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Expression of SARS-CoV-2 cell entry genes, *ACE2* and *TMPRSS2*, in the placenta across gestation and at the maternal-fetal interface in pregnancies complicated by preterm birth or preeclampsia

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Condensation

Placental *ACE2 and TMPRSS2* were expressed in higher levels in the first trimester and did not chance in preterm birth or pre-eclampsia.

Short Title

ACE2 and *TMPRSS2* in the maternal-fetal interface across pregnancy.

AJOG at a Glance….

A. Why was the study conducted?

To investigate the expression pattern of *ACE2* and *TMPRSS2*, associated with SARS-CoV-2 cell entry in the placenta across human pregnancy and at the maternal-fetal interface in pregnancies complicated by preterm birth or pre-eclampsia.

B. What are the key findings?

ACE2 and *TMPRSS2* are highly expressed in the human placenta in early pregnancy but their expression decreases significantly with advancing gestation. Expression of these genes at the maternal-fetal interface did not change in pregnancies complicated by either preterm birth or preeclampsia.

C. What does this study add to what is already known?

The decrease in expression of placental *ACE2* and *TMPRSS2* with advancing gestational age suggests the potential for differential risk of placental infection across pregnancy. Pregnancies complicated by preterm birth or preeclampsia are not associated with changes in the expression of these SARS-CoV-2 cell entry genes.

Abstract

Background: While there is some evidence that SARS-CoV-2 can invade the human placenta, limited data exist on the gestational-age dependent expression profile of the SARS-CoV-2 cell entry mediators, *ACE2* and *TMPRSS2* at the human maternal-fetal interface. There is also no information as to whether the expression of these mediators is altered in pregnancies complicated by pre-eclampsia (PE) or preterm birth (PTB). This is important since the expression of decidual and placental *ACE2* and *TMPRSS2* across gestation may impact susceptibility of pregnancies to vertical transmission of SARS-CoV-2.

Objectives: To investigate the expression pattern of specific SARS-CoV-2 cell entry genes, *ACE2* and *TMPRSS2*, in the placenta across human pregnancy and in paired samples of decidua and placenta in pregnancies complicated by PTB or PE compared to term, uncomplicated pregnancies.

Study Design: Two separate cohorts of patients, totalling 87 pregnancies were included. The first cohort comprised of placentae from first (7-9 weeks), second (16-18 weeks), third-trimester preterm (26-31 weeks) and third-trimester term (38-41 weeks) pregnancies (n=5/group), whereas, the second independent cohort, included matched decidua and placentae from pregnancies from term, uncomplicated pregnancies (37-41 weeks; n=14) as well as pregnancies complicated by PTB (26-37 weeks, n=11) or PE (25-37 weeks n=42). Samples were subjected to qPCR and next-generation sequencing (NGS)/RNAseq for *ACE2* and *TMPRSS2* mRNA expression quantification, respectively.

Results: In the first cohort, the SARS-CoV-2 cell entry genes *ACE2* and *TMPRSS2* exhibited a gestational-age dependent expression profile, i.e. *ACE2* and *TMPRSS2* mRNA was higher ($p<0.05$) in the first trimester compared to second trimester, PTB and term

placentae ($p<0.05$) and exhibited a negative correlation with gestational age ($p<0.05$). In the second cohort, RNAseq demonstrated very low/undetectable expression levels of *ACE2* in PTB, PE and term decidua and in placentae from late gestation. In contrast, *TMPRSS2* was expressed in both decidual and placental samples but did not change in pregnancies complicated by either PTB or PE.

Conclusions: The increased expression of these SARS-CoV-2 cell entry associated genes in the placenta during the first trimester compared to later stages of pregnancy suggest the possibility of differential susceptibility to placental entry to SARS-CoV-2 across pregnancy. Even though there is some evidence of increased rates of PTB associated with SARS-CoV-2 infection, we found no increase in mRNA expression of *ACE2* or *TMPRSS2* at the maternal-fetal interface.

