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Periodontal Bacteria–Derived Vesicles Disrupt Maternal and Fetal Health

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Abstract

Periodontitis is a chronic inflammatory disease driven by a dysbiotic subgingival microbiome that has been consistently associated with adverse pregnancy outcomes (APOs). However, the molecular mechanisms linking pathogenic periodontal bacteria to gestational complications remain incompletely understood. Outer membrane vesicles (OMVs) released by gram-negative periodontal bacteria have emerged as key mediators of host–microbe interactions due to their systemic disseminating properties, endowing them the capacity of delivering virulence factors to distant tissues and modulating immune-inflammatory and metabolic responses in the absence of live bacteria. In this study we investigated whether systemic administration of OMVs derived from 2 major periodontal pathogens, *Porphyromonas gingivalis* (*Pg*) or *Fusobacterium nucleatum* (*Fn*), alters pregnancy outcomes, maternal inflammation, glucose tolerance, and fetal growth in a murine model. Periodontitis-affected pregnant mice were systemically exposed to *Pg*- or *Fn*-derived OMVs throughout gestation. Pregnancy success, alveolar bone loss, OMV biodistribution, systemic and placental inflammation, glucose metabolism alterations, and fetal growth were assessed. *Pg*-OMVs significantly impaired pregnancy success ($P = 0.0055$), with only 2 *Pg*-OMV–exposed females reaching the gestational endpoint with viable pregnancies, precluding subsequent gestational outcome analyses. In contrast, *Fn*-OMVs primarily affected established pregnancies, exacerbating alveolar bone loss, as evidenced by reduced BV/TV ($P = 0.0003$), and inducing hepatomegaly ($P = 0.037$) and splenomegaly ($p = 0.009$), accompanied by increased splenic interleukin (IL)–1β and IL-6 expression ($P = 0.045$ and $P = 0.035$, respectively). Metabolically, *Fn*-OMVs impaired maternal glucose tolerance ($P = 0.017$) and preferentially accumulated in the placenta, where NF-κB activation ($P = 0.012$) and IL-1β and tumor necrosis factor–α expression ($P = 0.036$ and $P = 0.025$, respectively) were upregulated. Furthermore, offspring displayed reduced fetal weight ($P = 0.044$) and anthropometric alterations consistent with fetal growth restriction (FGR) ($P < 0.05$). Notably, these organ-specific responses occurred without overt plasmatic inflammatory cytokine elevation but with increased circulating IL-4 levels ($P = 0.023$). In sum, periodontal bacteria–derived OMVs differentially disrupt pregnancy, with *Pg*-OMVs impairing pregnancy success and *Fn*-OMVs inducing maternal metabolic and placental alterations compatible with an FGR phenotype.

Keywords: periodontitis, outer membrane vesicles, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, pregnancy loss, fetal growth restriction

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Background

Periodontitis, a chronic inflammatory disease triggered by a dysbiotic subgingival microbiome, has been associated with a wide range of pathological systemic conditions (Hajishengallis 2022), including adverse pregnancy outcomes (APOs) (Karimi et al 2023). APOs encompass a range of perinatal complications, such as miscarriage, preterm birth, preeclampsia, intrauterine growth restriction, low birth weight, stillbirth, and gestational diabetes mellitus (GDM) (Xu and Han 2022).

Two primary mechanisms have been proposed to explain how periodontitis may contribute to APOs: (1) the direct translocation of oral micro-organisms or their products to the fetal–placental unit and (2) the indirect impact of inflammatory mediators produced in the oral cavity, disseminated systemically reaching the fetal–maternal unit (Figuero et al 2020). Periodontal bacteria, such as *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*), have been implicated not only in periodontitis but also in the development of systemic inflammation and multiple comorbidities, including APOs (Choudhury et al 2022; Xu and Han 2022; Yoshida et al 2022). Notably, both *Pg* and *Fn*, as well as their bacterial components, have been associated with impaired gestations (Fischer et al 2019).

Pg has been detected in the amniotic fluid from women at risk of preterm labor (León et al 2007) and linked to meconium-stained amniotic fluid (Jin et al 2022). Furthermore, exposure to its subproducts has been associated with placental inflammation (Bendek et al 2026), fetal growth restriction (FGR) (Tavarna et al 2020) and preterm birth (Ao et al 2015). Similarly, *Fn*, a commensal bacterium that can opportunistically acquire pathogenic traits under dysbiotic conditions (Sakanaka et al 2022), modulates host immune responses to facilitate tissue invasion and persistence, contributing not only to periodontitis progression but also tumorigenesis (Yang et al 2025). *Fn* has also been extensively detected in fetal tissues and associated to APOs, including preterm birth, premature rupture of membranes, and chorioamnionitis (Vander Haar et al 2018).

Accumulating evidence has shown that outer membrane vesicles (OMVs) are key mediators of bacterial–host interactions, representing a novel mechanism by which periodontal pathogens exert systemic effects (Chen et al 2023). OMVs are nanosized vesicles continuously shed from the outer membrane of gram-negative bacteria, carrying a wide range of virulence factors derived from their parental cells (Juodeikis and Carding 2022). Their lipidic bilayer protects OMVs cargo from degradation, facilitating immune evasion, systemic dissemination, and targeted delivery to host cells (Furuyama and Sircili 2021), thereby enhancing the ability of these bacteria to manipulate and disrupt essential host cellular processes (Ellis and Kuehn 2010). Moreover, OMVs can cross biological barriers including the placental syncytiotrophoblast (Wei et al 2025), enabling access to otherwise protected anatomical sites. These features allow OMVs to function as long-range "biological missiles" that can efficiently propagate bacterial virulence factors, mediate host–microbe interactions, and promote the disruption of host homeostasis.

Emerging evidence suggests that OMVs derived from oral periodontal bacteria contribute to systemic inflammation (Zhang et al 2025) and metabolic dysregulation (Takamura et al 2016; Seyama et al 2020). In this context, OMVs may represent a previously underappreciated mechanism linking periodontal disease to APOs. While periodontal bacteria have demonstrated the ability to disrupt maternal homeostasis during pregnancy (Gómez et al 2020), the contribution of their secreted OMVs to APO development remains unexplored. In this study, we hypothesized that *Pg*- and *Fn*-derived OMVs contribute to APOs by promoting inflammatory and metabolic disturbances. Using an *in vivo* approach, we assessed the impact of systemic exposure to *Pg*- and *Fn*-OMVs on pregnancy success, maternal organ–specific inflammatory responses, glucose tolerance, and fetal outcomes.

Methods

Detailed methods are provided in the Appendix Extended Methods.

