Brussels IVF

The microbiome and ART:

how close are we to clinical application?

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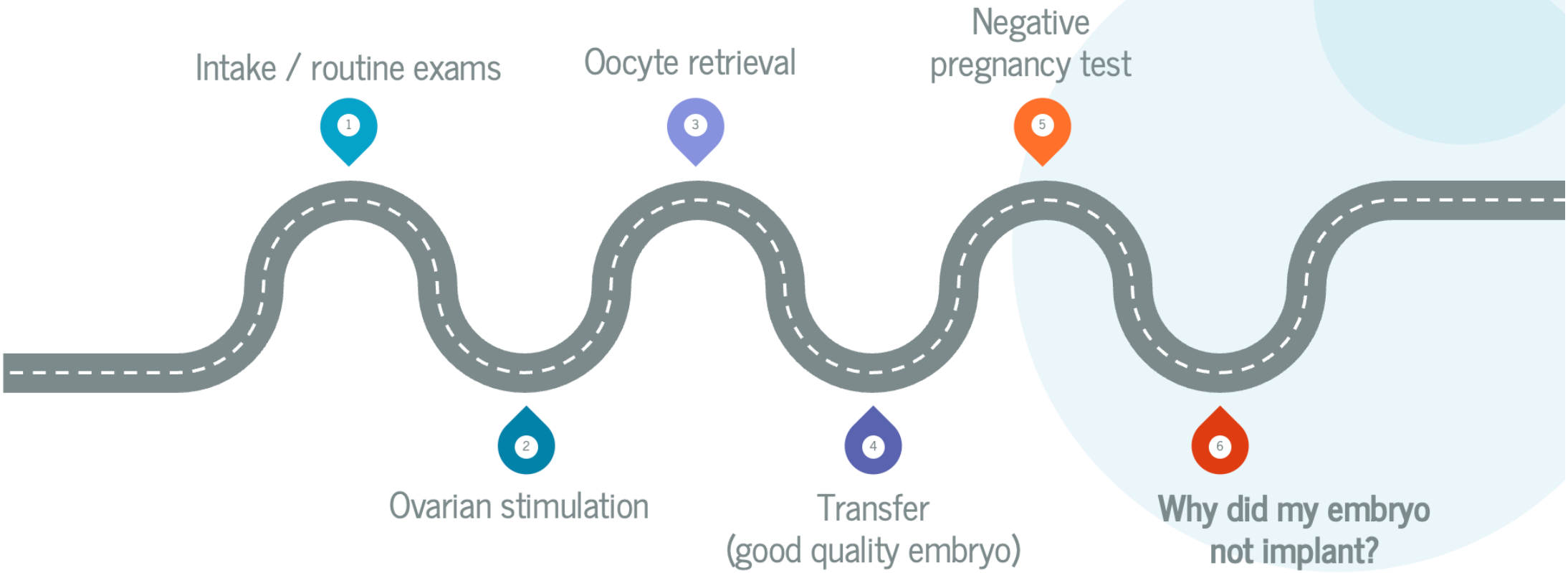
Brussels IVF, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel

BSRM, March 20th 2026

Universitair Ziekenhuis Brussel

VUB VRIJE UNIVERSITEIT BRUSSEL

IVF from the patient's perspective

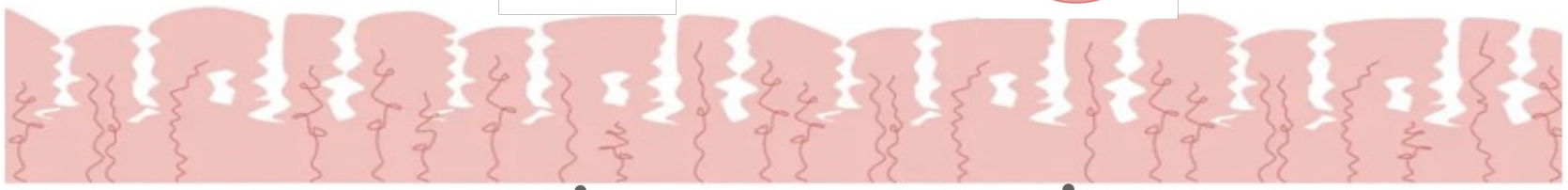


Embryo versus Endometrium

Craciunas et al., 2019

euploid

aneuploid

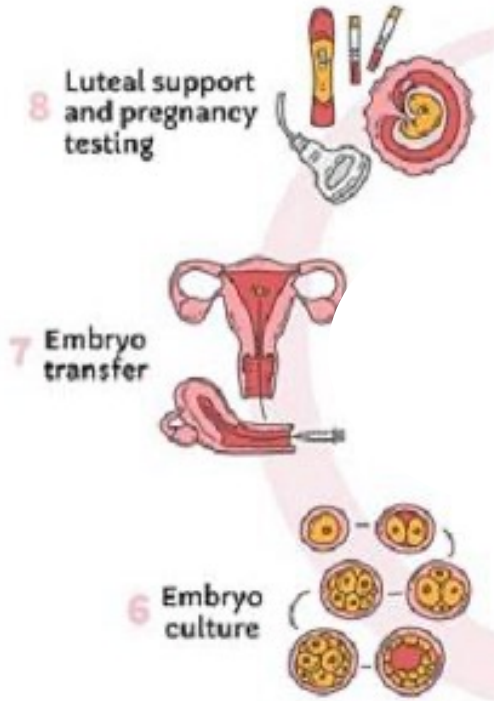


RIF

REPL

succesful pregnancy

denied implantation

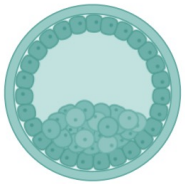


Repeated Implantation Failure

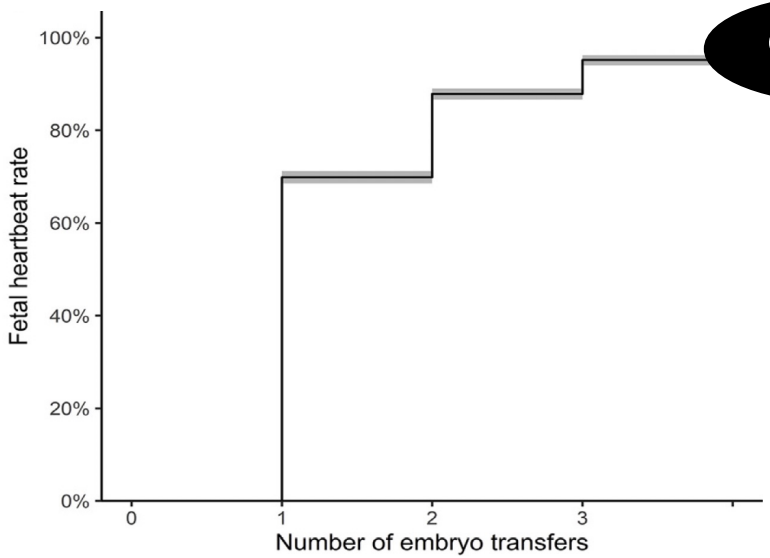
Pirtea et al., F&S 2021

Gil et al., HR 2024

euploid



(n=4429 patients)



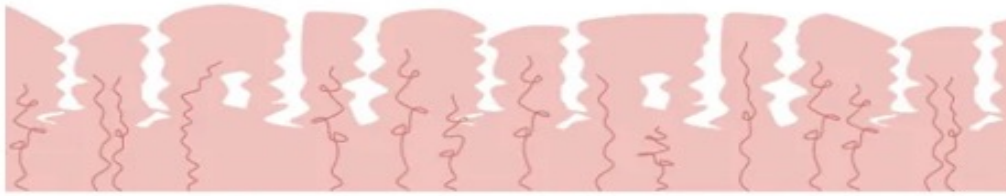
95.2%

(n=123 987 patients)

98.1%

n=105
with at least 1 additional
euploid blastocyst transfer

?



