A CENTRAL ROLE FOR MICROBIOTA IN PREGNANCY AND EARLY LIFE?

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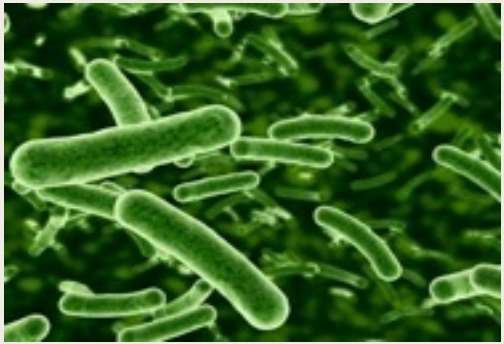
NASOM/GÉMOQ Annual Conference 2016



CHUM
Centre hospitalier de l'Université de Montréal

FARNCOMBE
Farncombe Family Digestive Health Research Institute

Disclosure

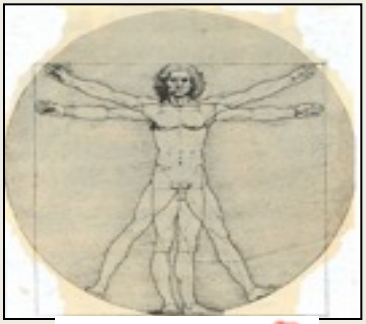
Nature of involvement	Company	Period
Advisory board	Actavis	2014-2015
Lecturer	Abbvie	2015-2016
Advisory board	Lupin	2015-2016

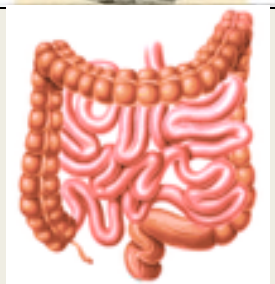
Objectives

- Review the basic concepts of microbiota structure and function
- Understand how the gut, vaginal and oral microbiotas may have a role in promoting a healthy pregnancy and early life
- Understand how dysbiosis may be associated with disease in pregnant women and newborn children

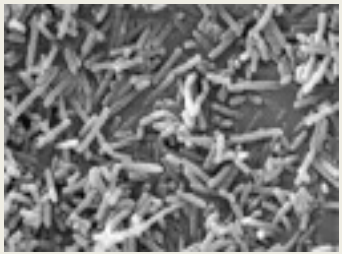
We are not alone…

10^{13} human cells





5 x10^{30} bacterial cells



Density of bacteria:
Water: 10^{4}-10^{7}/g
Sediment: 10^{8}/g
Soil: 10^{7}-10^{9}/g

10^{14} GI bacteria/ human host
Weight: 1-2 kg

Mammalian intestine: 10^{12}/g

Whitman, Coleman, and Wiebe, 1998

Definitions

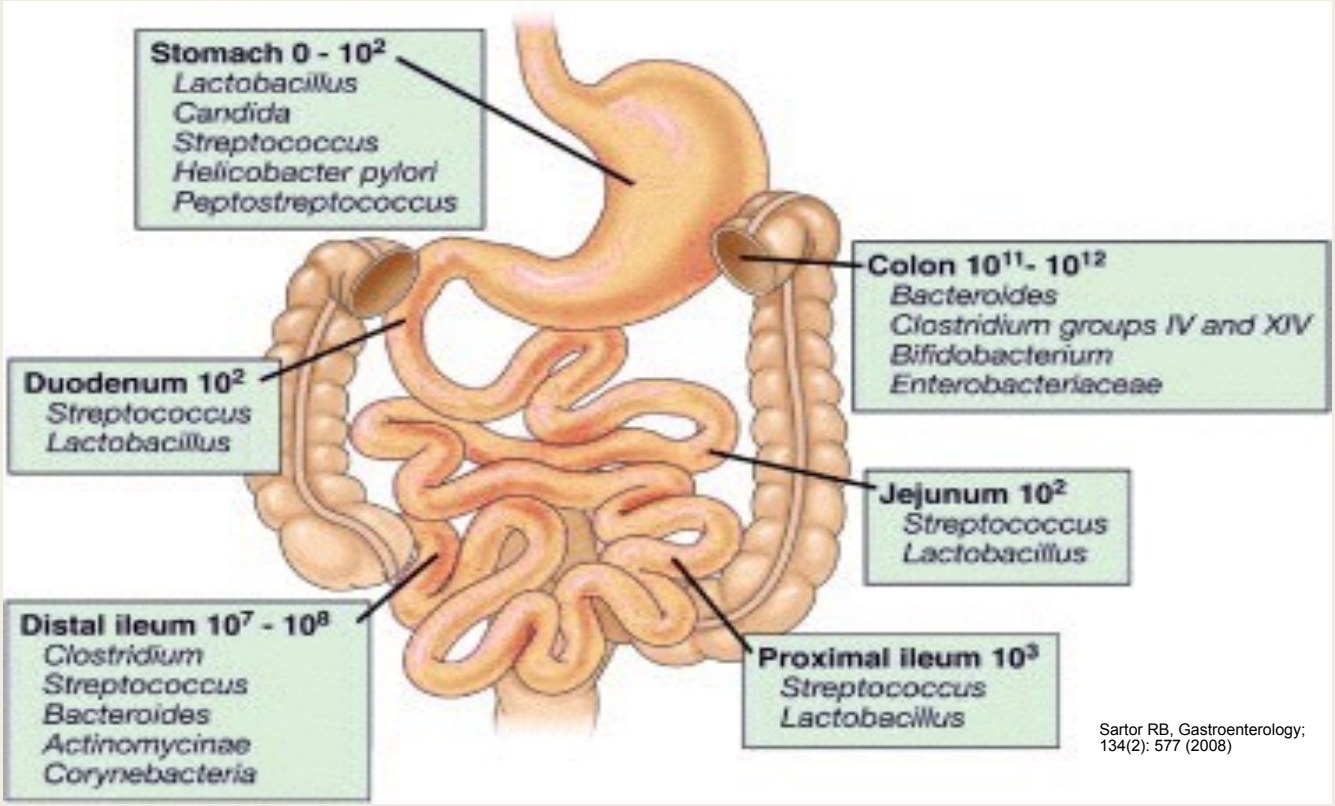
- Microbiota: Community of micro-organisms present in an organism
- Microbiome: Genetic material contained in the micro-organisms, potentially having an impact on the host. In the human gut: 10 millions of genes per host.
- Microbiome contained in the microbiota produces substances that interact with the host: metabolites.
- The structure of the microbiota is studied via metagenomics studies; the function via metabolomics studies.

Microbiota: composition

- Intestinal microbiota: most dense and better characterized
- Other microbiotas: respiratory, vaginal, oral cutaneous…
- Intestines contain members from all Kingdoms of Life
- Bacteria, Archaea, microeukaryotes (yeasts) and viruses
- Two big projects tackled the composition of the gut microbiota

Microbiota: composition

- Aerobic bacteria proximally – anaerobic distally:



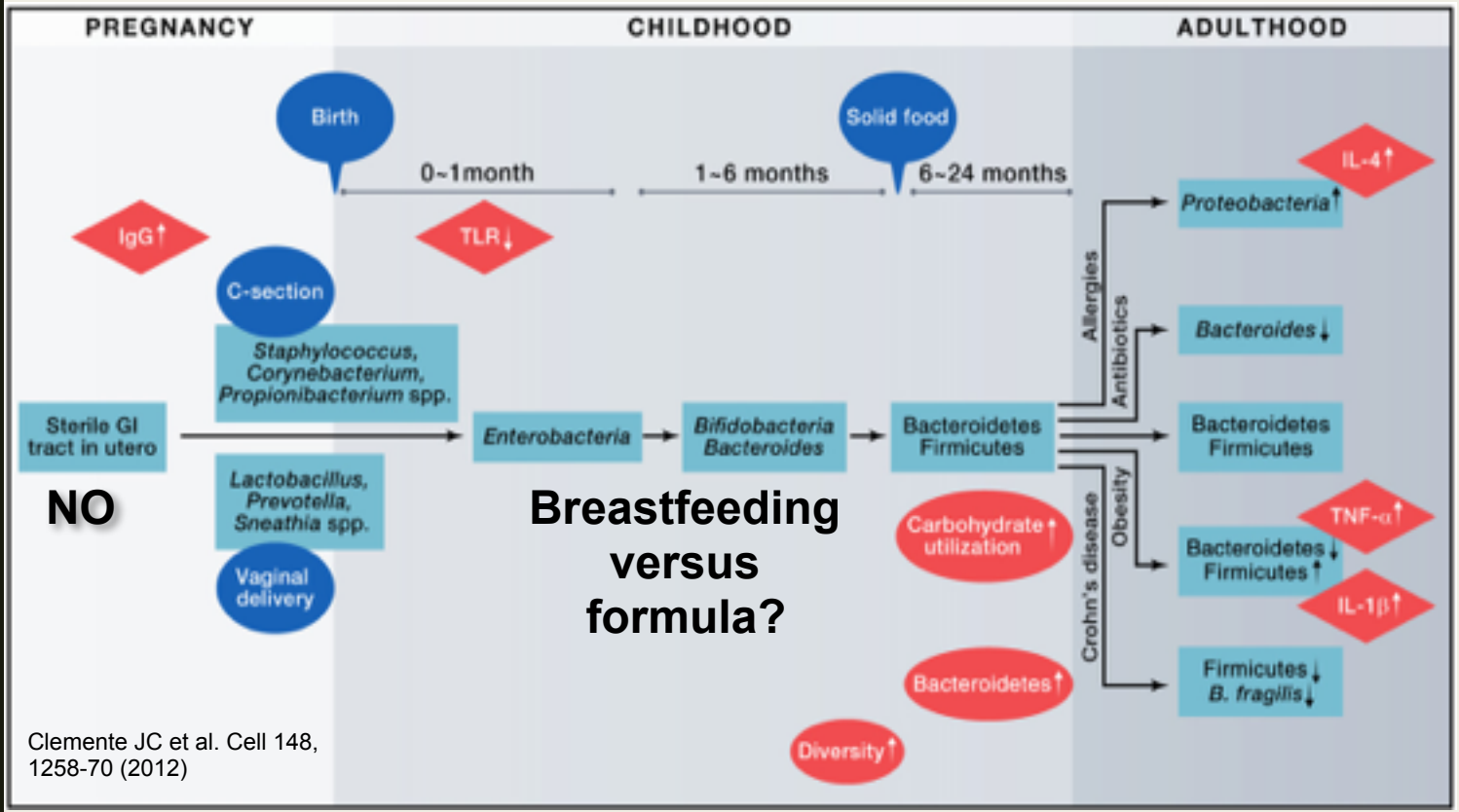
Sartor RB, Gastroenterology; 134(2): 577 (2008)

Microbiota: composition

- High variability in healthy individuals
- Homozygous twins share less than 50 % of bacterial genome
- Factors that influence composition (2016):
  - *Host genetics*
  - *Geography*
  - *Diet (stable or variable)*
  - *Pathological states*
  - *Social and personal history: significant stress, social interactions, travel, migration…*
  - *Pregnancy*
  - *Medication*

Microbiota: development

- Intestinal microbiota develops along with its host



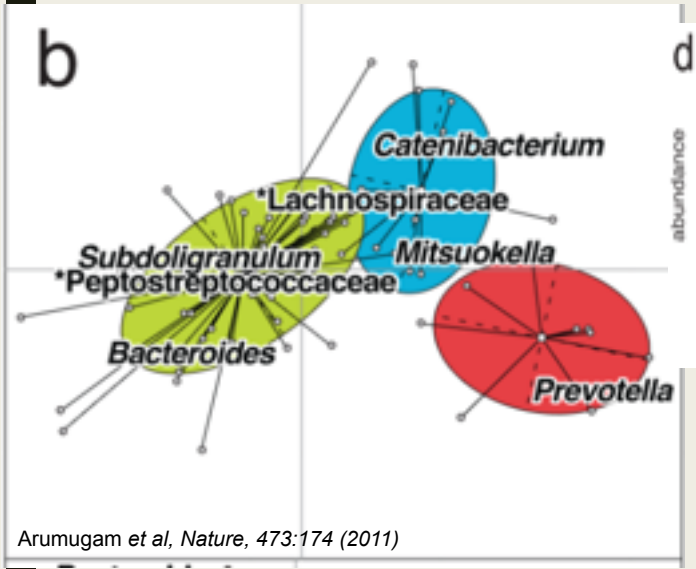
Clemente JC et al. Cell 148, 1258-70 (2012)

Intra-individual diversity

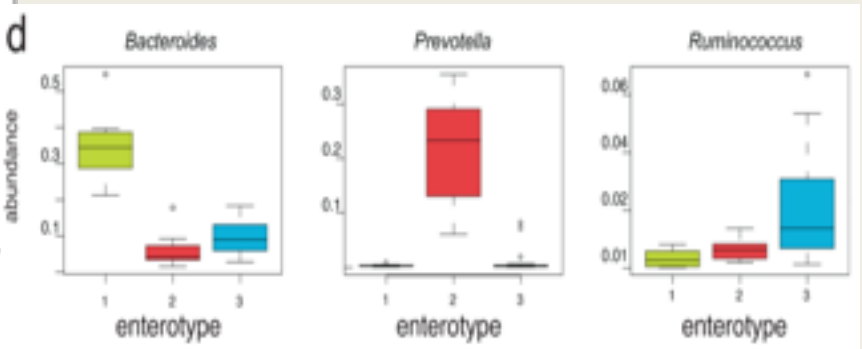
- Aerobic bacteria predominate in newborns
- In first year of life, microbiota resembles that of mother
- Variable and susceptible to physical and emotional stress
- At 11 months, specific to child and STABLE
- Normal microbiota:

Inter-individual diversity

- 3 types of intestinal microbiota in healthy humans = ENTEROTYPES
- Individual enterotype depends on geographical location and especially diet pattern:



Arumugam *et al, Nature, 473:174 (2011)*



Western or Japanese Diet

African (fiber rich) diet

Mixed diet

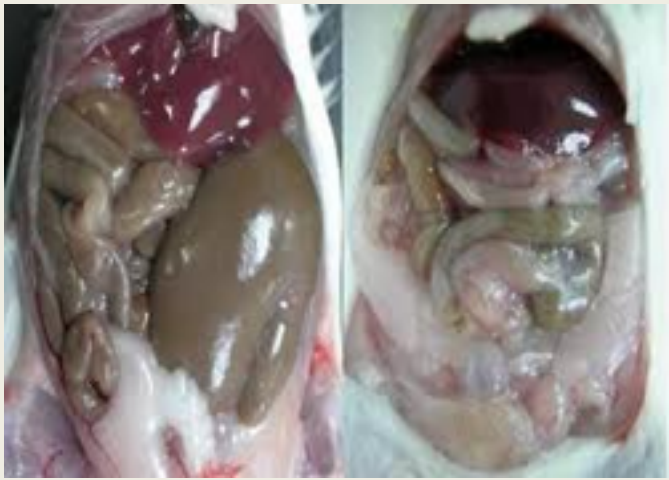
Why bother with the microbiota?

- Intestinal microbiota has evolved with animal hosts
- Prototype of symbiosis relationship
- Host-microbiota interactions essential to host homeostasis

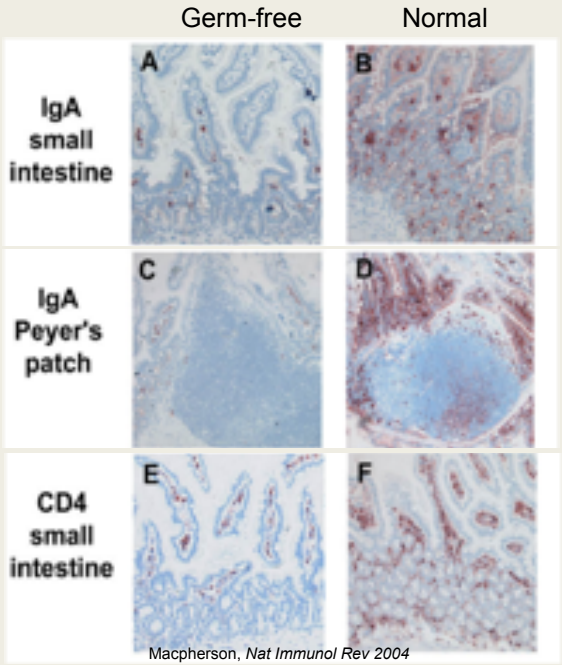
- Microbiota influences host activity by:
  - *Rgulating human gene expression*
  - *Producing active compounds (neurotransmitters, hormones, anti- or pro-inflammatory compounds)*
  - *Eliminating host substances*
  - *Other yet non elucidated effects*

Why bother with the microbiota?

