

Impatto del microbiota genitale femminile nell'outcome dei trattamenti di PMA



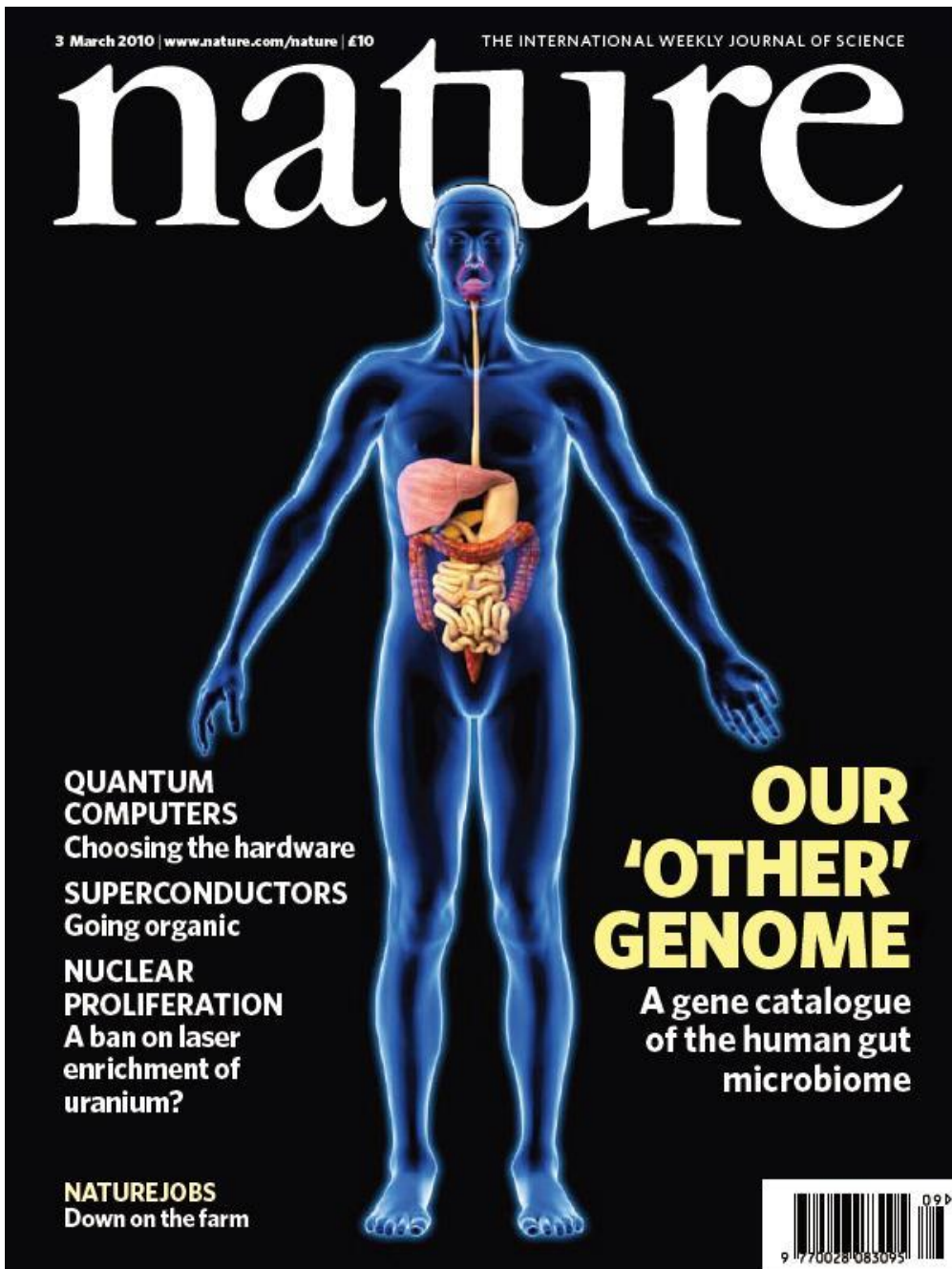
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Digestion of carbohydrates

(As microbes produce enzymes that are lacking in humans)

Suppression of harmful microbes

(by competitive exclusion)

