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ORIGINAL ARTICLE



# Effect of *Porphyromonas gingivalis* infection in the placenta and umbilical cord in pregnant mice with low birth weight

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**ABSTRACT**

**Objective:** Growing evidence indicates an association between periodontitis and delivery outcome; however, the mechanism is unclear. This study aimed to investigate the influence of *Porphyromonas gingivalis* (*Pg*) infection on delivery outcome in mice.

**Materials and methods:** Bacteremia was induced in pregnant Slc:ICR mice (8 weeks old) by intravenous injection of *Pg*. Mice were randomly divided into a control group (CO), and those receiving *Pg* injection at gestational day 1 (GD1), gestational day 15 (GD15) or every day (ED). Delivery outcome, *Pg* infection, and gene expression in the placenta and umbilical cord were evaluated.

**Results:** Birth weight was lower in the ED and GD15 groups than in the CO group. A remarkable increase in anti-*Pg* IgG antibody was observed in the ED and GD1 groups, although *Pg* was not detected in the placenta or umbilical cord. mRNA expression of *Tnfα* and *Il6* in the placenta, and *Hif1α* in the umbilical cord, was significantly increased in the ED group. Microarray analysis of the umbilical cord revealed increased expression of several genes including *Orm1*, *Mgl2*, *Rps6ka3* and *Trim15* in the ED group.

**Conclusions:** *Pg* infection during the third trimester caused low birth weight and inflammation in the placenta and umbilical cord.

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Periodontitis; low birth weight; *Porphyromonas gingivalis*; placenta; umbilical cord

## Introduction

Preterm birth is defined as a birth at less than 37 weeks of gestation, and low birth weight is defined as a birth weight of less than 2500 grams [1]. These adverse pregnancy outcomes are associated with increased morbidity and mortality in infants [2].

Periodontal disease is a chronic infectious disease caused by pathogenic oral bacteria that can lead to destruction of alveolar bone and connective tissues around the teeth [3]. Periodontal infection has long been associated with increase the risk for various diseases, such as atherosclerotic vascular disease [4], type 2 diabetes [5] and adverse pregnancy outcomes [6]. A meta-analysis of 17 studies concluded that periodontitis increased the risk of preterm birth and low birth weight by 2.3 and 4.0-fold, respectively [7].

There are two hypotheses how periodontitis causes adverse pregnancy outcome. First, local periodontal infection may lead to an increase in systemic inflammatory mediators [8,9]. Hasegawa et al. [10] reported that women with preterm birth had higher incidence of periodontal inflammation, increased number of periodontal bacteria in the saliva and increased level of serum inflammatory cytokines during pregnancy. Second, in patients with periodontitis, bacteremia may originate from periodontal pockets, as patients with generalized chronic periodontitis showed ulcers in inflamed periodontal pockets, and the total area of the ulcers was estimated to be as large as the size of the palm [11]. A previous study detected *Porphyromonas gingivalis* in the amniotic fluid in patients with threatened preterm labor, which supports the periodontal pocket origination hypothesis [12]. Previously, we also reported an association between *P. gingivalis* infection and threatened preterm labor and preterm birth [13].

Although numerous reports have demonstrated a relationship between periodontitis and adverse pregnancy outcome, it remains unclear how periodontitis causes adverse pregnancy outcome. The purpose of this study was to investigate whether *P. gingivalis* infection affects delivery outcome in mice.

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Supplemental data for this article can be accessed here.

## Material and methods

### Animals

Slc:ICR mice (8 weeks old; Sankyo Laboratory, Tokyo, Japan) were allowed free access to water and food throughout the experimental period. Gestational day (gd) was determined by the presence of a vaginal plug [14]. Mice were randomly divided into four groups: the control group (CO), *P. gingivalis* injection at gd 1 group (GD1), *P. gingivalis* injection at gd 15 group (GD15) and every day *P. gingivalis*-injection group (ED). Mice in the CO group were intravenously injected once daily with 100 μL of saline from gd 1 to gd 18. Systemic infection was induced by intravenous injection of *P. gingivalis* ATCC 33277. Mice in the ED group were injected once daily with 100 μL of *P. gingivalis* ($10^9$ CFU/mL) from gd 1 to gd 18. Mice in the GD1 and GD15 groups received a single 100-μL injection of *P. gingivalis* ($10^9$ CFU/mL) at gd 1 and gd 15, respectively. The body weight of pregnant mice, duration of gestation and birth weight of pups was recorded.

On gd 15, the mice underwent an operation under isoflurane anesthesia to determine the number of fetuses and verify pregnancy condition ($n = 4$–7 for each group). We also evaluated the body weight of fetuses and collected the placenta and umbilical cord at gd 17 ($n = 5$–6 for each group). Five umbilical cords from one pregnant mouse were pooled as one sample. Tissue samples were stored at −80 °C for RNA isolation. Plasma was also collected from mice at gd 17 and stored at −80 °C.

All protocols pertaining to animal use and euthanasia were reviewed and approved by the Animal Care Committee of the Experimental Animal Center at Tokyo Medical and Dental University (approval no. 0170246A).