- Absence of a gut microbiota has profound effects on host:
  - *Altered metabolism: weight loss and decreased weight gain during pregnancy*
  - *Decreased susceptibility to anxiety and depression*
  - *Decreased immunity*
  - *Altered GI motility*



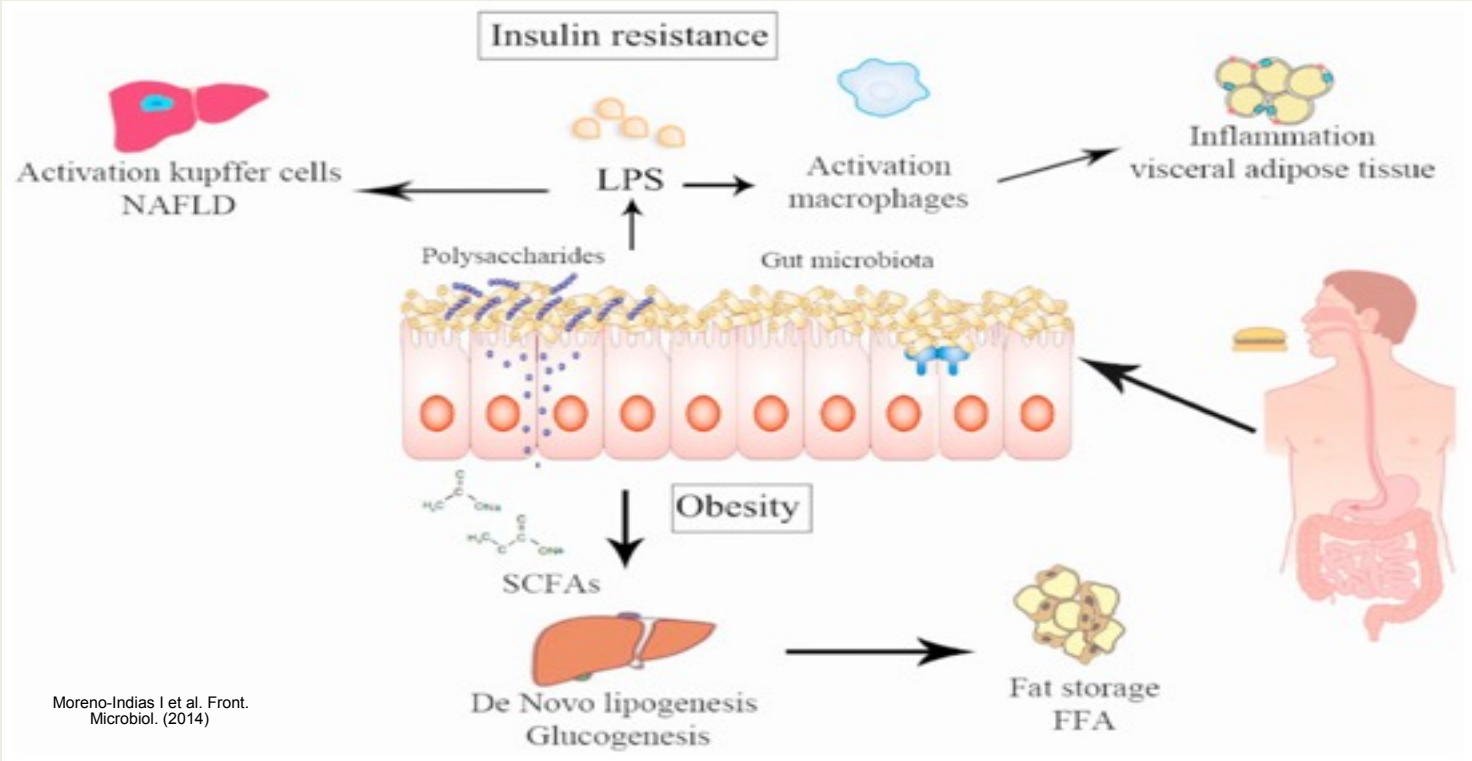
Adapted from physiologizing.blogspot.com



Macpherson, *Nat Immunol Rev 2004*

Obesity and metabolic syndrome

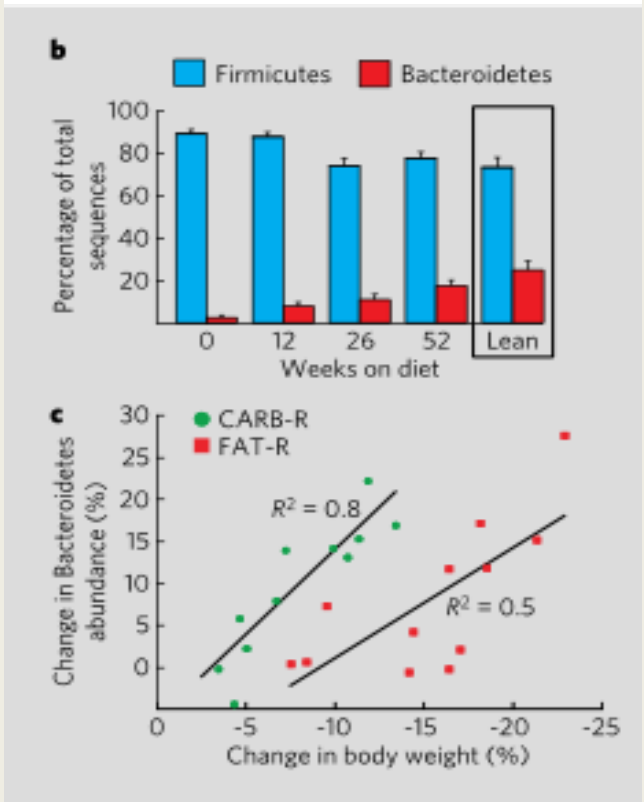
- Obesity = metabolic and inflammatory disease
- Bacterial components (LPS, SCFA) contribute to insulin resistance and inflammation



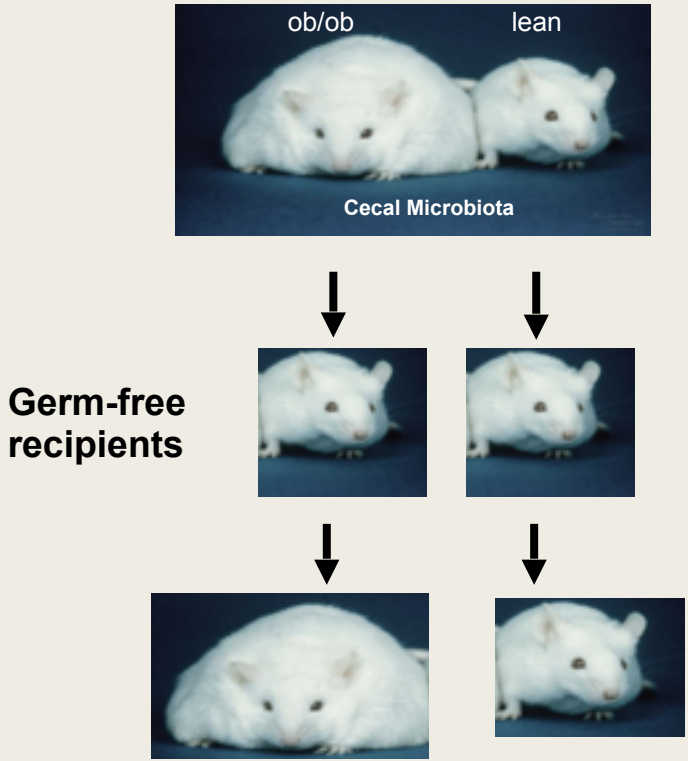
Moreno-Indias I et al. Front. Microbiol. (2014)

Obesity and metabolic syndrome

- Direct involvement of the microbiota in obesity:



Ley *et al*, *Nature 2006*



Mice receiving microbiota from obese donor mice become obese

Thurnbaugh *et al Nature 2006*

Healthy pregnancy and microbiota

Pregnancy: complex physiology

- During pregnancy, simultaneous changes in most physiological systems
- Including changes relevant to gut microbiota: hormonal changes, weight gain, immune system modulation, etc.
- 2 separate metabolic phases:

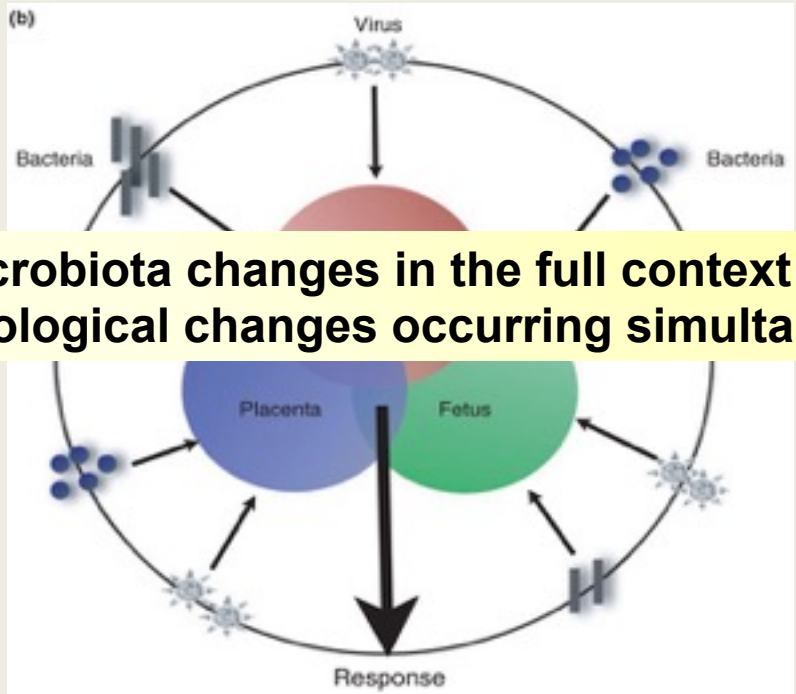
Table 4.6 Summary of maternal anabolic and catabolic phases of pregnancy[8–10]

Maternal Anabolic Phase 0–20 Weeks	Maternal Catabolic Phase 20+ Weeks
Blood volume expansion, increased cardiac output	Mobilization of fat and nutrient stores
Buildup of fat, nutrient, and liver glycogen stores	Increased production and blood levels of glucose, triglycerides, and fatty acids; decreased liver glycogen stores
Growth of some maternal organs	Accelerated fasting metabolism
Increased appetite, food intake (positive caloric balance)	Increased appetite and food intake decline somewhat near term.
Decreased exercise tolerance	Increased exercise tolerance
Increased levels of anabolic hormones	Increased levels of catabolic hormones

https://nutritionduringpregnancy.wordpress.com/physiological-changes/

Pregnancy: complex physiology

- Maternal immune system integrates stimuli from environment, placenta and fetus
- Emerging concept: pro-inflammatory stage at implantation and parturition, anti-inflammatory mid-pregnancy

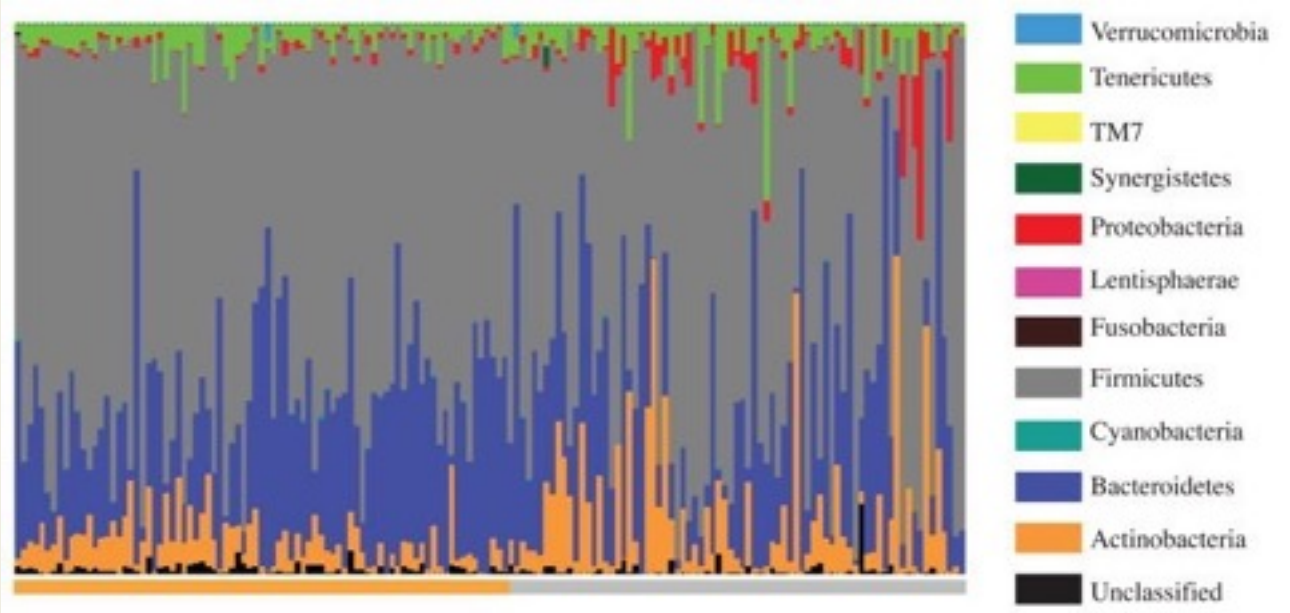


Microbiota changes in the full context of all physiological changes occurring simultaneously.

Mor *et al*, *Am J Reprod Immunol (2010)*

Gut microbiota in pregnancy

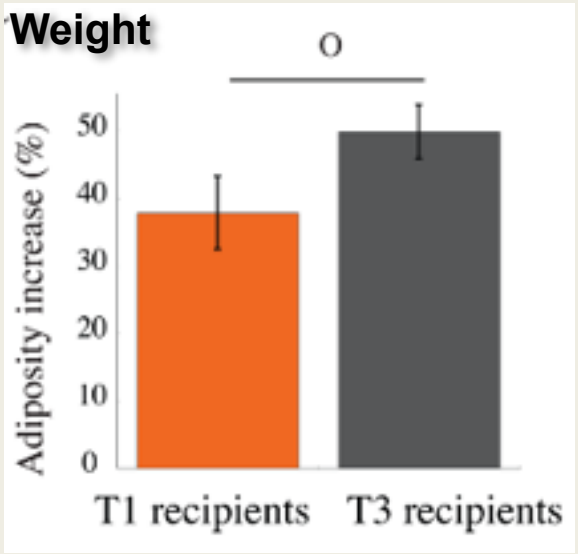
- Mother's intestinal microbiota changes progressively and significantly from conception to T3
- Increase in Proteobacteria and Actinobacteria
- Decrease in anti-inflammatory *Fusobacterium*, individual diversity and richness

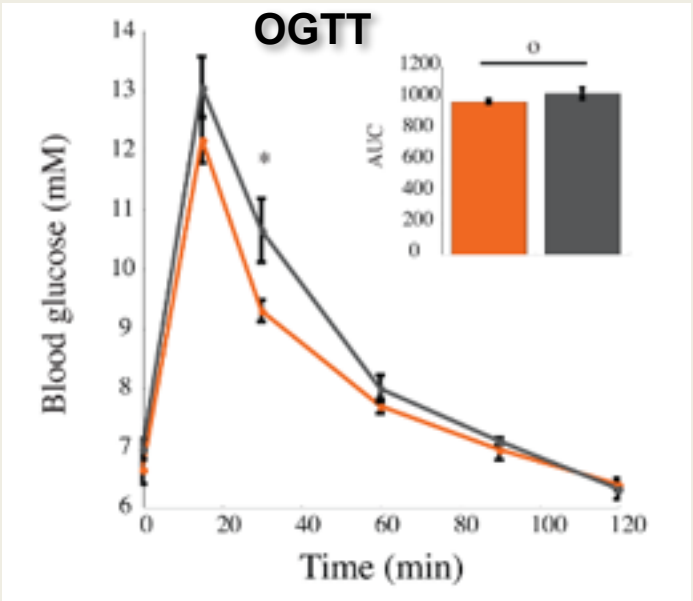


Koren O. *et al*. *Cell* (2013)

Gut microbiota in pregnancy

- Bacteria found in T3 are similar to those found in metabolic syndrome… Effect on host physiology?
- GF-mice transplanted with microbiota from either T1 or T3:





Koren O. *et al*. *Cell* (2013)

T3 microbiota induced changes consistent with metabolic syndrome, as well as an increase in markers of immune activation.

