

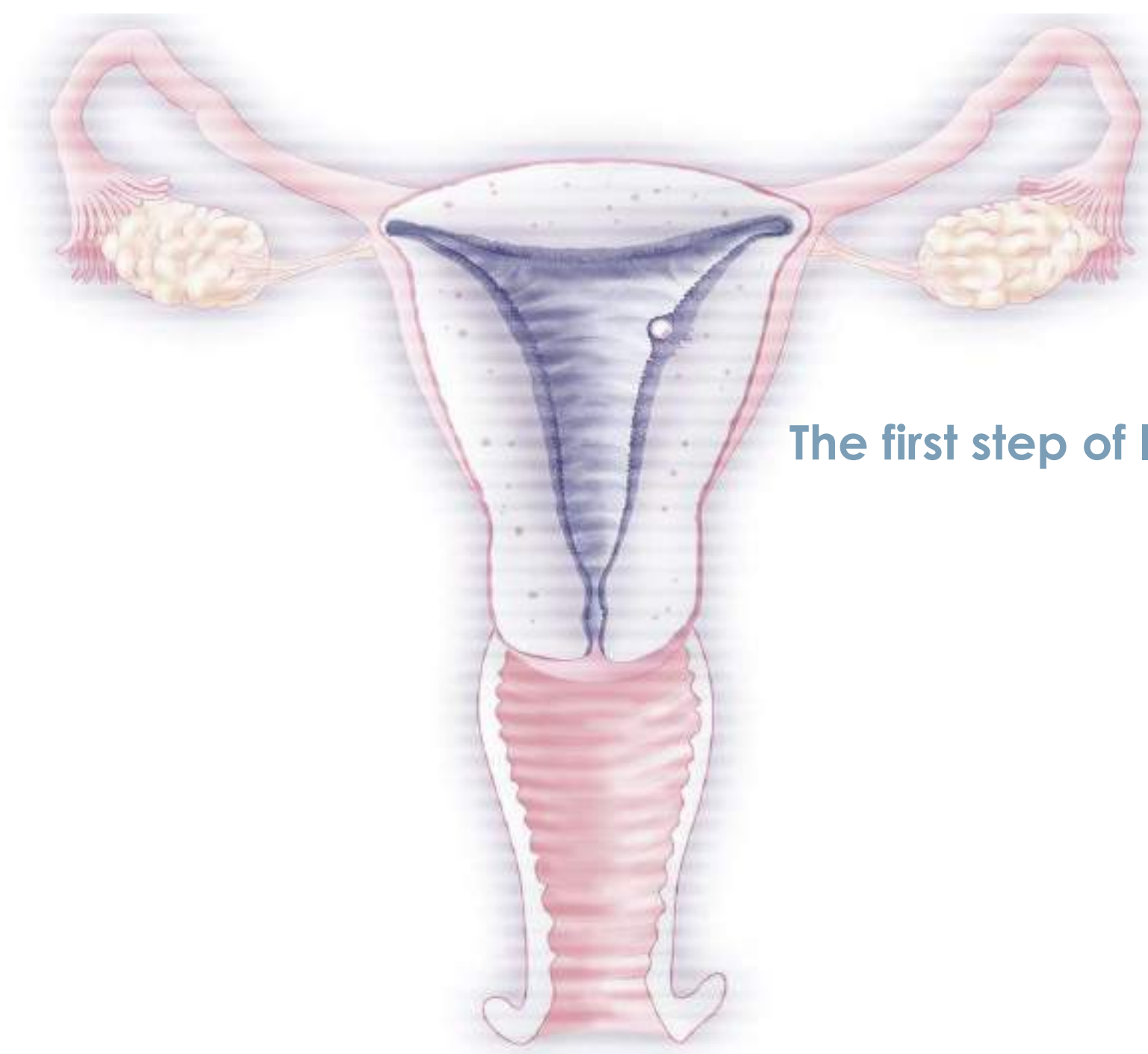
# Microbiota: La nueva era del análisis endometrial

Dra. Inmaculada Moreno

21 de abril de 2020

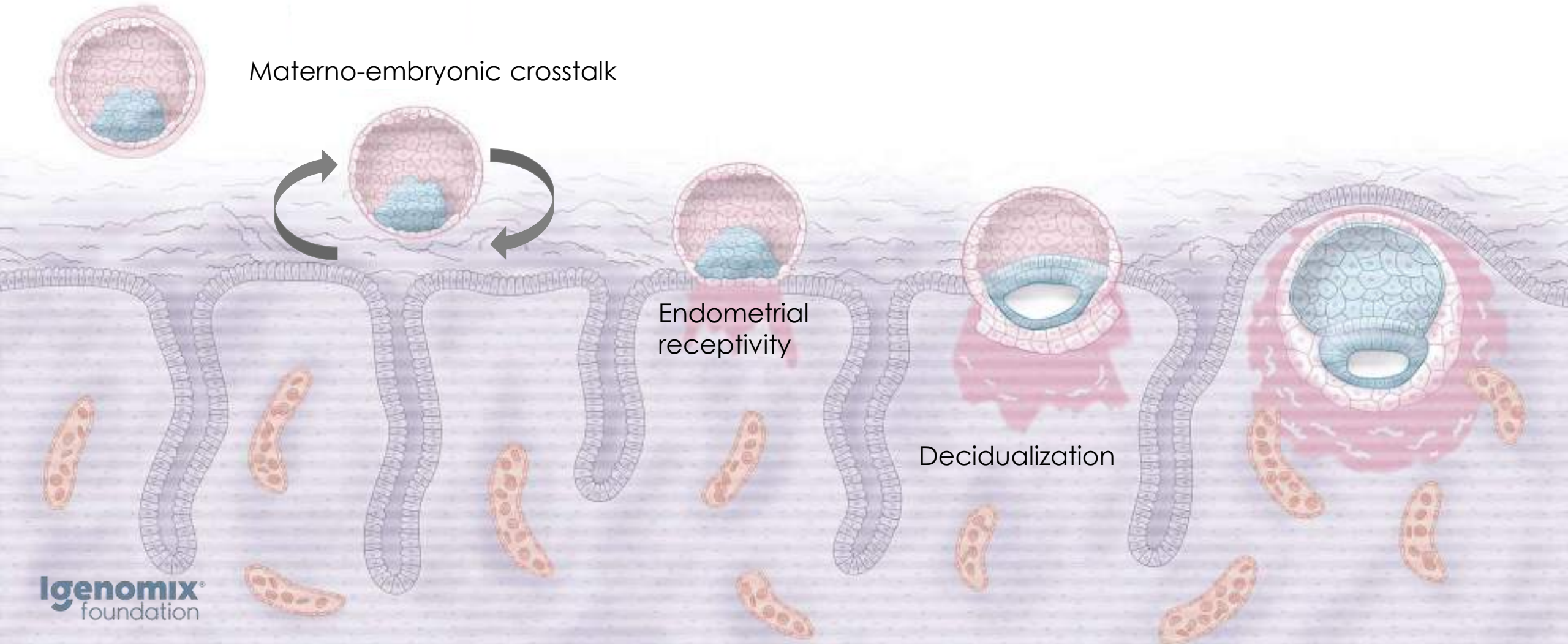
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THINKING

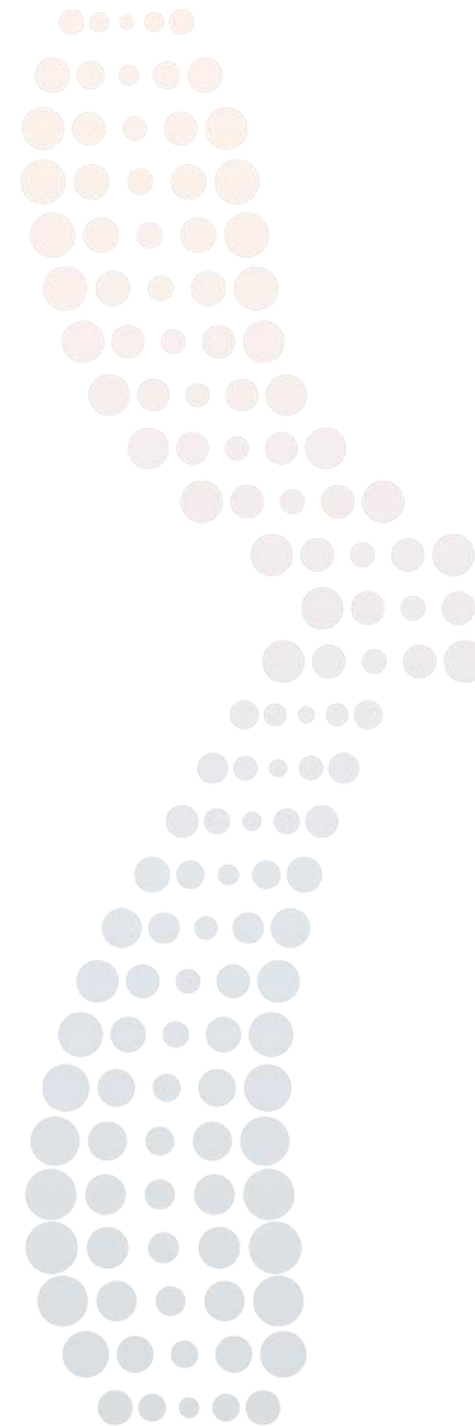
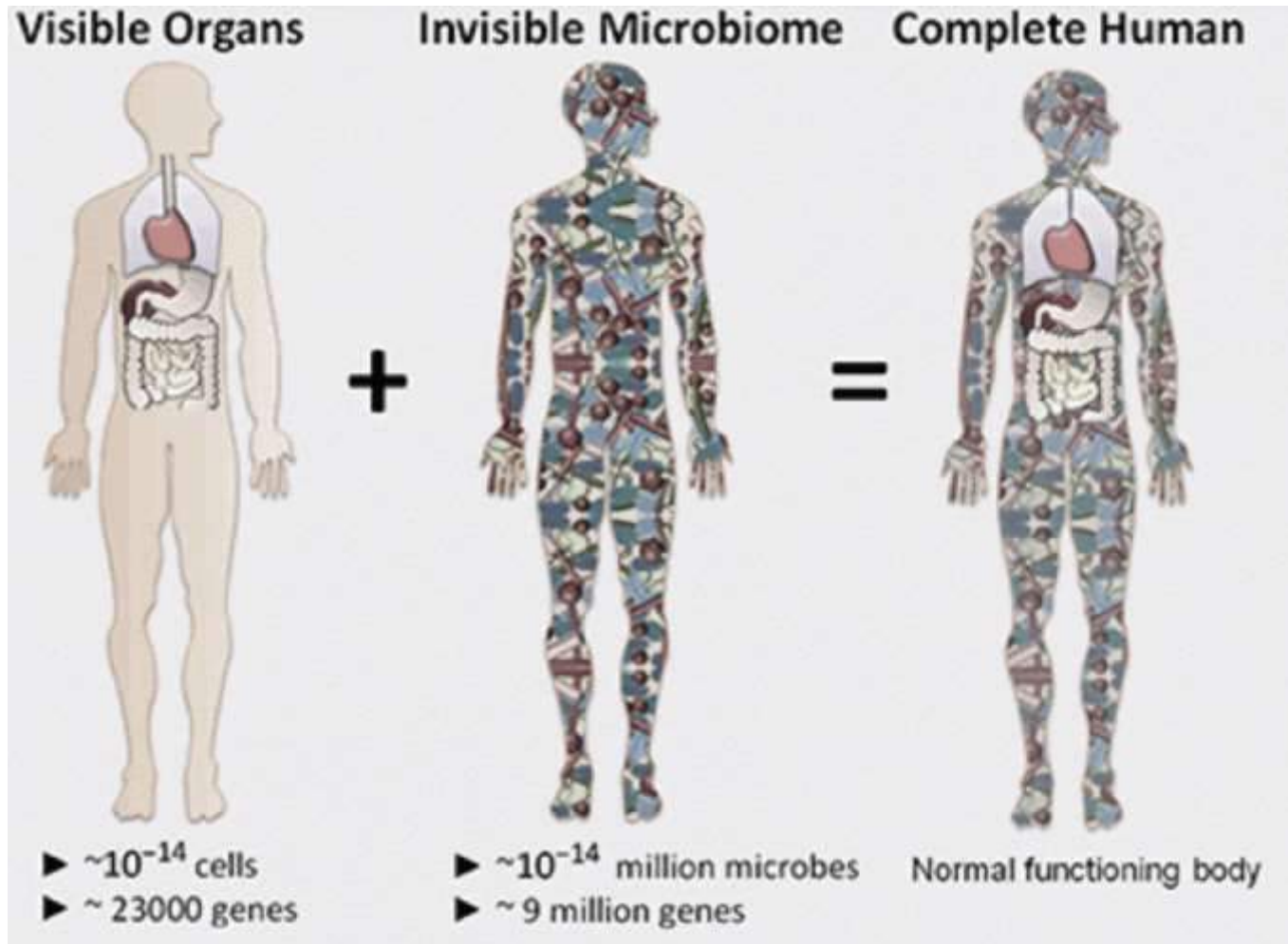


The first step of life's journey...

