taken together, these studies suggest that though ACE2 is located on the X sex chromosome, that ACE2 expression is stable and similar between the sexes from fetal development through middle age, and that only once late-stage life is reached will males experience significantly lower levels of ACE2 than female counterparts.[30,31] Furthermore a recently published review paper that shed light on the ACE2-estrogen pathway in COVID-19, which relates the loss of ACE2 to the development of venous thromboembolism in patients with SARS-CoV-2—adding evidence to the current trend that suggests the lower levels of ACE2 in SARS-CoV-2 patients may be associated to higher rates of negative patient outcomes.[32] Dalpiaz and colleagues showed that ACE2 expression is elevated in spontaneously hypertensive male mice compared to female mice, and that it correlated with cardiac hypertrophy. This was reversed by orchiectomy, followed by

improvement of cardiac performance, and females ovariectomized had more ACE2 expression and a higher incidence cardiac hypertrophy.[33]

Epidemiological studies have shown that aging can be an important risk factor of viral infection. A study on SARS-CoV showed that individuals under 25 years old had mild to moderate symptoms, whereas elderly people over 60 years old have a mortality rate greater than 50%.[34,35] The same profile was observed in the laboratory with animal models, where young B6 mice (6 to 10 weeks old) were resistant to SARS-CoV infection, and mice over 5 months of age were highly susceptible to infection.[36,37] An epidemiological study by Karlberg et al showed that incidence and mortality rates were lowest in young women (0-44 years old) and were increased in women between the ages of 45 and 74. Notably, the protective effect is completely lost in patients over 75 years old, where similar mortality rates are seen in both sexes.[38] These results suggest that there is a time-dependent loss of protection to viral infection in women. Likewise, data from MERS outbreaks showed that the age groups most affected were 45 to 59 years old and ≥60 years old. It is also important to highlight that the number of deaths in women is higher with age from 45 to 59 years old,[39] which typically corresponds to the beginning of the menopause.

A possible explanation for this age-related increase in the number of cases and mortality rate may be associated with the decline in immune response in older populations.[40] The immune system declines with age, a phenomenon also known as immunosenescence.[41] Age leads to reduced adaptive immune function and increased pro-inflammatory activity.[42] In addition, preexisting diseases common in older adults including hypertension, coronary heart disease, and diabetes can increase the risk of COVID-19.[43] However, these factors do not explain the potentially increased susceptibility of the male gender to these infections.

## 1.3 | Lung inflammation induced by COVID-19

Inflammation is a natural defense mechanism of the body to remove harmful stimuli such as pathogens and initiate the recovery process. SARS-CoV-2 induces COVID-19 that, in its extreme form, can induce severe pneumonia with intense lung inflammation and the release of high levels of cytokines. Acute lung injury present in COVID-19 is associated with coagulation alterations and pulmonary embolism that can impair gas exchange and quickly lead the patient to death.

The epithelial tissue of the respiratory system acts as a barrier that actively regulates local immunity with the ability to signal and produce cytokines when activated, and is essential for maintaining the respiratory system tissue homeostasis.[44,45] Lung tissue biopsy of SARS-CoV-2 patients revealed diffuse alveolar damage, epithelial cells peeling with AT2 (alveolar type 2) pneumocytes reactive hyperplasia, fibrinous exudate associated with interstitial fibrosis and chronic inflammation.[46] Immunohistochemistry stains revealed the presence of Rp3 NP SARS-CoV-2 protein mainly in alveolar epithelium cells, including those that were peeled and injured in the alveolar space.[46] The radiological findings of COVID-19 are variable, but there is a consensus that most of the patients have bilateral lung involvement with the presence of ground-glass opacity in computed tomography.[46-48] Radiological changes that occur in SARS-CoV-2-induced pneumonia are compatible with radiological findings of severe respiratory infection, in many cases similar to what is observed in acute respiratory distress syndrome (ARDS). Severe pneumonia, ARDS, sepsis, and septic shock[19] are the most common consequences of COVID-19 pulmonary infection.

Among patients with SARS-CoV-2 infection admitted in intensive care units (ICU), approximately 67%-85% developed ARDS[1] making it the leading cause of mortality.[49] ARDS is defined as an acute and diffuse inflammatory lung injury, which triggers pulmonary vascular permeability, an increase in lung mass due to alveolar edema, and loss of pulmonary tissue due to tissue destruction. The pathophysiology of ARDS is marked by the recruitment and the activation of PMN (polymorphonuclear cells), especially neutrophils, and the consequent release of pro-inflammatory mediators, such as cytokines and chemokines, as well as ROS (reactive oxygen species) and RNS (reactive nitrogen species). Importantly, alveolar edema and the cytokine storm have a major impact on respiratory failure observed in ARDS. According to the American-European Consensus Conference (AECC), ARDS is classified by oxygenation level as mild ($PaO_2/FiO_2$ 200 ≤ 300 mm Hg), moderate ($PaO_2/FiO_2$ 100 ≤ 200 mm Hg), and severe ($PaO_2/FiO_2$ ≤ 100 mm Hg).[50] Similar symptoms have been observed in patients with SARS-CoV-2-induced respiratory failure with impairment of both lungs and severe acute respiratory failure with oxygen-refractory hypoxemia. The incidence of ARDS in the US population among adolescents is relatively low when compared with elderly populations,[51,52] which may contribute to the higher mortality rate due to SARS-CoV-2 the elderly and immunosuppressed individuals.

