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SARS-CoV-2 infection risk assessment in the endometrium: viral infection-related gene expression across the menstrual cycle

Ismael Henarejos-Castillo, M.Sc.,^{a,b,c} Patricia Sebastian-Leon, Ph.D.,^{a,b} Almudena Devesa-Peiro, M.Sc.,^{a,b,c} Antonio Pellicer, M.D., Ph.D.,^{a,b,c} and Patricia Diaz-Gimeno, Ph.D.^{a,b}

^a Department of Genomic and Systems Reproductive Medicine, IVI-RMA IVI Foundation; ^b Instituto de Investigación Sanitaria Hospital Universitario y Politécnico La Fe; and ^c Department of Pediatrics, Obstetrics and Gynaecology, University of Valencia, Valencia, Spain

Objective: To determine the susceptibility of the endometrium to infection by—and thereby potential damage from—SARS-CoV-2.

Design: Analysis of SARS-Cov-2 infection-related gene expression from endometrial transcriptomic data sets.

Setting: Infertility research department affiliated with a public hospital.

Patient(s): Gene expression data from five studies in 112 patients with normal endometrium collected throughout the menstrual cycle.

Intervention(s): None.

Main Outcome Measure(s): Gene expression and correlation between viral infectivity genes and age throughout the menstrual cycle.

Result(s): Gene expression was high for *TMPRSS4*, *CTSL*, *CTSB*, *FURIN*, *MX1*, and *BSG*; medium for *TMPRSS2*; and low for *ACE2*. *ACE2*, *TMPRSS4*, *CTSB*, *CTSL*, and *MX1* expression increased toward the window of implantation. *TMPRSS4* expression was positively correlated with *ACE2*, *CTSB*, *CTSL*, *MX1*, and *FURIN* during several cycle phases; *TMPRSS2* was not statistically significantly altered across the cycle. *ACE2*, *TMPRSS4*, *CTSB*, *CTSL*, *BSG*, and *MX1* expression increased with age, especially in early phases of the cycle.

Conclusion(s): Endometrial tissue is likely safe from SARS-CoV-2 cell entry based on *ACE2* and *TMPRSS2* expression, but susceptibility increases with age. Further, *TMPRSS4*, along with *BSG*-mediated viral entry into cells, could imply a susceptible environment for SARS-CoV-2 entry via different mechanisms. Additional studies are warranted to determine the true risk of endometrial infection by SARS-CoV-2 and implications for fertility treatments. (Fertil Steril® 2020;114:223-32. ©2020 by American Society for Reproductive Medicine.)

El resumen está disponible en Español al final del artículo.

Key Words: ACE2, coronavirus, COVID-19, endometrial transcriptomics, SARS-CoV-2

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The rapid international spread of novel coronavirus disease 2019 (COVID-19) (1) has resulted in 5,103,006 confirmed cases and 333,401 deaths worldwide as of May 23, 2020 (2). COVID-19 is caused by

SARS-coronavirus 2 (SARS-CoV-2) infection in the lower respiratory tract (3), but the mechanisms underlying infection remain poorly understood (4). The rapid spread of SARS-CoV-2, genetically closely related to severe

acute respiratory syndrome coronavirus (SARS-CoV-1) (5), has resulted in a global health emergency with long-term consequences on economics markets, nutritional habits, and mental and physical well-being (5–9).

As information is still emerging about the health consequences of COVID-19, assisted reproductive treatments (ARTs) have been delayed due to fear of the unknown impact of SARS-Cov-2 on fertility (10, 11). Fertility is compromised by age, and the longer treatments are delayed, the less likely that successful outcomes will be achieved (12). Furthermore, effects of SARS-CoV-2 infection increase in severity with host age (13–15),

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Reprint requests: Patricia Diaz-Gimeno, Ph.D., IVI-RMA IVI Foundation, Biopolo La Fe, Instituto De Investigación Sanitaria La Fe, Av. Fernando Abril Martorell 106, Torre A, Planta 1ª, 46026 Valencia, Spain (E-mail: patricia.diaz@ivirma.com).

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meaning that women of more advanced reproductive age undergoing ART could be at higher risk of infection. It is therefore critical to determine exactly how the virus affects reproductive physiology. Although the studies to date have been discordant, vertical transmission during pregnancy seems to occur infrequently, ranging from 0 to 11% (11, 16–18). To date there is no information about how infection affects early implantation and development.

The ability of SARS-CoV-2 to damage tissue is determined by its capacity to enter and infect cells in that tissue (4). The SARS-CoV-2 entry point on the cell is angiotensin-converting enzyme 2 (ACE2) (19, 20), which plays a key role in the renin-angiotensin system, cleaving angiotensin II to angiotensin 1–7. The system is disrupted after SARS-CoV-2 gains cell entry and down-regulates the expression of ACE2, leading to up-regulation of the proinflammatory response by angiotensin II (21–23). ACE2 exhibits moderately increased expression with age (24), which could explain disease severity in older people. Another path of entry, using Basigin (BSG) as receptor instead of ACE2, has been proposed (25).