Gut microbiota in pregnancy

- Proposed mechanisms leading to enhanced metabolic syndrome:
  - *Enhanced absorption of glucose and fatty acids*
  - *Increased fasting-induced adipocyte factor secretion*
  - *Induction of catabolic pathways*
  - *Stimulation of immune system*
- Microbiota: cause or consequence? Bi-directional communication
- Other factors also influence changes in gut microbiota:
  - *Maternal diet prior to and during pregnancy*
  - *Pre-conception weight*
  - *Environment*
  - *Events during pregnancy (illness, antibiotics…)*

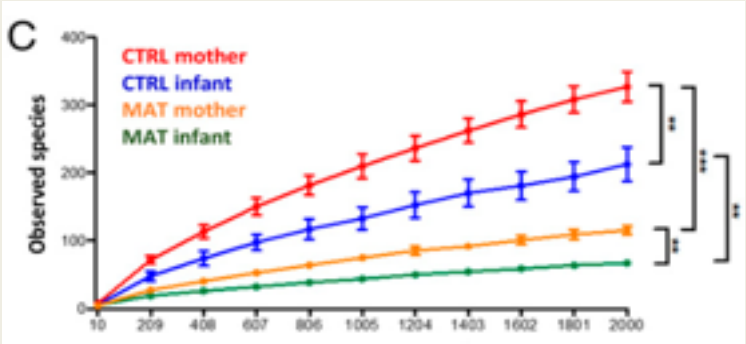
Antibiotics during pregnancy

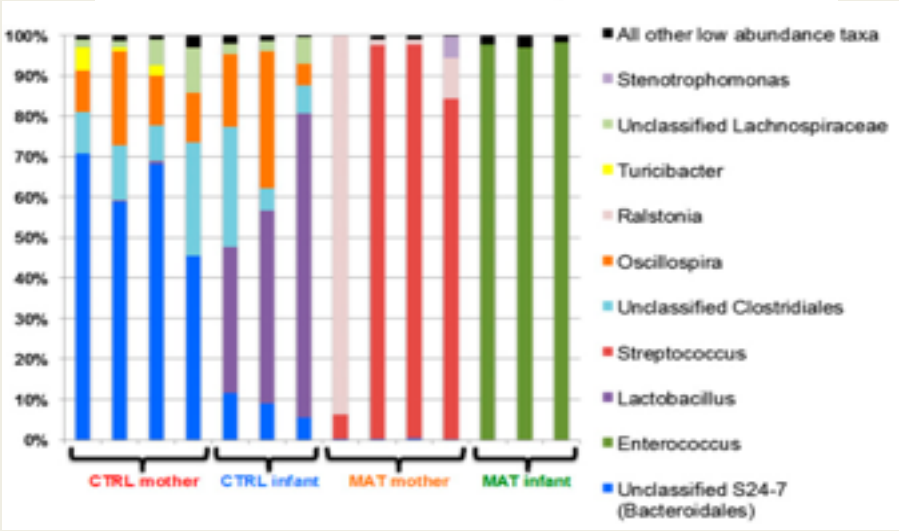
- All antibiotics (except rifaximin) disrupt gut microbiota
- Antibiotics studied: azithromycin, amoxicillin, cefaclor…
- Reduction in bacterial diversity
- Promotion of weight gain

- Many reports of consequences of antibiotics on immune system in animal models and humans
- Gut dysbiosis may be involved in immune-mediated diseases: Inflammatory Bowel Disease, Celiac Disease…
- What is the consequence of antibiotic-induced dysbiosis on pregnancy and newborn?

Antibiotics during pregnancy

- Systemic antibiotics during pregnancy induce changes in microbiota composition of mother and pup (mouse model):





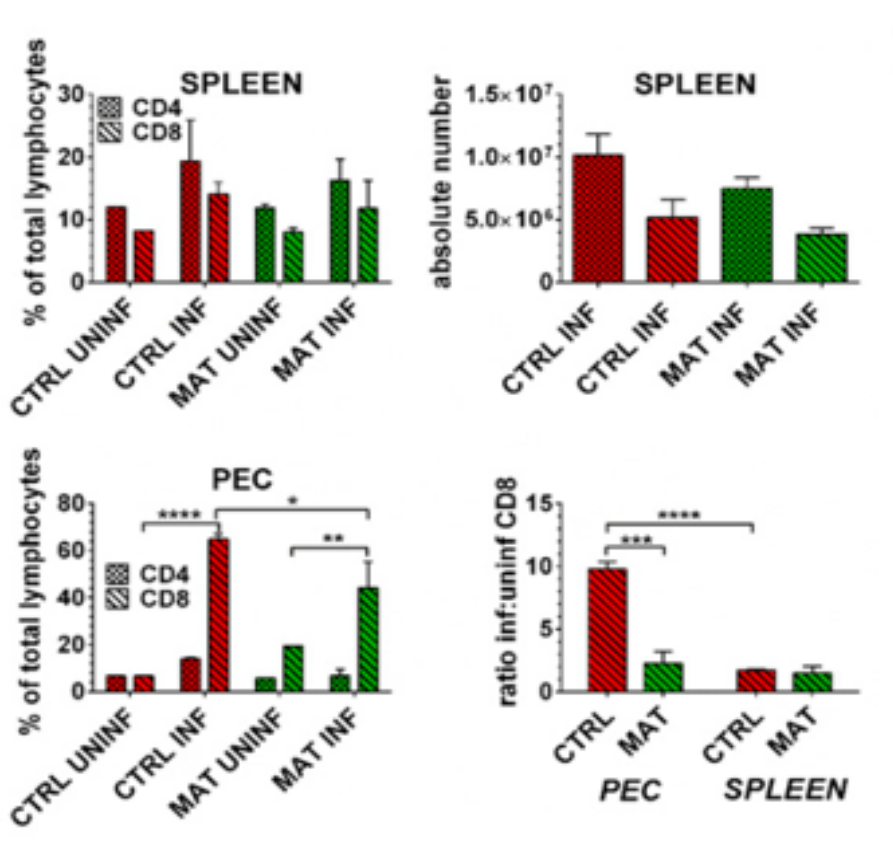
Ampicillin + Streptomycin+ Clindamycin

Reduced microbial diversity and composition changes in gut microbiota of mothers and infants.

Gonzalez-Perez G et al. *J Immunol* (2016)

Antibiotics during pregnancy

- Maternal antibiotics enhance susceptibility to viral infection:

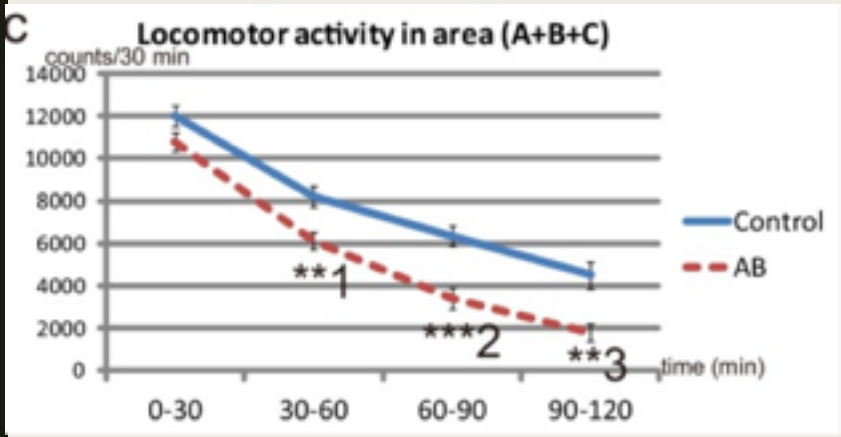


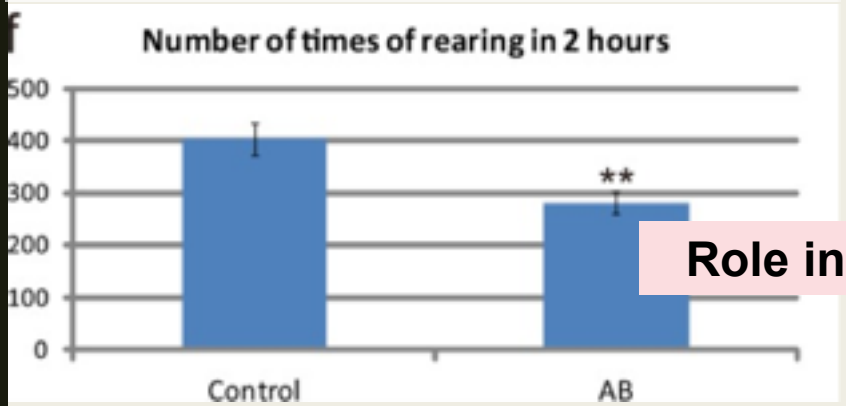
Antibiotic treatment in pregnancy yields pups that have lower lymphocytes and less immune activation following viral infection.