It has been recognized that epithelial dysfunction is an important contributor to pulmonary injury in patients with ARDS.[53] Airway epithelial cells are involved in the secretion of several molecules as surfactant components and pro-inflammatory mediators.[54,55] Interleukin 6 (IL-6) and Interleukin 8 (IL-8) are strongly involved in respiratory acute lung injury. Pires-Neto et al showed that airway epithelial cells from patients with ARDS have increased expression of IL-8 and IL-6 compared with controls.[56] In ARDS patients, these cytokines are found in high levels in both serum and

bronchoalveolar lavage.[57,58] In sepsis, IL-6 is one of the cytokines that is initially released in acute phase[59] and the chemotactic cytokine IL-8 is correlated to neutrophil recruitment and severity of lung injury.[60] Is important to consider that epithelial cells can also interact with immune cells such as neutrophils, influencing the signaling pathways. Once in the lung, SARS-CoV-2 also can activate immune cells and cytokines.

Several studies have analyzed the bronchoalveolar lavage of patients with ARDS and also observed an increase in Tumor Necrosis Factor-α (TNFα) levels.[61,62] Interestingly, in patients who developed severe SARS-CoV-2-induced infection, high levels of several cytokines such as IL-2 (Interleukin-2), IL-7 (Interleukin-7), IL-10 (Interleukin-10), G-CSF (Granulocyte colony-stimulating factor), IP10 (Inducible protein 10), MCP1 (monocyte chemoattractant protein-1), MIP1a (Macrophage Inflammatory Protein 1 alpha), and TNF-α were observed.[1] Some evidence described the occurrence of cytokine storm syndrome in patients who developed severe forms of COVID-19.[63] Lagunas-Rangel and Chávez-Valencia suggested that high ratio of IL-6/IFN-γ can be associated with the severe form of COVID-19.[64]

It was proposed that T lymphocytes are involved in the pathogenesis of COVID-19 and provide defense against SARS-CoV-2. Wan et al showed that the CD4 + T and CD8 + T were more reduced in the group that developed the most severe form of the disease compared with those that developed the mild form of COVID-19.[65] This has been previously reported in SARS-CoV by Chen et al.[66]

It should be noted that ARDS as well as SARS-CoV-2-induced severe pneumonia and acute lung inflammation and ARDS do not have a specific treatment, which reinforces the importance of several research groups seeking therapeutic alternatives to reduce mortality and impact on national health systems.

## 1.4 | Estrogen role in lung inflammation

The progress of COVID-19 plays a major role in the lungs—therefore, targeted modulation of cytokine secretion and hyperinflammation can be an important therapeutic strategy. Evidence from both clinical and experimental studies strongly suggests that estrogen modulates innate and adaptive immune responses. Estrogens can act in either pro or anti-inflammatory roles depending on the cell type or dose, but it is recognized that this hormone interferes with the prevalence and severity of lung diseases.[67] 17β-estradiol, the predominant circulating estrogen, can modulate both immune cells as well as cytokine release. ERs (estrogen receptors) were detected in immune cells such as neutrophils and macrophages[68] and ERα was detected in both resident lung and inflammatory cells.[69] Low doses of 17β-estradiol can enhance pro-inflammatory cytokines production (IL-1, IL-6, and TNF-α), whereas high or sustained concentrations were able to reduce pro-inflammatory cytokines release.[70] The activation of the ERs can modulate pro-inflammatory cytokines due to inhibition of NF-κB, an important nuclear factor for cellular signaling, limiting the severity of the inflammation.[71,72]

In ARDS, one of the severe complications of COVID-19, experimental studies have suggested a protective role of estrogen. Rats submitted to acute lung injury induced by seawater aspiration have pulmonary edema reductions by downregulation of aquaporins after the administration of 17β- estradiol.[73] Doucet et al showed a reduction in lung injury in ovariectomized rats treated with 17β-estradiol or agonist receptors,[74] and Vieira et al demonstrated that lung injury is attenuated by 17β-estradiol in brain-dead rats and this effect is related to the regulation of NO (nitric oxide) synthases by estrogen.[75] In addition, Fantozzi et al showed that treatment with 17β-estradiol administered before the induction of acute lung injury induced by intestinal ischemia and reperfusion prevented the systemic and pulmonary release of pro-inflammatory cytokines.[76]

In virus-induced lung inflammation, exogenous estrogen treatment in female mice infected with H1N1 reduced pulmonary inflammation and the levels of pro-inflammatory genes, protecting females from a severe form of influenza.[77] High estrogen concentrations in females with SARS-CoV was able to reduce cytokine storm and eliminated the inflammatory cells.[78]

Neutrophils are the central cells to host defense against viral infection. Infected female mice that received the administration of 17β-estradiol showed elevated chemo attractants recruiting neutrophils into the lungs and adaptive T cell responses.[79] In COVID-19 patients, T cells seem to be downregulated and are correlated to hospital death and severity of lung damage.[80] Estrogen-regulated T cell-mediated autoimmune inflammatory diseases reduced genes associated with pro-inflammatory cytokines in the lungs and reduced antibody titers during Influenza infection.[77]

Together, these data suggest that estrogen can be a tool to be considered in the treatment of COVID-19 since estrogen not only acts on virus receptors, but also affects acute lung inflammation by the modulation of immune responses.

