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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. This is puzzling because systemic immune changes predispose pregnant women to increased severity of respiratory viral infections, especially influenza A. 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, 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 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]), 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).

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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
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

JANUARY 2021 American Journal of Obstetrics & Gynecology 119

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

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