Keywords: SARS-CoV-2, COVID-19, placenta, decidua, *ACE2*, *TMPRSS2*, preterm birth, pre-eclampsia, term pregnancies, gestation-age dependent gene expression.

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) can induce the life-threatening Coronavirus disease 2019 (COVID-19), which emerged in the Wuhan, Hubei Province, China, in December 2019[1]. The pathogenesis of COVID-19 is complex, but it may involve cell viral replication and in severe cases, a resulting “cytokine storm”; a systemic pro-inflammatory response that seriously harms the brain, heart, kidneys, liver and lungs and leads to organ failure and ultimately death[1-5].

At present, the pathogenesis of SARS-CoV-2 infection in pregnancy is poorly

understood. However, to date, there has been limited evidence of its maternal to fetal transmission, in contrast to other viruses[6-9]. The most likely path for the virus to access to the fetus would be via the placenta, however, little is known about the tropism of the SARS-CoV-2 for the decidua and placenta. Emerging reports suggest that SARS-CoV-2 can invade the human placenta[10] and the occurrence of a second trimester miscarriage in a patient with symptomatic coronavirus disease, exhibiting SARS-CoV-2 positivity in the placental submembranes and cotyledons; associated with mixed inflammatory infiltrates and funisitis[11]. Furthermore, potential vertical transmission in pregnant women with COVID-19 has been reported. In one case of second trimester preterm delivery, the amniotic fluid and infant tested positive (by PCR) for SARS-CoV-2[12] and one neonate exhibited elevated IGM antibody levels 2 hours after birth[13]. In another case, a third trimester (35^{+5} weeks gestation) neonate was born to a mother exhibiting clincal symptons and being tested positive (by PCR) for SARS-CoV-2 genes. The neonate (blood and non-bronchoscopic bronchoalveolar lavage fluid-first day of life), placenta and clear amniotic fluid (collected prior to rupture of membranes during cesarean section) also tested positive for SARS-CoV-2 genes. Placental pathological examination, identified diffuse peri-villous fibrin deposition with associated infarction and acute and chronic intervillositis. Of importance, SARS-CoV-2 N-protein immunostaining in the placenta, revealed strong immunosignals concentrated in the syncytial layer[14]. This suggests that the syncytial layer is enriched with SARS-CoV-2 cell entry receptors, and highlights the need to investigate potential routes and associated mechanisms of placental SARS-CoV-2 infection and vertical transmission.

Compared to the general population, pregnant women are particularly susceptible to

specific viral infections including, cytomegalovirus (CMV), herpes simplex (HSV) and zika (ZIKV) viruses, and exhibit greater complications and mortality rates associated with varicella, rubeola and H1N1 infections. Importantly, the cause of this susceptibility is poorly defined [6], but it may be related to the immunological adaptations inherent to pregnancy or to the tissue distribution of cell entry viral mediators at the maternal-fetal interface[6]. Indeed, it is estimated that approximately one-third of pregnant women died after Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection[15-19]. Recent epidemiological data suggest that maternal mortality rates and pregnancy complications such as miscarriage/stillbirth and intrauterine growth restriction (IUGR) are not as prevalent in cases of COVID-19 when compared to SARS-CoV and MERS-CoV infections. However, of importance, emerging evidence points to probable increased rates of preterm birth (PTB) in COVID-19 disease[7,17,20], with reports showing high percentages of PTB in COVID-19 pregnancies that range from 23.8 to 39%[17,21], or no increased PTB rates[7].

The best described mediators of SARS-CoV-2 cell entrance, are angiotensin-converting enzyme 2 (ACE2) and the transmembrane protease serine 2 (TMPRSS2) protein receptors [22-24]. SARS-CoV-2 cell entry comprises the binding of the N-terminal portion of its spike (S) protein attached to the "corona" like viral envelope, to a pocket of the cell membrane ACE2 receptor. In an important second step, TMPRSS2 cleaves and detaches the S1 from the S2 portion to allow a conformational rearrangement of the viral membrane and subsequent fusion and entry of the virus into the targeted cell[22,25]. Tissue identification and the expression dynamics of these two cell-entry associated proteins are crucial for a better understanding of the SARS-CoV-2 cell tropism and COVID-19 pathogenesis,

treatment and prevention.

