Il Microbiota Umano : lo stato di equilibrio chiamato eubiosi

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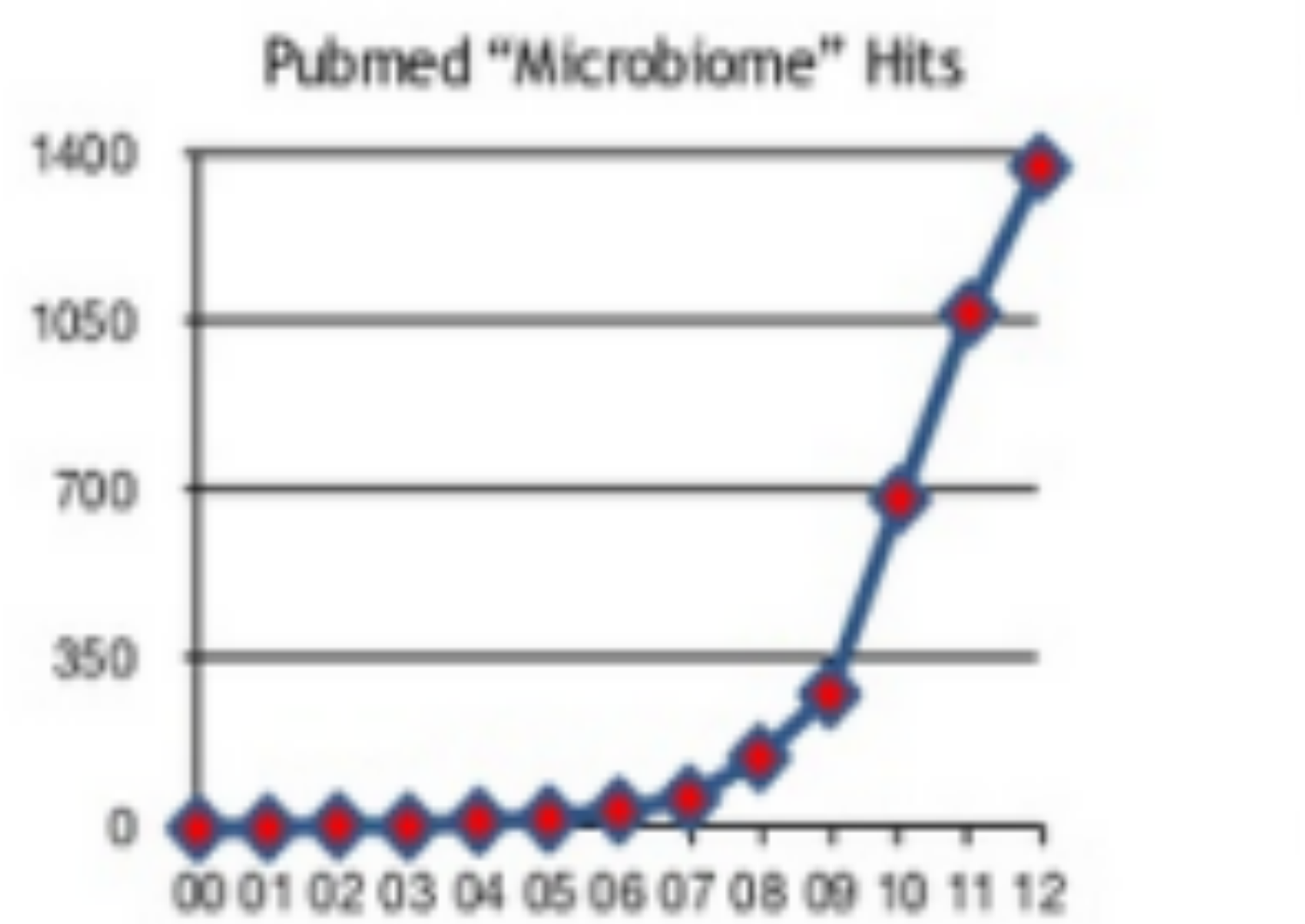
NIH Human Microbiome Project (HMP) was ~~established in 2008~~

Mission - generating resources that would enable the comprehensive characterization of the human microbiome and analysis of its role in human health and disease

The Rise of the Microbiome



Future Research Directions

Longitudinal studies across the lifespan

Are microbiome changes a cause or consequence

of the disease?

Understanding the functionality of the microbiome

Metabolomics

Transcriptomics

Proteomics

Strategies to modify the composition of the microbiota

- **Probiotics** – Microbes that confer health benefits
  - Probiotics produce food for beneficial bacteria
  - Probiotics increase the number of good bacteria at the expenses of bad bacteria
- **Prebiotics** – non-microbial entities to elicit favorable microbial activity
- **Antibiotics** – targeted or nonspecific antimicrobial


WINNER
LIFE SCIENCES
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MICROBIRTH
REVEALING THE MICROSCOPIC EVENTS DURING CHILDBIRTH
THAT COULD HOLD THE KEY TO THE FUTURE OF HUMANITY

COSA E’ IL MICROBIOTA UMANO

THE HUMAN BODY….

- Consists of trillions of human cells

&

- Trillions of microorganisms

10-100 trillion

Number of symbiotic microbial cells harbored by each person, primarily bacteria in the gut, that make up the human microbiota

10X

There are 10 times as many outside organisms as there are human cells in the human body

> 10,000

Number of different microbe species researchers have identified living in the human body

22,000

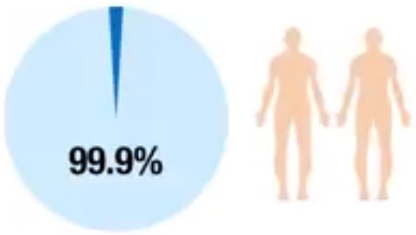
Approximate number genes in the human gene catalog

3.3 million

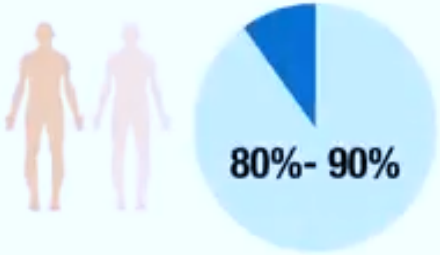
Number of non-redundant genes in the human gut microbiome

100 to 1

The genes in our microbiome outnumber the genes in our genome by about 100 to 1

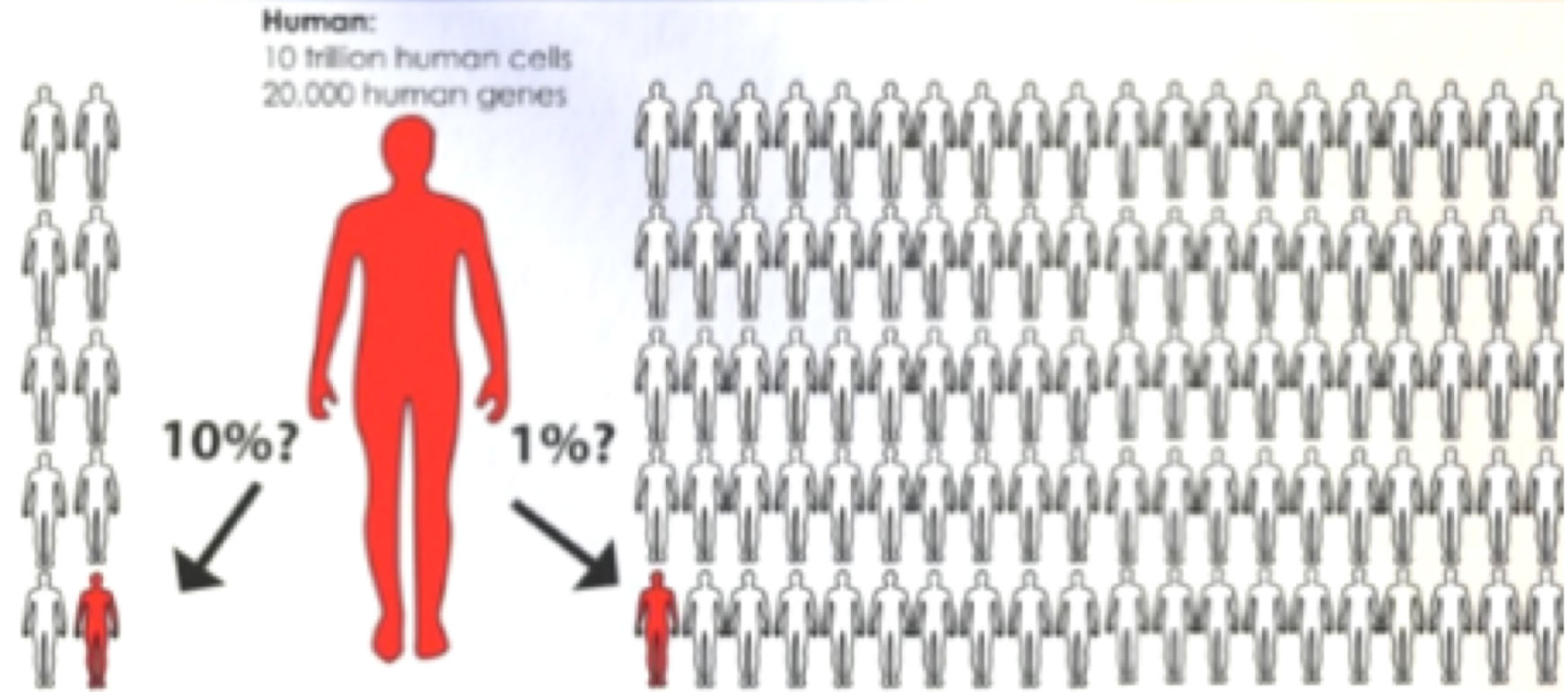


Percentage individual humans are identical to one another in terms of host genome



Percentage individual humans are different from another in terms of the microbiome

Microbial abundance raises the question: how human are we?



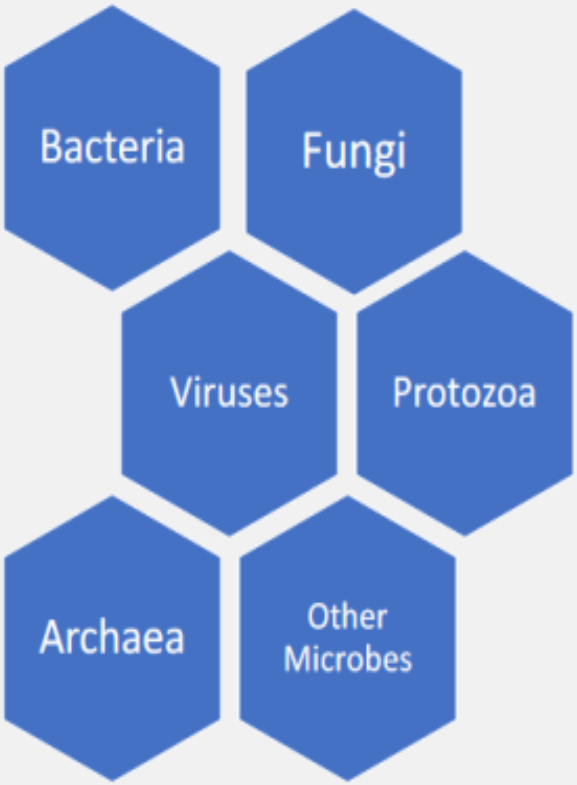
99.9% of our genomes the same, but our microbes...?

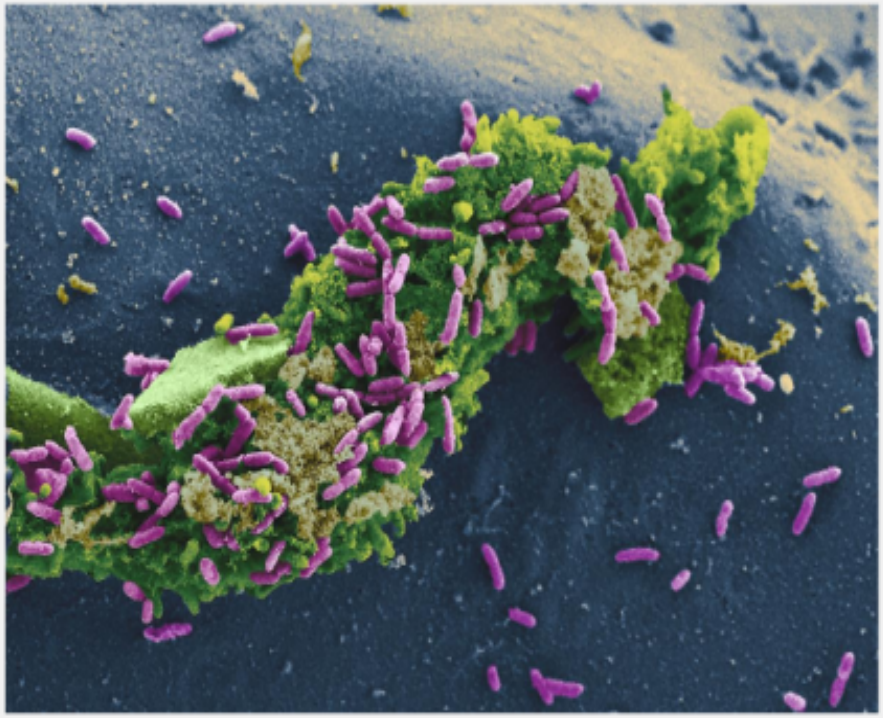
Micah Hamady, PhD thesis, 2009

- **Microbiota** e **microbioma** sono due termini spesso usati come sinonimi. Ma non lo sono. Nella maggior parte dei casi questo utilizzo “intercambiabile” non compromette la comprensione del testo, tuttavia è importante riflettere sulla profonda **differenza di significato** tra le due parole.

- Microbiota si riferisce a una **popolazione di microrganismi** che colonizza un determinato luogo.
- Il termine microbioma invece indica la totalità del **patrimonio genetico** posseduto dal microbiota, cioè i geni che quest'ultimo è in grado di esprimere.

THE MICROORGANISMS LIVING ON AND IN US INCLUDE...



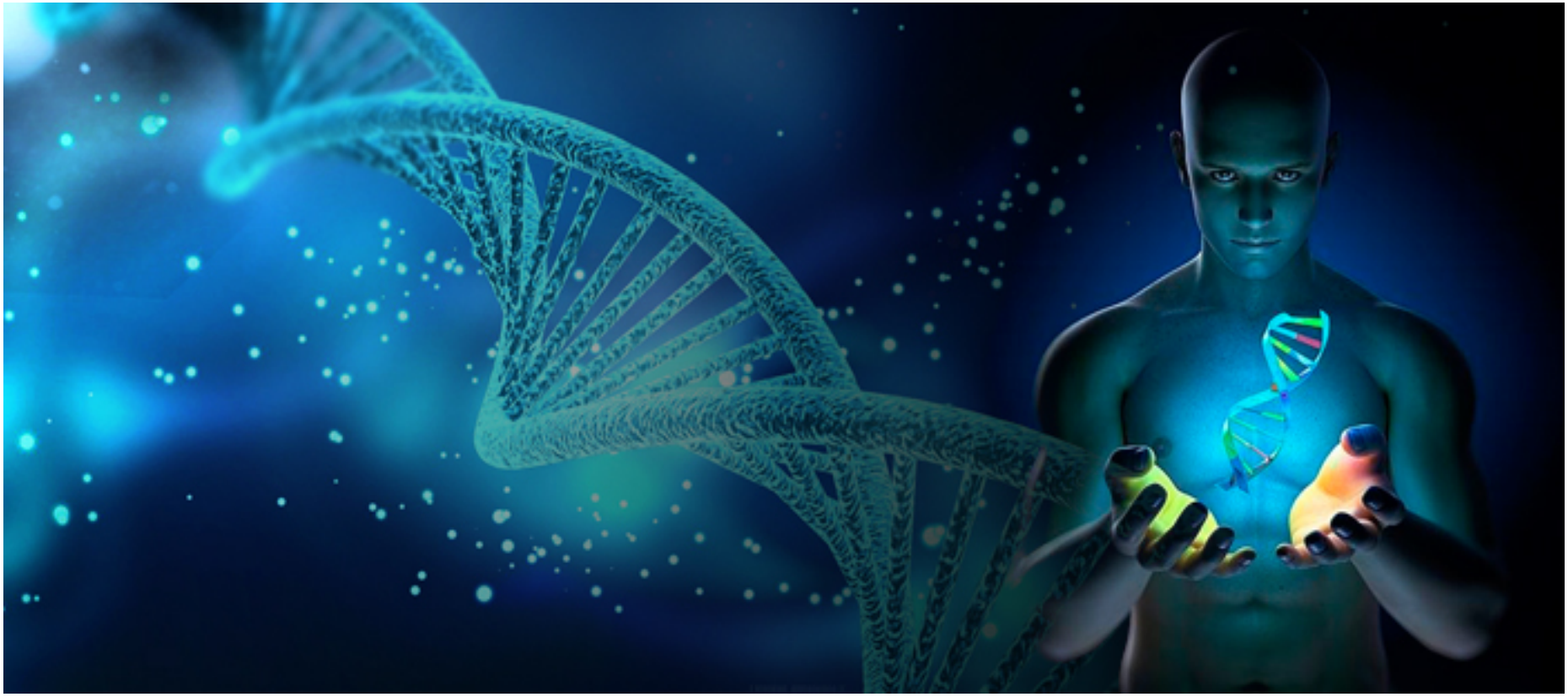


Bacterial sample prepared and imaged by Dr. Adam Jakus of Prof. Ramille Shah's TEAM Laboratory, Simpson Querrey Institute for BioNanotechnology, Northwestern University

- Quando si parla di microbiota si fa riferimento alla totalità dei singoli **microrganismi** – batteri, funghi, archeobatteri e protozoi – e dei **virus** che vivono e colonizzano uno specifico ambiente in un determinato tempo, vivendo in simbiosi con il corpo umano .
- Tutto il corpo ospita differenti nicchie microbiotiche per un totale di circa **38.000 miliardi di batteri**. Le tribù (chiamati phyla) più abbondanti sono *Firmicutes* e *Bacteroidetes*.

- Il termine microbioma indica invece la totalità del **patrimonio genetico** posseduto dal microbiota, cioè i geni che quest'ultimo è in grado di esprimere.
- Se consideriamo il **microbioma umano**, tali geni codificano per alcune molecole che il corpo non riesce a produrre autonomamente : Il **99%** della nostra componente genetica deriva dai batteri, che rappresentano un **secondo genoma aggiuntivo simbiotico** .

Patrimonio genetico umano: circa 20 mila geni



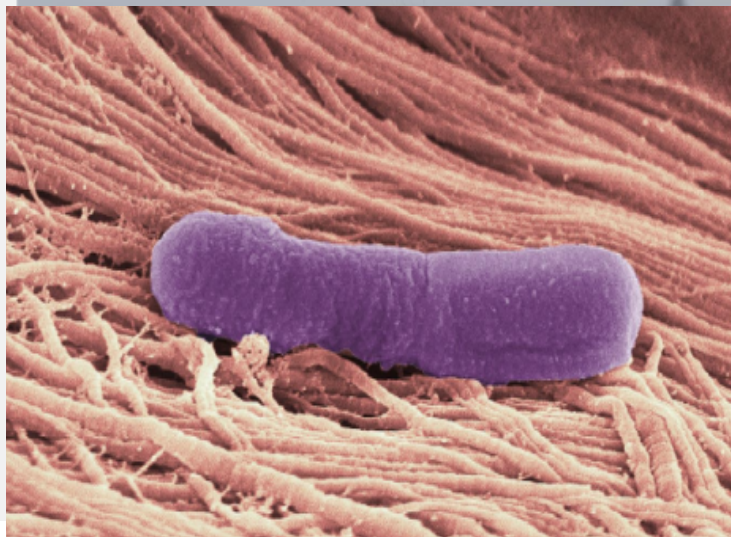
Patrimonio genetico batterico: circa 20 milioni di geni milioni di geni

Possiamo affermare che abbiamo 2 genomi:

- Primo (fisso e immutabile) ereditato dai genitori
- Secondo (molto più dinamico) acquisito dai batteri che compongono il Microbiota

TOGETHER, HUMAN CELLS AND MICROBES FORM A 'HUMAN SUPERORGANISM'

- *"We form what's called a symbiotic superorganism, because we do things for each other. And those things are very important for the health of the total organism"*

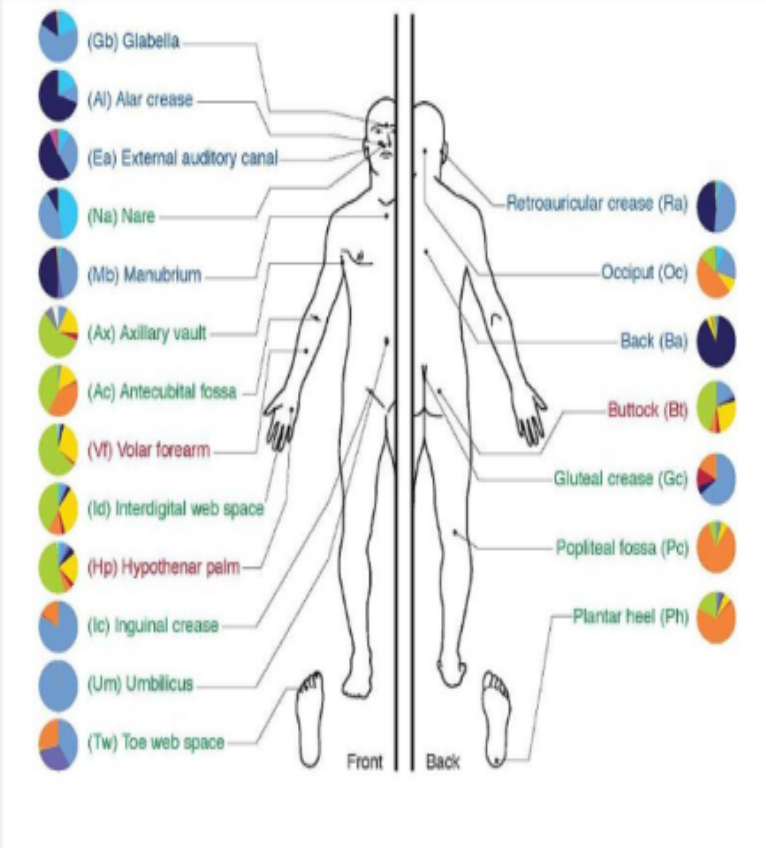


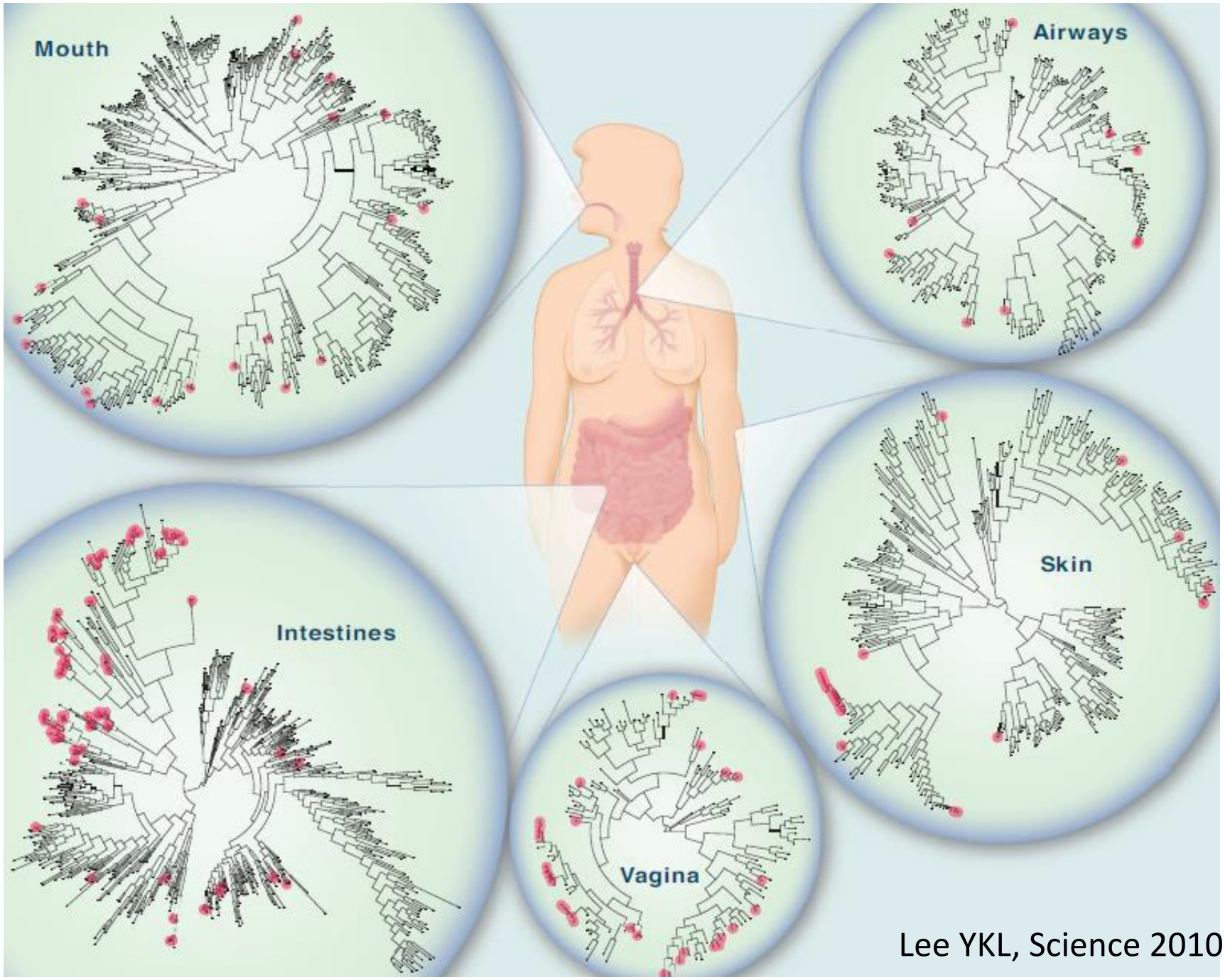
A person's microbiome is as unique as a fingerprint

DOVE E’ LOCALIZZATO IL MICROBIOTA UMANO

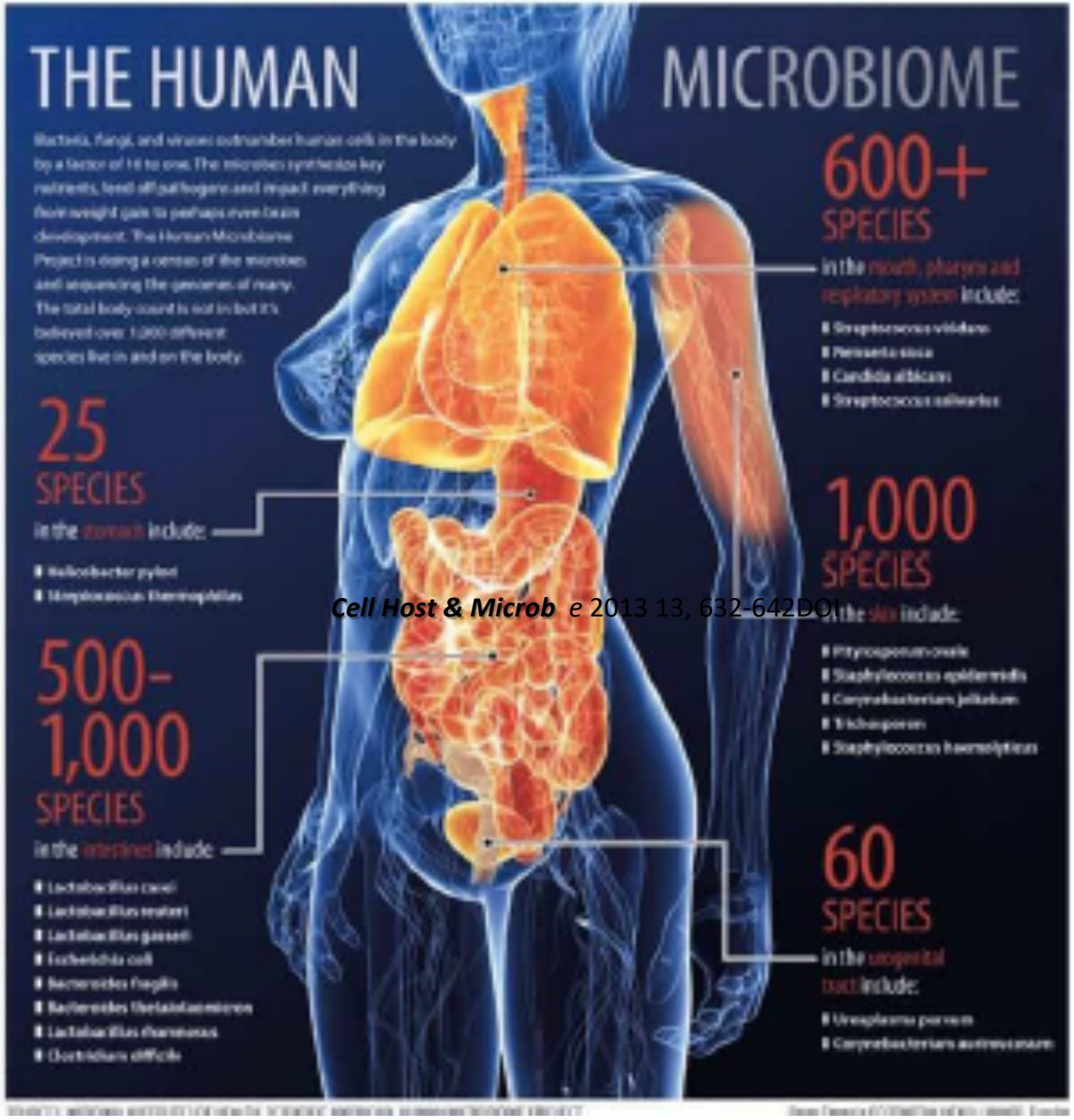
WHERE ARE THE MICROBES IN OUR BODIES?

- On your skin, in your mouth, nose, eyes, ears, lungs, vagina, gut…





Lee YKL, Science 2010

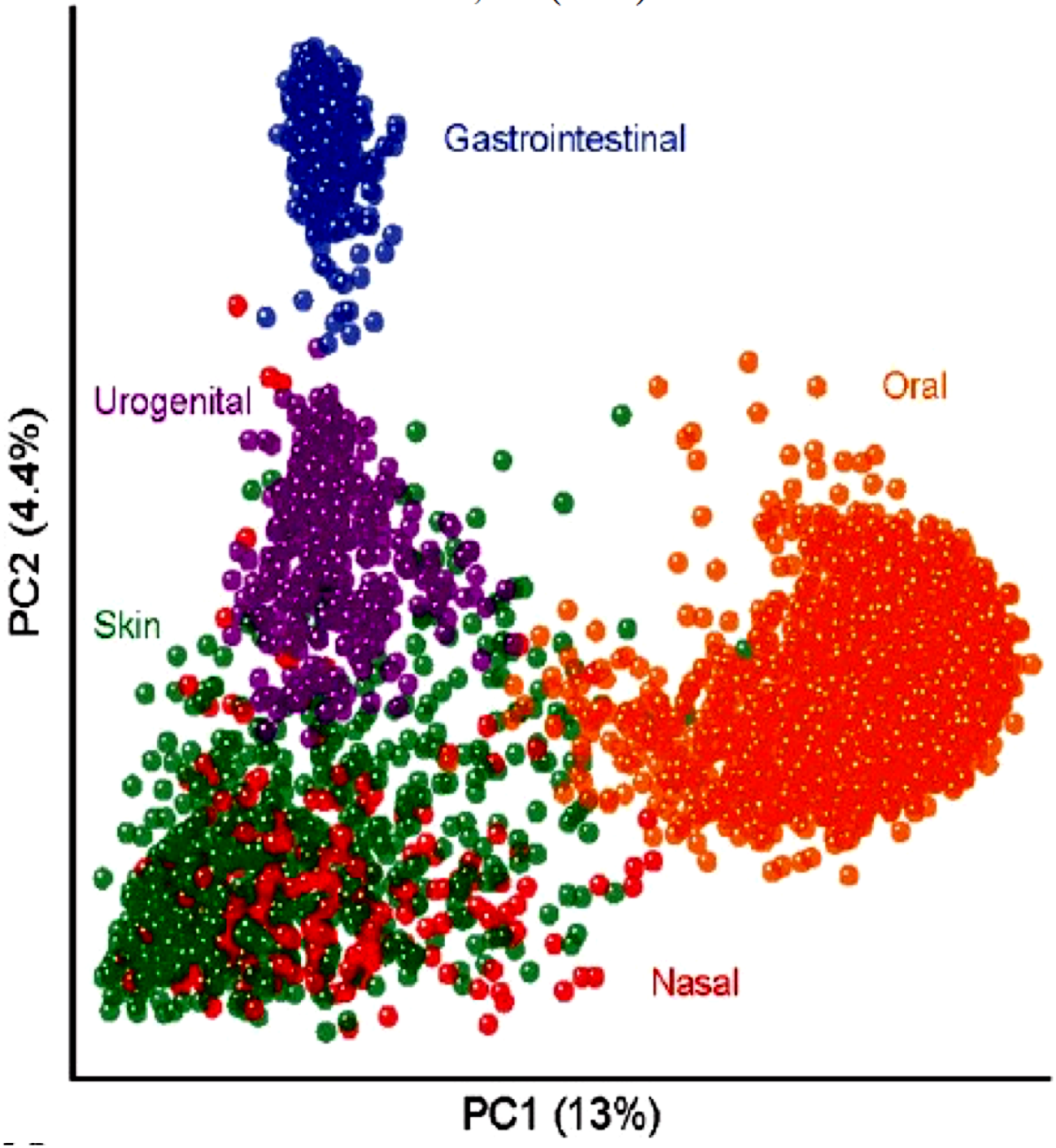


Cell Host & Microb *e* 2013 13, 632-642DOI

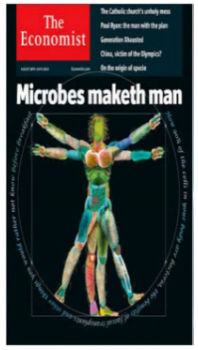
Cell Host & Microb *e* 2013 13, 632-642DOI

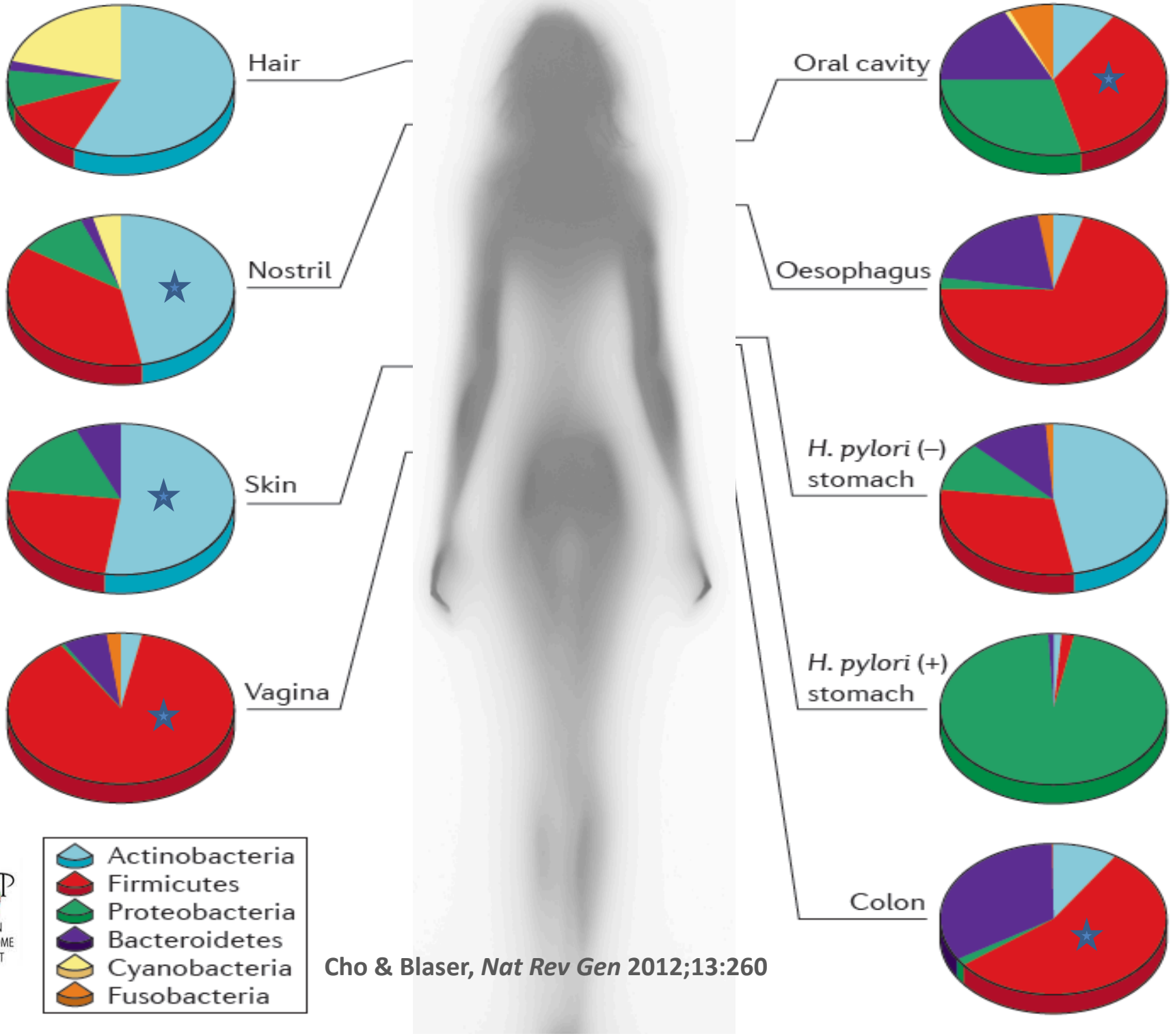
- We estimate there are approximately 1000 species in the intestine, approximately 700 in the mouth, approximately 300-400 in the vagina, approximately 700 in the skin.

] *Nature*. ; 486(7402): 207–214. doi:10.1038/nature11234.



Human microbiota(s)





Cho & Blaser, *Nat Rev Gen* 2012;13:260

Possiamo classificare i microbioti in :

-***Primari*** : fecale, vaginale, orale, cutaneo e nasale

-***Derivati*** : polmonare, endometriale, vescicale, mammario, intestinale fetale

I microbioti sono interconnessi e le interconnessioni sono strutturate :

- all'interno dell'ospite
- tra ospiti
- con l'ambiente circostante (terreno , atmosfera , cibo e animali)

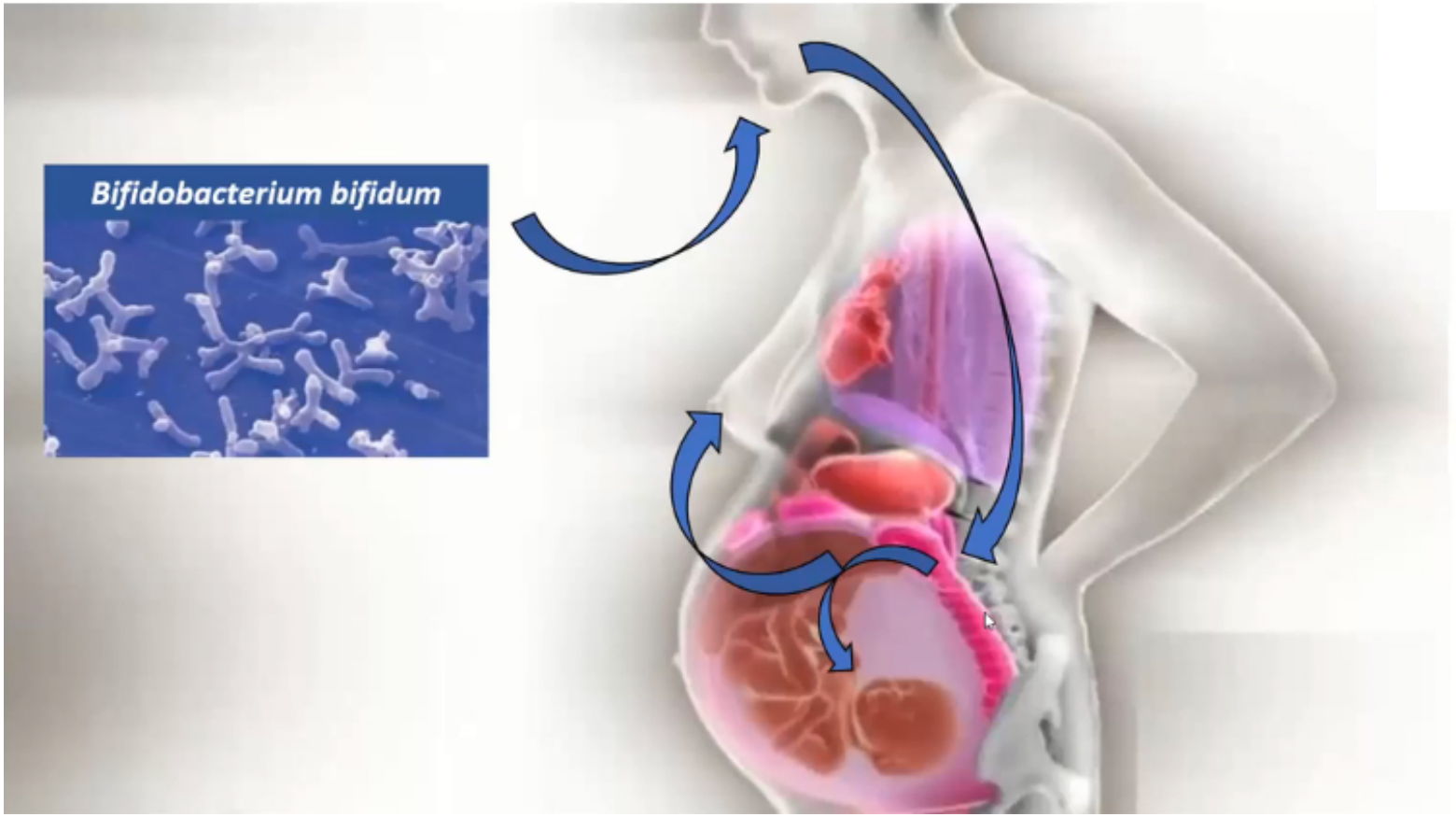
MICROBIOTI

Consorzi microbici (batterici)
in movimento nello spazio e nel tempo

Interconnessioni all'interno dell'ospite

- L'intervento sui microbioti primari influenza l'eubiosi del microbiota secondario, attuare una terapia batterica indicata per il microbiota primario, di conseguenza, ci darà vantaggi per quello secondario

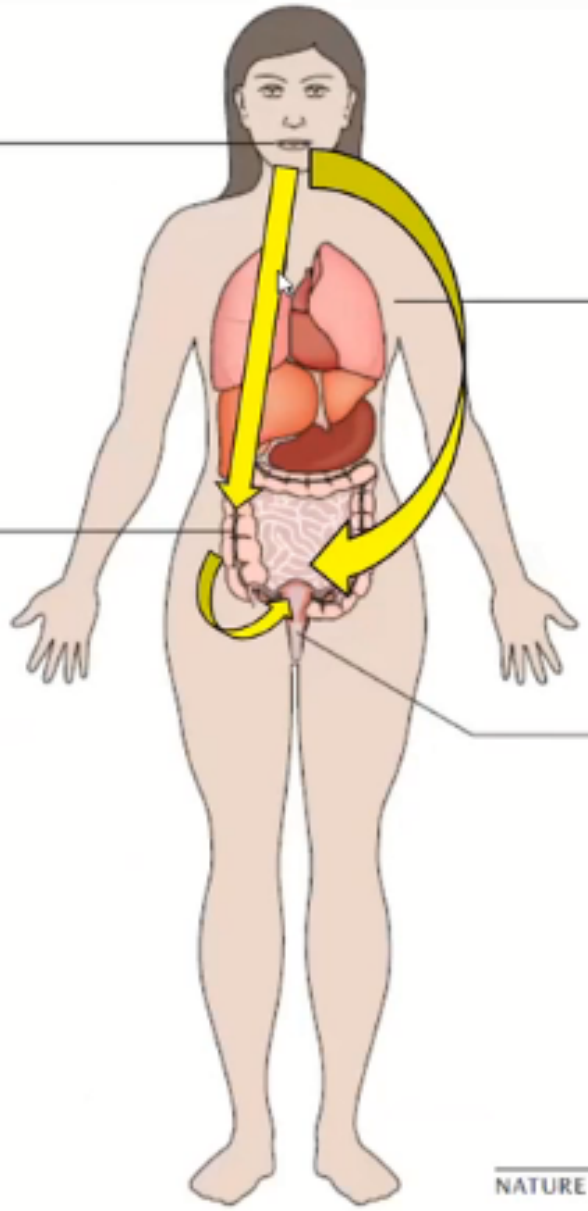
- L’intervento con lo Streptococcus salivariusK12 nel cavo orale ci darà vantaggi anche sul microbiota polmonare e placentare
- L’intervento con il Lactobacillus crispatusM247 sul microbioma intestinale porterà vantaggi all’ambiente vaginale e di conseguenza anche a quello endometriale e vescicale.


Bifidobacterium bifidum

Fusobacterium nucleatum

Oropharyngeal
- Periodontitis*
- Endodontic infections*
- Gingivitis*
- Tonsilitis
- Head and neck cancers

Gastrointestinal tract
- Appendicitis
- Inflammatory bowel diseases
- Colorectal cancer



Other
- Endocarditis
- Atherosclerosis
- Respiratory tract infections
- Brain abscess
- Liver abscess
- Osteomyelitis

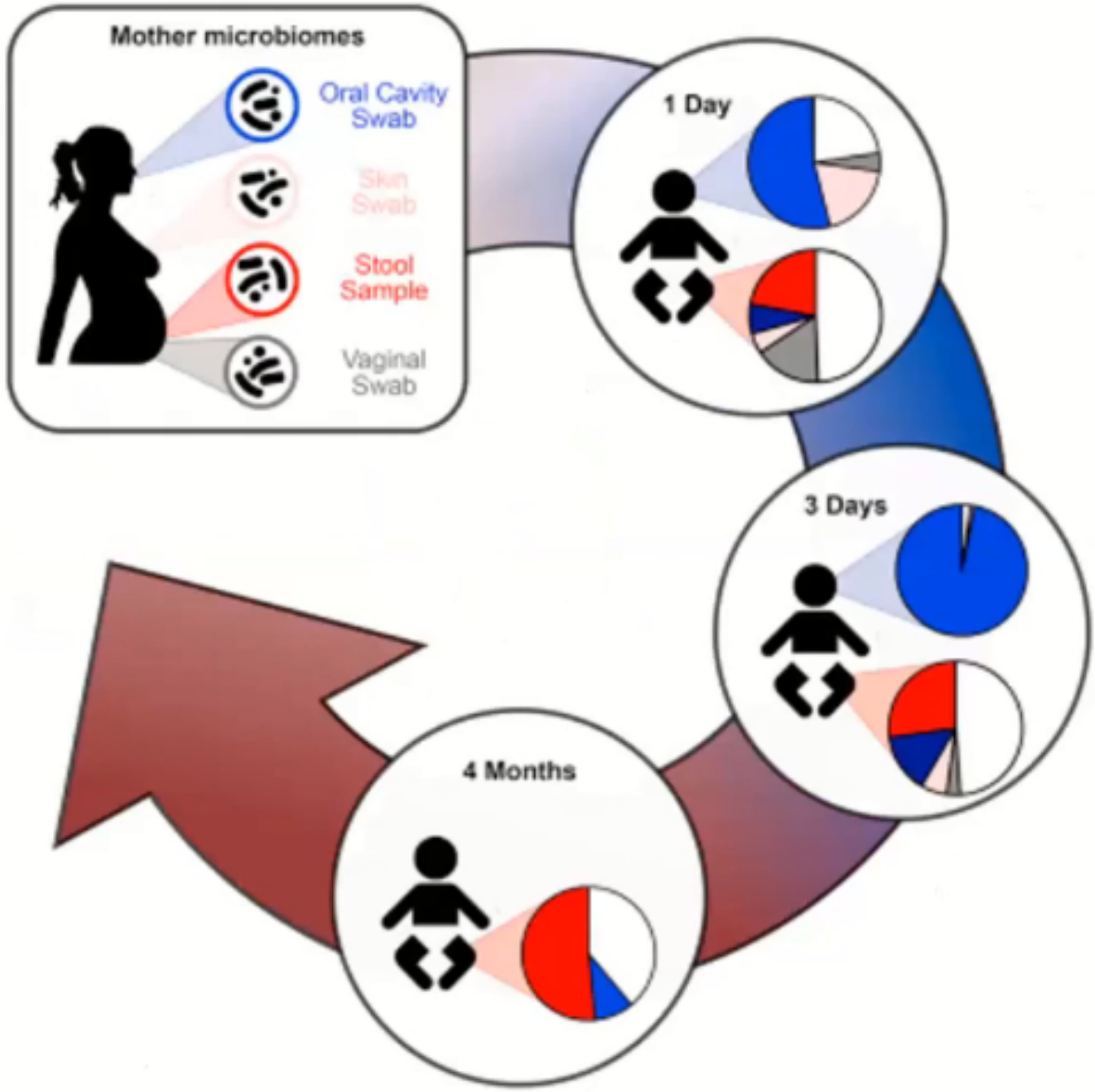
Urogenital tract
- Adverse pregnancy outcomes (pre-term labour, stillbirth and chorioamnionitis)
- Urinary tract infections

- Moderate evidence
- Some evidence
- Associative evidence

*Polymicrobial infections

Interconnessioni tra ospiti

- Una giusta colonizzazione del microbiota intestinale materno durante l'ultimo trimestre di gravidanza con il Bifidobacterium bifidumPRL2010 ne garantisce sicuramente il trasferimento durante il parto e tutto l'allattamento al seno al nascituro , che ne gioverà in termini metabolici e immunitari , certamente nei suoi primi 2 anni di vita , ma anche nel suo futuro


Mother microbiomes
Oral Cavity Swab
Skin Swab
Stool Sample
Vaginal Swab
1 Day
3 Days
4 Months

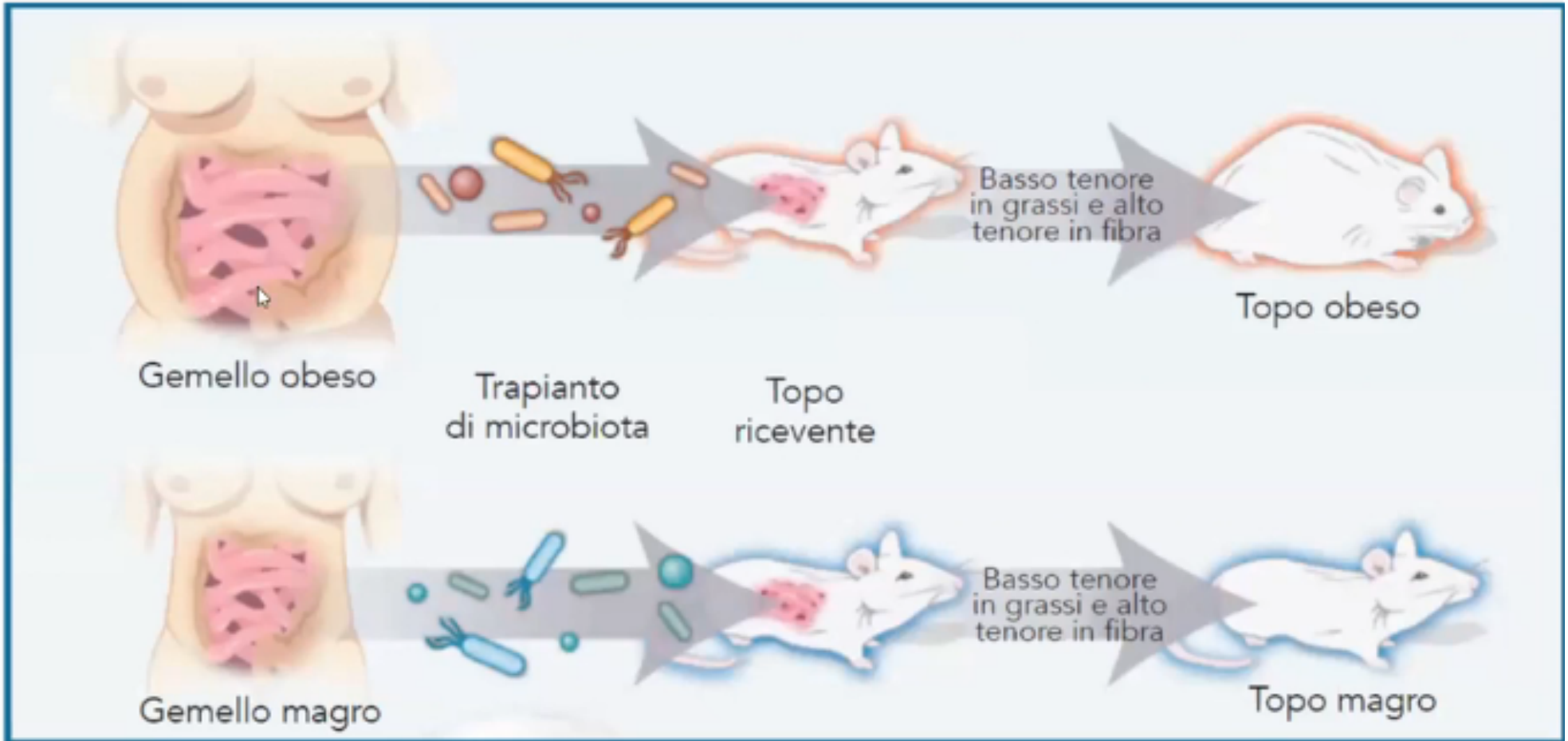
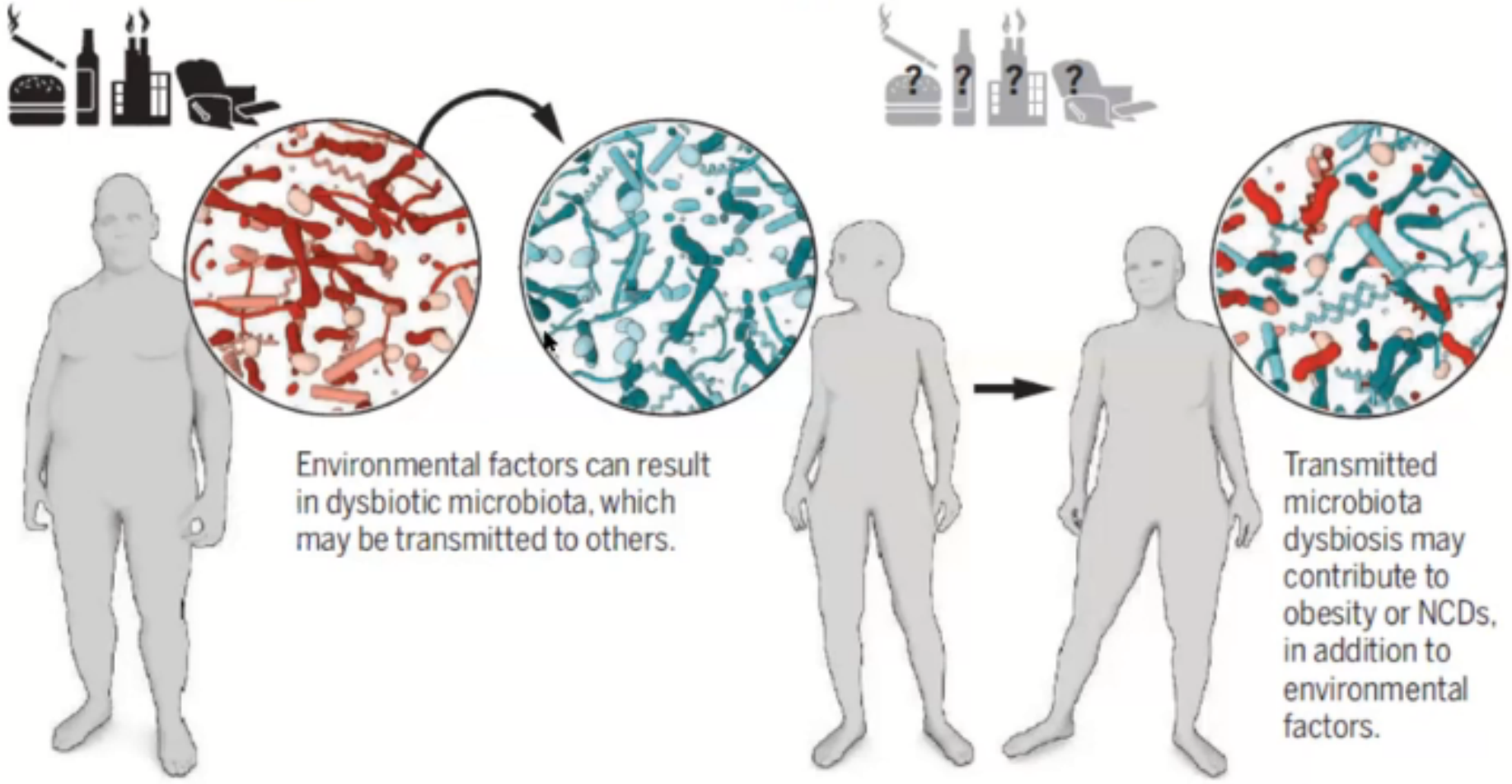


Figura 2 - Il trasferimento del microbiota fecale da donatore obeso e da donatore magro trasferisce, nel topo gnotobiotico, i fenotipi obeso e magro anche se i due donatori sono gemelli omozigoti.

Da: Walker AW *et al.* Science. 2013;341(6150):1069-70 (adattato)

Microbial transmission in NCDs?

The microbiota of an individual is affected by environmental factors such as diet, smoking, alcohol intake, and exercise. Dysbiotic microbiota can influence NCDs or their risk factors, such as obesity, and might be transmitted between individuals, potentially contributing to the spread of disease.



Interconnessioni con l'ambiente

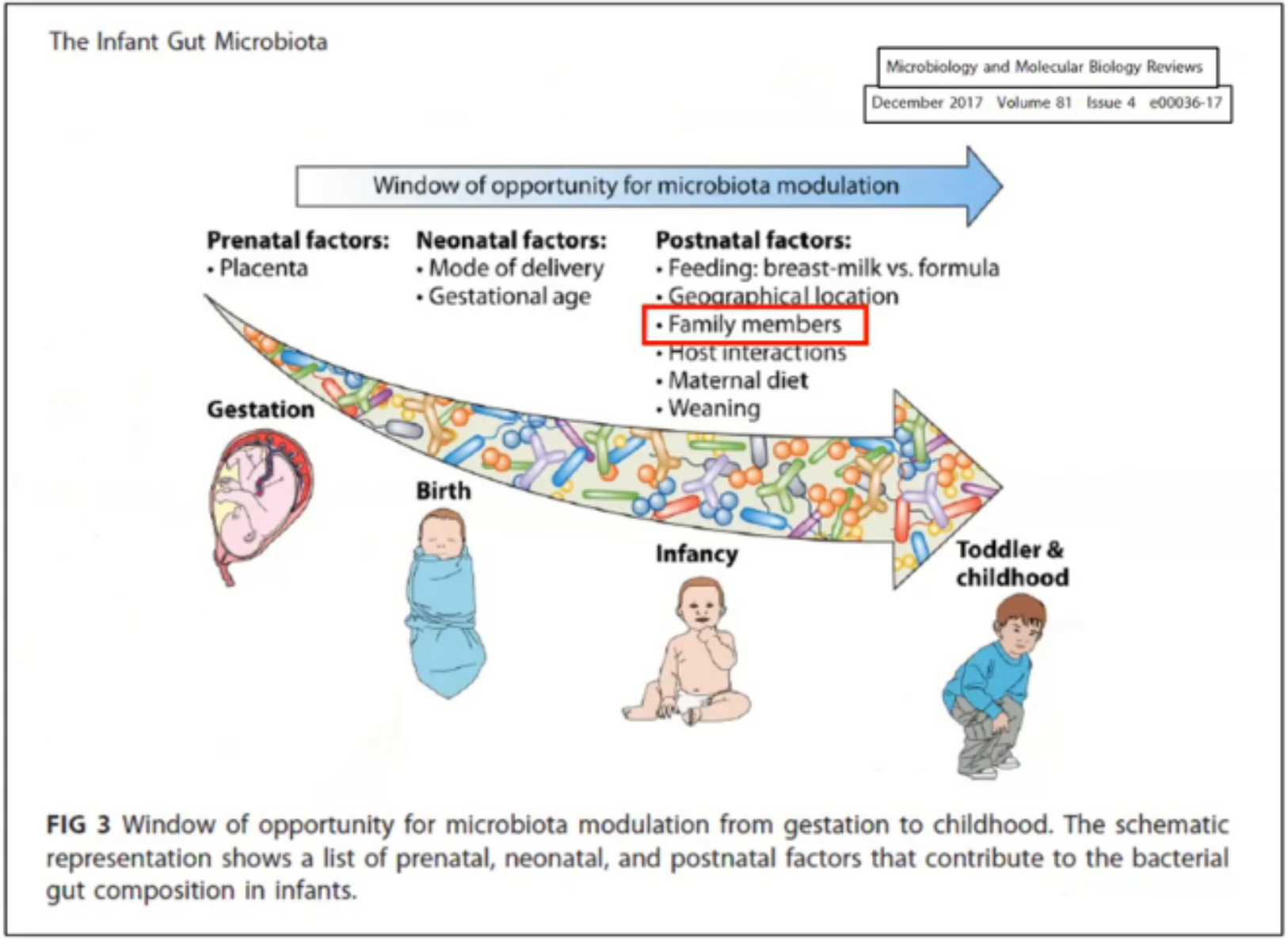
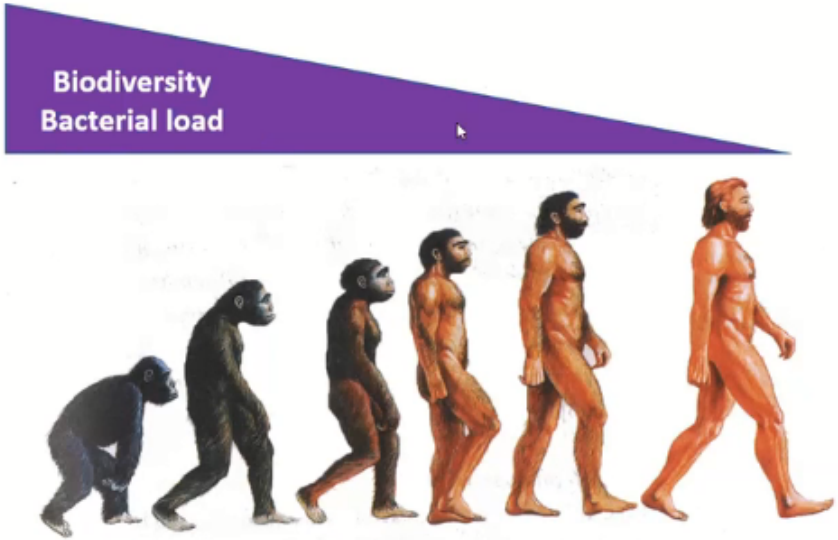
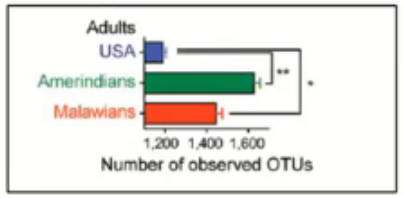
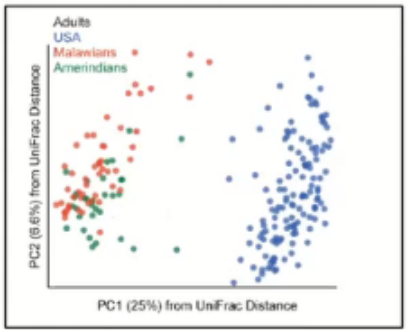


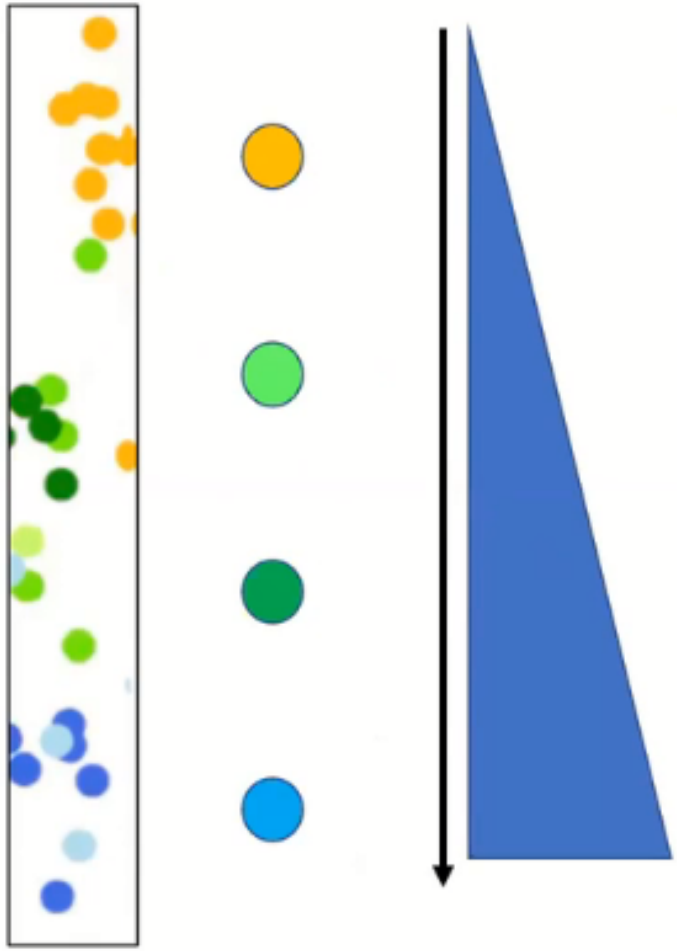
FIG 3 Window of opportunity for microbiota modulation from gestation to childhood. The schematic representation shows a list of prenatal, neonatal, and postnatal factors that contribute to the bacterial gut composition in infants.







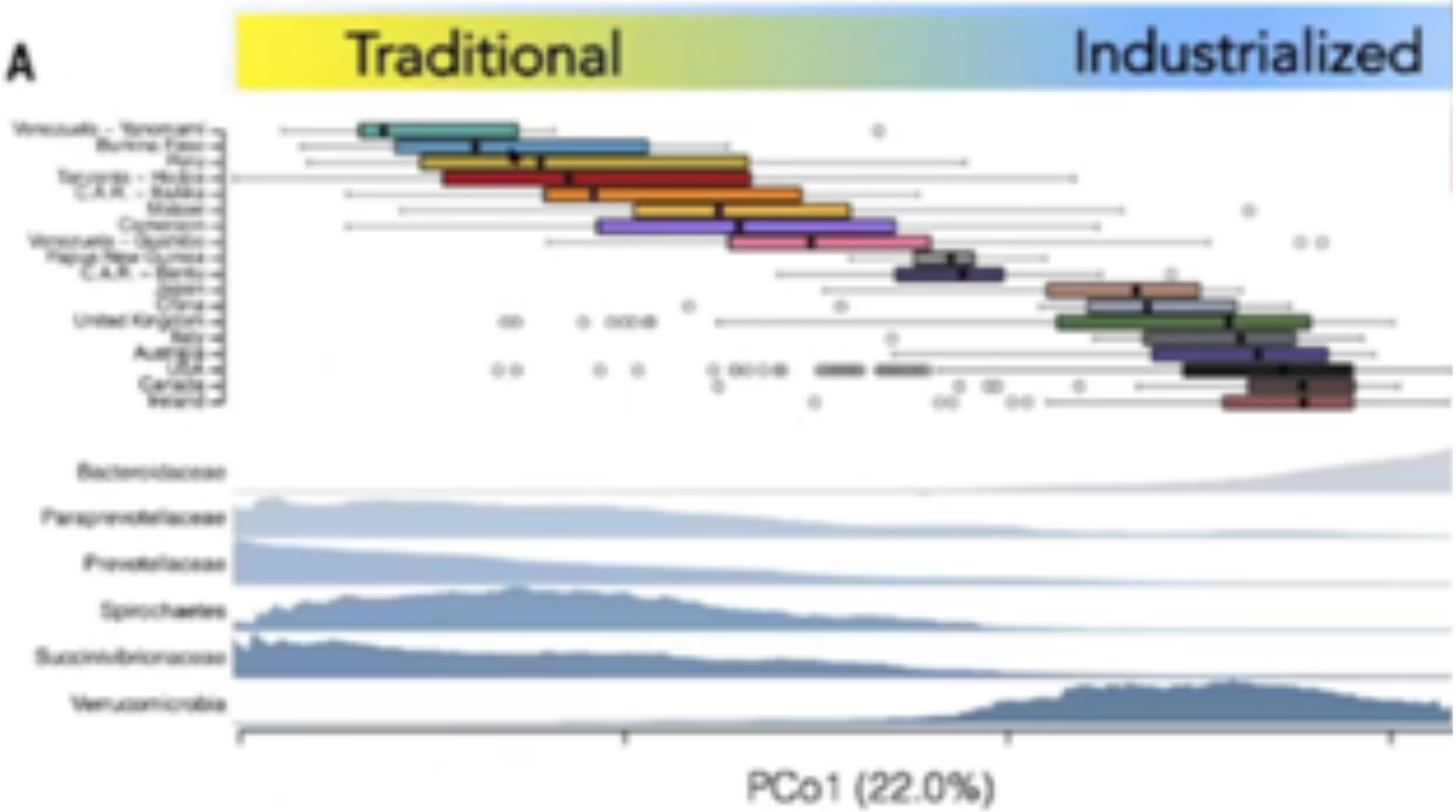
Nature. ; 486(7402): 222–227. doi:10.1038/nature11053.



α-biodiversity

Bacterial load

Dunn RR, Amato KR, Archie EA, Arandjelovic M, Crittenden AN and Nichols LM (2020) The Internal, External and Extended Microbiomes of Hominins. Front. Ecol. Evol. 8:25. doi: 10.3389/fevo.2020.00025

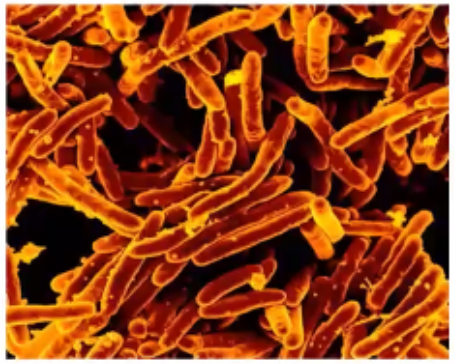


Vulnerability of the industrialized microbiota

J.L. Sonnenburg , *Science* 25 Oct 2019

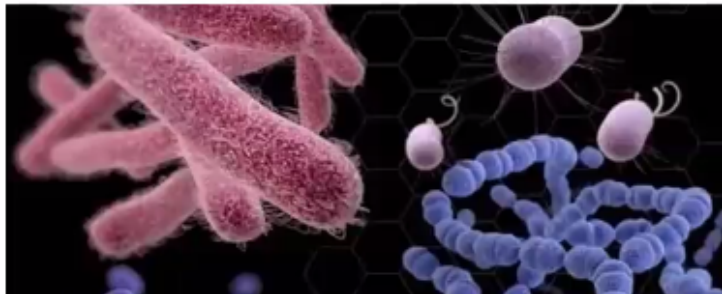
Vol. 366, Issue 6464, eaaw9255

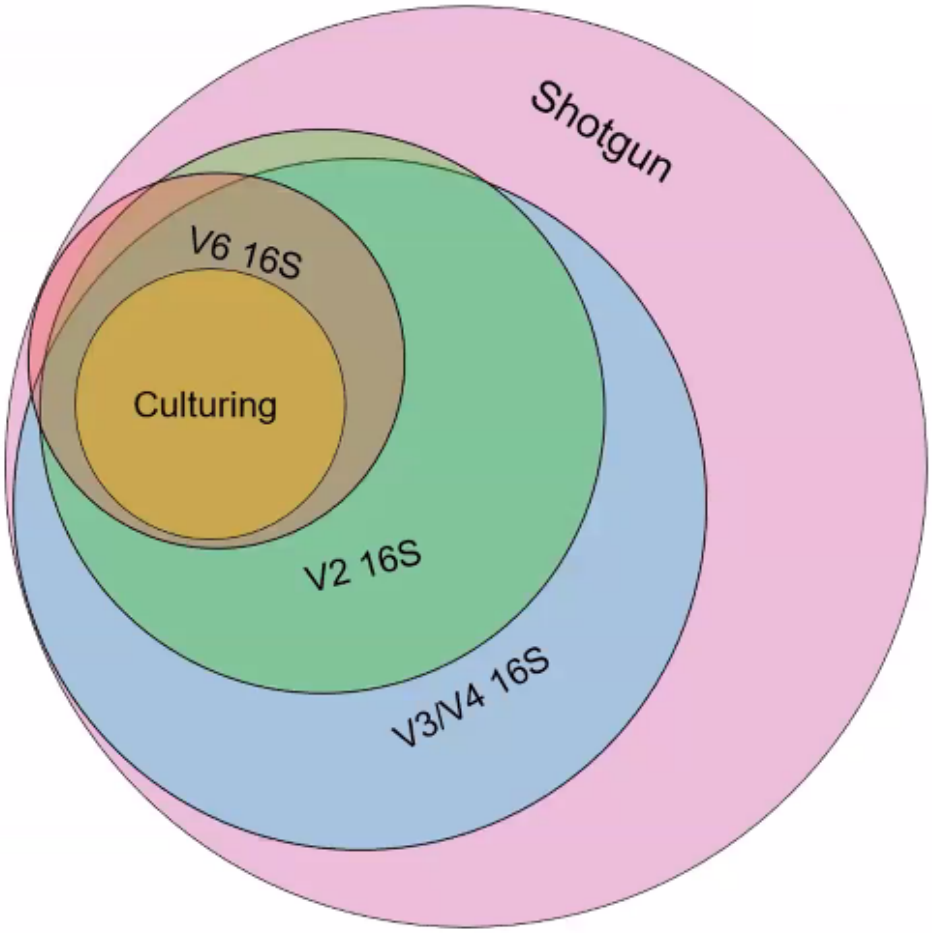
TASSONOMIA BATTERICA



Tecniche colturali

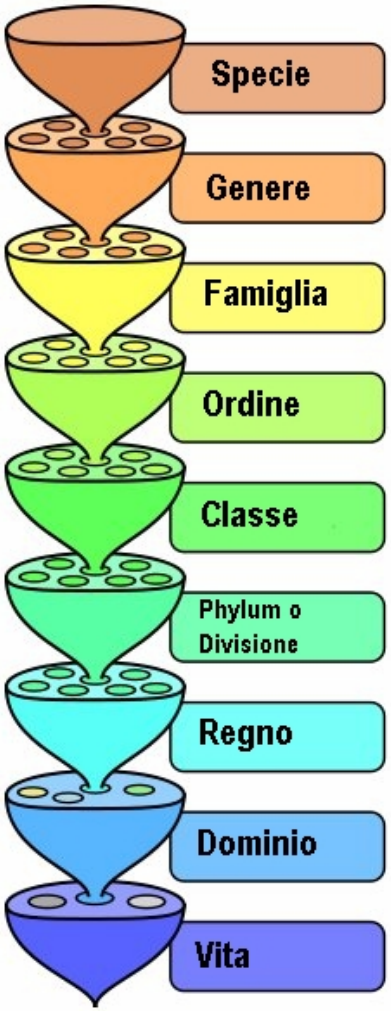
NGS





- Se oggi possiamo studiare (sebbene solo per quanto riguarda la parte batterica) la composizione del microbiota lo dobbiamo alla **metagenomica**, la quale basa le proprie indagini sul microbioma.
- In particolare, l'esame in grado di indagare la popolazione batterica è il **sequenziamento genomico del 16S rRNA**, un gene dell'RNA specifico di ogni batterio che serve a produrre i ribosomi, responsabili della sintesi proteica. Identificarlo significa risalire alla singola specie batterica.

TECNICHE DI SEQUENZIAMENTO GENOMICO

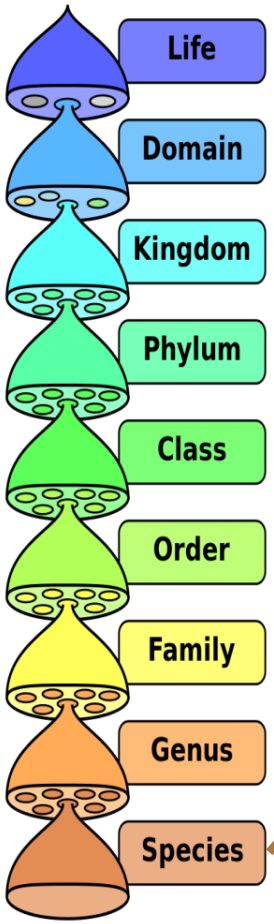


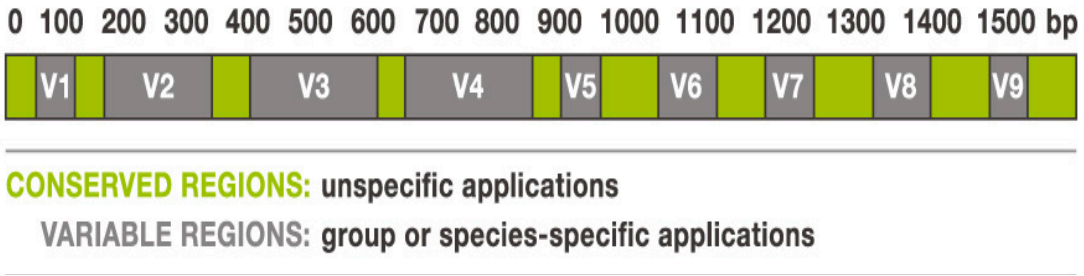
Mediante l'uso del gene 16 SrRNA, unico per ora che identifica i procarioti quali sono i nostri batteri intestinali.

Le tecniche genomiche hanno permesso di:

- Identificare altre popolazioni batteriche presenti nell'intestino
- Di raggruppare in Phylum (tribù) gli ordini e le specie

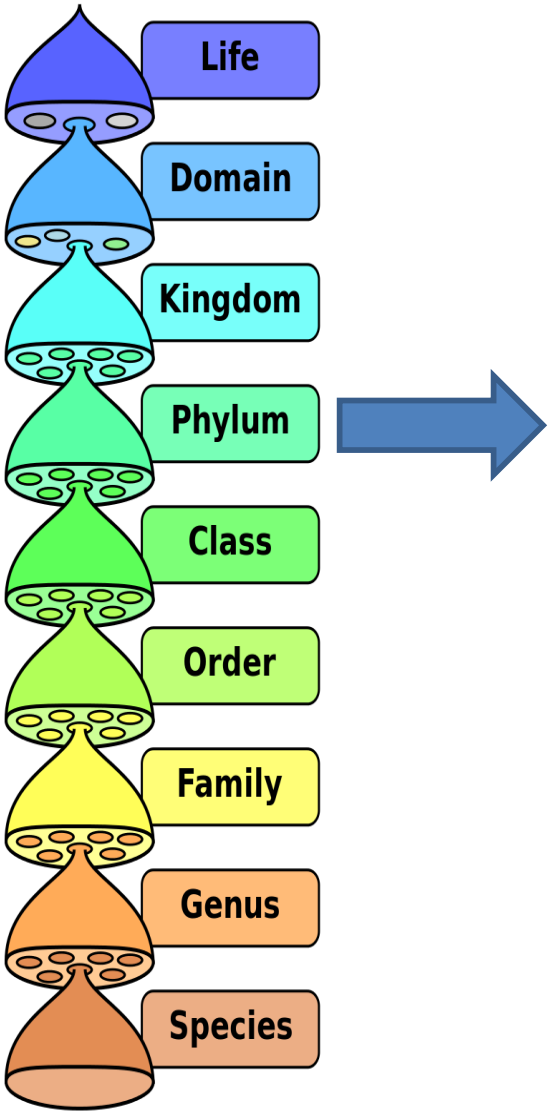
16S ribosomal RNA gene sequences





- The 16S rRNA is part of the 30S (small) subunit of the ribosome. Its sequence is highly conserved.
- The 16S rRNA has blocks of conserved regions (green blocks) interspersed with variable sequences (grey). This makes designing primers for PCR amplification possible.
- Due to this conservation across the genus *Bacteria* it is possible to **design primers that can amplify any bacterial species.**
- <u>Bacterial taxonomy based on 16S rRNA sequence:</u> Variable regions are nearly identical within species, but also similar within a genus and family. **Therefore, analysis can be performed to identify the genus (e.g. *Bacteriodes*), species (*Bacteriodes fragilis*).**

Gut Microbiome Composition



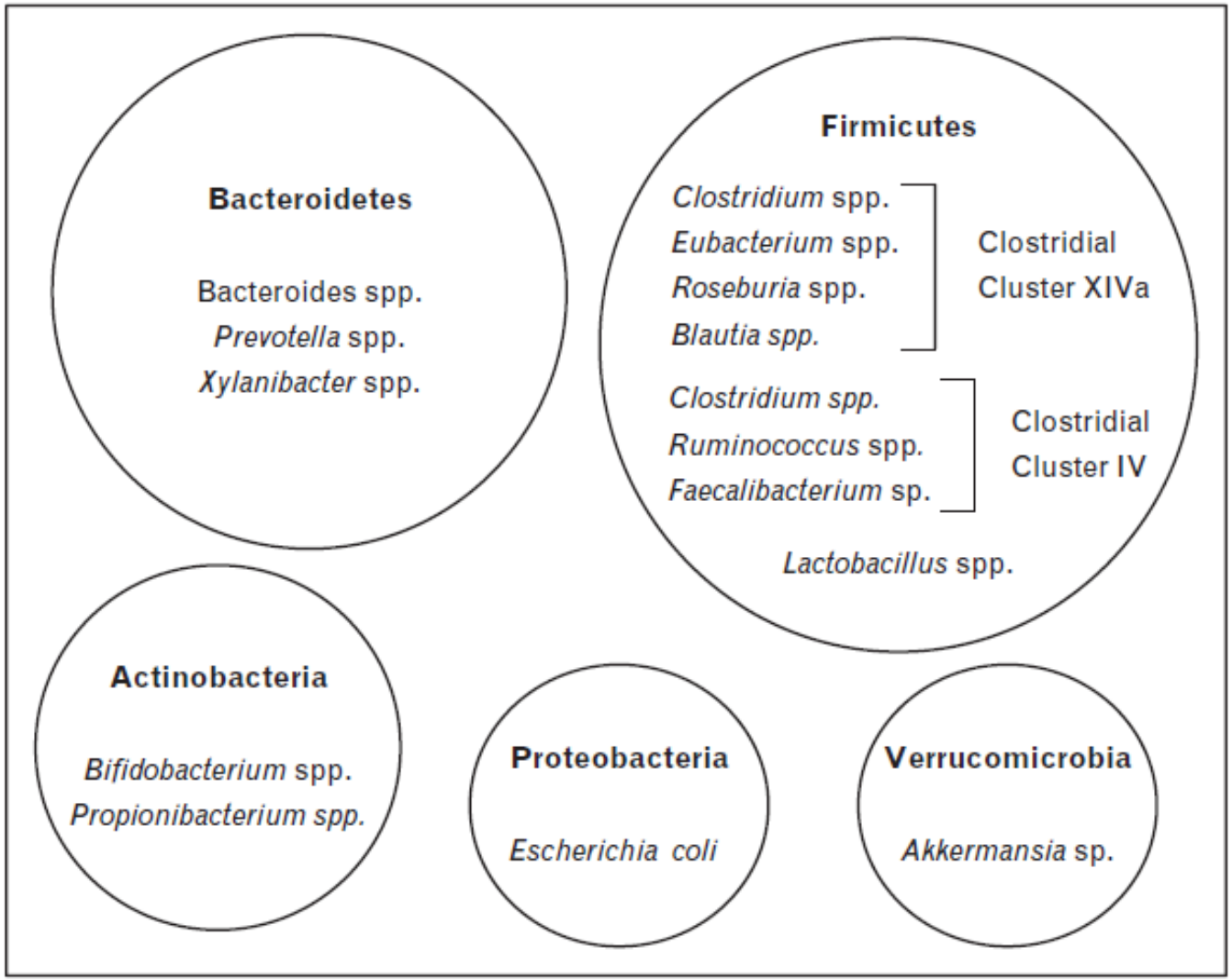
Composed of 5 main phyla:

- *Bacteroidetes*
- *Firmicutes*

90%

- *Actinobacteria*
- *Proteobacteria*
- *Verrumicrobia*

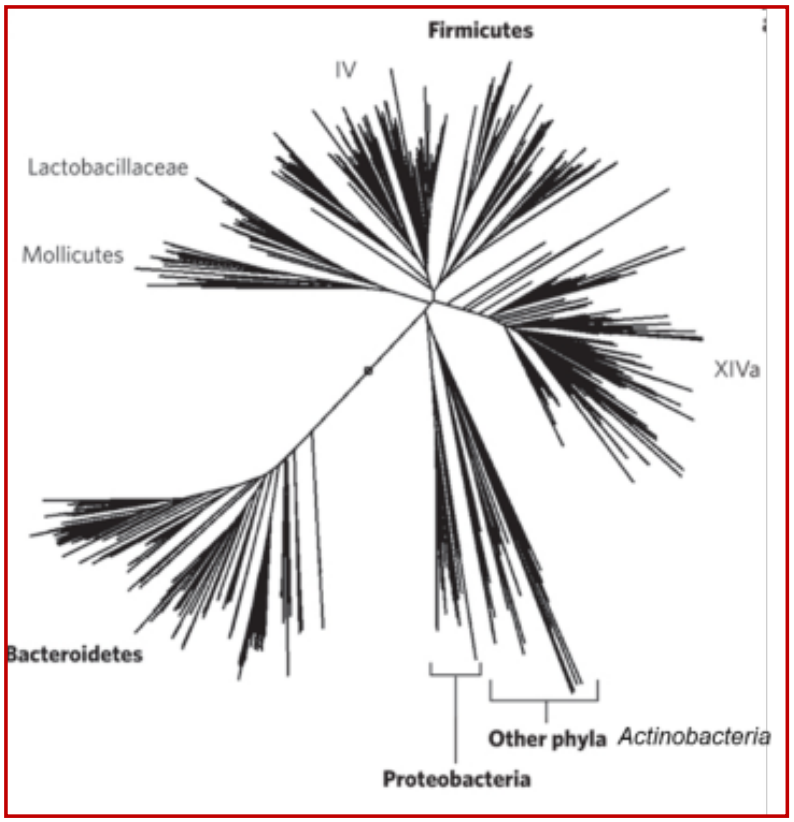
Qin, J., et. al (2010). *Nature,* 464, 59-65.



Predominant species of the human colonic microbiota.

Chassard et Lacroix, 2013

PHYLOGENETIC DIVERSITY



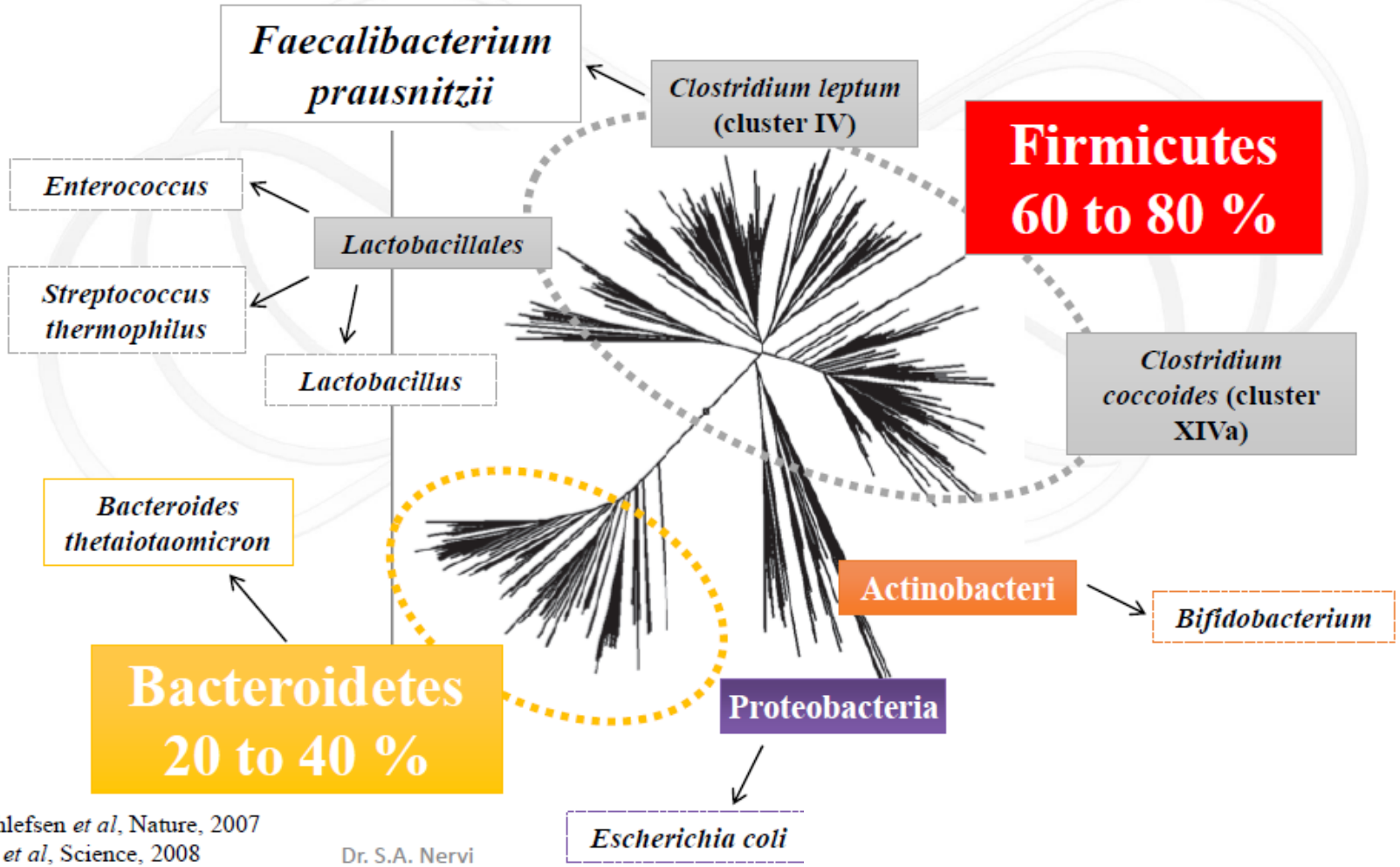
> 1000 species

5 (out of 100) bacterial phyla

- *Firmicutes* (65%), *Bacteroidetes* (25%)
- *Actinobacteria* (5%), *Proteobacteria* (<8%), *Fusobacteria* (1%) and *Verrucomicrobia* (1%)

Phylogenetic Diversity of Intestinal Microbiota

2 major phyla: Firmicutes and Bacteroidetes (80 to 90 % of the microbiota)



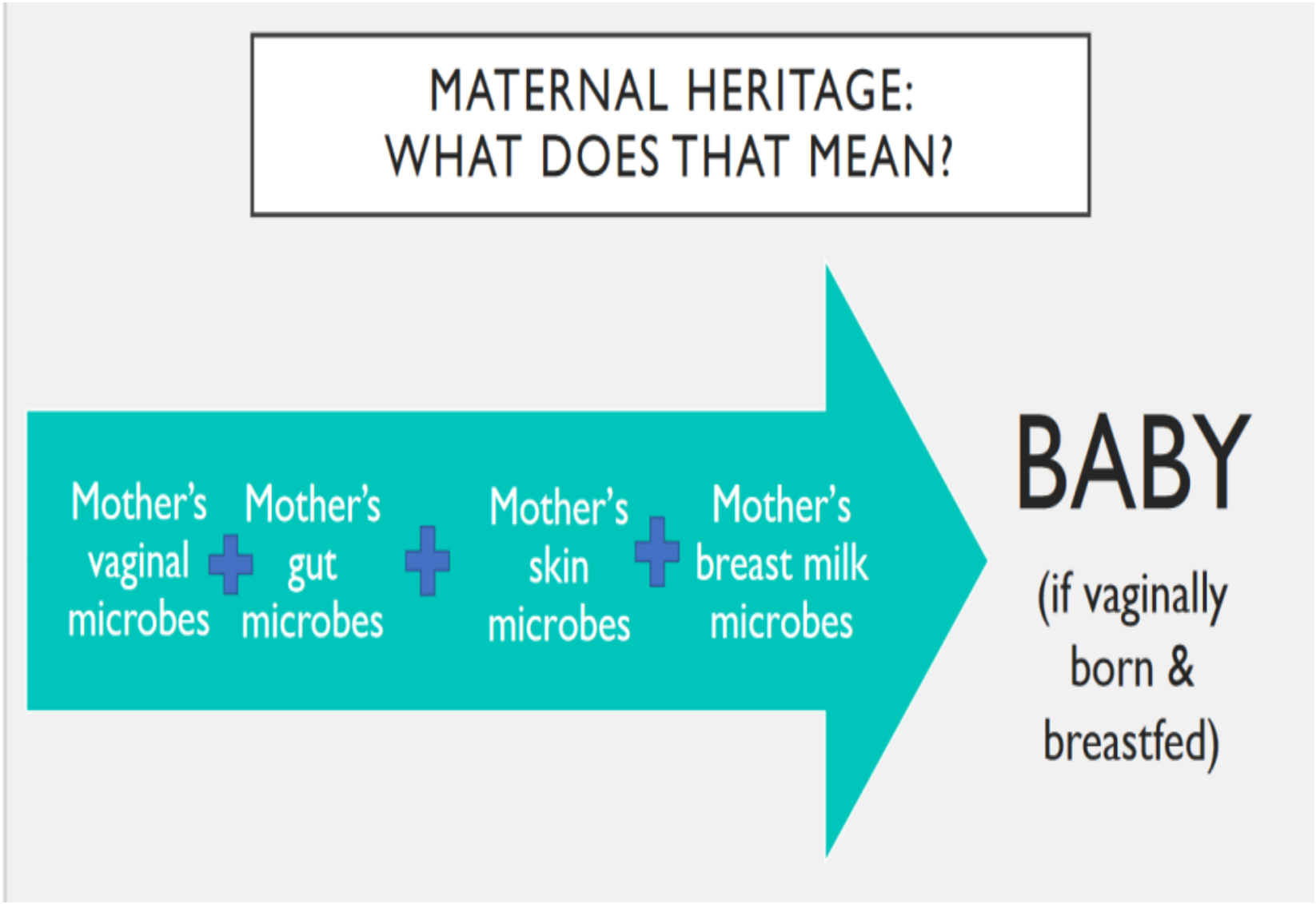
Dethlefsen *et al*, Nature, 2007
Ley *et al*, Science, 2008
Tap *et al*, Environ Microbiol, 2009

Dr. S.A. Nervi

COME SI FORMA IL MICROBIOTA

THE MOTHER'S UNIQUE MICROBIOME IS PASSED TO THE NEXT GENERATION

Through
vaginal birth
& exclusive
breastfeeding


MATERNAL HERITAGE:
WHAT DOES THAT MEAN?
Mother's vaginal microbes
Mother's gut microbes
Mother's skin microbes
Mother's breast milk microbes
BABY
(if vaginally born & breastfed)

IF VAGINALLY BORN AND BREASTFED.....