Animals

Female C57BL/6 wild-type mice (8 to 12 wk old) were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee (protocol CEC2021021) and complied with ARRIVE guidelines.

Bacterial Strains and OMV Isolation

Fusobacterium nucleatum subsp. *nucleatum* ATCC 25586 *(Fn)* and *P. gingivalis* ATCC 33277 *(Pg)* OMVs were isolated from bacterial culture supernatants by sequential filtration and ultracentrifugation as previously described (Sepúlveda-Pontigo et al 2025). OMVs were characterized according to MISEV guidelines (Welsh et al 2024) by transmission electron microscopy, nanoparticle tracking analysis (Longjohn and Christian 2022), and endotoxin quantification, following standard procedures.

In Vivo Model and Subsequent Analyses

Experimental periodontitis was induced by bilateral ligation of the second maxillary molars as previously described (Rojas et al 2021). Five days after ligature placement, baseline glucose tolerance was assessed by intraperitoneal glucose tolerance test (ipGTT) (Pedro et al 2020). Females were then mated overnight, and mating was confirmed by the presence of a vaginal plug (E0.5). Following mating confirmation, females received daily intravenous injections of sterile Dulbecco's phosphate-buffered saline (DPBS) or 1×10^8 *Pg*- or *Fn*-derived OMVs from E1.5 to E17.5. Body weight was monitored throughout pregnancy (Appendix Figs 2A and 3A). Animals

were euthanized at E18.5 for collection of maternal blood, maxillae, liver, spleen, placentae, and fetuses.

At E18.5, females were classified as pregnant or nonpregnant according to the presence of viable fetuses. Animals with successful pregnancies were included in gestational outcome analyses, whereas females that failed to establish or maintain pregnancy were analyzed as a separate nonpregnant cohort. Because *Pg*-OMV administration markedly reduced pregnancy success, with only 2 *Pg*-OMV–treated females reaching E18.5 with viable pregnancies, those animals were included exclusively in the pregnancy success analysis and excluded from comparative gestational outcome analyses. To enable appropriate comparisons within the nonpregnant cohort, additional PBS-treated nonpregnant females were included. The final study comprised pregnant PBS ($n=8$) and *Fn*-OMV ($n=9$) groups and nonpregnant PBS ($n=9$), *Pg*-OMV ($n=8$), and *Fn*-OMV ($n=8$) groups. Gestational outcome analyses were performed using 15 litters (104 fetuses), and an additional unligated pregnant PBS cohort (UL+PBS; $n=3$; 21 fetuses) was included to evaluate the contribution of ligature-induced periodontitis. A detailed summary of cohort allocation, sample sizes, and outcome assessments is provided in Appendix Table 1.

Alveolar bone loss was assessed by micro–computed tomography (micro-CT) (Cafferata et al 2020), pregnancy outcomes by fetal and placental morphometric analyses (Mu et al 2008), metabolic status by ipGTT, inflammatory responses by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) using specific primers (Appendix Table 2), and multiplex bead-based assay, tissue morphology by histology, and OMVs biodistribution using DiR-labeled vesicles (Yanten-Fuentes et al 2025). All procedures were performed as previously described. Image acquisition, fetal morphometric analyses, and outcome assessments were performed by investigators blinded to treatment allocation whenever feasible. Statistical analyses were performed using GraphPad Prism 8.0.2, applying the appropriate parametric or nonparametric tests according to data distribution.

Results

Pg *and* Fn *Release Nano-Sized OMVs*

To determine the main features of the isolated OMVs, we characterized them using complementary approaches (Appendix Fig 1A–G). Transmission electron microscopy revealed a characteristic cup-shaped morphology for both *Pg*- and *Fn*-OMVs (Appendix Fig 1C). Nanoparticle tracking analysis showed that *Pg*-OMVs displayed a broader size distribution, with 2 major size peaks, whereas *Fn*-OMVs exhibited a predominantly unimodal size distribution (Appendix Fig 1D). Nonetheless, mean particle size and protein content per particle did not differ between groups (Appendix Fig 1E – F). Both preparations contained detectable endotoxin levels (Appendix Fig 1G). Endotoxin content per particle was assessed in OMV preparations, with values of 4.72×10^{3} EU/particle for *Fn*-OMVs and $4.51\times10^{-5}\pm3.32\times10^{-5}$ EU/particle for *Pg*-OMVs. However, given the limited sample size, these values are reported descriptively, and no formal statistical comparison between preparations was performed. These findings confirm the successful isolation of gram-negative bacterial OMVs and reveal distinct biophysical characteristics between *Pg*- and *Fn*-derived vesicles.

Exposure to Periodontal Bacteria–Derived OMVs Differentially Affect Pregnancy Success and Enhance Alveolar Bone Resorption

To evaluate the impact of circulating OMVs on pregnancy outcomes, we established an *in vivo* model of periodontitis-affected pregnant mice systemically exposed to *Pg*-OMVs or *Fn*-OMVs. As outlined in Figure 1A, periodontitis was induced prior to mating, and OMVs were administered throughout gestation. This experimental design enabled the assessment of the effects of systemically disseminated periodontal pathogen–derived OMVs in the context of established periodontitis.

Systemic exposure to *Pg*- and *Fn*-OMVs differentially affected pregnancy success. While *Fn*-OMVs moderately reduced pregnancy success (53.6%), *Pg*-OMVs markedly impaired this outcome, with only 20% of females achieving a successful pregnancy after mating, compared with 80% in the control group ($P=0.0055$) (Fig 1B). Because only 2 *Pg*-OMV–exposed females reached E18.5 with viable pregnancies, this group was excluded from subsequent gestational analyses, whereas nonpregnant animals were analyzed as a separate cohort (Appendix Fig 2). Animals from the nonpregnant cohort exhibited no significant body weight changes throughout the study period (Appendix Fig 2A). In contrast, pregnant females exhibited a progressive body weight increase from gestational day 10 onward, with no significant differences between conditions (Appendix Fig 3A).

Periodontitis induction was confirmed by micro-CT, showing alveolar bone loss in both *Fn*-OMVs and the control group (Fig 1C). However, *Fn*-OMVs–exposed mice exhibited exacerbated bone loss, evidenced by reduced bone volume fraction (BV/TV, $P=0.0003$), trabecular number (Tb.N, $P=0.017$), and trabecular thickness (Tb.Th, $P=0.0144$), together with increased trabecular separation (Tb.Sp, $P=0.0033$) (Fig 1D). Together, these results show that systemic administration of *Pg*- and *Fn*-OMVs differentially affect pregnancy outcomes. Whereas *Pg*-OMVs markedly reduced pregnancy success, *Fn*-OMVs partially impaired this parameter and exacerbated periodontal bone loss.