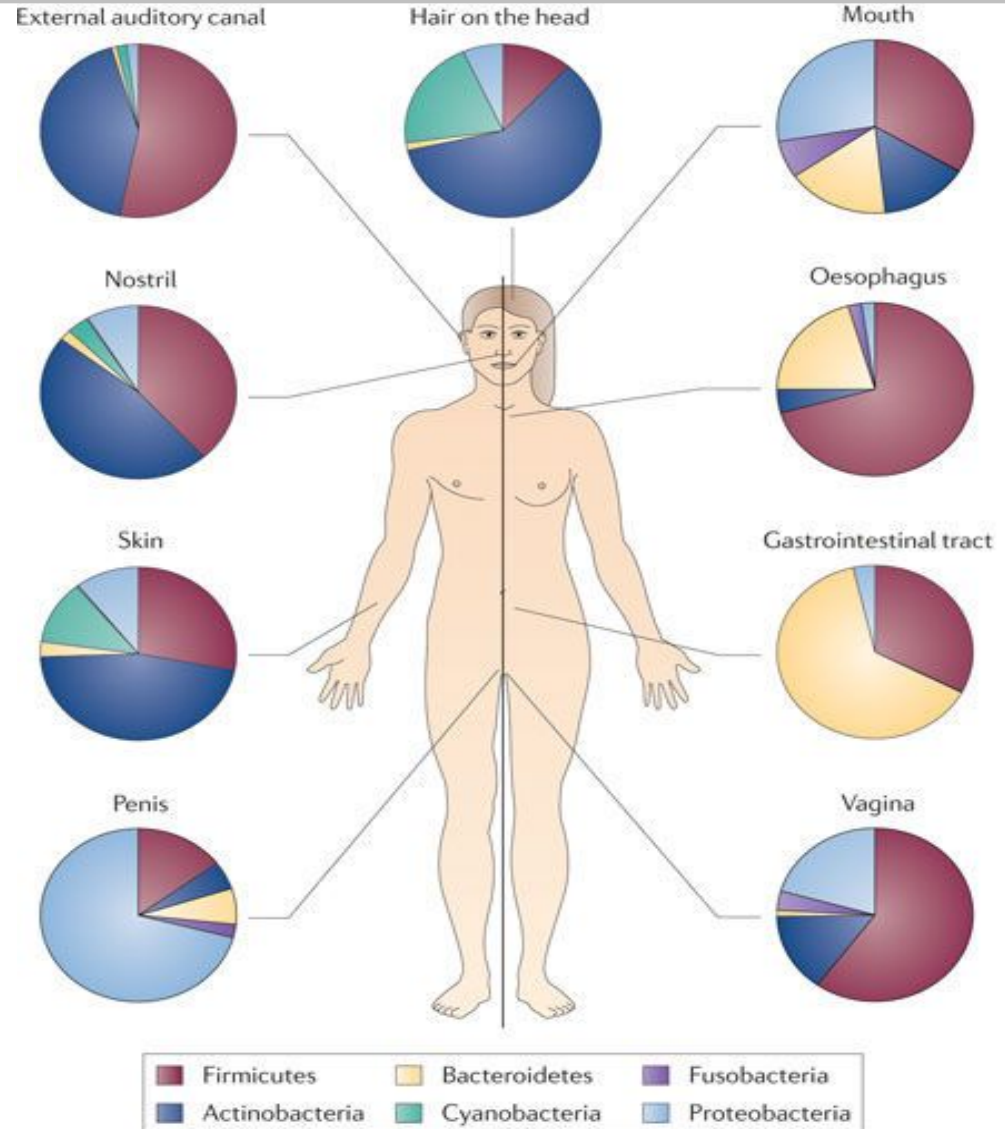
DRUG Metabolism

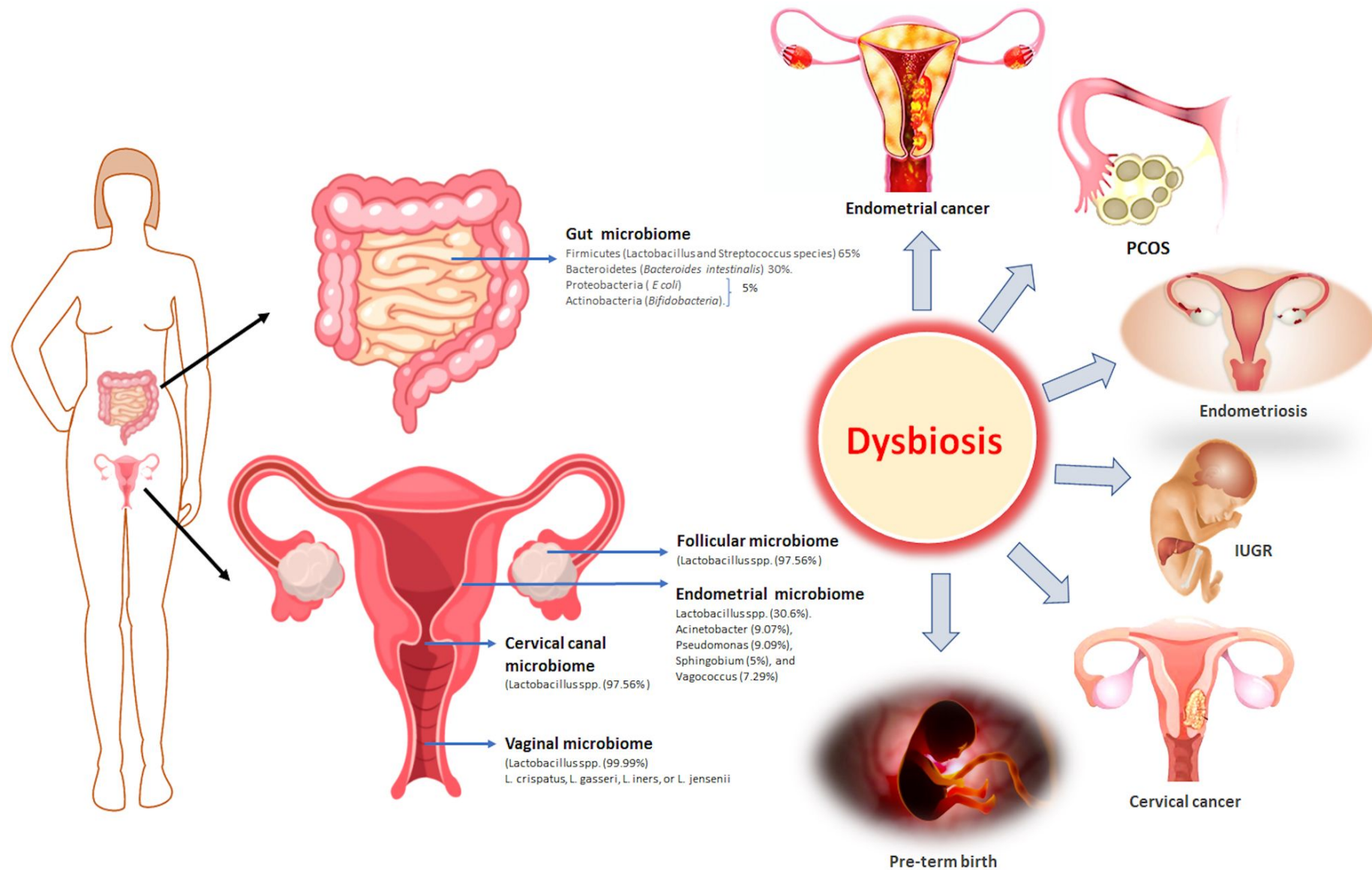
Metabolic Function

(Vitamin synthesis)