- Mother's Microbiome

- Baby's Microbiome

(Resembles)

FROM WHICH SOURCES COULD FETUS BE EXPOSED?

- Amniotic fluid

Science ref: 11. Oh K.J. et al, *J Perinat Med* 2010

- Umbilical cord blood

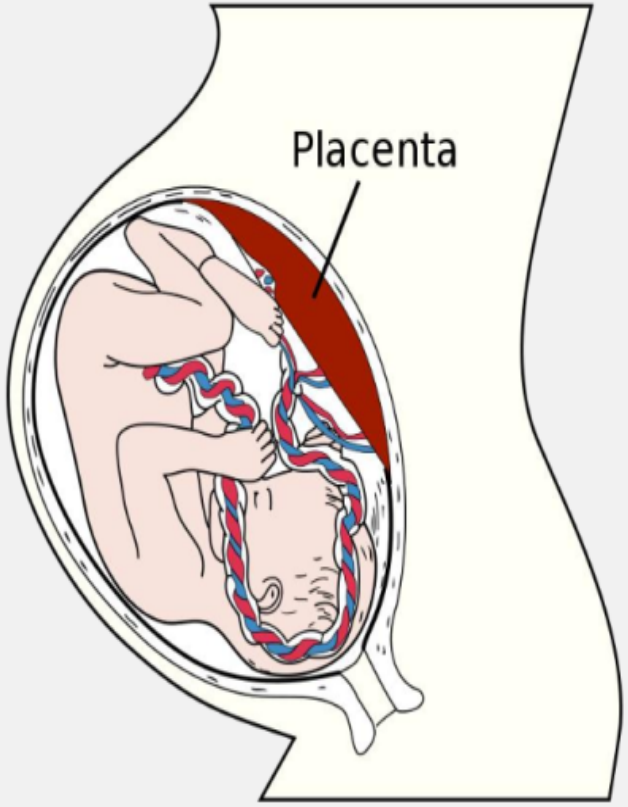
Science ref: 12. Jiménez E. et al, *Curr Microbiol.* 2005

- Fetal membranes

Science ref: 13. Steel JH, *Pediatr Res.* 2005

- Placenta

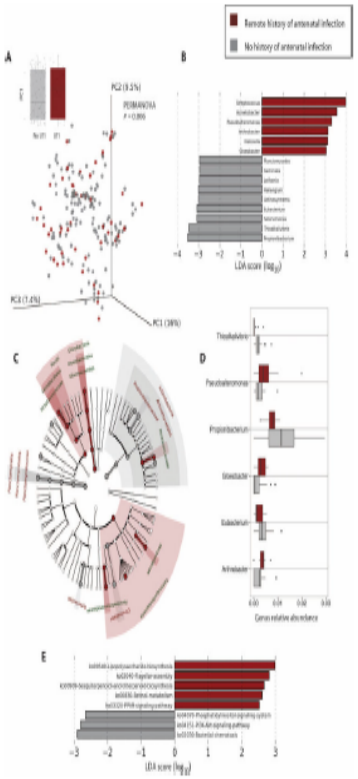
Science ref: 14. Aagaard K, *Sci Transl Med.* 2014



The Placenta Harbors a Unique Microbiome

K. Aagaard, J. Ma, K.M. Antony, R. Ganu, J. Petrosino, J. Versalovic

PMC 2016 July 01. Published final edited : Sci Transl Med. **2014** May 21; 6(237): 237ra65.
doi:10.1126/scitranslmed.3008599



Abbiamo identificato un specifico microbioma placentare unico, composto da:
Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes e Fusobacteria phyla.

i profili del microbioma placentare erano più affini (dissomiglianza <0,3) al microbioma orale umano.

⇒ Burkholderia, Actinomycetales e Alphaproteobacteria erano preponderanti tra le gravide pretermine

⇒ Paenibacillus era preponderante nei campioni placentari a termine

REVIEW: MATERNAL HEALTH AND THE PLACENTAL MICROBIOME

E.Pelzer, L.F.Gomez-Arango, H.L.Barrett, M. Dekker Nitert **Placenta** Volume 54, **June 2017**, Pages 30-37 https://doi.org/10.1016/j.placenta.2016.12.003

Il microbioma ha un ruolo centrale nella regolazione del metabolismo, della funzione immunitaria e del comportamento nell'uomo.

È diventato chiaro che la placenta non è un organo sterile, ma ha il suo microbioma endogeno.

La composizione del microbioma placentare è diversa da quella della vagina mentre si sovrappone, come specie batteriche, al microbioma orale.

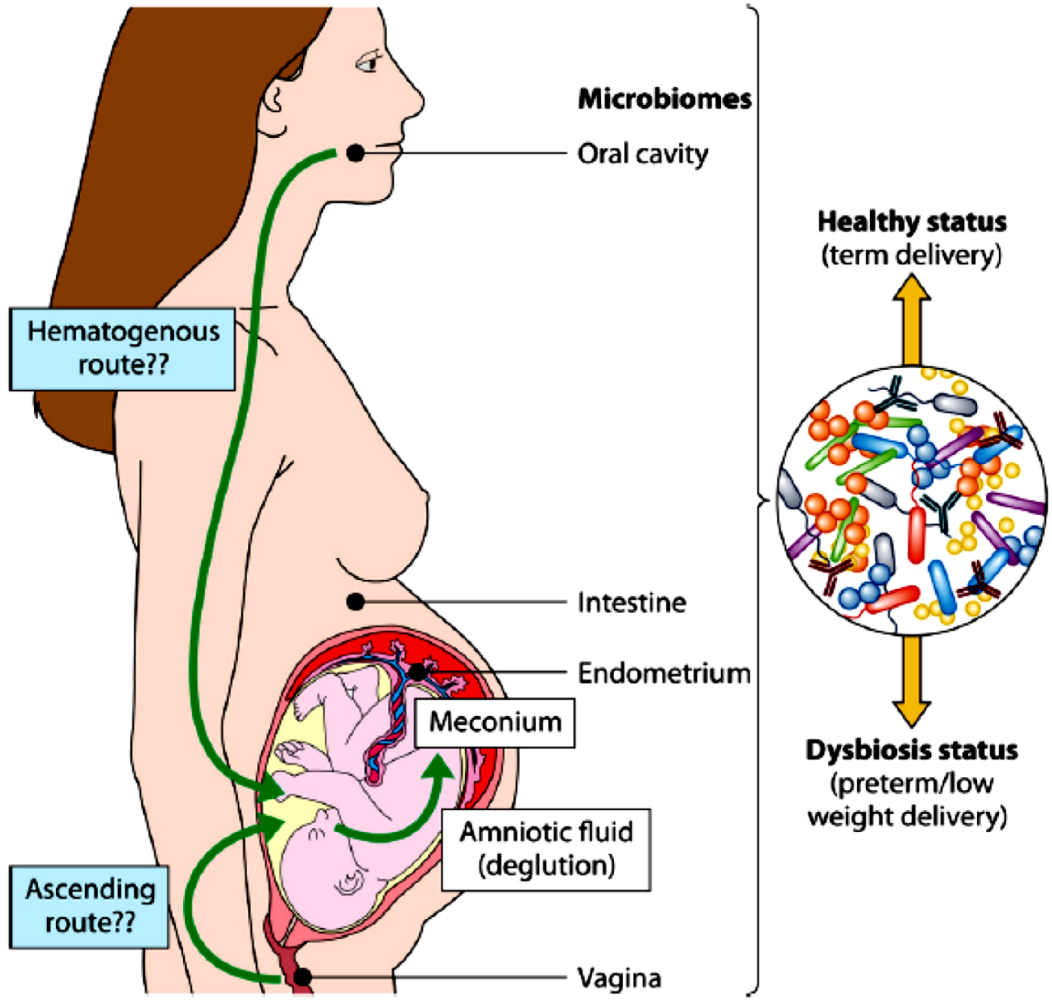


FIG 4 Colonization routes of maternal microbiomes to the infant. The mother portrayal exhibits the maternal microbiome locations and the related routes that result in the vertical transmission of the microbiota to the infant.

nature medicine

ARTI

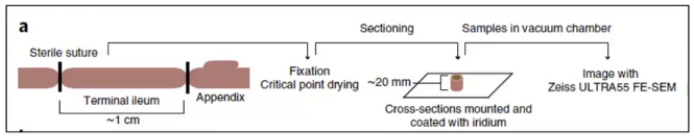
https://doi.org/10.1038/s41591-

Viable bacterial colonization is highly limited in the human intestine in utero

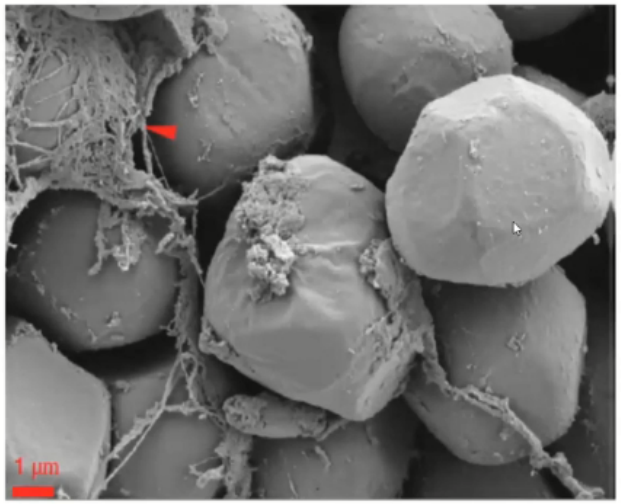
E. Rackaityte[1,2], J. Halkias[3,4], E. M. Fukui[1], V. F. Mendoza[3,4], C. Hayzelden[5], E. D. Cra
K. E. Fujimura[1,9], T. D. Burt[8] and S. V. Lynch[1]

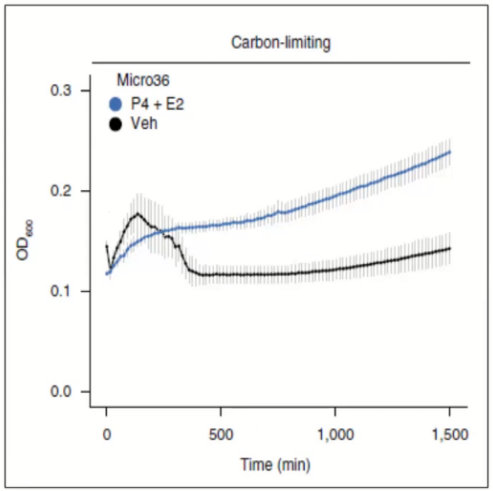
Mucosal immunity develops in the human fetal intestine by 11–14 weeks of gestation, yet whether viable mi and interact with the intestinal immune system is unknown. Bacteria-like morphology was identified in po meconium at mid-gestation by scanning electron microscopy ($n = 4$), and a sparse bacterial signal was de sequencing ($n = 40$ of 50) compared to environmental controls ($n = 87$). Eighteen taxa were enriched in f Micrococcaceae ($n = 9$) and *Lactobacillus* ($n = 6$) the most abundant. Fetal intestines dominated by Micro distinct patterns of T cell composition and epithelial transcription. Fetal *Micrococcus luteus*, isolated onl monocytes, grew on placental hormones, remained viable within antigen presenting cells, limited inflan possessed genomic features linked with survival in the fetus. Thus, viable bacteria are highly limited in t mid-gestation, although strains with immunomodulatory capacity are detected in subsets of specimens.

NATURE MEDICINE | VOL 26 | APRIL 2020 | 599–607 | ww

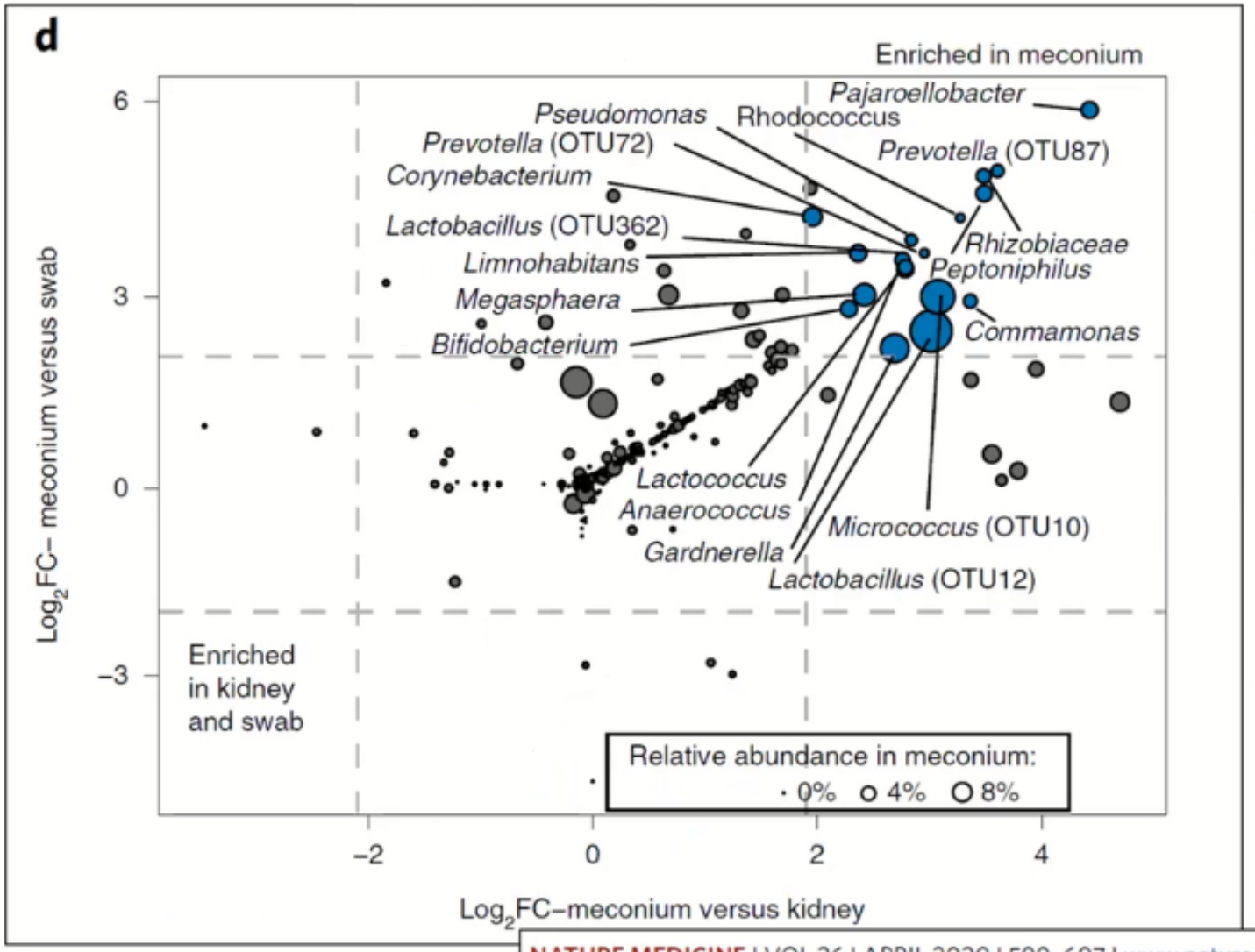


NATURE MEDICINE | VOL 26 | APRIL 2020 | 599–607 | www.nature





Effects of 10^{-5} M progesterone (P4) and 10^{-6} M β-estradiol (E2) on the growth of Micro36

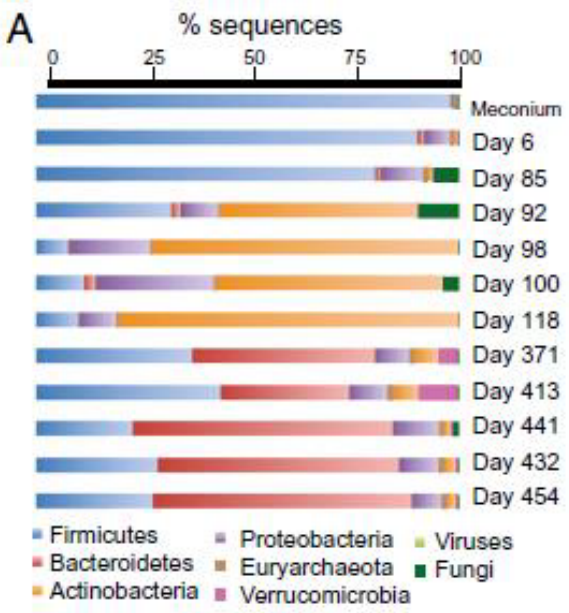


NATURE MEDICINE | VOL 26 | APRIL 2020 | 599–607 | www.nature.com/naturemedicine

COSA AVVIENE AL MOMENTO DEL PARTO ….

IMPIANTO O TRAPIANTO DEL MICROBIOTA MATERNO

Alla nascita il neonato è esposto a:



Microbiota Vaginale (materno)

Microbiota Fecale (materno)

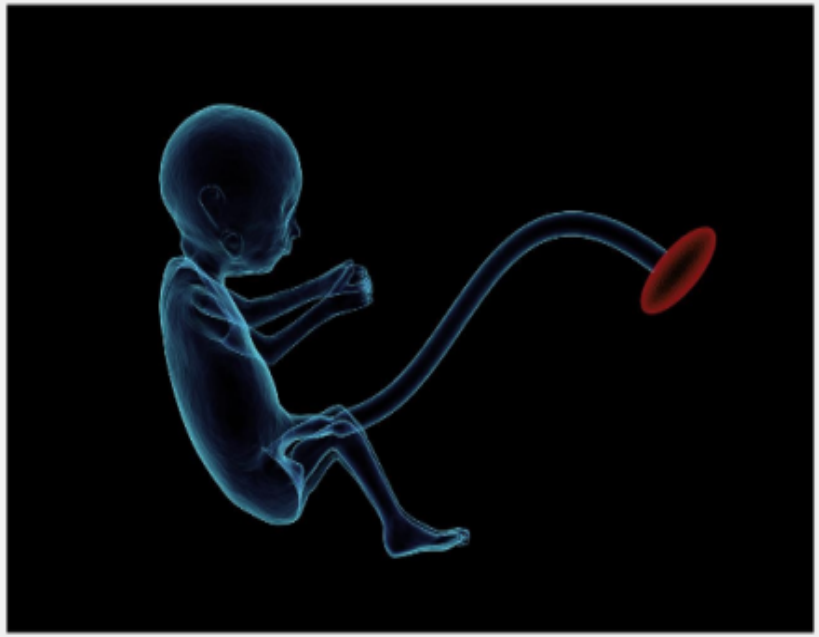
Microbiota Cutaneo (madre,padre,parenti, babysitter, animali)

Dieta Ambiente

Il Native CORE microbiota si sviluppa nei primi 12-24 mesi di vita

MATERNAL HERITAGE

- *'Gestational changes in the vaginal and intestinal microbiome are of particular relevance because these body sites are responsible for vertical microbial transmission to the newborn'*
  - - Quote from science ref: 1. *Mueller N.T. et al, Trends in Molecular Medicine 2015*



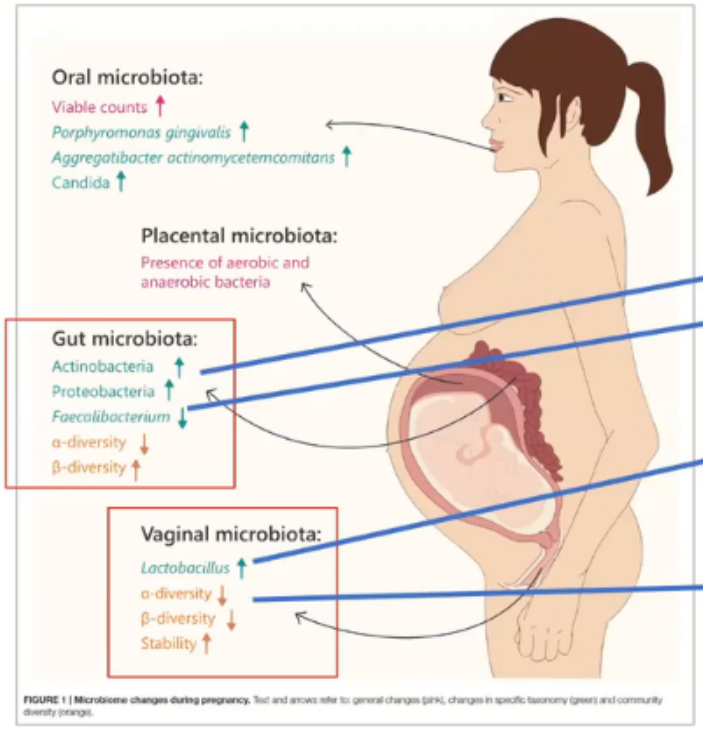


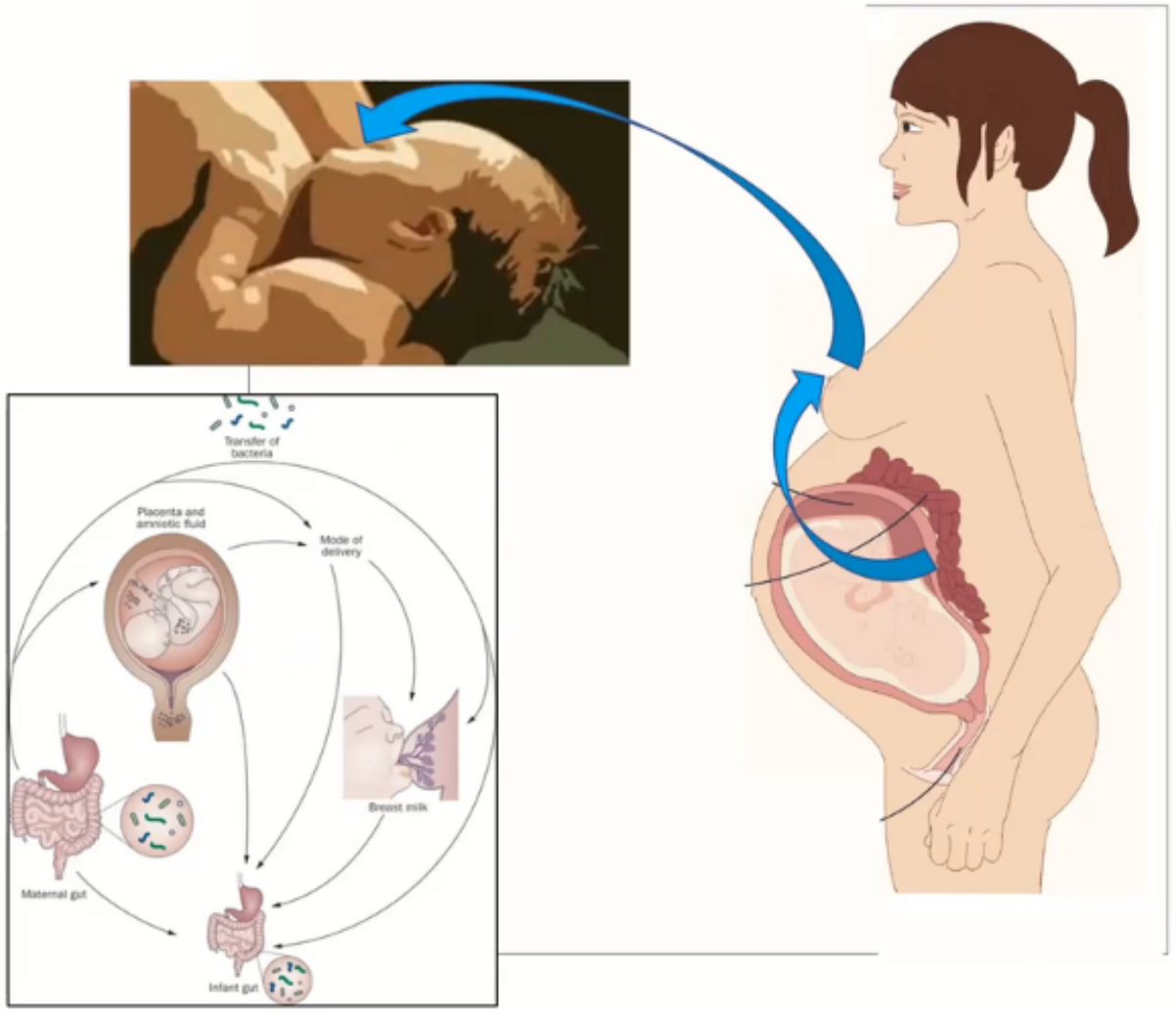
FIGURE 1 | Microbiome changes during pregnancy. Text and arrows refer to: general changes (pink), changes in specific taxonomy (green) and community diversity (orange).

Infiammazione
Insulino-resistenza

Bonifica della nicchia vaginale

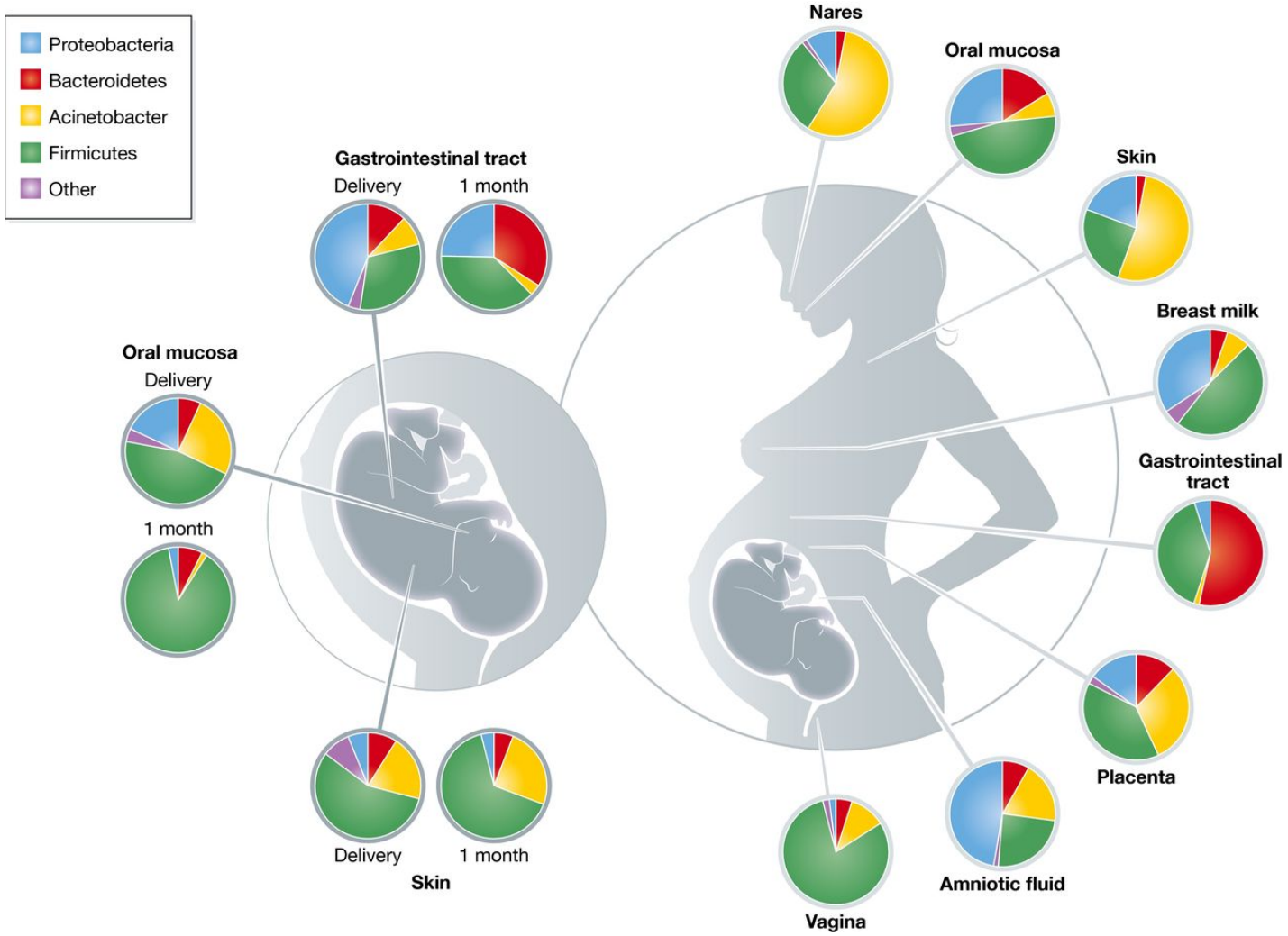
«fornitura» di beta-galattosidasi

Frontiers in Microbiology, 2016;7:1031

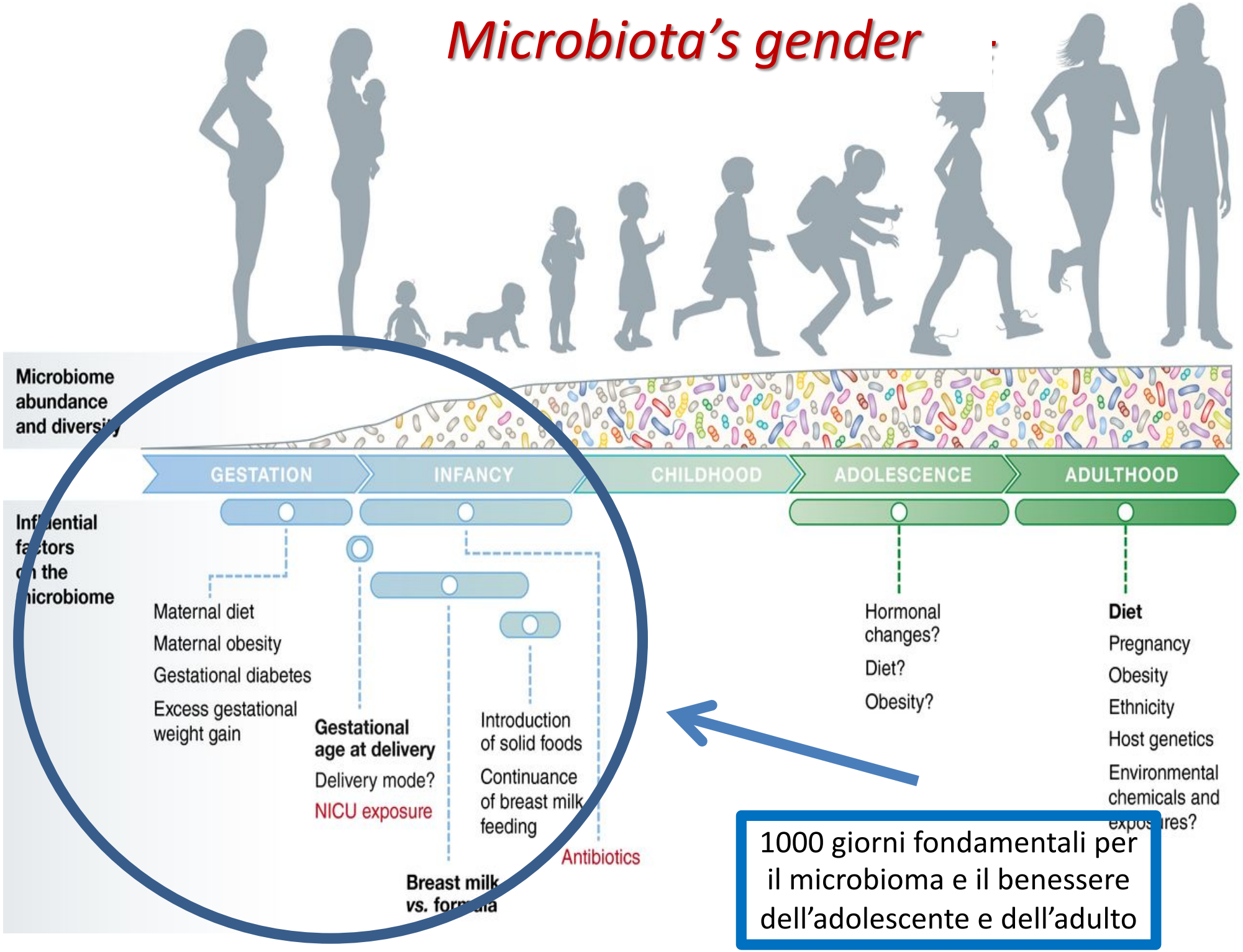

Transfer of bacteria
Placenta and amniotic fluid
Mode of delivery
Breast milk
Maternal gut
Infant gut

Alta biodiversità del microbiota enterico

Bassa biodiversità del microbiota vaginale



Microbiota's gender

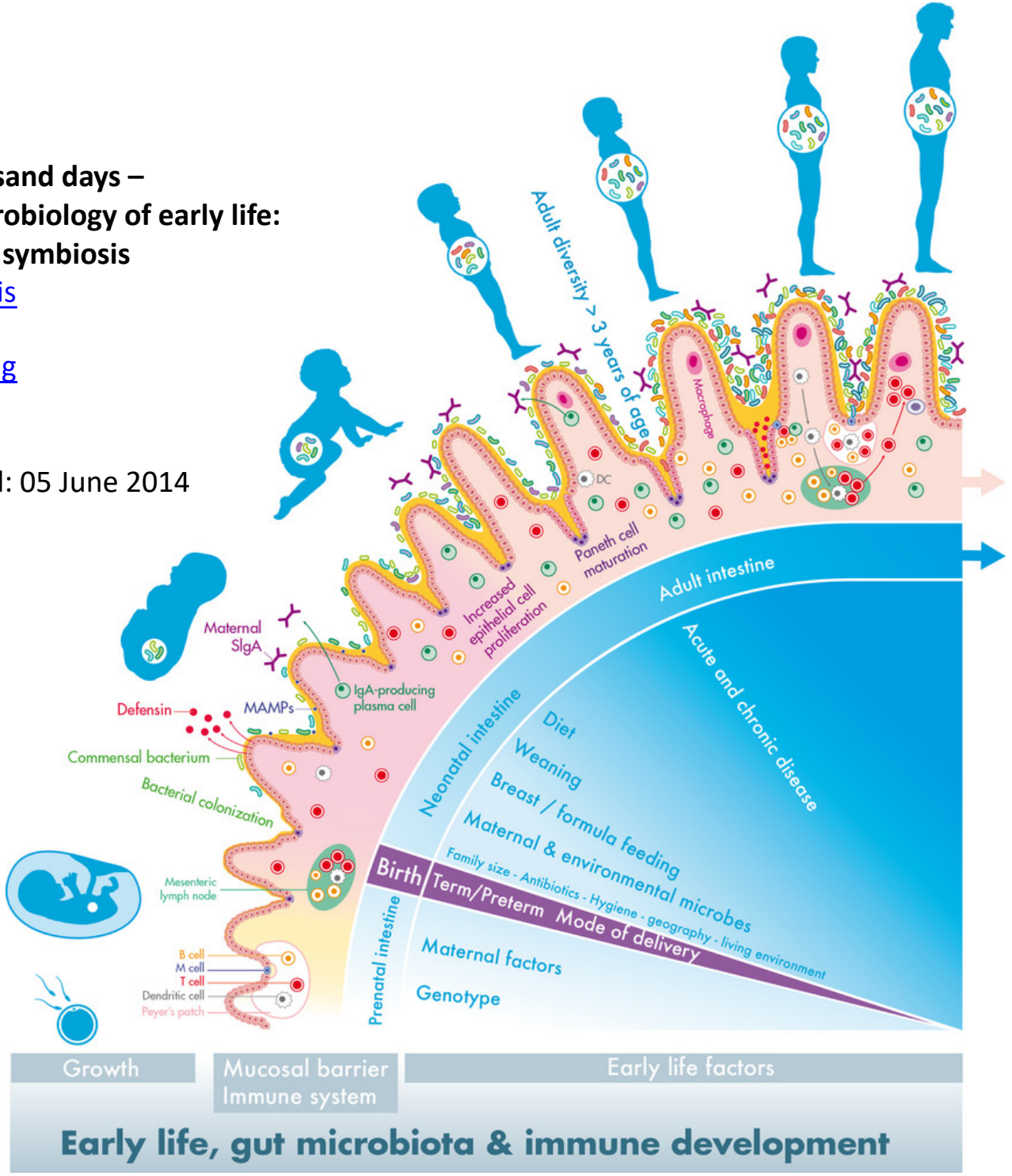


1000 giorni fondamentali per il microbioma e il benessere dell'adolescente e dell'adulto

The first thousand days – intestinal microbiology of early life: establishing a symbiosis

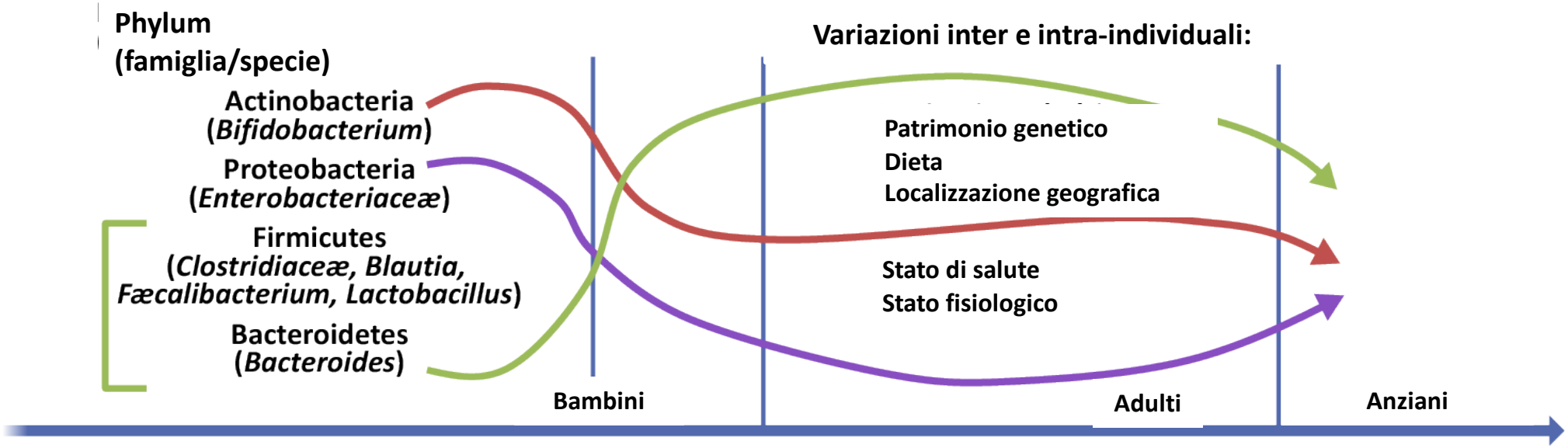
Harm Wopereis
Raish Oozeer
Karen Knipping
Clara Belzer
Jan Knol

First published: 05 June 2014



https://doi.org/10.1111/pai.12232

Fino ai 2 anni il microbioma è instabile, mentre dopo diventa stabile con un equilibrio modificabile da antibiotici, dieta o stili di vita



Neonati **6 mesi** **3 anni**

Variations inter- et intra-individuelles :

Modalità di parto
Alimentazione (allattamento materno vs latte in polvere)
Stato di salute

Tojo et al., 2014

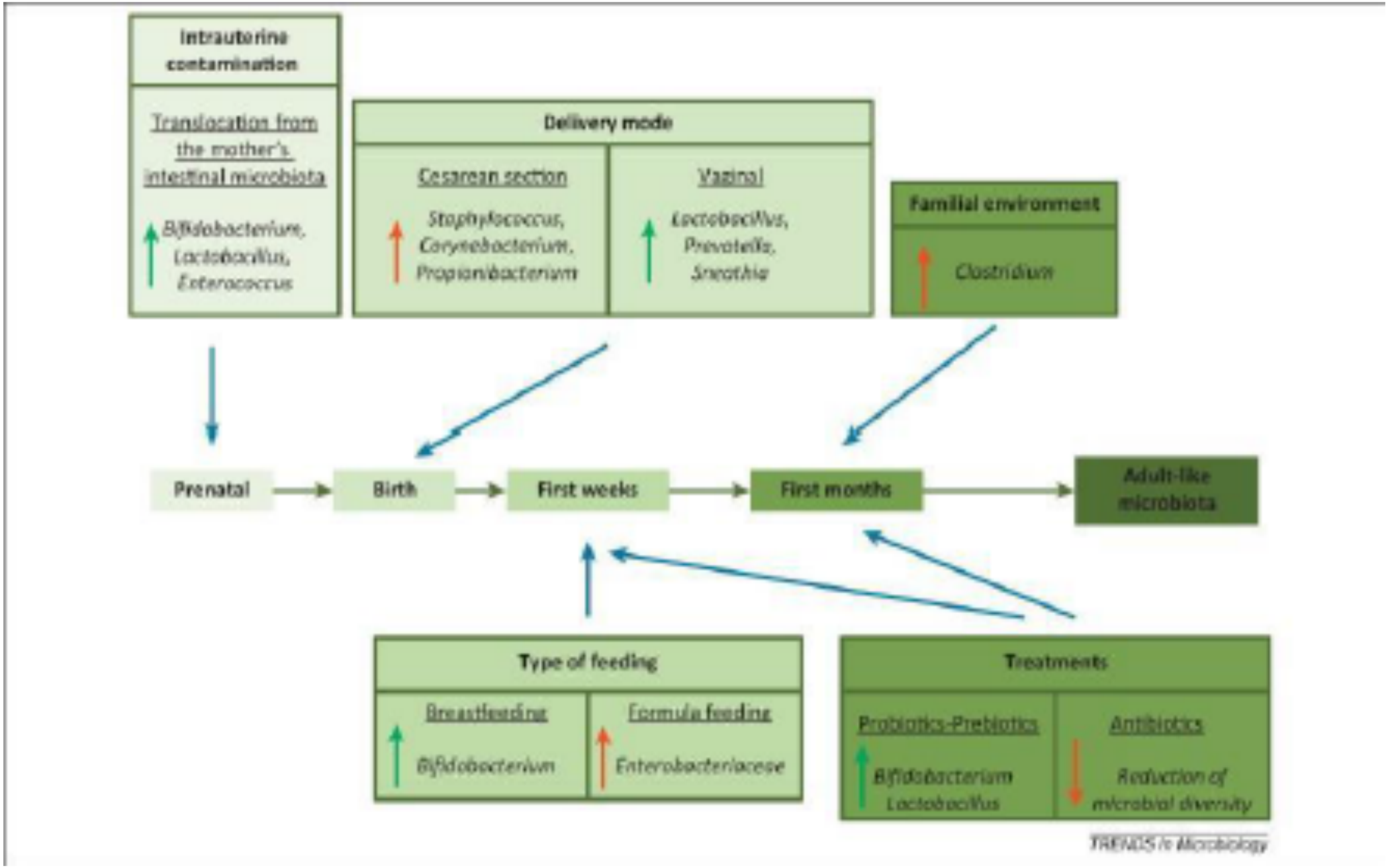
Un andamento cronico e protratto nel tempo può determinare una pericolosa disbiosi

- Establishment of complete colonisation at birth, primarily in the gut, and the gradual diversification into a stable microbial ecosystem during infancy, is essential for this symbiotic relationship to develop

- At the age of 2-5 years, the gut possesses a microbial profile, which fully resembles ‘adult-like’ microbiota in terms of composition and diversity
- Optimal symbiosis may profoundly help to maintain health throughout infancy and childhood and a complete colonisation and development of the microbiota during early life play important roles in maintaining health during infancy , childhood and adult life .

- The most important perinatal environmental factors that largely affect the developing infant microbiota are pre- and postnatal medical treatments, including caesarean delivery, maternal intrapartum antibiotic prophylaxis (IAP) and postnatal infant antibiotic treatment , hospitalisation post birth and formula feeding

A.L. Kozyrskyj, S.L. Bridgman and M.H. Tun



"Development of intestinal microbiota in infants and its impact on health"S. Matamoros et al., review in Trends in Microbiology, 2013.

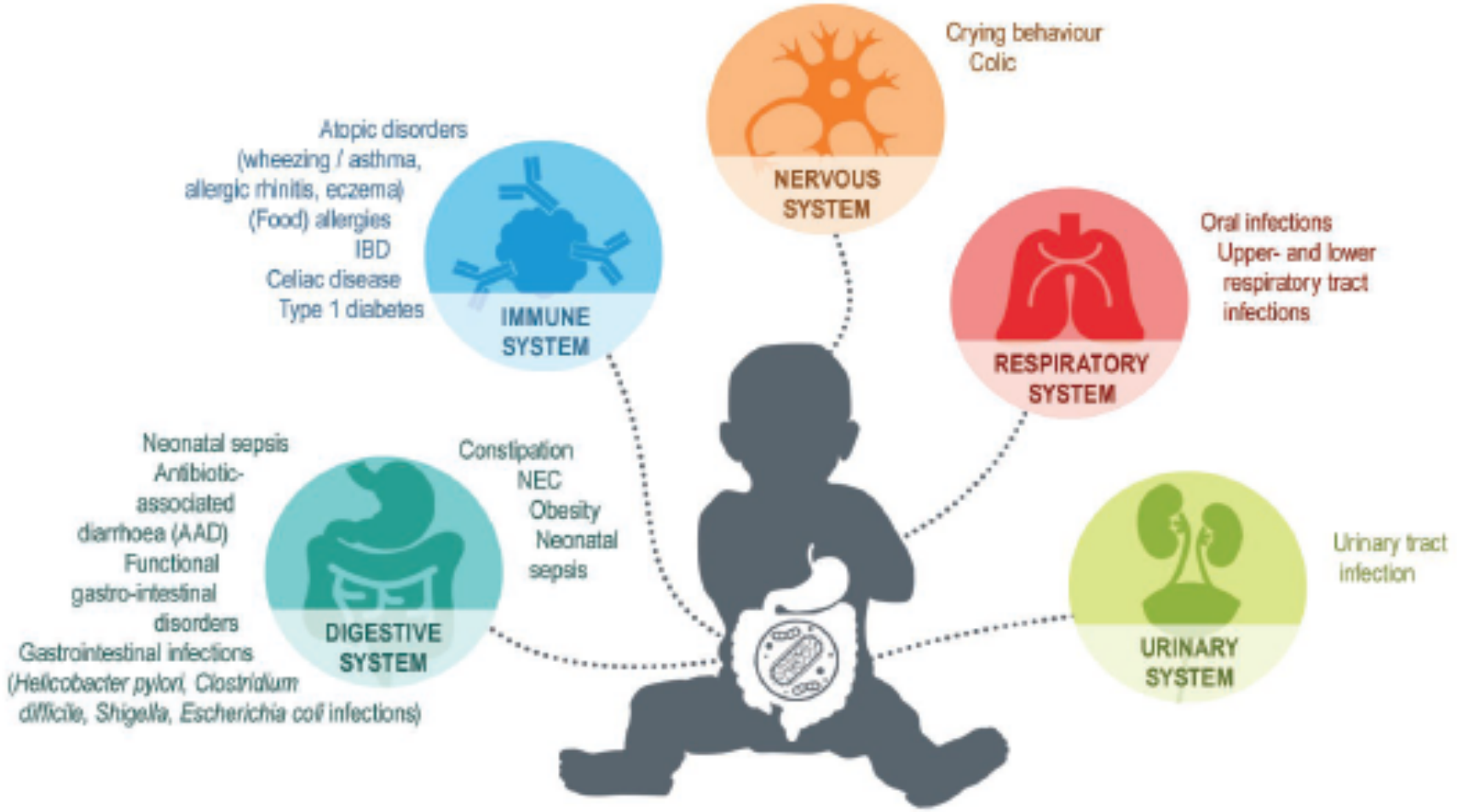


Figure 1.1. The interplay between microbiota, body systems and paediatric diseases. The human microbiota are important for the development and functioning of various physiological systems in the child's body, including the digestive system, immune system, nervous system, urinary system and respiratory system. Disruption of microbial communities may thus affect the child's health and behaviour.

QUALE E’ IL RUOLO DEL MICROBIOTA

- Conoscere il microbiota e le sue funzioni comporta **un cambiamento di prospettiva**: l'uomo appare come un **ecosistema** costituito da un aggregato di geni umani e geni microbici; il nostro metabolismo e quello delle specie che ci abitano si intrecciano, interagiscono ed evolvono parallelamente.

- Il microbiota dovrebbe essere considerato come **un vero e proprio organo metabolico** squisitamente convertito alla nostra fisiologia, che si occupa di funzioni che non siamo in grado di svolgere altrimenti.

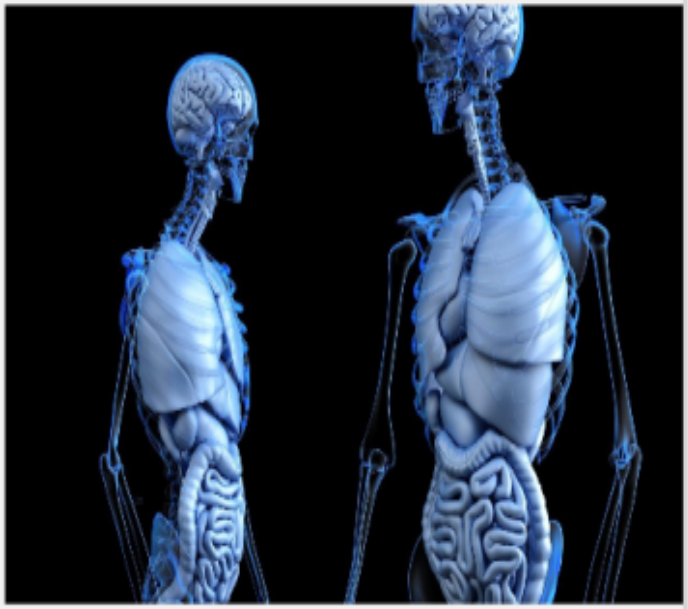
- Tali **funzioni** includono la capacità di assimilare componenti altrimenti indigeribili della nostra dieta, sintetizzare alcune vitamine indispensabili, disgregare e detossificare sostanze che il nostro organismo non è in grado di smantellare, regolare l'espressione del sistema immunitario (di cui, non a caso, il 70% risiede nell'intestino), proteggere la mucosa intestinale dall'attacco di specie patogene.

- L’influenza del microbiota nella regolazione dell’**attività metabolica** è oggi riconosciuta con sempre più evidenze a supporto. Allo stesso modo, è stato scoperto anche un impatto del microbiota sugli **stati psicologici** per via dell’influenza sull’asse ipotalamo-ipofisi-surrene e sul sistema serotoninergico.

- Un’altra caratteristica del microbiota umano è il ruolo nello sviluppo del **sistema immunitario** durante la prima parte dell’infanzia e, di conseguenza, sullo stato di infiammazione del corpo.

ROLE OF BACTERIA WITHIN HUMAN HEALTH

- They help with human digestion
- They help with metabolism
- They manufacture vitamins
- They produce neurochemicals
- They interact with our hormones
- They interact with our nervous system
- They have anti-infective properties
- They are integral to our immune system



Science refs: : 1. Ursell et al., *Allergy Clin Immunol* 2012
2. Turnbaugh et al., *Nature* 2006
3. Neuman et al., *FEMS Microbiol Rev.* 2015
4. Naik et al., *Science* 2012
5. Human Microbiome Project Consortium, *Nature* 2012
6. Cryan et al, *Nature* 2012

Il Microbiota sostiene e promuove importanti funzioni:

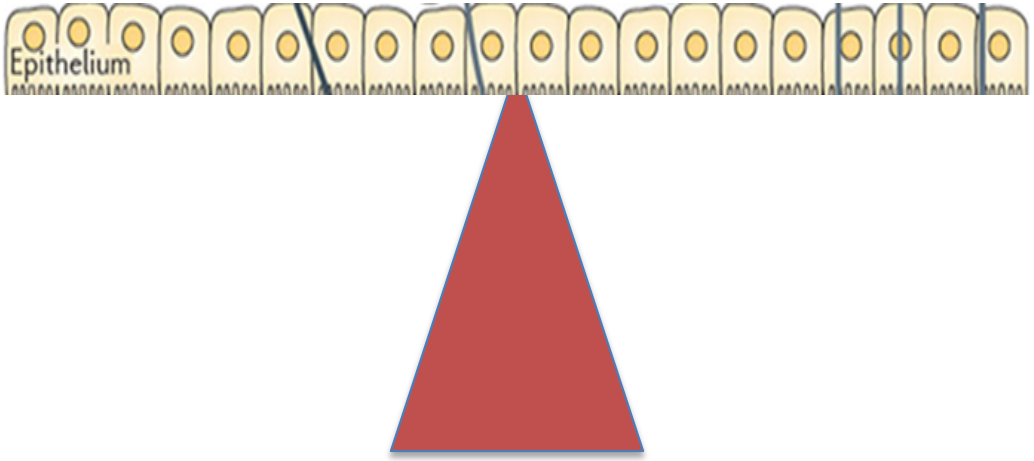
1. Produzione di enzimi, favorendo così i processi digestivi
2. Decomposizione fermentativa degli zuccheri con produzione di acidi organici e anidride carbonica
3. Decomposizione putrefattiva dei residui proteici con produzione di fenolo, indolo, cresolo, ammoniaca e acido solfidrico
4. Metabolizzazione dei grassi e regolazione plasmatica di colesterolo e trigliceridi
5. Sintesi di vitamine (vitamina K, B1, B6, B12, PP, Acido Folico e Acido Pantotenico)
6. Produzione di acidi grassi a catena corta, come l'acido acetico, propionico e butirrico
7. Decomposizione degli Acidi Biliari, Bilirubina e Ormoni Steroidei
8. Produzione di sostanze ad azione antimicrobica
9. Modulazione del sistema immunitario
10. Potenziamento della funzione di barriera intestinale, contro l’invasione di batteri patogeni
11. Regolazione della motilità del tubo digerente e della composizione dei gas intestinali e delle feci
12. Regolazione del pH dell’ambiente gastrointestinale.

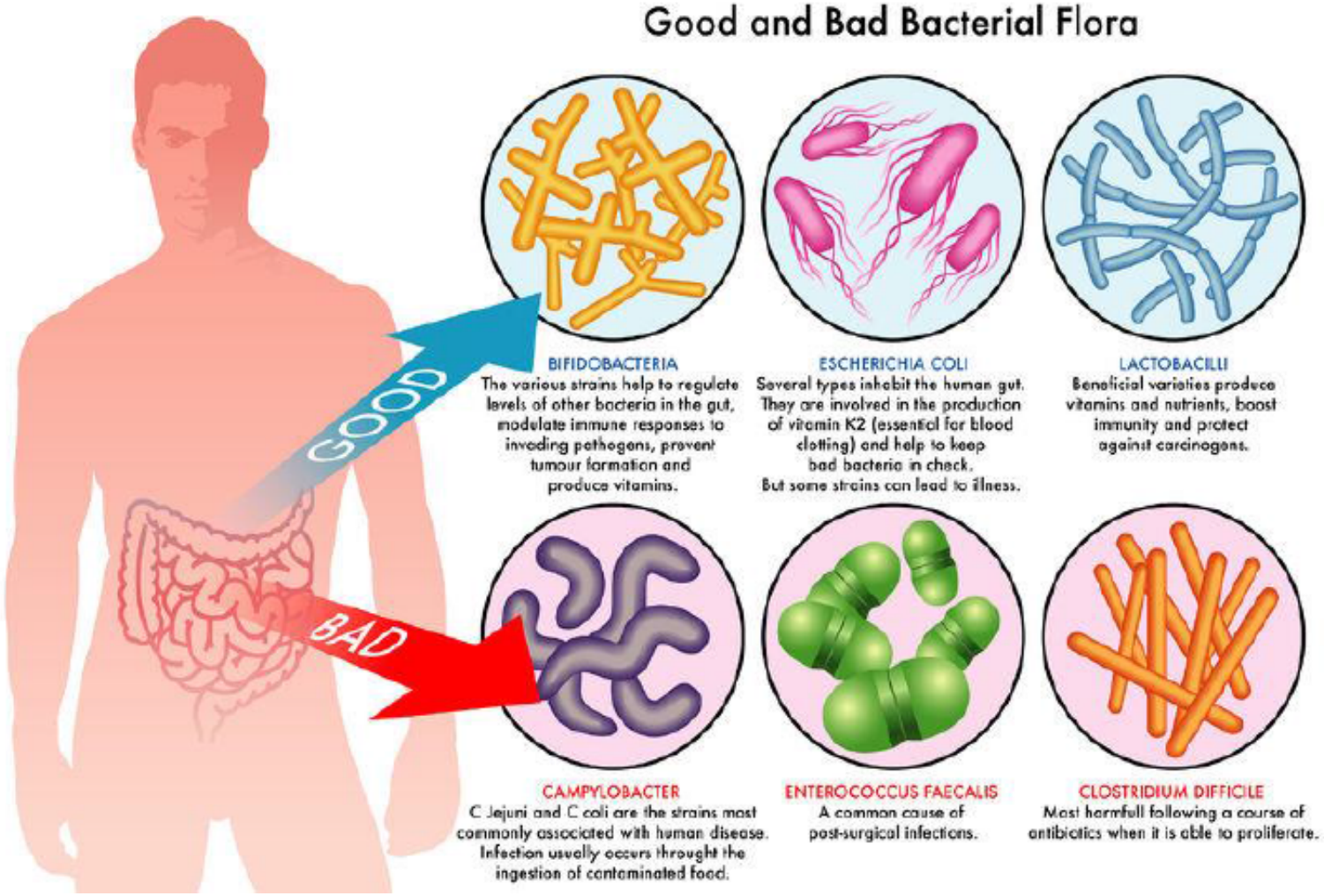
Microbiota EUBIOSIS

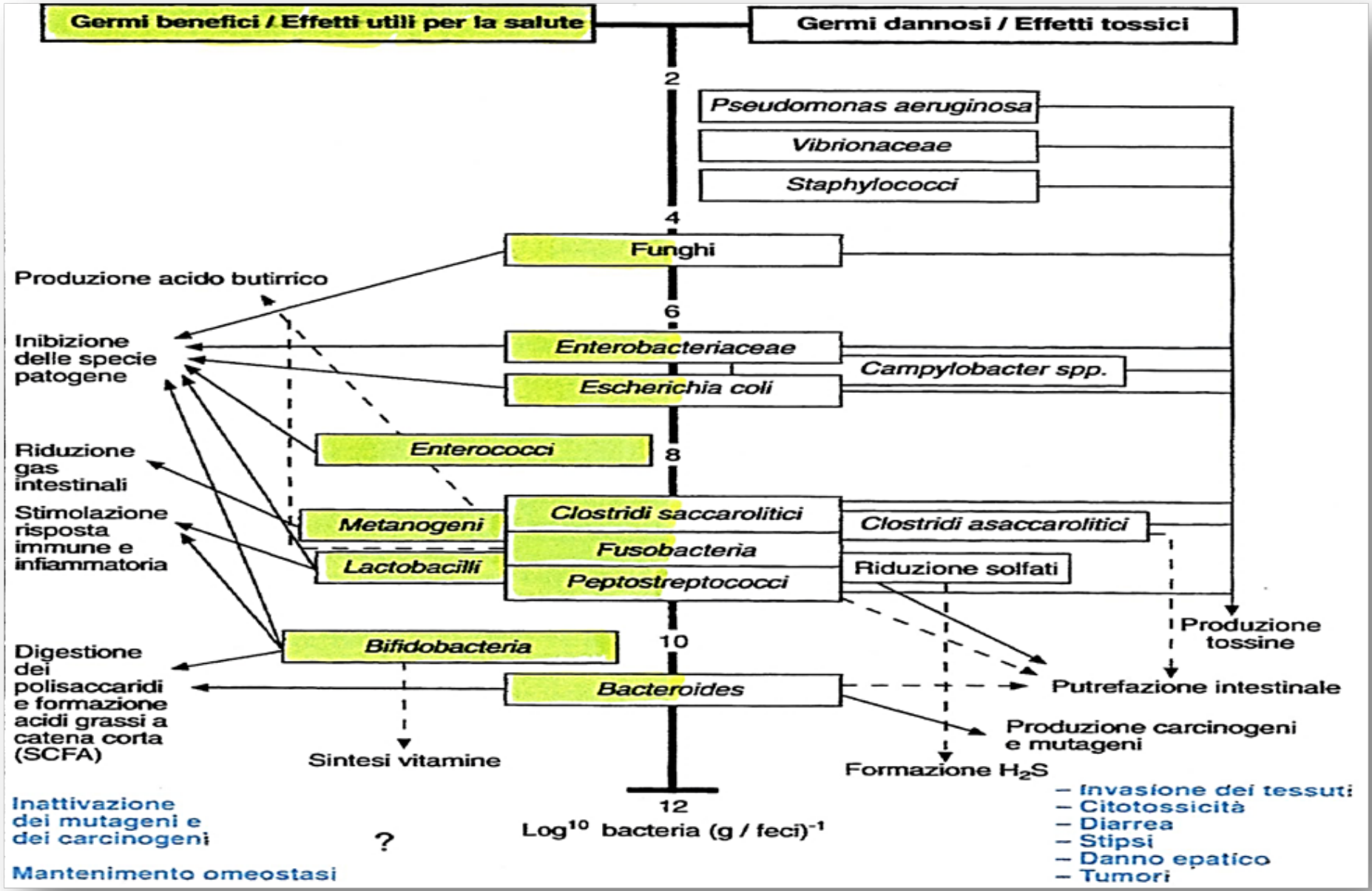
?

PRO-
INFLAMMATORY
MICROBES

IMMUNO-
MODULATORY
MICROBES




Good and Bad Bacterial Flora
GOOD
BAD
BIFIDOBACTERIA
The various strains help to regulate levels of other bacteria in the gut, modelate immune responses to invading pathogens, prevent tumour formation and produce vitamins.
ESCHERICHIA COLI
Several types inhabit the human gut. They are involved in the production of vitamin K2 (essential for blood clotting) and help to keep bad bacteria in check.
But some strains can lead to illness.
LACTOBACILLI
Beneficial varieties produce vitamins and nutrients, boost immunity and protect against carcinogens.
CAMPYLOBACTER
C Jejuni and C coli are the strains most commonly associated with human disease. Infection usually occurs throught the ingestion of contaminated food.
ENTEROCOCCUS FAECALIS
A common cause of post-surgical infections.
CLOSTRIDIUM DIFFICILE
Most harmfull following a course of antibiotics when it is able to proliferate.


Germi benefici / Effetti utili per la salute
Germi dannosi / Effetti tossici
2
Pseudomonas aeruginosa
Vibrionaceae
Staphylococci
4
Funghi
Produzione acido butirrico
6
Inibizione delle specie patogene
Enterobacteriaceae
Campylobacter spp.
Escherichia coli
Enterococci
8
Riduzione gas intestinali
Stimolazione risposta immune e infiammatoria
Metanogeni
Clostridi saccarolitici
Clostridi asaccarolitici
Fusobacteria
Lactobacilli
Riduzione solfati
Peptostreptococci
Produzione tossine
10
Bifidobacteria
Digestione dei polisaccaridi e formazione acidi grassi a catena corta (SCFA)
Bacteroides
Putrefazione intestinale
Produzione carcinogeni e mutageni
Sintesi vitamine
Formazione H_2S
12
Log^{10} bacteria (g / feci)$^{-1}$
Inattivazione dei mutageni e dei carcinogeni
?
Mantenimento omeostasi
– Invasione dei tessuti
– Citotossicità
– Diarrea
– Stipsi
– Danno epatico
– Tumori

- Batteri della stessa specie possono svolgere funzioni molto diverse, e addirittura all'interno della stessa specie possiamo ritrovare **ceppi molto patogeni** e **ceppi assolutamente salutari** (come nella Escherichia coli).

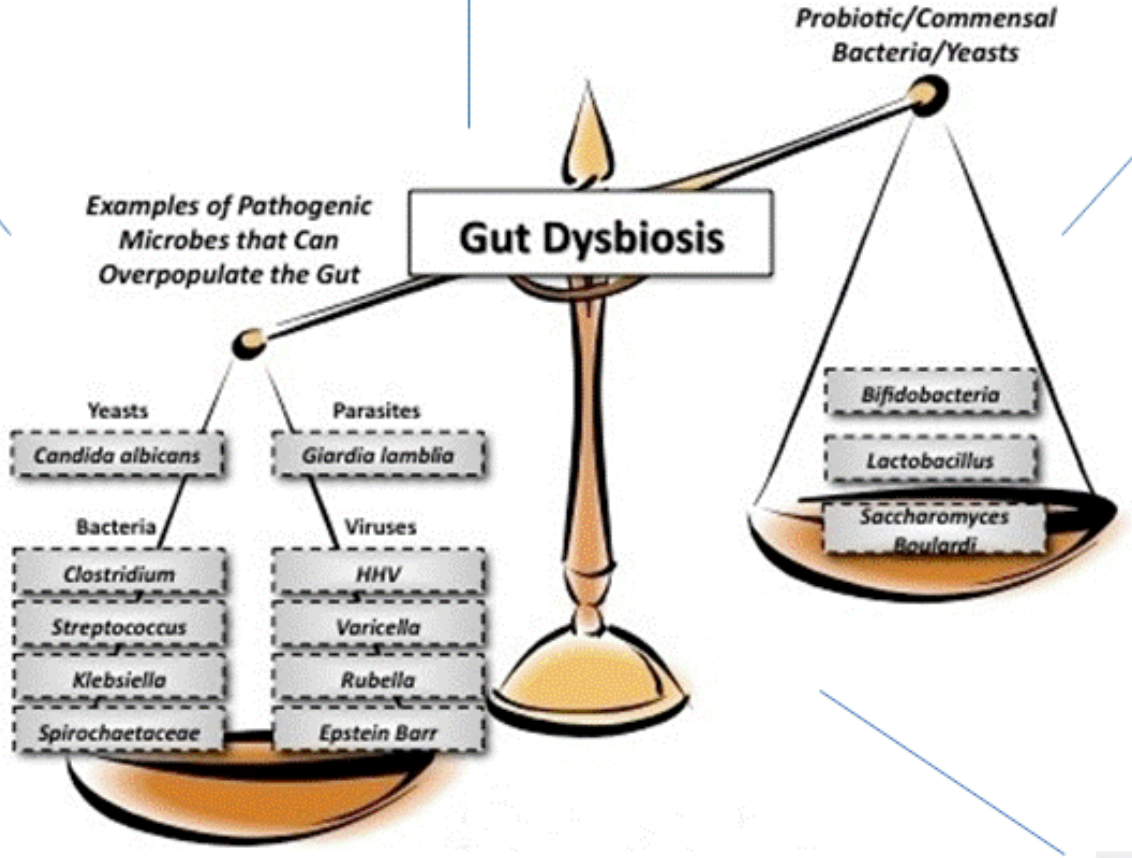
EUBIOSI:
EU = BUONA & BIOS = VITA / SALUTE

- Uno stato di equilibrio tra i batteri buoni e i batteri dannosi (eubiosi) è quindi fondamentale per garantire il buon funzionamento di tutto l'organismo.
- Microbiota ed Ospite , in qualunque nicchia biologica si incontrino , convivono mediante un modello di aggregazione cooperativo
- E' necessaria una corretta biodiversità , una adeguata ricchezza ed abbondanza delle diverse specie per ottenere uno stato di equilibrio microbico del microbiota che si traduca solo in effetti positivi per salute dell'organismo ospite .

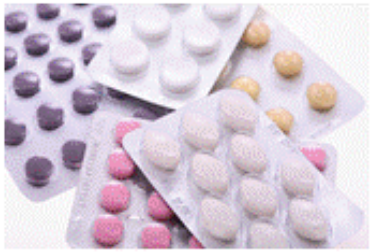
DIETA

STRESS

ETA’



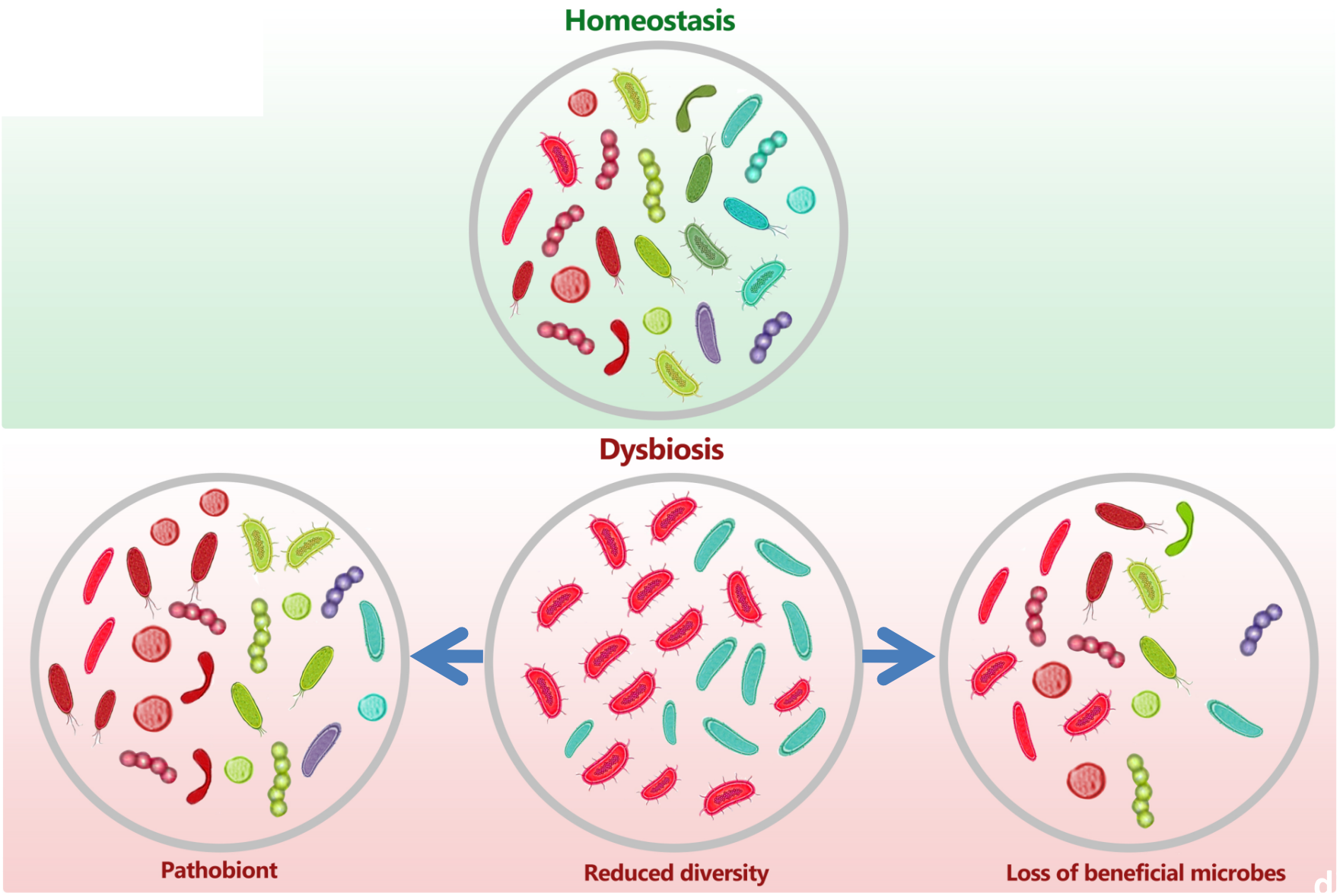
TRATTAMENTI



PATOLOGIE

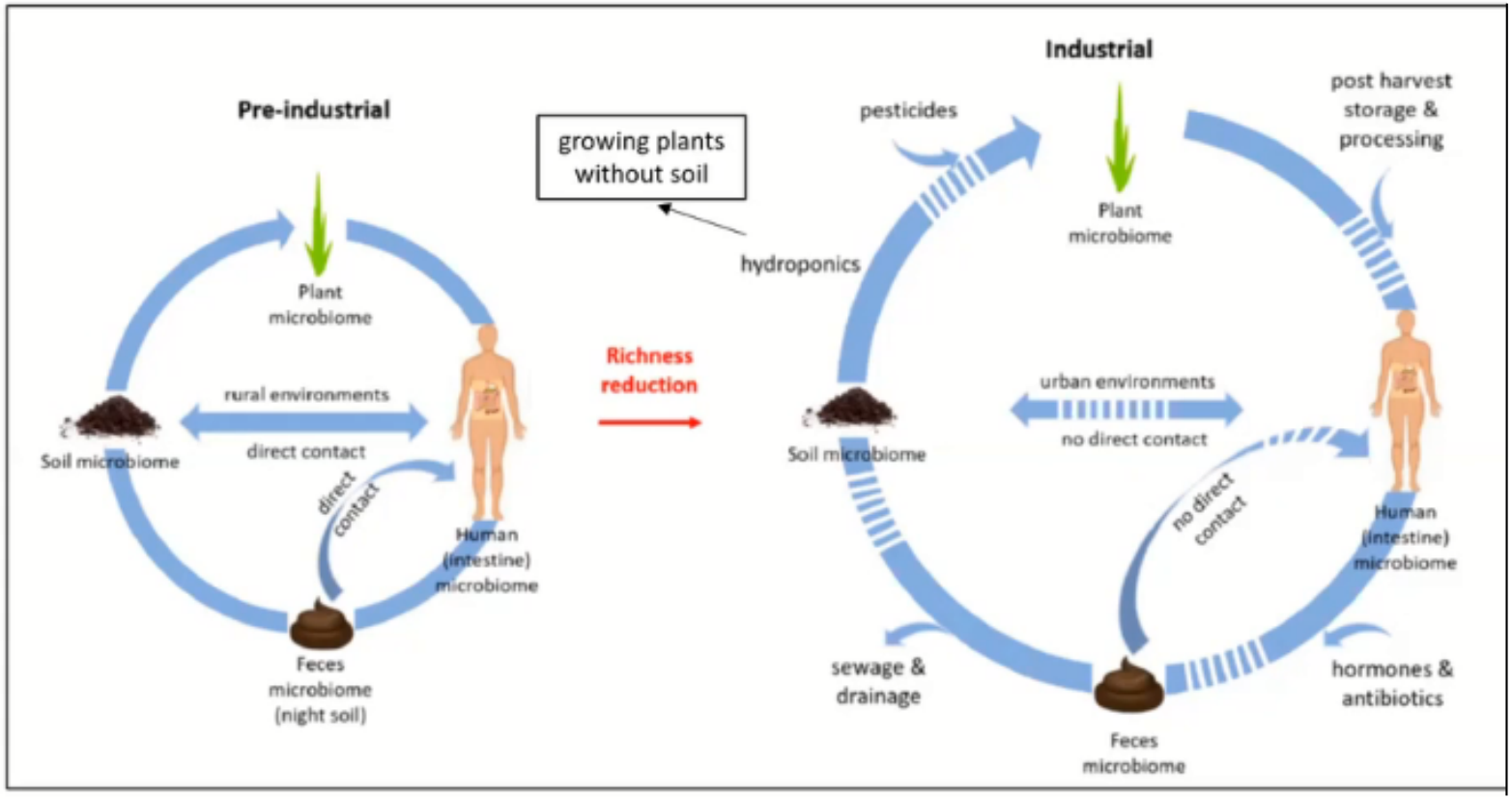
- Ogni volta che questo equilibrio si interrompe si crea invece una **disbiosi**. Nella condizione di disbiosi non solo viene meno la codifica genica delle molecole utili, ma vengono in parte metabolizzati composti dannosi da parte dei microrganismi patogeni, anch'essi parte del microbiota.

Eubiosi vs disbiosi



- I **fattori** che possono influenzare il microbiota in età adulta sono i più diversi: fattori ambientali, stress, assetto ormonale (come in gravidanza, in menopausa, o nel periodo premestruale), terapie farmacologiche; anche il cibo ha un ruolo centrale nel determinare la composizione individuale del microbiota.

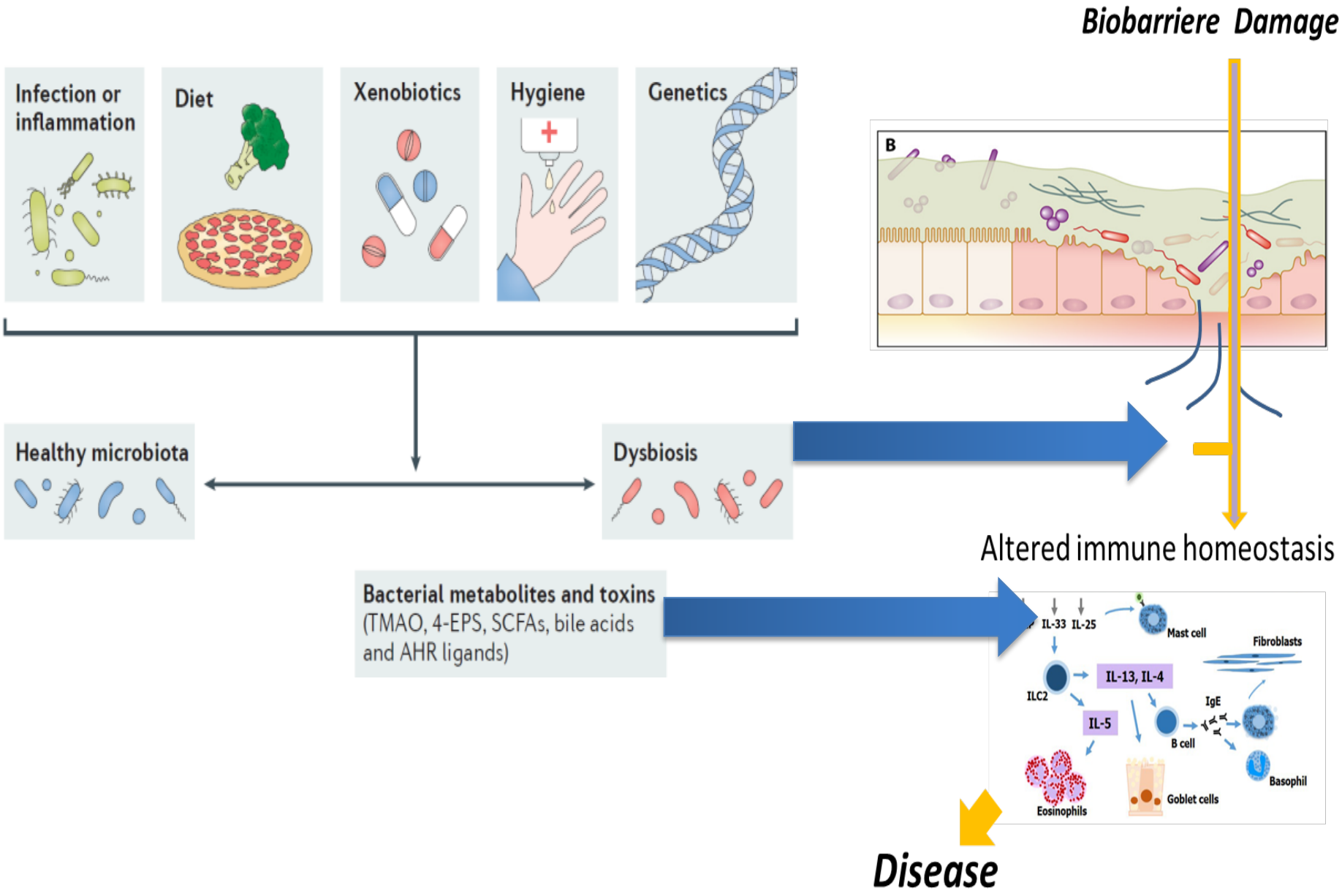
Interference exists with the microbial cycle
of urban human environments versus pre-industrial rural environments



We should consider the human intestinal microbiome as well as the soil/root microbiome as 'superorganisms' which, by close contact, replenish each other with inoculants

Microorganisms 2019, 7, 287; doi:10.3390/microorganisms70

Host-microbiota changes & modern lifestyle



- La rottura di questo equilibrio del microbiota (**disbiosi**) è all'origine di molti stati patologici del sistema digerente , (intestino irritabile, stipsi o diarrea, infiammazioni intestinali ,Sartor 2008)
- Si osserva la comparsa di malattie legate alla sfera immunologica, allergie, patologie autoimmuni, sovrainfezioni batteriche che possono essere la fonte di infezioni recidivanti ricorrenti dell'apparato urinario (cistiti, vaginiti, prostatiti)o di insufficienza renale cronica (Koeth 2013

- Si osserva la comparsa anche di patologie metaboliche (insulino-resistenza, ipercolesterolemia, obesità) (Ridaura 2013) e di importanti disturbi comportamentali e dell'umore (irritabilità, depressione, autismo) (Toh 2015; Hsiao 2013).

INSIGHTS | PERSPECTIVES

17 JANUARY 2020 • VOL 367 ISSUE 6475

sciencemag.org SCIENCE

HYPOTHESIS

Are noncommunicable diseases communicable?

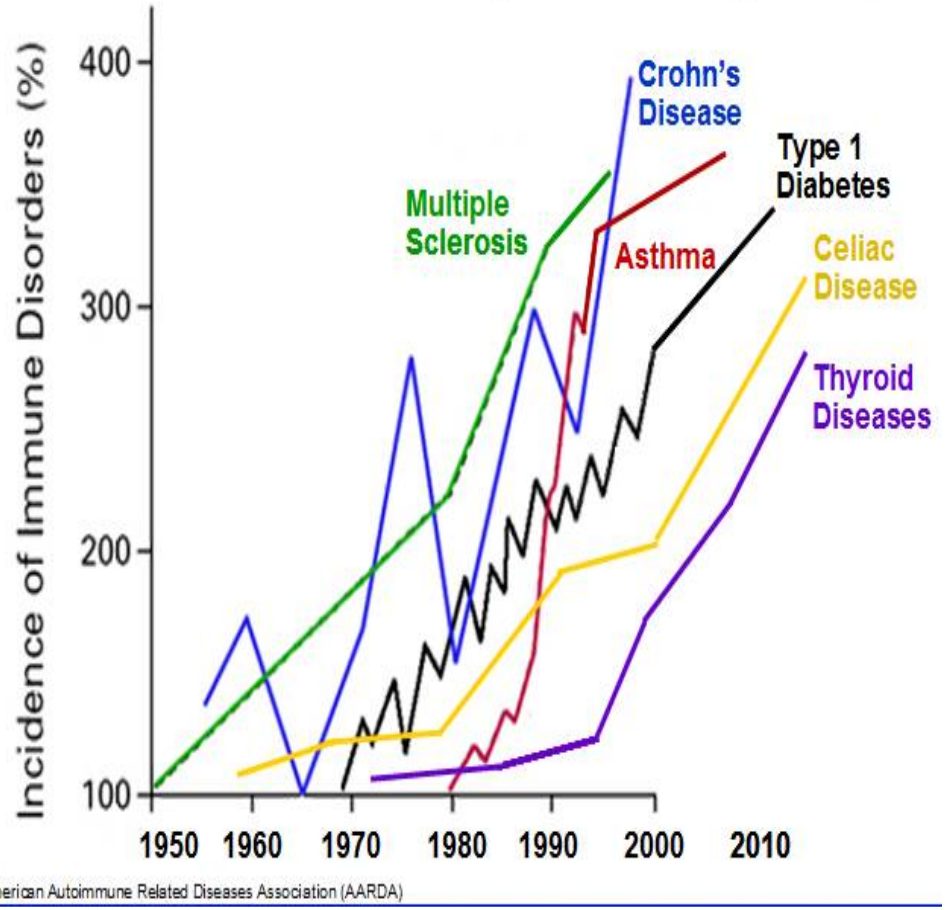
Numerous noncommunicable diseases could have a transmissible microbial component

By B. B Finlay[1,2] and CIFAR Humans and the Microbiome[2]

environments, their microbiota is expected to be similar. Thus, whether shared microbiota

accounted for by chance alone, although, like most infectious diseases, the "transmis-

DYSBIOSIS could be the reason for which we have been facing an epidemic, a rising epidemic of plagues. From asthma to obesity to allergic diseases.



American Autoimmune Related Diseases Association (AARDA)

- Let's say you have ten diseases that are rising at the same time. Each one might have a separate cause. Or, perhaps, there's one thing that's fuelling them all, that's causing all of them to rise. One possible reason is a change in our microbiome, in what I've called Missing Microbes, or The Disappearing Microbiota Hypothesis.

- That our ancient microbiome, which protected us against many diseases, is degrading. And with that degradation, these diseases are being fuelled.

GRAZIE DELLA ATTENZIONE

I vaginotipi nella donna in età fertile e le loro implicazioni ostetriche

Dott. Francesco Bernasconi
U.O.C. di Ostetrica e Ginecologia
Ospedale di Erba

NIH Human Microbiome Project (HMP) was ~~established in 2008~~

Mission - generating resources that would enable the comprehensive characterization of the human microbiome and analysis of its role in human health and disease

- the Human Microbiome Project has enabled the study of the structure and composition of the microbiome at different body sites, revealing that the female reproductive tract microbiota accounts for approximately 9% of the total bacterial load in humans.

Deciphering the effect of reproductive tract microbiota on human reproduction

Inmaculada Moreno| Carlos Simon

- The microbiome of the vagina in healthy reproductive-age women presents a biomass of approximately one billion bacteria per gram of vaginal fluid with low diversity, mainly composed of one or few *Lactobacillus* species, representing 90%-95% of the total bacteria in the reproductive tract.

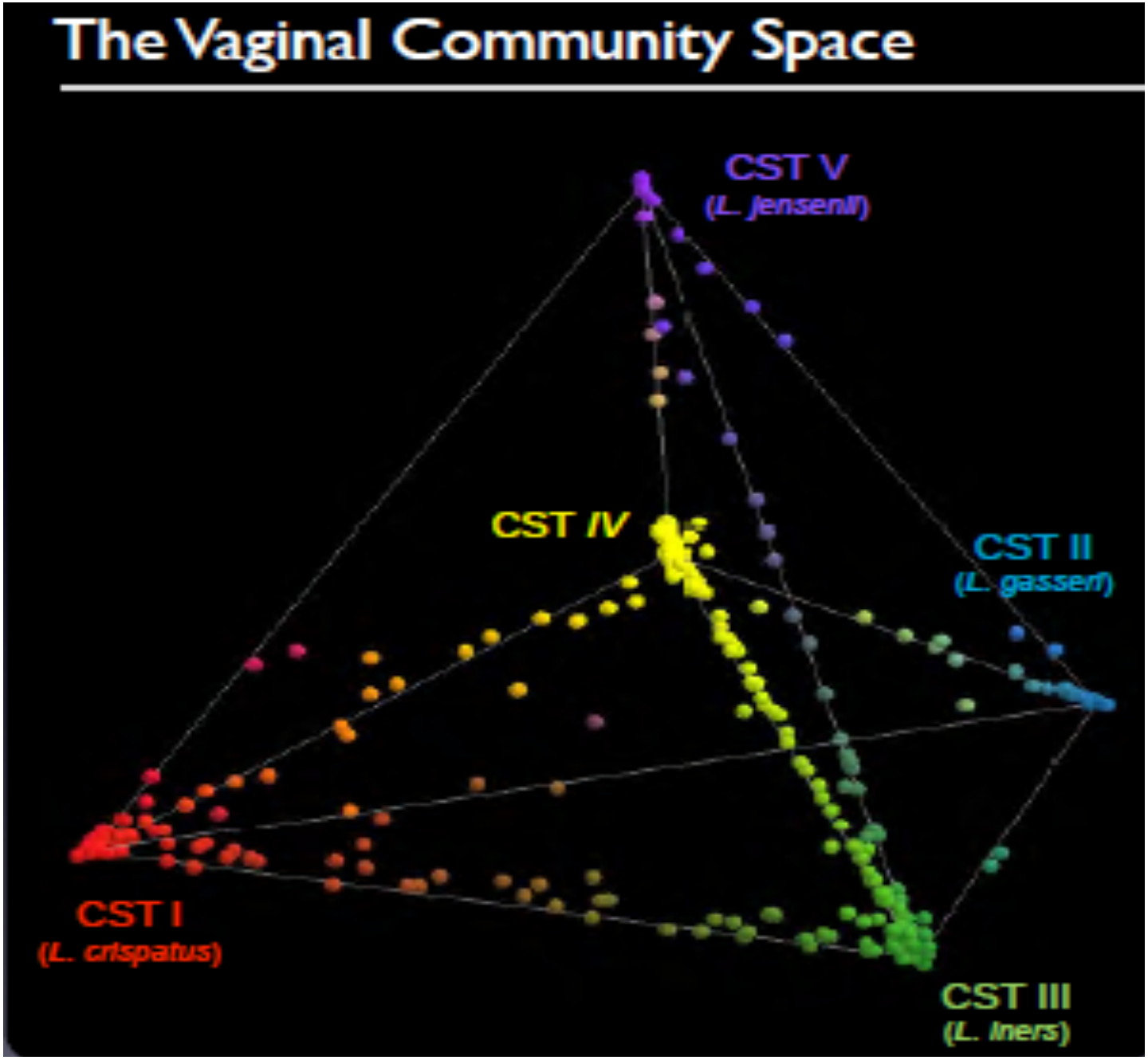
- The human vaginal microbiota is a key component in the defense system against microbial and viral infections, conferring protection against disease (Turovskiy et al., 2011).
- The vaginal microbiome is dominated by many species including *Lactobacillus and members of the Clostridiales, Bacteriodales, and Actinomycetales(Aagaard et al., 2012).*

- This community , however , predominantly comprises *Lactobacilli* in healthy women, although other genera have been identified, namely, *Prevotella*,*Gardnerella*, *Atopobium*, *Sneathia*, *Bifidobacterium*, *Megasphaera*, and *Anaerococcus*

- This is an interesting finding unique to the human reproductive tract microbiome, as other mammals present a vaginal microbiota not dominated by *Lactobacillus*.

- the bacterial communities in the reproductive tract play important roles at different stages of the reproductive process, starting with gamete formation, fertilization, pregnancy establishment and maintenance and even the microbial colonization of the newborn

- Based on this concept, various studies have sought to define the features and composition of a “normal/healthy” microbiome and establishing the potential shifts leading to a “dysbiotic/abnormal” microbiota.


The Vaginal Community Space
CST V
(L. jensenii)
CST IV
CST II
(L. gasseri)
CST I
(L. crispatus)
CST III
(L. iners)

- The normal vaginal microbiome in healthy women is generally dominated by Lactobacilli species , although variation due to age and hormonal milieu is evident .

Vaginal microbiome of reproductive-age women. Proc Nat Acad Sci 2011;

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al.

- The first description of the vaginal microbiome in a set of 396 reproductive-age women using NGS for the 16S rRNA bacterial gene revealed the existence of five distinct community state types (CSTs) depending on the abundance of the bacteria identified. CST-I, -II, -III, and -V are dominated by *Lactobacillus crispatus*, *Lactobacillus gasserii*, *Lactobacillus iners*, and *Lactobacillus jensenii*, respectively.

