UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

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Centro de Investigación en Ciencias de la Salud y Biomedicina (CICSaB)

”Efecto de la gingivitis materna en el establecimiento del microbioma intestinal del neonato.”

TESIS QUE PRESENTA

M. en C. ANA KARENINA ROCHA VIGGIANO

PARA OBTENER EL GRADO DE DOCTORA EN CIENCIAS BIOMÉDICAS BÁSICAS

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CRÉDITOS INSTITUCIONALES

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Resumen.

Durante el embarazo, las mujeres pasan por una serie de cambios fisiológicos, incluyendo variaciones hormonales importantes. La gingivitis del embarazo es una condición que afecta del 30 hasta el 100% de las mujeres y se ha relacionado con estas modificaciones hormonales, pudiendo tener un rol importante en la colonización del intestino fetal, así como el entrenamiento inmunológico para el recién nacido. Sin embargo, la salud oral no siempre es considerada como rutina en el cuidado prenatal. En este estudio se colectaron muestras de saliva de mujeres embarazadas con (PG) y sin (NPG) gingivitis del embarazo para analizar la microbiota presente por medio de secuenciación 16s. Adicional, se colectaron muestras de meconio de los correspondientes neonatos y también fueron analizados.

La microbiota oral de las mujeres embarazadas con y sin gingivitis del embarazo no mostraron diferencias significativas en cuanto a diversidad. Sin embargo, se encontraron diferencias en la composición de la microbiota. Adicional a esto, pareciera que la composición de la microbiota intestinal de los neonatos también tiene diferencias entre los correspondientes a las madres con (PG) y sin (NPG) gingivitis. No obstante, el número de muestras de meconio analizadas no permiten una conclusión definitiva. Es por esto, que se requiere una cohorte mayor y secuenciación de mayor profundidad para demostrar las diferencias en la microbiota oral y explorar la posibilidad de la traslocación bacterial de la encía materna hacia el intestino fetal.

Article

Effect of Pregnancy Gingivitis on Maternal Saliva Microbiota

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Abstract: Pregnant women undergo a myriad of physiological changes during this stage, including important hormonal variations. Pregnancy gingivitis is a condition that affects up to 30% to 100% of women, is related to hormonal modifications, and could play an important role in gestational gut colonization and immunological training in the newborn. Nonetheless, oral health is not always considered part of routine prenatal care. In this study, we collected saliva samples of pregnant women with and without pregnancy gingivitis and analyzed the oral microbiota through 16S sequencing. In addition, meconium from the infants of participating women was also analyzed. The oral microbiota of pregnant women with and without pregnancy gingivitis did not show significant diversity differences. However, significant differences in microbiome composition were observed. In addition, it appears that microbiome composition of the offspring of mothers with gingivitis may also differ from that of mothers without gingivitis, although the number of available samples did not allow definite conclusions. As such, a larger cohort and deeper sequencing methods are needed to demonstrate the differences in the oral microbiota of pregnant women with and without gingivitis and to explore the possibility of bacterial translocation from the maternal gingiva to the fetal gut.

Keywords: Oral microbiota, microbiota, metagenomics, pregnancy gingivitis, newborn.

1. Introduction

During pregnancy, women experience multiple modifications at the hormonal, immunological, and metabolic levels. The maternal oral microbiota experiences notable changes during pregnancy, particularly in the context of pregnancy gingivitis (PG). Balan et al. (2018) highlighted that hormonal, metabolic, and immunological factors influence the oral microbiome, leading to dysbiosis associated with PG [1]. Their findings reveal a pathogenic shift in the oral microbiome during pregnancy, which reverts to a healthy state postpartum. This change underscores the potential role of oral dysbiosis in adverse pregnancy outcomes. La et al. (2022) further elucidated this transition by characterizing the oral microbiota from preconception to late pregnancy [2]. Their study indicates a significant reduction in microbial diversity during the third trimester, with an increase in pathogenic taxa such as *Prevotella* and *Atopobium parvulum*. Notably, women with better oral hygiene practices exhibited lower richness and diversity of pathogens, suggesting that oral hygiene may mitigate the adverse effects of dysbiosis. Collectively, these studies suggest that the maternal oral microbiota is susceptible to changes during pregnancy, particularly in the presence of gingivitis, emphasizing the importance of maintaining oral health to support both maternal and fetal well-being.

Alterations in maternal oral microbiota during pregnancy, especially those related to PG, could influence the neonatal gut colonization. Whether microbial colonization occurs during the fetal stage of not is still a debate. At present, there is no consensus about the origin of the infant's intestinal colonization, and in this ongoing debate, bacteria from the intestinal, oral, vaginal, and skin from the maternal microbiota have been proposed as potential sources [1,2].

Recent studies have detected bacterial DNA in the placenta, umbilical cord blood, meconium, and amniotic fluid, indicating the presence of microbes before birth [3–5]. Some of these microbes, including species of *Streptococcus*, *Fusobacterium*, *and Porphyromonas*, are commonly found in the oral cavity, supporting the hypothesis of potential vertical transmission of the maternal oral microbiota to the fetus, especially in women with PG [6]. Maternal oral bacteria that colonize the fetus may prime the immune system, influencing susceptibility to infections, allergies, and metabolic conditions later in life. However, dysbiosis—an imbalance in microbial communities—may have negative consequences[7].

One possible mechanism of the origins of colonization was proposed by Zaura et al, who reported that moderate gingivitis during pregnancy is a physiological and non-pathological process that aims to translocate the oral bacteria via the bloodstream into the placenta, and finally to the fetus [8]. PG is the inflammation and bleeding of the gums during the gestational stage [9]. Of note, dysbiosis of the oral microbiota has been reported in the presence of gingivitis; if such changes occur during pregnancy, this might have a significant impact in the immune system training of the newborn [10]. However, the specific contribution of maternal oral microbiota on neonatal intestinal colonization remains poorly understood.

In this study, we describe the oral microbiota of pregnant women with and without PG. In addition, we analyzed the relationship between maternal oral

microbiota and meconium microbiota from their respective newborns, to assess the potential role of the oral cavity as a source for the first bacterial colonization in infants.

2. Materials and Methods

We recruited 30 pregnant women (15 with PG and 15 without it (NPG group)) at the Obstetrics and Gynecology Service at Hospital Central "Dr. Ignacio Morones Prieto". The study was approved by the Ethics and Research Committee from the hospital with approbation number: 35-19. The inclusion criteria were Mexican healthy pregnant women aged 18 or older, with a gestational age from 30 to 42 weeks. The non-inclusion criteria were women with systemic or chronic disease, gestational diabetes, preeclampsia or eclampsia, ongoing antibiotic treatment, orthodontic treatment, and periodontal disease.

2.1. Sample collection

Unstimulated saliva was collected during prenatal care visits, in a Zymo Research DNA/RNA shield Saliva Collection Kit (Zymo Research Corp, United States) from the recruited women; also, shortly after birth a meconium sample was collected from the diaper in a Fecal Collection tube DNA/RNA Shield ™ (Zymo Research Corp, United States) of their newborn infants.

2.2. DNA extraction, 16s amplicon PCR, and sequencing

DNA was extracted from saliva and meconium samples using the ZymoBIOMICS Miniprep Kit (Zymo Research Corp, United States), following the manufacturer´s instructions.

A 16S rRNA PCR was performed using the Illumina primers 16S Amplicon PCR (Forward TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and Reverse GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTA ATCC).

Cycle conditions were 95 °C (3 min), then 35 cycles of 95 °C (30 s), 55 °C (30 s), 72 °C (30 s), then a final extension of 72 °C (5 min). Libraries were purified using Select-a-Size DNA Clean & Concentrator MagBead Kit (Zymo Research Corp; United States) according to the Illumina 16S metagenomic sequencing library protocol. Dual indices and Illumina sequencing adapters from the Illumina Nextera XT index kits v2 B and C (Illumina, San Diego, USA) were added to the target amplicons in a second PCR step using GoTaq Colorless Master Mix (Promega, United States). The DNA sequences of the amplicon library were determined using the Illumina MiSeq platform at Laboratorio de Genómica Funcional y Comparativa at the Instituto Potosino de Ciencia y Tecnología (IPICYT).

2.3. Bioinformatic analysis

To perform the analysis, we used the FASTQ files generated by the Illumina MiSeq platform. Then, a quality check was performed using the FASTQC tool [11], and then the R package DADA2 v1.16.0 [12] was used to analyze the 16S sequencing

data obtained by first, trimming 10nt at the left and on the right sides of the fragments. The forward primer AGRGTTYGATYMTGGCTCAG and the reverse primer RGYTACCTTGTTACGACTT were removed by using the *removePrimers ()* function, with default parameters.

By using the dada2 function the sample composition was inferred using default parameters. Chimeras were removed using the *removeBimeraDenovo ()* function and then the *assignTaxonomy ()* function for the taxonomy assignment. To obtain the amplicon sequence variant (ASV), we used the silva_nr99_v138 database.

2.4. Taxa abundance analysis

Taxa abundances were processed using custom R v4.0.2 [13] scripts.

2.5. Diversity

R package vegan v2.5.6 [14] was used to calculate the alpha diversity index with the *diversity()* function with default parameters.

2.6. Pathway's Inference

We have used Tax4Fun2 [15] to perform the pathway prediction for the 16S sequencing data. First, we used the *runRefBlast ()* and then the *makeFunctionalPrediction()* functions, with default parameters. The input to these functions was the ASV quantification profiles. We used the reference RF99NR in both steps. The resulting pathway prediction table was used to perform a sparse Partial Least Squares Discriminant Analysis (sPLS-DA). To perform this, we used the function *spls-da()* from the R package MixOmics with default parameters [16]. We used in-house R scripts to generate the plots to represent the results of the sPLS-DA [17].

2.7. Statistical analysis

The frequency of detection of individual phyla and genera between mothers with and without PG were compared using Fisher's exact test of the chi-square test, as appropriate. Analyses were carried out using OpenEpi, Version 3.01 (Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 3.01. Available at: www.OpenEpi.com; Accessed 2025/11/08)

3. Results

We collected 30 maternal salivary samples (15 with PG and 15 NPG). We also collected meconium samples from the corresponding babies, but only 6 samples from the NPG group and 3 from the PG group were suitable for sequencing of the 16S rRNA gene. We sequenced the 30 maternal samples, as well as the 9 neonatal samples to describe the characteristics of microbiota, at phylum and genera level, and the differences in diversity and abundance between the study groups of mothers and newborns. Maternal characteristics are shown in Table 1.

Table 1. Baseline characteristics of study participants			
	Mothers without gingivitis (NPG) (n=15)	Mothers with gingivitis (PG) (n=15)	P
Age (years)	26.21 (±6.2)	28.13 (±5.9)	0.35
Gestation age at recruitment	33.5 (±1.9)	35.4 (±1.9)	0.77
Pregnancy number	2.5 (±1.2)	2.3 (±1.2)	0.72

3.1. The diversity between the two groups

Alpha diversity indexes were obtained to observe differences within the samples. Comparisons of the Chao1 and ACE indexes (Figure 1a, 1b) on the saliva samples showed no statistical differences between the two groups (PG and NPG).