To enter the cell, SARS-CoV-2 uses its spike S protein to bind to ACE2, which leads to fusion with the cell membrane and endocytosis (4, 20, 26). TMPRSS2, a transmembrane protease, cleaves the S protein (27). Cleavage is necessary for the virus to bind to ACE2 and spread through the infected host (19). Other proteases are under investigation as possible implications in SARS-CoV-2 infectivity related to S protein cleaving. TMPRSS4 increased virus infectivity on its own, at least in gut epithelial cells (28), while cathepsins B and L (CTSB and CTSL, respectively) had residual cleaving activity of viral S protein in TMPRSS2- cells (19). FURIN, another protease predicted to cleave S protein, presents alongside ACE2 in epithelial layers of several oral mucosal tissues (29, 30). MX dynamin-like GTPase 1 (MX1) regulates neutrophil infiltration, favoring infection through protein S modification by neutrophil elastase (31).

The clinical presentation of COVID-19 ranges from mild respiratory symptoms to severe progressive pneumonia, gastrointestinal symptoms, fecal shedding, multiorgan failure, and even death (32, 33), but few studies have focused on the virus's effect on fertility and damage to reproductive tissues, or on concerns regarding the use of reproductive treatments. Leydig and Sertoli cells in the testis (34–37), oocytes (38), and ovarian tissue (37) are likely to experience damage due to their medium-high expression of the ACE2 receptor. The endometrium is crucial for human reproduction and embryo implantation, but studies of the effect of SARS-CoV-2 infection on menstrual cycle progression have not been performed. Delineating the virus's impact on the tissue is important for determining risk to ART, given that a healthy endometrium is needed for embryo implantation and growth.

The endometrium is a complex tissue subjected to a cycle of cell death and renewal approximately every 28 days (39). Numerous transcriptomic studies have sought to understand gene expression changes throughout the menstrual cycle (40), and most of these data sets are available in public repositories, such as the Gene Expression Omnibus (GEO) database (41). According to the Human Protein Atlas (HPA) (42), ACE

transcript is in low abundance in endometrium and not present as protein. According to HPA expression levels, *TMPRSS4* and *FURIN* RNA expression is low, and protein levels are medium, whereas *CTSB*, *MX1*, and *BSG* have medium RNA expression and high protein expression (43). However, there is little information on how the virus could affect endometrial receptivity and embryo implantation.

We analyzed the impact of SARS-CoV-2 infection on the endometrium by measuring endometrial *ACE2*, *TMPRSS2*, *TMPRSS4*, *CTSB*, *CTSL*, *FURIN*, *MX1*, and *BSG* gene expression. Transcriptomic data sets available across the phases of endometrial progression were used to evaluate molecularly the risk of SARS-CoV-2 infection during the COVID-19 pandemic.

MATERIALS AND METHODS

Search and selection of SARS-CoV-2 infectivity-related proteins

A thorough literature search identified proteins related to the SARS-CoV-2 disease-causing mechanism, including genes related to cell entry and genes with implications in health and fertility, among others. The search for relevant genes associated with viral infectivity was made chronologically up to May 10, 2020. The keywords searched in PubMed (44) included all possible combinations between “SARS-CoV-2,” “COVID-19,” “coronavirus,” “cell entry mechanisms,” “long-term implications,” and “fertility.”

Search and selection of endometrial transcriptomic data sets

Public transcriptomic data sets were used to analyze expression of SARS-CoV-2 infectivity-related genes throughout the menstrual cycle. Endometrial transcriptomic experiments for control patients (without any known endometrial pathology) were systematically searched in the GEO database (41) with no restrictions on publication date or language and according to preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (45). Keywords were: *uter* OR endometr**, filtered by “*homo sapiens*.” Experiments were selected if

- RNA was extracted directly from human endometrial biopsies.
- Endometrial biopsies were collected at different times during the menstrual cycle.
- Cycle phase at the time of biopsy was available for all samples.
- Endometrial gene expression was evaluated by microarray or RNA sequencing using Affymetrix, Illumina, or Agilent platforms.
- Raw gene expression data were freely available to download from GEO.

Preprocessing and integrative analysis

Transcriptomic raw data were downloaded from the GEO database and processed according to the standards of the technology used (microarrays or RNA-seq) using *limma* R-package (version 3.34.9) (46). Expression data from each

experiment were annotated with biomaRt (version 2.30.0) (47), log transformed, and quantile normalized using limma (46). Principal components analysis (PCA) was done using the `prcomp()` function, and the scores were displayed using the `ggplot2` R-package (48) to look for possible batch effects and were corrected if using linear models. Outliers were deleted from posterior analysis if there was a distinct transcriptional behavior according to PCA.

To integrate the included endometrial transcriptomic experiments, several steps were followed as recommended by Tajti et al. (49): after being independently normalized, selected studies were joined in a unique data set, and batch effects were corrected using linear models (limma R package) (43). A relative expression value of low, medium, and high expression were established. The thresholds respectively correspond to 1% to 10%, 11% to 50%, and 51% to 100% categories of gene expression values of the entire integrated data set.

Pairwise differential expression analysis between experiments was performed using limma to identify genes with expression differences. *P* values were corrected using false discovery rates (FDR) (50), and genes differentially expressed between experiments were removed from the analysis (FDR < .05).