Fn-OMVs *Accumulate in Placental Tissues and Induce Hepatosplenomegaly Accompanied by Plasmatic Tolerogenic Responses*

To investigate the systemic distribution and downstream effects of *Fn*-OMVs during pregnancy, DiR-labeled *Fn*-OMVs biodistribution and systemic inflammatory responses were

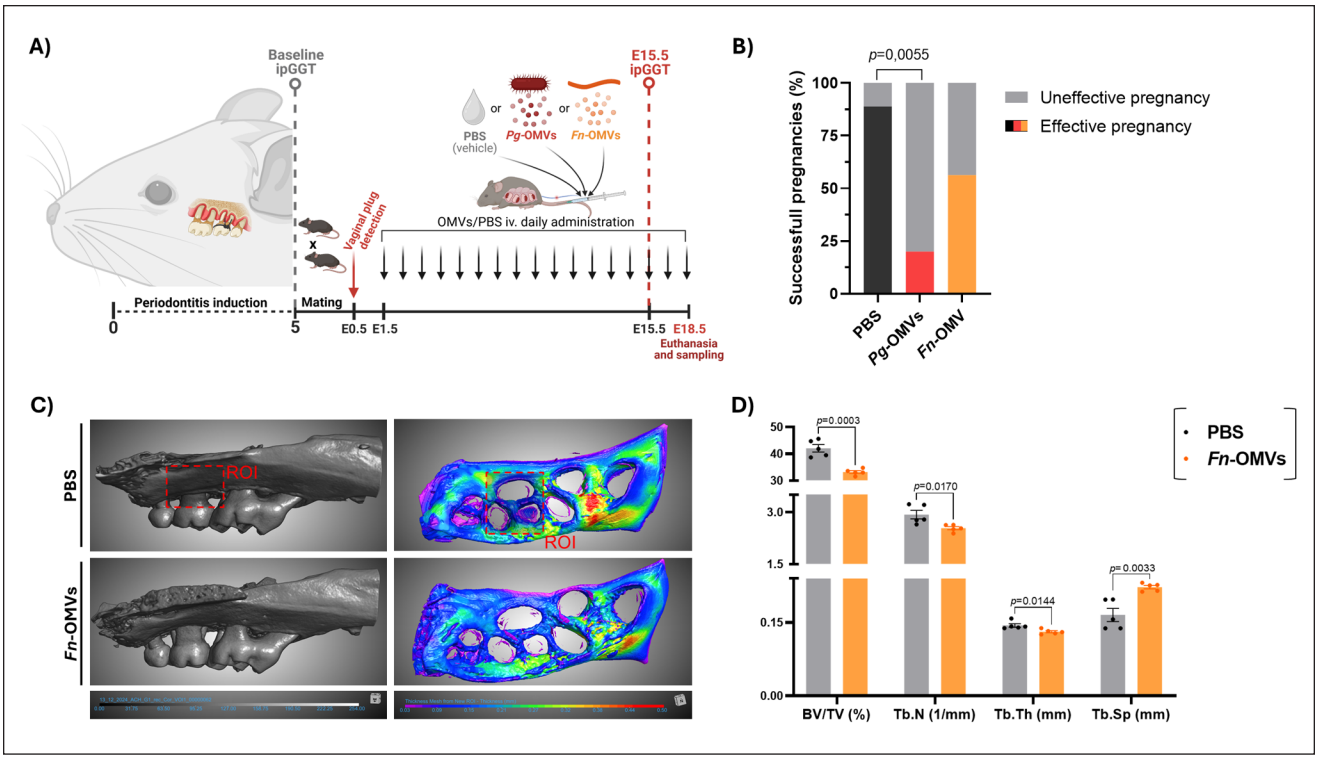


Figure 1. Experimental model and effects of periodontal bacteria outer membrane vesicles (OMVs) on pregnancy success and bone loss. (**A**) Schematic representation of the experimental design illustrating periodontitis induction (day 0), intraperitoneal glucose tolerance tests (ipGTTs) (baseline and E15.5), mating (day 5), vaginal plug detection, and daily administration of *Pg*- or *Fn*-derived OMVs (E1.5–E17.5). Control mice received daily intravenous injections of Dulbecco's phosphate-buffered saline (DPBS). At E18.5, mice were euthanized and samples collected. (**B**) Pregnancy success rate defined as the percentage of females with confirmed mating and pregnancy determined by the presence of a vaginal plug following mating and the presence of fetuses within uterine horns at euthanasia, respectively. Pregnant mice in the PBS group: $n=8$ of a total of 9 mice; *Pg*-OMVs group: $n=2$ of a total of 10 mice; *Fn*-OMVs group: $n=9$ of a total of 16 mice. Statistical significance was assessed using a chi-square test. Nonsignificant values are not displayed. (**C**) Representative 3-dimensional reconstructions from micro–computed tomography analysis and corresponding color-coded bone thickness maps. The region of interest is indicated by a red dashed square. (**D**) Quantification of bone morphometric parameters: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). PBS group: $n=5$; *Fn*-OMVs group: $n=5$. Data are presented as mean±standard error of the mean. Normality was assessed using the Shapiro–Wilk test, and statistical significance was determined using an unpaired *t* test.

evaluated. DiR-labeled *Fn*-OMVs showed high fluorescence in the liver, kidneys, and cervical lymph nodes, with no differences between groups (Appendix Fig 4A–B). Notably, placentas from *Fn*-OMVs–exposed dams exhibited increased fluorescence compared with controls ($P=0.017$; Appendix Fig 4C), whereas no differences were observed in yolk sacs or fetuses (Appendix Fig 4D–E).

Fn-OMVs–exposed pregnant mice developed hepatomegaly and splenomegaly, as evidenced by increased organ-to-body weight ratios (Fig 2A, B and D, E). The liver-to-body weight ratio was significantly increased in the *Fn*-OMVs group compared with controls (4.92%±0.118% vs 4.34%±0.233%, $P=0.037$; Fig 2B). However, hepatic gene expression of tumor necrosis factor–α (TNF-α), interleukin (IL)–1β, and IL-6 (Fig 2C) or protein levels (Appendix Fig 3D) did not differ significantly between groups. Consistently, hepatic histopathological evaluation revealed no major alterations or differences in the number of morphologically identified Kupffer cells following *Fn*-OMVs exposure (Appendix Fig 3B–C), suggesting that hepatomegaly developed in the absence of overt hepatic inflammation.

