



UNIVERSITÀ
DEGLI STUDI DI TRIESTE

VERSO UN PROTOCOLLO MICROBIOLOGICO NELLA PMA:

IL PARERE DEL GINECOLOGO

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ISTITUTO DI RICOVERO E CURA
a carattere scientifico
Burlo Garofolo di Trieste

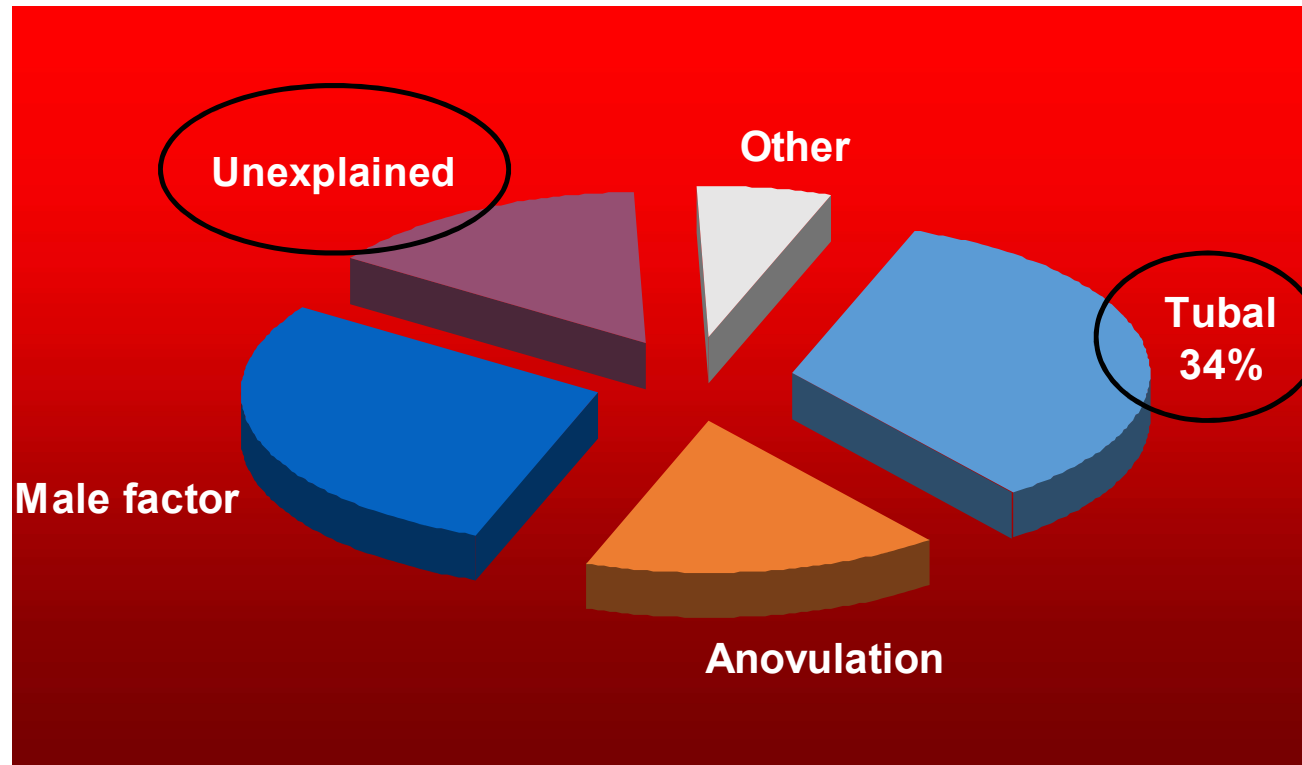


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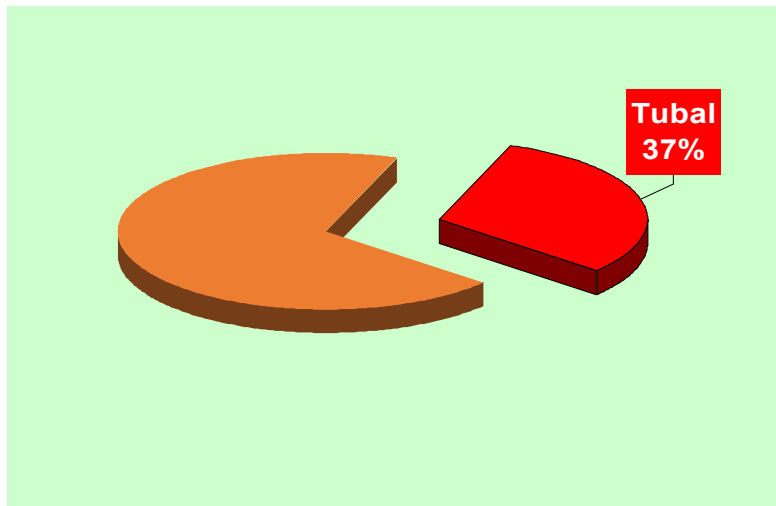
11 - 14 Novembre 2017

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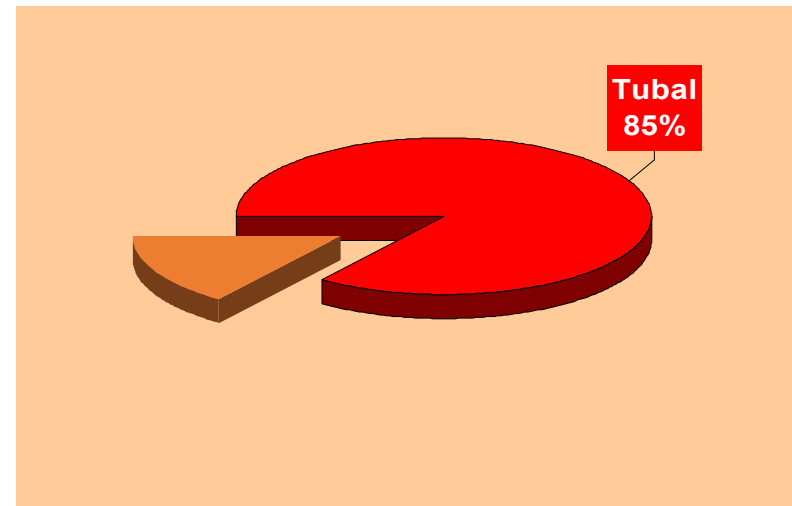
Eziologia dell'infertilità di coppia