Differential gene expression throughout the menstrual cycle

An analysis of variance (ANOVA) was performed for each selected gene related to SARS-CoV-2 infectivity to assess which genes showed statistically significant differences between endometrial phases. Analysis of variance was followed by a pairwise *t*-test to determine statistically significant differences between phases. Fold changes in gene expression from phase to phase were also calculated. The mean expression and confidence intervals for each gene in each phase were represented using `ggplot2` R-package (version 3.0.0) (45).

Coexpression of infectivity genes and the effect of age

Pearson's correlation (51) was used to assess coexpression between each pair of selected proteins related to SARS-CoV-2 infectivity and to analyze the effect of age (from 23 to 50 years) on the expression of each protein. All programming and statistical tests (*t*-test, Pearson's correlation, and analysis of variance) were implemented in the R environment (version 3.4.4, 2018-03-15) (52).

RESULTS

Menstrual cycle clock in integrated endometrial data sets

From a total of 694 studies retrieved from GEO, our search identified five unique studies that evaluated endometrial gene expression in women with normal endometrium, comprising 112 samples (Table 1; see Supplemental Fig. 1, available online, for detailed filtering steps). All studies were analyzed separately for correction of possible batch effects, and three samples were excluded from analysis (details in Supplemental Fig. 2, available online). A unique combined data set with a population of 109 patients was obtained,

comprising 29 samples in the proliferative phase, 29 in the early secretory phase, 43 in the medium secretory phase, and eight in the late secretory phase (Table 1).

The 109 samples were grouped depending on the experiment rather than by the menstrual cycle phase (Fig. 1A, left). To make data sets comparable after the integration of all samples, this batch effect was removed (Fig. 1A, right). The resulting gene expression samples showed a behavior based on menstrual cycle phases rather than study nature (Fig. 1B).

Viral infectivity genes show a different expression landscape across the menstrual cycle

The gene expression landscapes for each viral gene across the cycle are shown in Figure 2B (56). *TMPRSS4*, *CTSL*, *CTSB*, *FURIN*, *MX1*, and *BSG* were highly expressed throughout the cycle; *TMPRSS2* was moderately expressed, and *ACE2* was low (Fig. 2A). Gene expression of viral proteins depended on menstrual cycle phase. Calculated *P* values and adjusted *P* values are presented in Supplemental Fig. 3A (available online). All genes except *TMPRSS2* (*P* = .053) had statistically significant changes in expression across the menstrual cycle (Fig. 2A). Specifically, the genes most affected by menstrual cycle progression (*P* < .0001) were *ACE2*, which increased expression from early secretory to midsecretory; *TMPRSS4*, whose expression increased from proliferative to midsecretory and from early secretory to midsecretory (*P* < .0001); *CTSL* and *CTSB*, which increased from early secretory to midsecretory (*P* < .0001); *TMPRSS2* with decreased expression from proliferative to early secretory (*P* < .05); and *BSG* and *FURIN*, which increased from proliferative to midsecretory (*P* < .01) (Fig. 2A). All genes, including *TMPRSS2*, showed increased expression from early secretory to midsecretory, as indicated in Supplemental Figure 3B. Detailed expression changes across the menstrual cycle are provided for each gene in Supplemental Figure 4 (available online).

Correlations between genes showed activations and repressions between them throughout the menstrual cycle (Supplemental Table 1, available online). *ACE2* and *TMPRSS4* were positively correlated in the early secretory phase (0.58). A weak correlation (activation) was found for *ACE2* with *TMPRSS4* and *CTSL* in the window of implantation (0.17 and 0.25, respectively). Coactivations were also detected in the proliferative phase between *CTSB* and *TMPRSS4*; the early secretory phase between *FURIN* and *BSG*; the midsecretory phase between *CTSB* with *CTSL*, and *MX1* with *CTSB*, *CTSL*, and *TMPRSS4*; and the late secretory phase between *TMPRSS4* and *CTSB*, *FURIN*, *CTSB*, and *CTSL*. Based on our results, high coactivation values were prioritized to build a molecular scheme of SARS-CoV-2 plausible infection of the endometrium based on two mechanisms of entry: one via *ACE2* receptor and the other via *BSG* receptor. This scheme is shown in Figure 2B.

In this model, *TMPRSS4*, which is highly expressed in all phases of the menstrual cycle, especially during the window of implantation, is the protease responsible for protein S cleaving, giving SARS-CoV-2 the capacity to bind to *ACE2* and infect cells even if *ACE2* is lowly expressed. The model adds *CTSL*, *CTSB*, and *FURIN* as novel actors in the SARS-CoV-2 mechanism of cell entry. These proteases are highly

TABLE 1

Characterization of endometrial transcriptomic data sets with GEO data sets identifier, experiment name given for this study, cycle type, method of cycle phase dating, population from which samples were collected, age, transcriptomic platform used to measure gene expression, number of genes of each data set, number of samples in each data set, number of samples per cycle phase, and publication in which data were initially employed.