Using the Shannon and Simpson indexes (Figure 1c, 1d), we did not find significant differences between newborns or mothers. However, there was a higher diversity level in the meconium samples from infants of NPG than those from PG.

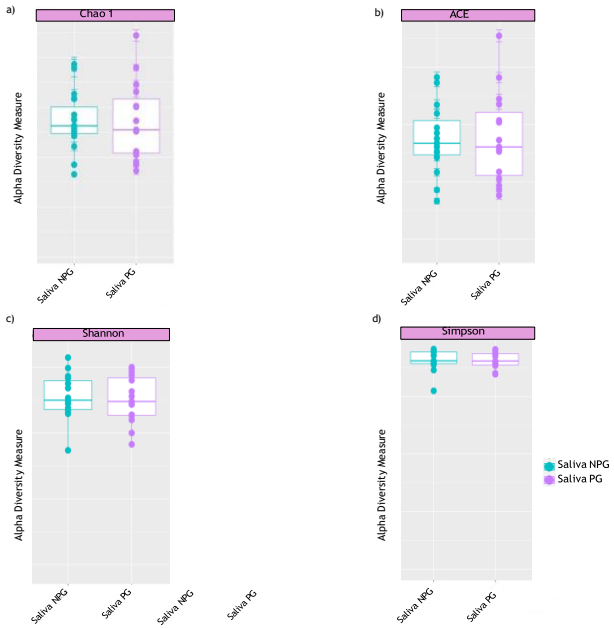

Figure 1. Alpha diversity. Diversity index. (a) Chao1 index (p value= 0.9). (b) ACE index (p value= 0.8). (c) Shannon index (0.7. (d) Simpson index (p value= 0.7).

3.2. Relative abundance of bacteria at phylum and genus level in saliva samples from mothers

Phylum composition on saliva samples from women in the NPG group were: Bacillota (85.5%), Actinomycetota (9.1%), Pseudomonadota (3.15 %), and Bacteroidota (2.11%) (Figure 2). Women with PG showed a similar composition, although there were differences in the contribution of each phylum: Bacillota (74.3%), Bacteroidota (13.7%), Pseudomonadota (10.5%), and Actinomycetota (1.2%) (Figure

2). Bacillota was the predominant phylum in all, but two mothers; these two mothers belonged to the PG group. Pseudomonadota contributed significantly (>5% of phyla) in 7 of 15 (46.7%) PG mothers compared with 1 of 15 (6.7%) NPG mothers (P=0.035). Bacteroidota were present (>5% of phyla) in 9 of 15 (46.7%) PG mothers compared with 5 of 15 (26.7%) NPG mothers (P=0.45). Overall, microbiota was composed almost entirely by Bacillus and Actynomycetota (>90% of phyla) in 10 of 15 (66.7%) NPG mothers, while such composition was identified in 6 of 15 (40%) PG mothers (P=0.27) The top 5 genera present in saliva samples from women from the NPG group were Streptococcus (85.5%), Rothia (8.1%), Haemophilus (3.1%) and Prevotella (2.1%), while in those having PG the top 5 were Streptococcus (73.5%), Prevotella (13.7%), Haeomophilus (10.5%), and Schaalia (1.2%) (Figure 3). Streptococcus was the predominant genus in most mothers (13 of 15 in both groups). Of note, Haemophilus contributed significantly to the microbiota (>5% of genera detected) of 7 of 15 (46.7%) mothers in the PG group compared with 1 of 15 (6.7%) mothers in the NPG group (P=0.035).

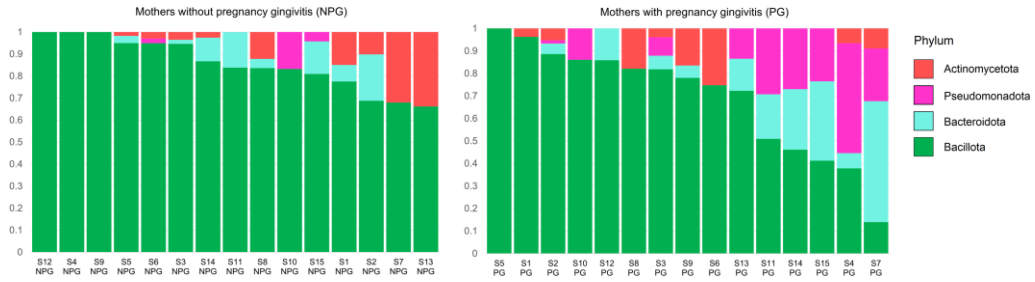

Figure 2. Barchart (a) and Heatmap (b) showing the relative abundance at phylum level in maternal saliva samples.

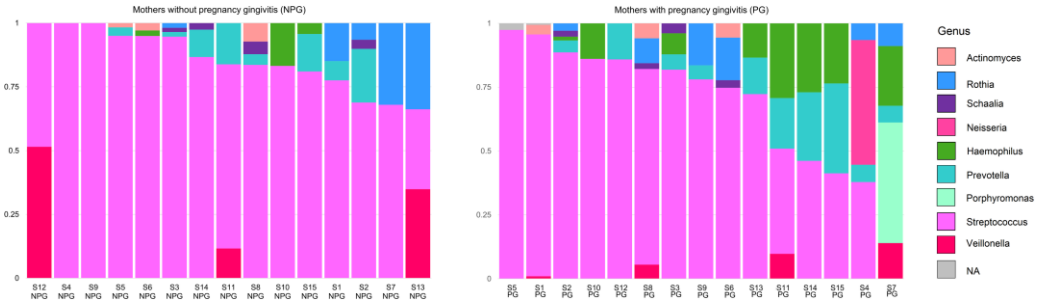

Figure 3. Relative abundance at the genus level. Cumulative relative abundance from saliva from NPG and PG groups, respectively.

3.3. Relative abundance of bacteria at phylum and genus level in newborn samples

In the meconium samples of newborns from NPG women, at phylum level the composition was: *Bacillota* (70.3%), *Pseudomonadota* (11.1%), *Bacteroidota* (10.2%), *Actinomycetota* (4.5%) and *Fusobacteriota* (0.3%), (Figure S1a), and in the meconium from the PG group: *Pseudomonadota* (64.5%), *Bacillota* (23.8%), *Actinomycetota* (6%), *Bacteroidota* (2.8%)and *Cyanobacteriota* (1%), (Figure S1b).

The five predominant genera in meconium samples of newborns from NPG mothers were *Streptococcus* (65.4%), *Lactobacillus* (15.1%), *Prevotella* (9.2%), *Haemophilus* (7.2%), and *Rothia* (2.1%) (Figure S2a). On the other hand, the predominant genera in the meconium samples of newborns from PG mothers were *Streptococcus* (52.1%), *Enterobacter* (33.3%), *Haemophilus* (14.2%), *Enhydrobacter* (11.3%), and *Brevudimonas* (7.6%) (Figure S3b).

3.4. Differential genera were found between groups

By using permutation and sPLS-DA analysis we obtained the differential genera between study groups.

Comparing the results of saliva samples from women between the two groups (NPG vs PG), differences in microbiota composition were identified with the following genera present predominantly in the NPG: *Actinomyces, Shwartzia, Rothia, Jeotbalibaca, Stomatobaculum, Campylobacter, Alloprevotela, Megasphaera,* and *Alloscardovia*. In contrast, *Catonella, Haemophilus, Mycoplasma, Peptococcus, Olsenella, Filifactor, Prevotela, Cutibacterium, Faucicola, Kingella, Odoribacter, Aggregatibacter, Neisseria, Phocaeicola, Bifidobacterium, Dialister, Tannerella, Lactobacillus,* and *Defluvitaleaceae UCG-10.* were the genera present predominantly in the PG group (Figure 4).

Analysis of the meconium samples of newborns from both groups (meconium NPG vs meconium PG) showed the presence of Granulicatella, Streptococcus, Gemella, Filifactor, Prevotellla 7, Bifidobacterium, Parabacteroides, TM7x, Candidatus Scharimonas, Megasphaera, Acholeplasma, Rothia, Abiotrophia, Veillonella, Campylobacter, Oceanivirga, Stomatobaculum, Oribacterium, Neisseria, and Prevotella in the NPG group, while Streptomyces, Sneathia, Ralstonia, Lentimicrobium, Klebsiella, Hydrogenobacter, Enterobacter, Brevudimonas, Enhydrobacter, Streptobacillus, Solobacterium, Lachnoanaerobaculum, Catonella, and Bergeyella were identified in the PG group (Figure S4).

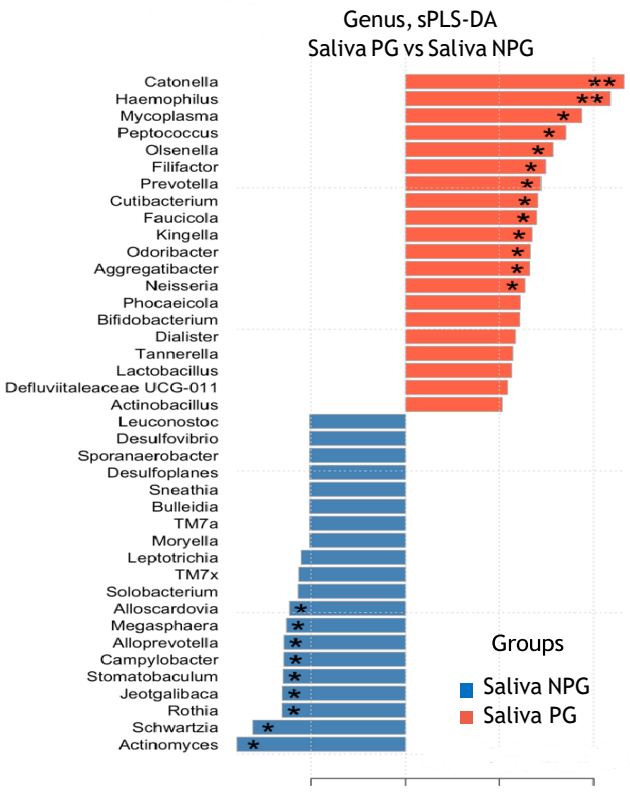

Figure 4. sPLS-DA at genera level. Saliva PG (red) vs Saliva NPG (blue).

3.5. Phylum and genera coincidence analysis by study group

At the phylum level, both at the meconium and saliva samples from the NPG and PG groups were characterized by presence of *Bacillota*, *Bacteroidota*, *Actinomycetota* and *Pseudomonadota*.

The coincidental genera present in both samples from at least one mother and infant pair were *Streptococcus*, *Prevotella*, *Haemophilus*, *Rothia*, and *Schaalia* in the NPG group. In the PG group, only *Streptococcus*, and *Prevotella* were found in both mother and infant in at least one pair.

3.6. Metabolic Pathway Prediction

Prediction of the metabolic pathways related to the main genera present in each study group was carried out using the Tax4Fun2 R package. The identified metabolic pathways were plotted using Sparse Partial Least Squares Discriminant Analysis (sPLS-DA).