Evidence suggests that placental *ACE2* expression decreases from early to late pregnancy[26], however, even though the expression of *TMPRSS2* has been demonstrated in the human placenta[27,28], the mRNA expression profile of *ACE2* and *TMPRSS2* in the human placenta across pregnancy (simultaneously comparing first, second and third trimester) and whether mRNA expression of these SARS-CoV-2 associated cell entry proteins are dysregulated in the decidua and placenta from pregnancies complicated by PTB or pre-eclampsia (PE) is unclear. We hypothesized that the placenta expresses *ACE2* and *TMPRSS2,* that encode proteins mediating infection of cells within the human maternal-fetal interface, in a gestational-age dependent manner, and that changes in the expression of these genes at the maternal-fetal interface may be associated with pregnancies complicated by PTB and/or PE. Information on SARS-CoV-2 expression dynamics at the maternal-fetal interface across pregnancy, and in cases of obstetric complications, may provide increased understanding of the potential for placenta and fetal infection and thus support management of patients who present with SARS-CoV-2 infection during pregnancy.

Material and Methods

Sample collection

This is a cross-sectional study, involving two different cohorts of patients, totalling 87 pregnancies. In the first cohort, we assessed the developmental expression profile of specific SARS-CoV-2 associated cell-entry genes, *ACE2* and *TMPRSS2,* in human placental tissue from 1) first trimester (7-9 weeks; n=5) or 2) second trimester (16-18

weeks; n=5) elective terminations, 3) third-trimester spontaneous onset PTB (26-35 weeks; n=5) 2 delivered vaginally and 3 by c-section indicated for fetal distress (2) or bleeding from fibroid (1), and 4) term delivery (38-41 weeks; n=5) following elective c-section delivery prior to labor (4) or following vaginal delivery (1) in otherwise uncomplicated pregnancies. In the second cohort, to evaluate whether the mRNA expression of specific SARS-CoV-2 cell-entry associated genes was altered at the maternal-fetal interface in pregnancies complicated by PTB or PE, a total of 67 patients were recruited. We collected matched decidual and placental tissue from patients experiencing 1) PTB (26-37 weeks) 8 patients experienced spontaneous onset preterm labor and 3 were delivered by c-section prior to labor onset, i.e. total n=11), 2) PE (25-37 weeks) 11 patients experience spontaneous onset labor and 31 were delivered by c-section prior to labor onset i.e. total n=42) or 3) term delivery (37-41 weeks) 12 patients experience elective delivery by c-section and 2 experienced a vaginal delivery following spontaneous onset of labor, i.e. total n=14) in otherwise uncomplicated pregnancies. PE was defined as new onset of high blood pressure (>140/90 mmHg) after 20 weeks with concurrent proteinuria, and/or end-organ dysfunction (renal dysfunction, liver dysfunction, central nervous system disturbances, pulmonary edema, and thrombocytopenia). Tissue samples were collected by the Research Centre for Women's and Infants' Health (RCWIH) BioBank at Mount Sinai Hospital (Toronto, Canada) following informed consent. Placental tissue was sampled and processed as previously described[29]. Briefly, placental villous from first trimester pregnancies, following dilation and curettage, were visually identified and dissected from decidua and other tissues, by specialized RCWIH staff. Second and third trimester placental villous tissue, as well as, placental fragments from PTB and PE pregnancies were dissected and

harvested immediately after birth in a similar way. Placental core sampling was undertaken by dissecting the maternal surface in quadrants in areas 1.5 cm away from: the center of the placental disc, the closest placental edge, the umbilical cord insertion site, from areas of infarcts, thrombosis or other abnormalities. All cuts were made to exclude the maternal decidua and the chorionic plate so only placental villous tissue were to be included in the study. For the RNAseq study, the decidual sample was isolated from the fetal membranes of delivered placenta by scraping and was stored at -80C until processing. This study was approved by the Institutional Research Ethics Board at Mount Sinai Hospital (Toronto, Canada), under the approval numbers: 20-0006-E; 20-0101-E.

