

Immune dysregulation, the vaginal microbiome, and sexual pain disorders

Bernard L. Harlow, Ph.D.

Professor of Epidemiology

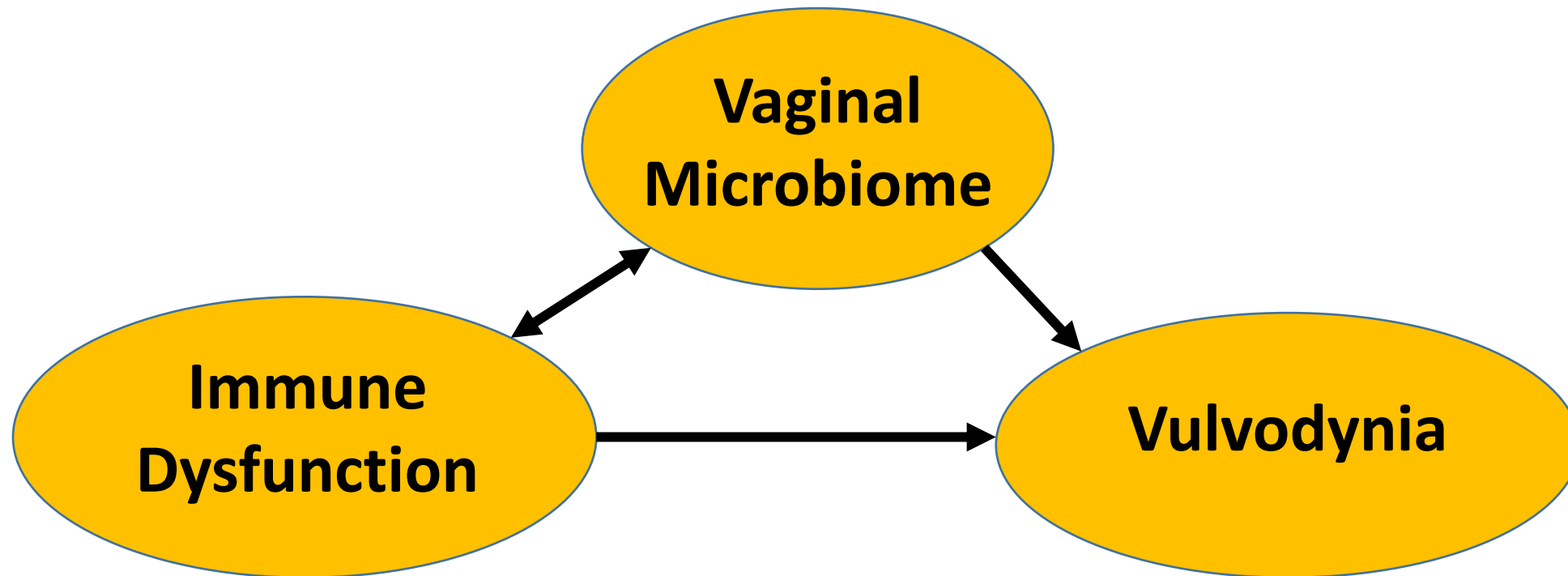
Boston University School of Public Health

Supported by Grants R01-HD038428 and R01-HD058608
From the National Institutes of Health

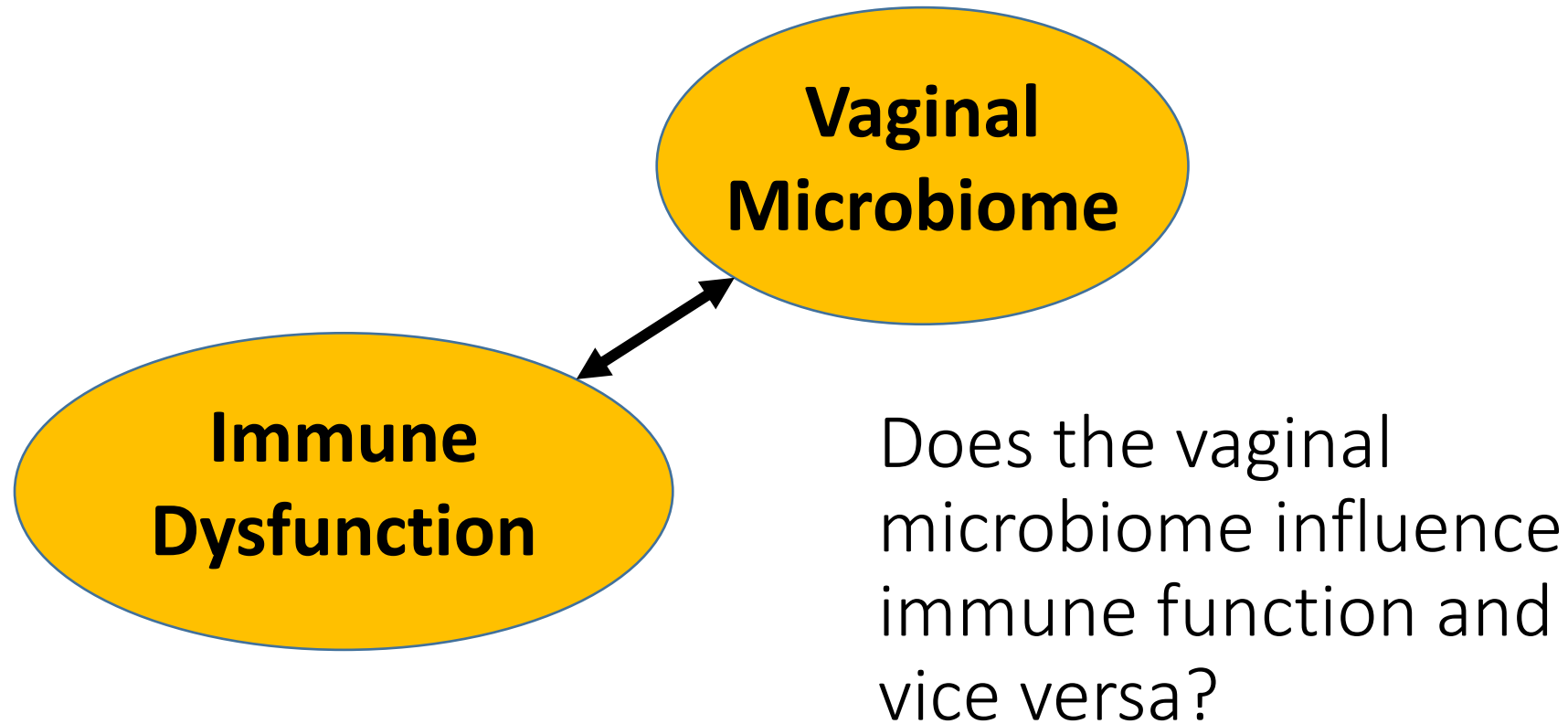
In the spirit of full disclosure

- I am **NOT** a clinician
- I am **NOT** a molecular biologist
- I am **NOT** an immunologist
- **I AM** an Epidemiologist