GEO ID	Experiment name	Cycle type	Cycle phase dating method	Population	Age	Platform	No. genes	No. samples	No. samples per cycle phase	Reference
GSE4888	Talbi 2006	Normo-ovulatory; regular (24–35 d); 3 mo since last hormone treatment	Noyes et al. (39) reviewed by four pathologists	Caucasian (n = 17); black (n = 6); Asian (n = 1); other (n = 2)	23–50	hgu133plus2 Affymetrix	19,361	27	PF (n = 6); ESE (n = 4); MSE (n = 9); LSE (n = 8)	(53)
GSE29981	Bradley 2010	Regular	Days from LH peak: PF (LH-14–LH-1), ESE (LH+1–LH+4), MSE (LH+6–LH+7)	Collected in Belgium	20–39	hgu133plus2 Affymetrix	19,361	19	PF (n = 10); ESE (n = 6); MSE (n = 3)	—
GSE98386	Altmäe 2017	Natural cycle	Days from LH peak. We classified it in ESE = LH+2; MSE = LH+8	Collected in Estonia	—	Illumina HiSeq 2500	16,426	38	ESE (n = 19); MSE (n = 19)	(54)
GSE86491	Sigurgeirsson 2017	Regular; 3 mo since last hormone treatment	Urinary LH ovulation predictor kit for MSE-LSE, days after the start of the subsequent menstruation for PF. Both confirmed by a gynecologic pathologist through histopathologic examination.	Collected in Iceland	24–30	Illumina HiSeq 2500	15,939	14	PF (n = 7); MSE (n = 7)	(55)
GSE119209	Kelleher 2017	—	—	Collected in U.S.	—	Illumina HiSeq 2500	17,934	11	PF (n = 6); MSE (n = 5)	—

Note: Last row indicates the total number of samples accounted and genes in common between all data sets for all menstrual cycle phases (53–55). ESE = early secretory; GEO = Gene Expression Omnibus; LH = luteinizing hormone; LSE = late secretory; MSE = medium secretory; PF = proliferative phase.

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expressed and coactivate with *TMPRSS4*. They could also help with the cleaving of protein S in different sites, thereby increasing infectivity. We also included *MX1*, which regulates neutrophil activity and is highly expressed, bringing neutrophils into the tissue with elastases to help cleave S protein. Finally, *BSG*, the alternative receptor for SARS-CoV-2, also showed high expression, which would favor infectivity in this model, and activation with *FURIN*, which would favor infectivity in this model.

Age influences the expression of viral genes throughout the menstrual cycle

For this analysis, only samples from the study Talbi et al. (53) were used because it was the only study that provided the age for each sample (in a range of 23–50 years old; n = 27). *ACE2* showed increased expression with age in the proliferative, early secretory, and midsecretory phases (0.4, 0.73, 0.44, respectively; Fig. 3A). Most correlations by age were stronger in early phases of the menstrual cycle (proliferative and early secretory) than in subsequent endometrial stages (Fig. 3B). *TMPRSS4* and *MX1* were highly correlated with age in the

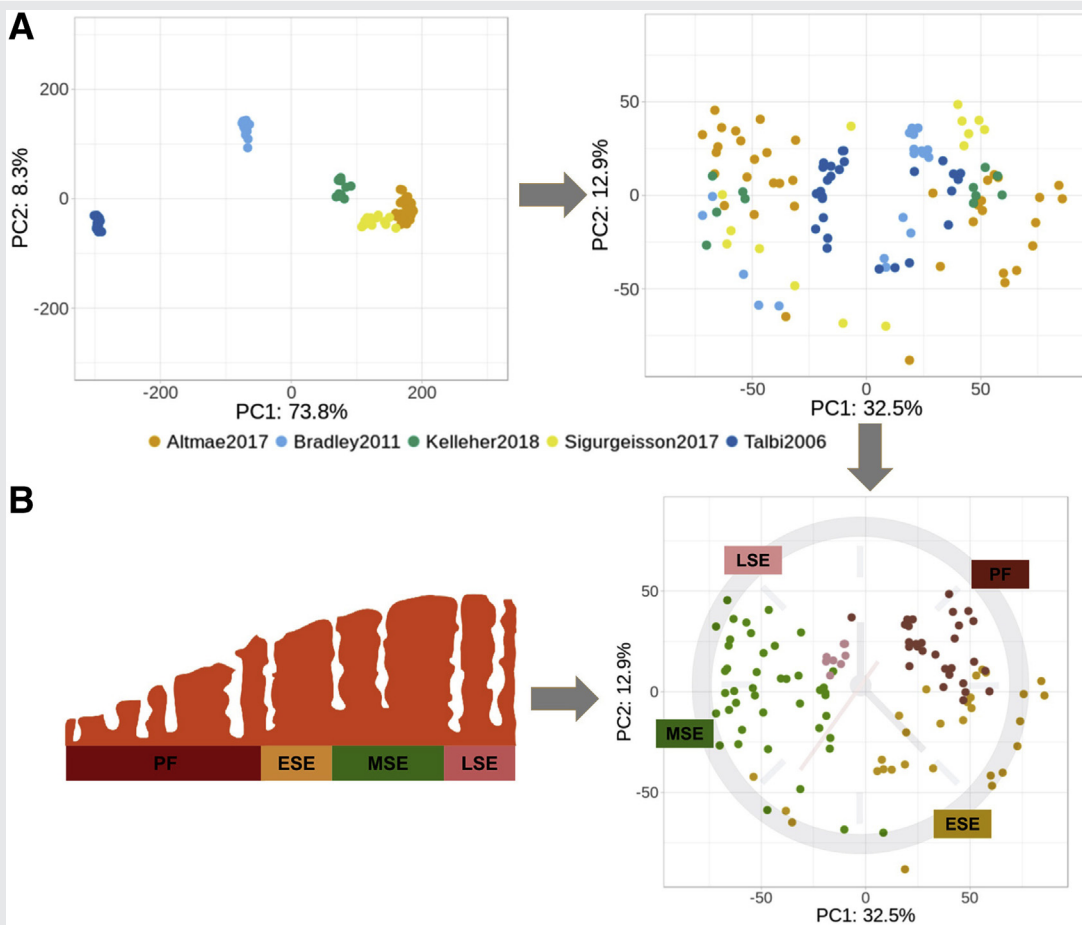
early secretory phase; *TMPRSS4*, *CTSL*, and *CTSB* had positive correlation in the proliferative phase; and *TMPRSS4* and *BSG* had positive correlation in the late secretory phase. In addition, high negative correlations were detected for *BSG* in the early secretory phase and for *FURIN* in the midsecretory phase.