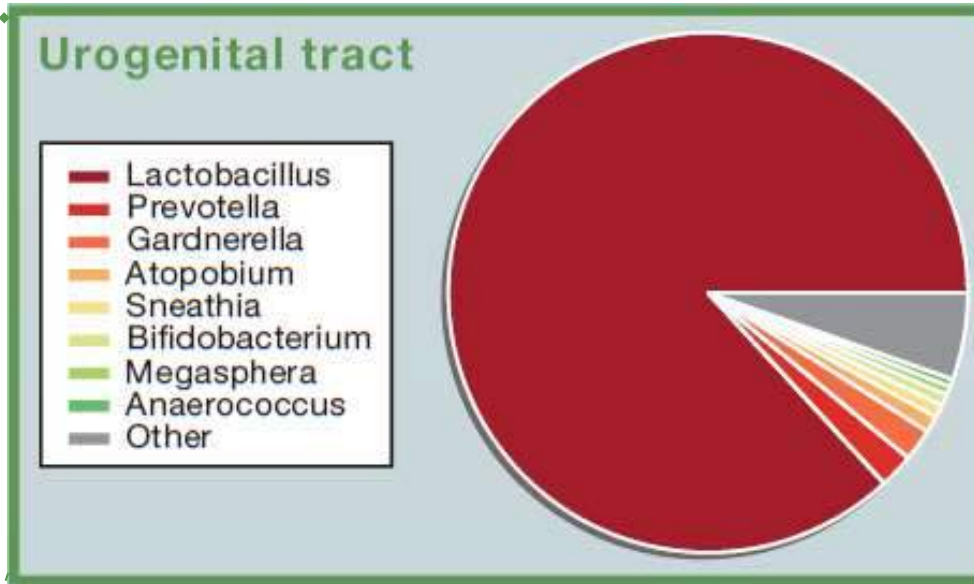
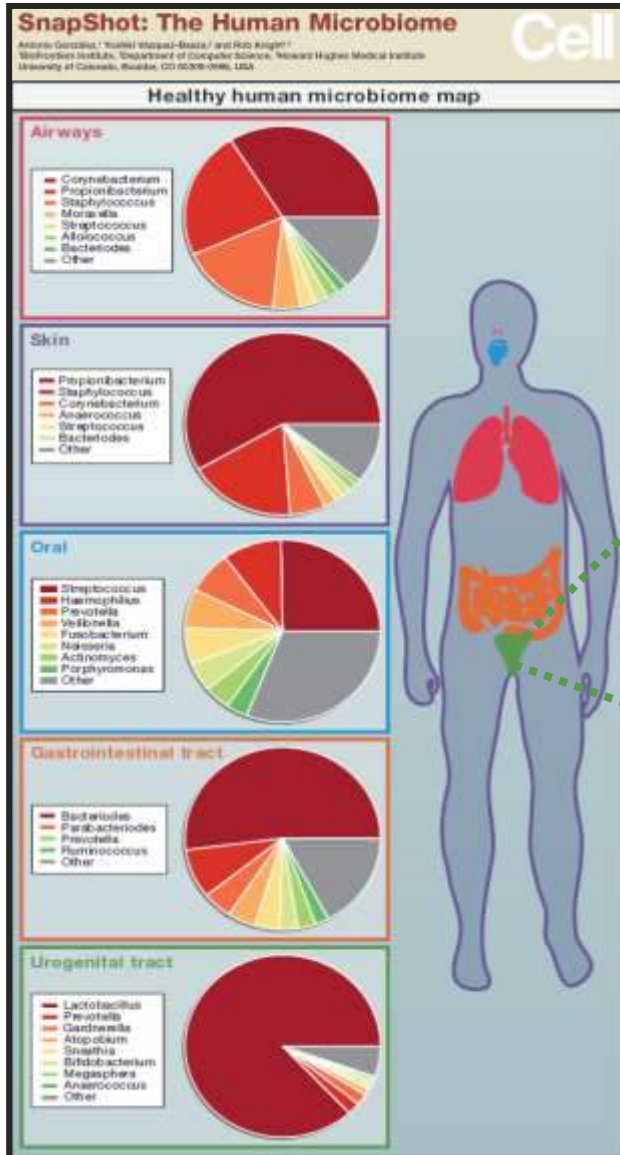
# The maternal contribution to pregnancy



# Human as a holobiont



# Urogenital tract microbiota



González et al., 2014. Cell 158: 690-690.e1  
The Human Microbiome Project (<http://hmpdacc.org>)



# Impact of vaginal microbiota in infertility and obstetrical complications

**DYSBIOSIS:** Several metagenomic studies associates low *Lactobacilli* with poor outcomes

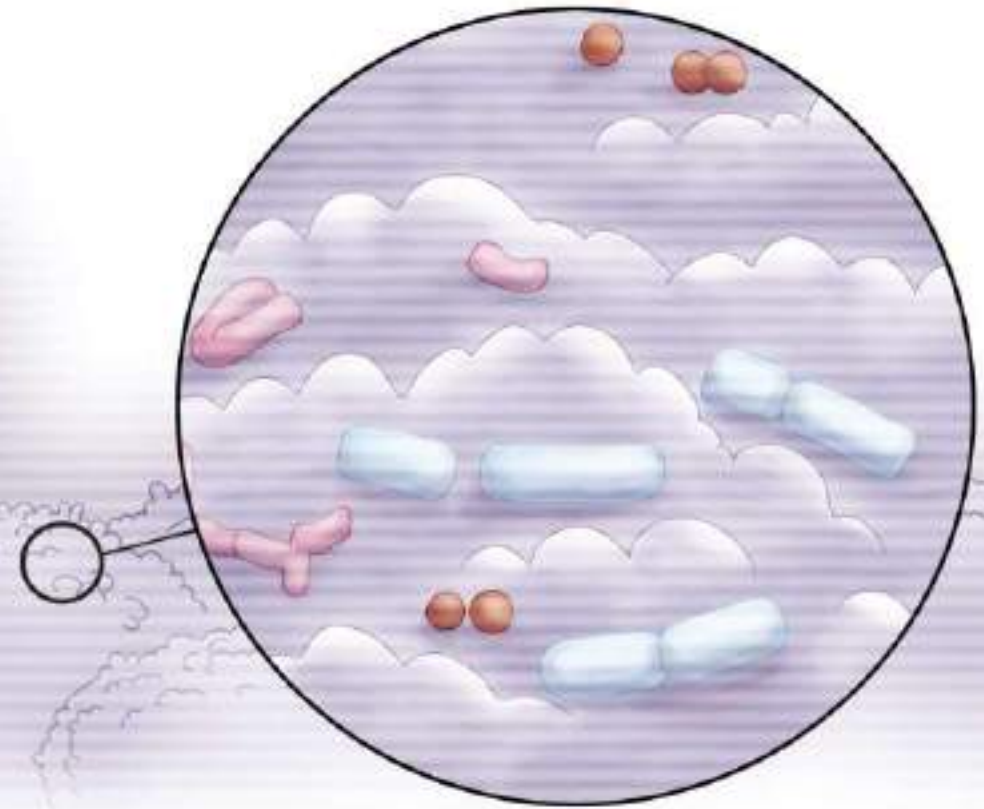
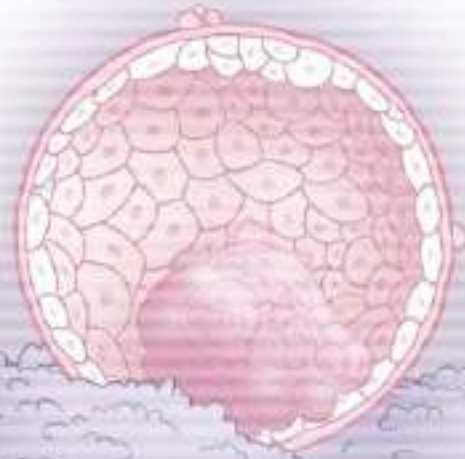
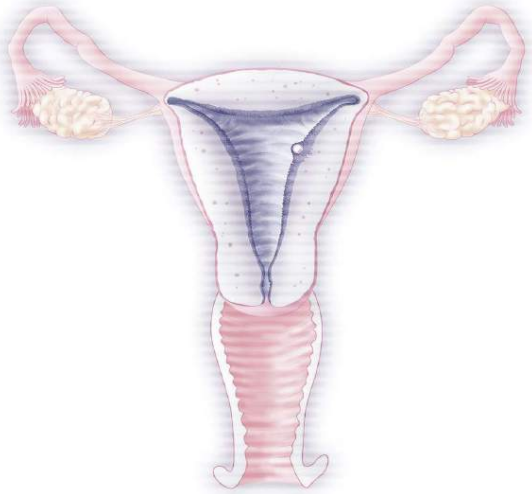
**RISKS:** Some pathogenic bacteria as *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and some *Mycoplasma spp.* are direct causes of infertility.

**PREVALENCE:** 20-30% of asymptomatic women present an altered vaginal microbiota with anaerobic pathogens, and up to 40% of patients undergoing IVF treatments present an abnormal vaginal microbiota.

**CONSEQUENCES:** bacterial vaginosis (BV) is responsible for:

- 2-fold increase risk of early miscarriage.
- >5-fold increased risk of late miscarriage.
- >3-fold increased risk of premature rupture of membranes.
- Up to 2-fold increased risk of preterm labor.

# What about the endometrial microbiome?



## Evidence that the endometrial microbiota has an effect on implantation success or failure

Inmaculada Moreno, PhD<sup>1</sup>; Francisco M. Codoñer, PhD<sup>1</sup>; Felipe Vilella, PhD<sup>1</sup>; Diana Valbuena, MD, PhD;  
Juan F. Martinez-Blanch, PhD; Jorge Jimenez-Almazán, PhD; Roberto Alonso; Pilar Alamá, MD, PhD;  
Jose Remohí, MD, PhD; Antonio Pellicer, MD, PhD; Daniel Ramon, PhD<sup>2</sup>; Carlos Simon, MD, PhD<sup>2</sup>

**BACKGROUND:** Bacterial cells in the human body account for 1–3% of total body weight and are at least equal in number to human cells. Recent research has focused on understanding how the different bacterial communities in the body (eg, gut, respiratory, skin, and vaginal microbiomes) predispose to health and disease. The microbiota of the reproductive tract has been inferred from the vaginal bacterial communities, and the uterus has been classically considered a sterile cavity. However, while the vaginal microbiota has been investigated in depth, there is a paucity of consistent data regarding the existence of an endometrial microbiota and its possible impact in reproductive function.

**OBJECTIVE:** This study sought to test the existence of an endometrial microbiota that differs from that in the vagina, assess its hormonal regulation, and analyze the impact of the endometrial microbial community on reproductive outcome in infertile patients undergoing in vitro fertilization.

**STUDY DESIGN:** To identify the existence of an endometrial microbiota, paired samples of endometrial fluid and vaginal aspirates were obtained simultaneously from 13 fertile women in prereceptive and receptive phases within the same menstrual cycle (total samples analyzed  $n = 52$ ). To investigate the hormonal regulation of the endometrial microbiota during the acquisition of endometrial receptivity, endometrial fluid was collected at prereceptive and receptive phases within the same cycle from 22 fertile women ( $n = 44$ ). Finally, the reproductive impact of an altered endometrial microbiota in endometrial fluid was assessed by implantation, ongoing pregnancy, and live birth rates in 35 infertile patients undergoing in vitro fertilization (total samples  $n = 41$ ) with a receptive endometrium diagnosed using the endometrial receptivity array. Genomic DNA was obtained either from endometrial fluid or vaginal aspirate and sequenced by 454 pyrosequencing of the V3–V5 region of

the 16S ribosomal RNA (rRNA) gene; the resulting sequences were taxonomically assigned using QIIME. Data analysis was performed using R packages. The  $\chi^2$  test, Student  $t$  test, and analysis of variance were used for statistical analyses.

**RESULTS:** When bacterial communities from paired endometrial fluid and vaginal aspirate samples within the same subjects were interrogated, different bacterial communities were detected between the uterine cavity and the vagina of some subjects. Based on its composition, the microbiota in the endometrial fluid, comprising up to 191 operational taxonomic units, was defined as a *Lactobacillus*-dominated microbiota (>90% *Lactobacillus* spp.) or a non-*Lactobacillus*-dominated microbiota (<90% *Lactobacillus* spp. with >10% of other bacteria). Although the endometrial microbiota was not hormonally regulated during the acquisition of endometrial receptivity, the presence of a non-*Lactobacillus*-dominated microbiota in a receptive endometrium was associated with significant decreases in implantation [60.7% vs 23.1% ( $P = .02$ )], pregnancy [70.6% vs 33.3% ( $P = .03$ )], ongoing pregnancy [58.8% vs 13.3% ( $P = .02$ )], and live birth [58.8% vs 6.7% ( $P = .002$ )] rates.

**CONCLUSION:** Our results demonstrate the existence of an endometrial microbiota that is highly stable during the acquisition of endometrial receptivity. However, pathological modification of its profile is associated with poor reproductive outcomes for in vitro fertilization patients. This finding adds a novel microbiological dimension to the reproductive process.