Pathways to be discussed

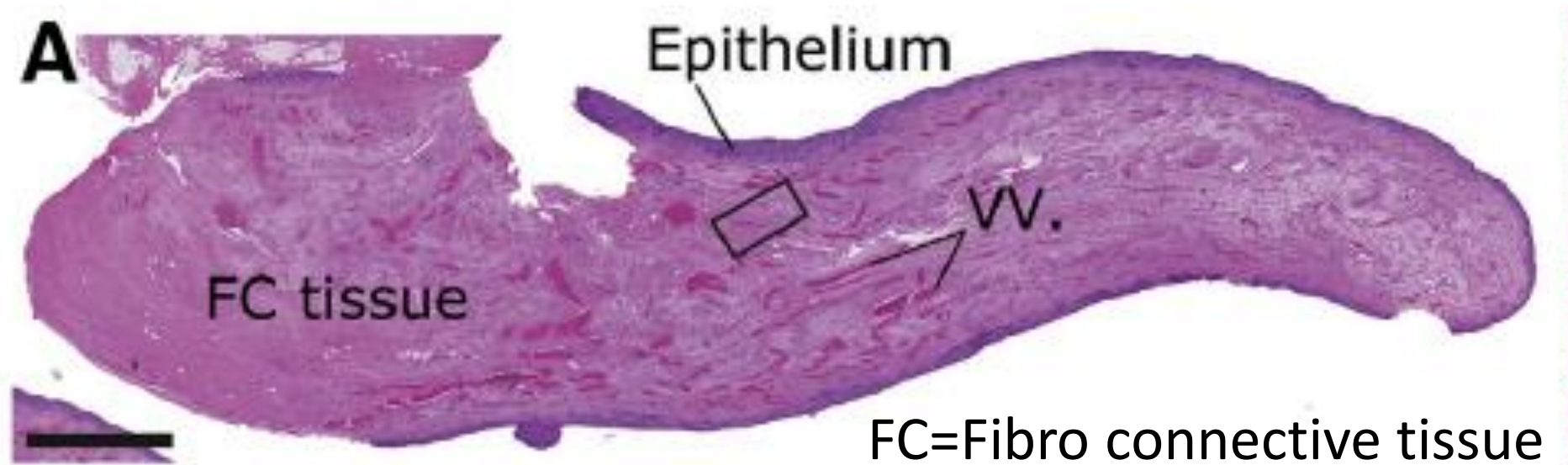


Pathways to be discussed



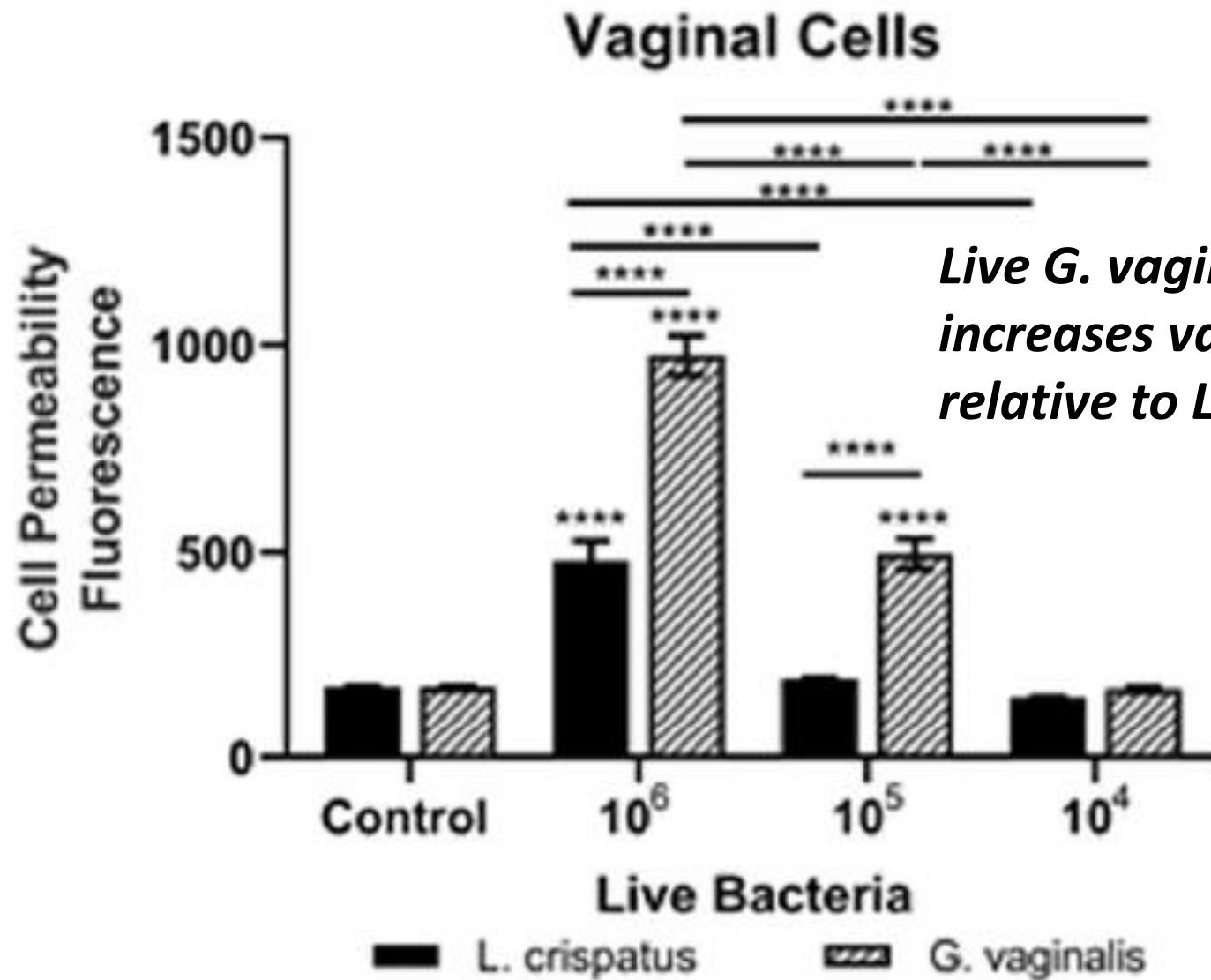
Vaginal Microbiome and Immune Function

- Mutual symbiosis
- Microbiomes impact immune activators and immune regulators when the epithelial barrier is breached
- There are specific vaginal epithelial cell immune responses to microbe-specific signals



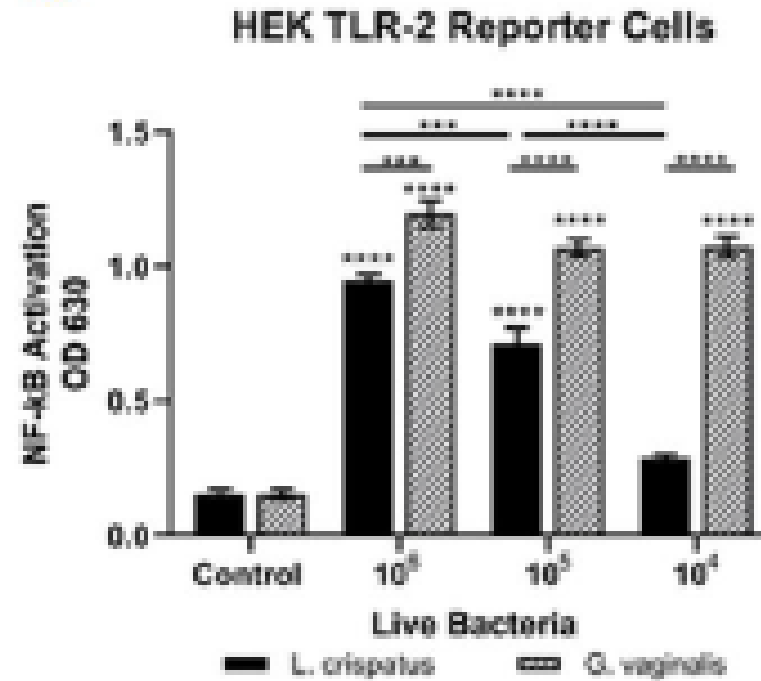
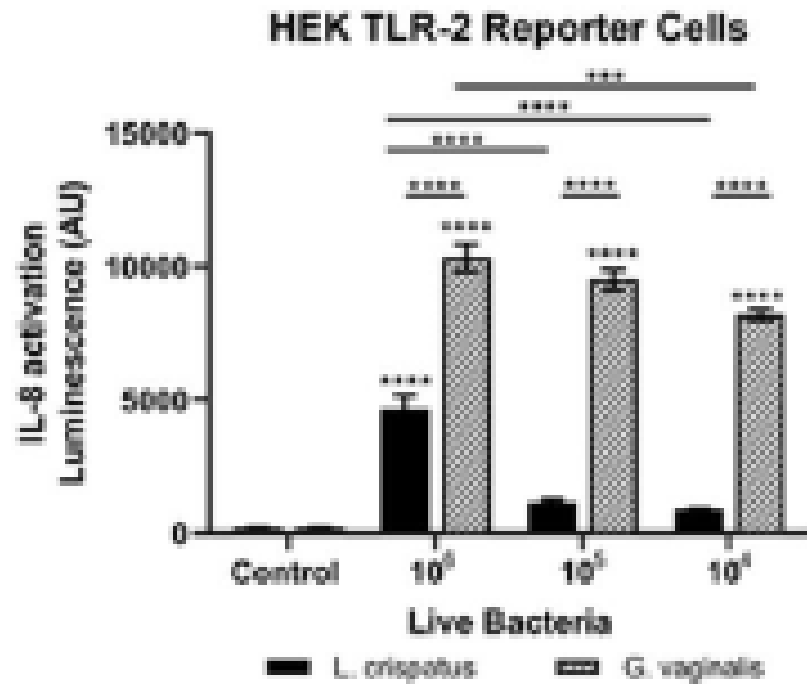
FC=Fibro connective tissue
VV=Interspersed blood vessels

Jackson LA et al, Am J Obstet Gynecol 2019;221:519.e1-10.