Eziologia dell'infertilità di coppia



Developed countries

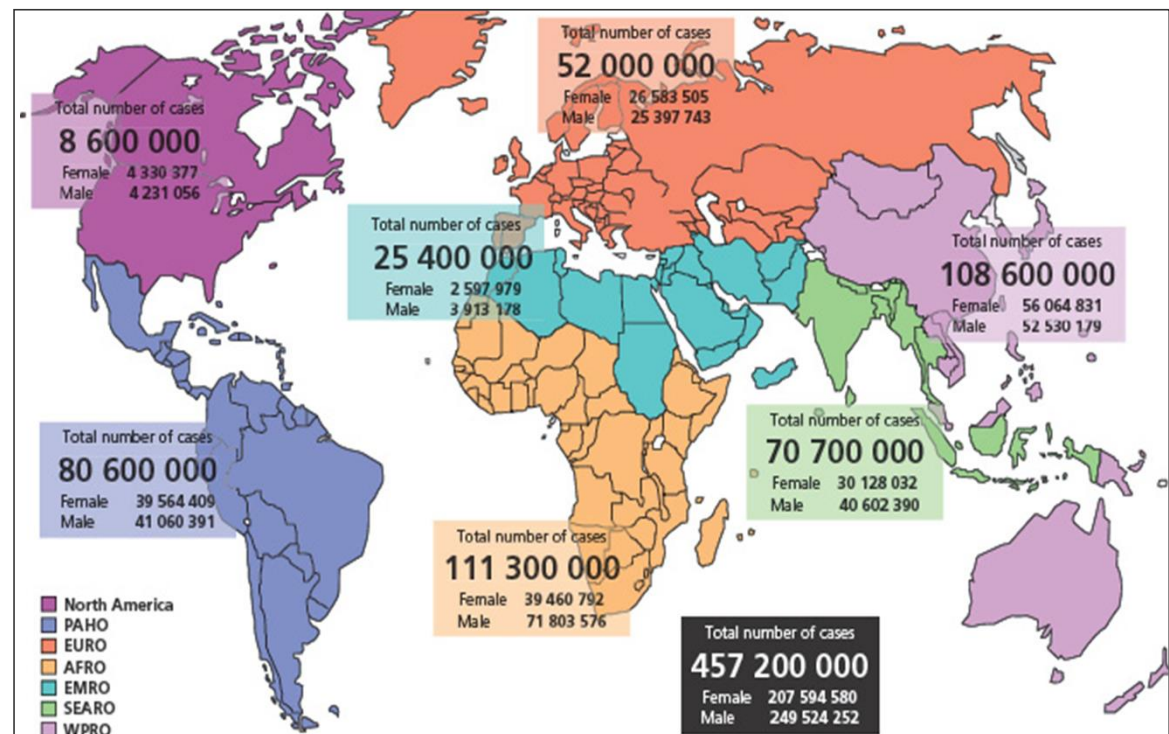


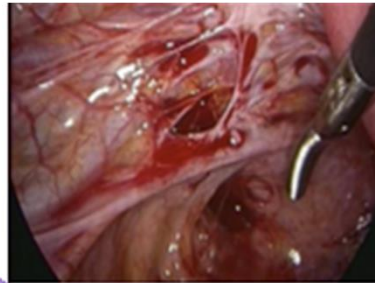
Developing countries

(WHO)

Sexual Transmitted Infections

- 340 milioni casi/aa
- 111 milioni/anno < 25 aa.
- Paesi piu' poveri: 85% degli adolescenti
- Clamidia, Gonococco, HSV, HPV





In addition to *N gonorrhoeae* and *C trachomatis*, organisms involved in PID include:

- *Mycoplasma hominis*
- *Mycoplasma genitalium*
- *Ureaplasma urealyticum*
- *Gardnerella vaginalis*
- Herpes simplex virus 2 (HSV-2)
- *Trichomonas vaginalis*

De Barberyrac, 2007
Honey, 2002
Kobayashi, 2006

Mycoplasma genitalium in Women: Current Knowledge and Research Priorities for This Recently Emerged Pathogen

Harold C. Wiesenfeld¹ and Lisa E. Manhart²

recognized microbial causes. Emerging data demonstrate an association between *M. genitalium* and PID, and limited data suggest associations with infertility and preterm birth, yet the attributable risk for female genital tract infections remains to be defined. Further investigations are needed to better define the impact of *M. genitalium* on women's reproductive health. Importantly, prospective studies evaluating whether screening programs and targeted treatment of *M. genitalium* improve reproductive outcomes in women are necessary to guide public health policy for this emerging pathogen.

Mycoplasma genitalium and infertility

- In 1981 it was first isolated from men with nongonococcal urethritis.
- Tubal factor infertility has been observed, independent of past chlamydial infection
- Subclinical or unrecognized PID
- The damage caused by *M. genitalium* tends to be moderate. However, when left untreated, damage may accumulate and yield serious long-term sequelae on fallopian tube function

Prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* in women undergoing an initial infertility evaluation

- The effect of the presence mycoplasmas in the genitourinary tract on the unexplained infertility remains still controversial
- For women undergoing IVF is still unclear whether or not test for these microorganisms or to prescribe antibiotics to colonised women

Sleha R et al., 2016

Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis



Noortje van Oostrum¹, Petra De Sutter¹, Joris Meys², and Hans Verstraelen^{1,*}

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MAIN RESULTS AND THE ROLE OF CHANCE: The estimated prevalence of BV (Nugent score >6) in infertile women is 19% [95% confidence interval (CI): 14–25%]. Abnormal microflora including BV and intermediate microflora (Nugent scores 4–10) occurs in 39% of the infertile patients (95% CI: 26–52%). BV is significantly more prevalent in women with infertility compared with antenatal women in the same population [OR (odds ratio) 3.32, 95% CI 1.53–7.20].

BV is significantly more prevalent in women with tubal infertility compared with women with other causes of infertility (OR 2.77, 95% CI 1.62–4.75). BV is not associated with decreased conception rates (OR 1.03, 95% CI 0.79–1.33). Similarly, none of the studies found an association between abnormal vaginal flora and conception rates following IVF treatment.

BV is associated with a significantly elevated risk of preclinical pregnancy loss (OR 2.36, 95% CI: 1.24–4.51). BV is not associated with an increased risk of first trimester miscarriage (OR 1.20, 95%CI: 0.53–2.75).