qPCR analysis in the placental ontogenetic study

Total RNA was isolated from human placental tissue using the RNeasy Plus Universal Mini Kit (73404, Qiagen, Toronto, ON, Canada), as described before[30,31]. RNA quality and concentration were determined using the NanoDrop1000 Spectrophotometer (Thermo Scientific). RNA integrity was assessed by calculating the RNA Integrity Number (RIN), using the Agilent Bioanalyzer 2100 and RNA 6000 Nano Labchip kit (Agilent Biotechnologies, CA, USA). 10 ng/µl of total RNA was reverse transcribed into cDNA using the iScript gDNA Clear cDNA Synthesis Kit (Bio-Rad). *ACE2* and *TMPRSS2* mRNA levels were assessed by qPCR using LuminoCt qPCR ReadyMix (Sigma-Aldrich) and the CFX384 Real-Time PCR Detection System (Bio-Rad). The cycling conditions were as follows: initial denaturation at 95^{o}C (2 min), followed by 40 cycles of denaturation at 95^{o}C (5s), and combined annealing and extension at 60^{o}C (20s). Gene expression was normalized to DNA topoisomerase 1 (TOP1) and succinate ubiquinone oxidoreductase (SDHA), which

presented stable expression between all groups. The primer sequences of all evaluated genes are shown in Table 1. Relative expression of target genes was obtained using the 2-ΔΔCT method[32].

Decidual and placental next-generation sequencing/RNAseq in patients with preterm birth or preeclampsia.

Total RNA was isolated from the decidual and placental samples with RNeasy Plus Universal Mini Kit (Qiagen, Canada), as previously described[30,31]. RNA concentration and quality were assessed with Fragment Analyzer systems (Agilent, USA). To identify the gene expression signature of *ACE2* and *TMPRSS2* mRNA levels, RNA sequencing technology (next-generation sequencing/NGS) in placental and decidual tissues was undertaken. RNA sequencing was conducted by a MGISEQ-2000 sequencer at Mount Sinai Hospital, Sinai Health System (Toronto). The RNA libraries were prepared using the MGIEasy RNA Directional Library Prep Set and MGIEasy rRNA Depletion kits (MGI Americas Inc. CA). Pair-end RNA sequencing was conducted at read-length of 100bp and 50 million reads per library. The workflow was conducted on the same platform by the same research team. We analyzed but found no significant variance due to batch effect.

Bioinformatics workflow

Sequencing data were analyzed using a high-performance clustering computing system (Galen; Sinai Health System, Toronto). Data quality of raw fastqc files was examined by FastQC (v0.11). After trimming off contaminated reads, Bowtie2 (v2.3), an ultrafast and memory-efficient tool, was used to align sequencing reads to the human reference genome

(hg38). Finally, binary alignment/mapping files were built up for the downstream analysis. Uniquely mapped reads were summarized to feature counts using GenomicAlignments (v1.23). Normalized RNA reads for TMPRSS2 expression were extracted by default settings of plotCounts (normalized counts plus a pseudocount of 0.5 as log2 scales) using the outcomes of the DESeqDataSet function provided by DEseq2 package (v1.27). TPM was calculated by the calculateTPM function from R package scater (v1.17) using mapped raw reads and effective gene length of the transcripts. The gene expressions of ACE2 and TMPRSS2 were identified by the ensemble ID ENSG00000130234 and ENSG00000184012, respectively. In this study, we found no evidence of a significant batch effect, therefore no batch correction was assigned.