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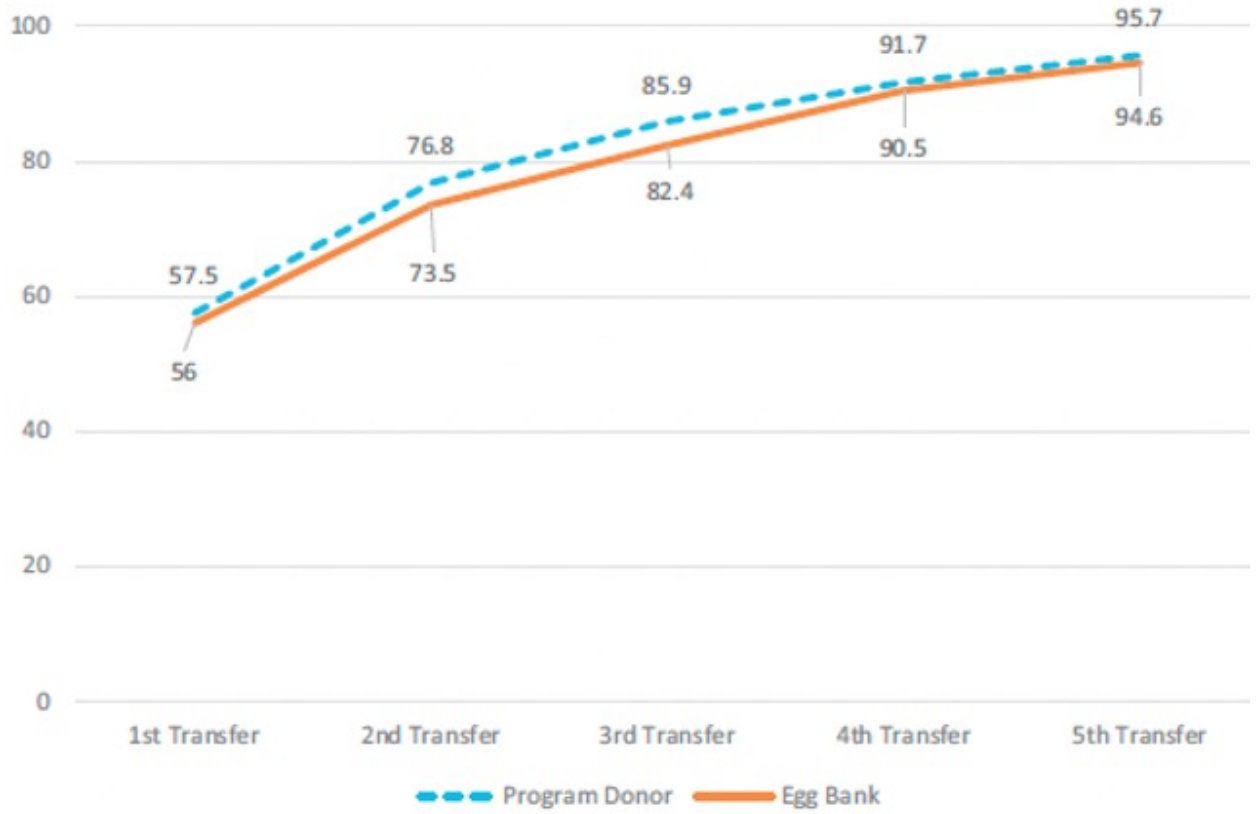
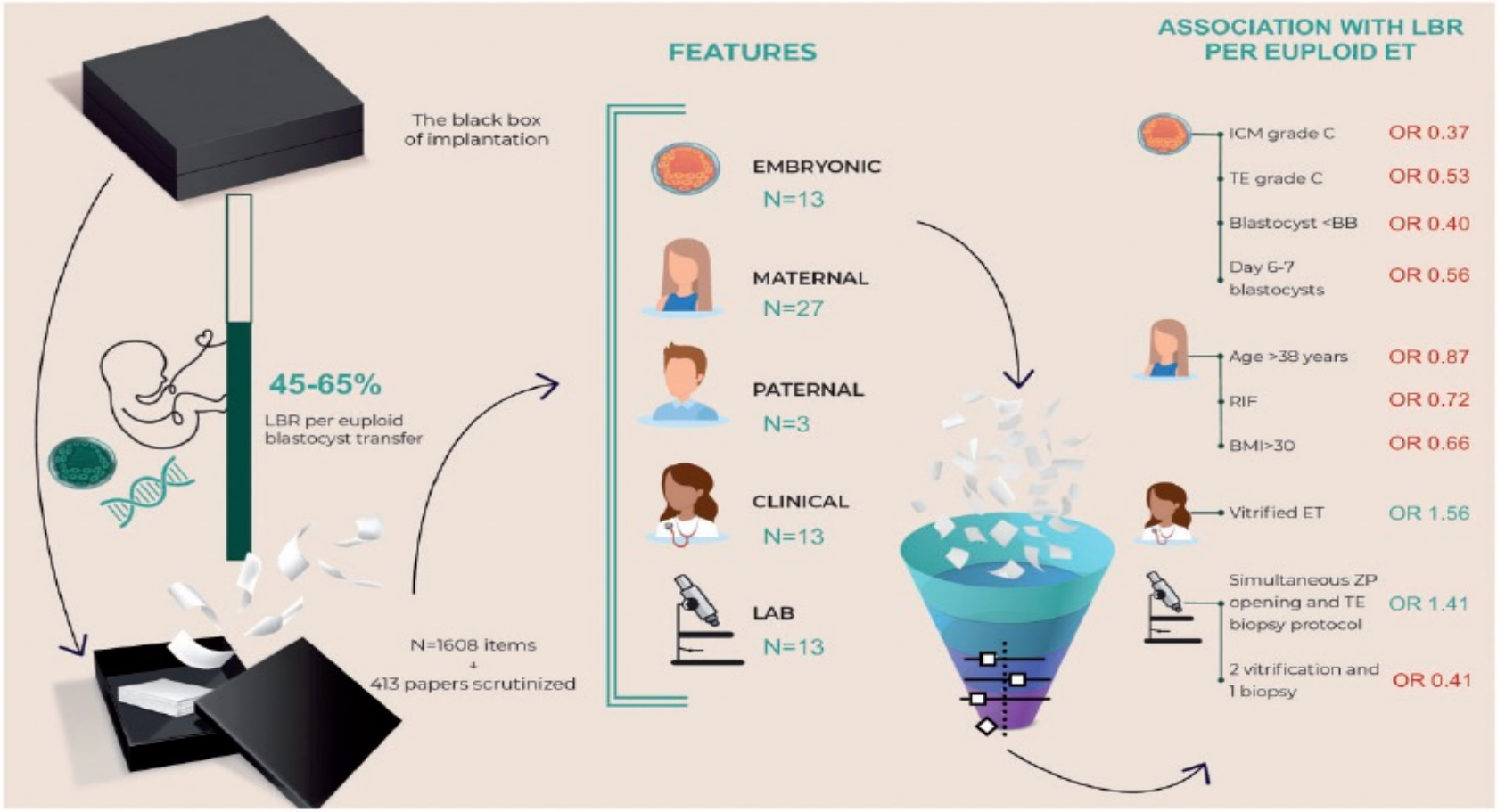
RIF following oocyte donation?

Williams et al., F&S 2022



Why do euploid blastocysts not implant?

Cimadomo et al., 2023



Opening the black box of implantation: low blastocyst quality and maternal aging, obesity or repeated implantation failures (RIF), as well as poor or excessive embryo manipulations may reduce the live birth rate per euploid blastocyst transfer.

Endometrial ‘pathology’?

Adenomyosis

Displaced/aberrant window of implantation

Immunological profile

Endometrial thickness

Microbiota - Microbiome

Chronic endometritis

BIVF microbiome - CE studies

ART MB	**Prospective observational cohort study (n=300)** **2016-2019**
CEREV	**Retrospective observational cohort study (n=786 patients)** **2020-2021**
FLORA	**Prospective observational cohort study (n=1000)** **Interim analysis n=335; 500 included; 2023-ongoing**

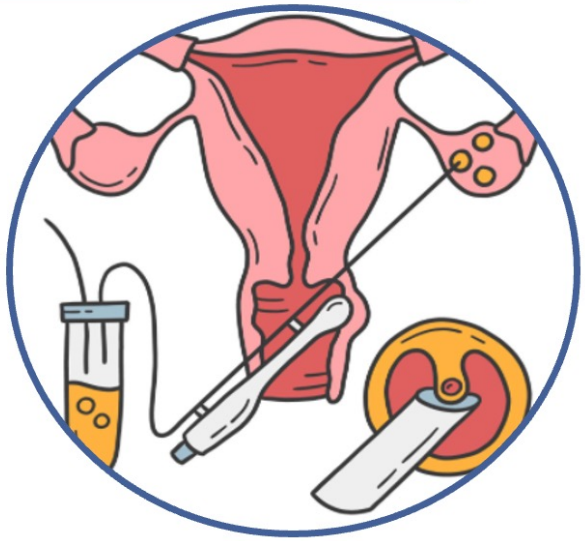
ART MICROBIOME

NCT03105453

Prospective observational cohort study (n=300)

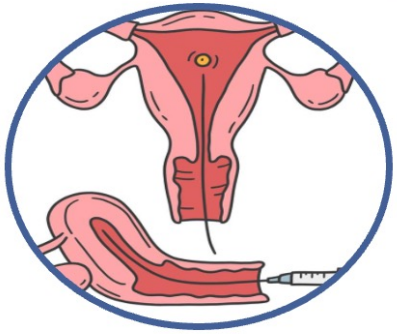
vaginal + cervical swab / questionnaire / blood sampling

1) What are the relevant confounders of the baseline LRT microbiota composition?



2) What is the impact of ovarian stimulation on these microbial communities?

HQ blastocysts/ LBR / CLBR



3) Are LRT microbiota associated with outcome? Are they biomarkers that can predict ART success?

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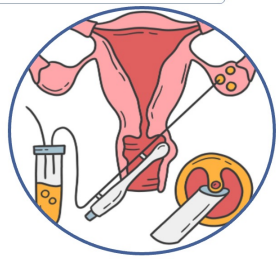

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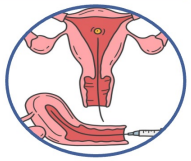


3) Are LRT microbiota associated with outcome? Are they biomarkers that can predict ART success?

Inclusion

Caucasian
<40 years
1st or 2nd cycle
Antagonist
Blastocyst SET
Normal AMH

Exclusion

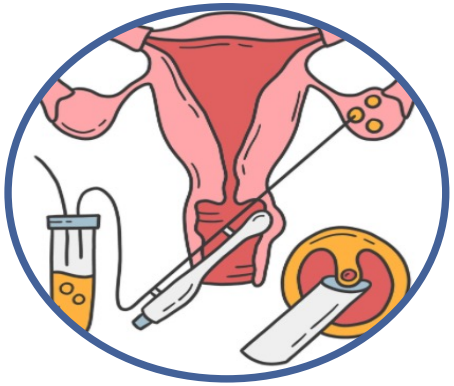
BMI $\geq$ 30 kg/m^2
AB < 3 weeks
Surgical sperm
IVM / PGT
Endometriosis
REPL
Chronic disease

ART MICROBIOME

NCT03105453

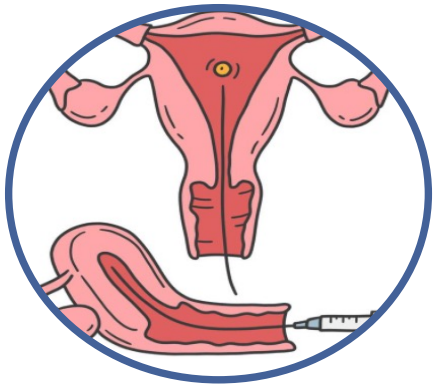
1) *What are the relevant confounders of the baseline LRT microbiota composition?*

→ ***cycle phase***
→ ***intercourse***



2) What is the impact of ovarian stimulation on these microbial communities?

→ ***confounder-independent effect of E2 rise on microbiome communities, i.e. increased diversity***



3) Are LRT microbiota associated with outcome? Are they biomarkers that can predict ART success?

→ ***baseline microbiota communities are associated to (C)LBR***
→ ***↑ Anaerococcus/Peptoniphilus ~ ↓ (C)LBR***
BUT: low accuracy for prediction

Clinical relevance of the LRT microbiota's impact on reproductive outcomes following ART is unclear.
Caution is warranted in case of microbiota-based decisions in ART.