### Cultivation of P. gingivalis

*P. gingivalis* was cultivated as previously described [15]. Briefly, *P. gingivalis* was maintained on trypticase soy agar (Difco Laboratories, Detroit, MI) supplemented with 10% defibrinated horse blood, hemin (5 μg/mL) and menadione (0.5 μg/mL) at 37 °C under anaerobic conditions (10% $CO_2$, 10% $H_2$ and 80% $N_2$). After 2 days of incubation, *P. gingivalis* was inoculated into trypticase soy broth supplemented with 0.5% yeast extract, menadione and hemin under anaerobic conditions. The bacterial suspension was cultured at 37 °C under anaerobic conditions to the mid-log phase (optical density at 660 nm of 0.6–0.9).

### Evaluation of cytokine levels in the plasma

Plasma levels of cytokines including interleukin (IL)-10, IL-17A, IL-6, IL-4, IL-2, tumor necrosis factor (TNF)-α and interferon (IFN)-γ were measured with the Cytometric Bead Array Mouse Th1/Th2/Th17 Cytokine kit (BD Biosciences, Franklin Lakes, NJ) according to the manufacturer's protocol. Data were analyzed with FCAP Array Software (BD Biosciences).

### Evaluation of anti-P. gingivalis IgG levels in the plasma

Antibody responses to *P. gingivalis* were determined by enzyme-linked immunosorbent assay using sonicated whole cell extracts of *P. gingivalis* ATCC 33277. Briefly, Costar 96-well enzyme immunoassay microplates (Corning Inc., Corning, NY) were coated with sonicated *P. gingivalis* (10 μg/mL) in carbonate buffer and incubated for 2 h at 37 °C. After blocking with 2% bovine serum albumin in carbonate buffer, plates were washed with phosphate-buffered saline (PBS) supplemented with 0.05% Tween 20 (pH 7.2). Serially diluted reference plasma ($2^5$–$2^{15}$; 200 μL/well) serving as a positive control and diluted sample serum ($2^{10}$; 200 μL/well) were added to each well, and the plates were incubated for 1 h at 37 °C. After washing, alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma-Aldrich, St. Louis, MO) was added (200 μL/well). The plates were incubated, washed and developed with a phosphatase substrate (Sigma-Aldrich). The optical density at 450 nm was measured with a SoftMAX microplate reader (Molecular Devices, Sunnyvale, CA). The mean optical density of mice in the CO group was set as '1' as the reference to calculate the fold change of the anti-*P. gingivalis* IgG level in the samples.

### RNA isolation and quantitative PCR analysis

Total RNA was extracted from the placenta and umbilical cord using NucleoSpin RNA (Takara Bio Inc., Shiga, Japan) after grinding using a Bio Masher IV (Wako Pure Chemical Industries, Osaka, Japan). The RNA samples were quantified by measuring the absorbance at 260 and 280 nm, and 500 ng of the RNA was reverse transcribed to cDNA using the PrimeScript RT Master Mix (Takara Bio). Quantitative PCR was performed on a Thermal Cycler Dice Real Time System II (Takara Bio). PCR mixtures were prepared using SYBR Premix Ex Taq II (Takara Bio), and reactions conditions were set according to the manufacturer's protocol. Gene expression levels were normalized to those of the reference gene *36b4*. PCR primers used in the study are listed in Supplementary Table S1.

### DNA isolation and detection of P. gingivalis

NucleoSpin DNA Tissue (Takara Bio) was used to extract DNA from the placenta and umbilical cord according to the manufacturer's instructions. To detect *P. gingivalis* DNA, extracted DNA was subjected to quantitative PCR using a TaqMan probe (5′-FAM-TGCGTAACGCGTATGCAACTTGCC-TAMRA-3′) on the Thermal Cycler Dice Real Time System II. Primers are listed in Supplementary Table S1.

### Microarray and data analysis

Quality of total RNA extracted from the umbilical cord of mice in the CO and ED groups was verified on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The Agilent Low Input Quick Amp Labeling kit was used to generate cRNA with a sample input of 200 ng of total RNA for

single-color (Cy3) microarray analysis according to the manufacturer's instructions. The cRNA was subsequently hybridized onto an Agilent SurePrint G3 Unrestricted Gene Expression 8 × 60K Microarray. The fluorescence signal from hybridized microarrays was detected on an Agilent Microarray Scanner System. Raw microarray data were extracted using Feature Extraction Software (ver. 11.0.1.1; Agilent Technologies).

The microarray data were 75% tile-normalized and log2-transformed according to the manufacturer's recommendations [16] using R software (ver. 3.3.2). The Limma Bioconductor package (ver. 3.30.4) [17] was used to identify differentially expressed genes (DEGs). Statistical significance was evaluated by Benjamin and Hochberg's false discovery rate (FDR) to account for multiple testing. DEGs were defined according to FDR $q < 0.05$ and $|\text{fold change}| > 2$.

### Histological analysis

Placenta samples were fixed with 4% paraformaldehyde in PBS for 24 h and embedded in paraffin. Subsequently, 5-μm-thick sections were prepared and stained with hematoxylin and eosin (HE). Quantitation of the areas of spongiotrophoblasts was performed every 5 pictures for each sample using Adobe Photoshop CC 2017 Software (San Jose, CA).