**Key words:** assisted reproductive techniques, bacterial pathogens, embryo implantation, endometrial microbiota, endometrial receptivity array



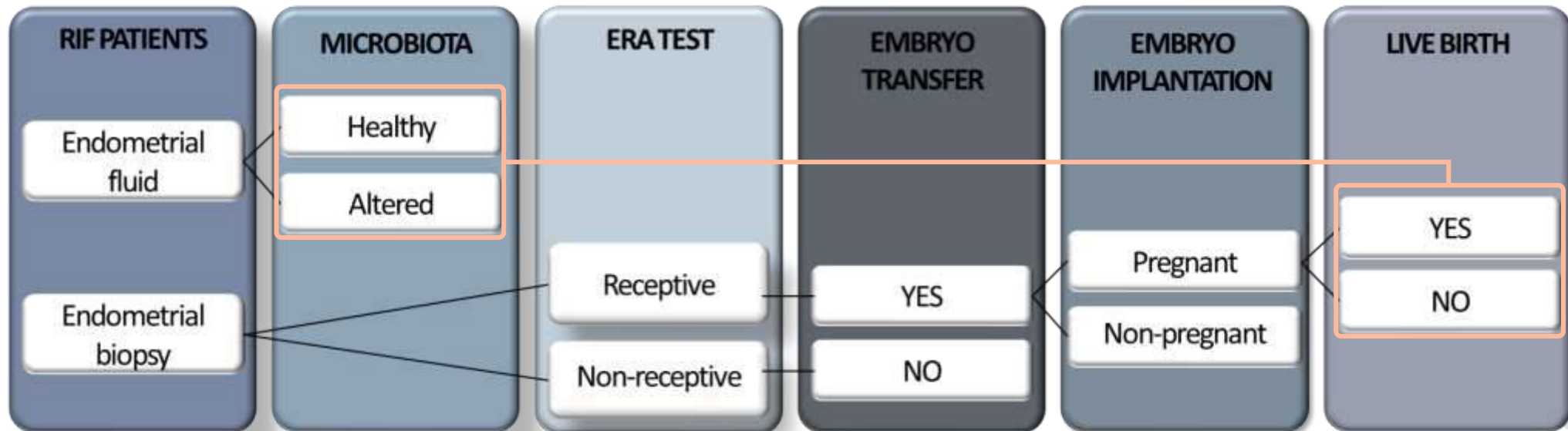
# Endometrial microbiome

Report of Major Impact

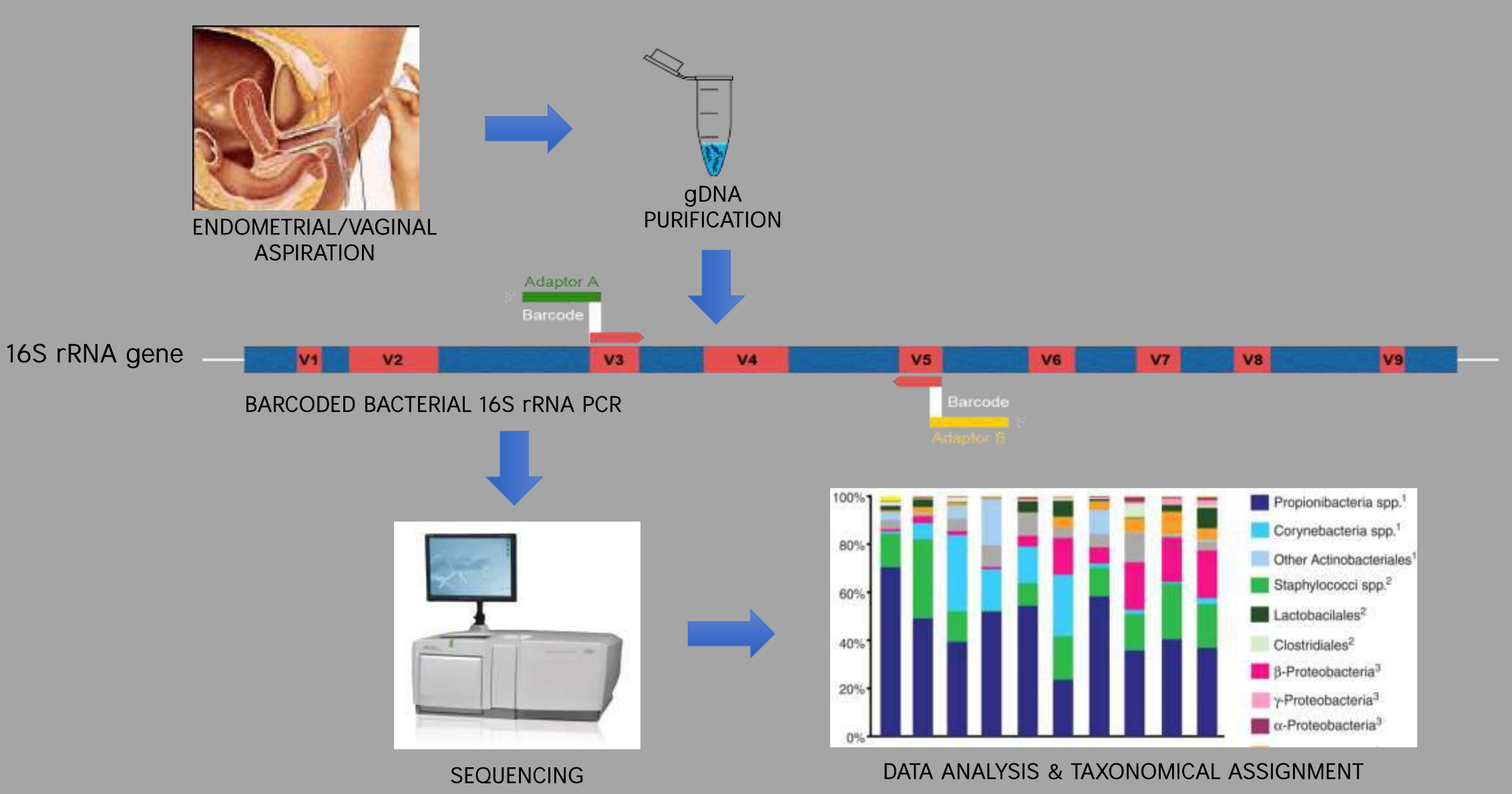
ajog.org

## Evidence that the endometrial microbiota has an effect on implantation success or failure

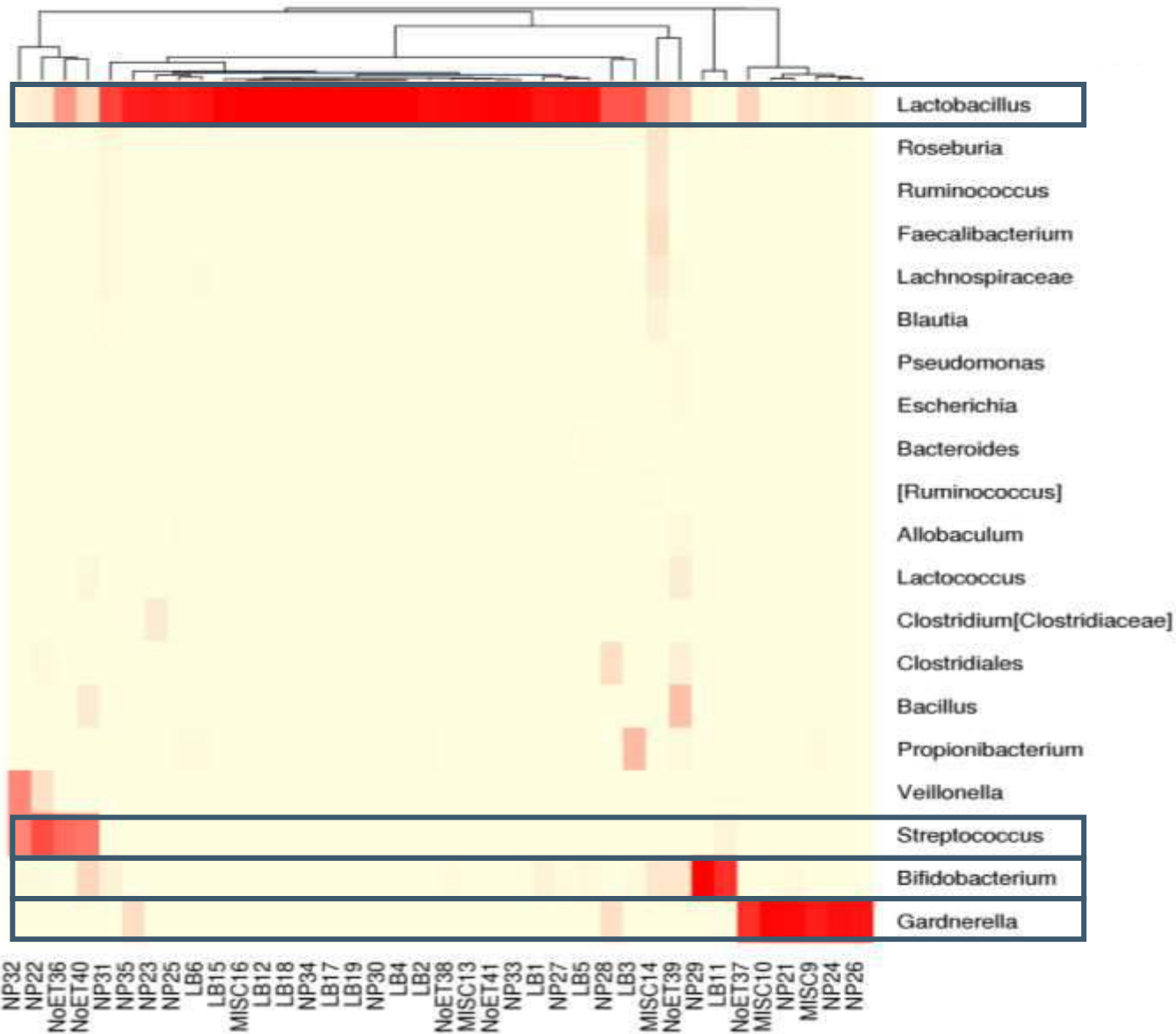
Inmaculada Moreno, PhD<sup>1</sup>; Francisco M. Codoñer, PhD<sup>1</sup>; Felipe Vilella, PhD<sup>1</sup>; Diana Valbuena, MD, PhD;  
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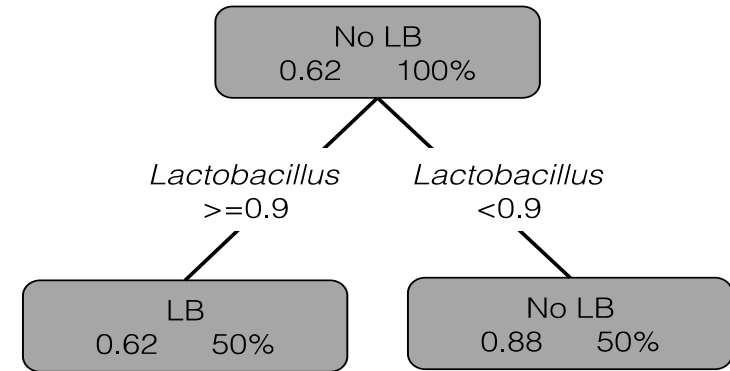
# Molecular assessment of endometrial microbiota by NGS



# Endometrial microbiota profile of infertile patients



## METHOD A: Classification and Regression Trees (CART)



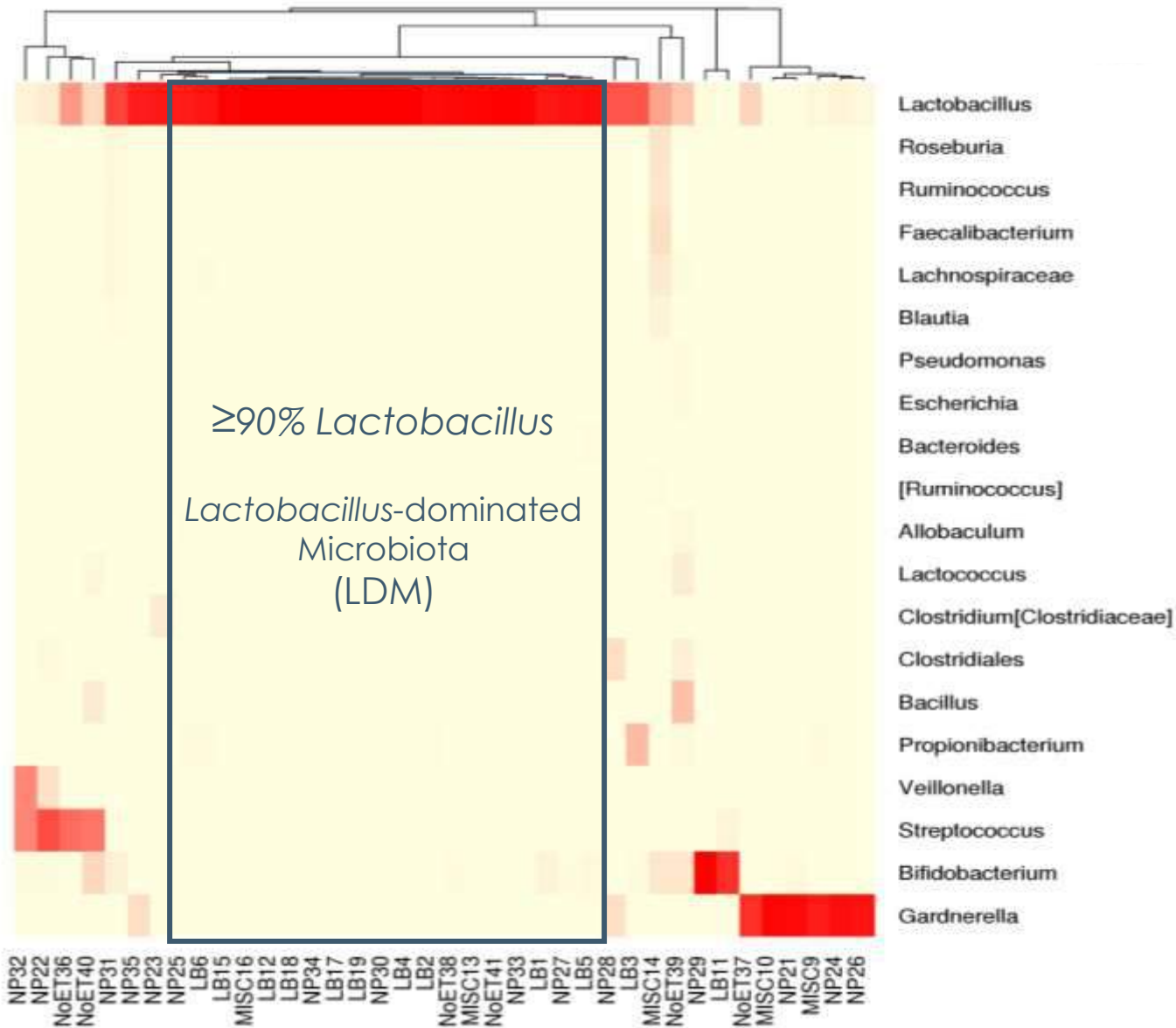
## METHOD B: Generalized linear model (GLM)