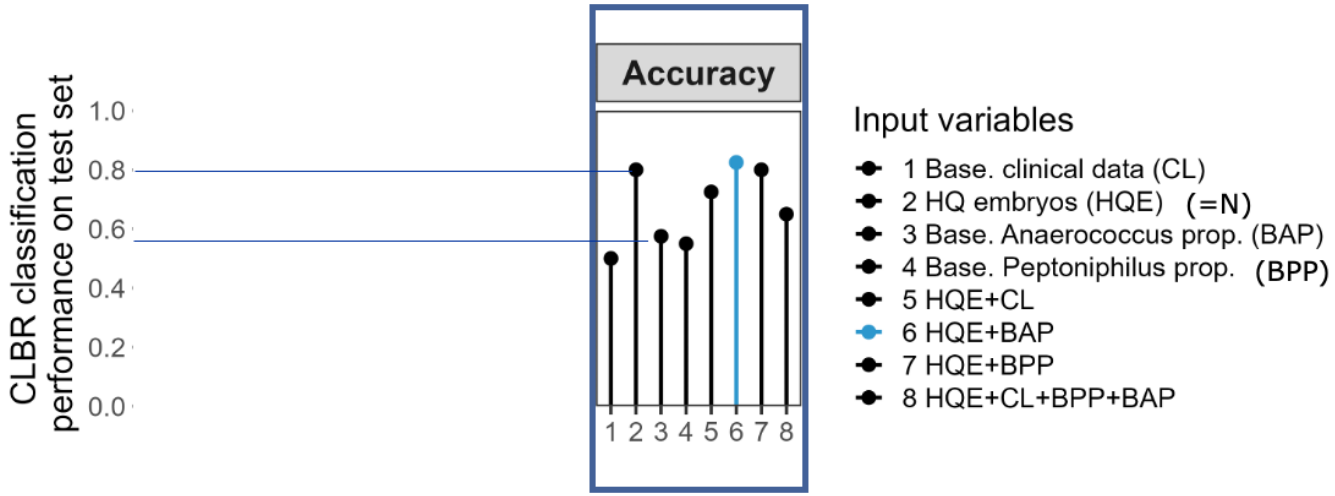
ART MICROBIOME

NCT03105453

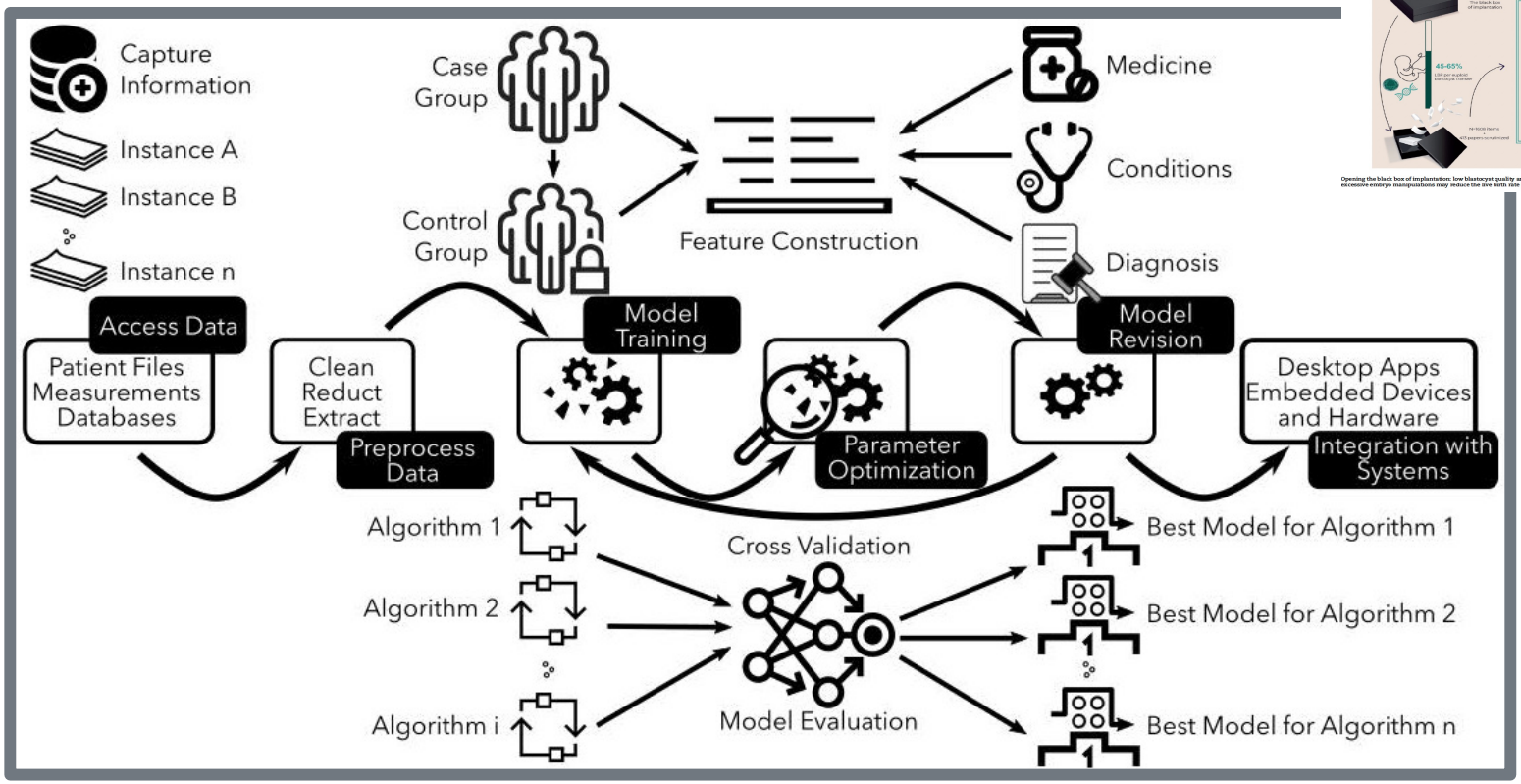
Pre-OS microbiota communities are not accurately predictive of CLBR

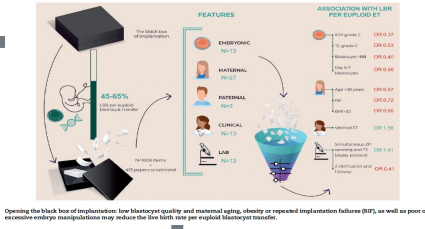
Machine-learning models determined that :

- Anaerococcos and Peptoniphilus proportions alone did not have a good accuracy
- the number of HQ embryos showed a good accuracy
- adding microbiota only slightly improved the model's accuracy



ASSOCIATION IS NOT PREDICTION



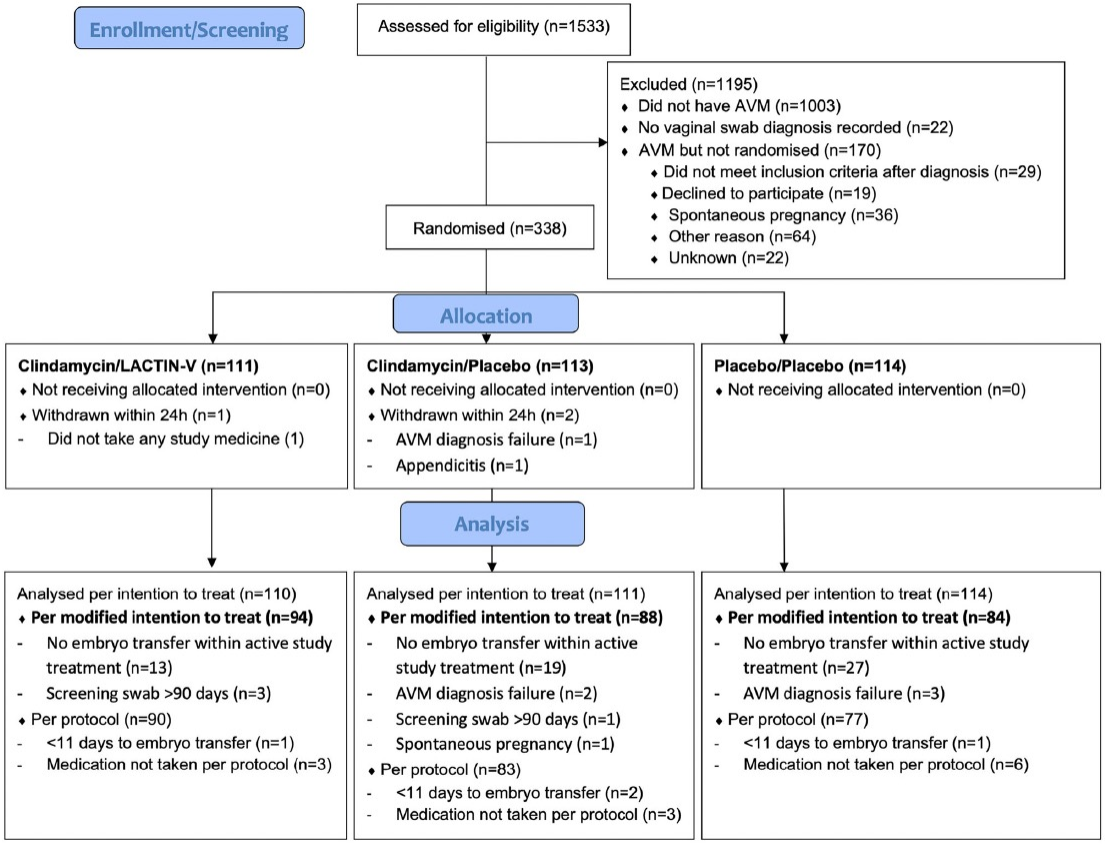


Opening the black box of implantation: low blastocyst quality and maternal aging, obesity or repeated implantation failures (RIF), as well as poor or excessive embryo manipulations may reduce the live birth rate per euploid blastocyst transfer.