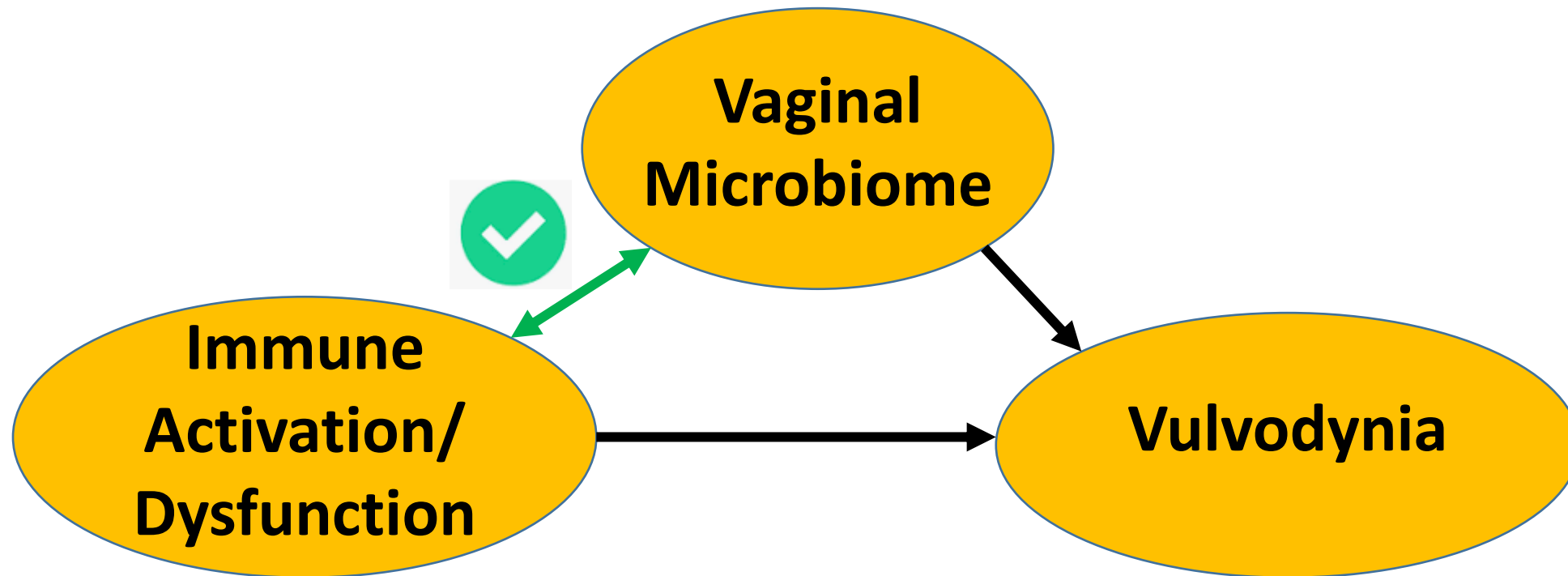
Live G. vaginalis significantly increases vaginal cell permeability relative to L Crispatus

Anton et al. Microbiome (2022) 10:119

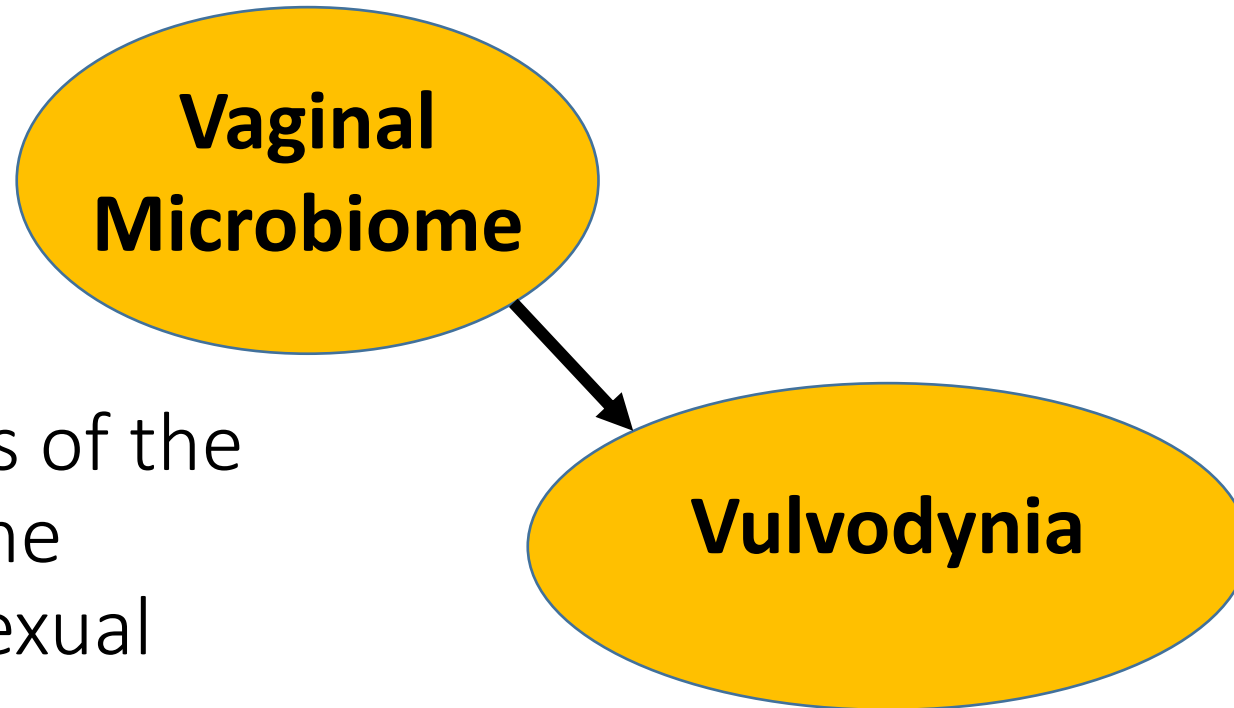


Live G. vaginalis significantly increases cytokine production relative to L Crispatus

Pathways to be discussed



Pathways to be discussed



Are characteristics of the vaginal microbiome associated with sexual pain disorders?

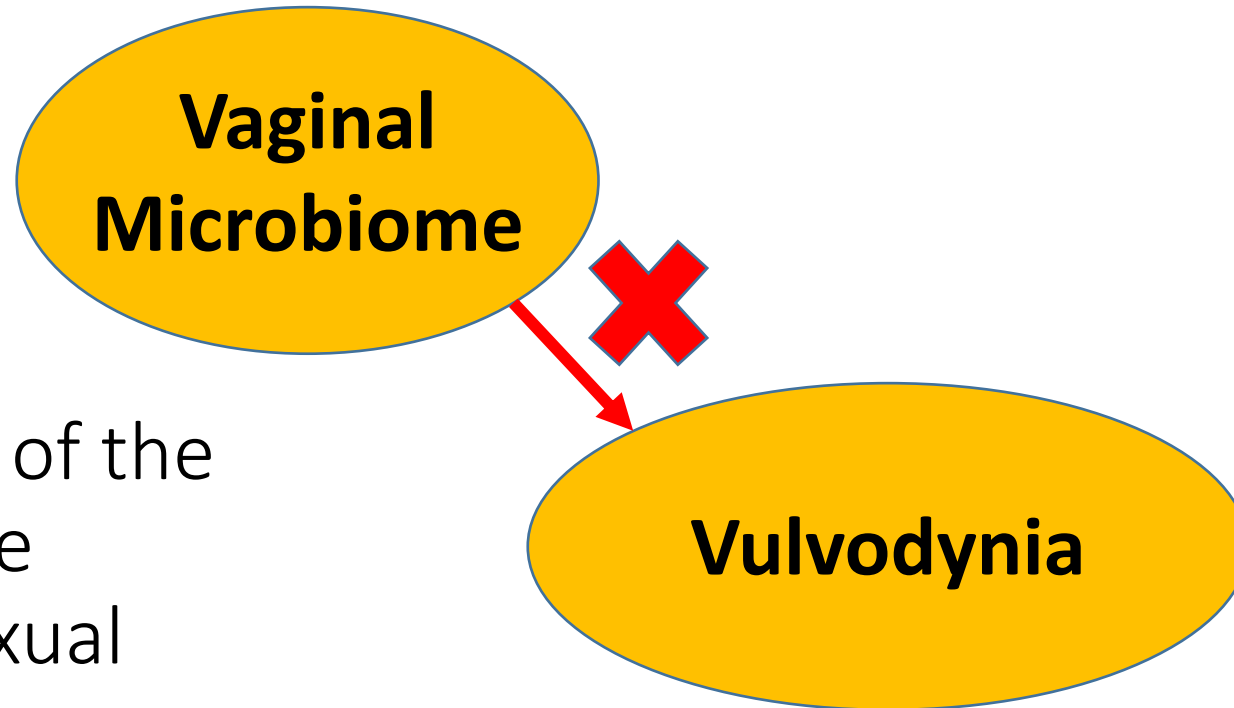
Systematic Review of case-control studies on vaginal microbiota and vulvodynia