DISCUSSION

It is important to understand the consequences of SARS-CoV-2 infection as the virus continues to spread worldwide. Fertility, specifically embryo implantation, is impacted by changes in endometrial gene expression throughout the menstrual cycle. Between menstrual cycle phases, gene expression varies to accompany physiologic changes (57). The differences in gene expression from one phase to another create a changing landscape for viral infection, and viral proteins themselves alter their expression throughout the menstrual cycle, setting the stage for a cyclical landscape of viral infectivity risk.

ACE2, the receptor for SARS-CoV-2 cell entry, showed low expression in the endometrium in our study, as reported previously in the Human Protein Atlas (HPA) (42). *ACE2* is also reduced in critical tissues such as lungs (24);

FIGURE 1



Endometrial data set integration and menstrual cycle clock. (A) Data set experiment effect and correction. Each point of the principal component analysis (PCA) plots represents endometrial gene expression of one sample and is colored by the endometrial transcriptomic data sets to which it belongs. Principal component 1 (PC1) and principal component 2 (PC2) explain the percentage of variability due to these components for each PCA. Integration of the transcriptomic studies showed a clear batch effect by the nature of each experiment (left PCA plot). After correction (right PCA plot), all genes in common between data sets were retained, amounting to a total of 13,437 genes, as no differentially expressed genes were detected between experiments. (B) Menstrual cycle effect. The samples of the integrated data sets are colored by the menstrual cycle phase to show how they are grouped by phases of the menstrual cycle. ESE = early secretory endometrium; LSE = late secretory endometrium; MSE = midsecretory endometrium; PF = proliferative phase.

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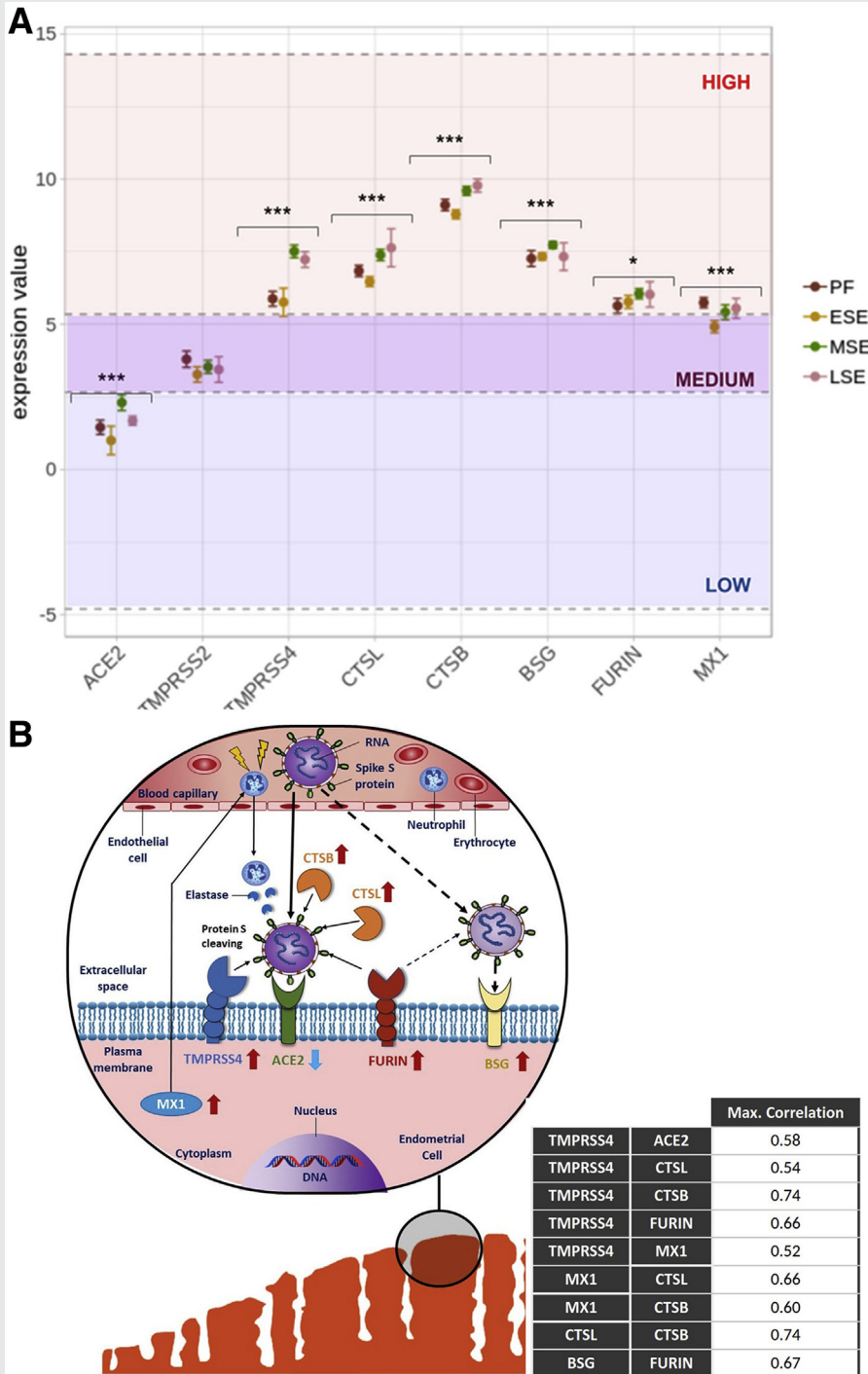
however, we cannot suggest that low levels of *ACE2* expression imply no effect of the virus on the tissue. Further studies are needed to elucidate the effect of decreased *ACE2* in the endometrium. It is interesting that *ACE2* increased (fold change = 2.47) from the early secretory to midsecretory phases, implying an increase in *ACE2* in the window of implantation and a high risk of viral infectivity at this stage of the menstrual cycle.