Toma and Wei, 2023

Vaginal dysbiosis in IVF patients: RCT study flow

Haarh et al., Nature Com 2025



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Vaginal dysbiosis in IVF patients: RCT results

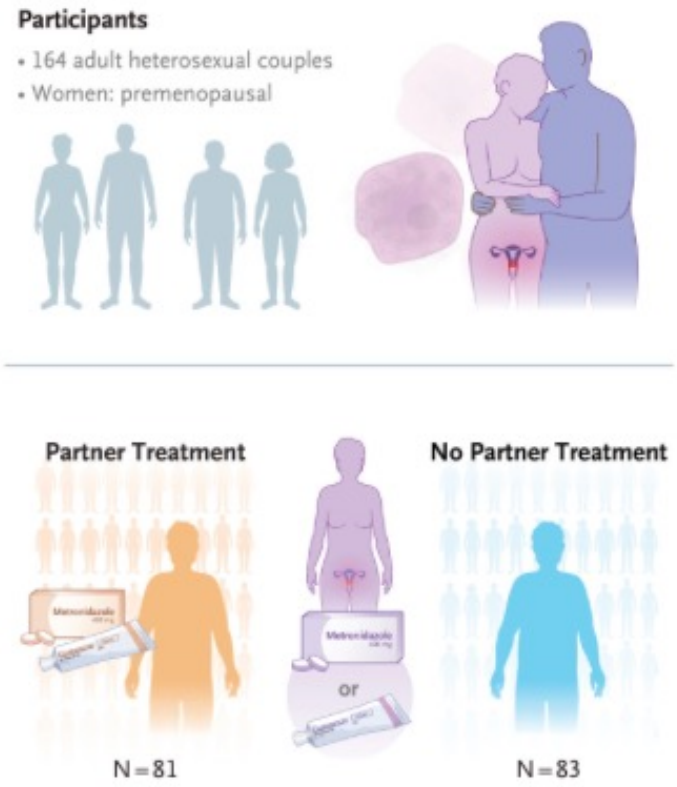
Haarh et al., Nature Com 2025

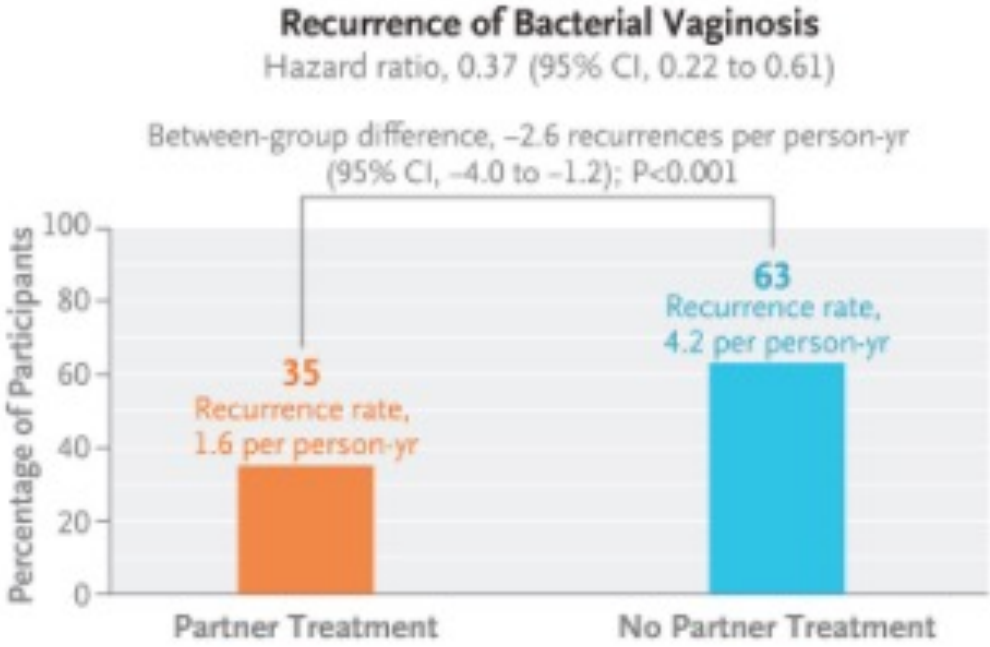
Table 2 | Crude and adjusted chance/risk of reproductive outcomes based on modified intention to treat criteria[a]

	Clindamycin/LACTIN-V (*N* = 94)	Clindamycin/Placebo (*N* = 88)	Placebo/Placebo (*N* = 84)	Both active arms versus Placebo/Placebo[b]
HCG positives	57 (61%) 62% (52–71%)	58 (66%) 65% (56–75%)	50 (60%) 59% (49–69%)	aRR 1.07 (0.87–1.32)
Clinical pregnancy week 7–9	39 (41%) 42% (32–52%)	41 (47%) 46% (36–56%)	38 (45%) 45% (35–56%)	aRR 0.98 (0.74–1.29)
Ongoing pregnancy week 10–12	38 (40%) 41% (31–51%)	40 (45%) 45% (35–55%)	37 (44%) 44% (34–54%)	aRR 0.97 (0.73–1.29)
Early pregnancy loss	19 (33%) 34% (21–46%)	18 (31%) 31% (19–43%)	13 (26%) 26% (14–38%)	aRR 1.24 (0.73–2.11)
Live birth	37 (39%) 40% (30–50%)	40 (45%) 45% (35–55%)	34 (40%) 40% (30–51%)	aRR 1.05 (0.77–1.42)

Bacterial vaginosis treatment – partner?

Vodstrcil et al., NEJM 2025





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BIVF microbiome - CE studies

ART MB	**Prospective observational cohort study (n=300)** **2016-2019**
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BIVF digital image-based CE scoring algorithm

Score 0	No positive stained plasma cells
Score IPC	Less than 5 isolated, scattered positive stained plasma cells
Score 1	≥1 cluster of 5-19 PCs/0.25mm^2
Score 2	≥1 cluster of 20-49 PCs/0.25mm^2
Score 3	≥1 cluster of 50 or more PCs/0.25mm^2

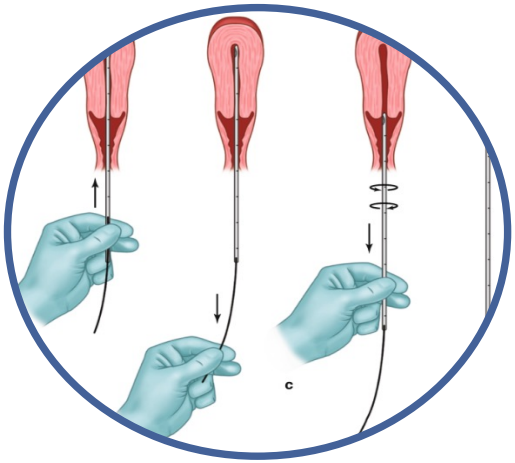
Excellent interobserver agreement between pathologists
+
Machine learning algorithm outperformed pathologists in the clinically most relevant 2-tier-system

Manuscript in preparation

CEREV

Submitted @ ESHRE 2026
Petrone et al.

Retrospective observational cohort study (n=786 patients)
2020-2021



HISTOLOGY

ROUTINE

2020
1 x AB treatment
(followed by treatment continuation)

2021
1 x AB treatment
(followed by rebiopsy)
+ 2nd course if indicated

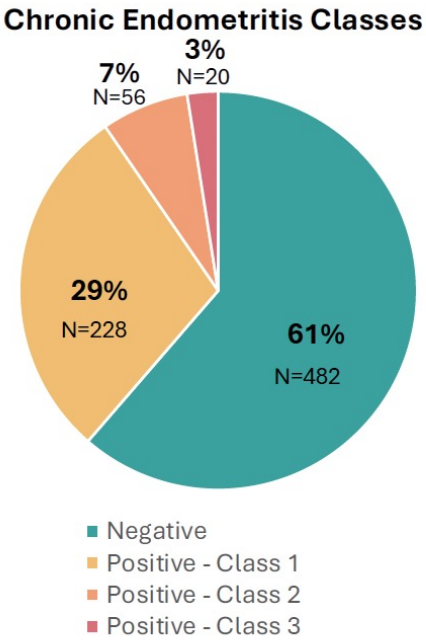
1-YEAR FU

n=585
with at least 1 subsequent ET

CEREV

Submitted @ ESHRE 2026
Petrone et al.