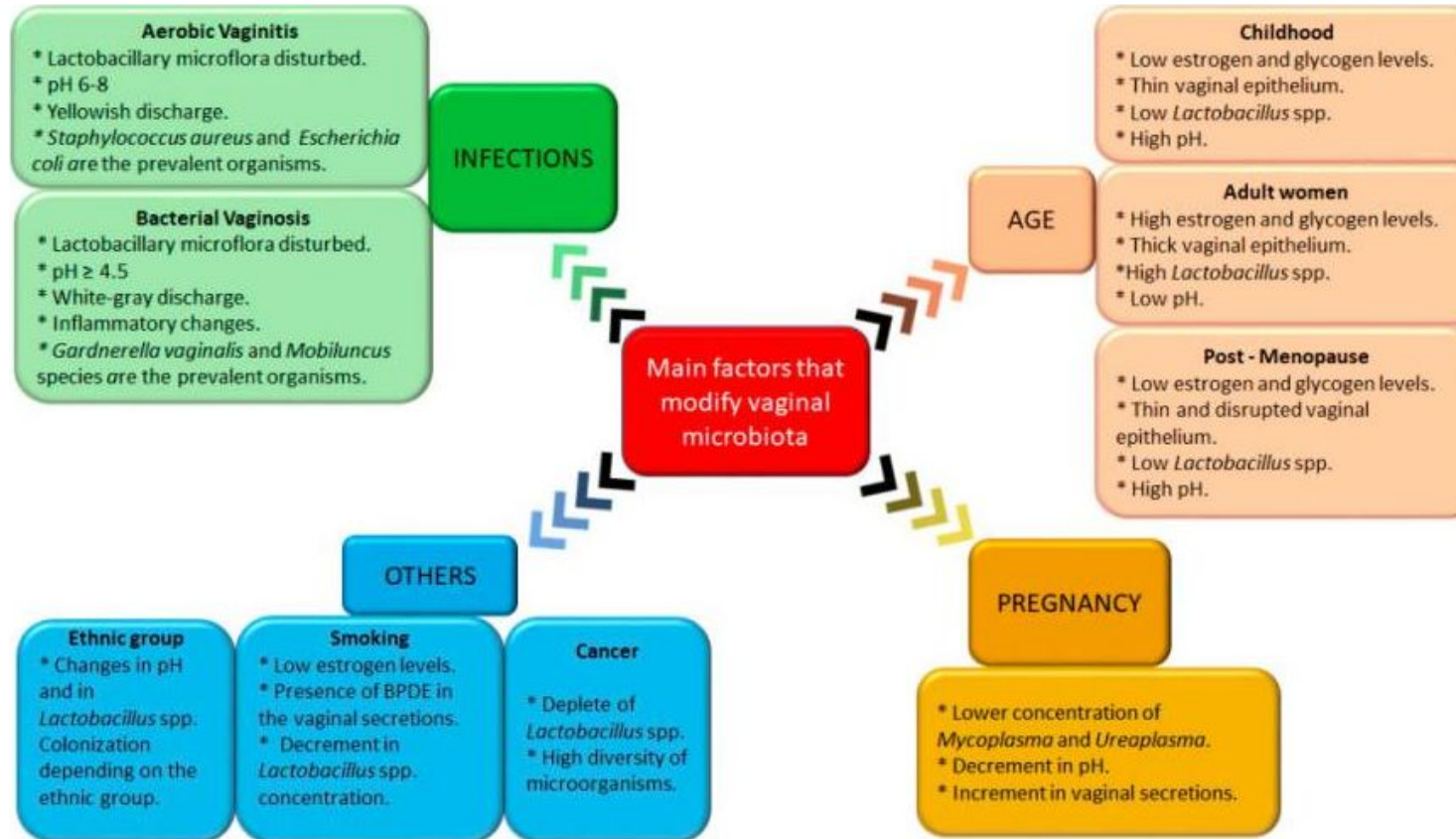
Immune System

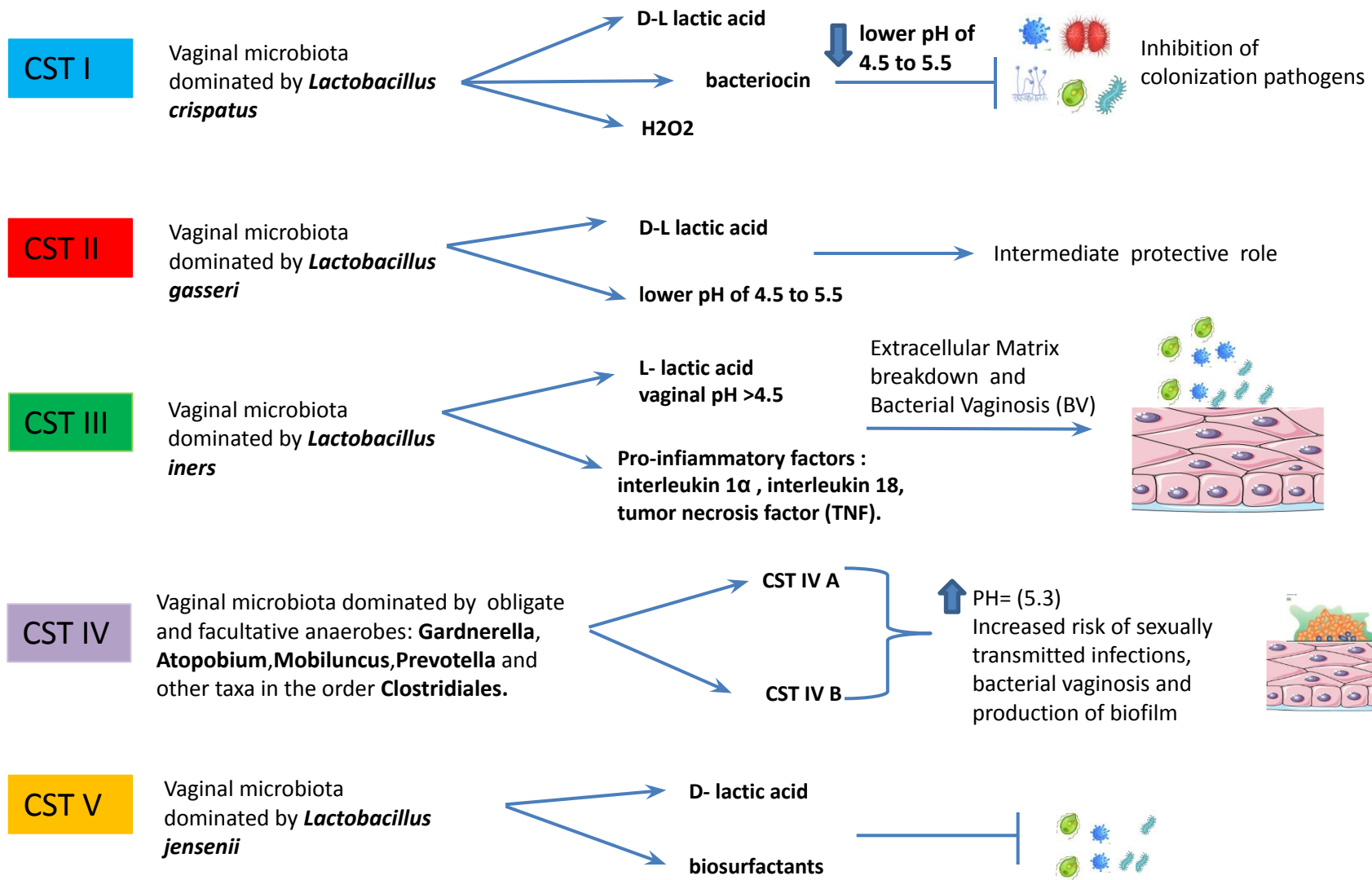
Identifying predictive bacterial markers from cervical swab microbiota on pregnancy outcome in woman undergoing assisted reproductive technologies





The factors that modify the vaginal microbiota





Identifying predictive bacterial markers from cervical swab microbiota on pregnancy outcome in woman undergoing assisted reproductive technologies



**L'epidemiologia per la sanità
pubblica**
Istituto Superiore di Sanità

- In 2019 about 99.062 ART procedures (Assisted Reproductive Technologies) II and III levels were performed in Italy with 14.162 live births
- CDC's 2019 Report 330.773 ART cycles performed in US resulting in 77.998 live births
- Success rate decrease from 24% (for patients with less than 35y/o) to 4,5% for patients with more than 43y/o