Comparison between maternal NPG vs PG saliva samples showed that lysine biosynthesis, glucagon signaling pathway, D-alanine metabolism, phenylalanine metabolism, retinol metabolism, arginine biosynthesis, fructose and manose metabolism, peptidoglycan biosynthesis, two-component system, glycerolipid metabolism, biosynthesis of aminoacids, degradation of aromatic compounds, atrazine degradation, naphthalene degradation, and MAPK signaling pathway were more predominant in the NPG group, while glycerophospholipid metabolism,

glutathione metabolism, pyruvate metabolism, phosphatidylinositol signaling system, cationic antimicrobial peptide (CAMP) resistance, bacterial secretion system, carbon fixation in photosynthetic organisms, monobactam biosynthesis, lipopolysaccharide biosynthesis, mismatch repair, biotin metabolism, oxidative phosphorylation, NOD-like receptor signaling pathway, one carbon pool by folate, sulfur relay system, fatty acid metabolism, fluid shear stress and atherosclerosis, riboflavin metabolism, and metabolic pathways were more frequent in the PG group (Figure 5).

Analyses based on results from newborn meconium samples showed that biofilm formation-vibrio cholerae related, biotin metabolism, plant-pathogen interaction, biofilm formation- pseudomonas aeruginosa, two-component system, prodigiosin biosynthesis, nitrogen metabolism, and bacterial secretion system pathways were more relevant in the meconium NPG group, while glucagon signaling, RNA polymerase, central carbon metabolism in cancer, lysine biosynthesis, nucleotide excision repair, base excision repair, longevity regulating pathway-multiple species, homologus recombination, staphylococcus aureus infection, D-alanine metabolism, aminoacyl-tRNA biosynthesis, purine metabolism, photosynthesis, DNA replication, ribosome, glycerophospholipid metabolism, pyrimidine metabolism, and D-glutamine and D-glutamate metabolism pathways were relevant in the PG group (Figure S5).

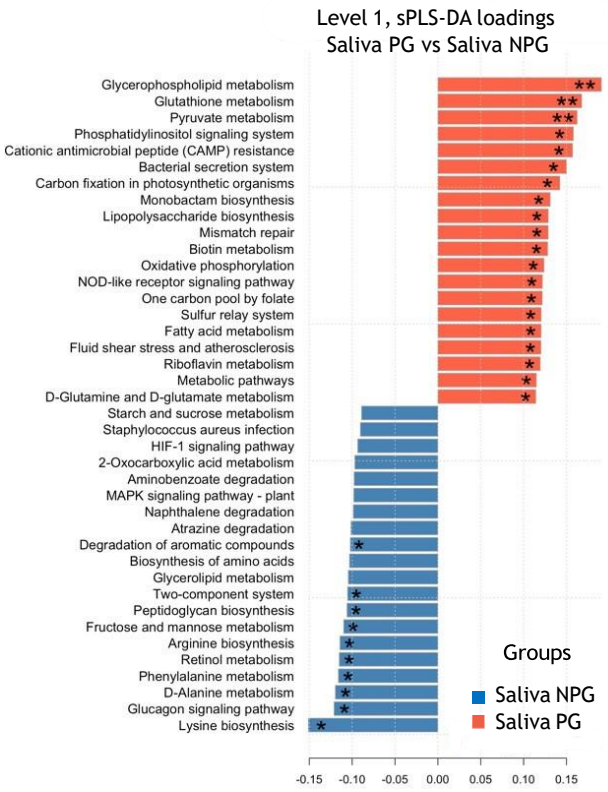

Figure 5. Metabolic pathways. Saliva PG (red) vs Saliva NPG (blue). .

4. Discussion

In recent years, the composition of the microbiome has been associated with diverse health outcomes, and it is possible that these effects could begin from early infancy. Therefore, understanding the mechanisms leading to intestinal colonization in infants is of great interest. Diverse factors (such as mode of delivery) and potential sources (including vaginal secretions, breastmilk, skin, and saliva) may contribute to shaping each individual microbiome [18,19]. Several studies have suggested that enteral colonization by bacteria may start during the prenatal period, and the maternal oral microbiome may contribute significantly to this process [20–22] [23,24]. While this hypothesis is still controversial, this would imply that conditions that alter the microbiome during pregnancy could have an important effect on the composition of the microbiome in neonates, with the consequent short and long-term health effects. In the present study we sought to determine if PG, a frequent condition during pregnancy, is associated with significant changes in the oral microbiome, and explore if this could have an impact on the offspring's microbiome.

The oral microbiota is constantly changing due to environment exposures, through eating and drinking, and oral hygiene habits. During pregnancy, gingivitis has been associated with the presence of higher levels of the genera *Prevotella*, *Streptococcus*, *Veilonella*, and *Neisseria* [25]. Balan et al. previously described that pregnancy leads to significant changes in the oral microbiota, with pregnant women (with or without gingivitis) exhibiting higher diversity than healthy non pregnant women [9]. In addition, gingivitis (in general) is associated with microbiome changes characterized by dominance of species such as *Streptococcus vestibularis*, *Treponema sp*., *Veillonella dispar*, *Fusobacterium naviformis*, and *Selenomonas* sp.[26] However, microbiome composition during PG shows distinct characteristics, as it is most often a result of hormonal changes, in contrast with gingivitis outside of pregnancy which is usually the result of poor oral hygiene.

In the present study, we identified differences in the microbiome of women with and without PG. *Streptococcus* was the most abundant genus in both groups, but its abundance was lower in the PG group compared with the NPG group; in addition, there was also a lower abundance of *Rothia*, while there was a higher abundance of *Haemophilus* in the PG group. Among the dominant genera, *Rothia* and *Schaalia* (from the Actinomycetota phylum) are typically associated with a healthy microbiota [27]. In contrast, *Haemophilus* (from the Pseudomonadota phylum) is a gram negative aerobic genus linked to plaque formation [28]. *Prevotella* and *Streptococcus* are generally associated with gingivitis [29]. Overall, the NPG group showed mostly commensal bacteria, while the PG group has presence of opportunistic pathogens.

We also predicted and compared the metabolic pathways between saliva from women with and without PG. Interestingly, despite the similarity in the top phyla and genera, the pathway analysis revealed significant functional differences. This indicates that microbiome changes associated with PG have an impact on microbial activity, networks, and functions [1,7,30,31]. The microbiota in the NPG group, showed central metabolism, stress metabolism, and repair mechanisms, while the PG group had activated energy metabolism and bacterial growth, lipid metabolism, and virulence pathways. The PG group also showed activation of the NOD-like pathway, which would indicate a response of the host immune system to bacterial components

like peptidoglycans [32]. This suggest a pro-inflammatory metabolic state in the PG group, consistent with previous reports on maternal oral microbiota associated with gingivitis [33].

Unfortunately, we obtained sufficient DNA for sequencing in only 6 meconium samples from infants in the NPG group and 3 from infants in the PG group; this was likely due to the composition of meconium, which is a complex matrix that includes mucus, amniotic fluid, and other substances, that act as PCR inhibitors [37]. Nevertheless, comparison of the microbiome of meconium of infants from mothers with and without PG showed interesting findings. While *Streptococcus* was present in infants of both groups, the frequency was lower in the PG group. In addition, *Enterobacter w*as the 2nd most abundant genus in infants of the PG group, and *Enhydrobacter* and *Brevudimonas* wer identified only in this group. In contrast, *Lactobacillus* was found exclusively in the NPG group.

Overall, genera identified in each infant group showed important differential patterns: *Streptococcus, Prevotella, Granulicatella, Gemella, Veilonella, Campylobacter* and *Sneatia* distinguished newborn samples from the NPG group, while *Ralstonia, Estreptomyces, Brevusimonas, Enterobacter* and *Lachnoanaerobaculum,* were identified as differential in newborns from the maternal PG group. *Streptococcus* and *Rothia,* which are characteristic of the oral microbiota, have been previously described to be present in the meconium microbiota [34] [34,35]. In women with oral dysbiosis, hematogenous transport across the placenta or by extracellular vesicles, could be a route of fetal exposure of these bacteria [35,36]. *Prevotella*, *Granulicatella, Gemella, Veilonella, Campylobacter,* and *Sneatia* are also commonly found in oral microbiota; however, they can be found in gut microbiota mostly as commensal bacteria that can transition to opportunistic pathogens [36–39]. On the other hand, *Ralstonia* has been found abundant in meconium microbiota in some preterm infants [40], *Streptomyces* is known for its antibiotic production and immunoregulation [41], *Enterobacter* is a well-known member of the meconium microbiota as an opportunistic member [42], *Brevudimonas* is considered a potential opportunistic microbe [43], and *Lachnoanaerobaculum* is a contributor to saccharolytic and butyrate-producing functions of the early gut [44]. Thus, meconium from the NPG group contained mostly commensal bacteria, while the PG group featured bacteria that could generate a pro-inflammatory environment.

Predicted metabolic pathways from the meconium samples further supported this idea. The PG group showed activated processes related with bacterial infection and pathogenicity, like the two-component system, biofilm formation, and biotin metabolism. The two component system regulates the expression of the virulence genes [45], while biotin metabolism is essential for activation of carboxylases, with synthesis vital for survival and pathogenicity in bacteria [46]. In contrast, the NPG group displayed activation of pathways like glucagon signaling pathway, RNA polymerase, central carbon metabolism, and lysine biosynthesis. The glucagon signaling pathway is specific to eukaryotic physiology [47], but in here, it could be related to the ability to respond to nutritional stress [48], the RNA polymerase pathway could be due to high transcriptional and metabolic activity[49], the central carbon metabolism [50] and lysine biosynthesis [51]could be related to metabolic versatility from the bacteria [52]. This aligns with the genera present, depicting a pro-

inflammatory, potentially pathogenic ecosystem in the PG newborns, in contrast to a focus on growth and homeostasis in the NPG group.

Recent studies provide context to our findings, Park et al. (2023) published a study where they collected 160 meconium samples in a Korean hospital and described the microbiota, as well as the association with microbiota from several anatomic sites of the mother. The most frequent genera identified in meconium samples were *Lactobacillus*, *Staphylococcus*, and *Ureaplasma* [53]. A study performed in Taiwan, sequenced meconium samples to describe their composition. They reported that the top phylum levels were Proteobacteria, Firmicutes, Bacteroides, Actinobacteria, and Fusobacteria [54]. The observed differences with our study suggest that regional or population characteristics may also have a significant impact on bacterial gut colonization in the newborn period.

In conclusion, we observed a healthier oral microbiota in NPG mothers, and a potentially pro-inflammatory one in mothers with PG. This profile was consistent with the microbiota found in meconium of their newborns. This could be the result of vertical transmission of oral bacteria during pregnancy via bloodstream, as supported by previous studies [25,55,56]. While the number of meconium samples with available results was small, the potential impact of PG on microbial colonization of the fetus supports the need of additional studies to confirm our findings.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is

unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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Supplementary materials.