Logistic regression: 
$$P(LB) = \frac{e^x}{1 + e^x}$$

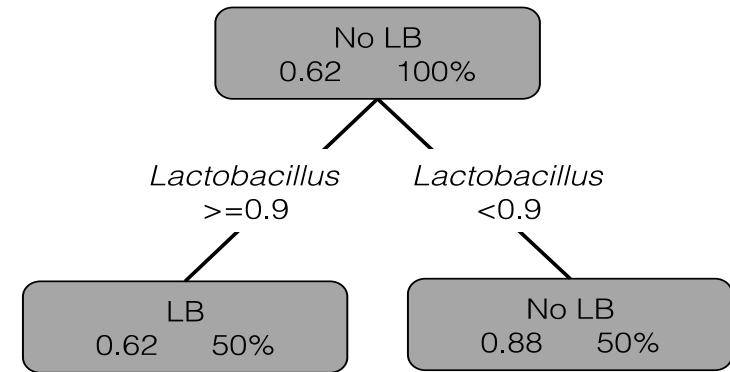
where: 
$$x = \frac{\ln p}{1 - p} = -2.359 + 2.554 * (\% \text{Lactobacillus})$$

COEFFICIENTS	Estimate	Std. Error	Z value	Pr(> z )
(Intercept)	-2.359	1.100	-2.145	*0.0320
Lactobacillus	2.554	1.277	2.001	*0.0454

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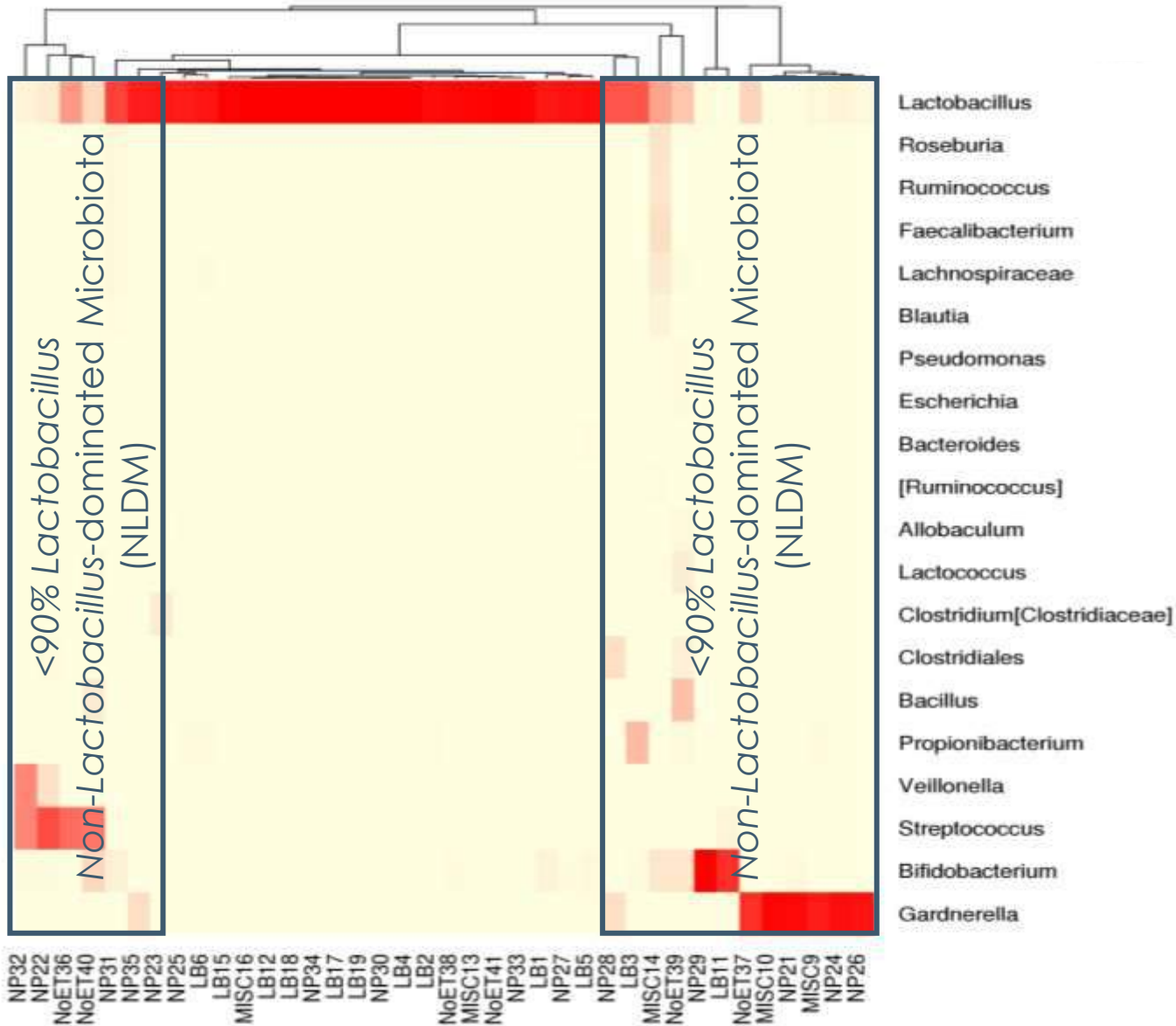
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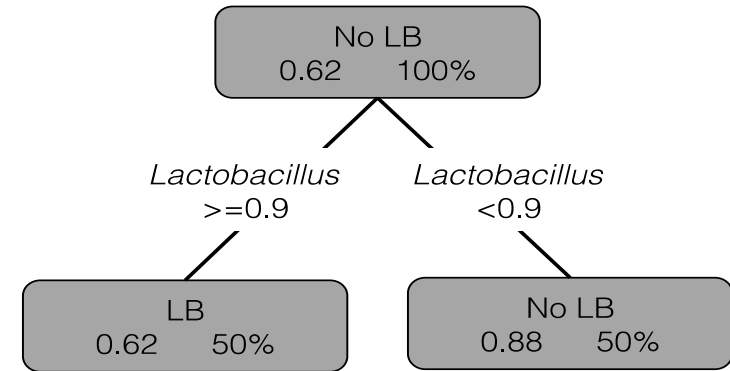
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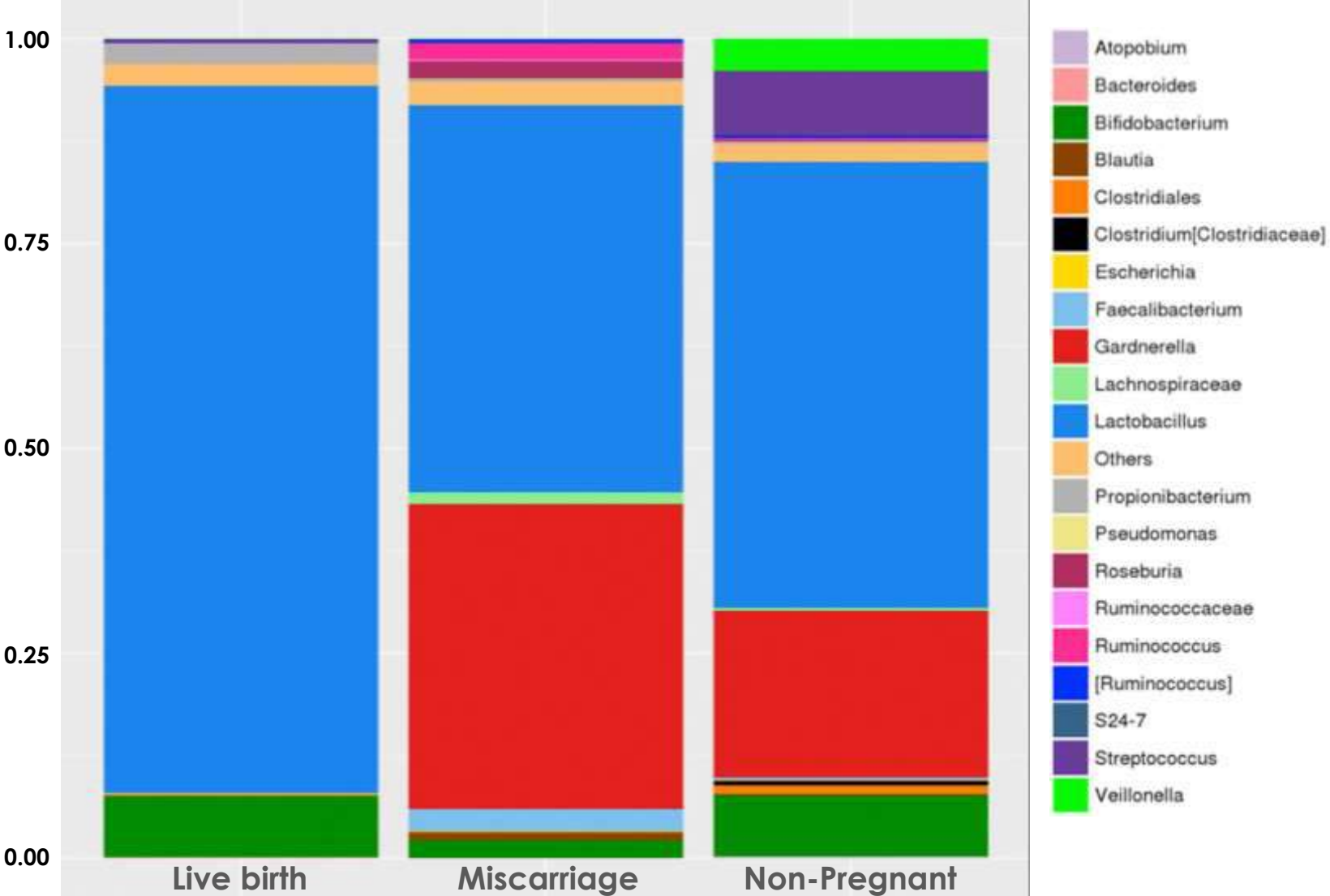
# Low *Lactobacillus* in EF associates with poor reproductive IVF outcomes

Characteristics and Outcomes	LDM (n=17)	NLDM (n=15)	p-value
Age (y)	40.06±3.47	39.00±5.09	0.49
BMI (kg/m <sup>2</sup> )	24.18±5.18	22.45±4.02	0.30
Previous pregnancies (n)	1.71±2.44	1.53±2.32	0.84
Previous miscarriages (n)	1.53±2.21	1.14±1.56	0.58
Metaphase II oocytes per cycle (n)	11.94±4.27	10.20±4.81	0.28
Fertilization rate per cycle	157/203 (77.34%)	118/153 (77.12%)	0.62
Transferred embryos per cycle (n)	1.65±0.49	1.73±0.59	0.65
Months between EF and transfer (n)	2.82±2.55	1.80±1.08	0.16
Pregnancy rate per transfer	12/17 (70.6%)	5/15 (33.3%)	0.03*
Implantation rate per transfer	17/28 (60.7%)	6/26 (23.1%)	0.02*
Ongoing pregnancy per transfer	10/17 (58.5%)	2/15 (13.3%)	0.02*
Miscarriage rates (%)	2/10 (16.7%)	3/5 (60.0%)	0.07
Live birth rate per transfer	10/17 (58.8%)	1 <sup>§</sup> /15 (6.7%)	0.002*

BMI: body mass index; LDM: *Lactobacillus*-dominated microbiota; NLDM: non-*Lactobacillus*-dominated microbiota;

\*Chi Square ( $\chi^2$  test) and Student's *t*-test were performed; \*p-value<0.05; §: Voluntary termination of pregnancy.

# Low *Lactobacillus* in EF associates with poor reproductive IVF outcomes



# Prospective MICROBIOME Study

**452** patients enrolled at **13** participant sites from **8** countries worldwide

<b>First IRB/EC approval</b>	May 29, 2017
<b>Last IRB/EC approval</b>	March 27, 2018
<b>Sample size</b>	434 patients
<b>FPII</b>	August 4, 2017
<b>LPLI</b>	February 26, 2019
<b>Recruitment length</b>	1.5 years (finished)
<b>Study length</b>	2.5 years



Register: 03330444  
First release: Nov 6, 2017  
Last Update: Mar 25, 2019  
Status: Active. Not recruiting






# Prospective MICROBIOME study



*Case Report*

# Taxonomical and Functional Assessment of the Endometrial Microbiota in A Context of Recurrent Reproductive Failure: A Case Report

Iolanda Garcia-Grau <sup>1,2</sup>, David Perez-Villaroya <sup>3</sup> , Davide Bau <sup>3</sup>, Marta Gonzalez-Monfort <sup>1,4</sup>, Felipe Vilella <sup>1</sup>, Inmaculada Moreno <sup>1,4,\*</sup> and Carlos Simon <sup>1,4,5,6,\*</sup>

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<sup>2</sup> Department of Pediatrics, Obstetrics and Gynecology, School of Medicine, University of Valencia, 46010 Valencia, Spain

<sup>3</sup> Bioinformatics Department, Igenomix R&D, 46980 Valencia, Spain; david.perez@igenomix.com (D.P.-V.); davide.bau@igenomix.com (D.B.)