- More than 70% of women demonstrated vaginal microbiota dominated by L. crispatus, L. gasseri, L. iners, or L. jensenii, corresponding to CST-I, -II, -III, and -V.
- A smaller proportion of women exhibit CST-IV, characterized by lower percentage of Lactobacilli and dominance of anaerobic bacteria including Aerococcus, Atopobium, Dialister, Gardnerella, Megasphaera, Prevotella, and Sneathia

Vagino-tipi (CST)

Community state types (CST) in the vaginal microbiota.[a] Vaccine 32 (2014) 1543–1552

CST	Dominant bacterial species
I	*L. crispatus*
II	*L. gasseri*
III	*L. iners*
IV-A[b]	Low-*Lactobacillus*
IV-B[b]	Low-*Lactobacillus*
V	*L. jensenii*

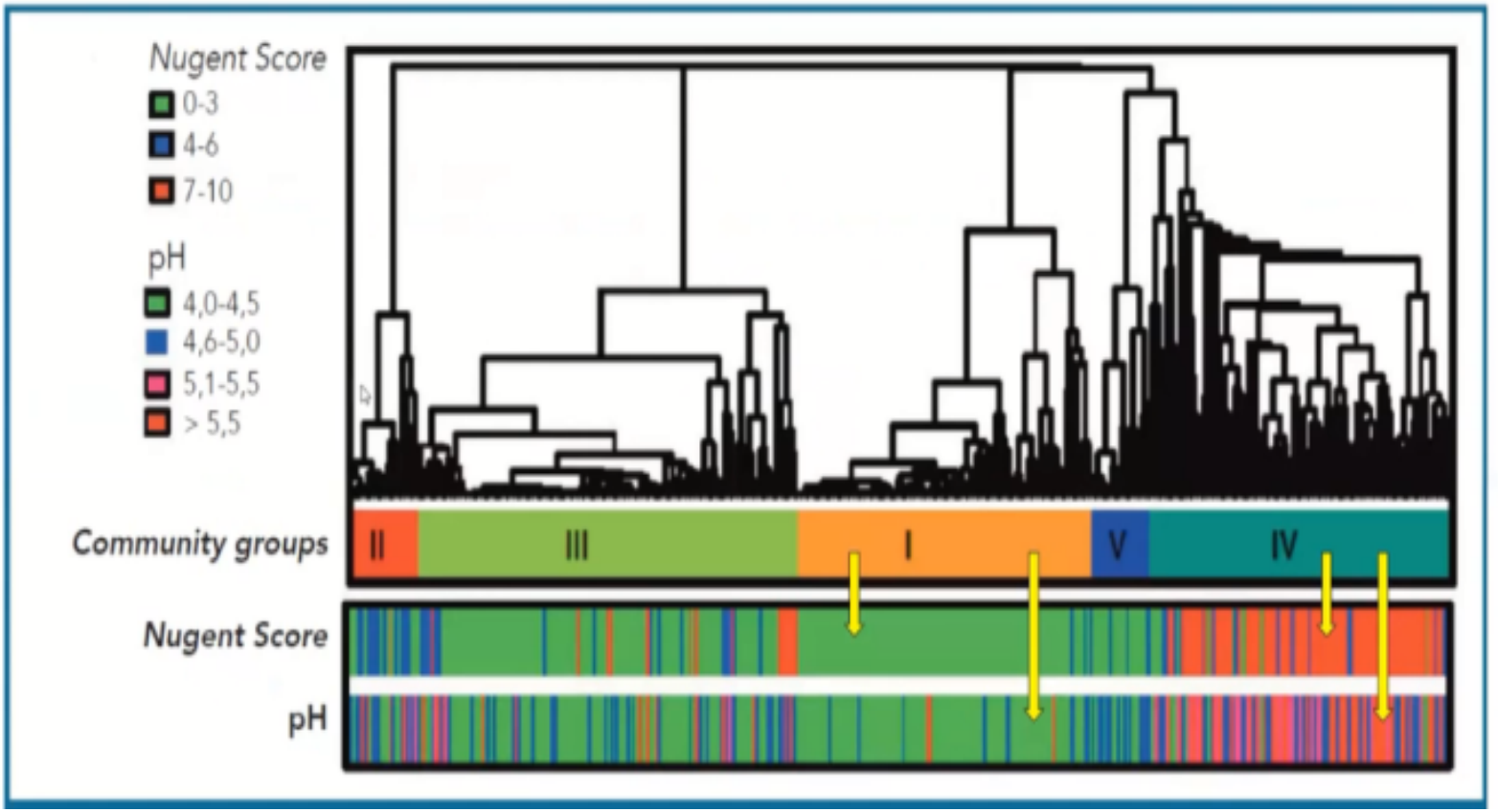
[a] CST IV-A is characterized by various species of anaerobic bacteria including *Anaerococcus*, *Peptoniphilus* and *Prevotella* spp., whereas CST IV-B had higher proportions of bacteria from the genera *Atopobium* and *Megasphaera* among others.

[b] CSTs reflect the clustering of samples based on bacterial composition and abundance. Gajer et al. previously reported on these 6 CSTs among women in Baltimore, MD [54].

Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductiveage women. *Proc Natl Acad Sci USA. 2011;108(Suppl 1):4680-4687.*

Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. *Sci Transl Med. 2012;4(132):132ra52-132ra52.*

- This CST-IV has been further divided into two sub-states CST IV-A and CST IV-B.
- CST IV-A contains species of genera *Anaerococcus, Peptoniphilus,Corynebacterium, Prevotella, Finegoldia and Streptococcus.*
- *CST IV-B is characterised by Atopobium, Gardnerella, Sneathia, Mobiluncus, Megasphera and* other taxa of order *Clostridiales*



Relazione tra CST , Nugent score e ph

Ravel J, Gajer P, Abdo Z, et al. *Proc Natl Acad Sci USA. 2011*

BACTERIAL VAGINOSIS : Amsel criteria

In clinical practice BV can be diagnosed using the Amsel criteria:

- Thin, white, yellow, homogeneous discharge
- Clue cells on microscopy
- pH of vaginal fluid >4.5
- Release of a fishy odor on adding alkali—10% potassium hydroxide (KOH) solution.
- At least three of the four criteria should be present for a confirmed diagnosis.[34] A modification of the Amsel criteria accepts the presence of two instead of three factors and is considered equally diagnostic.

BACTERIAL VAGINOSIS :
Nugent score

- The Nugent Score is more used by physicians even if it takes much time to read the slides and requires the use of a trained microscopist. A score of 0-10 is generated from combining three other scores. The scores are as follows:
- 0–3 is considered negative for BV
- 4–6 is considered intermediate
- 7+ is considered indicative of BV.
- At least 10–20 high power (1000× oil immersion) fields are counted and an average determined.

SCORE DI NUGENT

Quantità per campo (obiettivo ad immersione)	> 30	6 - 30	1 - 5	< 1	0
Morfotipo lattobacilli (L) Bacilli Gram +	0	1	2	3	4
Morfotipi Gardnerella e anaerobi (G) : Bacilli Gram variabile	4	3	2	1	0
Morfotipo Mobiluncus (M) : Bacilli ricurvi, Gram variabili (taglio di unghia)	2	2	1	1	0

- Based on Nugent score, CST-IV represents the most common dysbiosis state *i.e. bacterial vaginosis* .
- However, this state was also found to be reported in various healthy individuals, predominantly (40%) in black and Hispanic women : it is still debateable whether this CST represents a healthy state or an asymptomatic state of BV.

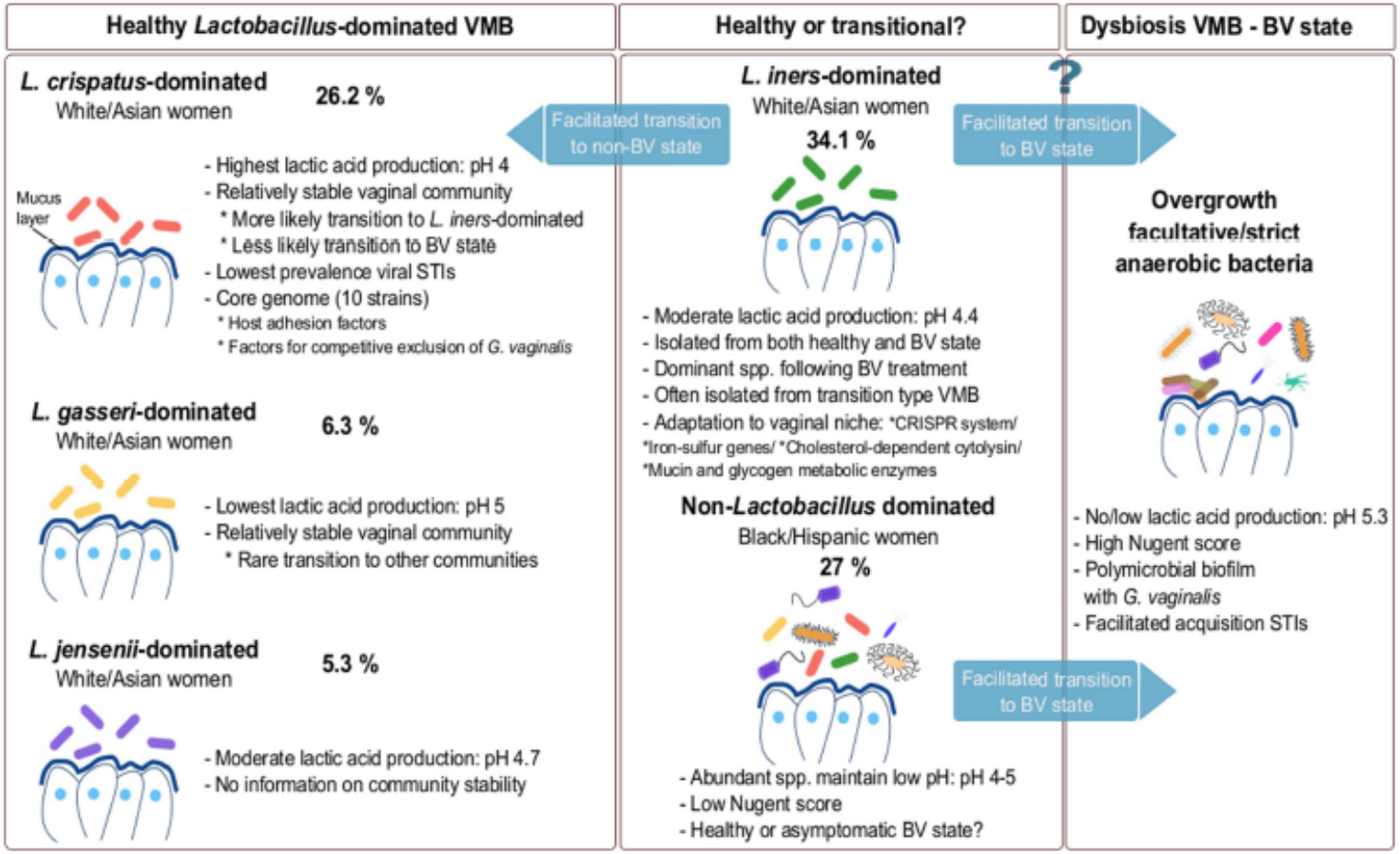


FIGURE 1 | Composition of VMB during healthy and dysbiotic states. The vaginal microbiota in healthy adult premenopausal women can be divided into different community groups. The exact number and type of community groups is still under debate (Verhelst et al., 2005; Ravel et al., 2011; Gajer et al., 2012; Santiago et al., 2012). The most commonly isolated dominating species belong to *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*. The vaginal community group dominated by *L. iners* is also often isolated during menstruation and in the transitional microbiota between healthy and BV state or vice-versa. Because this species is often isolated during BV, *L. iners* may not be able to effectively protect against pathogens. Additionally, a non-*Lactobacillus* dominated VMB is also documented in various healthy individuals (Zhou et al., 2004; Ravel et al., 2011; Santiago et al., 2012). For each compositional state, we have added an example of their abundance based on the study of Ravel et al. (2011). However, these numbers are only exemplary and should be considered with caution, as they clearly depend on the study population (size and characteristics) and they certainly need to be substantiated in further studies.

- The role of *Lactobacillus* is to maintain vaginal homeostasis by producing lactic acid to lower the vaginal pH, the production of hydrogen peroxide, bacteriocins, and other antimicrobial compounds, facilitates the adhesion of *Lactobacilli* to the vaginal epithelial cells and competition for the nutrients in the niche to deter the growth of pathogenic bacteria.

Lactic acid have been shown to affect host immune responses through different mechanisms including :

- eliciting significant increases in the production of the anti-inflammatory mediator i.e. interleukin-1 receptor antagonist (IL-1RA) from vaginal epithelial cells
- inhibiting production of pro-inflammatory mediators i.e. IL-6, IL-8, tumor necrosis factor alpha (TNFα), RANTES (regulated on activation, normal T cell expressed and secreted) and macrophage inflammatory protein-3 alpha (MIP3α)

- releasing transforming growth factor beta (TGF-β) to stimulate antiviral response [20], stimulating the T helper 17 (Th17) T lymphocyte pathway via IL-23 production on exposure to bacterial lipopolysaccharide
- building up cytosolic lactic acid that blocks the production of cyclic adenosine monophosphate (cAMP) leading to increased autophagy in epithelial cells for the degradation of intracellular microbes and upholding homeostasis .

- In contrast, CST-IV is characterized by increased diversity due to polymicrobial colonization with facultative anaerobic bacteria such as *Gardnerella*, *Prevotella*, *Megasphaera*, *Atopobium*, and *Dialister* to the detriment of *Lactobacilli and is associated to a BV*.

MICROBIAL ECOLOGY IN HEALTH AND DISEASE, 2017
VOL. 28, 1303265
http://dx.doi.org/10.1080/16512235.2017.1303265

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RESEARCH ARTICLE

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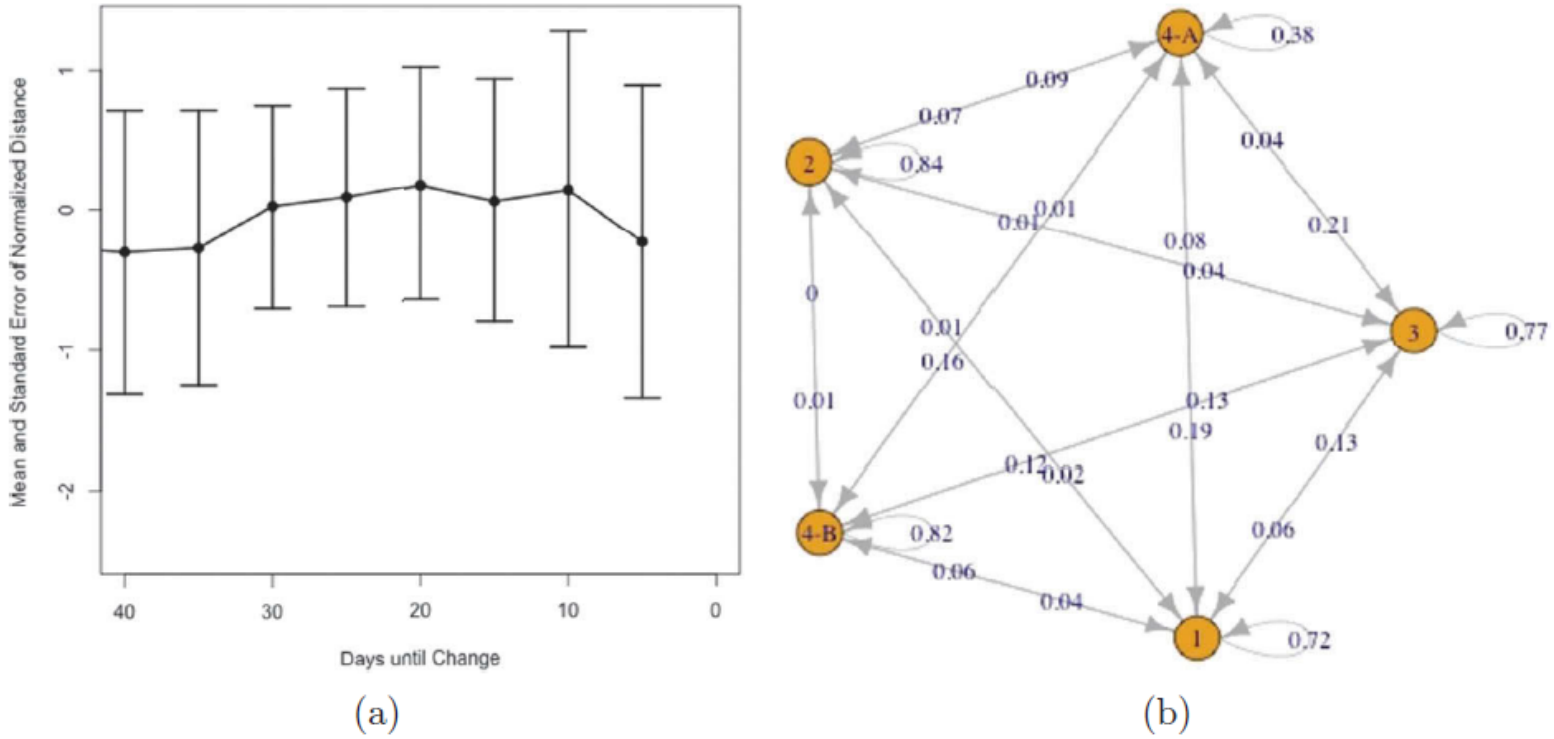
Changes in vaginal community state types reflect major shifts in the microbiome

J. Paul Brooks[a,c], Gregory A. Buck[b,c], Guanhua Chen [d], Liyang Diao[e], David J. Edwards[a], Jennifer M. Fettweis[c], Snehalata Huzurbazar[f], Alexander Rakitin[g], Glen A. Satten[h], Ekaterina Smirnova[f,i], Zeev Waks[j], Michelle L. Wright[k], Chen Yanover[j] and Yi-Hui Zhou[l]

- Shifts between different CSTs occur in women,sometimes involving acquisition of the CST-IV microbiome.
- Healthy subjects persist in a CST for two to three weeks or more on average, while those with evidence of dysbiosis tend to changemore often.
- Changes in CST can be gradual or occur over less than one day. Upcoming CST changes and switches to high risk CSTs can be predicted with high accuracy in certain scenarios



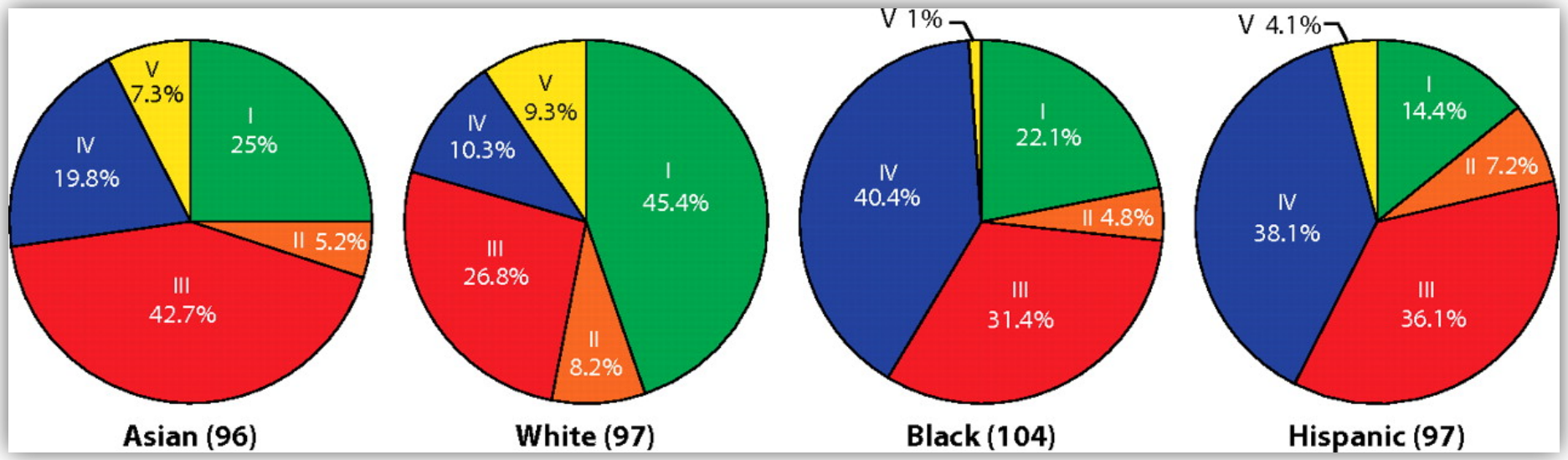
The probability of remaining in a given state over the course of a week is 0.38 (0.22,0.50) for CST 4-A and at least 0.72 (0.61,0.79) for other CSTs (Figure 2(b)).

.

Cervicovaginal microbiota and reproductive health: the virtue of simplicity.

Anahtar MN, Gootenberg DB, Mitchell CM, Kwon DS.*Cell Host Microbe*. 2018;23(2):159-168.

- the CST-IV profile is also common in asymptomatic women depending on their racial background.
- The percentage of women with a lower genital tract dominated by *Lactobacillus* is 90%, 80%, 60%, and 60%-37% in White, Asian, Hispanic, and Black populations, respectively.



L. crispatus

L. gasseri

L. iners

Low lactobacilli

L. jensenii

Vaccine 32 (2014) 1543–1552

- The variation of microbiota profiles in these populations may reflect not only racial or genetic predisposition to one or other types of bacteria, but also geographic, social, and/or economic factors

Effects of combined oral contraceptives, depot medroxyprogesterone acetate and the levonorgestrel-releasing intrauterine system on the vaginal microbiome

J. Paul Brooksa,b, David J. Edwardsa, Diana L. Blithec, Jennifer M. Fettweisb,

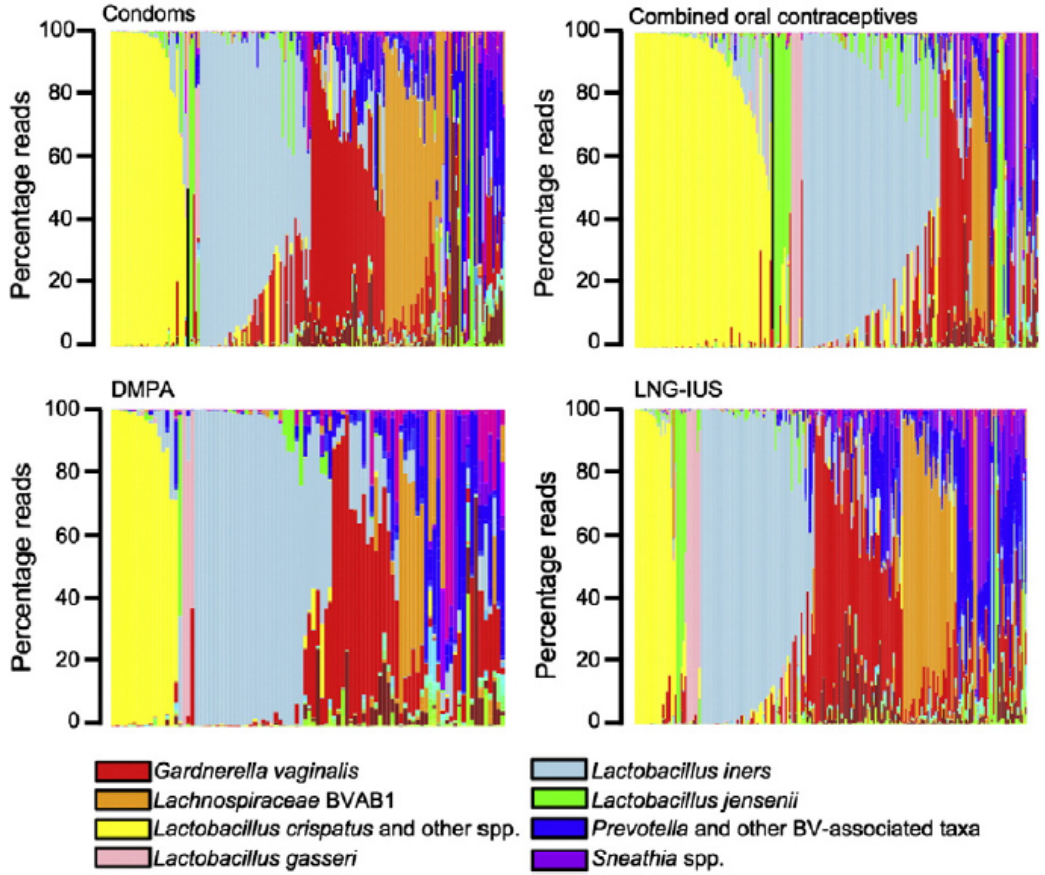


Fig. 1. Microbial community profiles of women using different methods of contraception. Stacked bar plots showing vaginal microbial community profiles from 186 women who reported only using condoms, 206 women who reported only using COCs, 94 women who reported using only DMPA injections and 196 women who reported using only LNG-IUS for contraception. The profiles are grouped by the most abundant species and are ordered by decreasing proportion of the dominant bacterium. An abbreviated color code showing the most abundant taxa is shown. Complete color codes for bacterial taxa appear in Supplementary Data file.

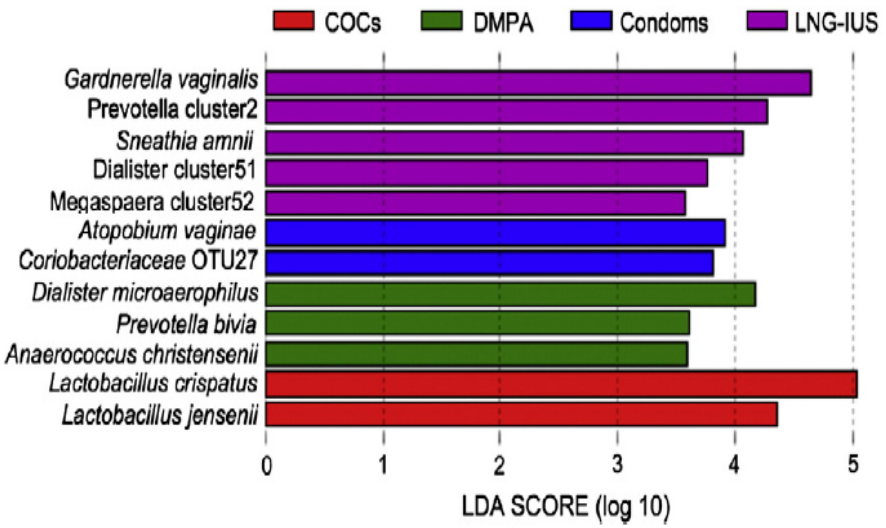


Fig. 2. Association between bacterial taxa and contraceptive method. LEfSe analysis of microbial profiles detected significant associations between the abundance of a number of bacterial taxa and contraceptive method when all methods were compared.

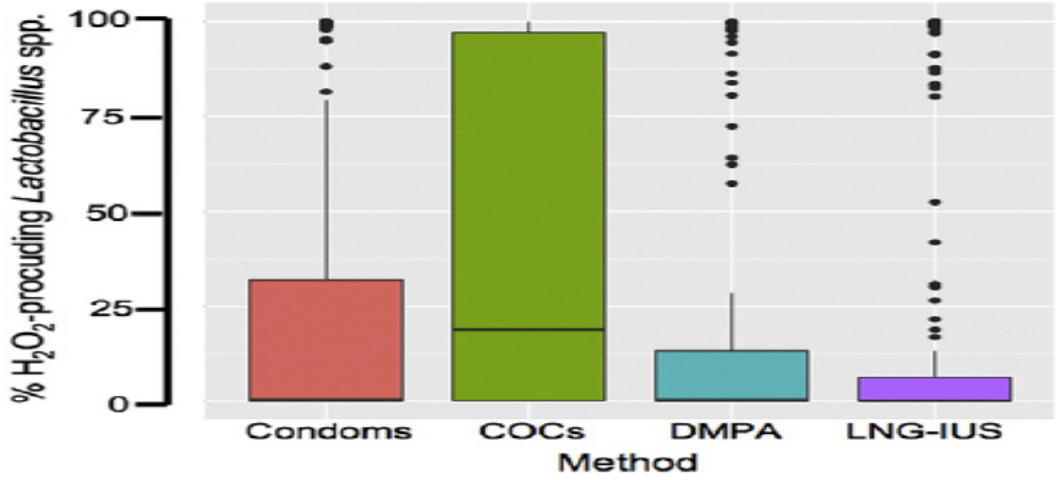


Fig. 4. Relationship between the percentage of H_2O_2-producing *Lactobacillus* species and contraceptive method. The analysis includes healthy women and women with BV. Subjects were grouped based on self-reported contraceptive method. Within each group, the proportion of H_2O_2-producing *Lactobacillus* species, including *L. crispatus*, *L. gasseri* and *L. jensenii*, was plotted. The boxes indicate the interquartile range, and the horizontal line in each box indicates the median.

- Women using COCs and DMPA, but not LNG-IUS were less likely to be colonized by BV-associated bacteria relative to women who used condoms.
- Women using COCs were more likely to be colonized by beneficial H2O2-producing Lactobacillus species compared with women using condoms, while women using DMPA and LNG-IUS were not.

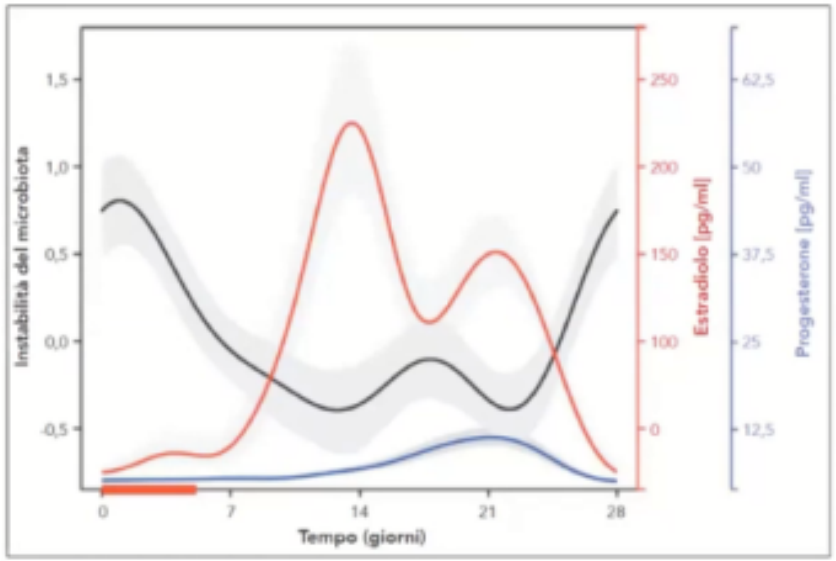
Research ArticleGenomics

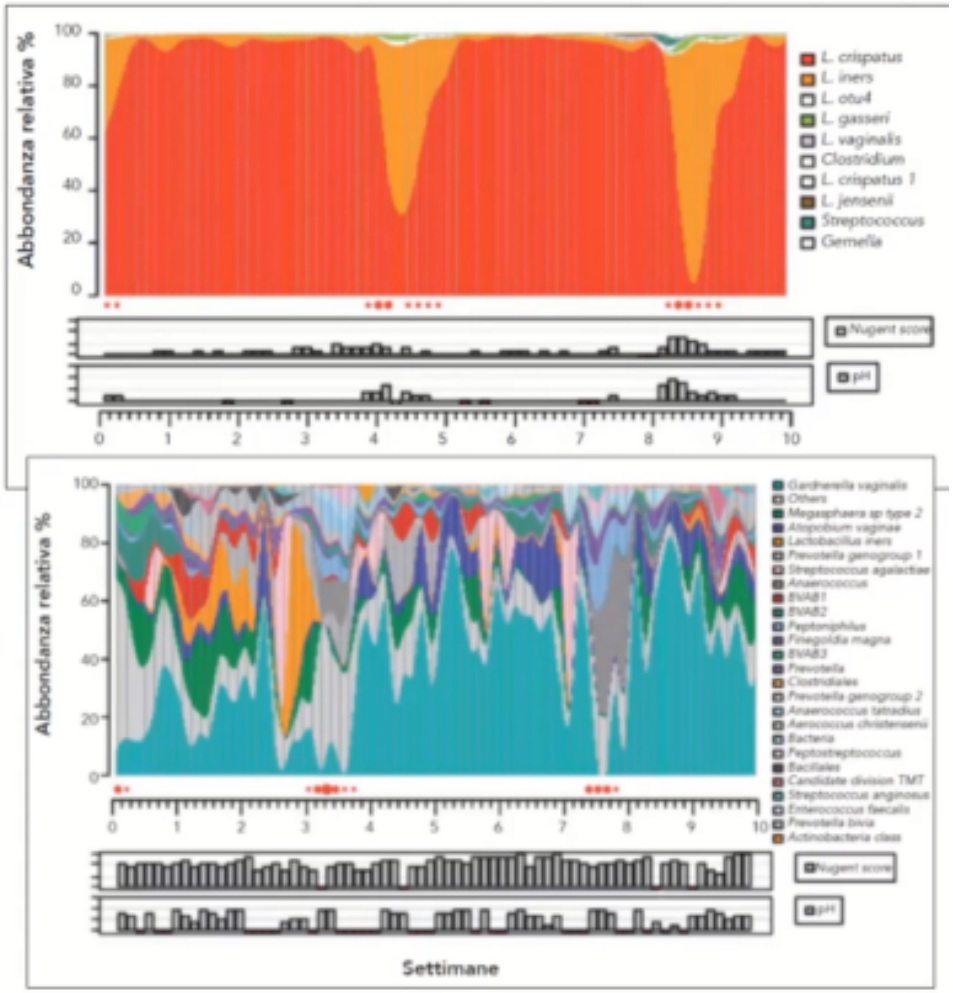
Temporal Dynamics of the Human Vaginal Microbiota

Pawel Gajer, Rebecca M. Brotman

Science Translational Medicine 02 May 2012:

- Among the endogenous factors known to contribute to microbiome changes are hormonal changes during the menstrual cycle.
- These changes are associated with shifts in vaginal bacterial content, with menses representing the phase in which the microbiome is more diverse, while the oestradiol and progesterone peaks are more bacterially stable times.


Instabilità del microbiota
1,5
1,0
0,5
0,0
-0,5
0
7
14
21
28
Tempo (giorni)
250
200
150
100
0
Estradiolo [pg/ml]
62,5
50
37,5
25
12,5
Progesterone [pg/ml]


Abbondanza relativa %
100
80
60
40
20
0
L. crispatus
L. iners
L. otu4
L. gasseri
L. vaginalis
Clostridium
L. crispatus 1
L. jensenii
Streptococcus
Gemella
Nugent score
pH
0
1
2
3
4
5
6
7
8
9
10
Abbondanza relativa %
Gardnerella vaginalis
Others
Megasphaera sp type 2
Atopobium vaginae
Lactobacillus iners
Prevotella genogroup 1
Streptococcus agalactiae
Anaerococcus
BVAB1
BVAB2
Peptoniphilus
Finegoldia magna
BVAB3
Prevotella
Clostridiales
Prevotella genogroup 2
Anaerococcus tetradius
Aerococcus christensenii
Bacteria
Peptostreptococcus
Bacillales
Candidate division TM7
Streptococcus anginosus
Enterococcus faecalis
Prevotella bivia
Actinobacteria class
Nugent score
pH
Settimane

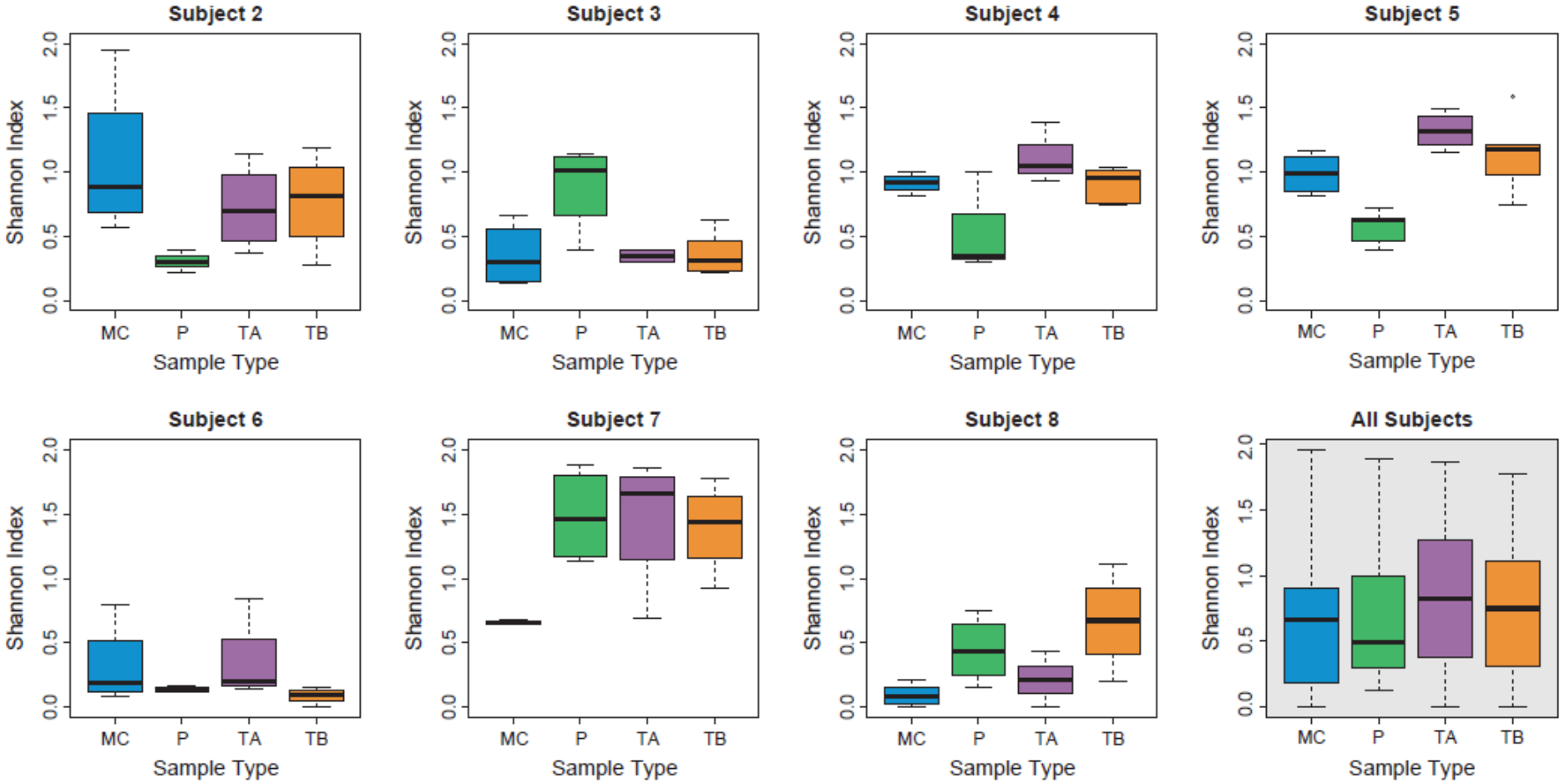


Figure 4. Boxplots of Shannon index values by treatment per participant. The *y*-axis of each plot indicates the value of the Shannon index. The *x*-axis labels indicate nonmenstrual mid-cycle samples (MC) or samples collected during catamenial product use (P, pad; TA, tampon A; TB, tampon B). The first seven panels (left to right, top to bottom) are organised by individual participant, whereas the last panel summarises the Shannon indices across all seven participants.

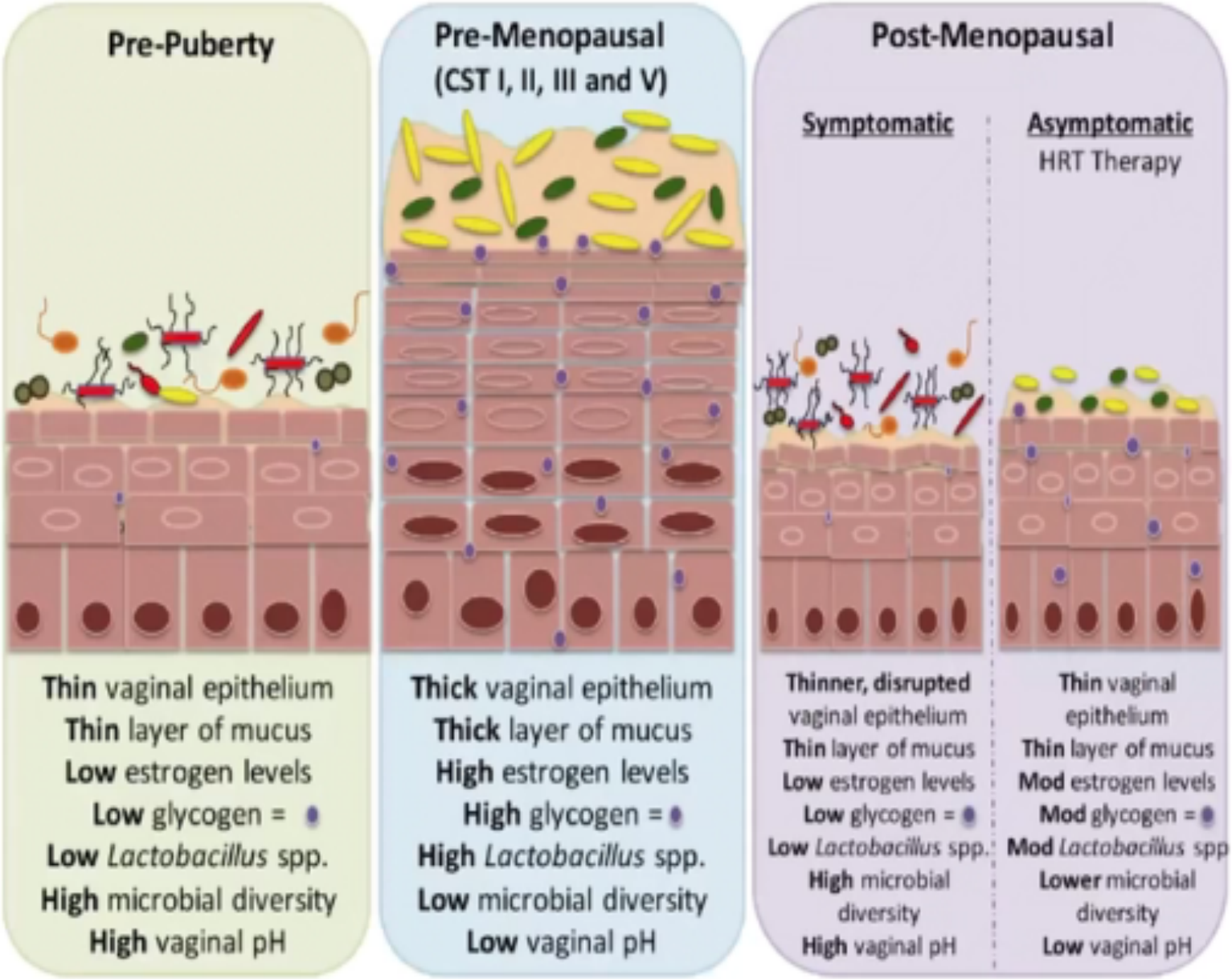
Menopause and the vaginal microbiome

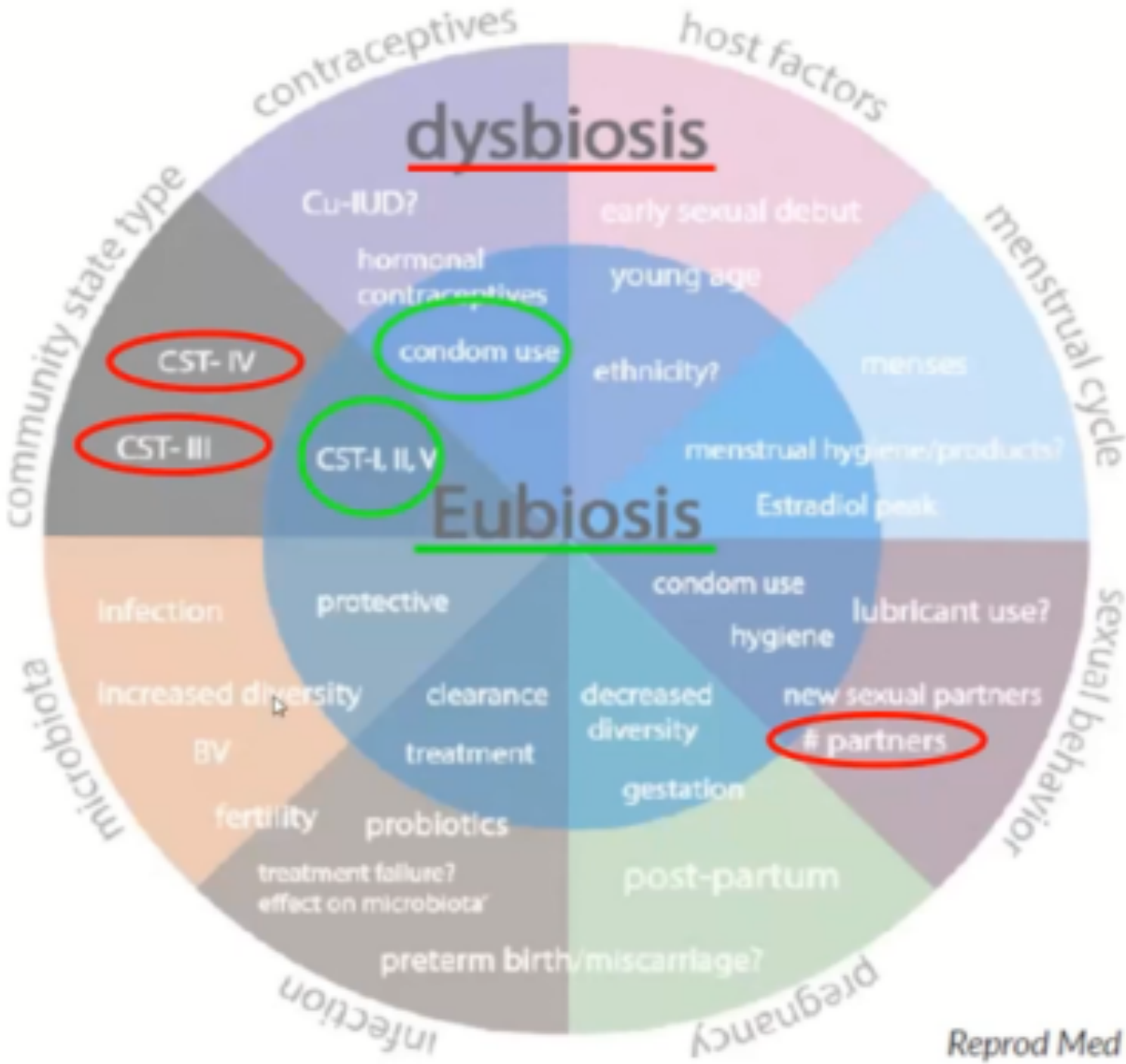
AliciaL.Muhleisena,b,MelissaM.Herbst-Kralovetzb

Maturitas91(2016)42–50

- The vaginal flora during infancy is a mixture of aerobic and anaerobic bacterial populations including Prevotella, Enterobacteria, Streptococcus, and Staphylococcus species
- After puberty, the estrogen rise leads to the production and accumulation of glycogen, which is essential for *Lactobacillus* growth and the colonization of the vaginal epithelium and pH starts to decrease ; the dominance of *Lactobacillus* is maintained during the reproductive years

- Finally, after menopause, the proportion of *Lactobacillus* species decreases again due to the drop in endogenous estrogen.
- Interestingly, the *Lactobacillus* content, as well as a low vaginal pH, is maintained in women receiving hormonal replacement therapy during menopause.


Pre-Puberty
Thin vaginal epithelium
Thin layer of mucus
Low estrogen levels
Low glycogen =
Low Lactobacillus spp.
High microbial diversity
High vaginal pH
Pre-Menopausal
(CST I, II, III and V)
Thick vaginal epithelium
Thick layer of mucus
High estrogen levels
High glycogen =
High Lactobacillus spp.
Low microbial diversity
Low vaginal pH
Post-Menopausal
Symptomatic
Thinner, disrupted vaginal epithelium
Thin layer of mucus
Low estrogen levels
Low glycogen =
Low Lactobacillus spp.
High microbial diversity
High vaginal pH
Asymptomatic
HRT Therapy
Thin vaginal epithelium
Thin layer of mucus
Mod estrogen levels
Mod glycogen =
Mod Lactobacillus spp
Lower microbial diversity
Low vaginal pH



Reprod Med Biol. 2019;18:40–50.

Ricchezza batterica e assenza di dominanza lattobacillare
sono cause prime di **potenziale instabilità**

Oscillazioni ormonali, rapporti sessuali, terapie antibiotiche,
riduzione risposta immune sono *trigger*, **inneschi di patologia**

Il concetto stesso di CST (IVB – IVA – III – II/V – I)
dovrebbe essere considerato
come **fattore di rischio**

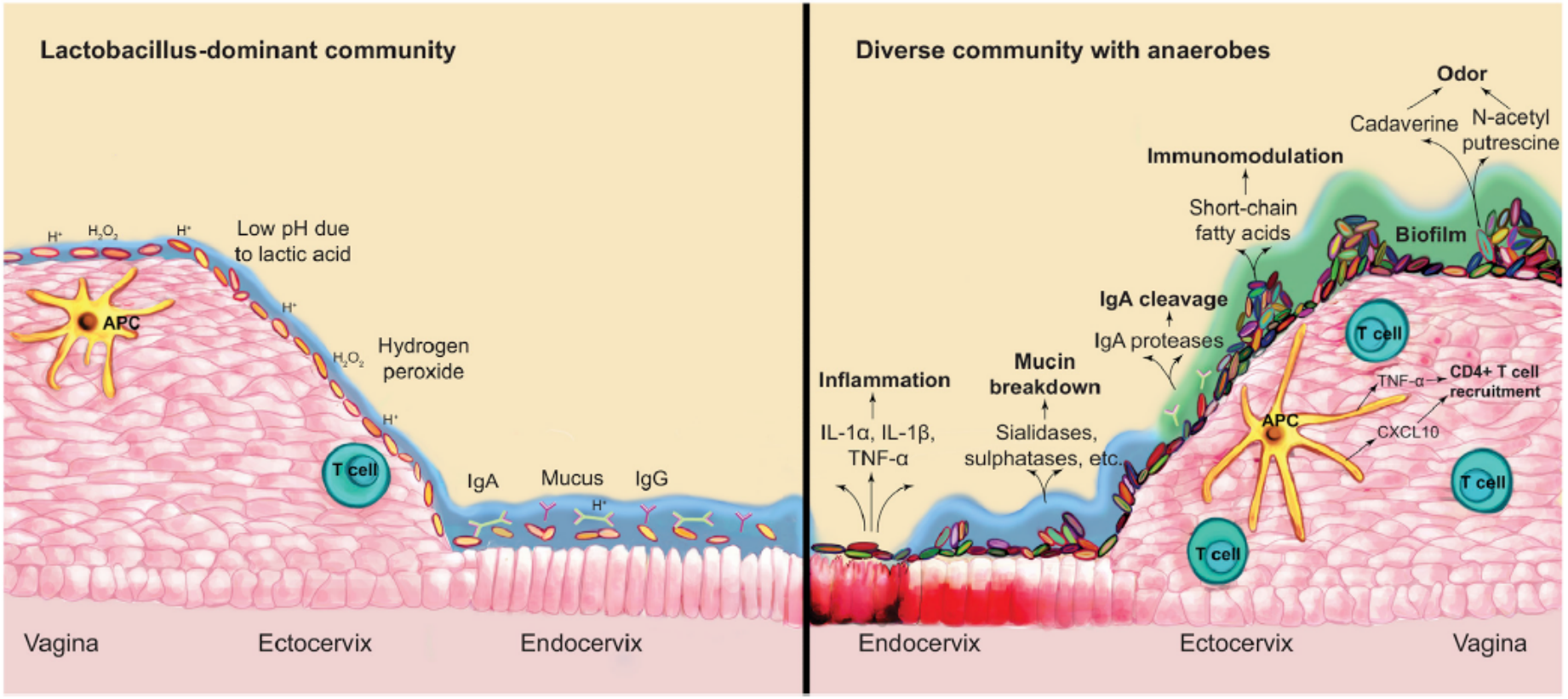


Figure 1. Modulation of the Female Genital Tract Microenvironment by Resident Bacterial Communities

Left: dominance by lactobacilli, particularly *Lactobacillus crispatus*, is associated with a low inflammatory cervicovaginal microenvironment. Lactobacilli protect their niche through the production of lactic acid, which maintains a hostile acidic environment with a pH around 4.0, and hydrogen peroxide, which inhibits the growth of catalase-negative bacteria. The endocervix contributes an innate immune barrier through the maintenance of a thick mucus layer containing IgG, secretory IgA, lactoferrin, lysozyme, and other antibacterial compounds. In this quiescent environment, there are sparse T cells and antigen-presenting cells in the lower reproductive endothelium. Right: in contrast, replacement of lactobacilli with a diverse community of anaerobes can result in a variety of changes to the microenvironment. One of the first signs of bacterial vaginosis is foul-smelling vaginal discharge, which is caused by the bacterial production of chemicals such as cadaverine and N-acetyl putrescine. *Gardnerella*, *Prevotella*, and others can produce sialidases, IgA proteases, and short-chain fatty acids to improve their adherence to epithelial cells, evade antibody-mediated inhibition, and modulate the immune environment, respectively. Epithelial cells and underlying antigen-presenting cells (APCs) respond to *Prevotella*, *Mobiluncus*, and *Sneathia*, for example, by producing pro-inflammatory cytokines including IL-1α, IL-1β, and TNF-α. APCs also produce CXCL10, which can attract additional activated $CD4^+$ T cells to the tissue and may be a key mechanistic link between these diverse communities and increased HIV acquisition risk. These diverse communities become particularly difficult to eradicate after the formation of biofilms.

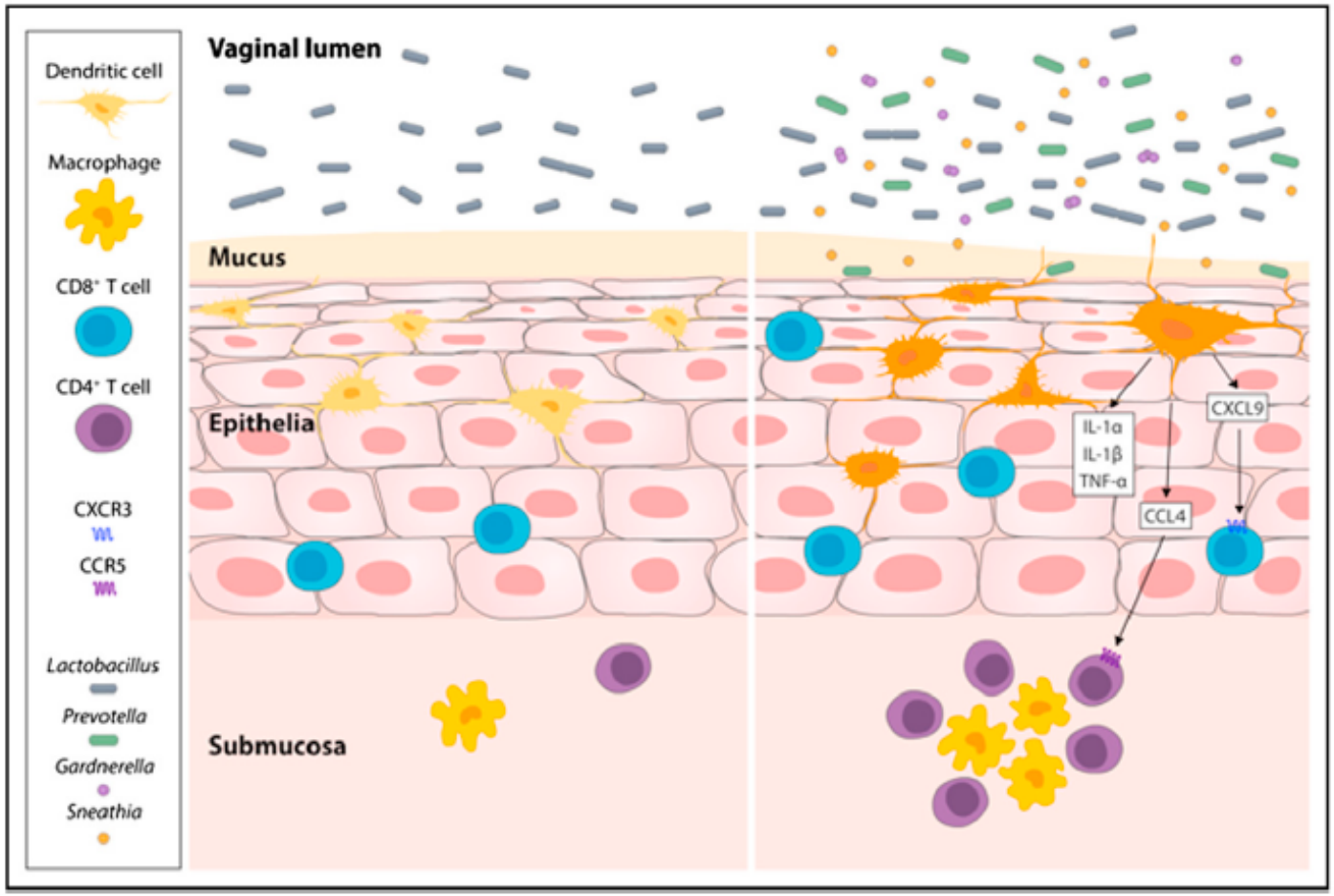


Figure 1. Vaginal Microbial Composition Influences Inflammation and T Cell Abundance

(Left) Low complexity vaginal flora represented by cervicotype CT1 and CT2 are *Lactobacillus* dominant and are not associated with inflammatory cytokine production. (Right) High-diversity vaginal flora, (specifically a cervicotype CT4 enriched for multiple bacterial genera including *Prevotella*, *Sneathia*, and *Gardnerella*) is strongly correlated with increased inflammatory cytokine production by both epithelial cells and activated vaginal APCs. These APCs are potentially directly activated by the vaginal microbiota and secrete both inflammatory cytokines, as well as chemokines resulting in T cell recruitment. Increased numbers of $CCR5^+$ CD4 T cells in hosts with this cervicotype might explain the previously reported link between high-diversity vaginal flora and increased HIV susceptibility. Memory CD4 T cells in the female reproductive tract are often found in close proximity with macrophages in "memory lymphocyte clusters" (Iijima and Iwasaki, 2014). The induction of a localized inflammatory milieu by high-diversity microbiota will likely influence the formation of memory lymphocyte clusters and will be an important consideration in the design of vaccines against STIs, as well as acquisition of HIV-1.

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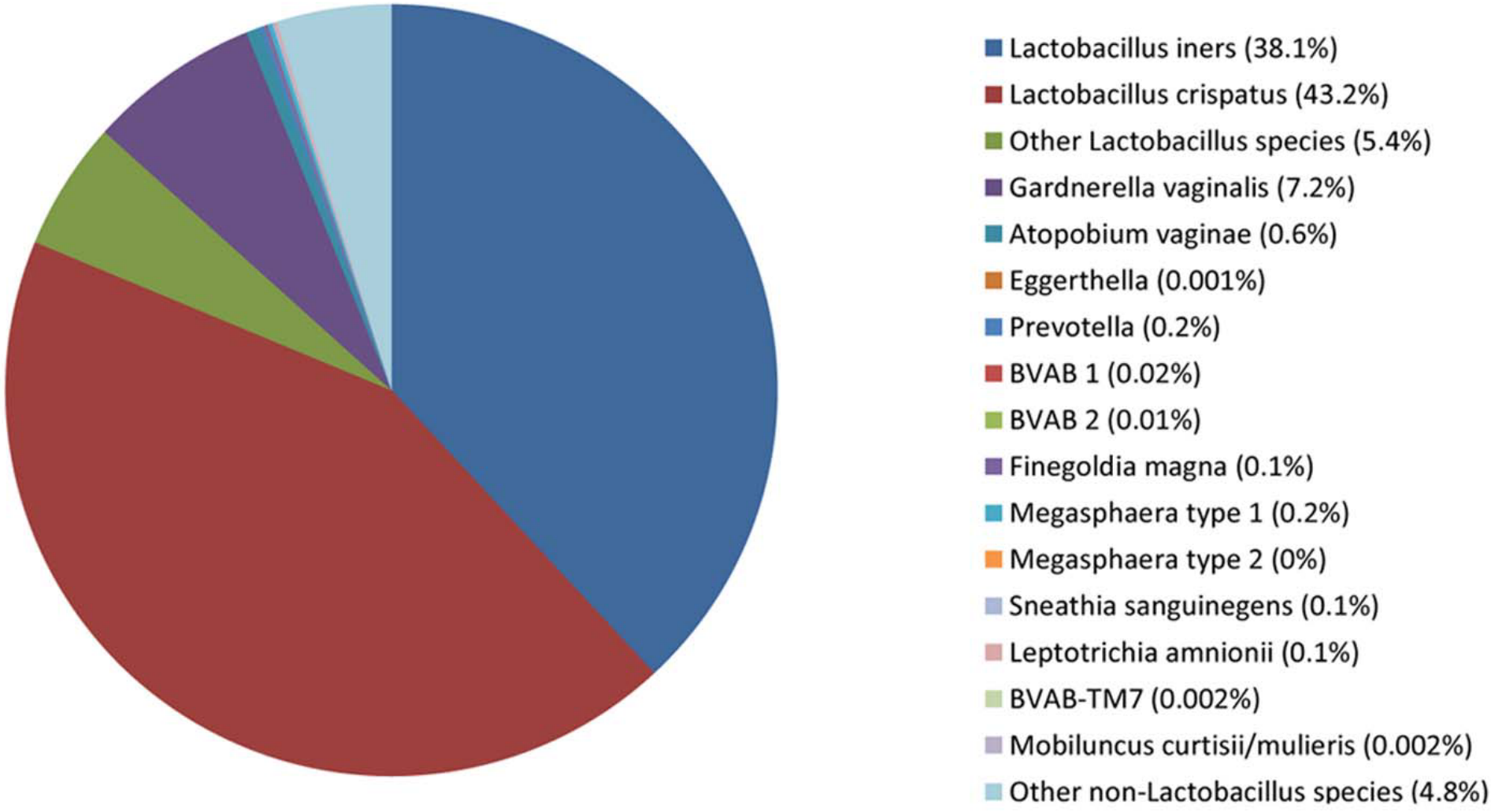
PLOS ONE

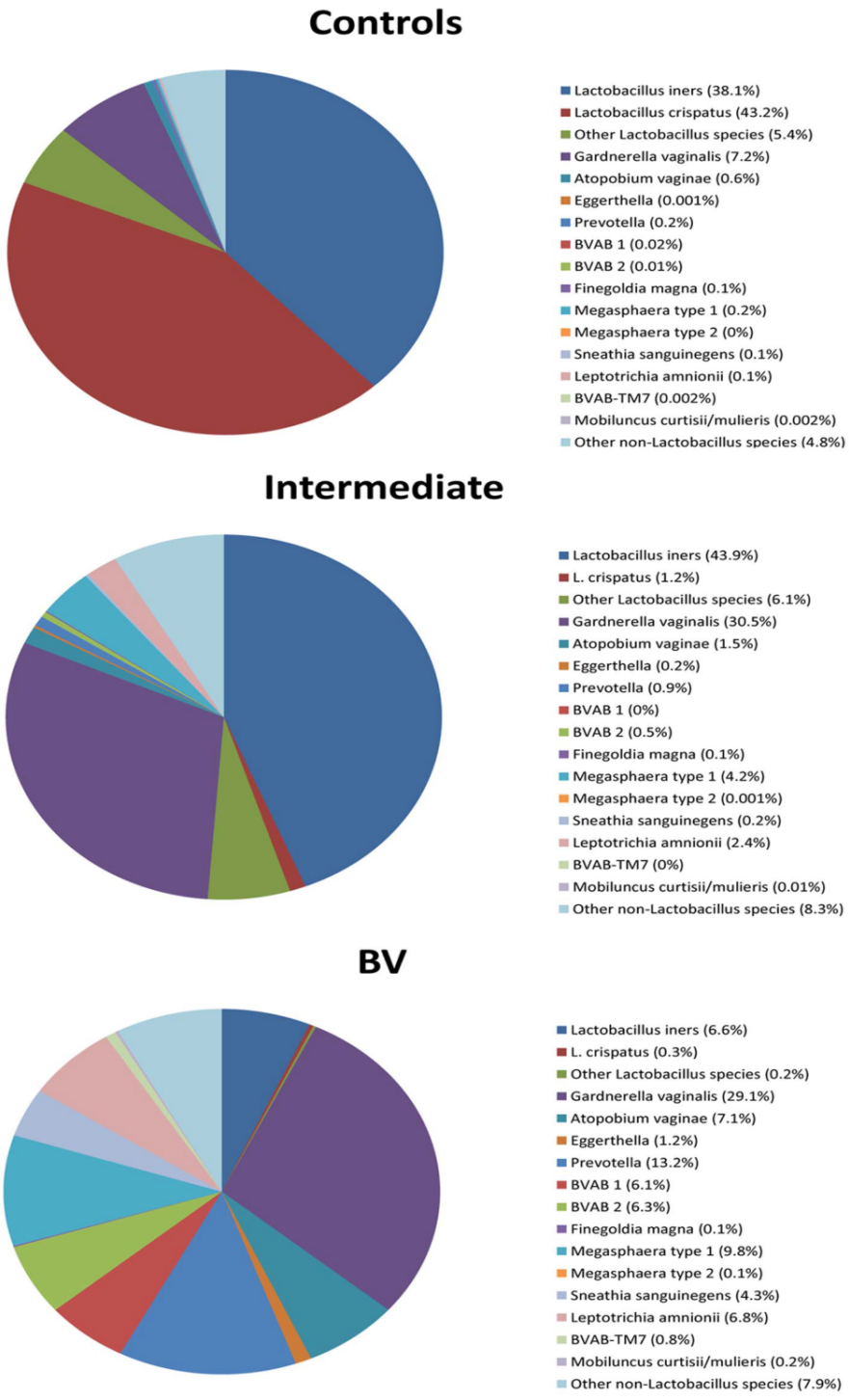
Composition of the Vaginal Microbiota in Women of Reproductive Age – Sensitive and Specific Molecular Diagnosis of Bacterial Vaginosis Is Possible?

Elena Shipitsyna[1], Annika Roos[2], Raluca Datcu[3], Anders Hallén[4], Hans Fredlund[5], Jørgen S. Jensen[3], Lars Engstrand[2], Magnus Unemo[5]*

- Vaginal samples from 163 women (79 control, 73 BV and 11 intermediate (Lactobacillary grade II flora) cases) were analyzed using 454 pyrosequencing of the hypervariable regions V3–V4 of the 16S rRNA gene and 16 quantitative bacterial species/genus-specific real-time PCR assays.

Controls


Lactobacillus iners (38.1%)
Lactobacillus crispatus (43.2%)
Other Lactobacillus species (5.4%)
Gardnerella vaginalis (7.2%)
Atopobium vaginae (0.6%)
Eggerthella (0.001%)
Prevotella (0.2%)
BVAB 1 (0.02%)
BVAB 2 (0.01%)
Finegoldia magna (0.1%)
Megasphaera type 1 (0.2%)
Megasphaera type 2 (0%)
Sneathia sanguinegens (0.1%)
Leptotrichia amnionii (0.1%)
BVAB-TM7 (0.002%)
Mobiluncus curtisii/mulieris (0.002%)
Other non-Lactobacillus species (4.8%)



- Relative to the healthy women, the BV patients had in their vaginal microbiota significantly higher prevalence, loads and relative abundances of the majority of BV associated bacteria. However, only Gardnerella vaginalis, Atopobium vaginae, Eggerthella, Prevotella, BVAB2 and Megasphaera type 1 detected at or above optimal thresholds were highly predictable for BV, with the best diagnostic accuracy shown for A. vaginae.
- The depletion of Lactobacillus species combined with the presence of either G. vaginalis or A. vaginae at diagnostic levels was a highly accurate BV predictor.

Vaginal microbiota composition and association with prevalent Chlamydia trachomatis infection: a cross-sectional study of young women attending a STI clinic in France.

Tamarelle J et al. Sex Transm Infect. 2018 Jan 22

- Microbiota composition CTS were for 132women
- CST: Lactobacillus Crispatus CST-I 37.1%, L. Iners CST-III 38.6%, and CST-IV 22.0% diversity of anaerobes represented.
- 21/132 women were CT positive.
- Proportions of CT-positive women were higher for samples belonging to CST-III (21.6%) than to CST-I (8.2%) with CST-IV (17.2%).
- 5CST were found in 132 young women from a STI clinic in France. These CSTs were not significantly associated with CT but higher proportions of CT-positive women were found in CST-III and CST-IV, consistent with a previous study in the Netherlands.

Characterization of cervico-vaginal microbiota in women developing persistent high-risk Human Papillomavirus infection

Monica Di Paola[1], Cristina Sani[2], Ann Maria Clemente[3], Anna Iossa[4], Eloisa Perissi[3], Giuseppe Castronovo[3], Michele Tanturli[6], Damariz Rivero[5], Federico Cozzolino[6], Duccio Cavalieri[5], Francesca Carozzi[2], Carlotta De Filippo[7] & Maria Gabriella Torcia[3]

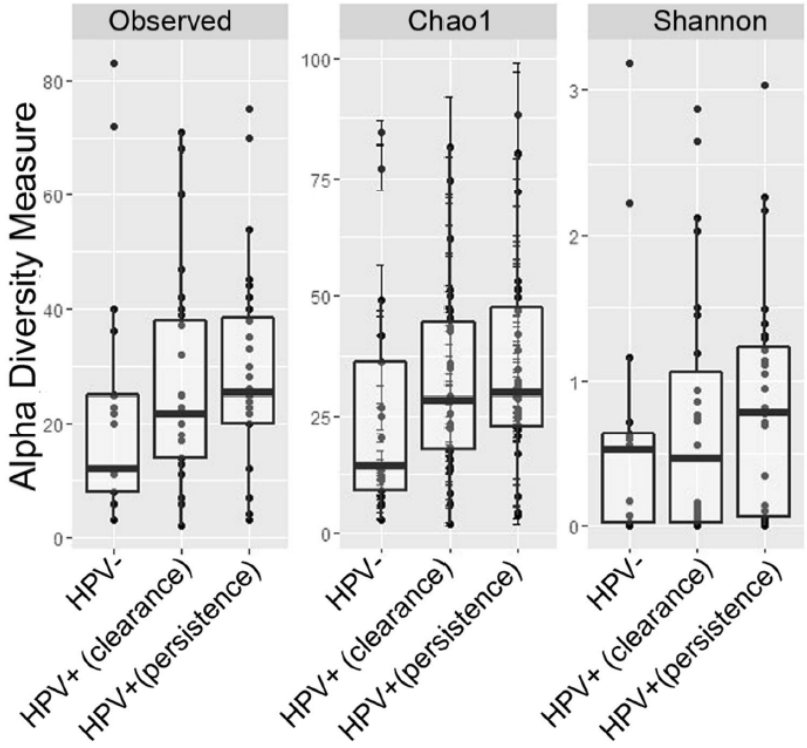
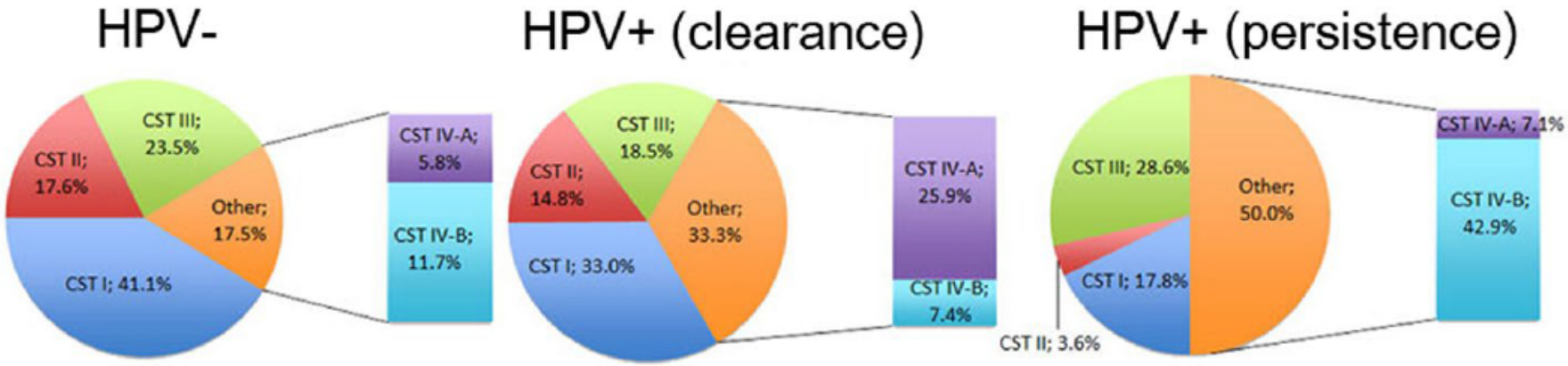


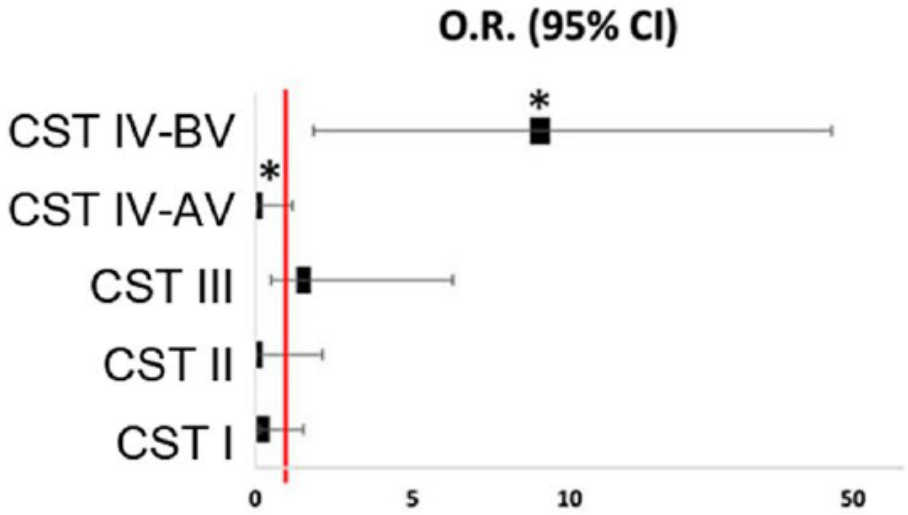
Figure 1. Alpha diversity measures. Box plots of observed OTUs, Chao 1, and Shannon index in the three groups of women. Pairwise comparisons by using the Wilcoxon rank sum test were not significant.

HPV- (Control) HPV + (Clearence) HPV+ (Persistence)

A



B



O.R.	p value	95% CI
9.38	**0.026**	1.85-47.52
0.11	**0.018**	0.01-0.93
2.08	0.2462	0.59-7.30
0.21	0.1471	0.02-2.04
0.43	0.1878	0.12-1.53

C

Anti-HIV-1 Activity of Lactic Acid in Human Cervicovaginal Fluid

David Tyssen,a Ying-Ying Wang,b

- The *Lactobacillus-dominated vaginal microbiota is associated with a* reduced risk of acquiring and transmitting HIV and other sexually transmitted infections (STIs).
- Lactic acid is a major organic acid metabolite produced by lactobacilli that acidifies the vagina and has been reported to have inhibitory activity *in vitro* against bacterial, protozoan, and viral STIs, including HIV infections.

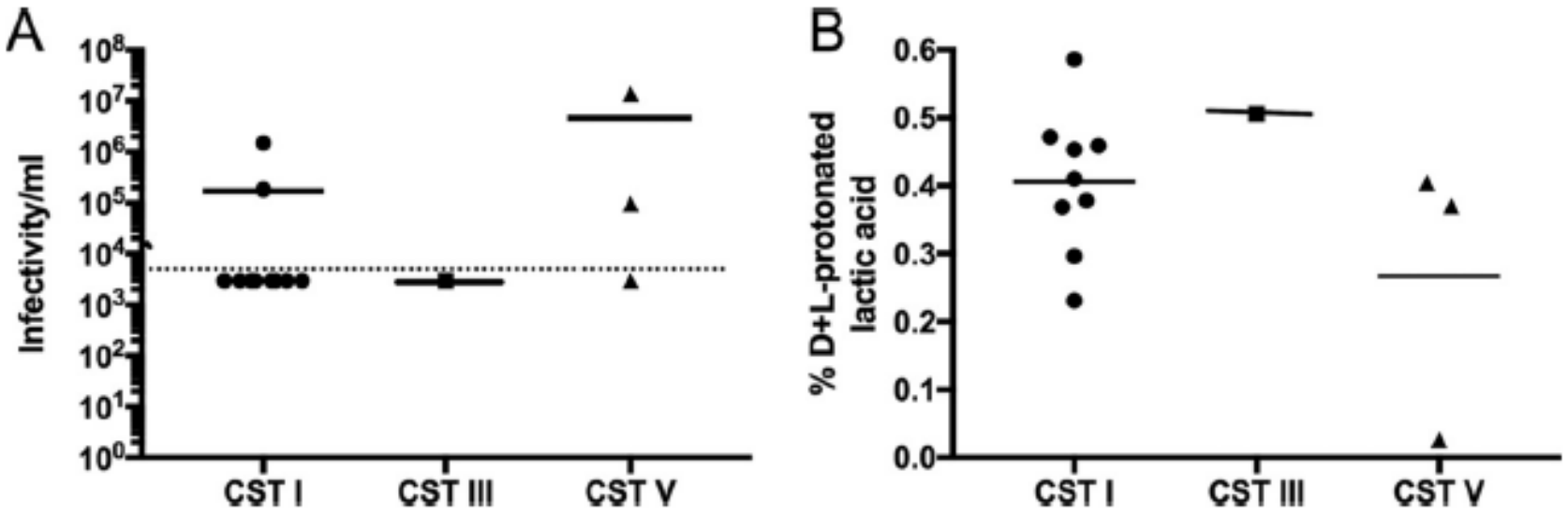


FIG 6 Anti-HIV_{Ba-L} activity and percent D+L-protonated lactic acid (wt/vol) levels of CVF samples with distinct vaginal microbiota dominated by *Lactobacillus* spp. (A) HIV_{Ba-L} infectivity after incubation in CVF samples (HIV_15 to HIV_27) with community state types (CST) dominated by *L. crispatus* (CST I), *L. iners* (CST III), or *L. jensenii* (CST V). Samples represented below the dotted horizontal line were below the detection limit of the infectivity assay. (B) Levels of percent D+L-protonated lactic acid in CVF samples (HIV_15 to HIV_27) categorized as CST (I), CST III, and CST V. Solid horizontal lines represent the means.

- Vaginal colonization with *Lactobacillus crispatus is associated* with reduced risk in women of acquiring HIV .
- HIV-infected women with microbiota dominated by *Lactobacillus spp., particularly L. crispatus, have a lower risk of* HIV genital shedding, which is thought to be associated with reduced HIV transmission to their sexual partners or neonates during vaginal birth

- Women with vaginal microbiota characterized by a high diversity of anaerobes (e.g., *Gardnerella,Prevotella, and Atopobium spp.) and a paucity of vaginal lactobacilli, as exemplified in* cases of bacterial vaginosis (BV) are at higher risk of acquiring HIV from and transmitting HIV to their male partners.

Possiamo classificare i microbioti in :

-***Primari*** : fecale, vaginale, orale, cutaneo e nasale

-***Derivati*** : polmonare, endometriale, vescicale, mammario, intestinale fetale

I microbioti sono interconnessi e le interconnessioni sono strutturate :

- all'interno dell'ospite
- tra ospiti
- con l'ambiente circostante (terreno , atmosfera , cibo e animali)

MICROBIOTI

Consorzi microbici (batterici)
in movimento nello spazio e nel tempo

- The upper reproductive tract is not sterile.
- For example, an active uterine microbiome has been characterized in healthy reproductive-age women, but bacteria have also been found to inhabit the fallopian tubes and the ovaries,with *Lactobacillus* the most abundant genus throughout the female reproductive tract.

Received: 11 November 2016 | Revised: 5 January 2017 | Accepted: 17 January 2017

DOI 10.1002/jcp.25806

ORIGINAL RESEARCH ARTICLE

WILEY Journal of Cellular Physiology

Subclinical alteration of the cervical–vaginal microbiome in women with idiopathic infertility

Giuseppina Campisciano[1] | Fiorella Florian[2] | Angela D'Eustacchio[3] | David Stanković[2] | Giuseppe Ricci[1,3] | Francesco De Seta[1,3] | Manola Comar[1,3]

Our results suggest that, in the Idiopathic, after a leading alteration caused by a decrease of *L. crispatus* and *L. iners*, and an increase of *L. gasseri*, a synergic action of different anaerobic bacteria, including *Atopobium*, *Prevotella*, *Veillonella*, *Ureaplasma*, and *Escherichia* (Biagi et al., 2009), rather than a predominant species, is involved in the pathogenesis of idiopathic infertility. The drop of some spp. of *Lactobacilli* corresponds to a decrease in acid lactic production and the metabolic byproducts of the anaerobic bacteria lead to an increase of normal vaginal pH (pH > 4.5), favoring a more hospitable niche for opportunistic pathogens.

TABLE 4 The relative abundances (%) of the *Lactobacilli* in the four cohorts of women

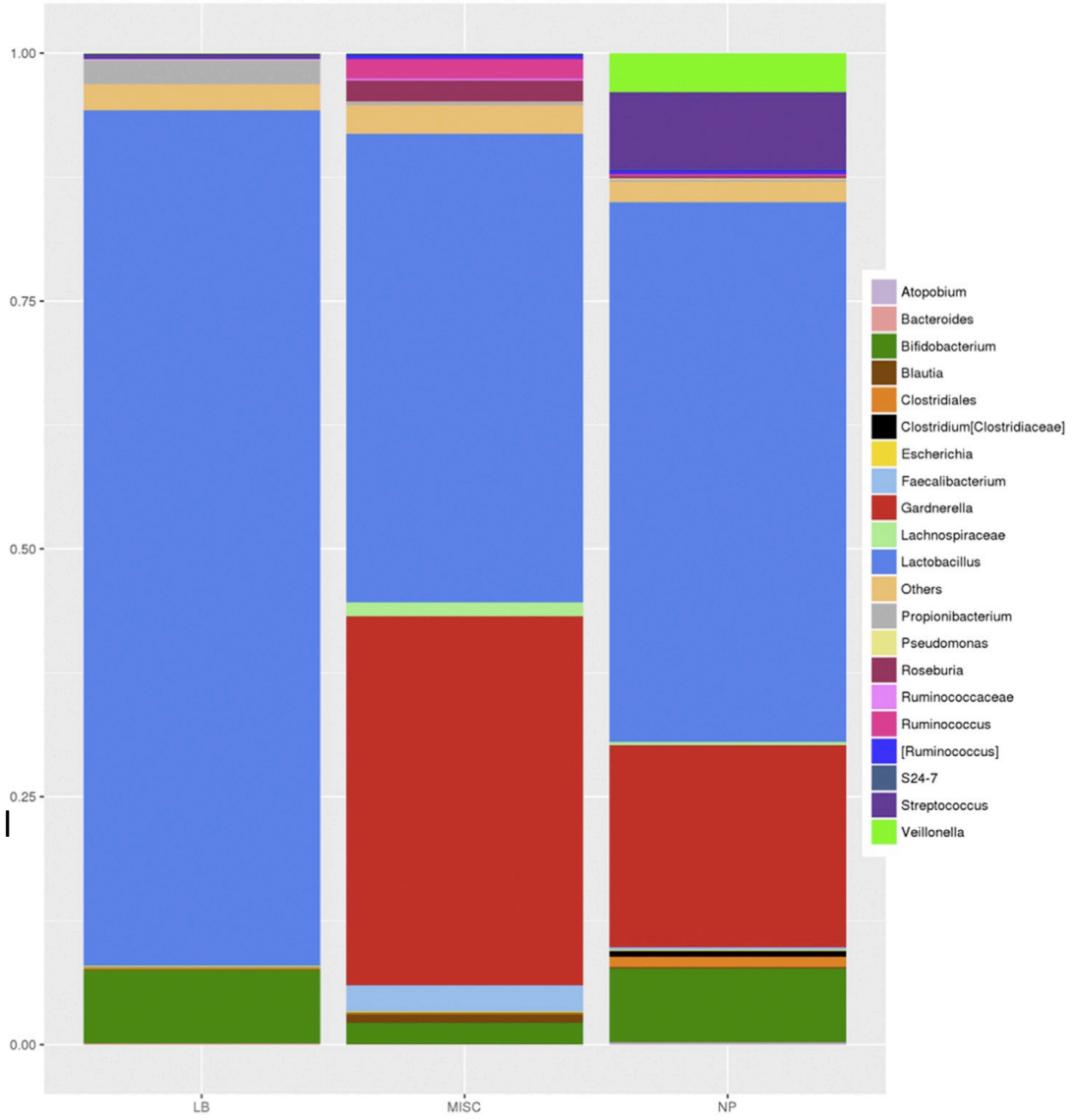
	Idiopathic	Control	Vaginosis	Infertile
L. iners	29	51	15	18
L. crispatus	31	36	6	25
L. gasseri	21	4	14	13
L. acidophilus	0	3	2	0
L. delbrueckii	0	4	0	0
L. johnsonii	0	1	0	6
L. vaginalis	0	0.7	0	0

Endometrial microbiota—new player in town

Inmaculada Moreno, Ph.D.a,b and Jason M. Franasiak, M.D.

- Average abundance of the 20 most abundant taxa in endometrial fluid samples from IVF patients with receptive endometrium.
- Patients are grouped by their reproductive outcome. LB live births; NP nonpregnant; MISC biochemical or clinical miscarriage.
- Low abundance of endometrial Lactobacillus is associated with poor reproductive outcome.

- Moreno I, Martinez-Blanch JF, Jimenez-Almaz_an J, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure.AmJ Obstet Gynecol 2016;215:684–703

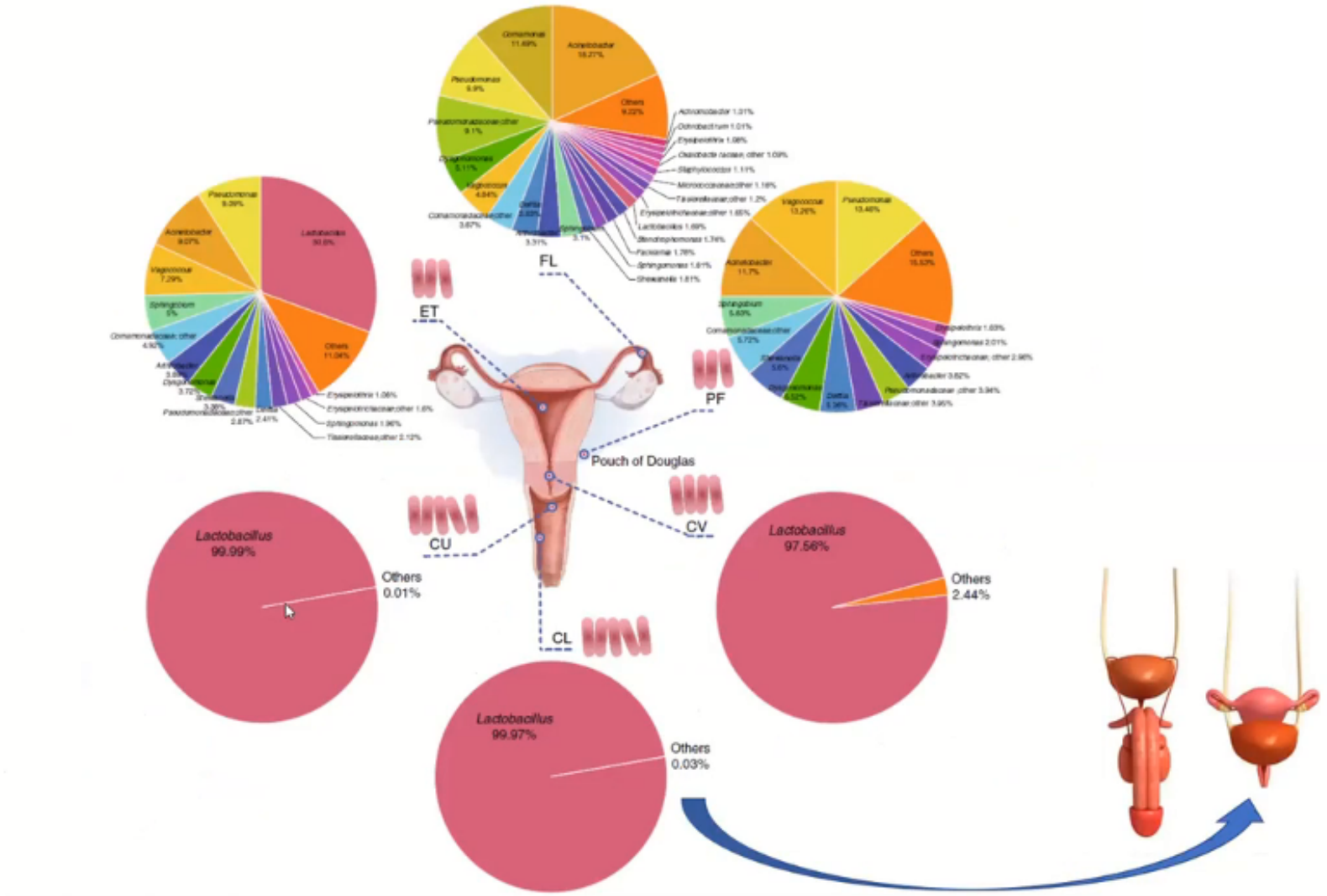


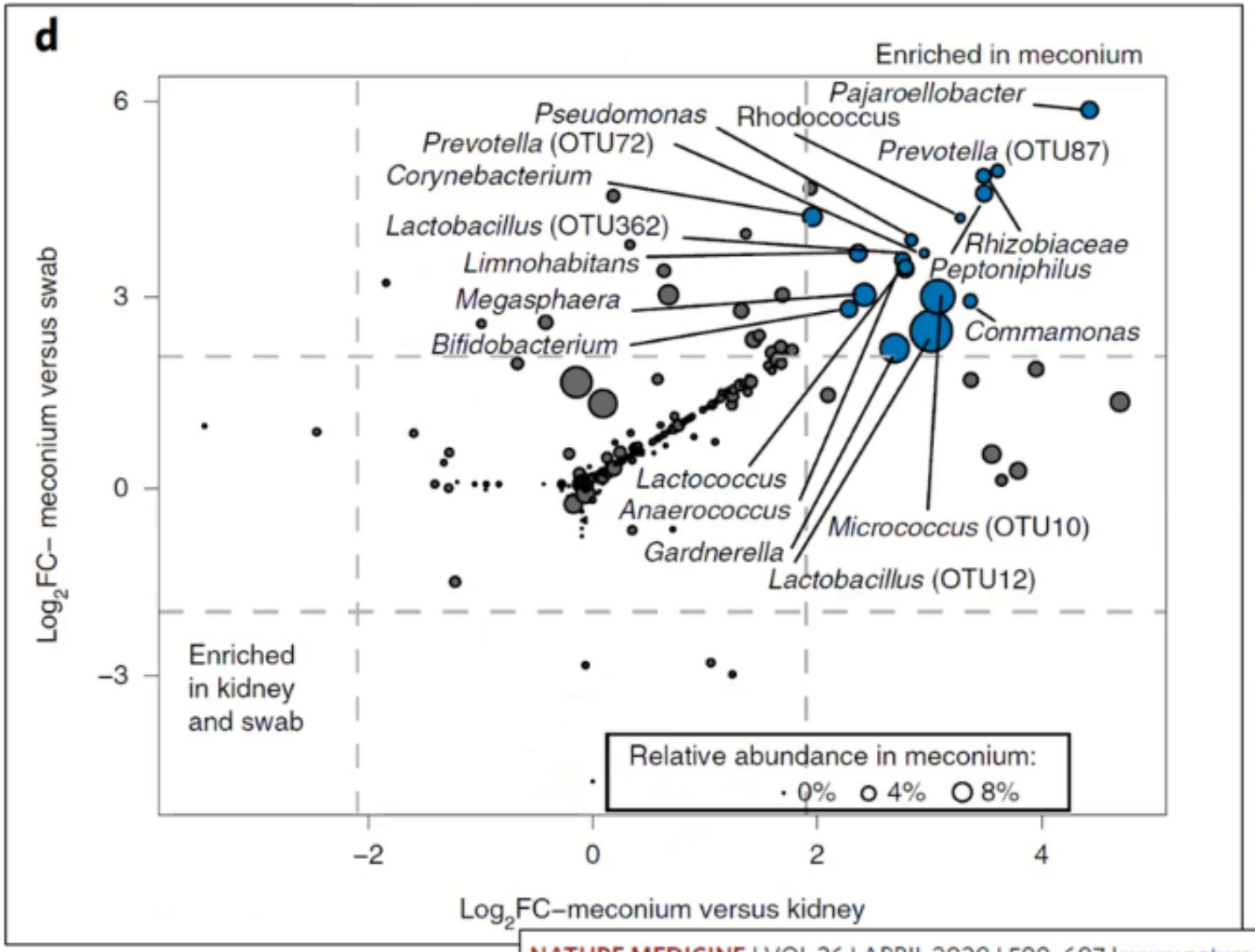
ARTICLE

DOI: 10.1038/s41467-017-00901-0 OPEN

The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases

Chen Chen[1,2], Xiaolei Song[1,3], Weixia Wei[4,5], Huanzi Zhong[1,2,6], Juanjuan Dai[4,5], Zhou Lan[1], Fei Li[1,2,3], Xinlei Yu[1,2], Qiang Feng[1,7], Zirong Wang[1], Hailiang Xie[1], Xiaomin Chen[1], Chunwei Zeng[1], Bo Wen[1,2], Liping Zeng[4,5], Hui Du[4,5], Huiru Tang[4,5], Changlu Xu[1,8], Yan Xia[1,3], Huihua Xia[1,2,9], Huanming Yang[1,10], Jian Wang[1,10], Jun Wang[1,11], Lise Madsen[1,6,12], Susanne Brix[13], Karsten Kristiansen[1,6], Xun Xu[1,2], Junhua Li[1,2,9,14], Ruifang Wu[4,5] & Huijue Jia[1,2,9,11]


FL
ET
PF
CU
CV
CL
Pouch of Douglas
Lactobacillus
99.99%
Others
0.01%
Lactobacillus
97.56%
Others
2.44%
Lactobacillus
99.97%
Others
0.03%



NATURE MEDICINE | VOL 26 | APRIL 2020 | 599–607 | www.nature.com/naturemedicine

FROM WHICH SOURCES COULD FETUS BE EXPOSED?