	Cas/Ctls	Overall associations
Ventolini et al 2013	17 total	Similar microbiota; L. Crispatus only in controls
Jayaram et al 2014	30/15	Similar microbiota
Mitchel et al 2020	30/52	Similar microbiota
Murina et al 2020	20/18	Similar microbiota
Bedford et al 2020	215/222	Similar microbiota
Park et al 2021	22/22	Similar diversity; variation in dominant genera
Panzarella et al 2022	29/26	Similar microbiota
Awad-Igbaria et al 2022	9/21	Similar microbiota

- No taxa aligned with vulvodynia
- No predominant Lactobacillus
- No difference in alpha diversity
- No difference in beta diversity

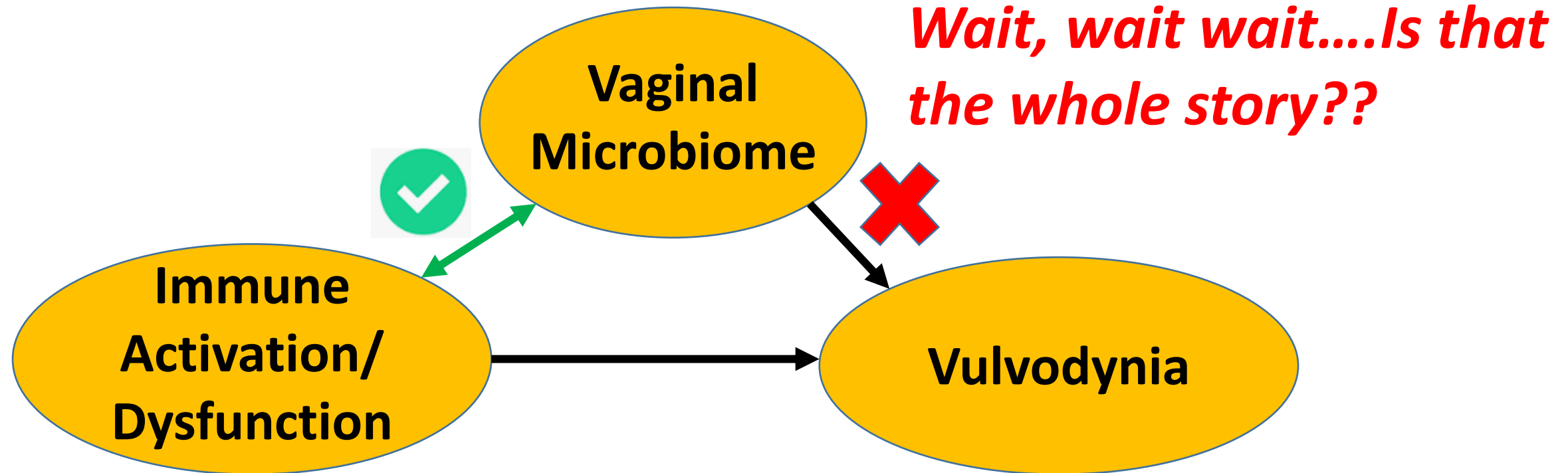
All sampled using vaginal swabs and all used 16s rRNA amplification

Pathways to be discussed



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GYNECOLOGY

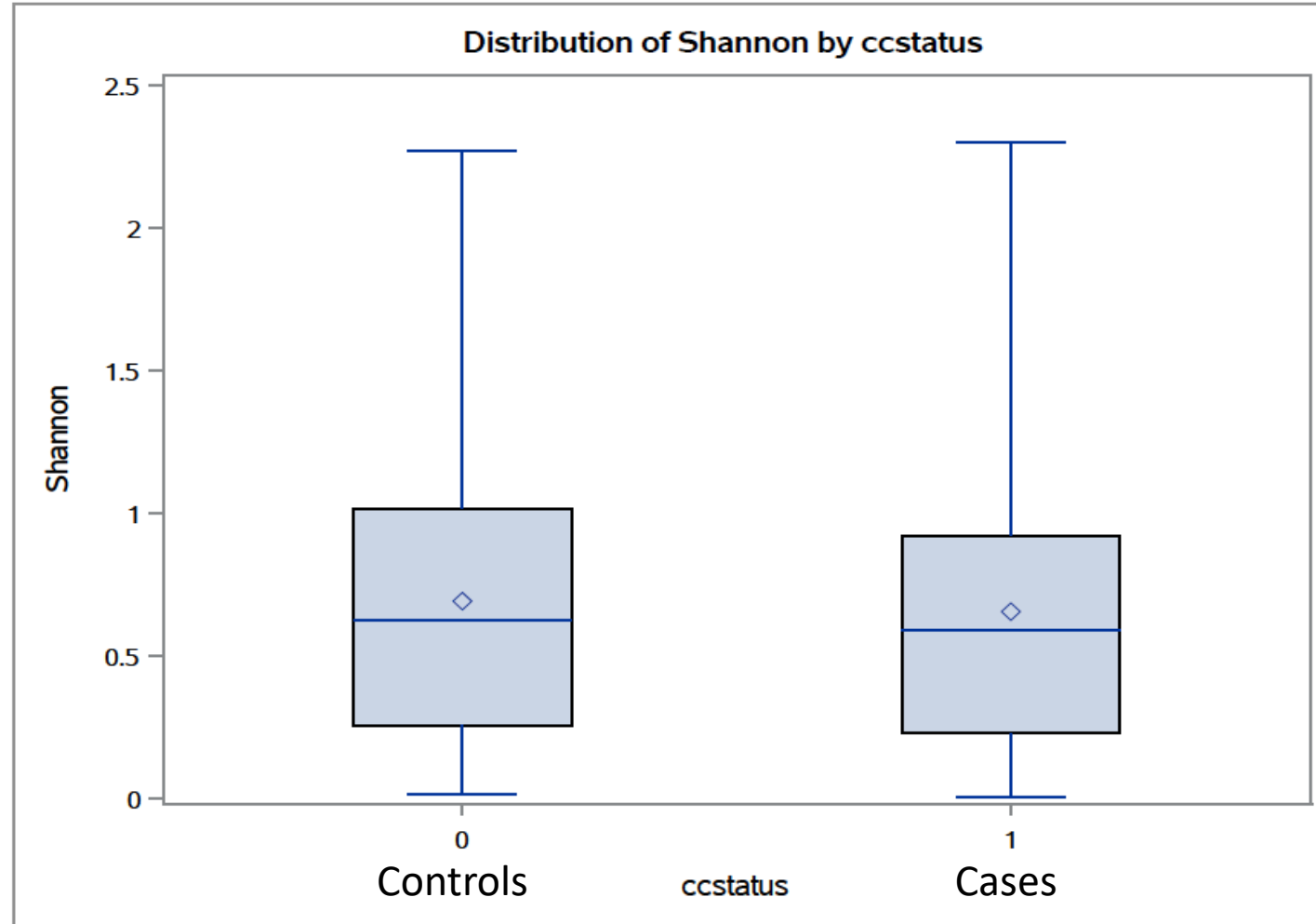
Characteristics of the vaginal microbiome in women with and without clinically confirmed vulvodynia



Lisa Bedford, MS; Samantha E. Parker, PhD; Elyse Davis, MPH; Elizabeth Salzman, MS; Sharon L. Hillier, PhD; Betsy Foxman, PhD¹; Bernard L. Harlow, PhD¹

- Assessed Alpha diversity of lactobacillus and clustering of microbiomes into categorizations of community state types
- How these microbiome characteristics impact known risk factors for vulvodynia

Distribution of Shannon Alpha Diversity in Microbiomes among Vulvodynia Cases and Controls



Recurrent Yeast Infections and Vulvodynia: Can We Believe Associations Based on Self-Reported Data?

