Expression of Coagulation-Related Protein Genes During LPS-Induced Preterm Delivery in the Pregnant Mouse

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Abstract

Preterm delivery (PTD) has been associated with inflammation along with activation of the coagulation pathway. These studies sought to characterize the expression of several coagulation pathway genes including plasminogen activator inhibitor 1 (*PAI-1*), tissue factor (*TF*), protease-activated receptor 1 (*Par1*), protease-activated receptor 2 (*Par2*), fibrinogen-like protein 2 (*Fgl2*), and thrombomodulin (*TM*) during lipopolysaccharide (LPS)-induced PTD in day 15 pregnant CD-1 mice. Western blot studies confirmed protein expression for *PAI-1*, *Par1*, *Par2*, *Fgl2*, and *TM* in the mouse uterus. Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) confirmed increased *PAI-1* messenger RNA (mRNA) in the uteri, lung, kidney, and liver tissues at 2 to 6 hours after LPS injection. In contrast, *TF* expression significantly decreased by 12 hours in uterine tissue; whereas, its expression was unchanged in the other maternal tissues. The uterine mRNA for *Par1*, *Par2*, *Fgl2*, and *TM* remained stable. In summary, these studies have confirmed expression of coagulation pathway genes within the pregnant uterus; some of which are modulated during LPS-induced PTD.

Keywords

plasminogen activator inhibitor 1, tissue factor, lipopolysaccharide, preterm delivery, CD-1 mouse

Introduction

Preterm birth affects over 12% of US pregnancies and significantly contributes to infant mortality and childhood morbidity. Its effects can extend far beyond the neonatal period, leading to considerable challenges for preterm children, their families, the health care system and our educational institutions.^{1,2} The pathophysiologic mechanisms underlying preterm birth are multifactorial and include proinflammatory pathways, along with possible activation of components of the coagulation cascade; phenomena that are comparable to those occurring during the systemic inflammatory response syndrome (SIRS).³⁻⁶

Numerous animal models of preterm birth have utilized an activated inflammatory cascade to help decipher the mechanisms leading to preterm delivery (PTD). Selected models include intra-amniotic Group B *Streptococcus* (GBS) in rhesus monkeys,⁷ endoscopic inoculation of live *Escherichia coli* through the cervix in rabbits,⁸ and renal abscess caused by *E coli* injection in CD-1 mice.⁹ Lipopolysaccharide (LPS), derived from the bacterial cell walls of *E coli*, causes a pronounced SIRS in animal and human tissues both in vivo and in vitro. Examples include intraperitoneal injection in transgenic and wild-type mice^{10,11} and rats¹²; intravenous injection in rats,¹³ guinea pigs,¹⁴ and sheep¹⁵; and inhalation of nebulized LPS in human volunteers.¹⁶ Multiple reports have

demonstrated that the LPS-induced proinflammatory response also stimulates premature delivery in mice.^{4,17-20} A 2003 report by Elovitz et al⁴ demonstrated the ability of LPS to induce PTD within 24 hours after intrauterine injection in pregnant CD-1 mice; an effect mediated predominantly by the activation of Toll-like receptor 4 (TLR4).

Activation of the coagulation cascade during SIRS is driven by stimulation of the proinflammatory response. As leukocytes migrate to the site of inflammation, proteins bound to their cell membranes, along with the activation of platelets, result in the initiation of the coagulation cascade.²¹ Stimulation of the innate immune response leads to increased expression and membrane exposure of tissue factor (*TF*), resulting in the activation of factor VII and the coagulation cascade.^{16,22} Plasminogen activator inhibitor 1 (*PAI-1*) produced during activation of the coagulation cascade enhances thrombosis through the

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inhibition of the plasminogen activators.²³ Previously reported studies using primate models and human volunteers have confirmed increased *PAI-1* expression during endotoxemia, with levels increasing 2 to 3 hours after challenge.^{24,25} Lipopolysaccharide-stimulated inflammation has been shown to increase *PAI-1* expression in vascular endothelial cells²⁶ and to upregulate *PAI-1* production in the lungs of human volunteers.¹⁶

The studies described in this report present novel data integrating current knowledge of the LPS-induced proinflammatory response and its effects on components of the coagulation cascade associated with LPS-induced PTD in the mouse. Specifically, these studies sought to characterize the expression of several coagulation pathway mouse genes including *PAI-1*, TF, protease-activated receptor 1 (*Par1*), protease-activated receptor 2 (*Par2*), fibrinogen-like protein 2 (*Fgl2*), and thrombomodulin (*TM*) within the pregnant uterus during LPS-induced PTD.

