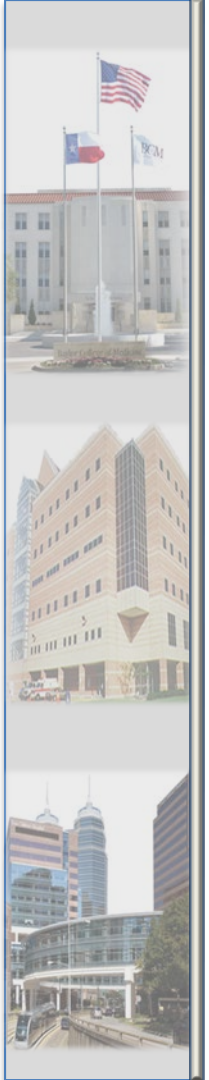


Maternal Diet During Pregnancy & Lactation and the Interaction with the (Developing) Infant Microbiome

Kjersti Aagaard, MD PhD MSCI
Henry & Susan E. Meyer Endowed Chair
Professor & Vice Chair
Department of Obstetrics & Gynecology,
Division of Maternal-Fetal Medicine,
&
Departments of Molecular & Cell Biology, Molecular Physiology & Biophysics,
Molecular & Human Genetics
HGSC Bioinformatics Research Lab,
National School for Tropical Medicine, and the
Centers for Reproductive Medicine and
Center for Metagenomics & Microbiome Research

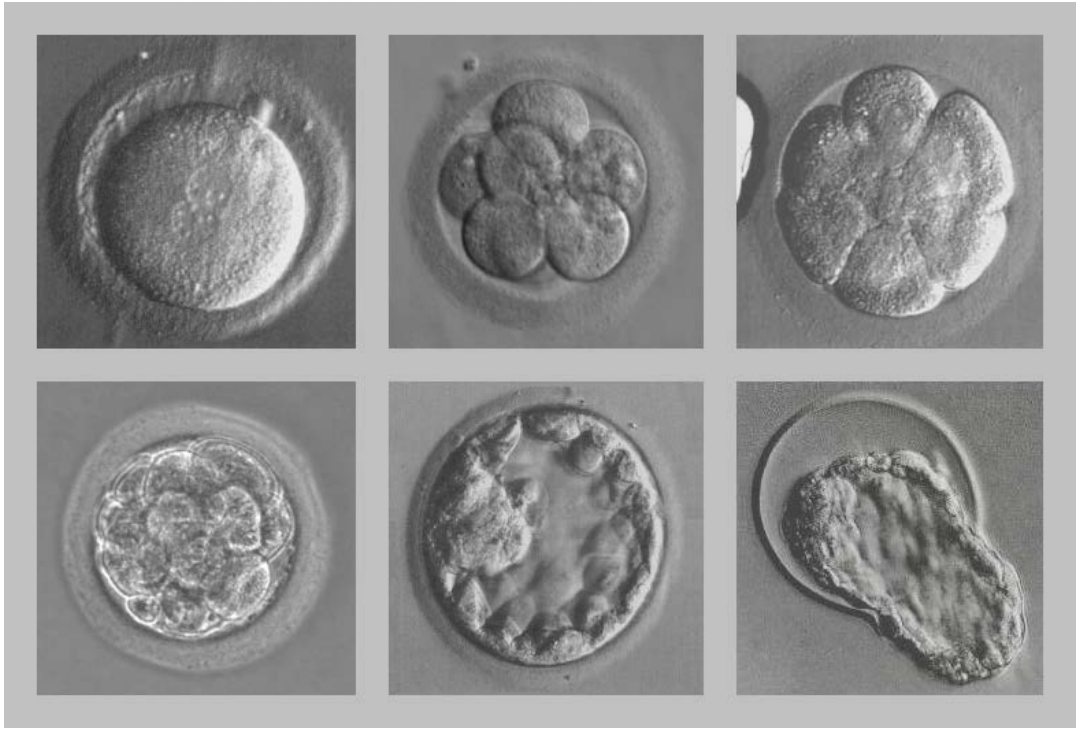
Baylor College of Medicine, Houston, Texas.

Texas Children's Hospital

Baylor College of Medicine

HARRIS HEALTH SYSTEM

Metabolic Heredity is Programmed in Fetal & Early Infant Life (DoHAD Hypothesis)



The Central Dogma of Human Development

Genomic Variation & Epigenomics Only Partially Explains Metabolic Programming: Role of the microbiome?

Peripartum

- **Maternal & paternal genomic variation**
  - Nuclear DNA (SNPs/CNVs)
  - Mitochondrial DNA (heteroplasmy)
- **Placental genome**
  - Confined placental mosaicism
- **Perinatal epigenome**
  - Histone variants (fetal & placental)
  - Methylome (entirely rewritten)
  - miRNA/ncRNA (placental)
  - Maternal diet & nutrition
  - 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 methylome
- **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 heritability in unexpected ways.

Aagaard-Tillery & Jelinek, *J Immunol* & *JCI* (1994,1994,1995,1996,1997), Aagaard-T *et al, Obstet Gynecol* (2005); Aagaard-T *et al, Obstet Gynecol* (2006); Aagaard-T *et al, Fetal Diagn Ther* (2006); Aagaard-T *et al, AJOG* (2006); Aagaard-T *et al, AJOG* (2006); Aagaard-T *et al, J Mol Endocrin* (2008); McCurdy *et al, J Clin Invest* (2009); Cox *et al, Am J Obstet Gynecol* (2009); Turgeon *et al, PNAS* (2009); Suter *et al, Metabolism* (2010); Aagaard-T *et al, Obstet Gynecol* (2010); Harris *et al Prenat Diagn* (2010); Abramavici *et al, Ped Endo* (2010); Suter *et al, FASEB* (2011); Aagaard*et al, Ann Reproduction* (2011); Suter *et al., Epigenetics* (2011); Aagaard *et al.*, *PNAS* (2012); Aagaard *et. al., PLoS One* (2012); Munch *et. al., PLoS One* (2012); Aagaard *et al., FASEB J* (2012); Riehle *et al., BMC Bioinformatics* (2012): Suter *et al., FASEB* (2012); Suter *et al., FASEB* (2013); Suter *et al.,* FASEB (2013); Suter *et al., Mol Endo* (2013) O'Neil *et al, Mol Genet Met* (2013); The Marmoset Consortium, *Nature Genetics* (2014); Harris et al., *PNAS* (2014); Goodspeed *et al., FASEB* (2014); Seferovic *et al., FASEB* (2015) Ma *et al, Nature Comm* (2014); Ma *et al.*, *BMC Genomics* (2014); Aagaard *et al, Science TM* (2014); Suter *et al, AJOG* (2014); Racusin *et al, Endocrine* (2014); Antony *et al, AJOG* (2015); Racusin *et al, AJOG* (2015); Cuevas-Guamn *et al, Peds Res* (2015); Li *et al, Epigenetics* (2015); Kahr *et al, AJOG* (2016); Pew *et al., AJOG* (2016); Gonzalez-Rodriguez *et al, AJOG* (2016); Chu *et al., Nature Medicine* (2017); Harris *et al*, *Nature Sci Rep* (2016); Aagaard et al, *et al*, *Nature Sci Rep* (2016); Human Twin Consortium, *Am J Hum Genet* (2016); Kappil *et al, Environ Epigenet* (2016); England *et al., Environ Health* (2016); McCurdy *et al, JCI* (2017); O'Neil *et al, AJOG* (2017); Labus *et al, Microbiome* (2017); Pace *et al BMC Microbiol* (2018); Pheiffer *et al, Exp Clin Endorcinol* (2018); Seferovic *et al AJPEM* (2018); Wesolowski *et al, Mol Metab* (2018); Elsakr *et al, Mol Metab* (2019); Prince *et al* AJP (2019); Seferovic *et al, AJOG* (2019); Suter et al, EJHI (2019).

There Are Several Ways the Maternal Diet During Pregnancy & Lactation could Influence the Developing Microbiome

- **Intrauterine colonization**

(available & present maternal microbes colonizing the fetus)

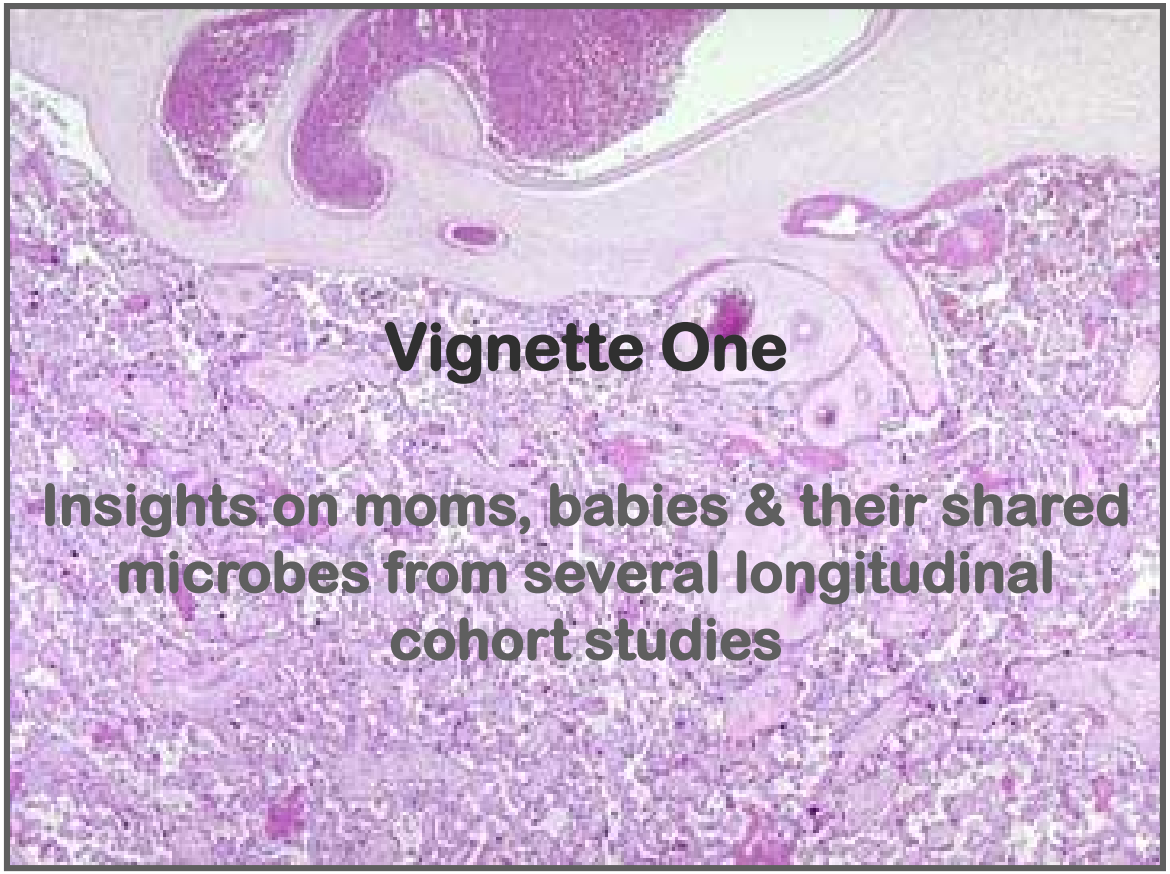
- **Immune education, enabling differential postnatal tolerance of commensal microbes**

(maternal diet alters the metabolic milieu, enabling tolerance to niche microbes to live and prosper early on in development)

- **Colonization resistance**

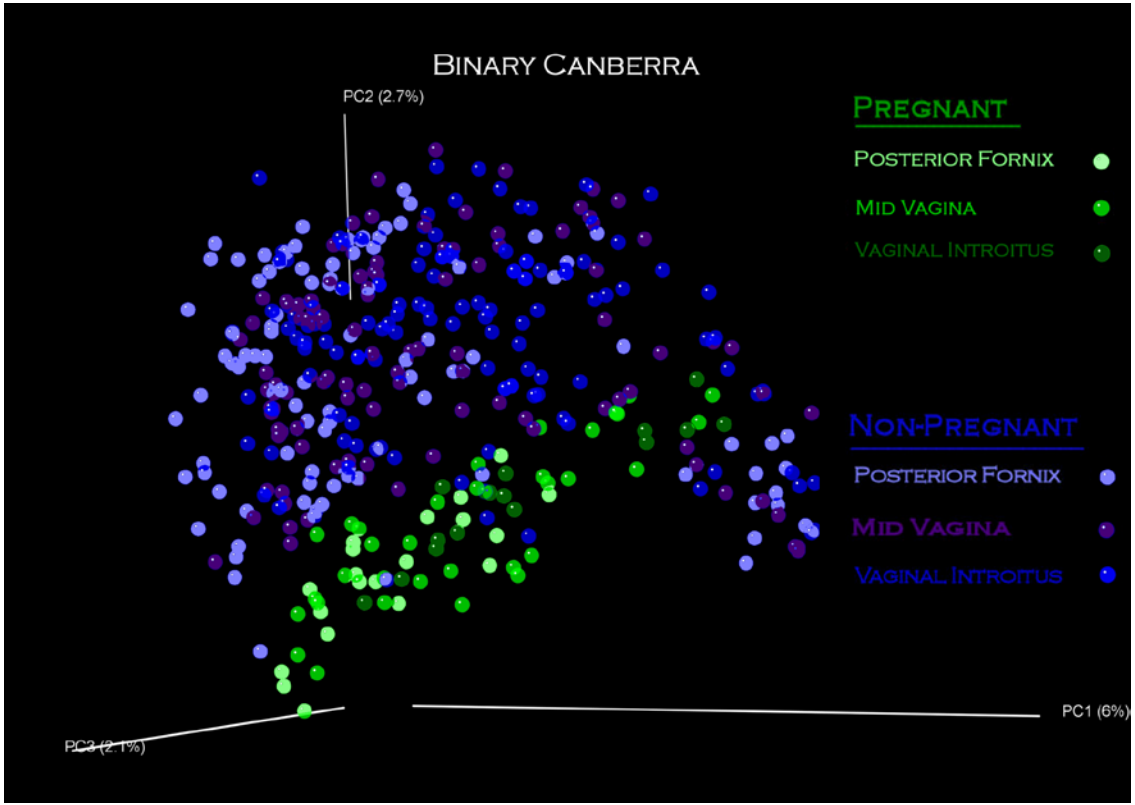
(be it through host immunity or microbe-microbe interactions, the presence of a few key microbes in the fetus/neonate prohibits colonization by others—pathogen or beneficial commensal)