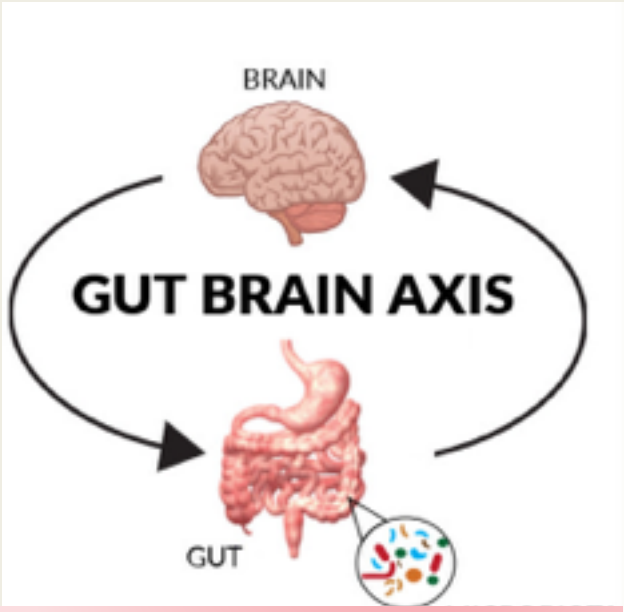
Gonzalez-Perez G et al. *J Immunol* (2016)

Antibiotics during pregnancy

- Antibiotics during pregnancy are associated with altered behavior in offspring (mouse model):







Role in development of autism??

Tochitani S *et al. PLoS ONE* (2016)

Probiotics during pregnancy

- Emerging studies on probiotics in all aspects of health
- Do probiotics have a beneficial immunomodulatory effect on pregnant women and/or offspring?
- RCT with healthy pregnant women randomized to 14 days:
  - *Bifidobacterium lactis (n=10)*
  - *B. lactis + Lactobacillus rhamnosus (n=9)*
  - *Placebo (n=10)*

- Aim: To determine whether probiotics alter immune gene expression in placenta and meconium (effect on baby?)
- NOT a clinical outcome

Probiotics during pregnancy

- Bacterial DNA was detected in ALL placenta and fetal gut (meconium)
- Probiotics induced significant changes in expression of specific immune-related genes:

Placenta

	B. lactis	p	*B. lactis* + *Lactobacillus* GG	p
TLR1	0.70 (0.54–0.86)	0.040	0.67 (0.56–0.78)	0.014
TLR2	0.87 (0.77–0.97)	0.076	0.92 (0.72–1.13)	0.543
TLR3	1.12 (1.08–1.17)	0.0052	0.79 (0.58–0.99)	0.110
TLR4	0.76 (0.61–0.92)	0.053	0.76 (0.53–0.99)	0.164
TLR5	0.85 (0.68–1.02)	0.196	0.77 (0.53–1.01)	0.181
TLR6	1.33 (0.85–1.81)	0.209	1.14 (0.60–1.67)	0.555
TLR7	0.68 (0.57–0.79)	0.014	0.72 (0.51–0.93)	0.087
MD-2	0.57 (0.47–0.68)	0.0016	0.78 (0.69–0.86)	0.0075
TIRAP	0.95 (0.75–1.15)	0.761	0.85 (0.76–0.94)	0.0337

The data are expressed as fold change compared to the placebo group with 95% CIs. The p values correspond to those obtained using Student's two-tailed t test.

Fetal gut

	B. lactis	p	*B. lactis* + *Lactobacillus* GG	p
TLR6	6.37 (0.00001–24.26)	0.266	0.09 (0.00001–019)	0.049
TLR7	0.30 (0.09–0.51)	0.029	1.46 (0.02–2.90)	0.497
TIRAP	0.79 (0.04–1.54)	0.654	2.24 (0.00001–5.10)	0.282

mRNA levels for TLR1-5 and MD-2 in meconium were not sufficient for reliable analyses. The data are expressed as fold change compared to the placebo group with 95% CIs. The p values correspond to those obtained using Student's two-tailed t test.

Rautava S *et al. Neonatology* (2012)

Probiotics during pregnancy

- Does immune supplementation have a beneficial effect on offspring?
- Effect of probiotics on reducing risk of allergies in babies?
- RCT of 241 mother-infant pairs
- Mothers with atopic disease and allergic sensitization randomized to:
  - *L. rhamnosus LPR + B. longum 999 (n=81)*
  - *L. paracasei ST11 + B. longum 999 (n=82)*
  - *Placebo (n=78)*
- Started 2 months before delivery, until 2 months after delivery
- Infants followed for 24 months

Probiotics during pregnancy

- Probiotic supplementation reduces occurrence of eczema in early life:

	Placebo	LPR+BL999	ST11+BL999
Eczema			
Ratio (%)	44/62 (71)	21/73 (29)	20/70 (29)
OR (95% CI)*		0.17 (0.08-0.35)	0.16 (0.08-0.35)
P value†		<.001	<.001
Chronically persistent eczema			
Ratio (%)	16/62 (26)	7/73 (10)	4/70 (6)
OR (95% CI)*		0.30 (0.12-0.80)	0.17 (0.12-0.80)
P value†		.016	.003
Skin prick test positive			
Ratio (%)	17/65 (26)	17/76 (22)	19/73 (26)
OR (95% CI)*		0.81 (0.38-1.76)	0.99 (0.46-2.13)
P value†		.60	.99

*Compared with placebo group.

†Corresponds to logistic regression analyses.

Rautava S *et al. J Allergy Clin Immunol* (2012)

Vaginal microbiota

- Less dense and less studied than gut
- Known to have a role as defense system against microbial and viral infections
- Mostly aerobic flora: *Lactobacilli*, Clostridiales, Bacteroidales and Actinomycetales
- Contains many strains found in probiotics…
- Antimicrobial effect:
  - *Lactobacilli keep low pH (<4.5)*
  - *Microbiota secretes inhibitors of bacterial and viral pathogens*
- Changes during pregnancy:

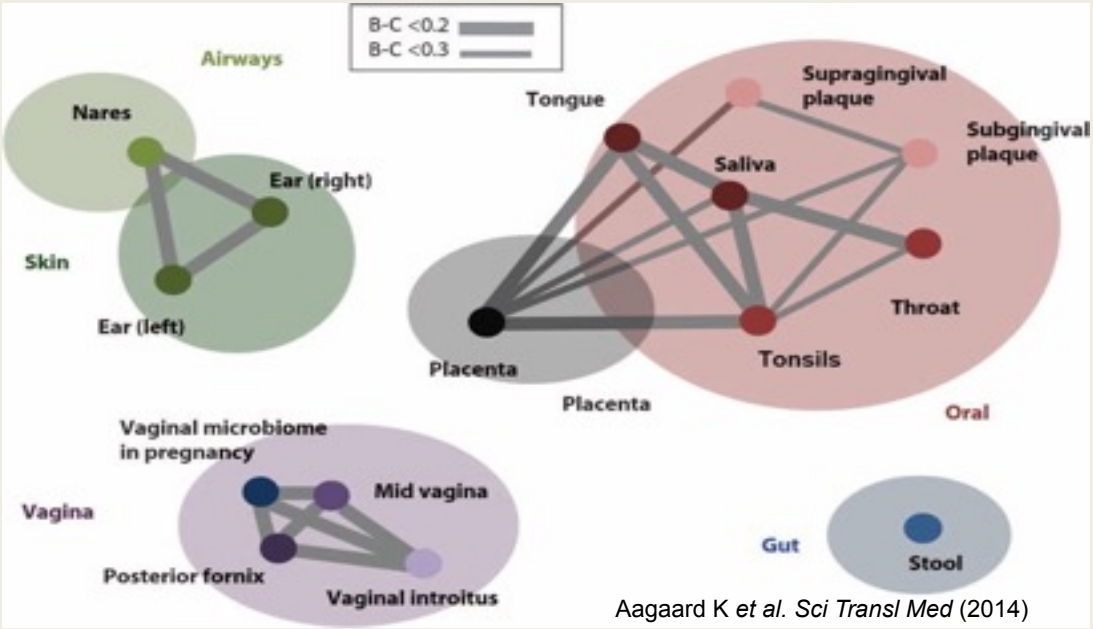
More ***Lactobacilli*** (antimicrobial)
More **stability**
Decreased diversity

Oral microbiota

- Even less well characterized
- Topographically diverse: teeth, tongue, palates, etc.
- Up to 600 species identified: *Lactobacilli*, *Staphylococci*, *Corynebacteria*, etc.
- In pregnancy:
  - *Increased total bacterial count*
  - *Increased periodontal pathogens: Poprphyromonas gingivalis, Candida…*
- Mechanisms unclear
- Clinical relevance?