Review

What fertility specialists should know about the vaginal microbiome: a review

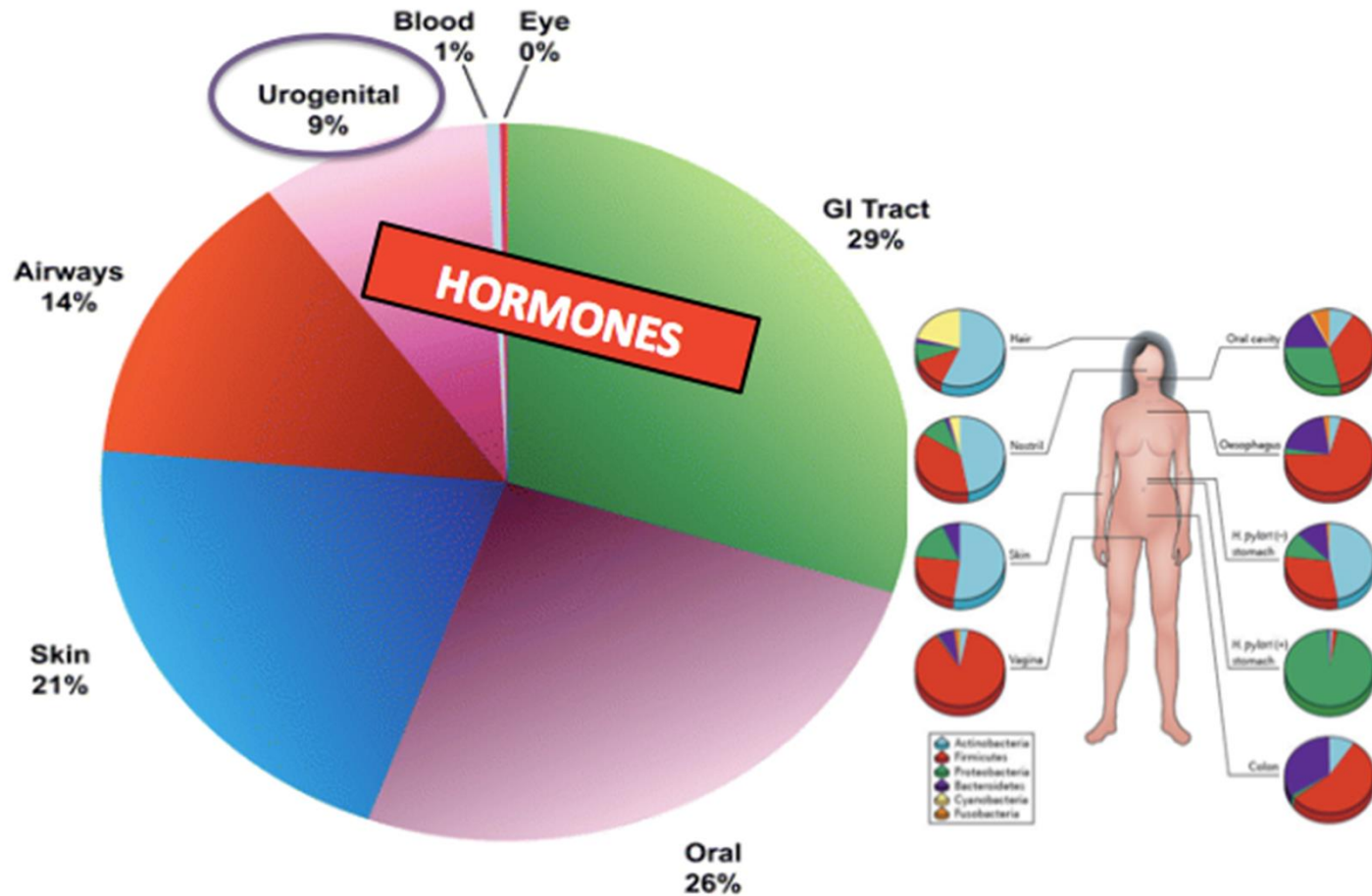
**Juan Antonio García-Velasco ^a, Marco Menabrito ^{b,*},
Isidoro Bruna Catalán ^c**

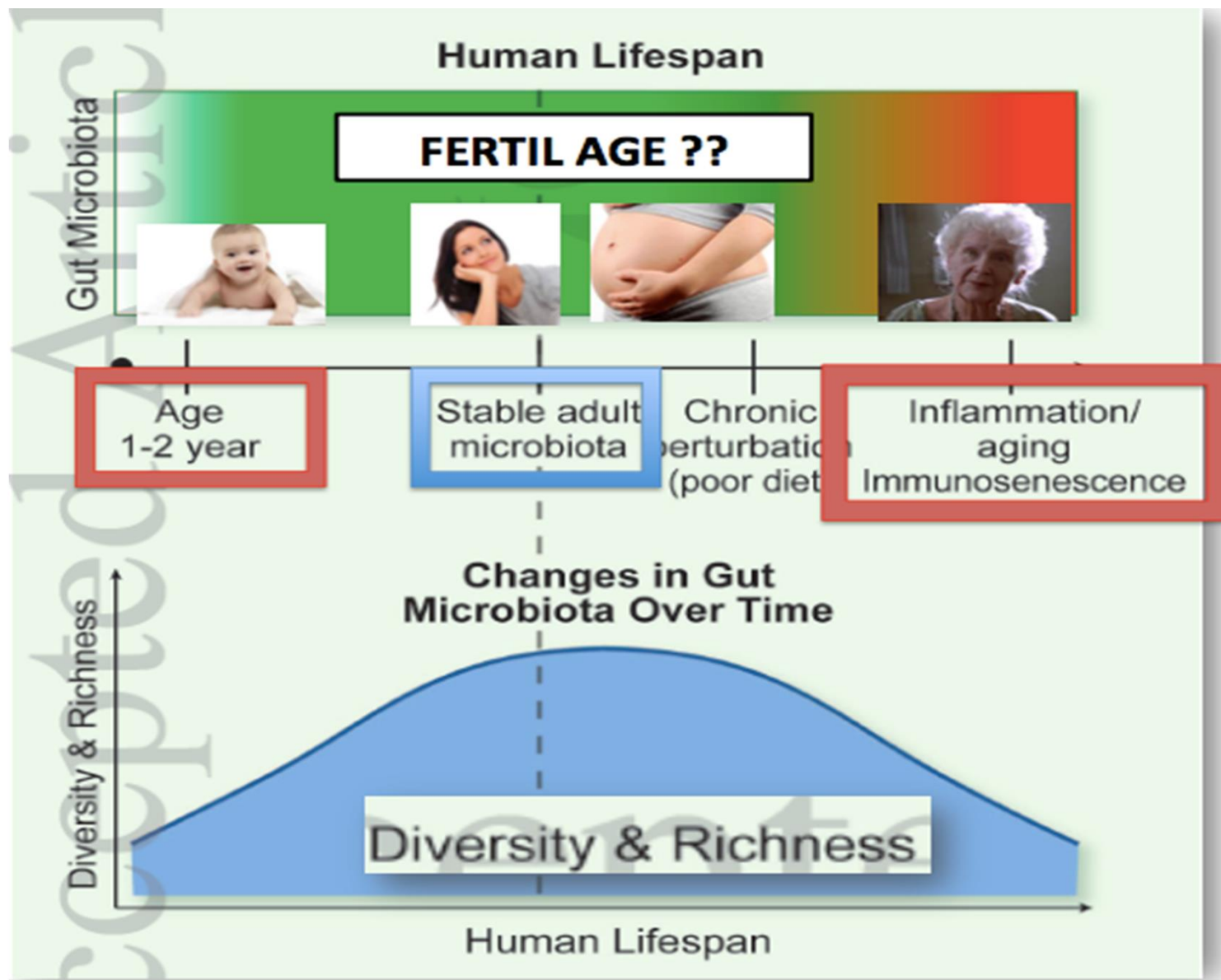
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host. Disturbances in the composition of bacterial communities have been shown to contribute to various disease states, and there is a growing body of evidence that the vaginal microbiota, which is unique to each woman, plays an important role in determining many facets of reproductive health.