Three Vignettes Which Illustrate Certainly the Latter Two Mechanisms & Hint at the First



Aagaard *et al., PLoS One* (2012); Koren *et al., Cell* (2012); Ma et al *BMC Genomics* (2014); Ma et al *Nature Commun* (2014); Chu *et al., Genome Medicine* (2016); Pace *et al., BMC Microbiology* (2018); Chu *et al., Nature Medicine* (2018); Pace *et al, under revised submission* (2019).

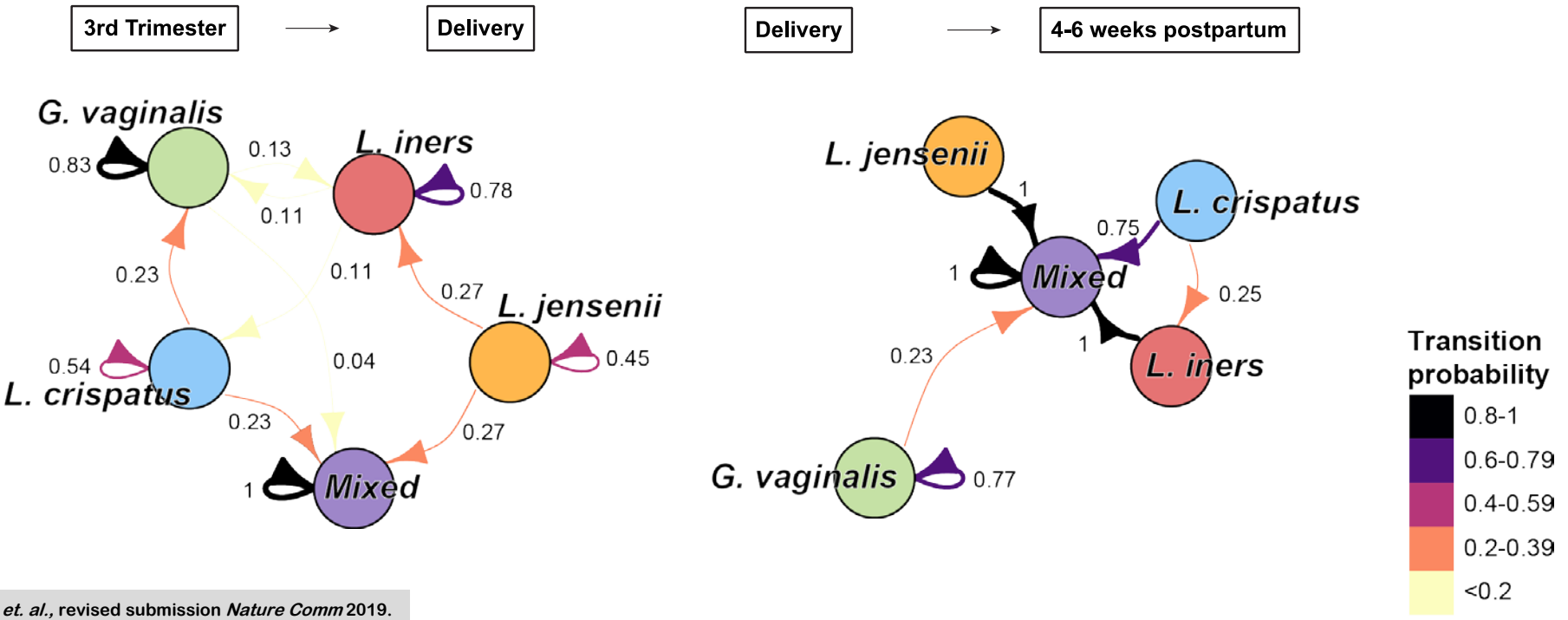
Early Observations: Pregnancy structures the vaginal microbiome to be less rich & less diverse at delivery



Beta Diversity Measures

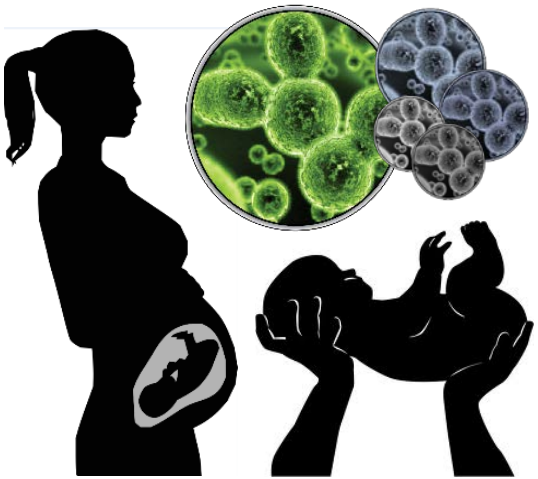
Aagaard *et. al., PLoS One* 2012.

Community Stability is Reached by co-Exclusion (remarkable restoration post pregnancy occurs naturally)



Pace *et. al.,* revised submission *Nature Comm* 2019.

True Fact: Vaginal microbes are not meant to (nor do they) stably populate the maternal nor infant gut



Gut phyla in the 1st week of life

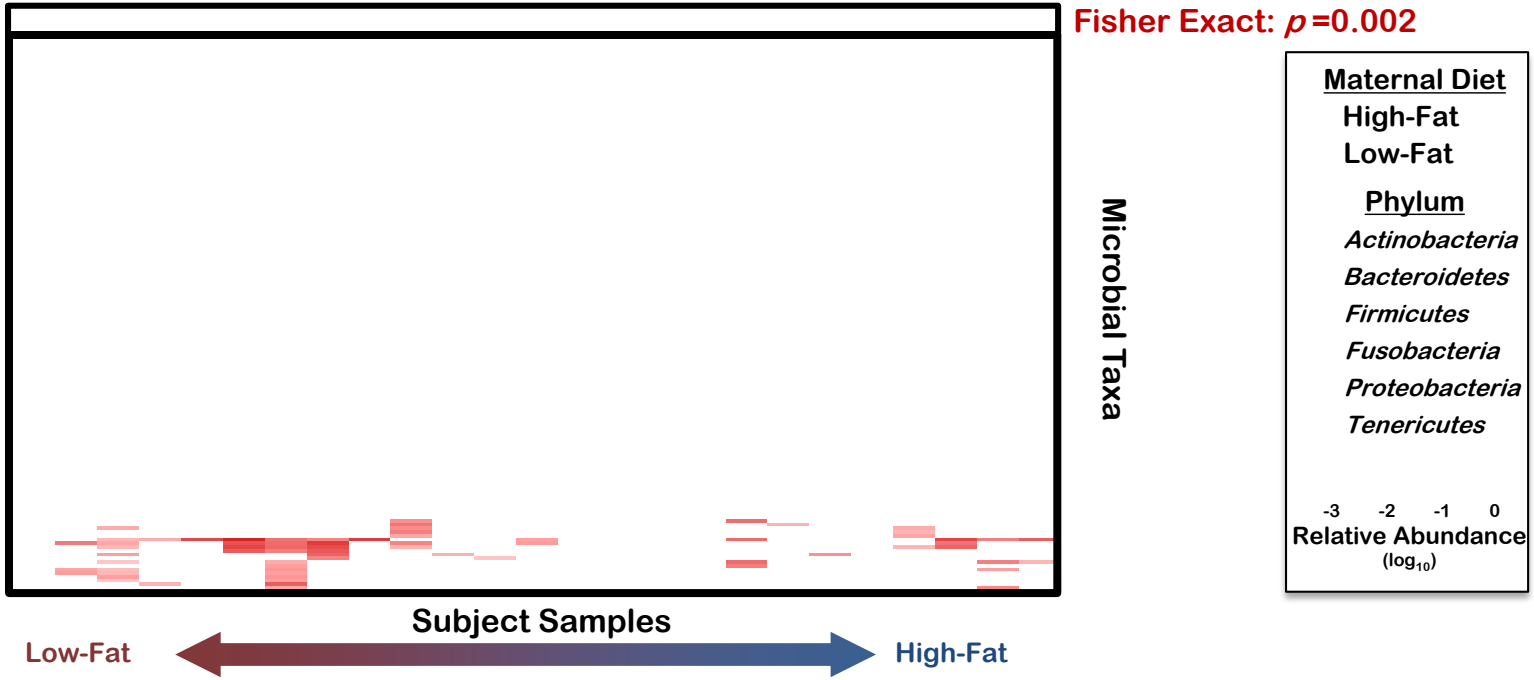
- **Firmicutes (10-20%)**
  Enterococcus, Clostridium, Lactobacillus, Staphylococcus, Streptococcus
- **Bacteroidetes (10-20%)**
  *Bacteroides**
- **Proteobacteria (20%)**
  Escherichia/Shigella
  Klebsiella
- **Actinobacteria (50%)**
  *Bifidobacterium **
  Propionibacterium

(this is actually where our journey interrogating other sources of the infant microbiome began)

Aagaard et al, *PLoS One* (2012); Jost *et. al., PLoS One*, 2012; Chu et al *Nature Medicine*, 2017.

Detailed & Validated Dietary Questionnaires Demonstrated that the Neonatal Meconium Microbiome Varies by Amount of Fat In Maternal Diet

