سدرة للطب
Sidra Medicine

MENA Microbiome

From Biomarkers Discovery
to Microbiota-based Therapeutics

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Venue: The Plaza Doha

Maternal immunoglobulins differentially bind the bacterial community in human colostrum and stool of breastfed neonates.

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In newborns, the successional establishment of the primordial microbiota strains in the gut is an interesting topic of investigation, where the IgA1, IgA2, IgM, and IgG immunoglobulins provided by the mother during breastfeeding play a primordial role. The objective of this work was to explore the functional role of the colostrum's maternal immunoglobulins, which bind differently, a diverse bacterial community in the intestine of breastfed neonates. We sequenced V3-16S rRNA gene libraries prepared with DNA extracted from single IgA1, IgA2, IgM, and IgG fluorescence-activated cell sorting fractions from meconium or colostrum. Our results show that in colostrum, the bacteria are already differentially bound by these immunoglobulins. We determined that IgA2 and IgM bind alfa and beta Proteobacteria at early breastfeeding stages, which might stimulate the immune system in the gut of neonates. In addition, it was found that IgG mostly binds facultative anaerobes of the Firmicutes phylum, which are reported as part of the human milk microbiota and pioneer elements of the neonatal gut. In the case of the neonatal stool, the immunoglobulins supplied by the mother, bind a wide diversity of bacteria. For example, IgA2 and IgM bind more bacteria of the phylum Bacteroidetes in comparison to what IgG binds. Bacteroidetes and some Firmicutes have been reported as late colonizers in the successional population of the neonatal gut since they can produce short-chain fatty acids like propionate and butyrate. Our results support the current view that joint microbial and immunoglobulin transference is fundamental for the normal development of the neonate's immune system and the establishment of a functional gut microbiota.

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CERTIFICATE OF ATTENDANCE

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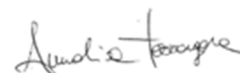## Jaime García Mena