## 1.5 | ACE2 and estrogen interaction

SARS-CoV-2 has been associated with higher mortality rate in male patients than in female patients.[81] According to New York City Health officials, updated data from August 11th 2020 the death rate per 100 000 people was over 283 men vs 173 women (https://github.com/nychealth/coronavirus-data/blob/master/by-sex.csv, accessed on 08/11/2020 14:00

GMT). Some hypotheses have been raised, such as a greater tendency among men to become smokers or adopt detrimental health habits though this is contradicted by a study conducted in 2017 by Channappanavar et al with SARS-CoV, which showed that in experiments with mice this proportion is repeated.[78] In this study, experiments performed on young and elderly mice demonstrated that the susceptibility to infection is age-dependent. This group also tested the hypothesis that the infection may be sex-dependent, which showed that mice ovariectomized or treated with ERs antagonist (fulvestrant (ICI)) presented increased susceptibility to infection, evolution to severe cases, and even lethality similar to male mice. This susceptibility may be related to a more aggressive and less specialized immune response and a greater sensibility to infectious agents in men, as well as a stronger adaptive innate response and a greater resistance to viral infections in women. These characteristics are attributed to steroidal hormones and to different numbers of copies of immune response genes linked to the X chromosome. Estrogen in low concentrations has an immune-stimulation function and its signaling pathway blocks viral replication by modulating genes that regulate metabolic functions.[78] Moreover, as discussed above estrogens also modulate pulmonary inflammation and lung damage in several models of acute inflammation including lung inflammation induced by virus exposure.[76,77] Thus, these findings suggest that estrogen can have a protective effect against SARS-CoV-2-induced pneumonia.

As already described, the presence of ACE2 is necessary for virus entry inside host cells. Higher expression of the ACE2 may cause a more efficient viral infection, which could make diabetics and people who use antihypertensive drugs more susceptible.[82] Recent studies demonstrate that G1 ((±)-1-[(3aR*,4S*,9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]- ethanone), a selective GPER1 (G protein-coupled estrogen receptor 1) agonist, reduced ACE2 activity, expression of angiotensin II receptor type 1 (ATR1), and immunoreactivity to angiotensin II.[83] Lu et al showed that *ACE2* silencing was able to reduce the replication of SARS-CoV in vitro, suggesting a possible combined therapy between RNAi and non-RNAi strategies could reduce viral infection.[84] A network map showing the interactions among *ESR1, ESR2, RAGE* (receptor for advanced glycation end-products, see below), *ACE2, and TMPRSS2* are shown in Figure 1.

Angiotensin I and II is cleaved by ACE2 in angiotensin 1-9 and angiotensin 1-7 (with vasodilator actions), respectively. For this reason, ACE2 has been pointed to as a potential participator in the regulation of heart function and target in cardiovascular diseases, hypertension.[85] Although ACE2 has a protein structure that is similar to ACE, it is not inhibited by ACE inhibitors. Instead, ACE inhibitors and angiotensin receptors blockers, usually prescribed to treat hypertension, may increase the amount of ACE2 formation.[86] Therefore, an issue of intense debate has been raised in the medical

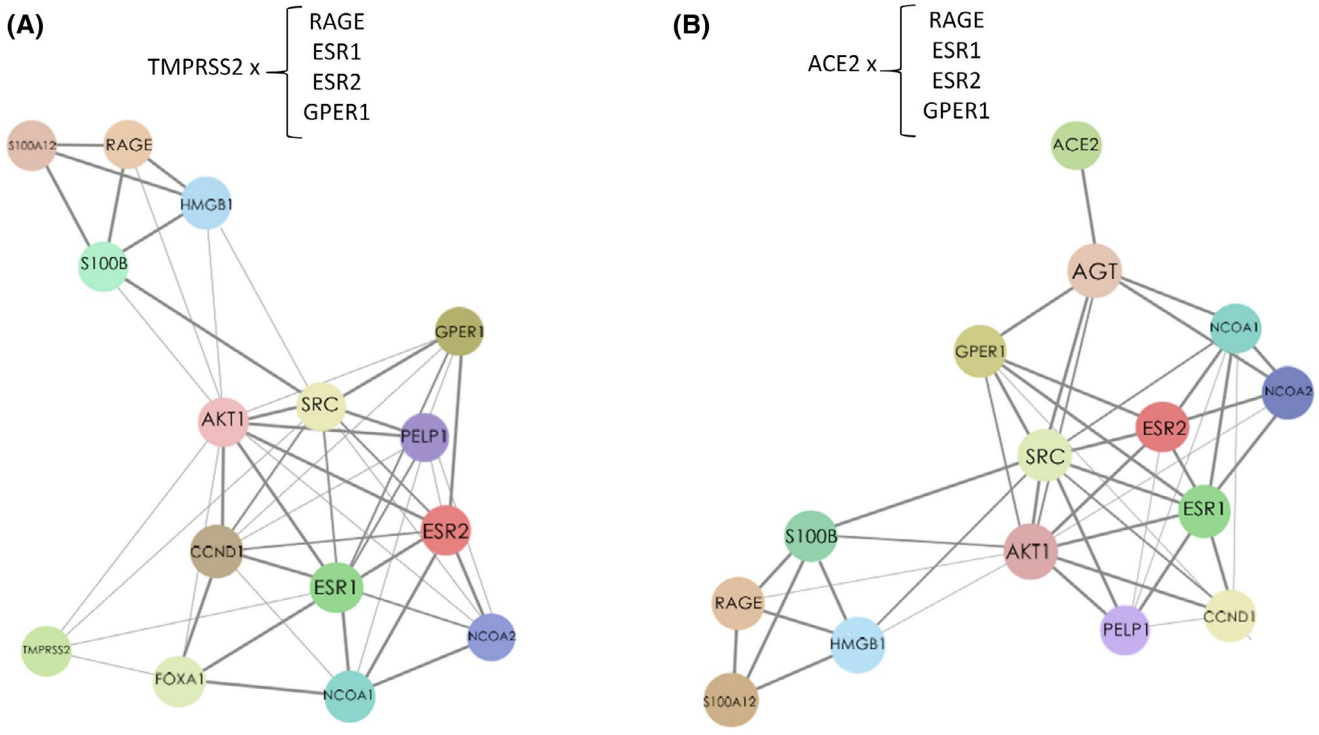


**FIGURE 1** Network map of correlative expression of ERs (estrogen receptors) and related signaling genes (*GPER1*, *ESR1,* and *ESR2*) with *RAGE*, also associated to *TMPRSS2* (A) and *ACE2* (B) expression. Adapted from https://string-db.org/[144,145]