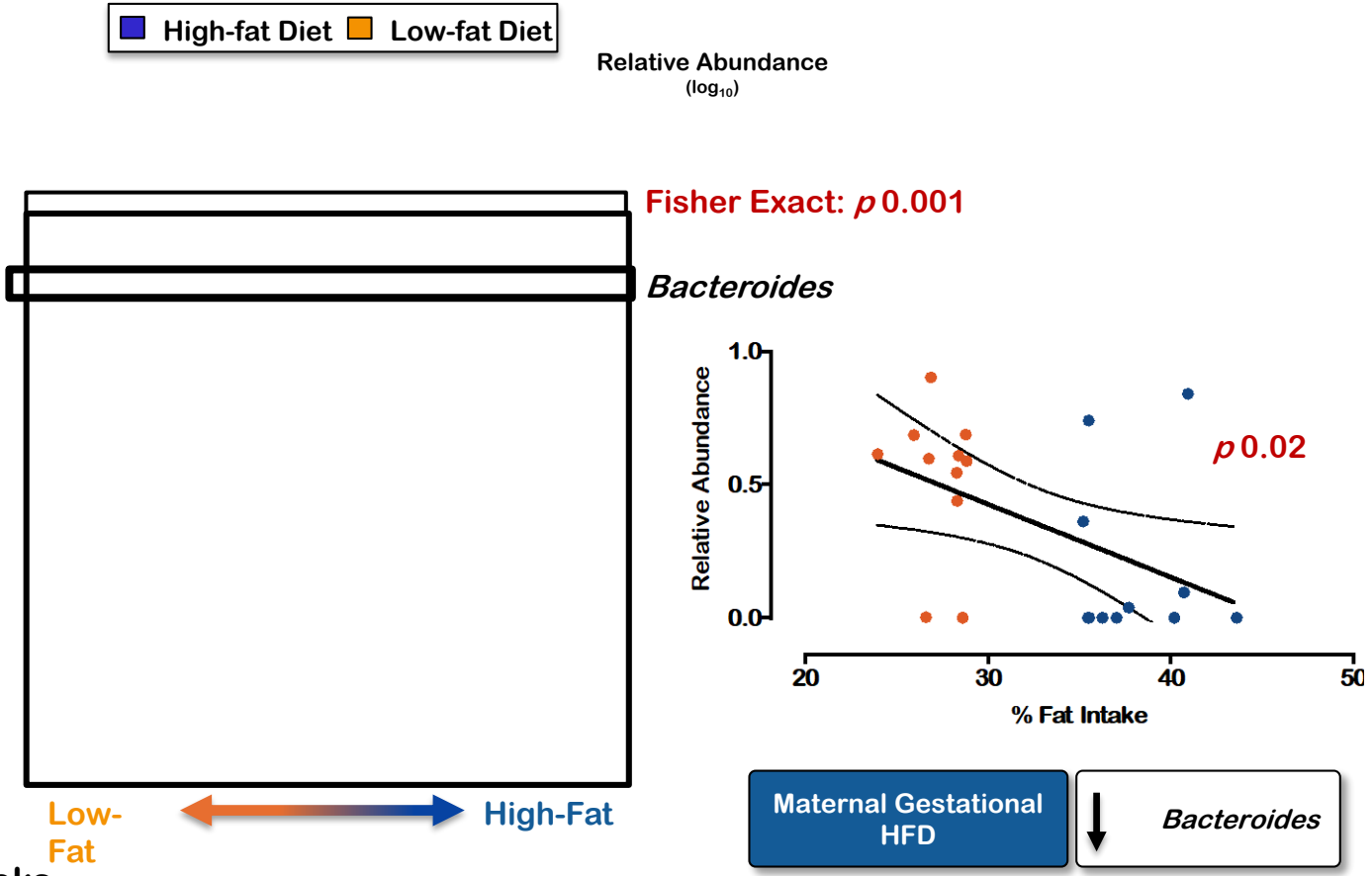
Neonatal Meconium



Neonatal meconium – at birth

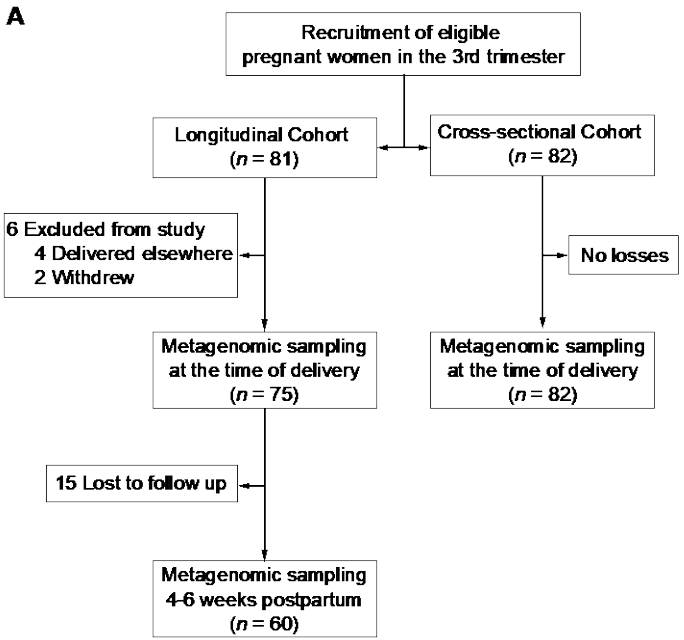
Chu *et al.*, Genome Medicine (2016).

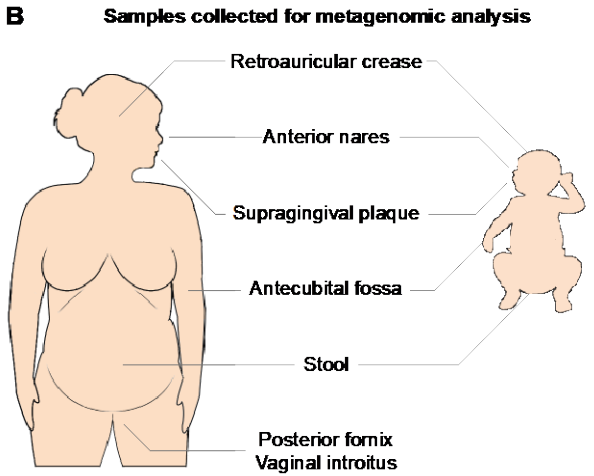
Persistent Association of Amount of Fat in the Maternal Diet with Infant Gut Microbiome at 6 Weeks



Infant Stool – 6 Weeks

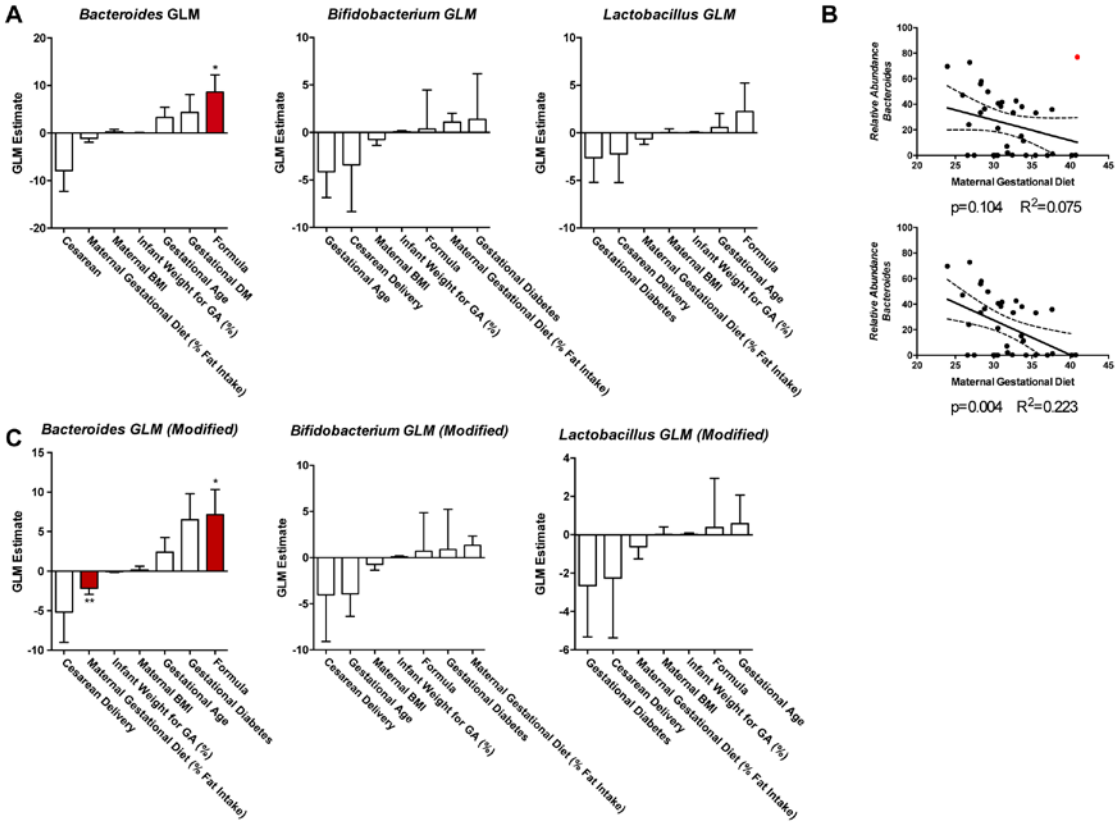
That was interesting, so we expanded with a second population-based prospective, longitudinal cohort





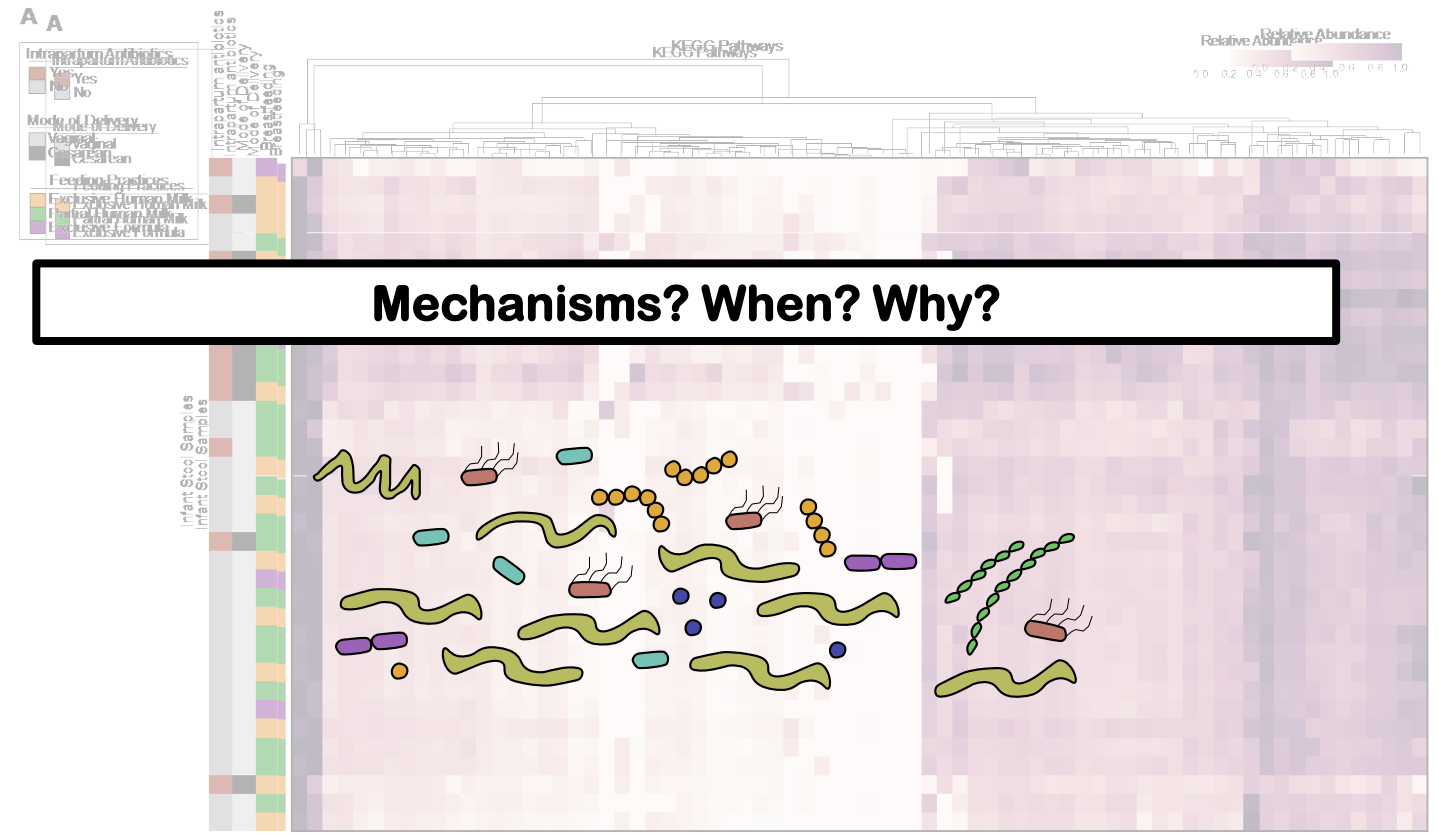
Chu *et al., Nature Medicine* (2017).

Moms Diet & Formula Feeding Matter at a Taxonomic Level, but Cesarean Does Not



Infant Stool – 6 Weeks

At a Functional Level, Moms Diet, Weight Gain & Breast Milk Feeding Matter, but Cesarean Does Not



Infant Stool – 6 Weeks

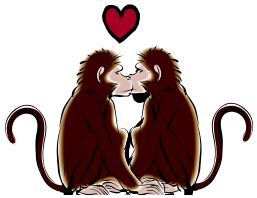
Ma *et al., Nature Communications* (2014); Prince *et al., AJOG* (2016); Chu *et al., Genome Medicine* (2016); Pace *et al., Microbiology* (2017); Seferovic *et al., Sci Reports* (2018); Dudley *et al, Nature Medicine* (2018); Prince *et al., Am J Primat* (2019); Chu *et al.,* in preparation (2019); Chu & Pace *et al.,* in preparation (2019).

Japanese Macaque Model of Maternal Obesity

Aagaard, Friedman & Grove (ONPRC)

Controls (*n* 64) : 13% fat (standard)

High Fat (*n* 90) : 35% fat (caloric dense)



Year 1

1st pregnancy

1st birth

overweight

natural cohorts

Years 2 - 5

HFDS

Obese on control diet

diet reversal

HFDR

lean

(7-24)

Maternal Caloric Dense Diet (isocaloric) in the Japanese Macaque

- Hyperinsulinemic, hyperglycerolemic, and euglycemic
- Increased leptin/body weight ratio
- Normal serum lipid and triglyceride levels
- Undergo cesarean with fetal necropsy at day 130 (term 167 days)
- 20-30% of dams do not become obese (“resistant” cohort)
- In year 7, obese dams are reverted to control diet (“diet reversal” cohort)

Aagaard *et al* (2008) *J Mol Endocrin*, McCurdy *et al* (2009) *J Clin Invest*, Cox *et al* (2009) *Am J Obstet Gyneco*l 201:281.e1-9), Suter *et al* (2011) *FASEB* 25: 714-726, Frias et al (2011) *Endocrine* 152: 2456-2464, Suter *et al* (2012) *FASEB J* 26: 5106-5114, Suter *et al* (2012) *Mol Endocrinol* 26: 2071-2080, Suter *et al* (2013) *Pediatr Res* 74: 252-258, Aagaard *et al* (2012) *PLoS One;* Sullivan *et al* (2011) *Neuroendocri;* Frias *et al* (2014) *FASEB J,* Aagaard *et al* (2014) *Science TM,* Ma *et al* (2014) *Nature Comm;* McCurdy *et al* (2016) *J Clin Invest*, Harris *et al* (2016) *Nature Sci Reports;* McCurdy *et al, JCI* (2017)*;* Weslowski *et al* (2018) *Mol Metab.*

Moms Diet Influences the Offspring Microbiome: Assessing the Fetal Microbiome & its Origins in Japanese Macaques

Japanese Macaque dam

High-Fat Diet (36%) | Control Diet (13%)

Control Diet (13%) | Control Diet (13%)

Pregnancy | Nursing | Post-weaning

Delivery | 6 mo. | 1 yr. | 3 yr.