Statistical analyses

Statistical analysis was performed using Prism (version 7.0; GraphPad Software, Inc., San Diego, CA). qPCR data were assessed for normal distribution using D'Agostino and Pearson or the Shapiro-Wilk test; outliers were identified using "QuickCalcs" Outlier calculator program (version 7.0; GraphPad Software, Inc., San Diego, CA). Gene expression in the ontogenetic study was analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Next-generation sequencing/NGS statistical analyses were performed by R software (v3.6) and RStudio (v1.3). Multiple comparisons of decidual and placental from PE, preterm and term pregnancies were conducted using the Kruskal–Wallis test and followed by pairwise Wilcoxon rank sum tests. The Spearman correlation was used to evaluate the linear relationship between gestational age and given gene. Statistical significance was assumed when $p<0.05$.

276

277 **Results**

278 ***The expression of specific SARS-CoV-2 associated cell-entry genes in human placenta is***
279 ***dependent on gestational-age***

280 The placental expression of *ACE2* and *TMPRSS2* mRNA was relatively high in the
281 first trimester of pregnancy (7-9 weeks) but exhibited a significant decrease ($p<0.05$) in
282 samples collected during the second trimester (16-18 weeks), during the third trimester,
283 preterm (26-31 weeks) and at term (Fig, 1A & C). Spearman correlation analysis, identified
284 a negative correlation between placental *ACE2* and *TMPRSS2* mRNA expression ($p<0.05$)
285 with advancing gestational age (Fig, 1, B & D).

286

287 ***ACE2 and TMPRSS2 expression at the maternal-fetal interface in term pregnancies and***
288 ***pregnancies complicated by preterm birth or preeclampsia***

289 We accessed unpublished RNAseq data generated from paired placental and
290 decidual samples in order to investigate the expression of *ACE2* and *TMPRSS2* in a large
291 cohort of patients experiencing a PTB, or diagnosed with PE, as well as in patients at term
292 undergoing an elective c-section but otherwise uncomplicated term delivery. All samples
293 from the RNAseq cohort exhibited 0-1 *ACE2* read count (raw values). Of the 134 samples,
294 only 9 exhibited a single *ACE2* read count, when the mean coverage of RNA sequencing
295 ranged around 60 million reads. The remainder of the samples did not exhibit any *ACE2*
296 mapping out (data not shown). By evaluating the TPM reads counts (Fig. 2 A&B), we
297 conclude that the expression of *ACE2* in decidua and placenta is undetectable by RNA Seq
298 (Fig. 2 C&D). In contrast, *TMPRSS2* was expressed in both decidual and placental samples

(Fig. 2A-B), although expression of this gene did not change across the patient groups (Fig. 3A & B) or with labor status (Fig, 1A & C).

Comments

Principal Findings

In the present study, we determined, for the first time, the mRNA expression of two key SARS-CoV-2 cell entry associated proteins in placentae from first, second and third-trimester pregnancies and in pregnancies complicated by PTB or PE. We found that placental expression of the genes that promote SARS-CoV-2 cell entry, *ACE2* and *TMPRSS2*, is downregulated as gestation progresses. Expression of *ACE2* or *TMPRSS2* at the decidual interface (placenta and decidua) did not change in pregnancies complicated by PTB (irrespective of labor status) or PE.

Clinical Implications

The higher placental *ACE2* mRNA levels in earlier stages of pregnancy, raise the possibility of a higher vulnerability to SARS-CoV-2 infection in the first trimester placenta. SARS-CoV and MERS-CoV infections during pregnancy are associated with increased rates of miscarriage/stillbirth[16,17], and there is limited evidence of miscarriage/stillbirth and fetal malformations in COVID-19 infected pregnancies [7,17]. The higher levels of placental *ACE2* and *TMPRSS2* in earlier stages of pregnancy is consistent with evidence showing the placental presence of SARS-COV-2[10], as well as the reported case of miscarriage during second trimester in which the amniotic fluid and infant tested positive (by PCR) for SARS-CoV-2[12]. The lower or absence of expression of *ACE2* from mid-pregnancy onwards is also

consistent with the limited evidence of vertical transmission of SARS-CoV-2 during pregnancy; as Dashraath[17] note, the majority of reports relate to women who acquired SARS-CoV-2 in the third trimester.

