VAGINAL MICROBIOME, PREGNANCY, BIRTH MODE

UO-CHC 441H/431H: Microbes + Social Equity

Lecture 3

Dr. Sue Ishaq Pellegrini

Learning objectives

- The vaginal microbiome
- Microbial and metabolic changes during pregnancy
- Transfer of microbes in utero and during birth
- Effect of birth method
- Socioeconomic status and prenatal care

THE VAGINAL MICROBIOME

The human vagina

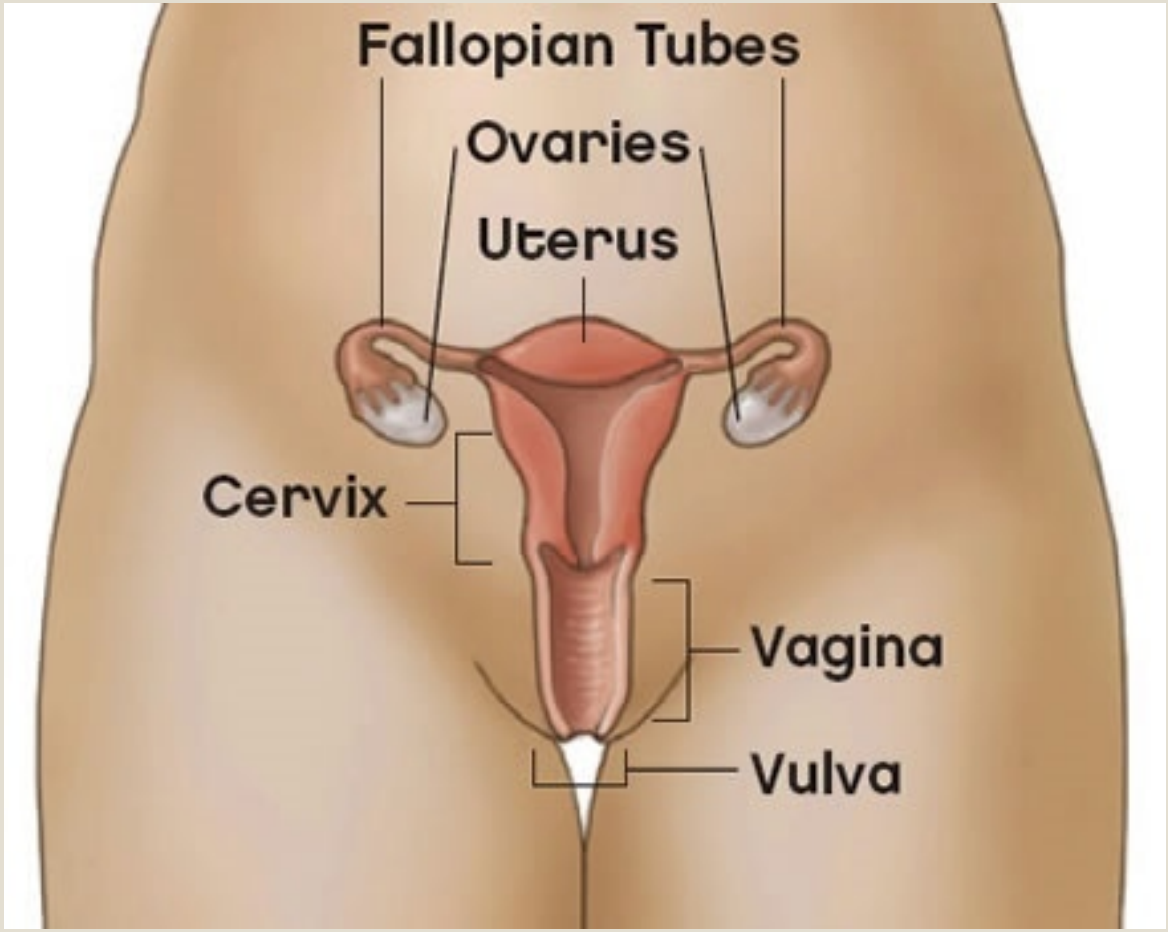
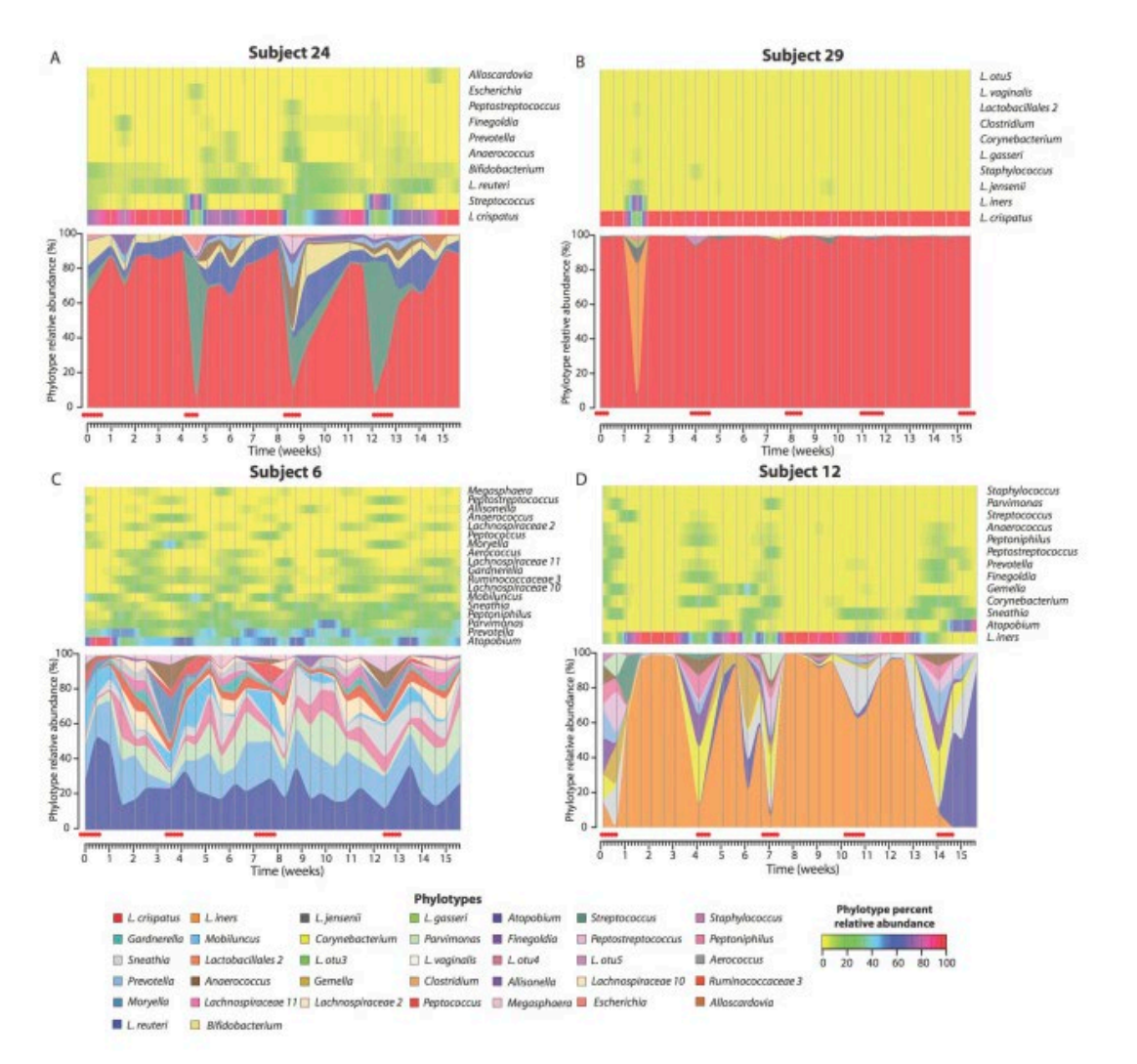


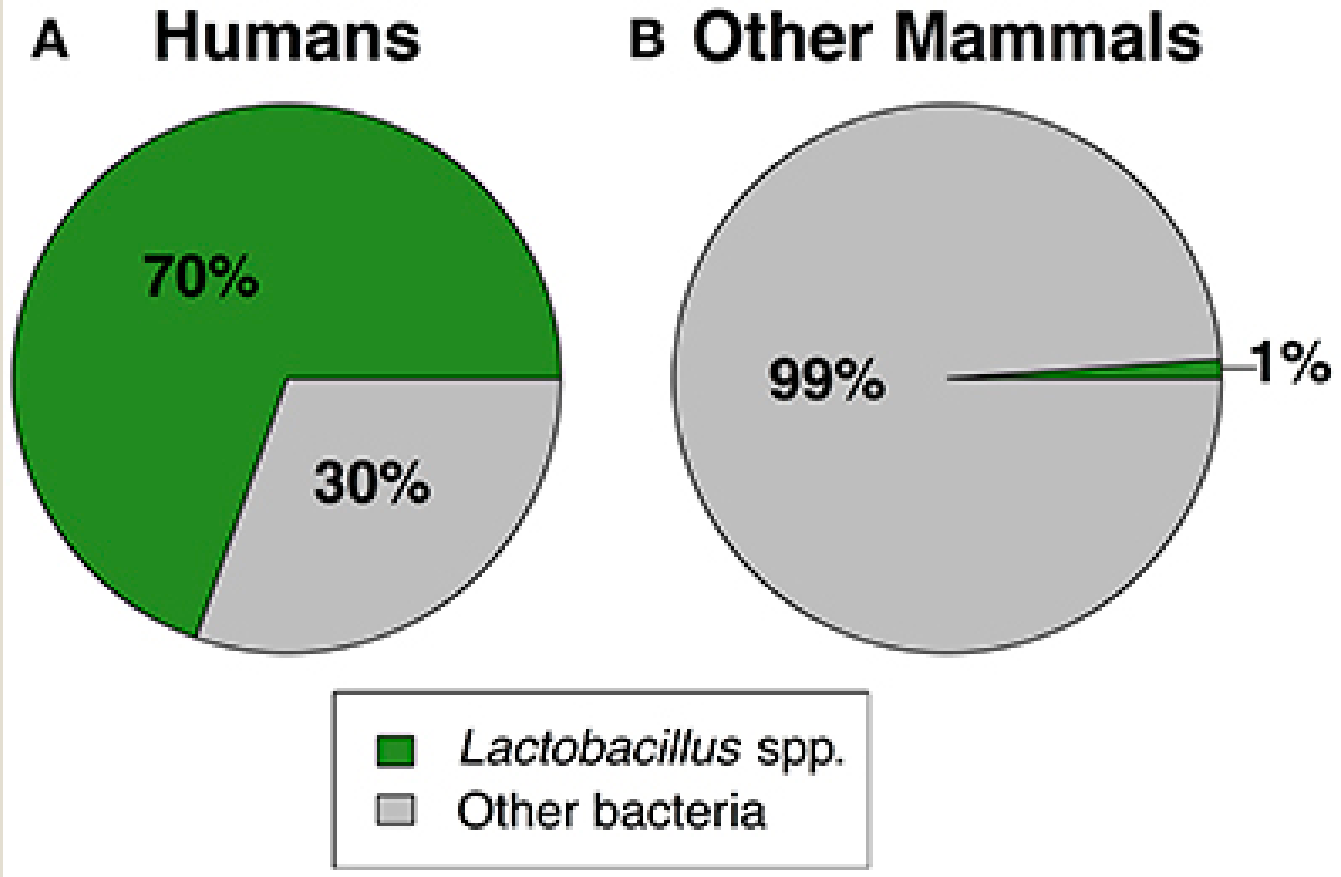
Image: News-Medical.Net

Bacterial community undergoes temporal variation related to estrous cycle and changing hormones



Nunn and Forney, 2016

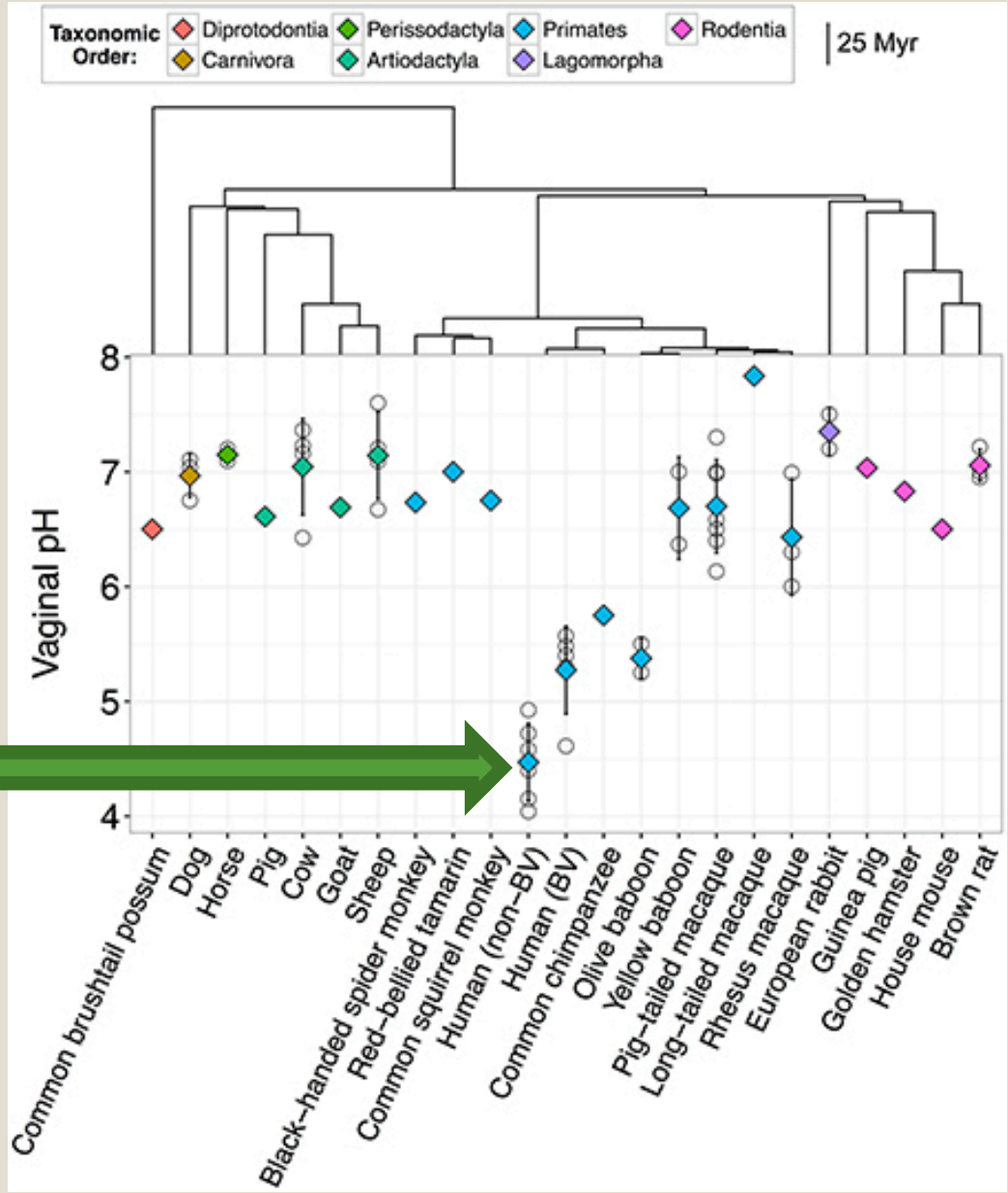
Typically, In the human vagina, a low-diversity microbial community is healthy