A

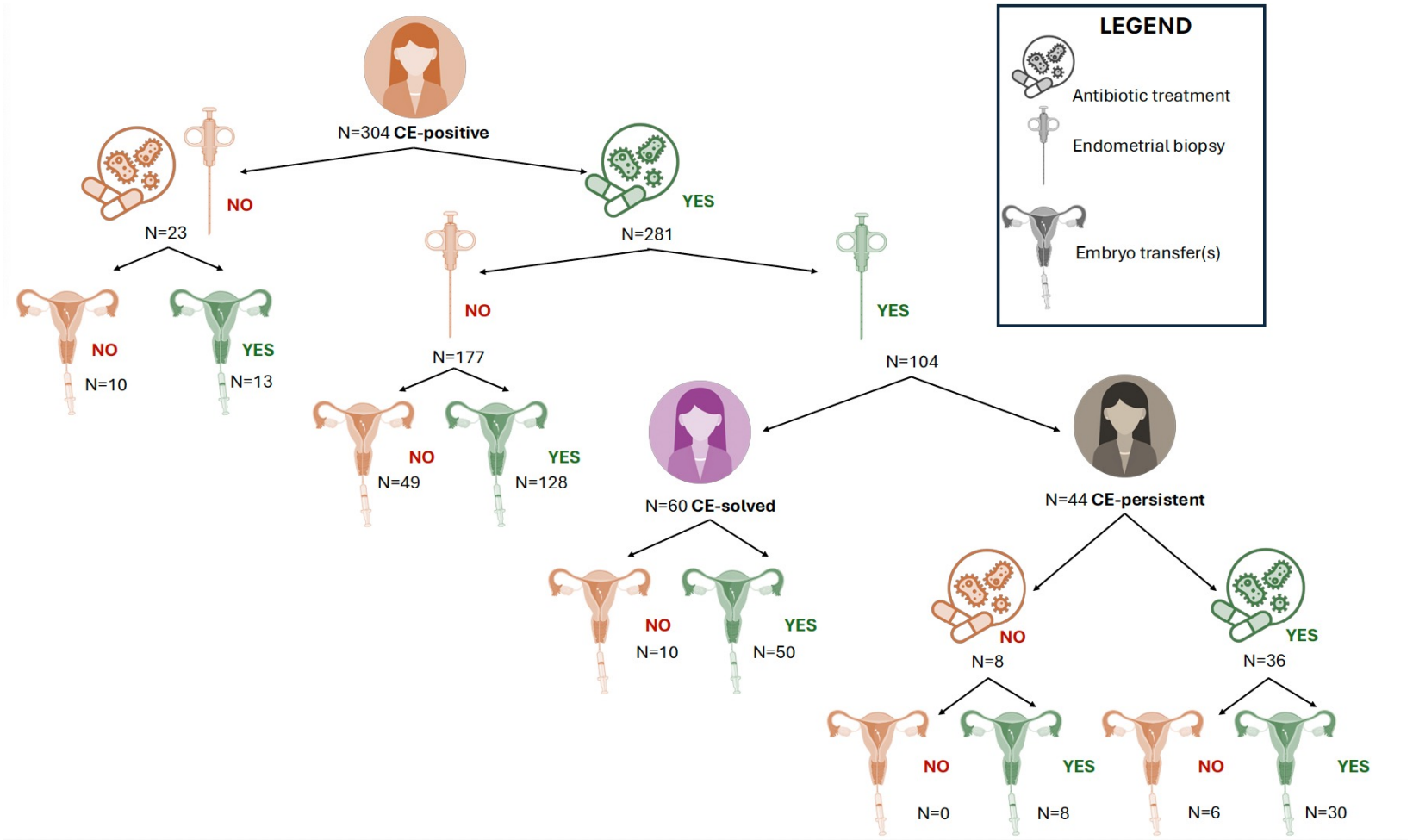


B

Variable		CE positivity	univariate OR, 95%CI, p-value
Findings at HSC			
	Normal	39.4% (N=250/634)	Control
	Trophoblast retention	69.6% (N=16/23)	**OR 3.5, 95% CI 1.4-8.7 p=0.006**
	Intracavitary pathology	32% (N=32/100)	OR 0.7, 95% CI 0.5-1.1 p=0.157
	Congenital pathology	50% (N=4/8)	OR 1.5, 95% CI 0.4-6-2 p=0.547
	Adhesiolysis/previous curettage	0% (N=0/10)	-
	Others	18.2% (N=2/11)	OR 0.3, 95% CI 0.1-1.6 p=0.171
Previous miscarriages			
	0	36% (N=165/455)	
	1	37.5% (N=54/144)	**OR 1.13 95%CI 1-1.3 p=0.015**
	2	43.5% (N=37/85)	
	≥3	47% (N=48/102)	
Ethnicity			
	Caucasic	37.8% (N=242/641)	Control
	African	36.7% (N=36/98)	OR 0.9, 95% CI 0.6-1-5 p=0.846
	Arab	61.3% (N=19/31)	**OR 2.6, 95% CI 1.2-5.4 p=0.011**
	Asian	43.8% (N=7/16)	OR 1.3, 95% CI 0.5-3.5 p=0.626

CEREV

Submitted @ ESHRE 2026
Petrone et al.



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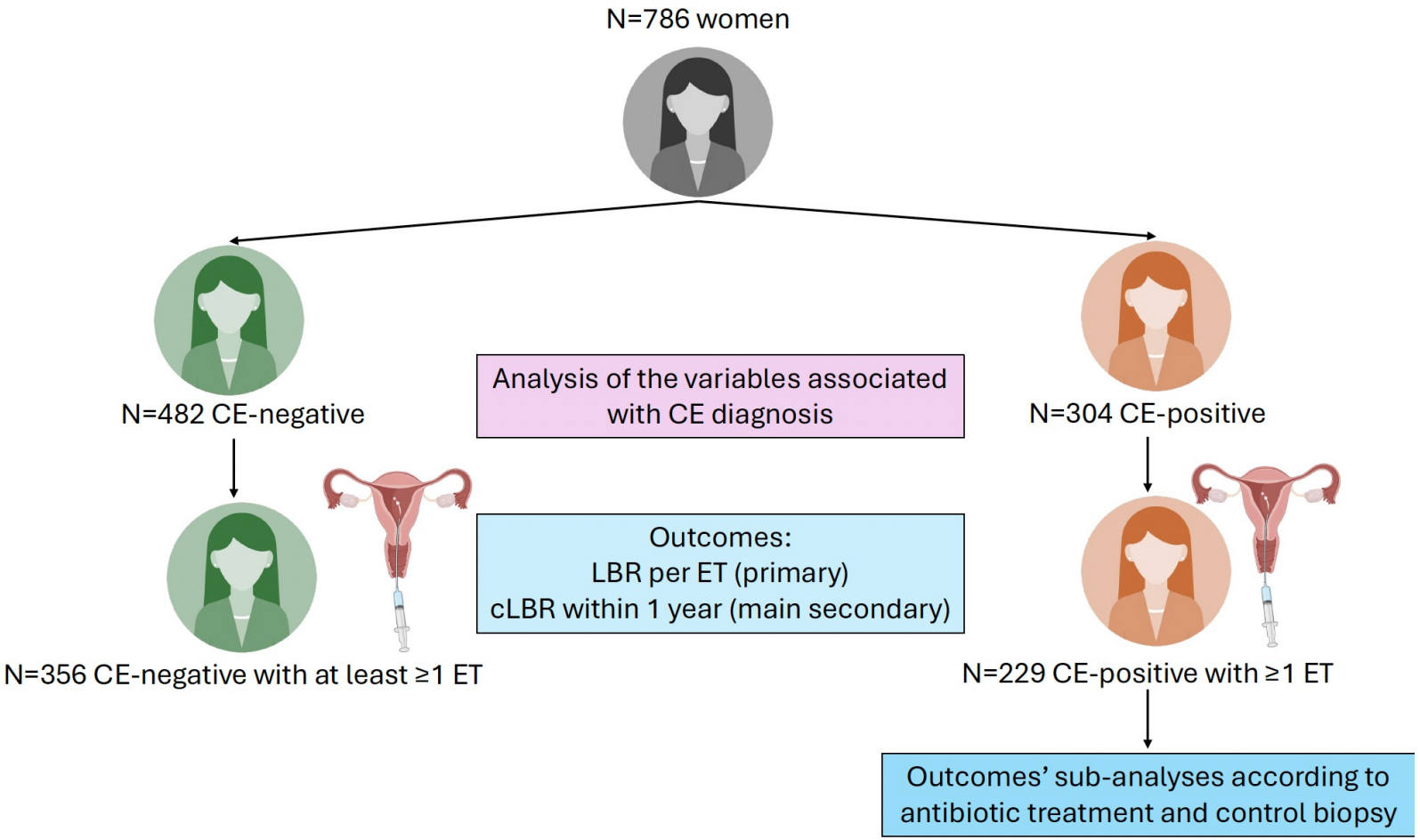


CEREV

Submitted @ ESHRE 2026
Petrone et al.

	CE Negative	CE positive	p value
Positive pregnancy test rate per first transfer	N=172/356 48.3%	N=121/229 52.8%	p=0.310
Biochemical pregnancy loss rate per positive pregnancy	N=16/172 9.3%	N=11/121 9.1%	p=0.999
Ectopic pregnancy rate per clinical pregnancy	N=7/156, 4.5%	N=3/110 2.7%	p=0.531
Miscarriage rate per clinical pregnancy	N=26/149 17.4%	N=25/107, 23.4%	p=0.269
Live birth rate per first transfer	N=123/356, 34.6%	N=82/229, 35.8%	p=0.790
Live birth rate within one year after endometrial biopsy	N=200/356, 56.2%	N=127/229, 55.5%	p=0.865

	CE negative	CE positive				
		Antibiotic NO Control biopsy NO	Antibiotic YES			
			Control biopsy NO	Control biopsy YES		
				CE solved	CE persistent	
					Antibiotic NO	Antibiotic YES
Positive pregnancy test rate per first transfer	N=172/356, 48.3%	N=11/13, 84.6%	N=64/128, 50%	N=24/50, 48%	N=4/8, 50%	N=18/30, 60%
*p-value vs CE-negative Multivariate OR**	*Control*	*p=0.011 NS*	*p=0.758*	*p=0.999*	*p=0.999*	*p=0.256*
Biochemical pregnancy loss rate per positive pregnancy	N=16/172, 9.3%	N=0/11, 0%	N=5/64, 7.8%	N=2/24, 8.3%	N=0/4, 0%	N=4/18, 22.2%
p-value vs CE-negative	*Control*	*p=0.602*	*p=0.803*	*p=0.999*	*p=0.999*	*p=0.103*
Ectopic pregnancy rate per clinical pregnancy	N=7/156, 4.5%	N=0/11, 0%	N=1/59, 1.7%	N=1/22, 4.5%	N=1/4, 25%	N=0/14, 0%
p-value vs CE-negative	*Control*	*p=0.999*	*p=0.451*	*p=0.999*	*p=0.187*	*p=0.999*
Miscarriage rate per clinical pregnancy	N=26/149, 17.4%	N=2/11, 18.2%	N=15/58, 25.9%	N=3/21, 14.3%	N=1/3, 33.3%	N=4/14, 28.6%
p-value vs CE-negative	*Control*	*p=0.999*	*p=0.179*	*p=0.999*	*p=0.446*	*p=0.292*
Live birth rate per first transfer	N=123/356, 34.6%	N=9/13, 69.2%	N=43/128, 33.6%	N=18/50, 36%	N=2/8, 25%	N=10/30, 33.3%
*p-value vs CE-negative Multivariate OR**	*Control*	*p=0.016 NS*	*p=0.913*	*p=0.874*	*p=0.720*	*p=0.999*
Live birth rate within one year after endometrial biopsy	N=200/356, 56.2%	N=9/13, 69.2%	N=72/128, 56.3%	N=26/50, 52%	N=5/8, 62.5%	N=15/30, 50%
p-value vs CE-negative	*Control*	*p=0.406*	*p=0.999*	*p=0.649*	*p=0.999*	*p=0.568*

In line with upcoming research?