In contrast, the spleen-to-body weight ratio increased (0.505%±0.033% vs 0.37%±0.026%; $P=0.009$; Fig 2E), together with splenic IL-1β ($P=0.045$) and IL-6 ($P=0.035$) gene expression in the *Fn*-OMVs exposed group as compared with controls (Fig 2F), whereas protein levels remained unchanged (Appendix Fig 3E). Accordingly, systemic proinflammatory markers remained largely unchanged. Plasma CRP and IL-1β levels were unaltered (Fig 2G, H), whereas TNF-α and IL-6 remained below detection limits (data not shown). In contrast, IL-4 was significantly elevated in the *Fn*-OMV–exposed group ($P=0.0236$), while IL-10 remained unaltered (Fig 2I, J). Notably, hepatosplenomegaly was absent in nonpregnant mice (Appendix Fig 2B, C), suggesting that the systemic effects of *Fn*-OMV exposure are pregnancy dependent.

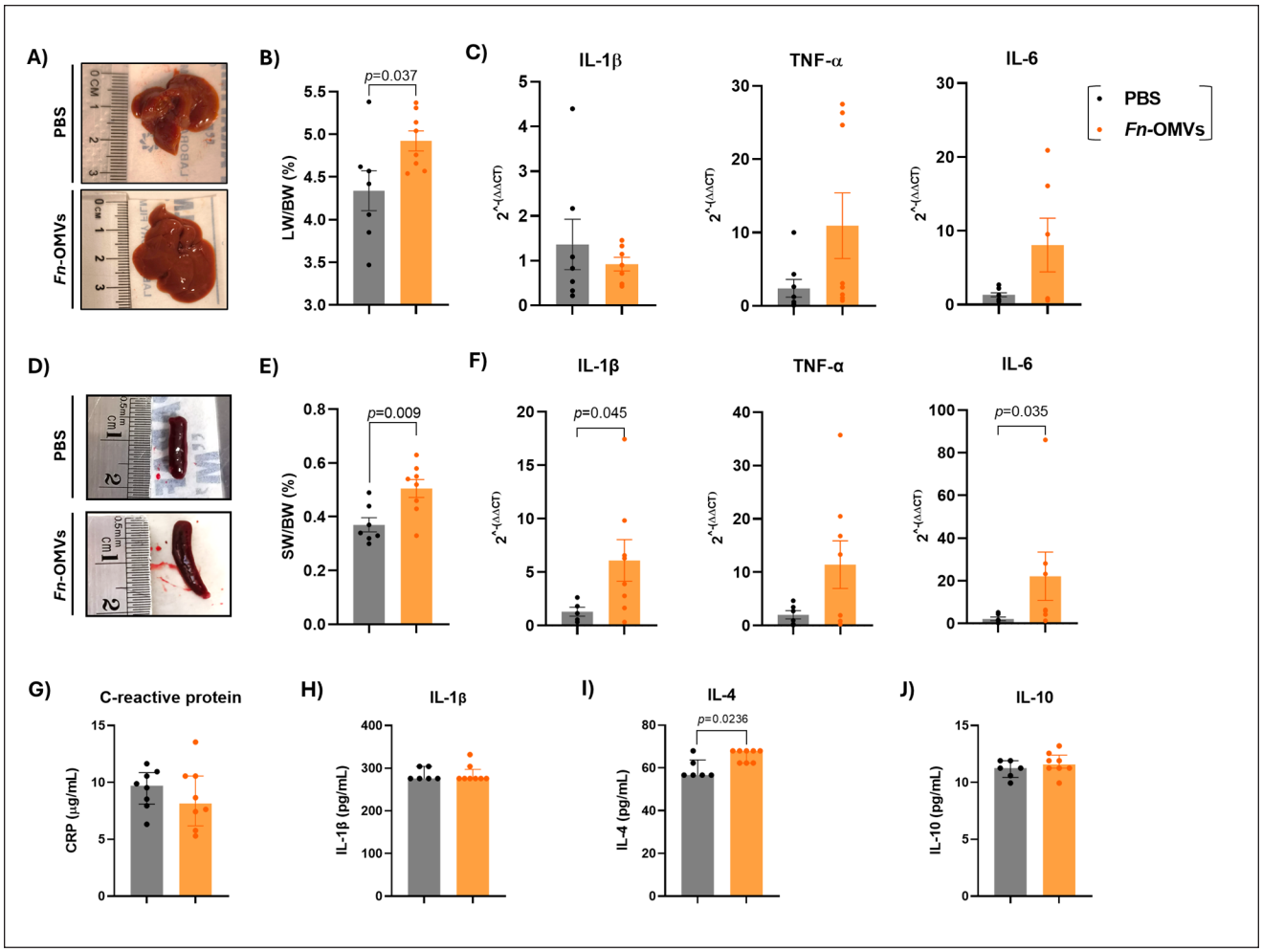


Figure 2. *Fn*–outer membrane vesicles (OMVs) administration induces hepatosplenomegaly and a tolerogenic plasmatic cytokine profile during pregnancy. (**A**) Representative macroscopic images of the liver of phosphate-buffered saline (PBS) or *Fn*-OMVs–exposed mice at E18.5. (**B**) Liver weight-to-body weight (LW/BW) ratio. (**C**) Hepatic mRNA expressions of interleukin (IL)–1β, tumor necrosis factor–α (TNF-α), and IL-6, determined by reverse transcription–quantitative polymerase chain reaction (RT-qPCR). (**D**) Representative macroscopic images of the spleen of PBS or *Fn*-OMVs–exposed mice at E18.5. (**E**) Spleen weight-to-body weight (SW/BW) ratio. (**F**) Splenic mRNA expression of IL-1β, TNF-α, and IL-6, determined by RT-qPCR. Plasmatic levels of (**G**) C-reactive protein (CRP), (**H**) IL-1β, (**I**) IL-4, and (**J**) IL-10. PBS group: $n = 7$; *Fn*-OMVs group: $n = 8$. Data are presented as mean ± standard error of the mean or median with interquartile range for cytokines. Normality was assessed using the Shapiro–Wilk test, and statistical significance was determined using an unpaired *t* test. Only significant *P* values are shown.

Systemic Fn-*OMVs Exposure Impairs Maternal Glucose Tolerance during Pregnancy*

To determine whether *Fn*-OMVs affect maternal metabolic homeostasis during pregnancy, ipGTTs were performed at baseline (prior to OMV administration) and at day 15 from the beginning of treatment (Fig 3 and Appendix Fig 2D–I). In the pregnant control group, mice exhibited similar glycemic curves and area under the curve (AUC) values at baseline and E15.5 (Fig 3A, B). In contrast, *Fn*-OMVs–exposed pregnant mice displayed impaired glucose tolerance at E15.5, with elevated glycemic values following glucose administration across multiple time points (Fig 3C), which was further confirmed by a significantly higher AUC ($P = 0.017$; Fig 3D). No differences in glucose tolerance were observed in nonpregnant mice (Appendix Fig 2D–I), indicating that exposure to *Fn*-OMVs disrupts maternal glucose homeostasis specifically during pregnancy. Notably, circulating insulin levels remained unchanged along all conditions (Fig 3E and Appendix Fig 2J).