G130

- Fetal Colon
- Placenta
- Maternal Anus, Mouth, Vagina

6 mo.

- Anus

10 mo.

- Anus

3 yr.

- Anus

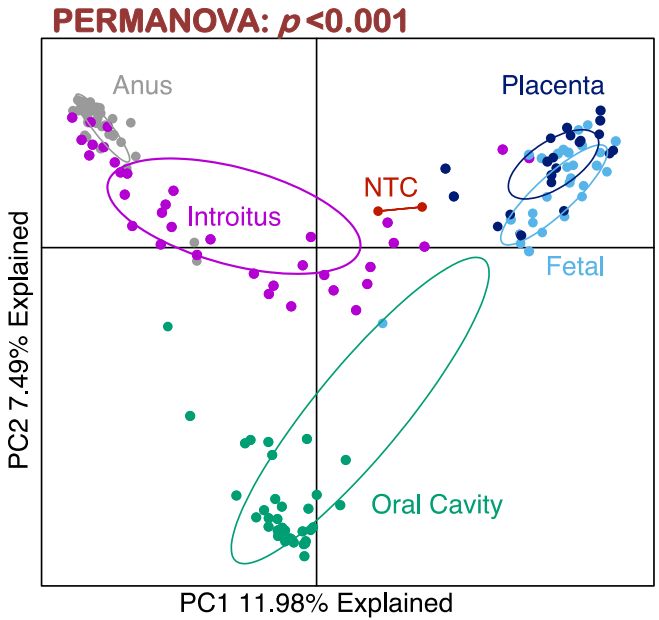
G130: Gestational age day 130 (~32 weeks gestation in humans)

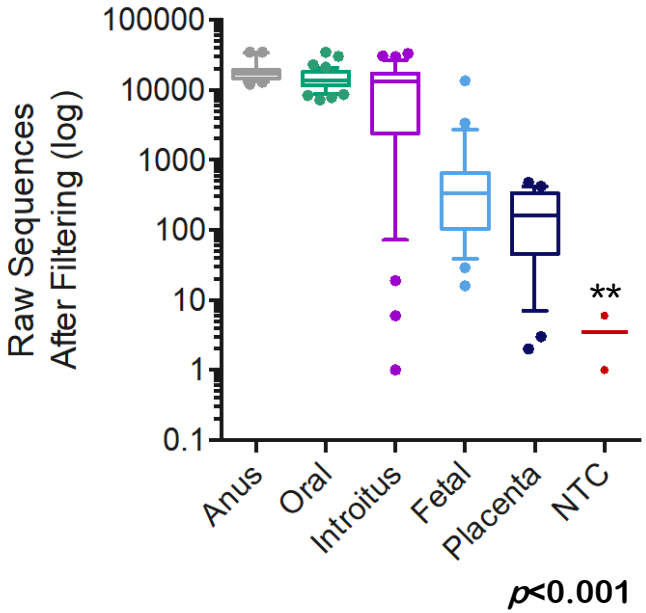
NTC (Non-template Sequencing Controls)

WGS & 16S rRNA Gene Sequencing (Microbial Profiling)

Ma *et al., Nature Communications* (2014); Prince *et al., AJOG* (2016); Chu *et al., Genome Medicine* (2016); Pace *et al., BMC Microbiology* (2017); Seferovic *et al., Sci Reports* (2018); Dudley *et al, Nature Medicine* (2018); Prince *et al., Am J Primat* (2019); Severovic *et al.,* in preparation (2019); Chu *et al.,* in preparation (2019).

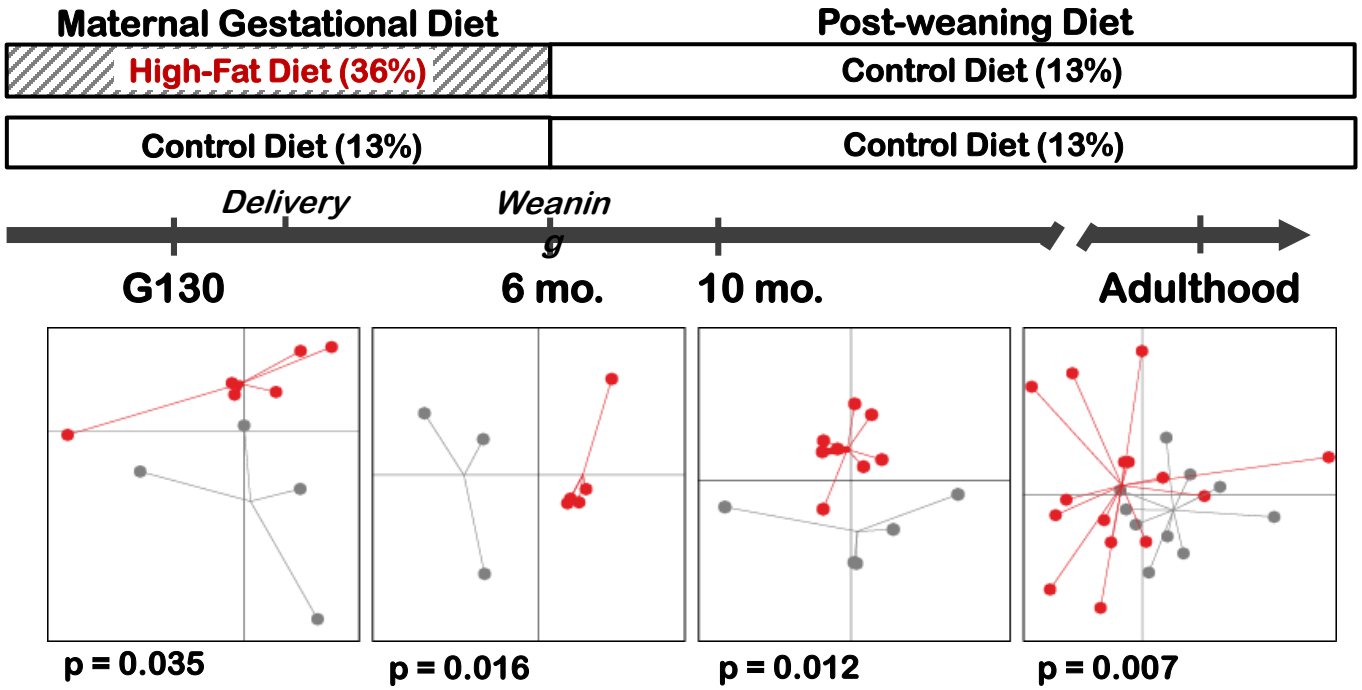
Microbial Profiles in Primate Low-Biomass Samples are Distinct from “kitome” & Sequencing Controls



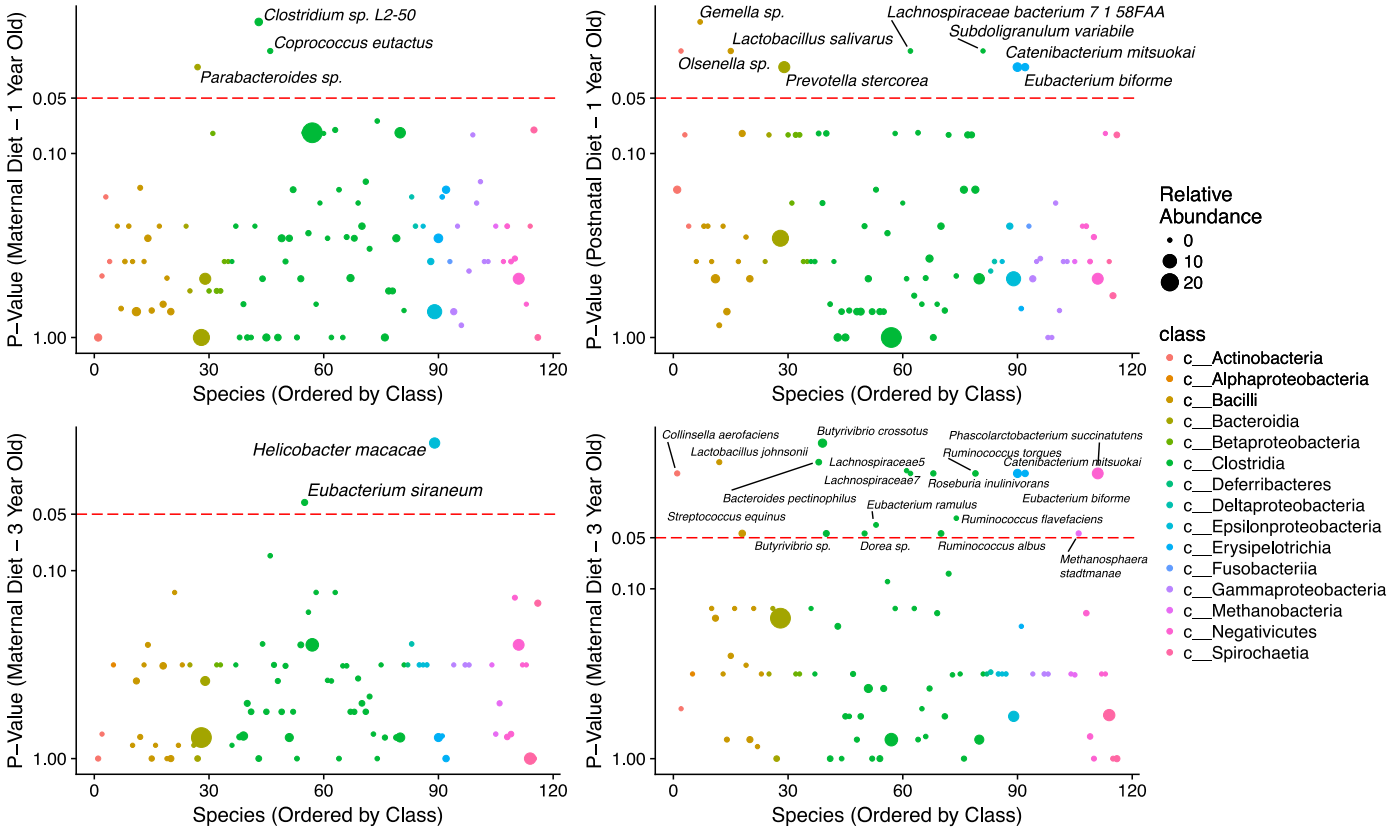


NTC: Negative Template Control

Maternal High-fat Diet Persistently Alters the Offspring Gut Microbiome from Fetal Life



Maternal High Fat Diet Persistently Alters Abundance of *Clostridia* & *Lachnospiraceae* spp. in Offspring Stool

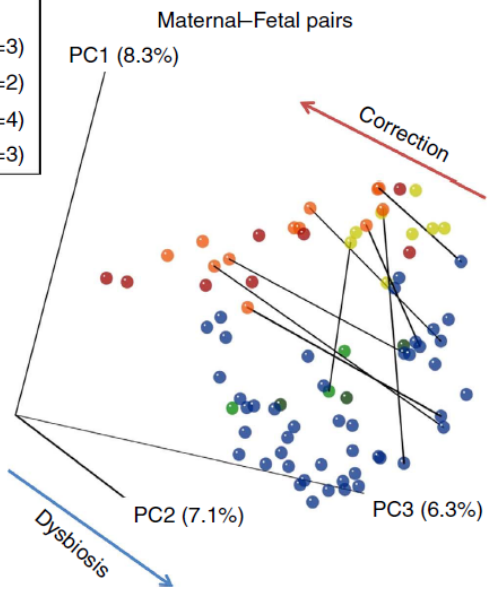


Gestational Influence of the Maternal Diet is not Fully Correctable with a Normal Postnatal (post-weaning) Diet nor Probiotics