<sup>4</sup> Research Department, Igenomix R&D, 46980 Valencia, Spain

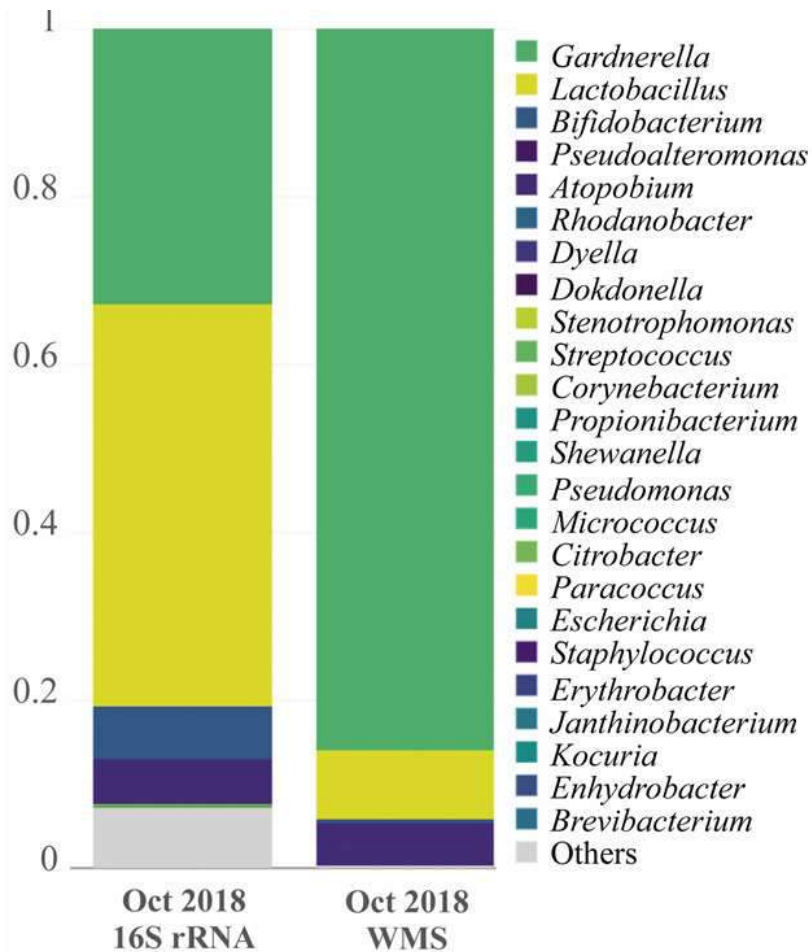
<sup>5</sup> Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77030, USA

<sup>6</sup> BIDMC, Harvard University, Boston, MA 02138, USA

\* Correspondence: inmaculada.moreno@igenomix.com (I.M.); carlos.simon@igenomix.com (C.S.); Tel.: +34-963905310 (I.M.); +34-963905310 (C.S.)



# Gardnerella vaginalis associated to recurrent reproductive failure



ADAPTATION TO ENVIRONMENT	
<i>Mobile elements and horizontal gene transfer:</i>	Recombinase (RecA); Transposase IS3509a; HK97 family phage major capsid protein; Site-specific recombinase phage integrase family; Helicase UvrD/REP; Phage related protein.
<i>Competence:</i>	Putative competence-damage inducible protein (CinA); ABC-type antimicrobial peptide transporter permease component; Glycoside hydrolase (GH) family; Lysozyme; Penicillin-binding protein; Fic-family protein; M13 family peptidase; ATP-binding subunit of Clp protease.
<i>Toxin-antitoxin system:</i>	RelB toxin/antitoxin family; Antitoxin/DNA-binding transcriptional repressor DinJ.
VIRULENCE	
<i>Biofilm formation and exopolysaccharide formation:</i>	Glycosyltransferase (GT) type II; Sortases; LPxTG domain; Actinobacterial surface anchored protein domain.
<i>Epithelial adhesion:</i>	Type I fimbrial major subunit precursor; Pilus assembly protein (PilY1); Tfp pilus.
<i>Antimicrobial resistance:</i>	Efflux transporter; ABC-type multidrug transport system; ABC-type bacteriocin/lantibiotic; Multidrug resistance transporter EmrB/QacA; Bleomycin hydrolase; SalY-type ABC-antimicrobial peptide transport system; Cadmium resistance transporter CadD family protein.
<i>Mucin degradation:</i>	Alpha-mannosidase; Beta-galactosidase.
<i>Cytotoxicity and hemolysis:</i>	Hemolysin-like protein.
<i>Iron intake and utilisation:</i>	FTR1-family iron permease; FtsK/SpoIIIE family protein.
<i>Other virulence factors:</i>	G-related albumin-binding (GA) modules; Virulence-associated E family; Oxygen-insensitive NAPDH nitroreductase (RdxA).

## The first glimpse of the endometrial microbiota in early pregnancy

Inmaculada Moreno, PhD; Iolanda García-Grau, BS; Davide Bau, PhD; David Perez-Villaroya, BS; Marta Gonzalez-Monfort, BS; Felipe Vilella, PhD; Roberto Romero, MD, DMedSci; Carlos Simón, MD, PhD

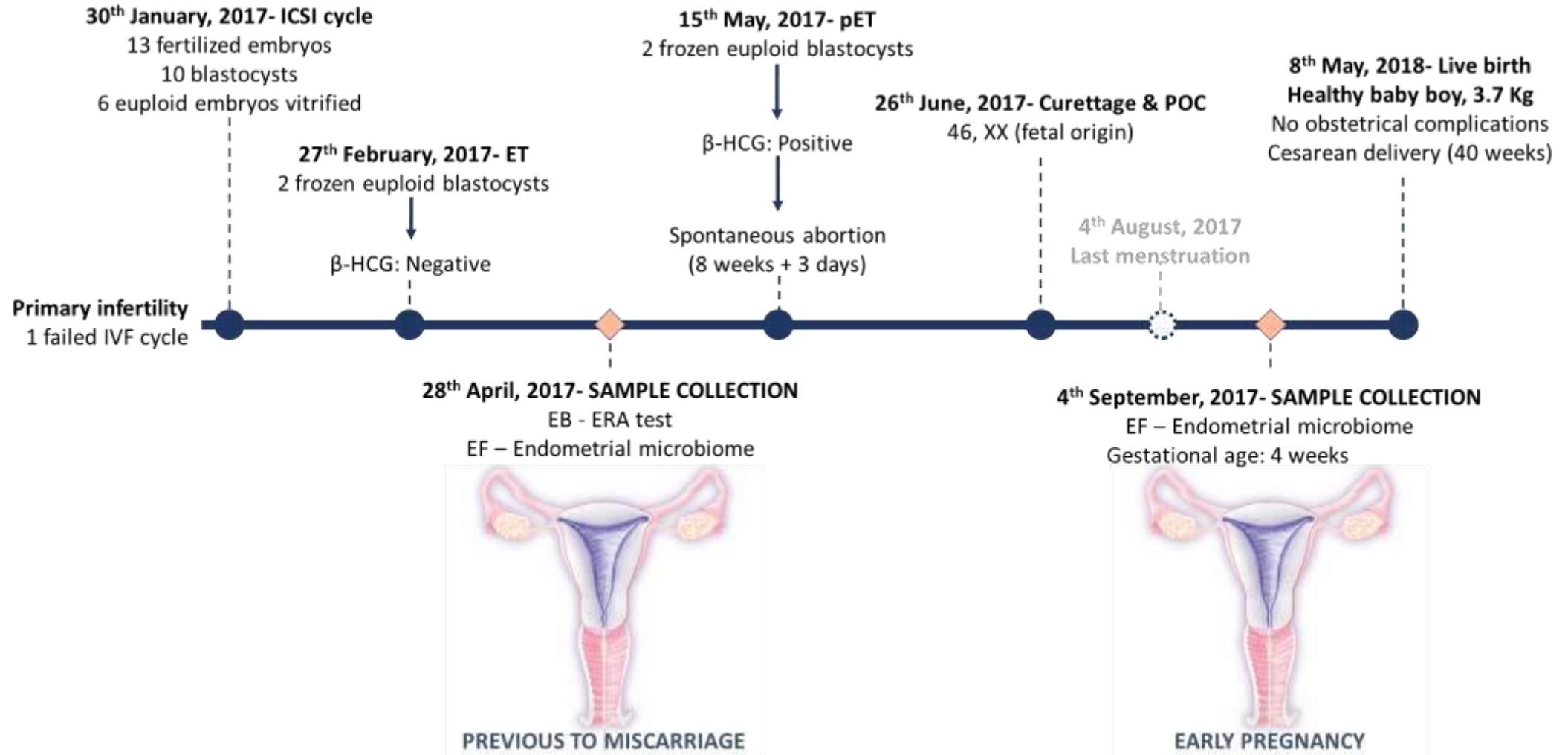
The efforts of the Human Microbiome Project have highlighted the importance of microorganisms and their genomes in several human niches and emphasized the importance in human health and disease.<sup>1</sup> The female reproductive tract contributes up to 90% of the human microbiota.<sup>2</sup> Until recently, the main research focus has been on the vaginal microbiota.<sup>3</sup> However, accumulating evidence suggests the existence of a different bacterial ecosystem in the endometrium,<sup>4–6</sup> challenging the traditional dogma of the sterility of the human uterus.<sup>9,10</sup>

The vaginal microbiota has been investigated for years with the use of microbial culture, microscopy, and culture-independent techniques, which show that the predominant bacteria are *Lactobacilli*.<sup>3</sup> The endometrial cavity has traditionally been considered sterile, and the isolation of Enterobacteriaceae, *Streptococcus*, *Staphylococcus*,

Investigation of the microbial community in the female reproductive tract with the use of sequencing techniques has revealed that endometrial samples obtained through a transvaginal catheter are dominated by *Lactobacillus* species. Dysbiotic changes in the endometrial microbiota may be associated with implantation failure or early spontaneous abortion in patients who undergo assisted reproductive technology treatment. Whether or not there is an endometrial microbiota in early pregnancy is unknown. Herein we describe the human endometrial microbiota in a patient who subsequently had an 8th week spontaneous clinical miscarriage with euploid embryos in the next cycle and, for the first time, during a successful pregnancy in which the endometrial fluid was sampled at 4 weeks of gestation. The microbial profile found on the endometrial sample before the spontaneous abortion had higher bacterial diversity and lower *Lactobacillus* abundance than the endometrial fluid from the healthy pregnancy. Functional metagenomics detected different *Lactobacillus* species between the 2 samples. *Lactobacillus crispatus* was present in the endometrium before the spontaneous abortion, as were other bacteria involved in dysbiosis, which had an unstable functional pattern characterized by transposases and insertion elements. *Lactobacillus iners* was the most prevalent microbe found in the endometrium during early pregnancy; its presence was associated with defense mechanisms and basal functions. These novel observations prompt future investigations to understand the potential implications of microbiology on healthy and pathologic human pregnancy.

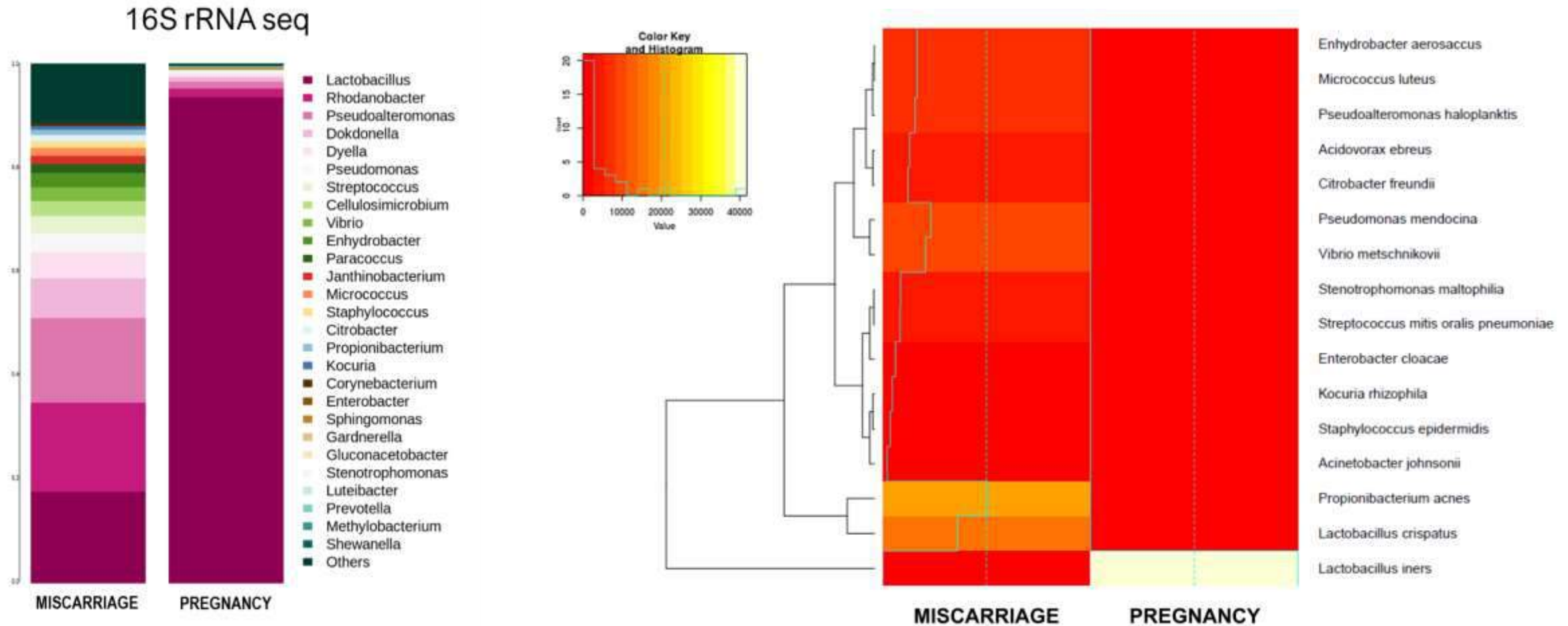
**Key words:** 16S rRNA, endometrial microbiota, *Lactobacillus crispatus*, *Lactobacillus iners*, metagenomic, microbiome, pregnancy, reproductive tract, sequencing, spontaneous abortion