Fn-*OMVs Exposure Induces FGR and Placental Inflammation during Pregnancy*

Finally, we assessed the impact of systemic *Fn*-OMVs on fetal outcomes and the placenta. No differences in litter sizes were observed between groups (Fig 4A). However, *Fn*-OMVs–exposed pregnancies exhibited a significant reduction in average

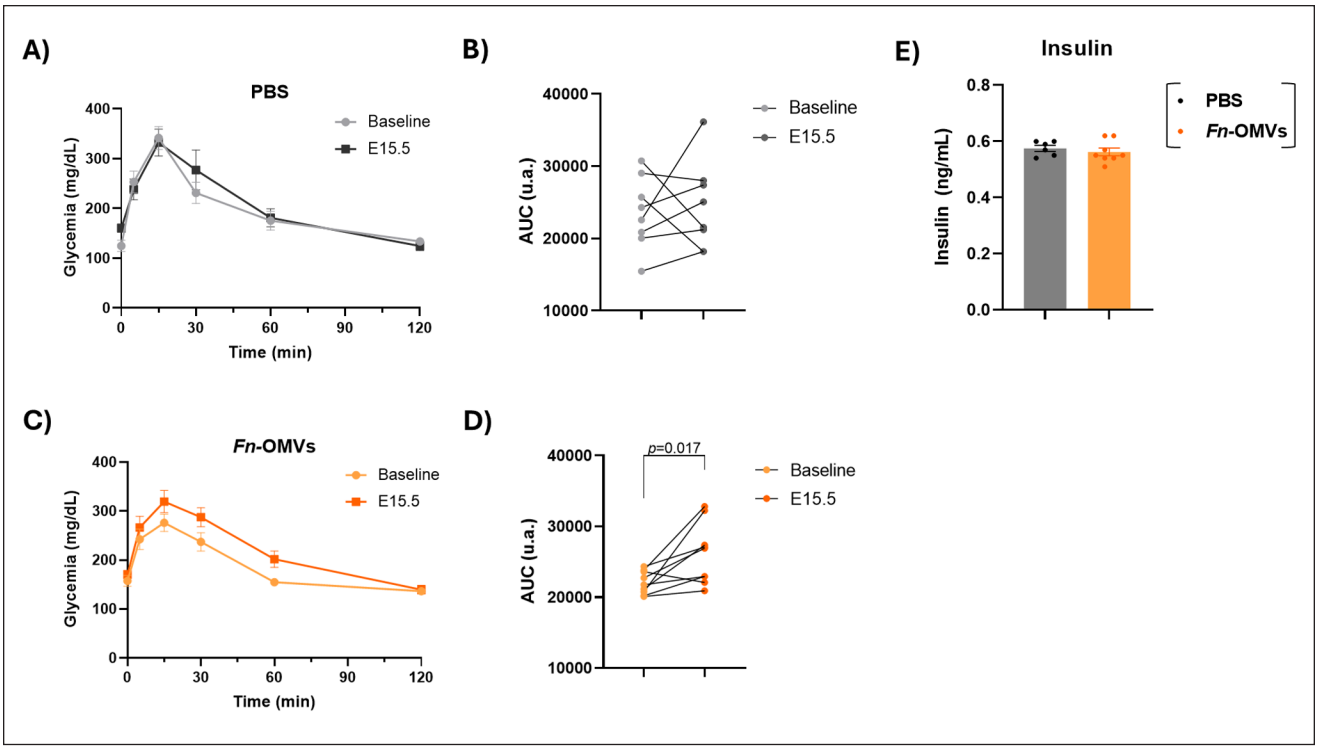


Figure 3. *Fn*–outer membrane vesicles (OMVs) exposure induces gestational glucose intolerance without altering insulin levels. (**A**) Intraperitoneal glucose tolerance test (ipGTT) curves at baseline and E15.5 in control dams ($n=8$). (**B**) Area under the glucose curve (AUC) for control dams ipGTT. (**C**) ipGTT curves at baseline and E15.5 in *Fn*-OMVs–exposed dams ($n=9$). (**D**) AUC for ipGTT in the *Fn*-OMVs–exposed group (phosphate-buffered saline [PBS group: $n=7$; *Fn*-OMVs group: $n=9$). (**E**) Fasting plasma insulin levels at E18.5 (PBS group: $n=6$; *Fn*-OMVs group: $n=8$). Two-way analysis of variance with Sidak's post hoc test was used for ipGTT curves (**A, C**). AUC and insulin analyses (**B, D, E**) were performed using an unpaired *t* test. Data are presented as mean ± standard error of the mean. Only significant *P* values are shown.

fetal weight ($P=0.044$; Fig 4B), without affecting placental weight or placental efficiency (placental-to-fetal weight ratio) (Fig 4C, D). Fetal anthropometric analysis further revealed reduced crown–rump length ($P=0.011$; Fig 4F), anteroposterior cranial length ($P=0.040$; Fig 4G), abdominal anteroposterior diameter ($P=0.014$; Fig 4H), without differences in the biparietal length ($P=0.130$; Fig 4J).

Given the relevance of placental fitness in fetal development, we then evaluated whether alterations in fetal morphometry were associated with placental inflammation. To determine placental inflammatory status, we analyzed NF-κB signaling and inflammatory marker expression on placental tissues. *Fn*-OMVs–exposed placentas showed increased phospho-p65 levels and reduced total p65 protein, resulting in a significantly higher p-p65/p65 ratio ($P=0.012$; Fig 5A, B). In addition, IL-1β and TNF-α gene expression levels were significantly upregulated ($P=0.036$ and $P=0.025$, respectively; Fig 5C and D), whereas IL-6 expression showed a nonsignificant increasing trend (Fig 5E).

Histological examination of E18.5 placentas revealed preserved overall placental architecture in both groups (Fig 5F, G), with no evidence of extensive necrosis, hemorrhage, thrombosis, or major inflammatory cell infiltrates (Fig 5F). Although *Fn*-OMV–exposed placentas showed a modest increase in cellular density and a trend toward larger labyrinth area (Fig 5G), no major structural abnormalities were detected.