community, suggesting that hypertensive patients may be more vulnerable to SARS-CoV-2 infection, potentially by the use of those drugs.[82] It is important to highlight that this is still controversial and there is currently no consensus in the field regarding these medications.[87] Carey et al showed that the use of angiotensin II receptor blockers and ACE inhibitors in hypertensive patients should not be discontinued during the pandemic induced by COVID-19.[88] There is evidence that shows that estrogen agonists are capable to reduce ACE2 activity.[83] Considering these findings and given the long time and expensive costs associated with licensing new therapies, the study and use of drugs that modulate the estrogenic route as a possible therapeutic target for COVID-19 is a promising strategy.

Experimental and clinical studies have shown that the renin-angiotensin system undergoes sex-related changes.[89,90] 17β-estradiol may be involved in this effect since it regulates the expression of ACE2.[91-93] In a clinical study, it was observed that treatment with estrogen increased the gene expression of *ACE2* and reduced *ACE* in the atrial tissue of male donors, while treatment with the ERα antagonist (MPP—1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinyl ethoxy) phenol]-1H-pyrazole) reversed this effect.[94] Similarly, a discussion was conducted in a paper by da Silva et al, which showed that in ovariectomized female rats there was a reduction in the expression and activity of ACE2.[95] The direct effect of estrogen to increase ACE2 expression can occur through the interaction of ERα and ERE on the ACE2 promoter.[96] However, these effects may change depending on the tissue involved, since in human endothelial cells there was an estrogen-mediated increase in the protein expression of ACE1, but not ACE2.[97] The lung tissue of young and middle-aged male and female rats (3 and 12 months) have a similar expression of ACE2. However, in old animals (24 months) there was a more significant decline in ACE2 expression in male rats.[30]

Of note, the exact role of estrogen in ACE2 expression is not fully elucidated. Recently, Stelzig et al reported that 17β-estradiol is able to reduce the ACE2 mRNA expression in normal human bronchial epithelial cells.[98] In addition, according to Kuba et al, ACE2 knocked out mice showed less SARS-CoV infection rate induced and demonstrated an attenuation in lung pathological alterations.[99] However, these authors also showed that the infection with SARS-CoV in wild-type mice reduced the ACE2 expression in the lung. It is known that ACE can be involved in the protection of acute lung injury,[100] including the potential association of angiotensin II to lung injury and viral load found in COVID-19 infected patients.[101] Although ACE2 is relevant to the

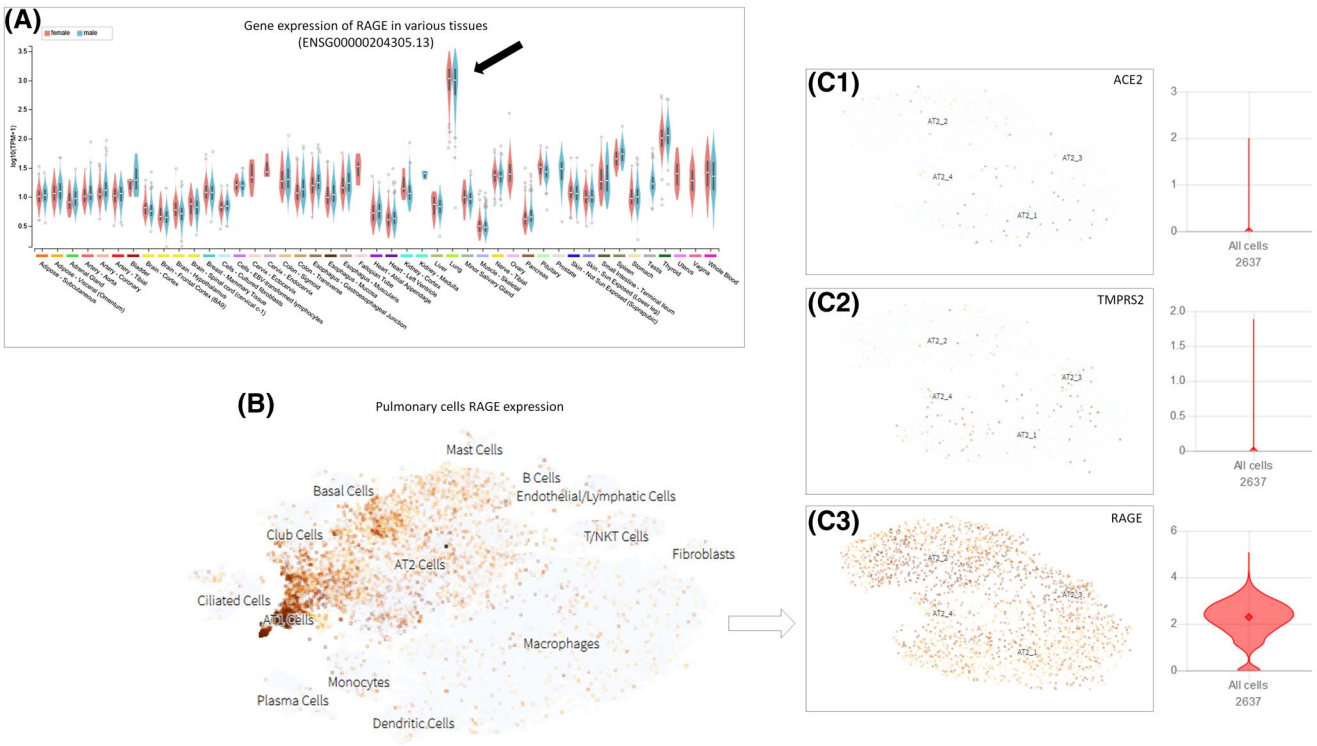


**FIGURE 2** Expression of *RAGE* in different tissues, mostly expressed in AT2 (alveolar type 2 pneumocyte) cells in lung. A, Values and correlations of *RAGE* gene expression in many organs, mostly in the lung (dark arrow). Adapted from https://gtexportal.org/.[146,147] B, Data set Genes collected from integrated single-cell RNA-Seq analysis of patients with pulmonary fibrosis. *RAGE* (C3) detectable expression is far more pronounced in AT2 cells compared to other important proteins in SARS-CoV-2 infection process, such as *ACE2* (C1) and *TMPRSS2* (C2). Adapted from https://www.nupulmonary.org/[148]

