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IMPACT OF *Porphyromonas gingivalis* VESICLES ON TROPHOBLASTIC CELLS AND BACTERIAL INTERACTIONS

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Periodontitis is a common chronic inflammatory disease in pregnant women, linked to pregnancy complications. *Porphyromonas gingivalis* (Pg) is a key pathogen due to its secretion of outer membrane vesicles (OMV) containing virulence factors. Although there is evidence that OMV influences the pathogenesis of periodontitis, the interaction between OMV and trophoblastic cells (tb), and the underlying mechanisms affecting invasion, have not been studied. Additionally, bacteria respond to stress conditions which can alter OMV production and composition. However, the impact of these conditions on Pg's interaction with host cells and its potential role in gestational complications remains unexplored. Furthermore, microorganisms form communities where interactions can influence pathogenesis. Pg is present in the human oral cavity in both healthy individuals and those with other infections. Particularly, cystic fibrosis (CF) patients experience recurrent and chronic infections caused by various bacterial species. The most frequent species contributing to morbidity and mortality in these patients are *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA), both in mono and coinfections. This study aims to investigate the effect of OMV-Pg on interactions with both tb cells and other bacterial species that may be present in infection sites.

OMVs from Pg cultures were purified by ultracentrifugation, characterized by protein analysis, and observed using Transmission Electron Microscopy (TEM). Additionally, the effect of stressors like H₂O₂ and GSNO on OMV-Pg production was analyzed by treating Pg cultures 24 hours before OMV harvest. The TEM analysis of the vesicles showed differences in their general characteristics. Then we analyzed the impact of untreated OMV-Pg on tb cells. Untreated OMV-Pg were used to pre-treat tb cells, followed by Pg invasion assays. Results showed a bacterial invasion rate nearly 6 times higher in OMV-prestimulated cells compared to the untreated control ($p < 0.005$). These results demonstrate that OMVs increase the susceptibility of tb cells to Pg infection, which has implications for virulence and tissue colonization. Additionally, untreated vesicles were used to initially assess the effect of OMV-Pg on other microorganisms. PAO1 cultures were treated with OMV-Pg at concentrations above and below 4 µg/ml for 24 hours. Rhamnolipid production, lipase, and protease activities on blood, milk, and egg agar plates showed no significant virulence differences. Competition with USA300 on TSA plates also revealed no significant differences. Bacterial biomass quantification by dry weight measurement revealed reduced

PAO1 growth when the culture was pre-treated with OMV-Pg, indicating relevance for polymicrobial infections. Our findings highlight the impact of OMVs from a periodontal pathogen like Pg on eukaryotic cells and other bacterial species, emphasizing the importance of understanding these interactions and their impact on health.

Palabras clave: OMV - periodontitis - pregnancy complications - bacterial interaction