Finally, to determine whether these effects were associated with OMV exposure rather than ligature-induced periodontitis, we compared inflammatory, metabolic, and pregnancy outcomes between the ligature-induced periodontitis control group receiving vehicle (Lig+PBS) with a small cohort of unligated pregnant dams receiving PBS throughout gestation (UL+PBS) (Appendix Fig 5). No significant differences were observed in liver- or spleen-to-body weight ratios, maternal glucose tolerance (baseline or E15.5), litter size, fetal or placental weight, placental efficiency, or fetal morphometric parameters (Appendix Fig 5A–L). These findings suggest that the observed maternal and fetal phenotypes may be primarily attributable to OMV exposure rather than to ligature-induced periodontitis per se. However, given the limited size of the unligated control group ($n=3$ dams), this conclusion should be interpreted with caution and warrants confirmation in future studies.

Discussion

This study provides preliminary mechanistic evidence that periodontal pathogen–derived OMVs differentially affect

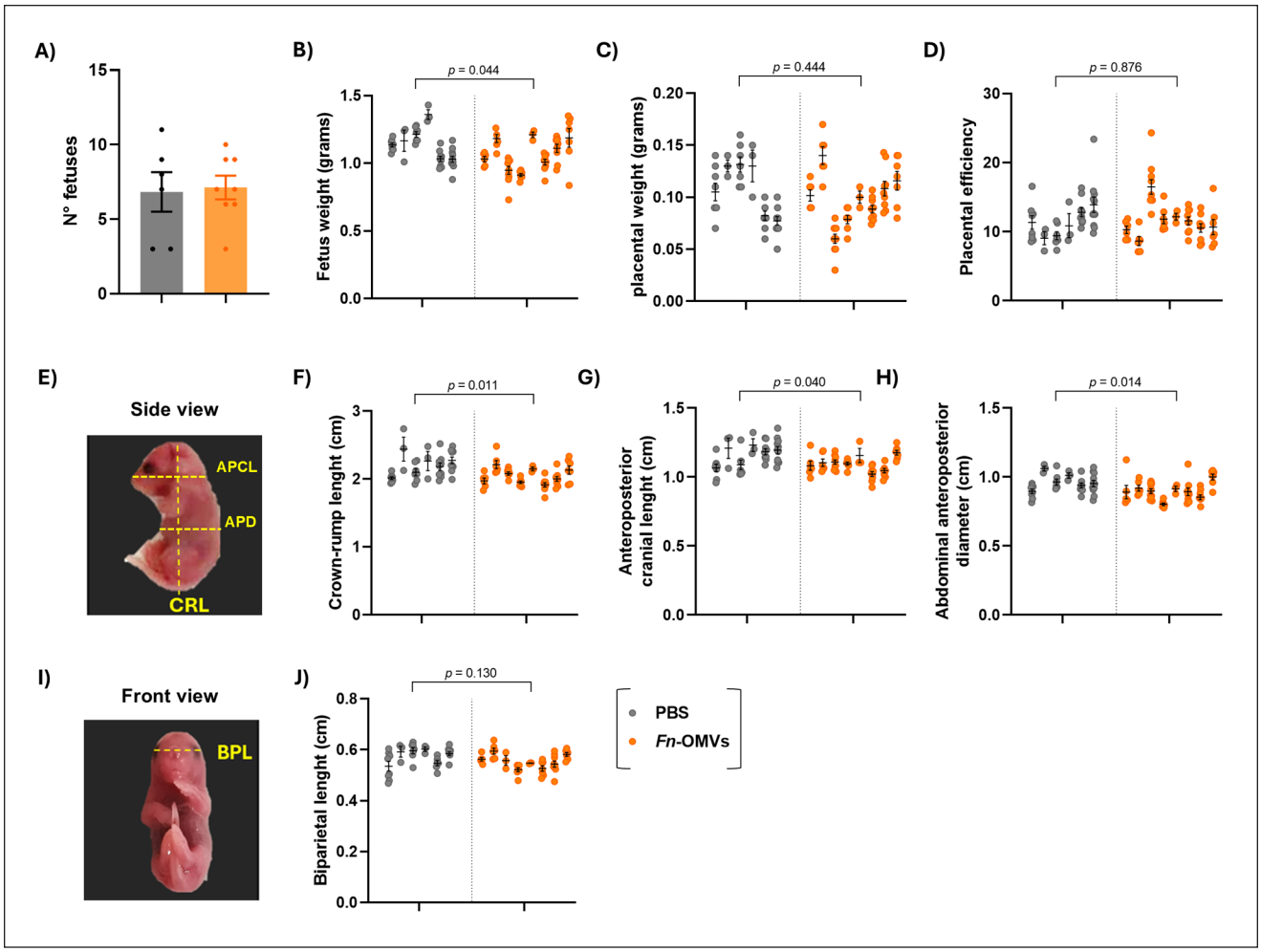


Figure 4. Systemic *Fn*–outer membrane vesicles (OMVs) exposure induces fetal growth restriction. (**A**) Number of fetuses per mother. (**B**) Fetal weight (g). (**C**) Placental weight (g). (**D**) Fetal-to-placental weight ratio. (**E**) Representative lateral view of fetuses indicating morphometric measurements. (**F**) Crown–rump length (cm). (**G**) Anteroposterior cranial length (cm). (**H**) Abdominal anteroposterior diameter (cm). (**I**) Representative frontal view of fetuses indicating biparietal measurement. (**J**) Biparietal length (cm). Each dot represents one dam in panel A (phosphate-buffered saline [PBS] group: $n = 7$; *Fn*-OMVs group: $n = 8$) or one fetus in panels B–J (fetuses PBS group: $n = 41$; fetuses *Fn*-OMVs group: $n = 57$). Data are presented as mean ± standard error of the mean. Normality was assessed using the Shapiro–Wilk test. Fetal and placental weight, placental efficiency, and morphometric outcomes were analyzed using a linear mixed-effects model, with litter included as a random intercept to account for nonindependence of fetuses/placentas within the same litter.

pregnancy outcomes according to their bacterial origin. Whereas *Pg*-OMVs markedly impaired pregnancy establishment, *Fn*-OMVs induced organ-specific inflammation, maternal glucose intolerance, and FGR. These findings identify periodontal bacteria–derived OMVs as candidate mediators linking periodontitis with APOs independently of live bacteria.

Our findings showed that *Pg*-OMVs markedly reduced pregnancy success, supporting a role for these vesicles in early gestational failure. Notably, this occurred without evidence of overt systemic inflammation, suggesting localized mechanisms affecting critical early reproductive processes, including fertilization, implantation, or decidualization. Consistently, *Pg*-OMVs have been shown to impair trophoblast migration and invasiveness (Lara, Loureiro, et al 2023), and promote immune overactivation by inducing neutrophil chemoattraction and the loss of trophoblast tolerogenic features (Lara, Sassot, et al 2023), all processes essential for successful implantation and placentation. However, because these early gestational processes were not directly assessed in this study, the mechanisms underlying the marked reduction in pregnancy success remain to be determined, representing an important priority for future investigation.