Miller et al. 2016

Vaginal pH drives the bacterial community

Healthy human vagina



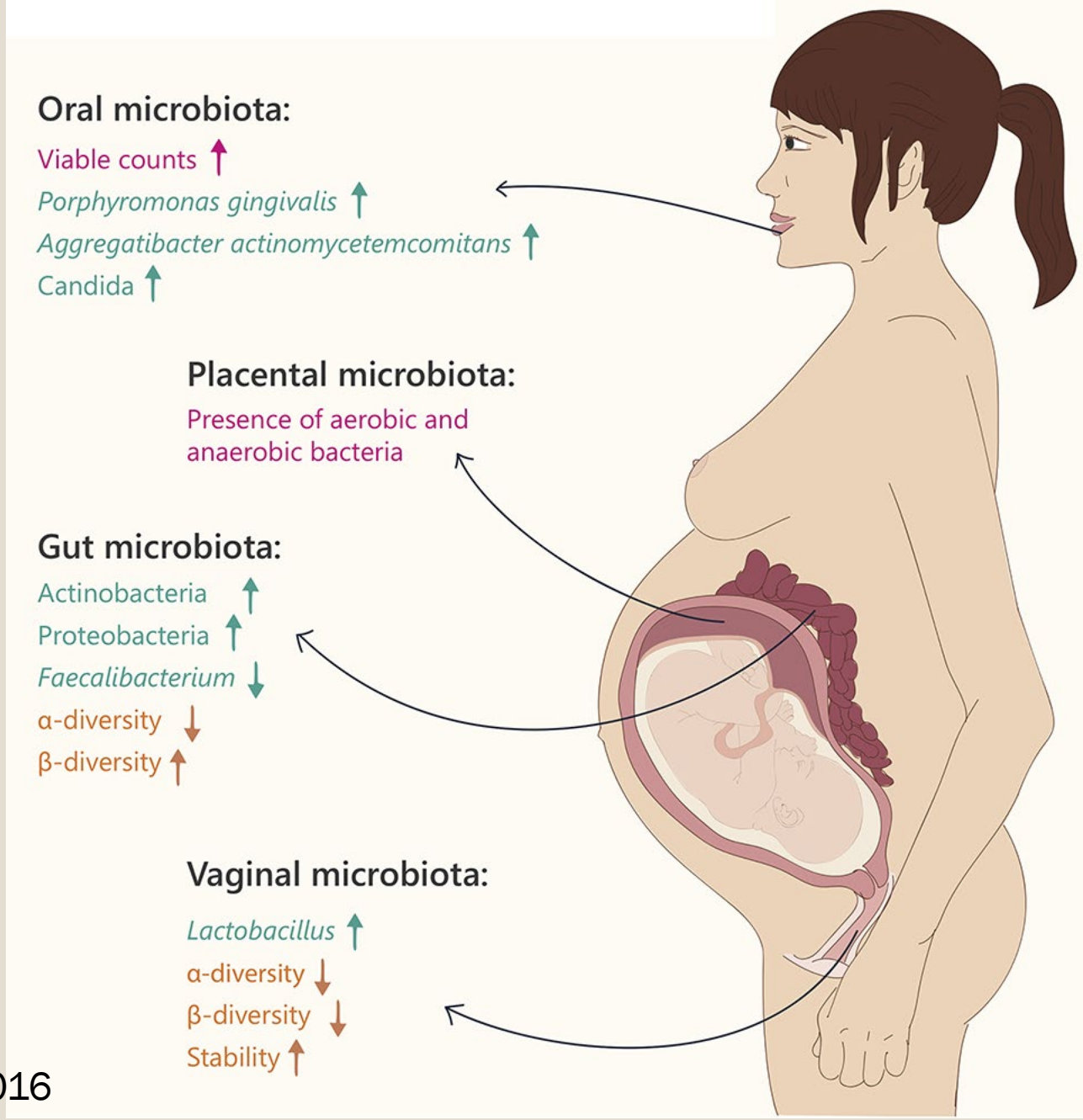
Miller et al. 2016

TL;DR

- Hormones regulate the vaginal tissue and function,
- which changes host-microbe interactions and stops feeding the helpful ones that keep the pH low,
- which can allow unhelpful microbes the chance to survive,
- which changes the vaginal ecosystem and function

MICROBIAL CHANGES DURING PREGNANCY

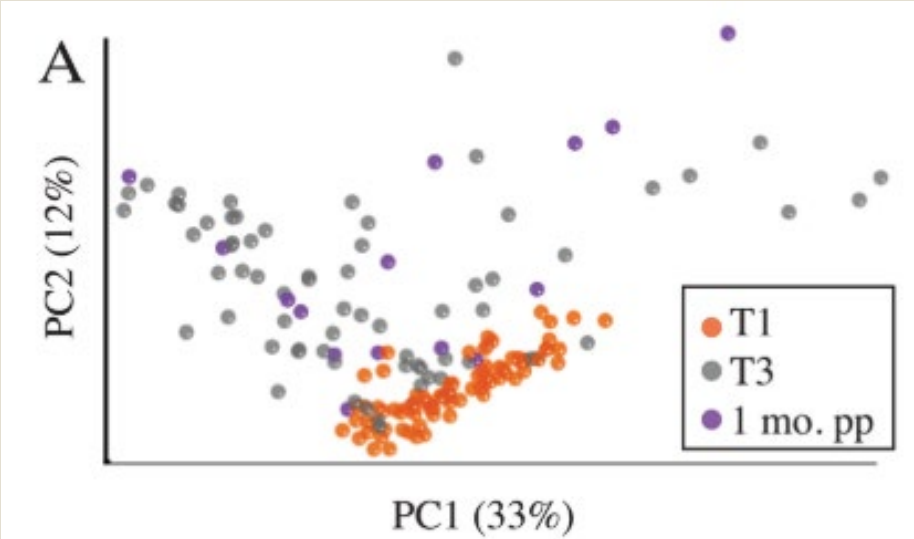
Hormonal changes in pregnancy drive changes in host metabolism and microbiota



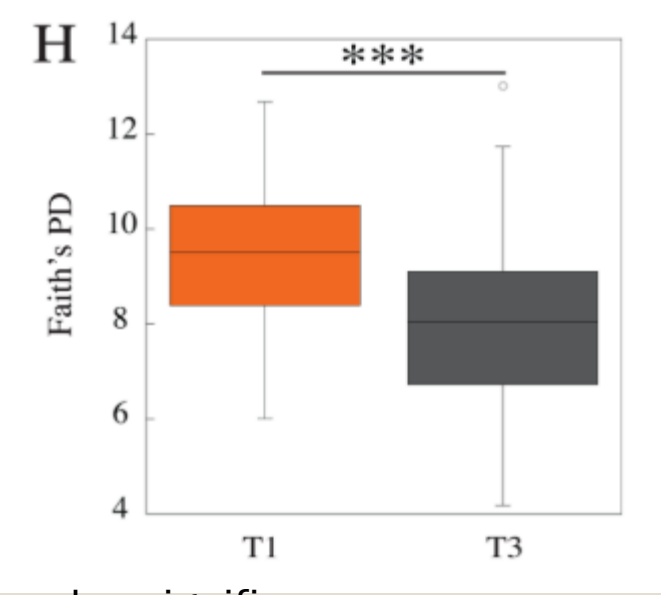
Nuriel-Ohayon et al. 2016

Maternal gut bacterial community changes during pregnancy

T1 is different from 3rd Trimester (T3) and 1 mo. post-partum (pp)



Lower bacterial diversity at T3 (fewer species present)

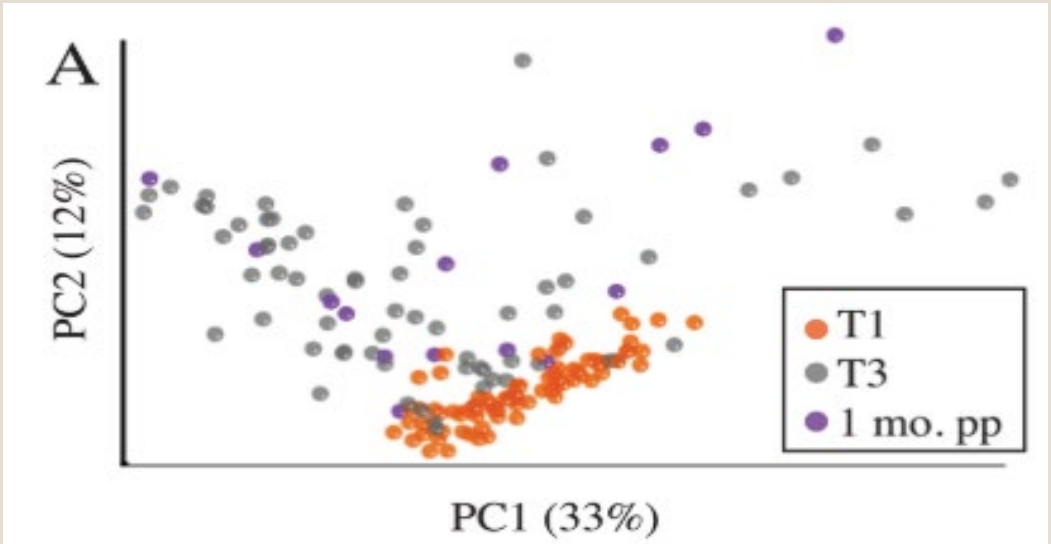


Ordination plot: each dot is 1 sample, proximity of samples signifies relatedness (in the microbial community)

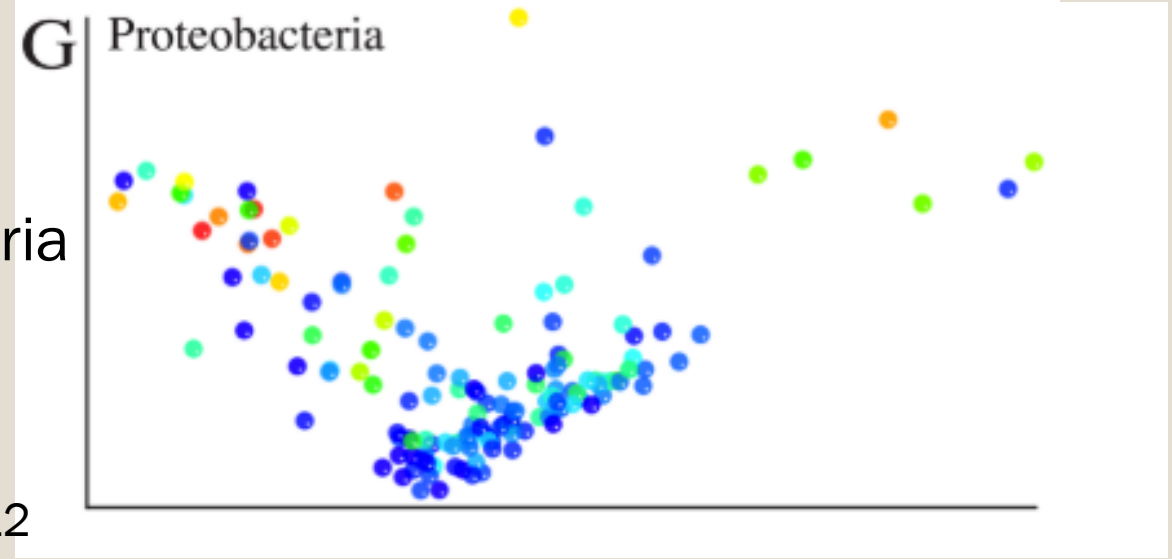
Koren et al. 2012

Maternal gut changes during pregnancy

Shift in the community at 3rd Trimester (T3) and 1 mo. post-partum (pp)



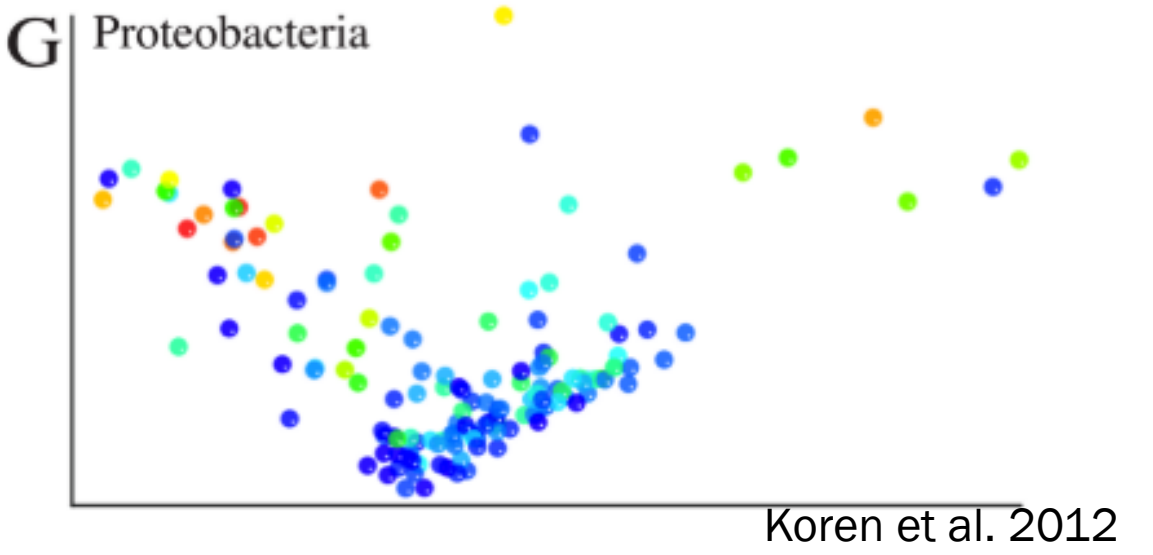
More Proteobacteria at T3



Koren et al. 2012

Maternal gut changes during pregnancy

More Proteobacteria at T3



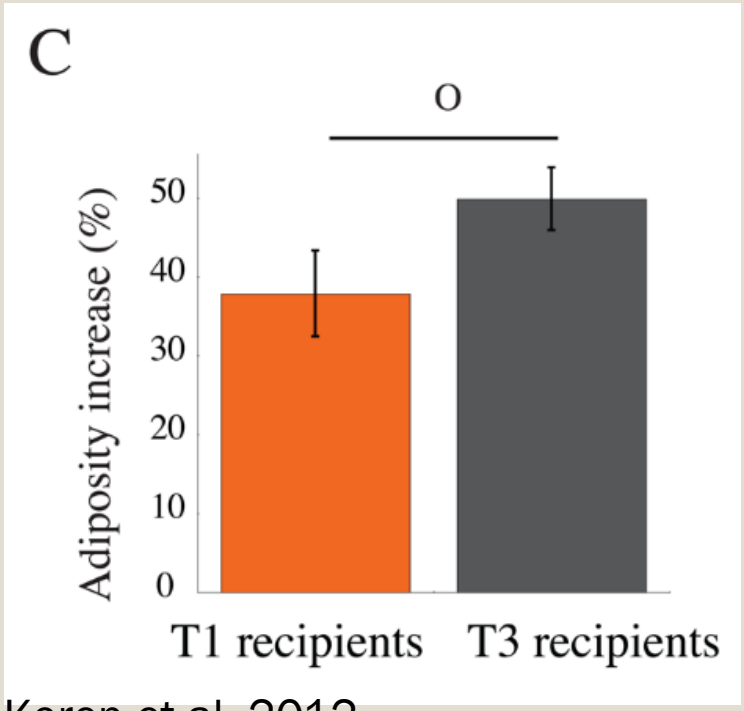
Koren et al. 2012

- Proteobacteria is a phylum of very diverse bacteria
- Generally the host-associated ones are good at:
  - Interacting with host epithelial and immune cells
    - In good and bad ways
  - Scavenging oxygen from low-oxygen places
  - Eating mucin (mucus)