### Statistical analysis

Data distribution was assessed with the Shapiro–Wilk test. Student's *t*-test was used for two-group comparisons. Pearson's $\chi^2$ test with Bonferroni correction or one-way analysis of variance followed by Dunnett's test was used for multiple-group comparisons versus the CO group. For comparison of anti-*P. gingivalis* IgG levels, one-way analysis of variance, followed by a *t*-test with Bonferroni correction, was performed for multiple-group comparisons. Data were analyzed using SPSS v.22.0 software (SPSS Inc., Chicago, IL). $p < .05$ was considered statistically significant.

## Results

### Evaluation of delivery outcome

Although *P. gingivalis* infection did not affect the average gestational period (i.e., all mice delivered on gd 19), continuous or gd 15 *P. gingivalis* infection resulted in low birth weight; the average birth weight of pups in the ED (1.53 ± 0.01 g) and GD15 (1.55 ± 0.02 g) groups was lower than that of pups in the CO group (1.72 ± 0.03 g) (Figure 1(A,B)). There were no significant differences in body weight between pups in the CO and GD1 groups. The body weight of fetuses in the ED group appeared to be lower than that of fetuses in the CO group ($p = .05$) at gd 17 (Figure 1(C)). There were no significant differences in the body weight of pregnant mice (Figure 1(D)) or in fetal survival rate (Figure 1(E)) at gd 17.

### Effect of P. gingivalis infection

The plasma levels of IL-10, IL-17A, TNFα, INF-γ, IL-6, IL-4 and IL-2 were below the assay's limit of detection at gd 17, suggesting that *P. gingivalis* injection did not induce a strong inflammatory response in mice. However, a significant increase of anti-*P. gingivalis* IgG was observed in the ED and GD1 groups. Remarkably, the level of anti-*P. gingivalis* IgG in the ED group was dramatically increased when compared to

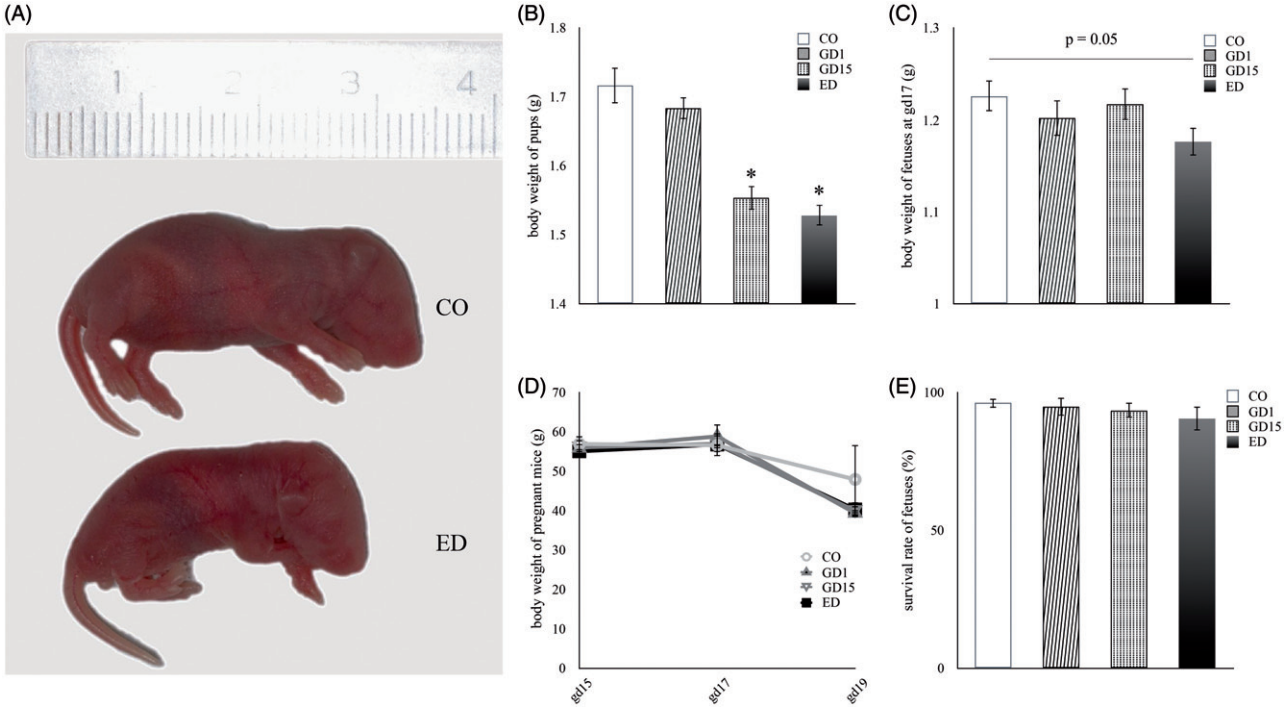


**Figure 1.** Evaluation of delivery outcomes after *P. gingivalis* infection ($n = 4$–7 pregnant mice). (A) Photograph of pups from CO and ED groups of mice after delivery. (B) Body weight of pups after delivery. (C) Body weight of fetuses at gd 17. (D) Body weight of pregnant mice. (E) Survival rate of fetuses at gd 15. *, $p < .05$ compared to the CO group.

that of GD1 group (Figure 2). Nevertheless, *P. gingivalis* was not detected in the placenta or umbilical cord by quantitative PCR using a TaqMan probe.

