The Role of the Metagenome and Microbiome During Pregnancy and Lactation on the Risk of Immune-Related Disease

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Baylor College of Medicine, Houston, Texas
No disclosures of conflict nor appearance of conflict.
Objectives

DOHAD KEY CONCEPTS

METAGENOMICS SCIENCE

TRANSLATIONAL IMPLICATIONS

KNOWLEDGE GAPS
The Breadth of Mammalian Adaptations to Maternal Exposures in Placentation, Gestation, and Lactation is Remarkable

- Marsupials
- Eutherians

- Conception
- Initial placentation
- Birth
- Lactation 1
- Lactation 2
- Lactation 3
- Placentation & gestation
- Weaning & nutritional independence
How Does Maternal-Fetal Communication Enable Adaptation and Fitness in a Changing World?

**Peripartum**
- Maternal & paternal genomic variation
  - Nuclear DNA (SNPs/CNVs)
  - Mitochondrial DNA (heteroplasmy)
- Placental genome
  - Confined placental mosaicism
- Perinatal epigenome
  - Histone variants (fetal & placental)
  - Methylation (entirely rewritten)
  - miRNA/ncRNA (placental)
  - Environmental chemicals & tobacco exposure (fetal & placental)
  - Maternal metabolic disease
- Perinatal metagenome
  - Placental microbiome
  - Maternal microbiome
  - Preterm vs term delivery
  - Mode of delivery: weak modifier

**Infancy & Childhood**
- Developmental epigenome
  - Stable histone variants, refractory methylation
- Developmental metagenome
  - Breastfeeding & diet, NICU, antibiotics, disease, immune modulation, early adolescent exposures

**Adulthood**
- Acquired genomic variants
  - Rare: Nuclear DNA; mtDNA (acquired heteroplasmy)
- Acquired metagenomes
  - More common: diet, aging, medications, disease, immune modulation, reproductive course

The *in utero* environment shapes our metabolic, immune & behavioral heritability in unexpected & creative ways.

Tip of the Iceberg

Environmental Chemicals, the Human Microbiome, and Health Risk

Microbes and their functional metabolites & small molecules

Susceptibility to environmental chemicals & "stress"

Clinically evident disease

Life Stage & Population Variation and Variability

Aagaard, Stewart & Chu *EMBO* (2016)
Fundamental Paradox in Development:
If the womb is sterile or the placenta is a barrier and not a communicator, how do we tolerate commensal microbes requisite to immunity and adapt to an ever-changing world?

- Simply active or passive transfer of microbial antigens or metabolites (would not explain lifelong changes and lasting impact)

- Intrauterine colonization?
  (available & present maternal microbes colonizing the fetus during pregnancy)

- Immune education, enabling differential postnatal tolerance of commensal microbes?
  (maternal exposures alter the intrauterine metabolic milieu, enabling tolerance to niche microbes resulting in early postnatal colonization)

- Colonization resistance & community resilience?
  (be it through host immunity or microbe-microbe interactions, the presence of a few key microbes in the fetus/neonate prohibits colonization by others—pathogen or beneficial commensal)

Are there a few vignettes that might help us understand potential mechanisms conveying the view from the womb?
The Female Upper Reproductive Tract is Not Sterile and Endometrial Microbial Community Fitness Affects Implantation


the placenta implants into the maternal uterine decidua (with its own functional immune repertoire)
Vignette 1: Maternal Microbial Ecology During Pregnancy

Lessons in vaginal microbial (re)colonization & community ecology

Early Observations: Pregnancy Structures the Vaginal Microbiome to be Less Rich and Less Diverse at Delivery

How does the vaginal microbiome repopulate? Could this provide clues on community establishment and pregnancy outcomes?

HMP Companion Manuscript--
It Took Us Another 10 Years to Understand How Pregnancy Structures the Vaginal Community Ecology

Vaginal diversity & richness is modulated during pregnancy across all subsites by gestational age

Pace et. al., Cell Med 2021.