Methods

These studies utilized 36 CD-1 timed-pregnant mice (from an ongoing series of over 100 intrauterine LPS-treated mice) purchased from Charles River Laboratories (Wilmington, Massachusetts). The animals were shipped on day 9 after mating and acclimated in the University of Vermont's animal care facility for several days before undergoing surgery on day 15 of gestation. All experiments were performed in accordance with the National Institutes of Health guidelines for laboratory animals and had approval from the Institutional Animal Care and Utilization Committee (IACUC) at the University of Vermont. After induction of isoflurane anesthesia, a laparotomy was performed using sterile technique to expose the right uterine horn. Lipopolysaccharide (extracted from *E coli* 055:B5; Sigma-Aldrich Co, St. Louis, MO L2880) dosed at 250 µg per mouse (diluted in 100 µL sterile normal saline) was injected intrauterine between gestational sacs 2 and 3 in the right uterine horn, as previously described by our laboratory.¹⁸ Control mice received injections with 100 µL sterile normal saline alone. At the completion of the surgery, the mice received one dose of buprenorphine (100 µg/kg) analgesic subcutaneously and were allowed to recover. Subsequently, the mice were euthanized at 2, 6, 12, 18, and 24 hours after intrauterine injection using isoflurane anesthesia and a lethal sodium pentobarbital injection (200 mg/kg intraperitoneal). The time zero control mice were euthanized without undergoing surgery or intrauterine injection. For these studies, maternal uteri, liver, lungs, and kidneys were harvested.

For the Western blots, mouse uterine tissues were homogenized in a protease inhibitor solution (containing 50 mmol/L Tris pH 7.4, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 5 µg/mL aprotinin, 3 µg/mL leupeptin, 1 mmol/L sodium orthovanadate, and 1 mmol/L phenylmethylsulfonyl fluoride). The homogenate solutions were centrifuged at 800g to remove the cellular debris; and the protein concentrations of the supernatant solutions (crude

tissue homogenates) were determined by bicinchoninic acid (BCA) protein assay. Subsequently, 100 µg protein aliquots were resolved using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and the Biorad Mini-PROTEAN 3 Electrophoresis system (BioRad Laboratories, Hercules, California). The resolved proteins were then electrophoretically transferred to nitrocellulose (NC) membranes. The NC membranes were blocked with 5% powdered milk and subsequently incubated overnight in a primary antibody solution (ie, polyclonal antisera for *PAI-1*, BD Biosciences, San Jose, California; *Par1* and *Par2*, Santa Cruz Biotech, Santa Cruz, California; *TM*, RD Systems, Minneapolis, Minnesota; and tissue prothrombinase [*Fgl2*], custom prepared for our laboratory). Immunodetection was performed using horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (IgG) antiserum (Bio-Rad Laboratories) followed by incubation in Supersignal HRP Substrate (Calbiochem EMB Chem, Gibbstown, New Jersey). Visualization of the chemiluminescent protein bands was performed using the Bio-Rad ChemiDoc XRS chemiluminescence detection system.

Samples for RNA assay were rinsed in normal saline, placed in RNeasy Lysis Buffer (Qiagen, Crawfordsville, Indiana), and stored at 4°C for several days, then removed from RNeasy Lysis Buffer and stored at -80°C. Total RNA was extracted from mouse uterus, liver, lung, and kidney tissue using TRIzol reagent (Invitrogen Corp, Carlsbad, California). Subsequently, genomic DNA was removed from samples using TURBO DNA-free (Ambion Inc). RNA concentrations were quantified using a NanoDrop spectrophotometer (NanoDrop, Inc, Wilmington, Delaware); and intact total RNA was confirmed by analysis of the 18S and 28S band patterns after formaldehyde-agarose gel electrophoresis.