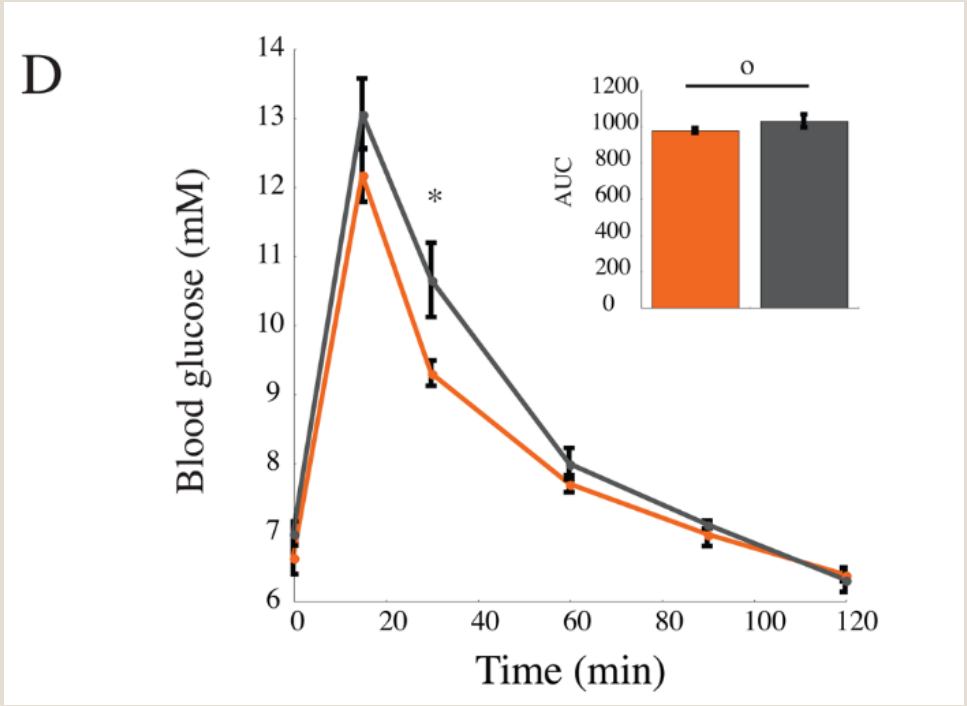
Maternal microbial community changes host metabolism

- Transfer of feces from pregnant women at T1 and T3 to mice

Increased fat deposition in mice



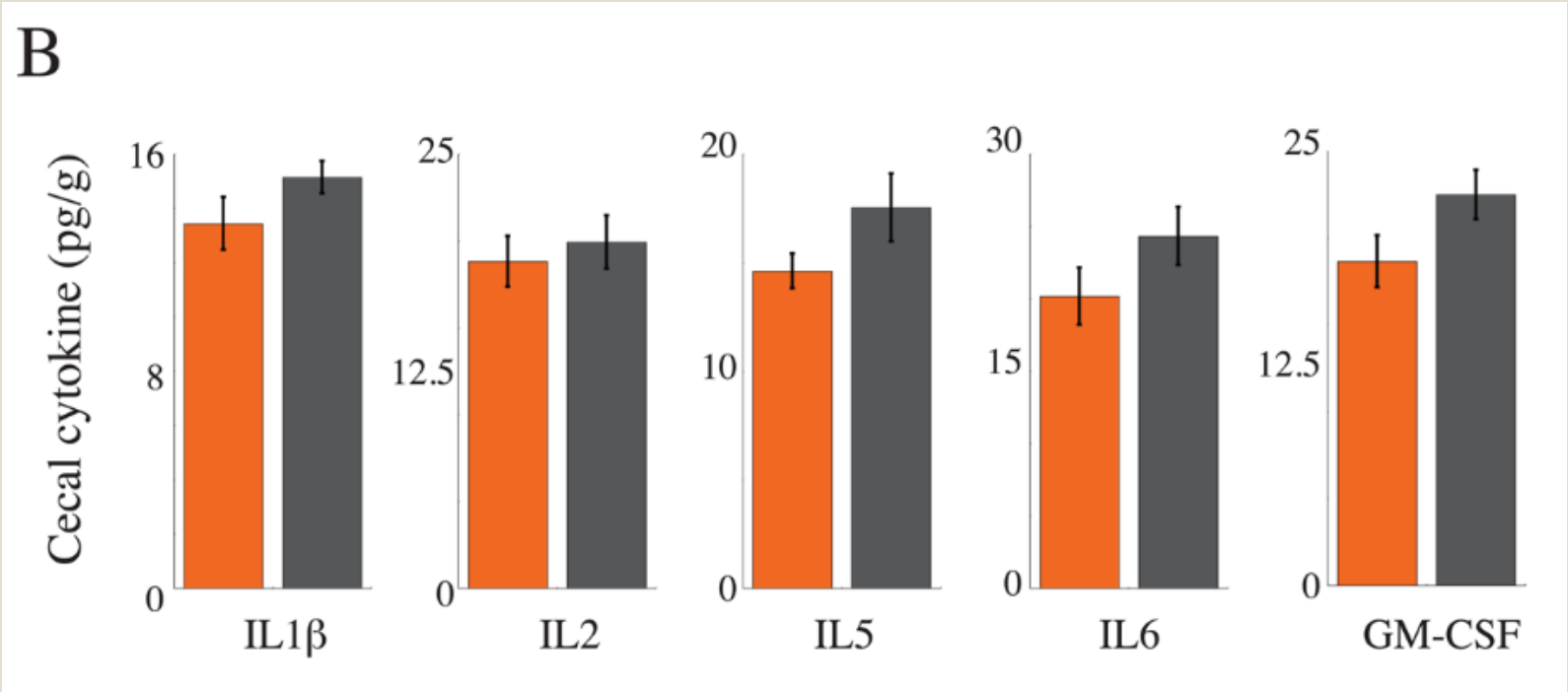
Increased blood glucose in mice (more in circulation and available for tissue growth)



Koren et al. 2012

Maternal microbial community changes host immune system

- Transfer of feces from pregnant women at T1 and T3 to mice increased expression of inflammatory factors



Koren et al. 2012

Benefits of maternal gut microbial change during pregnancy

- Increased feed efficiency and weight gain for mother
  - *Need extra 300 cal/day in T3 and 600 cal/day when lactating*
- Help offspring immune system learn to tolerate microbes
  - *Increase abundance of bacteria that thrive in a human host*
  - *Passed to offspring in utero, during labor, breastfeeding, contact*
- Pass on microbes that are good at interacting with the host directly
  - *Interact with host tissue surfaces (along GI tract epithelia cells)*
  - *Can turn host immune system on or off*
  - *Critical time period in first few weeks of life to learn this*
  - ***More on this in future lectures***

Vertical transmission in animals

- **Vertical transmission – transmission (of something) from female to offspring during gestation or birth**
  - *Microorganisms (maybe infectious)*
  - *Genes (and phenotypic traits)*
  - *Mitochondrial genes (and phenotypic traits)*

- Transferred via
  - *Placental transfer*
  - *Milk*
  - *Direct contact during birth*
    - Often via blood

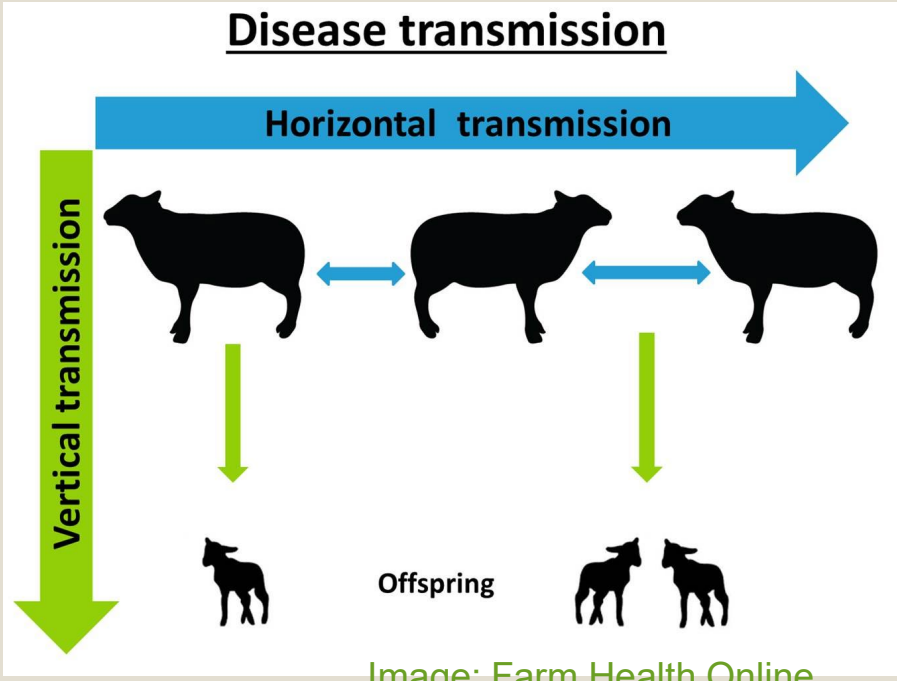
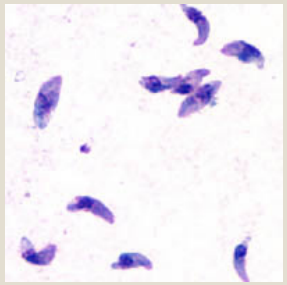


Image: Farm Health Online

IN UTERO TRANSMISSION OF MICROBES AND CONSEQUENCES

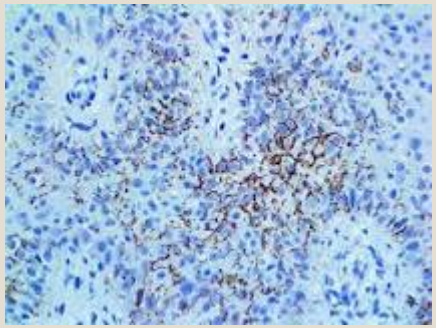
Placenta was presumed to be sterile

- Initial interest in transfer of pathogens
  - *Viruses*
  - *Toxoplasma gondii (protozoa parasite)*
  - *Listeria monocytogenes*
  - *Treponema pallidum (syphilis)*
- Unclear if beneficial microbes might cross as well



Toxoplasma gondii, Wikipedia

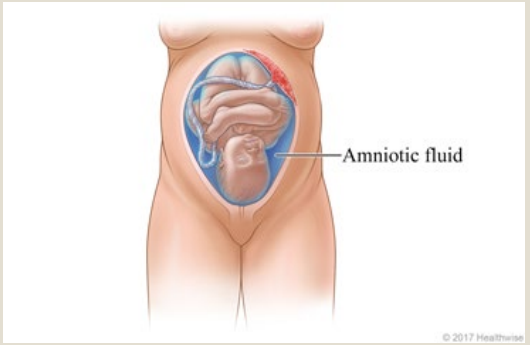
Listeria monocytogenes, Wikipedia



Treponema pallidum, Wikipedia

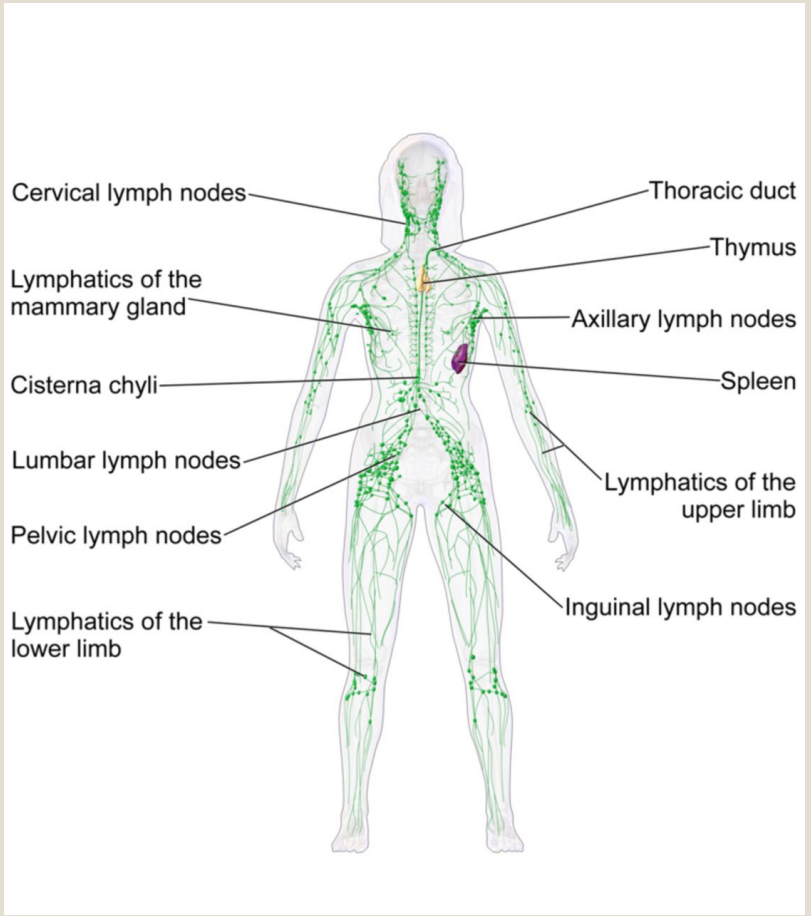
We now know, *in utero* environment not sterile

- Live bacteria isolated from amniotic fluid in healthy mothers
  - *Also umbilical cord and placenta*
- Living bacteria isolated from meconium
  - *First fecal waste produced by fetus while in utero and expressed right before/after birth*
  - *Do not recommend image search :(*
- After ingestion of genetically-labelled *Enterococcus faecium* by pregnant mice, same bacteria could be found in offspring meconium
  - *Reviewed by Francino 2014*