In contrast, *Fn*-OMVs had only a modest effect on pregnancy establishment, with their detrimental effects becoming apparent later in gestation as FGR. This temporal pattern suggests that *Fn*-OMVs primarily influence mechanisms regulating fetal and placental development rather than early pregnancy events, likely through local inflammatory alterations in the maternal host. *Fn*-OMVs also exacerbated alveolar bone loss in periodontitis-affected dams, likely reflecting enhanced local

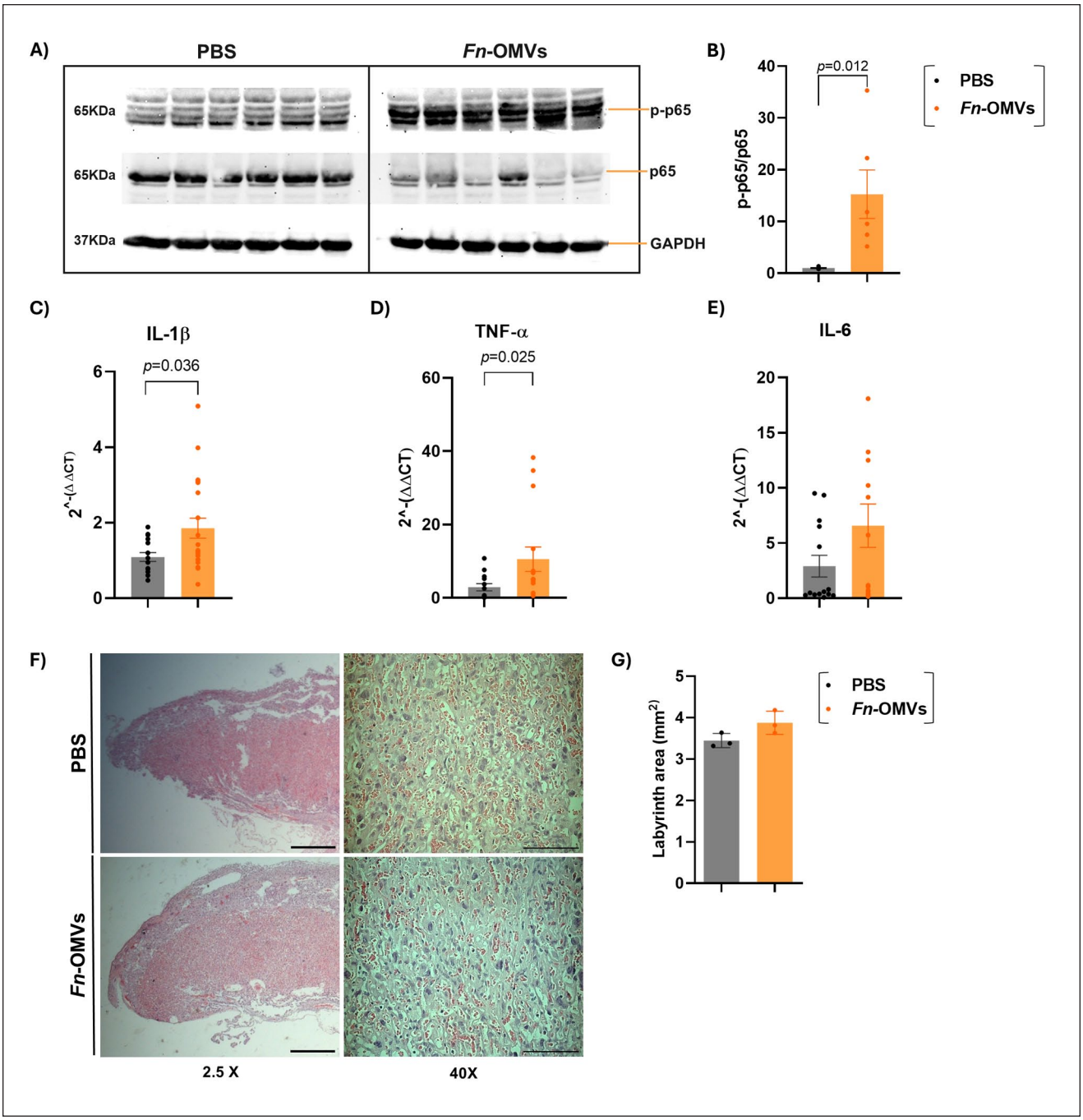


Figure 5. *Fn*–outer membrane vesicles (OMVs) induce NF-κB–mediated placental inflammation without altering architecture. (**A**) Representative immunoblot of placental tissues showing phosphorylated p65 (p-p65) and total p65 levels. GAPDH was used as loading control. Each lane corresponds to 1 placenta from an individual fetus. (**B**) Quantification of the p-p65/p65 ratio normalized to GAPDH ($n = 6$ per group). (**C–E**) Relative mRNA expression of placental inflammatory cytokines: (**C**) interleukin (IL)–1β, (**D**) tumor necrosis factor–α (TNF-α), and (**E**) IL-6, determined by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) (phosphate-buffered saline [PBS] group: $n = 15$; *Fn*-OMVs group: $n = 21$). (**F**) Representative hematoxylin and eosin–stained sections of E18.5 placentas from control and *Fn*-OMVs–exposed pregnancies. Low-magnification images (2.5×; left panels) show the overall placental morphology, while high-magnification images of the labyrinth region (40×; right panels) illustrate the cellular organization within the exchange compartment. (**G**) Quantification of the labyrinth area for individual placentas in each group ($n = 3$). For these analyses, placentas were randomly selected from the available litters, with not all litters represented in each assay; therefore, *n* represents individual placentas. Data are presented as the mean ± standard error of the mean. Statistical analysis was performed using an unpaired *t* test for protein quantification (B) and labyrinth area (G), and the Mann–Whitney test for gene expression (C–E). Only significant *P* values are shown.

immune activation, as previously reported following direct OMVs administration within the periodontium (Zhang et al 2024). Given the central role of immunity in bone homeostasis, whether this phenotype results from OMV-driven periodontal immunity overactivation warrants further investigation.

