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Vaginal Microbiota and HIV Susceptibility

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Disclosures

No (potential) conflict of interests

Vaginal Microbiome Affects HIV Risk in Young Women in Sub-Saharan Africa

INFECTIOUS DISEASE

Vaginal microbiome affects HIV risk

Unusual bacteria in vagina help explain high infection rates in South African women

Every week around 6200 adolescent girls and young women become infected with HIV

In sub-Saharan Africa, four in five new HIV infections among adolescents aged 15-19 years are in girls

A biological factor that contributes to increased HIV risk is the vaginal microbiome

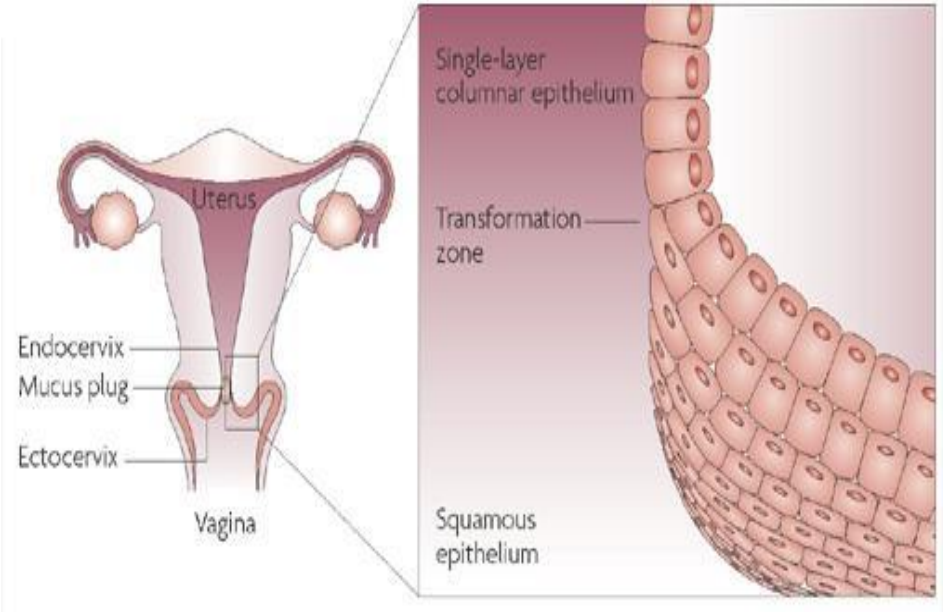
Cohen 2016 Science 353:6297 *Masson et al 2015 Clin Infect Dis 61: 260*
Anahtar et al 2015 Immunity 42: 965 *Gosmann et al 2017 Immunity 46:1*

Most HIV Infections in Women Occur by Entry via the Female Reproductive Tract (FRT)

>80% of new HIV infections in women occur by HIV entry via the FRT

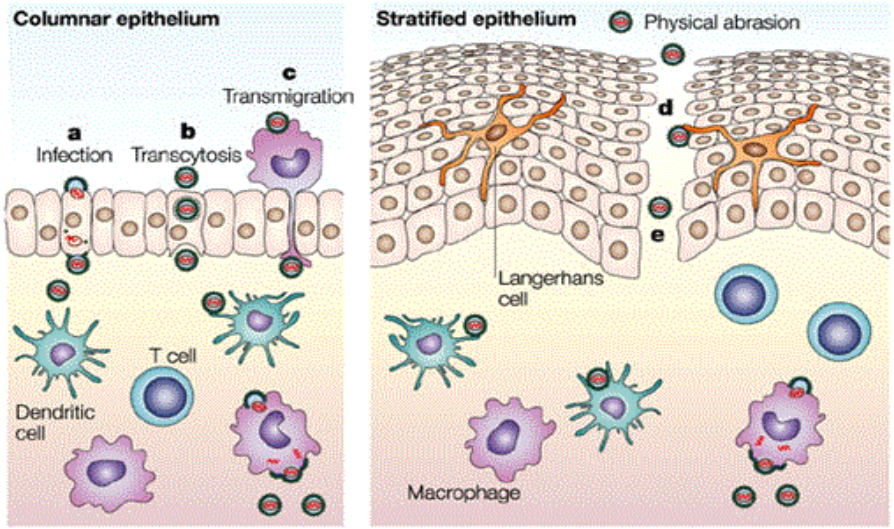
HIV can establish infection mainly by entry through vagina, ectocervix, and endocervix, and potentially other sites in the upper FRT (SIV reporter virus)

FRT: Probability of HIV transmission per exposure event is usually low relative to rectal/parenteral 1 in 1000 (semen)



Hladik and Hope 2009 Current HIV/AIDS Reports 6: 20 *Stieh et al 2014 PLoS Pathog e1004440*
Powers et al 2008 Lancet Infect Dis 8: 553 *Boily et al 2009 Lancet Infect Dis 9:118* *Buve et al 2014 AIDS July*

Antimicrobial Defense Mechanisms in the FRT



Endocervix

Ectocervix and Vagina
(preference for HIV infection)

Physical Defense
Mucous
Ciliary clearance
Epithelial cells

Biological Defense
Immune cells (epithelial cells) respond to MAMPs/PAMPs to produce immune mediators: antiviral and pro-inflammatory (paradoxically promote HIV infection in women)

Microbiota Communities of commensal bacteria (along with fungi and viruses)
Competition, bacteriocins, organic acid metabolites (i.e. lactic acid –produced by lactobacilli associated with HIV protection)

Shattock and Moore 2003 Nat Micro Reviews *Carias et al 2013 J Virol 87: 11388*
Wira et al 2011 Am J Rep Immunol *Stieh et al 2014 Plos Path 10:e1004440*

Vaginal Microbiota of 90% of Asymptomatic Reproductive-Age Women Is Dominated by *Lactobacillus* spp.(USA)

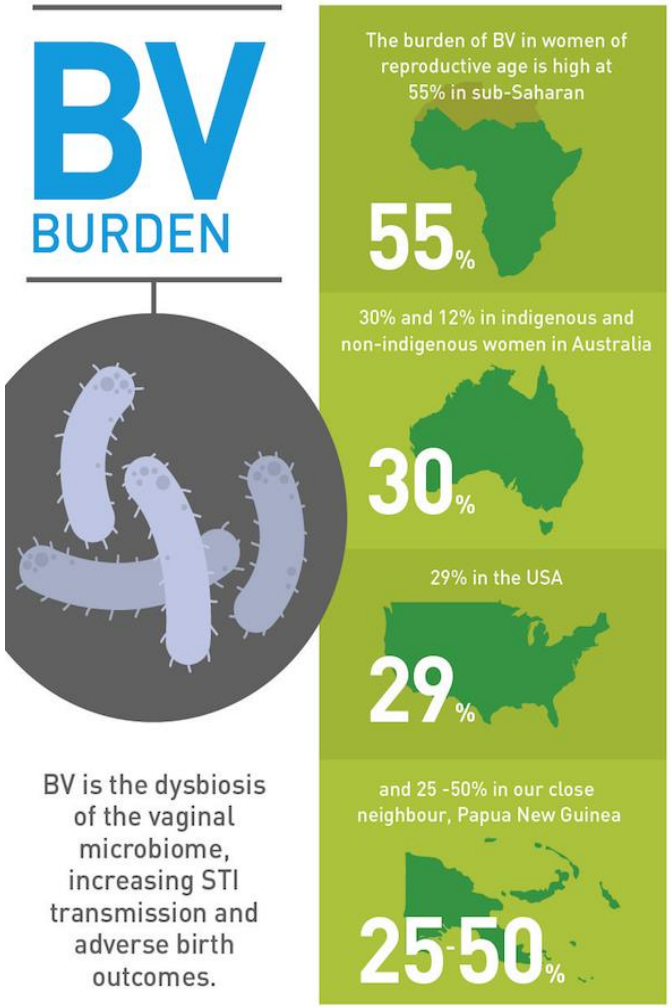
Group	Bacterial communities (CST)	
I	*Lactobacillus crispatus*	< pH 4.0*
II	*Lactobacillus gasseri*	pH 5.0
III	*Lactobacillus iners*	pH 4.4
IVA	Modest *Lactobacillus sp.*, *Anaerococcus, Corynebacterium, Finegoldia* and *Streptococcus*	pH 5.3
IVB	No *Lactobacillus sp.* detected, *Atopobium, Prevotella, Sneathia, Mobiluncus, Peptoniphilus* and several other taxa	pH 5.3
V	*Lactobacillus jensenii*	pH 4.7