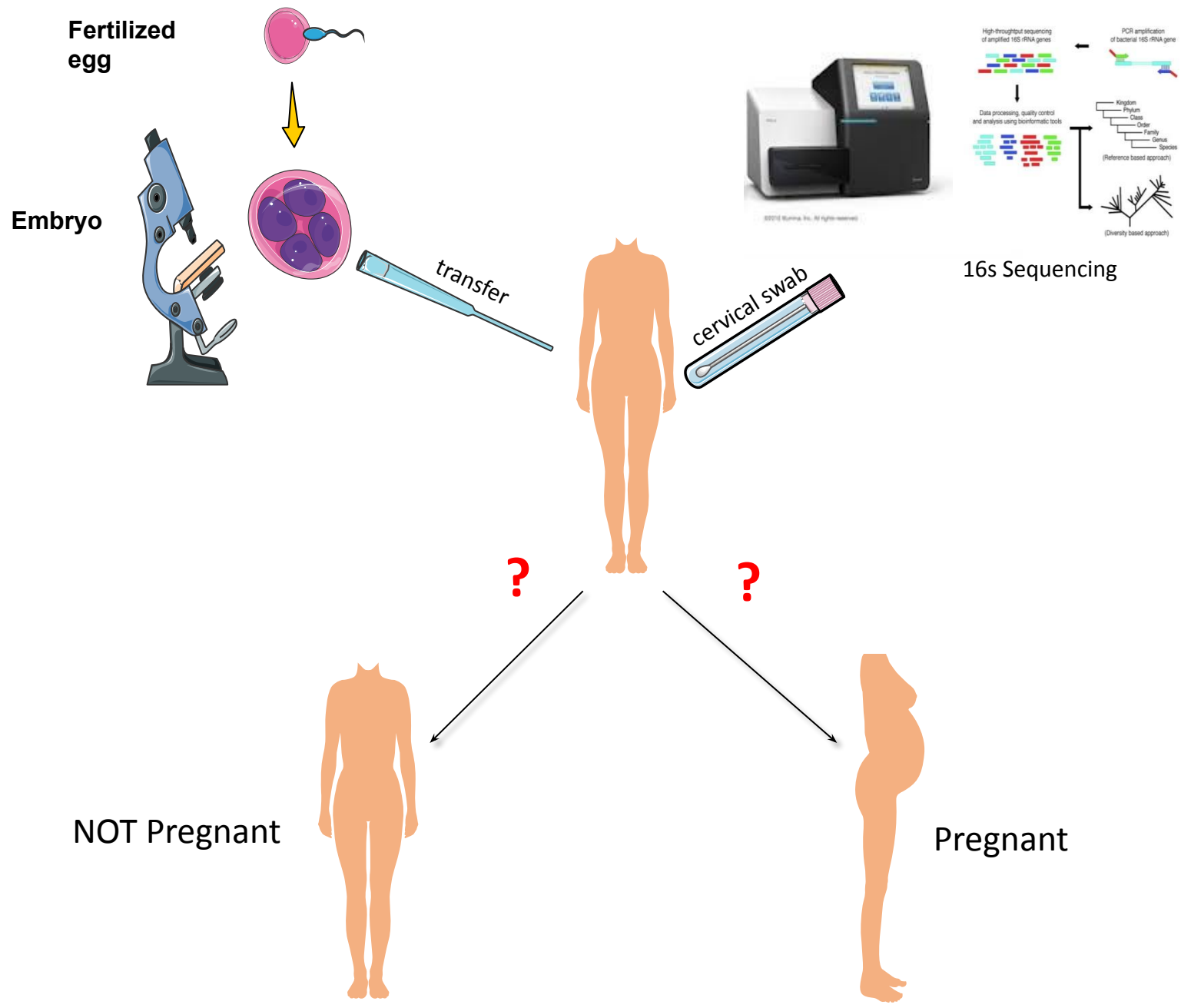


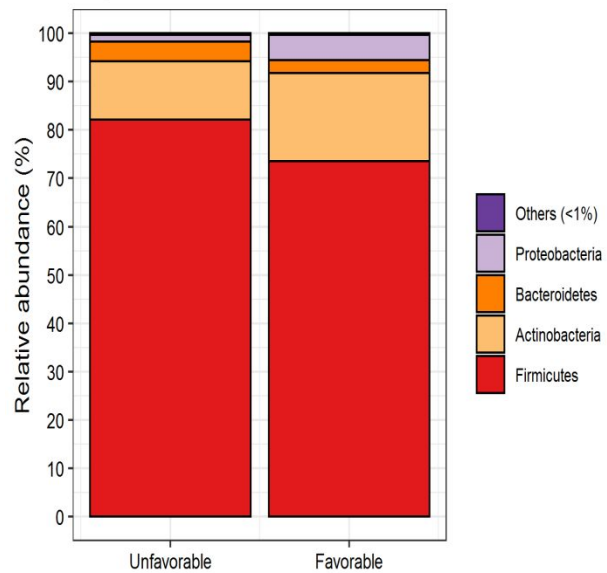
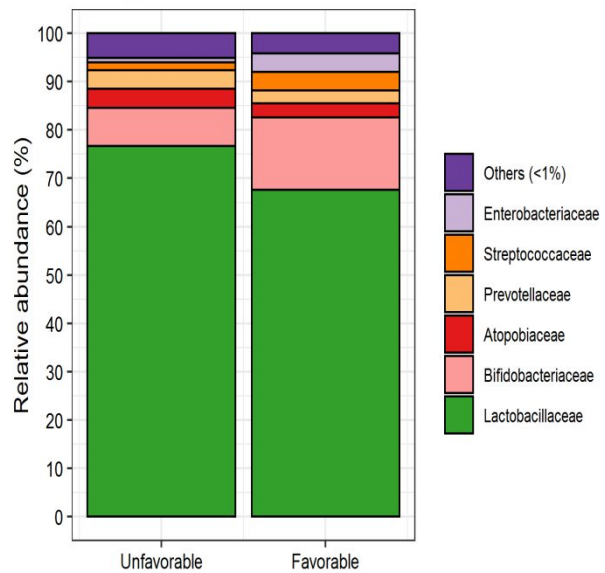
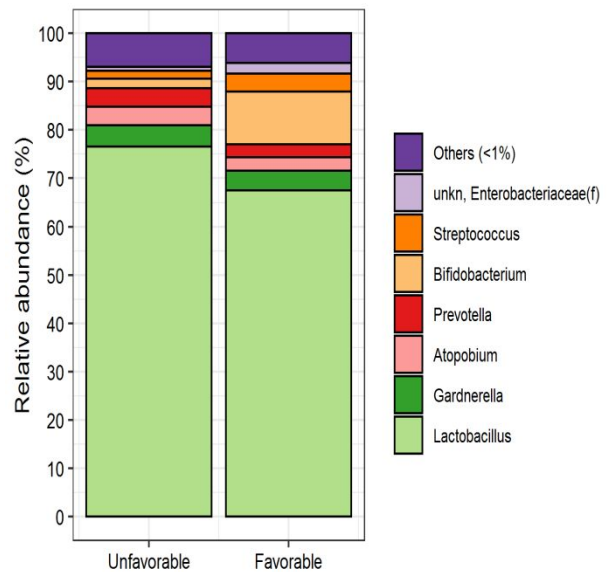
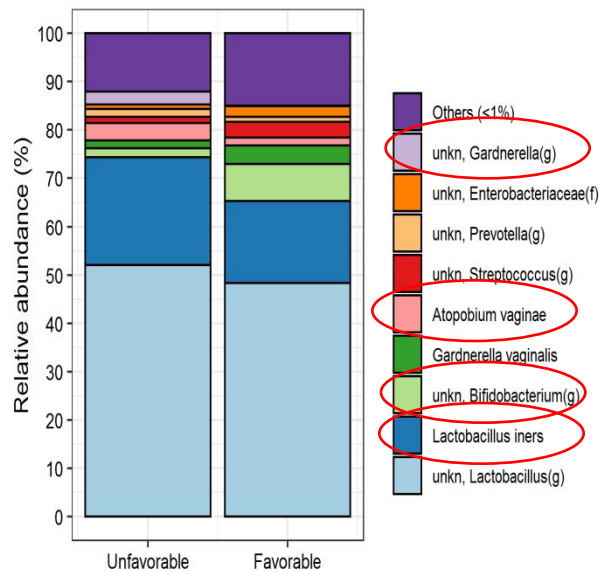
Table I. Demographic and clinical patients characteristics (overall and according to the pregnancy outcome)