Bernard L. Harlow, PhD^{1,2}, Rachel E. Caron, BS¹, Samantha E. Parker, PhD²,
Devavani Chatterjea, PhD³, Matthew P. Fox, DSc² and Ruby H.N. Nguyen, PhD¹

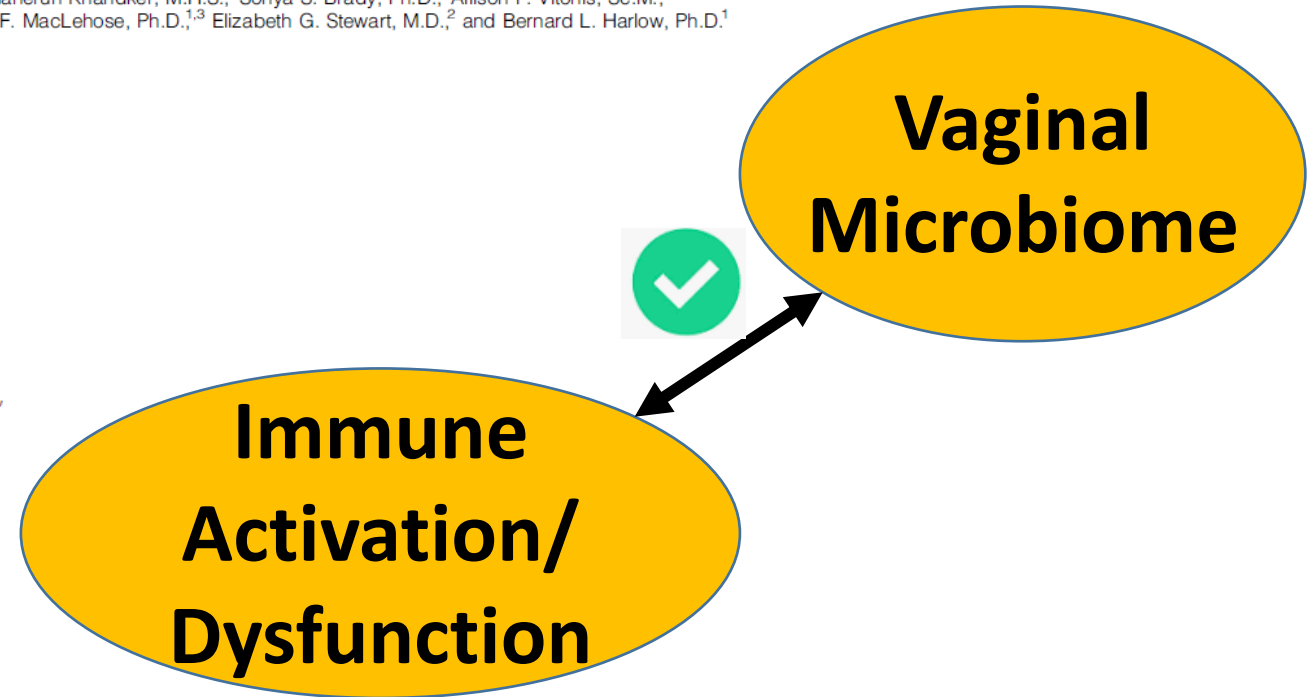
> J Sex Med. 2019 Jun;16(6):880-890. doi: 10.1016/j.jsxm.2019.03.010. Epub 2019 Apr 19.

Early-life Chronic Stressors, Rumination, and the Onset of Vulvodynia

Maheruh Khandker¹, Sonya S Brady², Sarah A Rydell², Rachel M Turner², Pamela J Schreiner²,
Bernard L Harlow³

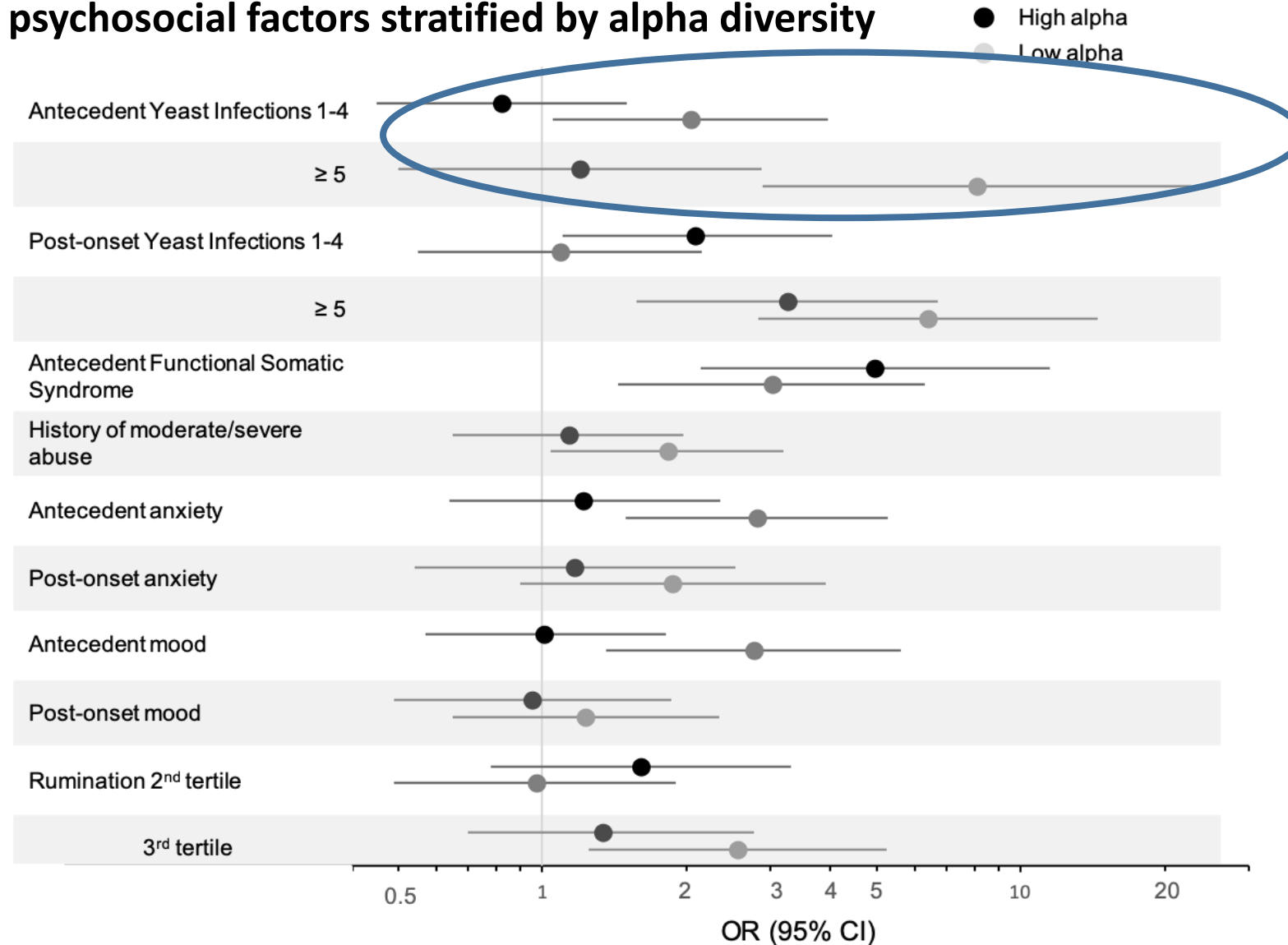
The Influence of Depression and Anxiety on Risk of Adult Onset Vulvodynia

Maheruh Khandker, M.H.S.¹, Sonya S. Brady, Ph.D.¹, Allison F. Vitonis, Sc.M.²,
Richard F. MacLehose, Ph.D.^{1,3}, Elizabeth G. Stewart, M.D.² and Bernard L. Harlow, Ph.D.¹