### *Inflammatory and hypoxia-related genes in the placenta and umbilical cord*

The mRNA expressions of inflammatory and hypoxia-related genes were evaluated in the placenta and umbilical cord.

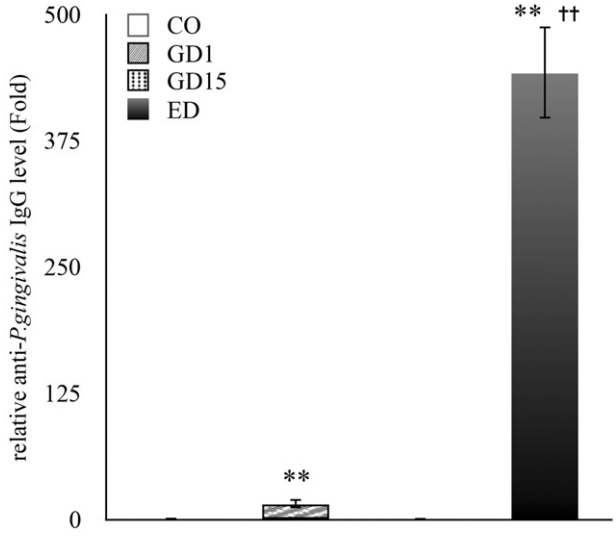


**Figure 2.** Relative anti-*P. gingivalis* IgG levels at gd 17 ($n = 5$–6). **, $p < .01$ compared to the CO group; ††, $p < .01$ compared to the GD1 group.

Continuous *P. gingivalis* infection led to an increase in the mRNA expressions of *Tnfα* and *Il6* in the placenta (Figure 3(A,B)). Pregnant mice injected with *P. gingivalis* on gd 15 showed upregulation of *Il6* and hypoxia-inducible factor 1α (*Hif1α*) genes in the placenta (Figure 3(B,C)). There were no differences in the mRNA levels of *Tnfα*, *Il6* and *Hif1α* in the placenta between the GD1 and CO groups (Figure 3(A–C)).

We observed an increasing trend of *Tnfα* and *Il6* mRNA expressions in the umbilical cord in response to *P. gingivalis* infection, although this was not statistically significant (Figure 3(D,E)). The mRNA expression of *Hif1α* was increased in the umbilical cord of mice in the ED group when compared to that of mice in the CO group (Figure 3(F)).

### *Microarray analysis of the umbilical cord*

To obtain a comprehensive overview of the gene expression profile in the umbilical cord following *P. gingivalis* infection, a microarray analysis was performed for mice in the CO and ED groups. No DEGs ($|\text{fold change}| > 2$; $q < 0.05$) were found in the gene sets. However, 104 genes showed $|\text{fold change}| > 2$ and $p < .05$; a heatmap for these genes, along with their Entrez Gene ID (81 genes), is shown in Figure 4(A).

We subsequently focused on genes related to inflammation, delivery and fetal growth. Compared to the CO group, the upregulation of orosomucoid 1 (*Orm1*), macrophage galactose N-acetyl-galactosamine-specific lectin 2 (*Mgl2*),