L crispatus most protective against STIs including HIV acidifies vagina to lower pH by lactic acid

L. iners least protective and less stable intrinsic/extrinsic factors and transitions to CST IV similar to bacteria vaginosis (BV) - "non-optimal" vaginal microbiota

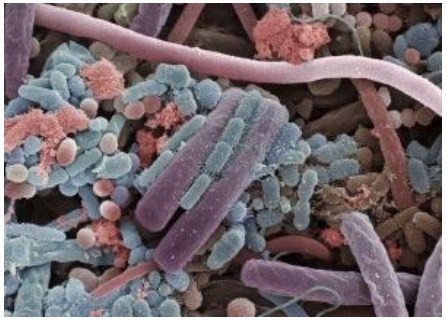
Gajer et al., 2012 STM 4:132ra52; Ravel et al., 2011 PNAS 108:4680
Marrazzo 2013 JID; Sha et al 2005 J Infect Dis 191:25; Mitchell et al 2013 AIDS Res Hum Retroviruses 29:1300

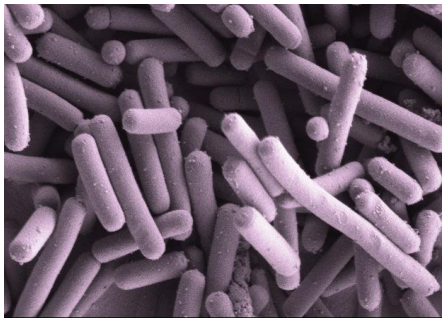
"Non-Optimal" Vaginal Microbiota: Bacterial Vaginosis (BV)



- Common yet poorly understood vaginal condition in reproductive age women
- Increase in load of obligate and facultative anaerobes and depletion of beneficial *Lactobacillus* spp.
- Adverse sexual and reproductive health outcomes

BV



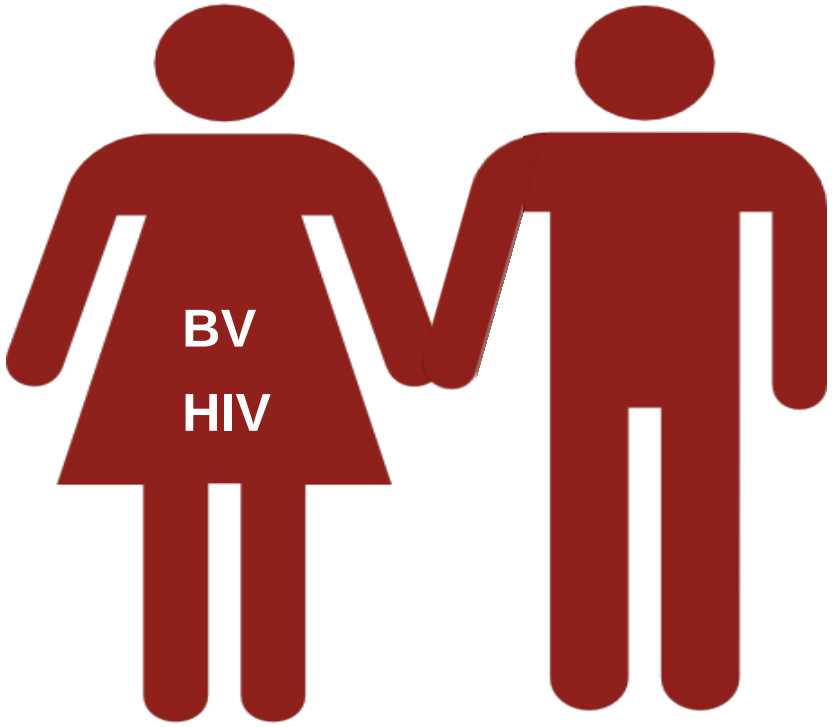


LB

Kenyon C et al 2013 Am J Obstet Gynecol 209:505

Bacterial Vaginosis (BV) is a Major Risk Factor for HIV

RISK OF HIV INCREASES 2-FOLD[1,2]



RISK OF HIV INCREASES 3-FOLD[3]

As defined by traditional techniques: Amsel's criteria and Nugent Score

[1] *Atashili et al 2008 AIDS 22:1493,* [2] *Low et al 2011 Plos Med 8:e1000416*
[3]*Cohen et al., 2012 PLoS Med 9:e1001251*

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 35, Number 3, 2019
Mary Ann Liebert, Inc.
DOI: 10.1089/aid.2018.0304

CLINICAL PERSPECTIVE

The Evolving Facets of Bacterial Vaginosis: Implications for HIV Transmission

Lyle R. McKinnon,[1,2] Sharon L. Achilles,[3,4] Catriona S. Bradshaw,[5,6] Adam Burgener,[7–9] Tania Crucitti,[10] David N. Fredricks,[11,12] Heather B. Jaspan,[13,14] Rupert Kaul,[15,16] Charu Kaushic,[17,18] Nichole Klatt,[19] Douglas S. Kwon,[20,21] Jeanne M. Marrazzo,[22] Lindi Masson,[23,24] R. Scott McClelland,[12,25,26] Jacques Ravel,[27] Janneke H.H.M. van de Wijgert,[28,29] Lenka A. Vodstrcil,[5,6] and Gilda Tachedjian[30–33]

BV is clinically and microbiologically heterogeneous – underscored by molecular techniques

However, advent of molecular techniques to characterise the vaginal microbiome has let to some confusion in the field regarding the role of BV in HIV acquisition

Prompted this Perspectives article – to clarify BV role in HIV acquisition
and to propose standardized nomenclature to describe the vaginal microbiome

Bacterial Vaginosis (BV) Diagnosis and Proposed Definitions- Define Method Used to Detect "Non-optimal" Vaginal Microbiota

Clinical and Microscopic (Traditional)

Amsel-BV	BV meets at least 3 of 4 Amsel's criteria: • Abnormal discharge • pH>4.5 • Clue cells • Fish odort Symptomatic or Asymptomatic
Nugent-BV	BV diagnosed by Gram Stain: • Nugent score 7 – 10 (Nugent-BV) • Nugent score 4 - 6 (Intermediate-BV) Nugent score 0 – 3 (Non BV), *Lactobacillus*-dominated^ Symptomatic or Asymptomatic