<table>
<thead>
<tr>
<th>PERMANOVA</th>
<th>p</th>
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<tr>
<td>Site</td>
<td>0.781</td>
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<tr>
<td>3rd Trimester/Site</td>
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<tr>
<td>Delivery/Site</td>
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<tr>
<td>Postpartum/Site</td>
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<tr>
<td>Time</td>
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<tr>
<td>Vaginal introitus/Time</td>
<td>0.005</td>
</tr>
<tr>
<td>Posterior fornix/Time</td>
<td>0.009</td>
</tr>
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Maternal Communities Are Dynamic: In the Vagina, Community Stability Is Reached by Co-exclusion (Remarkable Restoration Post Pregnancy Occurs)
Keystone Species Are Present as Multiple Strains

A

G. vaginalis

L. iners

L. crispatus

L. jensenii

B

<table>
<thead>
<tr>
<th>G. vaginalis</th>
<th>Gv1</th>
<th>Gv1a</th>
<th>Gv1b</th>
<th>Gv2</th>
<th>Gv2a</th>
<th>Gv2b</th>
<th>Gv3</th>
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<tbody>
<tr>
<td>L. crispatus</td>
<td>Lc1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. iners</td>
<td>Li1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. jensenii</td>
<td>Lj1</td>
<td></td>
<td></td>
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</tbody>
</table>

Yellow: Negative  
Orange: Random  
Blue: Positive
Probabilistic Modeling of Species Co-occurrence: *Lactobacillus* spp Are Relatively Exclusionary in the Vagina during Pregnancy

Lactobacilli, particularly *L. crispatus*, also excludes pathobionts such as Group B Strep.

Vignette 1 take away message: pregnancy imparts a unique and dynamic vaginal community ecology.
Vignette 2: Are there Concrete Examples of Fetal Immune Modulation with Maternal Exposures?
What Evidence for Active Fetal Immune Development *In Utero*?

- Fetal immune system is present prior to mid-gestation
- In fetal specimens, microbes present in mid-gestation, trigger activation and tolerance**

Mid-gestation fetal samples from ongoing pregnancies

Fetal T Cell Epitopes Map to Known Antigens

TRA CDR3 Amino Acid Sequence

Pathology
- 0.05% Allergies
- 0.81% Autoimmunity
- 98.61% Pathogen
- 0.53% Cancer

Total=2093

TRB CDR3 Amino Acid Sequence

Pathology
- 5.36% Autoimmunity
- 87.50% Pathogen
- 7.14% Cancer

Total=56

McPAS-TCR epitope database
Unprecedented Resolution of Antigen-Specific Fetal Immune Development

scRNA-seq of 22,058 cells from paired CSF and AF (n=14 subjects)

1,210 T cells subset and analyzed by pseudotime trajectory analysis
Expansion of Shared Clonotypes in Amniotic Fluid and Fetal CSF

Additional 3 subjects 5’ scRNA-seq + hTCR V(D)J sequencing
Increased Dual-Expansion of T cells with Maternal SARS-CoV-2 Infection or Vaccination during Pregnancy
Term Placental Atlas with Single-Cell and Spatial Resolution

291,871 transcriptomes
13 placentae = 4 control and 9 mSARS-CoV-2+
Evidence for Acquired Immune Tolerance in Placenta

Sparse and highly positive SARS-CoV-2 niches

Differential macrophage polarization and histiocytic intervillositis with highly positive placentae
Vignette 3: Maternal Exposures and Her Infant’s Microbiome

Maternal diet, gestational age and milk feeding are likely key in establishing the early primate microbiome.
Trends in Molecular Medicine

Opinion

Crucial nuances in understanding (mis)associations between the neonatal microbiome and Cesarean delivery