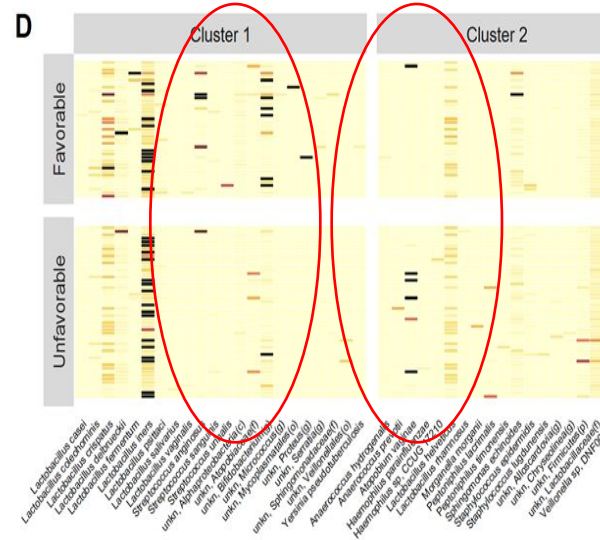
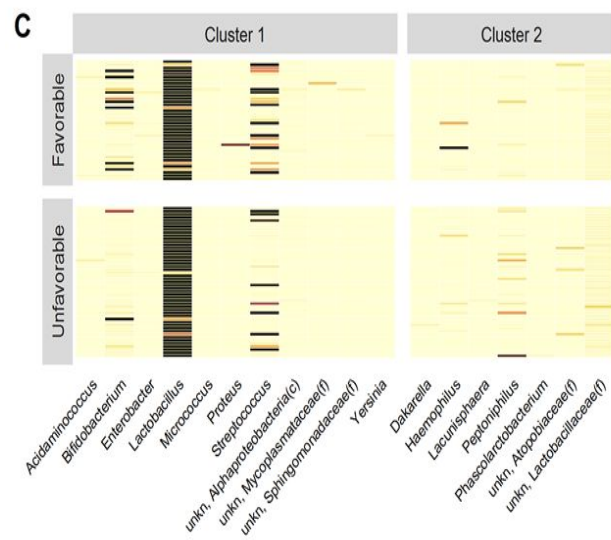
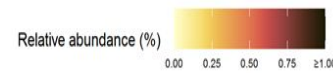
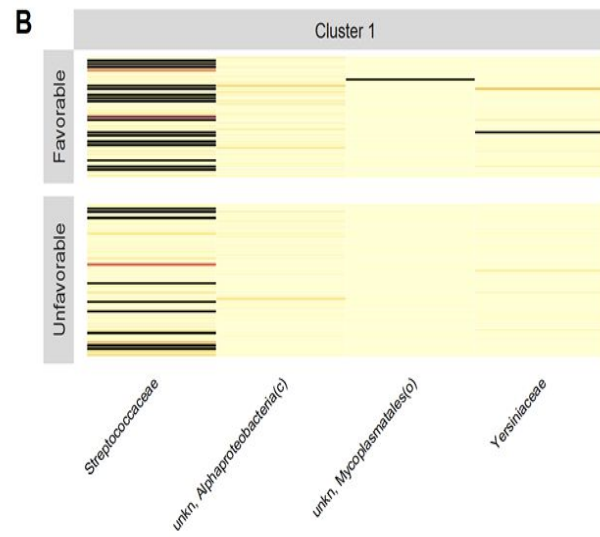
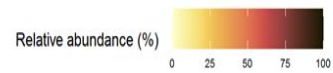
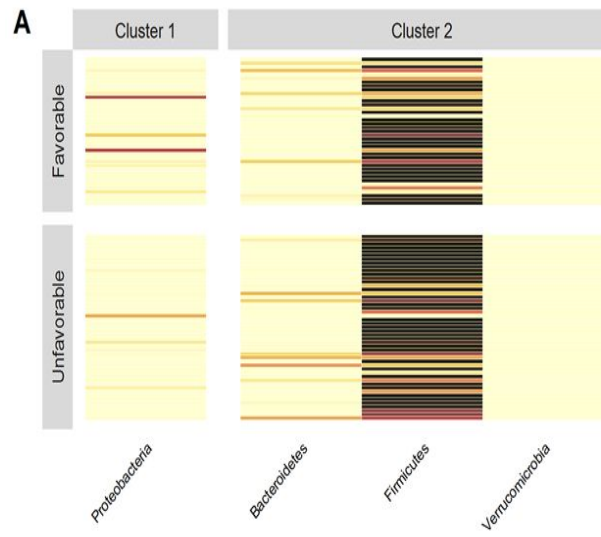
Variable	Category	All subjects (N=88)	Unfavorable (N=49)	Favorable (N=39)	p-value
Age (years)	Mean±SD	35.1±3.0	35.3±3.4	35.0±2.6	0.659*
	Median [IQR]	36 [33-37]	36 [32-38]	35 [33-37]	
	Range (min-max)	24-40	29-39	24-40	
BMI (Kg/m ²)	Mean±SD	22.1±3.2	22.2±3.1	22.0±3.4	0.770*
	Median [IQR]	21.5 [19.9-23.1]	21.6 [20-22.6]	21.3 [19.9-23.3]	
	Range (min-max)	16.1-32.8	16.1-32.5	17.6-32.8	
Infertility - N(%)	1=Male infertility	20 (22.7)	13 (26.5)	7 (17.9)	0.627 [#]
	2=Idiopathic	13 (14.8)	8 (16.3)	5 (12.8)	
	3=Low ovarian reserve	18 (20.5)	10 (20.4)	8 (20.5)	
	1+3=Male and Low ovarian reserve	3 (3.4)	1 (2.0)	2 (5.1)	
	4= Ovulatory endocrine	10 (11.4)	6 (12.2)	4 (10.3)	
	5=Endometriosis	3 (3.4)	2 (4.1)	1 (2.6)	
	6=Multifactorials	15 (17.0)	8 (16.3)	7 (17.9)	
7=Tubal infertility	6 (6.8)	1 (2.0)	5 (12.8)		
FSH - N(%)	1=Meropur	17 (19.3)	10 (20.4)	7 (17.9)	0.518 [#]
	2=Pergoveris	27 (30.7)	13 (26.5)	14 (35.9)	
	3=Bemfola	43 (48.9)	26 (53.1)	17 (43.6)	
	4=Meropur+Ovaleap	1 (1.1)	0 (0.0)	1 (2.6)	
Diet - N(%)	1=Mediterranean	74 (84.1)	42 (85.7)	32 (82.1)	0.862 [§]
	2=Vegetarian/Vegan	14 (15.9)	7 (14.3)	7 (17.9)	
Physical activity - N(%)	1=Low-intensity	16 (18.2)	7 (14.3)	9 (23.1)	0.675 [#]
	2=Moderate-intensity	64 (72.7)	37 (75.5)	27 (69.2)	
	3=High-intensity	8 (9.1)	5 (10.2)	3 (7.7)	
Smoking habits - N(%)	1=Smoker	24 (27.3)	12 (24.5)	12 (30.8)	0.677 [§]
	2=Non-smoker	64 (72.7)	37 (75.5)	27 (69.2)	
Drink habits - N(%)	1=Drinker	20 (22.7)	12 (24.5)	8 (20.5)	0.835 [§]
	2=Non-drinker	52 (59.1)	29 (59.2)	23 (59.0)	
	3=Occasional-drinker	16 (18.2)	8 (16.3)	8 (20.5)	
Sexual activity – N(%)	1= <1 at week	33 (37.5)	18 (36.7)	15 (38.5)	0.777 [§]
	2= 1-2 at week	41 (46.6)	22 (44.9)	19 (48.7)	
	3= >2 at week	14 (15.9)	9 (18.4)	5 (12.8)	

Exclusion criteria:

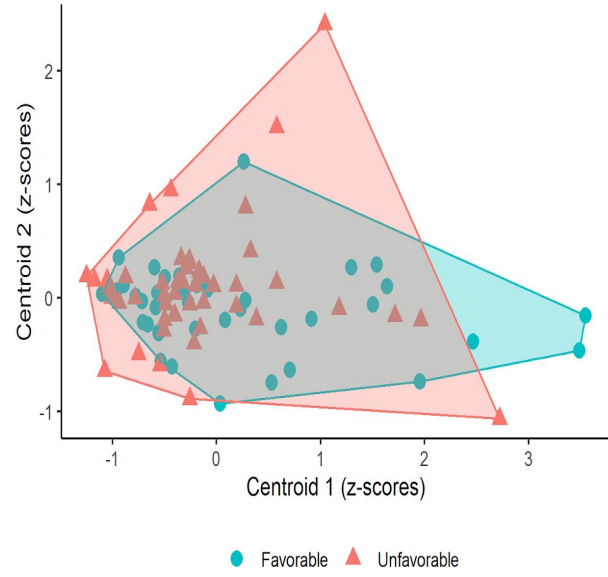
- ❖ vaginal infection and antibiotic use 30 days before ART protocol
- ❖ no previous pregnancy, previous history of pelvic inflammatory disease (PID)
- ❖ body mass index more than 30
- ❖ Preimplantation test (PGT) positive for genetic diseases