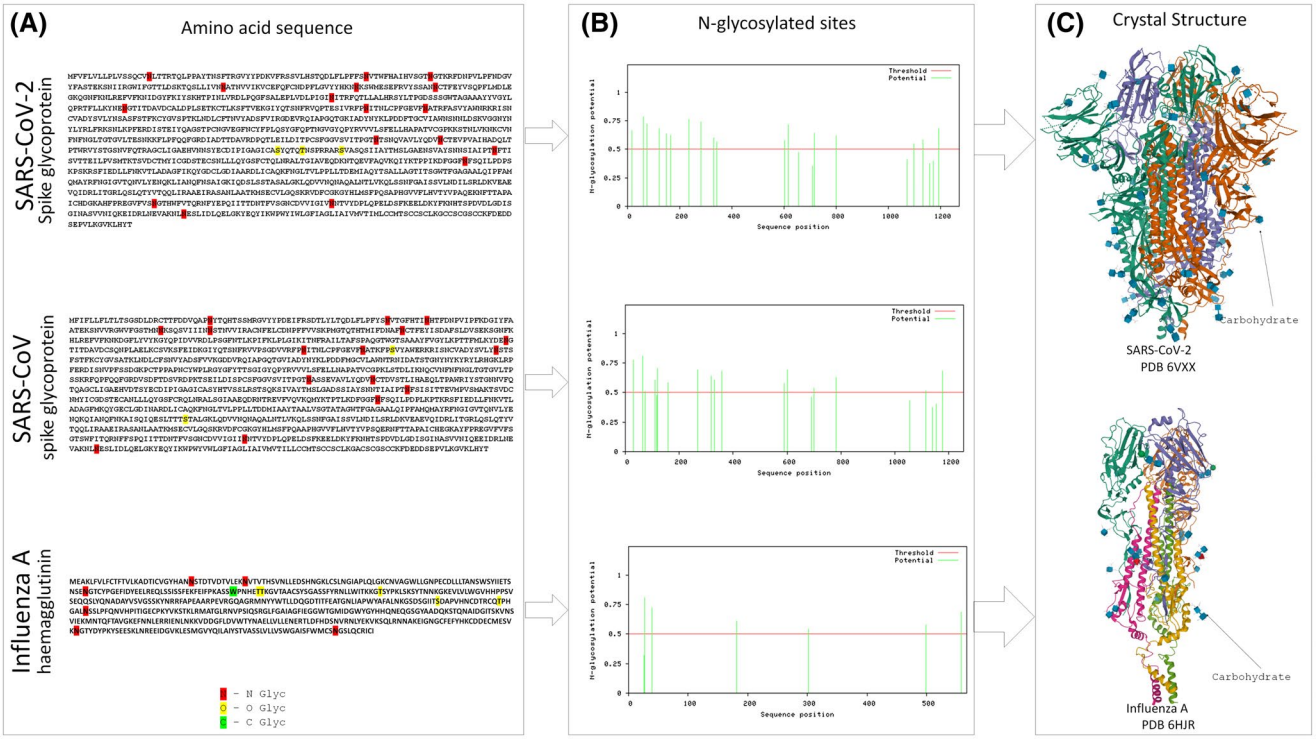


**FIGURE 3** Comparison of potential glycosylation sites in amino acid sequence (A) of the spike protein of SARS-CoV-2 (GenBank QIC53213.1) and SARS-CoV (GenBank AAU04646.1) vs hemagglutinin Influenza A (GenBank BAA01280.1), shows similar numbers of N, O, C glycosylation in coronavirus and fewer glycosylation points in H1N1, as illustrated in histograms of N-glycosylation sites (B). Adapted from www.cbs.dtu.dk/services/NetNGlyc (Gupta, Jung and Brunak. In preparation, 2004) www.cbs.dtu.dk/services/NetCGlyc[149]; www.cbs.dtu.dk/services/NetOGlyc.[150] C, The crystallography structure of spike protein SARS-CoV-2 and hemagglutinin of Influenza A extracted from Protein Data Bank (https://www.rcsb.org; PDB ID: 6VXX,[151] PDB ID: 6HJR1[152]) shows dark arrows pointing carbohydrate sites, which are more frequent in SARS-CoV-2

coronavirus cell entry, the reduction in ACE2 levels after the infection can be associated with severe lung injury.[99]

Thus, it is not clear yet why women are less susceptible to COVID-19 infections if the expression of ACE2 is greater in the lung tissue. For example, estrogen and androgen compounds reduce TMPRSS2 levels in MCF-7 cells,[102] suggesting its potential role of steroid hormones in COVID-19 therapeutic strategy.