Alexa M. Sassis, Grace J. Johnson, Allison N. Goulding, and Hyemt M. Aggarwal

As ratios of Cesarean delivery and common non-communicable disorders (NCDs), such as obesity, metabolic disease, and autoimmunities, have concurrently increased in recent decades, investigations have attempted to discern a causal link. One line of research has led to a hypothesis that Cesarean birth disrupts the preserved normal process of colonization of the neonatal microbiome with vaginal microbes, existing NCDs later in life. However, a direct link between a disrupted microbiota transfer at time of delivery and acute and/or chronic illness in infants born via Cesarean has not been causally established. Microbiota seeding from maternal vaginal or stool sources has been preliminarily evaluated as an interventional design to compensate for the lack of (or limited) exposure to such sources among Cesarean-delivered neonates. However, to date, clinical trials have yet to show a clear health benefit with neonatal vaginal seeding practices. Until the long-term effects of these microbiota alterations can be fully determined, it is paramount to conduct parallel meaningful and mechanistic-minded interrogations of the impact of clinically modifiable material, nutritional, or environmental exposure on the functional microbiome over the duration of pregnancy and lactation to determine their role in the mitigation of childhood and adult NCDs.

The Developmental Origins of Health and Disease

The Developmental Origins of Health and Disease (DOHaD) hypothesis[1] envisions a substantial body of evidence that the temporality and functionally fine-tuned exposure to adverse outcomes in utero shaping larger NCDs. Given a variety of obesity, metabolic diseases, cardiovascular diseases, and behavioral conditions, DOHaD hypothesis-driven studies in animal models demonstrated that maternal nutrition deprivation or high-fat diet feeding during pregnancy may influence and even cause adverse outcomes in the offspring. The weight of evidence suggests a mechanism-driven view of the DOHaD theory, which emphasizes the maternal and fetal environmental factors that influence the development and expression of NCDs. This suggests that, in addition to maternal exposure, genetic factors also contribute to NCDs. The effects of maternal nutrition, environmental factors, and even the time of birth on the development of NCDs have been studied extensively.

One of the key factors that may influence the development of NCDs is the microbiome. The gut microbiome is known to play a crucial role in the development of the immune system and in the regulation of various physiological processes. The gut microbiome is established during the first few months of life and can be influenced by various factors, including the mode of delivery. Cesarean delivery, which is associated with a disrupted maternal-fetal microbial transfer, may alter the colonization of the gut microbiome in the newborn, leading to an increased risk of NCDs later in life.

Figure 2. Maternal and environmental factors that can affect the development of the infant microbiome. Several maternal and environmental factors have been shown to have varying degrees of impact on the development of the neonatal, infant, and/or early childhood microbiome. Current evidence supports the notion that gestational age at delivery, environmental chemical exposures, perinatal antibiotic exposure, perturbations in the intrauterine environment, maternal and infant feeding practices, and/or maternal diet all significantly impact the neonatal microbiome. Additionally, many factors are not independent and can be additive or have a cumulative effect (i.e., maternal diet can influence gestational weight gain, which, in turn, can affect the breast milk microbiome and subsequently the neonatal microbiome).
Gut phyla in the 1st week of life

- **Firmicutes** (10-20%)
  - *Enterococcus, Clostridium, Lactobacillus, Staphylococcus, Streptococcus*
- **Bacteroidetes** (10-20%)
  - *Bacteroides*
- **Proteobacteria** (20%)
  - *Escherichia/Shigella, Klebsiella*
- **Actinobacteria** (50%)
  - *Bifidobacterium*, *Propionibacterium*


It is increasingly unclear how much of a role vaginal microbes play in strain-by-strain colonizing the infant gut....

(this is actually where our journey interrogating other sources of the infant microbiome began)
Observations from a Large Population-Based Prospective, Longitudinal Cohort

The Neonatal Microbial Community is Present but Relatively Homogenous at Birth
By 6 Weeks, the Infant Community Membership Has Expanded and Differentiated

Microbiome Expansion & Diversification

- Neonate: 78.8 taxa; 6,577 metagenomes
- Infant: 391 taxa; 66,147 metagenomes
- Maternal: 716 taxa; 384,290 metagenomes
What Influences this Community Expansion? Mom’s Diet and Formula Feeding (but not Cesarean) Matter in Linear and Mixed Effect Models

Infant Stool – 6 Weeks
Orthogonal approaches: maternal diet as a principal driver is faithfully recapitulated in our primate models.