MENA Microbiome: From Biomarkers Discovery to Microbiota-based Therapeutics

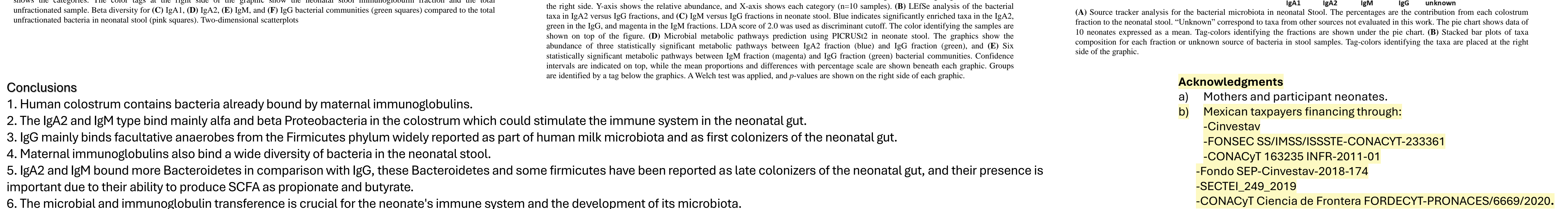**Date:** 28-29 September 2024   **Place:** Doha, Qatar
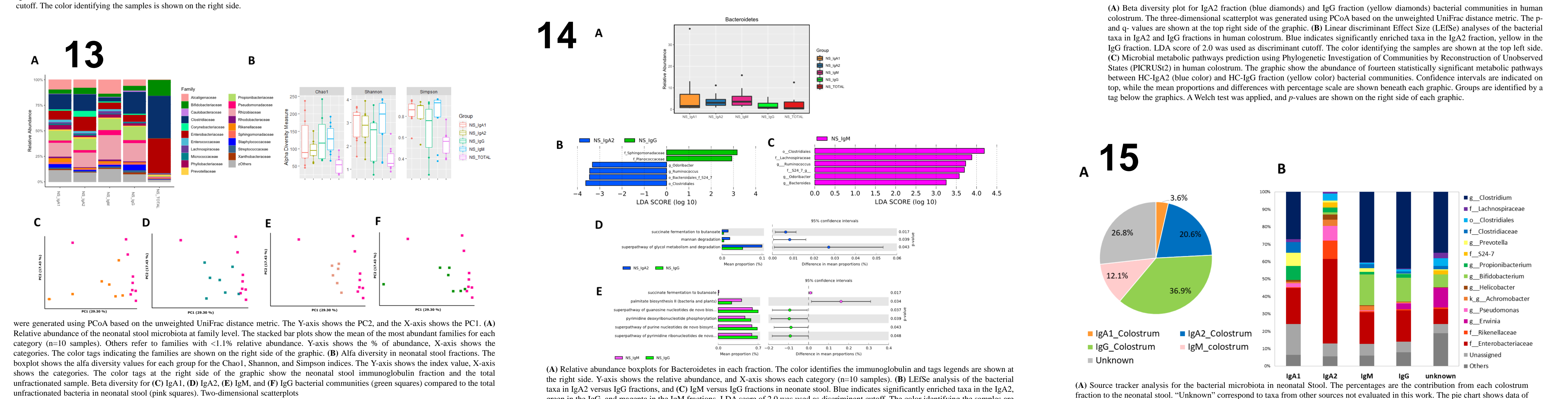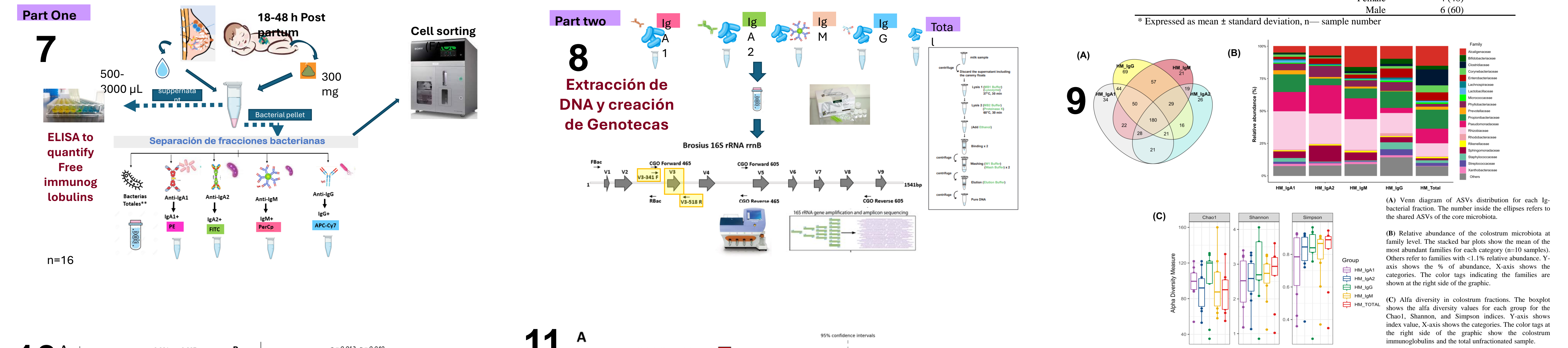
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Maternal immunoglobulins differentially bind the bacterial community in human colostrum and stool of breastfed neonates

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1 The mother perinatally transfers microbiota from different sources to the newborn.

2 One important source of vertical transmission of microbiota is breastfeeding.

3 Important human milk components.

4 ## Our hypothesis

Human colostrum/milk immunoglobulins of the class IgA1, IgA2, IgM, and IgG bound to commensal bacteria, are transmitted through lactation to the newborn to contribute to the establishment and maintenance of functional gut microbiota.

5 Dr José María Rodríguez public hospital (Ecatepec, Edo. México).

Sampling from July 2021-January 2022

Regional Hospital of High Specialty, Ixtapaluca (Ixtapaluca, Edo. México).