Molecular

Molecular-BV	General term for "non-optimal" bacterial communities depleted of lactobacilli with abundant anaerobes* characterized by molecular techniques
Seq BV	• 16S rRNA gene sequencing or broadrange PCR. Shotgun sequencing approaches High relative abundance of anaerobes* depleted of *Lactobacillus* spp. associated with increased genital inflammation and/or HIV risk*
qPCR-BV	• Taxon specific quantitative PCR "Non-optimal" taxa demonstrating concentration dependent associations with increased genital inflammation and/or odds of HIV risk Symptomatic or Asymptomatic

^Depends on the population studied[22]
*Polymicrobial/diverse or *G. vaginalis*-dominated
#May also be associated with other adverse sexual as well as reproductive health outcomes

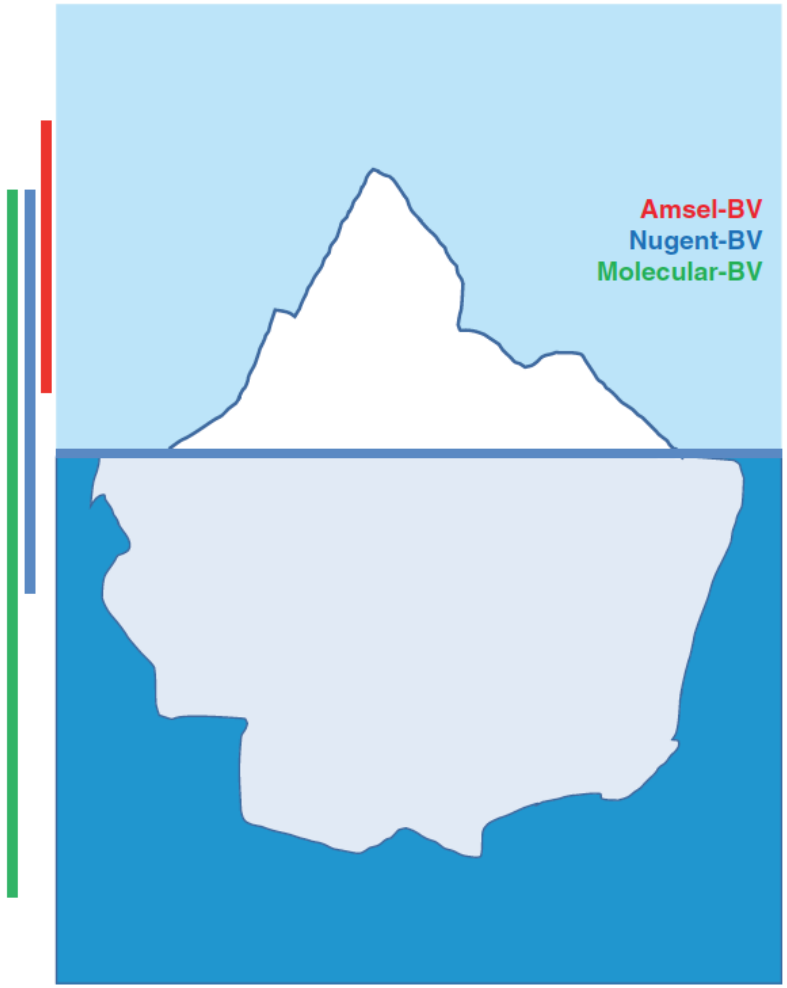
Increased genital inflammation and/or HIV risk

McKinnon et al 2019AIDS Res Hum Retroviruses 35: 219

BV by Amsel's and Nugent do not Capture High Proportion of Women with "Non-Optimal" Vaginal Microbiota Associated with Increased Risk of HIV Acquisition

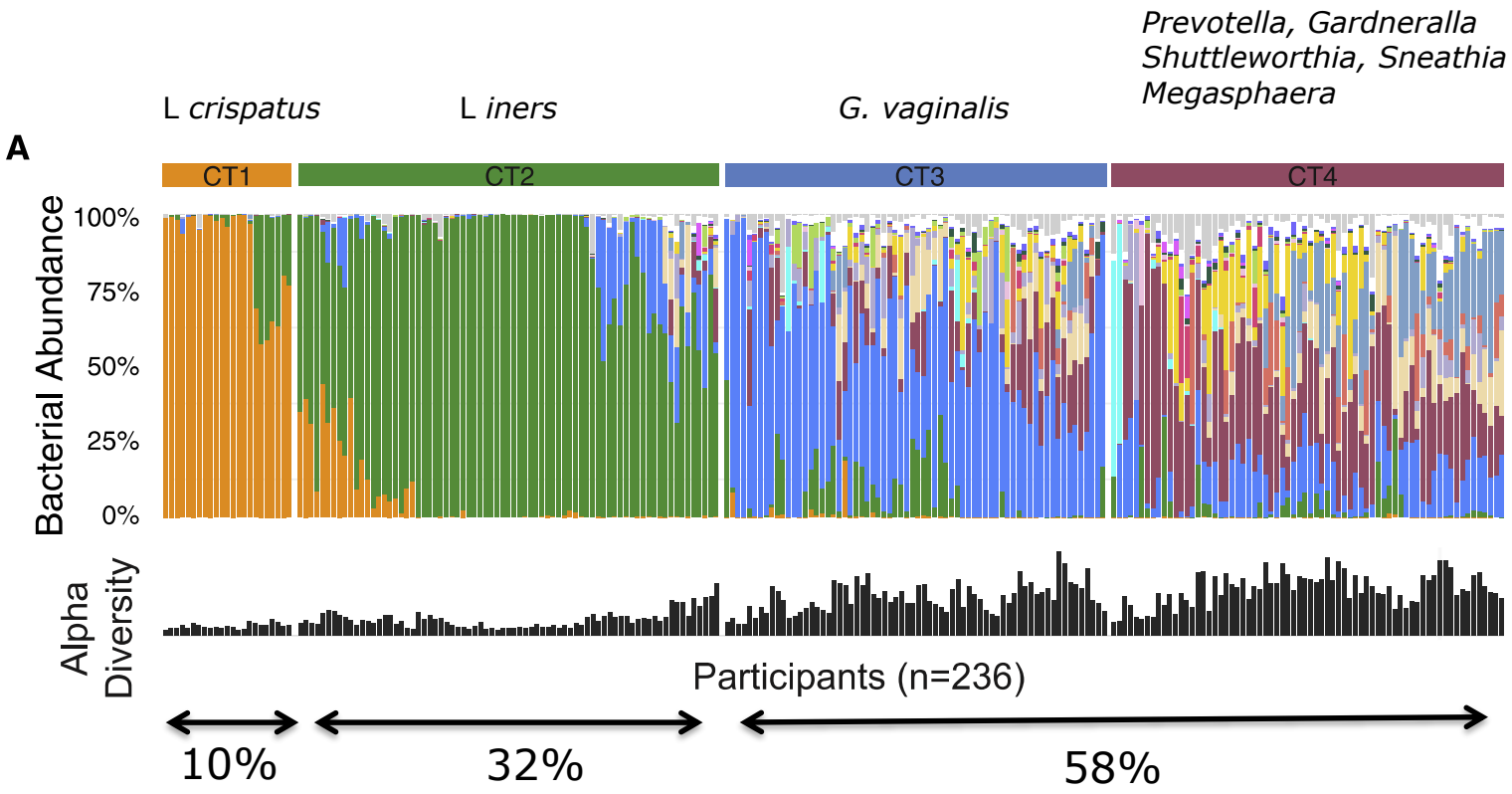
Amsel-BV Nugent-BV important diagnostic in clinic and epidemiological studies

Tip of iceberg – "non-optimal' vaginal microbiota"