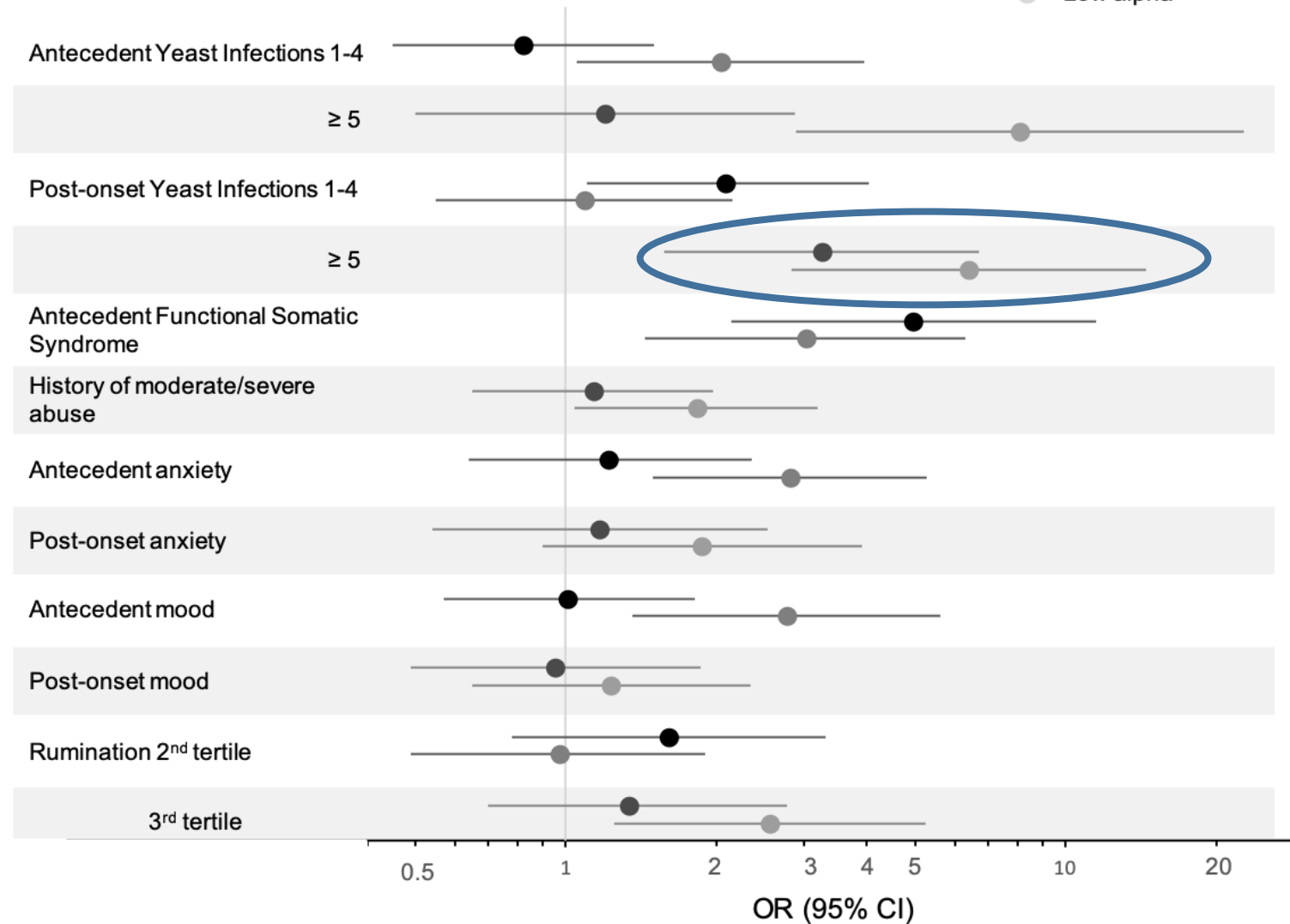
*Is it possible that characteristics of the microbiome
could influence these associations?*

Age-adjusted odds ratios for yeast infections and psychosocial factors stratified by alpha diversity

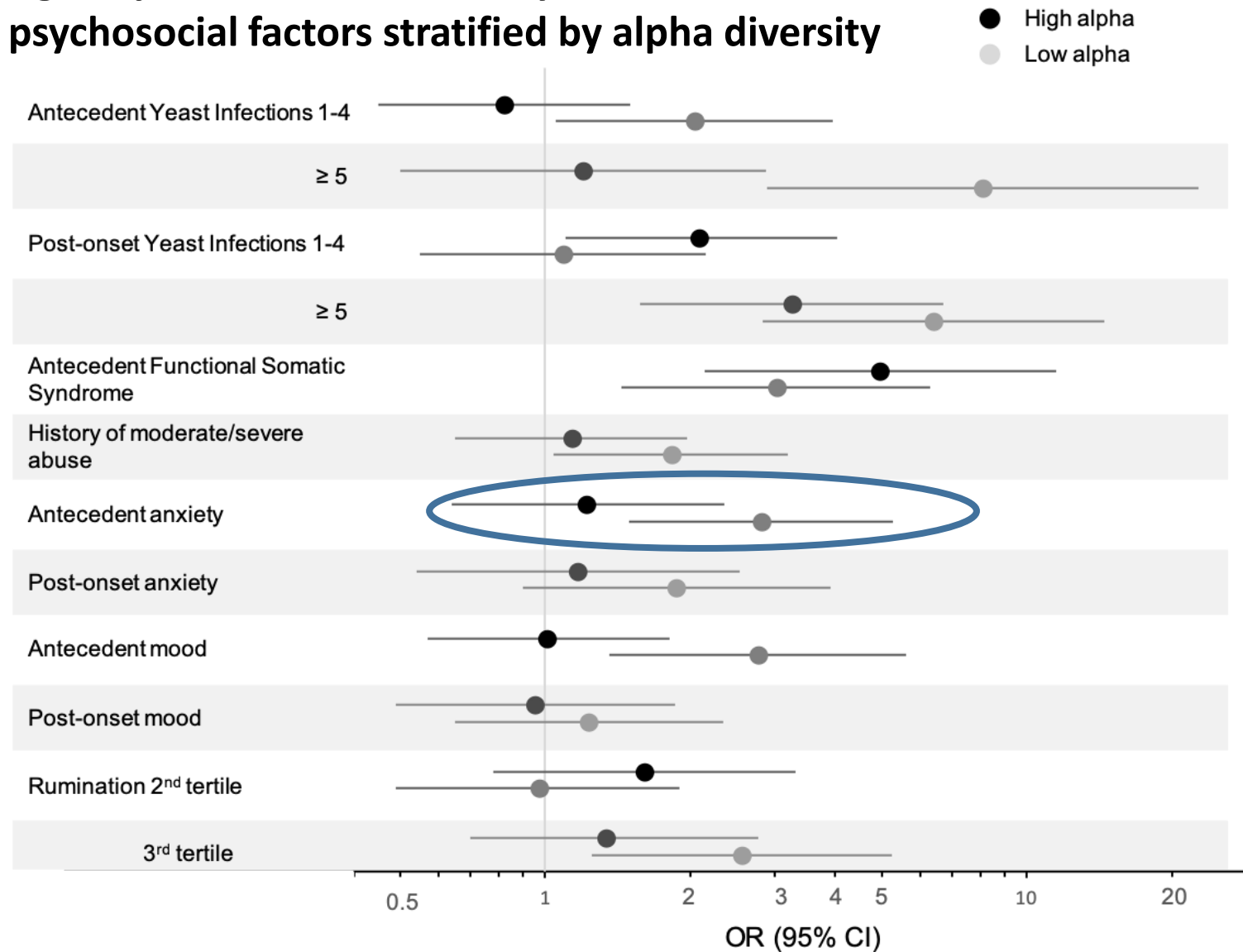


Age-adjusted odds ratios for yeast infections and psychosocial factors stratified by alpha diversity