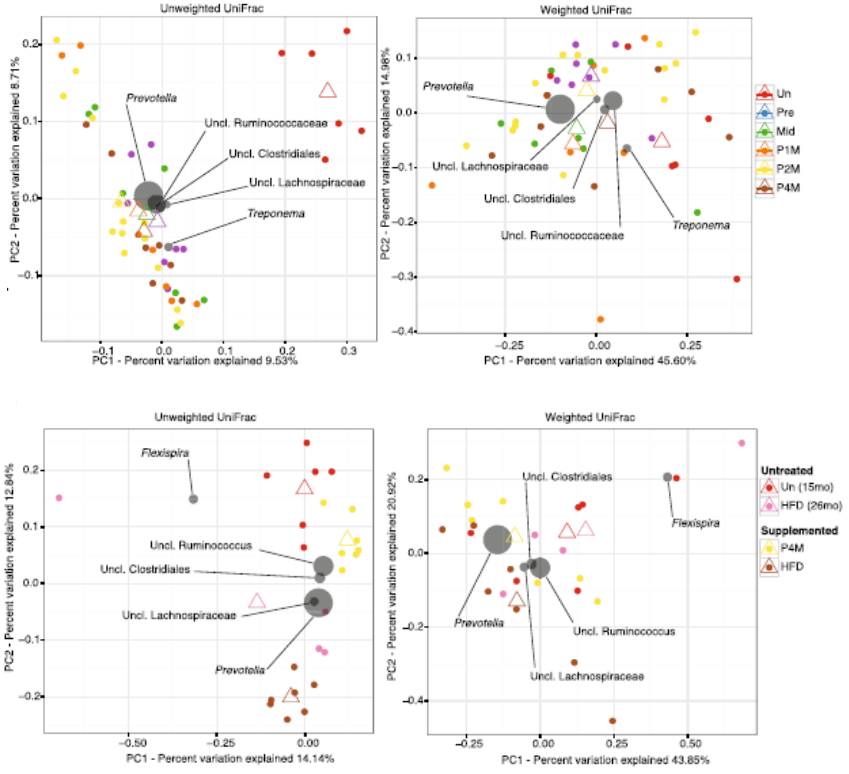
Maternal (n=34)
- CTD (n=6)
- HFD (n=28)

Juvenile (n=12)
- CTD/CTD (n=3)
- HFD/HFD (n=2)
- HFD/CTD (n=4)
- CTD/HFD (n=3)

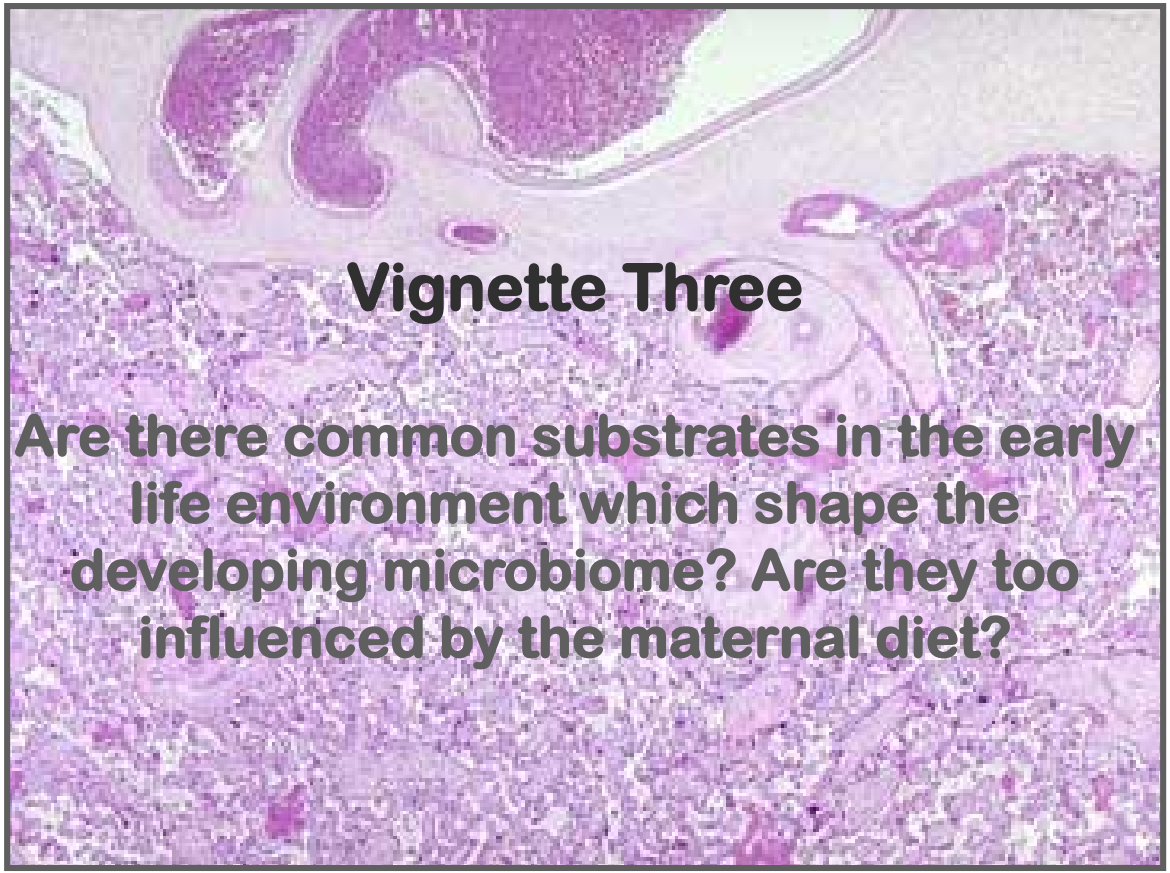
Post-weaning Diet Correction



Post-weaning Probiotics (symbiotic)



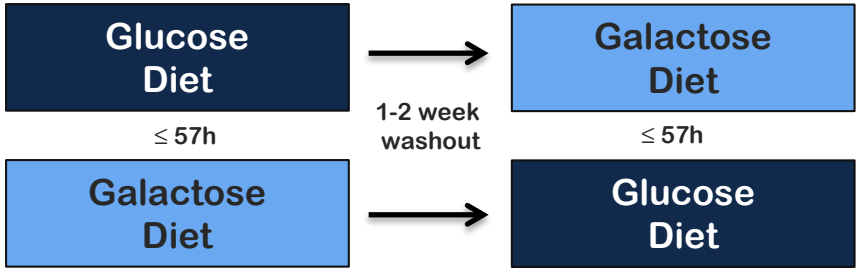
Ma *et al., Nature Communications* (2014); Chu *et al., Genome Medicine* (2016); Pace *et al., BMC Microbiology* (2018); Prince *et al., AJP* (2019).

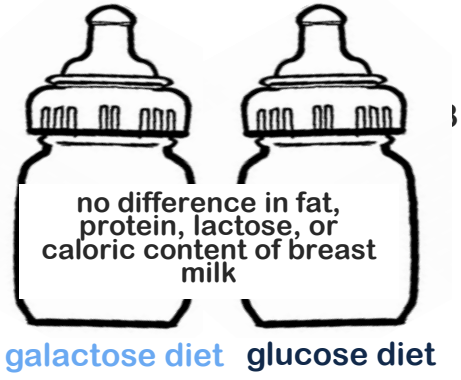


Seferovic, Mahmoud, Engevik, Pace *et al.,* in revised submission (2019); Jochum & Seferovic, in preparation (2019).

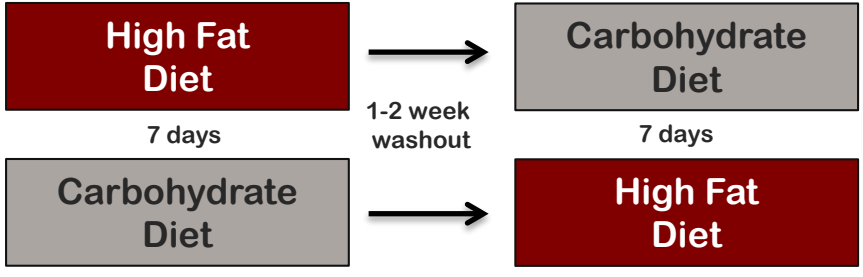
Study Design: Paired cross-over with defined dietary interventions (each person is their own control)

Glu/Gal Cohort (*n* 7)



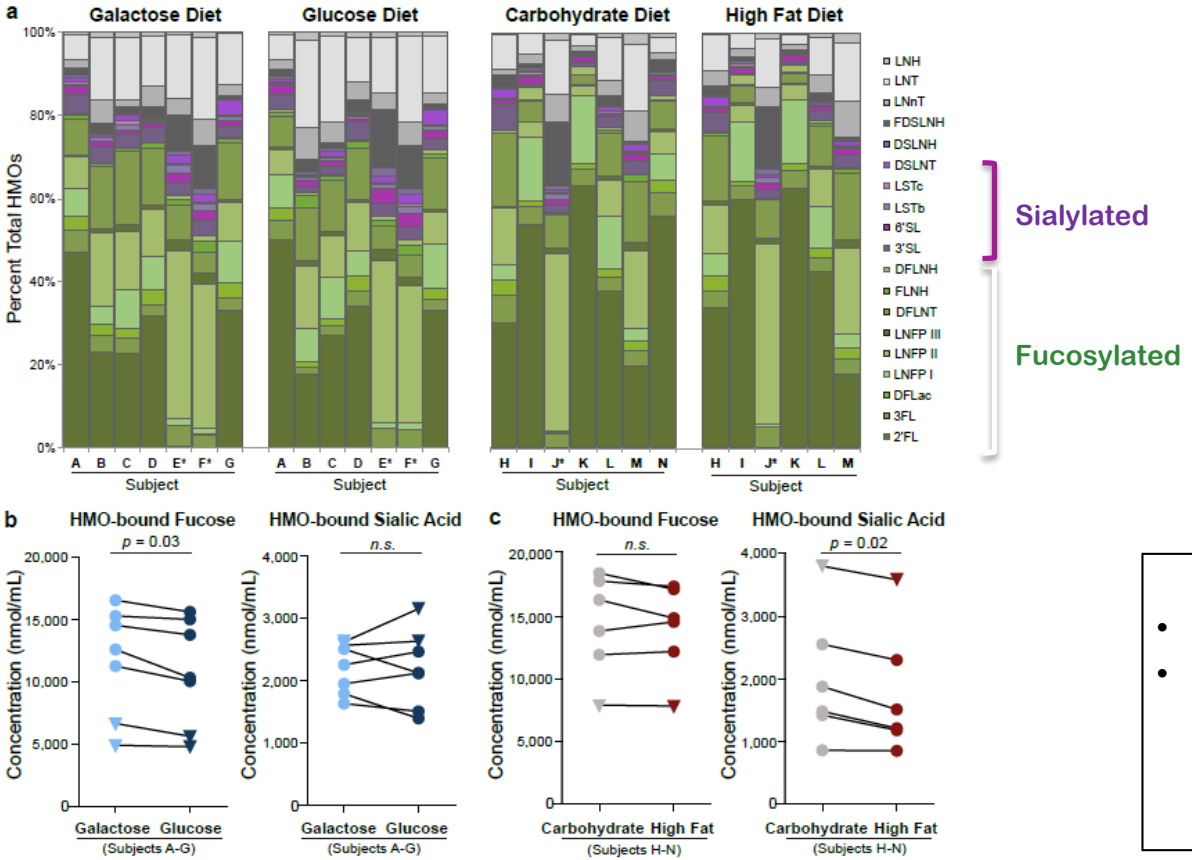


Carb/Fat Cohort (*n* 7)