- Amniotic fluid

Science ref: 11. Oh K.J. et al, *J Perinat Med* 2010

- Umbilical cord blood

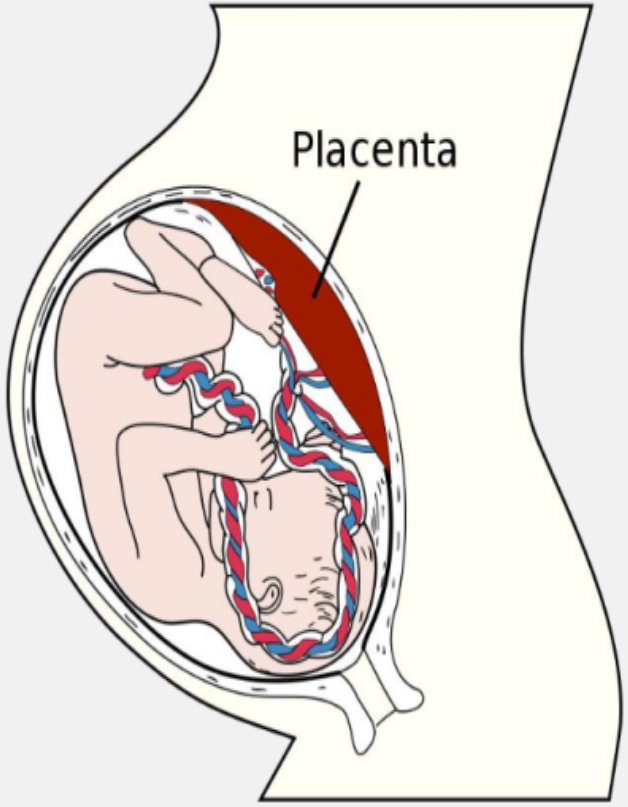
Science ref: 12. Jiménez E. et al, *Curr Microbiol.* 2005

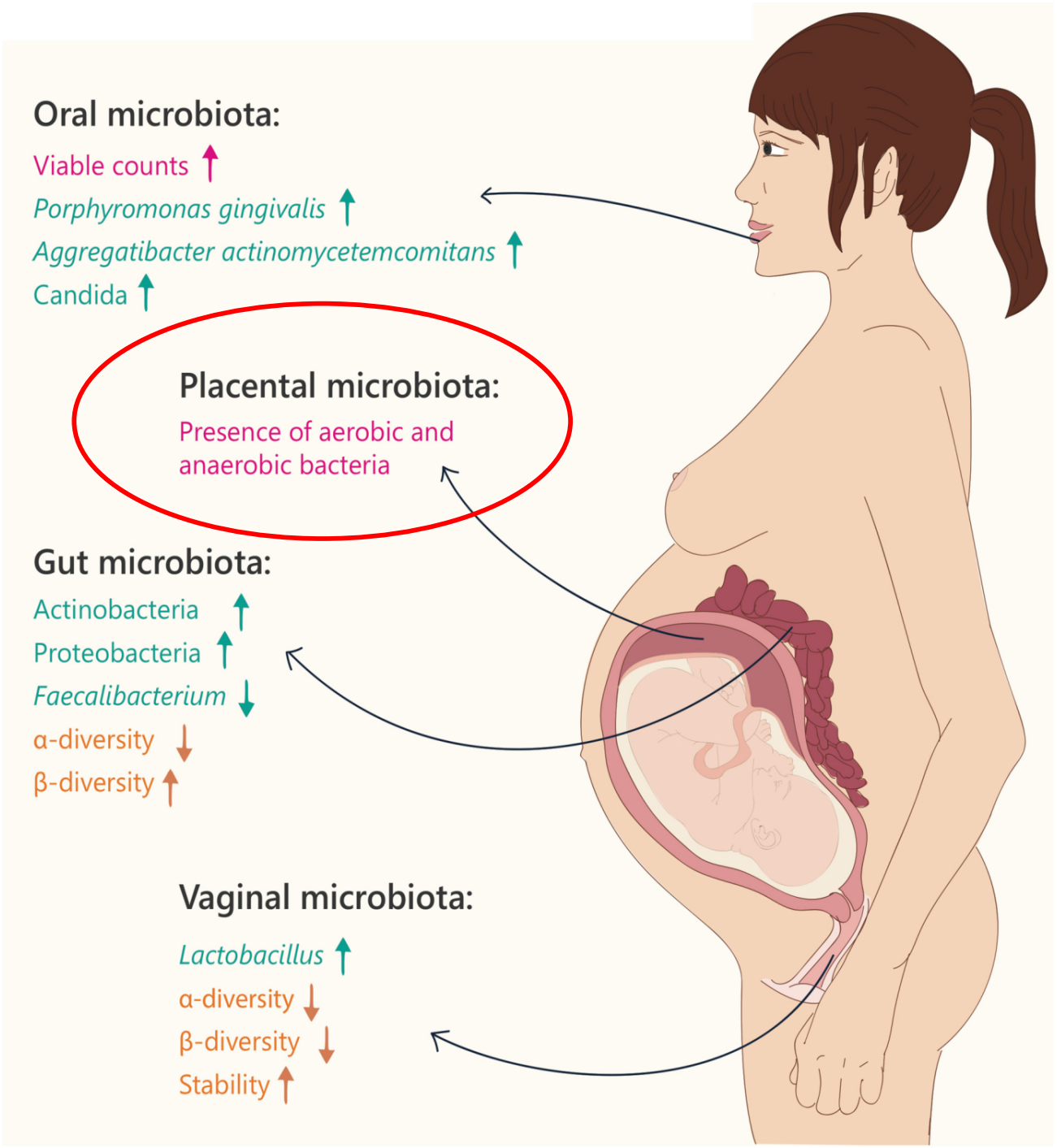
- Fetal membranes

Science ref: 13. Steel JH, *Pediatr Res.* 2005

- Placenta

Science ref: 14. Aagaard K, *Sci Transl Med.* 2014




Oral microbiota:
Viable counts ↑
Porphyromonas gingivalis ↑
Aggregatibacter actinomycetemcomitans ↑
Candida ↑
Placental microbiota:
Presence of aerobic and anaerobic bacteria
Gut microbiota:
Actinobacteria ↑
Proteobacteria ↑
Faecalibacterium ↓
α-diversity ↓
β-diversity ↑
Vaginal microbiota:
Lactobacillus ↑
α-diversity ↓
β-diversity ↓
Stability ↑

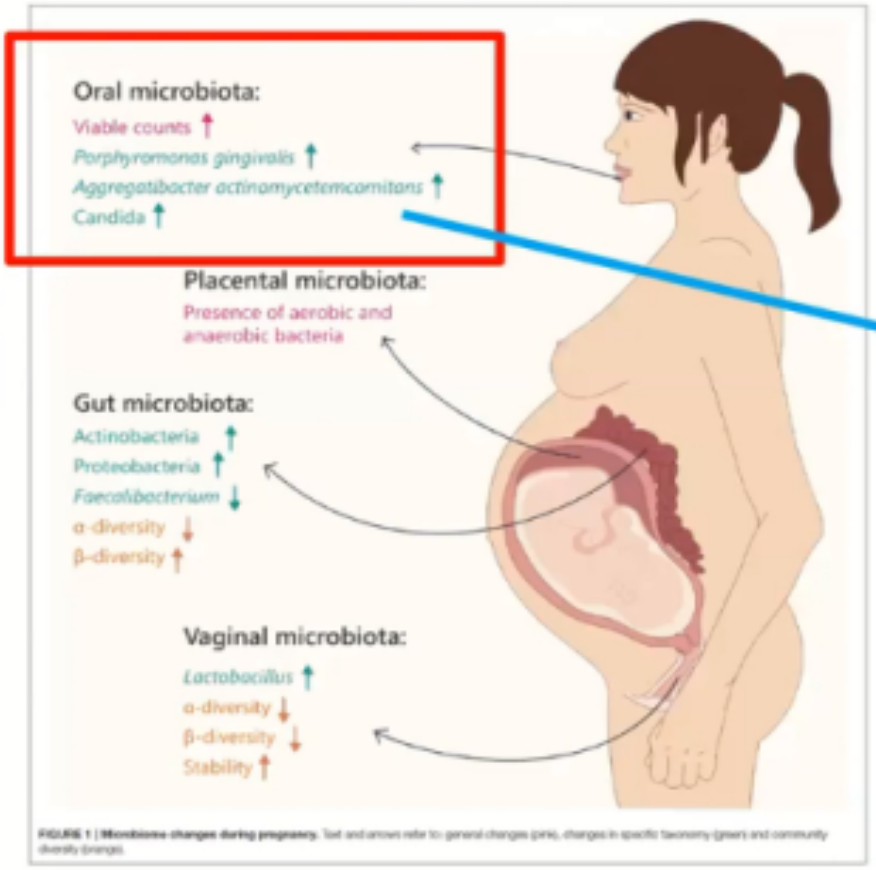
- Several additional reports described bacteria in the placenta (Goldenberg et al., 2000; Dominguez-Bello et al., 2010; Hyman et al., 2014; Romero et al., 2014a; MacIntyre et al., 2015)

- Using whole genome shotgun sequencing (WGS) of samples from 320 subjects, Aagaard et al. reported that the placenta contains a unique microbiome (Aagaard et al., 2014)
- The major phylum was Proteobacteria, and compared to all other organs,the composition was most similar to the oral microbiota,including species such as *Prevotella tannerae and Neisseria (Aagaard et al., 2014).*

- The oral microbiome includes up to 600 diverse species including *Streptococci, Lactobacilli, Staphylococci,Corynebacteria, etc., residing in different microenvironments within the oral cavity (teeth, tongue, palates, etc., Dewhirst et al., 2010).*

- The total viable microbial counts in all stages of pregnancy were higher than those of the non-pregnant women, especially in early pregnancy (Fujiwara et al., 2015), and levels of the pathogenic bacteria *Porphyromonas gingivalis and Aggregatibacteractinomycetemcomitans in the subgingival plaque, were significantly higher during the early and middle stages of pregnancy, compared to the non-pregnant group(Fujiwara et al., 2015).*

Microbiota changes in pregnancy



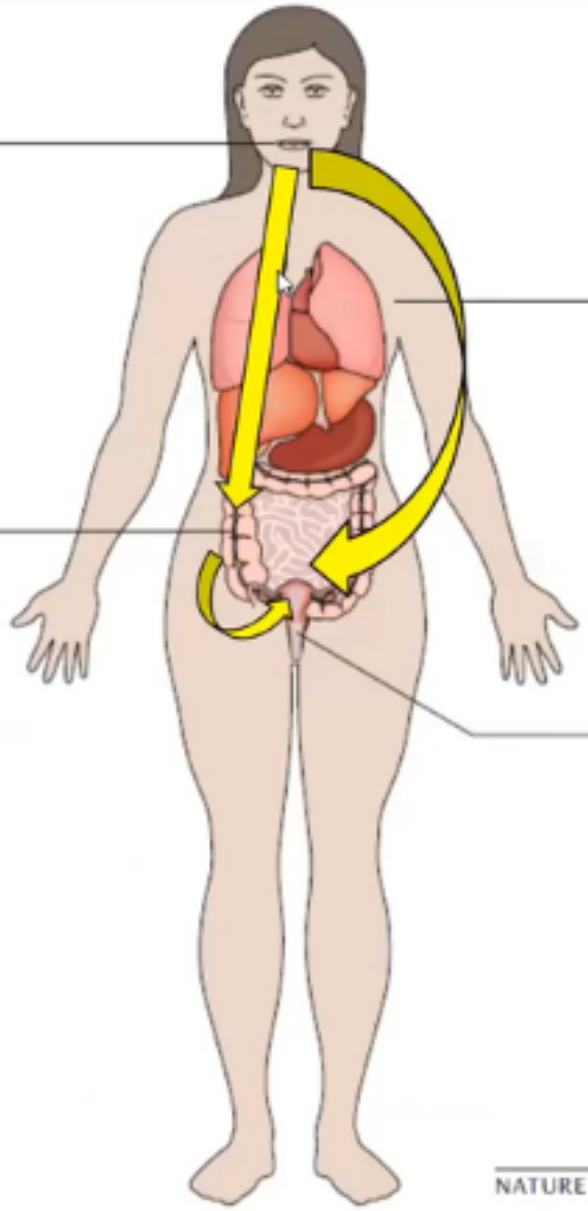
Parodontopatie
PROM e PPROM

Frontiers in Microbiology, 2016;7:1031

Fusobacterium nucleatum

Oropharyngeal
- Periodontitis*
- Endodontic infections*
- Gingivitis*
- Tonsilitis
- Head and neck cancers

Gastrointestinal tract
- Appendicitis
- Inflammatory bowel diseases
- Colorectal cancer



Other
- Endocarditis
- Atherosclerosis
- Respiratory tract infections
- Brain abscess
- Liver abscess
- Osteomyelitis

Urogenital tract
- Adverse pregnancy outcomes (pre-term labour, stillbirth and chorioamnionitis)
- Urinary tract infections

- Moderate evidence
- Some evidence
- Associative evidence

*Polymicrobial infections

RECENT RESEARCH INDICATES....

- Could be link between oral microbiome (from mother's mouth) & preterm birth
- Could be link between placental microbiome and oral microbiome (from mother's mouth)

Science Refs:
14. Aagaard K, et al. *Sci Transl Med.* 2014
15. Jefferson, *Adv Appl Microbiol*. 2012

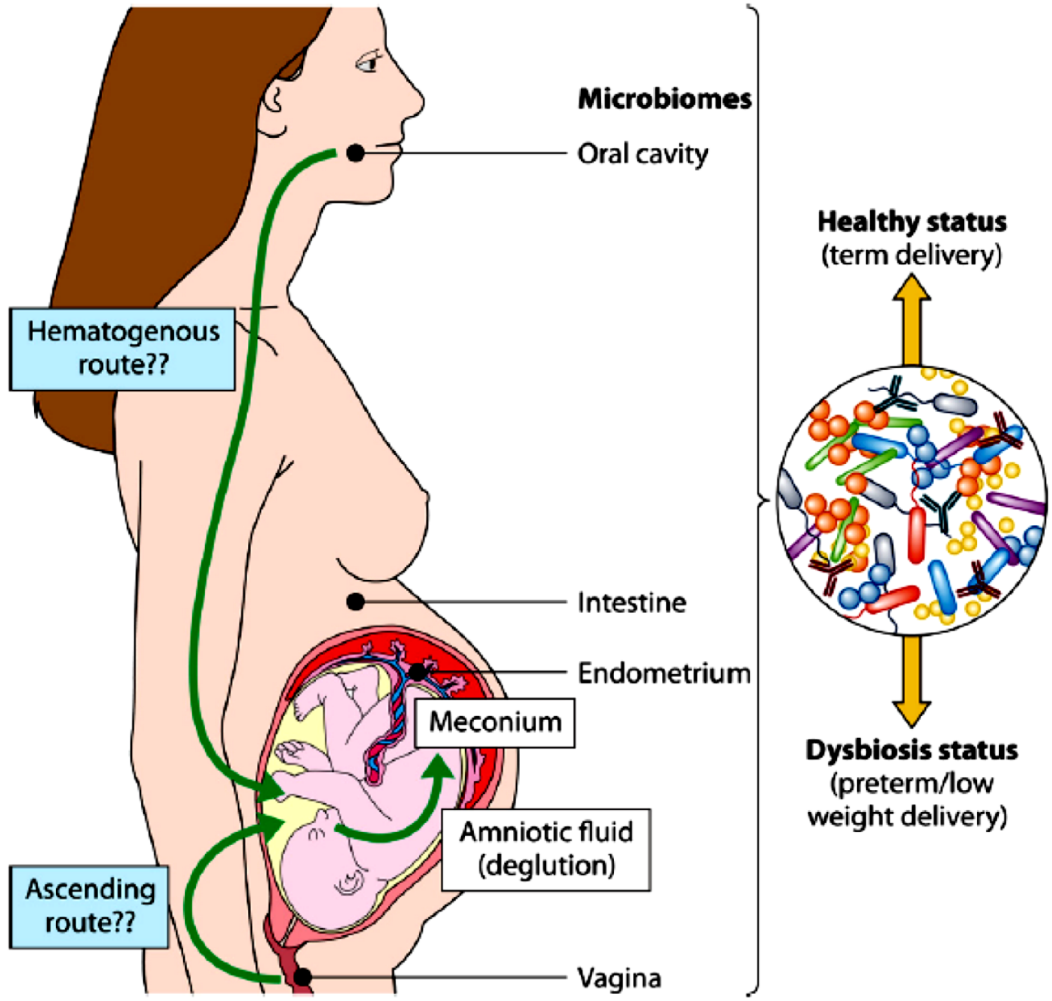


FIG 4 Colonization routes of maternal microbiomes to the infant. The mother portrayal exhibits the maternal microbiome locations and the related routes that result in the vertical transmission of the microbiota to the infant.

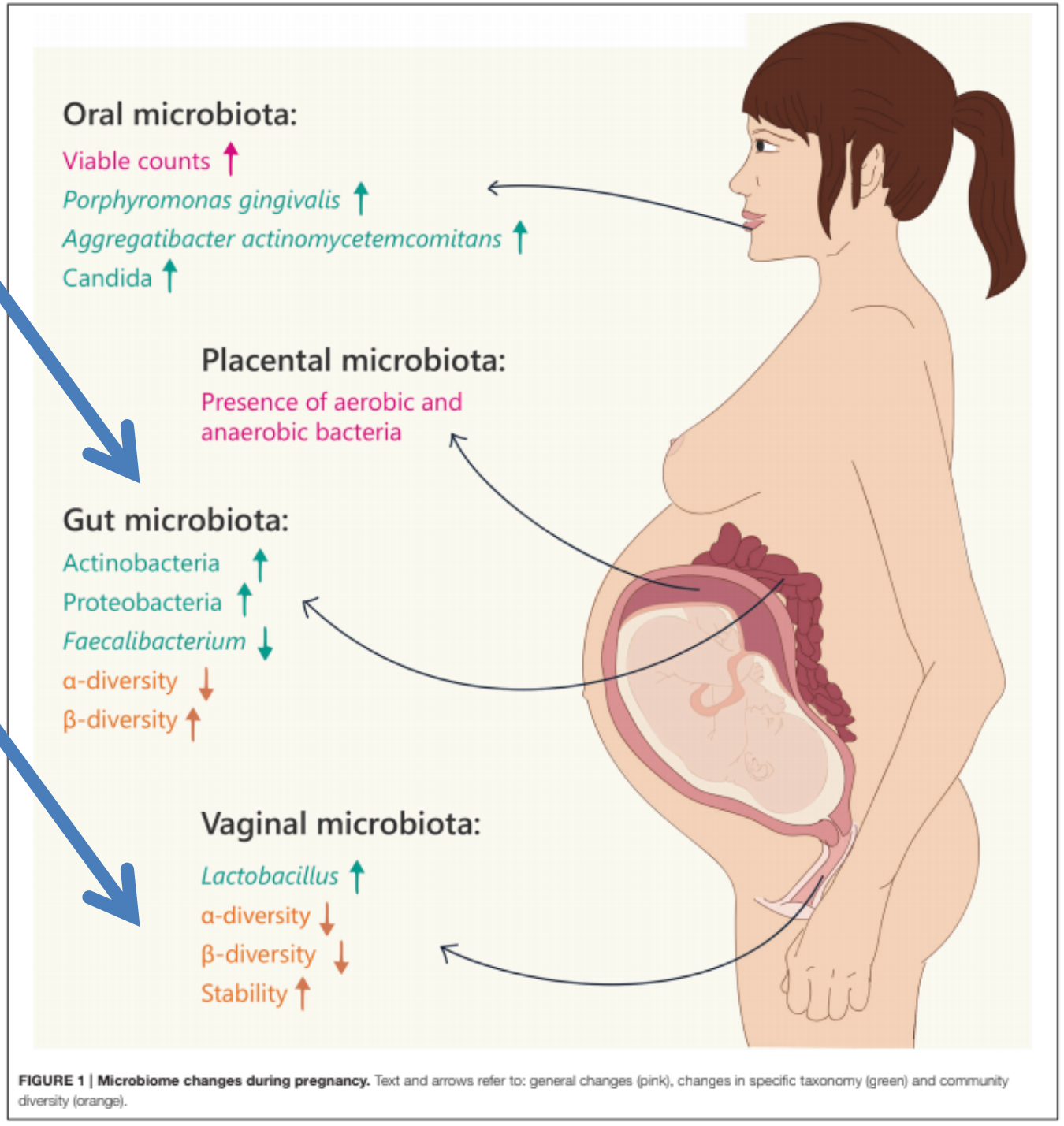
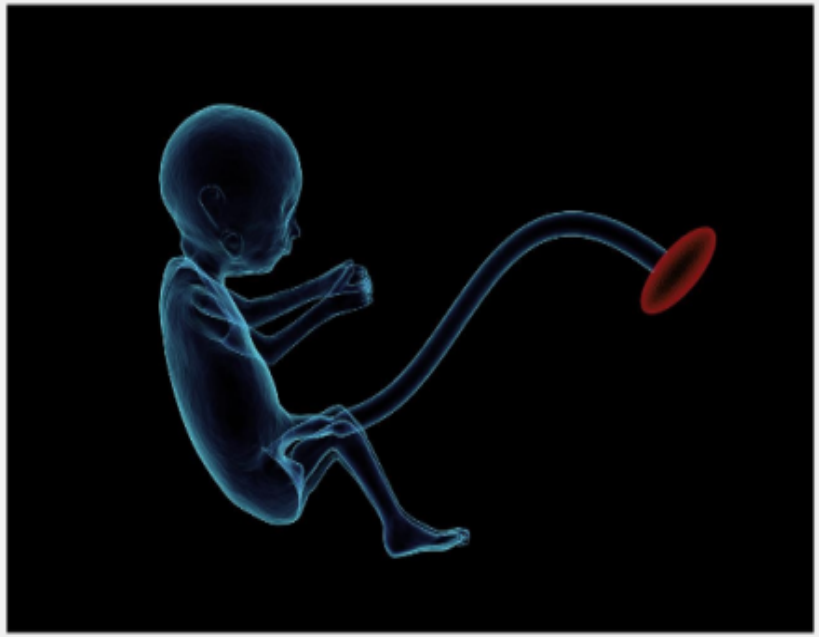


FIGURE 1 | Microbiome changes during pregnancy. Text and arrows refer to: general changes (pink), changes in specific taxonomy (green) and community diversity (orange).

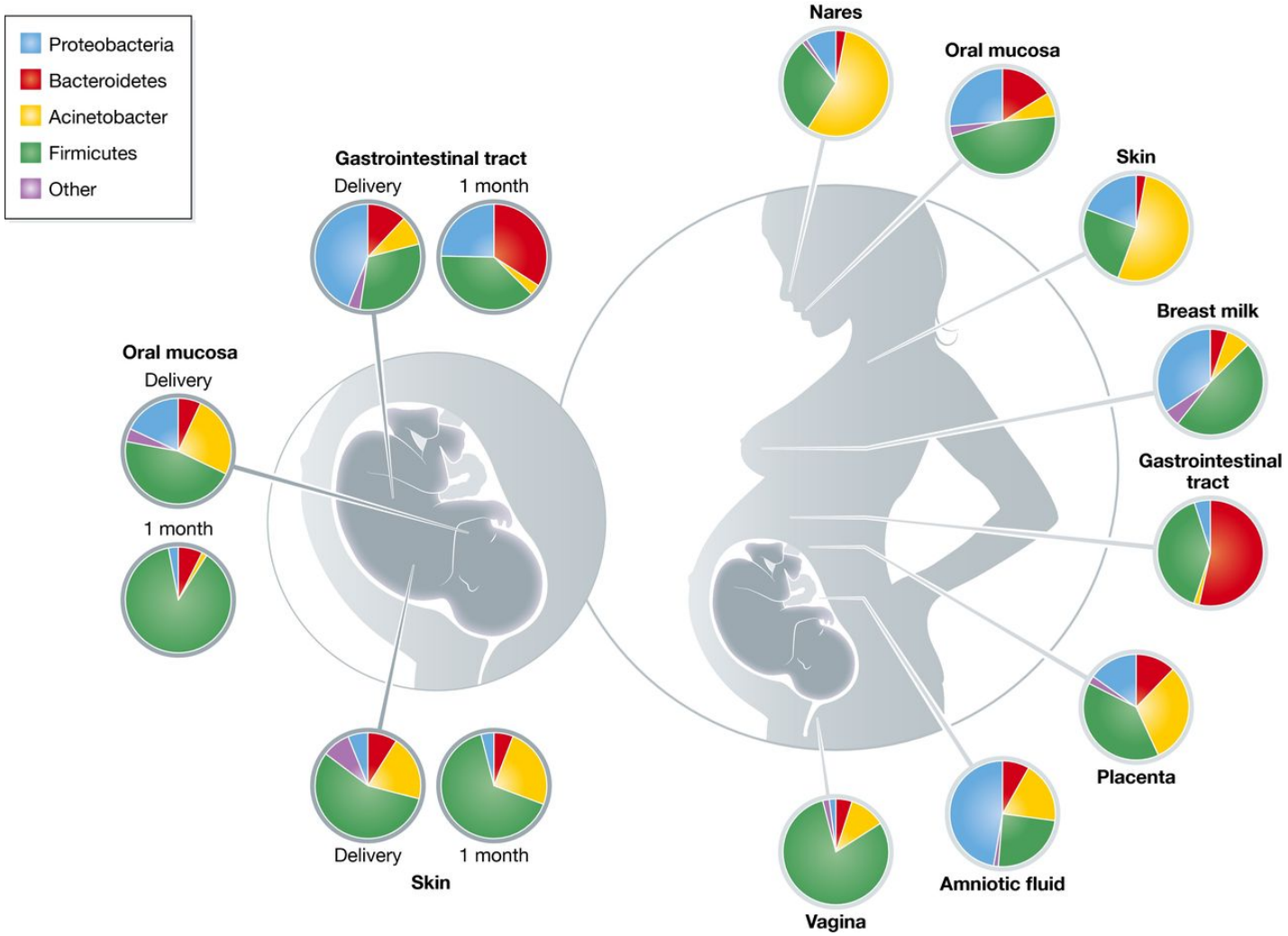
MATERNAL HERITAGE

- *'Gestational changes in the vaginal and intestinal microbiome are of particular relevance because these body sites are responsible for vertical microbial transmission to the newborn'*
  - - Quote from science ref: 1. *Mueller N.T. et al, Trends in Molecular Medicine 2015*



Alta biodiversità del microbiota enterico

Bassa biodiversità del microbiota vaginale



HOW DOES MOTHER'S VAGINAL MICROBIOME CHANGE DURING PREGNANCY?

Decrease in diversity of species of bacteria

HOW DOES MOTHER'S VAGINAL MICROBIOME CHANGE DURING PREGNANCY?

Increase in *Lactobacilli*

(a species of bacteria)

Science ref: 8. Aagaard et al. *PLoS ONE* 2012

- The vagina lmicrobiome undergoes significant changes during pregnancy, including a significant decrease in overalldiversity, increased stability (the community composition changes overtime),and enrichment with *Lactobacillus species (Aagaard et al., 2012).*
- *These correlate with a decrease in the vaginal pH and an increase in vaginal secretions (Prince et al., 2014a).*

- Vaginal microbial compositions were found to differ according to gestational age, while the communities at the later stages of pregnancy resembled those of the non-pregnant state (Aagaard et al., 2012).
- A study characterizing the vaginal microbiota of pregnant and non-pregnant African-Americans reported that during pregnancy, one of the changes is dominance of a single *Lactobacillus species over others(Romero et al., 2014a).*

- Some *Lactobacillus species have bactericidal activities against other species,ensuring their predominance and low variability, which may help in protection against infections during pregnancy(Spurbeck and Arvidson, 2010).*

Gruppo	Dominanza	Frequenza media
CST I	*Lactobacillus crispatus*	25%
CST II	*Lactobacillus gasseri*	5%
CST III	*Lactobacillus iners*	35%
CST IV	Scarsità di lattobacilli	30%
CST V	*Lactabacillus jensenii*	5%

Tabella 1 - CST I-V: tipologia e frequenza in donne nord-americane sane e in età fertile e di etnia mista ma equamente rappresentate (98 caucasiche, 104 di colore, 97 asiatiche, 97 ispaniche).
Da: Ravel J *et al.* Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4680-7 (adattato)

CST	Gravidanza	*Post-partum*
I	60 (43%)	3 (20%)
II	13 (9%)	1 (7%)
III	42 (30%)	1 (7%)
IV	3 (2%)	9 (60%)
V	20 (14%)	1 (7%)

Tabella 3 - Frequenza dei vari CST prima e 6 settimane dopo il parto in donne europea di diversa etnia.
Da: 66) MacIntyre DA *et al.* Sci Rep. 2015; 5:8988

- The postpartum vaginal microbiome was also characterized by gradual depletion of *Lactobacillus species, increased alpha-diversity, and enrichment of bacteria associated with vaginosis, such as Actinobacteria, in 40% of the subjects at 6 weeks after birth (as opposed to only 2% of the subjects during pregnancy; MacIntyre et al., 2015).*

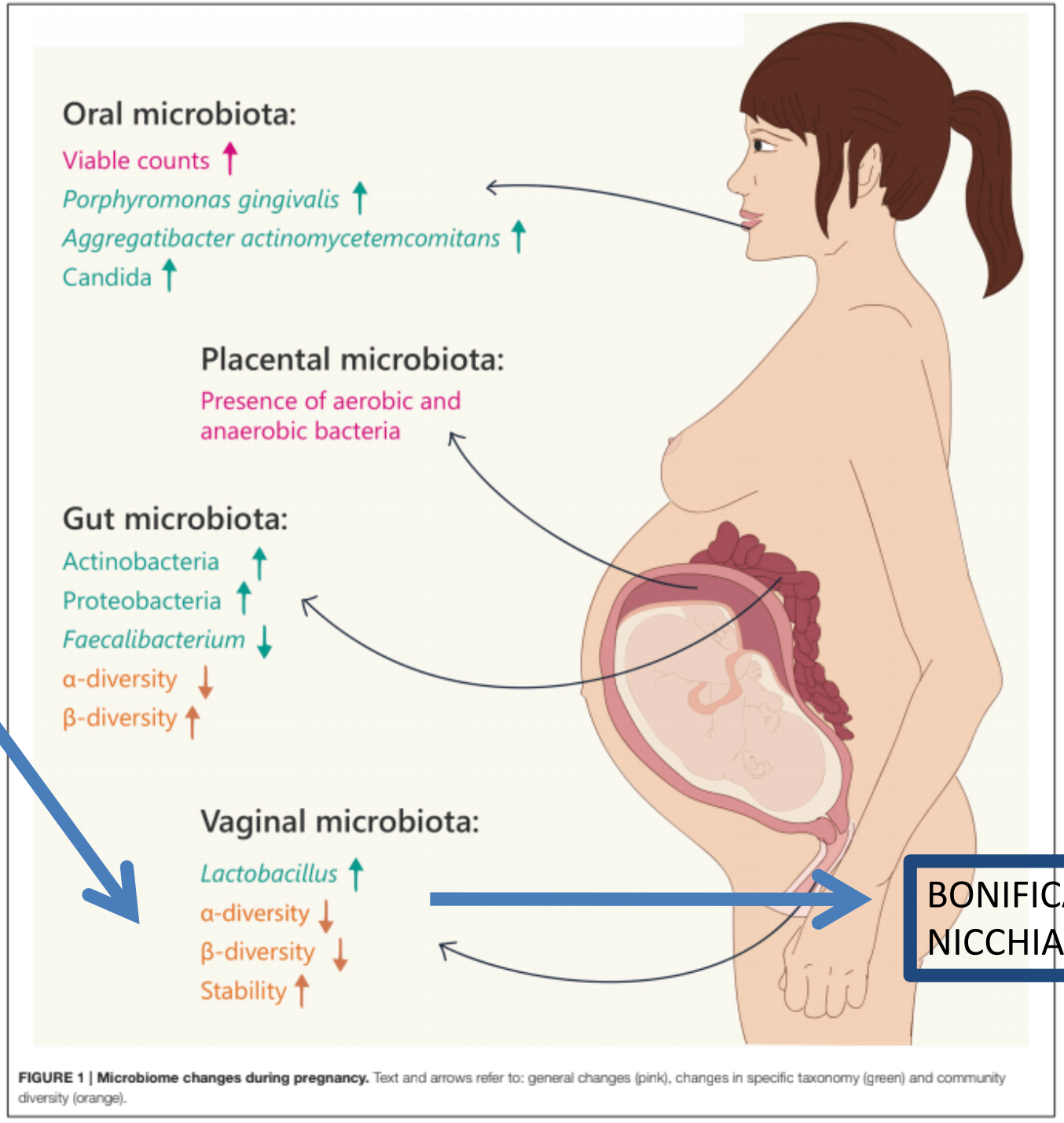


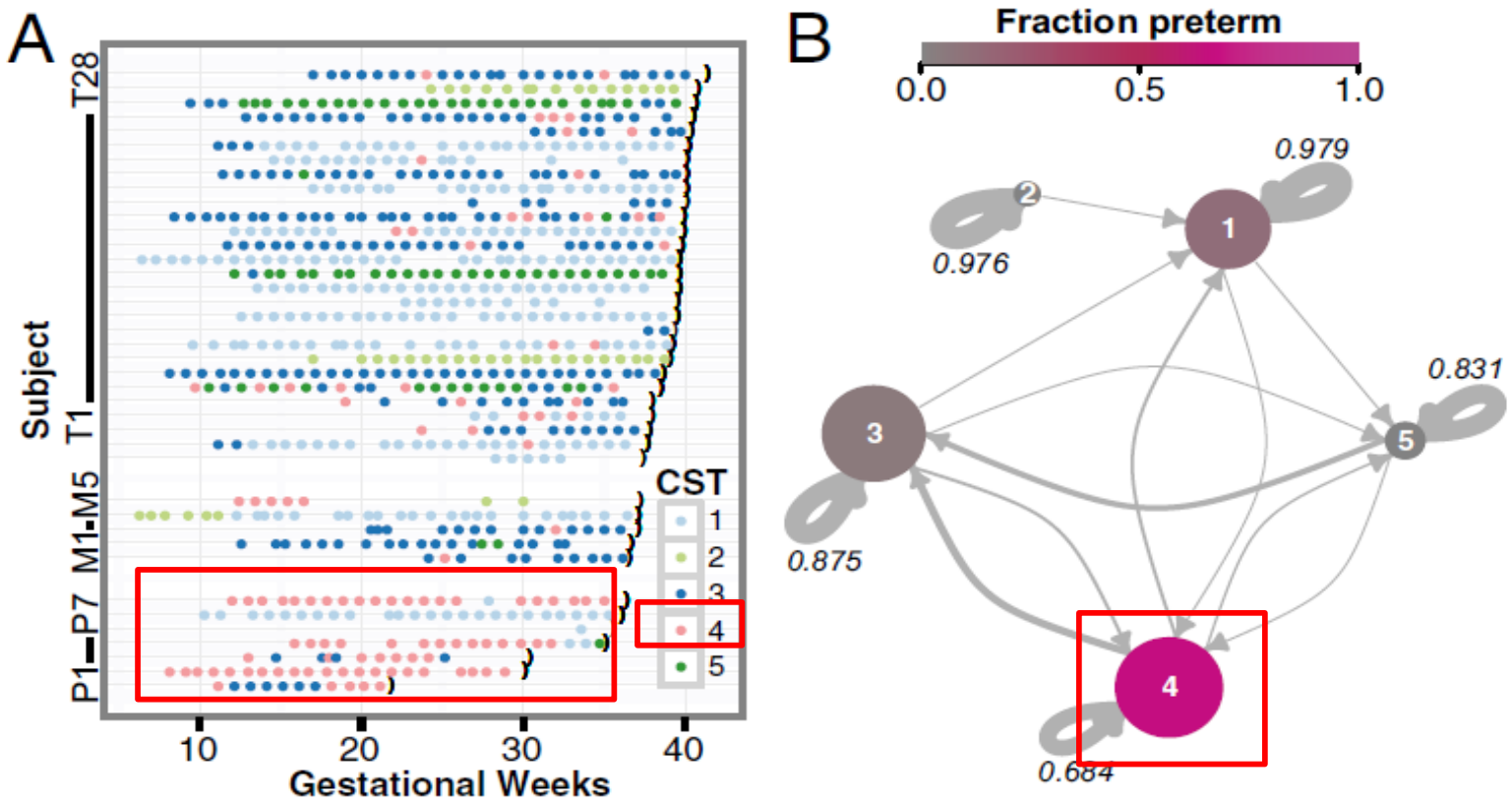
FIGURE 1 | Microbiome changes during pregnancy. Text and arrows refer to: general changes (pink), changes in specific taxonomy (green) and community diversity (orange).

Temporal and spatial variation of the human microbiota during pregnancy

Daniel B. DiGiulio, Benjamin J. Callahan

PNAS | September 1, 2015 | vol. 112 | no. 35

- We conducted a case-control study of 49 pregnant women, 15 of whom delivered preterm. From 40 of these women,we analyzed bacterial taxonomic composition of 3,767 specimens collected prospectively and weekly during gestation and monthly after delivery from the vagina, distal gut, saliva, and tooth/gum.



- Our model indicated that the four Lactobacillus-dominated CSTs (CSTs 1, 2, 3, and 5) were more stable (had higher self-transition probabilities) than the diverse CST (4). This finding is qualitatively similar to the observations of Gajer et al. of CSTs in nonpregnant women (25); however, the Lactobacillus-dominated CSTs were more stable in our cohort

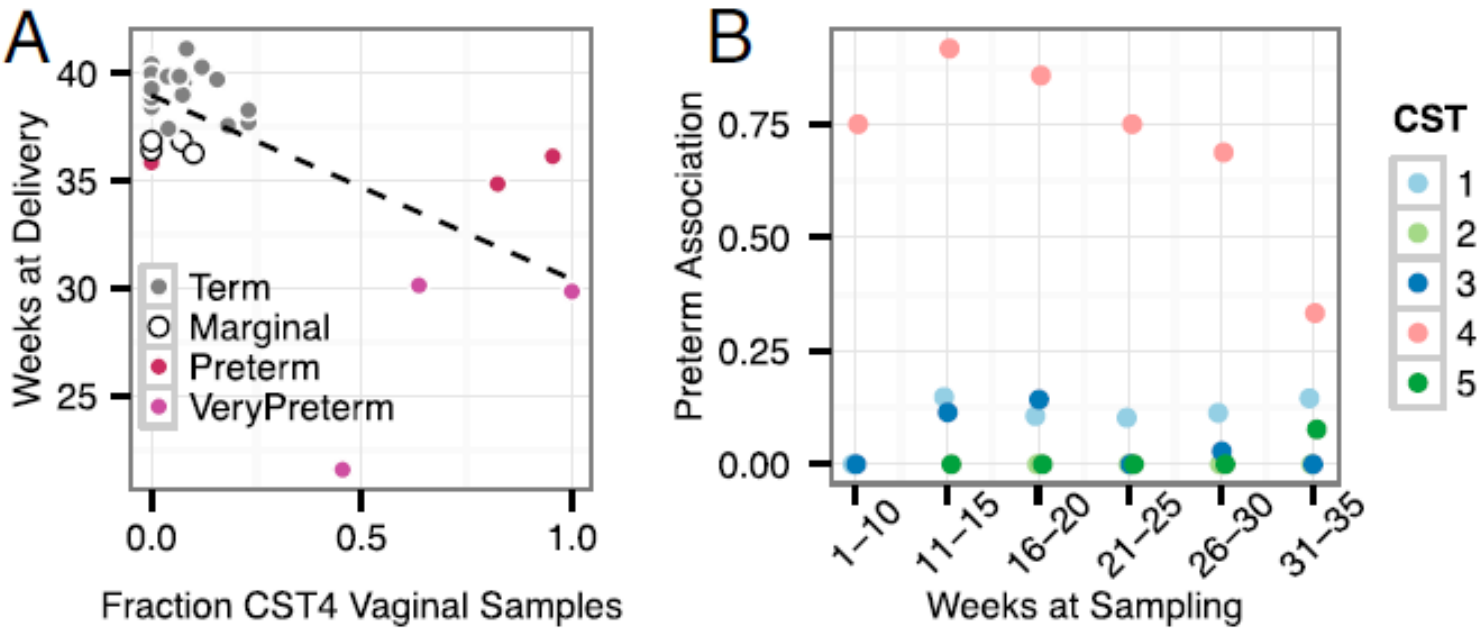


Fig. 4. The high-diversity vaginal CST 4 was associated with earlier deliveries and a higher likelihood of preterm birth in the first group of 40 women. (*A*) Gestational age at delivery is plotted against the fraction of vaginal specimens assigned to the high-diversity CST 4 for the 33 subjects for whom at least 10 vaginal specimens were collected. The dashed line indicates the linear fit. Increased prevalence of the diverse vaginal CST was significantly correlated with earlier delivery ($P = 1.1 \times 10^{-4}$, Pearson; $P = 0.0147$, Spearman). (*B*) The fraction of specimens collected from subjects who delivered preterm is shown by specimen CST and the gestational period during which the specimens were collected. CST 4 specimens collected at any time during pregnancy were associated with a higher proportion of preterm birth.

ARTICLES
https://doi.org/10.1038/s41591-019-0450-2

nature medicine
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The vaginal microbiome and preterm birth

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NATURE MEDICINE | VOL 25 | JUNE 2019 | 1012–1021 | www.nature.com/naturemedicine

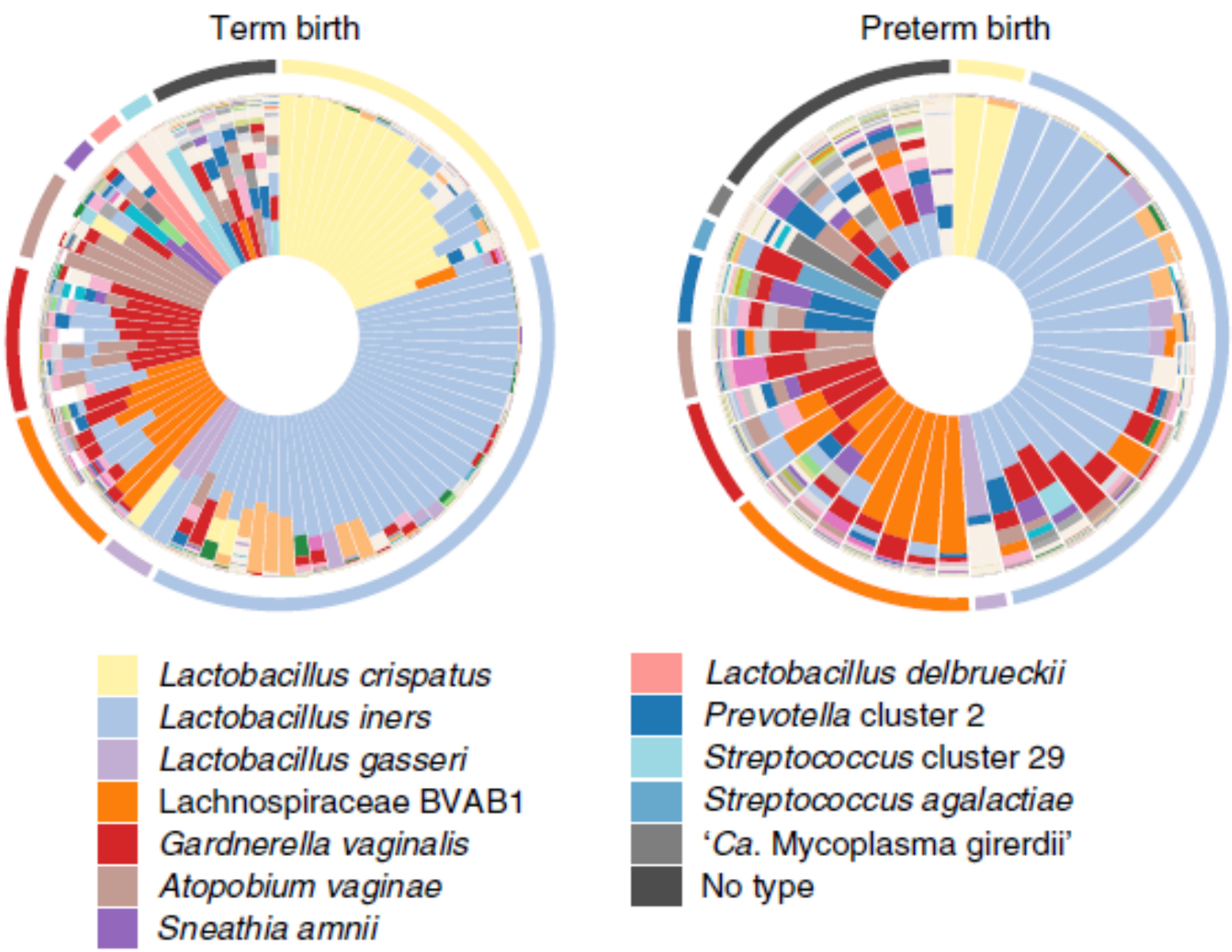
The Multi-Omic Microbiome Study: Pregnancy Initiative (MOMS-PI) includes a total of 1,572 pregnancies, with 992 pregnancies from clinics associated with the Research Alliance for Microbiome Science (RAMS) Registry, based at Virginia Commonwealth University (VCU) in Virginia, and 580 pregnancies from sites associated with the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) in Washington State.

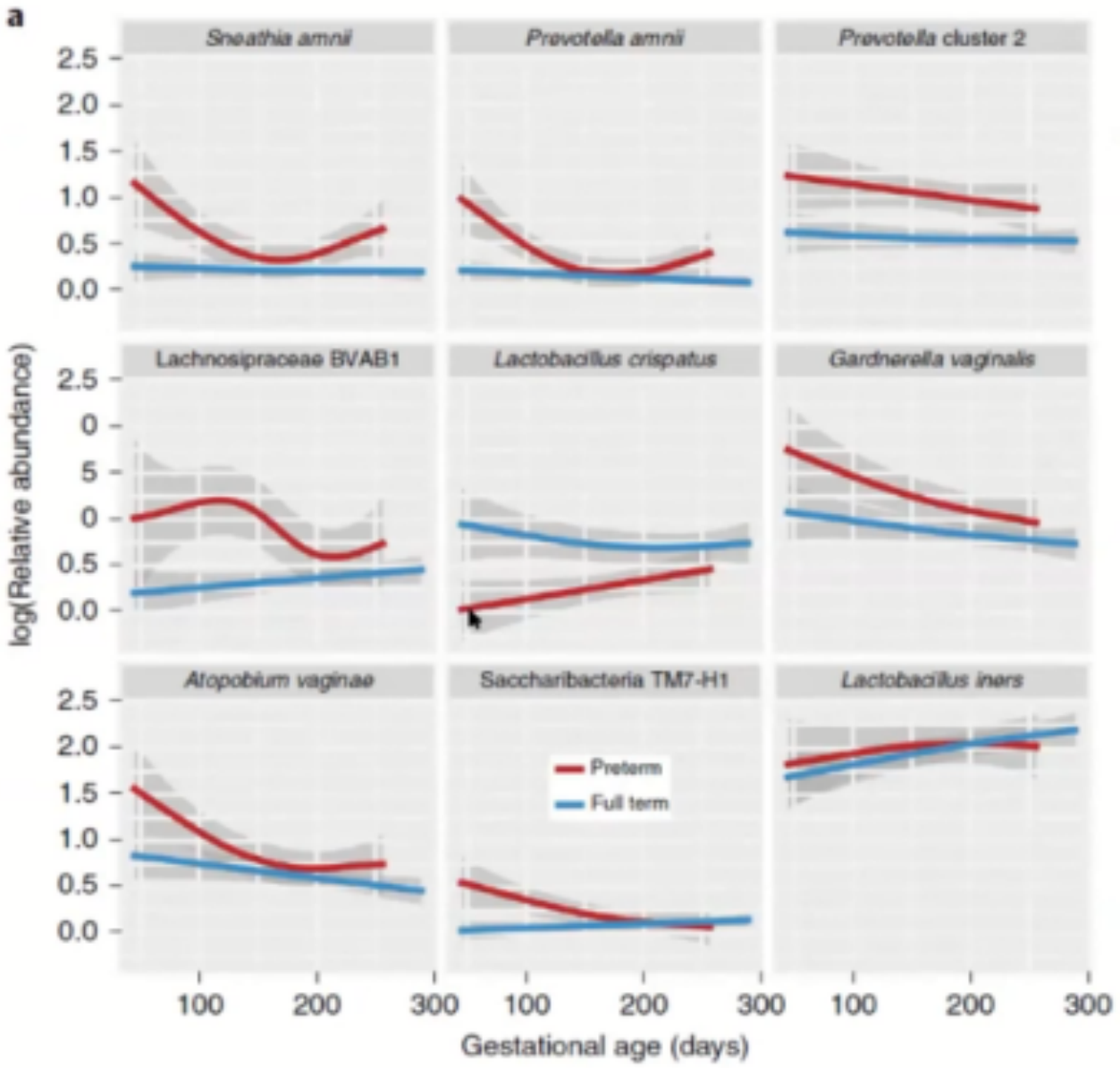
Table 1 | Description of cohort studied in this project

	Preterm delivery <37 weeks ($n=45$)	Term delivery ≥39 weeks ($n=90$)
Mean age (years)[a]	26 (5.68)	25.9 (5.43)
Ancestry/ethnicity (no. (%))		
African	35 (77.8)	71 (78.9)
European	6 (13.3)	13 (14.4)
Hispanic	3 (6.7)	5 (5.6)
Native American	1 (2.2)	1 (1.1)
Household income (no. (%))[b]		
<US$20,000	29 (72.5)	66 (77.7)
US$20,000–59,999	9 (22.5)	15 (17.6)
US$60,000+	2 (5.0)	4 (4.7)
Vaginal delivery (no. (%))	38 (84.4)	74 (82.2)
Previous preterm (no. (%))	14 (31.1)	9 (10.0)
Preterm premature rupture of the membranes (no. (%))	26 (57.8)	0 (0)

[a]Standard deviation listed in parentheses. [b]Missing values $n=5$ (PTB), $n=5$ (TB).

a


Term birth
Preterm birth
Lactobacillus crispatus
Lactobacillus iners
Lactobacillus gasseri
Lachnospiraceae BVAB1
Gardnerella vaginalis
Atopobium vaginae
Sneathia amnii
Lactobacillus delbrueckii
Prevotella cluster 2
Streptococcus cluster 29
Streptococcus agalactiae
'Ca. Mycoplasma girerdii'
No type


a
Sneathia amnii
Prevotella amnii
Prevotella cluster 2
Lachnospiraceae BVAB1
Lactobacillus crispatus
Gardnerella vaginalis
Atopobium vaginae
Saccharibacteria TM7-H1
Lactobacillus iners
log(Relative abundance)
Preterm
Full term
Gestational age (days)

Diversity and composition of vaginal microbiota of pregnant women at risk for transmitting Group B Streptococcus treated with intrapartum penicillin

Luiz Fernando Wurdig, PLoS One. 2017; 12(2)

- Penicillin administration was associated with an altered vaginal microbial community composition characterized by increased microbial diversity.
- Lactobacillus sp. Contributed only 13.1% of the total community in the women that received penicillinc ompared to 88.1% in the controls.
- Streptococcus sp. Were present in higher abundance in GBS positive woman compared to controls, with 60% of the total vaginal microbiota in severe cases identified as Streptococcus sp.

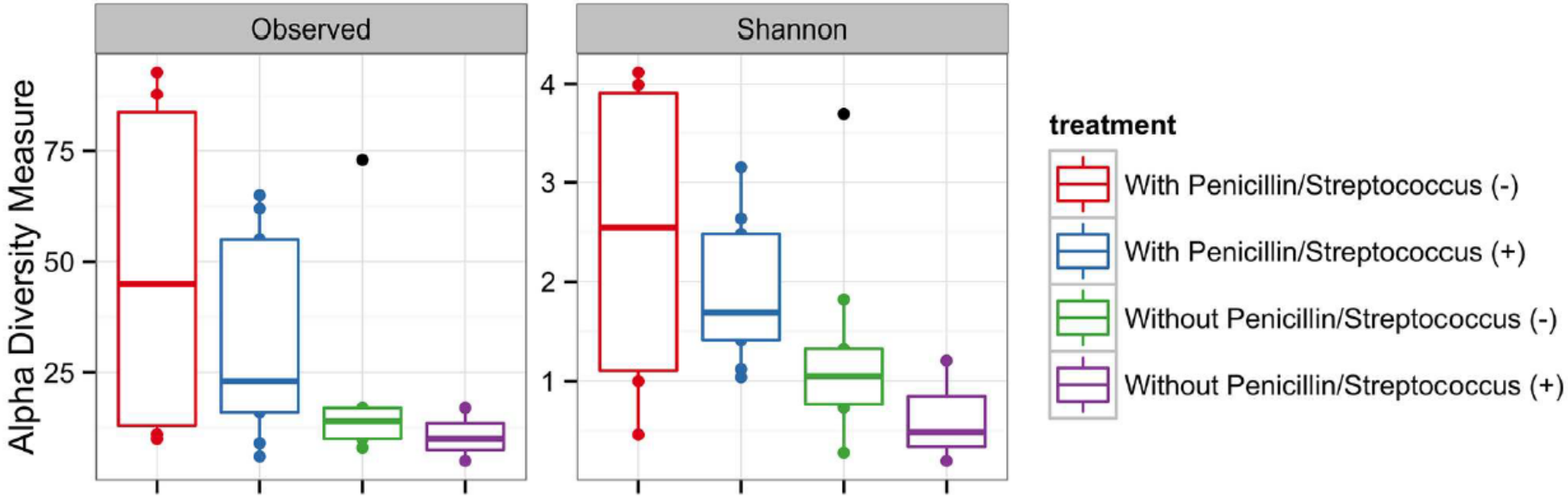


Fig 2. Alpha diversity measurements of vaginal microbial communities from pregnant women under different treatments. The boxes spans the first quartile to the third quartile, the horizontal line inside the boxes represents the median. Lines extending vertically from the boxes indicate variability outside the upper and lower quartiles and the single black circles indicate outliers. After detecting overall differences in beta and alpha diversity among samples, an analysis of the specific taxonomic composition of mothers' vagina was performed in order to identify the microbes responsible for differences among treatments. Significant differences ($p < 0.05$) were observed in the abundance of *Lactobacillus* (Fig 3). *Lactobacillus* spp. was the most abundant genus found in the swab samples from the group of pregnant women without use of penicillin contributing 88.1% and 68.5% with positive and negative *Streptococcus* screening, respectively. In contrast, *Lactobacillus* contributed only 13.1% and 6.0% of the total vaginal microbial community found in the cases with use of penicillin with positive and negative screening for GBS, respectively. *Pseudomonas* was the second most abundant genus among samples contributing up to 6.0% of the total community in those women without use of penicillin. On the other hand, *Pseudomonas* contributed up to 17.5% of the total community in the cases with use of penicillin. Although the average abundance of *Pseudomonas* suggested differences between treatments, such difference was not significant ($p = 0.134$).

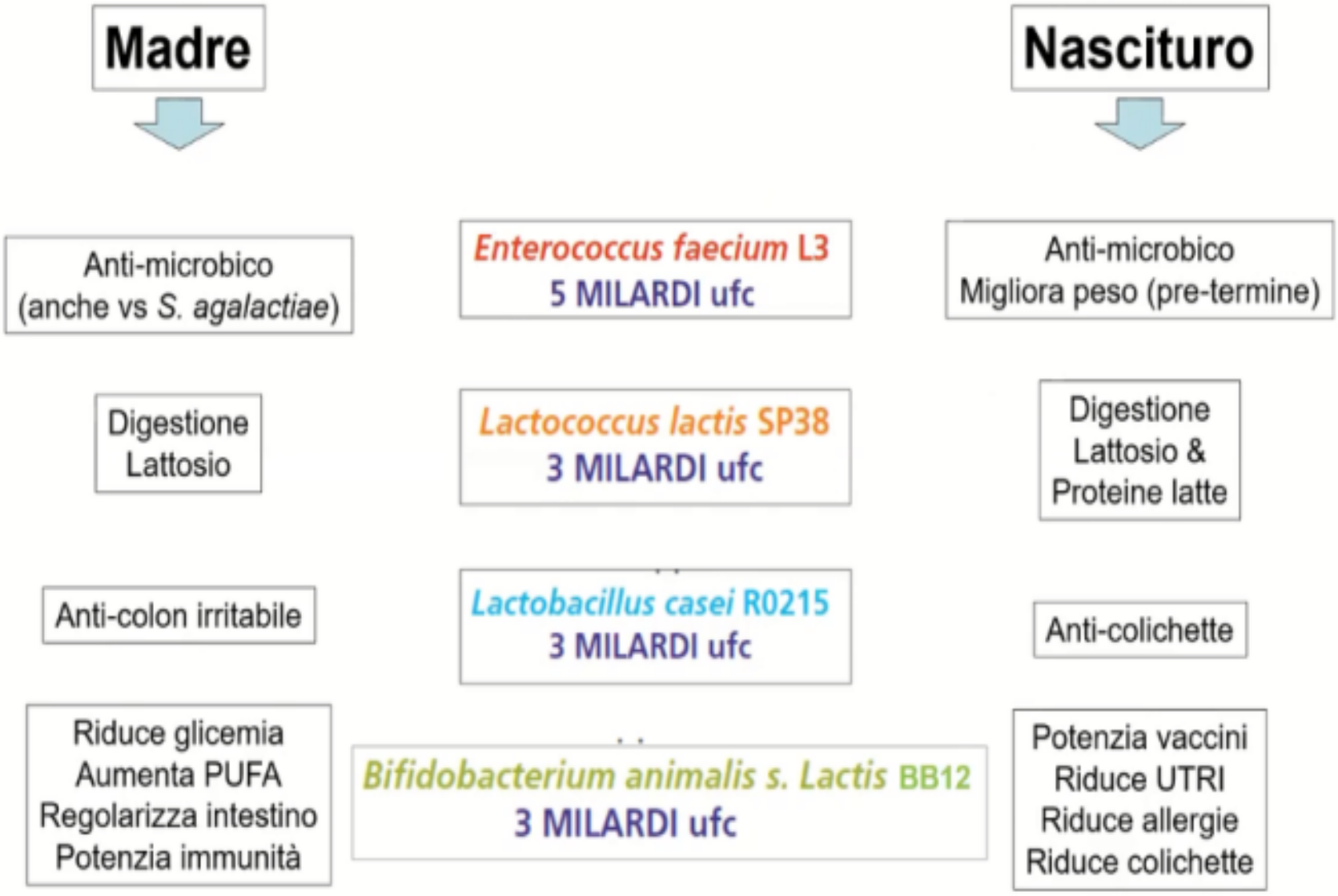
- Vaginal communities of healthy pregnant women were dominated by Lactobacillus sp. and contained low diversity, while GroupBStreptococcus positive women receiving intrapartum antibiotic prophylaxis had a modified vaginal microbiota composition with low abundance of Lactobacillus but highermicrobial diversity

Positive clinical outcomes derived from using a proprietary mixture of selected strains during pregnancy

F. Di Pierro; A.M. Parolari; B. Brundu; R. Nigro

Acta Biomed 2016; vol 87, n°3: 1-7

- ***Enterococcus faecium*** *L3 : LMG P-27496 / 5mld/UFC*
- ***Bifidobacterium animalis subsp.*** *Lactis (BB-12*) : DSM 15954 / 3mld/UFC*
- ***Lactobacillus casei*** *R0215: CNCM I-3429 / 3mld/UFC*
- ***Lactobacillus lactis*** *SP38: DSM 26868 / 3mld/UFC*


Madre
Nascituro
Anti-microbico
(anche vs S. agalactiae)
Enterococcus faecium L3
5 MILARDI ufc
Anti-microbico
Migliora peso (pre-termine)
Digestione
Lattosio
Lactococcus lactis SP38
3 MILARDI ufc
Digestione
Lattosio &
Proteine latte
Anti-colon irritabile
Lactobacillus casei R0215
3 MILARDI ufc
Anti-colichette
Riduce glicemia
Aumenta PUFA
Regolarizza intestino
Potenzia immunità
Bifidobacterium animalis s. Lactis BB12
3 MILARDI ufc
Potenzia vaccini
Riduce UTRI
Riduce allergie
Riduce colichette

- We tested an L3-based probiotic formula (iNatal®) on 127 pregnant women attending our gynaecological unit in 2015. We compared the study subjects with 279 pregnant women enrolled in the same year and with 892 other pregnant women who attended our gynaecological unit in 2013 and 2014.

Table 2. Adverse events (number of global episodes) during 10-week treatment with the proprietary mixture of selected strains and in the control group

	Gastric[1]	Gut[2]	Skin[3]	Headache	Insomnia
Treated (127)	60	11*	1	15	68
Control (279)	136	75	3	39	152

[1]Pain, reflux, nausea, vomiting, spasm; [2]pain, constipation, colitis, meteorism, flatulence, diarrhoea; [3]rash, erythema, dermatitis, acne-like reaction.
*$p<0.01$ versus control.

Table 3. Number of women attending our gynaecological department in 2013-2015 with rectal-vaginal swabs positive for *Streptococcus agalactiae*

	Treated 2015	Untreated 2015	All 2014	All 2013
Positive/total	27/127*	76/279	125/390	138/502
%	21.3	27.3	32.05	27.5

*p<0.05 versus Untreated 2015, All 2014 and All 2013.

Table 4. Episodes of PROM° in all women attending our gynaecological department, 2013-2015

	Treated 2015	Untreated 2015	All 2014	All 2013
PROM/total	0/127*	87/279	156/390	180/502
%	0.0	31.2	40	35.9

*p<0.001 versus Untreated 2015, All 2014 and All 2013.
°PROM: premature rupture of membranes.

- La supplementazione probiotica materna può rappresentare una efficace prevenzione primaria di numerose patologie ostetriche e neonatali ……

GRAZIE DELLA ATTENZIONE !!!

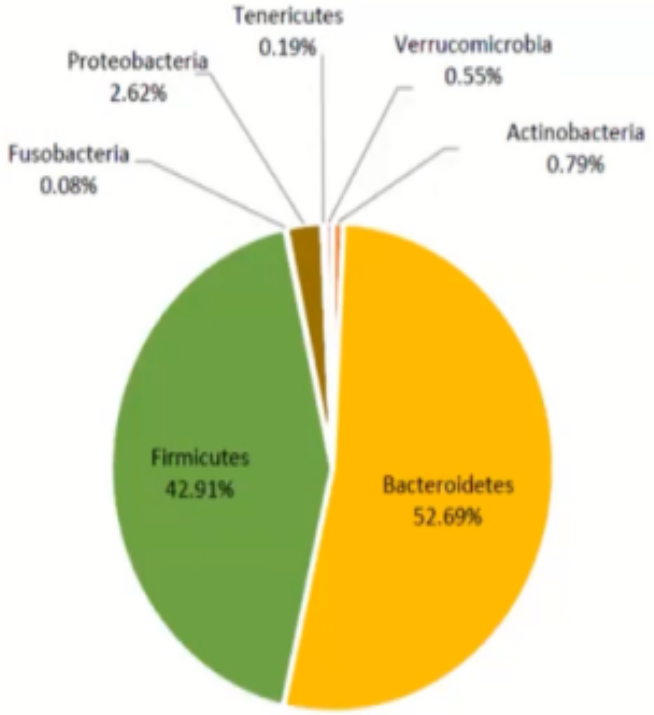
IL MICROBIOMA NELLA DONNA GRAVIDA E IL TRAPIANTO MATERNO-FETALE

Dott. Francesco Bernasconi
U.O.C. di Ostetricia e Ginecologia
Ospedale Sacra Famiglia di Erba

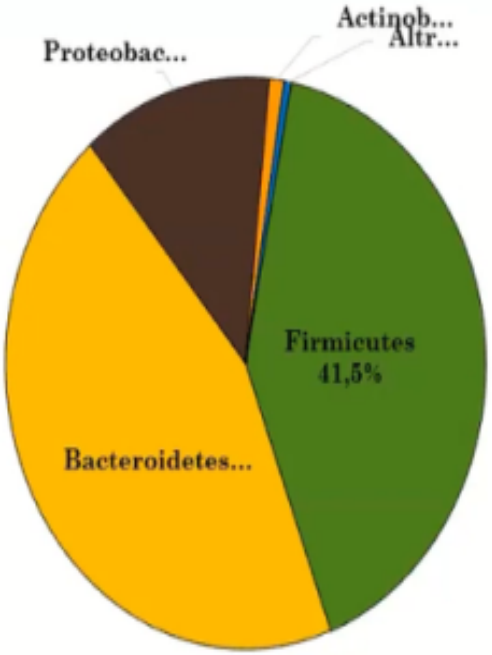
- Pregnancy is a remarkable biological process involving simultaneous changes in many physiological systems to support the development of healthy progeny.
- These changes include hormonal changes, weight gain, immune system modulation, and others, which must all be synchronized to preserve the health of both the mother and the offspring (Dunlop et al., 2015).

- While some of the pregnancy- associated hormonal and metabolic changes have been known for decades (Kumar and Magon, 2012)
- The dramatic changes in maternal microbiome composition that take place during gestation have only recently been appreciated.

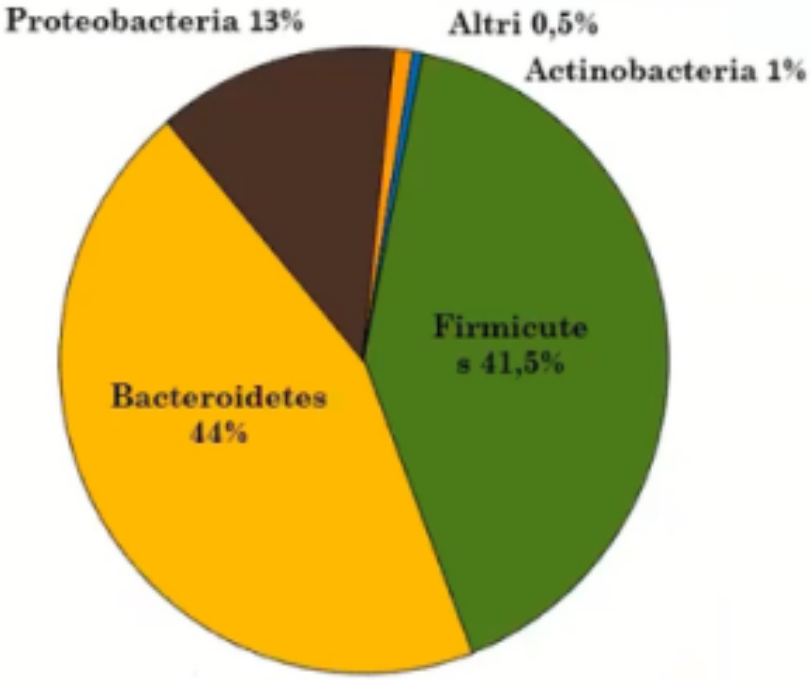
Adulto



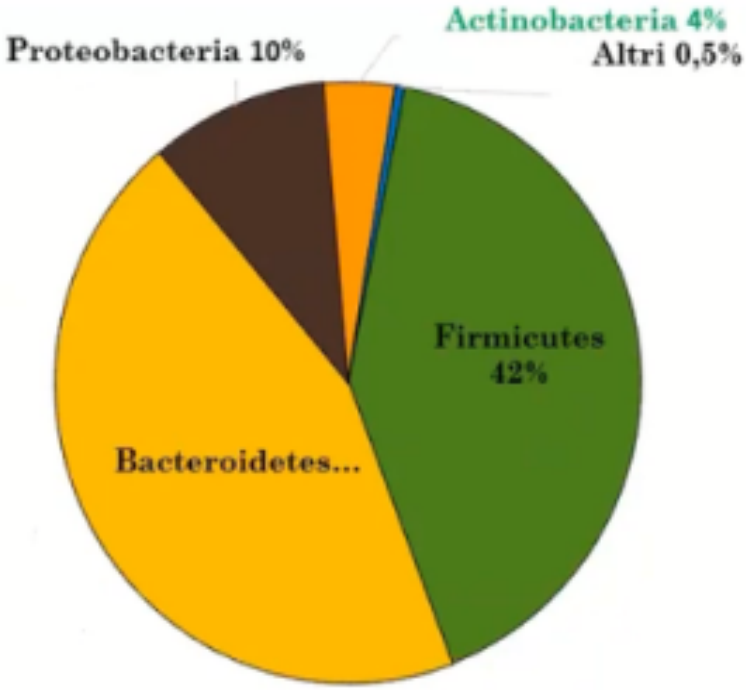
Donna in gravidanza 32 w



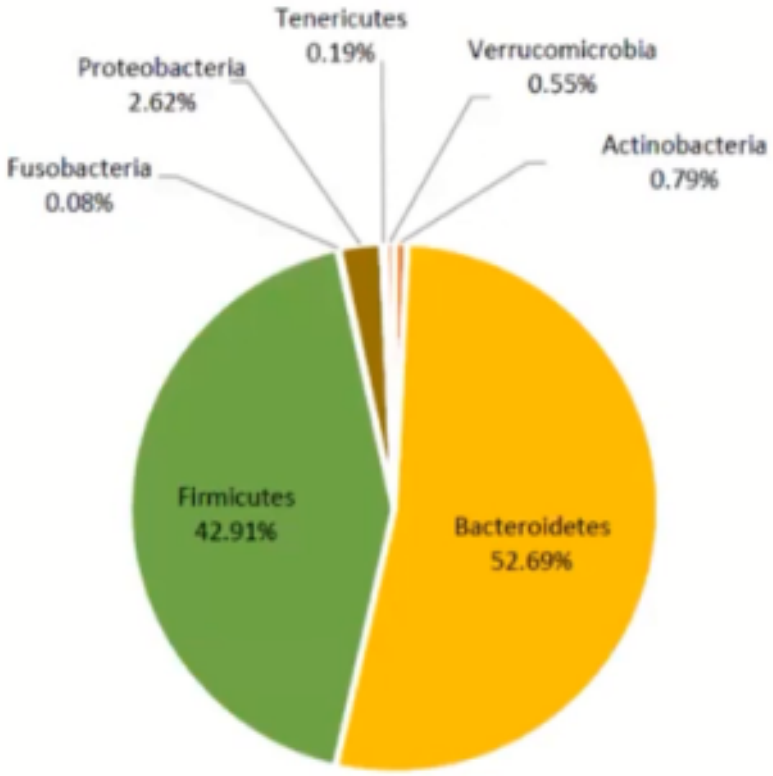
Gravidanza 32 w



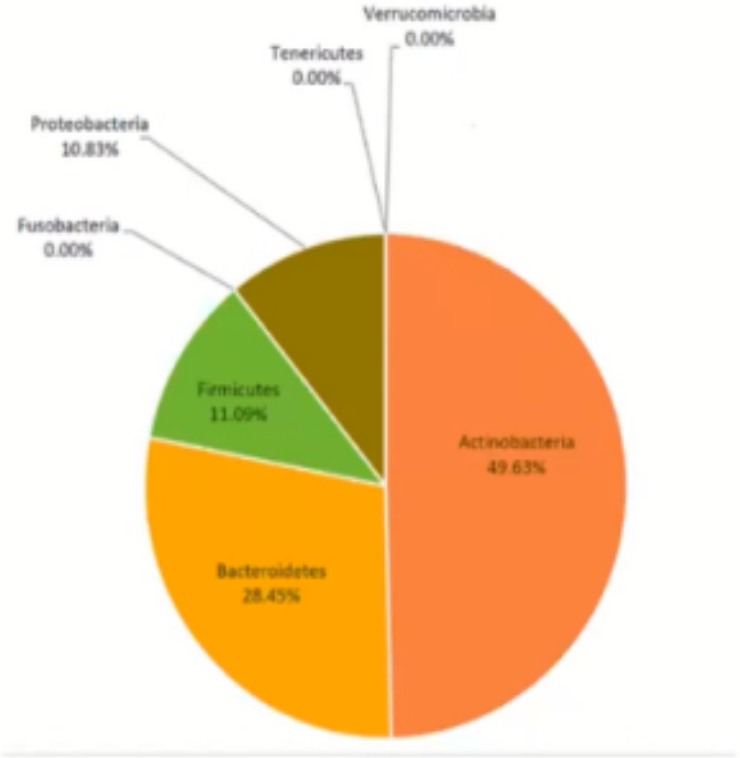
Gravidanza a termine



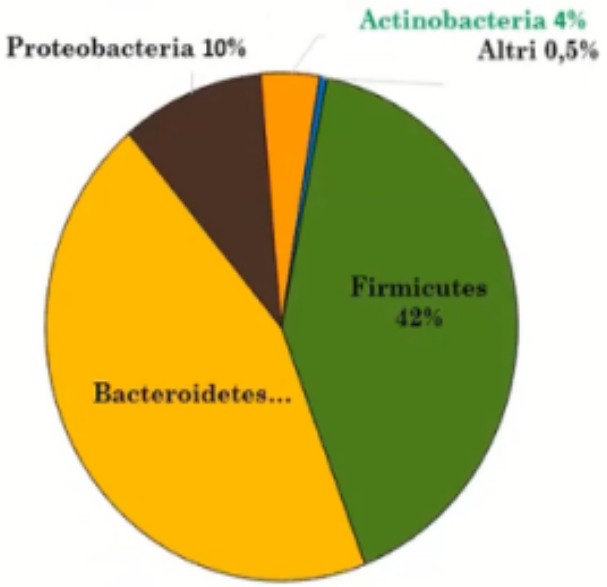
Adulto



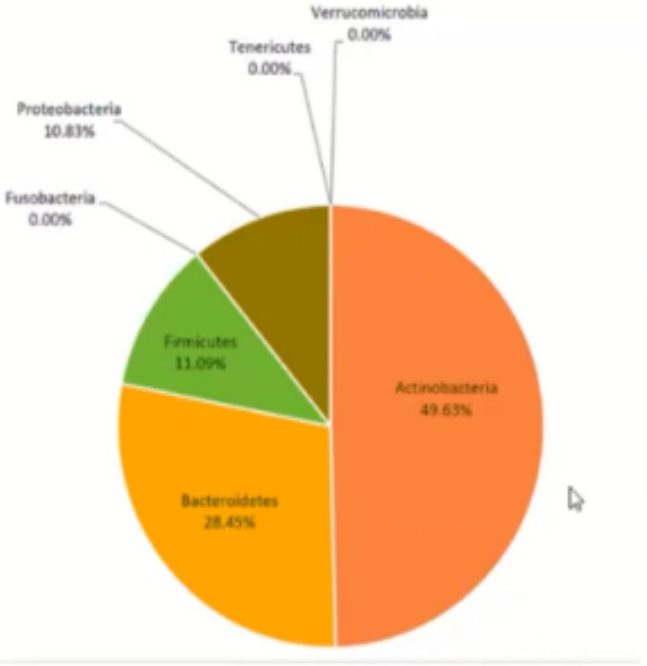
Lattante nato da PS allattato al seno

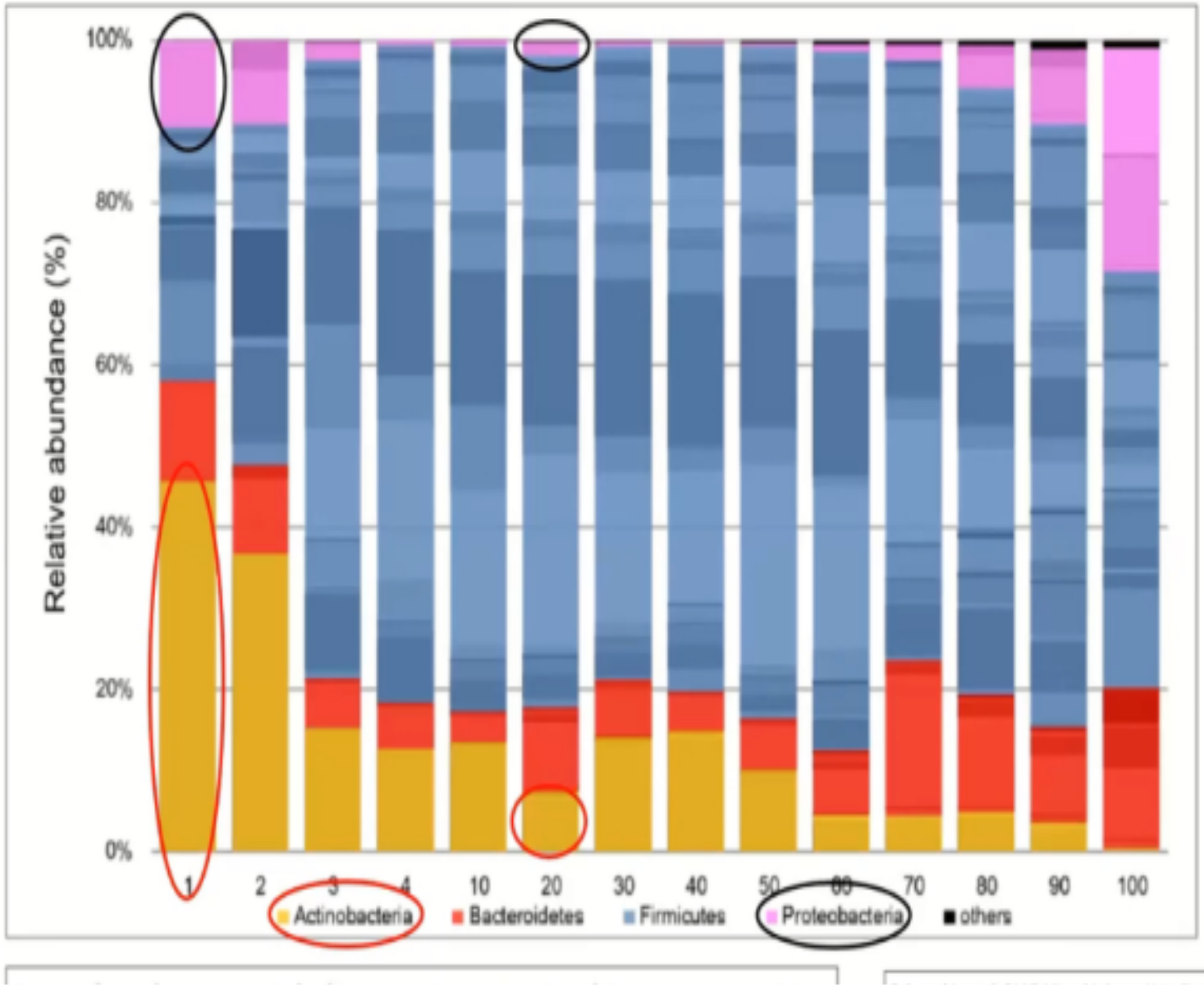


Gravidanza a termine



Lattante nato da PS allattato al seno



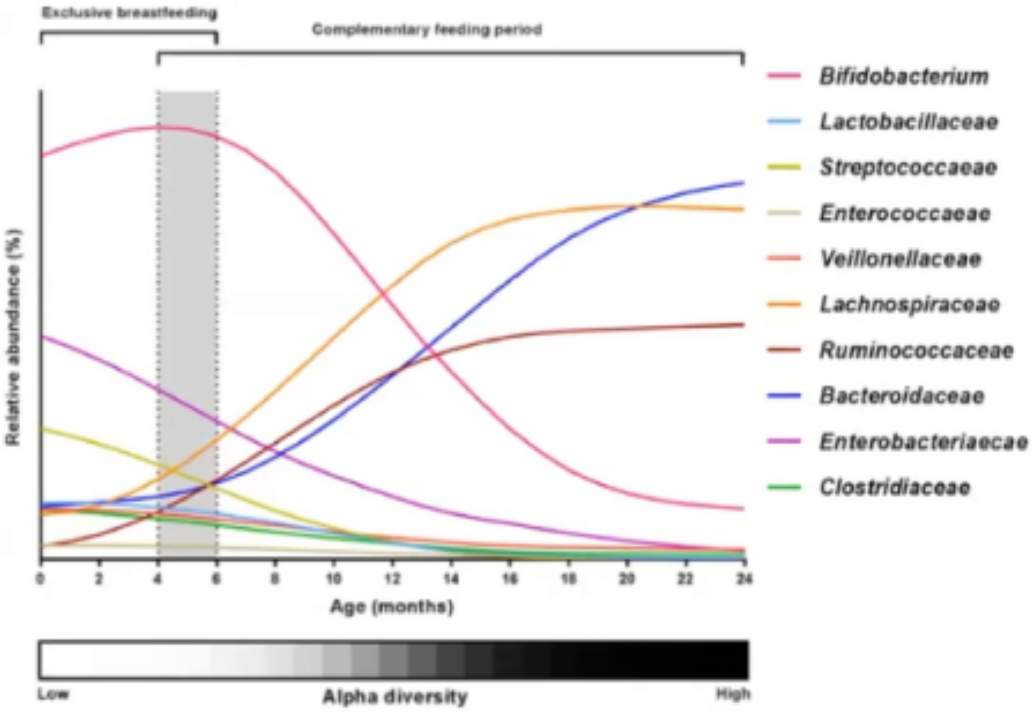

100%
80%
60%
40%
20%
0%
Relative abundance (%)
1
2
3
4
10
20
30
40
50
60
70
80
90
100
Actinobacteria
Bacteroidetes
Firmicutes
Proteobacteria
others

First Foods and Gut Microbes

frontiers in Microbiology

*Martin F. Laursen[1], Martin I. Bahl[1], Kim F. Michaelsen[2] and Tine R. Licht[1]**

[1] National Food Institute, Technical University of Denmark, Søborg, Denmark, [2] Department of Nutrition, Exercise and Sports, University of Copenhagen, Frederiksberg, Denmark



Variazioni del microbiota intestinale
nei **primi mesi di vita**

Enterobacteriaceae (proteobatteri) nella fase iniziale (conseguenza dell'acquisizione da parte della madre)

Obiettivo: creare nell'intestino neonatale l'ambiente microaerofilo necessario per lo sviluppo di specie anaerobie, tra cui i bifidobatteri

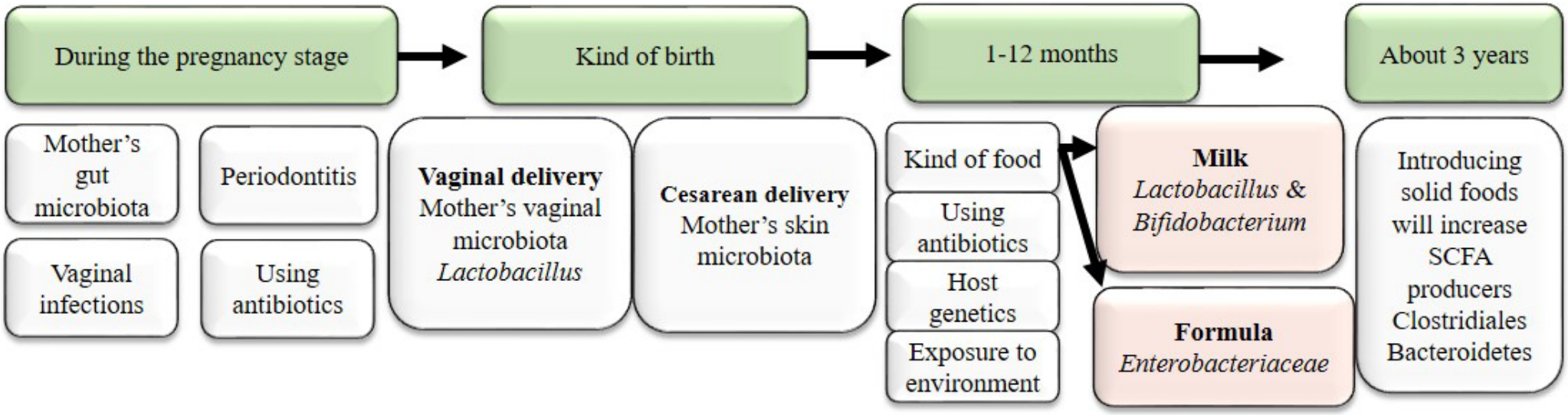
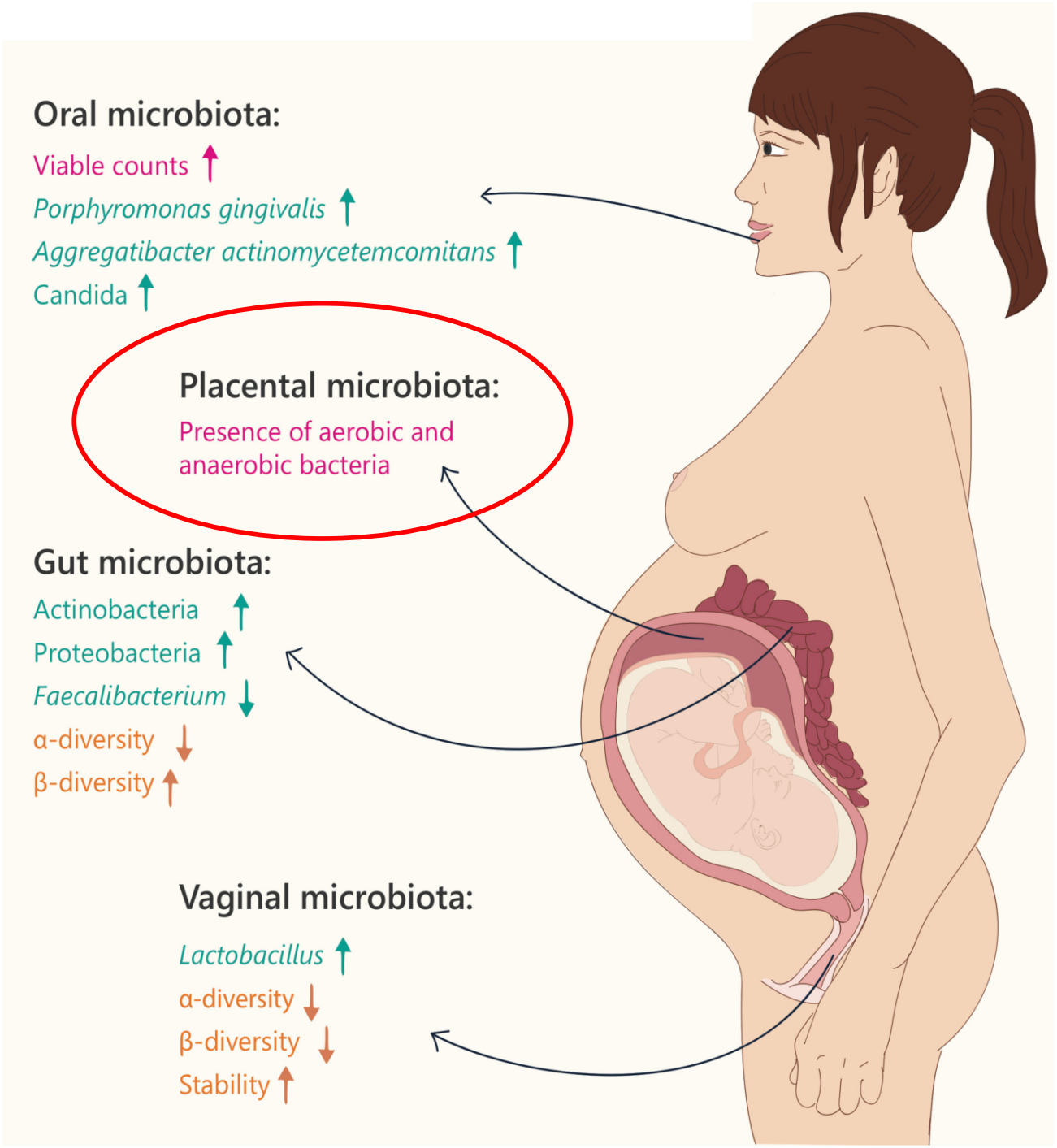


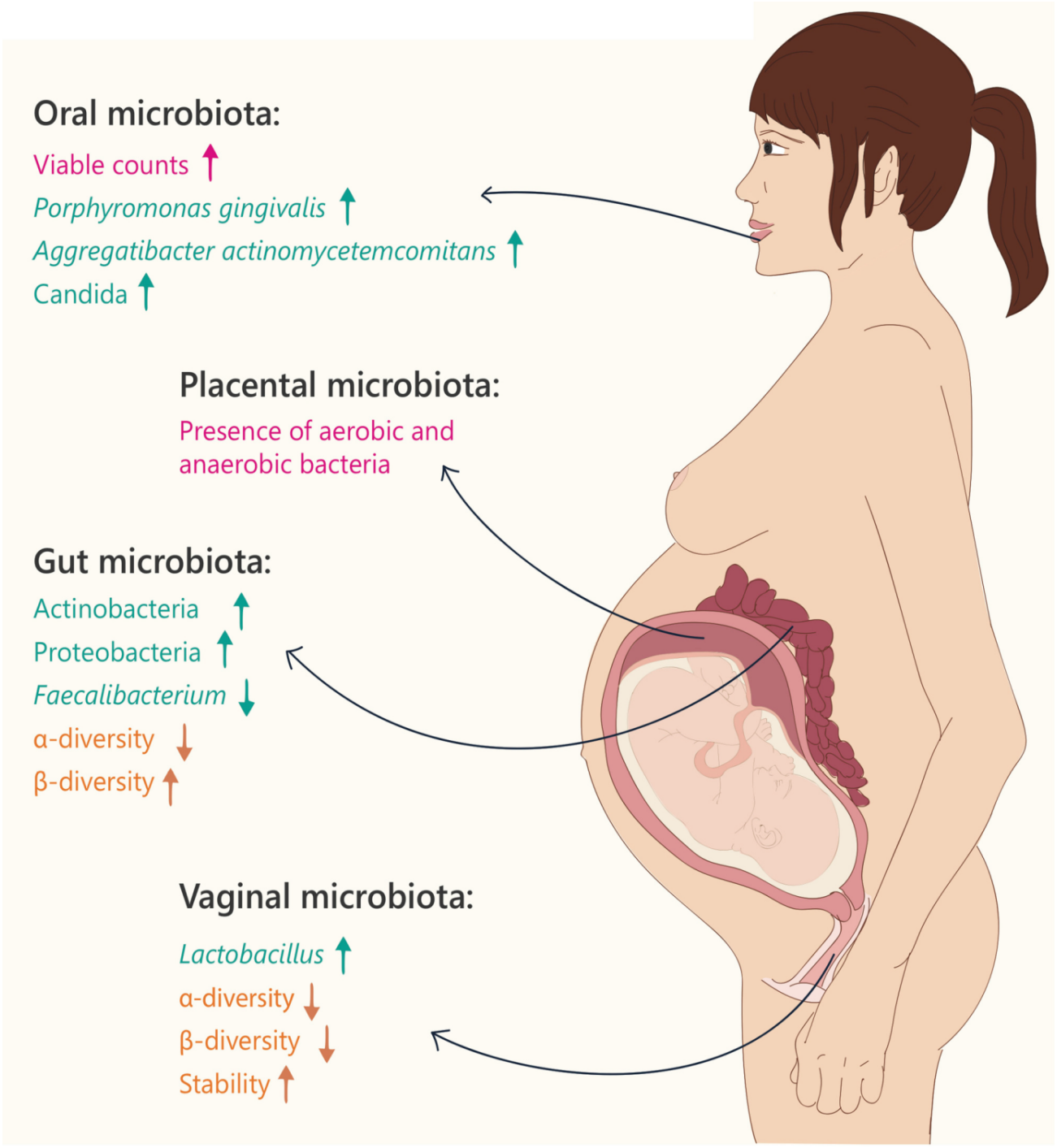
Fig. 1. Factors influencing the pediatric microbiota up to 3 years of age.

