Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

motivation and engagement that exists. In addition, patient activation was similar across all of the participants regardless of maternal demographic traits, gestational age, or health literacy level. Future work should address the limitations of this cross-sectional investigation, specifically the lack of a non-pregnant comparison group, the limitation of a sample of individuals who were engaged in perinatal care, the single time point of investigation, a small sample size, and the lack of power to detect small differences. ■

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Reduced severe acute respiratory syndrome coronavirus 2 entry factors and enhanced innate immune gene expression in the nasal epithelium of pregnant rats

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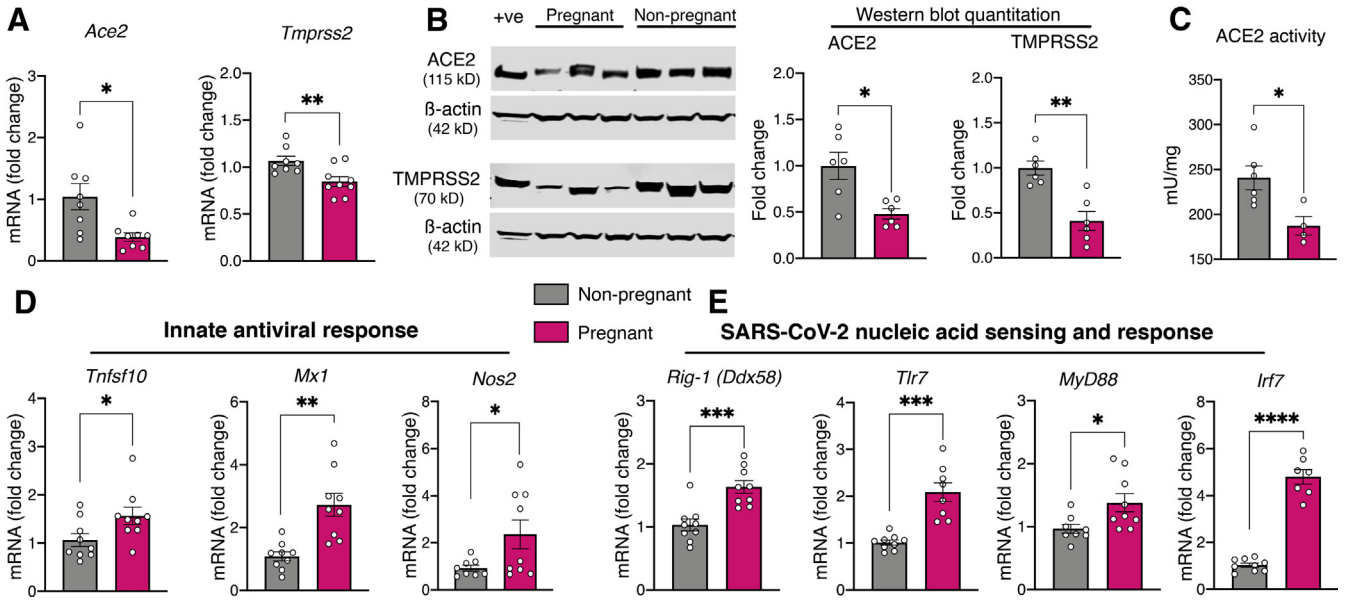
OBJECTIVE: An enigmatic epidemiologic feature of the ongoing coronavirus disease 2019 pandemic is the high rate of asymptomatic infection in pregnant women.[1] This is puzzling because systemic immune changes predispose pregnant women to increased severity of respiratory viral infections, especially influenza A.[2] A major roadblock in understanding this atypical clinical presentation is the poor characterization of cellular entry factors for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—angiotensin-converting enzyme 2 (ACE2) and the androgen-sensitive transmembrane protease serine 2 (TMPRSS2)—in the respiratory tract during pregnancy. Motivated by a recent report showing an estradiol-mediated down-regulation of ACE2 in the airway epithelium,[3] we hypothesized that the hormonal changes of pregnancy will decrease the expression of SARS-CoV-2 cell entry factors. Here, we compare their expression and examine the innate immune system in the nasal epithelium of term pregnant (20 days' gestation) vs nonpregnant 2-month-old female rats.