# Endometrial MICROBIOME in a Successful Early Pregnancy



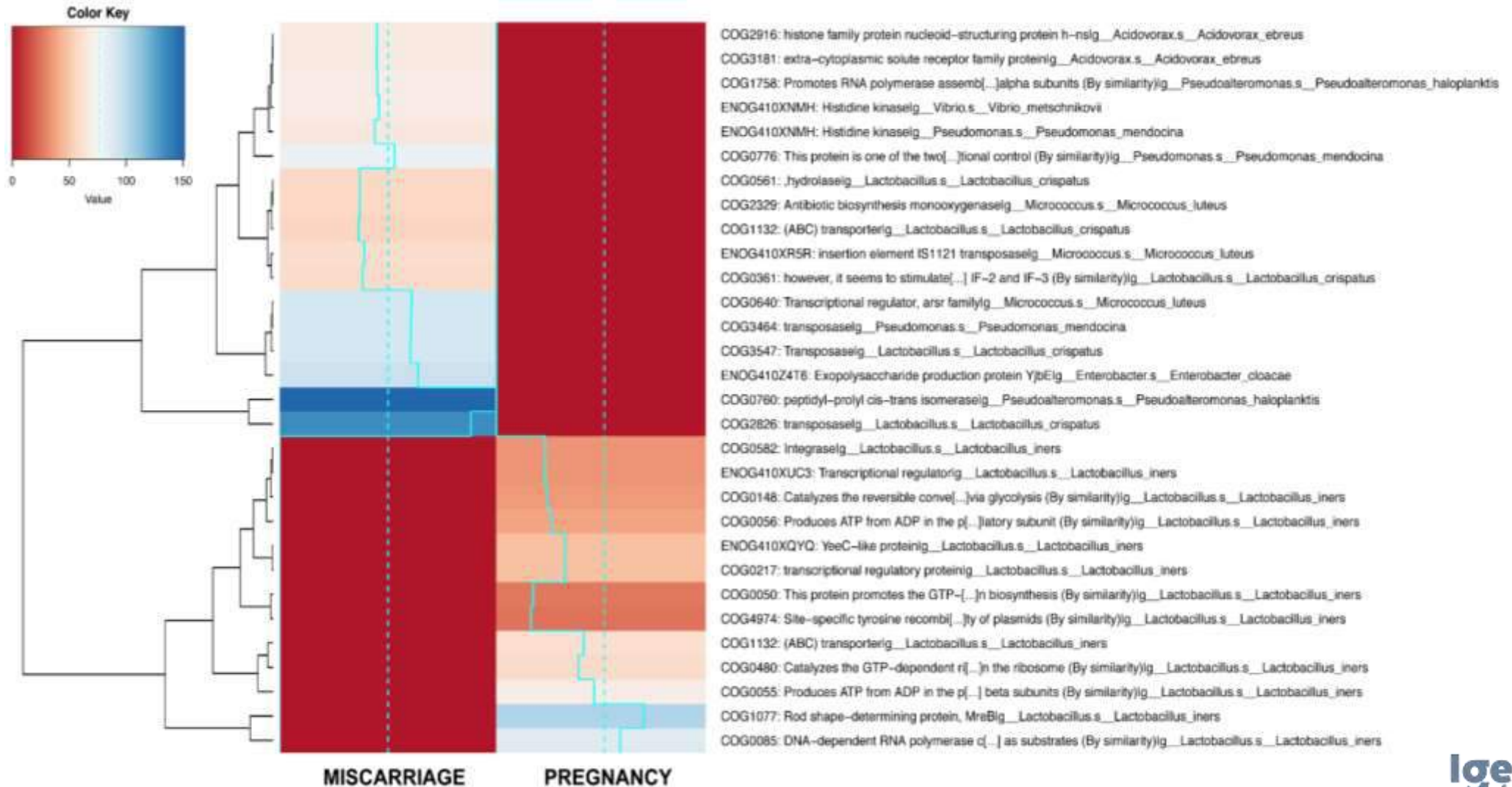
# Endometrial MICROBIOME in a Successful Early Pregnancy

*L. iners* is the only bacterium found in early successful pregnancy



# Endometrial MICROBIOME in a Successful Early Pregnancy

## Positive impact of *Lactobacillus* in a successful pregnancy





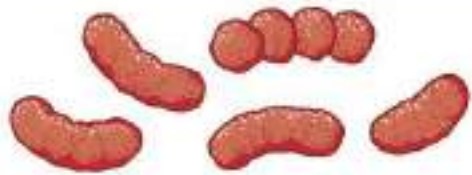
# CONCLUSIONS

- The percentage of *Lactobacillus* together with specific pathogens are significant variables to predict reproductive success.
- These results expand the evaluation of endometrial factor not only at the morphological and molecular levels but also at the microbiological viewpoint.
- Our observations leads us to propose new diagnostic tools and personalized therapeutic procedures through molecular microbiological evaluation.



# The balance of bacteria in the endometrium is a key factor for reproductive success.

Higher reproductive success →



## Pathogenic bacteria

These bacteria cause infection, which is linked to implantation failure and recurrent miscarriage



## Dysbiotic bacteria

Microbial imbalance is linked to embryo implantation failure



## Optimal microbiome

A balanced microbiome improves the reproductive prognosis, resulting in increased chance of pregnancy and live births



## EMMA

Endometrial Microbiome  
Metagenomic Analysis  
by Igenomix®



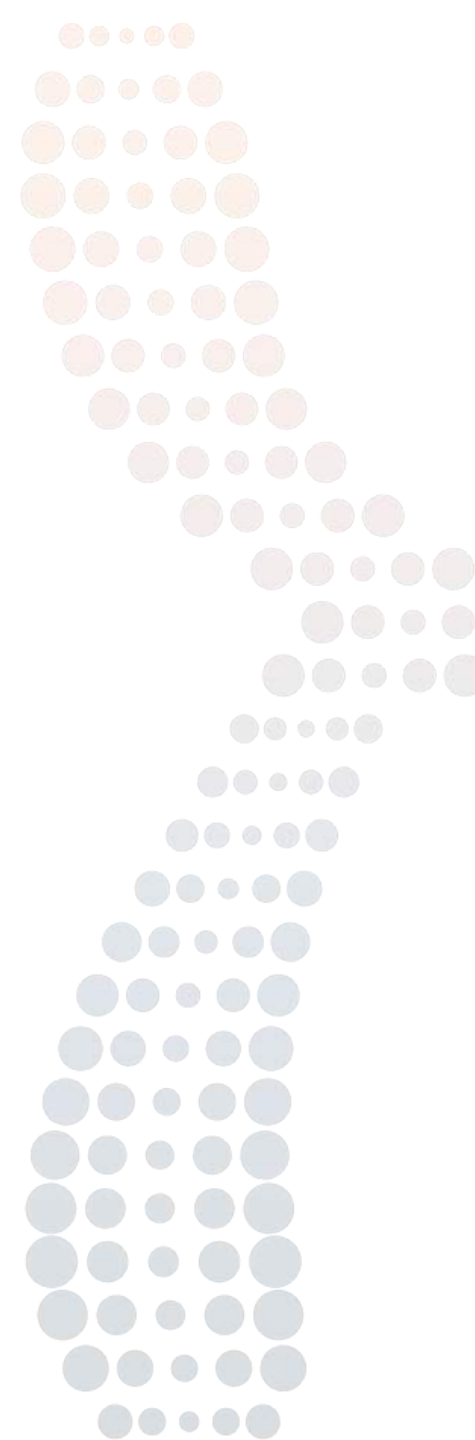
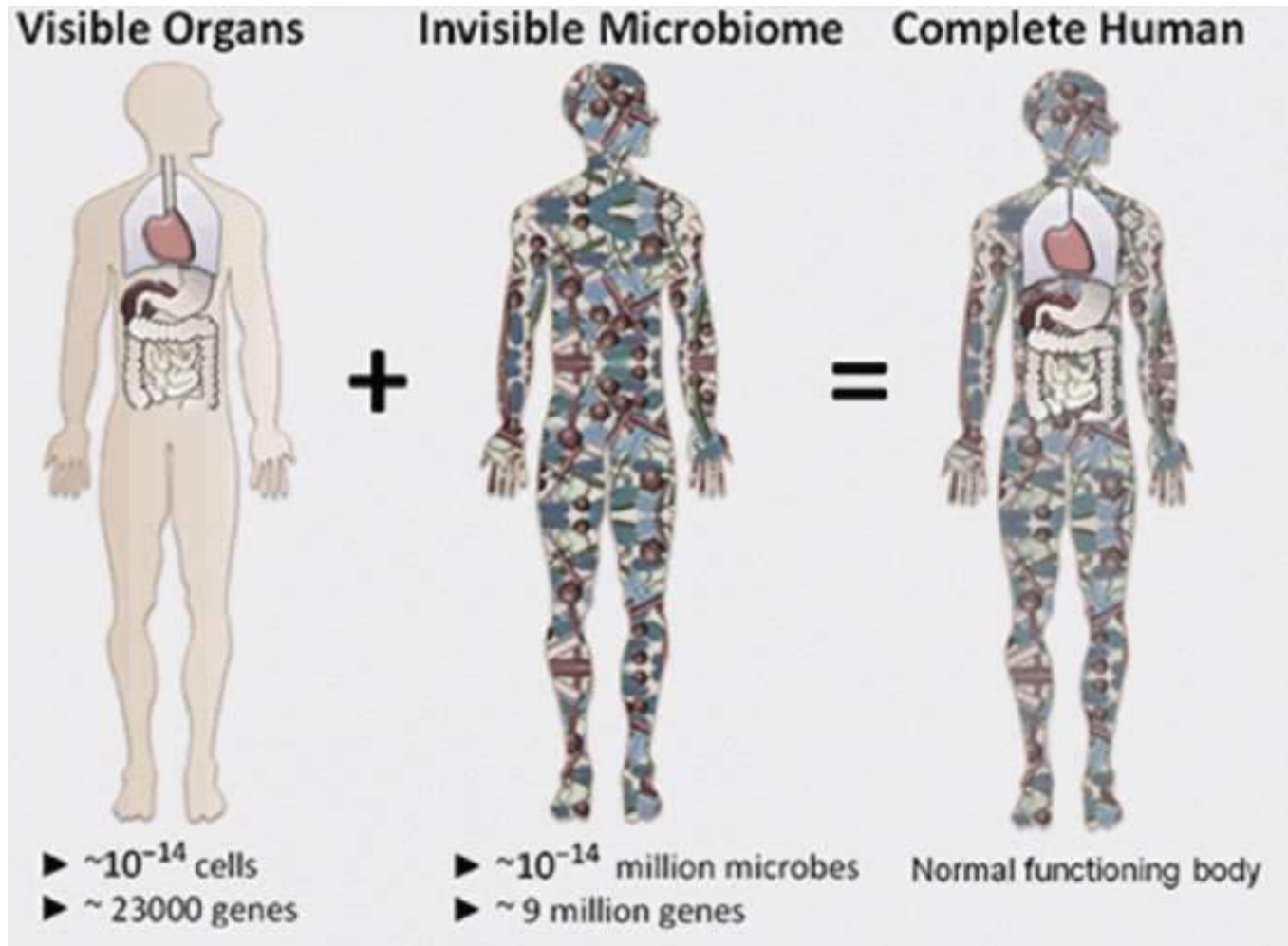
## ALICE

Analysis of Infectious  
Chronic Endometritis  
by Igenomix®

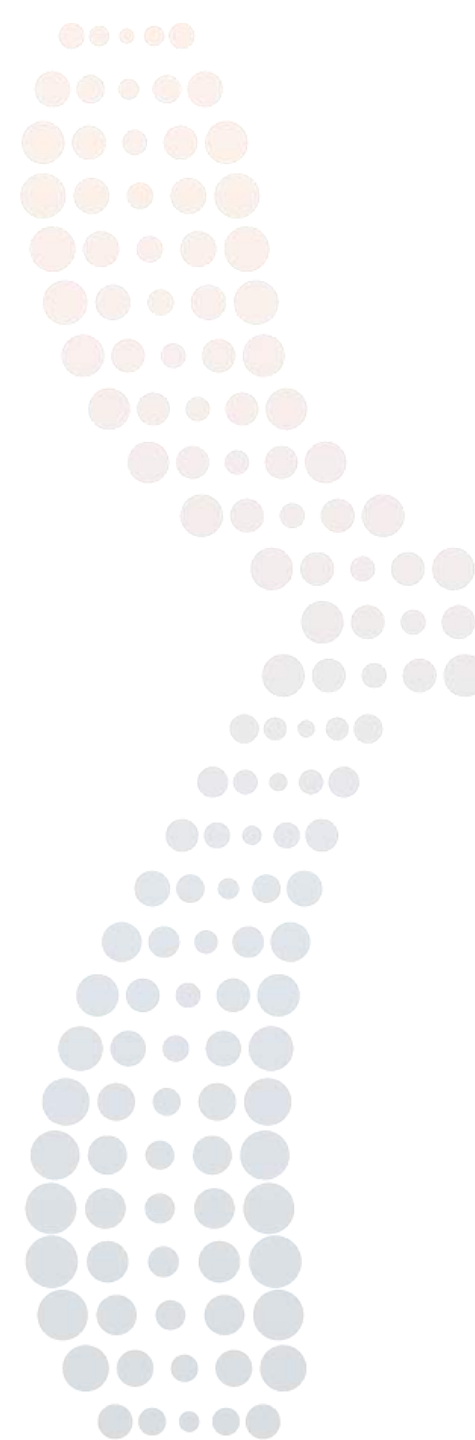
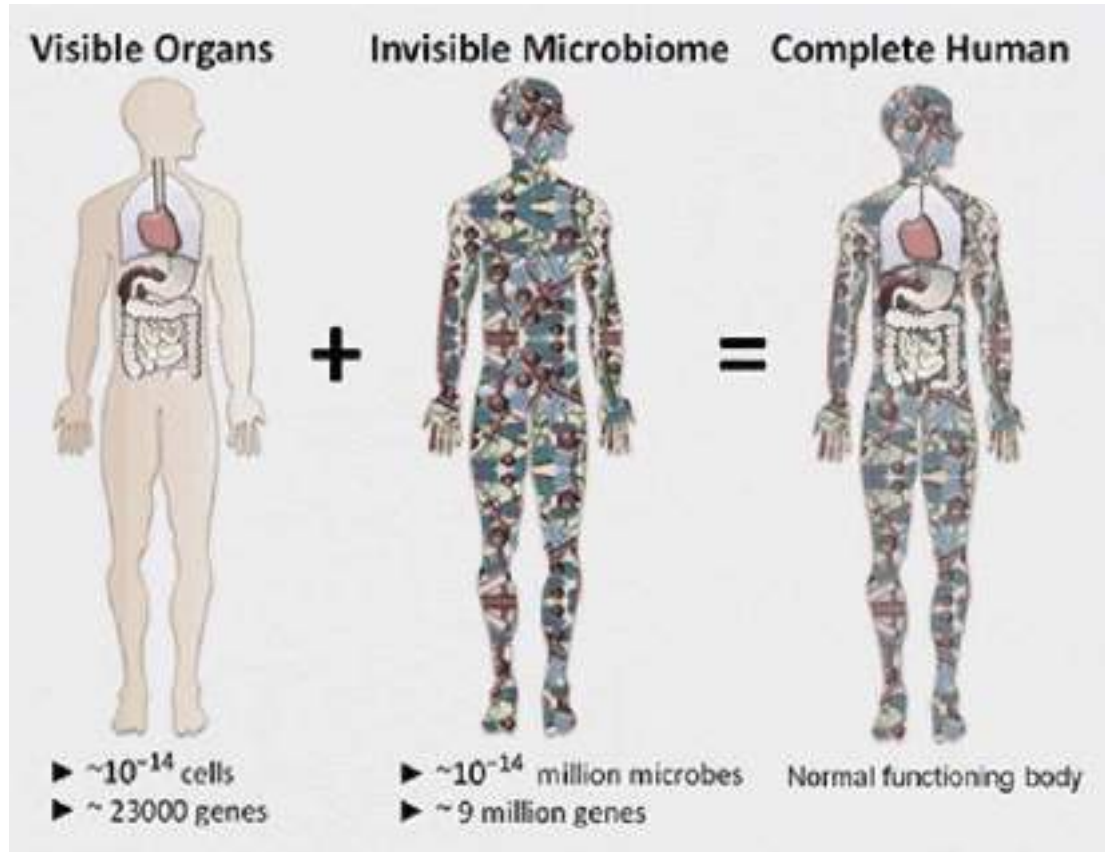
# Virus and bacteria: a wider view of the microbiome