Several possible mechanisms of SARS-CoV-2 cell entry have been proposed (19, 28). *TMPRSS2*, the most-reported protease involved with SARS-CoV-2 infectivity alongside *ACE2*, had medium endometrial expression in our study. However, there was no correlation between *TMPRSS2*, *ACE2*, and the rest of the genes studied. These results imply that the endometrium should be safe against SARS-CoV-2 infectivity mediated by *TMPRSS2*, though the expression of other proteases associated with S protein cleavage show a different landscape than

TMPRSS2. *TMPRSS4* statistically significantly changes its expression throughout the menstrual cycle, showing a statistically significant increase in the midsecretory phase. This protein also showed an interesting correlation with other genes, including an increase alongside *ACE2* in the early secretory phase. Although *TMPRSS2* is implicated in cell entry, the fact that S proteins could be targeted by *TMPRSS4* and that *TMPRSS4* increased infectivity in gut epithelial cells (26, 28) could mean a vulnerability of the endometrium to infection by SARS-CoV-2 mediated by *TMPRSS4*. Furthermore, *TMPRSS4* up-regulation was correlated with *CTSL* and *CTSB* in all phases except the early secretory phase. Cell infection by SARS-CoV-2 was observed in *TMPRSS2*- cell lines expressing both *CTSL* and *CTSB*, so these proteins may have a residual function of cleaving viral S protein (19).

We propose that a synergy of *CTSL* and *CTSB* with *TMPRSS4* through most of the cycle favors SARS-CoV-2