Possible means of microbial transit from maternal tissue to fetal tissue

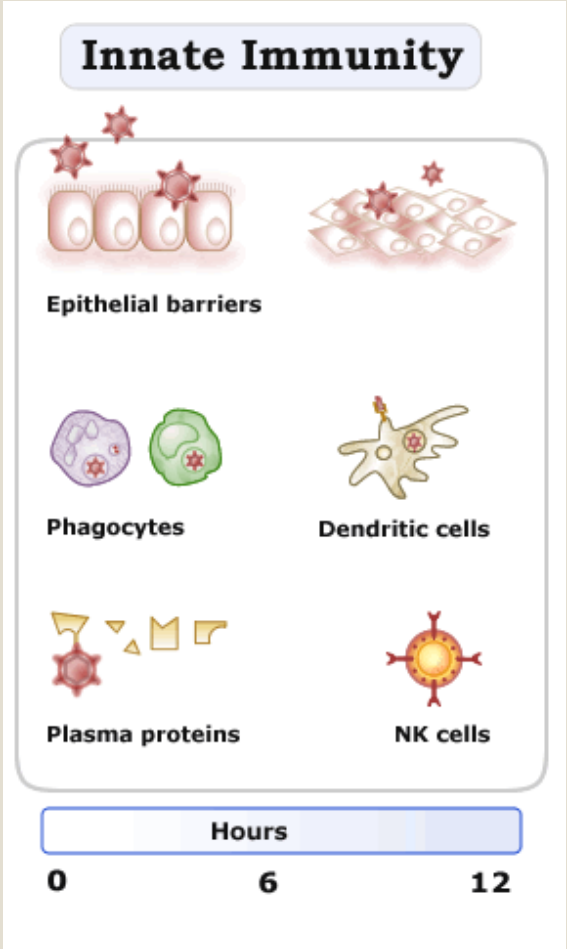
- Bacterial translocation from the gut
  - *to mesenteric (digestive tract) lymph nodes*
  - *Lymph system empties into circulatory system*
  - *takes place during late pregnancy*
- Maternal immune cells have been shown to carry whole bacterial cells or bacterial DNA
  - *Specifically peripheral blood mononuclear cells*
  - ***Bacterial cells in lymph might be using host immune cells for transport through the body***
- Perez et al. 2007



Organ Systems by Eli Wilson

Effects of transplacental exposure of microbes

- Lactobacilli in the placenta induce a systemic immune response in neonates after preterm delivery
  - *Jumpstart immune system?*
- DNA from nonpathogenic bacteria detected in the placenta increased innate immunity in the fetal gut
  - *Jumpstart immune system?*
  - *Innate immunity is ready immediately, doesn't need to learn good or bad*
    - Creating barrier to keep microbes out of your tissue
    - Inflammation to recruit immune cells
    - Fever
    - Antimicrobial proteins
- Discussed in Rautava et al. 2012



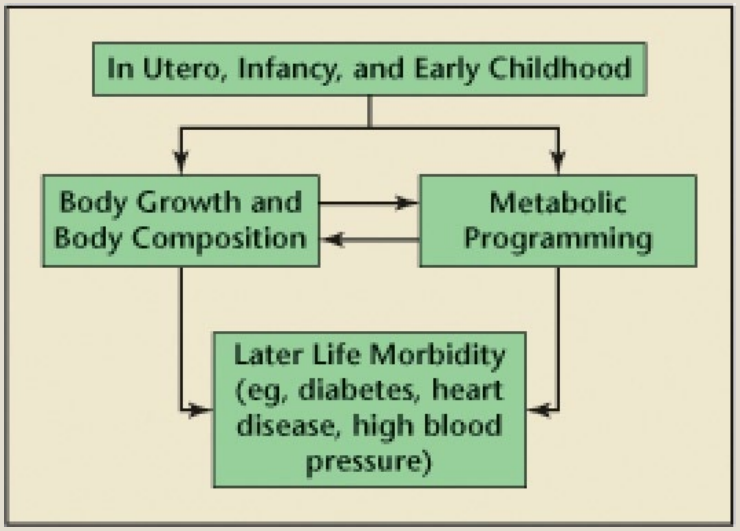
The innate and adaptive immune system

Effects of transplacental exposure of microbes

- Maternal obesity can change gene expression in the placenta
  - *Changes how mother/fetus recognize microbes and label them as "good" or "bad"*
  - *In science terms: modulate innate microbial pattern-recognition receptor gene expression in the placenta in an experimental sheep model*
  - *Result: increased inflammation in the placenta*
  - *"Collectively, these emerging new data suggest that manipulation of maternal microbial exposure during pregnancy might provide a novel means to modulate early immune development and to reduce the risk of immunoinflammatory or metabolic disease both in the mother and the child " - in Rautava et al. 2012*
- Discussed in Rautava et al. 2012

Maternal obesity alters gut microbiota of infants

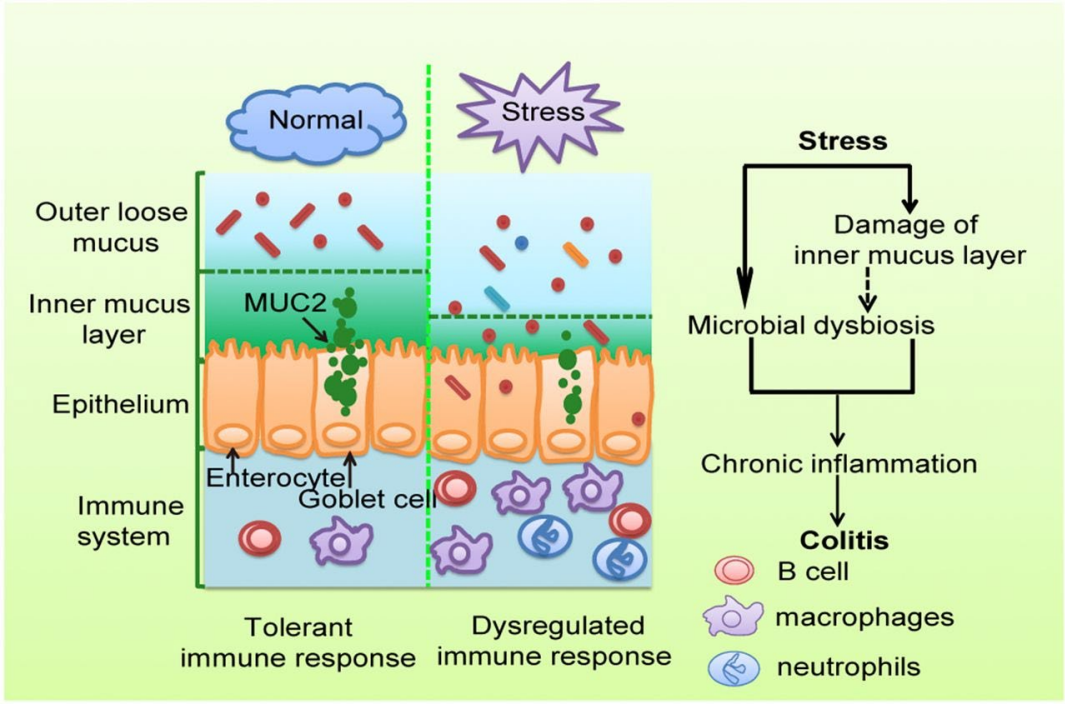
- Maternal obesity
  - *decreased Bifidobacterium longum (good bacteria)*
  - *Increased Clostridioides difficile (bad bacteria)*
  - *Collado et al. 2010*



Leddy et al. 2008

Definition of stress

- Physiological (stress, disease, infection, malnutrition, pregnancy, obesity) or psychological
  - *Body responds in similar ways*
  - *Changes hormones, immune system vigilance*
    - These change how you interact with microbes



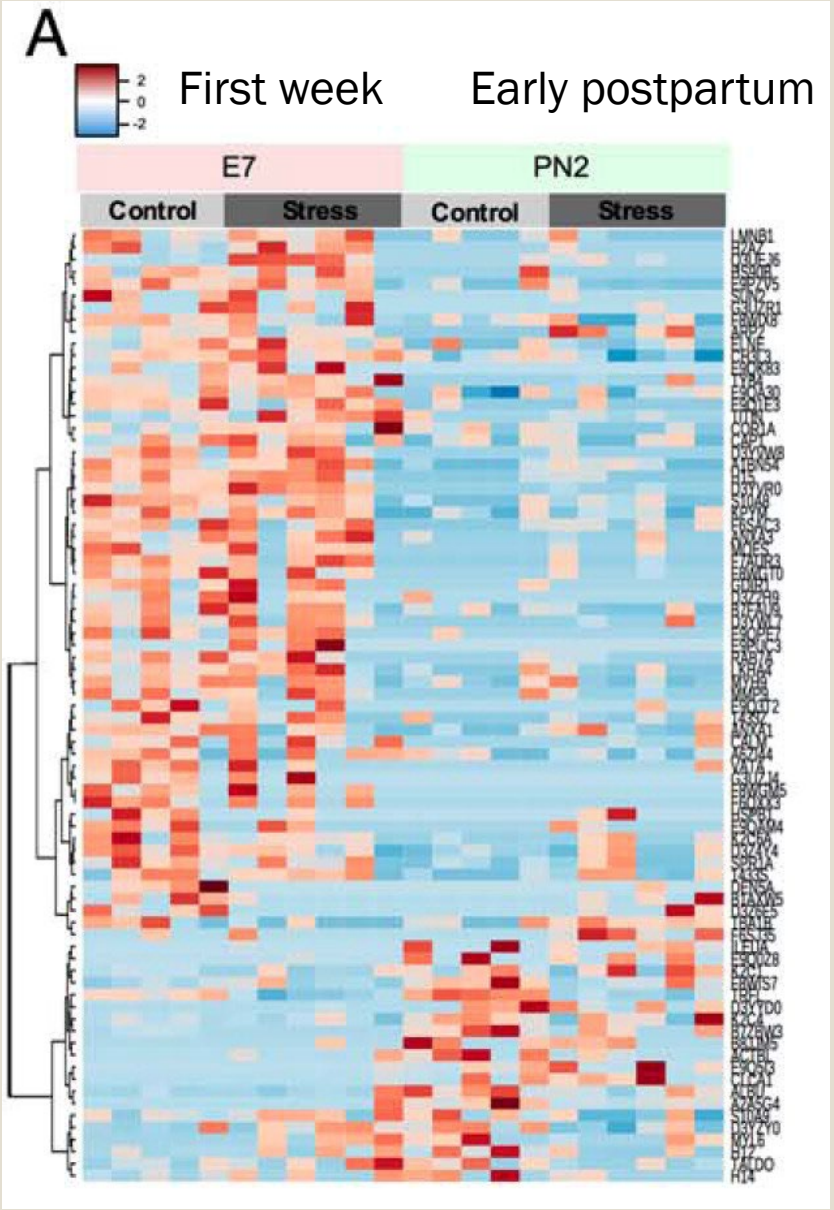
Chronic stress promotes colitis by disturbing the gut microbiota ...PNAS

Early-Pregnancy Stress changes maternal cell gene expression in vagina (in mice)