*p-value from two-sample test;
[§]p-value from Chi-Square test;
[#]p-value from Fisher exact test.
 Abbreviations: SD: Standard deviation

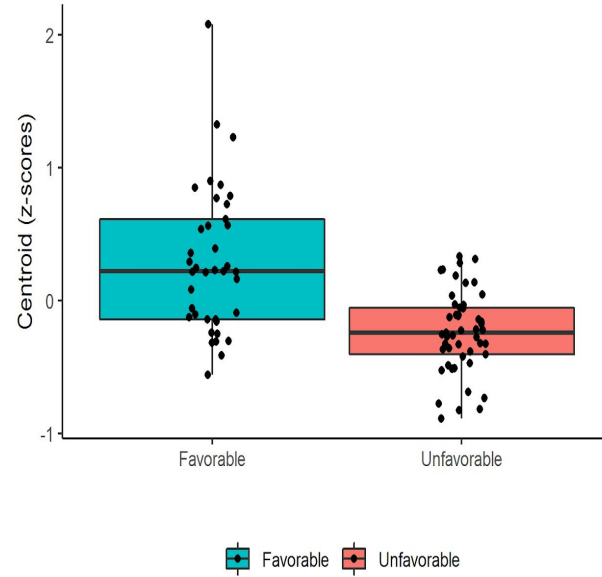
A Phylum**B** Family**C** Genus**D** Species



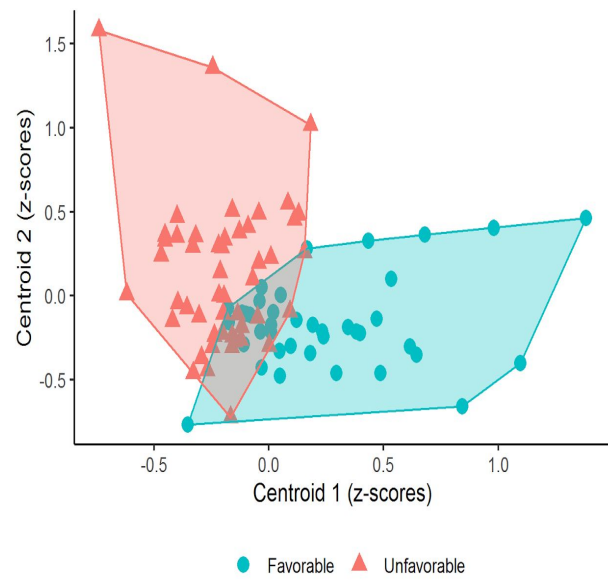
A Phylum



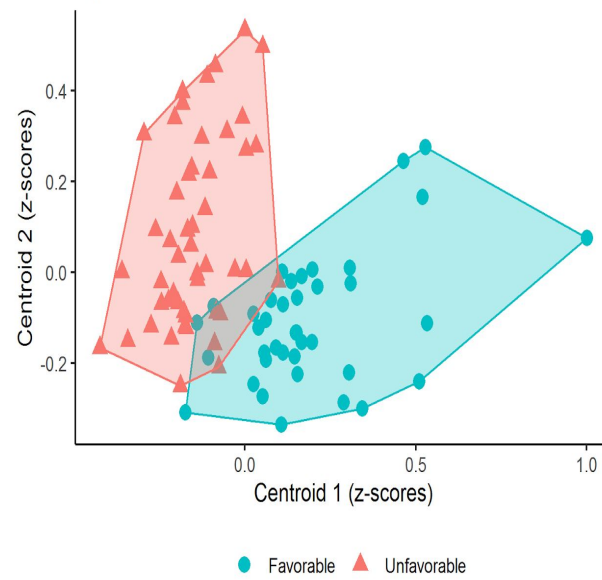
B Family



C Genus



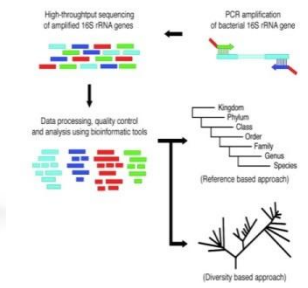
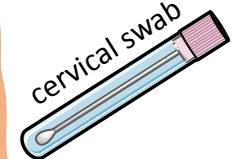
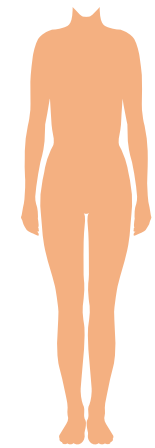
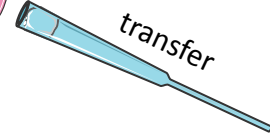
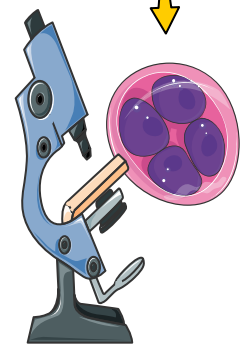
D Species



Fertilized egg



Embryo

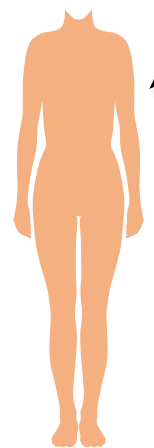


16s Sequencing



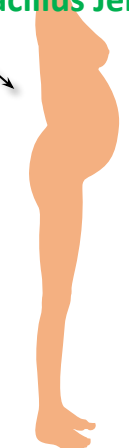
Atopobium vaginae
Lactobacillus iners
Peptoniphilus lacrimalis
Peptoniphilus timonensis
Gardnerella