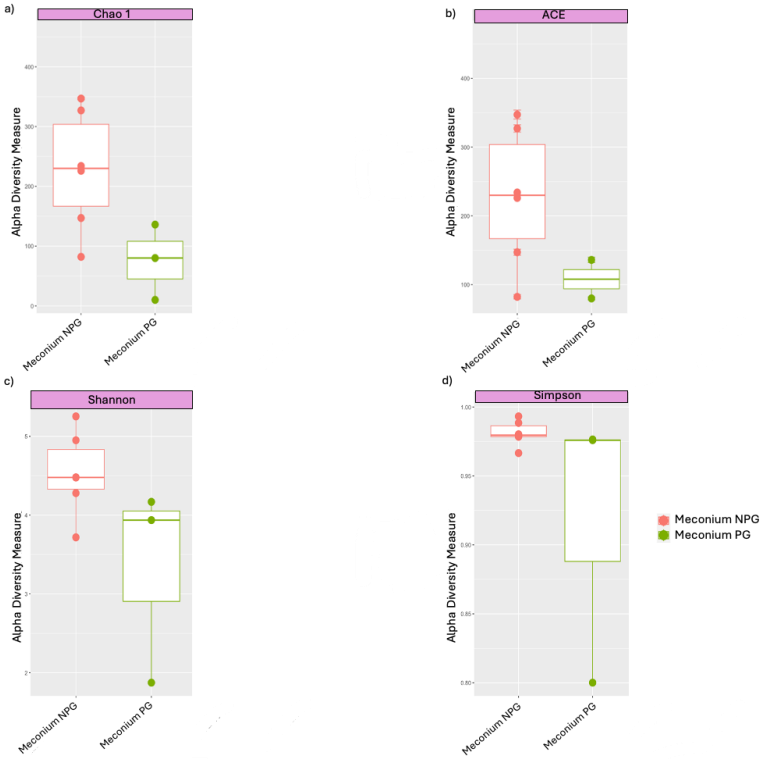

Figure S1 Alpha diversity. Diversity index. (a) Chao1 index (p value= 0.048). (b) ACE index (pvalue= 0.048). (c) Shannon index (p value= 0.095). (d) Simpson index (p value= 0.095). .

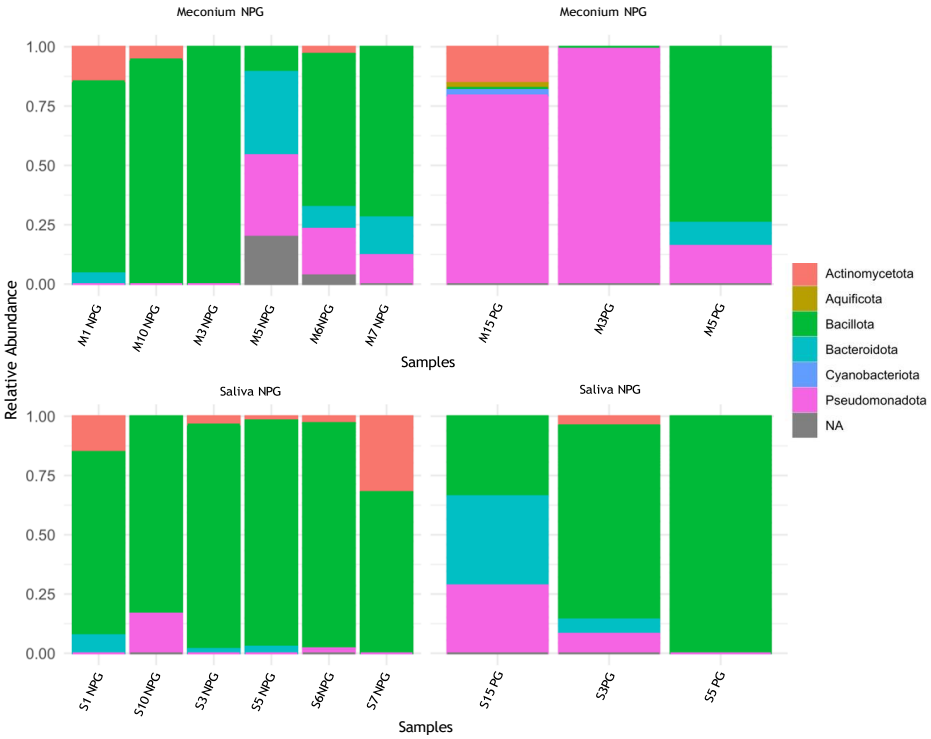

Figure S2 Barplot showing the relative abundance at phylum level in meconium and maternal saliva matching dyads.

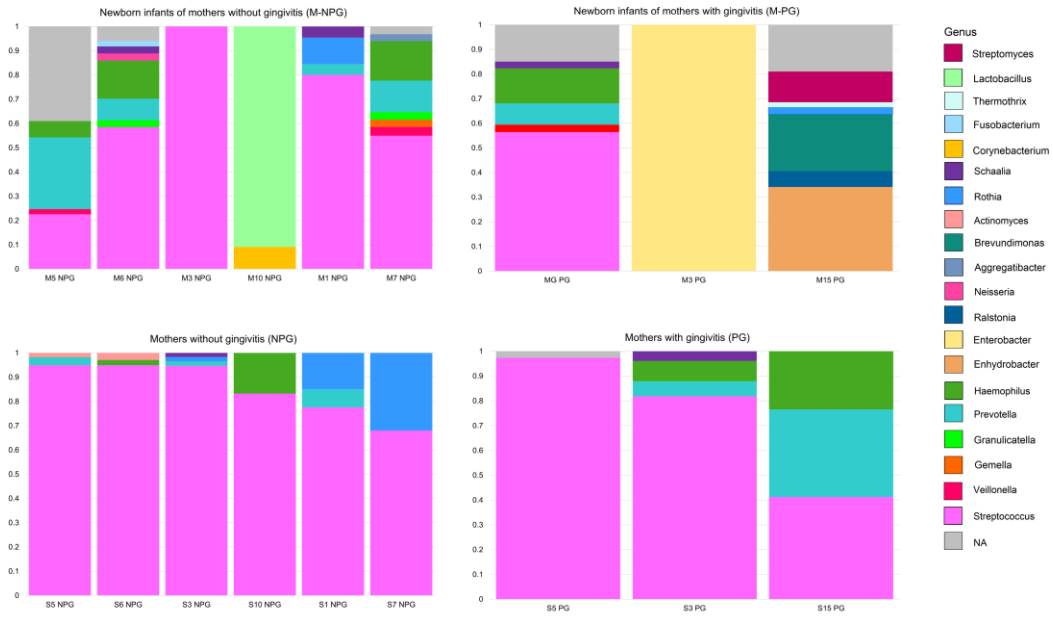

Figure S3 Barplot showing the relative abundance at genus level in meconium and maternal saliva matching dyads.

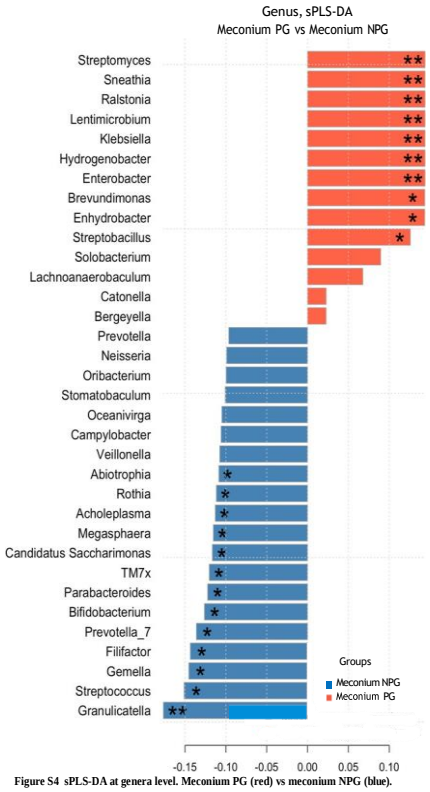

Figure S4 sPLS-DA at genera level. Meconium PG (red) vs meconium NPG (blue).

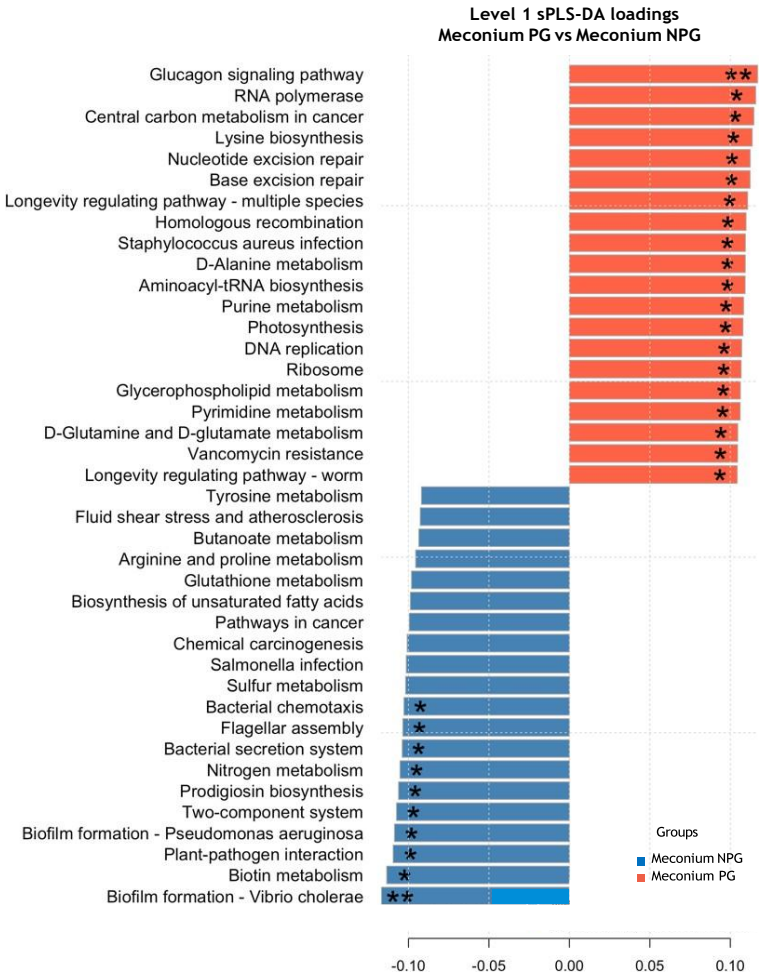

Figure S5 Metabolic pathways. Meconium PG (red) vs Meconium NPG (blue)

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Research Article

Meconium Microbiota Composition and Association with Birth Delivery Mode

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Recently, the intrauterine sterile environment theory has been questioned. Growing evidence shows that microbial *in utero* pioneer gut colonization could occur prebirth, and this initial colonization may play an important role in the development of the neonate immune system and setting up a niche for the adult-like microbiota. In this study, we compared the microbiota networks from public available meconium datasets from different countries. The findings showed differences at the genera level and were country-dependent. We generated and analyzed bacterial networks, at the genera level of meconium samples from c-section and vaginally delivery modes. Interestingly, bacterial networks from the c-section-delivered meconium samples tended to have a bigger diameter but fewer correlations, whereas the vaginally delivered meconium networks were smaller and with a higher number of correlations. Even more, the networks were similar in the delivery mode, even between countries, at the genera level. The c-section networks suggest incomplete colonization or important lack of bacteria, promoting the susceptibility of the network to receive new members, beneficial or pathogens. These results suggest that the network analysis contributes to the knowledge of microbiota composition, identifying microbial associations, despite the differences between the environment and country habits, and obtaining a better understanding of microbial gut colonization.