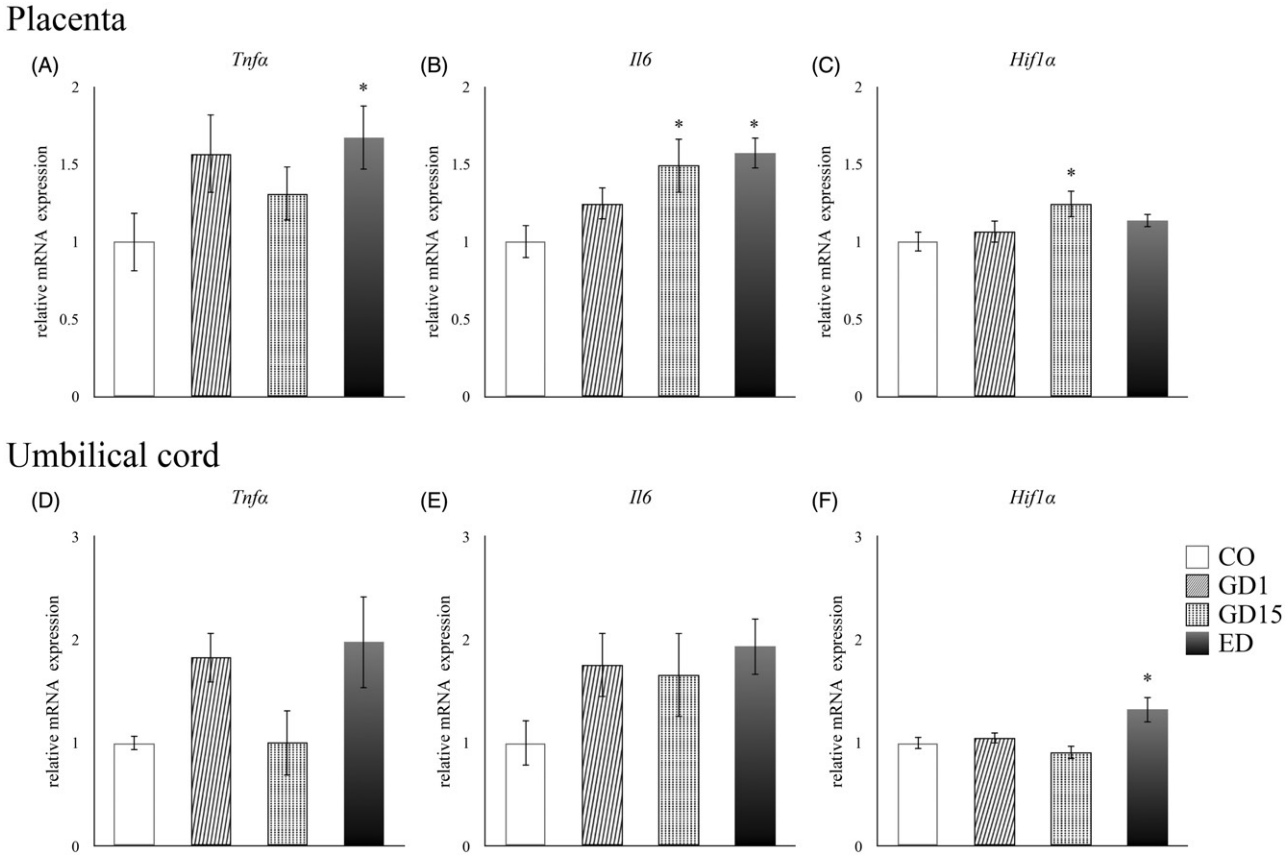


**Figure 3.** mRNA expressions in the placenta and umbilical cord among mice in CO, GD1, GD15 and ED groups ($n = 5$–6). (A) *Tnfα*, (B) *Il6* and (C) *Hif1α* mRNA expression in the placenta. (D) *Tnfα*, (E) *Il6* and (F) *Hif1α* mRNA expression in the umbilical cord. *, $p < .05$ compared to the CO group.