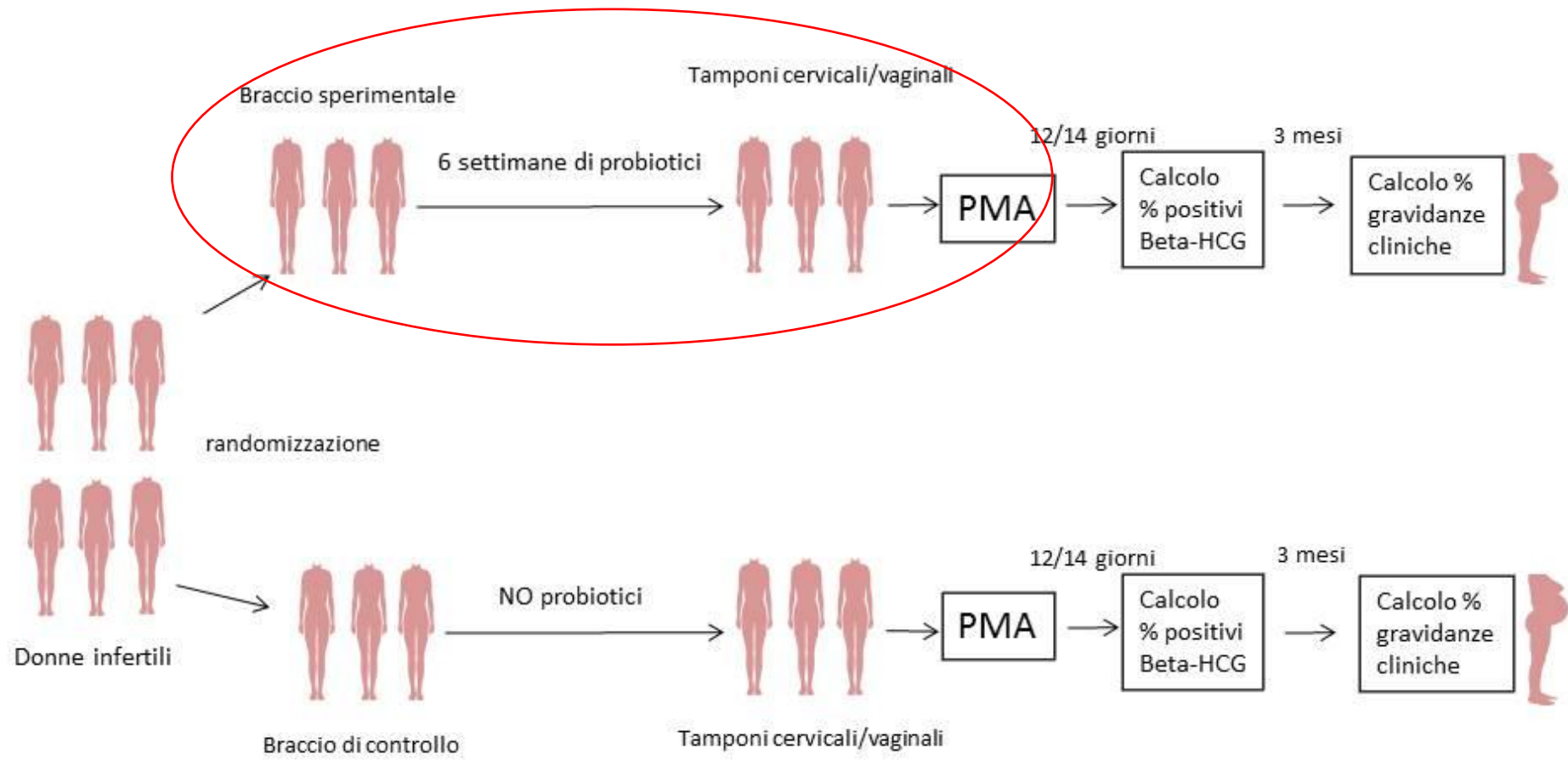
NOT Pregnant



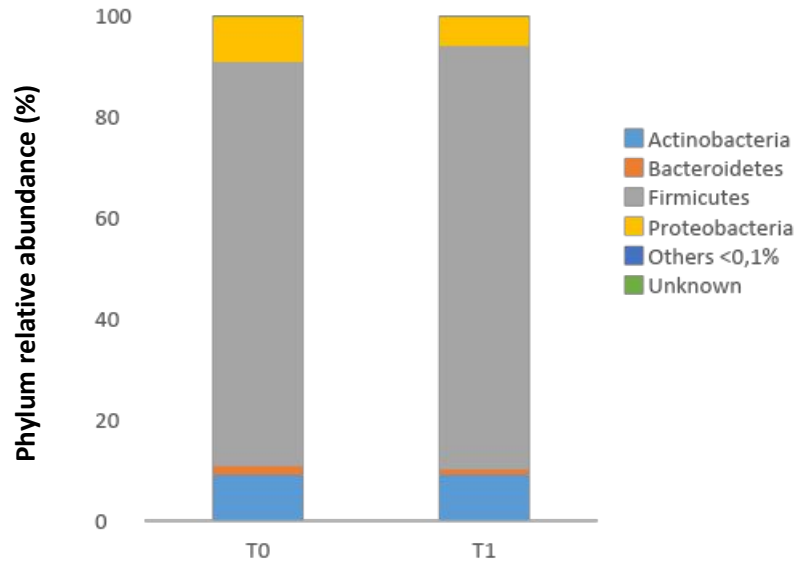
Bifidobacterium breve
Lactobacillus Crispatus
Lactobacillus casei
Lactobacillus acidophilus
Lactobacillus Gasseri
Lactobacillus Jensenii

Pregnant





SOSTANZE NUTRITIVE CON VALENZA FISIOLGICA Tenore giornaliero medio		
Componenti	Per Dose	*VNR%
FOS	1 g	---
Fibra di Acacia	1 g	---
Fermenti Lattici Probiotici	83 mg	
di cui:		
→ <i>L. gasseri</i> (SGL 09)	2,5 Mld UFC	---
→ <i>L. acidophilus</i> (SGL 11)	2,5 Mld UFC	
→ <i>L. casei</i> (SGL 15)	1,5 Mld UFC	
→ <i>B. breve</i> (SGB 01)	2,5 Mld UFC	
→ <i>L. crispatus</i> (6272)	1,5 Mld UFC	



MEAN	T0 (%)	T1 (%)
Actinobacteria	9,09803	9,082657273
Bacteroidetes	1,64165	1,043468182
Firmicutes	80,0867	83,89149273
Proteobacteria	8,84362	5,798191818
Others <0,1%	0,0505	0,009325455
Unknown	0,27949	0,174871818



T1 vs T0 (used as reference)

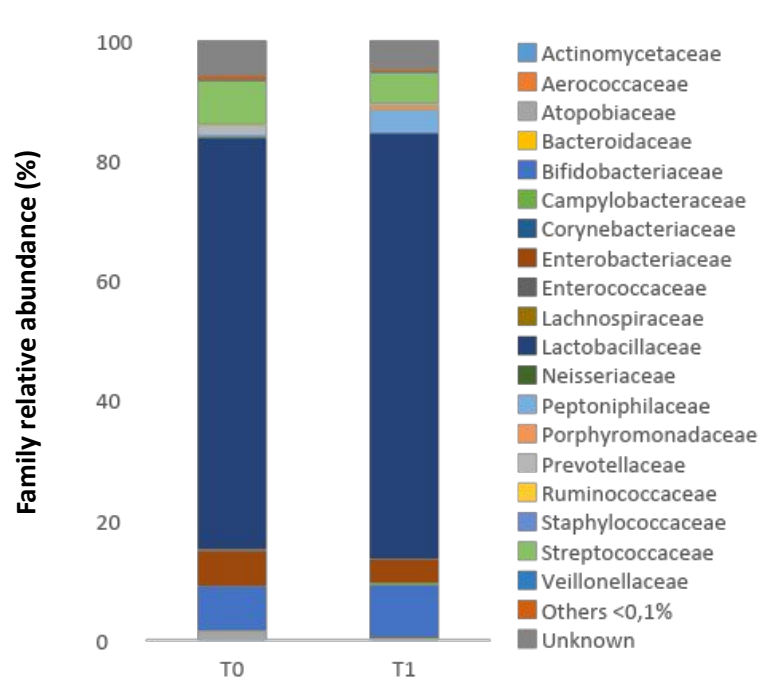
UP-represented in T1 vs T0

OTUs	logFC.deseq2	P-value
Bacteria;Candidatus Saccharibacteria	5,068353453	0,0072128
Bacteria;Candidatus Parcubacteria	4,805278187	0,009581312
Bacteria;Chloroflexi	3,78160048	0,032924866

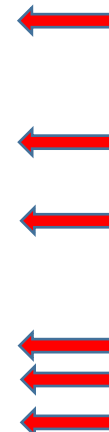
DOWN-represented in T1 vs T0

OTUs	logFC.deseq2	P-value
Bacteria;Fusobacteria	-2,990697207	0,009674589
Bacteria;Tenericutes	-3,827800185	0,011053603