For quantitative analysis of mRNA expression using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), complementary DNA (cDNA) was synthesized from 1 µg samples of RNA template using the iScript cDNA Synthesis kit (Bio-Rad Laboratories) with a mix of oligo-dT and random sequence primers. Next, the PCR was performed using the iTaq DNA polymerase kit (Bio-Rad Laboratories) and mouse-specific sense and antisense primers for *PAI-1*, *TF*, *Par1*, *Par2*, *Fgl2*, and *TM* genes (see Table 1 for primer sequences). In addition, 3 constitutively expressed genes were used as controls for the qRT-PCR studies: β -2-microglobulin (B2M), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide (Ywhaz), and hypoxanthine guanine phosphoribosyl transferase (Hprt). The PCR amplicons were visualized using Tris borate EDTA (TBE) horizontal gel electrophoresis; gels were made with 1.0% to 1.2% agarose and stained with GelRed (Biotium, Inc, Hayward, California). Imaging of gels was performed using the Bio-Rad ChemiDoc XRS chemiluminescence detection system.

Real-time qRT-PCR studies were performed using an ABI Prism 7000 Sequence Detection System and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, California). The quantitative PCRs were performed in triplicate for each sample. Subsequently, the PCR standard curves were generated for each primer set and used to calculate the relative

Table 1. Mouse Sense and Antisense PCR Primers^a

Gene	Primer Sequence	Amplicon Length	NCBI Accession #
<i>PAI-1</i> sense	TCCTCATCCTGCCTAAGTTCTCT	128 bp	NM_008871.1
<i>PAI-1</i> antisense	CTGCTCTTGGTCGGAAAGACTT		
<i>TF</i> sense	TTTCCTGGGAGAAACACTCATCA	160 bp	NM_010171.2
<i>TF</i> antisense	GCTTCAGCCTTTCTCTATGC		
<i>Par1</i> sense	CCCTATGAGCCAGCCAGAATC	162 bp	NM_010169.2
<i>Par1</i> antisense	TAGACTGCCCTACCTCCAGC		
<i>Par2</i> sense	CGGGACGCAACAACAGTAAA	125 bp	NM_007974.2
<i>Par2</i> antisense	GAGGATGGACGCAGAGAACT		
<i>Fgl2</i> sense	ATTAGATGTTGAACTGGCTGTGA	97 bp	NM_008013
<i>Fgl2</i> antisense	TGGCAAATCTAACCGTTGTGG		
<i>TM</i> sense	CCTGCCCTTGTAGTCCCGAAATA	199 bp	NM_009378.1
<i>TM</i> antisense	CTAGCCAGATCCCAAGCGAGGTC		
<i>B2m</i> sense	ATGCTATCCAGAAAACCCCTCAA	79 bp	NM_009735.2
<i>B2m</i> antisense	CAGTTCAGTATGTTCCGGCTTCCC		
<i>Hprt</i> sense	CAGTCCCAGCGTCGTGAT	137 bp	NM_013556.2
<i>Hprt</i> antisense	CAAGTCTTTCAGTCTGTCCATAA		
<i>Ywhaz</i> sense	GCAACGATGTACTGTCTCTTTTGG	149 bp	NM_013556.2
<i>Ywhaz</i> antisense	GTCCACAATTCCTTTCTTGTCATC		

Abbreviations: *PAI-1*, plasminogen activator inhibitor 1; *Par1*, protease-activated receptor 1; *Par2*, protease-activated receptor 2; *Fgl2*, fibrinogen-like protein 2; *TM*, thrombomodulin; *B2m*, β -2-microglobulin; *Hprt*, hypoxanthine guanine phosphoribosyl transferase; *Ywhaz*, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide; RT-PCR, reverse transcriptase polymerase chain reaction.

^a Sense and antisense primer sequences used for the quantitative RT-PCR studies. Primers were designed over at least one exon-exon junction to avoid inadvertently amplifying genomic DNA remaining after DNase treatment.

quantities of all samples. The geometric mean of the quantities of the 3 constitutively expressed genes was used to normalize the target gene quantities. Negative (water) controls were run for each primer set in the qRT-PCR reaction to ensure the reagents were not contaminated and that no secondary primer structures were amplified. Specific sense and antisense primers used in qRT-PCR runs were designed based on published cDNA sequences (see Table 1). Note that the amplicon sequences were designed to span at least one exon-exon junction, thereby preventing the inadvertent amplification of genomic DNA. The resulting PCR amplicons for all genes were sequence verified by the University of Vermont's DNA Analysis Core using an ABI 3130x1 Genetic Analyzer (16 capillary; Applied Biosystems).