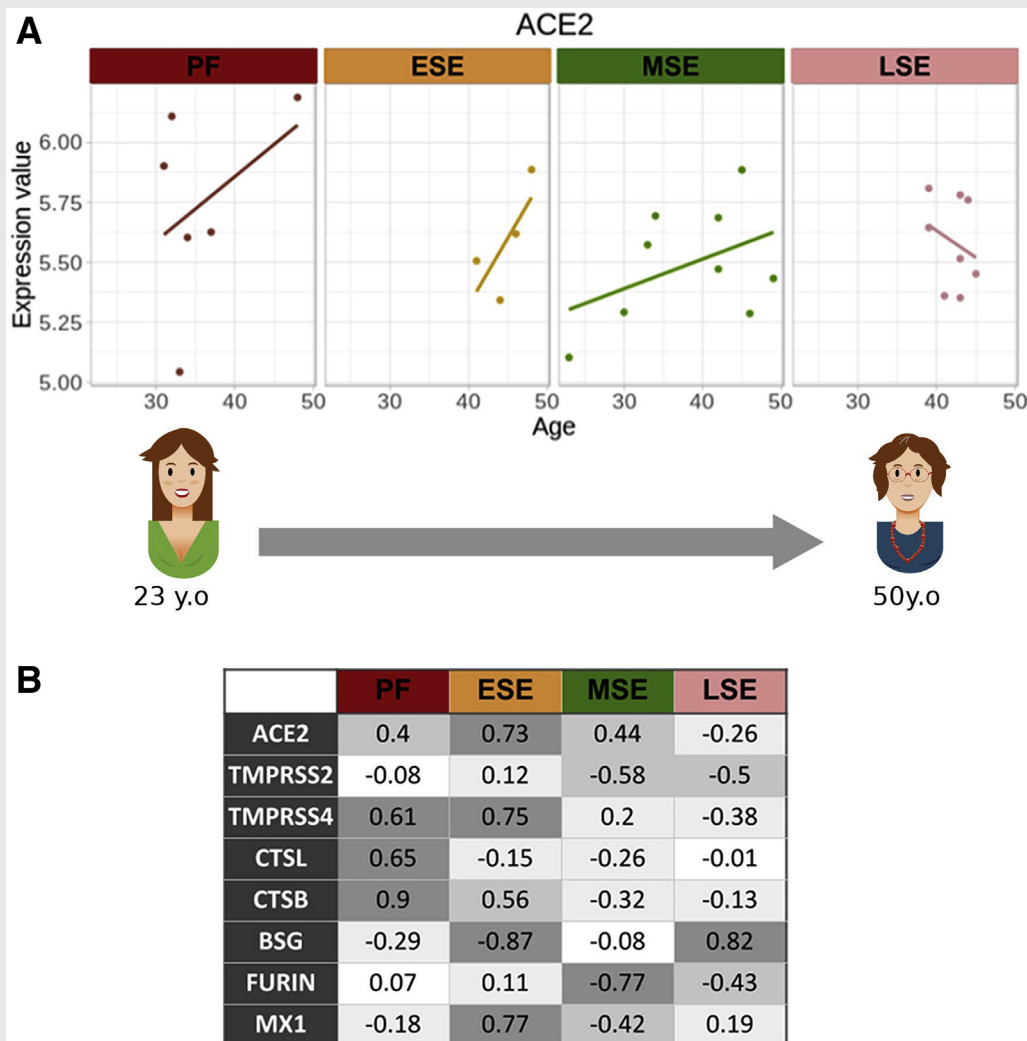
FIGURE 2



Gene expression of viral infection-related genes throughout the menstrual cycle. (A) Landscape of expression changes. Genes were located depending on their relative expression against the whole set. Low, medium, and high expression thresholds correspond to 1% to 10%, 11% to 50%, and 51% to 100% categories of gene expression values of the entire integrated data set, respectively. Analysis of variance results for overall change of expression during cycle are shown for each gene. * $P < .05$; *** $P < .0001$. (B) Molecular scheme of SARS-CoV-2 endometrial infection. ACE2, TMPRSS4, FURIN, and BSG are shown in plasma membrane of an endometrial cell (lower left figure). CTSL and CTSB are represented outside the cell. MX1 is shown in cytoplasm. Expression of viral genes in comparison to whole transcriptomic set is represented as arrows next to their names: up = highly expressed; down = lowly expressed. Viral genes are positioned in their schematized cell locations as established by GeneCards database > Localization section (release 4.14) (56). Only maximum confidence levels (5 and 4) for compartments-derived cell locations were used. Proteins were grouped considering the highest coactivation values between pairs of viral genes during the menstrual cycle, which are shown in the lower right table in the figure. Discontinuous arrow shows less evidence according to our results, given that only FURIN showed high activation with BSG and that further studies are needed to understand BSG-related mechanisms of SARS-CoV-2 entry. ESE = early secretory endometrium; LSE = late secretory endometrium; MSE = midsecretory endometrium; PF = proliferative phase.

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FIGURE 3



Impact of age on viral-related infectivity gene expression throughout the menstrual cycle. (A) Effect of age on *ACE2* expression. Gene expression is represented for *ACE2* in each phase of the cycle according to the age of the sample analyzed. The range of age from patients involved in this study was 23 to 50 years. (B) Effect of age on viral gene expression. Pearson correlation R^2 values are shown for each gene studied through of the phases of the menstrual cycle. Gray scale represents the magnitude of the correlation of increase or decrease in expression with age. High values are colored darker, and low values are colored lighter. ESE = early secretory endometrium; LSE = late secretory endometrium; MSE = midsecretory endometrium; PF = proliferative phase.

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infectivity in the endometrium. *FURIN*, another protease predicted to cleave protein S (37, 58, 59), also showed a positive correlation with *TMPRSS4* in the late secretory phase; with both targeting S protein, higher infectivity may occur. Finally, *TMPRSS4* was positively correlated with *MX1* in the midsecretory phase. *MX1* actively participates in SARS-CoV-2 infection by attracting neutrophils to infected tissue and employing their elastases to cleave protein S along with *TMPRSS2* (31); if *TMPRSS4* participates alongside *MX1*, a more severe infection could occur during the window of implantation. *TMPRSS4*, *CTSB*, *CTSL*, *FURIN*, and *MX1* showed statistically significant expression changes throughout the cycle. Protein expression of *TMPRSS4*, *CTSB*, *FURIN*, and *MX1* (42) supports a susceptible environment for infection in the early secretory and midsecretory phases. Future studies

are needed to fully understand the mechanism of SARS-CoV-2 cell entry and the roles of *TMPRSS4*, *CTSB*, *CTSL*, *FURIN*, and *MX1* in the endometrium.