Ma et al., Nature Communications (2014); Chu et al., Genome Medicine (2016); Pace et al., BMC Microbiology (2018); Prince et al., AJP (2019); Batterjee et al, in preparation (2022); Chu and Rochat et al, in preparation (2022); Bolte et al, in preparation (2022).

Vignette 3 take home message: the infant microbiome community structure & function is modulated by several key factors in gestation & lactation

Are there common in utero & ex utero metabolites?

Infant Stool – 6 Weeks
Vignette 3: Are there common microbial metabolic threads linking immune modulation and the microbiome?

Seferovic, Mahmoud, Engevik, Pace et al., Sci Reports (2020); Jochum & Seferovic, in preparation (2023).
Study Design: Paired Cross-Over with Defined Dietary Interventions (each person is their own control)

Glu/Gal Cohort \((n = 7)\)

- **Glucose Diet**: 1-2 week washout \(\leq 57h\)
- **Galactose Diet**: 1-2 week washout \(\leq 57h\)

Galactose diet glucose diet

no difference in fat, protein, lactose, or caloric content of breast milk

Carb/Fat Cohort \((n = 7)\)

- **High Fat Diet**: 7 days
- **Carbohydrate Diet**: 7 days
- **Carbohydrate Diet**: 1-2 week washout
- **High Fat Diet**: 1-2 week washout

Carbohydrate diet high fat diet

fat caloric content

Maternal Diet Alters Composition of Human Milk Oligosaccharides (HMOs)

- Indigestible by the infant
- Digested by bacteria, favoring proliferation of beneficial bacteria in the infant gut
- HMOs are immunomodulatory
Maternal Diet Alters HMO-bound Fucose, which Correlates with Abundance of Bacterial Fucosidase

HMO-bound Fucose

Glucose  Galactose

\[ p = 0.031 \]

Fucosidase

Glucose  Galactose

\[ p = 0.030 \]

Log-fold Increase in Fucosidase Inferred Relative Abundance

\[ R = 0.88 \]

\[ p = 0.048 \]
In vitro Mechanistic Evidence: HMOs (fucosylated) Enable Enhanced Growth of *Streptococcus mitis* and *oralis*

If HMOs are such great commensal bacterial substrates, why are they only found in milk? Or are they?

Maternal Diet Drives HMO Production & Selective Microbial Growth
Ethnically diverse group of older gravidae mostly presenting for genetic amniocentesis for advanced maternal age.

<table>
<thead>
<tr>
<th>Maternal Age (years)</th>
<th>Cohort (n 731)</th>
<th>U.S. National Averages</th>
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<tbody>
<tr>
<td></td>
<td>34.3 ± 5.4 (17 to 44)</td>
<td>25.6</td>
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</table>

### Ethnicity

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Cohort (n 731)</th>
<th>U.S. National Averages</th>
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<tbody>
<tr>
<td>Asian</td>
<td>227 (31.1%)</td>
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<tr>
<td>Hispanic</td>
<td>139 (19.1%)</td>
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</tr>
<tr>
<td>African American</td>
<td>144 (19.8%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>219 (30.0%)</td>
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<table>
<thead>
<tr>
<th>Nulliparous</th>
<th>Cohort (n 731)</th>
<th>U.S. National Averages</th>
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<tr>
<td>Nulliparous</td>
<td>200 (27.4%)</td>
<td>~40%</td>
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<th>History of PTB</th>
<th>Cohort (n 731)</th>
<th>U.S. National Averages</th>
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</thead>
<tbody>
<tr>
<td>History of PTB</td>
<td>78/729 (10.7%)</td>
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<tr>
<th>Preterm Delivery</th>
<th>Cohort (n 731)</th>
<th>U.S. National Averages</th>
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</thead>
<tbody>
<tr>
<td>Preterm Delivery</td>
<td>92/729 (12.6%)</td>
<td>9.6%</td>
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<table>
<thead>
<tr>
<th>Gestational Age at Sampling (weeks)</th>
<th>Cohort (n 731)</th>
<th>U.S. National Averages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age at Sampling (weeks)</td>
<td>17.5 ± 1.9 (14 to 29)</td>
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</table>