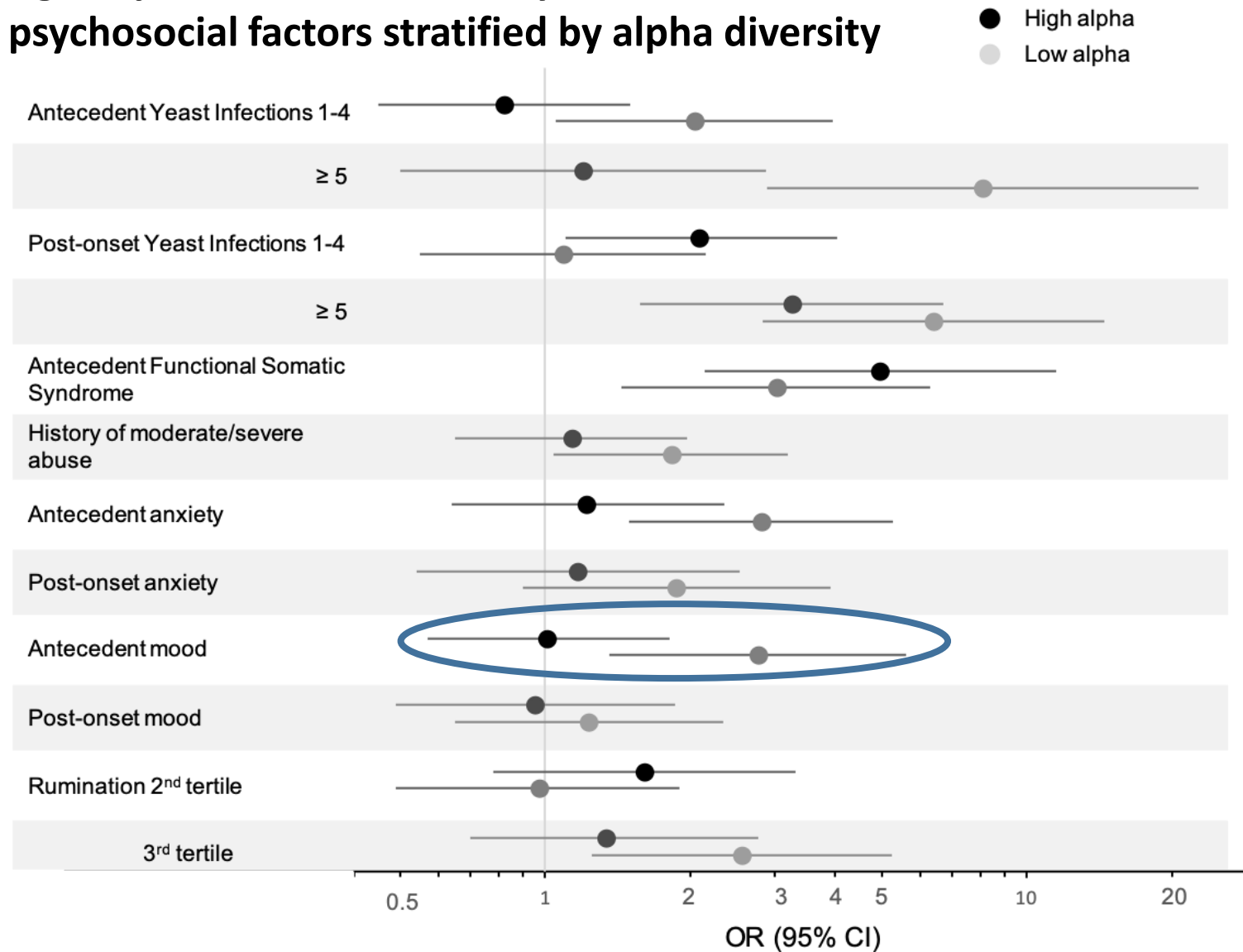
● High alpha
● Low alpha



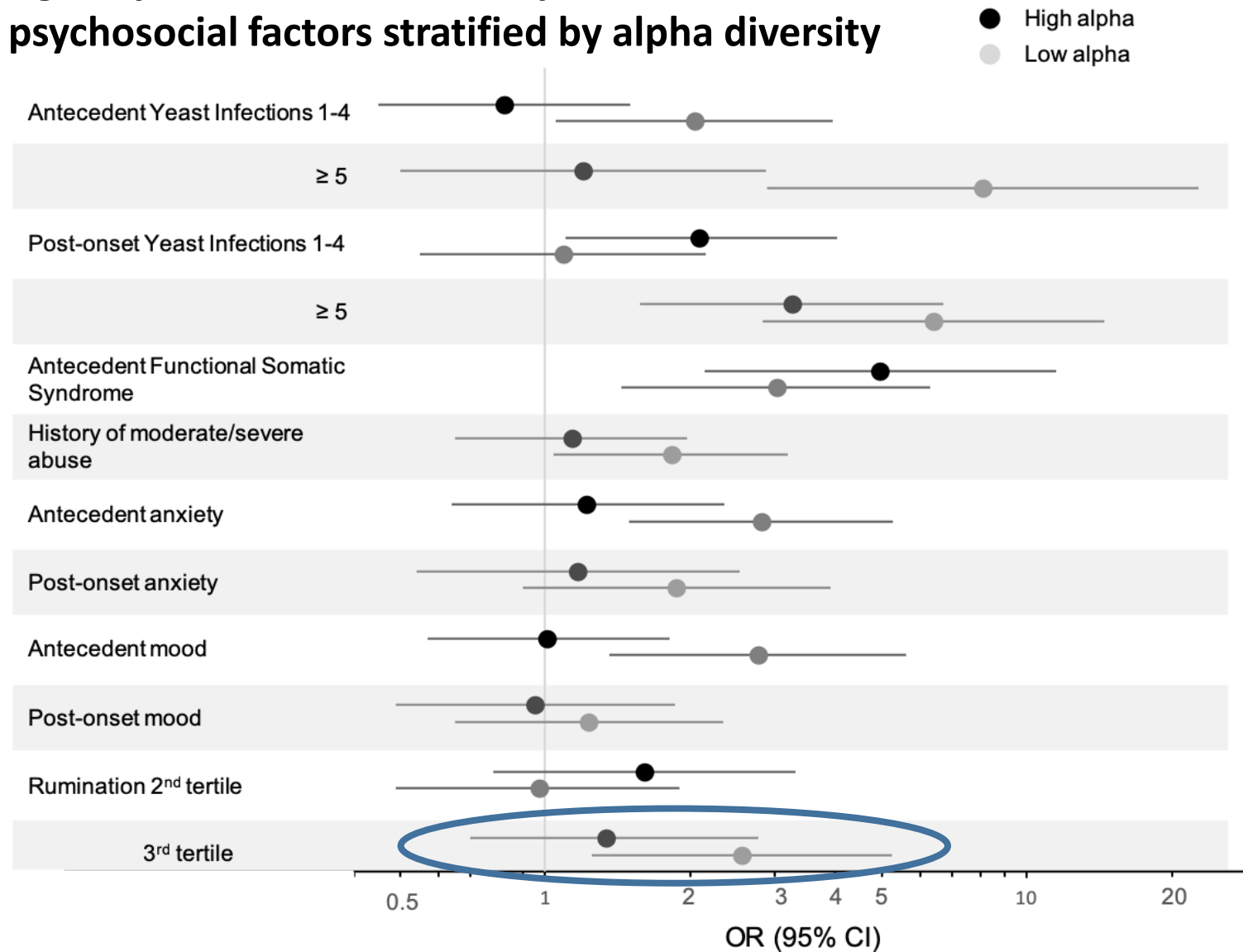
Age-adjusted odds ratios for yeast infections and psychosocial factors stratified by alpha diversity



Age-adjusted odds ratios for yeast infections and psychosocial factors stratified by alpha diversity



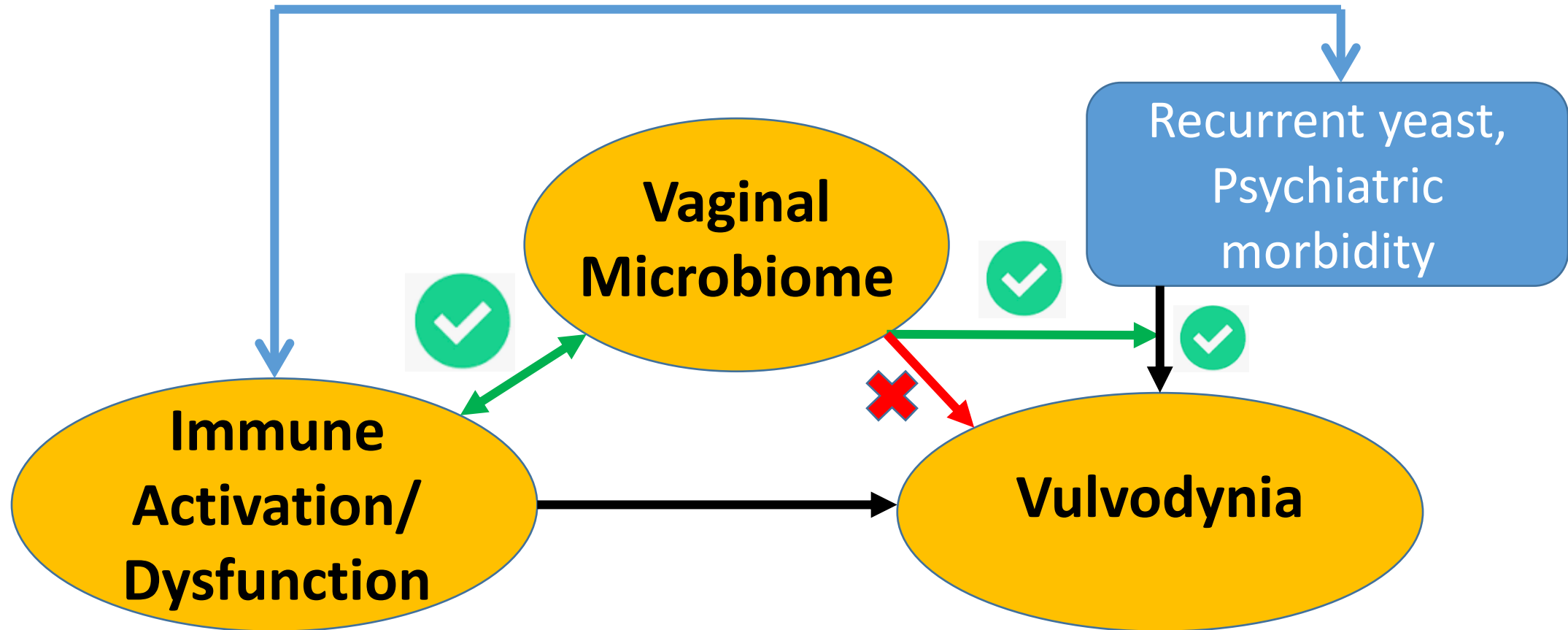
Age-adjusted odds ratios for yeast infections and psychosocial factors stratified by alpha diversity