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Table 1. Sociodemographic and clinical data of the study population

Maternal Data		n (%)
Age in years*		23.1 ± 4.14
Gestational Age		39 ±1
Birthplace	Ixtapaluca (State-of-Mexico)	3 (30)
	Ecatepec (State-of-Mexico)	7 (70)
Main Activity	Housewife	6 (60)
	Student	2 (20)
	General employee	2 (20)
Parity	Uniparous	4 (40)
	Multiparous	6 (60)
Delivery mode	Vaginal	8 (80)
	C-Section	2 (20)
SARS-CoV-2 vaccine	AstraZeneca/Sputnik/Pfizer	6 (60)
	None	3 (30)
Neonatal Data		**n (%)**
Age in hours		25.88 ± 12.32
Weight (g)		3091.44 ±422.85
Size (cm)		49.44±1.62
Sex	Female	4 (40)
	Male	6 (60)

* Expressed as mean ± standard deviation, n— sample number

7 Part One

18-48 h Post partum — Cell sorting — 500-3000 μL supernatant — 300 mg — Bacterial pellet

ELISA to quantify Free immunoglobulins

Separación de fracciones bacterianas

n=16

8 Part two

Extracción de DNA y creación de Genotecas

Brosius 16S rRNA rrnB

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(A) Venn diagram of ASVs distribution for each Ig-bacterial fraction. The number inside the ellipses refers to the shared ASVs of the core microbiota.

(B) Relative abundance of the colostrum microbiota at family level. The stacked bar plots show the mean of the most abundant families for each category (n=10 samples). Others refer to families with <1.0% relative abundance. Y-axis shows the % of abundance, X-axis shows the categories. The color tags indicating the families are shown at the right side of the graphic.

(C) Alfa diversity in colostrum fractions. The boxplot shows the alfa diversity values for each group for the Chao1, Shannon, and Simpson index. Y-axis shows index value, X-axis shows the categories. The color tags at the right side of the graphic show the colostrum immunoglobulins and the total unfractioned sample.

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Beta diversity plots for (A) IgA2 fraction (blue diamonds) and (B) IgM fraction (green diamonds) bacterial communities compared to the total unfractioned bacteria in human colostrum (red diamonds). Two-dimensional scatterplots were generated using PCoA based on the unweighted UniFrac distance metric. The Y-axis shows the PC2, and the X-axis shows the PC1. The p- and q- values are shown at the right side of the graphics. (C) Relative abundance boxplots for proteobacteria in each fraction. The color identifies the immunoglobulin and tag legends are shown on the right side. The Y-axis shows the relative abundance, and the X-axis see of bacterial class in the colostrum fractions. The stacked bar plots show the mean of the most abundant taxa with relative abundance hows each category (n=10 samples). (D) Relative abundan>1.0% for each category (n=10 samples). Others refer to families with <1.0% relative abundance. Y-axis shows the % of abundance, X-axis shows the categories. The color tags identifying the classes are shown on the right side of the graphic. Linear discriminant Effect Size (LEfSe) analyses of the bacterial taxa in IgA2 (E) and IgM fraction (F), compared to the total unfractionated bacteria in human colostrum. Blue indicates significantly enriched taxa in the IgA2 fraction, green in the IgM fraction, and red indicates taxa enriched in the total unfractionated bacteria. An LDA score of 2.0 was used as a discriminant cutoff. The color identifying the samples is shown on the right side.

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The graphics show the abundance of (A) twelve statistically significant metabolic pathways between HC fraction (red color) and HC-IgA2 fraction (blue color) bacterial communities. (B) Eleven statistically significant metabolic pathways between HC fraction (red color) and HC-IgM fraction (green color) bacterial communities. Confidence intervals are indicated on top, while the mean proportions and differences with percentage scale are shown beneath each graphic. Groups are identified by a tag below the graphics. A Welch test was applied, and p-values are shown on the right side of each graphic.