Placental microbiota

- First evidence in 1982 that bacteria are present in normal placenta
- Later studies: placental microbiota resembles oral, with predominance of Proteobacteria (Prevotella, Neisseria)
- Function of this microbiota still to be elucidated…



Aagaard K *et al. Sci Transl Med* (2014)

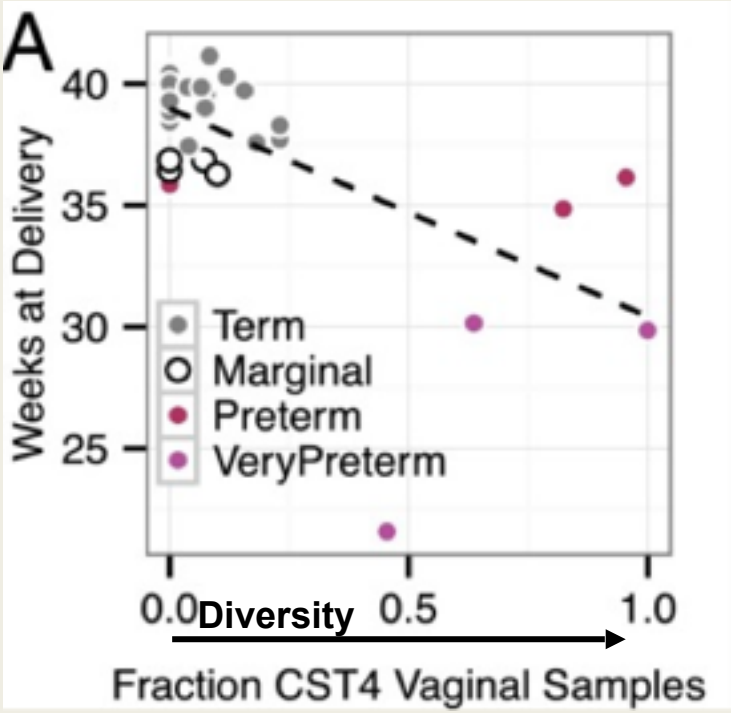
Microbiota and obstetric complications

Microbiota and complications: Rationale

- Longstanding belief that bacterial infections may be correlated with pregnancy complications
- Intestinal microbiota shown to treat and prevent infections: *C. difficile*
- Bacterial metabolites regulate metabolism and immunity
- Dysbiosis results in systemic release of pro-inflammatory mediators: LPS, TNF, IFN, interleukins…
- Release of prostaglandins may induce uterine contractions
- Search for microbial markers associated with pregnancy complications is ongoing
- Emerging evidence regarding preterm birth

Microbiota and preterm birth

- High diversity of vaginal microbiota during pregnancy associated with preterm birth:



DiGiulio DB *et al. PNAS* (2015)

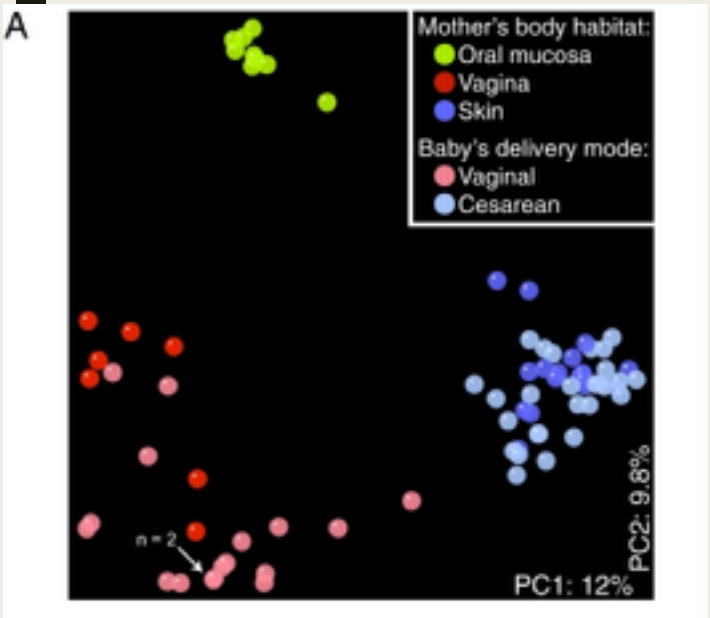
Higher abundance of *Gardnerella* and *Ureaplasma*, and lower *Lactobacillus*, associated with higher risk of preterm birth.

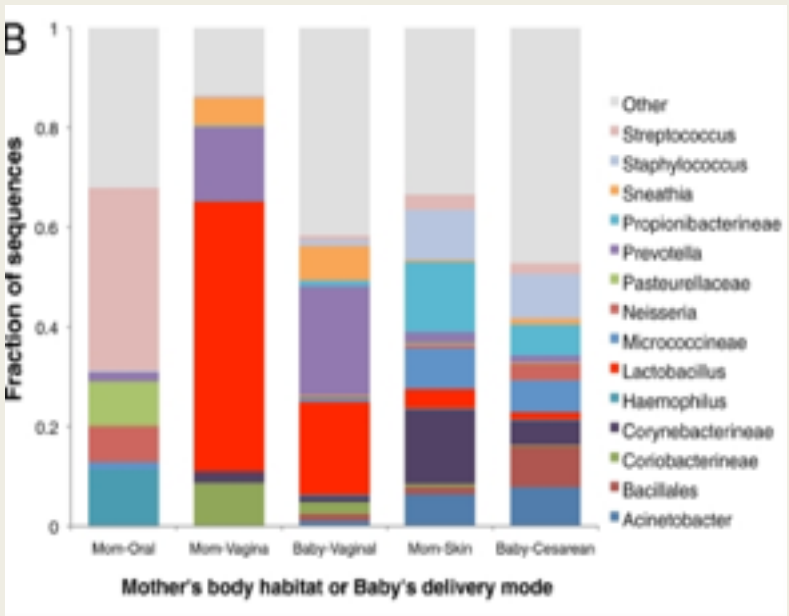
Presence of *Candida albicans* correlates with PTB.

Microbiota and mode of delivery

Infant gut microbiota and delivery

- Several studies show distinct effects of delivery mode on gut microbiota of newborn babies.





→ Babies born **vaginally** adopt gut microbiota similar to mother's **vaginal microbiota**.

→ Those born by **C-section** adopt a microbiota similar to mother's **skin microbiota**.

Dominguez-Bello MG *et al. PNAS* (2010)

Infant gut microbiota and delivery

- Gut microbiota of babies born by vaginal delivery contains mainly *Lactobacillus*, *Prevotella* and *Sneathia…*
- Mainly bacteria with PROBIOTIC properties
- Gut microbiota of babies born by C-section contains cutaneous flora: *Propionibacterium*, *Corynebacterium*, *Streptococcus*…
- Also delayed colonization of Bacteroidetes and lower diversity
- Survey from CDC (2006): 64-82 % of MRSA skin infections in newborns occur in C-section infants:
  - *Gut microbiota of Cesarean newborns is enriched in bacterial-resistance genes*
  - *Higher abundance of Staphylococcus*

C-section and disease

- C-section may increase incidence of medical conditions.
- Swedish Case-Control study (2012): Higher incidence of celiac disease in children born via C-section:

Marild K *et al. Gastroenterology* (2012)

Table 2. Risk of Celiac Disease After Cesarean Delivery

	Matched controls (%)	Celiac disease (%)	OR; 95% CI	*P* value	Adjusted OR,[a] 95% CI	*P* value
Cesarean delivery	5766/53,887 (10.7)	1299/11,749 (11.1)	1.04; 0.98–1.10	.232	1.06; 0.99–1.13	.074
Number of participants			65,636		65,493	
Emergency cesarean delivery[b]	2136/41,699 (5.1)	444/8827 (5.0)	0.99; 0.90–1.10	.857	1.02; 0.92–1.13	.749
Number of participants			50,526		50,415	
Elective cesarean delivery[b]	2125/41,688 (5.1)	508/8891 (5.7)	1.11; 1.01–1.22	.027	1.15; 1.04–1.26	.005
Number of participants			50,579		50,471	

➡ Slight increase in celiac disease incidence in children born only by **ELECTIVE C-section**.

➡ These children were never exposed to mother's vaginal microbiota.