The human vaginal microbiota seem to play a key role in preventing a number of urogenital diseases, such as bacterial vaginosis, yeast infections, sexually transmitted infections, urinary tract infections (2–9), and HIV infection (10, 11). Common wisdom attributes this to lactic acid-producing bacteria, mainly *Lactobacillus* sp., that commonly inhabit the vagina.

Bacteria distribution and gender



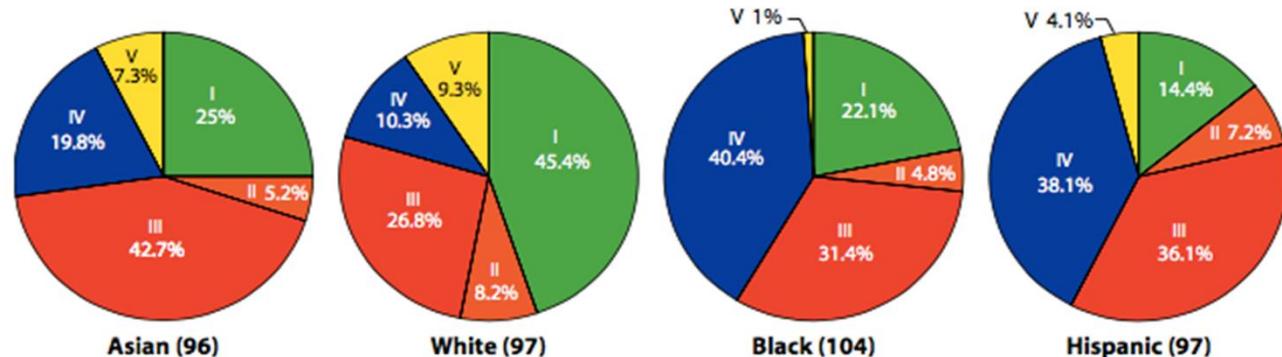


Vaginal microbiome of reproductive-age women

Jacques Ravel^{a,1}, Pawel Gajer^a, Zaid Abdo^b, G. Maria Schneider^c, Sara S. K. Koenig^a, Stacey L. McCulle^a, Shara Karlebach^d, Reshma Gorle^e, Jennifer Russell^f, Carol O. Tacket^f, Rebecca M. Brotman^a, Catherine C. Davis^g, Kevin Ault^d, Ligia Peralta^e, and Larry J. Forney^{c,1}

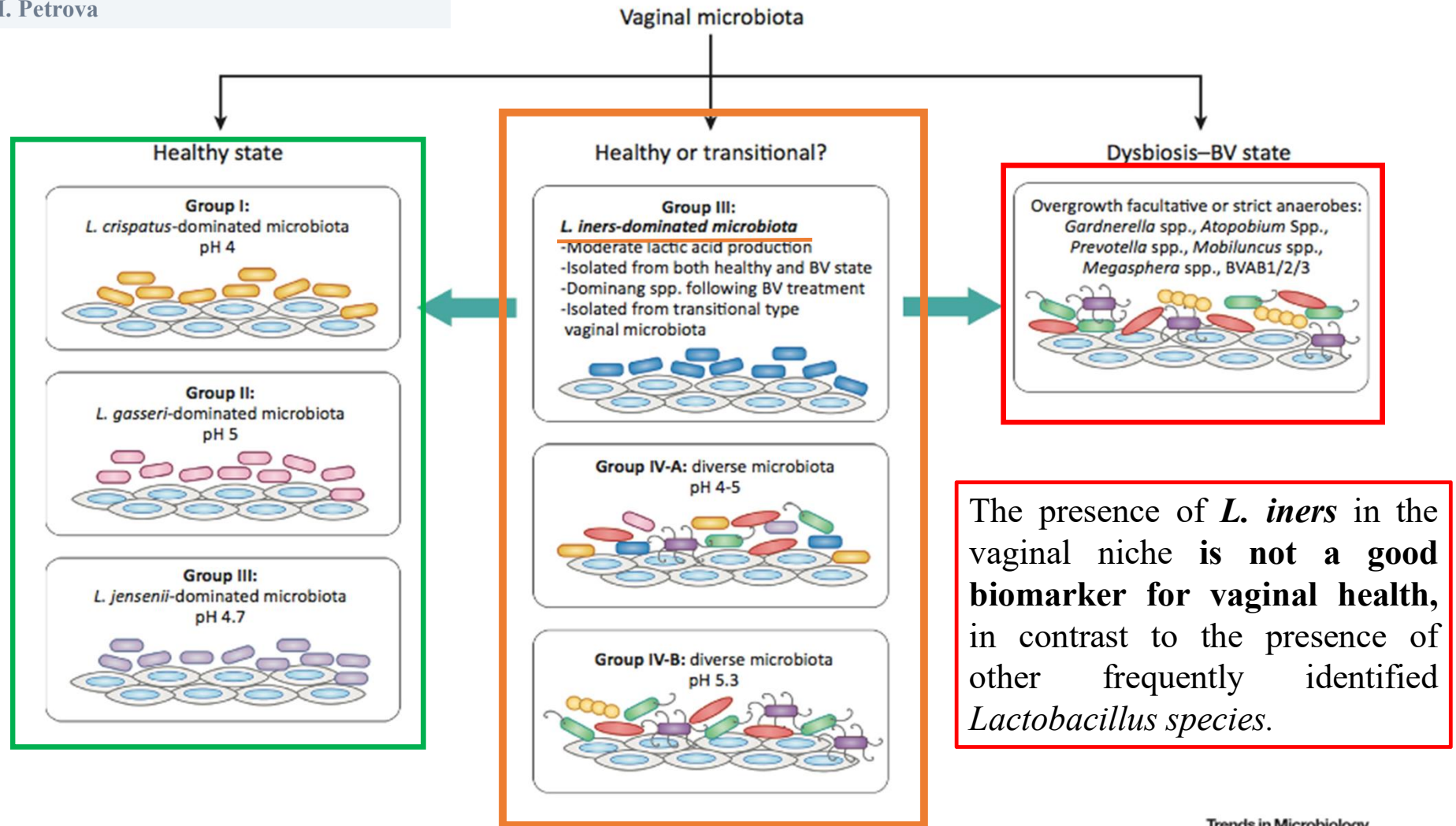
Table 1. pH of vaginal community groups in women of different ethnicities

Ethnic groups	Community groups*											
	I (<i>L. crispatus</i>)		II (<i>L. gasseri</i>)		III (<i>L. iners</i>)		IV (Diversity group)		V (<i>L. jensenii</i>)		All groups	
	Subjects [†]	pH [‡]	Subjects [†]	pH [‡]	Subjects [†]	pH [‡]	Subjects [†]	pH [‡]	Subjects [†]	pH [‡]	Subjects [†]	pH [‡]
Asian	24	4.4 ± 0.52	5	4.4 ± 0.44	41	4.0 ± 0.0	19	5.5 ± 0.44	7	5.0 ± 0.89	96	4.4 ± 0.59
White	44	4.0 ± 0.0	8	4.7 ± 0.44	26	4.3 ± 0.30	10	5.5 ± 0.74	9	4.85 ± 0.22	97	4.2 ± 0.30
Black	23	4.0 ± 0.0	5	5.0 ± 0.0	33	4.0 ± 0.0	42	5.3 ± 0.44	1	4.7 ± 0.44	104	4.7 ± 1.04
Hispanic	14	4.0 ± 0.0	7	4.7 ± 0.22	35	4.4 ± 0.59	37	5.3 ± 0.44	4	5.0 ± 0.59	97	5.0 ± 0.74
All ethnic groups	105	4.0 ± 0.3	25	5.0 ± 0.7	135	4.4 ± 0.6	108	5.3 ± 0.6	21	4.7 ± 0.4	394	4.4 ± 0.7