## 1.6 | The role of RAGE related to estrogen signaling: possible connection to risk factors in COVID-19

Many factors are associated with enhanced susceptibility to cardiovascular diseases and diabetes, which has recently been linked to declining clinical conditions in COVID-19 patients. One receptor involved in those actions is the receptor for advanced glycation end-products (RAGE), which is mostly expressed in the lungs (Figure 2). RAGE activation produces a pro-inflammatory response via NF-κB maintenance response by enhancing NF-kappaBp65 expression and degradation of IkappaB.[103] There are many endogenous ligands described, such as S100A,[104] and AGE (advanced glycation end-products),[105] produced by nonenzymatic glycation of proteins, which increases with age[106] and correlates to disease comorbidities.[107,108] These receptors also recognize pathogens and promote the activation of immune responses to infection. Other endogenous ligands of RAGE, such as HMGB1 could be potentially associated with viral infection such as SARS-CoV[109] (Figure 1). Other molecules, such Pathogen-associated molecular patterns (PAMP) also activate RAGE,[110] and SARS-CoV was previously suggested to be an antigen for RAGE activation.[111]

Some of SARS-CoV proteins, particularly M and 3a of coronavirus can be glycosylated by N-linked or by O-linked oligosaccharides,[112] and this glycosylation pattern could be potentially investigated in SARS-CoV-2 (Figure 3). Kumar et al analyzed the amino acid sequence of SARS-CoV and SARS-CoV-2 and found 23.6% variation in S glycoprotein receptor-binding domain, which suggests a significant difference in binding and infectivity of the new coronavirus.[113] Moreover, glycosylation is important in several viral infections such as influenza, Zika, and coronavirus, due to virus strategy to evade immune system and life cycle.[114]

**TABLE 1** Summary of possible effects of estrogen on RAGE and RAGE ligands

| Effects on RAGE ligands | Possible effects on inflammation | Reference |
|---|---|---|
| Estrogen inhibits RAGE expression and oxidative stress and for this reason, estrogen therapy did not have effect in diabetic woman | Estrogen has a protective effect on inflammation | 115 |
| Estrogen inhibits the synthesis of AGE, the substrate of RAGE in vaginal epithelial tissues of postmenopausal women | These findings indicate a potential anti-inflammatory and protective role for estrogen | 138 |
| Direct evidence of the regulation of RAGE by ERs (in addition to AGE and TNF-α) has been demonstrated in endothelial cells | Estrogen has a protector effect on inflammation | 116 |
| The inhibition of intracellular AGE accumulation with pyridoxamine may protect glomeruli against age-related oxidant stress by preventing an increase of TGFβ production and by regulation of the estrogen receptor | Inhibition of RAGE has a protector effect on inflammation | 117 |
| Lifestyle changes can affect AGE production which would benefit the patient, as breast cancer survivors who practiced physical activity had reduced circulating AGE levels | Reduced AGE production is beneficial in estrogen-positive breast cancer | 118 |
| Several aspects of estrogen therapy in vascular inflammation related to increased tyrosine nitration of proteins and the production of ROS and NO, including RAGE pro-inflammatory actions | Estrogen has a pro-inflammatory action by increasing RAGE | 121 |
| Postmenopausal women treated with conjugated estrogens combined with progestin had elevated levels of NO in serum | High NO levels may produce benefits in cardiovascular system | 122 |
| Female mice that overexpress S100A4/Mts1 (ligand for RAGE) presented greater expression of this protein in pulmonary arterial compared to male, which correlated to elevated pulmonary vascular remodeling and risk to develop pulmonary arterial hypertension in female mice, despite the similar levels of RAGE in both sexes | Treatment with ligands for RAGE is deleterious particularly in female animals | 139 |

*Note:* RAGE: receptor for advanced glycation end-products; AGE: advanced glycation end-products.

Abbreviations: ER, estrogen receptors; NO, nitric oxide.

The inhibition of RAGE-signaling pathway has been suggested to be in close association with estrogenic-mediated protection in cardiovascular diseases.[115] In addition to the effects on AGEs and TNF-α, direct evidence of the regulation of RAGE by ERs, which are mediated by Sp-1 protein complex, suggests that it might be a contributing factor for diabetic microvasculopathy.[116] To exemplify this possible association, pyridoxamine, an inhibitor of AGE production, was shown to affect the expression of kidney ERα in vivo,[117] one of the most affected systems by SARS-CoV-2 infection. Interestingly, lifestyle changes can affect AGE production which would benefit the patient, as breast cancer survivors who practiced physical activity had reduced circulating AGE levels,[118] and Vlassara et al showed that AGE dietary restrictions positively correlated to insulin resistance amelioration.[119] To our knowledge, the comparison of RAGE expression in male and female has not been fully addressed yet. However, males showed higher levels of HMGB1-mediated renal injury induced by ischemia and reperfusion compared to females,[120] and HMGB1 is a RAGE ligand.

The impact of estrogen in inflammation was reviewed by Chakrabarti et al, considering several aspects of estrogen therapy in vascular inflammation related to increased tyrosine nitration of proteins and the production of ROS and NO, including RAGE pro-inflammatory actions.[121] As an example, postmenopausal women treated with conjugated estrogens combined with progestin had elevated levels of NO in serum.[122] In a model of acute lung inflammation induced by intestinal ischemia and reperfusion, the anti-inflammatory effects of 17β-estradiol are dependent on the effects of NO produced by eNOS (endothelial nitric oxide synthase).[123] Therefore, considering the regulation of RAGE and its association to diabetes and hypertension, it is noteworthy that one possible cause of the gender disparity in COVID-19 cases could be linked to RAGE and steroid hormone signaling. The possible effects of estrogen on RAGE signaling are detailed in Table 1.