Mohammad *et al., Am J Clin Nutr.* 2011; Munch *et al., PLoS ONE. 2013.*

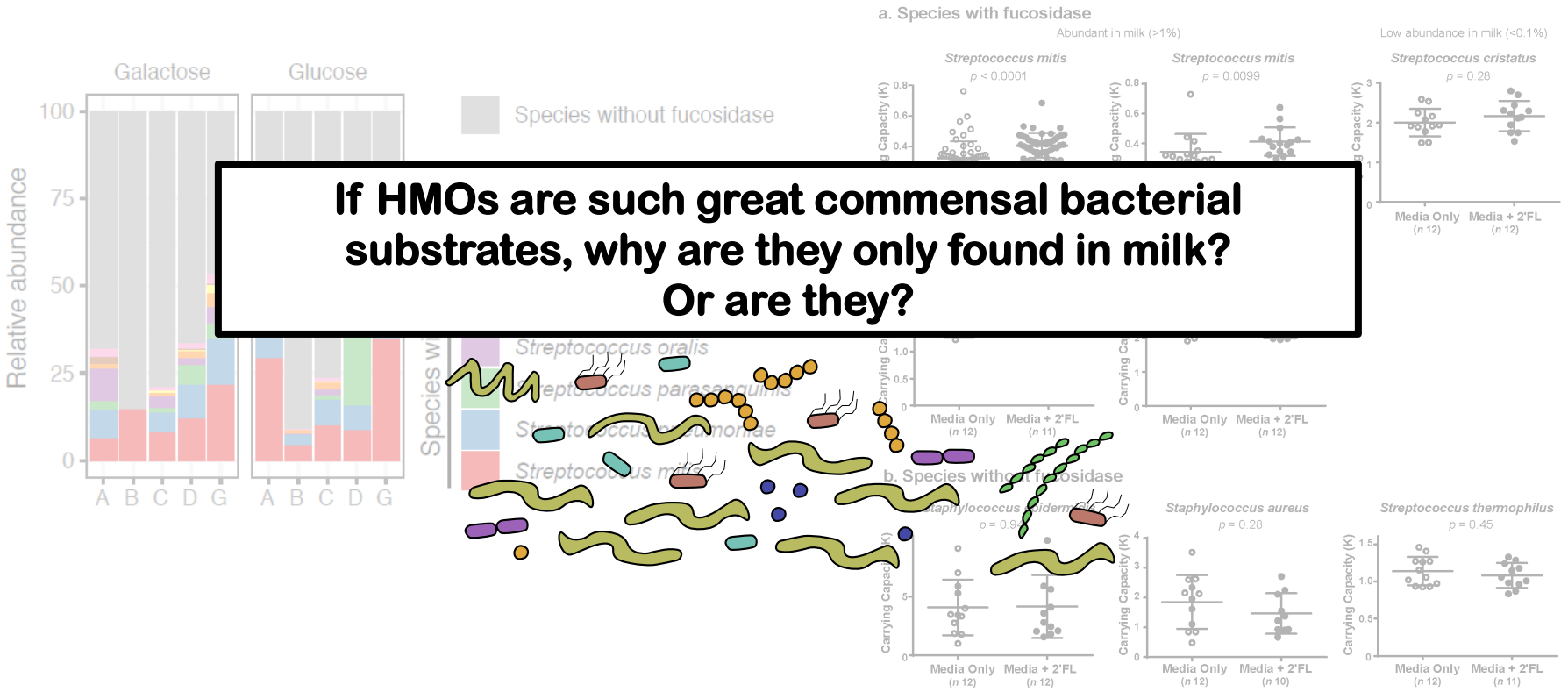
Maternal Diet Alters Composition of Human Milk Oligosaccharides (HMOs)



HMOs

- Indigestible by the infant
- Digested by bacteria, favoring proliferation of beneficial bacteria in the infant gut

In vitro Mechanistic Evidence: HMOs (fucosylated) enable enhanced growth of *Streptococcus mitis & oralis*



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

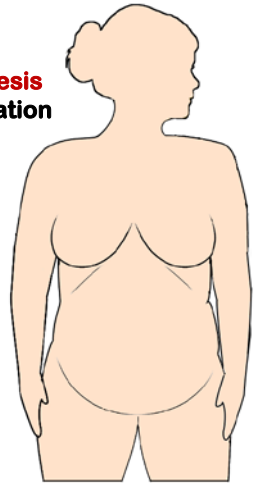
Maternal Diet Drives HMO Production & Selective Microbial Growth

Undertook Discovery Metabolomics in a Large Prospective Cohort with Midtrimester Amniotic Fluid Samples

	Cohort (*n* 731)	U.S. National Averages
Maternal Age (years)	34.3 +\- 5.4 (17 to 44)	25.6
Ethnicity		
Asian	227 (31.1%)	
Hispanic	139 (19.1%)	
African American	144 (19.8%)	
White	219 (30.0%)	
Nulliparous	200 (27.4%)	~40%
History of PTB	78/729 (10.7%)	
Preterm Delivery	92/729 (12.6%)	9.6%
Gestational Age at Sampling (weeks)	17.5 +\- 1.9 (14 to 29)	
Amniocentesis Indication		
Advanced Maternal Age (AMA)	389	
+Mat. Serum Screen (+MSS)	218	
Abnormal Ultrasound	40	
Multiples	82	

Ethnically diverse group of older gravidae mostly presenting for genetic amniocentesis for advanced maternal age

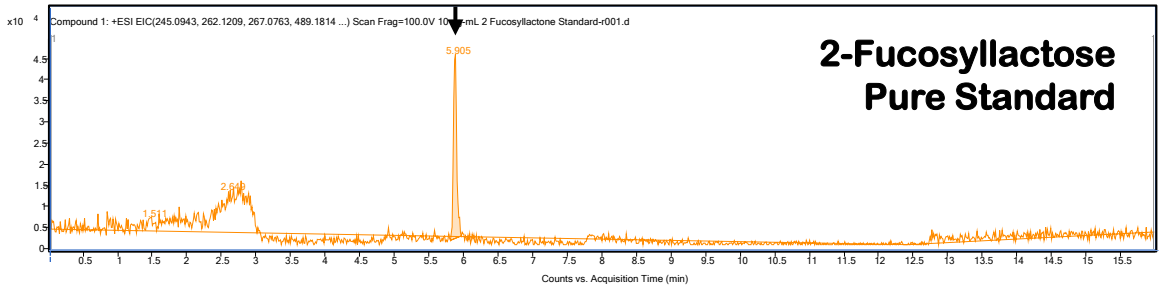
Genetic Amniocentesis at **14-29 weeks gestation**

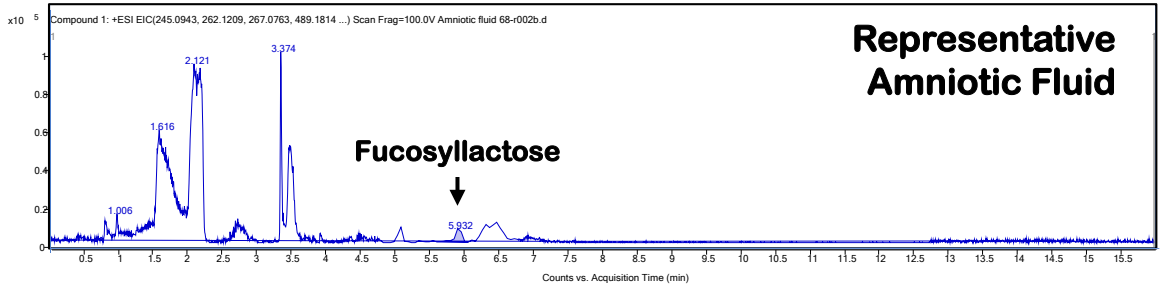


No signs of intraamniotic infection

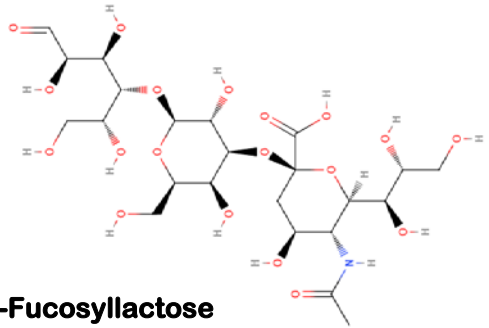
Seferovic & Joachum *et al.,* in preparation (2019).

Putative 2'-Fucosyllactose HMO Discovered in Unbiased GC-MS mid-trimester Amniocentesis





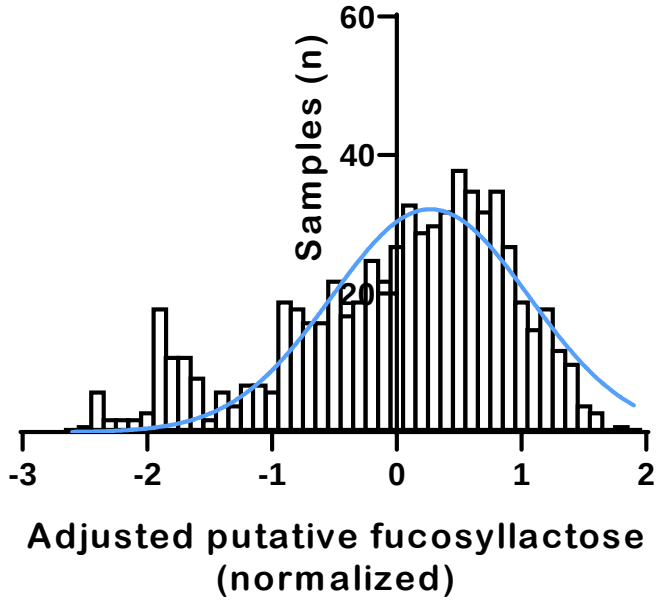
- Unusual compound *m/z* 511.164 Da (i.e $C_{18}H_{32}O_{15}Na+$)
- MS/MS fragmentation confirms trisaccharide
- Metlin database searches correspond to fucosyllactose variants
- Purified standard assessed in parallel confirms retention time & *m/z*

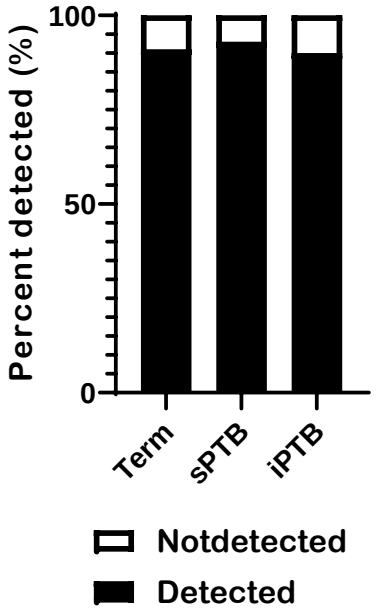


2-Fucosyllactose

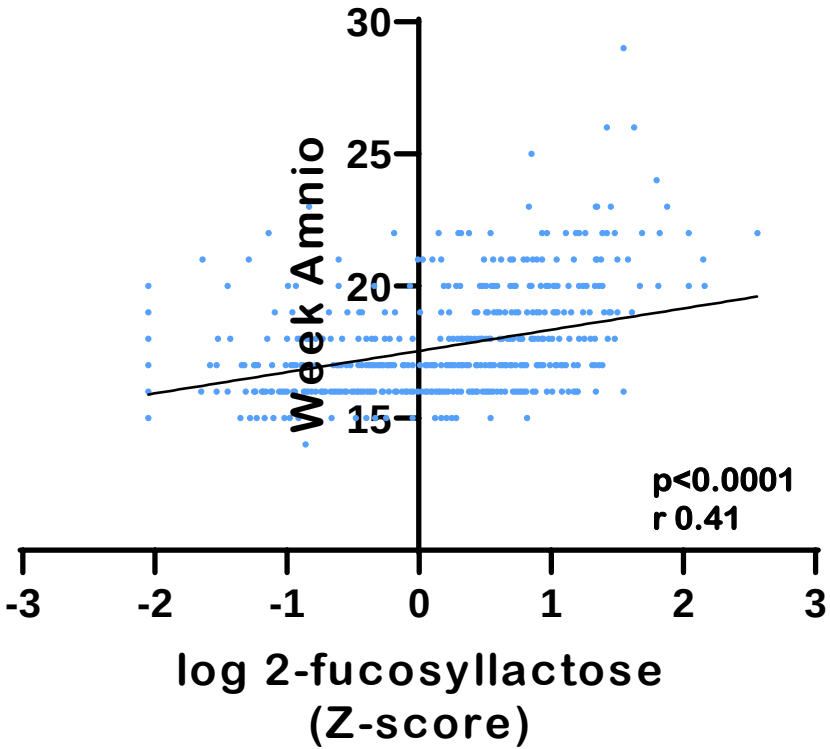
(Near) Universal HMO (FL-SL) Detection in 672 mid-Trimester Amniotic Fluid Samples

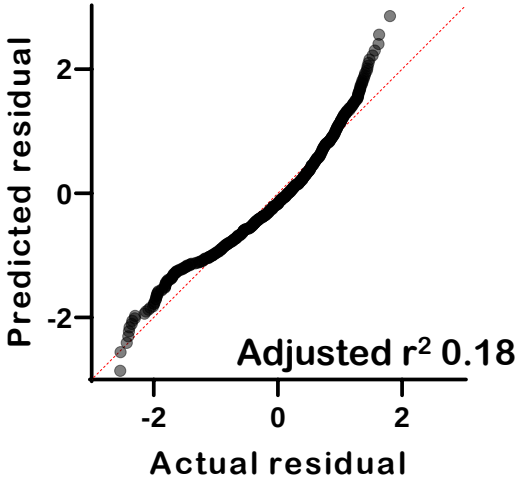
	Term	sPTB	iPTB
Total	595	56	21
Race/Ethnicity			
Asian	189	20	4
Hispanic	108	11	6
Black	114	12	3
White	182	13	8
Nullparity	164	14	5
hx PTD	54	10	6
Week Amnio	17.5 (1.9)	18.0 (2.5)	17.5 (2.1)
Maternal Age	34.4 (5.4)	32.2 (5.7)	34.7 (6.3)





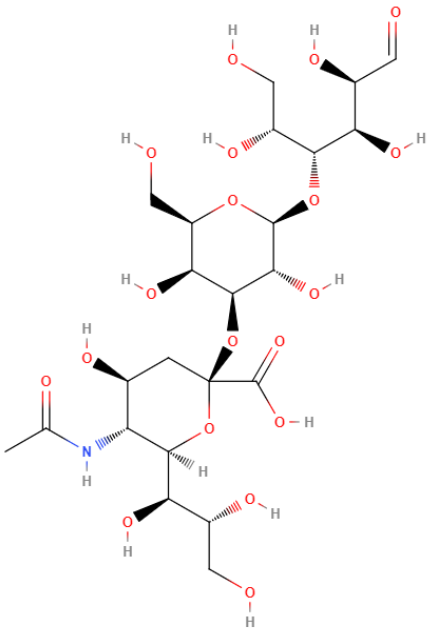
Moderate Increase with Gestational Age at Amniocentesis