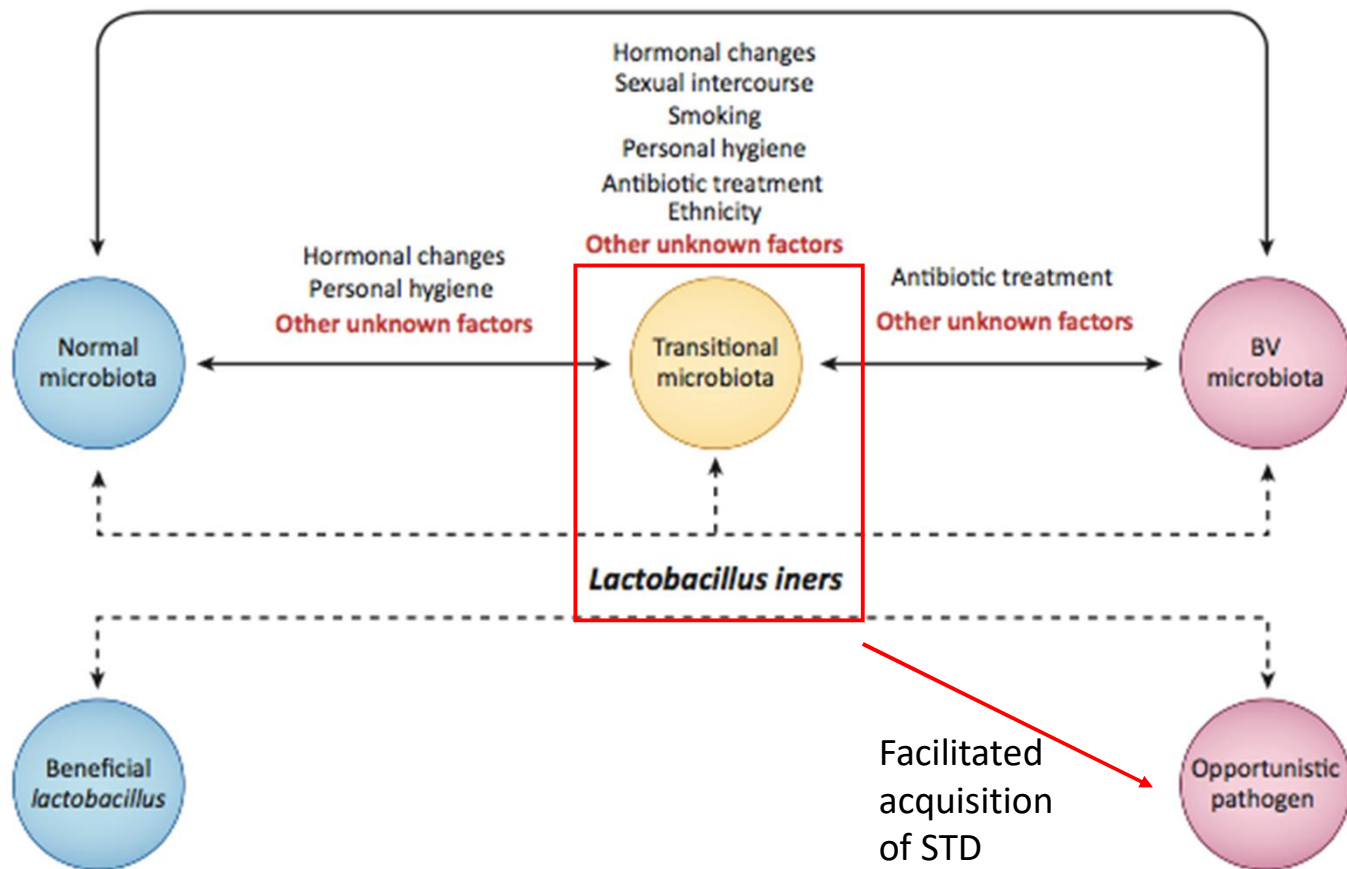


Lactobacillus iners: Friend or Foe?

Mariya I. Petrova



Lactobacillus iners: Friend or Foe?