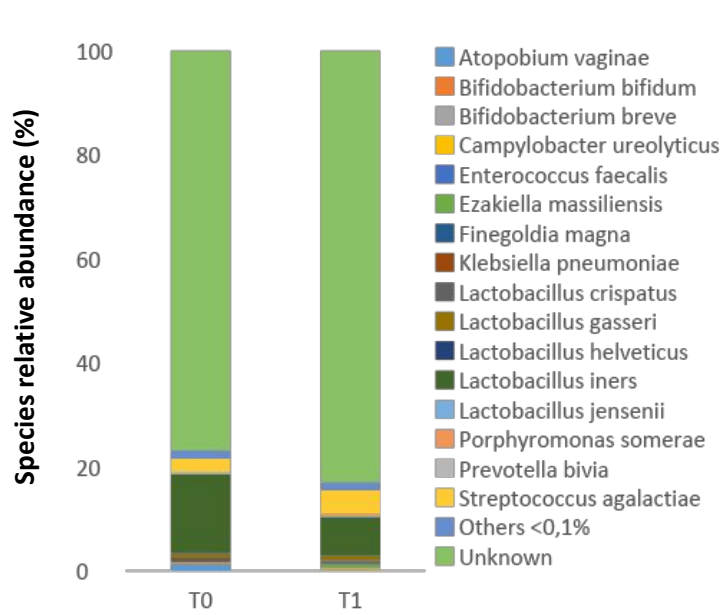


MEAN	T0 (%)	T1 (%)
Actinomycetaceae	0,107813	0,111468
Aerococcaceae	0,023535	0,125655
Atopobiaceae	1,42413	0,002985
Bacteroidaceae	0,080193	0,152699
Bifidobacteriaceae	7,305517	8,841122
Streptococcaceae	7,191028	5,002505
Corynebacteriaceae	0,134075	0,038712
Enterobacteriaceae	5,795551	3,876314
Enterococcaceae	0,179869	0,022365
Lachnospiraceae	0,118205	0,057599
Lactobacillaceae	68,58744	70,99918
Neisseriaceae	0,205879	0,000537
Prevotellaceae	1,497615	0,153896
Ruminococcaceae	0,087884	0,220402
Veillonellaceae	0,177681	0,119548
Others <0,1%	0,724951	0,364722
Unknown	5,873831	4,883475



DOWN-represented in T1 vs T0

OTUs	logFC.deseq2	P-value
Bacteria;Proteobacteria;Gammaproteobacteria;Pasteurellales;Pasteurellaceae	-5,123472916	1,19985E-05
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae	-3,082336364	4,58918E-05
Bacteria;Fusobacteria;Fusobacteriia;Fusobacteriales;Fusobacteriaceae	-4,174526838	0,000197416
Bacteria;Firmicutes;Clostridia;Clostridiales;Eubacteriaceae	-2,739425068	0,00225138
Bacteria;Actinobacteria;Actinobacteria;Corynebacteriales;Corynebacteriaceae	-2,81840038	0,002332479
Bacteria;Proteobacteria;Betaproteobacteria;Neisseriales;Neisseriaceae	-4,334729961	0,003963436
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae	-2,056958515	0,006606042
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Morganellaceae	-2,967802302	0,007214022
Bacteria;Firmicutes;Negativicutes;Selenomonadales;Selenomonadaceae	-2,064737637	0,010848609
Bacteria;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae	-2,058888356	0,026113141
Bacteria;Actinobacteria;unkn. Actinobacteria(p);unkn. Actinobacteria(p);unkn. Actinobacteria(p)	-2,14348444	0,033571625
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae	-1,368710664	0,037486215
Bacteria;Actinobacteria;Coriobacteriia;Coriobacteriales;Atopobiaceae	-1,839602528	0,041523527
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;unkn. Bacteroidales(o)	-1,165786081	0,043539806



MEAN	T0	T1
Atopobium vaginae	1,222808	0 ←
Bifidobacterium breve	0,243366	0,294763 ←
Enterococcus faecalis	0,109726	0,001517
Klebsiella pneumoniae	0,31551	0,204135
Lactobacillus gasseri	0,743521	0,773395
Lactobacillus iners	15,1045	7,360495 ←
Lactobacillus jensenii	0,031899	0,172225 ←
Prevotella bivia	0,446223	0,006175 ←
Others <0,1%	1,594583	1,436623
Unknown	76,89914	83,02902

DOWN-represented in T1 vs T0

OTUs	logFC.deseq2	P-value
Bacteria;Actinobacteria;Actinobacteria;Corynebacteriales;Corynebacteriaceae;Corynebacterium;Corynebacterium pyruviciproducens	-23,00242531	4,69982E-21
Bacteria;Firmicutes;Bacilli;Bacillales;unkn. Bacillales(o);Gemella;unkn. Gemella(g)	-8,007258913	1,15522E-05
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;Prevotella sp. HGH0351	-7,049853076	1,61649E-05
Bacteria;Firmicutes;Negativicutes;Veillonellales;Veillonellaceae;Veillonella;unkn. Veillonella(g)	-4,99984903	3,84645E-05
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;unkn. Prevotella(g)	-2,993794503	0,002004758
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;Prevotella bivia	-5,328824428	0,002204886
Bacteria;Fusobacteria;Fusobacteriia;Fusobacteriales;Fusobacteriaceae;Fusobacterium;unkn. Fusobacterium(g)	-4,414936293	0,002382154
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;unkn. Streptococcus(g)	-2,777828684	0,003575318
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Actinomycetaceae;Actinomyces;Actinomyces neuii	-7,569727482	0,003973844
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;Streptococcus pneumoniae	-5,858041474	0,006000633
Bacteria;Proteobacteria;Betaproteobacteria;Neisseriales;Neisseriaceae;Neisseria;unkn. Neisseria(g)	-5,609007753	0,007947454
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;unkn. Prevotellaceae(f);unkn. Prevotellaceae(f)	-2,472907431	0,013515525
Bacteria;Firmicutes;Negativicutes;Veillonellales;Veillonellaceae;Negativicoccus;unkn. Negativicoccus(g)	-2,801044143	0,014986211
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Roseburia;unkn. Roseburia(g)	-3,041358963	0,019839601
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Butyrivibrio;unkn. Butyrivibrio(g)	-3,426427967	0,025073937
Bacteria;Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus;Enterococcus faecalis	-3,571719302	0,027608887
Bacteria;Firmicutes;Bacilli;Bacillales;Staphylococcaceae;Staphylococcus;Staphylococcus hominis	-4,397112024	0,035875632
Bacteria;Actinobacteria;Coriobacteriia;Coriobacteriales;Atopobiaceae;Atopobium;unkn. Atopobium(g)	-2,842279693	0,037519779

CONCLUSION AND PERSPECTIVES

- PERFORMING VAGINAL MICROBIOTA PROFILE BEFORE ART PROCEDURE COULD BE USEFUL TO ASSESS TREATMENTS
- IT'S **NOT** ALL ABOUT *L. CRISPATUS*...A consortium of specific lactobacilli is necessary
- CLINICAL TRIALS TO ASSESS THE ABILITY OF PRECISION PROBIOTICS FORMULATION TO INCREASE THE SUCCESS RATE ARE UNDERGOING
- NEW INTEGRATIVE APPROACHES ARE URGENTLY NEEDED IN ORDER TO INCREASE THE SUCCESS RATE OF ART PROCEDURES
- PROBIOTICS COMSUPTION MUST BE ALWAYS PERSONALIZED BASED ON THE PATIENTS' NEEDS IN ORDER TO ACHIEVE REAL BENEFITS. The use of AI tools are suggested (pharmabiotix.it/questionario).

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