Statistical analyses were performed using the Kruskal-Wallis 1-way analysis of variance (ANOVA) on ranks, followed by multiple comparisons tests using the Dunn and Dunnett tests where appropriate. Statistical significance occurred when $P < .05$.

Results

As previously reported by our laboratory,¹⁸ the intrauterine LPS caused PTD in the majority of the treated mice by 12 hours after injection; in contrast, none of the saline-injected control mice had delivered by 24 hours. In this ongoing series of studies, no mice have delivered at 2 hours and only 10% have delivered by 6 hours; however, by 12 hours 67% of the mice have delivered, and by 18 to 24 hours 86% had delivered. Mouse pups that delivered were born either dead or nonviable, as is consistent with day 15 to 16 of gestation.

As observed in Figure 1, Western blots performed using mouse uterine tissue confirmed protein expression for *PAI-1*, *Par1*, *Par2*, *Fgl2*, and *TM*; blots for *TF* were unsuccessful because of the poor specificity of the commercial antibody (data not shown). Reverse transcriptase PCR studies confirmed the expression of mRNA for *PAI-1*, *TF*, *Par1*, *Par2*, *Fgl2*, and *TM* in the pregnant uterus at day 15 of gestation. The amplicons for these genes were all of the predicted size (see Table 1 and Figure 2); and cDNA sequencing studies performed at the UVM DNA core facility confirmed the identity of these amplicons.

The quantitative RT-PCR studies have shown early upregulation of *PAI-1* mRNA in uterus, kidney, lung, and liver tissues, followed by a tissue-specific pattern of return toward baseline over time as observed in Figure 3. Uterine tissue showed a 23-fold increase in *PAI-1* mRNA at 2 hours, 32-fold at 6 hours (both $P < .05$), 9-fold at 12 hours, and 10-fold at 18 hours ($n = 6$ for each time point) as shown in Figure 3A. In contrast, *PAI-1* levels in normal saline-treated mice were significantly lower than the LPS-treated mice at 24 hours after intrauterine injection; and these levels were not significantly different from the time zero values (Figure 3A). For the other maternal tissues, *PAI-1* mRNA also increased significantly 53-fold in lung (Figure 3B), 120-fold in kidney (Figure 3C), and 60-fold in liver tissue (Figure 3D) at 2 hours after LPS injection (all $P < .05$; $n = 4$ for each time point).

The quantitative RT-PCR studies also confirmed *TF* expression in all of the mouse tissues examined (Figure 4); interestingly, *TF* expression in uterine tissue was markedly decreased at 12, 18, and 24 hours after LPS injection, compared with time zero (control) mice as observed in Figure 4A. At

Figure 1. Western blots demonstrating protein expression for *PAI-1*, *Par1*, *Par2*, *Fgl2*, and *TM* in the pregnant mouse uterus at day 15 of gestation (time 0 controls) and during the 24 hours after intrauterine LPS treatment. *PAI-1* indicates plasminogen activator inhibitor 1; *Par1*, protease-activated receptor 1; *Par2*, protease-activated receptor 2; *Fgl2*, fibrinogen-like protein 2; *TM*, thrombomodulin; LPS, lipopolysaccharide.

12 hours, *TF* had decreased significantly to 10% of baseline (time zero) levels, to 3% at 18 hour, and to 10% at 24 hours after LPS injection (all P s < .05). In contrast, *TF* levels in saline-treated mice were not significantly different from the time zero values; and these levels were significantly higher than those found in the LPS-treated mice at the 24-hour time point (Figure 4A). In the lung tissues, *TF* expression appeared to increase at 2 hours (Figure 4B); however, this upward trend was not statistically significant. In contrast, *TF* expression in the kidney and liver tissues remained stable during the 24 hours after LPS injection as observed in Figure 4C and 4D.

The quantitative RT-PCR studies also confirmed the expression of other coagulation-related genes including *Par1*, *Par2*, *Fgl2*, and *TM* in pregnant mouse uterine tissue (Figure 5). Interestingly, the mRNA expression for these mouse genes was not found to significantly change during LPS-induced PTD as observed in Figure 5.