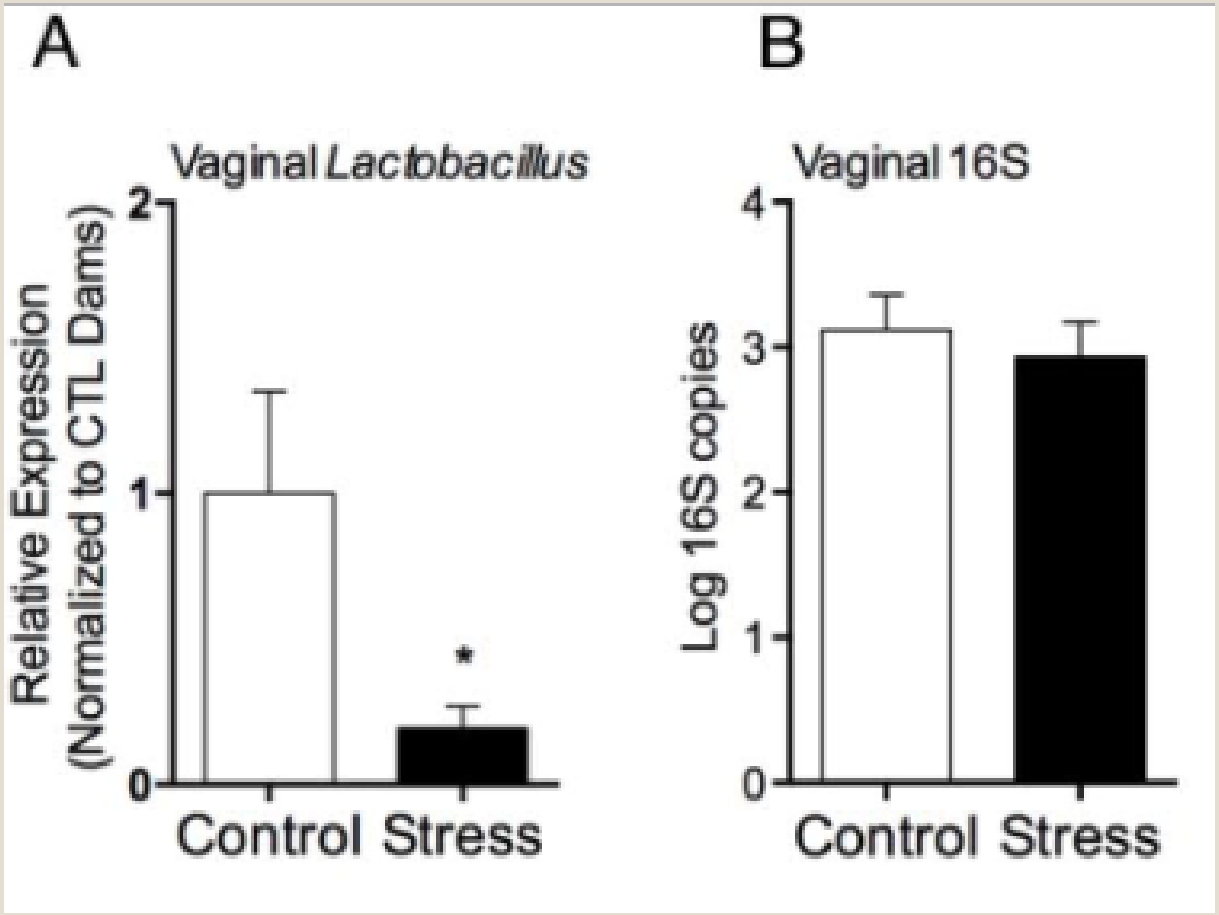
Blue: mouse gene expression decreased

Red: mouse gene expression increased

Jašarević et al. 2015

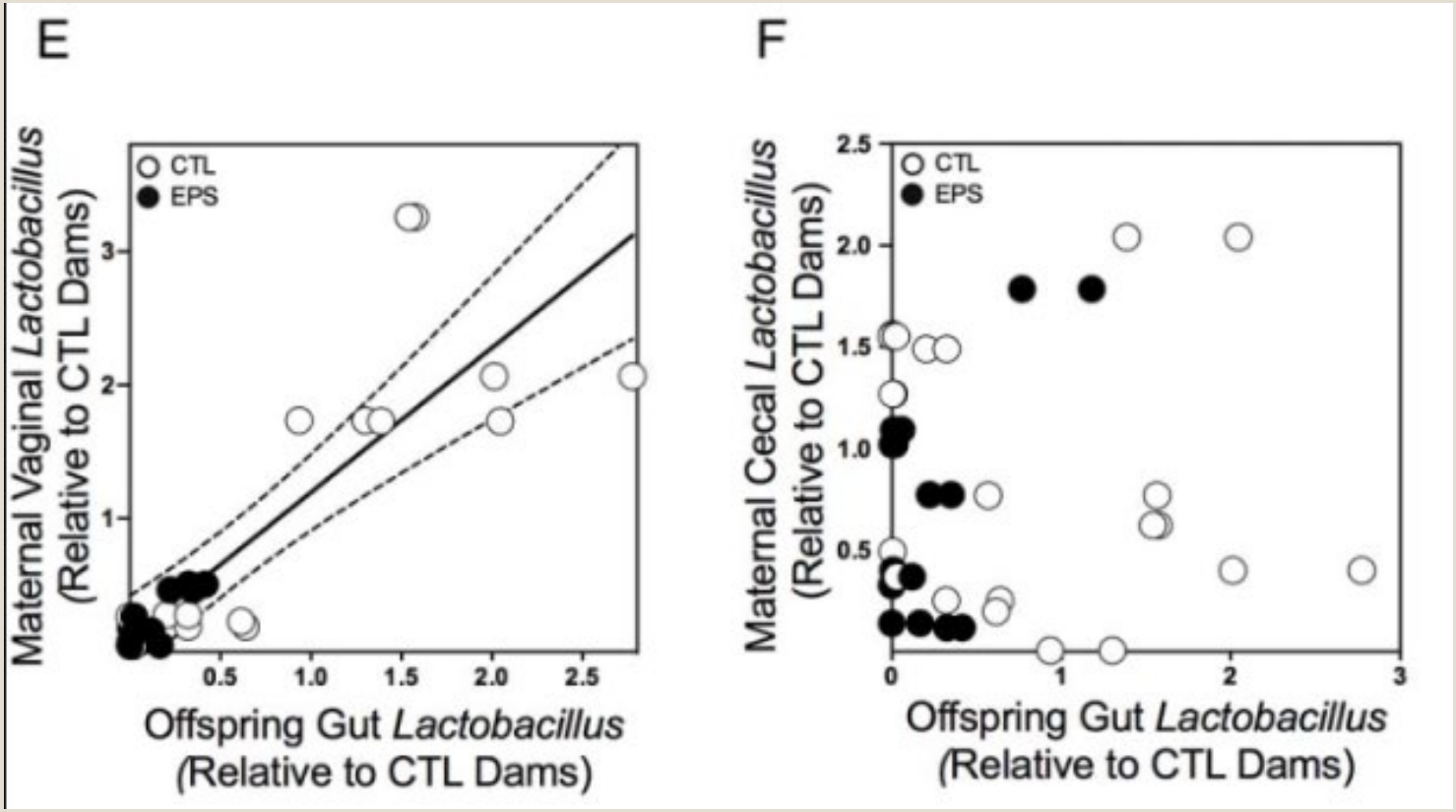


Stress reduces lactobacilli, but not all bacteria, in postpartum mice



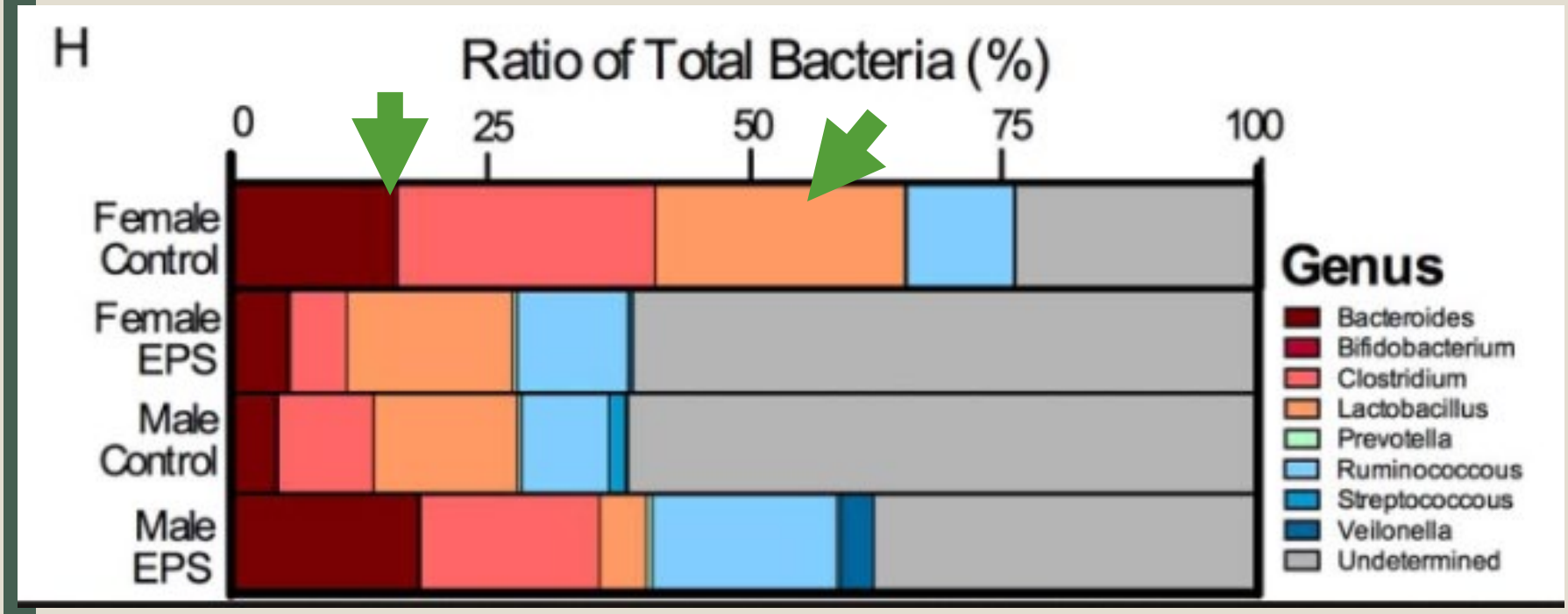
Jašarević et al. 2015

Vaginal lactobacilli related to offspring fecal lactobacilli (not maternal fecal lactobacilli)



Jašarević et al. 2015

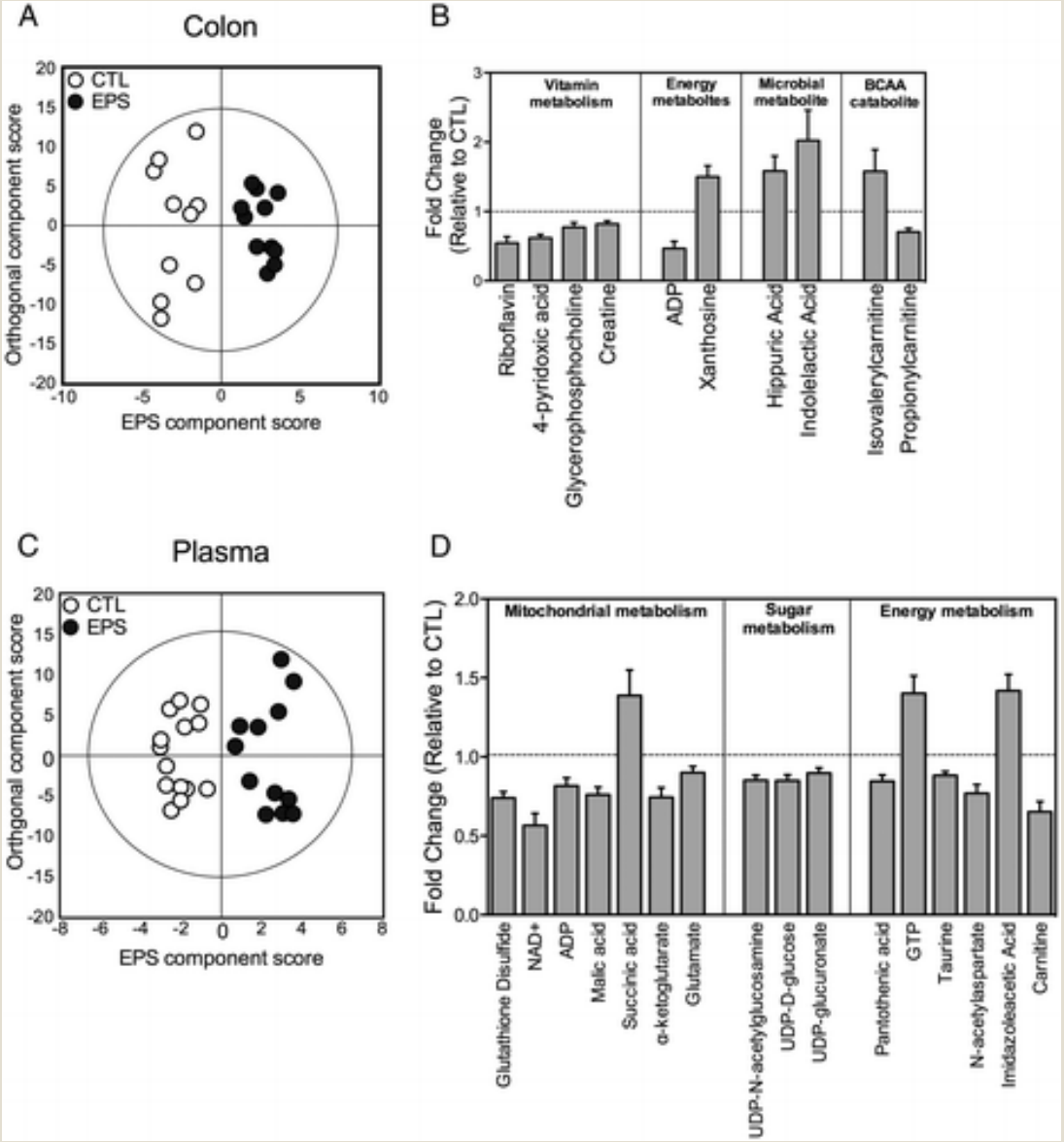
Early pregnancy stress (EPS) to dam altered gut bacteria in offspring – differently by sex



Jašarević et al. 2015

Early pregnancy stress (EPS) alterations to the community also alters function

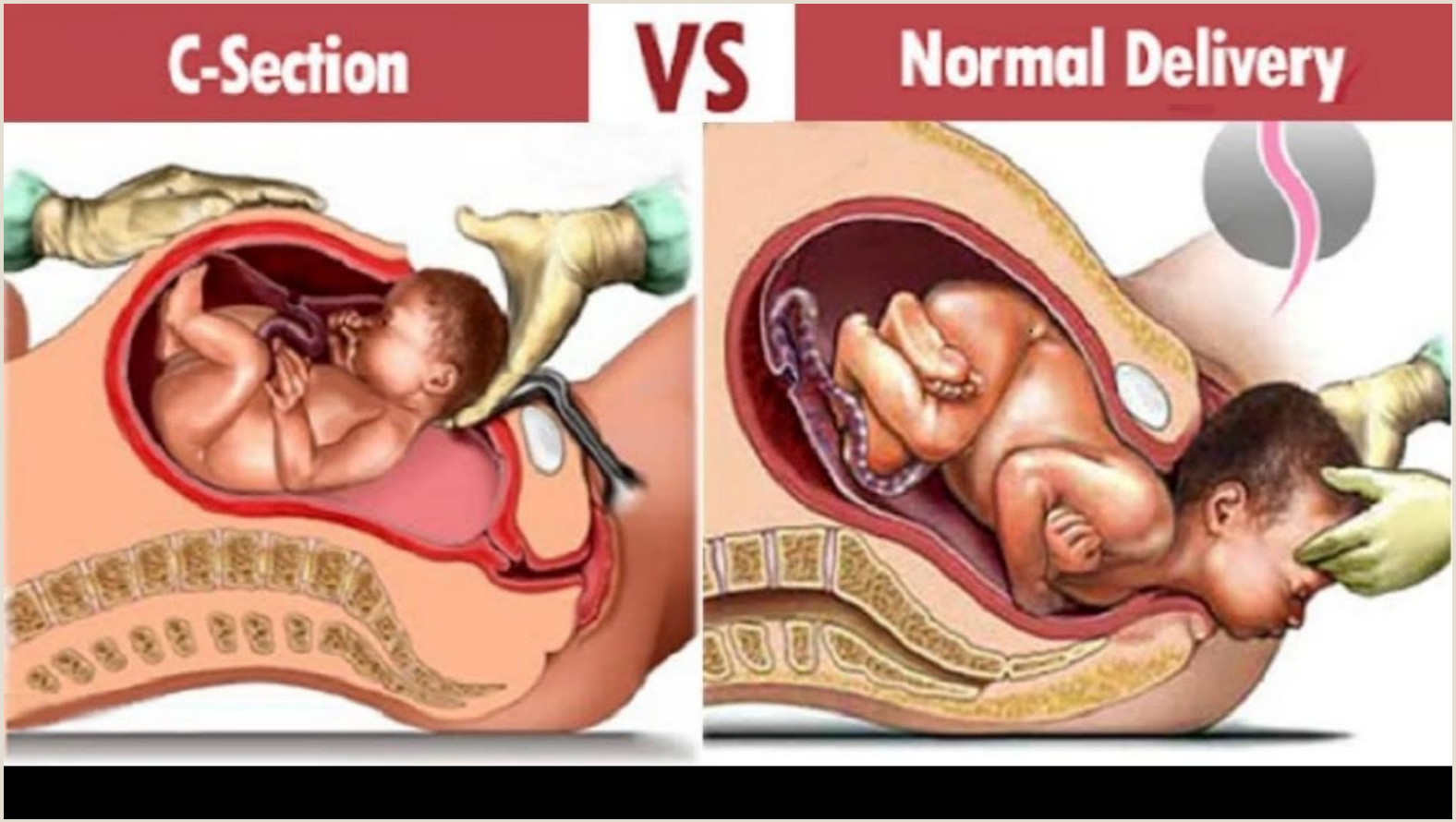
and metabolites they produce



Jašarević et al. 2015

BIRTH MODE AND MICROBIAL EXPOSURE

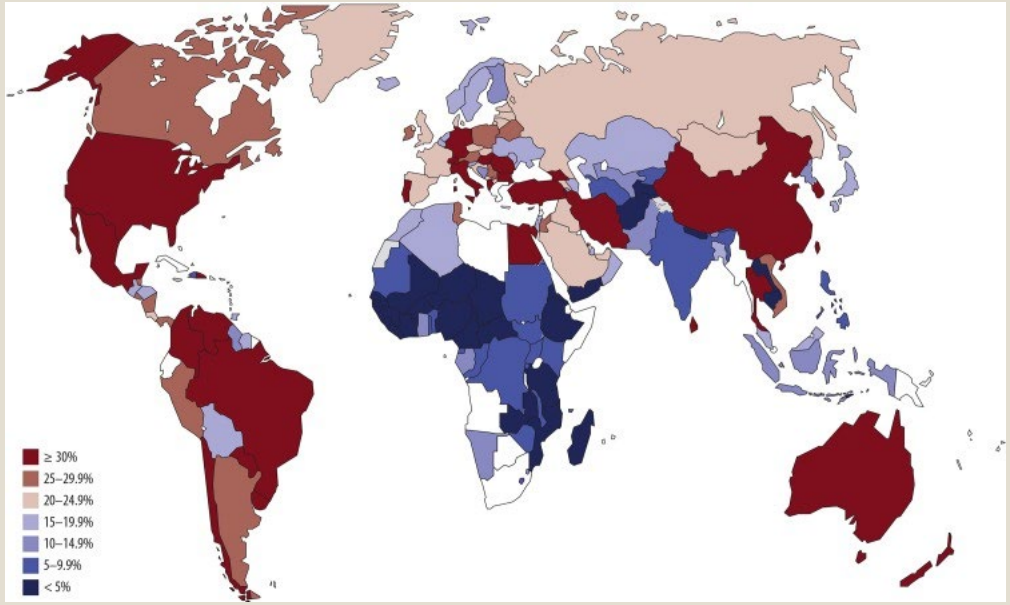
Birth mode



Reasons for C-section

- Medically indicated (planned or emergency)
  - *These are life saving and no one is arguing against them*
  - *Rates remain pretty stable*