BSG, the alternative receptor for SARS-CoV-2 entry (25), showed high expression with statistically significant changes between phases and strong activation with *FURIN* through most of the cycle; however, its repression was correlated with *ACE2* and *TMPRSS2*. Our results may indicate that only *FURIN* could be involved in S viral protein cleavage, if necessary, for SARS-CoV-2 binding to *BSG*, though other unknown proteases could also be involved. Further studies are needed to provide insight on *BSG* function in SARS-CoV-2 infection in the endometrium.

We also investigated the effect of age on viral gene expression throughout the menstrual cycle. In a recent study

in lungs, *ACE2* expression was detected as highly variable among individuals ranging from 0.17 transcripts per million to 14.5 transcripts per million in the same demographic group and highlighted a skewness of expression that statistically significantly increased with age (24). Our results showed a positive correlation between age and *ACE2* from the proliferative to midsecretory phases, especially in the early secretory phase, meaning that the endometrium in older women (to age 50) could be more susceptible to SARS-CoV-2 infection. *TMPRSS2* decreased with age, particularly in the midsecretory and late secretory phases, which may mean that cell-mediated entry of the virus is lowered with increased age. However, *TMPRSS4* increased in expression from the proliferative to early secretory phases similar to *CTSB*, *CTSL*, and *MX1*. These results, in conjunction with *ACE2* expression changes, highlighted the same behavior related to age as in the lungs (24), implying a higher risk of endometrial infection in older women, especially in the early phases of the menstrual cycle, as well as potential implantation problems. However, our results are limited to only one study with 27 patients, and there are no prospective studies showing the real clinical implications.

ACE2 plays an important role in endometrial changes because it cleaves angiotensin II and impacts spiral artery vasoconstriction, posterior proliferation, and renewal of tissue (60–62). *ACE2* down-regulation by SARS-CoV-2 could alter the endometrial balance (63); however, given that the landscape of the tissue is modified with each menstrual cycle renewal, the consequences of SARS-CoV-2 infection could be reset from cycle to cycle.

Because our study integrates data sets from GEO, this approach is limited by the study design. This also means that results depend on the experimental transcriptomic procedures of prior reported results and the sample cohort included in each study. However, the inclusion criteria for the selected studies were very careful, and uniform and raw data were pre-processed to control undesired effects. Likewise, the virus differentially affects individuals due to each person's genetic profile (31, 64). Further prospective research is needed to determine the mechanisms of SARS-CoV-2 infection in the endometrium and how it could affect the fertility of each patient.

CONCLUSION

Although *TMPRSS2* expression implies a safe environment against infection, the roles of proteases and neutrophil regulating proteins *TMPRSS4*, *CTSB*, *CTSL*, *FURIN*, and *MX1* could imply susceptibility of infection, especially during the early secretory and midsecretory phases. Additionally, the high expression of *BSG* could imply an alternative mechanism independent of *ACE2* for endometrial viral infection. Our findings show that viral gene expression increases with age, suggesting that the endometrium of older women undergoing ART is at higher risk of viral infection. A careful approach is advised, with precautions implemented when resuming ART.

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Evaluación del riesgo de infección por SARS-CoV-2 en el endometrio: expresión génica relacionada con la infección viral a lo largo del ciclo menstrual.

Objetivo: Determinar la susceptibilidad del endometrio a la infección -y el consiguiente daño potencial ocasionado- por el SARS-CoV2.

Diseño: Análisis del conjunto de datos de transcriptómica endometrial de expresión génica relacionados con la infección por el SARS-CoV2.

Entorno: Departamento de investigación en infertilidad afiliado a un hospital público.

Paciente(s): Datos de expresión génica de cinco estudios en 112 pacientes con endometrio normal, recogidas a lo largo del ciclo menstrual.

Intervención(es): Ninguna.

Medida del resultado principal: Expresión génica y correlación entre los genes de infectividad viral y la edad a lo largo del ciclo menstrual.

Resultado(s): La expresión génica fue alta para TMPRSS4, CTSL, CTSB, FURIN, MX1 y BSG; media para rTMPRSS2 y baja para ACE2. La expresión de ACE2, TMPRSS4, CTSB, CTS y dMX1 se incrementó hacia la ventana de implantación. La expresión de TMPRSS4 se correlacionó de manera positiva con ACE2, CTSB, CTSL, MX1 y FURIN durante varias fases del ciclo; TMPRSS2 no estaba alterado de manera estadísticamente significativa a lo largo del ciclo. La expresión de ACE2, TMPRSS4, CTSB, CTSL, BSG y MX1 se incrementó con la edad, especialmente en las fases tempranas del ciclo.

Conclusión(es): El tejido endometrial está probablemente a salvo de la entrada celular por el SARS-CoV2 basándonos en la expresión de ACE2 y TMPRSS2, pero la susceptibilidad se incrementa con la edad. Además, la entrada del virus al interior de las células mediado por TMPRSS4 junto con BSG podría implicar una susceptibilidad ambiental para la entrada del SARS-CoV2 a través de diferentes mecanismos. Se recomiendan más estudios para determinar el verdadero riesgo de infección endometrial por SARS-CoV2 y sus implicaciones para los tratamientos de fertilidad.