We did not observe altered placental or decidual expression of *ACE2* or *TMPRSS2* in pregnancies complicated by PTB (whether spontaneous onset or iatrogenic). This is consistent with clinical observations that while rates of preterm birth are increased, this is largely due to PPROM (there were no cases of PROM in our study) or iatrogenic indications[7]. While PE is often associated with placental inflammation[33], it was not associated with any changes in *ACE2* or *TMPRSS2* mRNA expression in the decidual or placenta.

Research Implications

Placental *ACE2* mRNA and protein expression have been previously investigated. ACE2 is an important component of the renin-angiotensin system (RAS), where it converts angiotensin II (Ang II) in Ang 1-7, an antagonist of Ang II that acts via Mas G-coupled protein receptor regulation [26,34]. Ang II regulates placental vascular tone and is thought to participate in the pathogenesis of gestational hypertension and PE [35,36]. In the first trimester placenta, ACE2 is abundantly immunolocalized to the syncytiotrophoblast and villous stroma, with lower levels in cytotrophoblasts[26]. This pattern of localization suggests that SARS-CoV-2 present in the maternal circulation has the potential to enter the maternal blood-bathed syncytiotrophoblast and infect the placenta via ACE2 binding. In fact, a case report depicted SARS-CoV-2 particles being predominantly present in the syncytiotrophoblast of a second-trimester pregnancy complicated with PE and placenta

abruption[37], whereas SARS-CoV-2 N-protein immunoreactivity was also concentrated in the syncytial layer in a case of third trimester SARS-CoV-2 vertical transmission[14]. However, and most importantly, ACE2 was not immunolocalized in the fetal vascular endothelium of the villous stroma[26], which theoretically, could prevent the SARS-CoV-2 penetration via ACE2 binding into the fetal circulation. Furthermore, SARS-CoV-2 syncytiotrophoblast entry has the potential to induce a potent inflammatory response and functionally disrupt the syncytiotrophoblast barrier, by negatively impacting nutrient and drug transport efficiency, hormonal output and cellular turn over. These possibilities clearly require further investigation.

Strengths and Limitations

It is possible that there are confounding factors associated with the gestational-age differences observed. In our early pregnancy cohort, we were unable (due to institution ethical policies) to collect further clinical information of the elective pregnancy terminations. As such, confounding factors may include maternal body mass index (BMI) status, fetal sex, ethnicity and the presence of unknown maternal infective and or inflammatory states and or endocrine and hypertensive disorders.

Using (NGS)/RNAseq, we were able to concomitantly screen the expression profile of *ACE2* and *TMPRSS2* in a large number of matched decidua and placentae from pregnancies complicated by PTB or PE. We observed very low levels of decidual and placental *ACE2* in all groups investigated in later gestation. This is somewhat similar to our findings using qPCR showing detectable but nevertheless lower, placental *ACE2* mRNA levels in later stages of pregnancy in healthy patients. The divergent detection of *ACE2* mRNA in our

cohorts is likely due to the differential sensitivity of techniques. In this context, data extracted from public datasets deposited at ArrayExpress or newly generated scRNA-seq, identified minimal levels of *ACE2* and *TMPRSS2* in first, second and third trimester placentae[28]. This is in agreement with our second and third trimester findings, but in disagreement with our first trimester results. Differences in the first trimester findings may be attributed to tissue collection protocols, patient inclusion criteria and or differences in the techniques used for gene expression assessment in these studies. Addititional studies are clearly required.