Quenby et al., ESHRE 2025

CERM trial

ABSTRACT TEXT

S. Quenby[1], J. Odendaal[1], N. Black[1], K. Fishwick[1], J. Thornton[1], K. Makwana[1], J. Fisher[2], R. Lall[2], A. Coomarasamy[3], I. Granne[4], M. Underwood[2], J. Guck[2], G. Bouliotis[2].
[1]University of Warwick, Division of Biomedical Sciences, Coventry, United Kingdom.
[2]University of Warwick, Warwick Clinical Trials Unit, Coventry, United Kingdom.
[3]University of Birmingham, Department of Metabolism and Systems Science, Birmingham, United Kingdom.
[4]University of Oxford, Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom.

Study question:
Does treatment with doxycycline in those with chronic endometritis (CE) and a history of recurrent miscarriage (RM) improve cumulative live-birth rates?

Summary answer:
Treatment with doxycycline did not increase live-birth rates in those with CE and a history of RM (Relative Risk (RR): 1.02; Credible Interval (CrI) 0.85-1.21).

What is known already:
RM affects up to 5% of couples trying to conceive. A commonly purported cause of RM is CE. This asymptomatic inflammation of the endometrium has been differentially attributed to both pathogenic organisms and derangement of the endometrial microbiota. CE has been associated with RM in numerous uncontrolled studies. Similarly, CE resolution has been demonstrated in women following treatment with antibiotic therapy in uncontrolled studies. Based on this antibiotics treatment of CE remains a common intervention for RM prevention and features in several national guidelines.

Study design, size, duration:
The CERM trial is an adaptive, multicentre, parallel-arm, double-blind, placebo-controlled, randomised trial. 2,178 women were screened for eligibility, with 728 women undergoing endometrial biopsy. Of these 505 screened positive for CE and 438 were randomised (219 doxycycline and 219 placebo). Randomised, participants were followed-up for a median of 525 (IQR: 329) days post-randomisation. 210 screen negative women were also followed-up.

Participants/materials, setting, methods:
Women aged ≥18 to <42 years with a history of ≥2 consecutive first trimester losses, attending 26 UK-wide clinics were screened for CE by endometrial biopsy and immunohistochemistry for CD138. Women who screened positive were randomised, 1:1, to pre-conceptual doxycycline 100mg or identical placebo commenced on day 1 of the menstrual cycle twice daily for 14 days. The primary outcome was cumulative live births and pregnancies ≥24+0 weeks gestation at cessation of the trial.

Main results and the role of chance:
No between group imbalances were detected in the baseline characteristics between screen positive and negative women and those randomised to doxycycline or placebo in terms of, age, number of previous miscarriages, BMI, previous live birth rate and ethnicity. 95% of randomised women met the criteria for treatment compliance. A small cohort of women (n=17) received doxycycline external to the trial of these 6 women received doxycycline after the first pregnancy outcome. No significant difference in time to first conception was demonstrated. 113 live-births or ongoing pregnancies occurred in the doxycycline group with 109 in the placebo group (RR 1.02; CrI 0.85-1.21). Similarly, no between group difference in miscarriage rate was demonstrated (RR 0.90; CrI 0.67-1.22). There were 7 ectopic pregnancies in the placebo group and 1 in the doxycycline group. A per-protocol sensitivity analysis excluding known treatment non-compliance additionally showed no significance (RR 1.05; CrI 0.87-1.26). Pre-specified subgroup analyses also showed no significance for the impact of age (35 vs ≥35), miscarriage history (≤3 vs >3) or CE severity. A comparison of first pregnancy outcome demonstrated more, (n=89) live-births or ongoing pregnancies in the placebo group compared to the CE negative group (n=62) (RR 0.73; CrI 0.56-0.95).

Limitations, reasons for caution:
The study was stopped prior to reaching target recruitment by the funder. This was for perceived futility based on the summation of the clinical and translational evidence available.

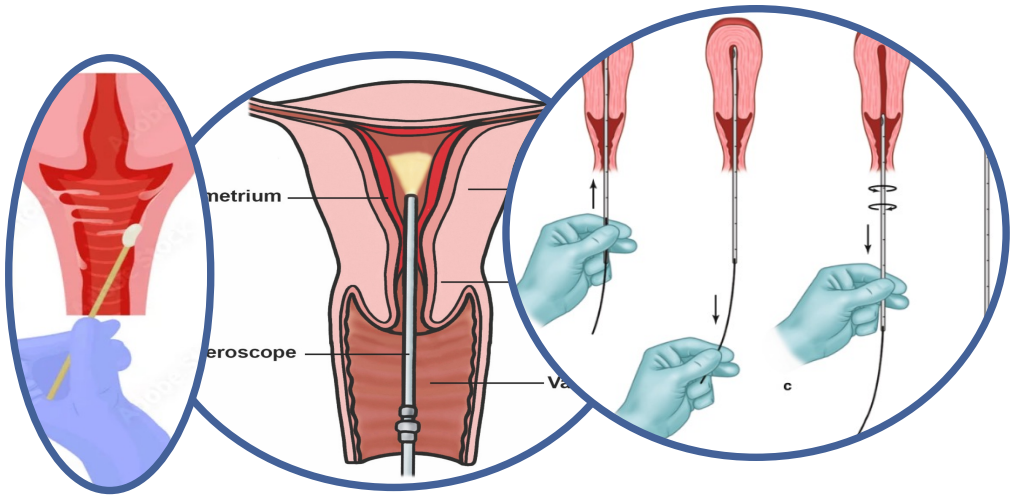
Wider implications of the findings:
Preconceptual doxycycline was not shown to be associated with improved live-birth rates in women with CE and RM. Screen negative women had worse pregnancy outcomes that screen positive women on placebo. The screening and treatment of CE should not form part of the routine management of RM.

FLORA

NCT05337072

Prospective observational cohort study (n=1000)
2023; ongoing - interim analysis n=335; 500 included

HYSTEROSCOPY // standardized score sheet

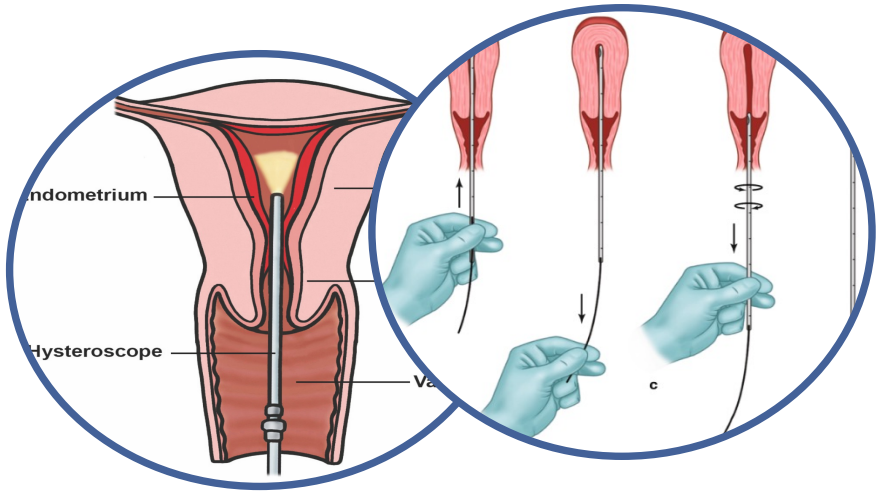


vaginal + endometrial microbiome sample // HISTOLOGY

FLORA

BIVF unpublished data

HYSTEROSCOPY // standardized score sheet



HISTOLOGY

Disconcordance hysteroscopy / biopsy

1st biopsy; n=335		CE diagnosis based on histology	
		+	-
CE diagnosis based on hysteroscopy	+	34 (10.1%)	72 (21.5%)
	-	45 (13.4%)	184 (54.9%)

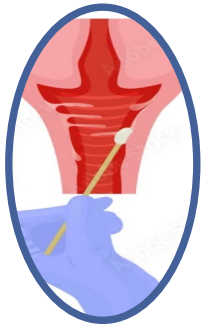
2nd biopsy; n=39		CE diagnosis based on histology	
		+	-
CE diagnosis based on hysteroscopy	+	4 (10.2%)	11 (28.2%)
	-	5 (12.8%)	19 (48.7%)

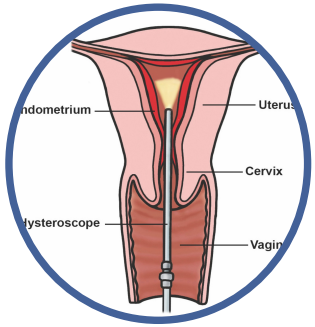
NCT05337072

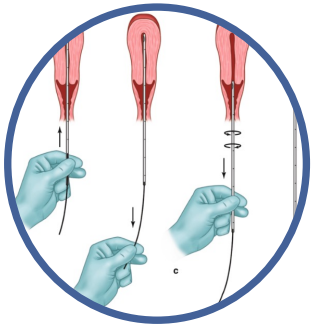
FLORA

Is there a specific CE microbiome signature?

