

Porphyromonas gingivalis impairs fetal development through macrophage extracellular vesicles

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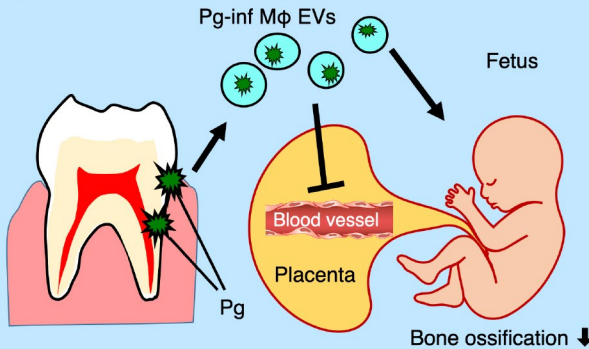
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Introduction

Periodontitis, caused mainly by *Porphyromonas gingivalis* (Pg), is correlated with increased abnormal pregnancy risks. We examined how Pg infected Macrophage extracellular vesicles (Pg-inf Mφ EVs) affects fetal development.

Graphical Abstract



Materials & Methods

Collection of Pg-inf Mφ EVs: Monocytic cell line (THP-1) was differentiated into Mφ through PMA treatment, and infected with Pg for 4 h. After culturing in an exosome-free medium for another 48 h, Pg-inf Mφ EVs were collected from the culture medium.

Animal Experiments: Pregnant mice were injected with Pg-inf Mφ EVs and sacrificed at embryonic day 18. The localization of Cy7 labelled-EVs was detected using In Vivo Imaging System (IVIS). The extracted placenta was used for HE staining.

Mouse skeleton preparation: The extract fetus were skinned and stained in Alcian blue and Alizarin red staining. Excess tissue were removed in 1%KOH / 20% glycerol solution.

Results

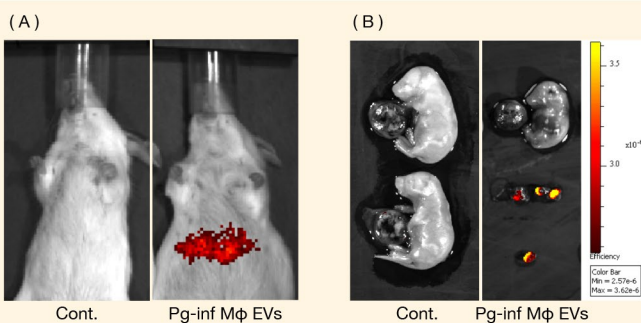


Fig. 1. Pg-inf Mφ EVs translocated to the placenta and fetus
A: The ventral view of pregnant mice using IVIS. Signals were detected in the (B) placenta and fetus of the experimental group. The signals represent the Cy7 labelled Pg-inf Mφ EVs.

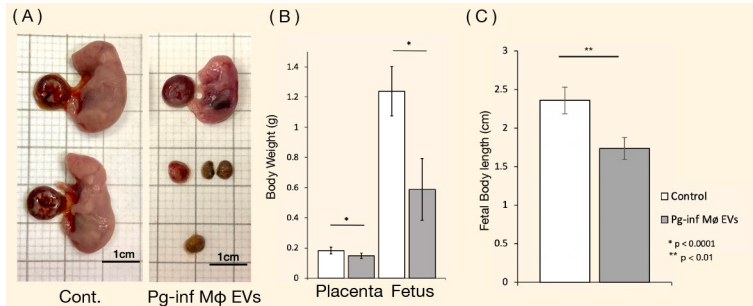


Fig. 2. Pg-inf Mφ EVs inhibited the growth of placenta and fetus
A: The placenta and fetus of the experimental group showed (B) decreased body weight and (C) fetal body length.

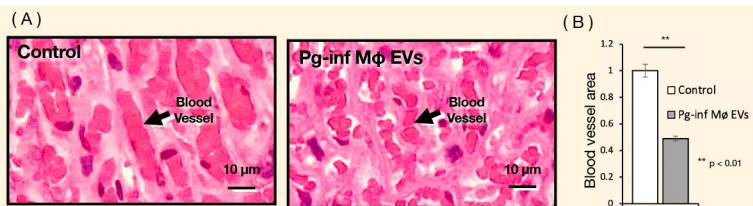


Fig. 3. Pg-inf Mφ EVs restricted placental angiogenesis

(A) HE staining of the placenta showed a decrease (B) blood vessel area.

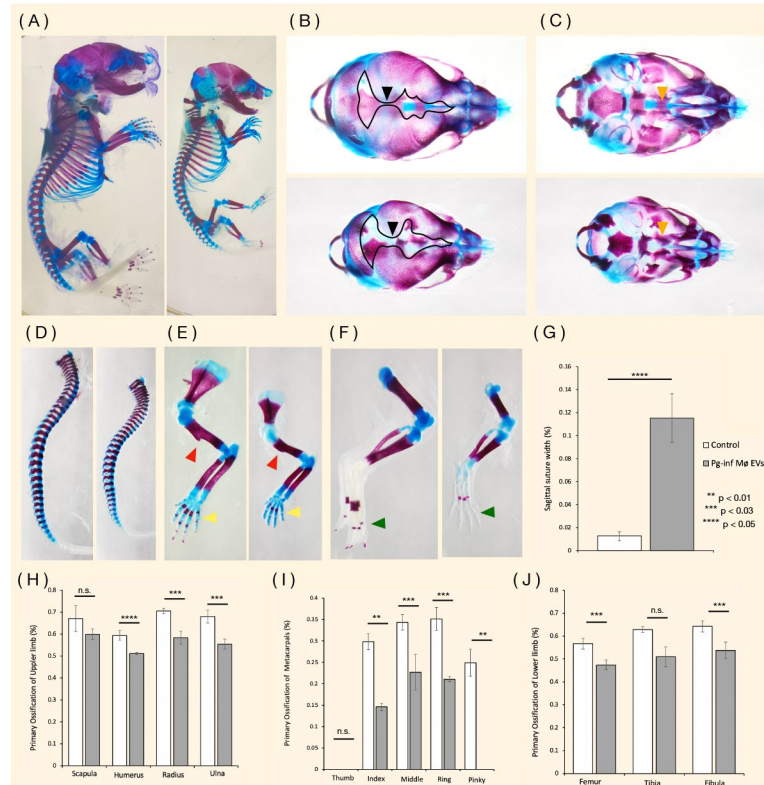


Fig. 4. Pg-inf Mφ EVs impairs primary bone ossification.

Alcian blue and Alizarin red staining of fetus A: Lateral view B: Superior view of cranium. ▶Sagittal suture C: Infernal view of cranium. ▶Palatine D: Vertebrae E: Upper Limb ▶Deltoid tuberosity ▶ Metacarpals F: Lower limb ▶Phalanges G: The width of the sagittal suture. Primary ossification was delayed in (H) upper limb, (I) metacarpals, and (J) lower limb.

Conclusion

Mφ EVs are an important factor for the transmission of Pg, which impairs fetal growth.