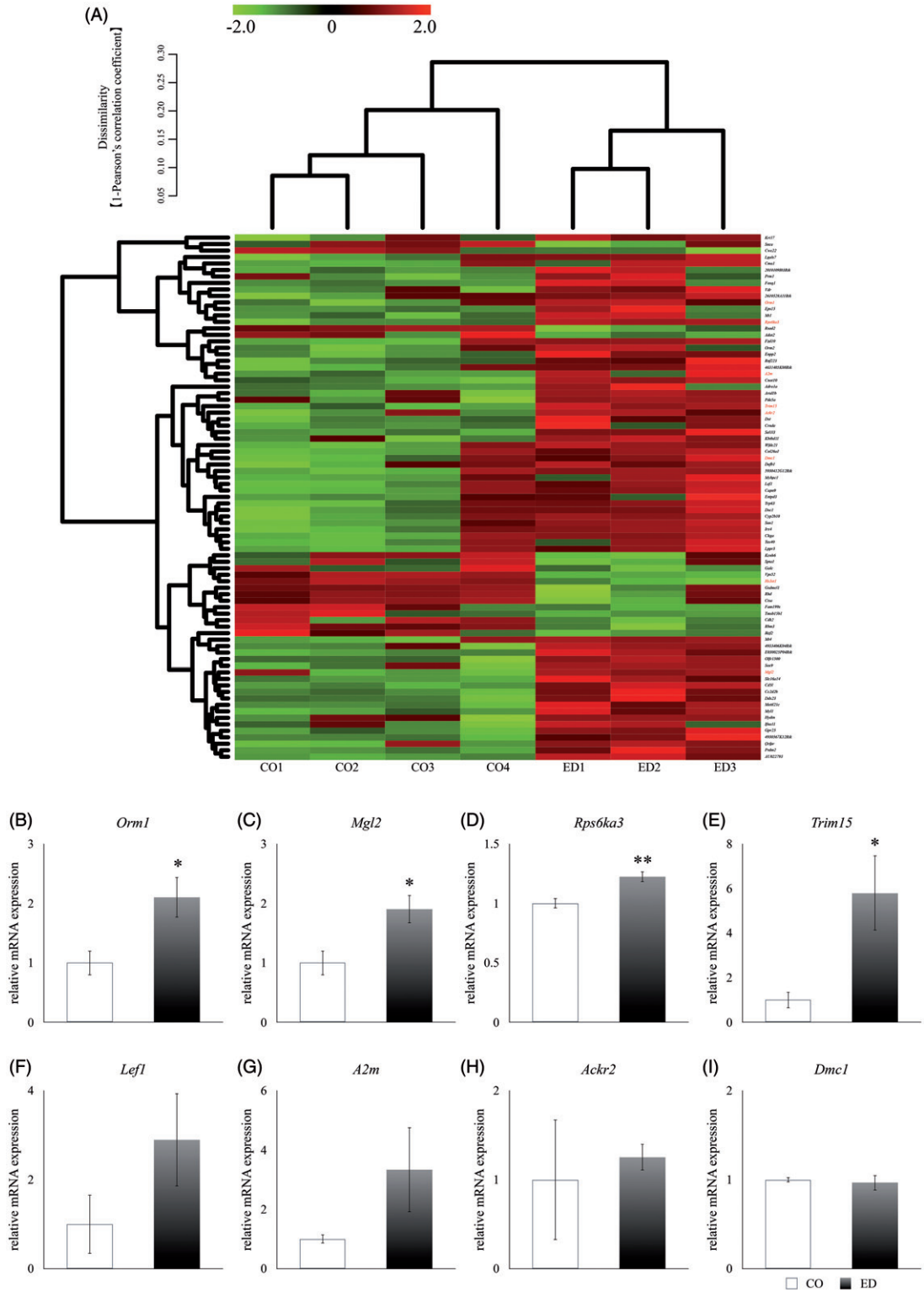


**Figure 4.** Microarray analysis of the umbilical cord of mice in the CO and ED groups ($n = 3$–4). (A) A heatmap of genes showing |fold change| > 2 and $p < .05$ along with their Entrez Gene ID. (B–I) Analysis of gene expression in the umbilical cord. (B) *Orm1*, (C) *Mgl2*, (D) *Rps6ka3*, (E) *Trim15*, (F) *Lef1*, (G) *A2m*, (H) *Ackr2* and (I) *Dmc1* mRNA expression. *, $p < .05$; **, $p < .01$ compared to the CO group.

ribosomal protein S6 kinase a3 (*Rps6ka3*) and tripartite motif-containing 15 (*Trim15*) mRNAs in the umbilical cord was validated in the ED group by quantitative PCR. The mRNA expression of lymphoid enhancer-binding factor 1 (*Lef1*) appeared to be increased in mice in the ED group, although this was not statistically significant (Figure 4(B–I)).

### *Histological analysis of the junctional zone of the placenta*

We observed notable alterations, the area of spongiotrophoblasts in the junctional zone of the placenta in the ED group; however, there were no apparent changes in the other layer of the placenta (the labyrinthine zone) and maternal decidual