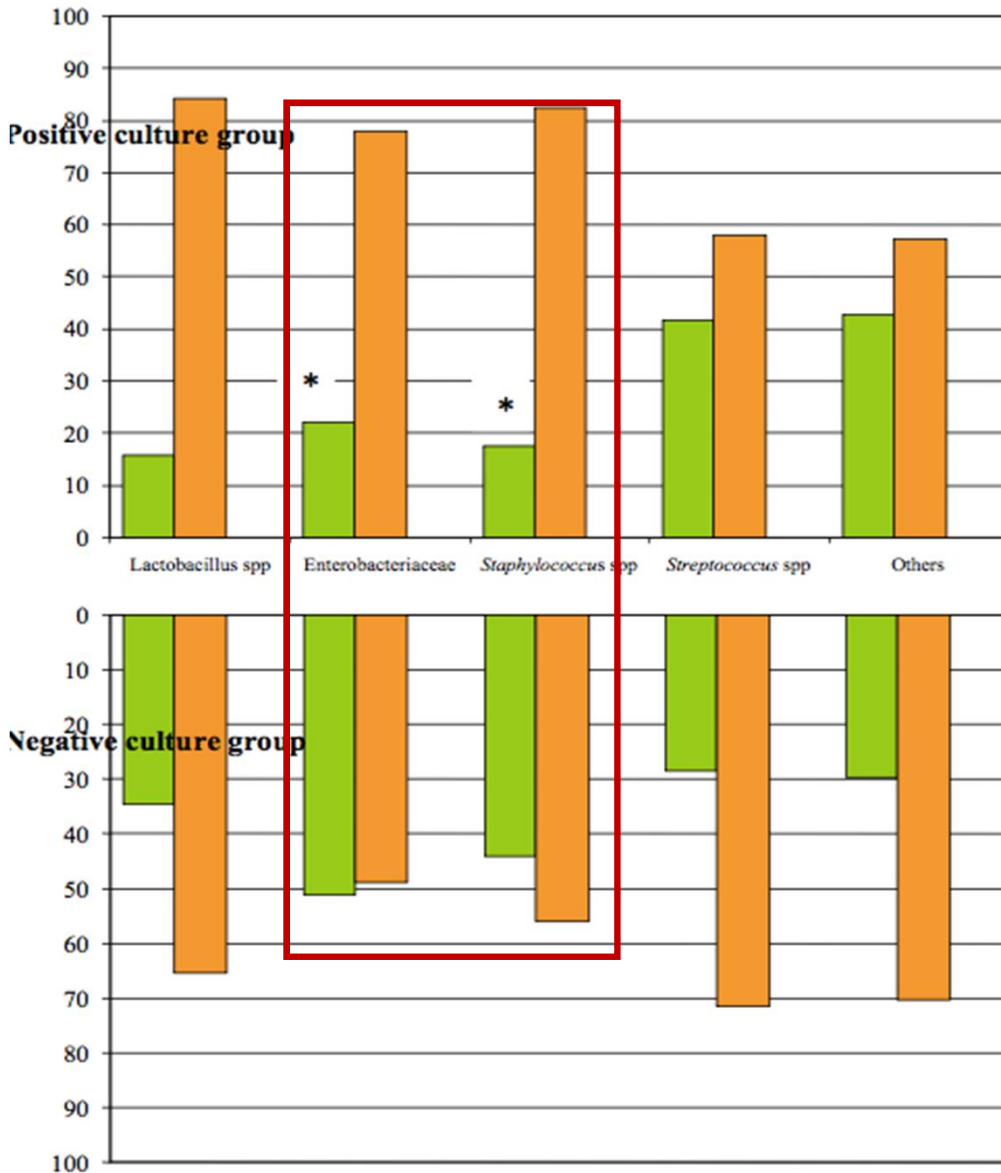


The dynamics of the vaginal microbiome during infertility therapy with in vitro fertilization-embryo transfer

Richard W. Hyman & Christopher N. Herndon & Hui Jiang & Curtis Palm & Marilyn Fukushima & Denise Bernstein & Kim Chi Vo & Zara Zelenko & Ronald W. Davis & Linda C. Giudice

- Pilot study to investigate the hormonal dependence of the microbiome in a typical IVF setting (only 30 pts)
- Vaginal swab in four different moments (baseline, triggering, ET, 6-8 gw)
- The presence of *Lactobacillus* on swab 1 appears to be favorable for a successful outcome of the IVF-ET procedure.
- Despite routine antibiotic prophylaxis before the IVF-ET cycles, ten patients had, at least, one bacteria on their vaginal swabs.
- Bias: long protocol (GnRH ago in luteal phase)

J Assist Reprod Genet, 2012



Examination of bacterial contamination at the time of embryo transfer, and its impact on the IVF/ pregnancy outcome

- Implantation rate significantly higher in favour of negative group

* Significantly lower pregnancy rate ($p < 0.001$) in patients positive to *Staphylococcus* and *Enterobacteriaceae* compared to negative patients

J Assist Reprod Genet (2007)

Abnormal vaginal microbiota may be associated with poor reproductive outcomes: a prospective study in IVF patients

Table IV qPCR classification of vaginal microbiota (VM) and reproductive outcome of IVF patients.

	Biochemical pregnancy	Clinical pregnancy
Normal VM (N = 62)	32 (52)	27 (44)
Abnormal VM (N = 22)	6 (27)	2 (9)

Data are n (percent of patients per row).

Table V Nugent score, reproductive outcome of IVF patients.

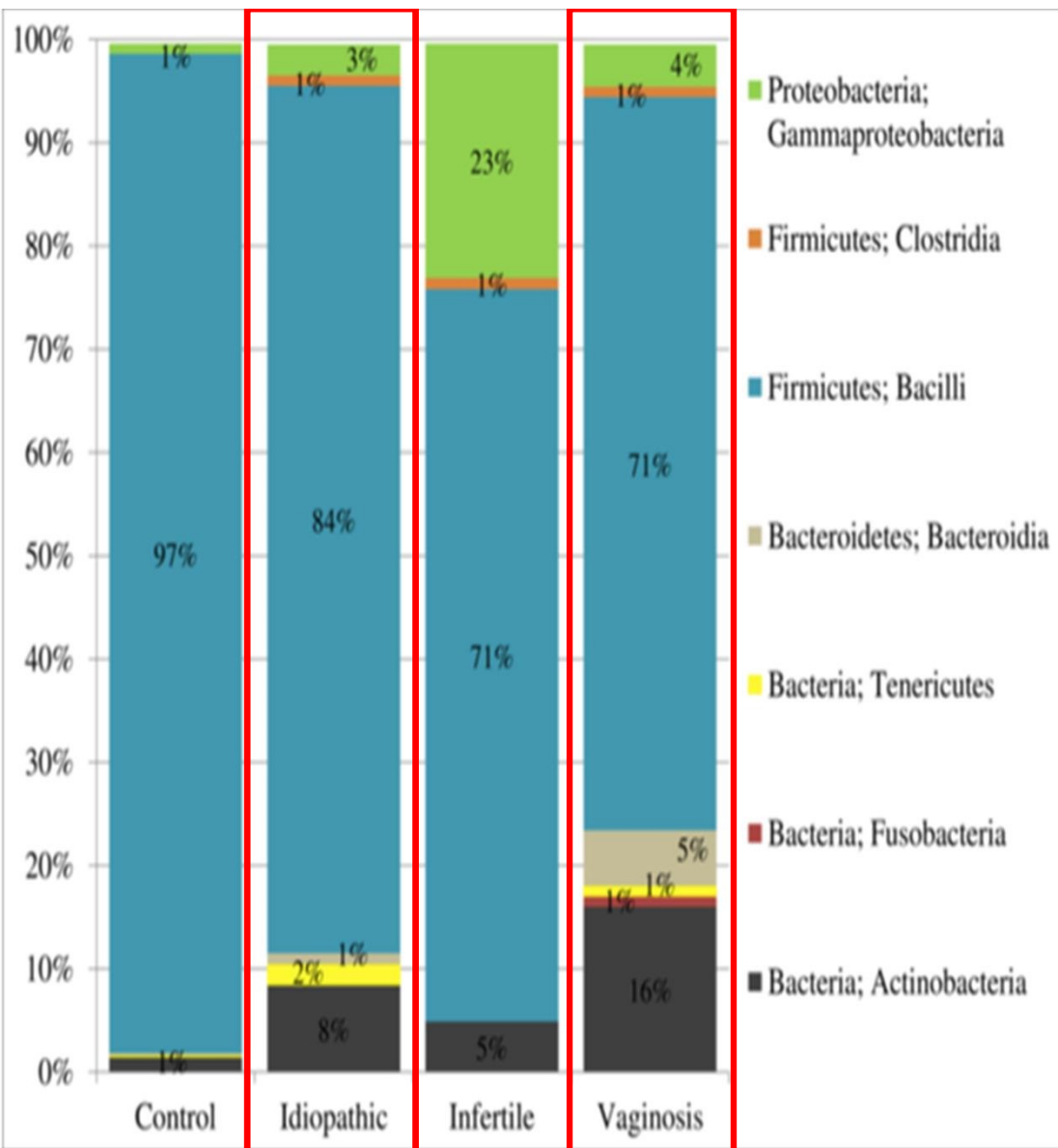
	Biochemical pregnancy	Clinical pregnancy
Normal flora (N = 60)	30 (50)	24 (40)
Intermediate (N = 12)	6 (50)	4 (33)
BV (N = 12)	2 (17)	1 (8)

Data are n (percent of patients per row).