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(A) Beta diversity plot for IgA2 fraction (blue diamonds) and IgG fraction (yellow diamonds) bacterial communities in human colostrum. The three-dimensional scatterplot was generated using PCoA based on the unweighted UniFrac distance metric. The p- and q- values are shown at the top right side of the graphic. (B) Linear discriminant Effect Size (LEfSe) analyses of the bacterial taxa in IgA2 and IgG fractions in human colostrum. Blue indicates significantly enriched taxa in the IgA2 fraction, yellow in the IgG fraction. LDA score of 2.0 was used as discriminant cutoff. The color identifying the samples are shown at the top left side. (C) Microbial metabolic pathways prediction using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) in human colostrum. The graphic show the abundance of fourteen statistically significant metabolic pathways between HC-IgA2 (blue color) and HC-IgG fraction (yellow color) bacterial communities. Confidence intervals are indicated on top, while the mean proportions and differences with percentage scale are shown beneath each graphic. Groups are identified by a tag below the graphics. A Welch test was applied, and p-values are shown on the right side of each graphic.

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were generated using PCoA based on the unweighted UniFrac distance metric. The Y-axis shows the PC2, and the X-axis shows the PC1. (A) Relative abundance of the neonatal stool microbiota at family level. The stacked bar plots show the mean of the most abundant families for each category (n=10 samples). Others refer to families with <1.1% relative abundance. Y-axis shows the % of abundance, X-axis shows the categories. (B) Alfa diversity in neonatal stool fractions. The Boxplot shows the alfa diversity value for each group for the Chao1, Shannon, and Simpson indices. The Y-axis shows the index value, X-axis shows the categories. The color tags at the right side of the graphic show the neonatal stool immunoglobulin fraction and the total unfractionated sample. Beta diversity plots for (C) IgA1, (D) IgA2, (E) IgM, and (F) IgG bacterial communities (green squares) compared to the total unfractionated bacteria in neonatal stool (pink squares). Two-dimensional scatterplots

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(A) Relative abundance boxplots for Bacteroidetes in each fraction. The color identifies the immunoglobulin and tags legends are shown at the right side. Y-axis shows the relative abundance, and X-axis shows each category (n=10 samples). (B) LEfSe analysis of the bacterial taxa in IgA2 versus IgG fractions in neonate stool. (C) IgM versus IgG fractions in neonate stool. Blue indicates significantly enriched taxa in IgA2, green in the IgG, and magenta in the IgM fractions. LDA score of 2.0 was used as discriminant cutoff. The color identifying the samples are shown on top of the figure. (D) Metabolic pathways predicted using PICRUSt2 in neonate stool. The graphics show the abundance of three statistically significant metabolic pathways between IgA2 fraction (blue) and IgG fraction (green), and (E) Six statistically significant metabolic pathways between IgM fraction (magenta) and IgG fraction (green) bacterial communities. Confidence intervals are indicated on top, while the mean proportions and differences with percentage scale are shown beneath each graphic. Groups are identified by a tag below the graphics. A Welch test was applied, and p-values are shown on the right side of each graphic.

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(A) Source tracker analysis for the bacterial microbiota in neonatal Stool. The percentages are the contribution from colostrum fraction to the neonatal stool. "Unknown" correspond to taxa from other sources not evaluated in this work. The pie chart shows data of 10 neonates expressed as a mean. Tag-colors identifying the fractions are shown under the pie chart. (B) Stacked bar plots of taxa composition for each fraction or unknown source of bacteria in stool samples. Tag-colors identifying the taxa are placed at the right side of the graphic.

Conclusions

1. Human colostrum contains bacteria already bound by maternal immunoglobulins.
2. The IgA2 and IgM type bind mainly and beta Proteobacteria in the colostrum which could stimulate the immune system in the neonatal gut.
3. IgG mainly binds facultative anaerobes from the Firmicutes phylum widely reported as part of human milk microbiota and as first colonizers of the neonatal gut.
4. Maternal immunoglobulins also bind a wide diversity of bacteria in the neonatal stool.
5. IgA2 and IgM bound more Bacteroidetes in comparison with IgG, these Bacteroidetes and some firmicutes have been reported as late colonizers of the neonatal gut, and their presence is important due to their ability to produce SCFA as propionate and butyrate.
6. The microbial and immunoglobulin transference is crucial for the neonate's immune system and the development of its microbiota.