STUDY DESIGN: All experiments were conducted after an appropriate institutional approval (protocol ID: 19-1071) and comply with Animal Research: Reporting of In Vivo Experiments guidelines. Briefly, the nasal epithelia from euthanized rats (n=9 each) were dissected according to the protocol described by Dunston et al[4] with modifications. Collected samples were assayed for the expression of SARS-CoV-2 entry factors (ACE2, TMPRSS2), innate antiviral immune genes that are highly coexpressed with ACE2 (TNFSF10, MX1, nitric oxide synthase 2 [NOS2]),[5] and genes involved in SARS-CoV-2 detection and defense RIG-1, TLR7, MYD88, interferon regulatory factor 7 [IRF7]) with TaqMan quantitative polymerase chain reaction (PCR) (Thermo Fisher Scientific, Waltham, MA). In addition, we determined the expression of ACE2 (LS-c763699, 1:1000 dilution; Lifespan Biosciences, Seattle, WA) and TMPRSS2 (sc-515727, 1:250 dilution; Santa Cruz Biotechnology, Inc, Dallas, TX) protein with immunoblots. Finally, we assayed ACE2 enzyme activity with a fluorometric assay (K897-100, BioVision Inc, Milpitas, CA).

FIGURE

Assessment of viral cell entry factors and innate immune response genes in the nasal epithelium during pregnancy



A, Scatter plots showing markedly decreased expression of ACE2 and TMPRSS2 in the nasal epithelium during pregnancy. **B,** Representative immunoblots showing markedly reduced ACE2 and TMPRSS2 protein expression along with quantification as scatter plots. Rat small intestinal lysate was used as positive control and β-actin as the loading control. **C,** Scatter plot demonstrating a marked reduction in enzymatic ACE2 activity in the nasal epithelium of pregnant rats. **D,** Scatter plots showing substantial up-regulation of innate immune genes highly coexpressed with ACE2 (TNFSF10, MX1, NOS2). **E,** Scatter plots showing up-regulation of genes involved with SARS-CoV-2 detection (RIG-1, TLR7, MYD88, IRF7) in pregnant nasal epithelial samples suggesting the possibility of heightened innate immune surveillance at baseline. Expression levels of genes of interest were assayed in duplicate along with 2 endogenous housekeeping control genes (EEF2 and ACTB). All TaqMan primers were acquired from Thermo Fisher Scientific, and thermal cycling was performed in 7500 Fast Real-Time PCR System (Applied Biosystems: Makrogiannis Phil, Foster City, CA). Relative mRNA expression, normalized to the geometric mean of EEF2 and ACTB, was calculated using the $2^{-\Delta\Delta CT}$ method. Data outliers were eliminated using robust regression and outlier analysis with Q set to 10% and normality of residuals was assessed with D'Agostino-Pearson omnibus test. Normally and nonnormally distributed data were analyzed with Welch's *t* test and Mann-Whitney *U* test, respectively, with $P \leq .05$ accorded statistical significance. Data were analyzed with Prism 8 for macOS (version 8.2.1; GraphPad Software Inc, San Diego, CA) and presented as mean±SEM; $^{a}P \leq .05$; $^{b}P \leq .01$; $^{c}P \leq .001$; and $^{d}P \leq .0001$ (n=9 each for all experiments except western blot where n=6 per group).

ACE2, angiotensin-converting enzyme 2; *mRNA*, messenger RNA; *MYD88*, myeloid differentiation primary response 88; *NOS2*, nitric oxide synthase 2; *RIG-1*, retinoic acid-inducible gene I; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2; *SEM*, standard error of mean; *TLR7*, toll-like receptor 7; *TMPRSS2*, transmembrane protease serine 2; *TNFSF10*, tumor necrosis factor ligand superfamily member 10.

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RESULTS: We observed a marked down-regulation of the expression of ACE2 and TMPRSS2 genes (Figure, A) along with concomitant changes in protein expression (Figure, B) and a marked decrease in ACE2 enzyme activity in the nasal epithelium during pregnancy (Figure, C). Innate immune genes with antiviral function that are highly coexpressed with ACE2 (TNFSF10, MX1, NOS2) were markedly elevated in the nasal epithelium from pregnant rats (Figure, D). Similarly, the expression of cytoplasmic (RIG-1) and endosomal viral sensors (TLR7, MYD88, and IRF7) involved in the detection of SARS-CoV-2 was substantially up-regulated with pregnancy (Figure, E). Collectively, our results show a decrease in cell entry factors for SARS-CoV-2 and a surprisingly robust expression of innate immune response genes in the nasal epithelium of pregnant rats.

CONCLUSION: Based on our preclinical findings, we surmise that the high rate of asymptomatic infection in pregnant women is likely caused by decreased SARS-CoV-2 tropism secondary to reduced expression of cell entry factors. Our observation of up-regulated innate immune defense in the nasal epithelium, in contrast to the immunologic indolence at the placental-fetal interface, was unexpected and novel. Considering the exquisite vulnerability of pregnant women to influenza A virus, another single-stranded RNA virus, but not SARS-CoV-2, our findings set the stage for

comprehensive characterization of respiratory mucosal immunology in pregnant women to better understand host-pathogen interaction in this unique demographic subset. ■

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