HYSTEROSCOPY // standardized score sheet







Vaginal 16S rRNAseq

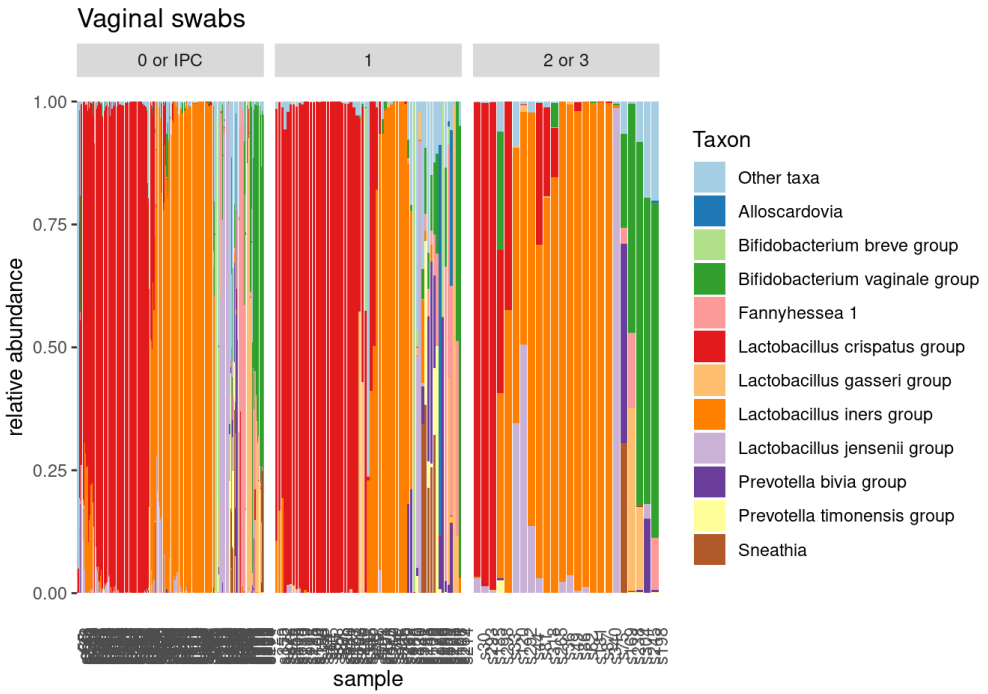
HISTOLOGY

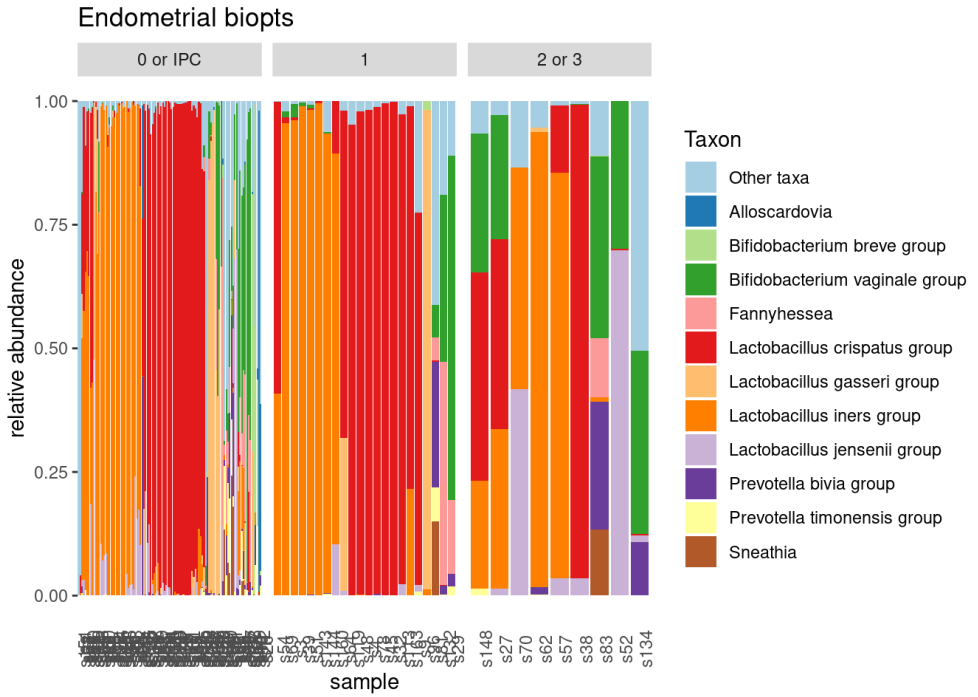
Endometrial 16S rRNAseq

FLORA

Relative abundace

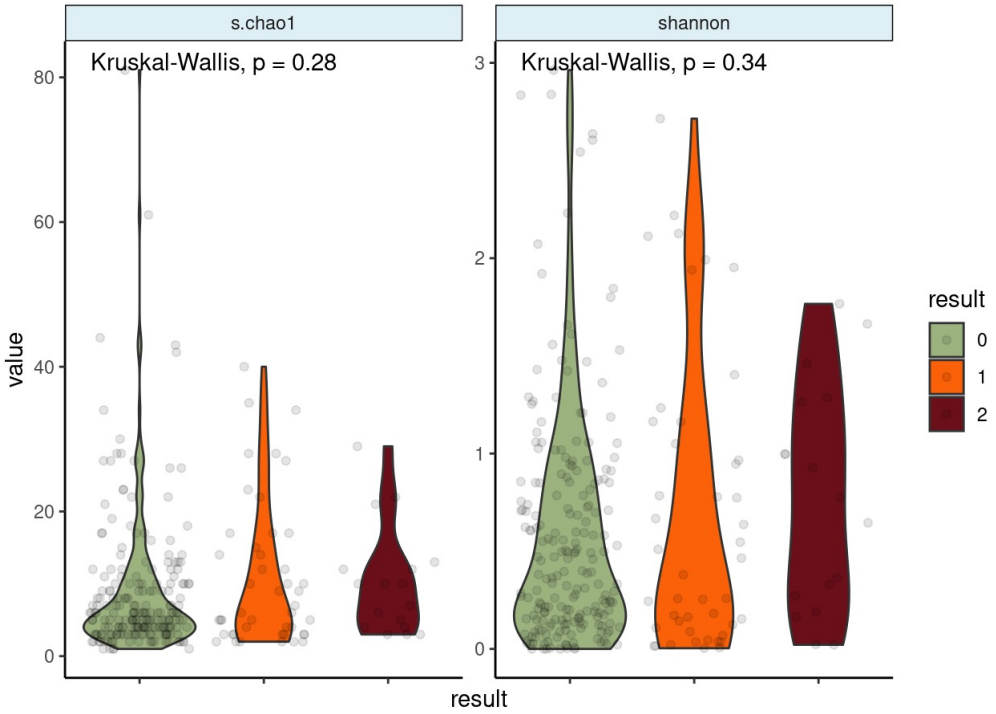
BIVF unpublished data



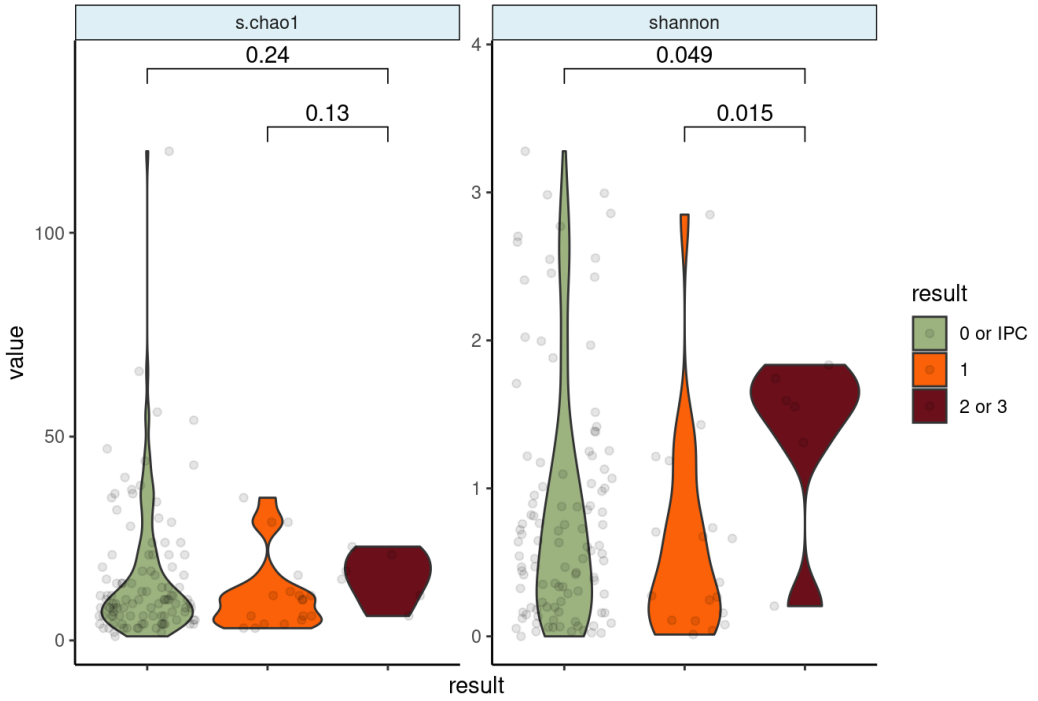


FLORA

Vaginal swabs

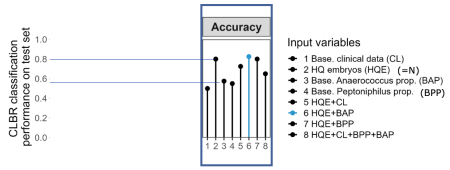


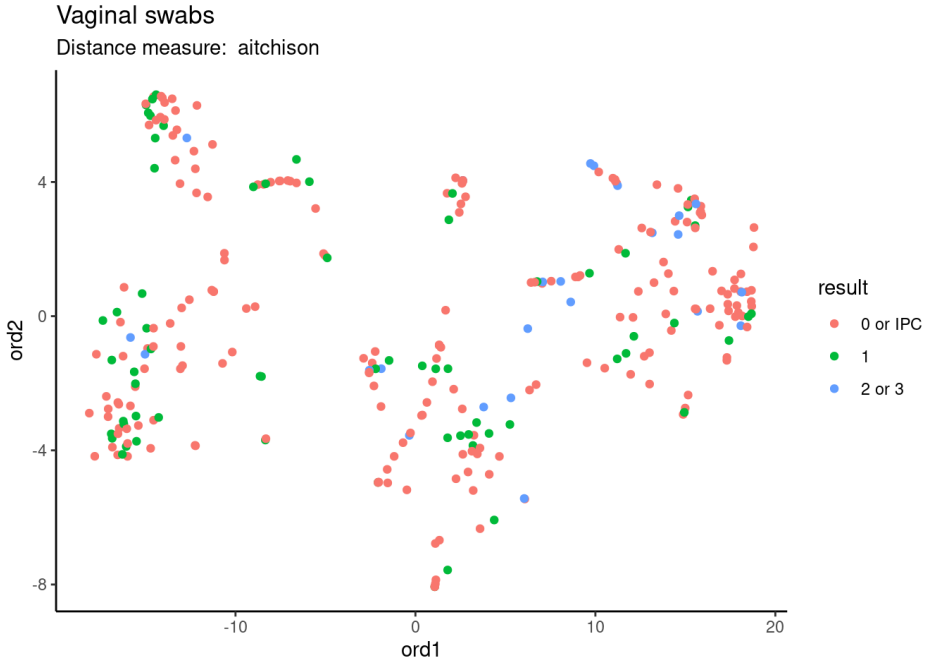
Endometrial biopts

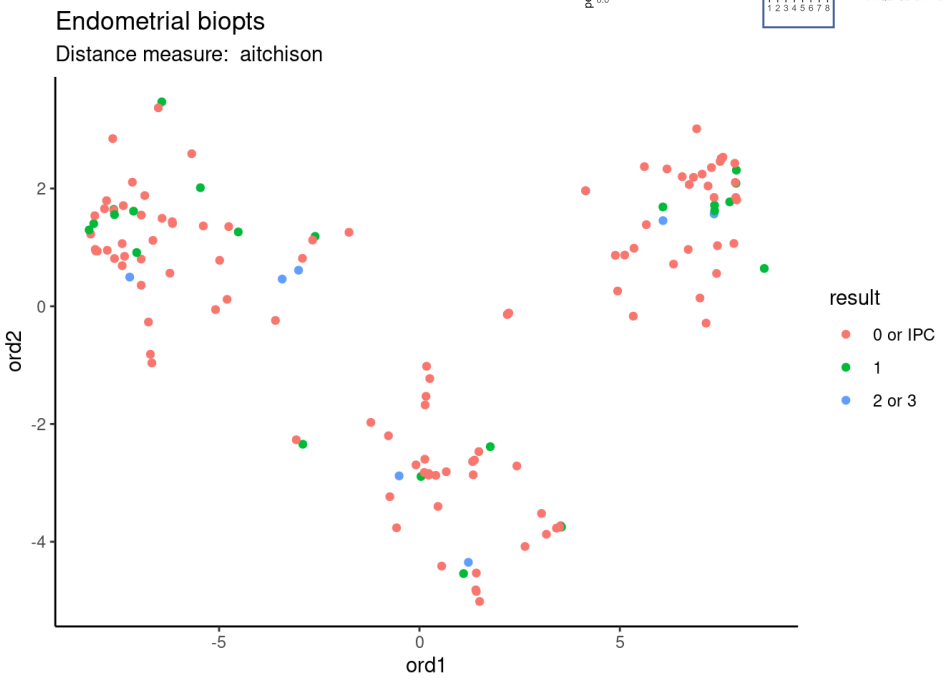


Beta diversity

▶ Code







Conclusion and future perspectives

Impact on reproductive outcomes is unclear

No clear relationship between female reproductive tract microbiome and CE

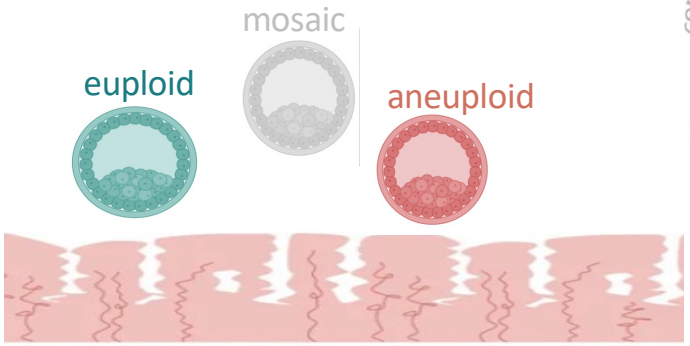
No standardized diagnostics
No standardized therapeutics