### Amniocentesis Indication

<table>
<thead>
<tr>
<th>Amniocentesis Indication</th>
<th>Cohort (n 731)</th>
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<tbody>
<tr>
<td>Advanced Maternal Age (AMA)</td>
<td>389</td>
</tr>
<tr>
<td>+Mat. Serum Screen (+MSS)</td>
<td>218</td>
</tr>
<tr>
<td>Abnormal Ultrasound</td>
<td>40</td>
</tr>
<tr>
<td>Multiples</td>
<td>82</td>
</tr>
</tbody>
</table>

*No signs of intraamniotic infection*

Undertook Discovery Metabolomics in a Large Prospective Cohort with Mid-Trimester Amniotic Fluid Samples

Putative 2’-Fucosyllactose “HMO” Discovered in Unbiased GC-MS Mid-Trimester Amniocentesis

- Unusual compound $m/z$ 511.164 Da (i.e. $\text{C}_{18}\text{H}_{32}\text{O}_{15}\text{Na}^+$)
- MS/MS fragmentation confirms trisaccharide
- Metlin database searches correspond to fucosyllactose variants
- Purified standard assessed in parallel confirms retention time & $m/z$

\[ \text{2-Fucosyllactose} \]

\[ \text{Representative Amniotic Fluid} \]

\[ \text{Fucosyllactose} \]

\[ \text{2-Fucosyllactose Pure Standard} \]
MRM Assay for Targeted Oligosaccharide Broad Profiling and “HMO” Validation in Amniotic Fluid from Ongoing Pregnancies

37 Oligosaccharides Identified

Confirmed Major HMO Variants Near Universally Represented

- LNH
- LNT & LNnT
- 6'SL

Number of samples vs [HMO] ng/mL

- 3'SL
- 2'FL
- LDFT

Oligosaccharides: 8_2_1_2_a, 7_2_1_2_a, 2_4_0_0_a, 2_2_0_1_a, 3_2_2_1_a, 4_0_0_0_a, 1_1_0_1.6'SL, 4_2_0_1_a, 5_3_0_0_a, 7_0_0_0_a, 1_1_0_1.3'SL, 2_0_0_1.3'SL, 5_3_0_1_a, 7_0_0_0_b, 6_0_0_0_a, 4_2_0_1_b, 5_3_0_0_b, 4_0_0_0_a, 5_4_0_1_a, 4_2_0_0_LNH, 3_1_0_0_LNT & LNnT, 4_2_0_0_b, 2_0_0_1.6'SL, 3_1_1_0_LNFP II & I, 3_0_0_0_c, 3_0_0_0_d, 2_0_1_0_2FL, 4_1_0_0_a, 3_0_0_0_b, 2_1_2_0_a, 2_0_2_0_LDFT, 3_1_1_0_LNFP II, 2_1_0_0_a, 2_1_0_0_b, 3_0_0_0_a, 3_0_1_0_a, 2_0_1_0_3'FL
Least Squares Multivariate Analysis Suggests Minimal Associations with Maternal Characteristics, Except for Blood Group (Secretor Status)