McKinnon et al 2019AIDS Res Hum Retroviruses 35: 219

Vaginal Microbiota of Most Young Healthy South African Women are Depleted of *Lactobacillus* spp



Prospective observational study (19 – 23 years of age HIV neg)
FRESH – Females rising through Education, Support and Health Cohort

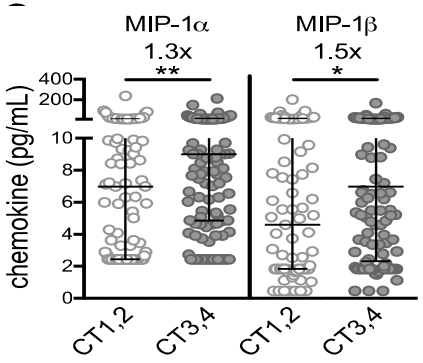
Majority of women who acquired HIV were asymptomatic and negative for Nugent-BV

o identifiable STI
ed sex acts, dry sex

Gosmann et al 2017 Immunity 46:1

Bacterial Communities Depleted of *Lactobacillus* spp: Increased Genital Inflammation and HIV Acquisition

Chemokines and cytokines



IL1α IL1β TNF IL-8

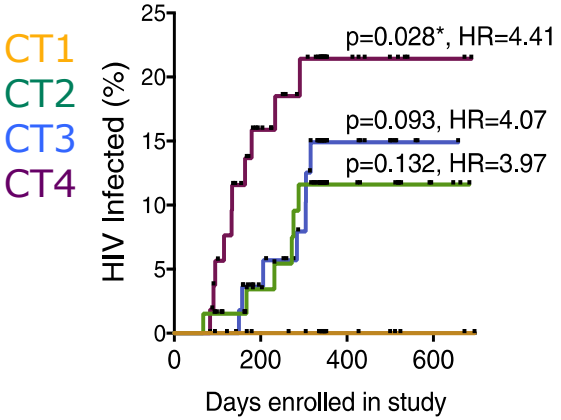
L. crispatus

L. iners

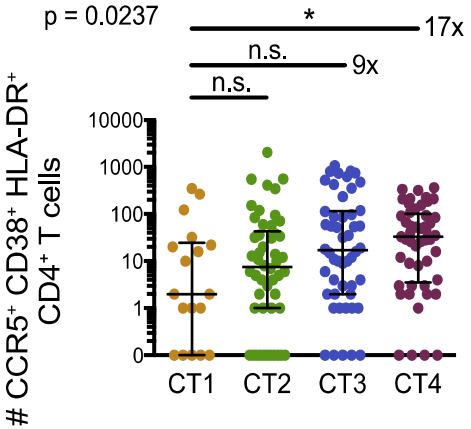
G. vaginalis

Highly diverse

Increased HIV risk



HIV target cells



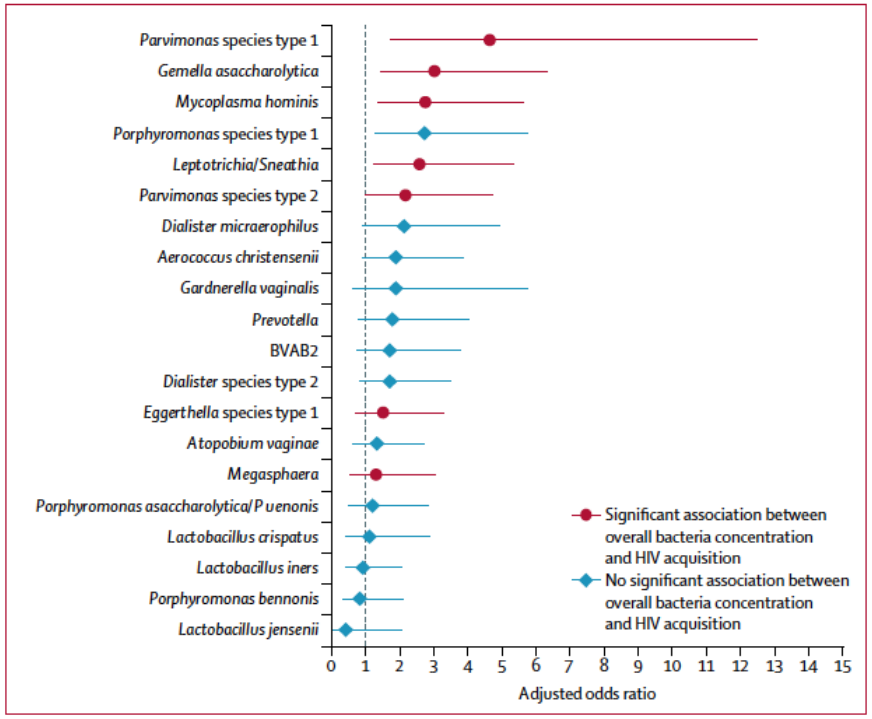
Cervical cytobrush

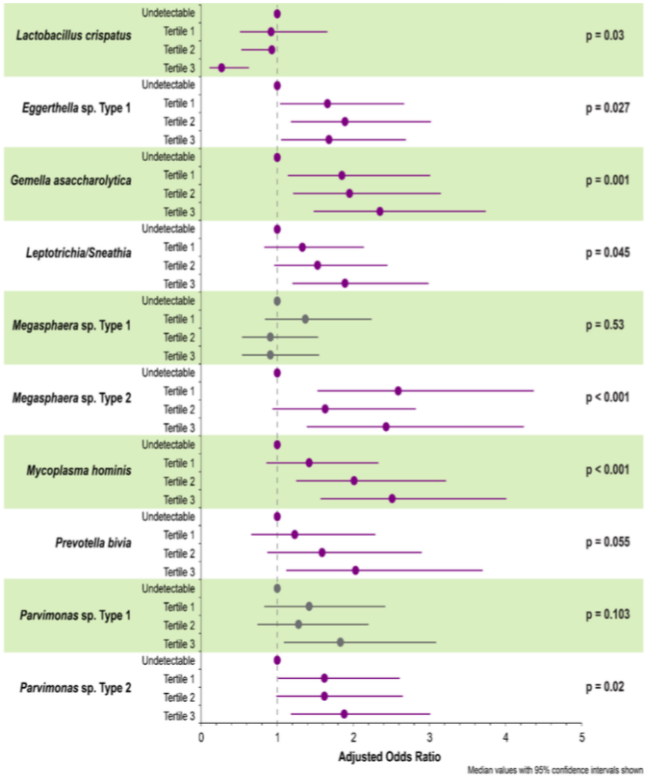
Seq-BV
"Broad-range" PCR

Gosmann et al 2017 Immunity 46:1

HIV Acquisition Associated with the Concentration of Specific Vaginal Microbial Taxa