Cesarean and disease

- Obesity and asthma/atopy associated with C-section birth
- Finnish study: RCT of 1223 infants at risk for allergy (mother has ≥ 1 family member with atopy)
- Intervention: Probiotics (2 *Lactobacilli*, *Bifidobacteria* and *Propionibacteria*) vs. Placebo during last month of pregnancy (mothers) and first 6 months of life (infants)
- Primary outcome: Cumulative incidence of allergic diseases at 5 years
- Study included vaginal and C-section deliveries
- Wide range of socio-economic background

Cesarean and disease

- Finnish study (2009): Outcomes of children in the overall study population

TABLE II. Allergic diseases and sensitization at 0 to 5 years in the probiotic and placebo groups

	Probiotic group		Placebo group		OR (95% CI)	*P* value
Primary end points						
Allergic disease	234/445	52.6%	245/446	54.9%	0.91 (0.70-1.18)	.482
Allergic disease, positive SPT response	138/443	31.2%	137/445	30.8%	1.02 (0.77-1.35)	.906
Allergic disease, specific IgE >0.7 kU/L	124/421	29.5%	106/398	26.6%	1.15 (0.85-1.56)	.370
Allergic disease, positive SPT response and/or specific IgE >0.7 kU/L	147/445	33.0%	146/446	32.7%	1.01 (0.77-1.34)	.925
Secondary end points						
Sensitization						
Any positive SPT response	165/443	37.4%	164/445	36.9%	1.02 (0.78-1.34)	.883
Any specific IgE >0.7 kU/L	152/375	40.5%	137/362	37.8%	1.12 (0.83-1.51)	.455
Any positive SPT response and/or specific IgE >0.7 kU/L	183/443	41.3%	184/445	41.3%	1.00 (0.77-1.33)	.987
Positive food SPT response and/or food-specific IgE >0.7 kU/L	92/443	20.8%	105/446	23.5%	0.85 (0.62-1.17)	.319
Positive inhalant SPT response and/or inhalant specific IgE >0.7 kU/L	169/444	38.1%	162/446	36.3%	1.08 (0.82-1.42)	.573
Allergic disease						
Eczema, all	175/445	39.3%	193/446	43.3%	0.85 (0.65-1.11)	.231
Eczema, IgE associated*	107/445	24.0%	112/446	25.1%	0.94 (0.70-1.28)	.711
Asthma, all	58/445	13.3%	63/446	14.1%	0.91 (0.62-1.34)	.634
Asthma, IgE associated*	43/445	9.7%	40/446	9.0%	1.09 (0.69-1.71)	.393

Cesarean and disease

- Finnish study (2009): Outcomes of subgroup of children born by C-section

TABLE III. Allergic diseases and sensitization at 0 to 5 years and in interim analysis at 0 to 2 years in cesarean-delivered children in the probiotic and placebo groups

	Age (y)	Probiotic group (n = 64-70; %)	Placebo group (n = 69-79; %)	OR (95% CI)
Primary end points				
Allergic disease, all	0-5	57.1	55.7	1.06 (0.55-2.03)
	0-2	26.7	36.7	0.63 (0.32-1.25)
Allergic disease, positive SPT response	0-5	24.3	40.5	0.47 (0.23-0.96)‡
	0-2	14.9	21.5	0.64 (0.28-1.47)
Allergic disease specific IgE >0.7 kU/L	0-5	25.0	30.4	0.76 (0.36-1.64)
	0-2	NA	NA	
Allergic disease, positive SPT response and/or specific IgE >0.7 kU/L	0-5	25.7	40.5	0.51 (0.25-1.02)
	0-2	12.2	22.8	0.47 (0.20-1.12)
Secondary end points				
Sensitization				
Any positive SPT response and/or specific IgE >0.7 kU/L	0-5	31.4	46.8	0.52 (0.27-1.02)
	0-2	23.0	35.4	0.54 (0.27-1.11)
Positive food SPT response and/or food-specific IgE >0.7 kU/L	0-5	10.0	25.3	0.33 (0.12-0.85)‡
	0-2	NA	NA	
Allergic disease				
Eczema, all	0-5	42.9	44.3	0.94 (0.49-1.80)
	0-2	22.7	35.4	0.53 (0.26-1.09)
Eczema, IgE associated*	0-5	15.7	30.4	0.43 (0.19-0.95)‡
	0-2	10.7	21.5	0.44 (0.18-1.08)
Asthma, IgE associated*	0-5	7.1	10.1	0.68 (0.21-2.19)
	0-2	NA	NA	

Cesarean and disease

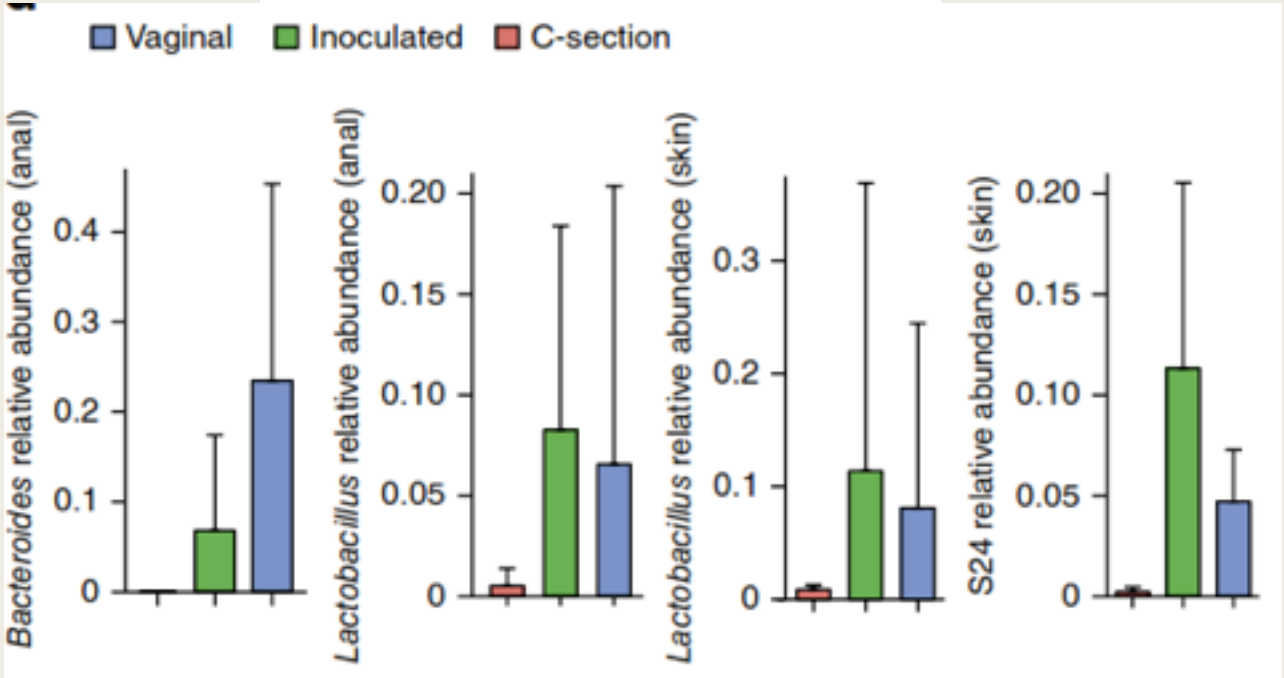
- No study has directly tested the role of bacteria in development of disease
- Mode of delivery may have effect on maturation of immune system
- Lower levels of T helper CXCL10 and CXCL11 chemokines with Cesarean delivery
- *Bacteroides*, known to stimulate maturity of immune system, is depleted in C-section born infants
- Probiotics may not be sufficient in restoring gut microbiota: role for vaginal microbiota transfer in infants?

Microbiota transfer

- Is gut microbiota of newborns delivered by Cesarean restored by transferring maternal vaginal microbiota peri-partum?
- Recent proof-of-principle study
- Babies exposed to their maternal vaginal contents within first 2 min of birth with gauze swab
- Mothers:
  - *Negative for Group B Streptococcus*
  - *No bacterial vaginosis*
  - *Vaginal pH<4.5 one hour before delivery*
- Multiple samples taken from babies in first month of life

Microbiota transfer

- Inoculation of newborns with vaginal contents induced restoration of gut bacteria near levels of vaginally delivered babies:



Dominguez-Bello MG *et al. Nat Medicine* (2016)

Conclusions

- Interplay between gut, vaginal, oral, placental microbiota and host during pregnancy
- Microbiota changes are complex and reminiscent of complex physiological changes in pregnancy
- Gut microbiota in mid-pregnancy promotes weight gain and insulin insensitivity: potential target for gestational diabetes?
- Gut microbiota in pregnancy promotes immune balance: protection against infections versus immune tolerance
- Modulation of gut microbiota in late pregnancy may protect against development of eczema
- Vaginal microbiota dramatically changes in pregnancy and is enriched with probiotic *Lactobacilli*

Conclusions

- Maternal microbiota changes culminate during parturition and mode of delivery is critical to composition of early life microbiota:

Vaginal delivery

Immediate contact with potentially *probiotic* bacteria: *Lactobacilli, Bacteroidetes*

C-Section

Immediate contact with bacteria similar to skin/oral microbiota
Less diversity
Potential increased risk of infectious/atopic/immune disease

Potential therapeutic role for microbiota modulation