<table>
<thead>
<tr>
<th>Least Squares Multivariate Analysis</th>
<th>Summary Statistics</th>
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<tbody>
<tr>
<td><strong>β0</strong> Intercept</td>
<td>0.254 0.800</td>
</tr>
<tr>
<td><strong>β1</strong> PTD</td>
<td>0.638 0.524</td>
</tr>
<tr>
<td><strong>β2</strong> Week Amniocentesis</td>
<td>1.588 0.113</td>
</tr>
<tr>
<td><strong>β3</strong> Maternal Age</td>
<td>1.531 0.127</td>
</tr>
<tr>
<td><strong>β4</strong> Asian [ref White]</td>
<td>1.602 0.110</td>
</tr>
<tr>
<td><strong>β5</strong> Hispanic [ref White]</td>
<td>3.127 0.002</td>
</tr>
<tr>
<td><strong>β6</strong> Black [ref White]</td>
<td>0.401 0.689</td>
</tr>
<tr>
<td><strong>β7</strong> nulliparity</td>
<td>0.174 0.862</td>
</tr>
<tr>
<td><strong>β8</strong> hx PTD</td>
<td>2.004 0.046</td>
</tr>
<tr>
<td><strong>β9</strong> Positive screening test [ref AMA]</td>
<td>1.072 0.285</td>
</tr>
<tr>
<td><strong>β10</strong> Abnormal ultrasound [ref AMA]</td>
<td>0.536 0.592</td>
</tr>
<tr>
<td><strong>β11</strong> AMA &amp; positive screening test [ref AMA]</td>
<td>1.159 0.247</td>
</tr>
<tr>
<td><strong>β12</strong> Spontaneous PTB</td>
<td>0.368 0.713</td>
</tr>
<tr>
<td><strong>β13</strong> Indicated PTB</td>
<td>0.719 0.473</td>
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However, there is a Significant Association with Advancing Gestational Age for the Abundant “HMOs”

<table>
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<tr>
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<th>2FL</th>
<th>LDFT</th>
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<tr>
<td>β0 Intercept</td>
<td>2.242 0.026</td>
<td>3.333 0.001</td>
</tr>
<tr>
<td>β1 PTD</td>
<td>1.019 0.309</td>
<td>0.237 0.813</td>
</tr>
<tr>
<td>β2 Week Amniocentesis</td>
<td>3.018 0.003</td>
<td>4.242 &lt;0.0001</td>
</tr>
<tr>
<td>β3 Maternal Age</td>
<td>0.295 0.768</td>
<td>0.656 0.512</td>
</tr>
<tr>
<td>β4 Asian [ref White]</td>
<td>0.170 0.865</td>
<td>0.972 0.332</td>
</tr>
<tr>
<td>β5 Hispanic [ref White]</td>
<td>0.163 0.871</td>
<td>1.325 0.186</td>
</tr>
<tr>
<td>β6 Black [ref White]</td>
<td>0.942 0.347</td>
<td>0.541 0.589</td>
</tr>
<tr>
<td>β7 nulliparity</td>
<td>1.131 0.259</td>
<td>0.082 0.935</td>
</tr>
<tr>
<td>β8 hx PTD</td>
<td>1.435 0.152</td>
<td>0.083 0.934</td>
</tr>
<tr>
<td>β9 Positive screening test [ref AMA]</td>
<td>0.086 0.932</td>
<td>0.364 0.716</td>
</tr>
<tr>
<td>β10 Abnormal ultrasound [ref AMA]</td>
<td>1.243 0.215</td>
<td>1.087 0.278</td>
</tr>
<tr>
<td>β11 AMA &amp; positive screening test [ref AMA]</td>
<td>1.007 0.315</td>
<td>1.282 0.201</td>
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<tr>
<td>β12 Spontaneous PTB</td>
<td>1.058 0.291</td>
<td>1.088 0.277</td>
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<tr>
<td>β13 Indicated PTB</td>
<td>0.488 0.626</td>
<td>0.157 0.876</td>
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Spearman’s
p=0.0000007
r=0.28
Metagenomic-Detected Microbes after Removing Putative Contaminants
(These are “sterile” pregnancies that continued for another 24 weeks or so)

- Eight kit contamination control blanks extracted and sequenced in parallel
- From a total of 8861 discrete taxa identified with shotgun (WGS) metagenomics:
  - 7919 taxa could be confidently identified at a probability threshold of 0.5
  - ~84.1% of amniotic fluid samples were retained by a threshold of at least 2 non-contamination reads.
- Median of ~16,647 reads for those retained
Whole Genome Metagenomic Sequencing of Amniotic Fluid Reveals Sparse and Decontaminated Bacterial Reads Associate with Lower Abundance HMOs

Minimal correlation between log-filtered metagenomics reads to most abundant HMOs