Concentration Dependent Associations with Increased Odds for HIV in Women





*

*

African women from five cohorts

Nested case control

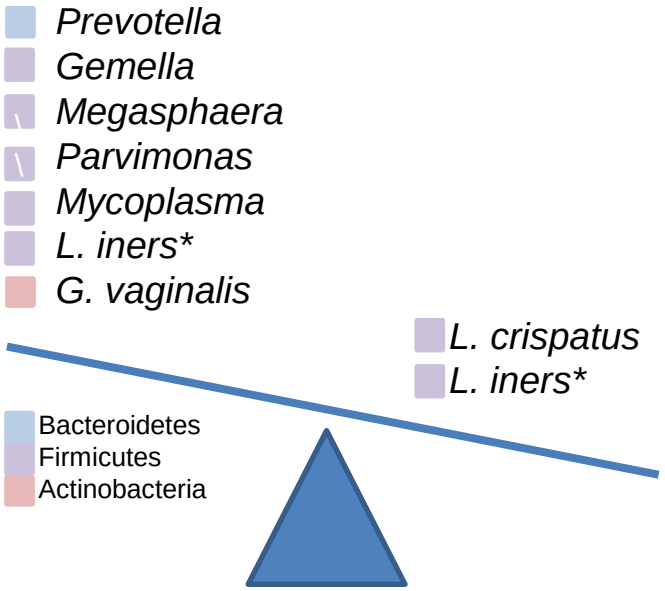
qPCR-BV
Taxon specific PCR

McClelland et al 2018 Lancet Infect Dis 18: 554 ; Srinivasan et al CROI 2018, Abstract 268

HIV Acquisition is Associated with the Abundance of Specific Vaginal Microbial Taxa

Cervicovaginal

Inflammation

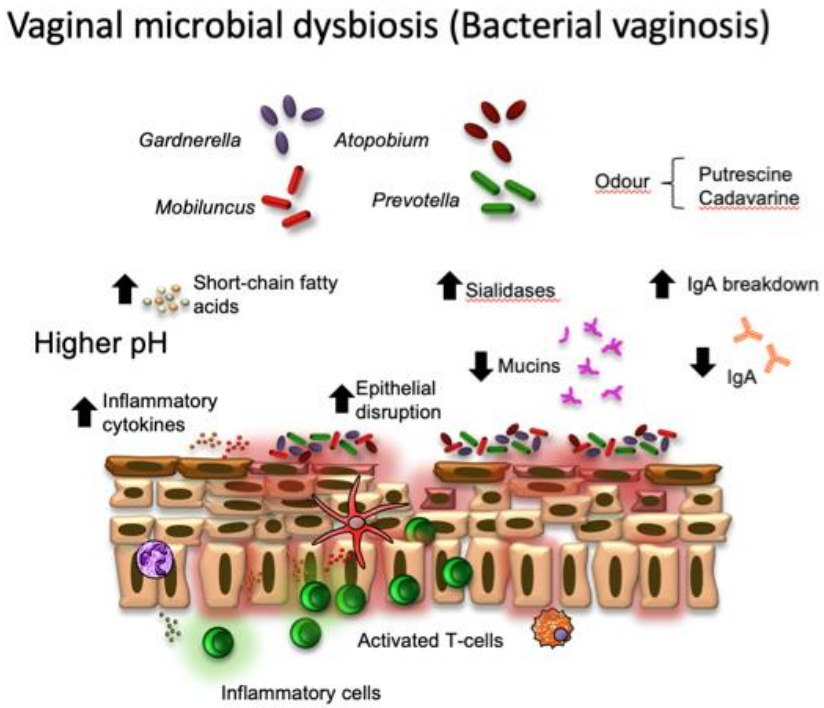


Non-inflammatory

Anahtar, et al 2015 Immunity; Gosmann et al 2017 Immunity;
McClelland et al 2018 Lancet Inf Dis; Srinivasan et al CROI 2018 ; Sabo et al 2019 STI
Masson L et al 2015 Clin Infect Dis

Vaginal Microbial Dysbiosis and Adverse Sexual and Reproductive Health Outcomes

Non-optimal



Recruitment/ activation of HIV target cells
Proinflammatory cytokines/chemokines

Disruption of epithelial barrier
Neutrophils-proteases cytokines

Disruption of protective cervicovaginal mucous
BV bacteria: sialidases, mucinases

Burgener et al 2015 Curr Opin Immunol 36:22;
McKinnon et al 2019 AIDS Res Hum Retroviruses 35:219

How do lactobacilli protect against HIV?

Protective Vaginal Microbiota Produces more Lactic Acid

	pH <4.5	pH ≥4.5
Acid	**Lactobacillus (LB)(mM)**	**BV (mM)**
Lactic	~120[1,2]	≤20[4]
Acetic	2-4[3]	≤120[1, 3]
Propionic	<1	2-4[3]
Butyric	<1	2-4[3]
Succinic	<1[4]	≤20[4]

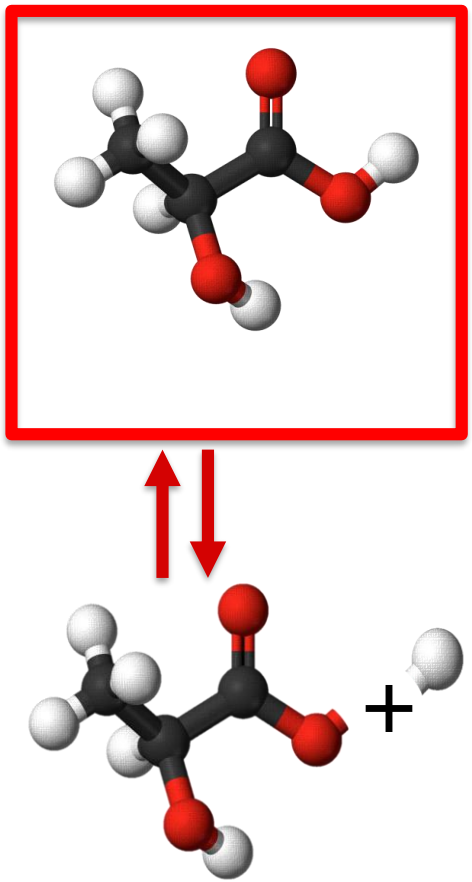
L. crispatus acidifies vagina to lower pH[5]

Does lactic acid have a protective role in the FRT?

[1]Gajer, 2012 STM 4:132ra52; [2]O'Hanlon, 2013 PLoS One 8:e80074;
[3] Mirmonsef, 2011/2012 Am J Reprod Imm. 65(3):190-5 and 67(5):391-400
[4]Al-Mushrif, 2000, J Med Microbiol, 49 (11); 1023-30 [5]Ravel et al., 2011 PNAS 108:4680

Lactic Acid (LA): Protonated form has Antimicrobial, Antiviral and Immunomodulatory Activities

- In the vagina 1.0 ± 0.2% DL-LA pH 3.5 ± 0.3[1]
- Uncharged LA (but not H_2O_2) Bactericidal BV-associated bacteria but not vaginal lactobacilli[2] HIV virucidal in vitro[3] and ex vivo CVF[9], ex vivo tissues[4]
- Inactivates N. gonorrhoeae[5], Chlamydia trachomatis[6], HSV[7]
- Uncharged LA mediates anti-inflammatory effects on cervicovaginal epithelial cells that could protect against HIV[8]
- Epithelial barrier function