1. Introduction

According to the Developmental Origins of Health and Disease (DOHaD) theory, the origin of adult health or disease could be established during fetal development [1]. During the intrauterine developmental phase, the environmental stimuli associated with maternal health like diet and nutritional state, weight, stress, physical activity, pollution-exposed habits like smoking, and alcohol consumption, among others, determine the conditions for developing the fetus. Otherwise, all that stimulus could lead to epigenetic markers that are part of fetal programming for health or disease [2].

Since the report of Aagaard and collegues, in 2014, about the placental microbiota composition [3], the belief of a sterile intrauterine environment was debated. The findings showed a particular and unique characteristic microbial niche with differences to others, such as the vagina, mouth, gut, and skin [3]. These results have been discussed due to possible contamination during the taking and processing of the sample and laboratory contamination [4].

In 2019, Li et al. published a study where using negative controls demonstrated that the first bacterial colonization happens prebirth and before the newborn can even have contact with any surface; therefore, the findings are not a contamination issue. Moreover, they proposed that the way

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REVISIÓN

REVISTA CIENTÍFICA ODONTOLÓGICA

IMPACTO DE LA LEUCEMIA LINFOBLÁSTICA AGUDA EN EL MICROBIOMA Y LESIONES BUCALES: REVISIÓN DE ALCANCE

IMPACT OF ACUTE LYMPHOBLASTIC LEUKEMIA ON THE MICROBIOME AND ORAL LESIONS: SCOPING REVIEW

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RESUMEN

Objetivo: Describir el conocimiento existente sobre las alteraciones del microbioma oral (MBO) y la presencia de lesiones orales (LO) en pacientes con leucemia linfoblástica aguda (LLA). **Materiales y métodos:** Se realizó una búsqueda electrónica en las bases de datos PubMed, SciELO y Google Académico, y se incluyeron artículos descriptivos, analíticos, observacionales sobre MBO, LO y LLA, se siguieron los criterios PRISMA. Se evaluaron 642, se eliminaron artículos duplicados, reportes de caso y aquellos donde solo reportaron los cambios durante o después del tratamiento quimioterapéutico. **Resultados:** Se evaluaron 10 artículos, publicados entre 1997 y 2021, 4 artículos coincidieron que el MBO de pacientes con LLA se encuentra en disbiosis mostrando un aumento significativo de firmicutes (0,1%), bacillus (0,05%) y bacterias oportunistas, como *Moraxella* spp., *Klebsiella* spp. (5,66%), *Pseudomona* spp. (3,77%), *Enterobacter* spp. (1,88%), *Acinetobacter* spp. (1,88%) y *E. coli* (1,08%). las LO más frecuentes reportadas en 5 artículos fueron sangrado gingival espontáneo (3,5%), gingivitis (25%) y úlceras (9,4%). **Conclusiones:** La cavidad oral de los pacientes con LLA se encuentra en disbiosis y se identifican LO asociadas. Es necesario establecer estrategias preventivas con un enfoque nicho-ecológico para restablecer el MBO, con la finalidad de disminuir el riesgo de infecciones oportunistas y otras LO durante el tratamiento de quimioterapia.

Palabras clave: microbiota, microbioma, leucemia linfoblástica aguda, bacterias orales, lesiones orales, disbiosis

ABSTRACT

Objective: To describe the existing knowledge about the alterations of the MBO oral microbiome and the presence of OL Oral Lesions in patients with Acute Lymphoblastic Leukemia ALL. **Materials and Methods:** An electronic search was carried out in the PubMed, SciELO, and academic Google databases, and descriptive, analytical, observational articles on MBO, OL, and ALL were included, following the PRISMA criteria. 642 were evaluated, duplicate articles, case reports, and those where only changes were reported during or after chemotherapy treatment were eliminated. **Results:** 10 articles were evaluated, published between 1997 and 2021, 4 articles agreed that the MBO of patients with ALL is in dysbiosis showing a significant increase in firmicutes 0.1%, bacillus 0.05%, and opportunistic bacteria such as *Moraxella spp*, *Klebsiella spp* 5.66%, *Pseudomonas spp* 3.77%, *Enterobacter spp* 1.88%, *Acinetobacter spp* 1.88% and *E. coli* 1.08%, the most frequent OL reported in 5 articles were spontaneous gingival bleeding 3.5%, gingivitis 25% and ulcers 9.4%. **Conclusions:** The oral cavity of patients with ALL is in dysbiosis and associated OL is identified. It is necessary to establish preventive strategies with a niche-ecological approach to restore the MBO, to reduce the risk of opportunistic infections and other OL during chemotherapy treatment.

Keywords: microbiota, microbiome, acute lymphoblastic leukemia, oral bacteria, oral lesions, dysbiosis

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NMCD
Nutrition, Metabolism & Cardiovascular Diseases

FULL LENGTH ARTICLE · Volume 31, Issue 11, P3210-3218, October 28, 2021

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Association of *BCAT2* and *BCKDH* polymorphisms with clinical, anthropometric and biochemical parameters in young adults

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Highlights

- The less common alleles frequencies were 15.2% for the *BCAT2* rs11548193 and 9.83% for the *BCKDH* rs45500792 polymorphisms.
- *BCAT2* polymorphism carriers presented lower isoleucine concentration than subjects homozygotes for the most common allele.
- *BCKDH* SNPs carriers displayed no differences in the evaluated parameters compared with non-carrier homozygotes subjects.
- Both SNPs carriers had higher BMI, blood pressure, glucose, Asp, Ile, Met, and Pro than non-carrier homozygotes subjects.

Berry Cactus Juice Concentrate: A Potential Modulator for Mitigating Metabolic Syndrome Markers in a High-Fat Diet Model

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Research Article

Keywords: Myrtillocactus geometrizans, metabolomic markers, Metabolic syndrome, 16S, Biomarker, Gut microbiota

Posted Date: October 16th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7688114/v1

Presentación para defensa de tesis:

Universidad Autónoma de San Luis Potosí
Facultad de Medicina
Posgrado en Ciencias Biomédicas Básicas

TESIS DOCTORAL

"Efecto de la gingivitis materna en el establecimiento del microbioma intestinal del neonato."

Presenta:
M. en C. Ana Karenina Rocha-Viggiano

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1

COMITÉ TUTELAR.

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- Asesor externo: Dr. Miguel Angel Brieño-Enríquez. (Magee-Womens Research Institute)

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ANTECEDENTES

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Gingivitis.

Es la enfermedad periodontal más frecuente.

Hasta un 70% de la población la padece.

Tipos de gingivitis.

- Asociada a placa.
- Asociada al uso de fármacos.
- Asociada a genética.
- **En el embarazo.**

Rocha Navarro, R. et al. Microbial plaque in children. 2016.

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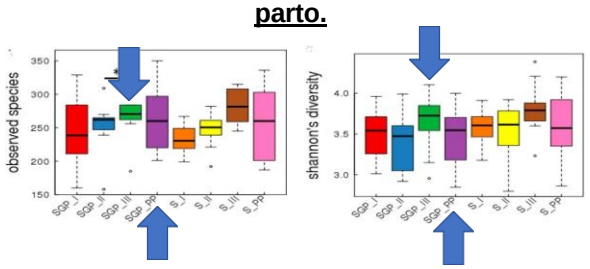
Microbioma oral durante el embarazo y post-parto.



Balan, P., et al. "Keystone species in pregnancy gingivitis: a snapshot of oral microbiome during pregnancy and pospartum period."(2 081).

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Diferentes redes microbianas durante el embarazo y en el post-parto.

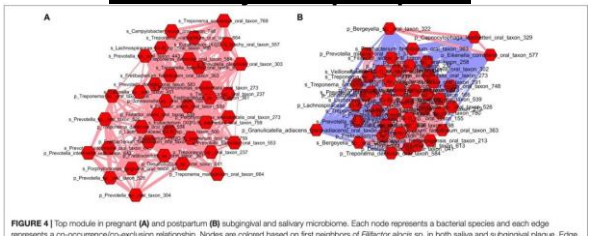

FIGURE 4 | Top module in pregnant (A) and postpartum (B) subgingival and salivary microbiome. Each node represents a bacterial species and each edge represents a co-occurrence/co-exclusion relationship. Nodes are colored based on first neighbors of *Filifactor alocis* sp. in both saliva and subgingival plaque. Edge width is proportional to the correlation coefficient and color indicates the sign of the association (blue: negative, pink: positive). Prefix 'p' and 's' denote species from subgingival plaque and saliva respectively.

Balan, P., et al. "Keystone species in pregnancy gingivitis: a snapshot of oral microbiome during pregnancy and pospartum period."(2018).

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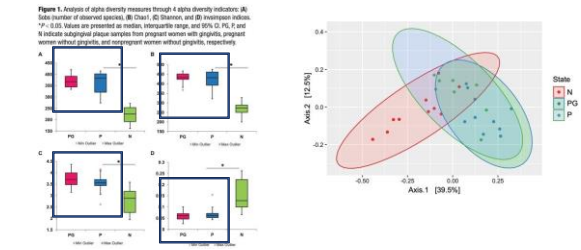
Microbioma oral durante el embarazo con y sin gingivitis.



Balan, P., Brandt, B., Chong, Y., Crielaard, W., Wong, M., Lopez, V., He, H., & Seneviratne, C. (2020). Subgingival Microbiota during Healthy Pregnancy and Pregnancy Gingivitis. *JDR Clinical & Translational Research*, 6, 343 - 351.

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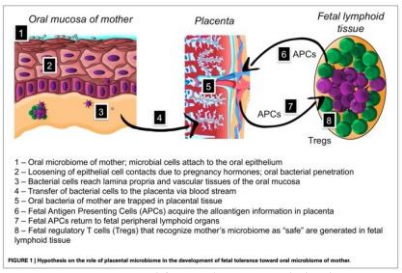
Hipótesis de entrenamiento inmunológico



Zaura et al., "Acquiring and Maintaining a Normal Oral Microbiome: Current Perspective."

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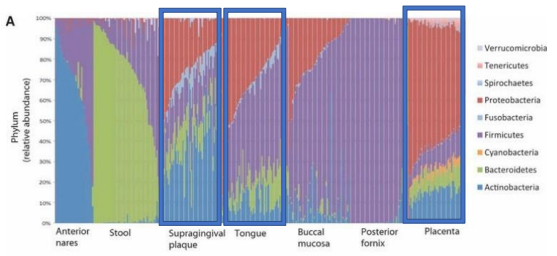
La placenta y su microbioma.



Aagaard, K. et al. "The placenta harbors a unique microbiome." (2014).

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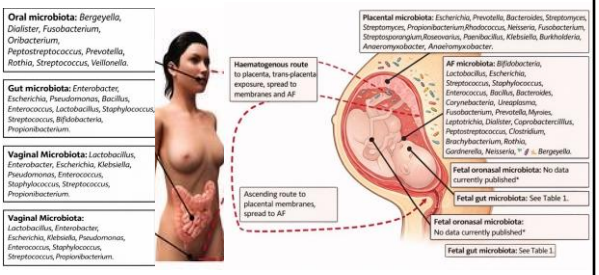
Posible origen de la microbiota neonatal.



Stinson, L. F.,et al. Planting the seed: Origins, composition, and postnatal health significance of the fetal gastrointestinal microbiota. (2017).