Specifically in the lung, treatment with either anti-RAGE mAb (monoclonal antibody) or sRAGE (soluble RAGE) is suggested to increase arterial oxygenation, reduce alveolar inflammation, and improve lung damage in acute lung inflammation[124,125] suggesting that the inhibition of RAGE can have a potential therapeutic effect in ARDS. Although there is evidence that RAGE signaling is involved in lung inflammation,[126] there is a lack of studies focused on inhibitors of this pathway in models of lung diseases.

Drucker et al have raised a possible association between diabetes and coronavirus infection by considering the impact of glucose-lowering therapies on the levels of ACE2 in urine samples, which are found to be increased in diabetes type 1 and 2.[127-129] Therefore, this disease are present in many cases

of severe COVID-19, and it has been suggested that particularly uncontrolled status of hyperglycemia may induce changes in glycosylation of the ACE2, together with glycosylation of the viral spike protein.[130] In addition, it has been discussed by Rojas et al the potential participation of RAGE in COVID-19's inflammatory response in lungs, exploring the role of AT1R-mediated signaling in RAGE transactivation, then, associating angiotensin to inflammation.[131] In this context, the role of RAGE and estrogens should be investigated to draw a comprehensive connection between metabolic activity and susceptibility to COVID-19. In pulmonary fibrosis, RAGE is located in alveoli, but not in fibrotic tissue,[132] suggesting that the inflammatory process is reduced over time.

## 1.7 | Theoretical effects of estrogen on COVID-19

A recent paper potentially correlates sex hormones to disease severity by relating preexisting chronic diseases and insulin resistance to defective ER signaling in humans.[133] Importantly, immune response can be also correlated to gender-associated viral infection.[133] In animal models, ERα knockout mice of both sexes present insulin resistance, glucose tolerance, and obesity,[134] all comorbidities associated with COVID-19 aggravation.

Selective estrogen receptor modulators (SERMs) that have been developed and approved as anticancer therapies,