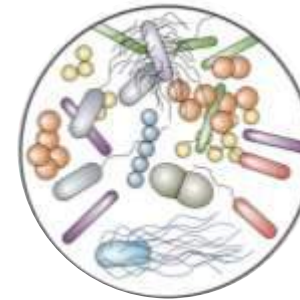
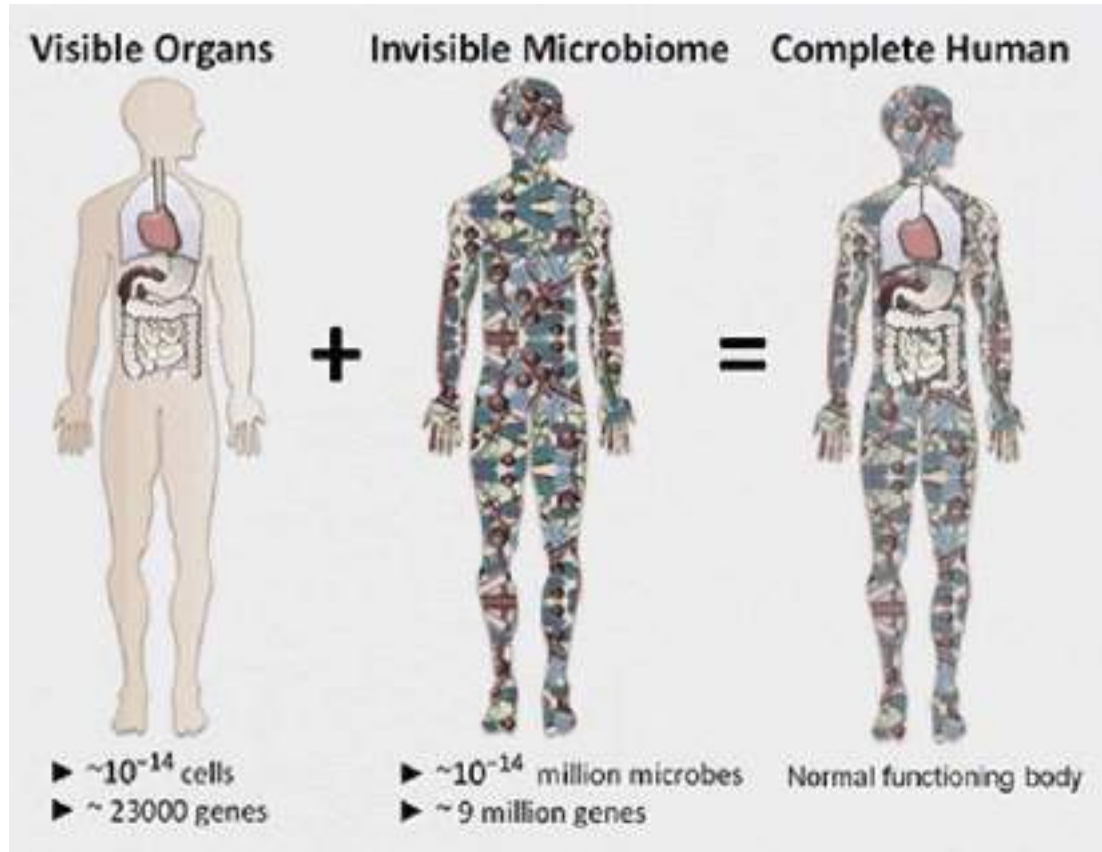
# Human as a holobiont



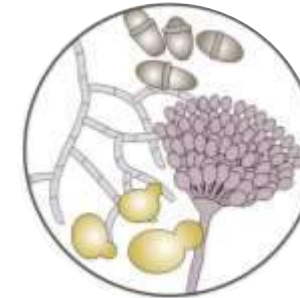
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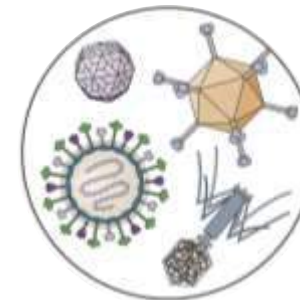
# Human as a holobiont



Bacteriome ( $10^{13}$ - $10^{14}$ )

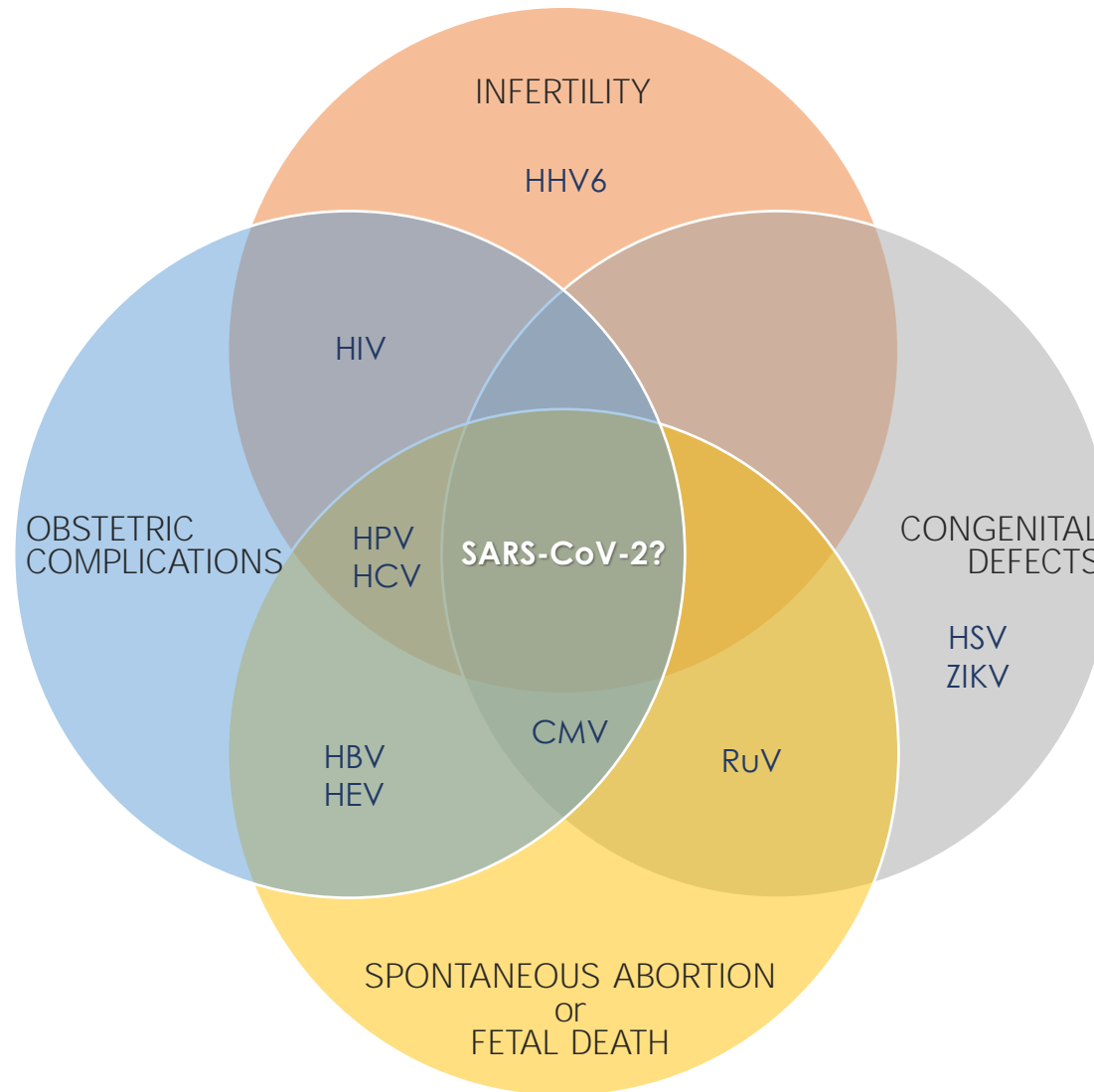


Mycobiome ( $10^{12}$ - $10^{13}$ )



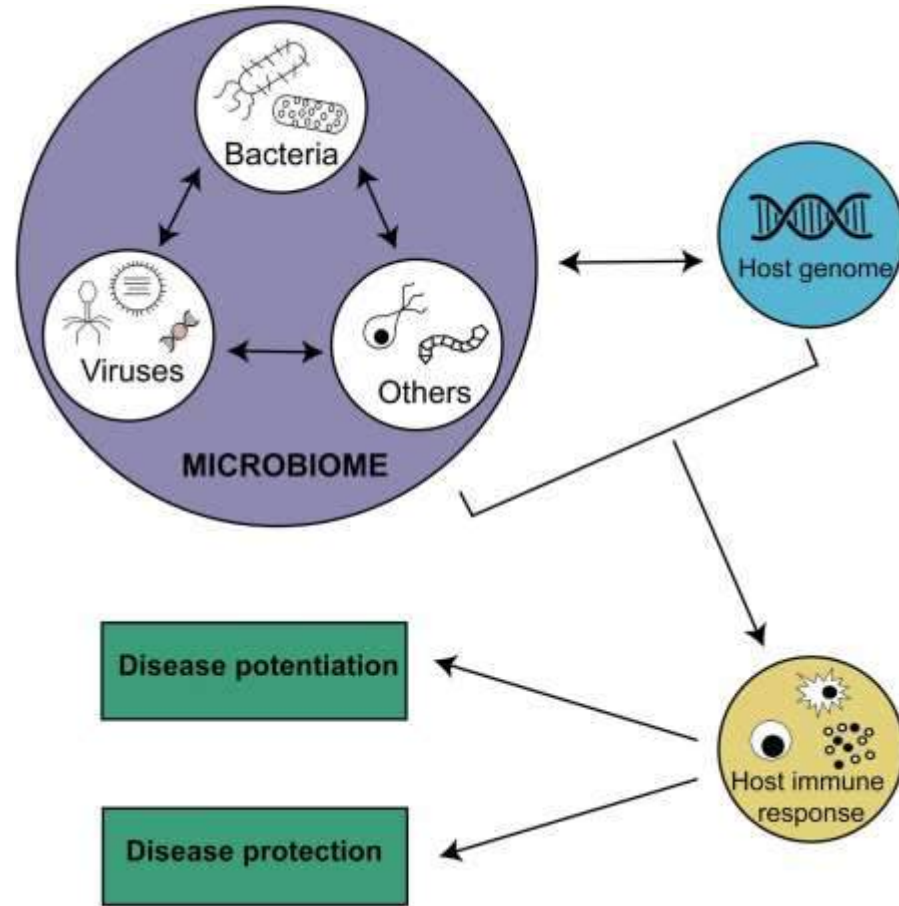
Virome ( $10^{14}$ - $10^{15}$ )

# Risks of viral infections in human reproduction



HHV6: Human Herpes Virus 6  
HIV: Human Immunodeficiency Virus  
HPV: Human Papillomavirus  
HCV: Hepatitis C Virus  
HBV: Hepatitis B Virus  
HEV: Hepatitis E Virus  
CMV: Cytomegalovirus  
RuV: Rubella Virus  
HSV: Herpes Simplex Virus  
ZIKV: Zika Virus