Fn-OMVs also induced hepatosplenomegaly without overt systemic inflammation, suggesting the involvement of alternative mechanisms underlying these organ-specific alterations. Although splenic IL-1β and IL-6 transcript levels increased in *Fn*-OMVs exposed dams, this was not reflected at the protein level. Moreover, hepatic inflammatory markers also remained unchanged. Interestingly, *Fn*-OMVs–exposed dams exhibited elevated circulating IL-4 concentrations. Since IL-4 levels are typically stable throughout uncomplicated pregnancies (Cemgil Arikan et al 2012), this increase may reflect a compensatory anti-inflammatory response (CARS), limiting sustained inflammation (Vergadi et al 2018), or a shift toward a tolerogenic Th2-like environment. Notably, splenomegaly may enhance erythrocyte clearance and promote anemia, a known risk factor for APOs (Wang et al 2025), potentially contributing to the observed phenotype. Whether *Fn*-OMVs promote the emergence of CARS and/or anemia or whether these responses contribute to gestational complications remains to be determined.

At the metabolic level, *Fn*-OMVs exposure impaired gestational glucose tolerance without altering circulating insulin levels, consistent with insulin resistance. This phenotype could arise from direct effects on glucose-regulating organs, as *Fn*-derived products have been shown to disrupt IRS1/AKT signaling, impair hepatic glycogen synthesis, and promote insulin resistance (Chang et al 2023), or from placental inflammation, particularly through NF-κB activation, a recognized contributor to gestational insulin resistance (Onori et al 2025). Accordingly, placental NF-κB activation together with increased IL-1β and TNF-α expression could account for the GDM-like metabolic phenotype observed in our study, consistent with inflammatory signatures reported in placentas from GDM pregnancies (Feng et al 2016). However, whether placental inflammation causally drives gestational insulin resistance, both arising in parallel from direct *Fn*-OMV effects, or metabolic dysfunction further exacerbates placental inflammation remains unclear.

Biodistribution analyses revealed preferential placental accumulation of *Fn*-OMVs, indicating placental tropism resembling that of the parental bacterium. *Fn* has been consistently identified in placental tissues and associated with APOs (Vander Haar et al 2018), partly through the adhesion-related virulence factors Fap2 (Coppenhagen-Glazer et al 2015) and FadA (Ikegami et al 2009), which are also present in OMVs, thereby promoting placental adherence and inflammatory overactivation (Zhang et al 2024) and supporting the concept that periodontitis-derived bacterial products can disrupt the tightly regulated maternal environment required for a healthy pregnancy.

Consistent with these alterations, *Fn*-OMVs exposure resulted in FGR, evidenced by reduced fetal weight and altered anthropometric parameters. Placental inflammation is increasingly recognized as a central mechanism linking maternal inflammation to FGR. The placental inflammatory response observed in our study, characterized by NF-κB activation and increased IL-1β and TNF-α expression, could underlie impaired fetal growth by disrupting trophoblast nutrient transport (Winterhager and Gellhaus 2017) or altering placental vascular tone (Tavarna et al 2020). Interestingly, low levels of *Fn* have also been associated with enhanced trophoblast function (Heusler et al 2021), suggesting dose-dependent effects. Whether *Fn*-OMVs reach the fetal–maternal unit at biologically relevant concentrations in periodontitis-affected human pregnancies and exert similar effects remains to be clarified.

A closely related study demonstrated that EVs released by *Pg*-infected macrophages induce APOs through placental vascular disorganization and impaired angiogenesis, without evidence of placental inflammation (Tanai et al 2025). The differences between their findings and ours likely reflect differences in vesicle origin, cargo composition, and gestational timing of exposure. Unlike host-derived EVs, bacterial OMVs are enriched in virulence factors that directly activate innate immune signaling (Chen et al 2023), whereas host-derived EVs primarily transfer regulatory molecules (Yue et al 2026). Importantly, anatomical and cellular differences between murine and human placentation, including placental architecture, trophoblast organization, spiral artery remodeling, and immune cell composition at the maternal–fetal interface (Georgiades et al 2002), underscore the need for validation in human samples.

Some limitations of the present study should be considered. First, the daily intravenous administration of 1×10^8 OMVs, selected based on similar *in vivo* studies (Seyama et al 2020), represents a potentially supraphysiological exposure scenario, as circulating periodontal pathogen-derived OMV concentrations during human pregnancy remain unknown. Second, implantation, decidualization, and early embryonic loss were not evaluated, precluding mechanistic interpretation of the marked reduction in pregnancy success following *Pg*-OMV exposure, and these remain a priority for future research. Furthermore, although our findings indicate that systemic *Fn*-OMV exposure was associated with maternal hepatosplenomegaly, gestational glucose intolerance, FGR, and placental inflammation, causal relationships between these processes cannot be established from the present data. Hence, to elucidate whether these outcomes are mechanistically linked or arise independently following systemic OMV exposure, pathway-specific interventional studies will be required.

It should be noted that OMV was standardized by particle number. However, differences in size distribution and endotoxin content between *Pg*- and *Fn*-OMVs may result in unequal delivery of immunogenic or virulence-associated cargo. Complementary normalization strategies, such as endotoxin content, should be further considered to improve comparisons across OMV preparations. Finally, the administration of OMVs from individual bacterial species does not recapitulate the polymicrobial vesicular environment of periodontitis. Because

Pg- and *Fn*-derived virulence factors engage shared and distinct signaling pathways (Lee and Tan 2014; Zou et al 2024), polymicrobial vesicle mixtures may elicit interactions not captured by our model. Future studies evaluating clinically relevant doses and polymicrobial OMV mixtures representative of the whole periodontal vesiculome may better reflect systemic exposure during periodontitis and help identify therapeutic targets to mitigate adverse maternal–fetal outcomes.

In summary, this study provides preliminary, *in vivo* evidence that periodontal pathogen-derived OMVs differentially disrupt pregnancy according to their bacterial origin. Whereas *Pg*-OMV exposure primarily impaired pregnancy establishment, *Fn*-OMVs induced compartmentalized organ-specific alterations, gestational glucose intolerance, placental inflammatory activation, and FGR. Together, these findings identify bacterial OMVs as candidate mechanistic mediators linking periodontitis with APOs and provide a framework for future translational studies targeting the oral–placental axis.

Author Contributions

C. Rojas, J. Concha-Cerda, contributed to conception and design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; M.L. Mizgier, F. Saavedra, M.J. Bendek, J. Valenzuela, C. Madrid, A. Sierra-Cristancho, P. Romo, contributed to conception, data acquisition, analysis, and interpretation, critically revised the manuscript; K. Schinnerling contributed to conception, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; C. Martínez, S. Illanes, contributed to conception, data interpretation, critically revised the manuscript; D. Busso, A. Chaparro contributed to conception and design, data analysis and interpretation, drafted and critically revised the manuscript. All authors gave their final approval to the manuscript and agreed to be accountable for all aspects of the work ensuring integrity and accuracy.

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Data Availability Statement

All raw data supporting the findings of this study are available in the Figshare repository at https://doi.org/10.6084/m9.figshare.31939074 and are also provided as supplementary material.

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