MAIN RESULTS : The prevalence of BV defined by Nugent score was 21% (27/130), whereas the prevalence of an abnormal vaginal microbiota was 28% (36/130) defined by qPCR with high concentrations of *Gardnerella vaginalis* and/or *Atopobium vaginae*. The overall clinical pregnancy rate was 35% (29/84).

Interestingly, only 9% (2/22) with qPCR defined abnormal vaginal microbiota obtained a clinical pregnancy (P 1/4 0.004).

Human Reproduction, 2016



Subclinical Alteration of the Cervical-Vaginal Microbiome in Women with Idiopathic Infertility[†]

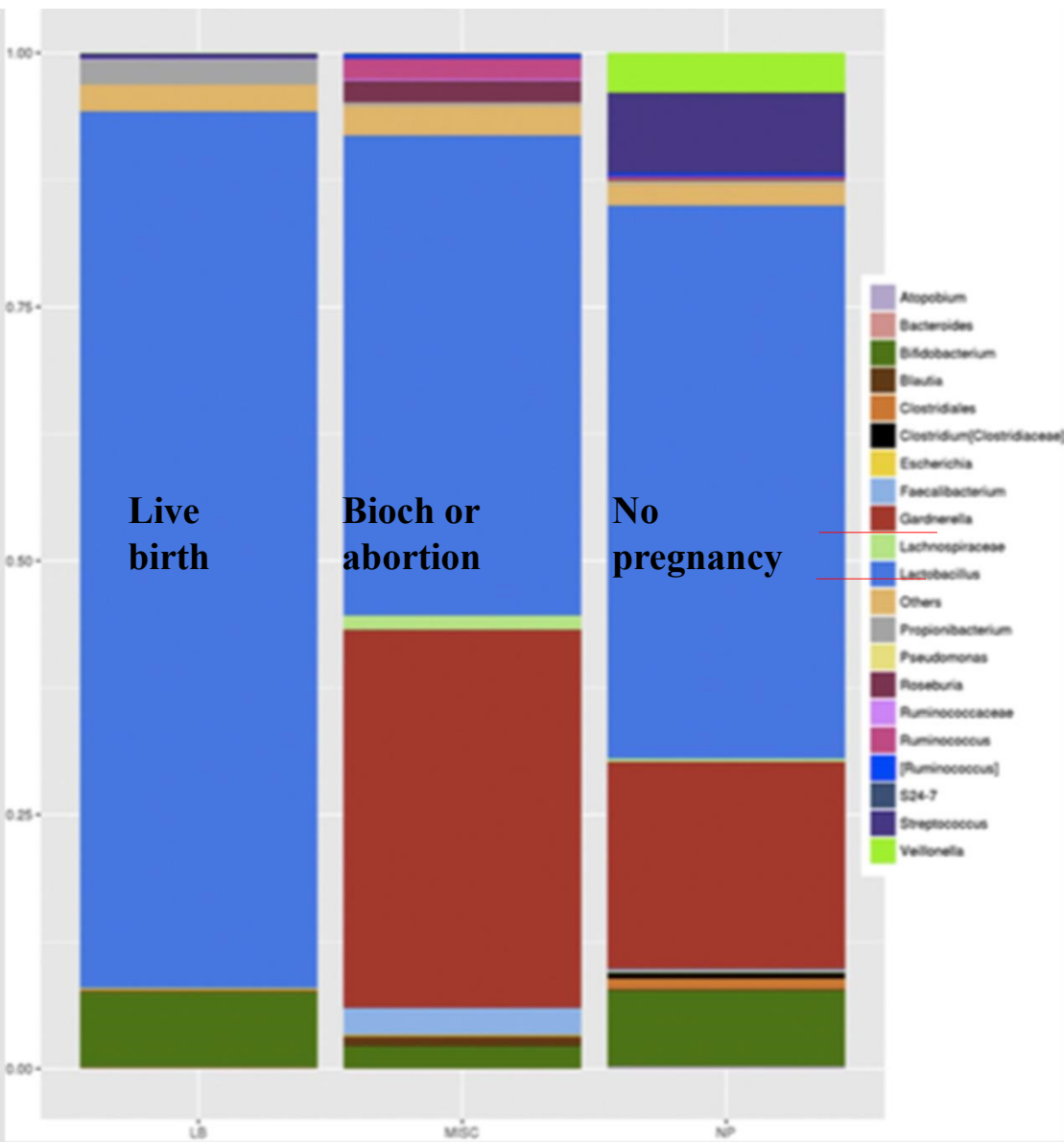
Campisciano G, Florian F, D'Eustacchio A, Stanković D, Ricci G, De Seta F, Comar M.

- *L. iners*, *L. crispatus*, and *L. gasseri* distinguished idiopathic infertile women from the other group, with microbial profile similar to that observed in bacterial vaginosis women has been detected.

Endometrial microbiota—new player in town

Detection of bacteria with molecular techniques has enabled the study of low biomass microbiomes in tissues and organs previously considered sterile, such as the endometrium. Subsequently, an abnormal endometrial microbiota has been associated with implantation failure, pregnancy loss, and other gynecological and obstetrical conditions. Further investigation of the reproductive tract microbiome will allow for a better understanding of bacterial communities' role in both physiology and pathophysiology, which in turn impacts the ability to achieve pregnancy and maintain a healthy pregnancy. Here we review the current literature that surrounds the endometrial microbiome and highlight the importance of assessing it as a future tool for improving reproductive outcomes in infertile patients. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)

Moreno I and Franasiak JD. *Fertil Steril* 2017



Low abundance of endometrial Lactobacillus is associated with poor reproductive outcome.

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

TABLE 2
Descriptive characteristics of subjects, cycles, transfers, and outcome results

Characteristics and outcomes	LDM, n = 17	NLDM, n = 15	P value
Age, y	40.06 ± 3.47	39.00 ± 5.09	.49
BMI, kg/m ²	24.18 ± 5.18	22.45 ± 4.02	.30
Previous pregnancies	1.71 ± 2.44	1.53 ± 2.32	.84
Previous miscarriages	1.53 ± 2.21	1.14 ± 1.56	.58
Metaphase II oocytes/cycle	11.94 ± 4.27	10.20 ± 4.81	.28
Fertilization rate/cycle	157/203 (77.34%)	118/153 (77.12%)	.62
Transferred embryos/cycle	1.65 ± 0.49	1.73 ± 0.59	.65
Time between EF and transfer, mo	2.82 ± 2.55	1.80 ± 1.08	.16
Pregnancy rate/transfer	12/17 (70.6%)	5/15 (33.3%)	.03 ^{a,b}
Implantation rate/transfer	17/28 (60.7%)	6/26 (23.1%)	.02 ^{a,b}
Ongoing pregnancy/transfer	10/17 (58.8%)	2/15 (13.3%)	.02 ^{a,b}
Miscarriage rates	2/12 (16.7%)	3/5 (60%)	.07
Live birth rate/transfer	10/17 (58.8%)	1 ^c /15 (6.7%)	.002 ^{a,b}

Values are mean ± SD unless otherwise noted.