Discussion

In this report, we have characterized the expression of several coagulation-related protein genes during LPS-induced PTD in

Figure 2. Representative DNA gels demonstrating the cDNA amplicons for *PAI-1*, *TF*, *Par1*, *Par2*, *Fgl2*, and *TM*. All of these amplicons were sequence verified to confirm their identity as the target mouse genes. *PAI-1* indicates plasminogen activator inhibitor 1; *TF*, tissue factor; *Par1*, protease-activated receptor 1; *Par2*, protease-activated receptor 2; *Fgl2*, fibrinogen-like protein 2; *TM*, thrombomodulin; cDNA, complementary DNA.

the day 15 pregnant CD-1 mouse. As reported previously, intrauterine LPS injection in day 15 pregnant mice caused the majority of the mice to deliver within 12 to 18 hours. We and others have previously reported that LPS injection induces a SIR in the mice characterized by a robust increase in proinflammatory cytokines and chemokines.^{4,17-19} The present study has added to these observations by demonstrating a robust increase in the expression of *PAI-1* in the pregnant uterus and other maternal tissues by 2 to 6 hours after LPS challenge. This time interval during which *PAI-1* expression was observed to increase after LPS injection was consistent with studies producing endotoxemia in chimpanzees²⁴ and in healthy humans.²⁵ Interestingly, we have observed significant downregulation of *TF* in uterine tissue, in contrast to its stable expression in mouse liver and kidney, and upward trend in the lung. The current studies have also confirmed the expression of *Par1*, *Par2*, *Fgl2*, and *TM* in the pregnant mouse uterus; however, the expression of these genes in uterine tissue was not modulated in response to intrauterine LPS injection.

During the SIRS, *TF* produced in response to inflammation is an important procoagulant that stimulates thrombin production through the activation of coagulation factor VII, leading to the formation of the activated factor X/factor V prothrombinase complex in the plasma.^{23,27} *Fgl2*, a procoagulant protein produced in response to tissue inflammation, has been reported to have similar prothrombinase activity and has been previously shown to be expressed in multiple tissues, including the pregnant uterus.²⁸ The membrane protease-activated receptors, especially *Par1* (the thrombin receptor) and *Par2* (the trypsin receptor), mediate cross talk during the inflammation and coagulation signaling cascades,^{27,29} while *TM* binding to thrombin leads to activation of protein C, an endogenous anticoagulant and anti-inflammatory agent.^{21,23}

The tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) stimulate the production of plasmin, which disrupts fibrin clots. Plasminogen activator inhibitor 1 inhibits tPA and uPA, thus promoting the generation of stable fibrin clots. Plasminogen activator inhibitor 1 is a

Figure 3. Quantitative RT-PCR results showing relative expression of *PAI-1* mRNA in uterus, lung, kidney, and liver tissues harvested from day 15 timed pregnant mice at 2, 6, 12, 18, and 24 hours after intrauterine LPS injection compared with time zero (control) animals. (*) denotes significance ($P < .05$), when compared with time zero levels (ANOVA on ranks, Dunn test); (†) denotes significance ($P < .05$), when compared to 24-hour LPS levels (ANOVA on ranks, Dunn test). Each bar represents the mean $\pm$ SE of mRNA from ($n = 6$) animals for each time point in uterus (A), and ($n = 4$) animals for each time point in lung (B), kidney (C), and liver (D). RT-PCR indicates reverse transcriptase polymerase chain reaction; *PAI-1*, plasminogen activator inhibitor 1; mRNA, messenger RNA; LPS, lipopolysaccharide; ANOVA, analysis of variance; SE, standard error.

single-chain glycoprotein in the serpin gene family that is consumed in the process of inhibiting the plasminogen activators.²⁶ Increased *PAI-1* consistently accompanies SIRS induced by sepsis or endotoxin^{10,16,24-26}; thus, *PAI-1* contributes to the prothrombotic state during the later stages of sepsis.³⁰ Plasminogen activator inhibitor 1 also appears to play a role beyond inhibiting plasminogen activators, including directly inhibiting interferon γ (IFN γ) production.¹⁰ Through its effects on surface protein and cellular adhesion, *PAI-1* appears to function as a “molecular switch” that can turn entire molecular pathways, such as inflammation or thrombosis, on or off.^{26,31} In septic human patients, higher blood levels of *PAI-1* protein have been correlated with disease severity, increased cytokine levels, hypotension, and risk of death.³⁰ Tumor necrosis factor α (TNF- α) and thrombin appear to contribute to enhanced