Uncharged form

Low pH
Lactic acid

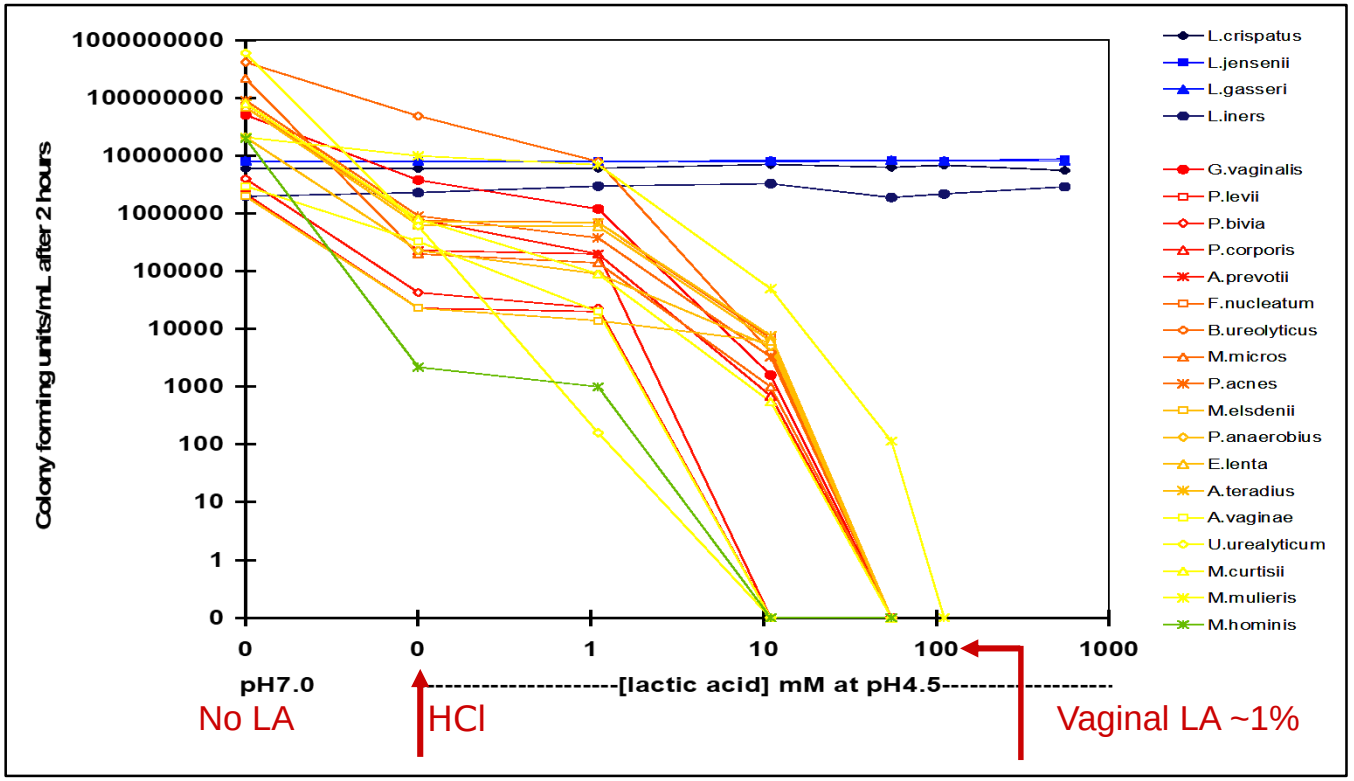
pKa 3.89

Neutral pH
Lactate anion

Charged form

[1]O'Hanlon et al 2011 BMC Infect Dis 11; [2]O'Hanlon et al 2013 PLoS One 8:e80074; [3]Aldunate et al 2013 J Antimicrob Chemother; [4]Palomino et al 2017 Front Microbiol 8 907 [5]Graver and Wade 2011 Clin Microbiol Antimicob 10:8; [6]Gong et al 2014 PLoS ONE 9:e107758; [7]Isaacs et al 2013 Antimicrob Agents Chemother 57: 3806; [8]Hearps et al 2017 Muc Immunol 10:1480; [9]Tyssen et al 2018 mSphere

Lactic Acid is Bactericidal Against 17 BV-Associated Bacteria



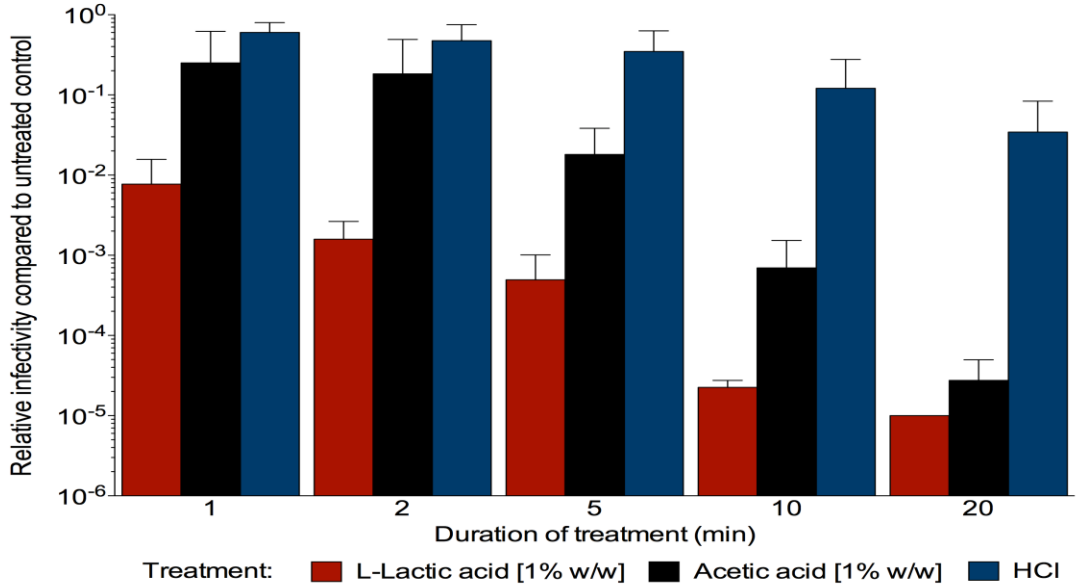
O'Hanlon, 2011 BMC Infect Dis 11:200

J Antimicrob Chemother 2013; **68**: 2015–2025
doi:10.1093/jac/dkt156 Advance Access publication 8 May 2013

Journal of Antimicrobial Chemotherapy

Vaginal concentrations of lactic acid potently inactivate HIV

Muriel Aldunate[1,2]†, David Tyssen[1]†, Adam Johnson[1], Tasnim Zakir[1], Secondo Sonza[1,2], Thomas Moench[3], Richard Cone[4] and Gilda Tachedjian[1,2,5]*



1% acetic acid = 179 mM

37°C pH 3.8 (n=4)
$p<0.05$

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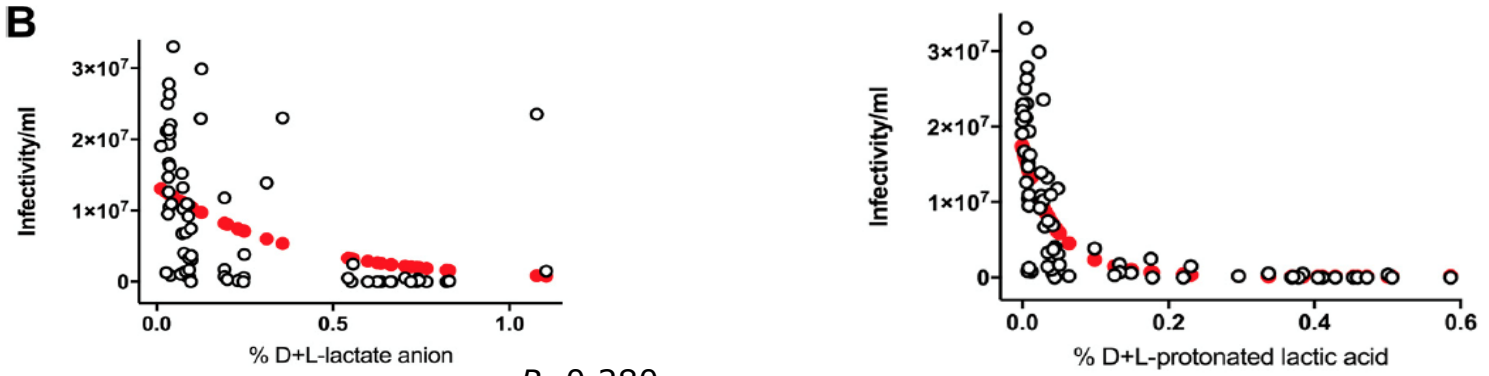
RESEARCH ARTICLE
Therapeutics and Prevention