Bioscience of Microbiota, Food and Health Vol. 39 (2), 23–32, 2020


Oral microbiota:
Viable counts ↑
Porphyromonas gingivalis ↑
Aggregatibacter actinomycetemcomitans ↑
Candida ↑
Placental microbiota:
Presence of aerobic and anaerobic bacteria
Gut microbiota:
Actinobacteria ↑
Proteobacteria ↑
Faecalibacterium ↓
α-diversity ↓
β-diversity ↑
Vaginal microbiota:
Lactobacillus ↑
α-diversity ↓
β-diversity ↓
Stability ↑

- Several additional reports described bacteria in the placenta (Goldenberg et al., 2000; Dominguez-Bello et al., 2010; Hyman et al., 2014; Romero et al., 2014a; MacIntyre et al., 2015)

- Using whole genome shotgun sequencing (WGS) of samples from 320 subjects, Aagaard et al. reported that the placenta contains a unique microbiome (Aagaard et al., 2014)
- The major phylum was Proteobacteria, and compared to all other organs,the composition was most similar to the oral microbiota,including species such as *Prevotella tannerae and Neisseria (Aagaard et al., 2014).*


Oral microbiota:
Viable counts ↑
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Placental microbiota:
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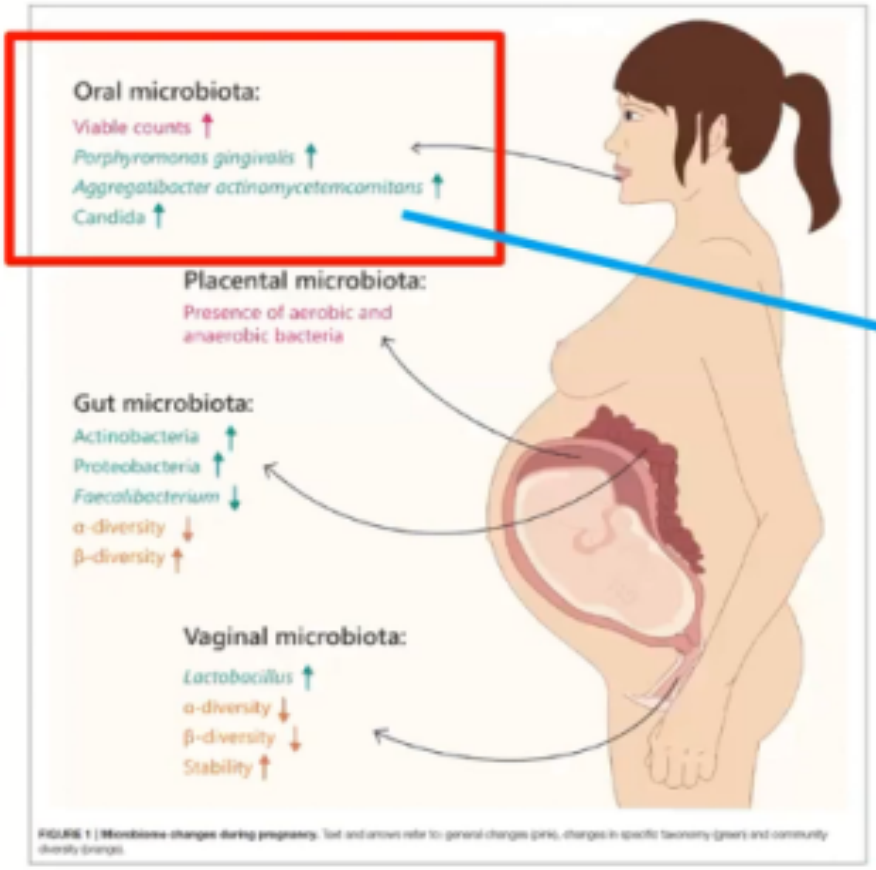
Gut microbiota:
Actinobacteria ↑
Proteobacteria ↑
Faecalibacterium ↓
α-diversity ↓
β-diversity ↑
Vaginal microbiota:
Lactobacillus ↑
α-diversity ↓
β-diversity ↓
Stability ↑

- The oral microbiome includes up to 600 diverse species including *Streptococci, Lactobacilli, Staphylococci,Corynebacteria, etc., residing in different microenvironments within the oral cavity (teeth, tongue, palates, etc., Dewhirst et al., 2010).*

- The total viable microbial counts in all stages of pregnancy were higher than those of the non-pregnant women, especially in early pregnancy (Fujiwara et al., 2015), and levels of the pathogenic bacteria *Porphyromonas gingivalis and Aggregatibacteractinomycetemcomitans in the subgingival plaque, were significantly higher during the early and middle stages of pregnancy, compared to the non-pregnant group(Fujiwara et al., 2015).*

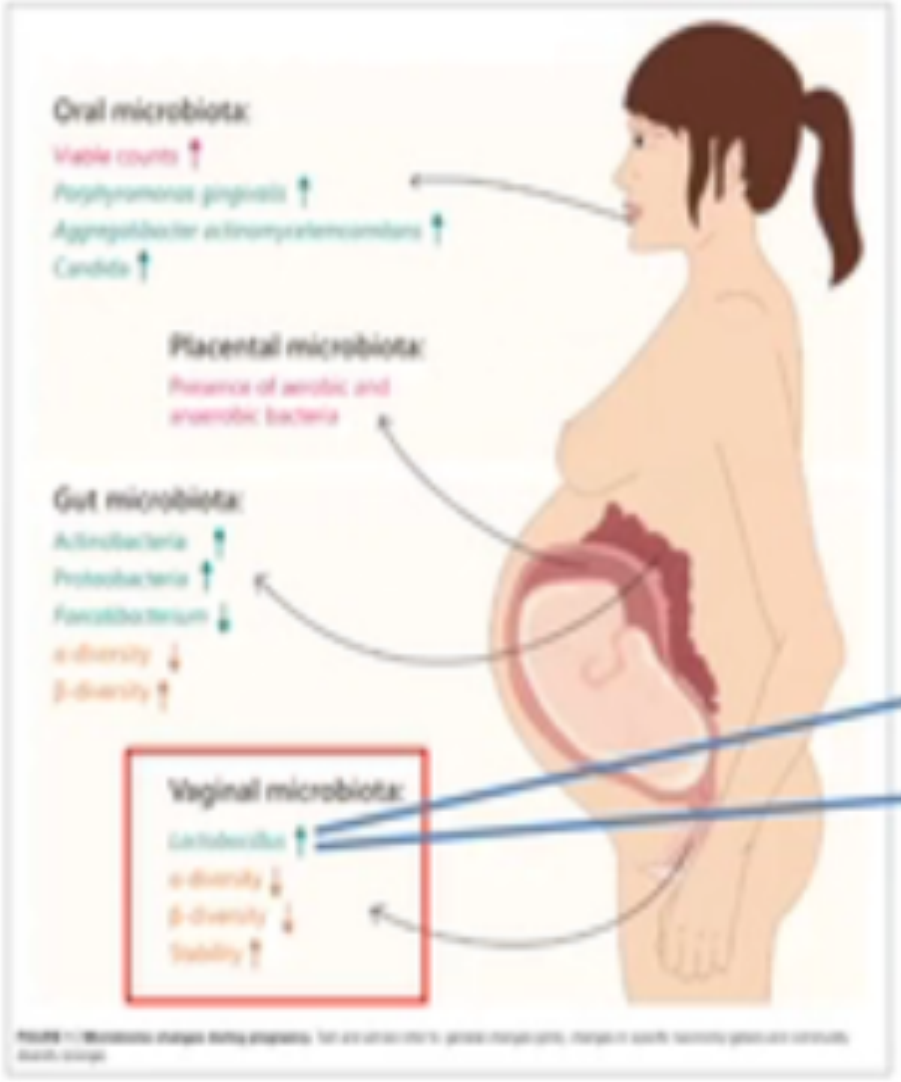
Microbiota changes in pregnancy



Parodontopatie
PROM e PPROM

Frontiers in Microbiology, 2016;7:1031

Microbiota changes in pregnancy



Bonifica della nicchia vaginale

«fornitura» di beta-galattosidasi

Frontiers in Microbiology, 2016;7:1031

- The human vaginal microbiota is a key component in the defense system against microbial and viral infections, conferring protection against disease (Turovskiy et al., 2011).
- The vaginal microbiome is dominated by many species including *Lactobacillus and members of the Clostridiales, Bacteriodales, and Actinomycetales(Aagaard et al., 2012).*

Vagino-tipi (CST)

Community state types (CST) in the vaginal microbiota.[a] Vaccine 32 (2014) 1543–1552

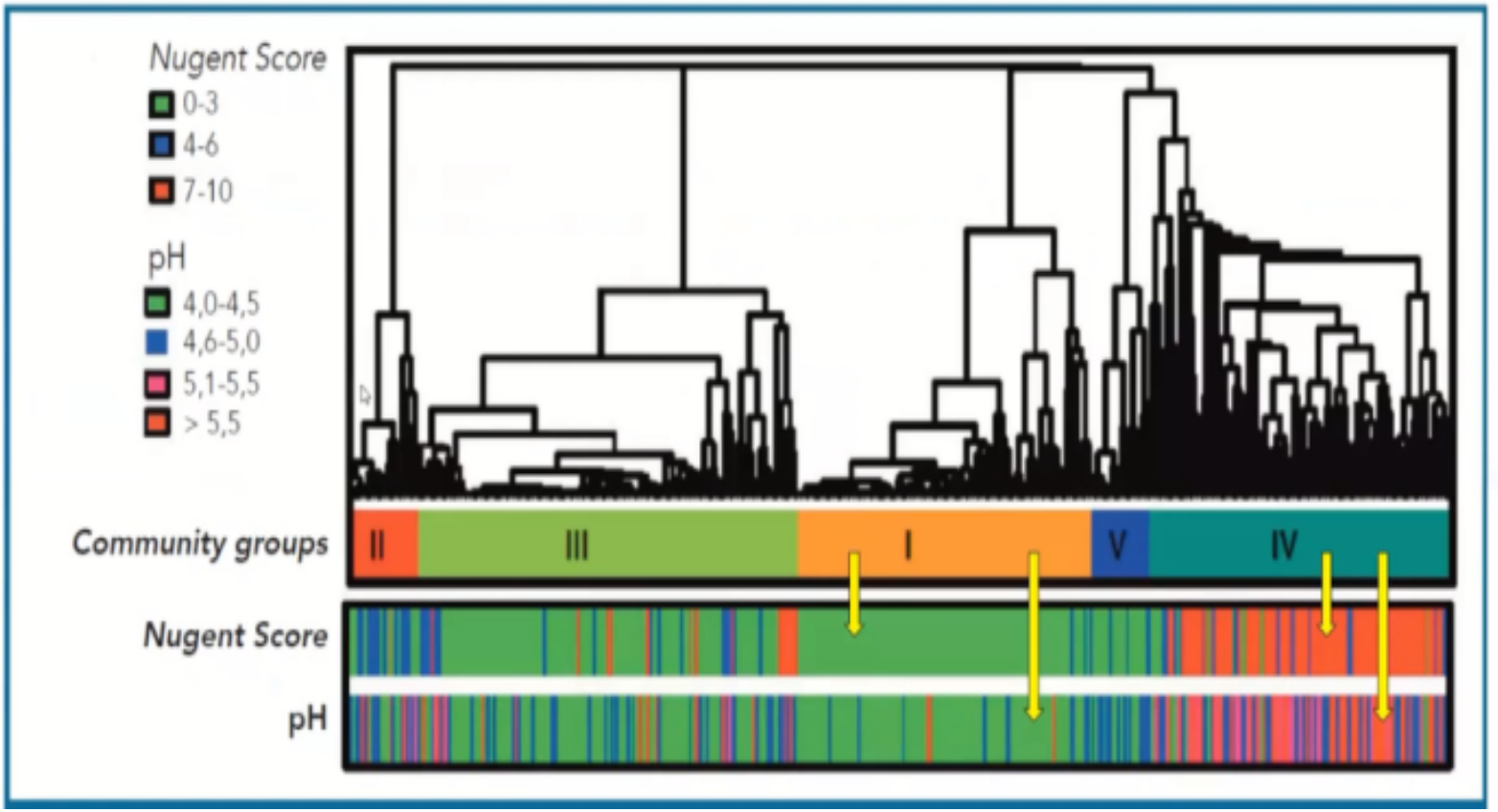
CST	Dominant bacterial species
I	*L. crispatus*
II	*L. gasseri*
III	*L. iners*
IV-A[b]	Low-*Lactobacillus*
IV-B[b]	Low-*Lactobacillus*
V	*L. jensenii*

[a] CST IV-A is characterized by various species of anaerobic bacteria including *Anaerococcus*, *Peptoniphilus* and *Prevotella* spp., whereas CST IV-B had higher proportions of bacteria from the genera *Atopobium* and *Megasphaera* among others.

[b] CSTs reflect the clustering of samples based on bacterial composition and abundance. Gajer et al. previously reported on these 6 CSTs among women in Baltimore, MD [54].

Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductiveage women. *Proc Natl Acad Sci USA. 2011;108(Suppl 1):4680-4687.*

Gajer P, Brotman RM, Bai G, et al. Temporal dynamics of the human vaginal microbiota. *Sci Transl Med. 2012;4(132):132ra52-132ra52.*



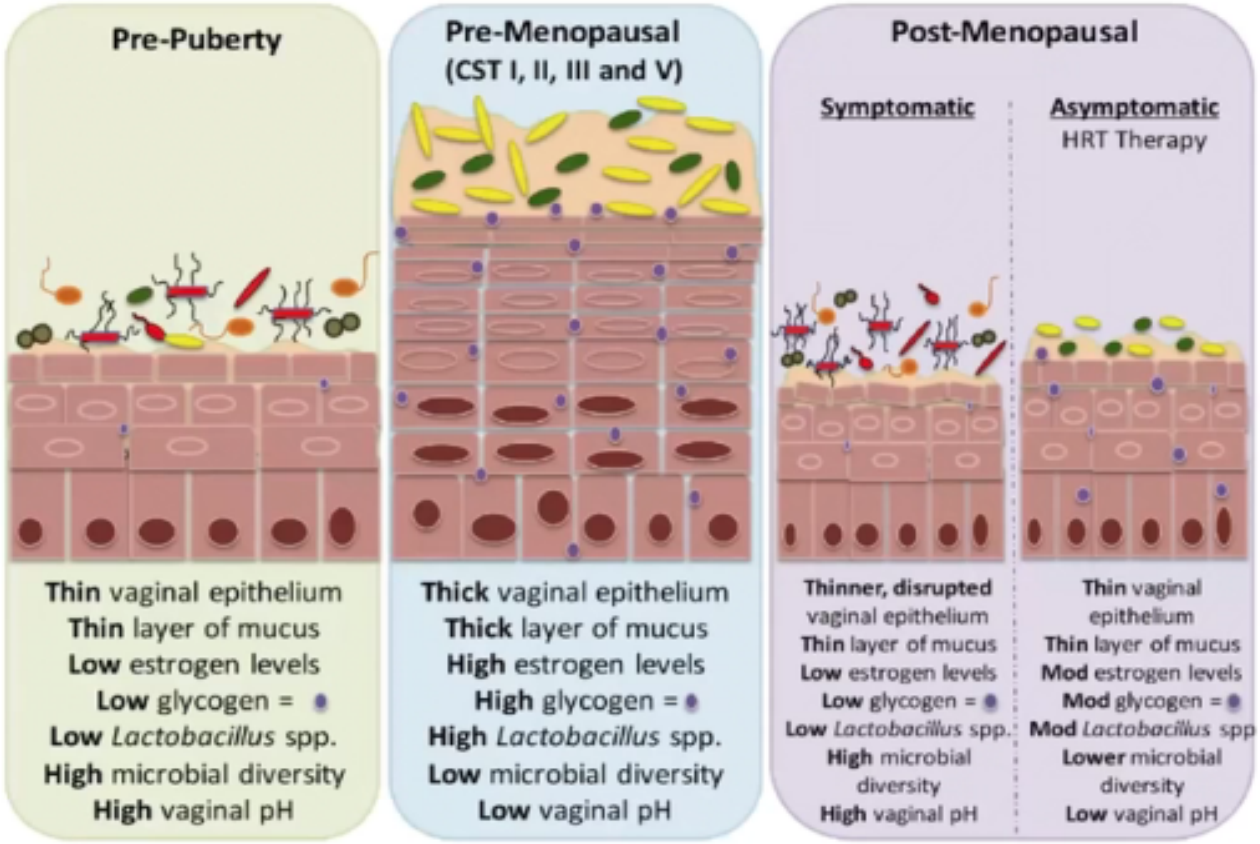
Relazione tra CST , Nugent score e ph

Ravel J, Gajer P, Abdo Z, et al. *Proc Natl Acad Sci USA. 2011*

Menopause and the vaginal microbiome

AliciaL.Muhleisena,b,MelissaM.Herbst-Kralovetzb

Maturitas91(2016)42–50



Gruppo	Dominanza	Frequenza media
CST I	*Lactobacillus crispatus*	25%
CST II	*Lactobacillus gasseri*	5%
CST III	*Lactobacillus iners*	35%
CST IV	Scarsità di lattobacilli	30%
CST V	*Lactabacillus jensenii*	5%

Tabella 1 - CST I-V: tipologia e frequenza in donne nord-americane sane e in età fertile e di etnia mista ma equamente rappresentate (98 caucasiche, 104 di colore, 97 asiatiche, 97 ispaniche).
Da: Ravel J *et al.* Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4680-7 (adattato)

CST	Gravidanza	*Post-partum*
I	60 (43%)	3 (20%)
II	13 (9%)	1 (7%)
III	42 (30%)	1 (7%)
IV	3 (2%)	9 (60%)
V	20 (14%)	1 (7%)

Tabella 3 - Frequenza dei vari CST prima e 6 settimane dopo il parto in donne europea di diversa etnia.
Da: 66) MacIntyre DA *et al.* Sci Rep. 2015; 5:8988

- The vagina lmicrobiome undergoes significant changes during pregnancy, including a significant decrease in overalldiversity, increased stability (the community composition changes overtime),and enrichment with *Lactobacillus species (Aagaard et al., 2012).*
- *These correlate with a decrease in the vaginal pH and an increase in vaginal secretions (Prince et al., 2014a).*

- Vaginal microbial compositions were found to differ according to gestational age, while the communities at the later stages of pregnancy resembled those of the non-pregnant state (Aagaard et al., 2012).
- A study characterizing the vaginal microbiota of pregnant and non-pregnant African-Americans reported that during pregnancy, one of the changes is dominance of a single *Lactobacillus species over others(Romero et al., 2014a).*

- Some *Lactobacillus species have bactericidal activities against other species,ensuring their predominance and low variability, which may help in protection against infections during pregnancy(Spurbeck and Arvidson, 2010).*

ARTICLES
https://doi.org/10.1038/s41591-019-0450-2
nature medicine
OPEN

The vaginal microbiome and preterm birth

Jennifer M. Fettweis[1,2,3], Myrna G. Serrano[1,3], J. Paul Brooks[3,4], David J. Edwards[3,5], Philippe H. Girerd[2,3], Hardik I. Parikh[1], Bernice Huang[1], Tom J. Arodz[3,6], Laahirie Edupuganti[1,3], Abigail L. Glascock[7], Jie Xu[3,8,9], Nicole R. Jimenez[1,3], Stephany C. Vivadelli[1,3], Stephen S. Fong[3,10], Nihar U. Sheth[11], Sophonie Jean[1], Vladimir Lee[1,3], Yahya A. Bokhari[6], Ana M. Lara[1], Shreni D. Mistry[1], Robert A. Duckworth III[1], Steven P. Bradley[1], Vishal N. Koparde[11], X. Valentine Orenda[1], Sarah H. Milton[2], Sarah K. Rozycki[12], Andrey V. Matveyev[1], Michelle L. Wright[13,14,15], Snehalata V. Huzurbazar[16], Eugenie M. Jackson[16], Ekaterina Smirnova[17,18], Jonas Korlach[19], Yu-Chih Tsai[19], Molly R. Dickinson[1], Jamie L. Brooks[1], Jennifer I. Drake[1], Donald O. Chaffin[20], Amber L. Sexton[20], Michael G. Gravett[20,21], Craig E. Rubens[20], N. Romesh Wijesooriya[9], Karen D. Hendricks-Muñoz[3,8,9], Kimberly K. Jefferson[1,3], Jerome F. Strauss III[2,3] and Gregory A. Buck[1,3,6*]

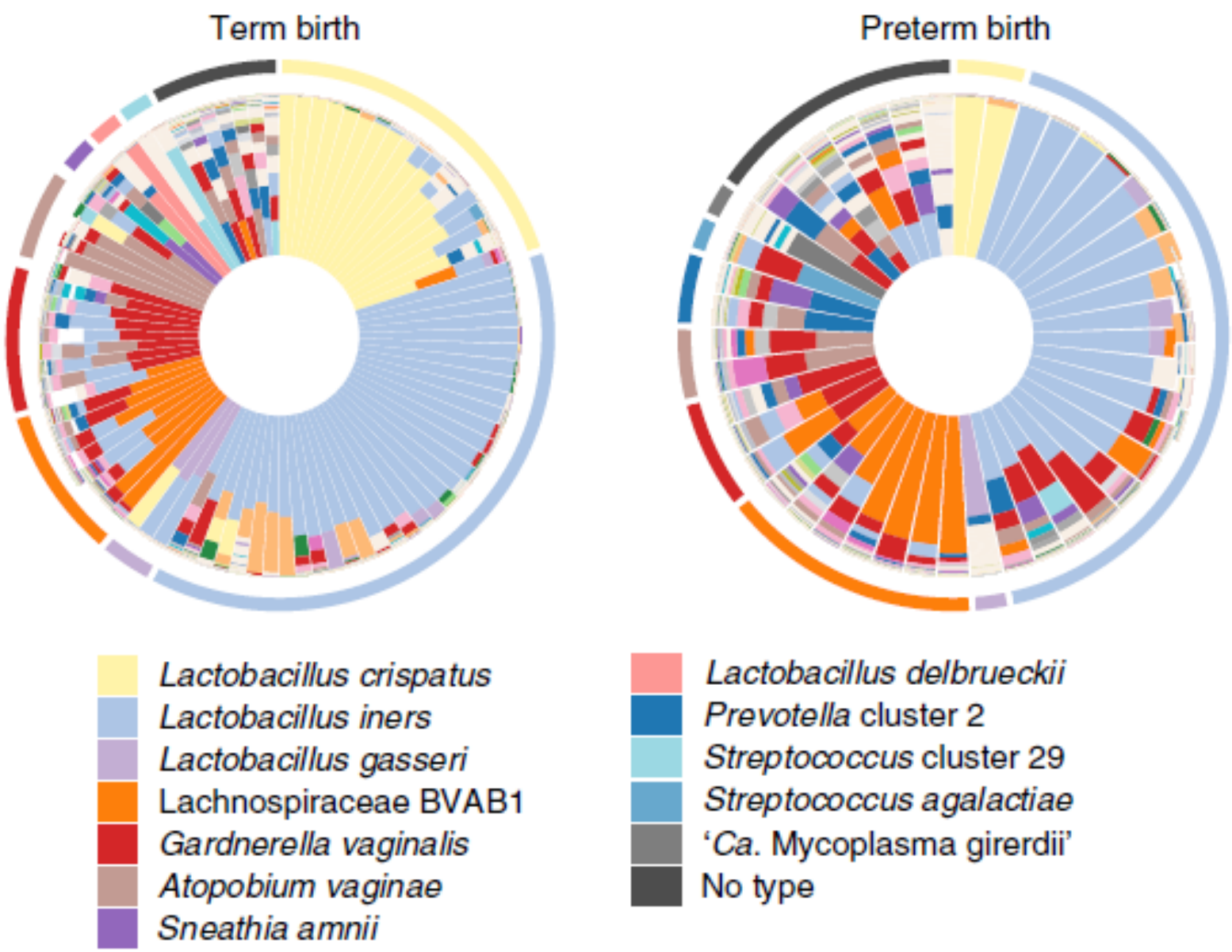
The Multi-Omic Microbiome Study: Pregnancy Initiative (MOMS-PI) includes a total of 1,572 pregnancies, with 992 pregnancies from clinics associated with the Research Alliance for Microbiome Science (RAMS) Registry, based at Virginia Commonwealth University (VCU) in Virginia, and 580 pregnancies from sites associated with the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) in Washington State.

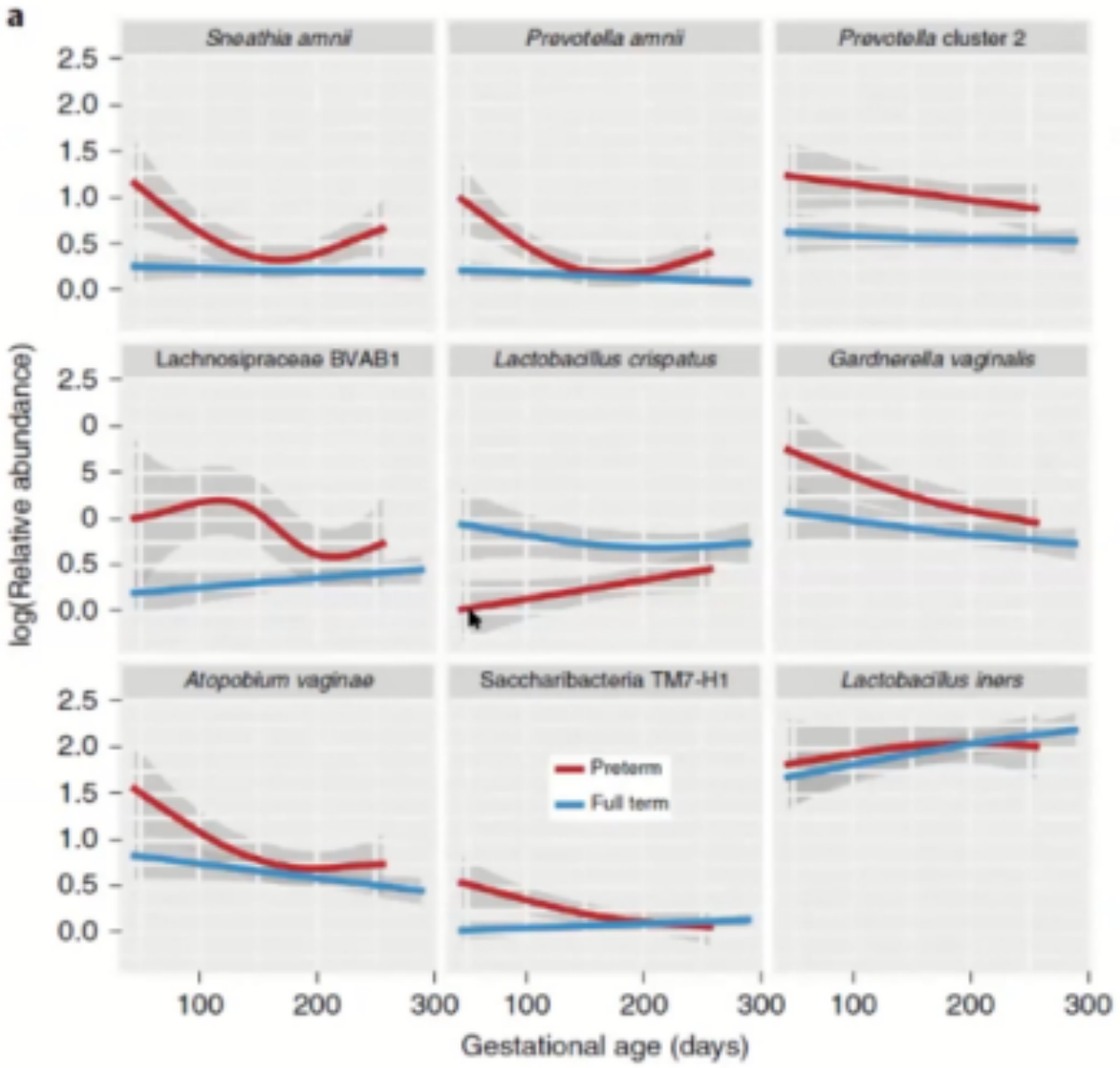
Table 1 | Description of cohort studied in this project

	Preterm delivery <37 weeks ($n=45$)	Term delivery ≥39 weeks ($n=90$)
Mean age (years)[a]	26 (5.68)	25.9 (5.43)
Ancestry/ethnicity (no. (%))		
African	35 (77.8)	71 (78.9)
European	6 (13.3)	13 (14.4)
Hispanic	3 (6.7)	5 (5.6)
Native American	1 (2.2)	1 (1.1)
Household income (no. (%))[b]		
<US$20,000	29 (72.5)	66 (77.7)
US$20,000–59,999	9 (22.5)	15 (17.6)
US$60,000+	2 (5.0)	4 (4.7)
Vaginal delivery (no. (%))	38 (84.4)	74 (82.2)
Previous preterm (no. (%))	14 (31.1)	9 (10.0)
Preterm premature rupture of the membranes (no. (%))	26 (57.8)	0 (0)

[a]Standard deviation listed in parentheses. [b]Missing values $n=5$ (PTB), $n=5$ (TB).

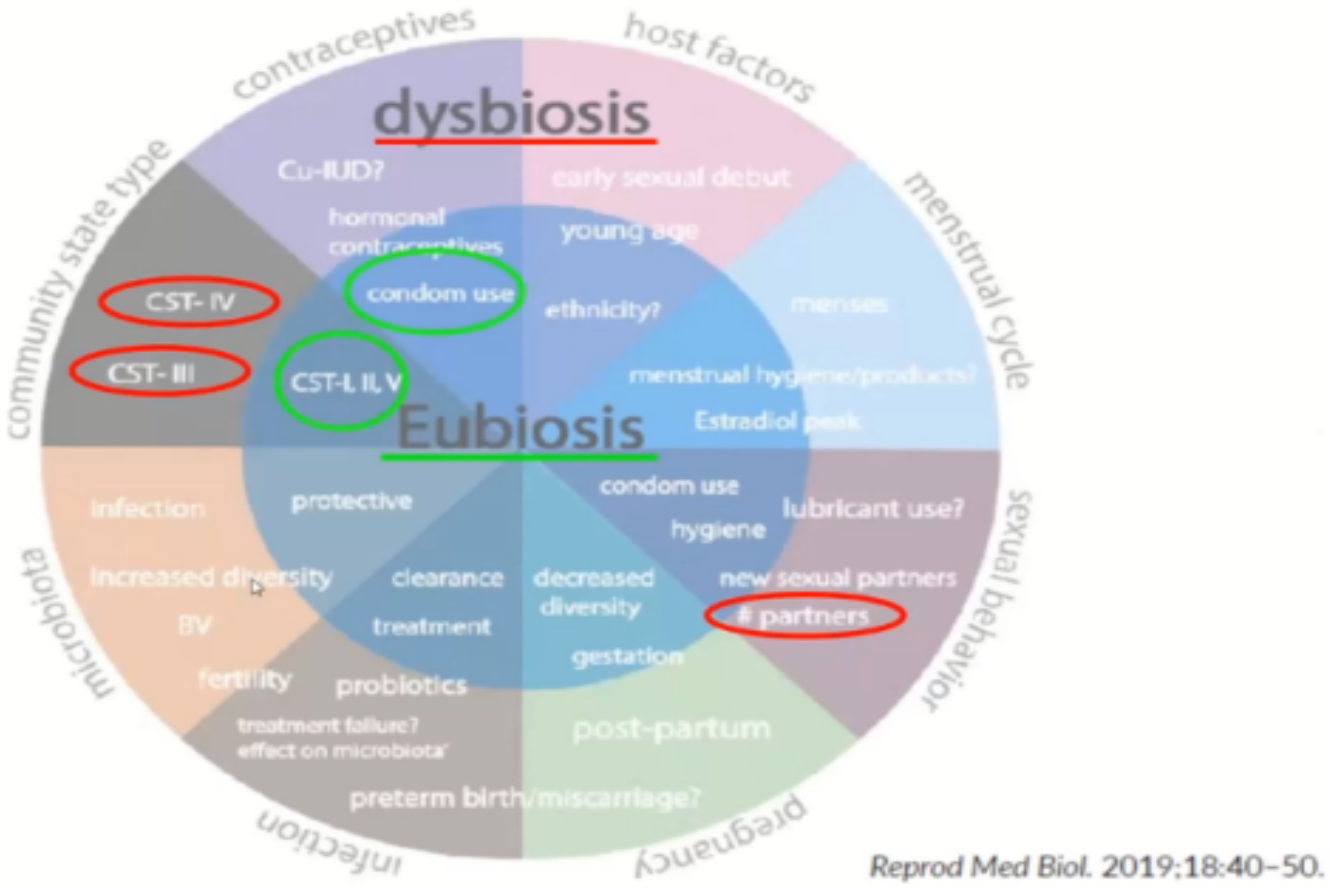
a


Term birth
Preterm birth
Lactobacillus crispatus
Lactobacillus iners
Lactobacillus gasseri
Lachnospiraceae BVAB1
Gardnerella vaginalis
Atopobium vaginae
Sneathia amnii
Lactobacillus delbrueckii
Prevotella cluster 2
Streptococcus cluster 29
Streptococcus agalactiae
'Ca. Mycoplasma girerdii'
No type


a
Sneathia amnii
Prevotella amnii
Prevotella cluster 2
Lachnospiraceae BVAB1
Lactobacillus crispatus
Gardnerella vaginalis
Atopobium vaginae
Saccharibacteria TM7-H1
Lactobacillus iners
log(Relative abundance)
Preterm
Full term
Gestational age (days)

Deciphering the effect of reproductive tract microbiota on human reproduction

Inmaculada Moreno,Carlos Simon



F I G U R E 1 Factors influencing the composition of the cervicovaginal microbiota.

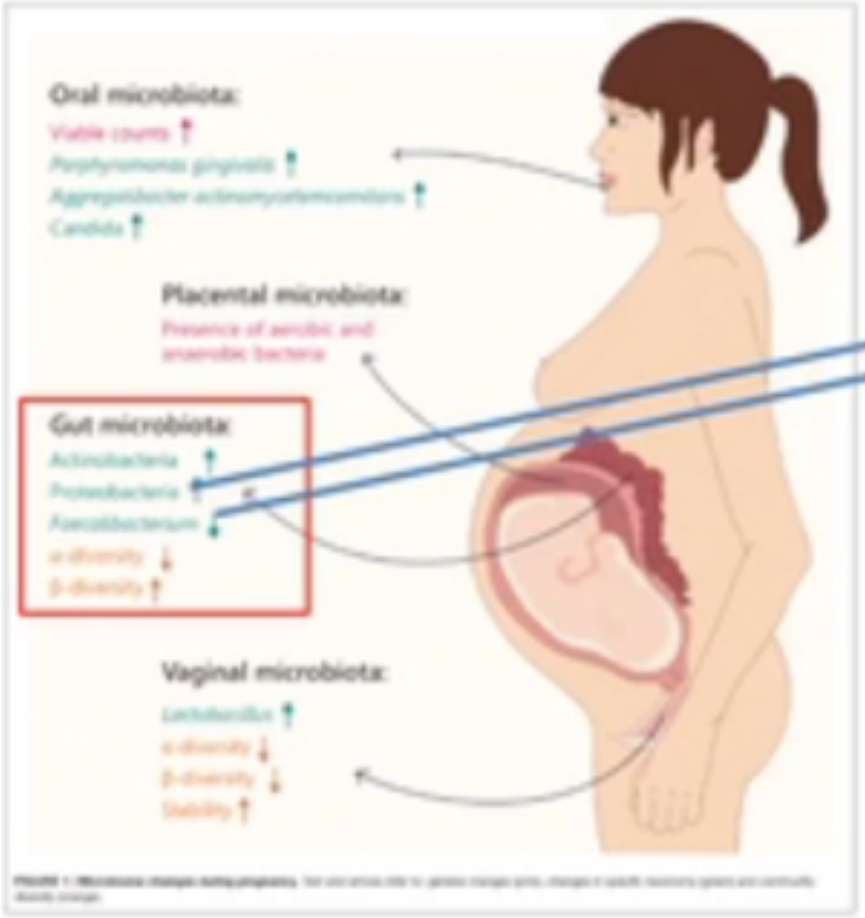
- Healthy pregnancy is characterized by an increase in the bacterial load and profound alterations in the composition of gut microbiota (Collado et al., 2008; Koren et al., 2012).
- In the first trimester of pregnancy, the gut microbial composition is similar to that of healthy, non-pregnant women.

- However,from the first to the third trimester,the gut microbiota composition changes dramatically.
- These changes are characterized by increased abundance of members of the Actinobacteria and Proteobacteria phyla, as well as a reduction in individual richness (alpha diversity; Koren et al., 2012).

- In addition,levels of *Faecalibacterium,* a butyrate-producing bacterium with anti-inflammatory activities, which is depleted in metabolic syndrome patients(Haro et al., 2015), are significantly decreased in the third trimester of pregnancy.

- Between-subject diversity (beta diversity), is increased in the third trimester, coupled with weight gain, insulin insensitivity, and higher levels of fecal cytokines, reflecting inflammation (Koren et al., 2012).

Microbiota changes in pregnancy

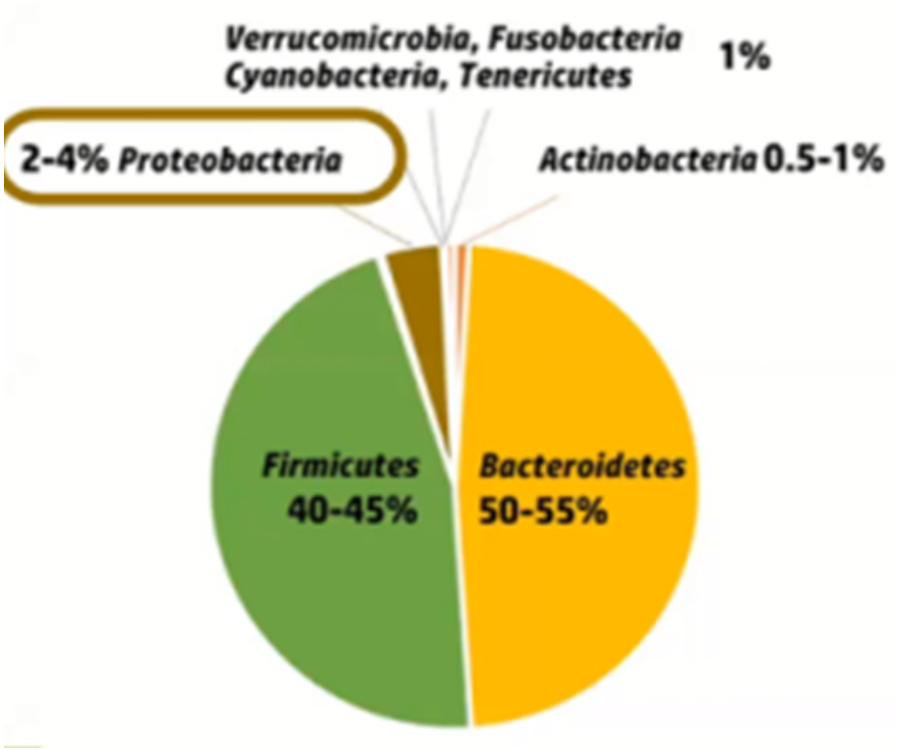


Infiammazione
Insulino-resistenza
Permeabilità

Frontiers in Microbiology, 2016;7:1031

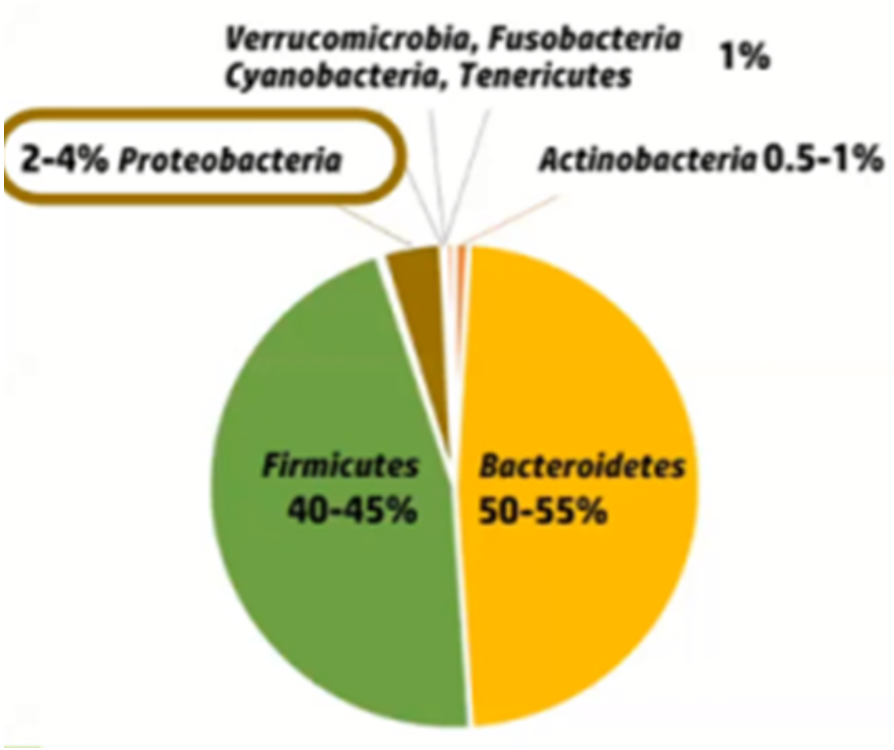
Phylum Proteobacteria

- Gram – pro-infiammatori
- Fisiologicamente abbondanti solo nei neonati e nelle donne in gravidanza , dove sotto la spinta ormonale possono arrivare oltre il 10%



Phylum Actinobacteria

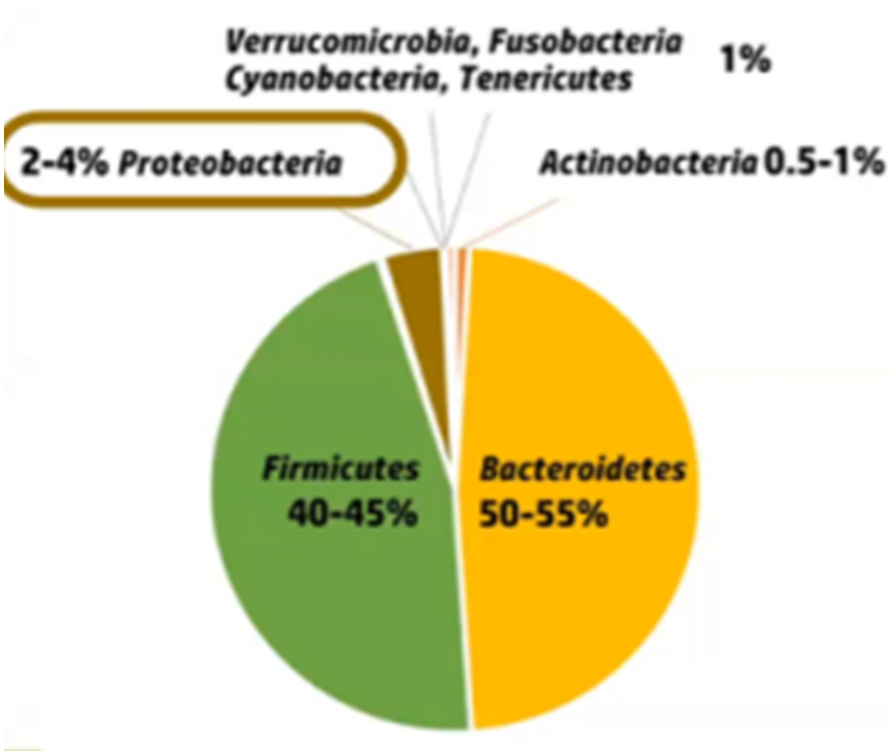
- Gram + anti-infiammatori
- Il genere Bifidobacterium nella fase finale della gravidanza aumenta in modo esponenziale fino al 10/14%
- Nei neonati e nel latte raggiunge il 50%



- In vagina il phylum è rappresentato dai generi Atopobium e Gardnerella , patogeni di origine intestinale
- I Bifidobatteri sono primariamente saccarolitici e quindi metabolizzano nell'adulto i glicani della mucina mentre nei neonati e nei lattanti gli HMO del latte materno

Phylum Verrucomicrobia

- Gram –
- La specie Akkermansia muciniphlia :
- modulo l'ambiente intestinale in senso anti-infiammatorio
- promuove l'integrità della barriera epiteliale attraverso la up regolazione dell'espressione delle proteine del le tight junctions
- Si alimenta del carbonio contenuto nel muco favorendone l'ulteriore ispessimento dello strato
- E' in grado di ridurre i meccanismi infiammatori LPS mediati



- La riduzione del Faecalibacterium Prausnifzil che è un potente batterio butirrato-produttore anti-infiammatorio , si accompagna ad una ulteriore modificazione in eccesso della permeabilità intestinale

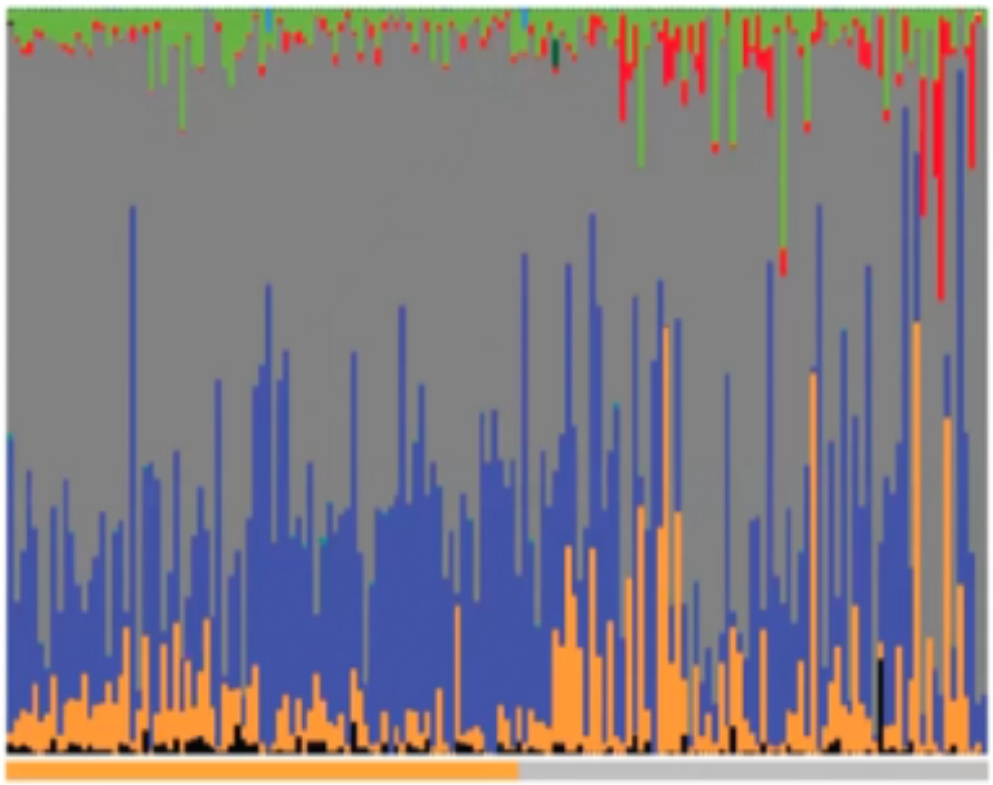
Microbiota e interazione metabolica

INFIAMMAZIONE SISTEMICA ED INSULINO RESISTENZA

SOLINI A. et alii, 2010
Dipartimento di Medicina Interna Università di Pisa;
Dipartimento di Medicina Clinica e Sperimentale Università di Ferrara; Sezione di Medicina Interna, Gerontologia e Nutrizione Clinica

- L'infiammazione è uno dei meccanismi cruciali nello sviluppo della insulino resistenza associata a malattie metaboliche quali l'obesità e il diabete tipo 2,
- rilascio di citochine pro-infiammatorie quali il tumor necrosis factor (TNF)-a e la interleuchina-6 (IL-6) ,
- attiva vie di segnale infiammatorie come risultato di una disregolazione cellulare dei pathways omeostatici, come la risposta allo stress del reticolo endoplasmico.

- In many ways, the metabolic changes associated with pregnancy are similar to those that occur in the metabolic syndrome, including weight gain, elevated fasting blood-glucose levels, insulin resistance, glucose intolerance, lowgrade inflammation, and changes in metabolic hormone levels (Fuglsang, 2008; Newbern and Freemark, 2011; Emanuela et al., 2012; Kumar and Magon, 2012).



Cell 2012 August 3; 150(3): 470–480. doi:10.1016/j.cell.2012.07.008.

Host remodeling of the gut microbiome and metabolic changes during pregnancy

Omry Koren[1], Julia K. Goodrich[1], Tyler C. Cullender[1], Aymé Spor[1,11], Kirsi Laitinen[3], Helene Kling Bäckhed[6,7], Antonio Gonzalez[8], Jeffrey J. Werner[2,12], Largus T. Angenent[2], Rob Knight[9,10], Fredrik Bäckhed[6,7], Erika Isolauri[4], Seppo Salminen[5], and Ruth E. Ley[1,*]

Biodegradation (2008) 19:683–693
DOI 10.1007/s10532-007-9173-z

ORIGINAL PAPER

17α-ethinylestradiol cometabolism by bacteria degrading estrone, 17β-estradiol and estriol

Bram Pauwels · Klaas Wille · Herlinde Noppe · Hubert De Brabander · Tom Van de Wiele · Willy Verstraete · Nico Boon

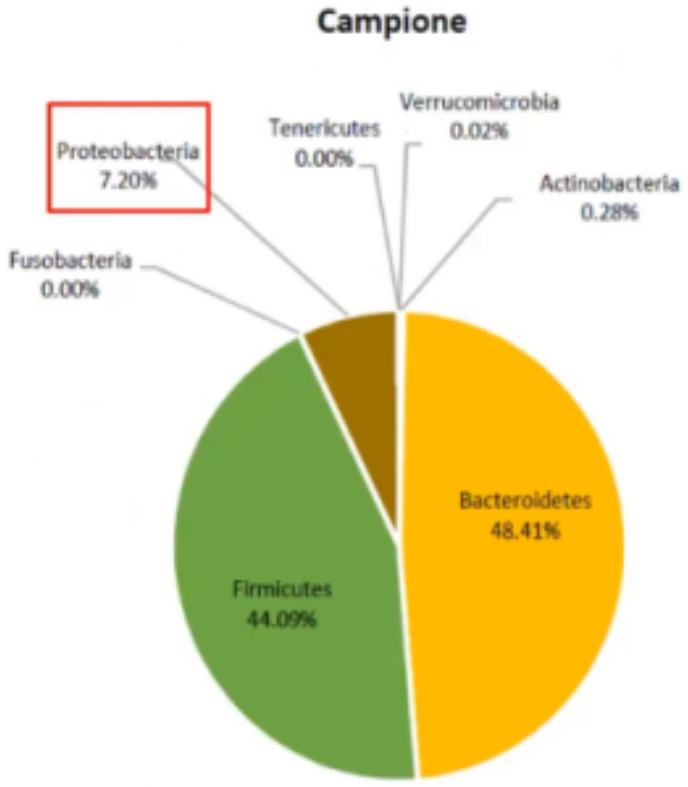
Table 1 Overview of the isolated bacterial species that can degrade E2 and EE2

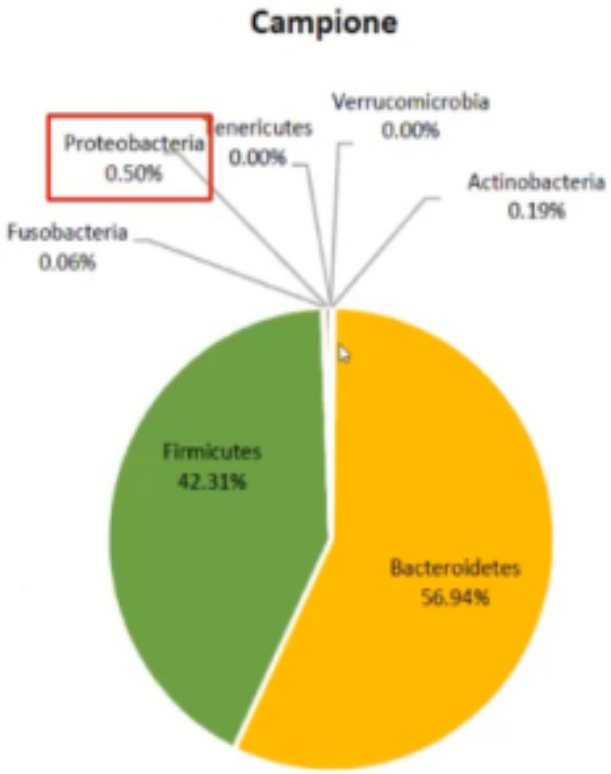
Isolate	Closest match[a] (accession nr.)	Classification[b]	Percentage similarity
BP1	*Phyllobacterium myrsinacearum* (AY785315)	Alpha-*Proteobacteria*	100 (929/929)
BP2	*Ralstonia pickettii* (AY268180)	Beta-*Proteobacteria*	99 (1247/1248)
BP3	*Pseudomonas aeruginosa* (AY738263)	Gamma-*Proteobacteria*	99 (1240/1241)
BP7	*Pseudomonas* sp. (DQ303435)	Gamma-*Proteobacteria*	99 (1238/1242)
BP8[c]	*Acinetobacter* sp. (CR543861)	Gamma-*Proteobacteria*	99 (1232/1244)
BP10[c]	*Acinetobacter* sp. (CR543861)	Gamma-*Proteobacteria*	99 (1222/1229)

[a] Based on nearest BLAST homology results

[b] Based on RDP-II query results

[c] Similarity between BP8 and BP10 was 99% (1224/1229)



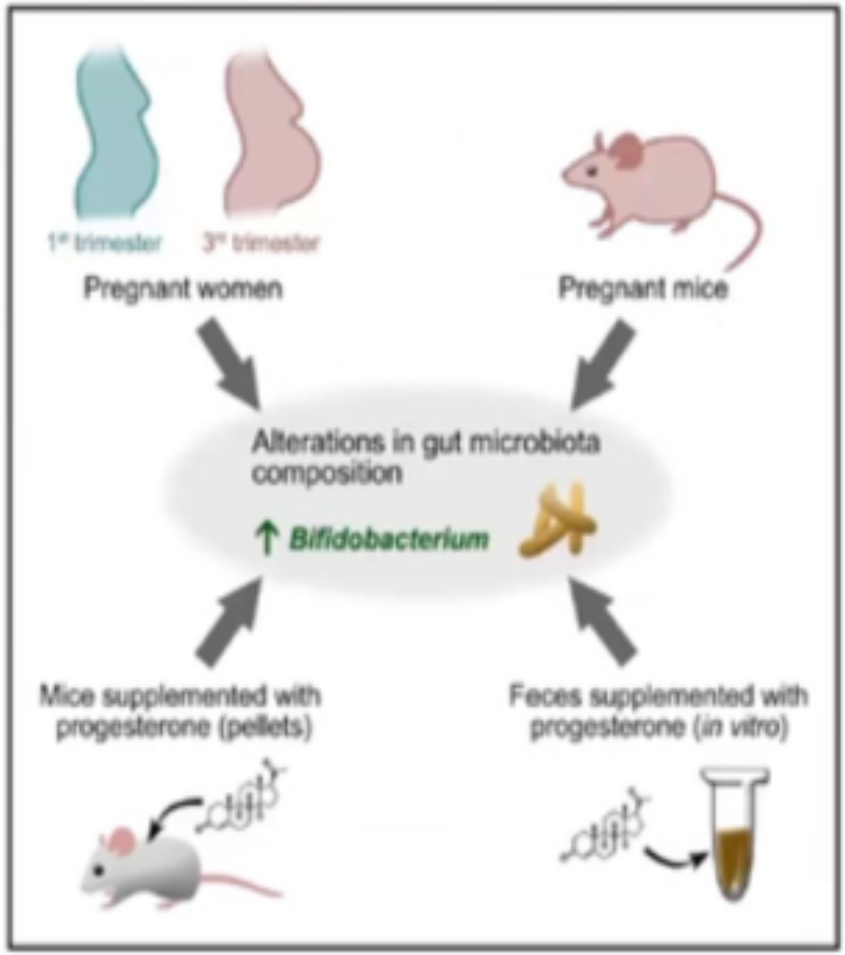


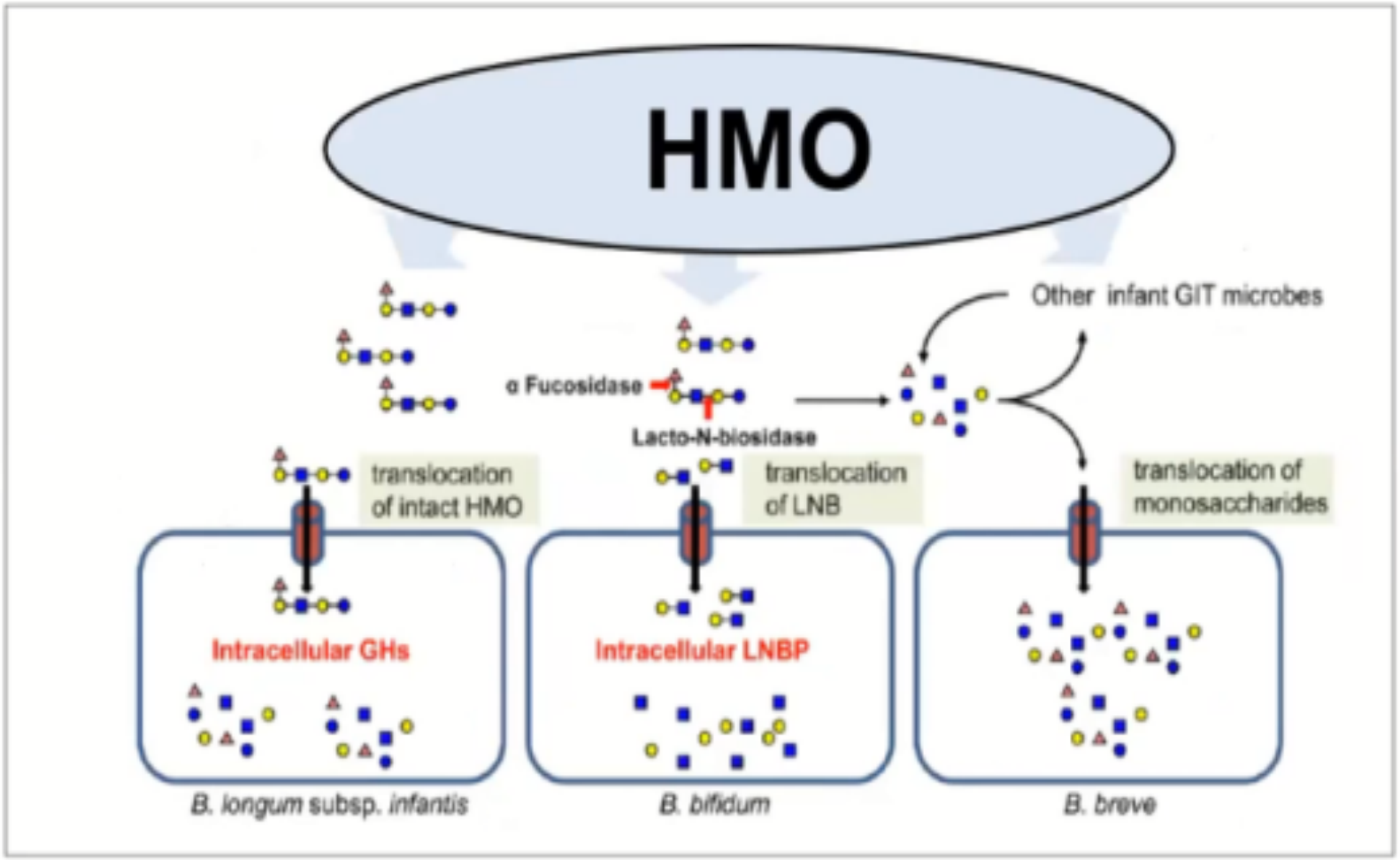
Soggetto B: pre-parto (7gg) **Soggetto B: post-parto (90gg)**

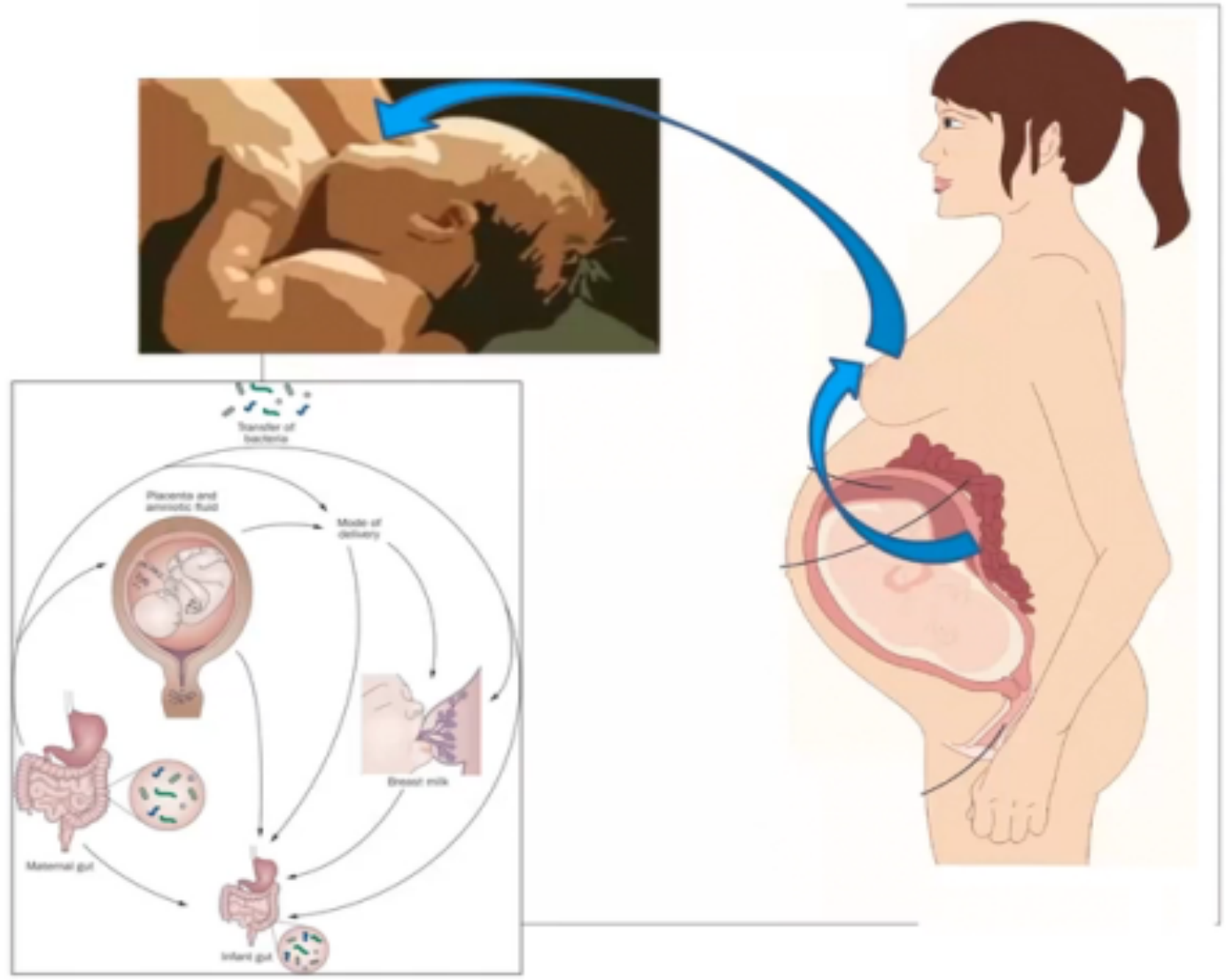
Nuriel-Ohayon et al., 2019, Cell Reports 27, 730–736

Cell Reports

Progesterone Increases *Bifidobacterium* Relative Abundance during Late Pregnancy




HMO
Other infant GIT microbes
α Fucosidase
Lacto-N-biosidase
translocation of intact HMO
translocation of LNB
translocation of monosaccharides
Intracellular GHs
Intracellular LNBP
B. longum subsp. infantis
B. bifidum
B. breve


Transfer of bacteria
Placenta and amniotic fluid
Mode of delivery
Breast milk
Maternal gut
Infant gut

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2011, p. 6788–6793 Vol. 77, No. 19
0099-2240/11/$12.00 doi:10.1128/AEM.05346-11

Transmission of Intestinal *Bifidobacterium longum* subsp. *longum* Strains from Mother to Infant, Determined by Multilocus Sequencing Typing and Amplified Fragment Length Polymorphism▽†

Hiroshi Makino,[1,2]* Akira Kushiro,[2] Eiji Ishikawa,[2] Delphine Muylaert,[1] Hiroyuki Kubota,[1,2] Takafumi Sakai,[1,2] Kenji Oishi,[1,2] Rocio Martin,[3] Kaouther Ben Amor,[3] Raish Oozeer,[3] Jan Knol,[3] and Ryuichiro Tanaka[1,2]

Yakult Honsha European Research Center for Microbiology, ESV, Technologiepark 4, 9052 Ghent-Zwijnaarde, Belgium[1]; Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan[2]; and Danone Research, Centre for Specialised Nutrition, Wageningen, The Netherlands[3]

Received 3 May 2011/Accepted 27 July 2011

The gastrointestinal tracts of neonates are colonized by bacteria immediately after birth. It has been discussed that the intestinal microbiota of neonates includes strains transferred from the mothers. Although some studies have indicated possible bacterial transfer from the mother to the newborn, this is the first report confirming the transfer of bifidobacteria at the strain level. Here, we investigated the mother-to-infant transmission of *Bifidobacterium longum* subsp. *longum* by genotyping bacterial isolates from the feces of mothers before delivery and of their infants after delivery. Two hundred seven isolates from 8 pairs of mothers and infants were discriminated by multilocus sequencing typing (MLST) and amplified fragment length polymorphism (AFLP) analysis. By both methods, 11 strains of *B. longum* subsp. *longum* were found to be monophyletic for the feces of the mother and her infant. This finding confirms that these strains were transferred from the intestine of the mother to that of the infant. These strains were found in the first feces (meconium) of the infant and in the feces at days 3, 7, 30, and 90 after birth, indicating that they stably colonize the infant's intestine immediately after birth. The strains isolated from each family did not belong to clusters derived from any of the other families, suggesting that each mother-

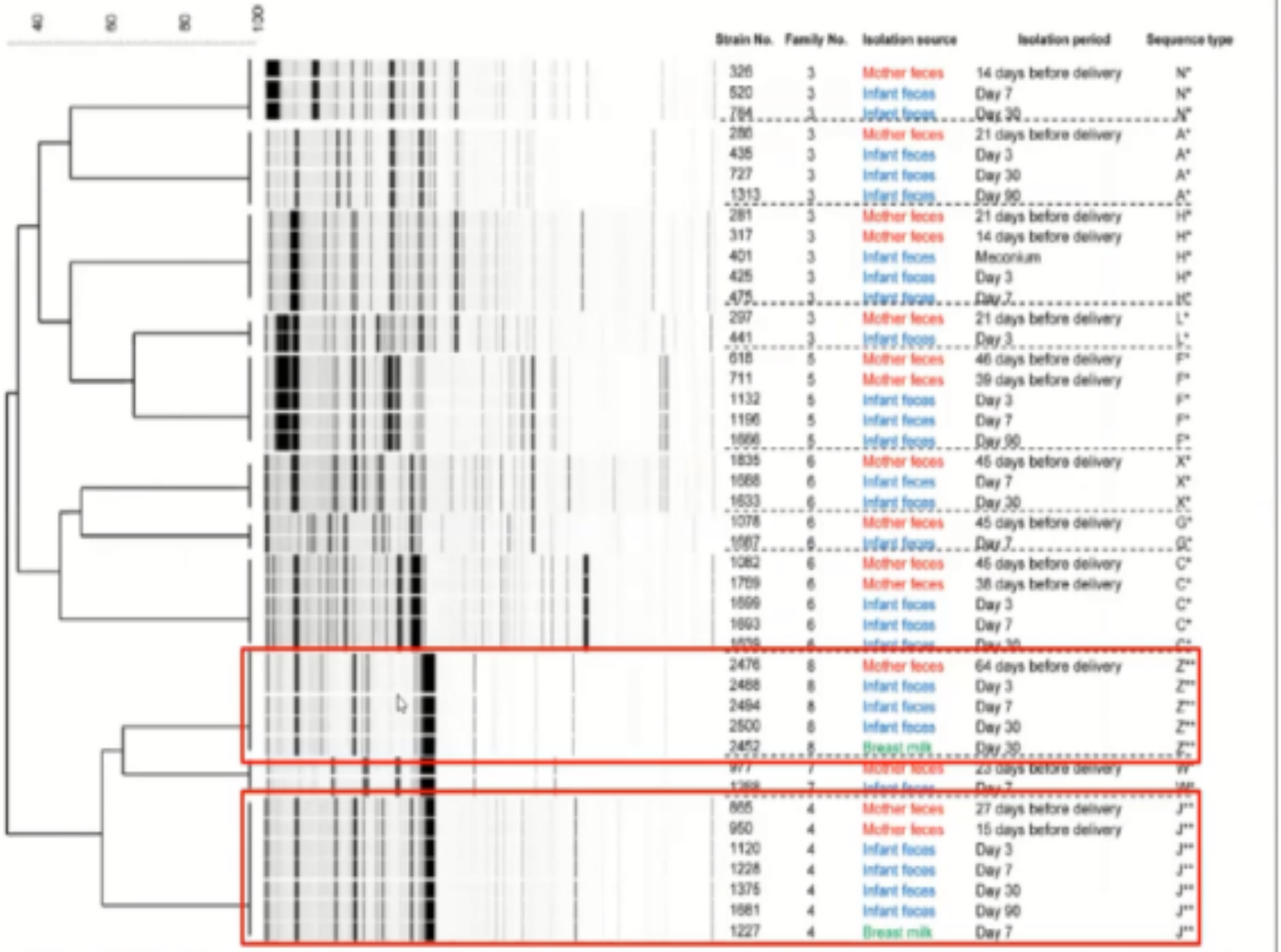
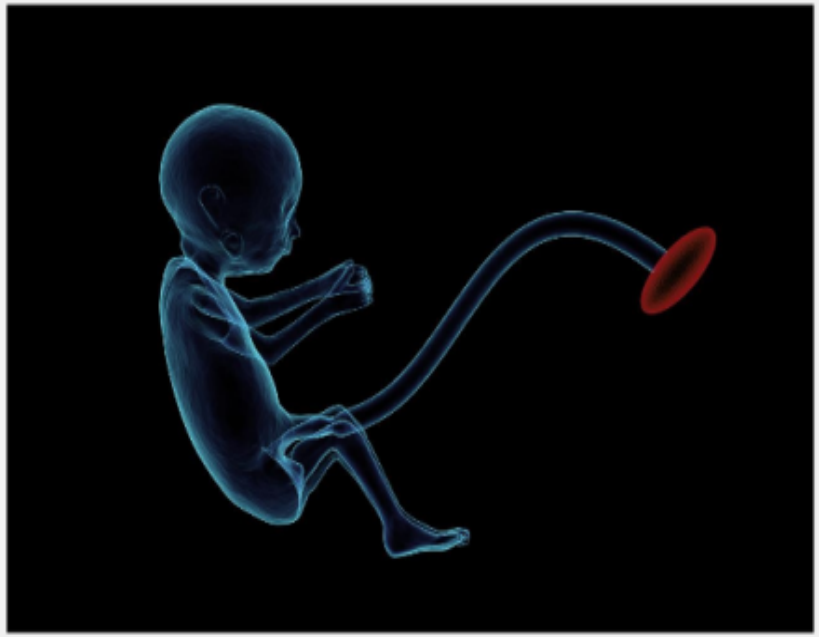
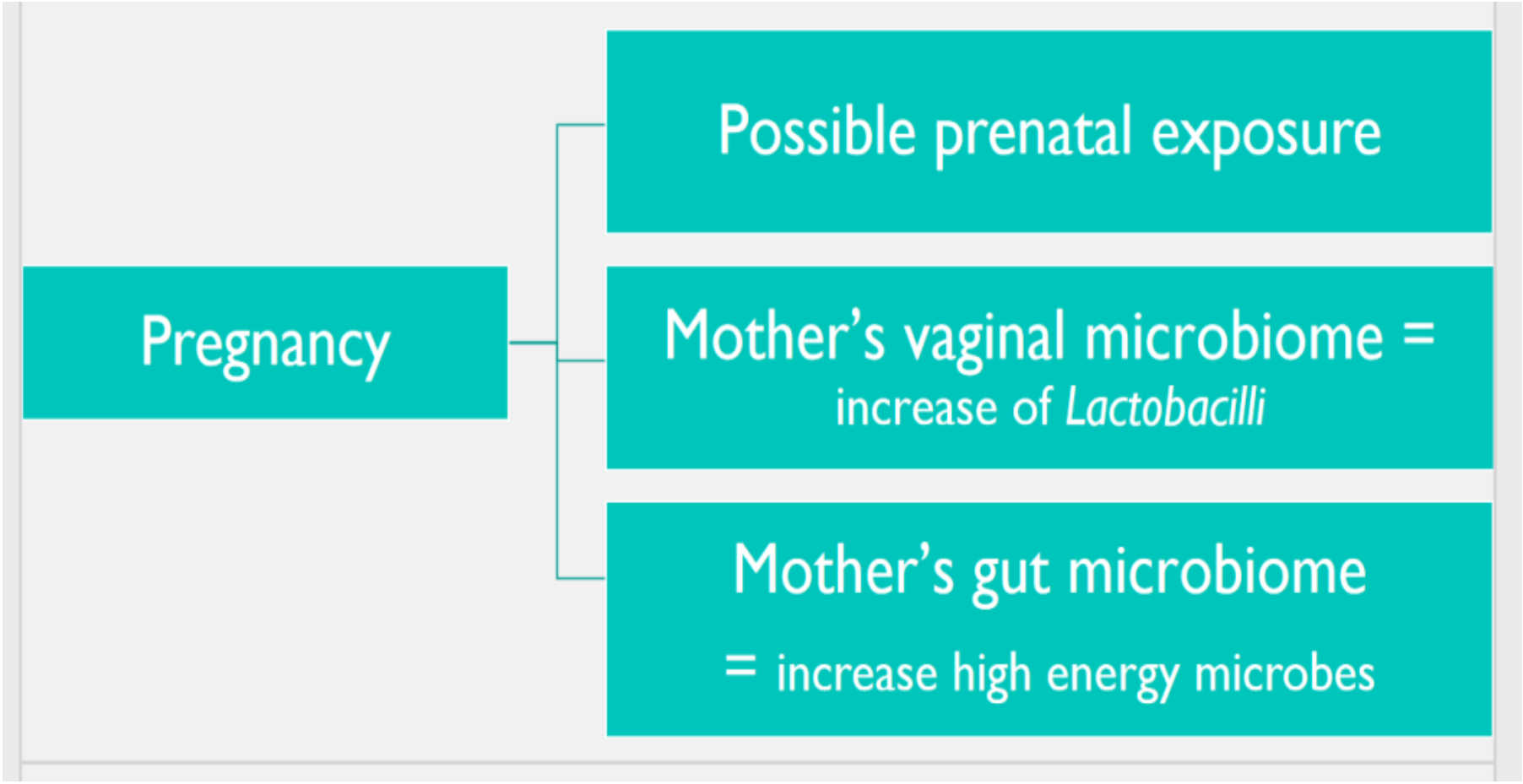


FIG. 2. AFLP profiles of the 11 *B. longum* subsp. *longum* strains found by MLST analysis to be monophyletic between feces from mothers and their infants. Dendrograms were generated with a multiscale setting for comparison and UPGMA for clustering.

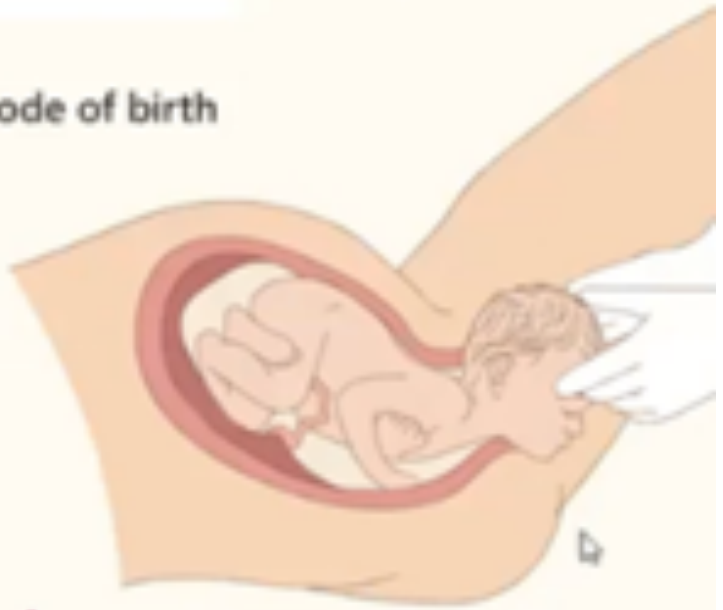
MATERNAL HERITAGE

- *'Gestational changes in the vaginal and intestinal microbiome are of particular relevance because these body sites are responsible for vertical microbial transmission to the newborn'*
  - - Quote from science ref: 1. *Mueller N.T. et al, Trends in Molecular Medicine 2015*




Pregnancy
Possible prenatal exposure
Mother's vaginal microbiome =
increase of Lactobacilli
Mother's gut microbiome
= increase high energy microbes

A. Changes associated with mode of birth (vaginal vs. C-section)



Prevotella ↑
Lactobacillus ↑
Propionibacterium ↓
Corynebacterium ↓
Enterobacteriaceae ↓
Streptococcus ↓
Earlier colonization of Bacteroidetes
Antibiotic resistance bacterial genes ↓
Resemblence to mother's gut microbiota ↑
Viable counts ↑
β-diversity ↓

B. Changes associated with feeding (breast milk vs. formula)

Bacteroides fragilis ↓
Bifidobacterium infantis ↑
Sneathia ↑
Staphylococcus ↓
α-diversity ↓

WHAT DOES THAT MEAN?

Importance of protecting the mother's microbiome during pregnancy

IMAGE CREDIT: Fotolia

WHAT COULD AFFECT A MOTHER'S MICROBIOME DURING PREGNANCY?

- Diet
- Lifestyle
- Exercise
- Stress
- Antibiotics
- Antibacterial practises

- Avoid all perinatal and postnatal environmental factors that may shape the infant microbiota after birth.

- Maternal prenatal psychosocial stress (PNS) and maternal obesity are two important prenatal risk factors that may affects the composition of offspring bacterial microbiota.
- PNS could alter immune development and may increase risk of allergies in childhood

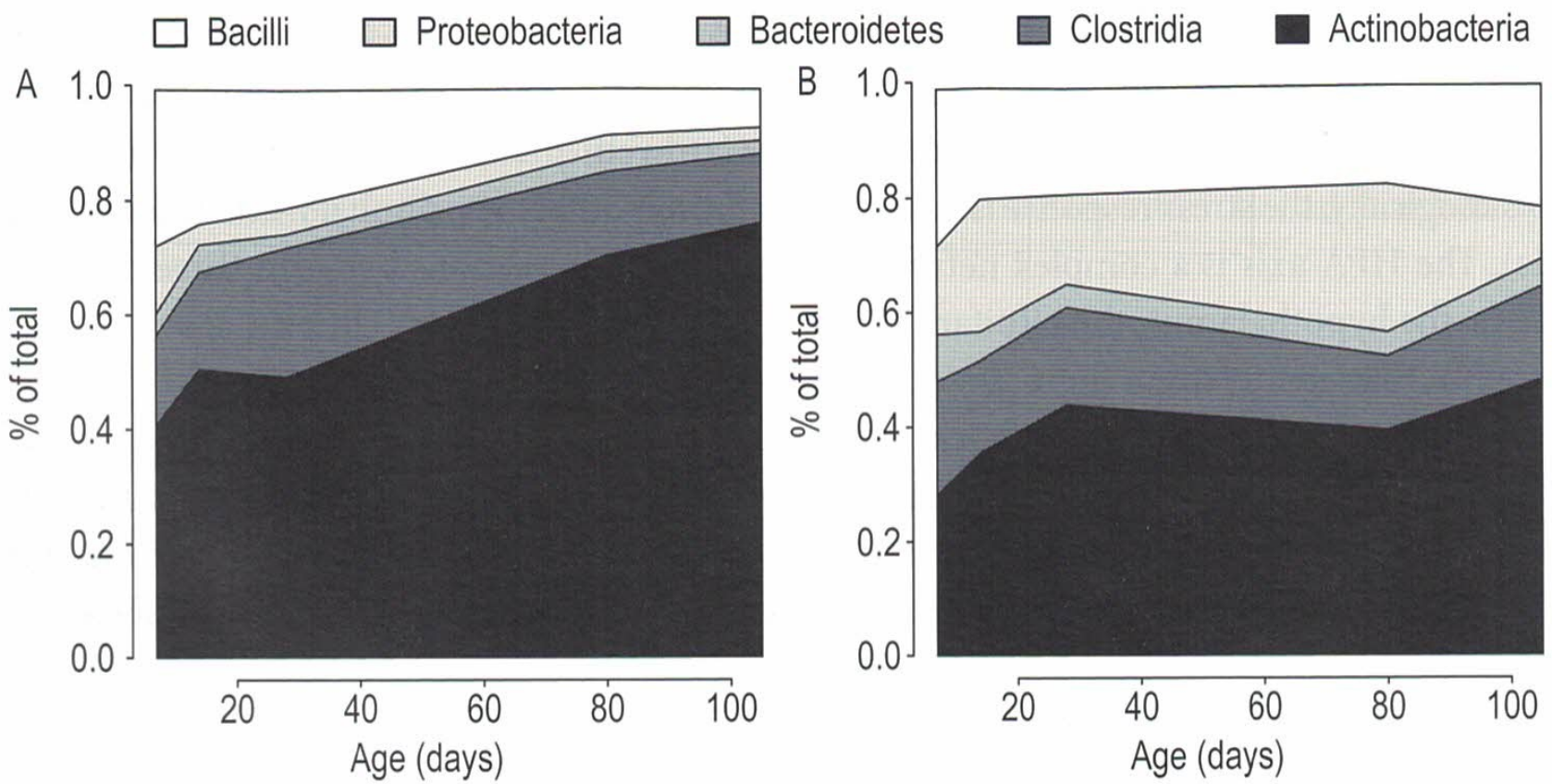


Figure 3.1. The average relative abundance of *Bacilli, Proteobacteria, Bacteroidetes*, *Clostridia* and *Actinobacteria* in infants with (A) exposure to low levels of maternal prenatal reported stress/anxiety as well as low levels of pregnancy cortisol, and (B) exposure to high levels of maternal prenatal reported stress/anxiety as well as high levels of pregnancy cortisol (Zijlmans *et al.*, 2015; Figure is reproduced with permission from Elsevier).

- A clear association exists between maternal obesity during pregnancy and risk of childhood obesity, with increased risk of obesity and diabetes type 2 (DM2) in offspring born to overweight and obese mothers

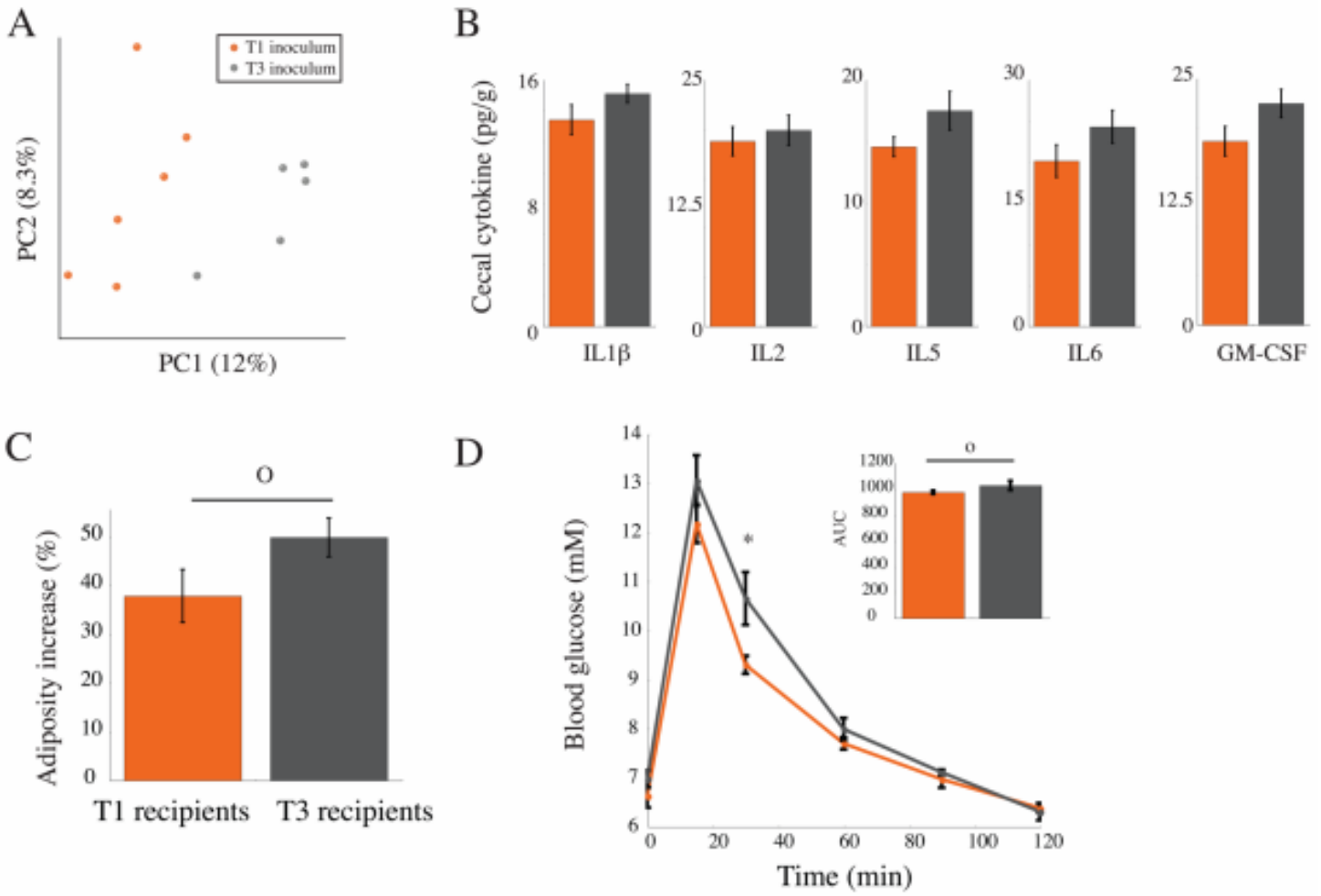
Published in final edited form as:
Cell. 2012 August 3; 150(3): 470–480. doi:10.1016/j.cell.2012.07.008.

Host remodeling of the gut microbiome and metabolic changes during pregnancy

Omry Koren[1], **Julia K. Goodrich**[1], **Tyler C. Cullender**[1], **Aymé Spor**[1,11], **Kirsi Laitinen**[3], **Helene Kling Bäckhed**[6,7], **Antonio Gonzalez**[8], **Jeffrey J. Werner**[2,12], **Largus T. Angenent**[2], **Rob Knight**[9,10], **Fredrik Bäckhed**[6,7], **Erika Isolauri**[4], **Seppo Salminen**[5], and **Ruth E. Ley**[1,*]

Gut microbiota changed dramatically from first (T1) to third (T3) trimesters, with vast expansion of diversity between mothers, an overall increase in Proteobacteria and Actinobacteria, and reduced richness

When transferred to germ-free mice, T3 microbiota induced greater adiposity and insulin insensitivity compared to T1.


A
T1 inoculum
T3 inoculum
PC2 (8.3%)
PC1 (12%)
B
Cecal cytokine (pg/g)
IL1β
IL2
IL5
IL6
GM-CSF
C
Adiposity increase (%)
T1 recipients
T3 recipients
D
Blood glucose (mM)
Time (min)
AUC

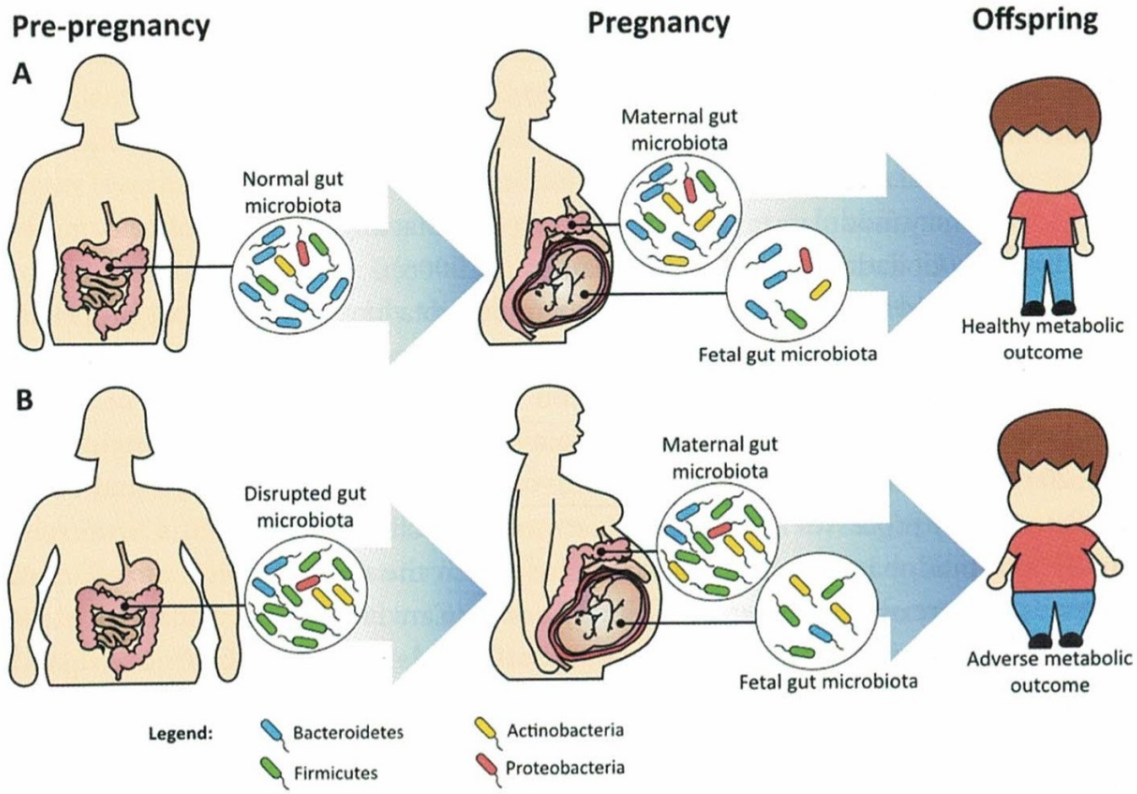
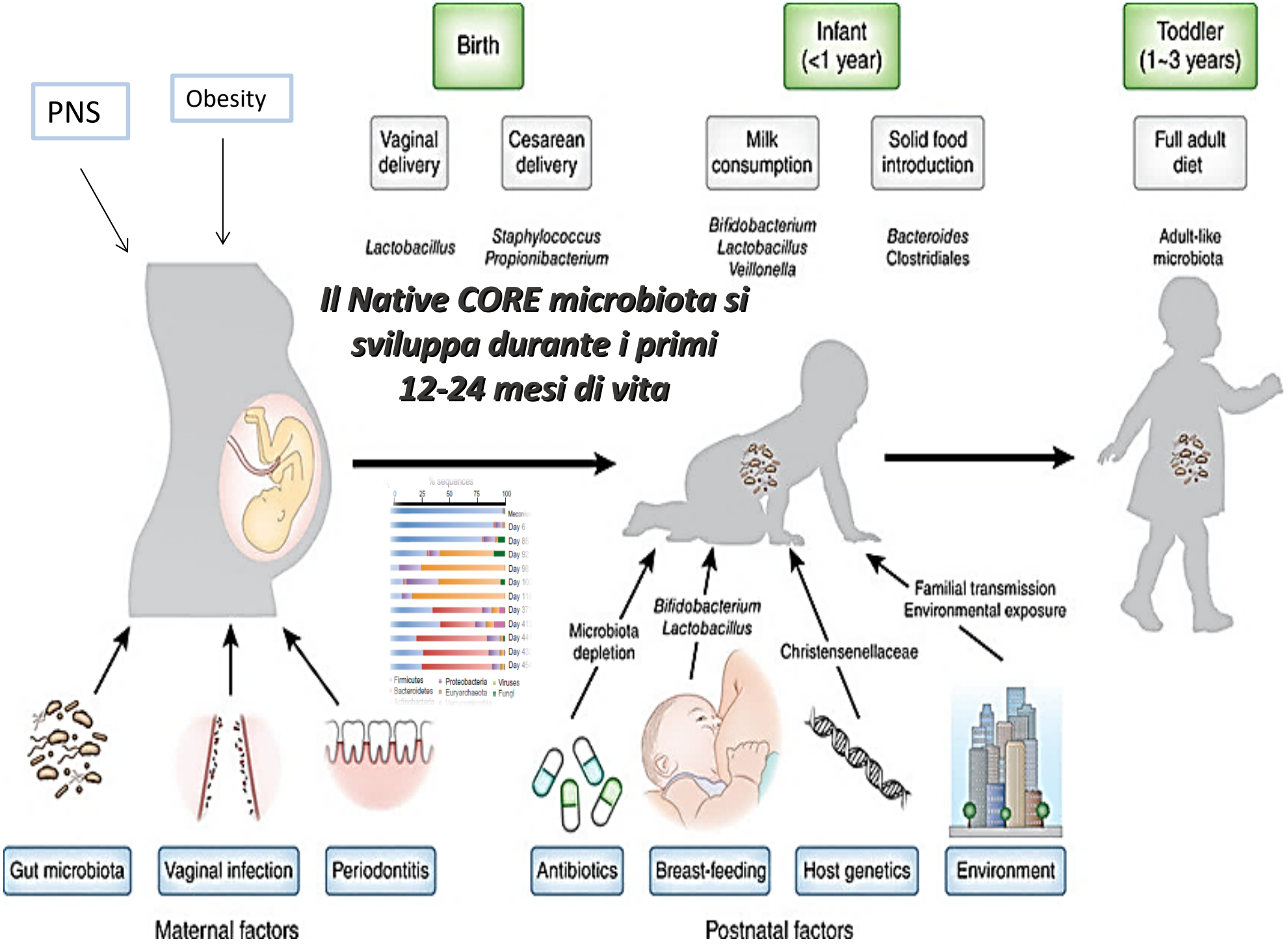


Figure 3.2. A theoretical model supported by empirical studies on microbial changes in the gut during pregnancy in lean versus obese women, and the consequences of maternal obesity on metabolic outcomes in offspring. The gut microbiota changes throughout pregnancy. Lean women, who are thought to possess a healthy microbial composition, maintain the same number of *Firmicutes* (green) and *Proteobacteria* (red) during pregnancy, paired with an increase in *Bacteroidetes* (blue) and *Actinobacteria* (yellow). Obese women with an aberrant gut microbiota, namely increased numbers of *Firmicutes* and *Actinobacteria* and decreased *Bacteroidetes*, display additional unfavourable modifications during pregnancy. *Actinobacteria* and *Firmicutes* further increase, whereas *Proteobacteria* and *Bacteroidetes* stay the same. The abnormal composition confers a disrupted intrauterine environment potentially leading to intestinal dysbiosis in infants with an obese mother, compared to the infants of lean mothers, consequently predisposing these children to adverse metabolic outcomes (Gohir *et al.*, 2015; Ollberding *et al.*, 2015; Scheepers *et al.*, 2015; Serino *et al.*, 2016). (Figure adapted from Gohir *et al.* (2015); reproduced with permission from Elsevier).