# Interplay between bacterial and viral components of the microbiome

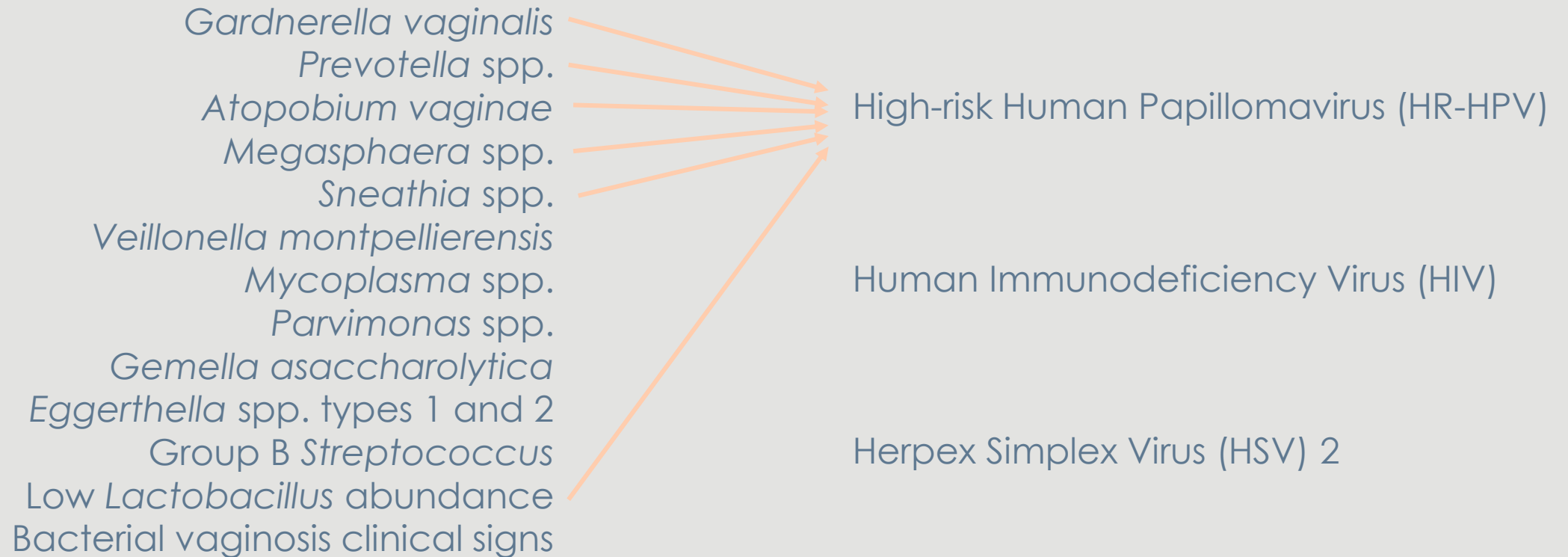


- Abnormal vaginal microbiota may impact the homeostasis of the immune system, leading to breakdown of the epithelial barrier dysfunction and favouring viral infection.
- This mechanism shows the importance of analyzing the bacteriome and the virome concurrently as their interactions can provide insight into new mechanisms of pathogenesis.



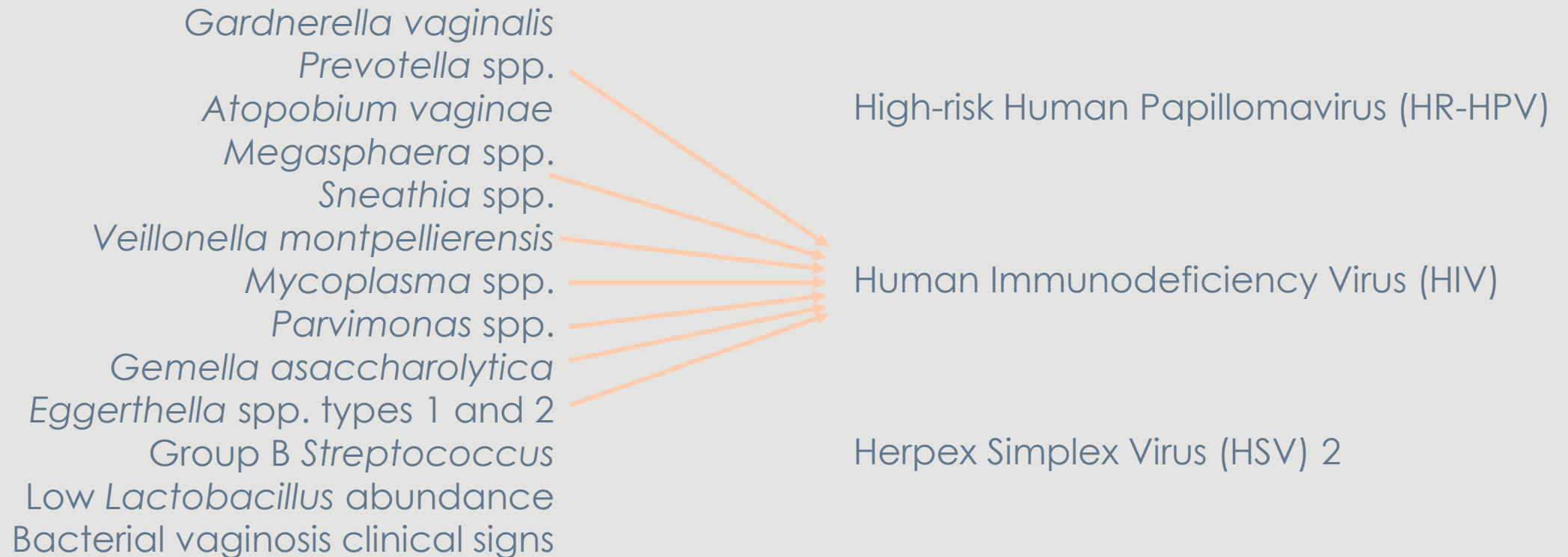
# Interplay between the cervicovaginal microbiota and viral infections

Vaginal dysbiosis is associated with increased risk of acquisition of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, Herpes Simplex Virus (HSV), Human Papilloma Virus (HPV), and Human Immunodeficiency Virus (HIV).



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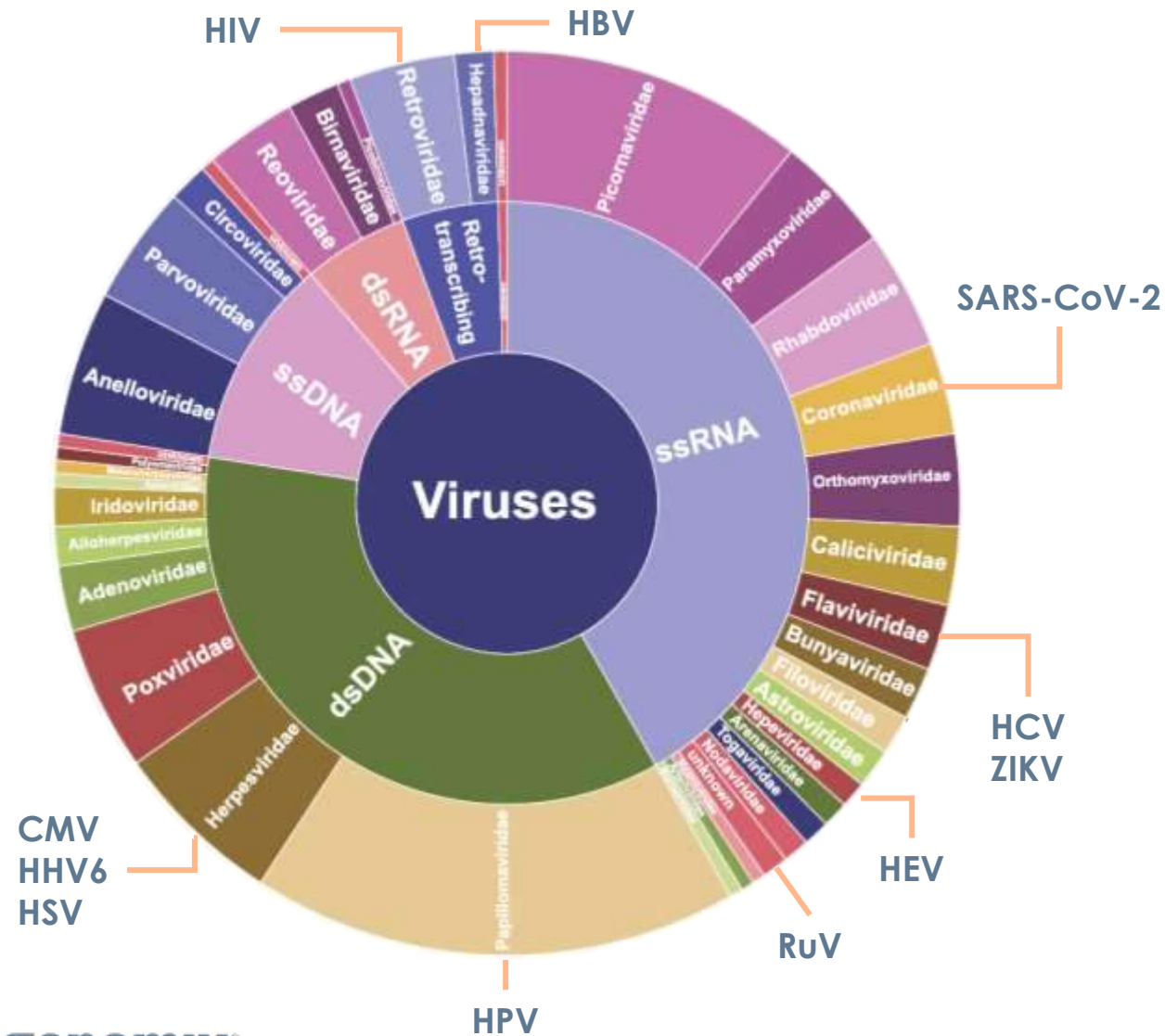


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Vaginal dysbiosis is associated with increased risk of acquisition of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, Herpes Simplex Virus (HSV), Human Papilloma Virus (HPV), and Human Immunodeficiency Virus (HIV).



# Studying the reproductive tract virome



- No universal marker for virus equivalent for the 16S rRNA gene or ITS markers.
- Specific tests and multiplexing is required to simultaneously analyse different types of virus.

# Future perspective



**EMMA**

Endometrial Microbiome  
Metagenomic Analysis  
by **Igenomix**

+

**REPRODUCTIVE VIROME**

SARS-CoV-2: Severe Acute Respiratory Syndrome-2

HHV6: Human Herpes Virus 6

HIV: Human Immunodeficiency Virus

HPV: Human Papillomavirus

HCV: Hepatitis C Virus

HBV: Hepatitis B Virus

HEV: Hepatitis E Virus

CMV: Cytomegalovirus

RuV: Rubella Virus

HSV: Herpes Simplex Virus

ZIKV: Zika Virus



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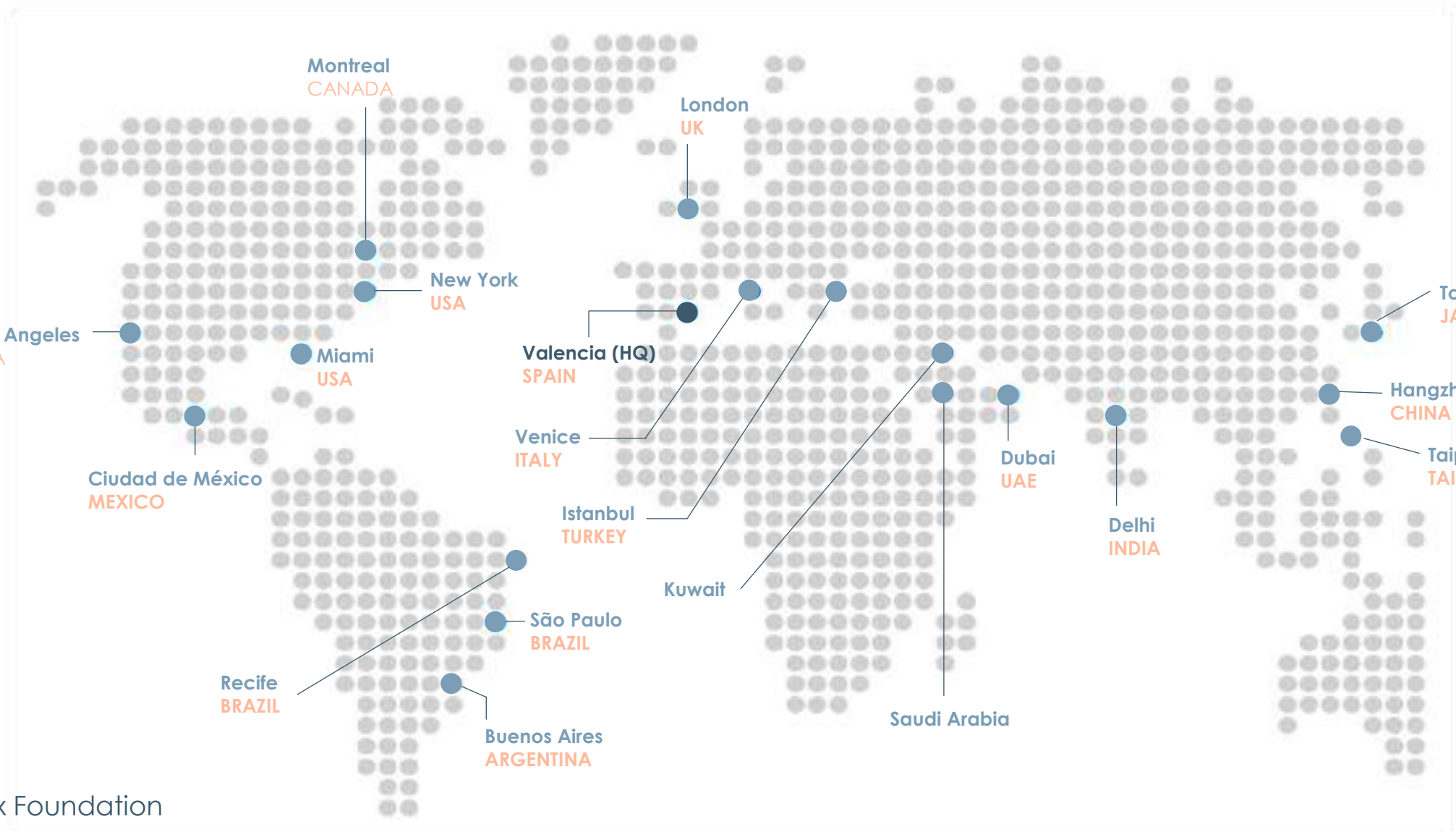
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# ¡GRACIAS!

Microbiota:

La nueva era del análisis endometrial

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