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

David Tyssen,[a] Ying-Ying Wang,[b] Joshua A. Hayward,[a] Paul A. Agius,[c,d] Kevin DeLong,[e] Muriel Aldunate,[a,f] Jacques Ravel,[g,h] Thomas R. Moench,[i] Richard A. Cone,[b,e] Gilda Tachedjian[a,f,j,k]



P=0.280

P<0.001

N= 20, 22 samples women 18 – 45 y
All with lactobacillus-dominated vaginal microbiota – majority CSTI, CSTIII, CSTV

Generalised linear mixed modeling

neat, 3-fold, 9-fold, 27-fold neutralized

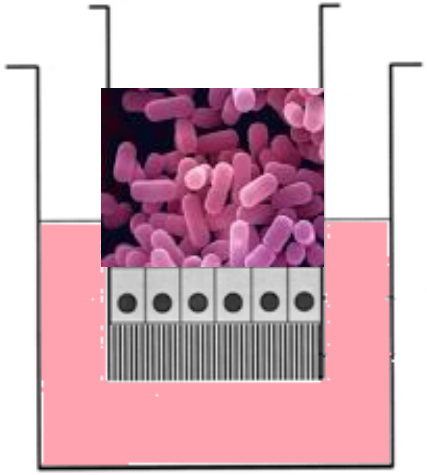
Vaginal lactic acid elicits an anti-inflammatory response from human cervicovaginal epithelial cells and inhibits production of pro-inflammatory mediators associated with HIV acquisition

AC Hearps[1,2], D Tyssen[1], D Srbinovski[1,3], L Bayigga[4], DJD Diaz[1,3], M Aldunate[1,3], RA Cone[5], R Gugasyan[1,6], DJ Anderson[4] and G Tachedjian[1,2,7,8]

L. crispatus Inhibits Production of Pro-Inflammatory Cytokines Produced by FRT epithelial cells

- *Lactobacilli* spp. (e.g. *L crispatus*) – largely non-inflammatory while BV-associated bacteria (e.g. *Atopobium vaginae*, *Prevotella amnii*) trigger a proinflammatory cytokine response from FRT epithelial cells[1,2,3,4]

- Lactobacilli but not BV-associated bacteria dampen TLR agonist response by reducing pro-inflammatory cytokines elicited by FRT epithelial cells[5,6]

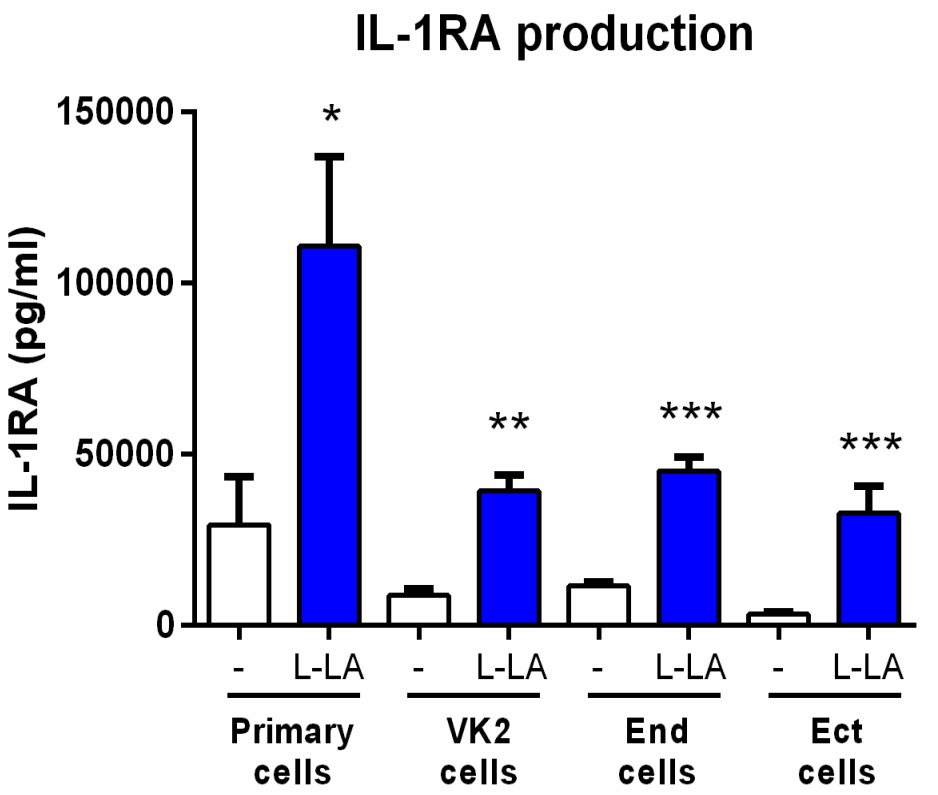


Does lactic acid contribute to this anti-inflammatory effect?

[1]Libby et al 2008 Microbes Infect 10:439; [2]Fichorova et al 2011 mBio; [3]Doerlinger et al 2014 JID; [4]Anahtar et al 2015 Immunity; [5]Rose et al 2012 PLoS ONE; Kyongo et al 2012 Plos ONE 7:e43951

0.3% L-Lactic Acid pH 3.9 Elicits an Anti-inflammatory Effect on Cervicovaginal Epithelial Cells

Anti-inflammatory Cytokine



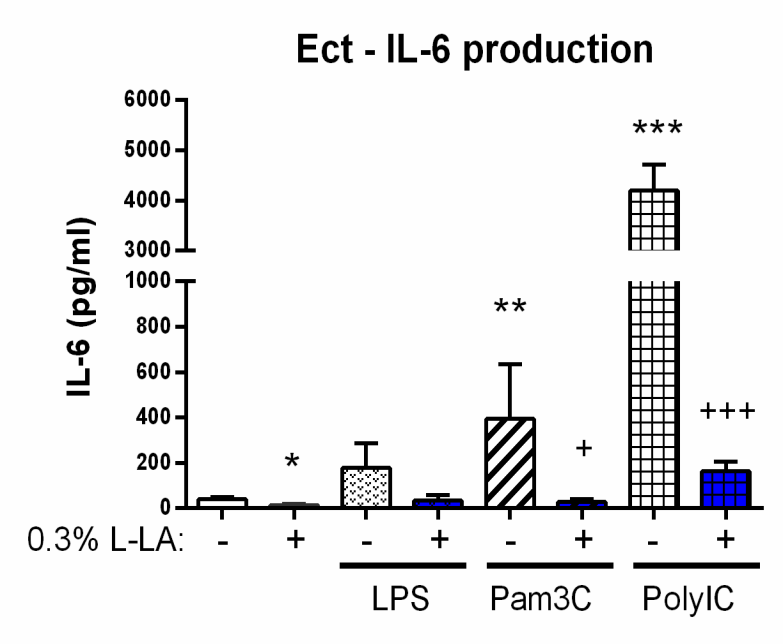
Similar increase
in IL-1RA with if add LA in presence of TLR agonists:
polyIC
LPS
Pam3C