- Non-medically indicated (elective)
  - *Gaining in popularity globally*
  - *Maternal request not as popular as you'd think*
  - *Influence of doctor suggestion*

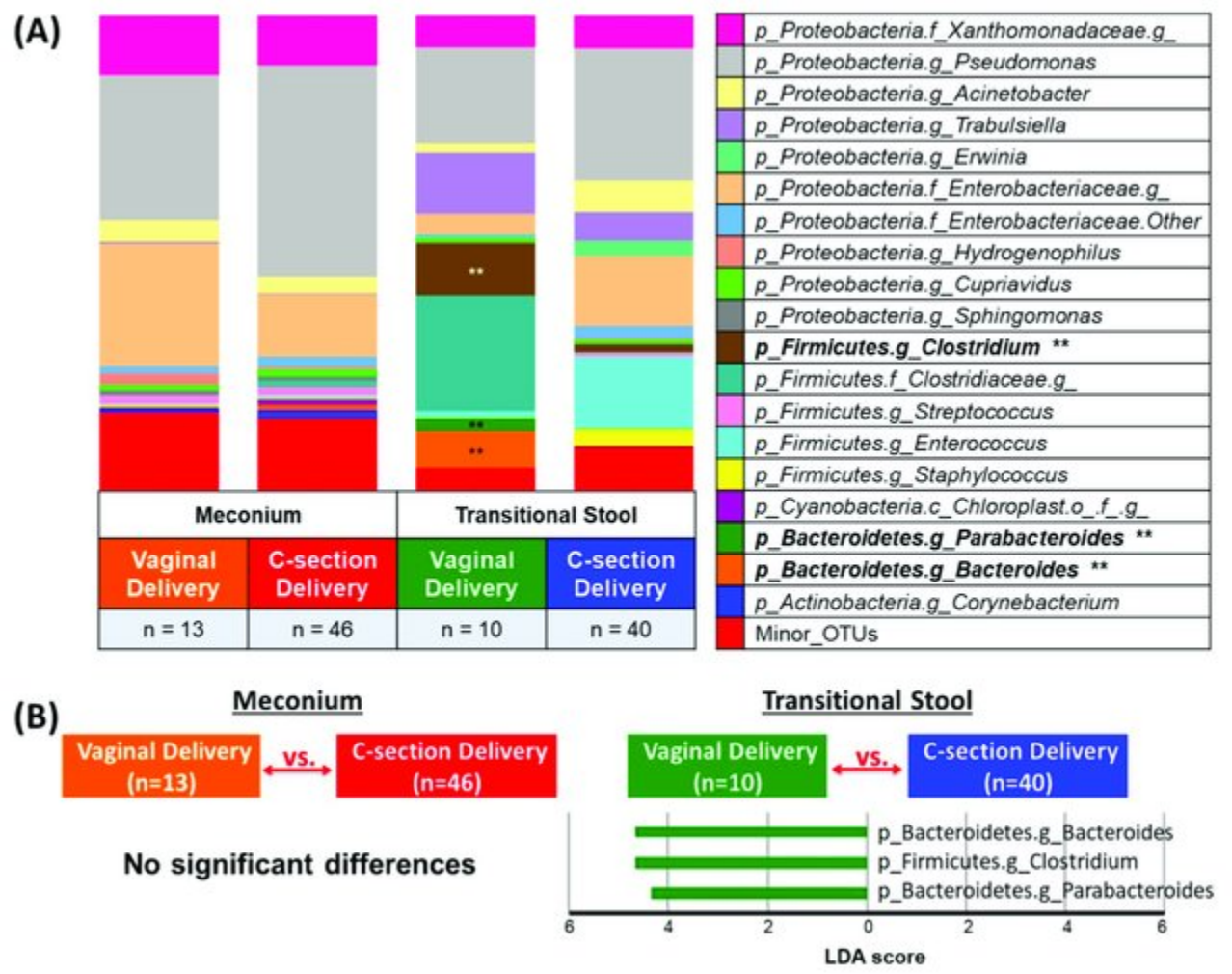


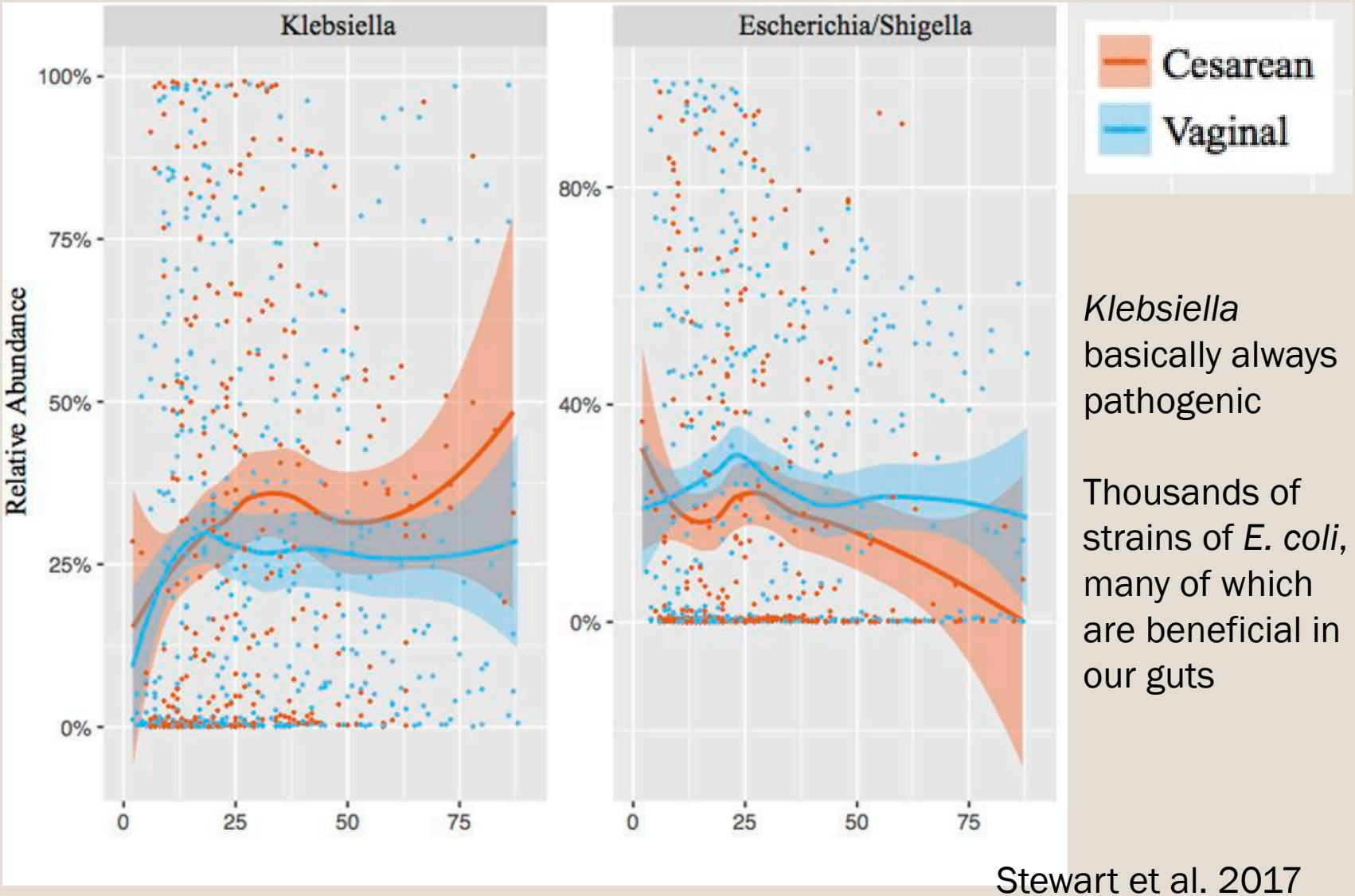
Betran et al. 2016

Vaginal delivery increased certain bacteria in infant feces, took several days to manifest

Meconium = first stool that develops while in utero from digested intestinal cells and mucus

Mueller et al. 2017





Klebsiella basically always pathogenic

Thousands of strains of *E. coli*, many of which are beneficial in our guts

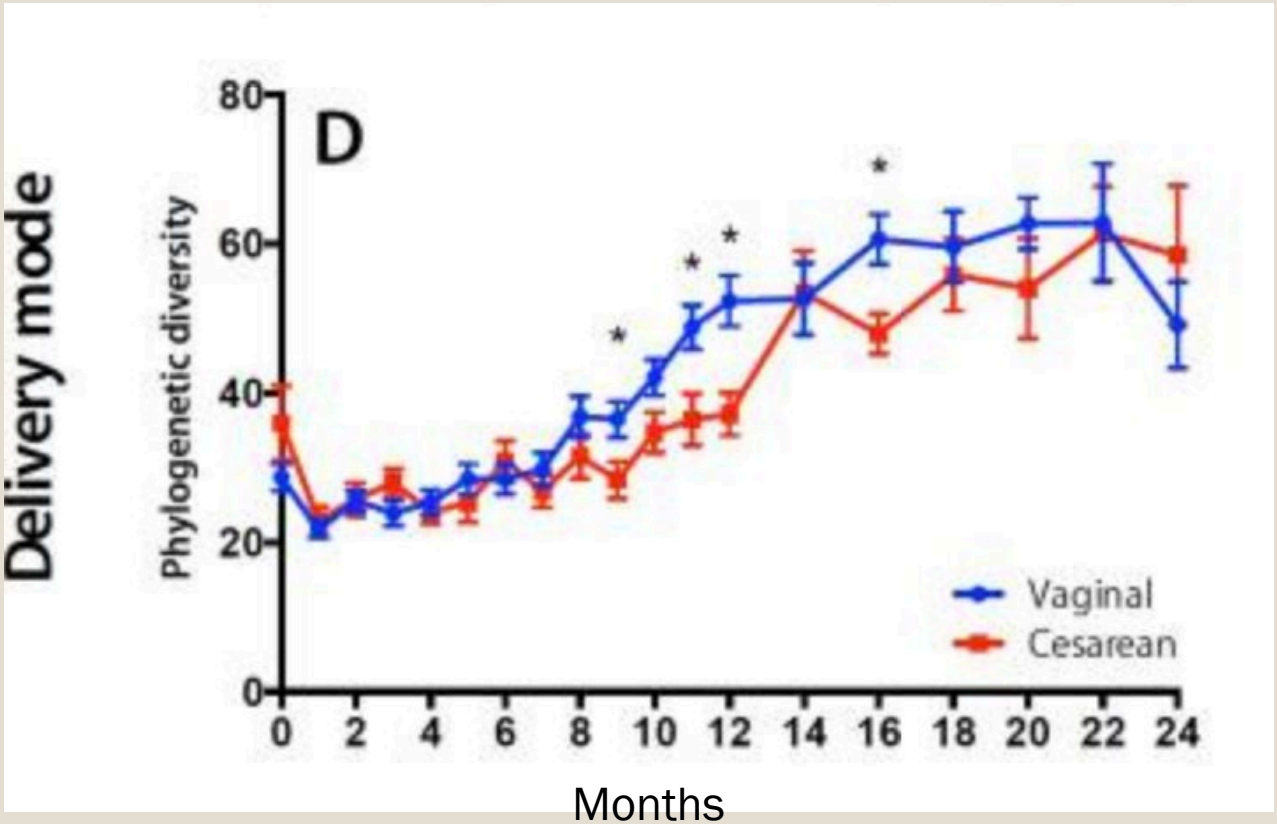
Stewart et al. 2017

Staphylococcus not that helpful in gut

Bifidobacterium extremely important in infant gut (more next lecture)