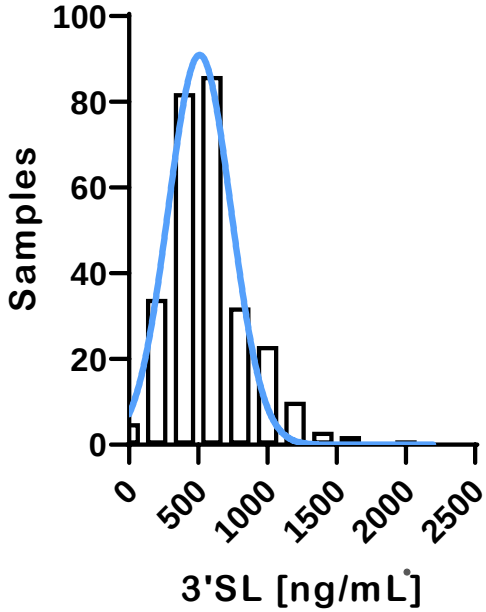


Variable	Parameter Estimate	SD	Units	VIF	P
Amnio week	**0.20**	0.02	Z/wk	1.12	**<0.0001**
Maternal age	**-0.02**	0.006	Z/y	1.15	**0.004**
Race/Ethnicity	**-0.07**	0.029	*	1.02	**0.01**
Nulliparity	-0.11	0.08	*	1.07	0.18
PTB prior	-0.12	0.12	*	1.05	0.30
Amnio reason	0.02	0.07	*	1.08	0.78

3-Sialyllactose Verified by MS/MS, Detected in a Subset of 279 Samples



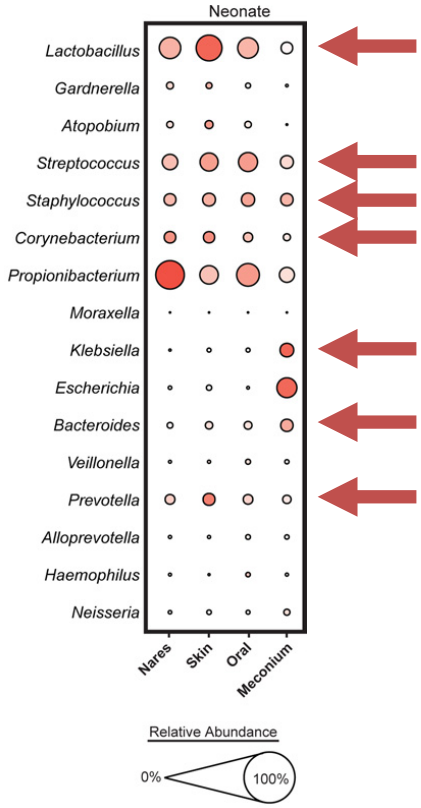
3-Sialyllactose



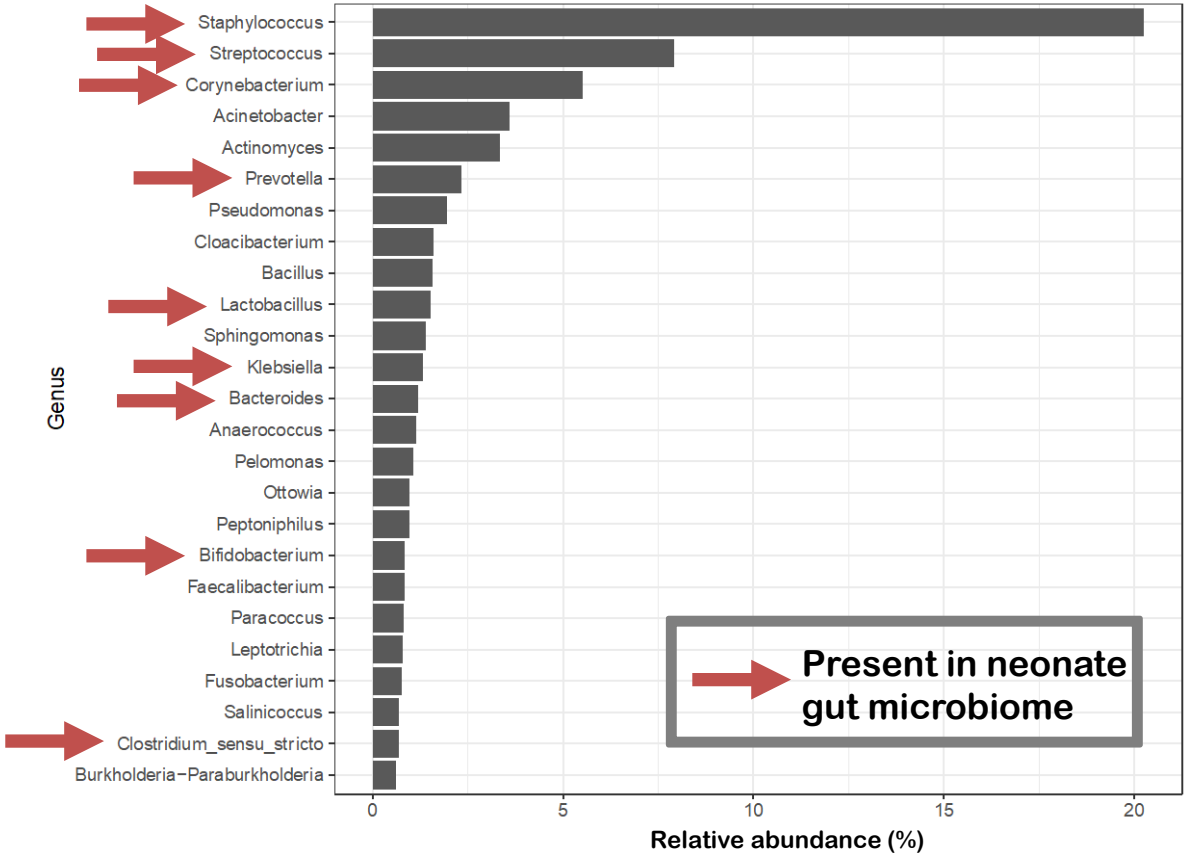
- Structural verification of peak ID by MS/MS
- Peak detected in 100% of a subset of 279 amniotic fluids
- Comparison to standards reveals absolute levels to be 0.5 mg/mL

Suggests that the food and fodder for bacteria (HMO) is actually present by 16 weeks… but is the bacteria? (form follows function)

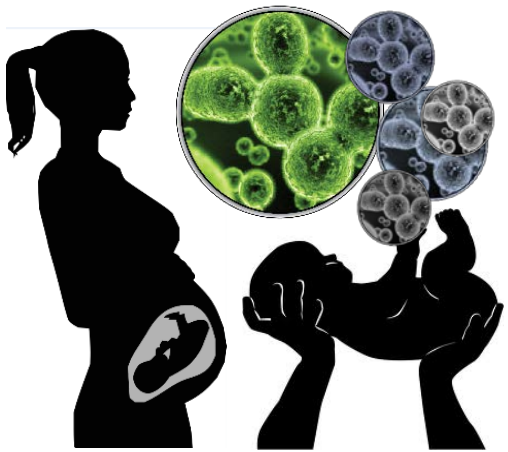
Striking Similarities Between the Amniotic Fluid Taxa at 17-22 weeks Gestation & the Neonate at Delivery



Chu et al., 2017



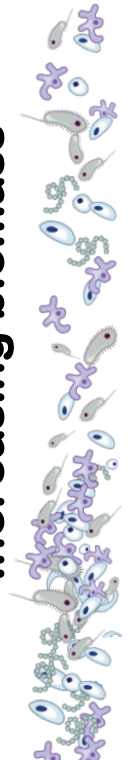
Interpretation: Early developmental communities are sparse, low abundance & low biomass—this is probably really important



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 (Propionobacteriaum& 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

Increasing biomass



(and we have always explicitly stated low biomass & low abundance, low diversity)

Where does this leave us?

Here's what I have shared with you today….

- **Intrauterine colonization—uncertain but probable (stay tuned)**

(present metagenomes in the primate fetus, which vary by moms diet)

- **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)

- **Colonization resistance—intriguing emerging data in moms & babies**

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

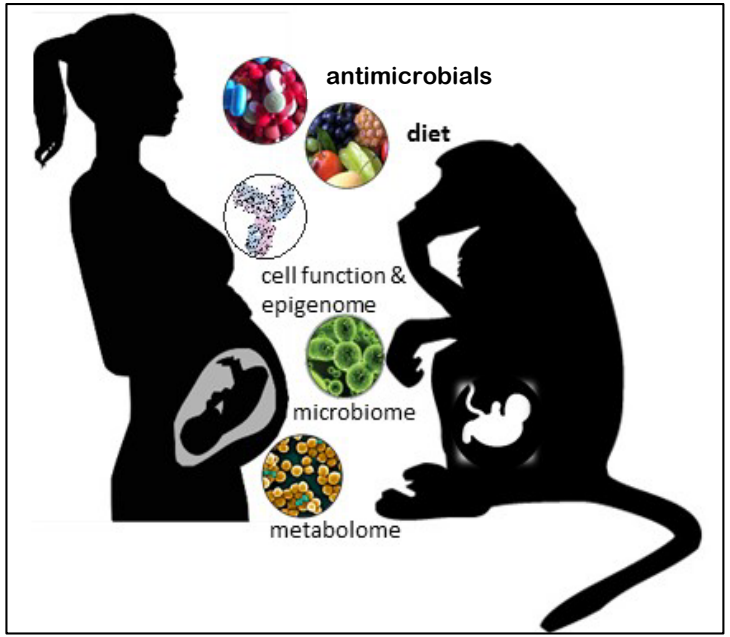
Rather Remarkable Convergence of Data from Many Labs….varying interpretation.

There are some clear truths, and quite a bit of ongoing uncertainty

- There is robust evidence from many labs using different techniques & approaches demonstrating a low biomass, low abundance microbiome in the uterus, placenta, amniotic fluid & membranes, fetus & milk
- Exposure to microbes during development are fundamentally important for normal behavior, metabolism & immunity
- Maternal nutrition during pregnancy and lactation matters in both the long & short term to her and her offspring's microbiome and metabolism
- Fetal programming of the microbiome does not have to involve colonization *per se*
- When in fetal or neonatal development true & robust microbial colonization actually occurs is undeniably uncertain <u>but low biomass, low abundance</u> communities are consistently present

(Solving the question of how we come to tolerate our commensal microbes & the impact of nutrition on this process is one of the most important public health challenges of our time)

Developmental exposures matter to the neonatal, infant, and later in life microbiome & disease risk



Across all mammalian species examined to date, later in life disease consistently follows several pregnancy exposures and are accompanied by changes in the metagenome, epigenome, & metabolome

- Maternal diet (low protein & high fat)
- Environmental chemical exposures (PAHs, metals, nicotine, particulate matter)
- Antimicrobials (antibiotics, metformin)
- Endocrine disruptors
- Altered maternal metabolic states (diabetes)

(and this is why this conference really, really matters)

Acknowledgements

Baylor College of Medicine
Aagaard Laboratory 2019

Angela Burgess, MD
Jun Ma, PhD
Min Hu, MD
Jennifer McKinney, MD
Angela Burgess, MD PhD
Mary Catherine Tolcher, MD
Lori Showalter, BSc
Cynthia Shope, MSc
Melissa Suter, PhD
Derek O'Neil, PhD
Erin Bolte, BSc
Derrick Chu, BSc
Braden Pew, BSc
Amanda Prince, PhD
Maxim Seferovic, PhD
Michael Jochum, PhD
Tiffany Kautz, PhD
Greg Valentine, MD
Ryan Pace, PhD
Stephen Saylor, BSc
Mark Hamilton, PhD

Division of Maternal-Fetal Medicine

Carey Eppes, MD MPH
Martha Rac, MD
Michael Belfort, MD PhD

Texas Children's Pathology & Immunology

James Versalovic, MD PhD
Daniel Lacorazza, PhD
Chun Shik Park, BSc
Jim Dunn, PhD
Eumenia Castro, MD PhD

Baylor College of Medicine Molecular Virology & Microbiology

Janet Butel, PhD
Mary Estes, PhD
Kristy Murray, DVM PhD
Rodion Gorkachov, PhD
Joe Petrosino, PhD

Baylor College of Medicine HGSC & Core Labs

Richard Gibbs, PhD
Lisa White, PhD

Baylor College of Medicine Molecular Imaging Core

Fabio Stassi, PhD
Michael Mancini, PhD

Current & Recent NIH Funding

NIH R01 & P50 Funding
NICHD/NIDDK 1RO1DK079194-4 & NIDDK 1R01DK080558-02
NIDDK/NICHD 1R01DK089201-01A1
NICHD 2P50HD044405-11
NINR RO1014792
NICHD Human Placental Project R01