- During pregnancy is a period where we really need management of the mother's microbiome. We need to be realising that she's going to pass that on. And what that looks like is really important for what the child will have in early life and the prognosis, then, for health versus disease as that child ages.


PNS
Obesity
Birth
Infant (<1 year)
Toddler (1~3 years)
Vaginal delivery
Cesarean delivery
Milk consumption
Solid food introduction
Full adult diet
Lactobacillus
Staphylococcus
Propionibacterium
Bifidobacterium
Lactobacillus
Veillonella
Bacteroides
Clostridiales
Adult-like microbiota
Il Native CORE microbiota si sviluppa durante i primi 12-24 mesi di vita
Microbiota depletion
Bifidobacterium
Lactobacillus
Christensenellaceae
Familial transmission
Environmental exposure
Gut microbiota
Vaginal infection
Periodontitis
Antibiotics
Breast-feeding
Host genetics
Environment
Maternal factors
Postnatal factors

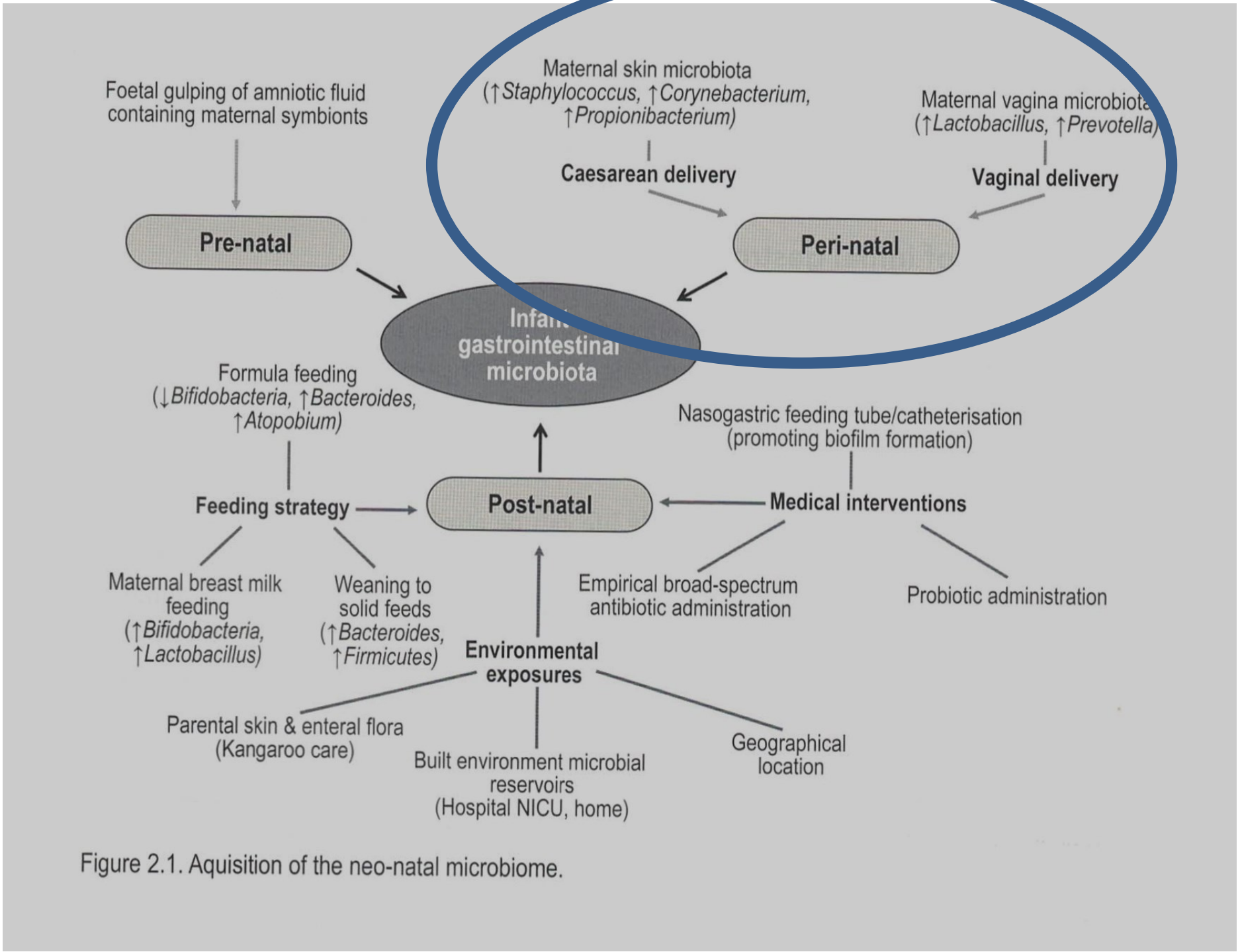


Figure 2.1. Aquisition of the neo-natal microbiome.

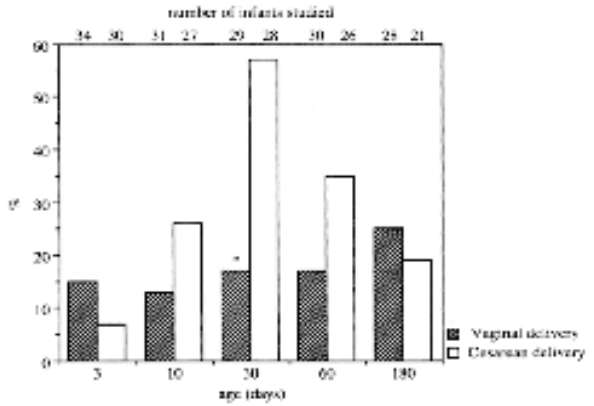
- The WHO recommendation that population rates of CS not exceed 15% has been in effect for almost 30 years (WHO, 1985).
- This benchmark remains appropriate to protect the woman and foetus from life-threatening complications, and also to prevent excess maternal and neonatal morbidity and mortality (Althabe and Belizan, 2006; Karlstrom et al., 2013).

- The consequences of CS birth should not be underestimated.
- Women undergoing CS are at greater risk for haemorrhage and uterine rupture (Armson, 2007; Liu et al., 2007), and their newborns have higher rates of respiratory distress and infection (Karlstrom et al., 2013; Magnus et al., 2011).

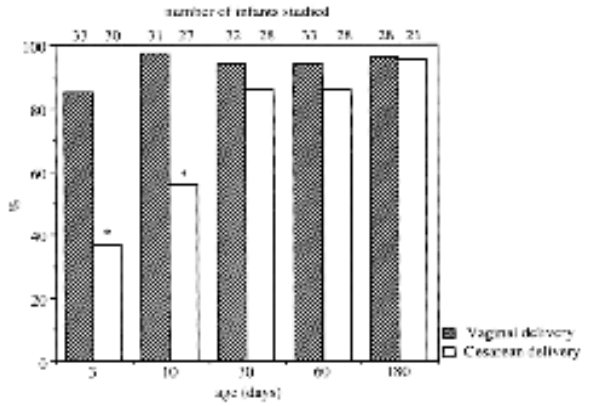
- Beyond maternal and neonatal risks, a number of risks to child health have been linked to CS birth, including food allergy (Sanchez-Valverde et al., 2009), asthma (Huang et al., 2015) and overweight (Li et al., 2013).

TC E COLONIZZAZIONE INTESTINALE

I neonati nati da taglio cesareo hanno un ridotto numero di *Bacteroides* e *Bifidobatteri*, mentre sono maggiormente colonizzati da *Clostridium difficile*, rispetto ai nati da parto spontaneo



The percentage of *Clostridium perfrigens* colonization *$p = 0.003$.



The percentage of *Bifidobacterium*-like bacteria (BLB) colonization *$p < 0.001$.

enders J et al. Pediatrics 2006

rolund el at. JPGN 1999

- Emergency and elective caesarean differ by indication, with foetal distress a common feature in the former. As well, many emergency caesareans are performed after a trial of labour, exposing newborns to maternal vaginal microbes.

- In the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, this difference was independent of breastfeeding status (Azad et al., 2016).
- Based on culture methods, a higher percentage of neonates were colonised with Citrobacter freundii and S. aureus one week after emergency than elective CS (or vaginal birth) in the COPSAC2010 cohort

- Klebsiella species were also found to be more abundant in the newborn gut following CS in the GUSTO cohort (Dogra et al., 2015). Finally, Enterococcus becomes more abundant in the infant gut at one or three months following emergency and elective CS (Azad et al., 2016; Stokholm et al., 2016); Stokholm et al. (2016) also reported greater colonisation of the infant hypopharyngeal microbiome with Enterococcus faecalis three months after CS delivery.

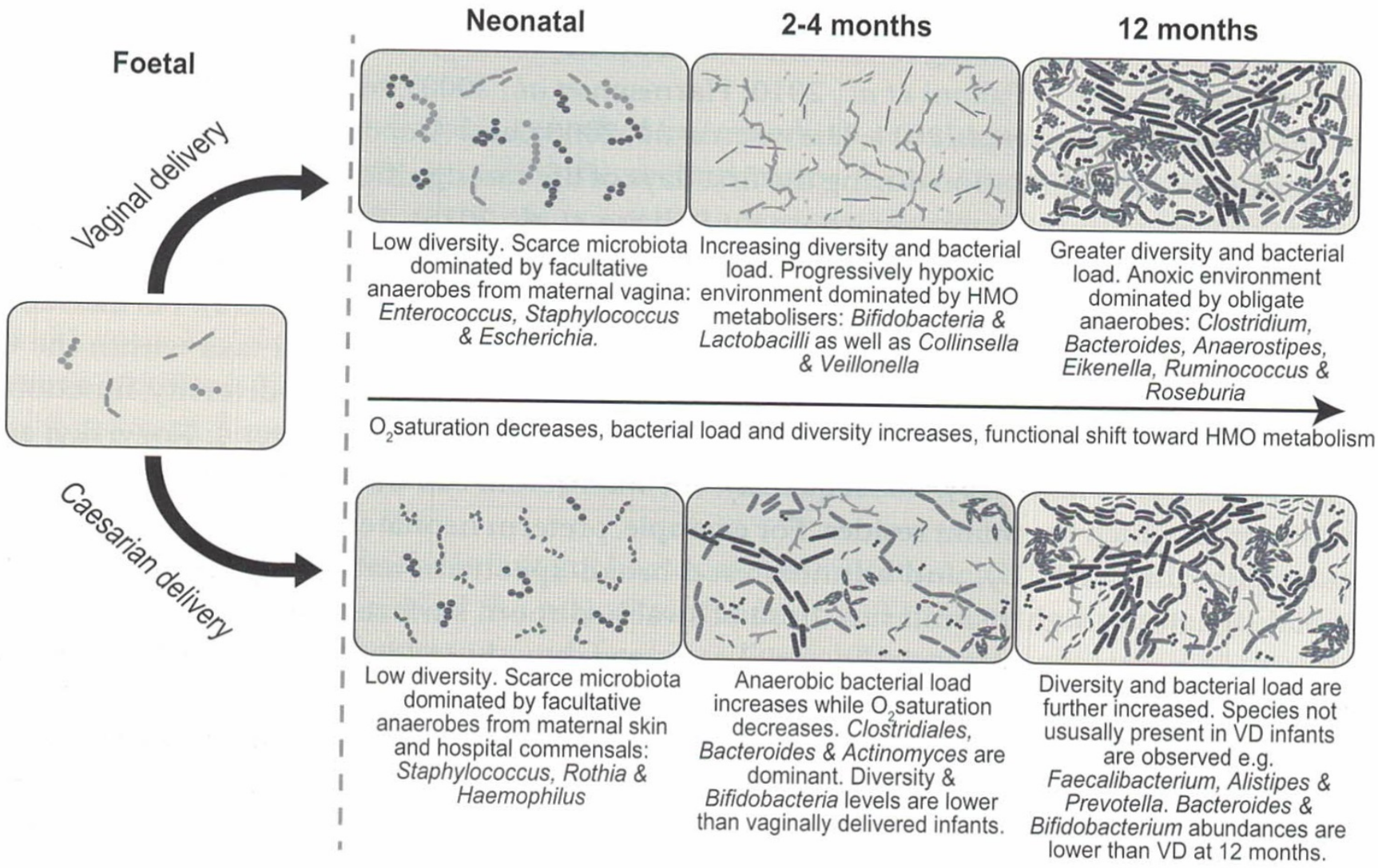


Figure 2.2. Signature of bacterial taxa per infant developmental stage.

Rutayisire et al. BMC Gastroenterology (2016) 16:86
DOI 10.1186/s12876-016-0498-0

BMC Gastroenterology

RESEARCH ARTICLE Open Access

The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review

Erigene Rutayisire[1], Kun Huang[1,2], Yehao Liu[3] and Fangbiao Tao[1,2*]

il taglio cesareo influenza il microbiota:

- basse prevalenza e diversità di *Bacteroides* e *Actinobacteria*
- alta prevalenza di *Firmicutes* fino ai 3 mesi di età

I neonati da TC sono più colonizzati da *Lactobacillus* e *Clostridium (Firmicutes)*

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-13014-7 OPEN

Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life

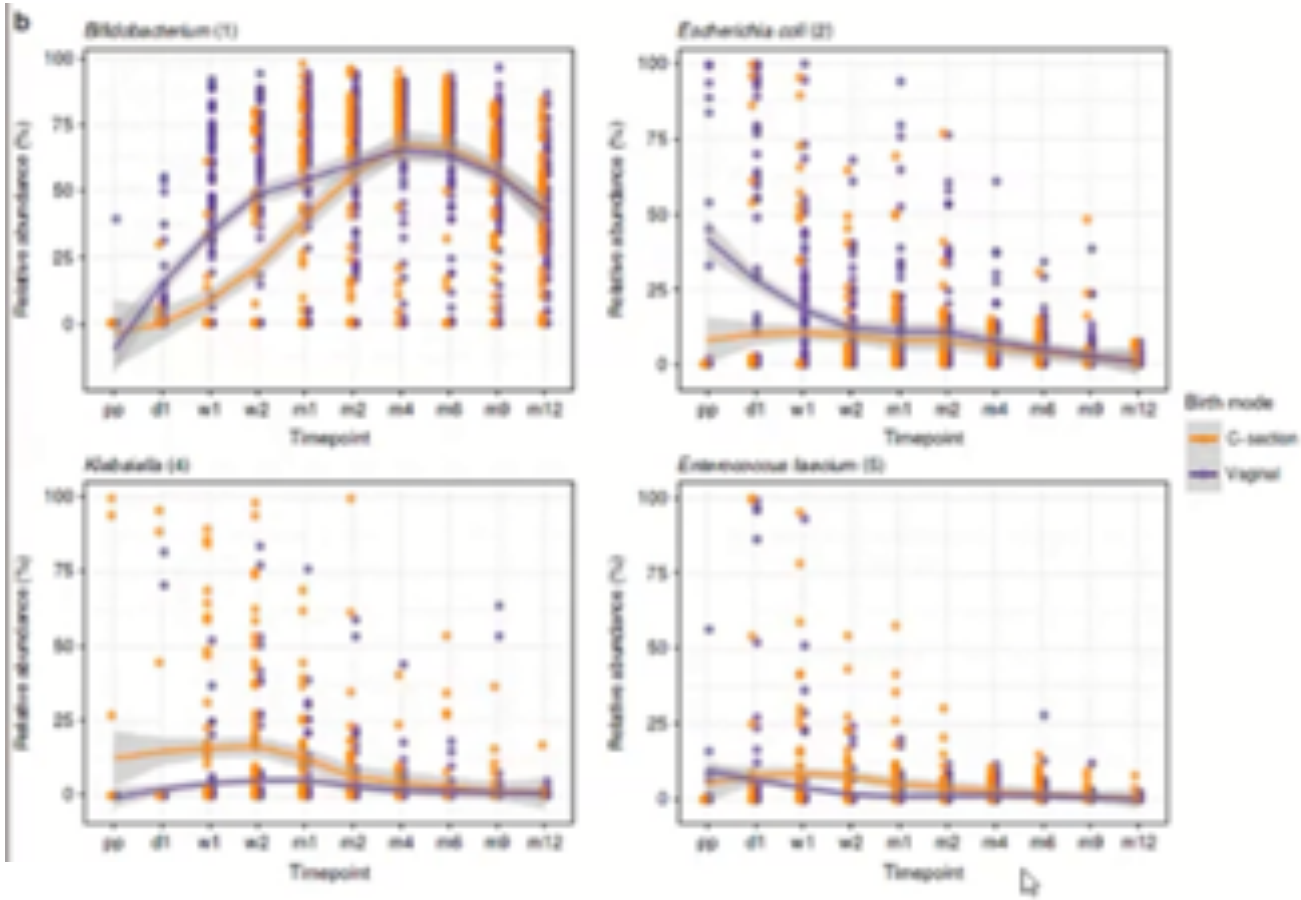
Marta Reyman[1,2], Marlies A. van Houten[2], Debbie van Baarle[3], Astrid A.T.M. Bosch[1], Wing Ho Man[1,2], Mei Ling J.N. Chu[1], Kayleigh Arp[1], Rebecca L. Watson[4], Elisabeth A.M. Sanders[1,3], Susana Fuentes[3,5] & Debby Bogaert[1,4,5*]

NATURE COMMUNICATIONS | (2019)10:4997 | https://doi.org/10.1038/s41467-019-13014-7 | www.nature.com/naturecommunications

120 pazienti, 74 PS 46 TC

Somministrazione di ATB alla madre dopo il clampaggio del cordone

- Concordanza del microbiota fecale del neonato con quello materno rispetto a quello di altre madri molto più alto nel gruppo PS, per l'intero anno di studio, a differenza del neonato nato da TC.
- Differenze nel microbiota tra TC e PS con effetto massimo ad una settimana di vita
- La differenza tra i due gruppi permane a due settimane anche tra gli allattati con formula (eliminazione confondente alimentazione), con maggior presenza di Bifidobatteri e minor presenza di *Klebsiella* e *Staphylococcus* nei nati da PS.
- L'abbondanza di bifidobatteri è associata all'età gestazionale, al parto spontaneo, all'allattamento al seno.
- L'allattamento al seno non compensa completamente la nascita per TC: i nati da TC allattati al seno hanno comunque meno bifidobatteri rispetto ai nati da PS allattati con formula.
- I nati da PS hanno anche più Bacteroides rispetto ai nati da TC.



Bifidobatteri più abbondanti in PS rispetto a TC per i primi 30 giorni, anche quando corretto per il fattore allattamento.
E. coli più abbondante nei neonati PS che nei TC per i primi 85gg
Klebsiella più abbondante nei TC fino al giorno 139
Enterococcus più presente nei TC tra 7 e 35 gg

Dominguez-Bello, M. G. et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl Acad. Sci. USA* **107**, 11971–11975 (2010).
Sevelsted, A., Stokholm, J., Bonnelykke, K. & Bisgaard, H. Cesarean section and chronic immune disorders. *Pediatrics* **135**, e92–e98 (2015).
Mueller, N. T. et al. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *Int. J. Obes.* **39**, 665–670 (2015).
Betrán, A. P. et al. The increasing trend in caesarean section rates: global, regional and national estimates: 1990-2014. *PLoS One* **11**, e0148343 (2016).
Chu, D. M. et al. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat. Med.* **23**, 314–326 (2017).

La nascita da taglio cesareo è stata associata ad effetti avversi sulla maturazione del sistema immune, predisponendo all'insorgenza di infezioni, allergie, manifestazioni atopiche, malattie infiammatorie, obesità.

PEDIATRICS
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Article

Cesarean Section and Chronic Immune Disorders

Astrid Sevelsted, Jakob Stokholm, Klaus Bønnelykke and Hans Bisgaard

Pediatrics January 2015, 135 (1) e92-e98; DOI: https://doi-org.bvsp.idm.oclc.org/10.1542/peds.2014-0596

Registro nazionale danese
35 anni: 1977-2012

RESULTS: Children delivered by cesarean delivery had significantly increased risk of asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel disease, immune deficiencies, and leukemia. No associations were found between cesarean delivery and type 1 diabetes, psoriasis, or celiac disease.

Clinical Epidemiology

Dovepress

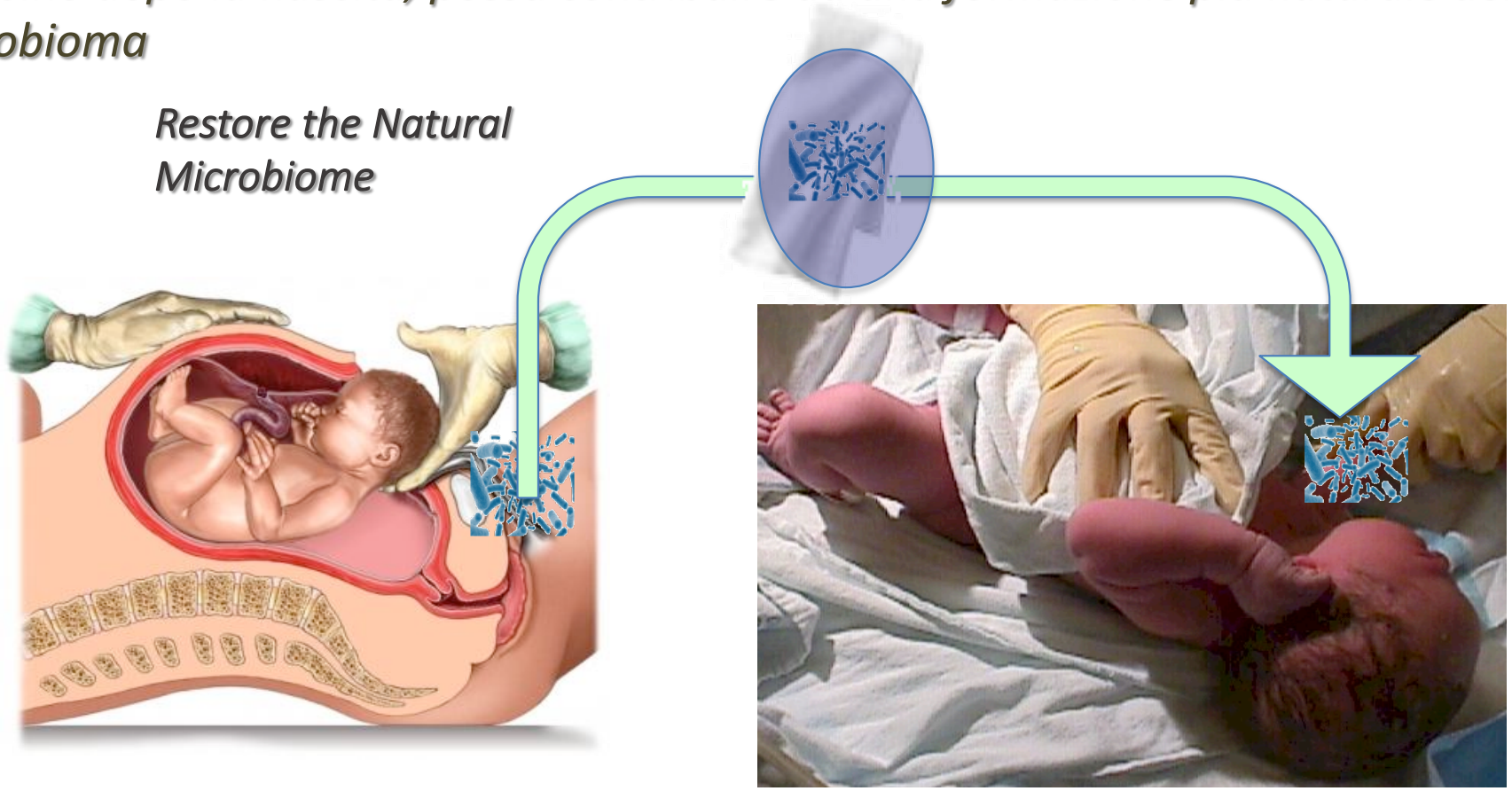
ORIGINAL RESEARCH

Caesarean Delivery and Risk of Chronic Inflammatory Diseases (Inflammatory Bowel Disease, Rheumatoid Arthritis, Coeliac Disease, and Diabetes Mellitus): A Population Based Registry Study of 2,699,479 Births in Denmark During 1973–2016

Vibeke Andersen[1–3]
Søren Möller[4]
Peter Bjødstrup Jensen[4]
Frederik Trier Møller[5,6]
Anders Green[4]

- Aumento di rischio di diabete, artrite, malattia celiaca, malattie infiammatorie intestinali
- Sia nei maschi che nelle femmine
- Sia TC elettivo sia TC urgente
- Il rischio più elevato è a 40 anni dal parto

*Per ovviare alla mancanza di batteri dei bambini nati con taglio cesareo, alcuni ricercatori fanno uno studio con lo scopo di verificare se una **semina artificiale**, tramite una garza che viene incubata un'ora prima dell'intervento nel canale vaginale della madre e poi strofinata sulla bocca, sul viso e sul resto del corpo del bambino dopo la nascita, possa contribuire ad una formazione più naturale del microbioma*



www.brooksidepress.org

Dr. Maria Gloria Dominguez-Bello, associate professor in the Human Microbiome Program at the NYU School of Medicine

http://commonhealth.wbur.org/2014/06/birth-canal-bacteria-c-section

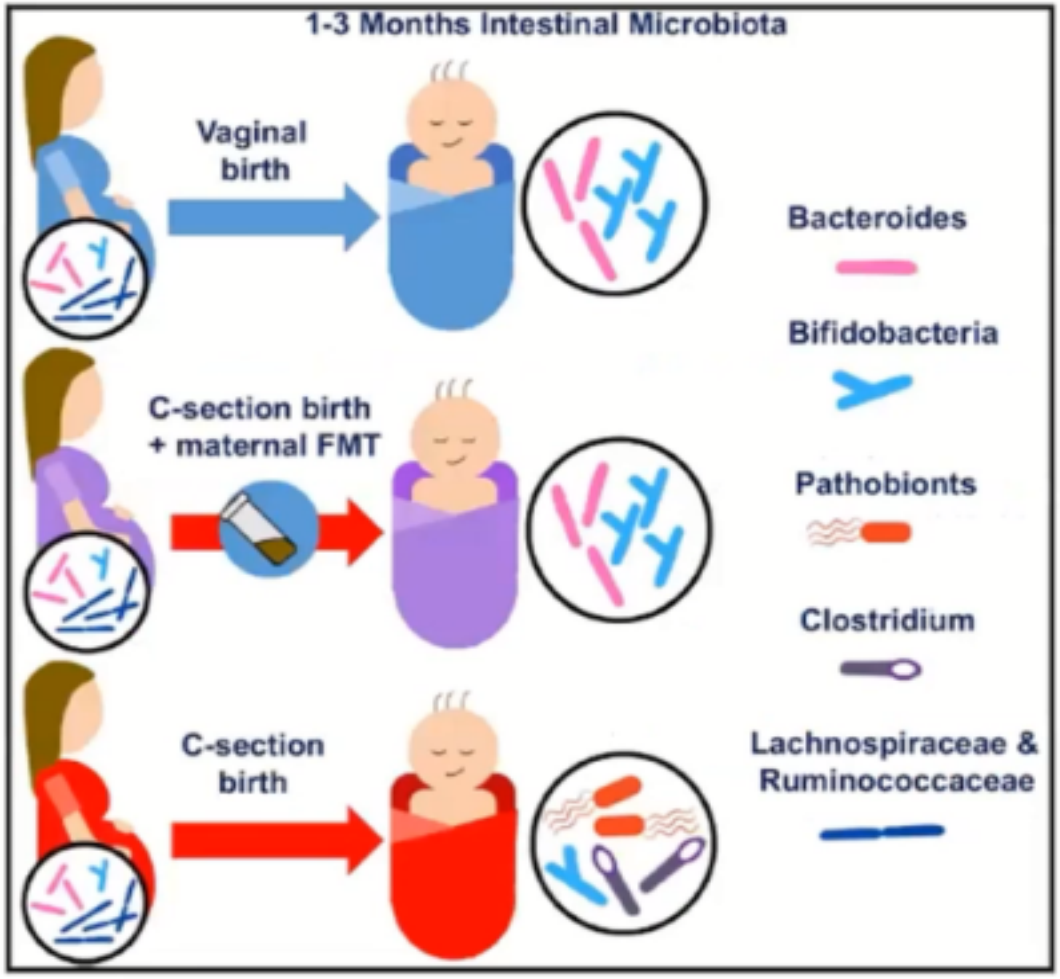
CellPress

Cell

Article

Maternal Fecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study

Katri Korpela,[1,7] Otto Helve,[2,7] Kaija-Leena Kolho,[2,3] Terhi Saisto,[4] Kirsi Skogberg,[5] Evgenia Dikareva,[1] Vedran Stefanovic,[4] Anne Salonen,[1] Sture Andersson,[2,8] and Willem M. de Vos[1,6,8,9,*]



Il trapianto fecale materno rende il microbiota dei bambini nati per cesareo simile a quello dei bambini nati per parto spontaneo dal 7° giorno (incremento di *Bacteroides* e *Bifidobatteri*)

E. faecium, E. faecalis, E. cloacae, K. pneumoniae, K. oxytoca, H. influenzae, C. jejuni, S. enterica più elevati nel gruppo TC ma non nel gruppo FMT

La metodica porta a risultati diversi da quelli ottenuti mediante vaginal seeding/swabbing (più simile a quelli dei bambini nati per TC)

Korpela et al. Microbiome (2018) 6:182
https://doi.org/10.1186/s40168-018-0567-4

Microbiome

RESEARCH Open Access

Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants

Katri Korpela[1,2*], Anne Salonen[1], Outi Vepsäläinen[3], Marjo Suomalainen[3], Carolin Kolmeder[4], Markku Varjosalo[5], Sini Miettinen[5], Kaarina Kukkonen[6], Erkki Savilahti[7], Mikael Kuitunen[7] and Willem M de Vos[1,8]

La somministrazione di una miscela di probiotici (*B.breve, L. rhamnosus, P. freundenreichii*) può mitigare ed addirittura annullare le differenze descritte nei gruppi

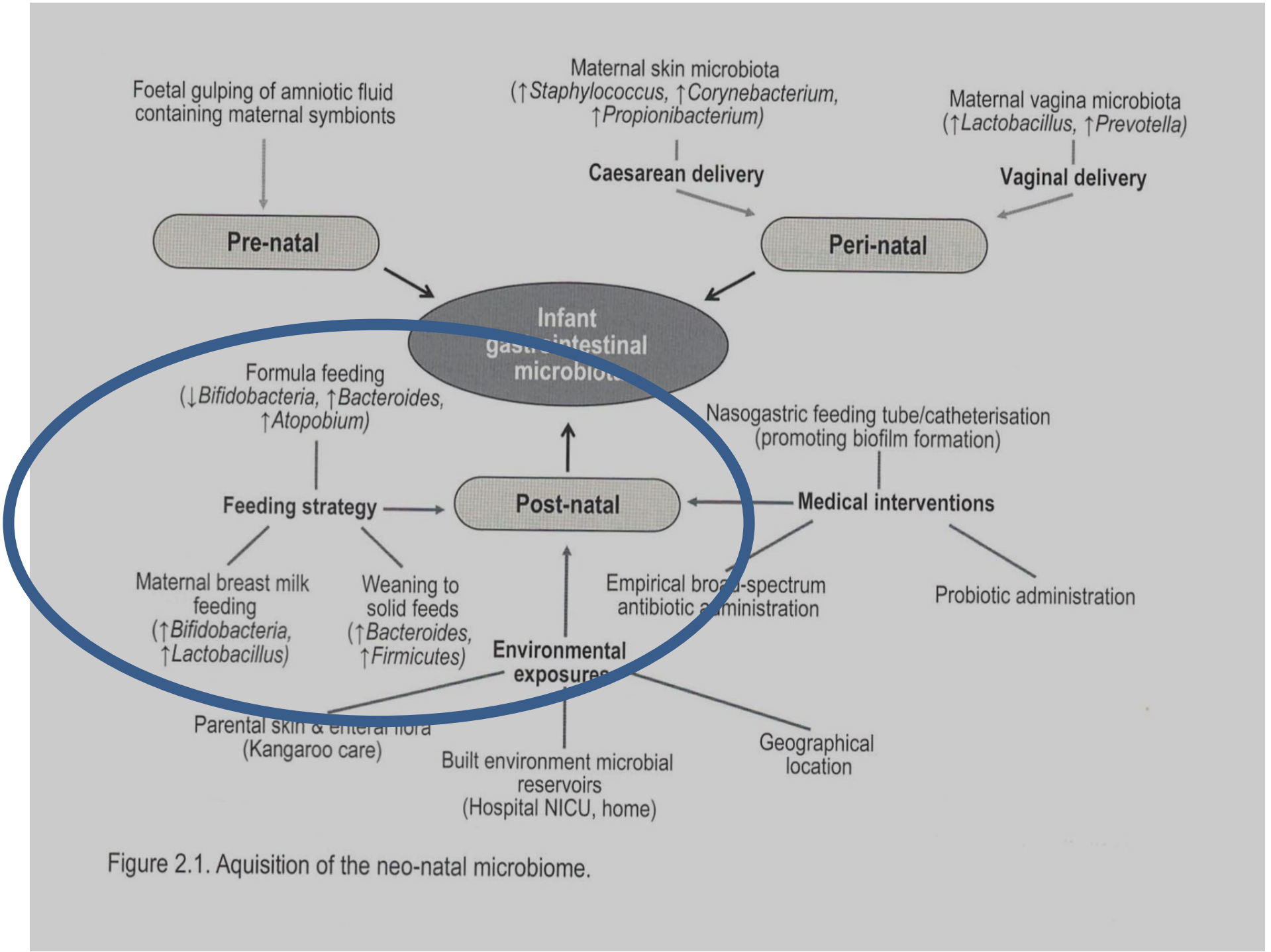


Figure 2.1. Aquisition of the neo-natal microbiome.

- Also, contrary to WHO guidelines, less than 30% of infants in middle-income countries are exclusively breastfed for the first 5 months of life (Victora et al., 2016), and less so if they are born by CS (Al-Sahab et al., 2010)

THE HUMAN MILK MICROBIOTA: ORIGIN AND POTENTIAL ROLES IN HEALTH AND DISEASE

L. Fernández, S. Langa, V. Martín, A. Maldonado, E. Jiménez, R. Martín, J. M.Rodríguez

Pharmacological Research Volume 69, Issue 1, March **2013**, Pages 1-10
https://doi.org/10.1016/j.phrs.2012.09.001

IL LATTE MATERNO rappresenta un rifornimento continuo di batteri commensali, mutualistici e / o potenzialmente probiotici nell'intestino infantile.

Questi hanno rivelato il predominio di stafilococchi, streptococchi, batteri dell'acido lattico e bifidobatteri in questo fluido biologico e il loro ruolo sulla colonizzazione dell'intestino infantile.

- Alcuni batteri presenti nell'intestino materno potrebbero raggiungere la ghiandola mammaria durante la tarda gravidanza e l'allattamento attraverso un meccanismo che coinvolge i monociti dell'intestino.
- *Ceppi selezionati isolati dal latte materno possono essere buoni candidati per l'uso come probiotici*.

Maternal obesity, environmental factors, cesarean delivery and breastfeeding as determinants of overweight and obesity in children: results from a cohort

Daniel S Portela1*, Tatiana O Vieira2, Sheila MA Matos3, Nelson F de Oliveira2 and Graciete O Vieira2
Portela et al. BMC Pregnancy and Childbirth (2015) 15:94 DOI 10.1186/s12884-015-0518-z

L'allattamento al seno è stato visto come un fattore protettivo contro obesità, tra cui alcune prove che dimostrano che questa protezione aumenta con il tempo trascorso l'allattamento al seno.

… il sistema metabolico è stato aggiustato, per mezzo del latte

materno, riprogrammando quei neonati che non avevano avuto contatto con il microbiota vaginale della madre durante la nascita.

Mother's Milk: A Purposeful Contribution to the Development of the infant Microbiota and immunity

K. Le Doare, B. Holder, A. Bassett, P.S. Pannaraj Frontiers in Immunology | www.frontiersin.org February 2018 | Volume 9 | Article 361

*Gli HMO (*oligosaccaridi del latte umano) *sono carboidrati complessi solubili che sono sintetizzati nelle ghiandole mammarie dipendenti dal genotipo materno, compresi i geni determinante l'antigene del gruppo sanguigno.*

Gli oligosaccaridi del latte umano (HMO) possono influenzare ulteriormente la costituzione di un microbioma sano:

- legando i batteri potenzialmente nocivi,
- modulando la risposta immunitaria
- promuovono la crescita di Batteri buoni come i

Bifidobacterium infantis prevenendo così le infezioni

- impedendo l'attaccamento alle cellule ospiti, prevenendo così l'adesione e l'invasione dei patogeni
- effetti antimicrobici diretti su alcuni patogeni
- modulano le risposte delle cellule epiteliali intestinali e agiscono da modulatori immunitari
- alterano l'ambiente del villo intestinale, riducendo la crescita cellulare e inducendo differenziazione e apoptosi
- alterano le risposte immunitarie spostando le risposte delle cellule T ad una produzione di citochine Th1 / Th2 bilanci*ata*

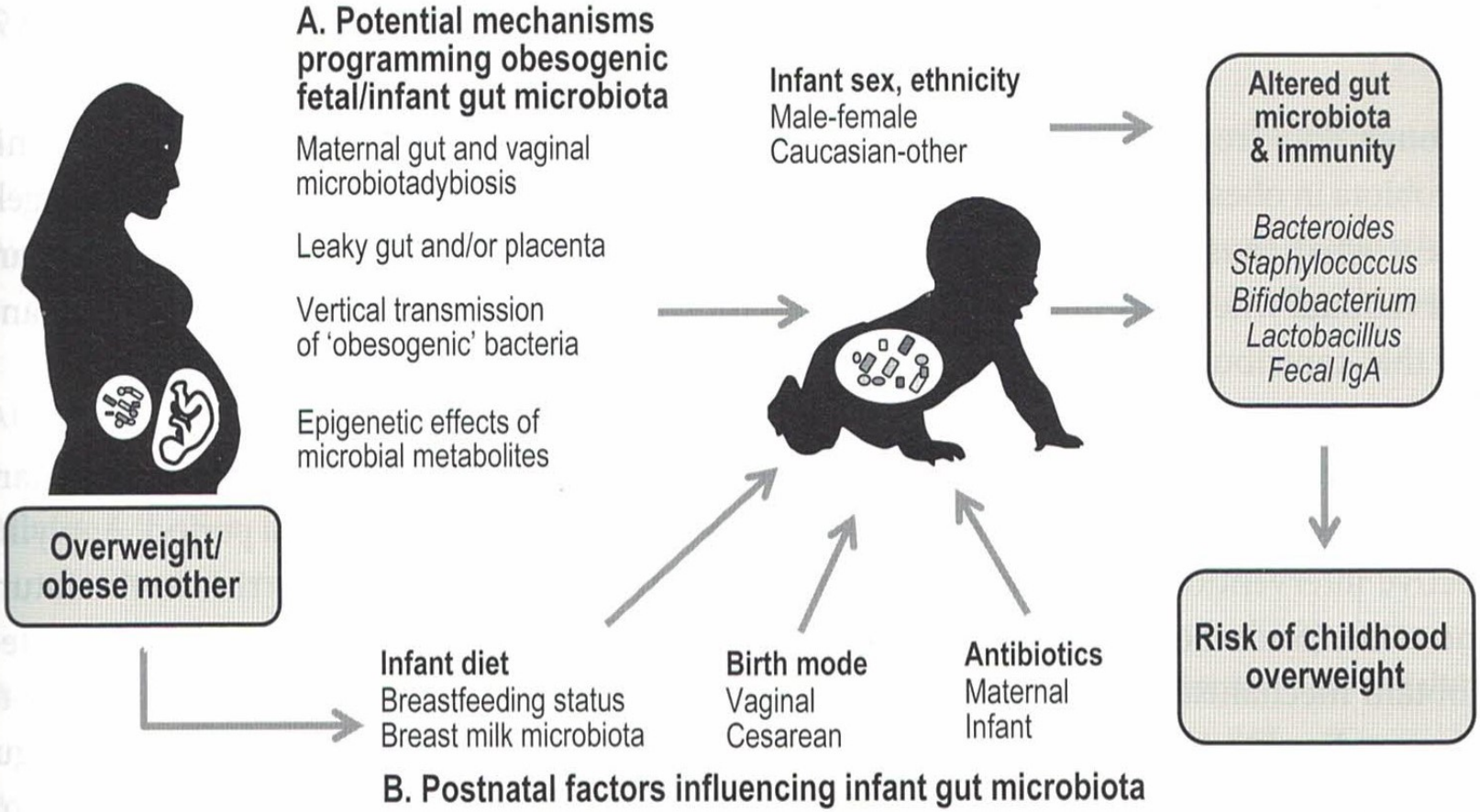


Figure 3.3. The figure presents an overview of the complex interactions between the diverse factors influencing the infant gut microbiota perinatally, and possibly leading to childhood overweight and obesity. A) Potential mechanisms involved in the programming of obesogenic foetal and infant gut microbiota, and B) Postnatal factors influenced by maternal overweight that may modify infant gut microbiota. (Adapted from Kozyrskyj *et al., 2016;* Reproduced with permission from Cambridge University Press).

- Antibiotics is a very meaningful, descriptive word. It means "anti life". Life of microbes. Westernised lifestyle is very anti microbiotics, against the healthy microbiome because a lot of practices are antimicrobial and affect our diversity.

ANTIBIOTICI 80% farmaci prescritti in GRAVIDANZA

ANTIBIOTICI INTRAPARTUM 40% GRAVIDE

TAGLIO CESAREO
IN ITALIA 33.7%

Bolzano 18%,
Campania 53.4%

OMS → 15%

GBS
25-35% donne Italiane

PROM

15% gravidanze a termine

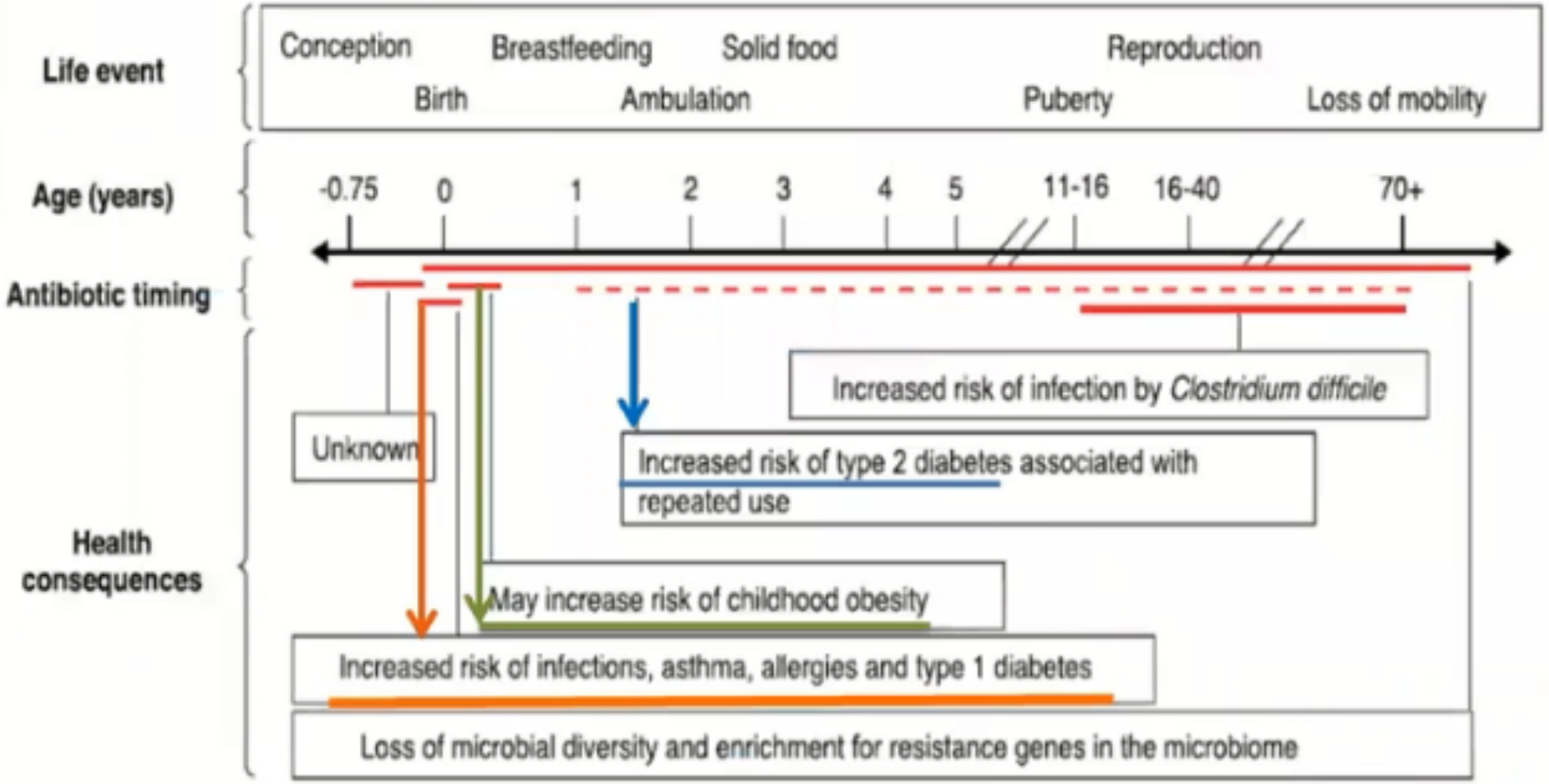
pPROM

Langdon et al. Genome Medicine (2016) 8:39
DOI 10.1186/s13073-016-0294-z

Genome Medicine

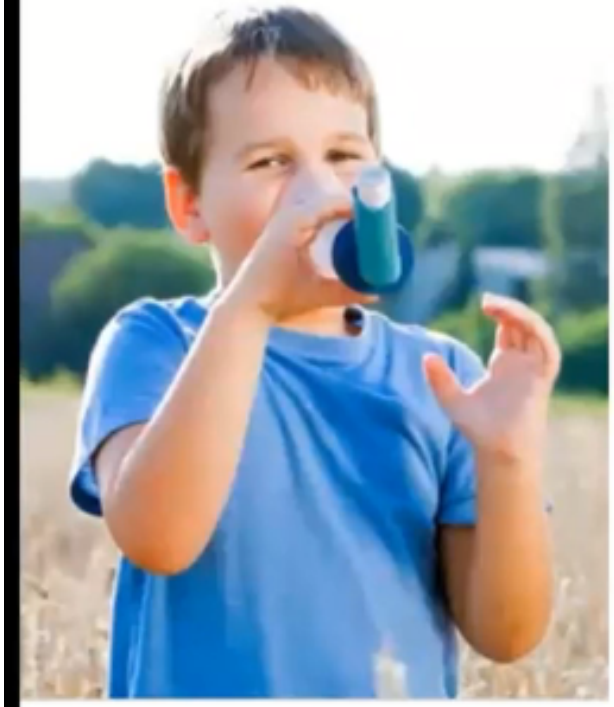
REVIEW Open Access

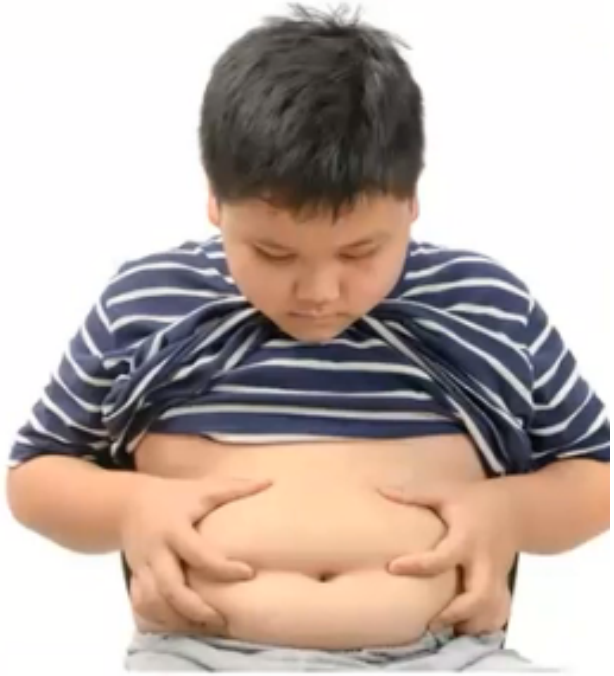
The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation



- In the long-term, infant antibiotic treatment is associated with childhood asthma and obesity (Azad et al., 2014; Penders et al., 2011), conditions also linked to gut dysbiosis in early life (Penders et al., 2007; Vael et al., 2011).
- In the preterm context (mean gestational age of 30 weeks, primarily CS birth and hospitalisation for 50 days), Arboleya et al. (2015) observed several changes to gut microbial composition following maternal IAP.

ANTIBIOTICO





ASMA

SOVRAPPESO e OBESITA'

RESEARCH ARTICLE

Prenatal antibiotics exposure and the risk of autism spectrum disorders: A population-based cohort study

Amani F. Hamad[1], Silvia Alessi-Severini[1,2☯], Salaheddin M. Mahmud[1,3,4☯], Marni Brownell[2,3☯], Ifan Kuo[1]*

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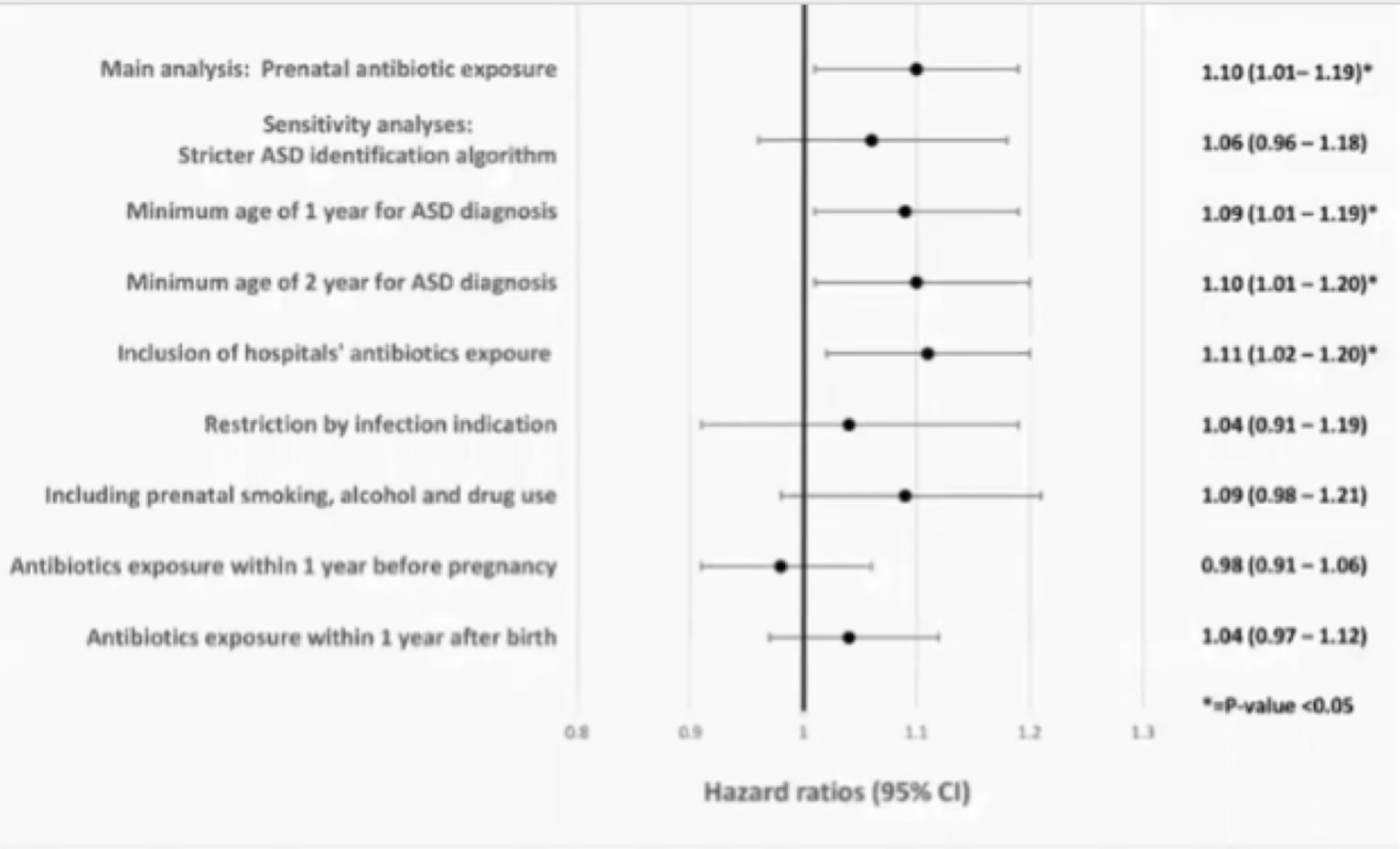
POPULATION BASED COHORT STUDY
ANTIBIOTICO PRENATALE e ASD

214834 nati in Manitoba CANADA-1998-2016
→80750 (37.6%) esposti ad **antibiotico prenatale**
→**2965 diagnosi ASD**
Rispetto ai non esposti ASD HR 1.10(95% CI 1.01-1.19)
→II trimestre HR 1.11(95% CI 1.010-1-23)
→ III trimestre HR 1.17 (95% CI 1.06-1.30)



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Journal of Infection 81 (2020) 190–204

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevier.com/locate/jinf

Review

The influence of prenatal and intrapartum antibiotics on intestinal microbiota colonisation in infants: A systematic review

T.H. Dierikx[a,b,*], D.H. Visser[c], M.A. Benninga[b], A.H.L.C. van Kaam[c], N.K.H. de Boer[d], R. de Vries[e], J. van Limbergen[b], T.G.J. de Meij[a,b]

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A total of 4.030 records were encountered. A total of 24 articles were included in the final anal- ysis. Infants from mothers exposed to antibiotics during delivery showed a decreased microbial diversity compared to non-exposed infants. The microbiota of infants exposed to antibiotics was characterised by a decreased abundance of *Bacteriodetes and Bifidobacteria , with a concurrent increase of Proteobacteria . These effects were most pronounced in term vaginally born infants.*

Published in final edited form as:
BJOG. 2020 January ; 127(2): 217–227. doi:10.1111/1471-0528.15799.

Specific class of intrapartum antibiotics relates to maturation of the infant gut microbiota: a prospective cohort study

MO Coker[a], AG Hoen[a,b], E Dade[a], S Lundgren[a], Z Li[c], AD Wong[d], MS Zens[a], TJ Palys[a], HG Morrison[e], ML Sogin[e], ER Baker[f], MR Karagas[a,b], JC Madan[a,f]

Table 2. Median relative abundance of dominant[†] taxa in faecal microbiota of infants at 3 months and 1 year, according to maternal IAP

Dominant taxa	Microbiota at 3 months (*n* = 176)				Microbiota at 1 year (*n* = 189)			
	no IAP Vaginal (82% BF) *n* = 96	IAP Vaginal (83% BF) *n* = 40	IAP Elective CS (78% BF) *n* = 17	IAP Emergency CS (80% BF) *n* = 23	no IAP Vaginal (50% BF) *n* = 108	IAP Vaginal (60% BF) *n* = 41	IAP Elective CS (39% BF) *n* = 16	IAP Emergency CS (38% BF) *n* = 24
Bacteroidetes	46.2	24.3*	0.4***	0.2***	55.0	54.2	52.1	38.1***
Bacteroidaceae	34.4	13.0*	0.3**	0.2***	46.9	44.8	45.6	33.1***
Firmicutes	20.1	16.8	42.6**	52.1***	32.5	36.0	27.6	48.0***
Clostridiales	12.8	12.7	27.6*	49.3***	30.2	32.8	25.7	43.4***
Clostridiaceae	0.08	0.96*	0.79	1.55**	0.10	0.24*	0.30	0.20*
Veillonellaceae	3.6	3.4	2.3	24.1***	5.0	4.4	2.3	3.6
Lachnospiraceae	1.8	0.9	5.9	0.2	13.1	13.5	13.0	22.4*
Ruminococcaceae	0.1	0.1	0.9˙	0.1	6.3	8.9	8.1	10.8*
Proteobacteria	15.5	21.9˙	25.9	30.0*	4.6	4.2	4.6	7.5*
Enterobacteriaceae	13.0	20.2˙	25.9	29.9*	1.0	1.0	0.7	1.7
Actinobacteria	5.4	4.8	8.0	4.0	1.6	2.4	1.3	1.4
Bifidobacteriaceae	5.3	4.7	7.8	3.9	1.6	2.4	1.3	1.4
Verrucomicrobia	0.01	0.00	0.00	0.00	0.01	0.01	0.10˙	0.03

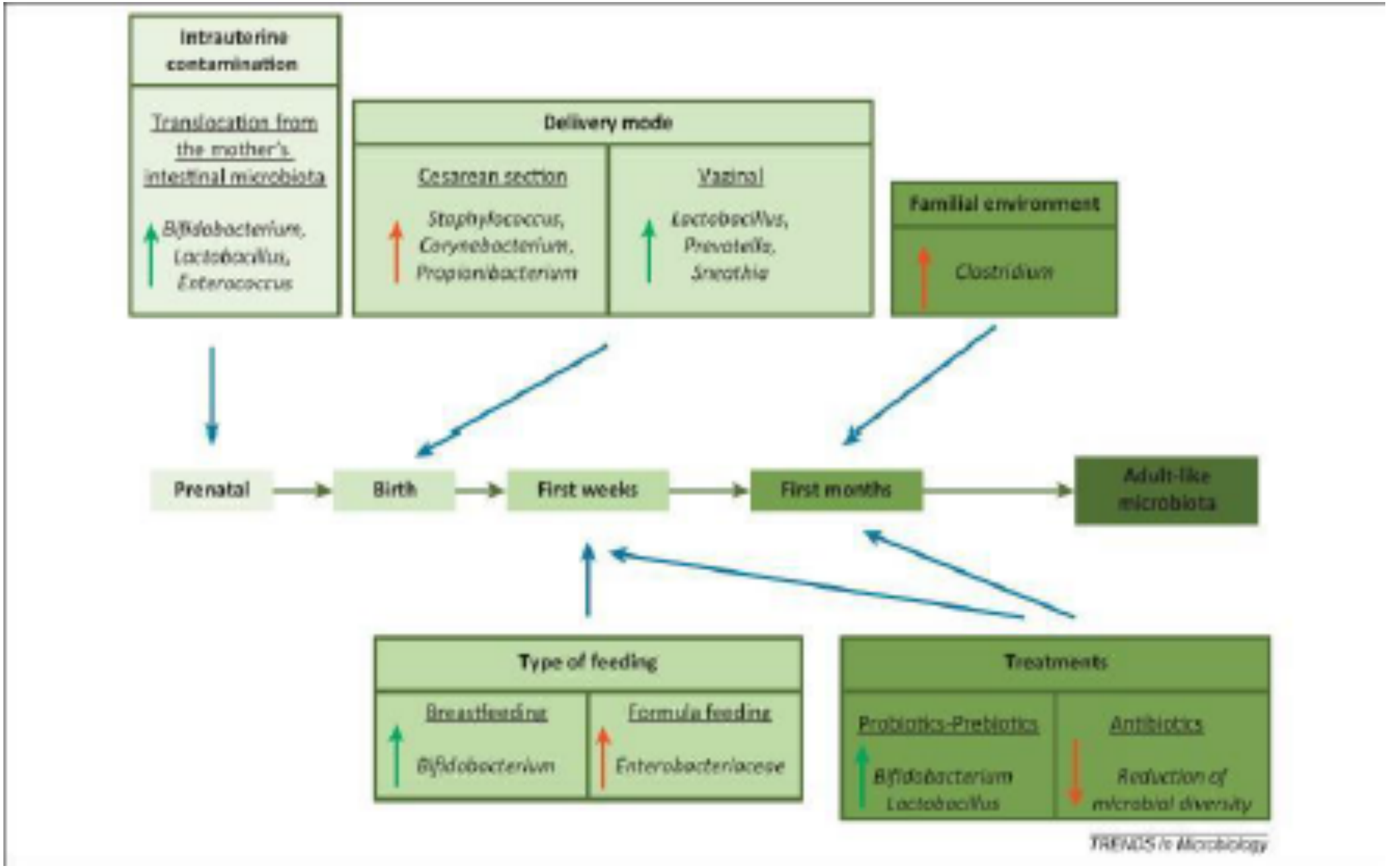
BF, any breastfeeding at sample collection (3 months or 1 year); CS, caesarean section; IAP, intrapartum antibiotic prophylaxis.

[†]Dominant taxa have overall median relative abundance >1% at 3 months and/or 1 year.

Comparisons by non-parametric Kruskal–Wallis test (versus no IAP, Vaginal). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ˙$P < 0.10$.

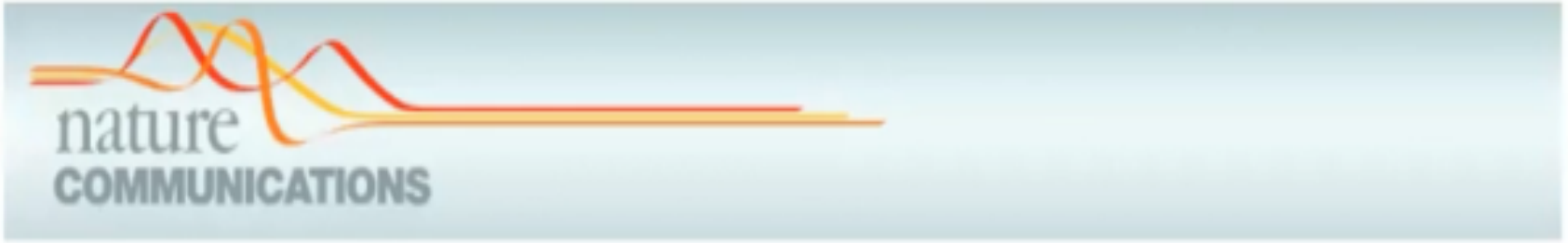
Bacteroidetes Firmicutes Proteobacteria Actinobacteria Verrucomicrobia Other.

Colours distinguish bacterial phyla and correspond to those used in Figure 1 and Table 3.



"Development of intestinal microbiota in infants and its impact on health"S. Matamoros et al., review in Trends in Microbiology, 2013.

E quanto a lungo dura tutto ciò?



ARTICLE

https://doi.org/10.1038/s41467-019-09252-4 OPEN

Perinatal factors affect the gut microbiota up to four years after birth

Fiona Fouhy[1,2], Claire Watkins[1,2], Cian J. Hill[1], Carol-Anne O'Shea[3], Brid Nagle[2], Eugene M. Dempsey[3,4], Paul W. O'Toole[1,5], R. Paul Ross[1,5], C. Anthony Ryan[1,3] & Catherine Stanton[1,2]

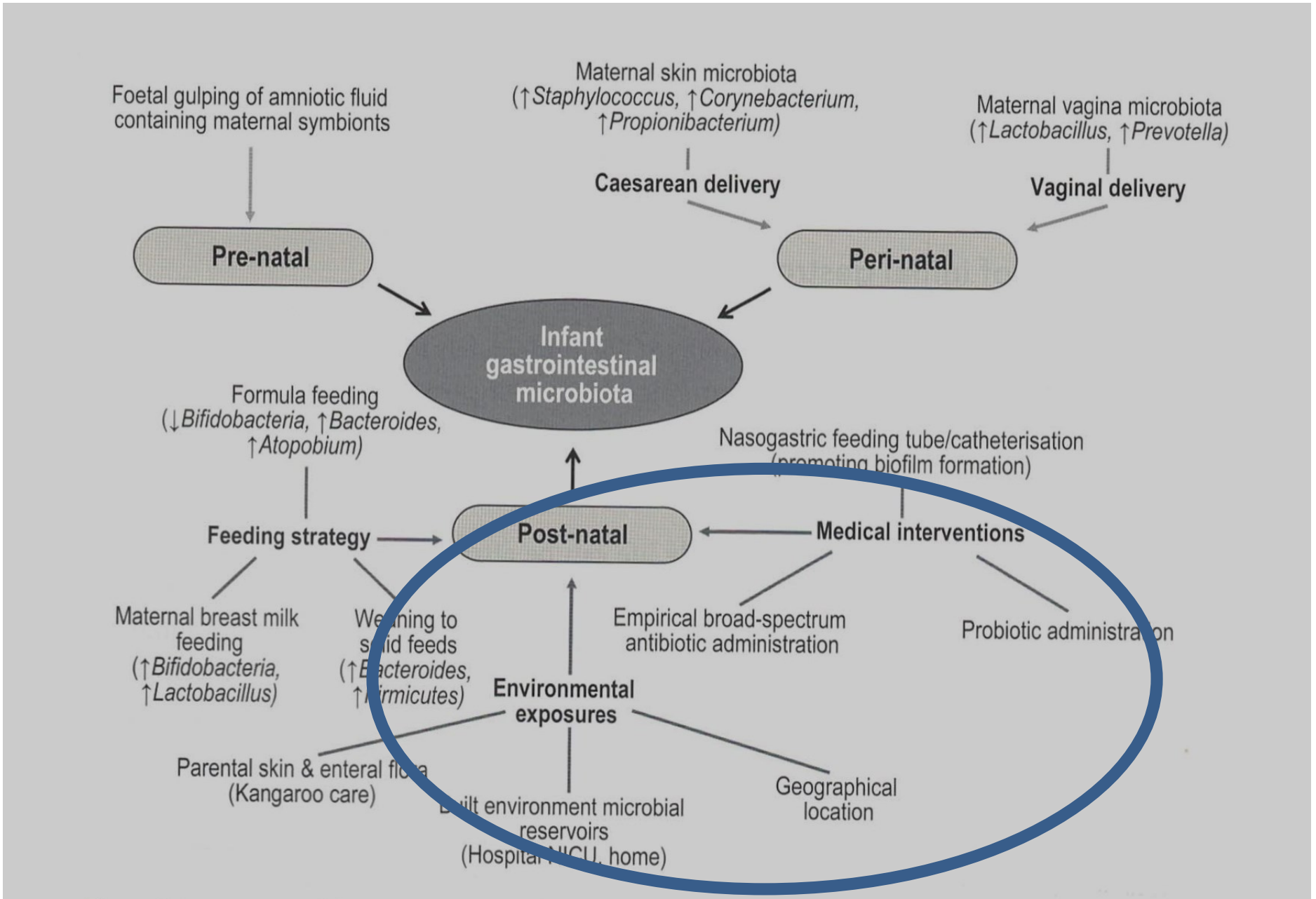
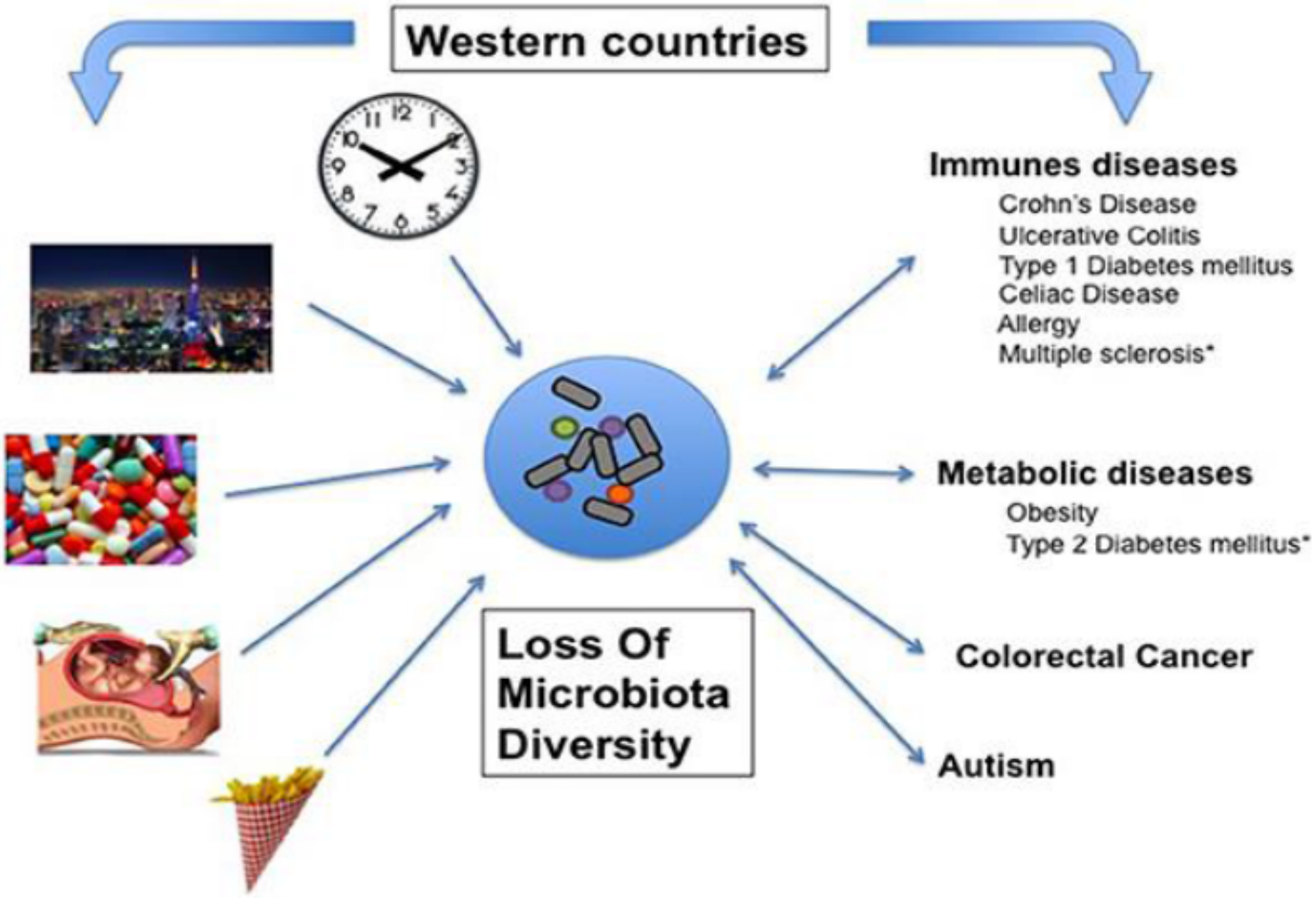


Figure 2.1. Aquisition of the neo-natal microbiome.


Western countries
Immunes diseases
Crohn's Disease
Ulcerative Colitis
Type 1 Diabetes mellitus
Celiac Disease
Allergy
Multiple sclerosis*
Metabolic diseases
Obesity
Type 2 Diabetes mellitus*
Colorectal Cancer
Autism
Loss Of Microbiota Diversity

FATTORI CHE MODIFICANO L'ECOSISTEMA INTESTINALE

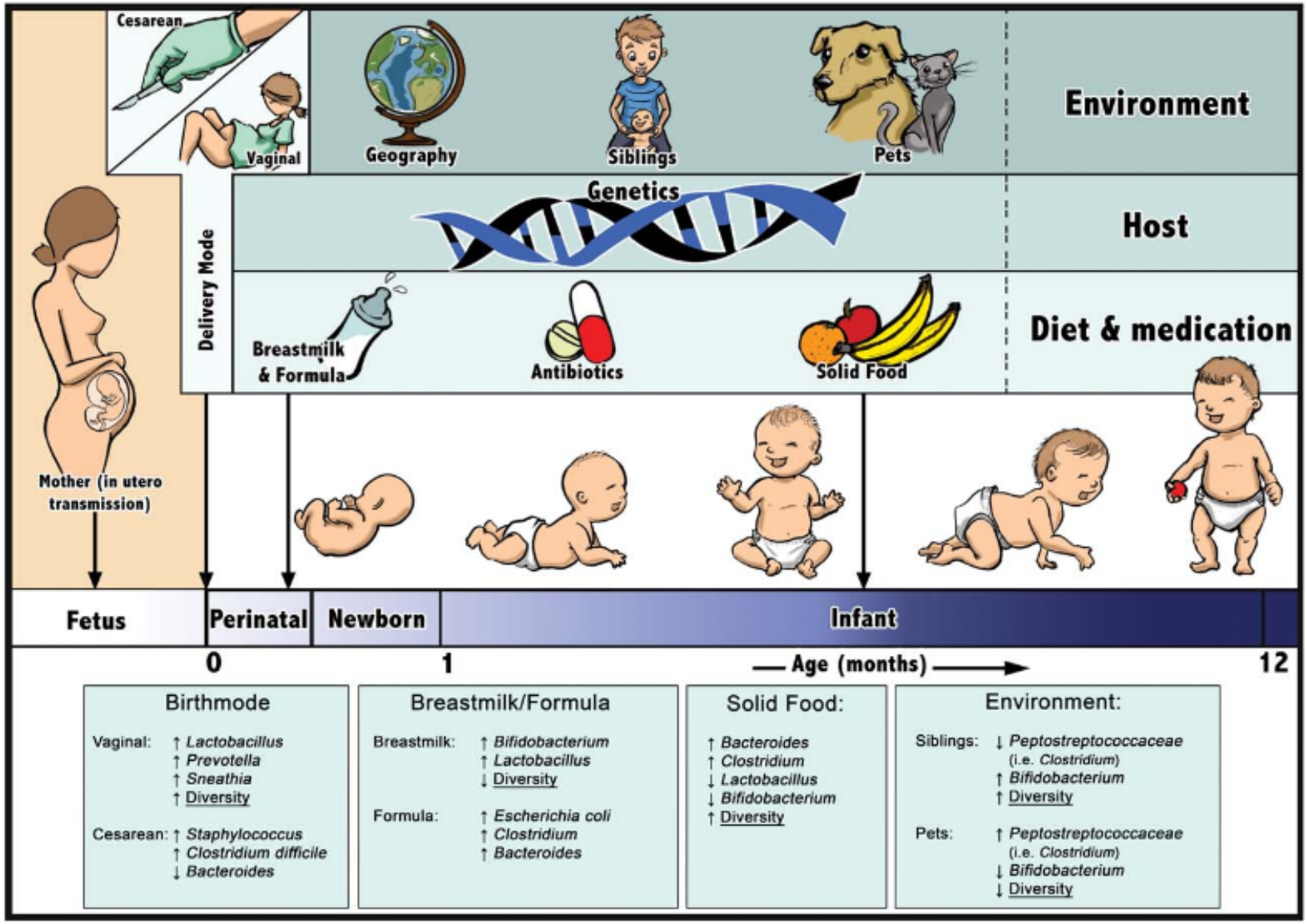


FIGURE 1. *Main maternal, environmental, and host determinants that impact the establishment of the intestinal microbiota during the first year of life. A non-exclusive listing of bacterial taxa affected by these determinants is depicted at the bottom of the figure. Note: the timeline is colored ranging from white to blue corresponding to a change in microbial diversity from low to high, respectively. Perinatal starts at rupture of the membranes.*

Grazie della attenzione !!

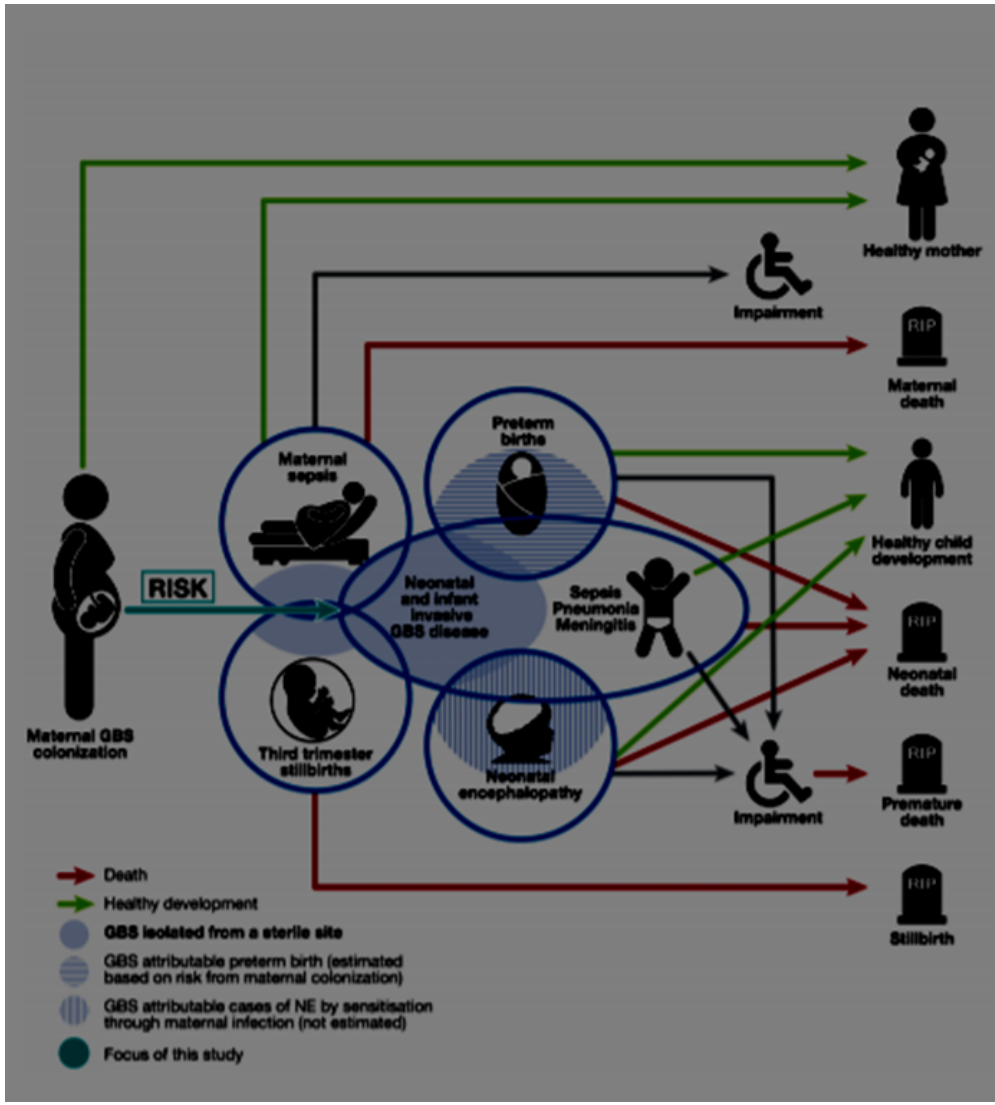
La sepsi neonatale è una infezione sistemica grave che può comportare non solo un esito infausto ma anche disabilità, morte precoce da disabilità, alterato sviluppo psicomotorio.
L'infezione materna a sua volta può comportare esiti negativi anche per le madri.

Si distingue in Early Onset (EOS), ad esordio nelle prime 72h (o 7 giorni) di vita e Late Onset (LOS) dopo le 72h (o 7 giorni)

FATEBENEFRATELLI

La trasmissione materno fetale del GBS: prevenzione e strategie di terapia a confronto

a cura di F. Focarile UO Pediatria e Neonatologia



Parameter	Case definitions used for estimates	Study
Group B *Streptococcus* maternal colonization	Isolation by culture of GBS from either the vagina (high or low), rectum or peri-anal region at any time during pregnancy	Russell et al. [23]
Maternal GBS disease	Laboratory isolation of GBS from sterile site in pregnant or postpartum woman (up to 42 days postpartum), with clinical signs of sepsis	Hall et al. [24]
Stillbirth GBS invasive disease	Birth of a fetus weighting >1000g and/ or ≥28 weeks' gestation age with no signs of life and evidence of GBS invasive disease from a normally sterile site such as fetal blood, lung aspirate or cerebrospinal fluid	Seale et al. [25]
Neonatal and infant GBS invasive disease	Laboratory isolation of Streptococcus agalactiae from a normally sterile site in an infant aged 0 to 89 days with signs of clinical disease, including meningitis, sepsis or bacteremic pneumonia	Madrid et al. [28]
Neonatal encephalopathy with invasive GBS disease	Laboratory isolation of GBS from a sterile site (blood, cerebrospinal fluid analysis or on a post mortem sample) in cases of neonatal encephalopathy, hypoxic-ischaemic encephalopathy and intrapartum-related death	Tann et al. [29]
Neurodevelopment impairment in children after GBS invasive disease	Cognitive and/or motor, vision or hearing impairment in survivors of invasive infant GBS disease isolated from a normally sterile site	Kohli-Lynch et al. [30]
Preterm birth associated with GBS maternal colonization	Delivery prior to completion of 37 weeks' gestation from mother with maternal GBS colonization isolated from vaginal, cervical and/or rectal swabs	Bianchi Jassir et al. [26]

La trasmissione materno fetale del GBS: prevenzione e strategie di terapia a confronto

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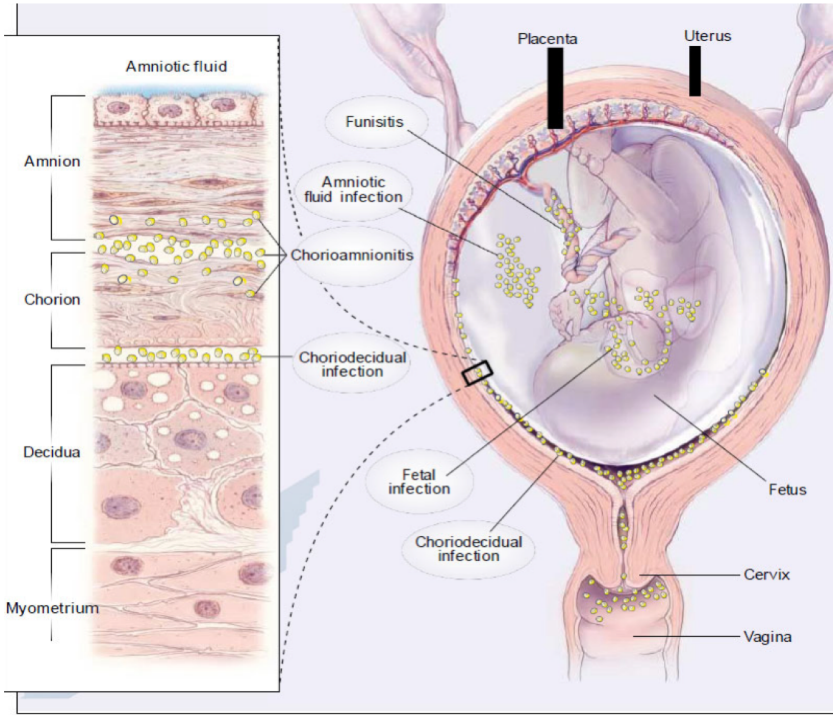
Le vie di trasmissione di infezioni dalla madre al feto o al neonato sono molteplici

- durante la vita fetale tramite lo scambio tra tessuti materni e membrane fetali, all'interno delle membrane fetali (amnion e chorion), tramite la placenta o il cordone ombelicale.
- durante il passaggio del neonato nel canale del parto



Si stima che **nel mondo** si verifichino ogni anno 6.9 milioni di infezioni neonatali e 400.000 decessi (WHO 2016)

In Italia 135-315 neonati/anno e 8-10 decessi per sepsi neonatale (Berardi, Ped Inf Dis , 2017)

L'incidenza è maggiore nei late preterm e 10-15 volte maggiore negli early preterm

La trasmissione materno fetale del GBS: prevenzione e strategie di terapia a confronto

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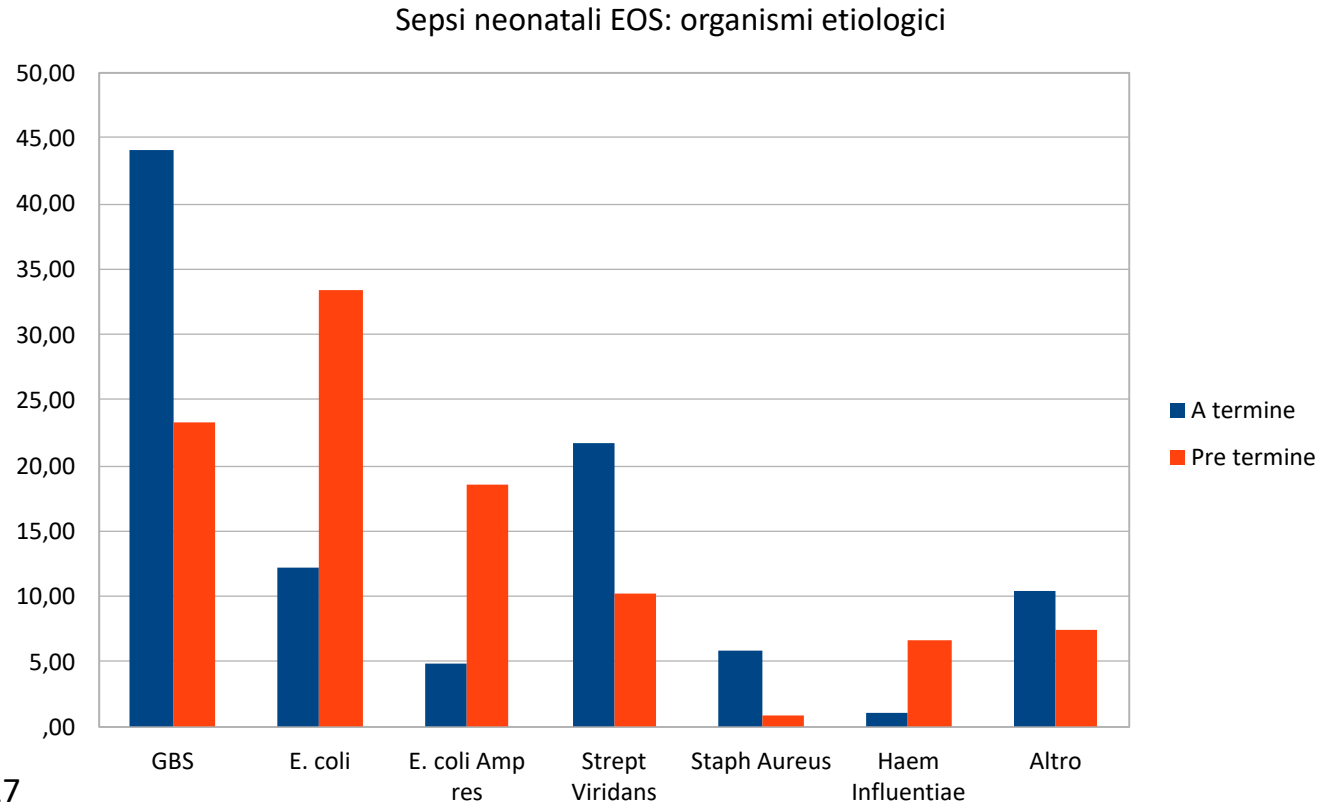
Lo streptococco agalactiae (GBS) è il principale ma non l'unico agente causale
Nella maggior parte delle casistiche la sua prevalenza è inferiore al 50%



% complessiva di EOS da GBS
sul totale dei nati=25%
(Berardi, J Matern Fetal Neonatal Med; 2017

Paese	Casi X 1000 nati	Morti x 1000 nati	Periodo
USA	0.3	0.04	2005-8
Italia	0.3-0.7	0.02	2008-10
Germania	0.3	0.01	2001-3
India	20	3.9	2008-10
Tailandia	44	?	2009-12

L'incidenza ed i tassi di mortalità nei paesi che hanno adottato strategie di profilassi e diagnosi precoce è comparabile

(Fleischmann-Struzek, Lancet, 2018; Creti ISTISAN, 2011)

Strategie finalizzate alla riduzione dell'incidenza delle sepsi neonatali

Strategie per la profilassi della infezione

Competenza Ostetrica

Strategie per la diagnosi precoce e profilassi secondaria della sepsi neonatale

Competenza Neonatologica

Strategie per la profilassi della infezione

Profilassi primaria

Vaccinazione anti streptococco agalactiae
(sperimentale, solo GBS)

Probiotici orali somministrati alla madre(sperimentale, solo GBS?)

Profilassi secondaria

Screening con tampone vagino rettale
(+)
Profilassi antibiotica intrapartum nelle gravide a rischio

Strategie per la diagnosi precoce della sepsi neonatale:

Screening ematochimico nei neonati con fattori di rischio

Neonatal sepsis calculator

Osservazione clinica sistematica

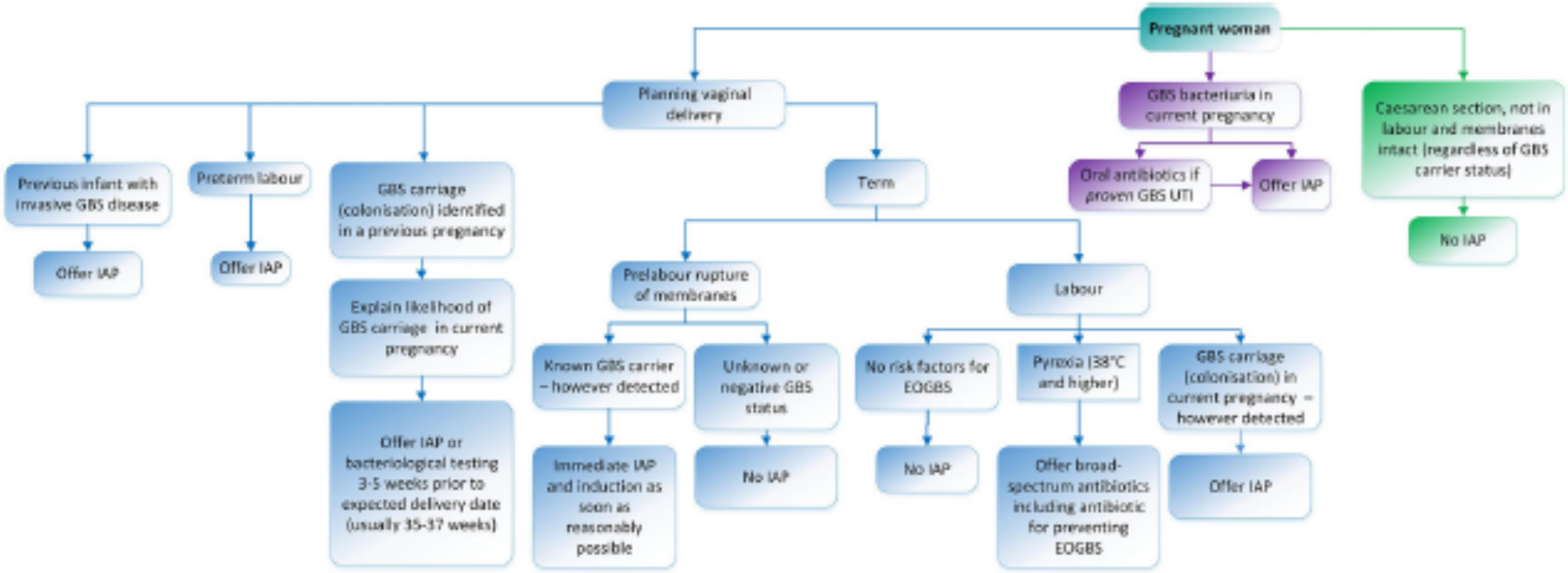
Strategie per la profilassi secondaria

Profilassi secondaria

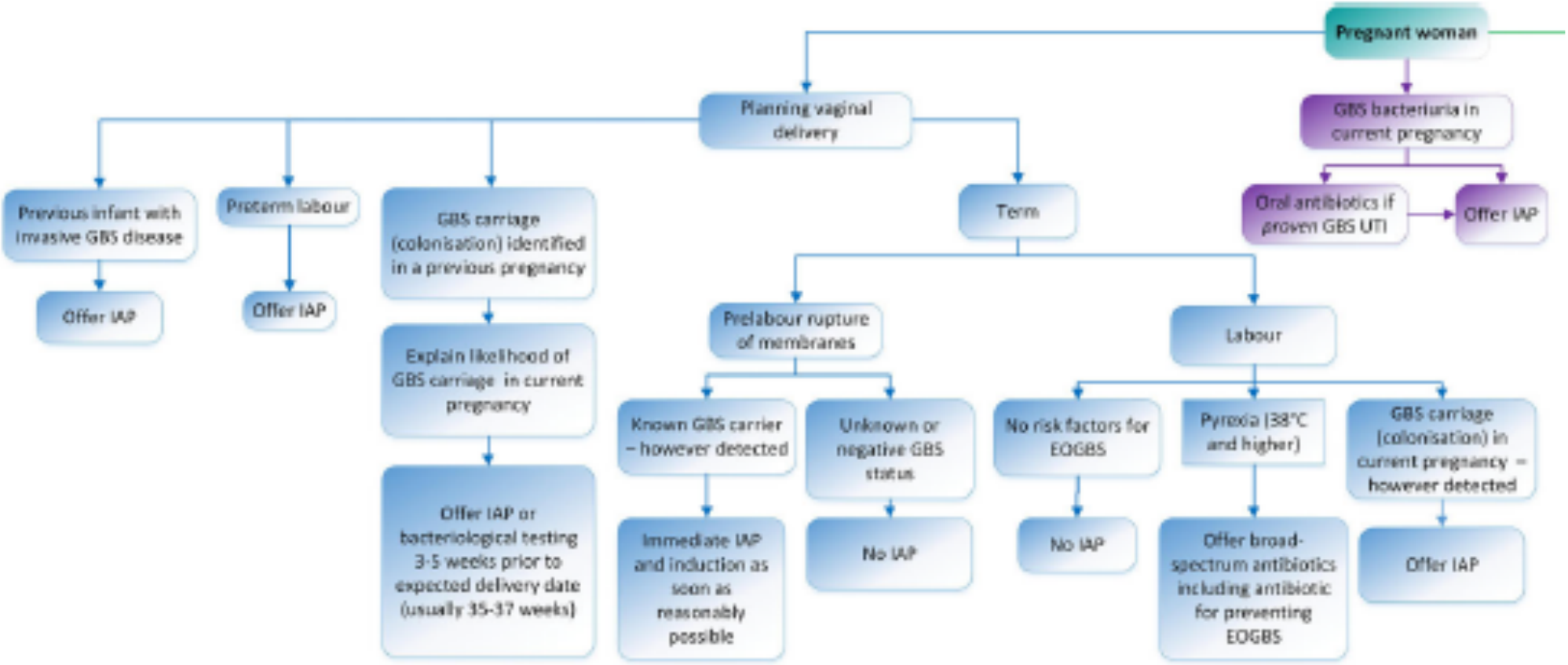
Screening con tampone vagino rettale

Profilassi antibiotica intrapartum (IAP) nelle gravide a rischio

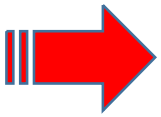
Flow Chart Prevention of Early-onset Neonatal Group B Streptococcal Disease (Royal College of Obstetrician & Gynecologists; NICE; 2017)



Flow Chart Prevention of Early-onset Neonatal Group B Streptococcal Disease (Royal College of Obstetrician & Gynecologists; NICE; 2017)



In Italia



In Italia

Raccomandazioni

- L'esecuzione dello screening dell'infezione da streptococco beta-emolitico gruppo B con tampone vaginale e rettale e terreno di coltura selettivo è raccomandata per tutte le donne a 36-37 settimane.
- Le donne in gravidanza con infezione da streptococco di gruppo B devono ricevere un trattamento antibiotico intraparto.