BMI, body mass index; EF, endometrial fluid; LDM, *Lactobacillus*-dominated microbiota; NLDM, non-*Lactobacillus*-dominated microbiota.

^a χ^2 test and Student *t* test were performed; ^b *P* value < .05; ^c Voluntary termination of pregnancy.

Moreno et al. Endometrial microbiota impacts reproductive potential. Am J Obstet Gynecol 2016.

Moreno et al., Am J Obstet Gynecol 2016

TABLE 1**Endometrial microbiota is related to pregnancy outcomes for in vitro fertilization patients** (continued)

Sample	Endometrial receptivity (d)	Shannon index	<i>Lactobacillus</i> OTUs, %	Non- <i>Lactobacillus</i> OTUs, %	Unassigned, %	Endometrial microbiota	Pregnancy	Ongoing pregnancy
39	NR (P+3)	4.651	22.38	63.72	13.91	NLD	No ET	NA
40	NR (P+5)	4.026	14.07	84.30	1.63	NLD	No ET	NA
41	NR (P+4)	2.013	97.94	1.77	0.29	LD	No ET	NA

Shannon diversity values, OTUs abundances, and microbiotic profiles from in vitro fertilization patients. Technical filtering was done from data coming from QIIME: ribosomal database project score <0.9 and ≤ 2 reads are filtered. All plots are based on these filtered data.

CD, cycle day; ET, embryo transfer; LD, *Lactobacillus* dominated; LH, luteinizing hormone; NA, not applicable; NLD, non-*Lactobacillus* dominated; NR, nonreceptive; OTU, operational taxonomic unit; P, progesterone; R, receptive; VTOP, voluntary termination of pregnancy.

Moreno et al. Endometrial microbiota impacts reproductive potential. Am J Obstet Gynecol 2016.

Moreno et al., Am J Obstet Gynecol 2016

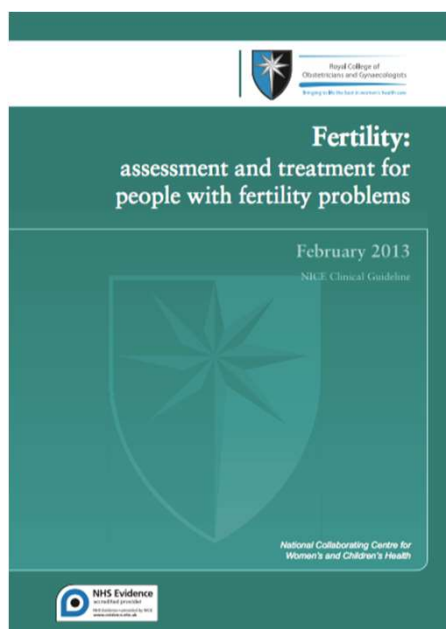
Conclusions

- STI have negative impact of reproductive outcome
 - Tubal infertility
 - Implantation failure
 - Miscarriage
 - Obstetrics Outcomes (SP vs ART pregnancy)
- Vaginal microbioma (BV)
- Endometrial microbioma (endometrial receptivity)

Conclusions

- Although we have recognized the existence of bacteria for >330 years, it is truly only within the past 7 years that we have begun to genetically characterize the bulk of that biome.
- At its current stage, there are no immediate clinical applications of microbiota-based research and clinical obstetrics.
- In IVF we need more attention to STI

STI screening in ART



Fertility: assessment and treatment for people with fertility problems

Recommendations

Number	Recommendation
80	Before undergoing uterine instrumentation women should be offered screening for <i>Chlamydia trachomatis</i> using an appropriately sensitive technique. [2004]
81	If the result of a test for <i>Chlamydia trachomatis</i> is positive, women and their sexual partners should be referred for appropriate management with treatment and contact tracing. [2004]
82	Prophylactic antibiotics should be considered before uterine instrumentation if screening has not been carried out. [2004]

NO MORE GUIDELINES

STI and ART

- First infertility assessment?
- Which type of patientes?
- Treatment ?
- Post treament test?
- Role of probiotics

How to screen infertile patients



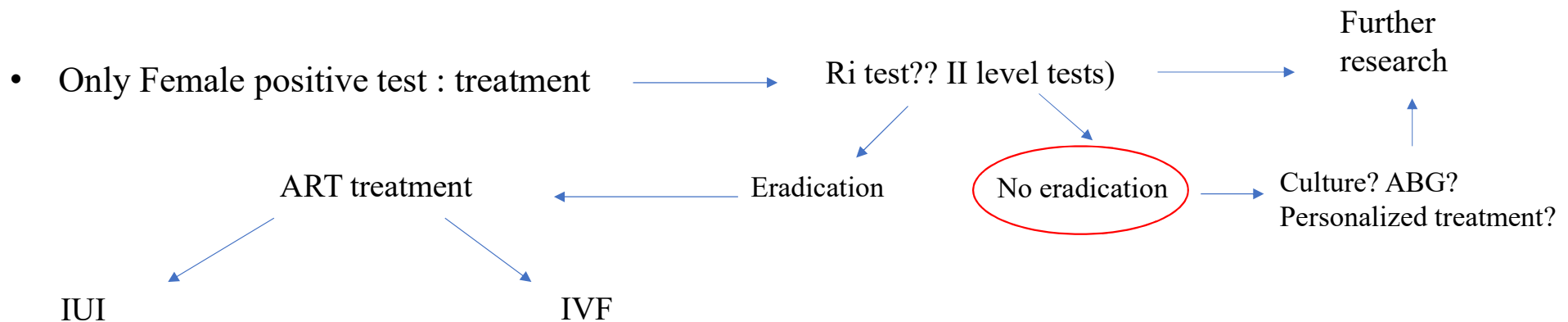
Protocol proposal

- Women who are undergoing investigations for infertility should be offered STI screening test

- Cervical swab (Chlamydia and micoplasmi)
- Vaginal swab (culture? PCR?) for BV or AVB
- GRAM; Nugent score
- Multiplex ?

I level investigation
prior TRA

- Positive test: partner investigation (spermiculture or???)



Protocol proposal

No pregnancy: new TRA

New test before new treatment?

- Re-test in RIF (repeated implantation failure)
 - II level (vaginal vs endometrial microbioma)
 - Egg donation receptors (POF vs Post menopause): how to study???
- How to treat??

Future research

Nothing has been published related to the microbiome's alteration and the utilization of antibiotics during IVF.

Vaginal microbiome during IVF stimulation (poor data)

Vaginal microbiome in endometrial preparation protocol (different E2 level)

Endometrial microbioma (IVF, egg donation – pre and post menopause)