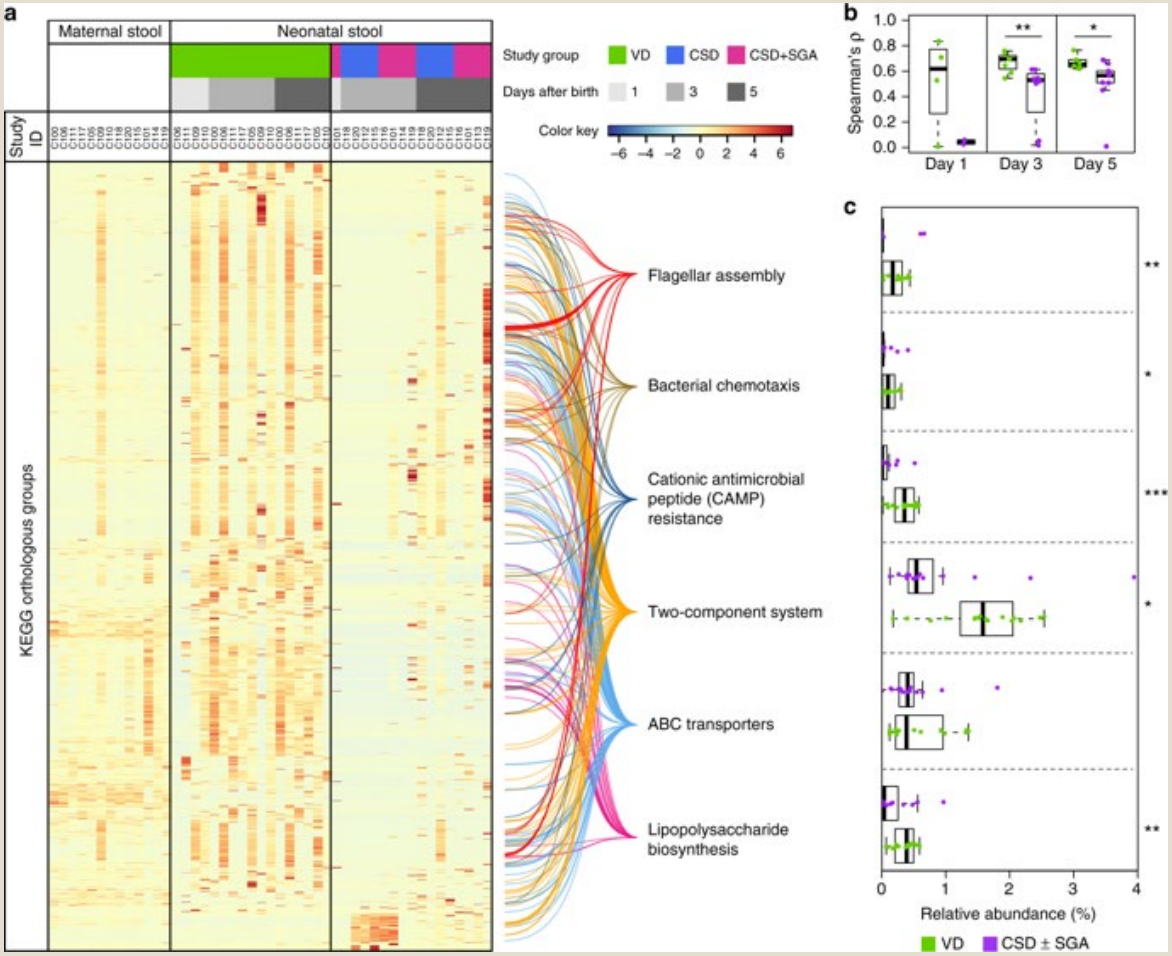
Stewart et al. 2017

Delivery mode can lead to long-term reductions in gut bacteria diversity



Bokulich et al. 2012

Birth mode affects functionality of gut bacterial community in neonates



Wampach et al. 2019

Birth mode and community function
Wampach et al. 2019

- Vaginal birth increased lipopolysaccharide (LPS) production in neonate gut
  - *Indicates more gram negative bacteria growth*
  - *Gram- linked to important microbial exposures that helps train immune system*

- LPS was extracted from neonate feces, and given to human cell culture to test immune reaction
  - ***LPS from vaginally-delivered neonate gut increased cell production of immune factors***
  - *tumor necrosis factor (TNF-α) and interleukin 18 (IL-18)*
    - Went back and found TNF-α and IL-18 higher in vaginally-delivered neonatal blood plasma

Summary of health outcomes for offspring by birth mode

- Affect on offspring physiology
  - *hormonal, physical, microbial, medical interventions*

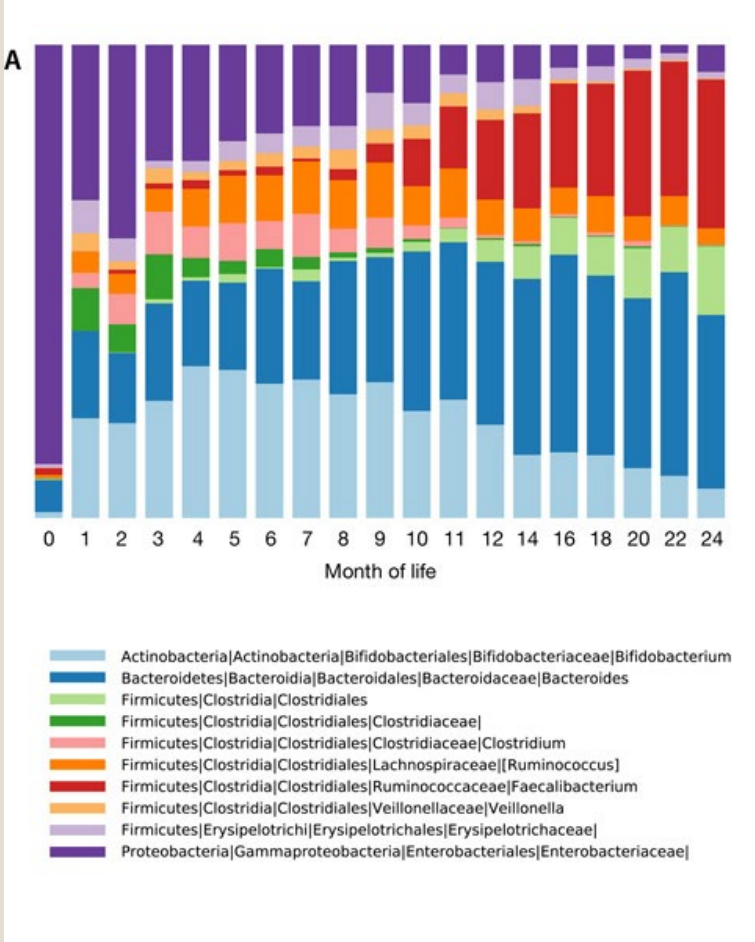
- Altered immune development
  - *increased likelihood of allergy, atopy, and asthma,*
  - *reduced intestinal gut microbiome diversity*

- Collective effect on metabolism
  - *greater incidence of late childhood obesity and asthma*
  - *cognitive and educational outcomes??*

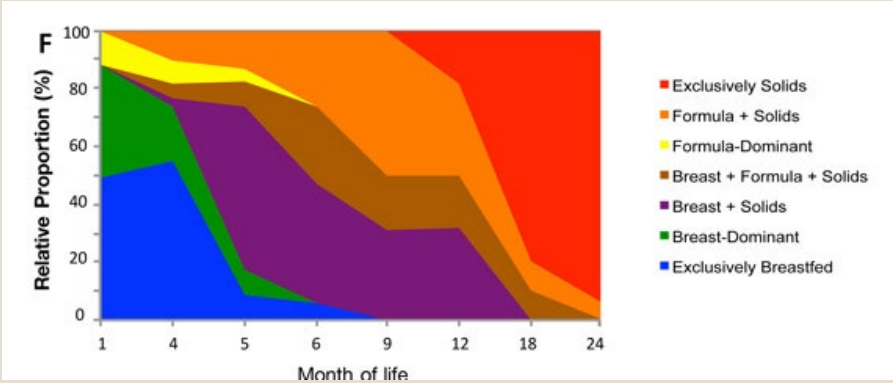
Discussed in Sandall et al. 2018

CAN WE RECOVER MICROBIAL DIVERSITY IN C-SECTION BABIES?

Gut microbes in early life change dramatically

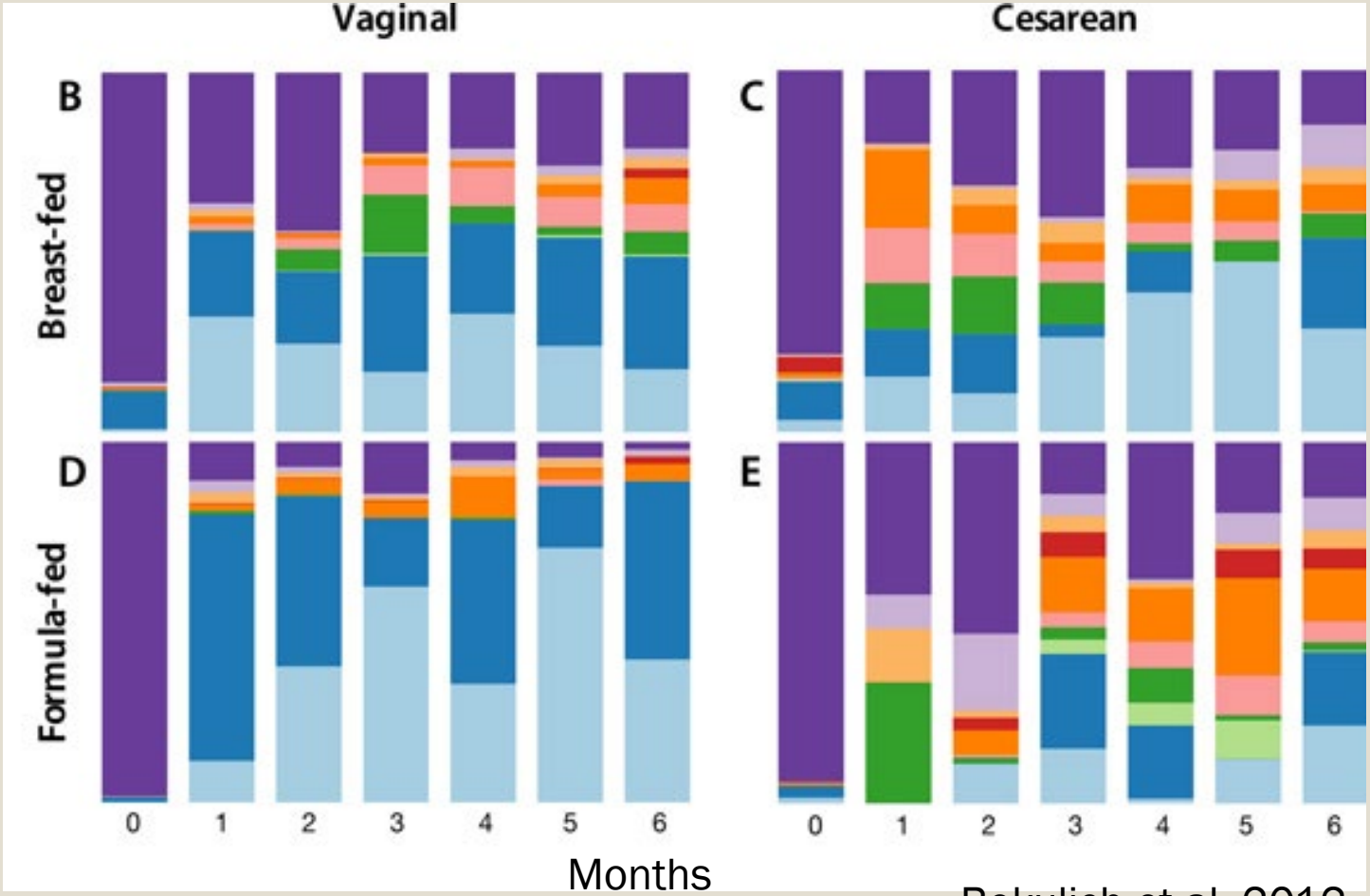


Change in first 6 months largely due to dramatic shifts in diet



Bokulich et al. 2012

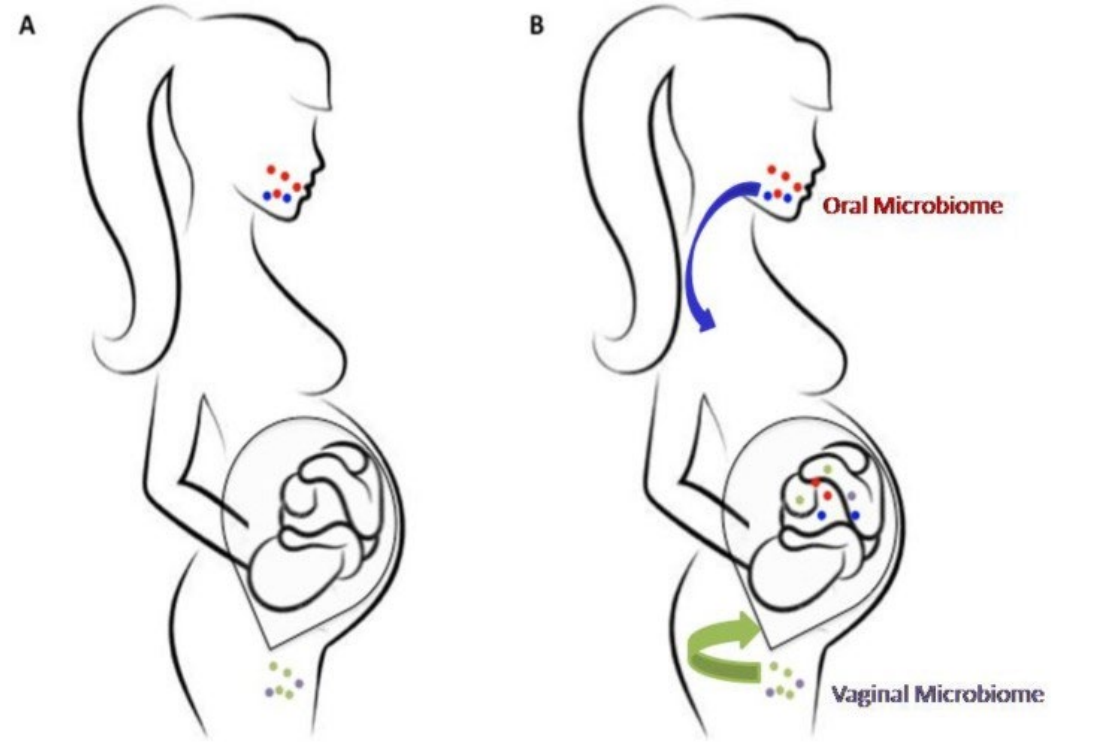
Effects of birth mode on gut microbes in offspring over first 6 months compounded or ameliorated by diet



Bokulich et al. 2012

The Prenatal Microbiome: A New Player for Human Health

Valeria D'Argenio



Generational health

- Stress and access to health care affects
  - *Maternal health*
  - *Offspring health*

- Successive generations of stressed mothers may cause dramatic shifts in microbial-immune system based conditions across demographic lines
  - *Obesity*
  - *Irritable bowel*
  - *Asthma*
  - *Allergies*

Gut microbiomes can be inherited

- Observed in 11 generations of mice in a laboratory
  - *Moeller et al. 2018, news post: https://www.the-scientist.com/news-opinion/mouse-microbiomes-are-mostly-inherited-64998*

- Discussed in **Generational Patterns of Stress: Help From Our Microbes?,** Bridget L. Callaghan

Summary

- Many factors alter transfer of microbes in pregnancy and birth
  - *Maternal heath and weight*
  - *Maternal stress*
  - *Birth mode*
  - *Amount of contact*
  - *Antibiotic use*
- Exposure to microbes in utero and first 6 months is extremely important (more on this in future lecture)
  - *Train immune system to tolerate microbes*
  - *Provide microbes that can turn down immune system*
  - *Provide microbes that can survive in neonate host*
  - *Provide microbes that can help with digestion*

SOCIAL IMPLICATIONS

Cost savings of prenatal care

- Prenatal care reduces the risk of a low birthweight infant
  - *Low birthweight increases morbidity and mortality*

- Henderson 1994:
  - "Females delivering normal-sized babies had lower cesarean-section rates than females delivering extremely low-birth-weight babies"
  - Better care means healthier fetus and carrying to term

Cost savings of prenatal care

- Henderson 1994:
- In 1989 in Texas: “net expected hospital cost savings for females who received prenatal care was over $1,000”
- “Annual cost savings of adequate prenatal care is approximately $230 per mother (1986 dollars). This includes the cost savings from neonatal intensive care and rehospitalization within the first year.”
- ”a Virginia program, if adopted statewide, could save the State almost $50 million annually by reducing the incidence of certain types of mental retardation due to low birth weight. If this program were adopted nationally, it would save between $14,000 and $30,000 for every low-birth-weight baby avoided.”
- **“estimated that for every dollar spent on prenatal care, $3.38 is saved in the cost of caring for low-birth-weight infants.”**

Barriers to prenatal care in urban women

Topic area	Themes and subthemes
Barriers	***Caregiver qualities***
	• Too busy/lack of time
	• Negative personality characteristics (e.g., rude, judgmental)
	Health care system barriers
	• Lack of public awareness of PNC services
	• Shortage of health care providers who provide PNC
	Personal barriers
	• Logistical difficulties related to transportation and child care
	• Financial problems
	• PNC not viewed as a priority, no interest, not seen as important
	• Previous negative experience with/distrust of health care system
	• Personal pressures (e.g., addictions, intimate partner violence)
	• Lack of social support
	Program and service characteristics: Inaccessible and/or inconvenient
	• Geographic distance
	• Lengthy office wait
	• Short visits; rushed appointments
	• Inflexible or inconvenient hours

Heaman et al. 2015

Those who need PNC the most aren't getting it

- Overall, ~75% of women in US get PNC in first trimester
  - *63% Black women*
  - *67.4% Hispanic women*
  - *(United States Department of Health and Human Services [US DHHS], 2011)*

- Increased use of PNC linked to
  - *Availability of insurance*
  - *Expansion of Medicaid coverage (Krans & Davis, 2012).*

  - *Gennaro et al 2017*

Those who need PNC the most aren't getting it

- Gennaro et al 2017:
- "Minority women are very likely to experience stress with 46.4% of Black women having high stress levels in one recent study (Gennaro, 2008)."
- "increased trait anxiety in Black women was also found to be related to preterm birth (Catov, Abatemarco, Markovic, & Roberts, 2010)"
- 85% of otherwise healthy Black pregnant women had increased depressive symptoms
- "Poor minority women are twice as likely to meet diagnostic criteria for major and minor depression during pregnancy as are middle class women (Grote et al., 2010)."
- "In both Hispanic women and Black women those who experience increased depressive symptoms have been shown to experience higher rates preterm birth (Field et al., 2006; Orr, James, & Prince, 2002)."