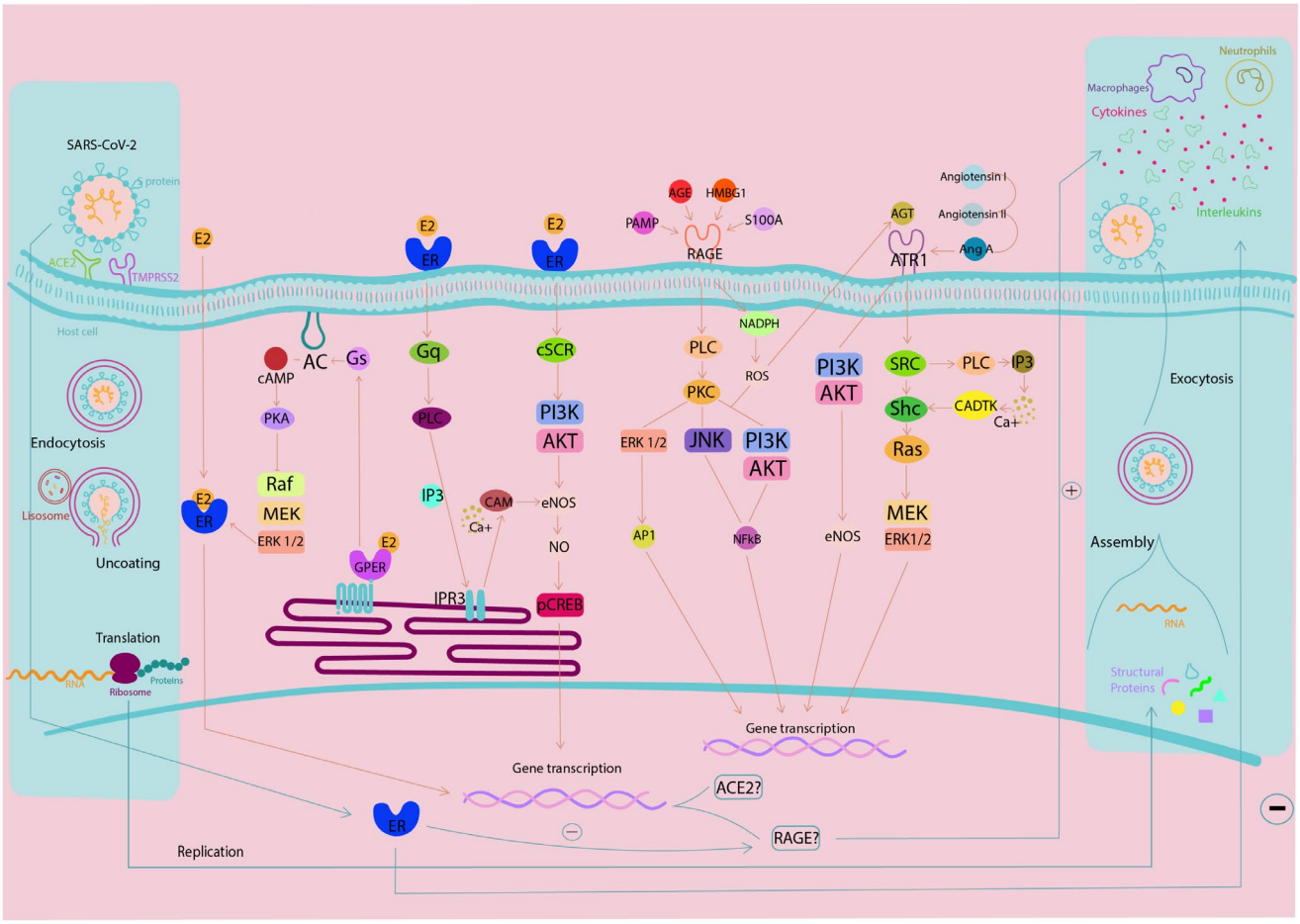


**FIGURE 4** Integrated signaling pathway and summary of events promoted by SARS-CoV-2 infection in human cells. This scheme describes the cellular response to SARS-CoV-2 entry into the cell mediated by ACE2, which can be modulated by estrogen signaling via transcriptional pathway. Under a pro-inflammatory response, RAGE can be stimulated by AGE, PAMP, S100A, or HMGB1, which activates NADPH and PLC (phospholipase C). NADPH elevates intracellular ROS levels, which may act in the PI3K-AKT or AGT (angiotensin) pathway. The PLC activates the PKC, which activates the signaling pathways of ERK 1/2, JNK, and PI3K-AKT. This results in activation of NF-κB and ERK 1/2 that ends in AP1 and gene transcription. Regarding renin-angiotensin signaling, AGT and AngA (angiotensin A) stimulate ATR1, leading to the activation of PI3K-AKT signaling pathway, promoting eNOS release. In turn, ATR1 also activates the SRC (proto-oncogene non-receptor tyrosine kinase), which activates Shc-Ras and finally MEK-ERK 1/2, heading to gene transcription. The estrogen pathway can also interfere with the internalization of SARS-CoV-2, due to its modulation of ACE2 gene transcription via Raf-MEK-ERK 1/2 pathway, which can be mediated by nuclear membrane receptors or associated with G protein. Adapted from KEGG (Kyoto Encyclopedia of Genes and Genomes)[153] and Xiang et al[154] Therefore, according to literature data, here, we propose that estrogen receptors activation may participate in modulation of ACE2 levels and modulation of RAGE cell response, which in turn could reduce inflammation in COVID-19

such as tamoxifen and toremifene, have demonstrated activity against SARS-CoV and MERS-CoV, HCV (hepatitis C virus) and Ebola virus and show low toxicity in cell lines.[135-137] The activity of SERMs can occur through gene transcription activation/inhibition after its interaction with classic ERs (ERα/ESR1; and ERβ/ESR2) or modulation of the GPER1 signaling. Watashi et al (2007) showed that tamoxifen inhibits HCV replication by classic estrogenic pathway blockage. It was observed that the ERα located in the endoplasmic reticulum promotes the interaction between HCV replication complex and NS5B polymerase and favors the replication of this virus, an effect that was revoked by tamoxifen treatment.[135] However, in Zika virus infections, ERα overexpression reduces the replication of this virus by 2000 times, suggesting a broader role for this receptor in viral replication.[138] Very importantly, female mice that overexpress S100A4/Mts1 (ligand for RAGE) presented greater expression of this protein in pulmonary arterial compared to male, which correlated to elevated pulmonary vascular remodeling and risk to develop pulmonary arterial hypertension in female mice, despite the similar levels of RAGE in both sexes.[139] Mukhopadhyay and Mukherjee (2005) reviewed several literature data and contrasted the conflicting aspects of RAGE-estrogen signaling association.[115] For instance, in vaginal epithelium estrogen promotes inhibition of the AGEs' synthesis,[140] but in endothelial cells cultures it upregulates RAGE expression.[116] Considering that estrogen exerts several anti-inflammatory effects in lungs, the role of RAGE is still elusive, keeping this issue under debate in literature, and could be further explored in COVID-19.

SERMs can also play important roles in viral replication by ER-independent pathways.[136] A study performed in HEK293T cells infected with Ebola virus showed that treatment with toremifene inhibited viral infection by membrane glycoprotein conversion blockage into its functional subunits, thus, preventing fusion between the cell and viral membrane. This result was not reproduced in treatments with tamoxifen, 4-hydroxy-tamoxifen and clomiphene for Ebola virus.[141]

How to correlate the production of oxygen-related species, such as ROS and NO, in RAGE/ER signaling is challenging.[115] As estrogen elevates NO levels, and considering that AGE leads to excessive generation of ROS,[142] this may result in peroxynitrite generation,[143] promoting deleterious effects on many organs and promoting a pro-inflammatory response.

RAGE evokes an inflammatory response in many types of cells. This pathway is also modulated by estrogen signaling, through the rapid activation of MAPK by estrogen membrane receptors. Figure 4 represents a proposed signaling pathway that could be further explored in SARS-CoV-2 infection. In this context, we are keen on investigating estrogen-related interactions with susceptibility to SARS-CoV-2 clinical condition aggravation, considering the role in modulating ACE2 levels and RAGE intracellular signaling response, which eventually may result in reduced inflammation and cell protection.

## 2 | CONCLUSIONS AND PERSPECTIVES

Overall, regarding COVID-19 risk factors, metabolic syndromes are exacerbated in the aging process, and therefore, may be closely associated with deteriorating clinical conditions of patients caused by SARS-CoV-2 disease progression. To date, no evidence exists to illuminate the role of RAGE or AGE to COVID-19, however, given that metabolic diseases are rampant in vulnerable aging populations, they should be the focus of further investigations regarding the complex SARS infection pathway given that both diabetes and hypertension are comorbidities associated with high fatality rates. Remarkably, the clinical condition aggravation in men compared to women opens the possibility to explore the role of estrogens in disease management, considering that experimental data correlates ERs stimulation to ACE2 levels. In addition, the well-known connection of estrogens to inflammation could be critical in attempting to balance immune responses to SARS infection. Furthermore, there is a long list of literature and clinical data that supports ER-targeting drugs in the treatment of different diseases, which opens the opportunity for drug-repurposing, with the main advantage of using drugs with human safety already determined. Therefore, future pharmacological directions should address the modulation of the estrogen pathway as a therapeutic strategy for COVID-19.

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### CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

### AUTHOR CONTRIBUTIONS

Designed research (R.P. Ureshino, R.S. Stilhano, C.M. Prado); Performed research (R.P. Ureshino, R.S. Stilhano, C.M. Prado,

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