Number of filtered bacterial reads in amniotic fluid correlates with low abundance [HMO]

Pearson’s $r = 0.20$  
$P=0.004$

Pearson’s $r = 0.12$  
$P=0.08$

Pearson’s $r = 0.15$  
$P=0.03$
Lactobacillus Metagenomes Characteristic of Infant Gut and Maternal Microbiomes Could Be Entirely Reconstructed in Mid-Trimester Amniotic Fluid Samples with Multiple ASV Hits

<table>
<thead>
<tr>
<th>Rank</th>
<th>Species</th>
<th>Total reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactobacillus crispatus</td>
<td>33289</td>
</tr>
<tr>
<td>2</td>
<td>Lactobacillus iners</td>
<td>26581</td>
</tr>
<tr>
<td>3</td>
<td>Lactobacillus delbrueckii</td>
<td>4981</td>
</tr>
<tr>
<td>4</td>
<td>Lactobacillus jensenii</td>
<td>2187</td>
</tr>
<tr>
<td>5</td>
<td>Lactobacillus gasseri</td>
<td>1679</td>
</tr>
</tbody>
</table>
Striking Similarities between the Amniotic Fluid Taxa at 17-22 Weeks Gestation and the Neonate at Delivery

Vignette 4 take-away: Coming back full circle to our xylitol trial....microbial substrates are potent modulators during pregnancy & lactation

Present in neonate gut microbiome
Take Home Message: Early Developmental Communities Appear to be Sparse, Low Abundance and Low Biomass but Functional

Key developmental niches with molecular, cultivation & histology data supporting the presence of a low biomass, low abundance community with remarkable taxonomic & functional overlap

- Placenta (parenchyma, villous tree; varies by preterm & term, with maternal antenatal infections)
  
  *Fusobacterium, Bacteroides, Lactobacillus, Staphylococcus, Streptococcus, Proteobacteria, Actinobacteria (Propionobacterium & Bifido)*

- Amnion & chorion membranes (preterm & term variation)
  
  *Fusobacterium, Bacteroides, Lactobacillus, Staphylococcus, Streptococcus, Proteobacteria, Actinobacteria, Atypicals (mycoplasma & ureaplasma)*

- Amniotic fluid (preterm & term variation)
  
  *Fusobacterium, Bacteroides, Lactobacillus, Streptococcus, Proteobacteria, Actinobacteria (Bifido)*

- Meconium (preterm & term variation, varies with multiple morbidities)
  
  *Fusobacterium, Bacteroides, Lactobacillus, Staphylococcus, Streptococcus, Proteobacteria, Actinobacteria (Bifido & Propionobacterium)*

- Milk (preterm & term variation, varies by maternal diet & comorbidities)
  
  *Streptococcus, Bacteroides, Lactobacillus, Staphylococcus, Actinobacteria*

(and we have always explicitly stated **low biomass & sparse**, but whether are live & colonizing remains to be determined)
Where Does this Leave Us in Resolving the Developmental Paradox?

Using vignettes from randomized controlled trials to discovery metabolomics, several intriguing and consistent messages emerge:

- While the metabolites themselves are not durable, their role in microbiome fitness likely is (finding which ones govern microbial niches is a key next step)

- **Intrauterine colonization**—uncertain & I remain agnostic (cultivatable & visualized microbes with demonstrable functional roles are detected in 2\(^{nd}\) trimester and beyond—but do they truly colonize the fetus?)

- **Immune education, enabling differential postnatal tolerance of commensal microbes**—more certain (maternal diet alters the metabolic milieu via HMOs, enabling tolerance to niche microbes to live and prosper early on in development; functional evidence emerging)

- **Colonization resistance**—*L. crispatus* & Group B strep exclusion as one example (be it through host immunity or microbe-microbe interactions, the presence of a few key microbes prohibits colonization by others—pathogen or beneficial commensal AND there is a highly predictable pattern to functional community restoration & fitness)

Let’s reframe the discussion away from “sterile” to “womb with a view”
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