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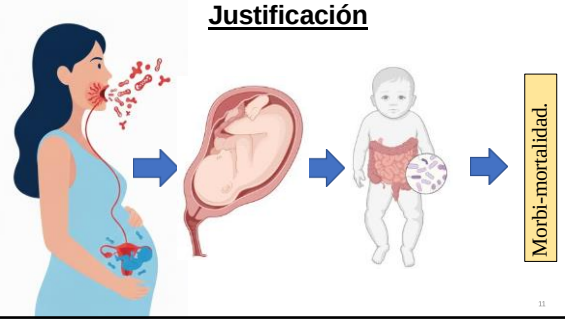
Justificación



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HIPÓTESIS.

La gingivitis y el microbioma oral materno tienen un efecto en el establecimiento del microbioma intestinal del neonato.

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PREGUNTA DE INVESTIGACIÓN.

¿De qué manera la presencia de gingivitis y la composición del microbioma oral en mujeres embarazadas influyen en la diversidad y el establecimiento del microbioma intestinal del neonato?"

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OBJETIVO.

- Determinar el efecto de la presencia de gingivitis materna sobre el establecimiento del microbioma intestinal del neonato.

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Objetivos específicos.

- Identificar, cuantificar y diferenciar el microbioma salival de mujeres embarazadas con y sin gingivitis mediante metagenómica.
- Identificar, cuantificar y diferenciar el microbioma en las heces de los neonatos provenientes de madres con y sin gingivitis,

mediante metagenómica.

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Diseño del estudio.

Estudio observacional, analítico de cohorte prospectiva.

- **Enfoque:** Cuantitativo, ya que se busca medir la abundancia y diversidad bacteriana mediante secuenciación genómica.
- **Diseño: Cohorte Prospectiva**. Se recluta a las madres durante el embarazo (exposición) y se sigue a los recién nacidos (resultado) para observar el establecimiento de su microbioma.

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Criterios de inclusión (materna)

Criterios de inclusión:

- Mayores de edad
- Consentimiento Informado (firmado)
- Mujeres que llegan a término del embarazo (34 a 36 semanas)
- Embarazo único

Criterios de exclusión:

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Criterios de inclusión (neonato)

Criterios de inclusión:

- Sanos
- Peso: mayor a 2500g
- Talla: 48 – 52 cm

Criterios de no-inclusión:

- Malformaciones congénitas mayores

Criterios de eliminación:

- No tener consentimiento informado firmado o que lo retiren
- Imposibilidad para tomar la muestra

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Carta de aprobación del Comité de Ética

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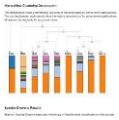
Plan de trabajo



Reclutamiento de mujeres embarazadas que cumplan con los criterios de inclusión. → Toma de muestras de saliva materna. → Toma de muestras a neonatos de meconio. → Secuenciación del fragmento 16s de muestras . → Obtención y análisis de datos.

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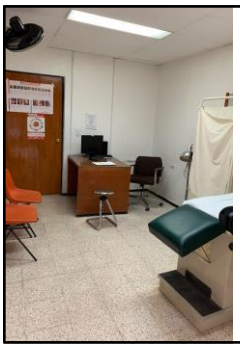

Consulta Vespertina (Bajo riesgo)

- Llegada de 2:00pm en adelante de lunes a viernes
- Revisión de expedientes para cálculo de SDG y descartar uso de antibióticos recientes
- Explicación del protocolo a las embarazadas que cumplen con las SDG (30 a 37) y firma de consentimiento informado

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Toma de muestra de saliva

- Revisión de IG y sondeo.
- Se tomó muestra de saliva sin estimular y fué almacenada en tubos colectores con DNA/RNA shield para preservar la integridad de ácidos nucléicos a temperatura ambiente.

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Toma de muestra en neonato

- Se realizó la toma de muestra del meconio (primera evacuación) de los neonatos provenientes de las mujeres participantes y fue almacenada en tubos colectores con DNA/RNA shield para preservar la integridad de ácidos nucléicos a temperatura ambiente.

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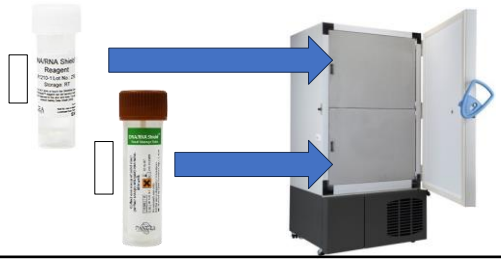
Almacenamiento a -80 hasta su procesamiento



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Resultados

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Muestras obtenidas

Participantes	30
Binomios completos	30
Parto	19
Cesárea	11
Sexo	16 Masc/ 14 Fem

Se dividieron en dos grupos

- Sin gingivitis (NPG)
- Con gingivitis (PG)

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Tabla demográfica mujeres gestantes

Table 1. Baseline characteristics of study participants

	Mothers without gingivitis (NPG) (n=15)	Mothers with gingivitis (PG) (n=15)	p
Age (years)	26.21 (±6.2)	28.13 (±5.9)	0.35
Gestation age at recruitment (weeks)	33.5 (±1.9)	35.4 (±1.9)	0.77

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Tabla demográfica neonatos

	Sana (NPG)	Gingivitis (PG)	p
No. Neonato	15	15	
SDG (Semanas de Gestacion al nacimiento)	39 ± 1.2	39 ± 1.2	0.77
Peso (gramos)	3,231 ± 440.7	3,217 ± 413.7	0.53
Sexo	7 fem (47%)	7 fem (47%)	0.33
Vía de nacimiento	9 vaginal (60%)	10 vaginal (67%)	0.33

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Extracción de DNA de saliva

- Se realizaron extracciones de las 30 muestras de saliva y utilizando el kit ZymoBIOMICS de Zymo

- Kit recomendado para extracción de DNA y ARN de muestras clínicas
- Elimina inhibidores de PCR
- Permite procesar hasta 50 muestras

Kazantseva J,Malv E, Kaleda A, Kallastu A,Meikas A.2021. Optimisation of sample storageandDNA extractio ;n) forhuman gut microbiota studies.BMC Microbiol 21.

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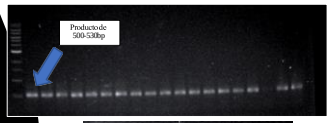
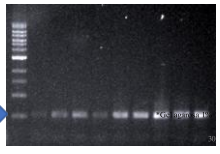
PCR 16s muestras de saliva con 45ng/ul





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Extracción de DNA de meconio

- Se realizaron extracciones utilizando el kit ZymoBIOMICS y el Fecal/soil microbe miniprep de Zymo

- Kit recomendado para extracción de DNA y su uso en secuenciación
- Elimina inhibidores de PCR

- Kit recomendado para extracción de DNA en heces y suelo
- Elimina inhibidores de PCR

- Kazmierczak J, Milev E, Kalesh A, Kallarin A, Meiksa A. 2021. Optimization of sample storage and DNA extraction for human gut microbiota studies. BMC Microbiol 21.
- Skrzypek K, Karamon J, Samorek-Pieróg M, Dąbrowska J, Kochanowski M, Sroka J, Bilska-Zając E, Cencek T. 2020. Comparison of two dna extraction methods and two pcrs for detection of echinococcus multilocularis in the stool samples of naturally infected red foxes. Antimi 10:1–8.

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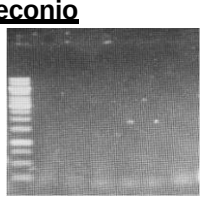
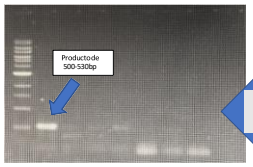
Extracción de DNA de meconio

- Se usaron diversas técnicas para mejorar el rendimiento, sin embargo, sólo se logró obtener material para 6 muestras del grupo NPG y 3 del grupo PG.





Re-amplificación con dilución 1:20

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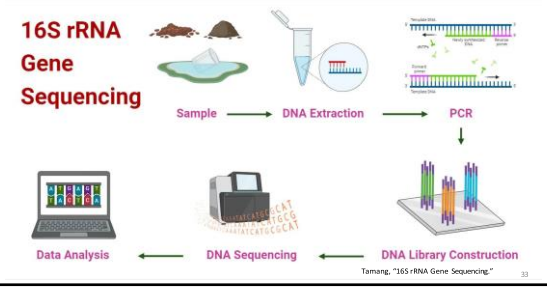
Secuenciación de las muestras



Tamang, "16S rRNA Gene Sequencing."

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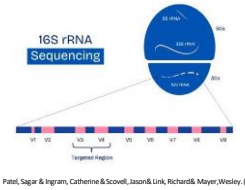
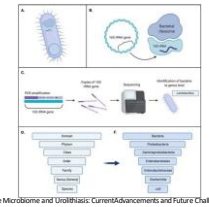
Secuenciación 16s

- Secuenciación de 16s es la mas comunmente utilizada en metagenómica
- Es aproximadamente de 1500pb y tiene 9 regiones variables.
- Las regiones variables de 16s rRNA son frecuentemente usadas para las clasificaciones filogenéticas tales como género en poblaciones microbiales diversas.





Patel, Sagar & Ingram, Catherine & Scovell, Jason& Link, Richard& Mayer, Wesley. (2022). The Microbiome and Urolithiasis: Current Advancements and Future Challenges.

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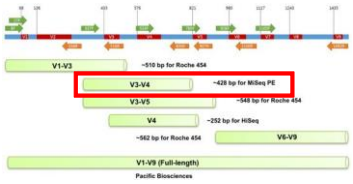
Región V3 y V4

Son ampliamente utilizadas para secuenciación con la Plataforma Illumina porque provee secuencias mas largas y detalladas (400-500bp) que únicamente utilizando V4. Ha mostrado alta eficiencia en captar la diversidad a nivel filo (phylum), clase y orden.



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PCR

- **PCR 1 (Amplicón):** Región **V3-V4** (341F/ 805R) con polimerasa de alta fidelidad.
- **PCR 2 (Indexación):** Adición de índices duales **Nextera XT** (8-10 ciclos).

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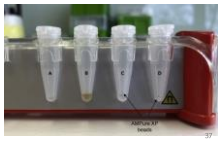
Purificación de amplicón

Proceso de Purificación con Perlas Magnéticas

- La limpieza se basa en la unión reversible del ADN a perlas paramagnéticas en presencia de polietilenglicol (PEG) y sal.
- **Incubación:** Mezclar por vórtex suavemente o pipeteo y dejar incubar a temperatura ambiente durante 5 minutos para que el ADN se una a las perlas.
- **Lavados con Etanol:** Realizar dos lavados con **Etanol al 80%** recién preparado. Es vital asegurar que las perlas permanezcan en el imán durante el descarte del sobrenadante para evitar pérdida de muestra.
- **Secado:** Dejar secar el pellet de perlas al aire (generalmente 2-3 minutos) hasta que pierda el brillo, pero sin que se agriete, ya que esto dificultaría la elución del ADN.
- **Elución:** Utilizar un buffer de elución o agua grado nuclease para resuspender las perlas y liberar el ADN purificado.



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Plataforma de secuenciación

- Plataforma: Illumina MiSeq (Tecnología SBS).
- Parámetros de Secuenciación (Kit v3)
- Reactivos: MiSeq Reagent Kit v3 (600 ciclos).
- Configuración: 2 x 300 pb (Paired-End) para maximizar el traslape y la resolución taxonómica.
- Diversidad: Adición de 10-15% PhiX (control interno) para balancear la baja complejidad del amplicón 16S.