Additionally, the interpretation of our data requires caution, because there may be other, yet undefined mechanisms linking SARS-CoV-2 obstetric outcomes in normal and in pathological conditions. Furthermore, future studies should investigate the protein expression pattern, localization and function of *TMPRSS2* in the decidua and placenta, as well as investigate the relationship of gene expression, protein levels and corresponding function, in order to better understand the potential routes by which SARS-CoV-2 could gain access to the maternal-fetal interface, especially during early pregnancy and the possibility of vertical transmission at this time.

Conclusions

The gestational-age expression pattern of SARS-CoV-2 cell entry associated proteins suggest a reduced likelihood of placental and decidual cell entry of the virus in later stages of pregnancy. However, earlier stages of pregnancy may be more susceptible to SARS-CoV-2 placental infection. Our data provide no evidence that pregnancies

complicated by PTB or PE are at increased risk of placental SARS-CoV-2 infection and vertical transmission.

Author Contributions

Conceptualization: EB, JZ, HH, GEI, PL, SGM, SJL; Funding acquisition: EB, SGM; SJL; Methodology: EB, JZ, JN, HH, CED, SL, MK, PL; Project administration EB, SGM, SJL; Supervision: EB, SGM, SGL; Validation: JZ, MK, PL; Writing - original draft: EB, JZ, GEI; Writing - review & editing: HH, CED, MK, PL, SGM, SJL.

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505

Table 1. Sequence of primers used in the present study.

Primer name	Sequence	Reference
ACE2 *	Forward: 5'- GGAGTGATAGTGGTTGGCATTGTC -3'	
	Reverse: 5'- GCTAATATCGATGGAGGCATAAGGA -3'	
TMPRSS2 *	Forward: 5'- AGCTGCAGAAGCCTCTGACTTTC -3'	
	Reverse: 5'- AGCGTTCAGCACTTCTGAGGTC -3'	
TOP1	Forward: 5'-GATGAACCTGAAGATGATGGC -3'	[38]
	Reverse: 5'-TCAGCATCATCCTCATCTCG -3'	
SDHA	Forward: 5'- TGGGAACAAGAGGGCATCTG -3'	[38]
	Reverse: 5'- CCACCACTGCATCAAATTCATG -3'	

506 *Gene specific primers were designed with Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast).

507 **Figure Legends**

508 **Figure 1: The expression of specific SARS-CoV-2 associated cell-entry genes in human**
509 **placenta is gestational-age dependent.** mRNA levels of *ACE2* (A) and *TMPRSS2* (C) in
510 first (7-9 weeks), second (16-18 weeks), third trimester spontaneous onset PTB (26-35
511 weeks) and third trimester term (38-41 weeks) pregnancies (n=5/group), A and C: ANOVA
512 followed by Tukey's multiple comparisons test. B and D: Spearman correlation analysis.
513 Data are presented as mean ± SD. Different letters if $p<0.05$.

514

515 **Figure 2: *ACE2* and *TMPRSS2* expression in human maternal-fetal interface in**
516 **preeclamptic, preterm and term pregnancies.** The values of transcripts per million
517 (TPM) were calculated by the number of mapped raw reads, the transcript's length and
518 sequencing depth. The TPM normalized reads count for *ACE2* and *TMPRSS2* was obtained
519 from decidual (A) and placental (B) samples in preeclampsia (n=42), preterm (n=11) and
520 term (n=14) pregnancies. Density plots (C & D) were constructed to examine the
521 probability of available reads numbers. Histograms were embedded in the graphs to
522 visualize the distribution of read counts.

523

524 **Figure 3: Expression of *TMPRSS2* in the human decidua and placenta.** The expression
525 profile of *TMPRSS2* were compared based on Deseq2 normalized RNA reads. Decidua- (A)
526 and placenta-specific (B) expressions were calculated by Kruskal-Wallis test, followed by
527 Wilcoxon Rank test. P values were listed when significant differences were detected at
528 $p<0.05$. n=42 (preeclampsia), n=11 (preterm) and n=14 (term) respectively.
529