IL1-RA – antagonises IL-1β

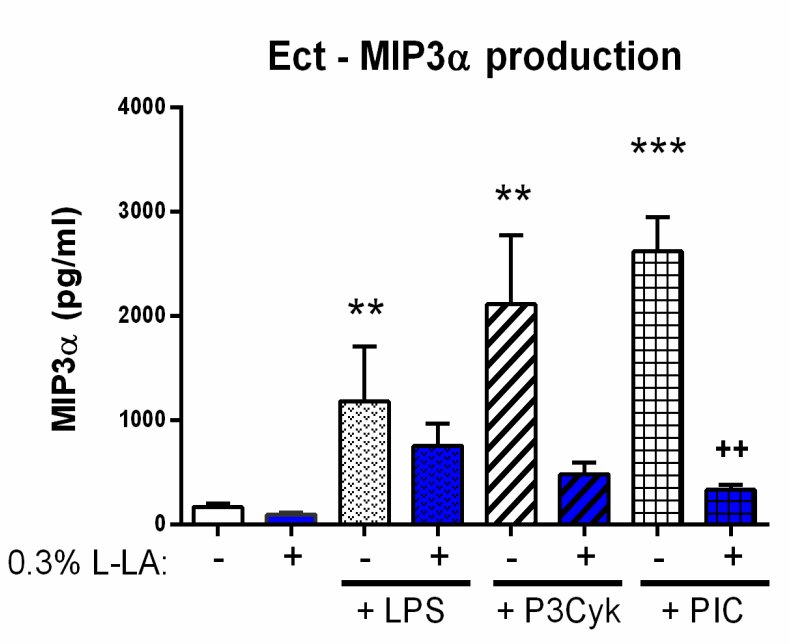
Hearps et al Mucosal Immunol 2017 10: 1480

0.3% L-Lactic Acid pH 3.9 Inhibits TLR-agonist Mediated Inflammation

Pro-inflammatory Cytokine



Pro-inflammatory Chemokine



n≥ 4

Similar effects IL-8, TNF, RANTES

Hearps et al Mucosal Immunol 2017 10: 1480

Summary – Lactic acid dampens inflammation

- Anti-inflammatory Cytokines: IL-1RA ↑
- Pro-inflammatory Cytokines: IL-6, TNF-α ↓
- Chemokines: IL-8, MIP-3α, RANTES ↓
- LA's immune modulatory effect mediated by protonated form and by both the L and D stereoisomers
- LA's effect not simply a low pH effect on cells
- LA pretreatment and post-treatment of epithelial cells inhibits pro-inflammatory mediators elicited by TLR agonists
- LA inhibits pro-inflammatory mediators elicited by genital secretions

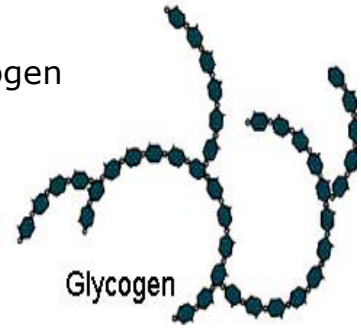
Summary

Lactic acid upregulates junctional molecules and increases barrier function of FRT epithelial cells that might retard HIV transepithelial migration and subsequent infection of target cells

Lactic acid – an Effector Molecule Produced by Vaginal Lactobacilli

Prebiotic

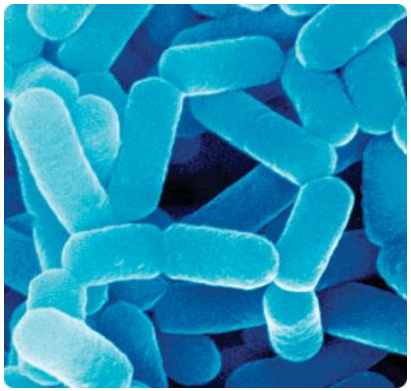
estrogen



α- amylase metabolites

Food source for probiotic

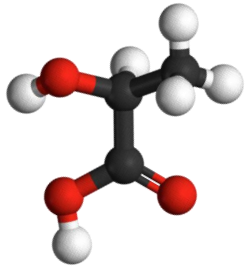
Probiotic



Vaginal Lactobacilli

Compete for binding sites on mucosa
Direct antagonism of pathogens
Reduce inflammation
Strengthen epithelial barrier

Postbiotic

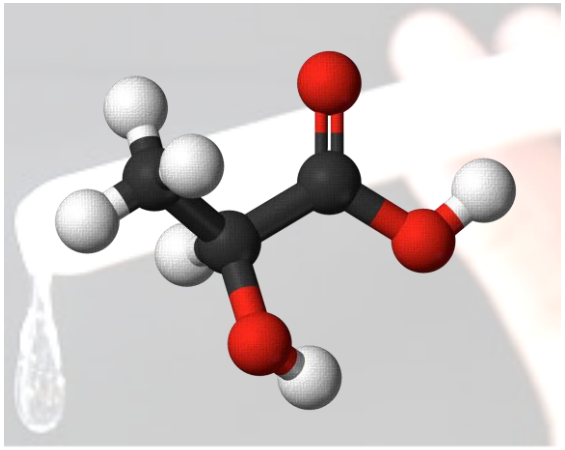


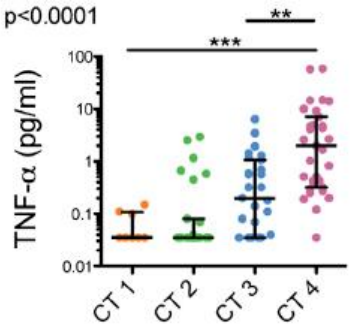
Lactic acid (organic acid metabolites)

Anti-BV bacteria activity
Anti-HIV activity
Anti-inflammatory activity
Epithelial barrier?

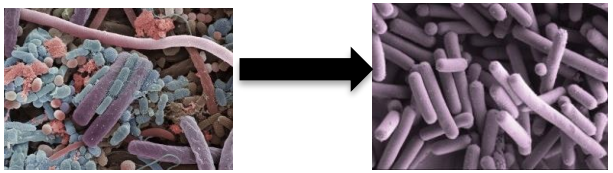
Effect of Lactic acid In Vivo Mechanistic Study in Women with BV

Bespoke LA Vaginal Gels

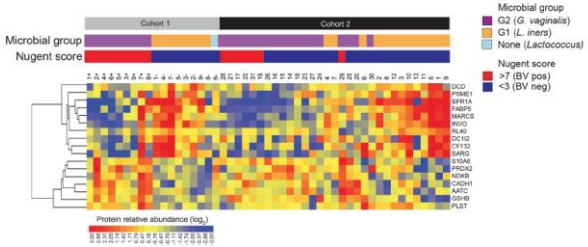




Inflammation



Vaginal microbiome



Vaginal proteome

US Patent:
Anti-inflammatory/BV

EVE-M

transforming women's health

Frontiers in Health and Medical Research Stage 1

Enhancing the vaginal environment and microbiome

Aim: Advance and commercialise a suite of novel interventions to revolutionise sexual and reproductive health products including intravaginal gels and intravaginal rings (MPT)

Burnet Institute Medical Research. Practical Action.

SWINBURNE UNIVERSITY OF TECHNOLOGY

MSHC MELBOURNE SEXUAL HEALTH CENTRE

MONASH University

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE INSTITUTE FOR GENOME SCIENCES

family planning nsw Reproductive & Sexual Health

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