→ *Queste raccomandazioni attribuiscono valore alla possibilità di identificare, attraverso lo screening, le donne nelle quali il trattamento antibiotico intraparto è potenzialmente in grado di ridurre una infezione neonatale da SGB a esordio precoce* (early onset GBS infection) *e alla possibilità di ridurre il numero delle donne che arrivano al parto con tampone eseguito da oltre 5 settimane (considerato l'intervallo ottimale), considerando che comunque, prima delle 37 settimane compiute, vi è indicazione alla profilassi antibiotica intraparto indipendentemente dal risultato del test.*

Negli USA
AAP & ACOG; Pediatrics; 2019
«Clinical report/Guidance»

Indicazioni per tampone GBS

- Gravide 36-37+6
- PROM <37

Indicazioni per IAP

- GBS + tampone vr o urine
- Precedente colonizzazione gravidica GBS
- Precedente neonato con infezione GBS
- PROM <37
- Tampone GBS non disponibile
- TC > 38°
- ROM >= 18

Madri a rischio di infezione GBS

- Precedente nato con infezione GBS
- Batteriuria materna in gravidanza anche se trattata
- Neonato <37 sett
- PROM >= 18h
- Tampone vaginale e/o rettale GBS+

ESCLUSO TC a membrane integre

- Corioamniosite (febbre materna >= 38°

durante il parto ESCLUSO altre cause note)

Strategie per la diagnosi precoce della sepsi neonatale:

Screening ematochimico nei neonati con fattori di rischio

Neonatal sepsis calculator

Osservazione clinica sistematica

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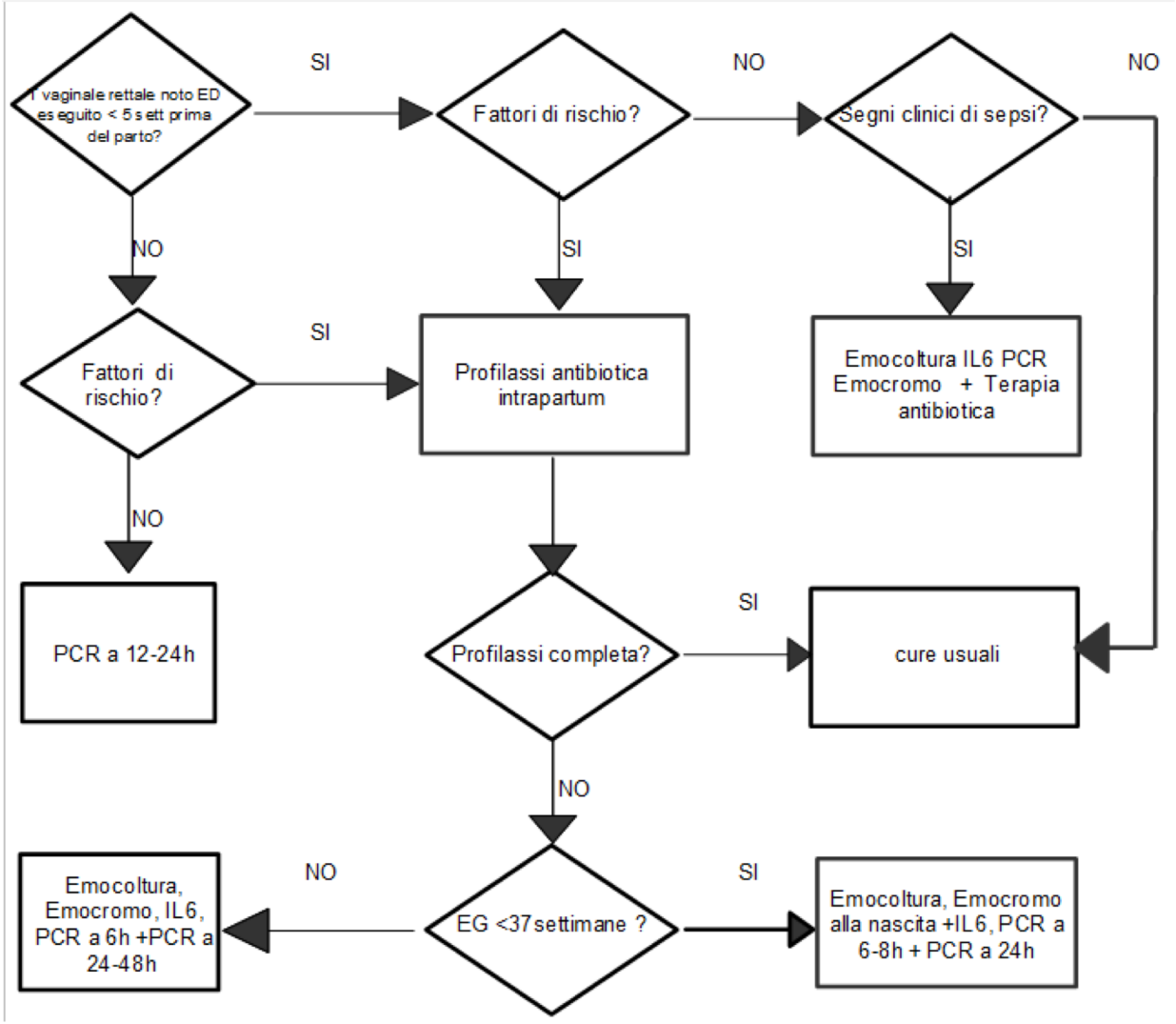

Guidance AAP 2012

Diagnosi e terapia precoci

Trattare i neonati con segni ematochimici precoci

Trattare i neonati sintomatici

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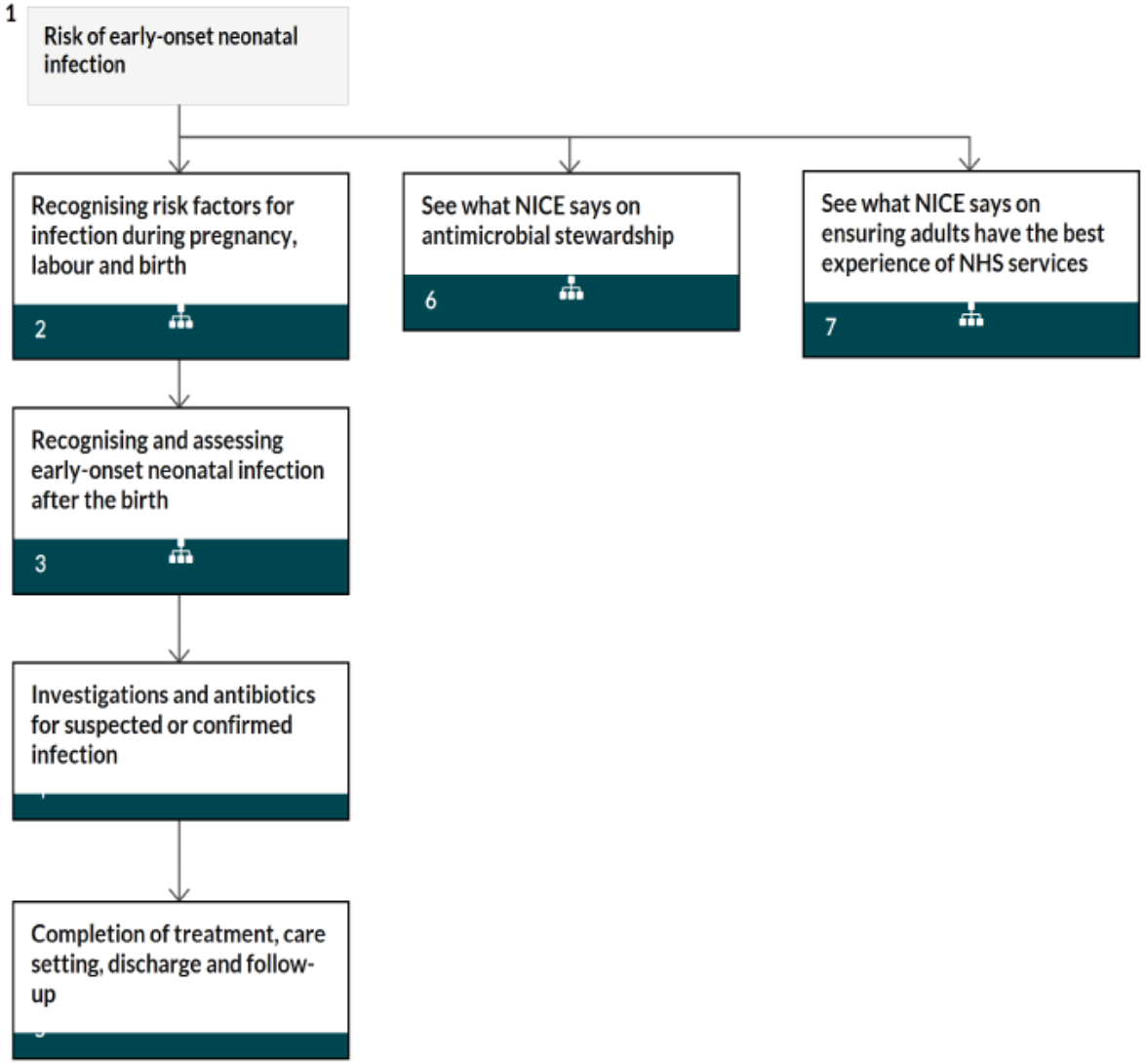

Linea guida NICE 2012

Diagnosi e terapia precoci

Trattare i neonati con segni ematochimici precoci

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Area Pediatrica

Visualizza 10 elementi

Cerca:

Argomento	Titolo originale	Ente produttore, anno di pubblicazione/ ultimo aggiornamento	Note	Data inserimento nell'SNLG	Link al full text
Infezione neonatale a esordio precoce: prevenzione e trattamento con antibiotici	Neonatal infection (early onset): antibiotics for prevention and treatment (CG149)	NICE, 2012	LG rivista a gennaio 2017 ed è in corso di aggiornamento; data pubblicazione non specificata	Febbraio 2019	Scarica file

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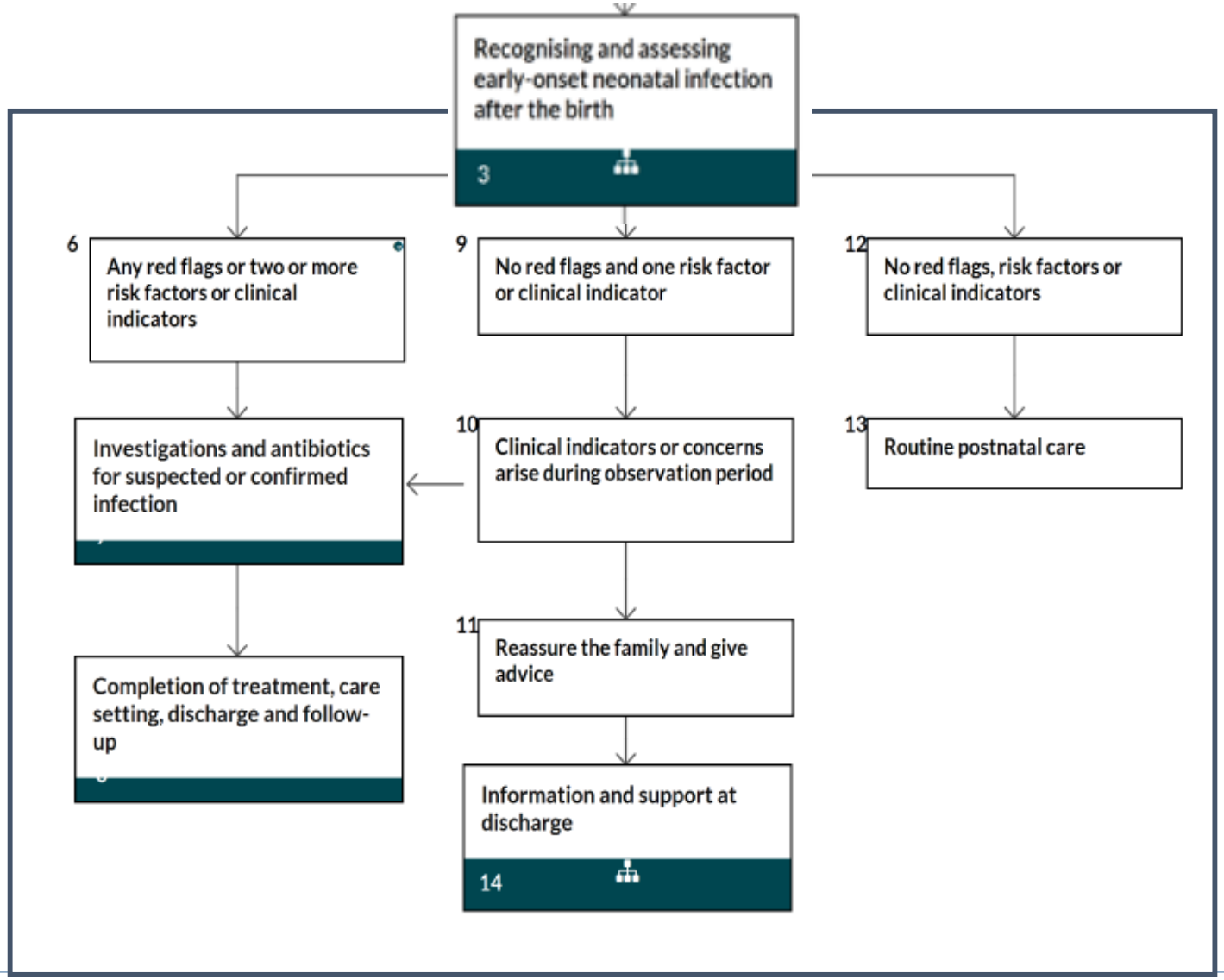

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Indicatori clinici per sepsi neonatale early onset

Clinical indicator	Red flag
Altered behaviour or responsiveness	
Altered muscle tone (for example, floppiness)	
Feeding difficulties (for example, feed refusal)	
Feed intolerance, including vomiting, excessive gastric aspirates and abdominal distension	
Abnormal heart rate (bradycardia or tachycardia)	
Signs of respiratory distress	
Respiratory distress starting more than 4 hours after birth	Yes
Hypoxia (for example, central cyanosis or reduced oxygen saturation level)	
Jaundice within 24 hours of birth	
Apnoea	
Signs of neonatal encephalopathy	
Seizures	Yes
Need for cardio-pulmonary resuscitation	

Negli USA
AAP & ACOG;
Pediatrics; 2019
«Clinical report/Guidance»

American Academy of Pediatrics
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Management of Infants at Risk for Group B Streptococcal Disease

Karen M. Puopolo, MD, PhD, FAAP,[a,b] Ruth Lynfield, MD, FAAP,[c] James J. Cummings, MD, MS, FAAP,[d] COMMITTEE ON FETUS AND NEWBORN, COMMITTEE ON INFECTIOUS DISEASES

Group B streptococcal (GBS) infection remains the most common cause of neonatal early-onset sepsis and a significant cause of late-onset sepsis among young infants. Administration of intrapartum antibiotic prophylaxis is the only currently available effective strategy for the prevention of perinatal GBS early-onset disease, and there is no effective approach for the prevention of late-onset disease. The American Academy of Pediatrics joins with the American College of Obstetricians and Gynecologists to reaffirm the use of universal antenatal microbiologic-based testing for the detection of maternal GBS colonization to facilitate appropriate administration of intrapartum antibiotic prophylaxis. The purpose of this clinical report is to provide neonatal clinicians with updated information regarding the epidemiology of GBS disease as well current recommendations for the evaluation of newborn infants at risk for GBS disease and for treatment of those with confirmed GBS infection. This clinical report is endorsed by the American College of Obstetricians and Gynecologists (ACOG), July 2019, and should be construed as ACOG clinical guidance.

The Centers for Disease Control and Prevention (CDC) first published

abstract

[a]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; [b]Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; [c]Minnesota Department of Health, St Paul, Minnesota; and [d]Departments of Pediatrics and Bioethics, Alden March Bioethics Institute, Albany Medical College, Albany, New York

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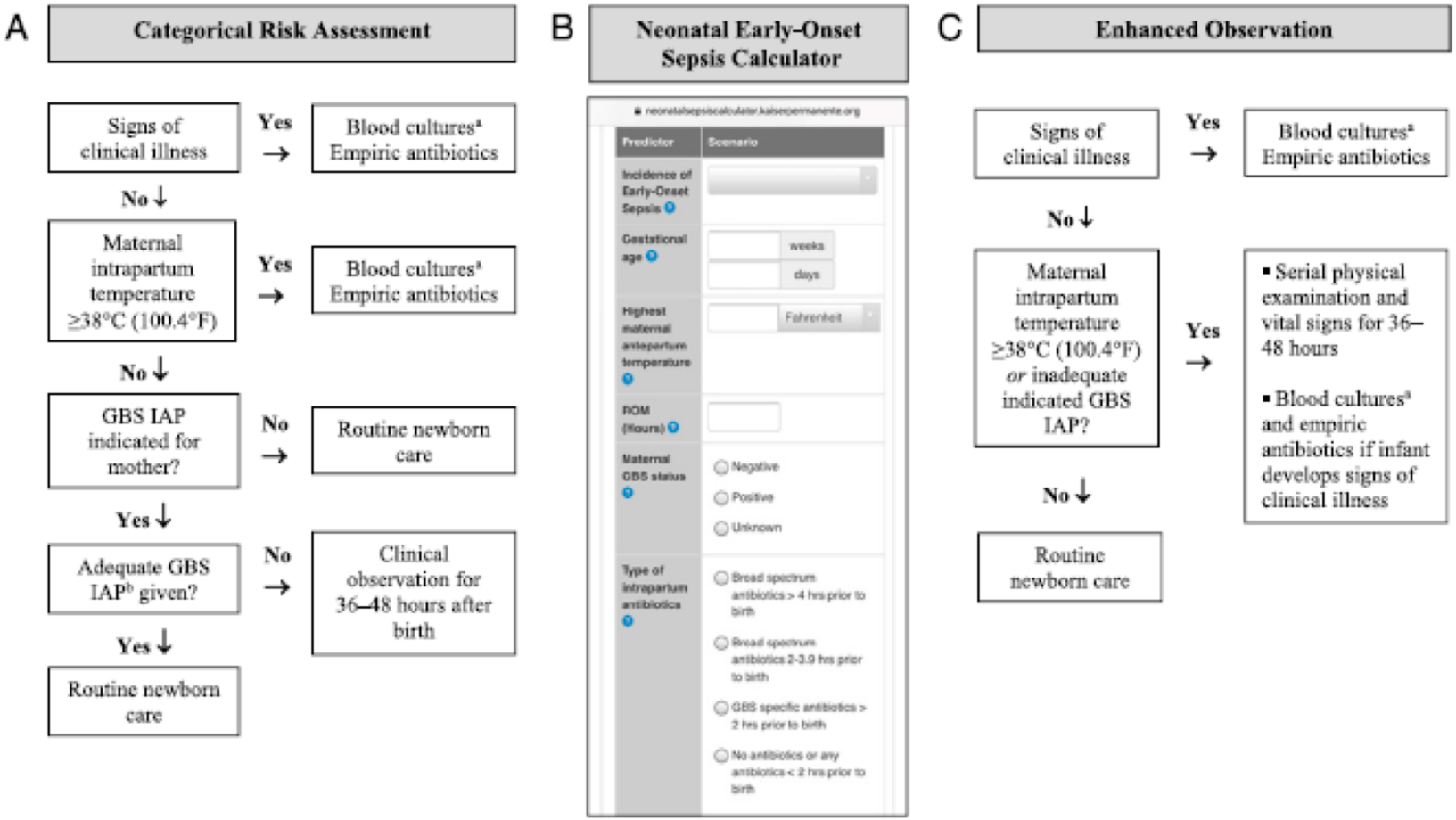
The guidance in this report does not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.

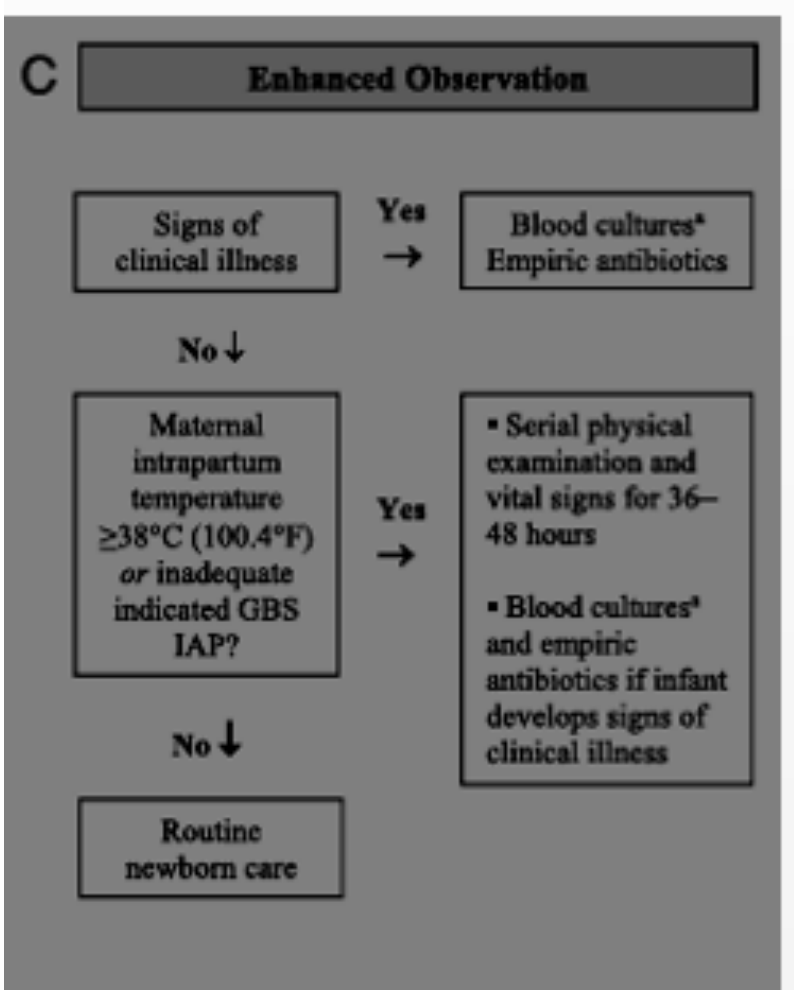
FATEBENEFRATELLI

La trasmissione materno fetale del GBS: prevenzione e strategie di terapia a confronto

a cura di F. Focarile UO Pediatria e Neonatologia

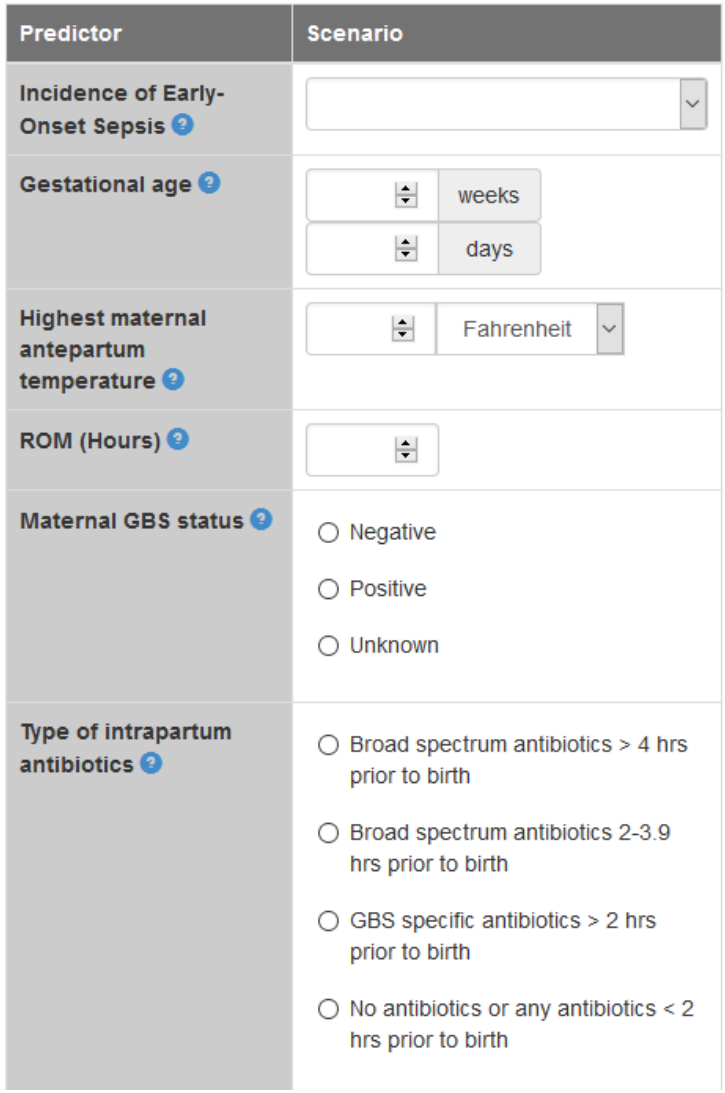


Osservazione intensiva
AAP e ACOG, Pediatrics, 2019



Sepsis Risk Score

- <1 cure normali
- 1-3 osservazione intensiva+emocoltura
- >3 terapia antibiotica



Predictor	Scenario
Incidence of Early-Onset Sepsis	
Gestational age	weeks days
Highest maternal antepartum temperature	Fahrenheit
ROM (Hours)	
Maternal GBS status	○ Negative ○ Positive ○ Unknown
Type of intrapartum antibiotics	○ Broad spectrum antibiotics > 4 hrs prior to birth ○ Broad spectrum antibiotics 2-3.9 hrs prior to birth ○ GBS specific antibiotics > 2 hrs prior to birth ○ No antibiotics or any antibiotics < 2 hrs prior to birth

Table 2 Comparison of antibiotic use as per NICE guidance vs recommendations of the SRC in the study population

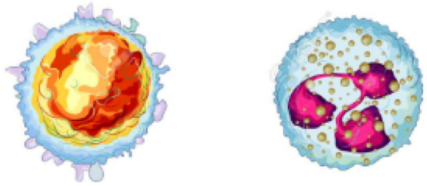
		Kaiser Permanente SRC		Total (% of study cohort)
		No antibiotics	Antibiotics	
Current guidance (NICE)	No antibiotics	3011	6	3017 (84)
	Antibiotics	426	150	576 (16)
Total (% of study cohort)		3437 (95.7)	156 (4.3)	3593 (100)

Sixteen per cent antibiotics as per NICE compared with 4.3% as per SRC. Absolute difference 11.7% (95% CI 10.64 to 12.74), $p<0.0001$, McNemar's test for matched proportions.
NICE, National Institute for Health and Care Excellence; SRC, sepsis risk calculator.

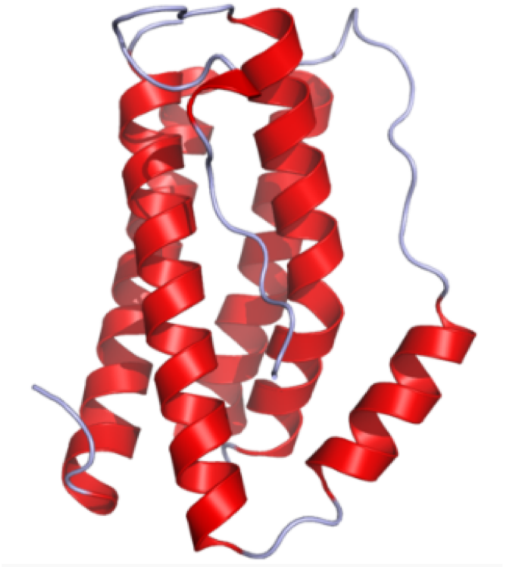
Nei neonati di età gestazionale >34wk l'utilizzo giudizioso dello strumento Neonatal Sepsis Risk Calculator può ridurre del 75% il numero di neonati Sottoposti a terapia antibiotica per sospetta sepsi

Segni ematochimici precoci nel neonato a rischio

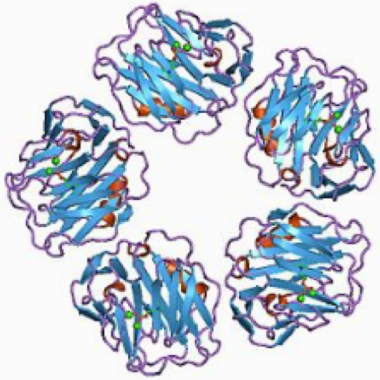
Linfociti e Neutrofili



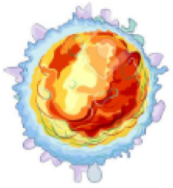
IL-6



PCR

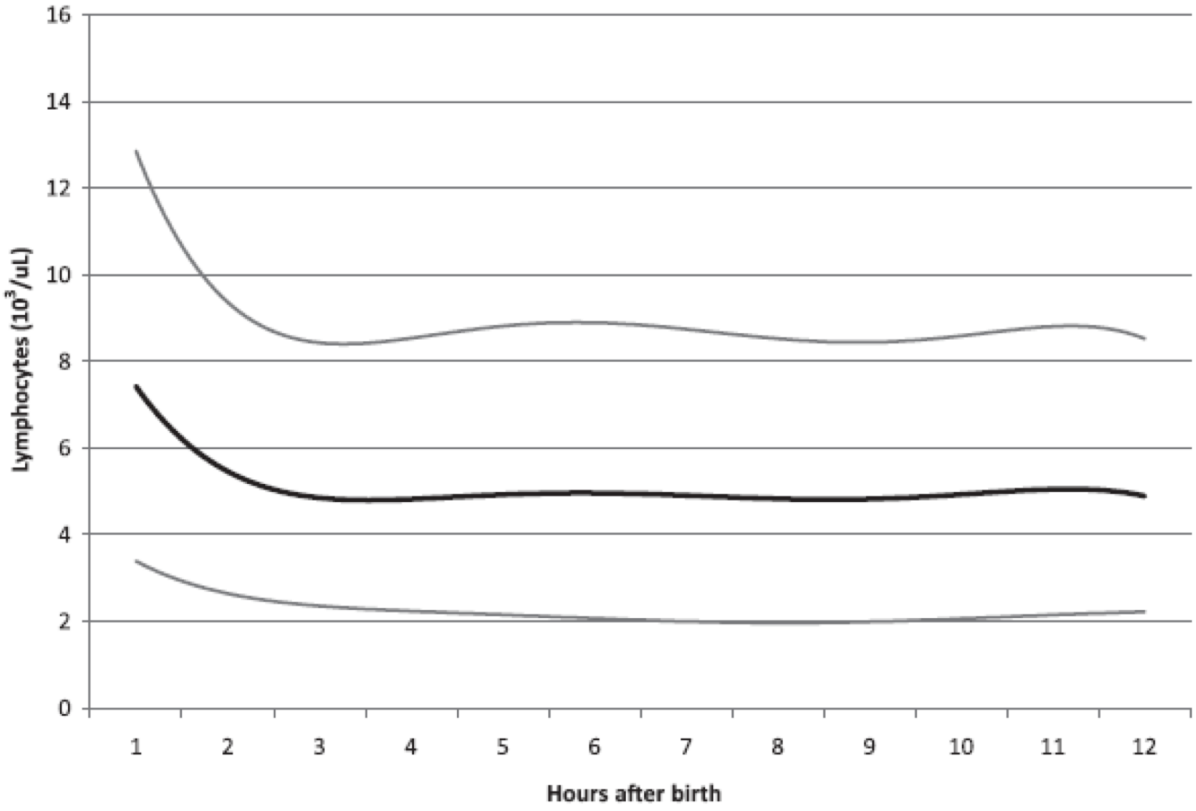


Linfociti

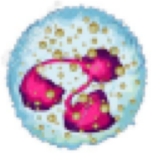


Nelle prime 12h di vita
Valori di linfociti
<2*10^9/L oppure
>8*10^9/L

Sono positivamante associati
a sepsi neonatale



Leucociti

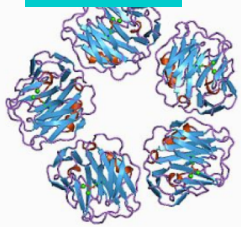


Nelle prime 12h di vita
Valori di neutrofili inferiori a
<5*10^9/L
sono positivamante associati
a sepsi neonatale

Alla 8ª ora il valore dei
neutrofili
Corrispondente al 95% è
28.5*10^9/L

Figure 1.

PCR



- La PCR ha un incremento tardivo dopo lo stimolo infettivo o di trauma tissutale

- Due determinazioni, a 8h e 24h o oltre hanno elevata sensibilità e specificità per la diagnosi di sepsi neonatale

- Il valore soglia (95°) aumenta a 12,24,48h

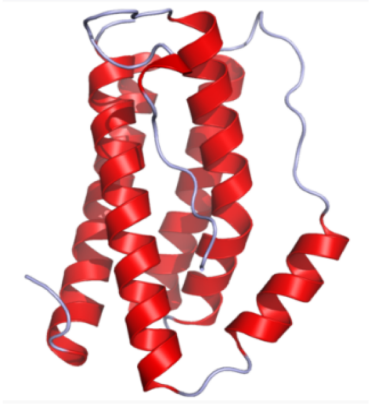
	PCR mg/dL 95°
12 ore di vita	0.26
24 ore di vita	0.69
48 ore di vita	1.33

- I neonati da parto cesareo elettivo hanno valori più bassi a 48h.

	PCR mg/dL 95° a 48h
Vaginale o Cesareo in emergenza	1.21
Cesareo in elezione	0.60

Perrone, Arch Dis Child 2018; Simonsen, Clin Microbiol Rev, 2014

IL-6



- La IL-6 interleukina 6 ha un incremento precoce dopo lo stimolo infettivo o di trauma tissutale e un rapido decremento dopo la cessazione dello stimolo infettivo/lesivo

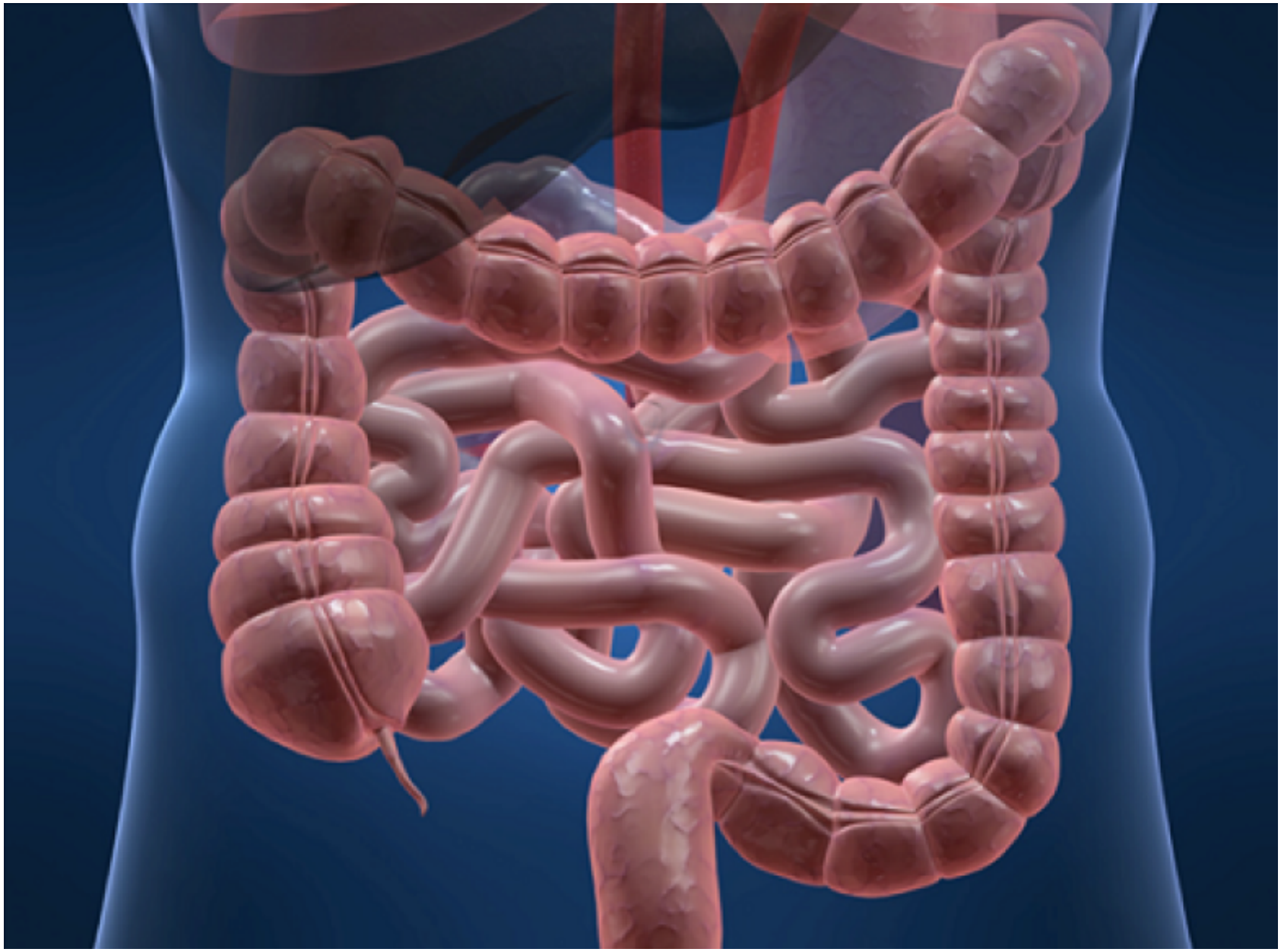
- Una determinazione a 6h dalla nascita ha elevata sensibilità e specificità per la diagnosi di infezione neonatale EOS

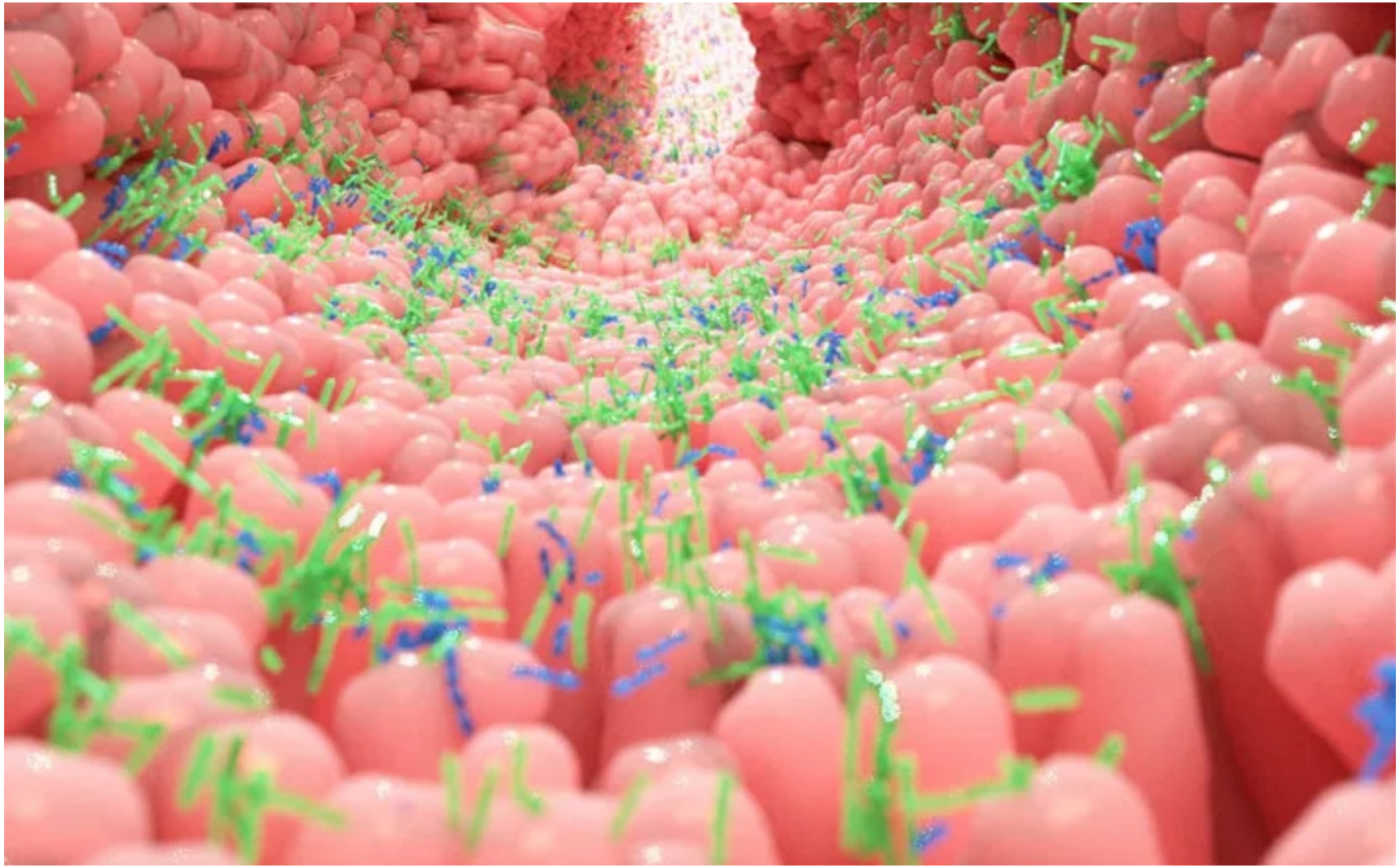
- Per le sue caratteristiche può essere utilizzato anche come marker di guarigione

Strategie per la riduzione della incidenza di sepsi neonatale early onset nei nati a termine e late preterm

Take home messages

- La sepsi neonatale a esordio precoce (EOS) è una patologia con possibili gravi conseguenze per il neonato e per la madre, con incidenza tuttora rilevante
- E' necessaria la combinazione di strategie di competenza ostetrica e neonatologica
- Lo screening universale di tutte le gravide tra la 36-37+6 settimane resta una raccomandazione forte
- Le più recenti Raccomandazioni per la diagnosi precoce nel neonato sono basate sulla osservazione clinica intensiva supportata dall'utilizzo di sistemi di punteggio, al fine di effettuare accertamenti diagnostici e terapie antibiotiche solo per i soggetti per i quali siano effettivamente necessarie
- Interventi di profilassi primaria dell'infezione da GBS sono in fase sperimentale e potrebbero offrire un valido strumento



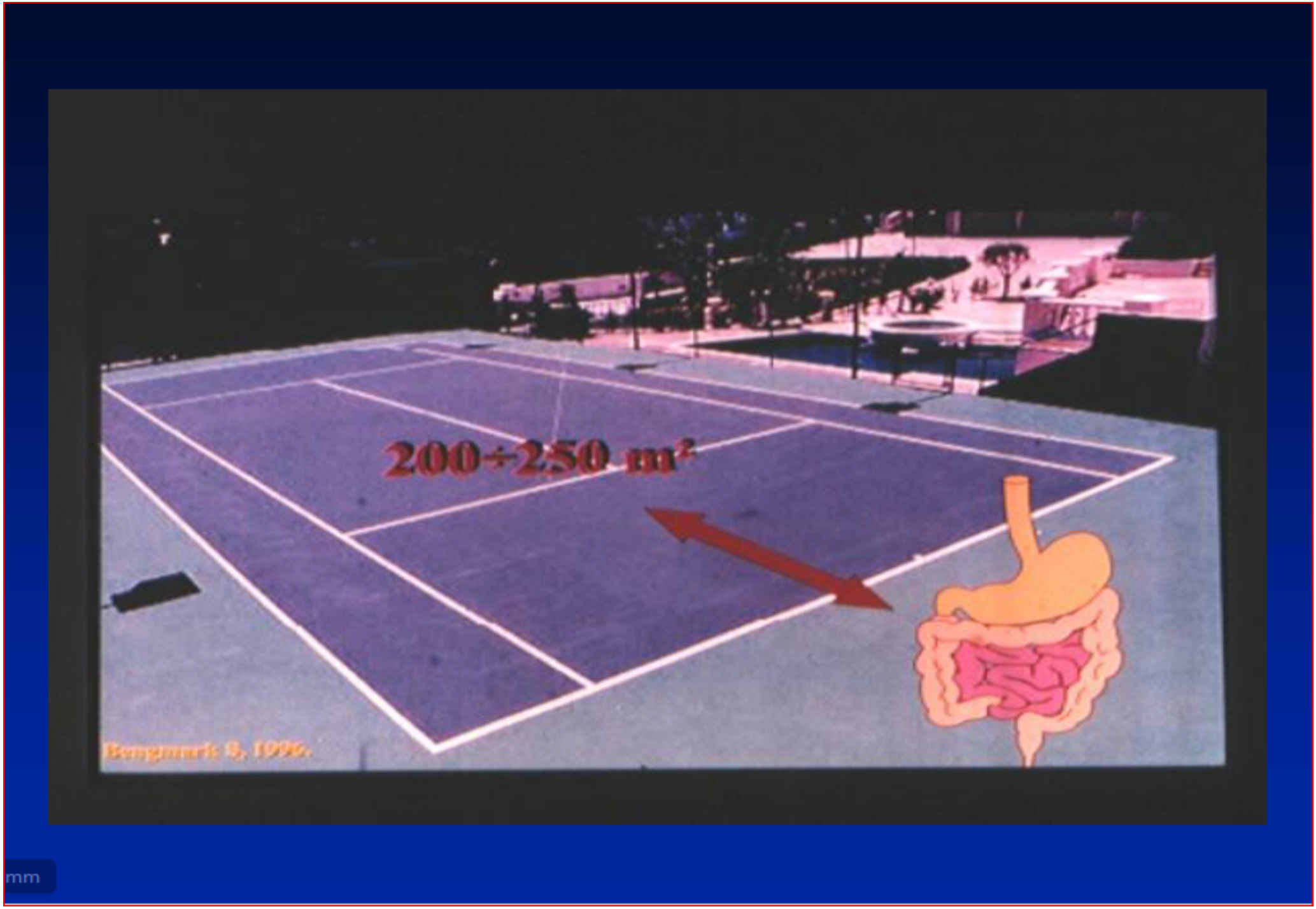


L'organismo umano, in condizioni normali, e' formato da

10^{13} cellule = 10.000 miliardi ed ospita una popolazione microbica di 10^{14} cellule = 100.000 miliardi

Ne consegue che per
ogni cellula eucariotica vi sono
10 cellule procariotiche

Bengmark S. Gut 1998; 42: 2-7.


200÷250 m²

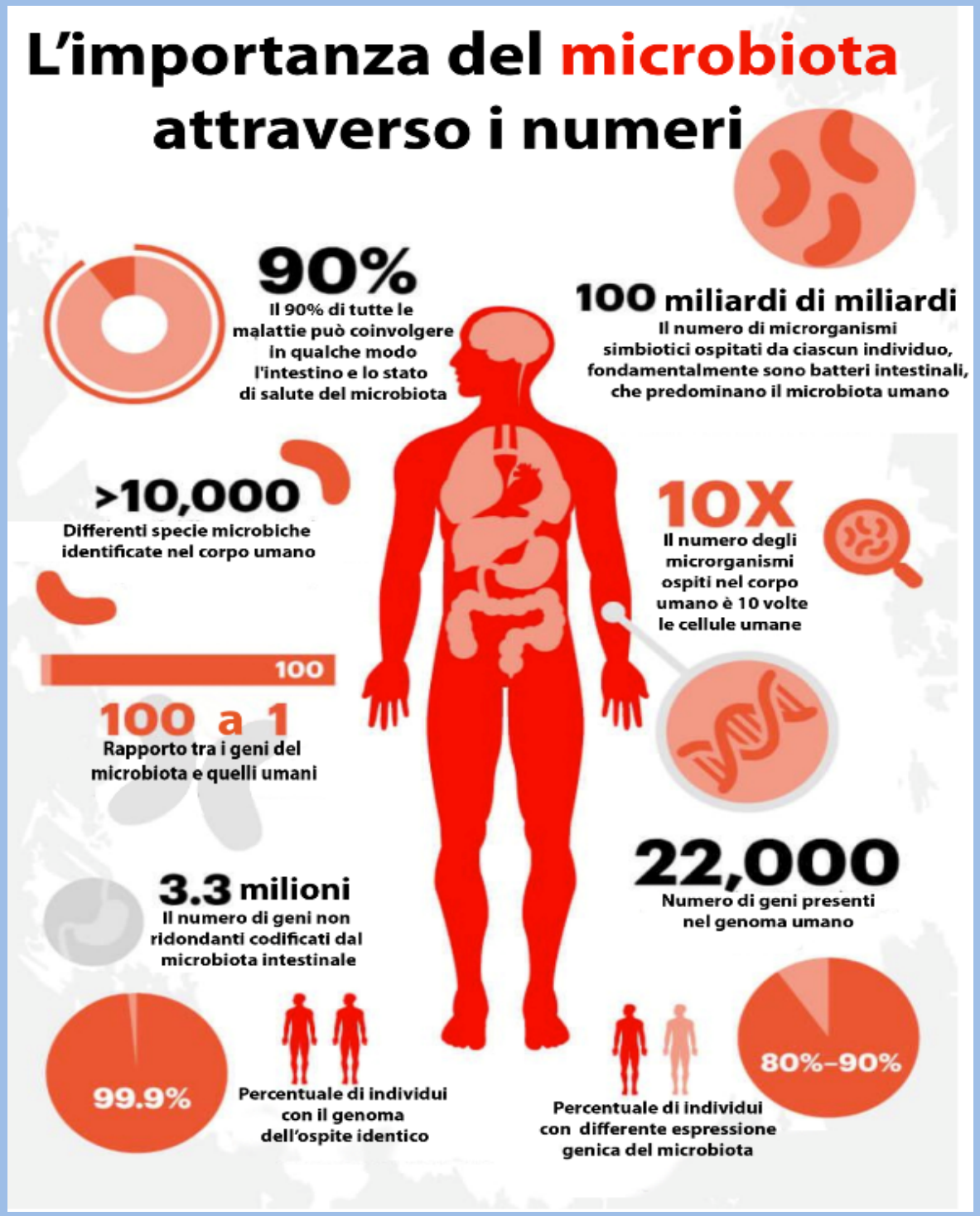
L'enorme importanza dell'intestino negli equilibri immunitari dell'organismo era già stata intuita da Ippocrate nel 2.500 a**.C. circa, al punto da spingerlo ad affermare che: "Tutte le malattie cominciano nell'intestino".**

Oggi sappiamo che l'intestino rappresenta uno dei più importanti baluardi immunitari dell'organismo.
Basti pensare che con la sua superficie di circa trecento metri quadrati, quest'organo ospita qualcosa come centomila miliardi di microrganismi e produce grossomodo il 60-70% delle cellule immunitarie dell'organismo.

A livello intestinale, infatti, sono presenti sistemi immunitari di **tipo innato (o aspecifico) e adattivo (o specifico)** che garantiscono una corretta risposta della mucosa intestinale in seguito all'ingresso di tutto ciò che viene riconosciuto come estraneo e pericoloso

Il fatto che i circa 100 trilioni di microrganismi appartenenti al microbiota non vengano attaccati dal nostro SI, al contrario di quanto avviene per i microbi patogeni, si deve alla complessa e dinamica interazione evolutasi nell'arco di quasi 500 milioni di anni, proprio tra questi speciali microrganismi e l'essere umano.

Questa interazione ha portato il sistema immunitario e il microbiota intestinale ad un adattamento reciproco molto vantaggioso per la nostra salute.


L'importanza del microbiota attraverso i numeri
90%
Il 90% di tutte le malattie può coinvolgere in qualche modo l'intestino e lo stato di salute del microbiota
100 miliardi di miliardi
Il numero di microrganismi simbiotici ospitati da ciascun individuo, fondamentalmente sono batteri intestinali, che predominano il microbiota umano
>10,000
Differenti specie microbiche identificate nel corpo umano
10X
Il numero degli microrganismi ospiti nel corpo umano è 10 volte le cellule umane
100
100 a 1
Rapporto tra i geni del microbiota e quelli umani
3.3 milioni
Il numero di geni non ridondanti codificati dal microbiota intestinale
22,000
Numero di geni presenti nel genoma umano
99.9%
Percentuale di individui con il genoma dell'ospite identico
80%–90%
Percentuale di individui con differente espressione genica del microbiota

Le principali funzioni del microbiota intestinale

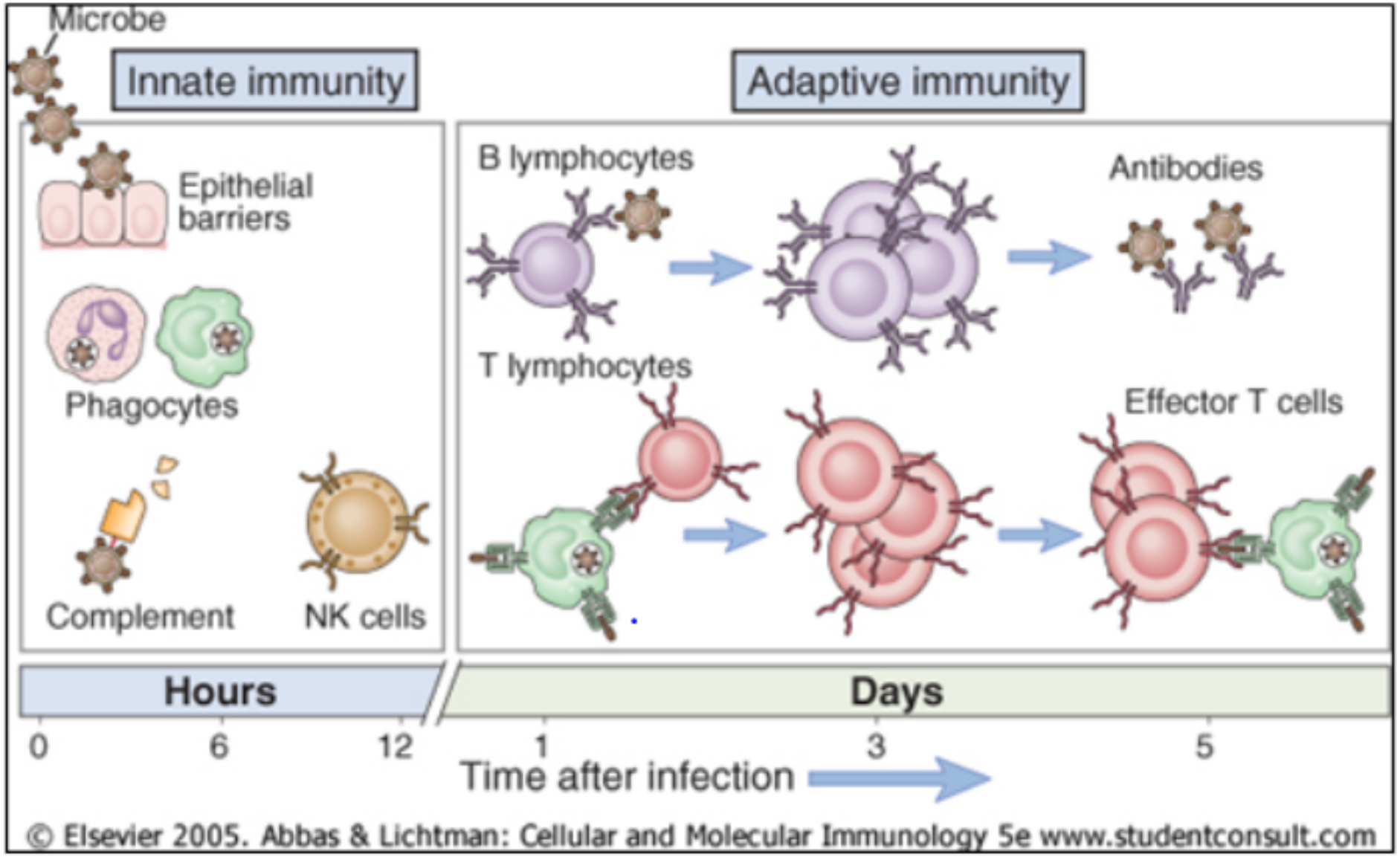
- Barriera contro la proliferazione dei patogeni, con un meccanismo noto come "*colonization resistance*" o "***effetto barriera***" o "***esclusione competitiva***". È il meccanismo utilizzato dai batteri già presenti nell'intestino per mantenere la loro presenza in questo ambiente, evitando la colonizzazione degli stessi siti intestinali da parte di altri microrganismi, ingeriti o già presenti
- Regolazione della maturazione del sistema immunitario e sua modulazione
- Produzione di vitamine (acido folico, vit. K, vit. del gruppo B)
- Regolazione della motilità intestinale
- **Parziale recupero di energia dalle fibre alimentari**

Il microbiota intestinale potrebbe essere inteso come un organo metabolicamente attivo del corpo umano

"It seems appropriate to consider ourselves as a composite of many species - human, bacterial, and archaeal - and our genome as an amalgamation of human genes and the genes in 'our' microbial genomes ('microbiome')"
Human Gut Microbiome Initiative (HGMI)

Durante la vita la mucosa intestinale viene a contatto con un numero incalcolabile di antigeni (batteri, virus, parassiti, antigeni alimentari, farmaci e diversi altri composti chimici); pertanto è di fondamentale importanza la presenza di difese che assicurino un’adeguata protezione.

Questo sistema complessivamente prende il nome di **barriera intestinale**.
Una parte fondamentale di questo effetto barriera viene svolto dal tessuto linfoide associato all'intestino (**GALT**), costituito prevalentemente da linfociti T, che costituiscono circa un sesto delle cellule dei villi, da linfociti B, cellule dendritiche e plasmacellule (secernenti prevalentemente **Ig A**) presenti nel connettivo della lamina propria e da follicoli linfatici isolati (più frequenti nel colon) o aggregati (placche del Peyer, nell'ileo).


Microbe
Innate immunity
Adaptive immunity
Epithelial barriers
Phagocytes
Complement
NK cells
B lymphocytes
Antibodies
T lymphocytes
Effector T cells
Hours
Days
0
6
12
1
3
5
Time after infection
© Elsevier 2005. Abbas & Lichtman: Cellular and Molecular Immunology 5e www.studentconsult.com

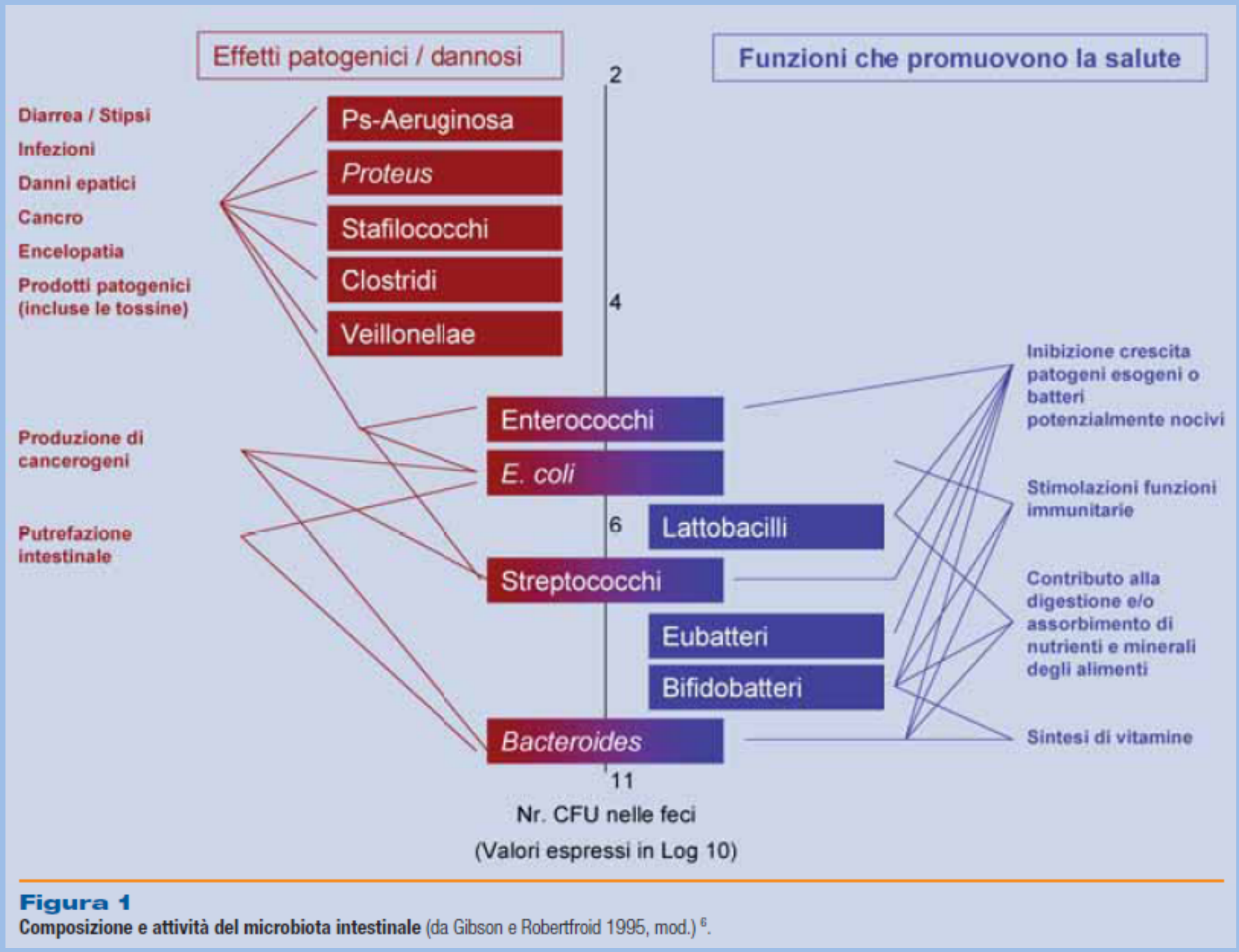
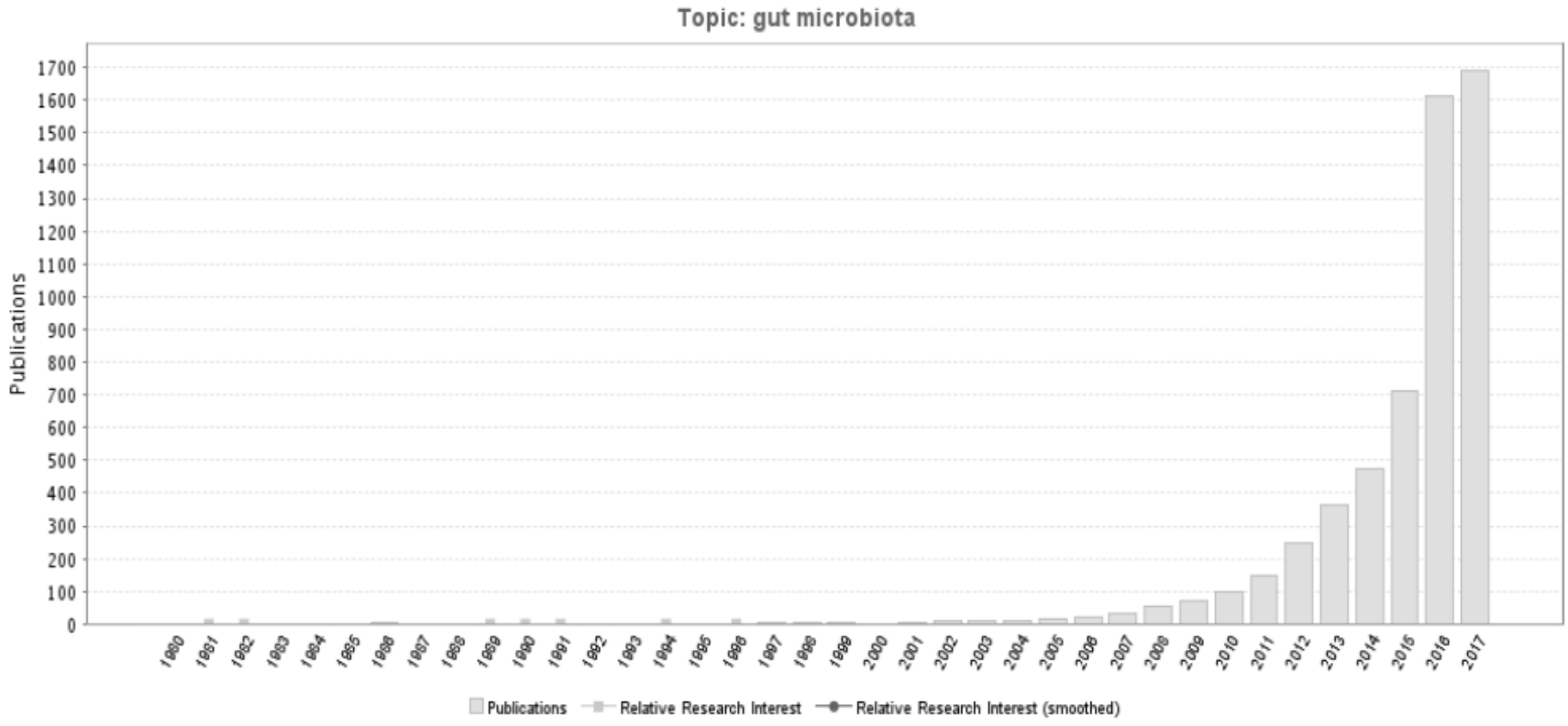


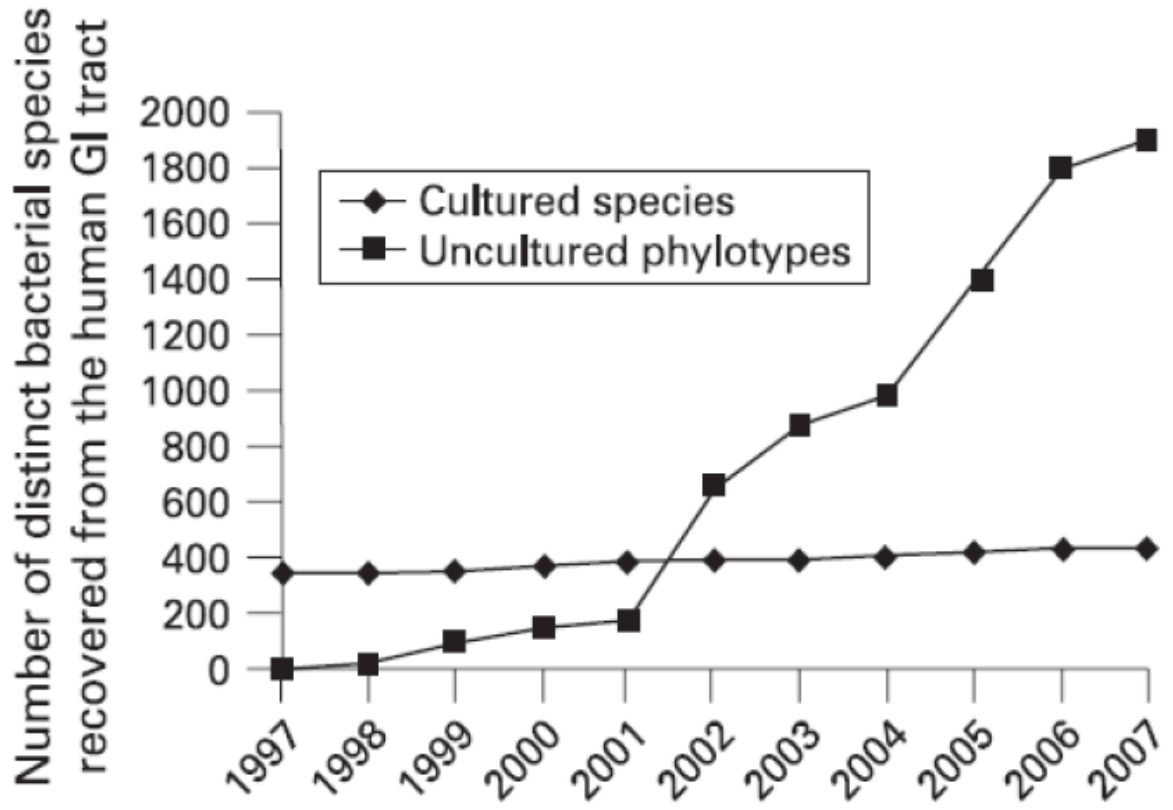
Figura 1
Composizione e attività del microbiota intestinale (da Gibson e Robertfroid 1995, mod.) [6].

N° di pubblicazioni/anno su Microbiota intestinale, 1980→2017



Fonte: GoPubMed, accesso 14/9/17

Metodi di identificazione coltura-dipendente vs coltura- indipendente



Zoetendall et al, *Gut* 2008;57:1605

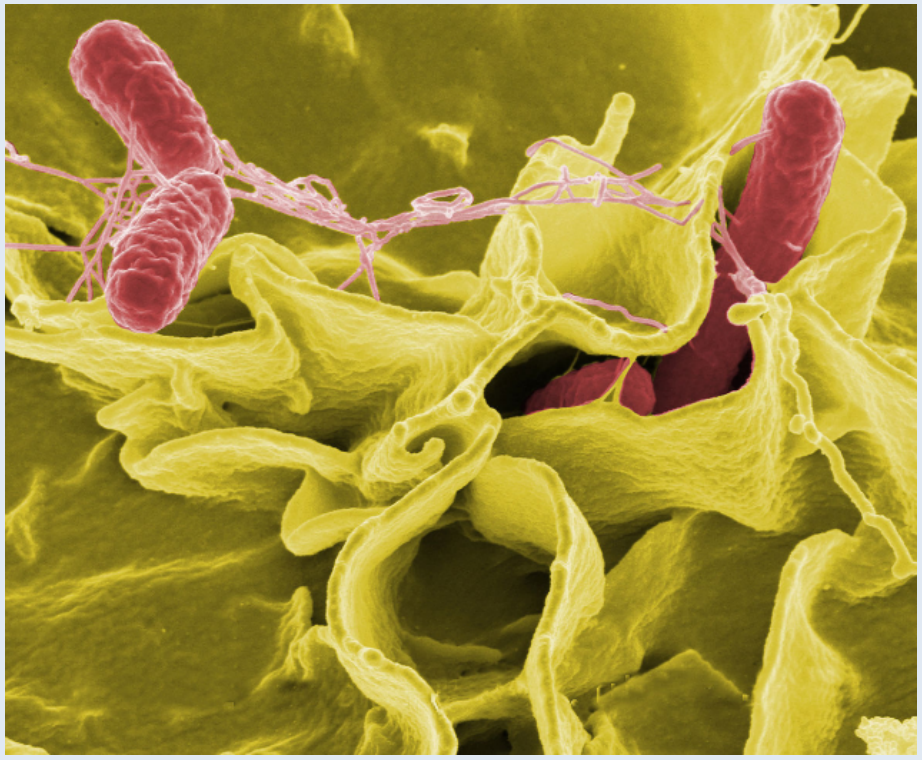
TECNICHE DI SEQUENZIAMENTO GENOMICO

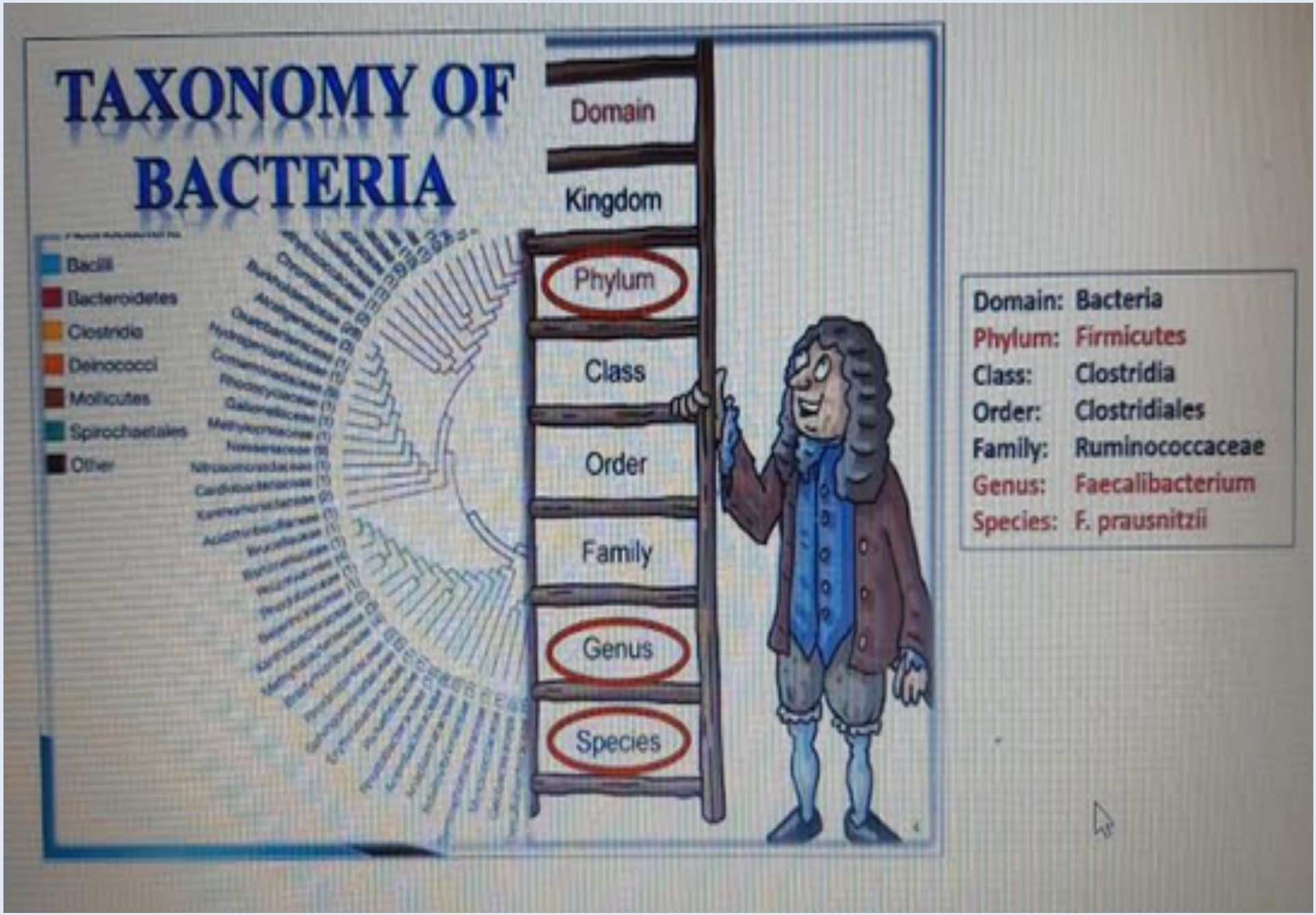
Mediante l'uso del gene 16 SrRNA, unico per ora che identifica i procarioti quali sono i nostri batteri intestinali.

Le tecniche genomiche hanno permesso di:

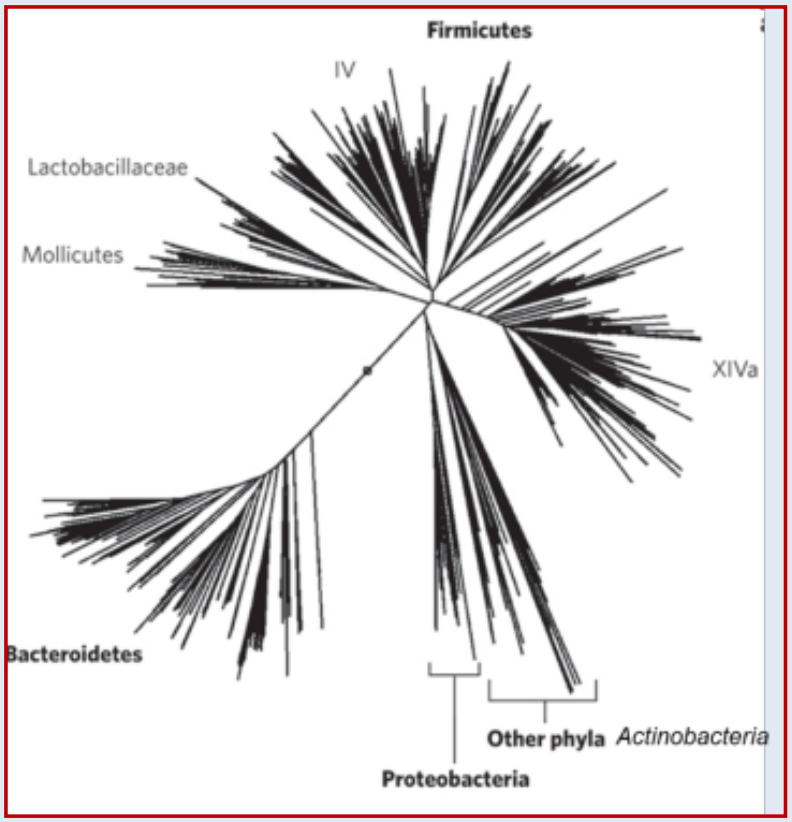
- Identificare altre popolazioni batteriche presenti nell'intestino
- Di raggruppare in Phylum (tribù) gli ordini e le specie

I Phylum sono inferiore al Regno e superiori alla Classe e si identificano in Ordini e Specie che raggruppati hanno caratteristiche comuni.




TAXONOMY OF BACTERIA
Bacilli
Bacteroidetes
Clostridia
Deinococci
Mollicutes
Spirochaetales
Other
Domain
Kingdom
Phylum
Class
Order
Family
Genus
Species
Domain: Bacteria
Phylum: Firmicutes
Class: Clostridia
Order: Clostridiales
Family: Ruminococcaceae
Genus: Faecalibacterium
Species: F. prausnitzii

PHYLOGENETIC DIVERSITY

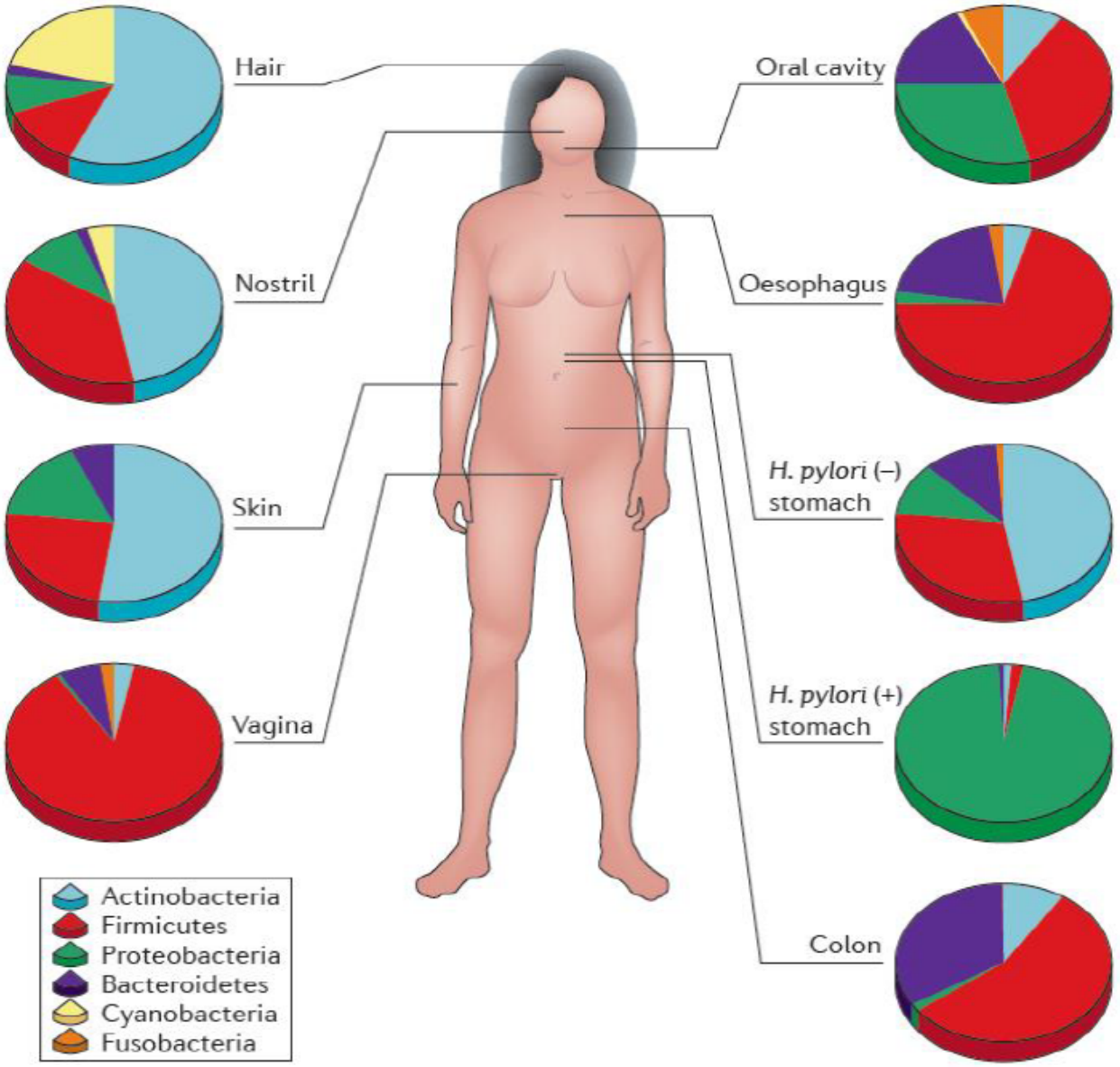


> 1000 species

5 (out of 100) bacterial phyla

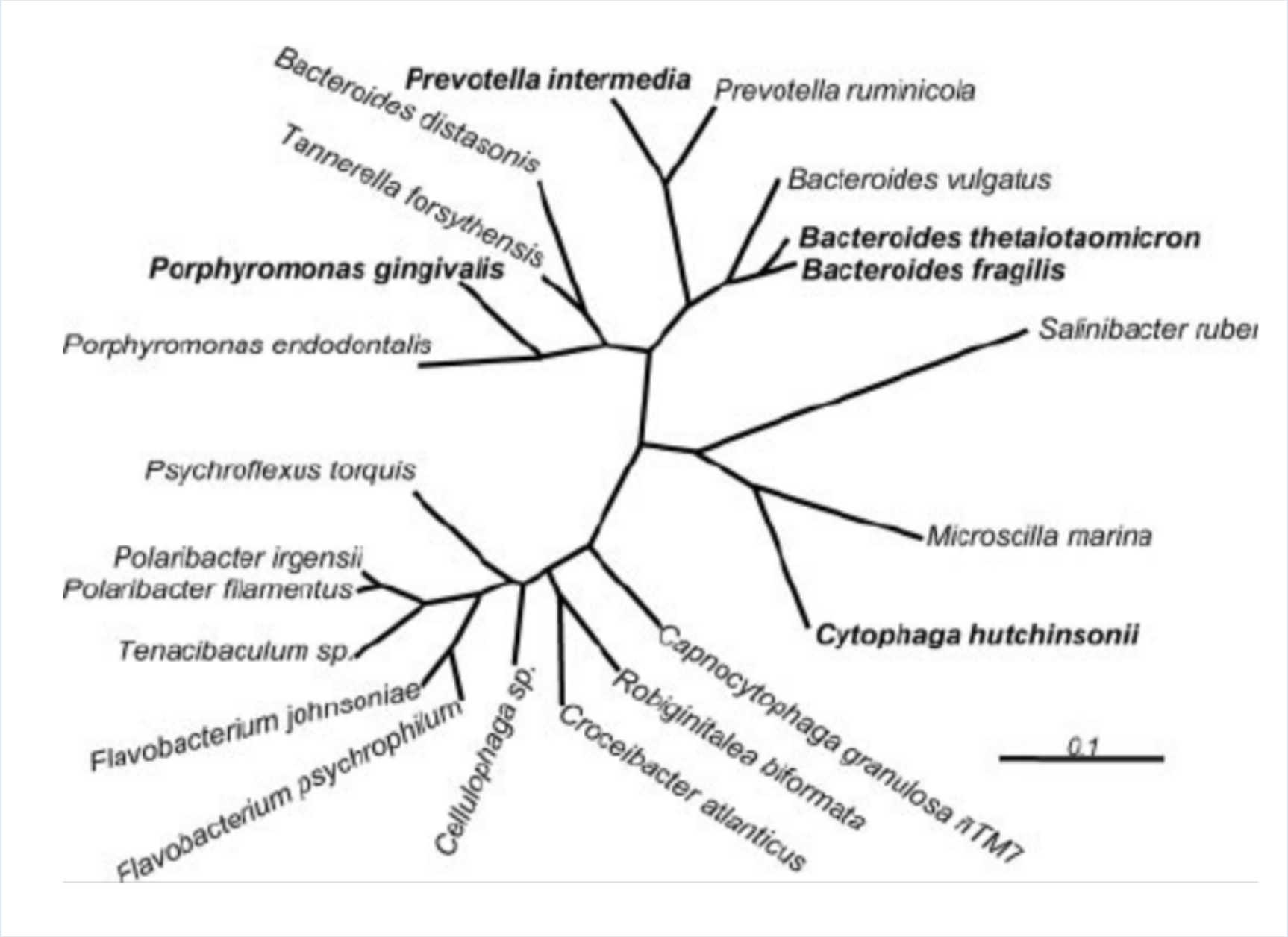
- *Firmicutes* (65%), *Bacteroidetes* (25%)
- *Actinobacteria* (5%), *Proteobacteria* (<8%), *Fusobacteria* (1%) and *Verrucomicrobia* (1%)

I diversi microbiota umani



Cho & Blaser, *Nat Rev Gen* 2012;13:260

Bacterioidetes



Firmicutes

Questi batteri sono dei gram-positivi che, insieme ai Bacteroides, abitano normalmente nel colon umano e sono naturali ospiti della flora microbica normale. Recenti studi dimostrano che hanno un'implicazione nell'assorbimento del glucosio, di conseguenza l'obesità potrebbe essere un fattore derivante da una flora batterica disbiotica e quindi potrebbe curarsi agendo su quest'ultima.