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Procesamiento de las lecturas

1. Control de Calidad Inicial

- **Programa: FastQC** o **MultiQC**.
- **Parámetros:** Evaluación de la puntuación de calidad (Phred score > 30), detección de adaptadores residuales y niveles de duplicación.

2. Limpieza y Filtrado (Trimming/Filtering)

- **Programas: Cutadapt** (para eliminar cebadores/primers)

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3. Inferencia de Variantes (Denoising)

- **Programas: DADA2**
- **Parámetros Críticos:**
  - **Truncado (truncLen):** Cortar las lecturas en el punto donde la calidad decae (ej. 280 pb para R1 y 220 pb para R2).
  - **MaxEE:** Número máximo de errores esperados (típicamente 2).
  - **Fusión (Merging):** Unir las lecturas *Paired-End* con un traslape mínimo de 12-20 pb.

4. Eliminación de Quimeras

- **Programa: DADA2**
- **Parámetros:** Comparación de las secuencias contra sí mismas para detectar artefactos de PCR (híbridos de dos bacterias distintas).

5. Asignación Taxonómica

- **Bases de Datos: SILVA.**

Amplicon Sequencing. **Exactly.**

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Análisis muestras de saliva materna

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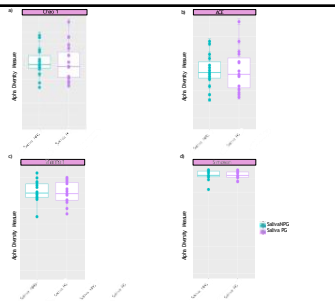
Índices de diversidad

- Chao1 mide riqueza, estima el total de especies real.
- ACE mide riqueza, pero estima la riqueza considerando especies con baja abundancia.
- Shannon y Simpson estiman diversidad, la primera estima la complejidad del ecosistema y la segunda mide las especies dominantes.
- Sin diferencias significativas.



Alphadiversity. Diversity index. (a) Chao1 index (pvalue= 0.9). (b) ACEindex (pvalue= 0.8). (c) Shannon index (p value= 0.7). (d) Simpson index (p value= 0.7).

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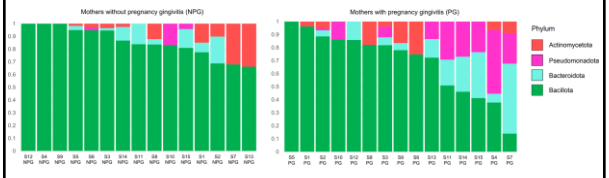
Abundancia relativa a nivel phylum



- Dominio de de phyllum Bacillota en el grupo NPG.
- En PG aumenta Pseudomonadota y Bacteroidota.

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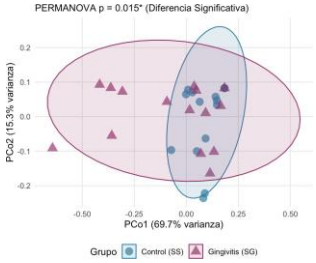
Análisis de Coordenadas Principales (PCoA). Distancia de Bray-Curtis (diversidad beta)



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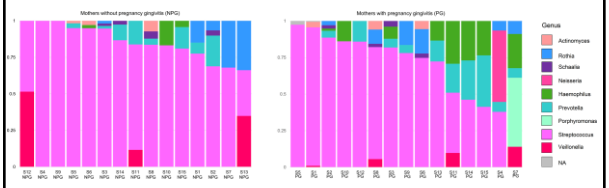
Abundancia relativa a nivel género



- Los 5 géneros mas abundantes en el grupo NPG son: *Streptococcus* (85.5%), *Rothia* (8.1%), *Haemophilus* (3.1%) y *Prevotella* (2.1%).
- Los 5 géneros mas abundantes en el grupo PG son: *Streptococcus* (73.5%), *Prevotella* (13.7%), *Haeomophilus* (10.5%), y *Schaalia* (1.2%).

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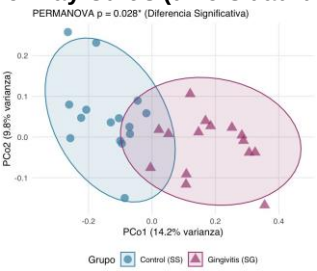
Análisis de Coordenadas Principales (PCoA). Distancia de Bray-Curtis (diversidad beta)



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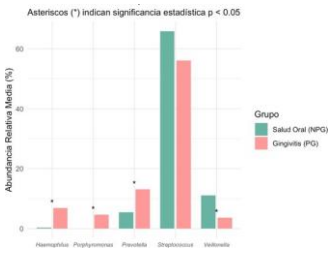
Comparativa de composicion con Wilcoxon (U de Mann-Whitney)

Genus	Media NPG (%)	Media PG (%)	Valor p
Streptococcus	65.8	56.1	0.18
Prevotella	5.5	13.1	**0.02***
Haemophilus	0.3	6.9	**0.01***
Porphyromonas	0.0	4.7	**0.01***
Veillonella	11.1	3.6	**0.04***



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Géneros compartidos entre saliva y meconio

Género	Compartido en NPG	Compartido en PG	Observación
Streptococcus	Si	Si	Disminuye en PG.
Prevotella	No	Si	Género asociado a inflamación.
Rothia	Sí	No (poco detectable)	Marcador de salud oral.
Haemophilus	No	Si	Biomarcador de gingivitis materna.
Actinomyces	Sí	Si	Colonizador oral persistente en ambos grupos.

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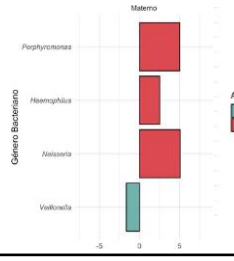
Análisis de efecto de tamaño Log2 Fold Change



Género	Grupo Asociado	Log2 Fold Change (L2FC)	Valor p (Wilcoxon)
Haemophilus	Gingivitis (PG)	+2.68	0.038*
Prevotella	Gingivitis (PG)	+1.12	0.334

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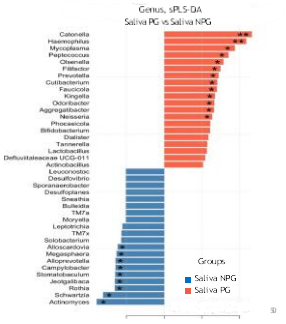
sPLS-DA a nivel género

- *Catonella, Haemophilus, Mycoplasma, Peptococcus, Olsenella, Filifactor y Prevotella* que tienen potencial patogénico, en el grupo PG.
- *Actinomyces, Schwartzia, Rothia, Jeotgalibaca, Stomatobaculum y Campylobacter* que son de predominio commensal, baja patogenicidad y potencial protector como el género *Rothia* en el grupo NPG.



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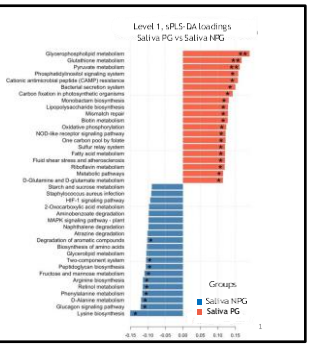
sPLS-DA con rutas metabólicas implicadas

- En el grupo NPG: metabolismo central, metabolismo de estrés y mecanismos de reparación.
- En el grupo PG: metabolismo energético y crecimiento bacteriano, metabolismo de lípidos y vías de virulencia.



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Discusión

- Aunque no se observan diferencias significativas a nivel de diversidad, ambos grupos presentan géneros bacterianos que caracterizan las diferencias entre ellos.
- Estos géneros se asocian a vías metabólicas que caracterizan al grupo NPG con un ambiente homeostático y al grupo PG con un ambiente pro-inflamatorio.

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Análisis muestras de meconio (neonatales)

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Índices de diversidad

- Mayor diversidad en grupo NPG.

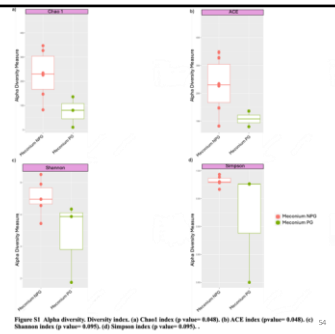

Figure S1 Alpha diversity. Diversity index. (a) Chao1 index (p value= 0.048). (b) ACE index (pvalue= 0.048). (c) Shannon index (p value= 0.095). (d) Simpson index (p value= 0.095). .

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Abundancia relativa a nivel phyllum

- Dominio de Bacillota en NPG.
- Dominio de Pseudomonadotaen PG.

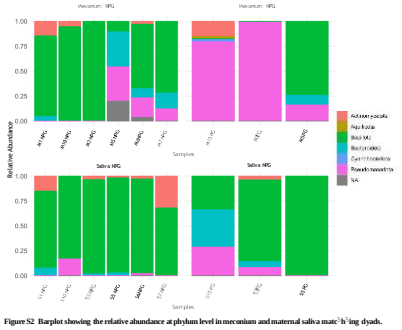

Figure S2 Barplot showing the relative abundance at phylum level in meconium and maternal saliva matched dyads.

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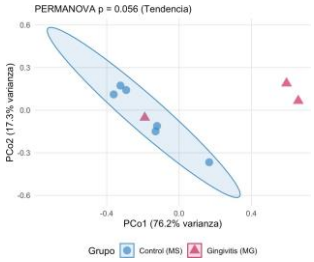
Análisis de Coordenadas Principales (PCoA). Distancia de Bray-Curtis (diversidad beta)



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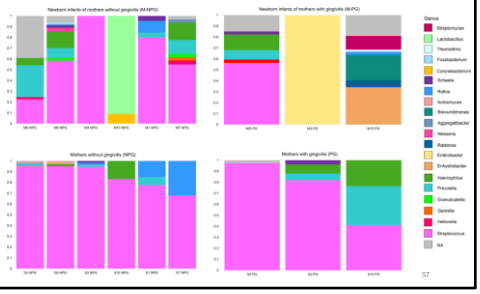
Abundancia relativa a nivel género

Dominio de NPG por:

- *Streptococcus* (65.4%)
- *Lactobacillus* (15.1%)
- *Prevotella* (9.2%)
- *Haemophilus* (7.2%)
- *Rothia* (2.1%)

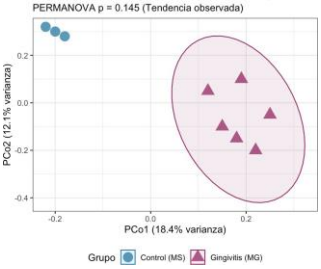
PG :

- *Streptococcus* (52.1%)
- *Enterobacter* (33.3%)
- *Haemophilus* (14.2%)
- *Enhydrobacter* (11.3%)
- *Brevudimonas* (7.6%).