Oregon National Primate Research Center
Primate Center Core Grant
NIDDK DK060685-0351
NIDDK 1R01DK079194-02
NIDDK R24 (Aagaard, Friedman, Grove, MPI grant)

Reproductive Scientist Development Program & WRHR
March of Dimes-NICHD Scholar of the RSDP
NIH 5K12HD00849
NIH 5K12HD050128

NIH Director New Innovator Pioneer Award
NIH DP21DP2OD001500-05

NIH Human Microbiome Project
NIH NIGMS U54 HMP NIH Common Fund RM08-05

March of Dimes
Preventing Prematurity Initiative
Global Prematurity Center of Excellence (Malawi)

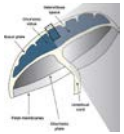
Burroughs Wellcome Fund
Preterm Birth Initiative

USAID/Bill & Melinda Gates Foundation
Grand Challenges
Saving Lives at Birth (Malawi)

Thrasher Foundation
Prevention of Prematurity and Xylitol (PPaX Trial, Malawi)

BioGaia
Unrestricted research funds

True Fact: The placenta is an inherently challenging microbiome niche



Excised placental & extraction blank ('kitome') samples

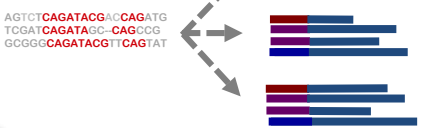
DNA extraction (microbial content in placenta ≈ 8 x 10^4/cm^3)

16S rRNA Sequencing

Amplify and Sequence V regions of 16S rRNA gene

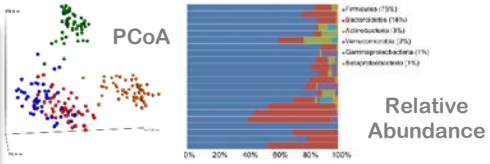
>1500 reads/sample

Cluster Similar Sequences & Assign Taxonomy (ASV or OTU)



img/hmp m GREENGENES The 16S rRNA Gene Database and Tools rdp silva

Determine Community Composition and Species Abundance



Challenge: Fine resolution of taxa calls (*i.e.*, sub-genera) in low abundance communities

Practical implication: Tossing out higher order taxa based on mere overlap with 'kitome' results in underestimation of bona fide members of a low biomass community

Whole Genome Shotgun Sequencing

Shotgun Sequence All (host & microbe) DNA

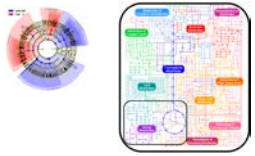
1-2 Gb/sample

Bin out human/eukaryotic reads

Align Remaining Sequences to Prokaryote Reference Genomes

img/hmp m bioBakery A metatomic analysis environment

Map Taxonomy and Functional Annotation

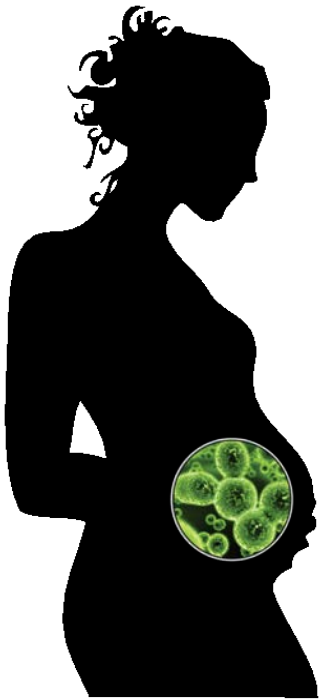


Challenge: Burning up *a priori* set sequencing depth with host (not microbial) DNA

Practical implication: Relies on chance and may underestimate diversity and biomass of bona fide members in a sparse community

(and the overwhelming majority of publications do take into account contamination)

True Fact: The weightedness of the published data shows a sparse placental microbiome distinguishable from contaminant controls



The human placenta *** harbors a low biomass microbiome that varies with preterm birth, and pregnancy health & disease

****Aagaard et al. *Science TM* (2014); Antony et al. *AJOG* (2015); Prince et al. *AJOG* (2016); Pace et al., *BMC Microbiology* (2017); Seferovic *et al AJOG* (2019); Prince *et al* in preparation (2019); Chu & Seferovic in preparation (2019).

***our published studies employ controls addressing the potential for environmental contamination

****we have always only described a low biomass, low abundance microbiome metagenomically; we say nothing about colonization in our work

Onderdonk et al *AJOG* (2008); Onderdonk et al *AJOG* (2008); Jimenez et al *Res Microbiol* (2008); Han et al *J Clin Microiol* (2009); Doyle et al, *Placenta* (2014); Dong et al, *Can J Infect Dis Med Microbiol* (2015); Amarasekara et al, *J Obstetr Gynecol Res* (2015); Zheng et al, *Nutrients* (2015); Doyle et al, *Placenta* (2014); Carlier Y *Acta Trop* (2015); Collado et al., *Sci Reports* (2016); Bassols et al, *Peds Res* (2016); Lal et al, *Nature Sci Reports* (2016); Gur et al, *Brain Behav* (2016); Puri et al., *PLoS One* (2016); Gomez-Arango et al, *Nature Sci Reports* (2017); Moore et al, *J Dairy Sci* (2017); Quereda et al., *Gut Microbes* (2017); Kuon et al, *J Reprod Immunol* (2017); Zheng et al, *Front Physiol* (2017); Gomez-Arango et al, *Sci Reports* (2017); Parnell et al, *Sci Reports* (2017); Elderman et al *Sci Reports* (2018); Tuominen et al *Sci Reports* (2018); Morimoto S et al *Jpn J Infect Dis* (2018); Dimova et al *Sci Reports* (2017); Tomlinson et al, *PLoS One* (2018); Zheng et al, *Oncotarget* (2018); Stout et al., *J Mat Fetal Neonatal Med* (2018); Amarsekara et al, *J Obstet Gynaecol Res* (2018); Cao et al *Placenta* (2018); Martinez et al *PLOS One* (2018); Borghi et al *Reprod Sci* (2018); Gohir et al *J Physiol* (2019); Fisher et al *Infect Immun* (2019); Luo et al *J Animal Sci* (2019); Zhu et al, *Front Microbiol* (2019) Peric et al, *IJMS* (2019); Fischer et al *Infect Immun* (2019); You et al, *Front Microbiol* (2019); Lannon et al *J Mat Fetal Neontal Med* (2019); You et al *Front Microbiol* (2019); Mortfly Smith et *al eBioM* (2019); Gohir et al *J Physiol* (2019); Tuominen et al *J Oral Micrbiol* (2019); Seferovic et al *AJOG* (2019); Younge et al *JCI Insight* (2019); Willis KA et al *FASEB J* (2019).

Exciting emerging evidence of the role of placental bacteria on fetal & maternal immune modulation in term, healthy pregnancies

Gomez de Aguero et al, *Science* (2016); Fayaerts et al. *Sci Reports* (2017); Weel et al. *J Repro Immunol* (2017); Erkers et al *J Leukoc Biol* (2017); Zhang et al. *J Cell Mol Med* (2017); Thion et al, *Cell* (2018); Tomlinson et al, *PLoS One* (2018); Dimova et al, *Sci Rep* (2018); Li N et al, *Nature Immunol* (2019); Stras et al, *Dev Cell* (2019).

Can't distinguish placental microbes from "contaminant" controls, even among preterm & chorioamnionitis cases

De Goffau et al, *Nature* (2019; erratum 2019); Theis et al. *AJOG* (2019); Leiby et al. *Microbiome* (2018); Lauder et al *Microbiome* (2017).

Multiple Lines of Evidence Suggesting the Placenta Functions Not as a Barrier but Rather as a Conduit for Maternal-Fetal Communication (I remain agnostic as to true colonization)

True Fact: Microbes <u>are</u> visualized in both preterm & term placentae

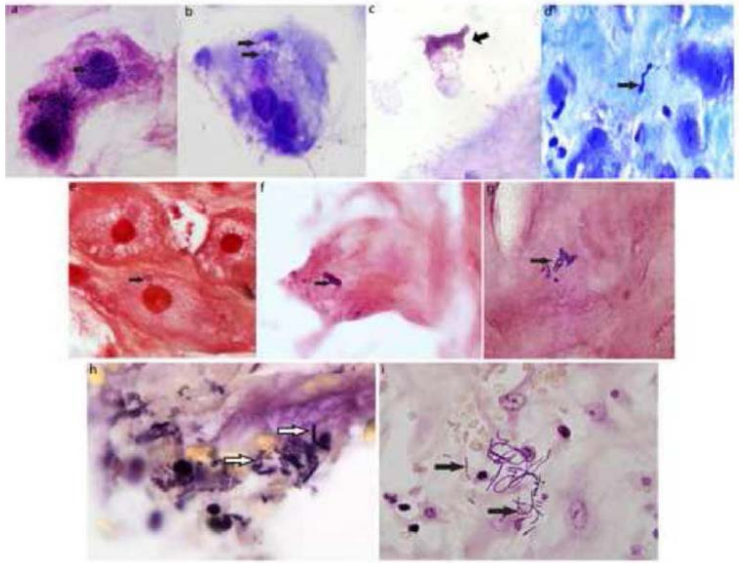
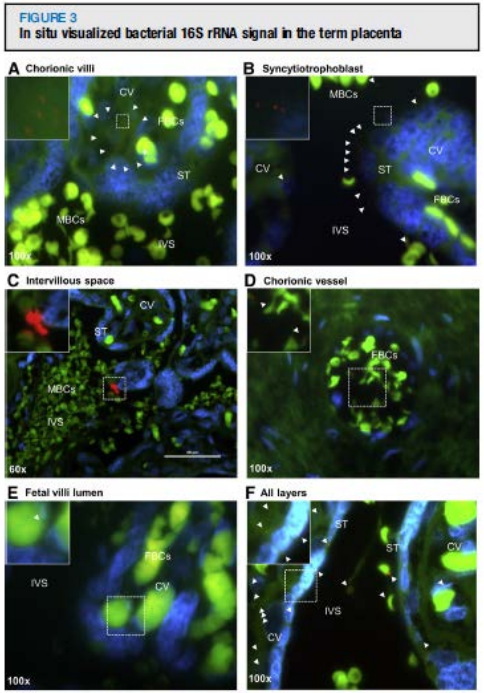


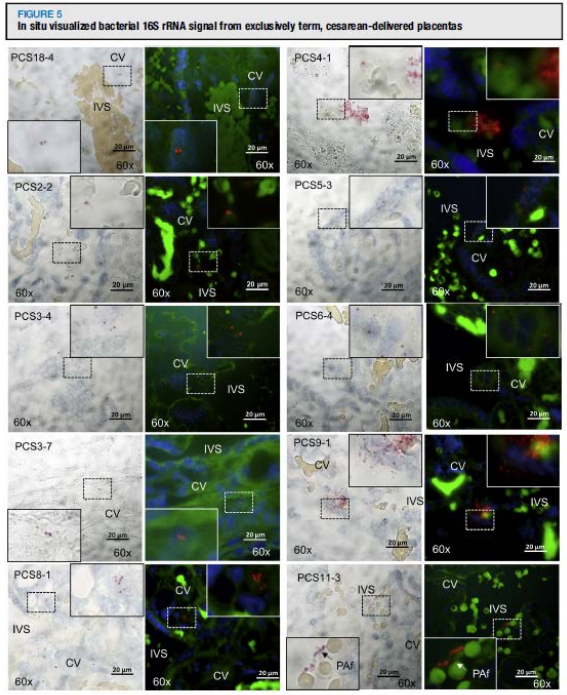
Figure 1. Intracellular bacteria in basal plate
(a–d) Hema 3 Geimsa stain (e–g) Gram stain (h–t) Brown and Hopps stain: all showing presence of single, clusters, chains, or filaments of intracellular bacteria (arrows).

Published in final edited form as:
Am J Obstet Gynecol. 2013 March ; 208(3): 226.e1–226.e7. doi:10.1016/j.ajog.2013.01.018.

Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations

Molly J. Stout, MD[1], Bridget Conlon[1,*], Michele Landeau[1,*], Iris Lee[1], Carolyn Bower[1], Qiuhong Zhao[1], Kimberly A Roehl[1], D. Michael Nelson, MD, PhD[1], George A. Macones, MD[1], and Indira U. Mysorekar, PhD[1,2,†]





16S bacterial ribosome, cell nuclei (DAPI), tissue, red blood cells

Seferovic et al. Visualization of placental microbiota. Am J Obstet Gynecol 2019.

(continued)

OBSTETRICS

Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection

Maxim D. Seferovic, PhD; Ryan M. Pace, PhD; Matthew Carroll, MD; Benjamin Belfort; Angela M. Major; Derrick M. Chu, PhD; Diana A. Racusin, MD; Eumenia C. C. Castro, MD, PhD; Kenneth L. Muldrew, MD, MPH; James Versalovic, MD, PhD; Kjersti M. Aagaard, MD, PhD

Red - 16S
Blue - DAPI
Green - Background