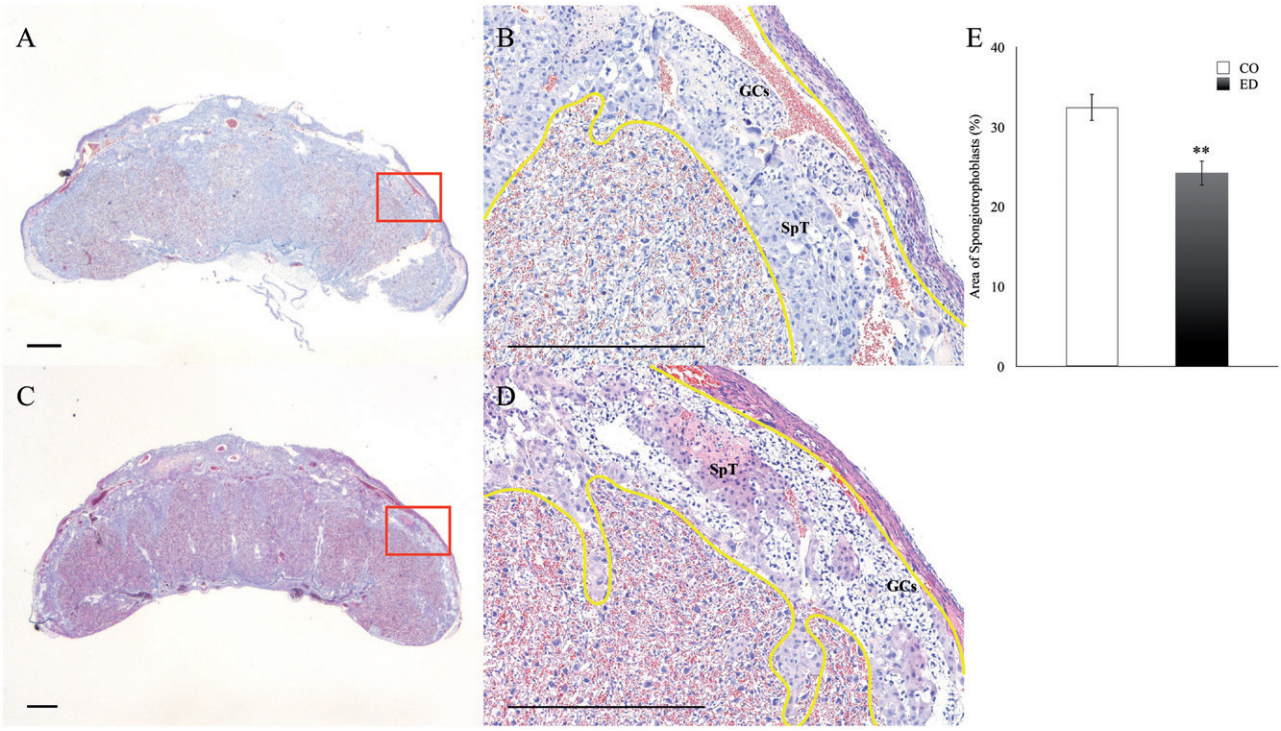


**Figure 5.** Hematoxylin and eosin-stained midline sections of the mature placenta at gd 17 from mice in the (A, B) CO and (C, D) ED groups ($n = 5$–6). (B, D) High-magnification images from the red box in panel A and C, respectively. The junctional zone is indicated between the yellow lines. GCs, glycogen cells; SpT, spongiotrophoblasts; black bar =500 μm. (E) Proportion of spongiotrophoblasts, **, $p < .01$ compared to the CO group.

component (Figure 5(A–D)). The proportion of spongiotrophoblasts was decreased in the junctional zone of mice in the ED group (Figure 5(E)).

## Discussion

The early life growth stage affects the risk for adult-onset diseases. Low birth weight is regarded as a risk factor for obesity [18], cardiovascular disease [19] and type 2 diabetes [20]. The global incidence of low birth weight is approximately 15.5% [21], and reducing this incidence is a major public health challenge. Meta-analysis studies have concluded that periodontitis is an independent risk factor for adverse delivery outcome [22,23]; however, it is not clear whether periodontitis directly causes low birth weight.

In this study, we demonstrated that *P. gingivalis* infection resulted in low birth weight, but not preterm birth. Pups born from mice injected with *P. gingivalis* at gd 15 exhibited low birth weight, although the pups from mice injected at gd 1 did not show low birth weight. We postulated that mice in the GD1 group were infected with *P. gingivalis* before implantation, whereas those in the GD15 group were infected during the third trimester. This suggests that it is critical to prevent *P. gingivalis* infection before implantation to reduce the incidence of low birth weight. Indeed, the aforementioned meta-analyses concluded that periodontal treatment was not effective to reduce the incidence of adverse delivery outcome [24,25], suggesting that periodontal treatment before pregnancy may be required to ensure safe delivery.

We observed that anti-*P. gingivalis* IgG levels were increased in the ED and GD1 groups. IgG is produced by acquired immunity at approximately 7 days after infection, and continuous infection can amplify the IgG production [26]. In this study, a dramatic increase of anti-*P. gingivalis* IgG was observed in the ED group, although similar trend was not observed in that of GD15 group. Although the infection was established, *P. gingivalis* was not detected in either the placenta or umbilical cord. In addition, the levels of inflammatory cytokines were not significantly different among mice in the CO, GD1, GD15 and ED groups.

We showed that in the ED group, mRNA expressions of *Tnfα* and *Il6* were significantly increased in the placenta, and tended to be elevated in the umbilical cord. Previous studies showed that inflammation in the placenta and umbilical cord affects delivery [27,28]. We also found that *Hif1α* mRNA expression was increased in the umbilical cord of these mice. *Hif1α* is a transcription factor induced under hypoxia [29], and activation of HIF-1α has an important role in myeloid cell infiltration and activation [30]. HIF-1α increased levels of pro-inflammatory cytokines, including TNF-α, IL-1, IL-4, IL-6 and IL-12 produced by macrophages in the presence of endotoxin [31]. Moreover, Peyssonnaux et al. [32] reported that HIF-1α expression was induced to a greater extent in the infected condition with Gram-positive or Gram-negative bacteria than in the no-infected condition in murine macrophages. Inflammation itself can cause hypoxia, which in turn induces further inflammation [33]. Therefore, the present study suggests that *P. gingivalis* infection is possible to induce HIF-1α expression and subsequent inflammation in the umbilical cord.

Our murine model developed fetal growth restriction (FGR), i.e., fetuses with an estimated fetal weight of less than the 10th percentile for the gestation age [34]. The etiology of

FGR can be broadly categorized into maternal, fetal and placental. Maternal factors include substance use and abuse, maternal nutrition, multiple gestation, teratogen exposure, infectious diseases, genetic and structural disorders, placental disorders and umbilical cord abnormalities [35]. Cotechini et al. [36] administrated low-dose lipopolysaccharide (LPS) to pregnant rats at gd 13.5–16.5, and showed that abnormal inflammation caused by LPS resulted in FGR. In our model, inflammation in the placenta and umbilical cord caused by *P. gingivalis* infection might also result in FGR in mice, mimicking the results previously obtained with LPS in rats.

Several studies have reported a relationship between periodontal bacterial infection and delivery outcome. Collins et al. [37] showed that hamsters infected at gd 8 with $10^7$ CFU of *P. gingivalis* via an implanted subcutaneous tissue chamber had increased maternal levels of both prostaglandin E2 and TNF-α, and developed adverse pregnancy outcomes such as FGR and embryo lethality. Additionally, continuous infection with $10^9$ CFU of *P. gingivalis* for 2 weeks and an additional infection with $10^7$ CFU of *P. gingivalis* at gd 7.5 into the subcutaneous tissue chamber increased the level of anti-*P. gingivalis* IgG and incidence of FGR [38]. Our results were in agreement with these findings. In another model, continuous infection with *P. gingivalis* (originally at $10^8$ CFU) via the dental pulp was shown to increase serum TNF-α, IL-6, and IL-17 levels, and resulted in both preterm birth and low birth weight [14]. Our infection model may have less impact on the delivery outcome than these models.