I Phylum Firmicutes comprende 274 generi tra cui:

Bacilli

- *Bacillus*
- *Listeria*
- *Lactobacillus*

Cocchi

- *Lactococcus*
- *Micrococcus*
- *Staphylococcus*
- *Streptococcus*
- *Sarcina*
- *Peptococcus*
- *Peptostreptococcus*

Clostridia

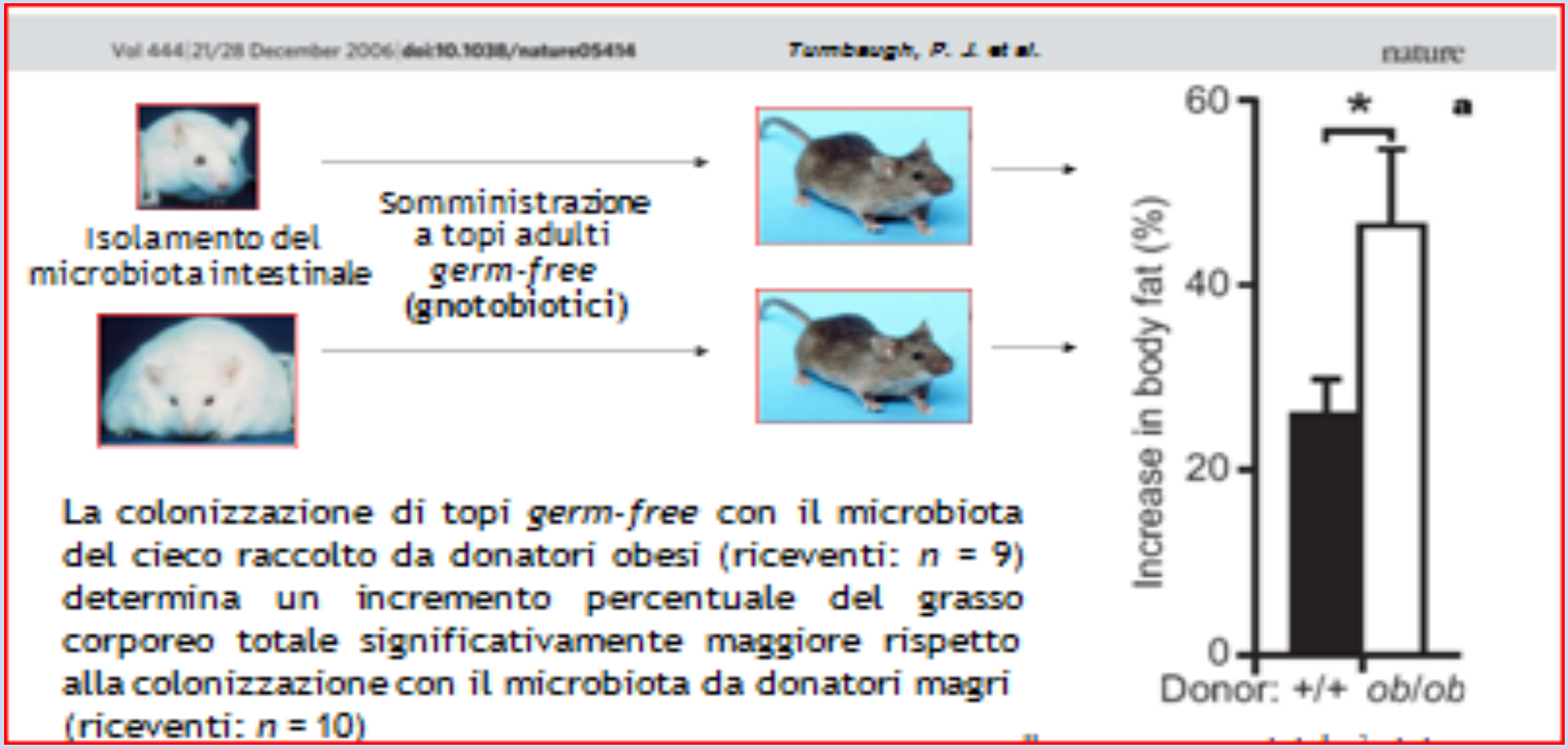
Rapporto standard tra

Firmicutes / Bacterioidites = 0,8

Se aumentano i Firmicutes andiamo incontro a obesità,

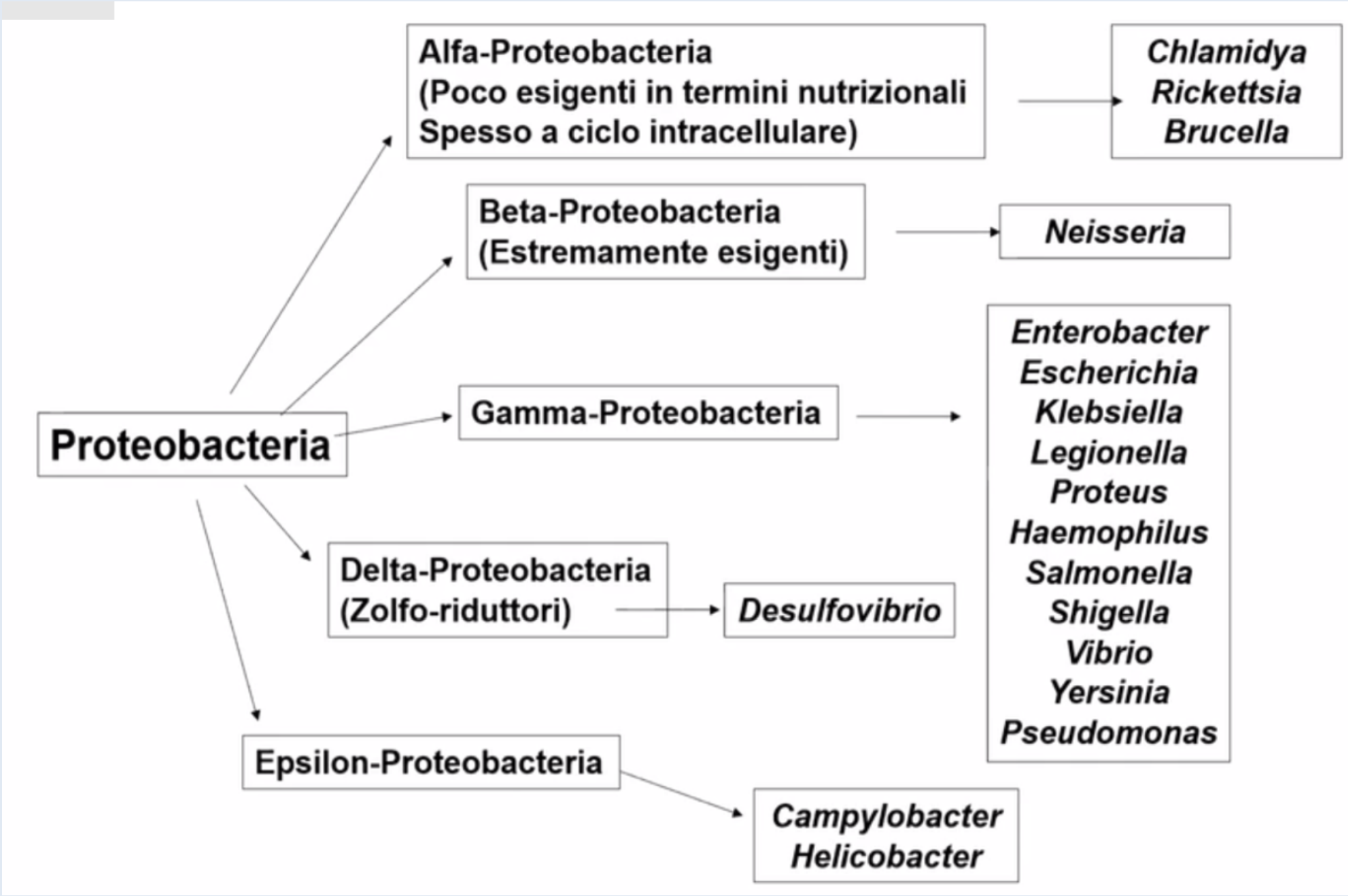
Se diminuiscono andiamo incontro ad infiammazione intestinale

I Bacterioidites proteggono dall'obesità non dal diabete



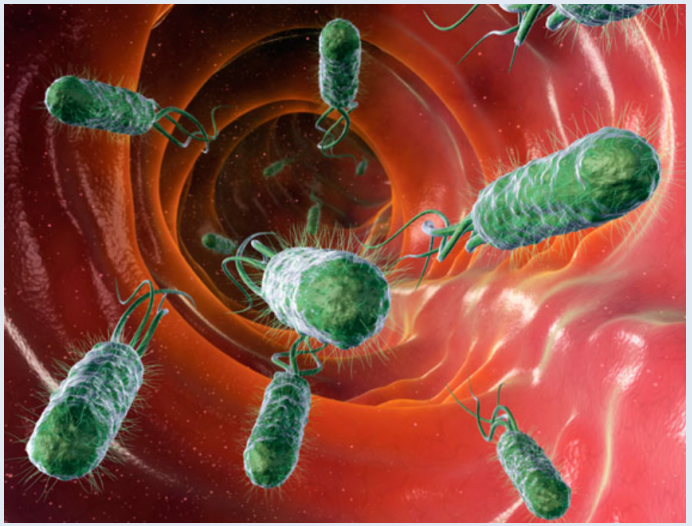
Il microbiota intestinale associato all'obesità ha una maggiore (e trasmissibile) capacità di indurre l'accumulo di grasso

a, Il grasso corporeo totale è stato misurato prima e dopo 2 settimane dalla colonizzazione, usando assorbimetria a raggi X a doppia energia. In grafico sono riportati i valori medi + errore standard s.e.m. Gli asterischi indicano le differenze significative (test *t* di Student, $*P < 0.05$)


Proteobacteria
Alfa-Proteobacteria
(Poco esigenti in termini nutrizionali
Spesso a ciclo intracellulare)
Chlamidya
Rickettsia
Brucella
Beta-Proteobacteria
(Estremamente esigenti)
Neisseria
Gamma-Proteobacteria
Enterobacter
Escherichia
Klebsiella
Legionella
Proteus
Haemophilus
Salmonella
Shigella
Vibrio
Yersinia
Pseudomonas
Delta-Proteobacteria
(Zolfo-riduttori)
Desulfovibrio
Epsilon-Proteobacteria
Campylobacter
Helicobacter

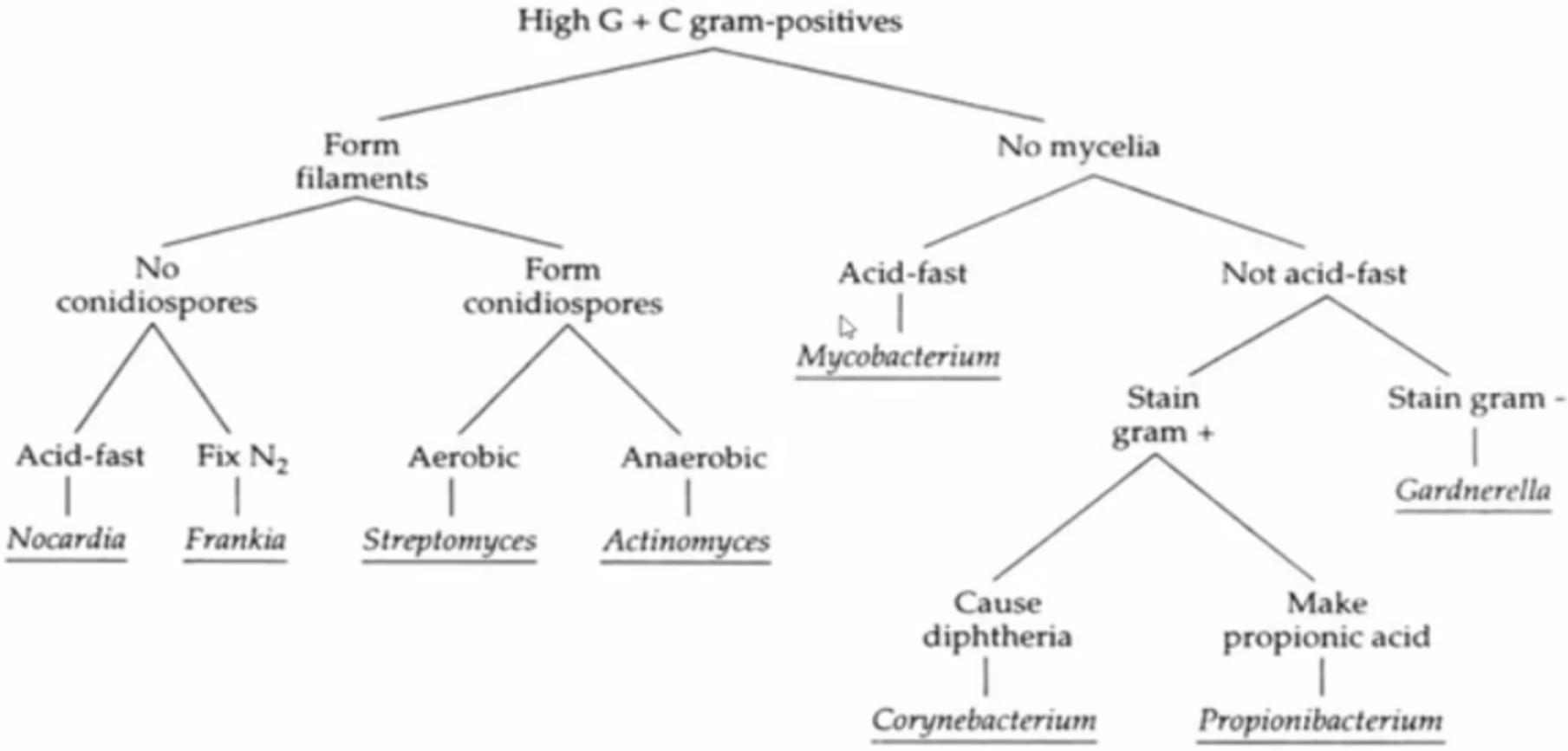
PHYLUM PROTEOBACTERIA

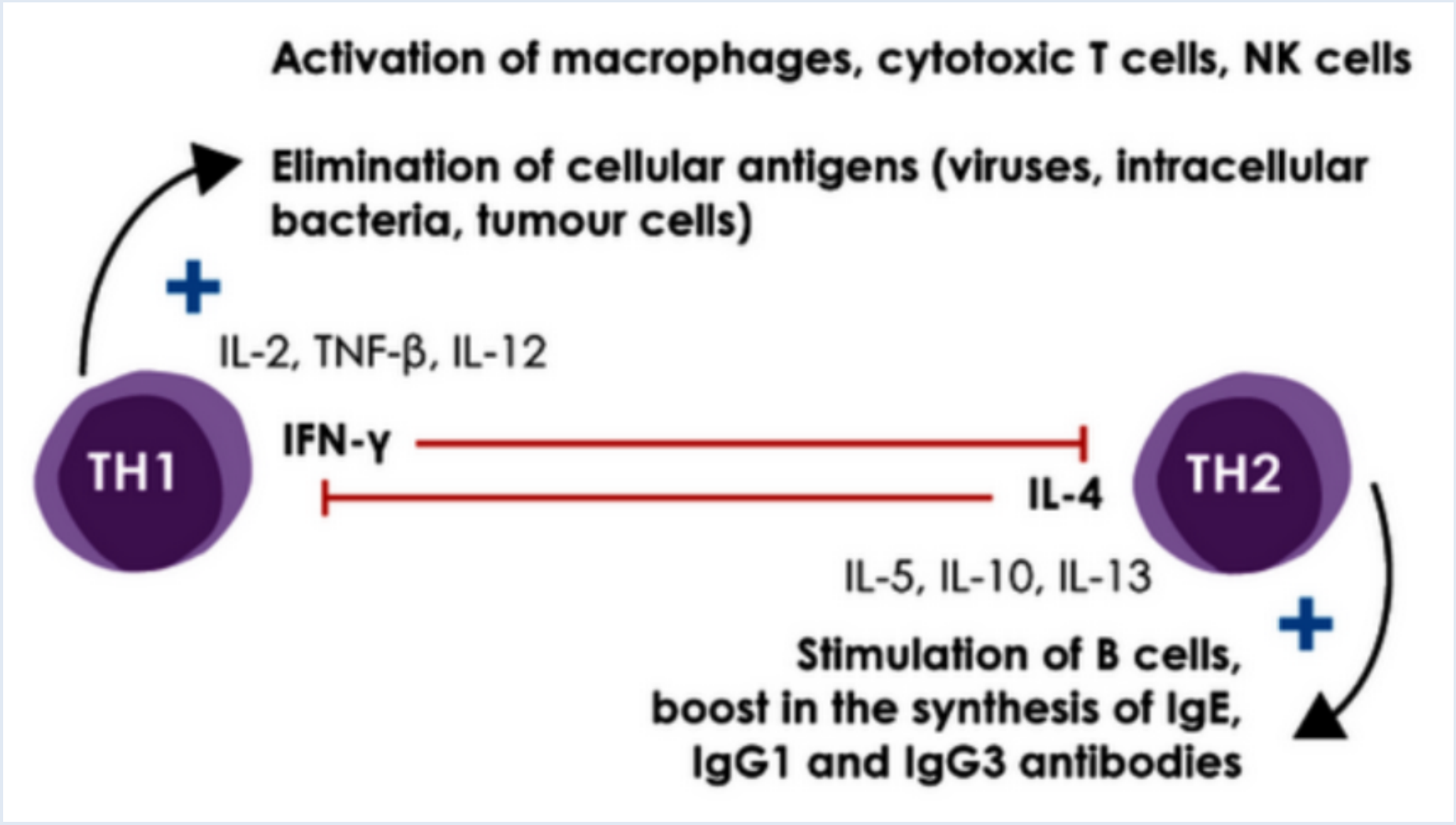
- I batteri che appartengono ai probacteria presentano una membrana esterna composta prevalentemente da LPS.
- Si spostano usando principalmente flagelli.
- Possono essere anaerobi obbligati o facoltativi.

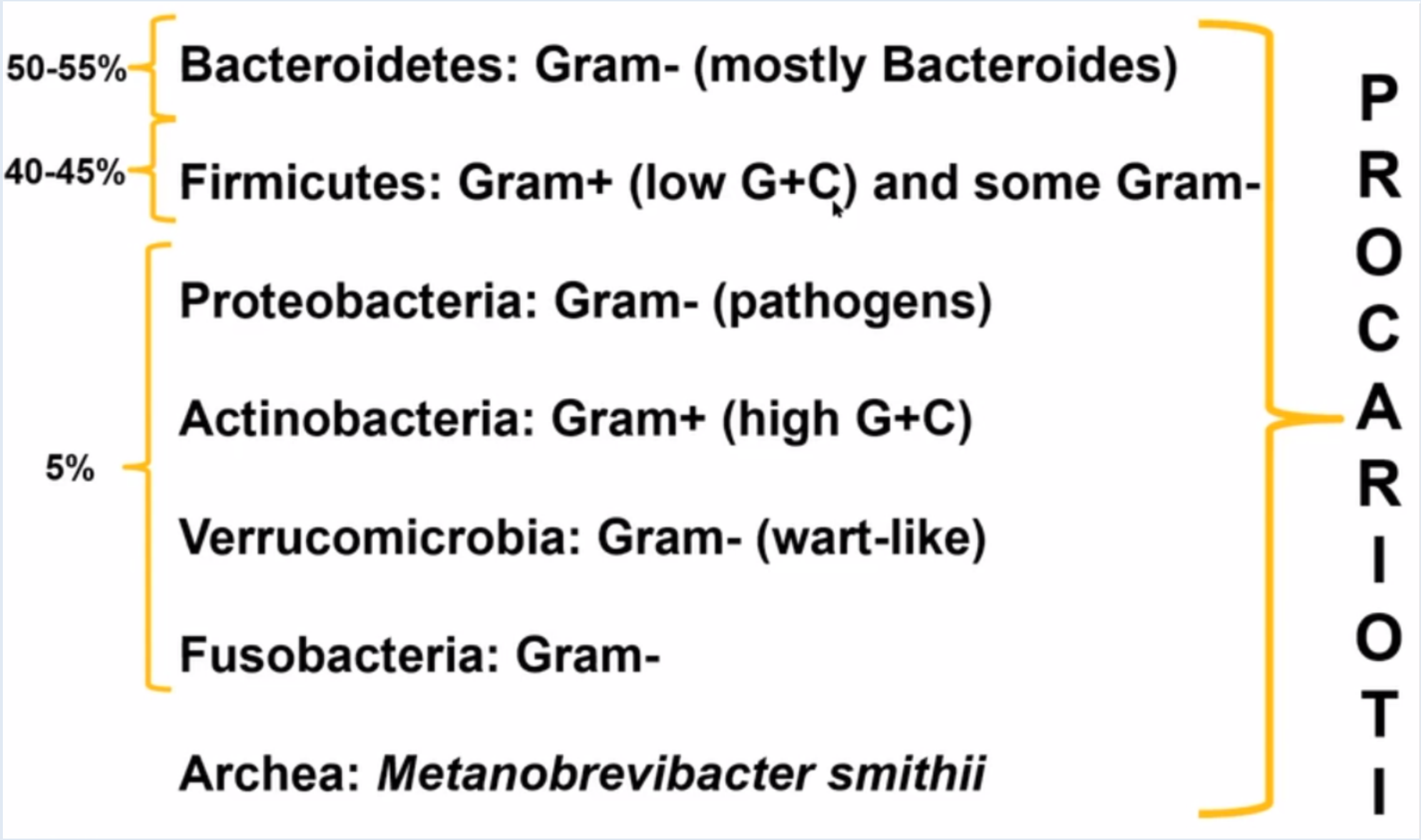


Phylum Actinobacteria

- Gram-positive - High G + C (more than 60%)
  - Coccoid, rod-coccoid , fragmenting hyphal forms, filamentous with permanent and highly differentiated branched mycelium
  - Physiologically very diverse
    - production of numerous **extracellular enzymes**, including **antibiotics**




Activation of macrophages, cytotoxic T cells, NK cells
Elimination of cellular antigens (viruses, intracellular bacteria, tumour cells)
IL-2, TNF-β, IL-12
TH1
IFN-γ
IL-4
TH2
IL-5, IL-10, IL-13
Stimulation of B cells, boost in the synthesis of IgE, IgG1 and IgG3 antibodies


50-55%
Bacteroidetes: Gram- (mostly Bacteroides)
40-45%
Firmicutes: Gram+ (low G+C) and some Gram-
5%
Proteobacteria: Gram- (pathogens)
Actinobacteria: Gram+ (high G+C)
Verrucomicrobia: Gram- (wart-like)
Fusobacteria: Gram-
Archea: Metanobrevibacter smithii
PROCARIOTI

FUNZIONE IMMUNOMODULATORIA

I batteri intestinali svolgono un ruolo essenziale nello sviluppo del sistema immune.
L'induzione e la regolazione del sistema immune avviene a livello del piccolo intestino.
Un disequilibrio della flora batterica intestinale conduce una inadeguata risposta del sistema immune che over-reagisce con gli antigeni esterni ed è responsabile dell'insorgenza di alterazioni locali e sistemiche.
Si ritiene che nelle forme di intolleranza e allergia alimentare la flora batterica intestinale possa svolgere un ruolo chiave in quanto induce una risposta iperimmune nei confronti degli antigeni alimentari.

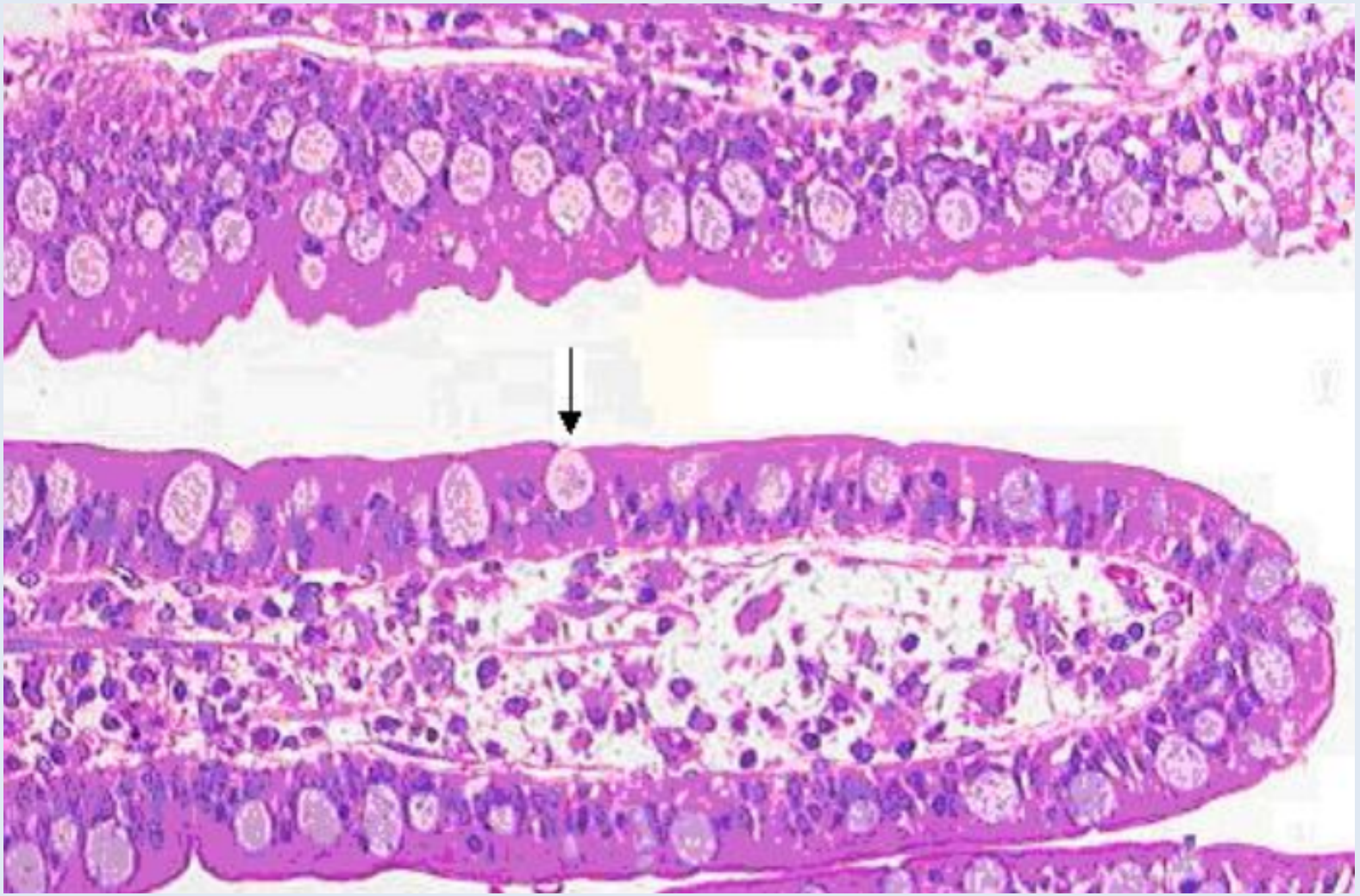
MUCO

Il microbiota è separato dalle cellule epiteliali da una rete di GLICANI (muco) prodotti dalle cellule mucipare intervallate dalle cellule epiteliali.

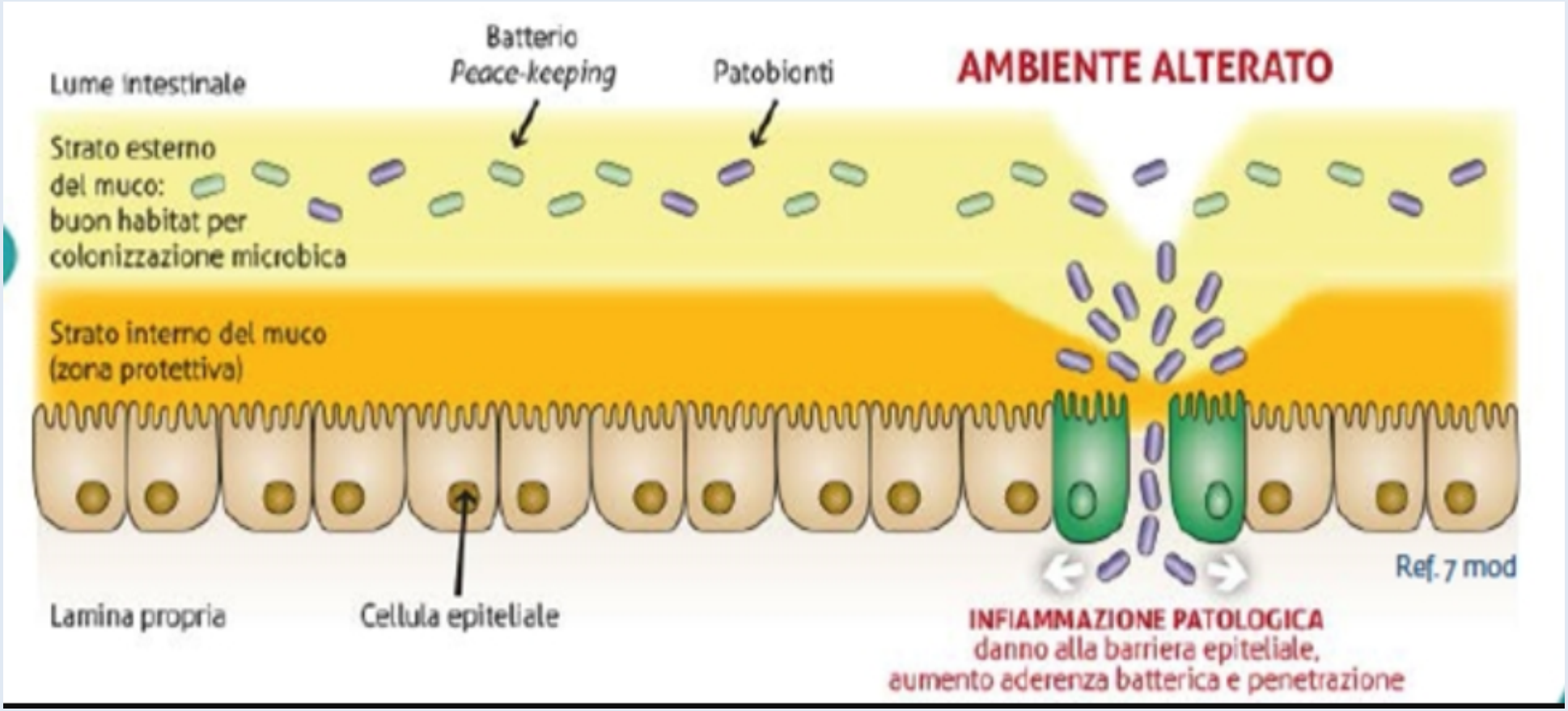
Lo strato di muco è essenziale per assicurare l'integrità della parete intestinale.

La produzione giornaliera di muco è di circa 5 litri.

CELLULA MUCIPARA



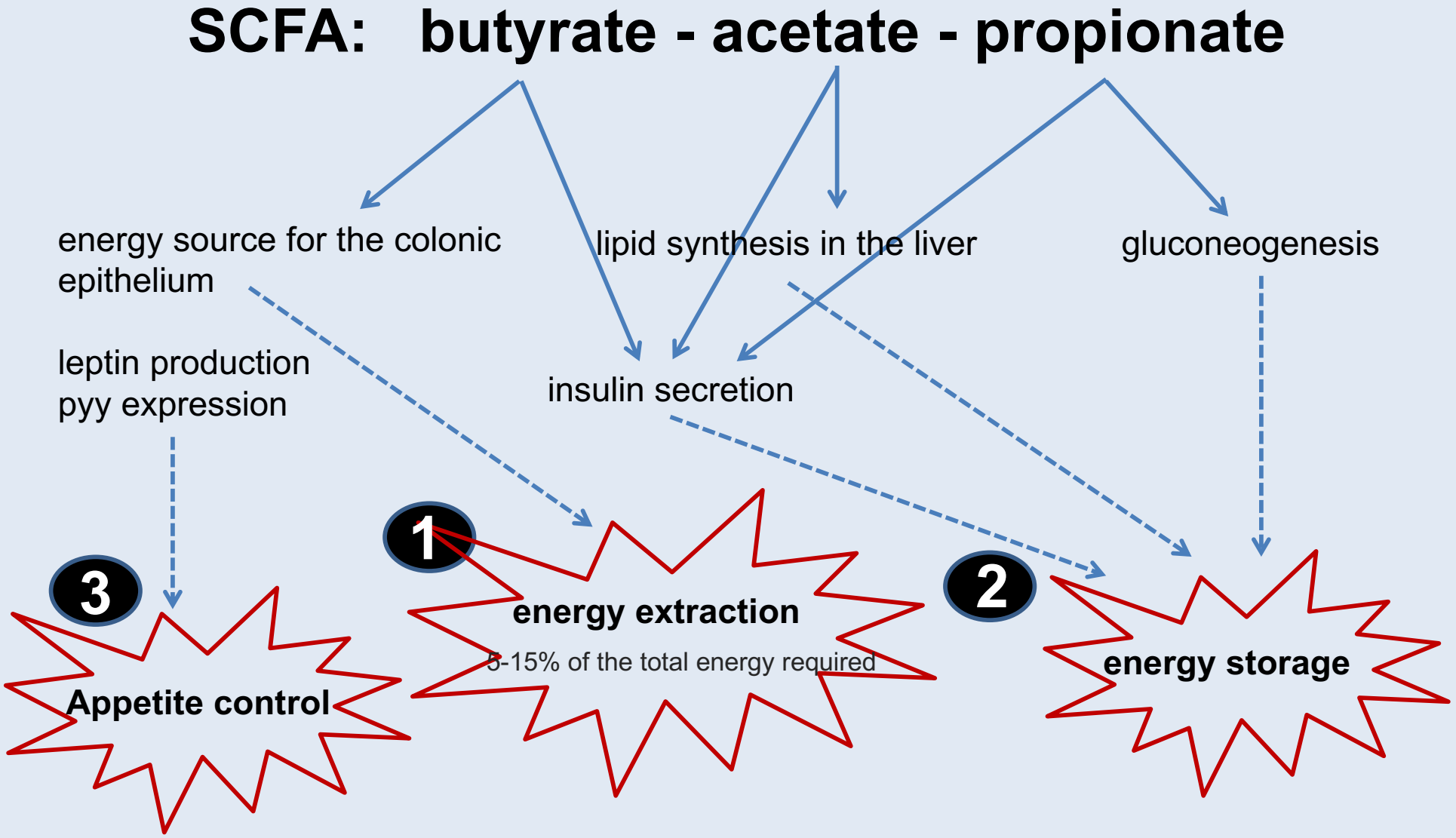
ALTERAZIONE DEL MUCO



Il microbiota contribuisce alla produzione di muco ed allo spessore dello strato mucoso.

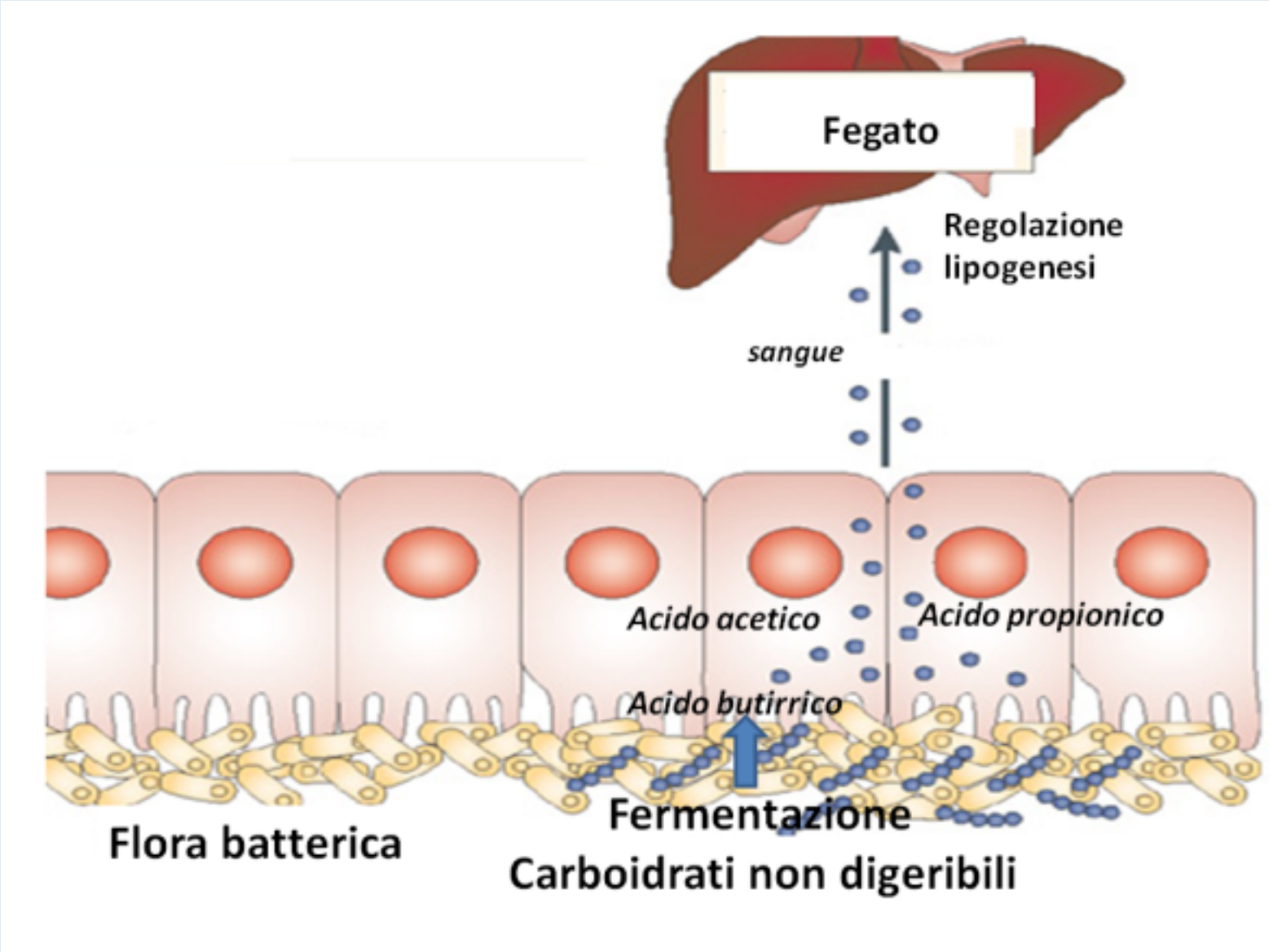
Questo avviene in parte per la stimolazione della sintesi di Mucina da parte di alcuni componenti di sintesi batterica quali SCFA (acido butirrico acetico, propionico).

SCFA, MICROBIAL METABOLITES WITH A KEY MULTIFACTORIAL ROLE IN HOST NUTRITION

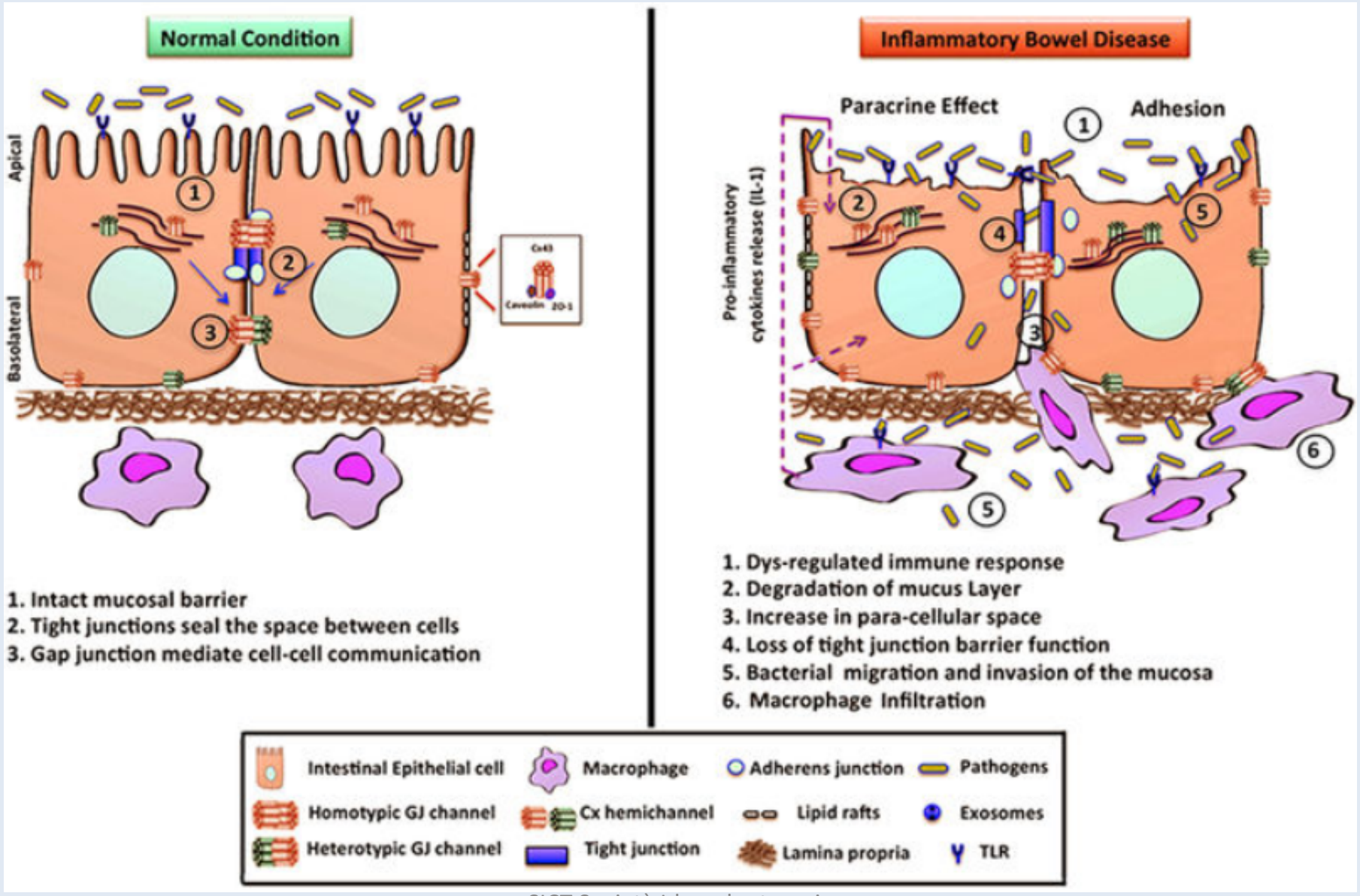


SCFA also possess immunomodulating and antimicrobial properties

ACIDO BUTIRRICO



1 - *Alterazione delle giunzioni serrate: passaggio di antigenici nello strato sottomucoso*



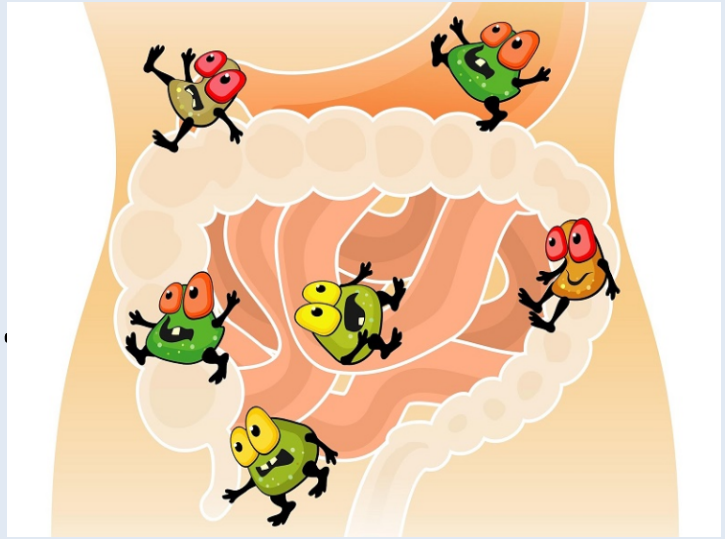
TRASLOCAZIONE BATTERICA

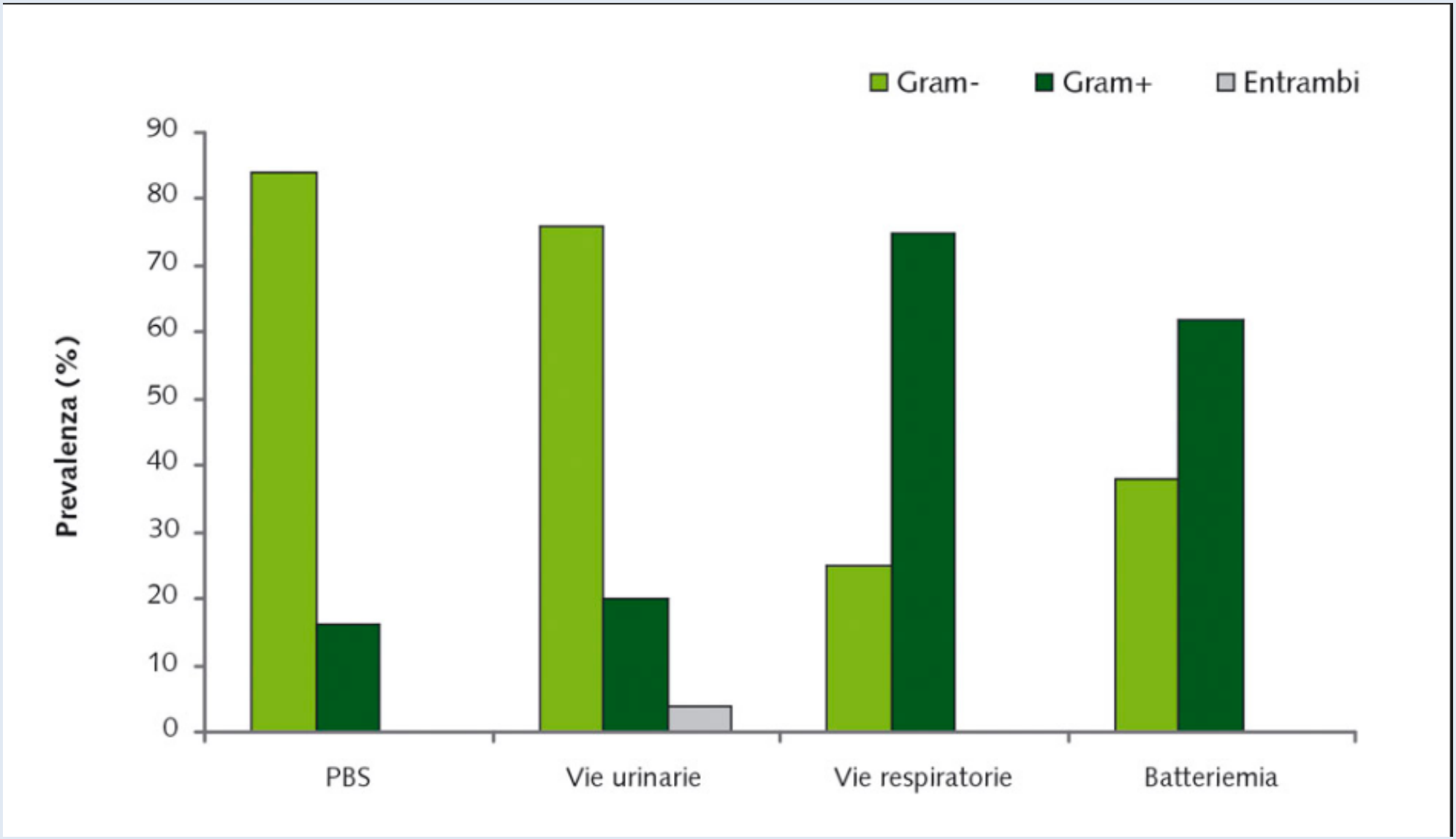
La condizione di disbiosi porta ad una disfunzione indiretta quindi non primaria, ma secondaria, della BARRIERA EPITELIALE INTESTINALE che crea una via di ingresso nell'organismo tramite il sangue di batteri, dando origine a fenomeni di

Traslocazione batterica

cioè di batteri in luoghi

diversi da quelli di origine.




Gram-
Gram+
Entrambi
Prevalenza (%)
90
80
70
60
50
40
30
20
10
0
PBS
Vie urinarie
Vie respiratorie
Batteriemia

ZONULINA

La individuazione della Zonulina avviene nel 2000 ad opera di A. Fasano, a capo della ricerca presso L'Università del Maryland Celiac Research Center, e del suo team presso l'Università del Maryland School of Medicine.

Ruolo della zonulina nelle modificazioni della permeabilità intestinale tipiche della fase acuta della malattia celiaca

T. NOT, I. BERTI, A. FASANO, S. FACCHINI, C. TREVISIOL, E. NERI, A. CITTÀ, A. VENTURA
Dipartimento di Pediatria, Università del Maryland, USA, IRCCS "Burlo Garofolo", Trieste, Italia

La gliadina rappresenta il primo ambito della ricerca che ha potuto mettere a fuoco il ruolo della zonulina sulla giunzione serrature. Il glutine influisce direttamente sulla mucosa intestinale favorendo la produzione di zonulina. E' una proteina che regola le giunzioni delle pareti intestinali e, se in eccesso, le allenta favorendo uno stato di permeabilità intestinale.

Minerva Med. 2018 Aug 28. doi: 10.23736/S0026-4806.18.05787-7. [Epub ahead of print]

Serum zonulin in patients with inflammatory bowel disease: a pilot study.

Caviglia GP[1], Dughera F[1], Ribaldone DG[2], Rosso C[1], Abate ML[1], Pellicano R[3], Bresso F[4], Smedile A[1,3], Saracco GM[1,3], Astegiano M[3].

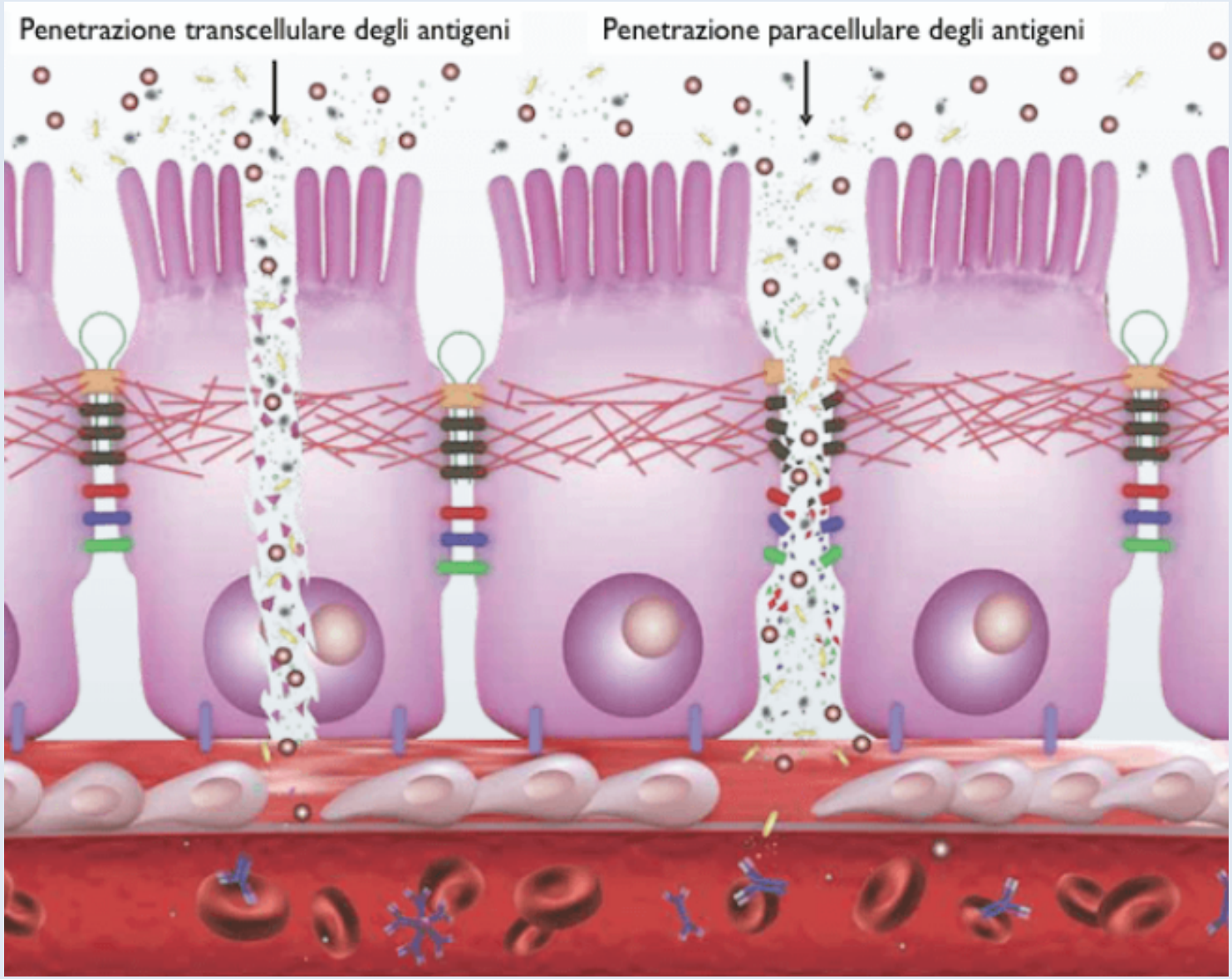
Tissue Barriers. 2016 Oct 21;4(4):e1251384. doi: 10.1080/21688370.2016.1251384. eCollection 2016.

Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases.

Sturgeon C[1], Fasano A[2].

Alcuni studi hanno evidenziato come la disbiosi del microbioma può causare il rilascio di zonulina che porta al passaggio di contenuti endoluminali attraverso la barriera epiteliale con conseguente rilascio di citochine pro infiammatorie.

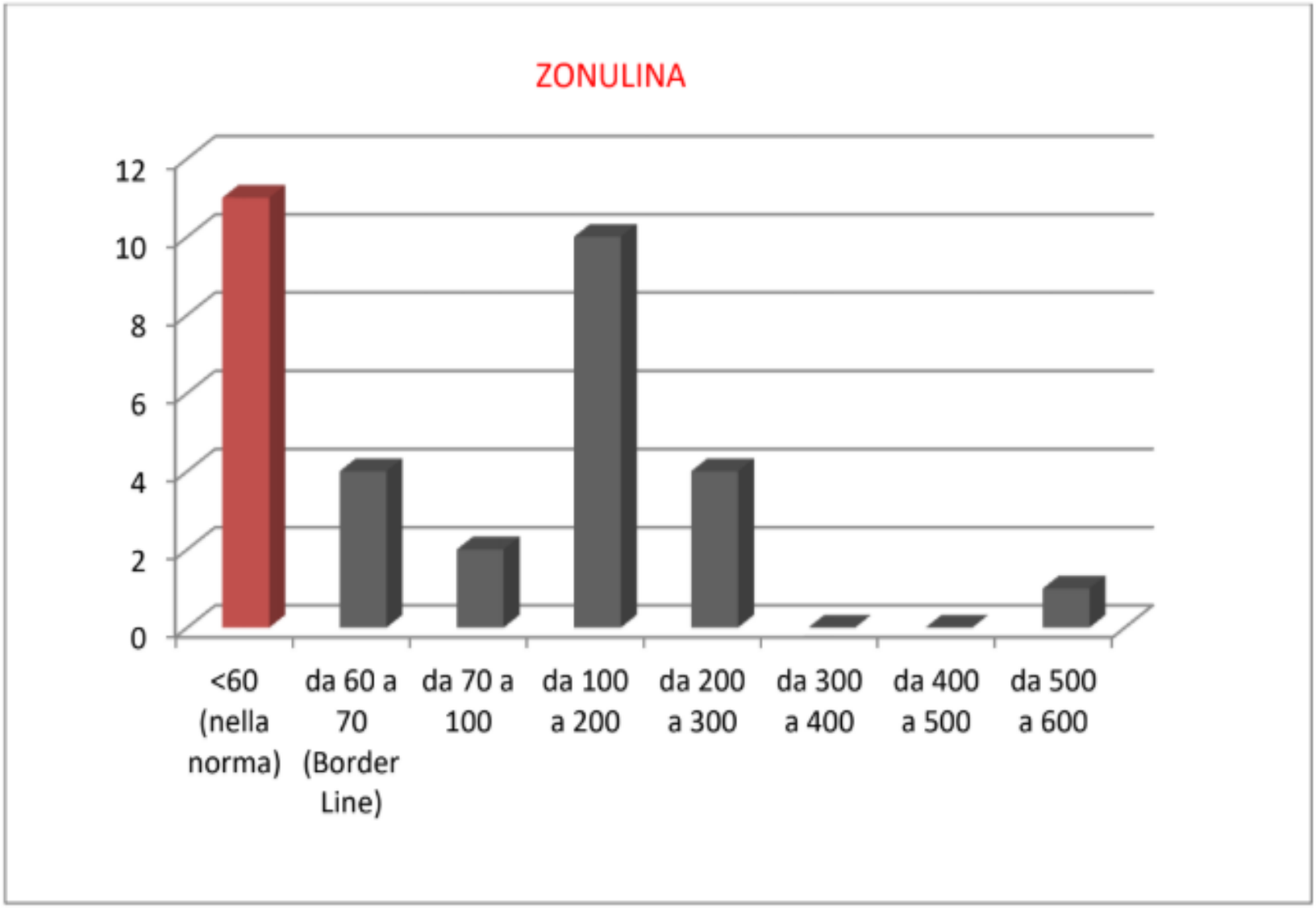
ZONULINA


Penetrazione transcellulare degli antigeni
Penetrazione paracellulare degli antigeni

I valori della zonulina possono rappresentare il parametro di riferimento per la classificazione dell'entità dell'alterazione della mucosa intestinale e conseguente stato di permeabilità.

I valori riscontrati sono significativi dello stato infiammatorio in atto e correlabili con i sintomi clinici.

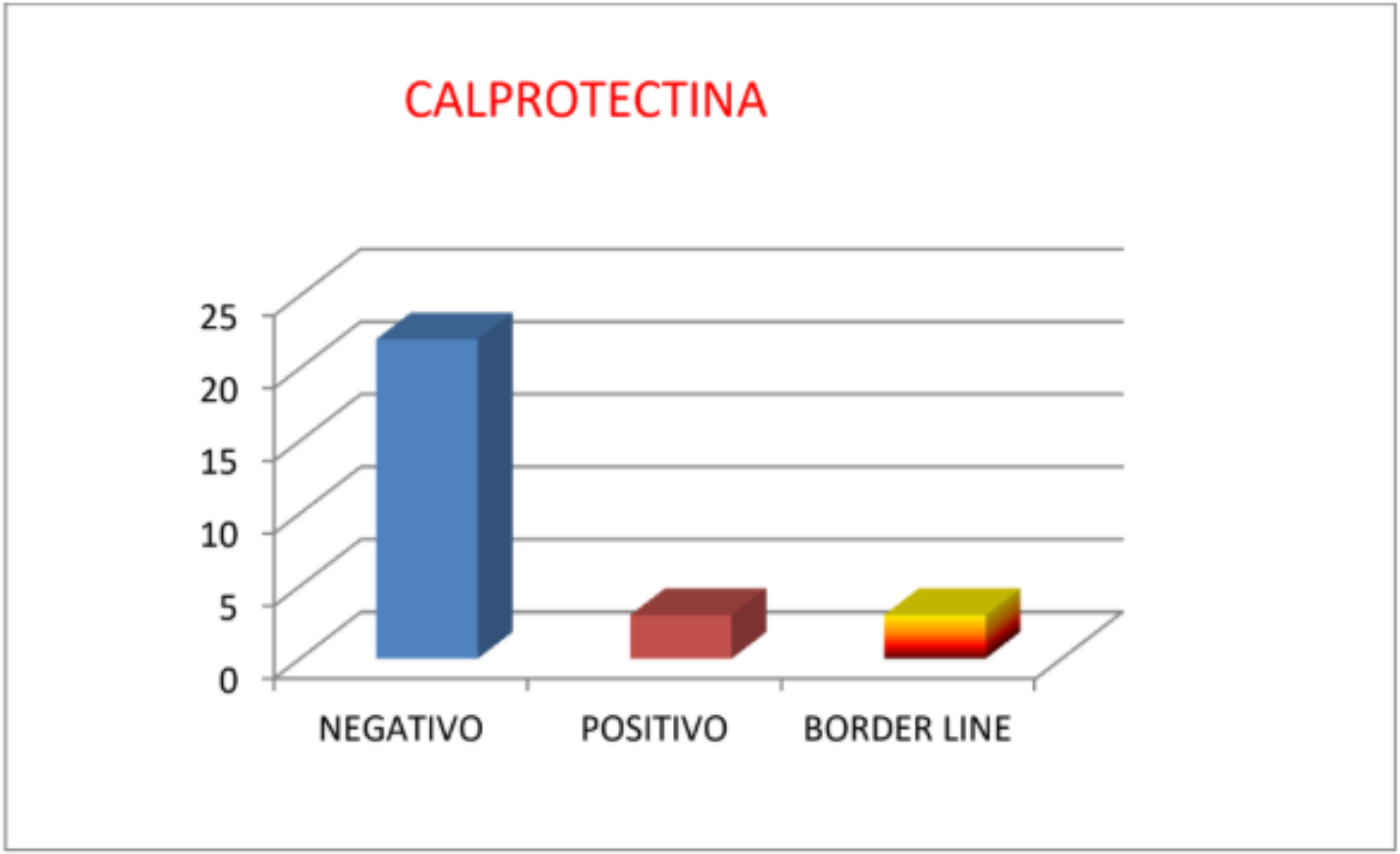
ZONULINA


ZONULINA
12
10
8
6
4
2
0
<60 (nella norma)
da 60 a 70 (Border Line)
da 70 a 100
da 100 a 200
da 200 a 300
da 300 a 400
da 400 a 500
da 500 a 600

ZONULINA

	VALORI	%
<60 (nella norma)	11	34%
da 60 a 70 (Border Line)	4	13%
da 70 a 100	2	6%
da 100 a 200	10	31%
da 200 a 300	4	13%
da 300 a 400	0	0%
da 400 a 500	0	0%
da 500 a 600	1	3%
	32	

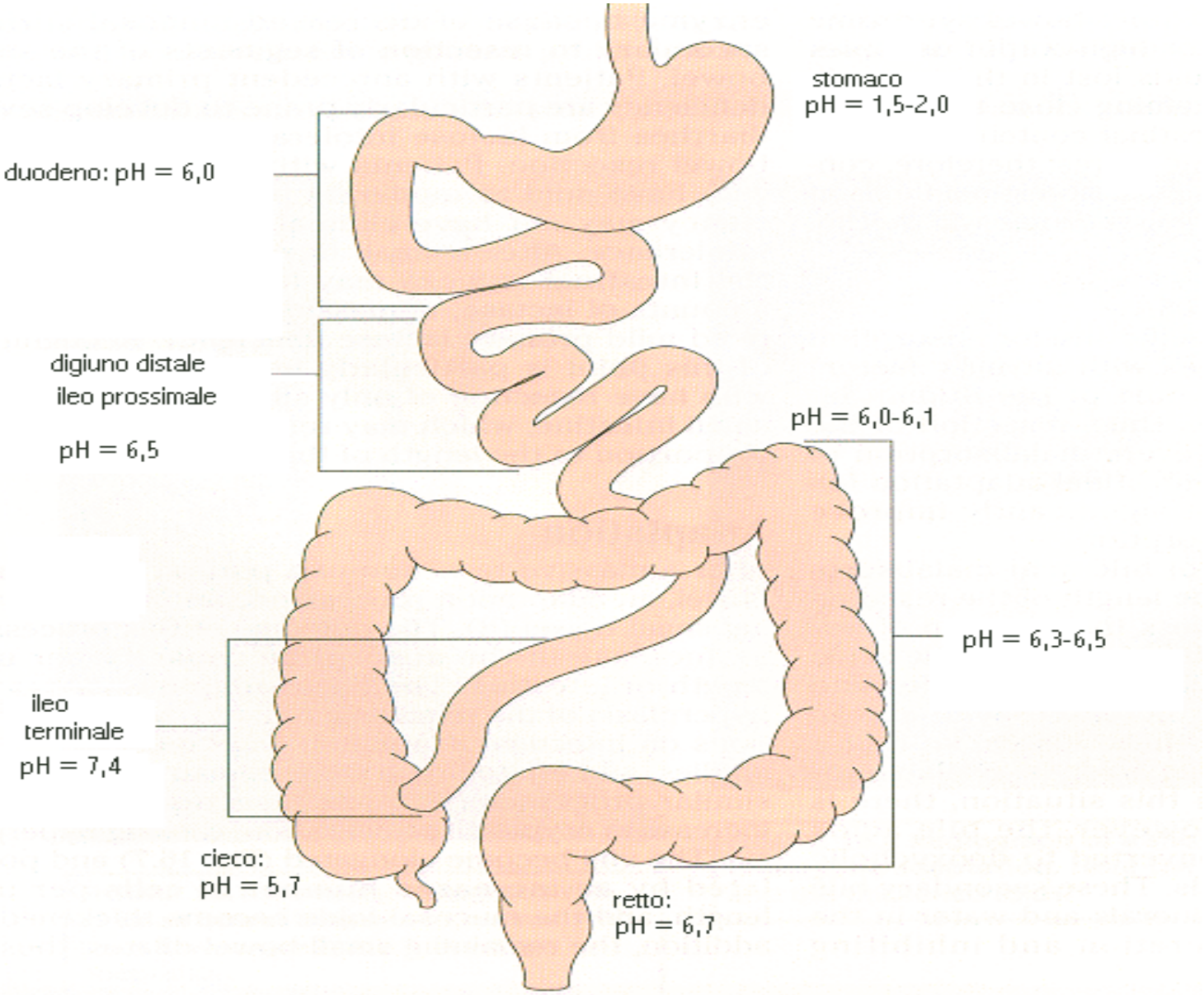
CALPROTECTINA



CALPROTECTINA

	valori	%
NEGATIVO	22	79%
POSITIVO	3	11%
BORDER LINE	3	11%
	28	

IL PH NEI SINGOLI DISTRETTI



PH *INTESTINALE*

Il Ph del colon ha un marcato effetto nella composizione delle specie batteriche presenti.

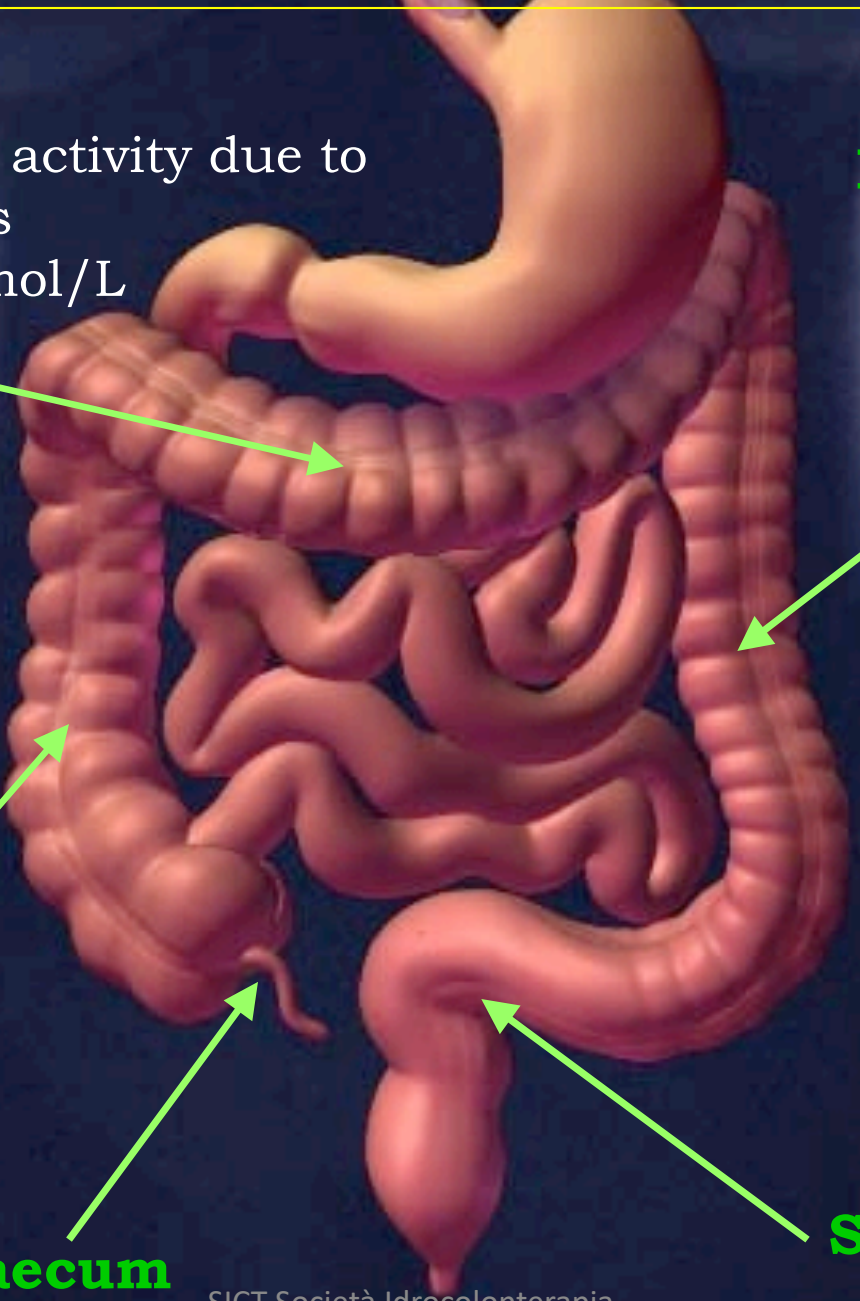
Walher et al (2005) hanno dimostrato una correlazione tra cambiamento nella composizione della specie dei microrganismi da:

- Ph 5.5 dove i batteri formanti butirrato sono il 20%
- Mentre a Ph 6.5 questo gruppo diventa impercettibile

PH *INTESTINALE*

- A Ph 6.5 la comunità dei fermentatori è dominata dai BACTEROIDI gram- patogeni.

REGIONAL DIFFERENCES IN FERMENTATIVE ACTIVITIES OF BACTERIA GROWING IN THE LARGE INTESTINE



Transverse colon

Reduction in bacterial activity due to depletion of substrates
Total SCFA *ca* 117 mmol/L
pH 6.2

Distal colon

Little carbohydrate fermentation
High concentration of products of protein fermentation (phenols, indoles, ammonia)
Total SCFA *ca* 90 mmol/L
pH 6.6 – 6.9

Proximal colon

Active fermentation
High bacterial growth rates
Total SCFA *ca* 127 mmol/L
pH 5.4 – 5.9

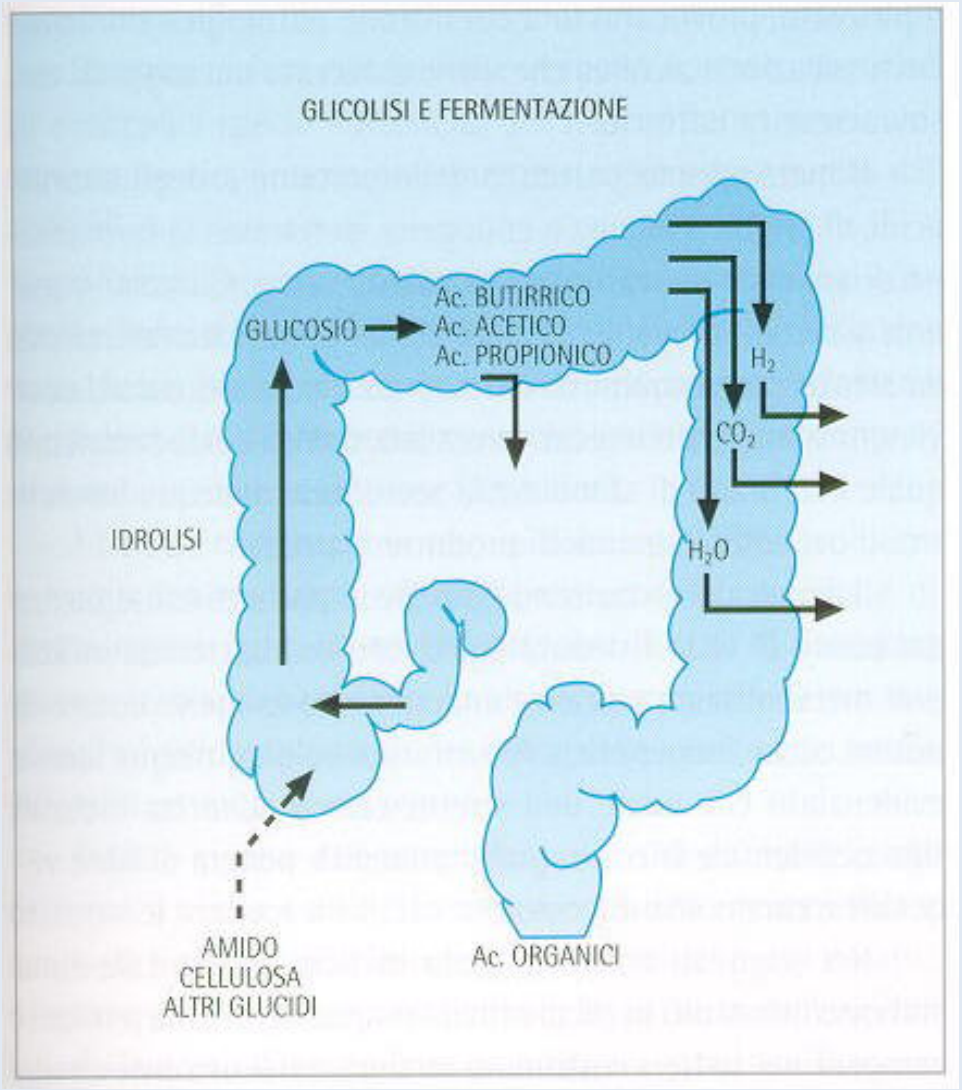
Caecum

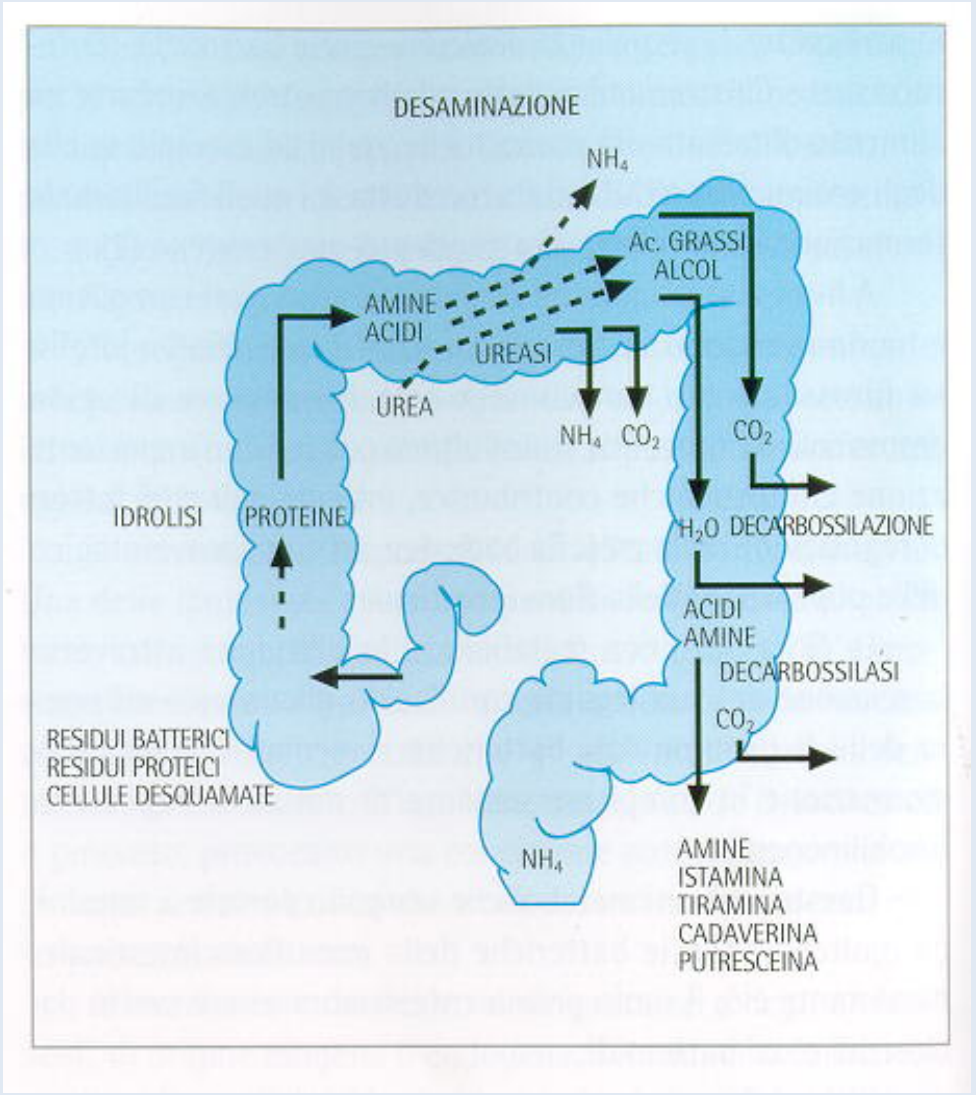
Sigmoid rectum

MODIFICAZIONI DELLE ABITUDINI ALIMENTARI NEI PAESI OCCIDENTALI NEGLI ULTIMI 100 ANNI

- ↑ apporto di zuccheri raffinati
- ↑ consumo di sodio (almeno 10 volte)
- ↑ consumo di grassi saturi (almeno 4 volte) e di cibi ricchi di colesterolo
- ↓ significativo dell'apporto di fibre alimentari e di minerali come potassio, magnesio, calcio e cromo.
- ↓ consumo di grassi omega3, lipidi di membrana, vitamine e antiossidanti.

LA FLORA FERMENTATIVA E PUTREFATTIVA





LA FLORA fermentativa

La fonte principale di carbonio e di energia per i batteri è costituita da carboidrati complessi (amido e altri polisaccaridi).
Il metabolismo dei carboidrati è di grande importanza a livello del grosso intestino, dato che i microrganismi sono prevalentemente *saccarolitici.*

La **fermentazione** è una reazione anaerobia nella quale le molecole di piruvato al posto di essere degradate nei mitocondri, rimangono nel citosol e – nel caso dei lieviti- vengono convertiti a **etanolo e CO_2**.

In mancanza di carboidrati fermentabili o in caso di aumentata presenza di proteine per una dieta ricca in grassi e carne e a basso contenuto in fibre la flora attua una degradazione enzimatica delle proteine e degli aminoacidi, detta **fermentazione proteica** o **putrefazione** con produzione di **ammonio e amine**.

LA FLORA Putrefattiva

La fermentazione proteica o *putrefazione* comporta
• un' aumentata concentrazione di Batteriodi
• aumento del pH fecale per aumento dell'ammonio fecale e con
• formazione di un certo numero di metaboliti potenzialmente tossici, ovvero di **fenoli, indoli e amine**.

• L'entità della fermentazione proteica si misura **dall'eliminazione urinaria di fenolo e di p-cresolo.**
• La produzione di queste sostanze è inibita o soppressa in molti microrganismi intestinali in caso di disponibilità di un substrato fermentabile di carboidrati *(6).*
• I processi putrefattivi diventano invece quantitativamente più **importanti nel colon distale**, ove i carboidrati fermentabili sono più scarsi. *(6)*

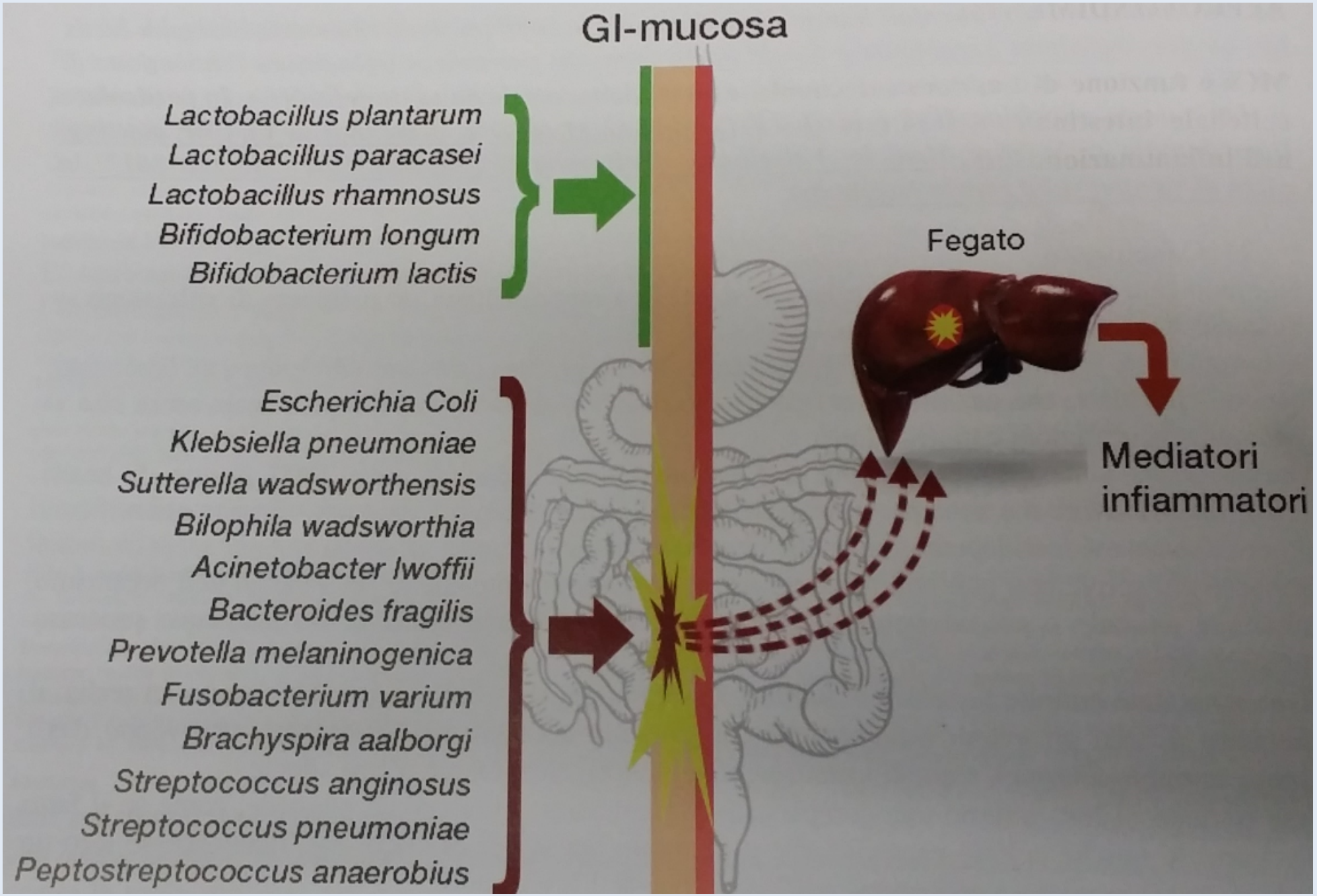
La disbiosi intestinale

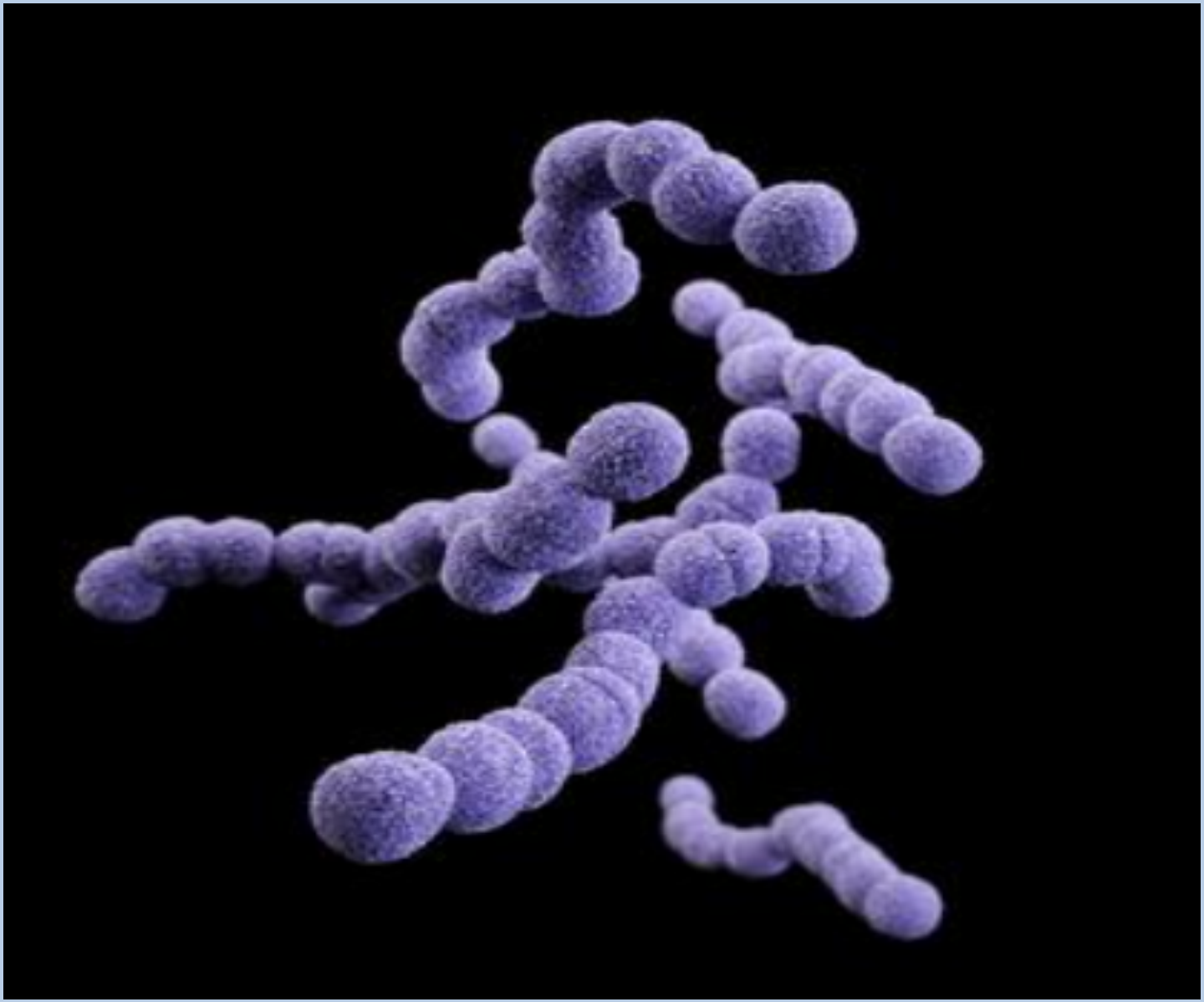
Definizione

Disbiosi è **uno stato di alterata ecologia della microflora che causa malattia.**
Può esistere a livello del cavo orale, del tratto gastroenterico e in vagina.
Il termine *disbiosi intestinale* tende a sostituire quello più vecchio di dismicrobismo e a cui si contrappone il termine di eubiosi.

In caso di disbiosi, organismi a bassa virulenza intrinseca, quali batteri, lieviti e protozoi, possono indurre malattie mediante alterazioni dello stato nutrizionale o la risposta immune dell'ospite *(9)*
Un'alterazione di quest'ecosistema può comportare importanti conseguenze sullo stato di salute con sintomi o patologie sia locali che sistemici.

La disbiosi **può interessare sia il tenue che il grosso intestino**, per cui il termine disbiosi non è da intendere come sinonimo di sovraccrescita batterica, la quale interessa soltanto il piccolo intestino, e che ne è quindi una delle forme possibili.


GI-mucosa
Lactobacillus plantarum
Lactobacillus paracasei
Lactobacillus rhamnosus
Bifidobacterium longum
Bifidobacterium lactis
Escherichia Coli
Klebsiella pneumoniae
Sutterella wadsworthensis
Bilophila wadsworthia
Acinetobacter lwoffii
Bacteroides fragilis
Prevotella melaninogenica
Fusobacterium varium
Brachyspira aalborgi
Streptococcus anginosus
Streptococcus pneumoniae
Peptostreptococcus anaerobius
Fegato
Mediatori
infiammatori



G B S

SICT Società Idrocolonterapia

STREPTOCOCCO AGALATIAE
GBS

E’ un cocco gram positivo

Appartiene al Phylum FIRMICUTES

PATOBIONTE

E’ principale responsabile:

- Severe infezioni batteriche del lattante
- Endometriti amniositi infezioni urinarie nelle gravide
- Stati infettivi nei diabetici e malati cronici

COLONIZZAZIONE GBS

Tra 4 e 36 % con maggioranza superiore al 20% nei Paesi Europei

In ogni Paese il 18-20% delle gravide è portatrice di GBS

Di solito la donna gravida con colonizzazione GBS è asintomatica ma costituisce il pre requisito per la trasmissione madre-neonato

La colonizzazione e la persistenza

nell’ambiente intestinale e vaginale

dell’ospite dipende

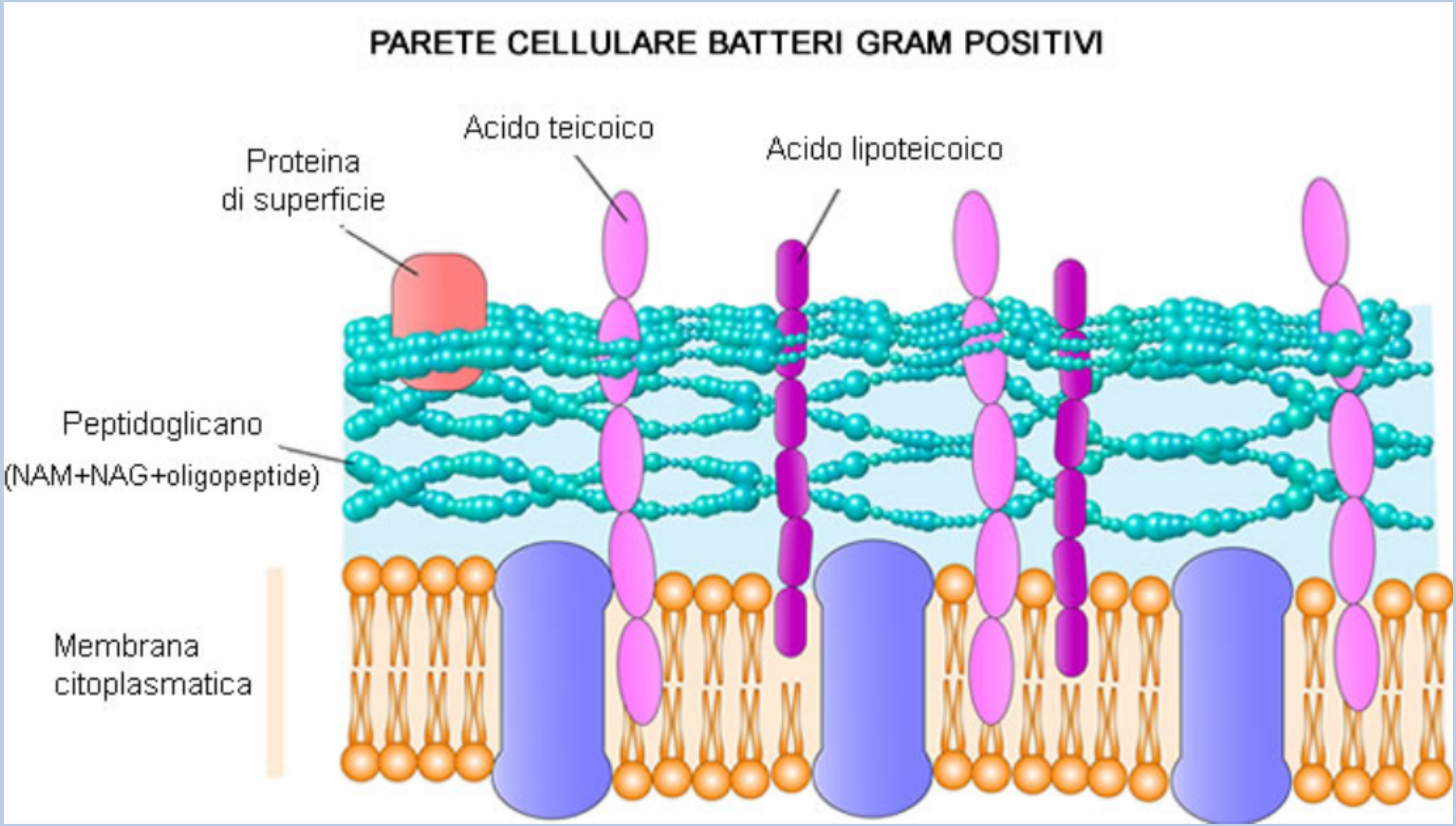
DALLA CAPACITA’ DI ADERENZA DEL GBS

SUGLI EPITELI

Le condizioni ambientali dell'ospite sono determinanti per lo sviluppo del biofilm batterico:

Una maggiore aderenza del GBS alle cellule epiteliali vaginali si verifica maggiormente in ambiente a PH neutro rispetto a un PH acido

La rigida struttura della parete cellulare caratteristica dei batteri Gram positivi consente loro di sopravvivere in ambiente ipotonico, modulando l’assunzione di acqua dall’esterno tale da impedire la lisi cellulare

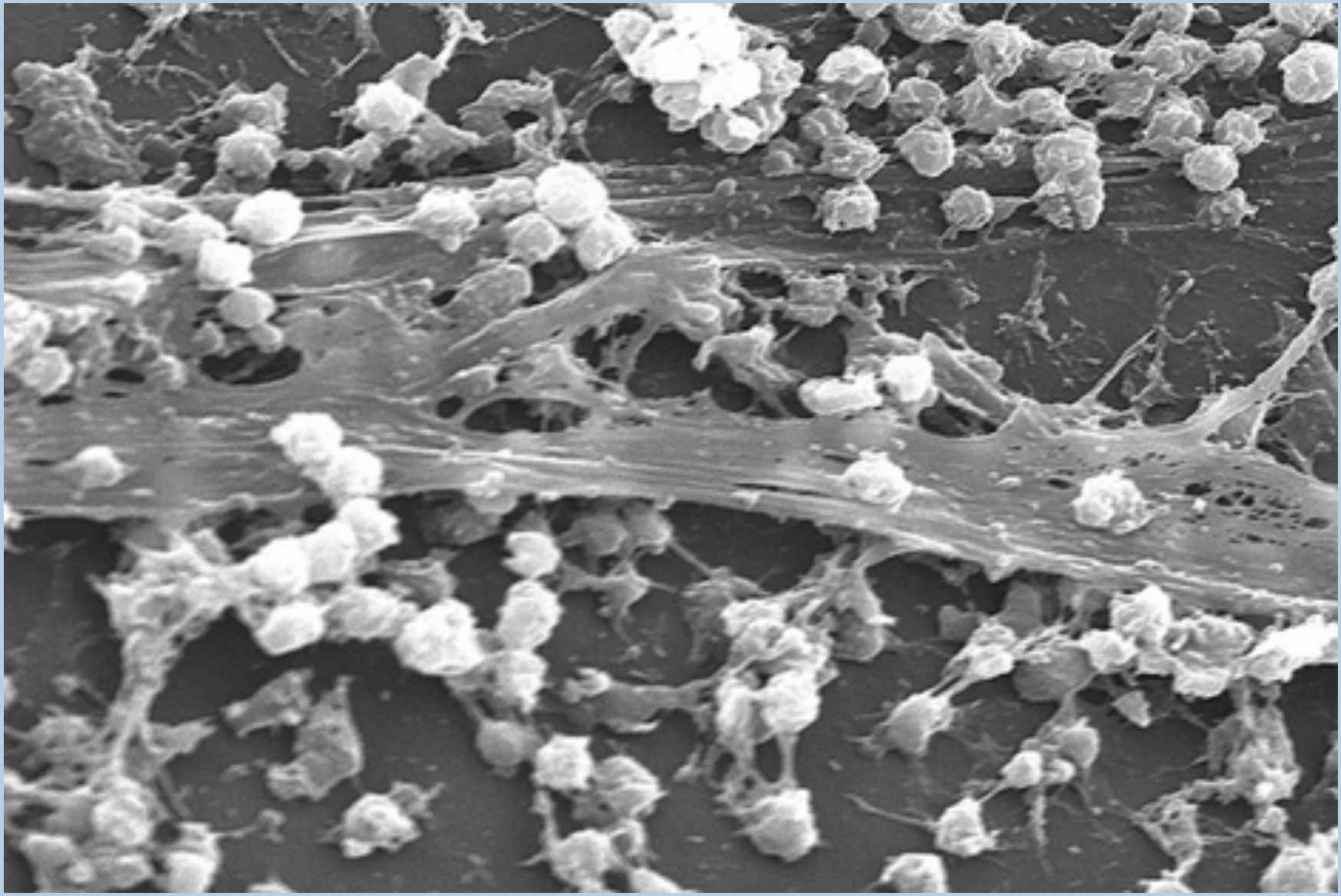

PARETE CELLULARE BATTERI GRAM POSITIVI
Acido teicoico
Acido lipoteicoico
Proteina
di superficie
Peptidoglicano
(NAM+NAG+oligopeptide)
Membrana
citoplasmatica

Il GBS presenta una capacità di adesione sia sulla matrice extra cellulare che sulle cellule ospiti svolta da una ADESINA BiBA

In grado di interferire con le proteine del COMPLEMENTO e inibire la fagocitosi neutrofila

Nell’ultimo decennio sono state scoperte strutture filamentose nel GBS che potrebbero esprimere un ulteriore ruolo nella adesione cellulare e nella colonizzazione e infezione dell’ospite.

Queste strutture favoriscono la formazione del biofilm



La presenza di pili e la formazione di Biofilm ha permesso di identificare un sottogruppo di ceppi del sierotipo III (tipo ST-17) in grado di formare biofilm più resistenti di altri e di essere la causa più frequenti di infezioni neonatali ad esordio tardivo

Review > FEMS Microbiol Rev. 2016 Jul;40(4):464-79. doi: 10.1093/femsre/fuw006.
Epub 2016 Mar 13.

Bacterial glycobiology: rhamnose-containing cell wall polysaccharides in Gram-positive bacteria

Michel-Yves Mistou [1], Iain C Sutcliffe [2], Nina M van Sorge [3]

Le variazioni della struttura del polisaccaride capsulare hanno determinato 10 varianti antigeniche ad oggi conosciute. In generale la capsula è composta da unità ripetute di glucosio, galattosio, ramnosio, N-acetilglucosammina, acido sialico.

Nei sierotipi Ia, Ib e III lo zucchero terminale è rappresentato dall'acido sialico che gioca un ruolo importante nella virulenza.

Infatti la rimozione di questo residuo porta ad una perdita di virulenza nel modello del ratto neonato e un aumentata fagocitosi da parte dei neutrofili umani.

Comparative Study

BMC Genomics. 2017 Jun 1;18(1):429. doi: 10.1186/s12864-017-3820-5.

Comparison of molecular serotyping approaches of Streptococcus agalactiae from genomic sequences

Georgia Kapatai [1], Darshana Patel [2], Androulla Efstratiou [2], Victoria J Chalker [3]

Infect Immun. 2005 May;73(5):3096-103. doi: 10.1128/IAI.73.5.3096-3103.2005.

Structural and genetic diversity of group B streptococcus capsular polysaccharides

Michael J Cieslewicz [1], Donald Chaffin, Gustavo Glusman, Dennis Kasper, Anup Madan, Stephani Rodrigues, Jessica Fahey, Michael R Wessels, Craig E Rubens

L’adesione e la colonizzazione del GBS è un complesso multifattoriale che porta al mantenimento del patobionte nell’ecosistema umano.

Il GBS possiede rigidi sistemi che favoriscono il suo mantenimento.

La colonizzazione e la persistenza dipendono anche dalla qualità del microbiota vaginale e intestinale.