HOMEWORK

Homework

- **Reading (pick 2):**
  - *Neu_2011_birth mode hygiene hypothesis*
  - *Milcent_2018_prenatal care, socioeconomic, C-section*
  - *Mueller_2015_birth mode, maternal weight, gut microbes*
  - *Rautava_2012_pregnancy_colonization_disease*
  - *Sandall_2018_birth mode and health outcomes*

- More info: IMM_11_vaginal_microbiome_2019

Citations

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Srinivasan et al. 2010 https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0010197

Stewart et al. 2017 https://europepmc.org/abstract/pmc/pmc5459931

Wampach et al 2019 https://www.nature.com/articles/s41467-018-07631-x

ADDITIONAL SLIDES WITH MORE TECHNICAL INFORMATION

Humans and mice have same placental type but different placental structures

Mice: labyrinthine placenta
- two layers of syncytiotrophoblost cells no blood contact
- one layer cells in direct contact with maternal blood

Humans: villous placenta
- one layer of syncytiotrophoblast cells bathed in maternal blood

- Arora et al. 2017

Pronounced like “sin-sitch-eo-tro-pho-blast”

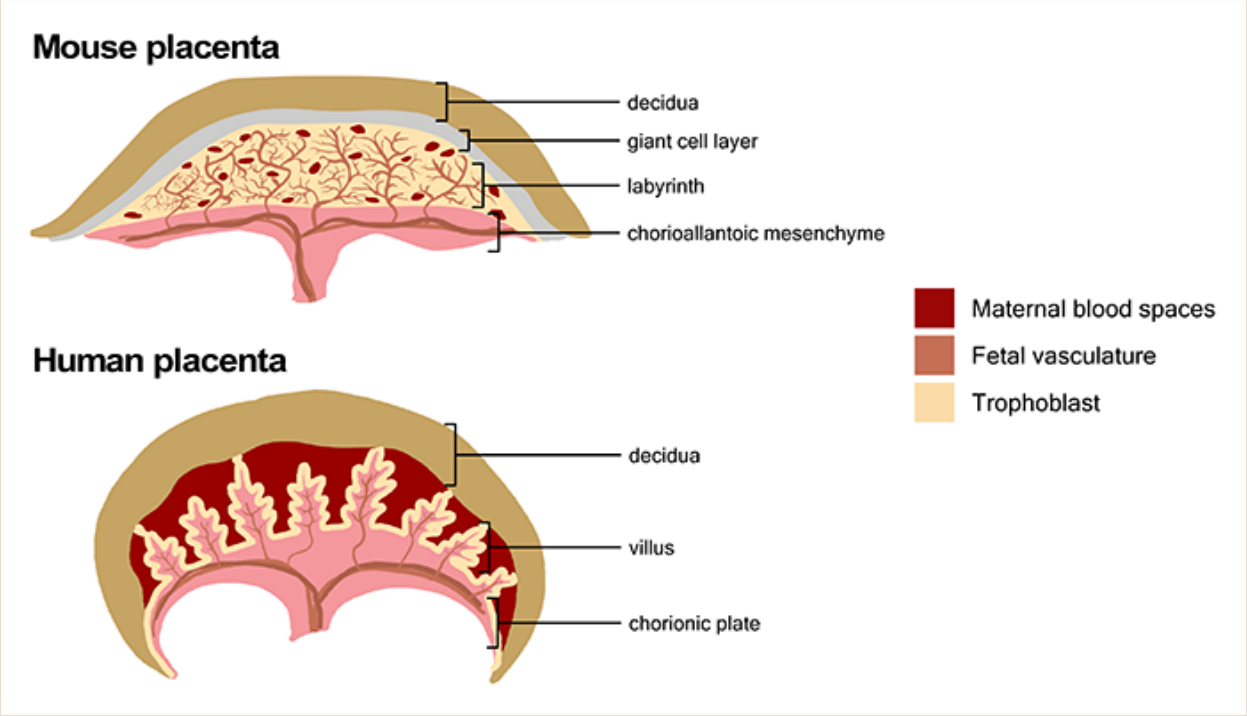


Image: katecholamine.org

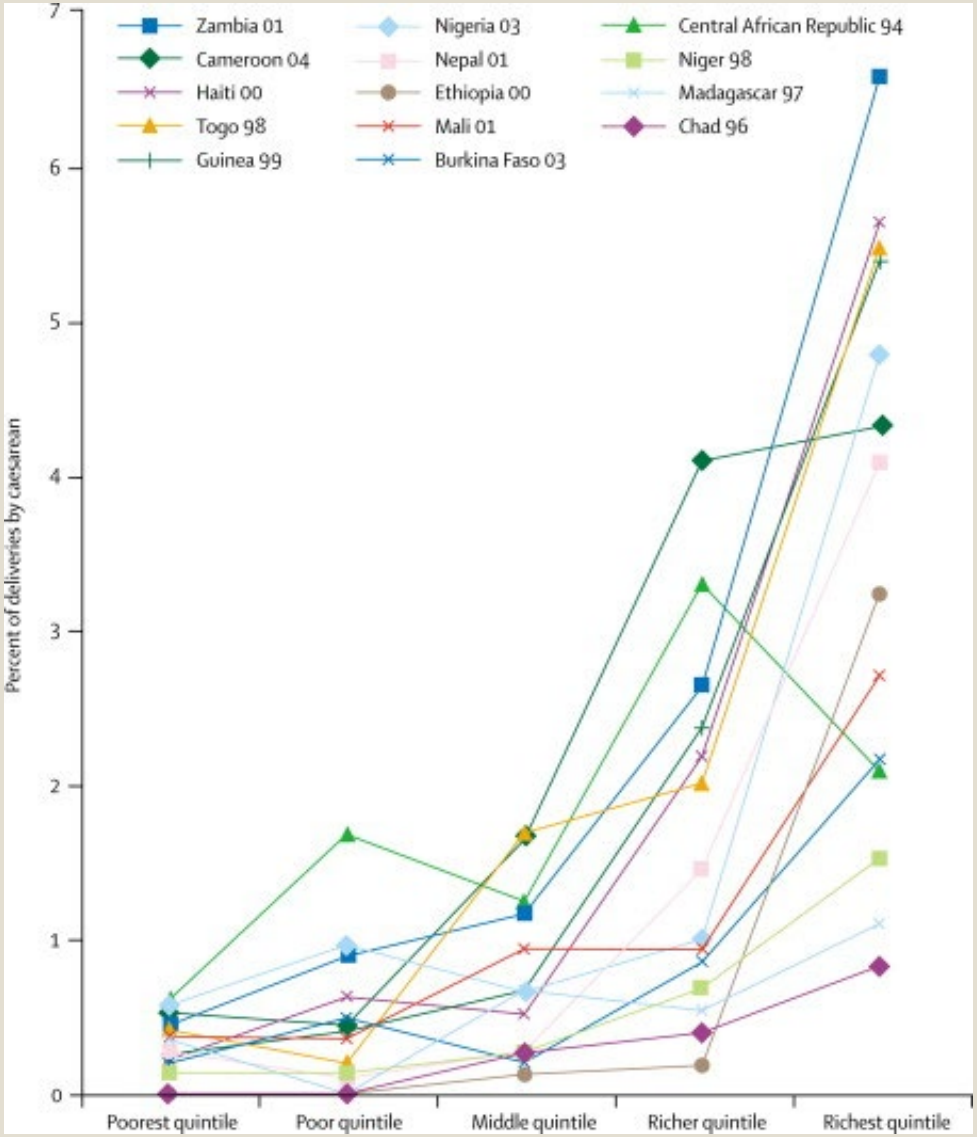
Syncytiotrophoblast layer resistant to viral and microbial transfer

- Mechanisms not very clear
- microRNAs (miRNAs) packaged in exosomes
  - *Containers outside the cell that release mini RNAs that target the RNA of invading cells and prevent those cells from getting anything done*
- type III interferons (IFNs)
  - *Proteins that inhibit viral replication*
- Unique physical properties that physically impede microbes
  - *dense, branched microvilli at the apical surface*
  - *complex cortical actin network*

- Syncytiotrophoblast resistant to infection, but other layers aren't
  - *As placenta thins during gestation, trauma, or secondary infection will make it vulnerable*
- Arora et al. 2017

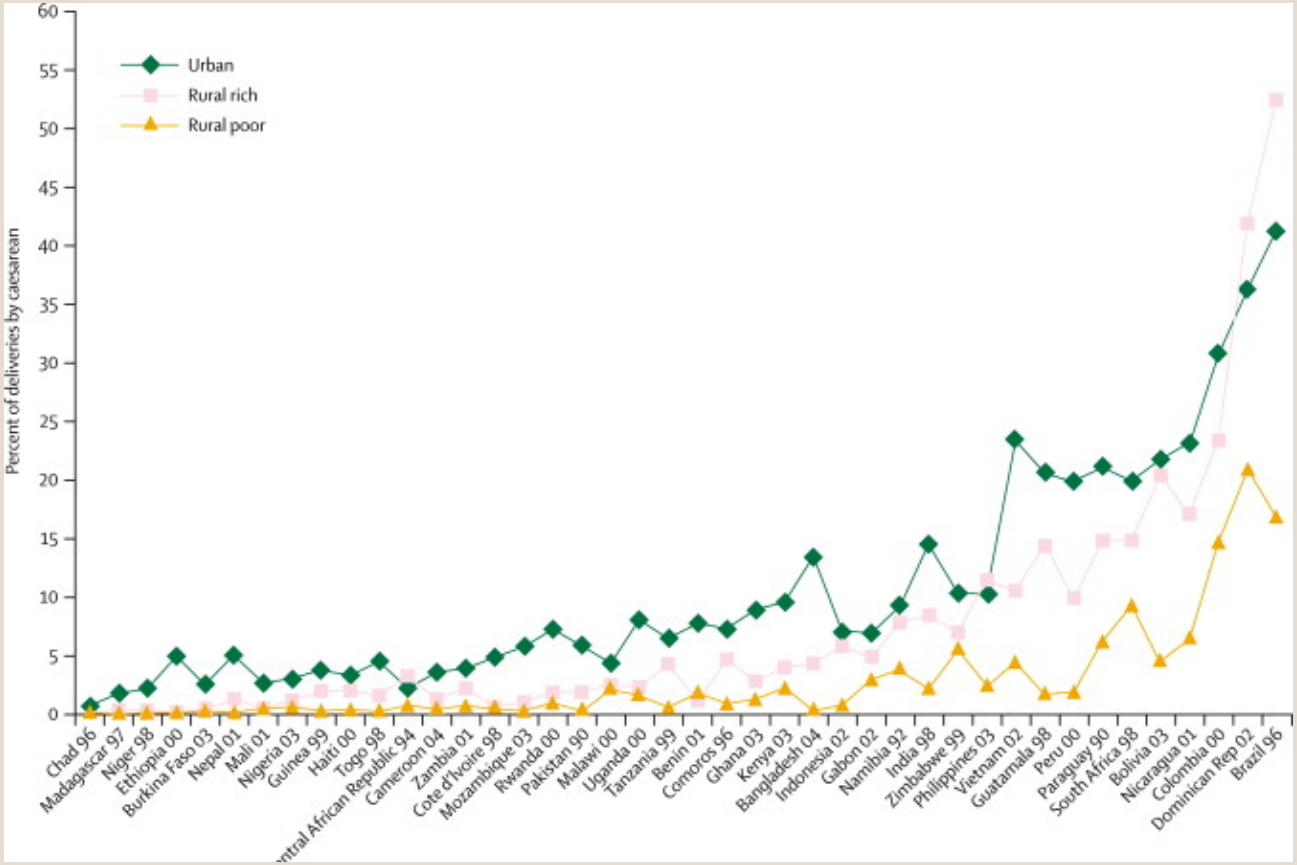
Elective C-section is increasing, but only at higher socioeconomic levels

- "developed" Räisänen et al. 2010 and "developing" Ronsmans et al. 2010



Ronsmans et al. 2010

Urban vs. rural differences in elective C-section trends



Ronsmans et al. 2010

Does it matter if it's elective or emergency C-section?

- Cohort of Vaginal, emergency C-section, elected C-section births
  - *Similar maternal BMI early in pregnancy*
  - Based on WHO 2006 Child Growth Standards, infant weight and length were computed into non–ethnic-specific BMI-for-age *z* scores (BAZs) using WHO Anthro software (version 3.2.2).[38] *At risk of overweight* was defined as a BAZ greater than 1 SD and less than or equal to 2 SDs and *overweight* was defined as a BAZ greater than 2 SDs.[39]

- Vaginal birth was the refence for overweight risk ratio (OR)
  - *Emergency C-section NOT associated with higher risk of being overweight at 12 months*
  - *Elective C-section associated with higher risk of being overweight at 12 months*

Table 2. Association of Delivery Mode

Delivery Mode	M C	Model 3[b] OR (95% CI)	*P* Value
Vaginal	1	1 [Reference]	
Emergency cesarean	1	0.95 (0.54-1.68)	.86
Elective cesarean	2	2.02 (1.05-3.89)	.04

Cai et al. 2018