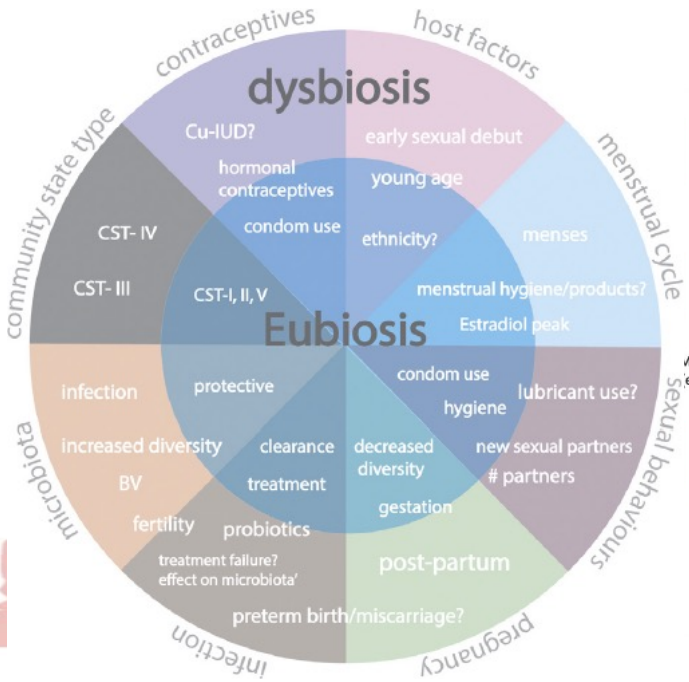
Association is not causation

Non-selection study to assess CE/microbiota and the impact on clinical outcomes (FLORA 2.0)

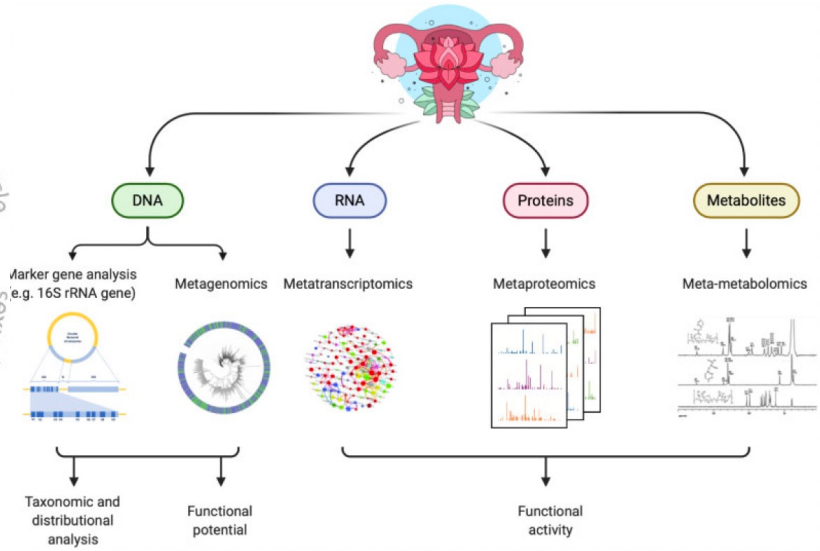
Correct selection of patient population (RIF – REPL?)
Large samples size and attention for confounders (PGT-A / EPL of non-chromosal origin)

Let’s not underestimate the complexity



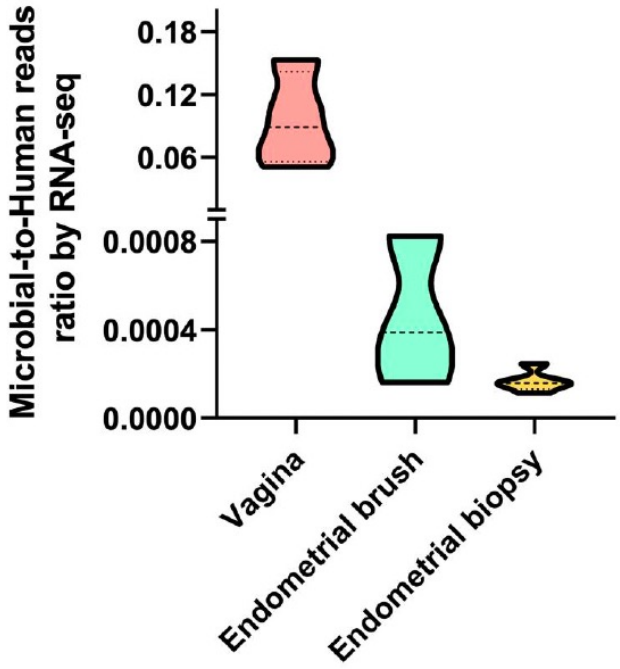


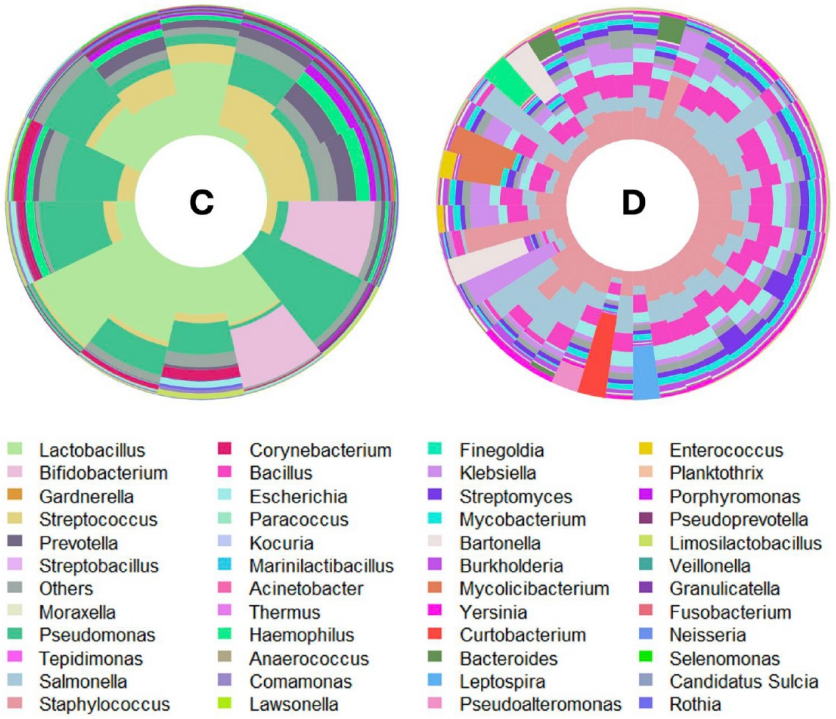
Koot et al., 2018



Molina et al., 2021

Which analysis (test) is most relevant?





Sola Leyva et al.,
Hum Rep Open 2026

Brussels IVF

The microbiome and ART:

how close are we to clinical application?

NOT YET THERE

THINKING MUST NEVER SUBMIT ITSELF.

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