57

Análisis de Coordenadas Principales (PCoA). Distancia de Bray-Curtis (diversidad beta)



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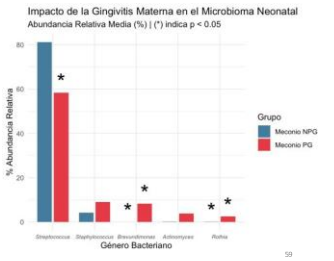
Comparativa de composicion con Wilcoxon (U de Mann-Whitney)

Genus	Media NPG (%)	Media PG (%)	Valor p
Streptococcus	81.2	58.4	**0.04***
Brevundimonas	0.0	8.2	**0.03***
Actinomyces	0.2	3.8	0.07
Rothia	0.1	2.5	**0.04***
Staphylococcus	4.2	9.1	0.15



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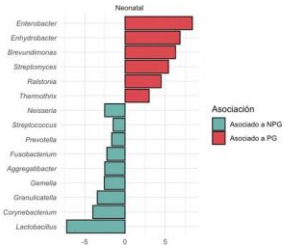
Análisis de efecto de tamaño Log2 Fold Change



Género	Grupo Asociado	Log2 Fold Change (L2FC)	Valor p (Wilcoxon)
Enterobacter	Gingivitis (PG)	+15.2	0.238
Streptococcus	Salud (NPG)	-1.54	0.237

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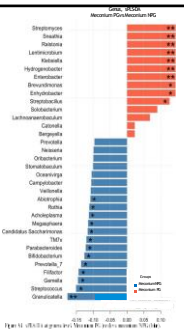
sPLS-DA a nivel género

- El grupo NPG contiene sobre todo bacterias comensales.
- El grupo PG contiene bacterias que podrían generar un ambiente pro-inflamatorio.



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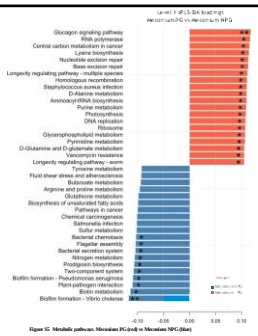
sPLS-DA de rutas metabólicas

- El grupo PG: procesos relacionados a la infección bacteriana y patogenicidad.
- El grupo NPG: muestra activación de vías relacionadas a crecimiento y homeostásis.



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Discusión

- En los grupos neonatales, se observa mayor diversidad en el grupo NPG, indicando una comunidad más rica en especies y distribuidas de manera equitativa.
- A nivel de phylum y género existen coincidencias entre las díadas materno-neonatales. Del mismo modo, el grupo NPG presenta predominantemente géneros comensales que favorecen un ambiente homeostático, mientras que en el grupo PG se observan géneros potencialmente patógenos que promueven un ambiente proinflamatorio.

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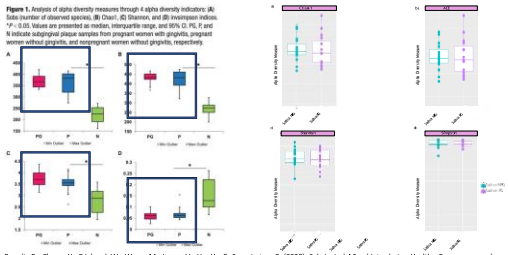
Microbiota oral con y sin gingivitis



Balan, P., Brandt, B., Chong, Y., Crielaard, W., Wong, M., Lopez, V., Ho, H., & Seneviratne, C. (2020). Subgingival Microbiota during Healthy Pregnancy and Pregnancy Gingivitis. *JDR Clinical & Translational Research*, 6, 343 - 351.

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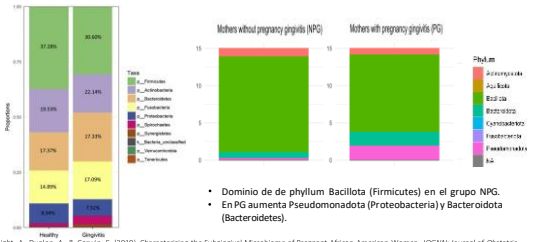
Microbiota oral con gingivitis



- Dominio de de phyllum Bacillota (Firmicutes) en el grupo NPG.
- En PG aumenta Pseudomonadota (Proteobacteria) y Bacteroidota (Bacteroidetes).

Yang, I., Knight, A., Dunlop, A., & Corwin, E. (2019). Characterizing the Subgingival Microbiome of Pregnant African American Women. *JOGNN: Journal of Obstetric, Gynecologic, and Neonatal Nursing*, 48, 140–152. https://doi.org/10.1016/j.jogn.2018.12.003.

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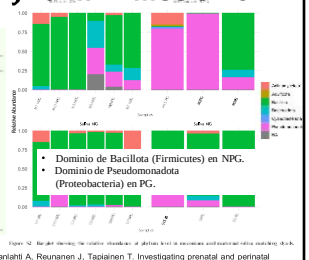
Comparativa a nivel phyllu m en mec onio

Ten most abundant species in the first-pass meconium according to the delivery mode.

Phylum	Vaginal (%)	C-section (%)	Genus	Vaginal (%)	C-section (%)
Firmicutes	74	40	*Staphylococcus*	36	23
Proteobacteria	22	20	*Escherichia-Shigella*	20	0.2
Actinobacteriota	1.3	18	*Lactobacillus*	18	1.0
Bacteroidota	1.6	11	*Streptococcus*	9.7	1.5
Acidobacteriota	0.3	2.9	*C Comamonadaceae*	0.4	7.9
Deinococcota	0.2	2.2	*Vibrionimonas*	0.3	6.7
K. Bacteria	0.01	1.7	*Corynebacterium*	0.4	4.7
Bdellovibrionota	0.01	1.5	*Cutibacterium*	0.4	4.5
Mycococcota	0.03	1.3	*Enterococcus*	4.5	0.2
Unassigned	0.03	0.8	*Bacillus*	0.3	2.7
Other	0.2	1.4	Other	11	47



- Dominio de Bacillota (Firmicutes) en NPG.
- Dominio de Pseudomonadota (Proteobacteria) en PG.

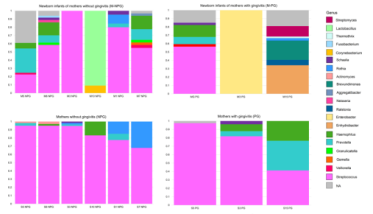
Turunen J, Tejesvi MV, Paalanne N, Pokka T, Amatya SB, Mishra S, Kaisanlahti A, Reunanen J, Tapiainen T. Investigating prenatal and perinatal factors on meconium microbiota: a systematic review and cohort study. Pediatr Res. 2024 Jan;95(1):135-145. doi: 10.1038/s41390-023-02783-z. Epub 2023 Aug 17. PMID: 37591927; PMCID: PMC10798900.

66

66

Comparativa a nivel género en meconio

Genus	Vaginal (%)	C-section (%)
Staphylococcus	36	23
Escherichia-Shigella	20	0.2
Lactobacillus	18	1.0
Streptococcus	9.7	1.5
f. Comamonadaceae	0.4	7.9
Vibrionimonas	0.3	6.7
Corynebacterium	0.4	4.7
Cutibacterium	0.4	4.5
Enterococcus	4.5	0.2
Bacillus	0.3	2.7
Other	11	47



Turunen J, Tejesvi MV, Paalanne N, Pokka T, Amatya SB, Mishra S, Kaisanlahti A, Reunanen J, Tapiainen T. Investigating prenatal and perinatal factors on meconium microbiota: a systematic review and cohort study. Pediatr Res. 2024 Jan;95(1):135-145. doi: 10.1038/s41390-023-02783-z. Epub 2023 Aug 17. PMID: 37591927; PMCID: PMC10798900.

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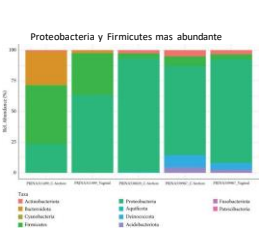
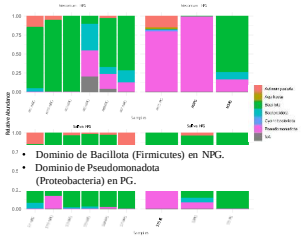
Comparativa a nivel phyllum en meconio

Proteobacteria y Firmicutes mas abundante





- Dominio de Bacillota (Firmicutes) en NPG.
- Dominio de Pseudomonadota (Proteobacteria) en PG.

Meconium Microbiota Composition and Association with Birth Delivery Mode Ana K. Rocha-Viegiano, Saray Aranda-Romo, Mariana Salgado-Bustamante, Cesaré Ovando-Vázquez First published: 07 November 2022 https://doi.org/10.1155/2022/6077912

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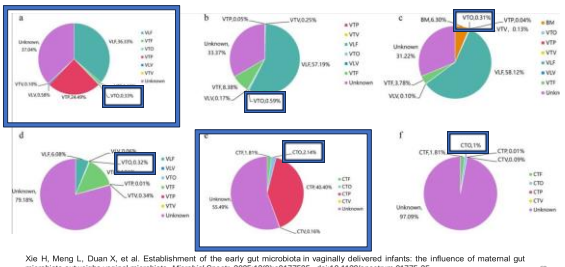
Posible origen usando Source Tracker



Xie H, Meng L, Duan X, et al. Establishment of the early gut microbiota in vaginally delivered infants: the influence of maternal gut microbiota outweighs vaginal microbiota. *Microbiol Spectr*. 2025;13(9):e0177525. doi:10.1128/spectrum.01775-25

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RESUMEN DE RESULTADOS

- No existen estudios que comparen la microbiota oral materna con la microbiota en meconio.
- Las madres sin gingivitis (NPG) mostraron un microbioma oral relacionado con salud oral.
- Las madres con gingivitis (PG) presentaron una microbiota potencialmente proinflamatoria.
- El perfil microbiano oral materno fue consistente con la microbiota encontrada en el meconio de sus recién nacidos.
- Los resultados apoyan la posible transmisión de bacterias orales maternas al feto vía torrente sanguíneo durante el embarazo.
- La gingivitis durante el embarazo podría influir en la colonización microbiana fetal.

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CONCLUSIONES

- Existe un efecto en la microbiota intestinal del neonato dependiente de la presencia o ausencia de gingivitis materna, generando un ambiente pro-inflamatorio en el grupo PG.
- La salud oral materna debe ser considerada parte de la atención médica pre-natal dadas las repercusiones en la salud neonatal.

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LIMITACIONES

- Hace falta una metodología que permita la extracción eficiente de DNA en muestras de meconio, ya que aunque se obtuvieron el total de muestras, sólo se obtuvo DNA para la secuenciación de 9 del total.
- Las vías metabólicas son predicciones que apuntan a las potenciales funciones que desempeñan los generos bacterianos presentes, para corroborarlas se requerirían estudios empleando técnicas como la metabolómica.

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Publicaciones

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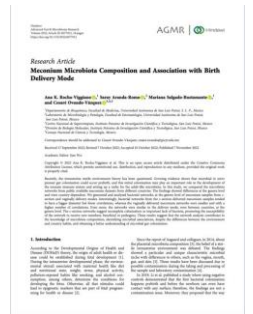

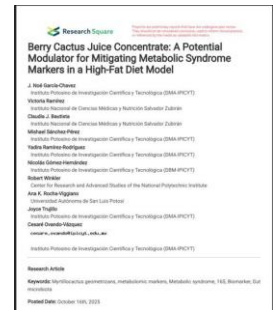

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Divulgación

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