PAI-1 expression and activation during inflammation, an effect that is further amplified by other proinflammatory cytokines including interleukin 1 (IL-1) and IL-6.^{21,23,24} Plasminogen activator inhibitor 1 has also been associated with other thromboembolic states, such as coronary artery disease, acute myocardial infarction, deep vein thrombosis, and atherosclerosis, as well as preterm preeclampsia.^{26,32} In normal term pregnancy, *PAI-1* peaks significantly between 32 and 36 weeks of gestation and decreases sharply after delivery, returning to prepregnancy levels within days.^{33,34} The 4G/4G genetic mutation associated with increase *PAI-1* production has been reported to be an independent risk factor for complications during pregnancy including preeclampsia, placental abruption, intrauterine growth restriction, and stillbirth.³⁵

Figure 4. Quantitative RT-PCR results showing relative expression of tissue factor (*TF*) mRNA in uterus, lung, kidney, and liver tissues harvested from day 15 timed pregnant mice at 2, 6, 12, 18, and 24 hours after intrauterine LPS injection compared with time zero (control) animals. (*) denote significance ($P < .05$), when compared with time zero levels (ANOVA on ranks, Dunn and Dunnett tests); (†) denote significance ($P < .05$), when compared to 24-hour LPS levels (ANOVA on ranks, Dunn test). Each bar represents the mean $\pm$ SE of mRNA from ($n = 6$) animals for each time point in uterus (A), and ($n = 4$) animals for each time point in lung (B), kidney (C), and liver (D). RT-PCR indicates reverse transcriptase polymerase chain reaction; mRNA, messenger RNA; LPS, lipopolysaccharide; ANOVA, analysis of variance; SE, standard error.

Several investigators have reported an increase in coagulation-related proteins in preterm labor and delivery. Multiple human clinical studies have documented elevated thrombin–antithrombin (TAT) complex levels, indicating thrombin generation and hence activation of the coagulation cascade, in women experiencing preterm labor with and without preterm premature rupture of the membranes.^{36–38} One of these studies has also suggested that elevated TAT levels predict preterm premature rupture of membranes (PPROM).³⁸ We have reported previously that protease-activated receptors are expressed in pregnant rat myometrium; *Par2* was observed to increase significantly, whereas *Par1* remained stable over the course of gestation in myometrial tissue.³⁹

The marked and ubiquitous increases in *PAI-1* mRNA expression after LPS injection and preceding PTD suggest that

PAI-1 potentially plays a role during the SIR initiated by LPS during PTD in the mouse. The lack of increased expression of *Fgl2*, *Par1*, *Par2*, and *TM* within the pregnant mouse uterus suggests that baseline levels of these genes are sufficient to respond to the inflammation induced by LPS. The apparent downregulation of *TF* in the mouse uterus after 6 hours might indicate a negative feedback response to the proinflammatory LPS challenge. The role, if any, of progesterone and its withdrawal during these phenomena are yet to be defined. Previous reports have described a decline in serum progesterone during inflammation-induced PTD in the mouse, which was sufficient, but not necessary, for the delivery to occur.^{40–42} Although the expression studies described in this report are descriptive, these observations are important because they provide the basis for future investigations to determine the precise roles of *PAI-1*

Figure 5. Quantitative RT-PCR results showing relative expression of mRNA levels of other coagulation-related proteins harvested from uterine tissue in day 15 timed pregnant mice at 2, 6, 12, 18, and 24 hours after LPS intrauterine injection compared with time zero (control) animals. Each bar represents the mean $\pm$ SE of mRNA from ($n = 6$) animals for each time point for *Par1* (A), *Par2* (B), *Fgl2* (C), and ($n = 3$) animals for each time point for *TM* (D). RT-PCR indicates reverse transcriptase polymerase chain reaction; mRNA, messenger RNA; LPS, lipopolysaccharide; SE, standard error.

and *TF* in preterm labor in the mouse and their interactions with other participants in the inflammatory and coagulation cascades.

Declaration of Conflicting Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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