In this study, we performed a microarray analysis to assess the comprehensive gene expression profile in the umbilical cord, and the mRNA expression of several genes was validated by quantitative PCR. We found that several genes, including *Orm1*, *Mgl2*, *Rps6ka3* and *Trim15*, were upregulated in mice that received daily *P. gingivalis* injections. The *Orm1* gene encodes an ORM protein, also known as α-1-acid glycoprotein, which is a highly glycosylated polyanionic protein [39]. ORM is an abundant plasma protein induced under stress such as tissue injury, inflammation and infections [40], and has a protection role in animals following lethal shock induced by TNF or LPS [41]. MGL2, encoded by the *Mgl2* gene, is a known marker of M2 macrophages. There are two well-established polarized phenotypes of macrophages: M1 macrophages as classically activated macrophages and M2 macrophages as alternatively activated macrophages [42]. In contrast to M1 macrophages, M2 macrophages play a role in polarization Th2-type immune responses, parasite clearance, resolution of or smoldering inflammation, tissue remodeling, angiogenesis, tumor progression and immunoregulation [43]. Sarr et al. [44] revealed that infection induced macrophage accumulation in the junctional zone of the placenta. *P. gingivalis* infection causes inflammation in the placenta and the proportion of M2 macrophages may be increased in the umbilical cord. *Rps6ka3* encodes the ribosomal S6 kinase (RSK) protein [45], which promotes the mammalian target of rapamycin complex-1 (mTORC1) signaling pathway [46]. The mTOR pathway regulates various major cellular processes such as cell proliferation, differentiation and cell survival [47] and is activated in pathological conditions. *Lef1* encodes the lymphoid enhancer-binding factor 1 (LEF-1) protein, a 54-kDa nuclear protein that is expressed specifically in pre-B and T cells [48]. LEF1 is a central regulator of iNKT cells, which are innate-like T cells that rapidly produce cytokines for antimicrobial immune responses [49]. Increased mRNA expression of *Orm1*, *Mgl2* and *Rps6ka3* in the umbilical cord suggests that bacteremia induced inflammation in the umbilical cord of mice in the ED group. Although not statistically significant, we observed an increased trend of *Lef1* mRNA expression in the ED group, which might suggest that iNKT cells accumulated in the umbilical cord in response to *P. gingivalis* infection. Therefore, the umbilical cord may have a role in protecting fetuses from infection.

Neonatal DNA methylation varies with gestational age, even in term deliveries [50]. *Trim15* is one of several genes associated with decreased methylation of CpG sites during the gestational period [51]. The role of TRIM15 is unknown; however, increased *Trim15* mRNA expression induced by infection may be related to preterm birth and low birth weight. A2M, also known as alpha-2 macroglobulin, is a member of the alpha-macroglobulin family of proteins that includes protease inhibitors [52]. A2M has a stronger affinity to plasmin than to fibrinogen [53]; therefore, elevated *A2m* mRNA expression may lead to failure of fibrinolysis and longer maintenance of blood clots. The increase in mRNA expression of the abovementioned genes in the umbilical cord thus shows that bacteremia induced by *P. gingivalis* affects maternal and fetal systems, and that the umbilical cord may have a crucial role in fetal protection.

The mouse placenta consists of three compartments, the labyrinthine zone, the junctional zones and the decidua basalis. The junctional zone/basal plate is required for fetal viability [54], and the junctional zone consists of spongiotrophoblasts, trophoblast giant cells and glycogen cells [55]. The functions of the glycogen cells and spongiotrophoblasts have not been thoroughly defined [56]; however, spongiotrophoblasts play a structural role and are key endocrine lineages of the mature placenta [57]. In this study, we showed a decrease in the proportion of spongiotrophoblasts in the junctional zone at gd 17 in the ED group compared to the CO group. Degeneration of trophoblasts and endothelial cells was previously reported in mice infected with *P. gingivalis* via the dental pulp [14]. It was also reported that *P. gingivalis* LPS upregulated inflammatory mediators in cultured trophoblasts [14]. The decrease in spongiotrophoblasts caused by *P. gingivalis* infection might affect the mature placenta. Abnormal placentation can cause hypoxia and upregulation of *Orm1*, *Mgl2* and *Rps6ka3* mRNA in the umbilical cord.

In conclusion, we showed that *P. gingivalis* infection, especially in the third trimester, caused low birth weight accompanied with inflammation in the placenta and umbilical cord. Our findings provide insight into the mechanism by which periodontitis during pregnancy can lead to adverse outcomes, and can inform strategies to reduce the risk of low birth weight.

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## Disclosure statement

The authors declare no potential conflicts of interest in this study.

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