Conclusions

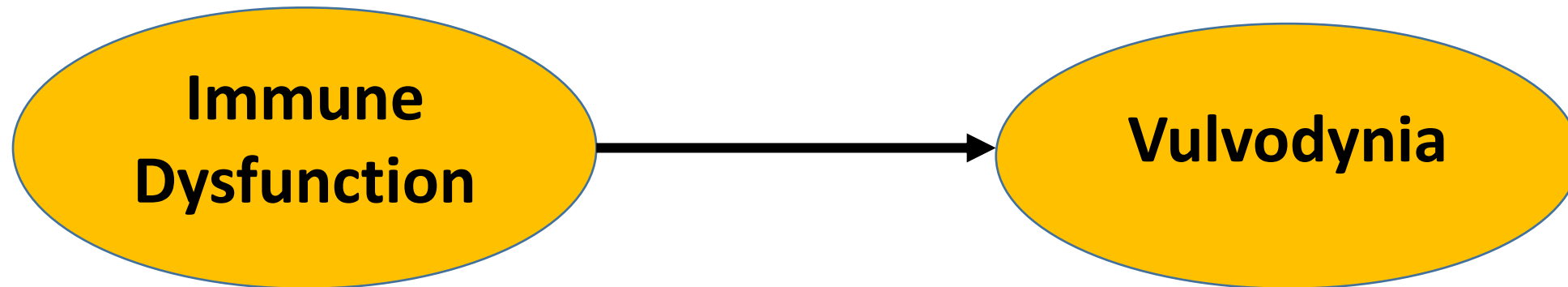
- Characteristics of the vaginal microbiome, in general, are not associated with risk of vulvodynia.
- Antecedent yeast infections and psychosocial risk factors are associated with vulvodynia only in women with alpha diversity below the median.
- Uncertain whether vaginal microbiome diversity modifies the association between these risk factors and vulvodynia or becomes altered as a consequence of these associations.
- Less diverse microbiome environments might indicate changes in bacteria with anti-inflammatory properties or pro-inflammatory signaling molecules; either might result in an altered immune inflammatory state that may facilitate risk factors to influence inflammation and nerve growth proliferation or alteration.

Pathways to be discussed



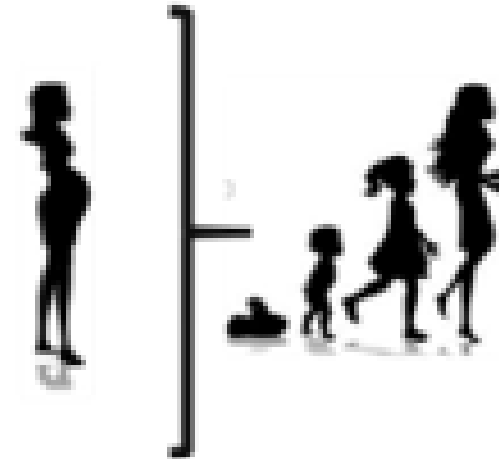
Pathways to be discussed

Is there evidence that immune dysfunction plays a role in the etiology of sexual pain disorders?



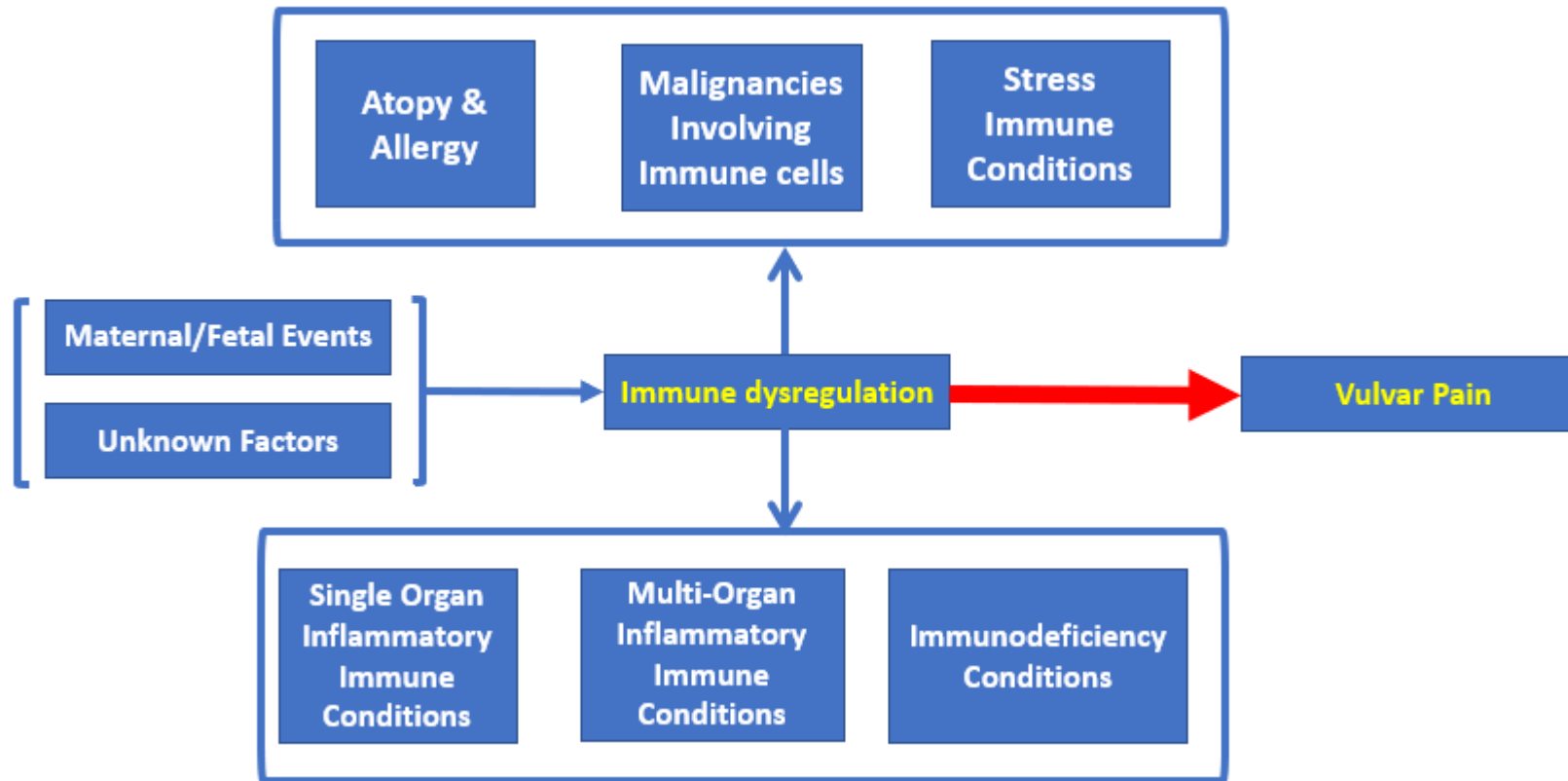
Immune dysfunction across the life-course

- ❖ Life Course Study
- ❖ Swedish National Registries
- ❖ Immune Related Events
 - ❖ At birth
 - ❖ Immune-specific conditions
 - ❖ Stress Immune Conditions



Swedish National Registry Study

Current Etiological Hypothesis



Swedish Registry Study Methods

- STUDY POPULATION

- Swedish Administrative Data from Medical Birth, Outpatient and Inpatient Registries
- All women born in Sweden between 1973-1996
- By 2018, these women were 22-45 years of age

- SAMPLE – Case Control Design

- **Cases:** All women from the Study Population with a diagnosis of Vulvodynia (ICD10 code=N94.2, N76.3, **N=4,787**) or Vaginismus (ICD10 code=F52.5, **N=2,063**), or both (**N=867**)
- **Controls:** Two randomly selected women per case with no vulvodynia or vaginismus codes (**N=15,434**)

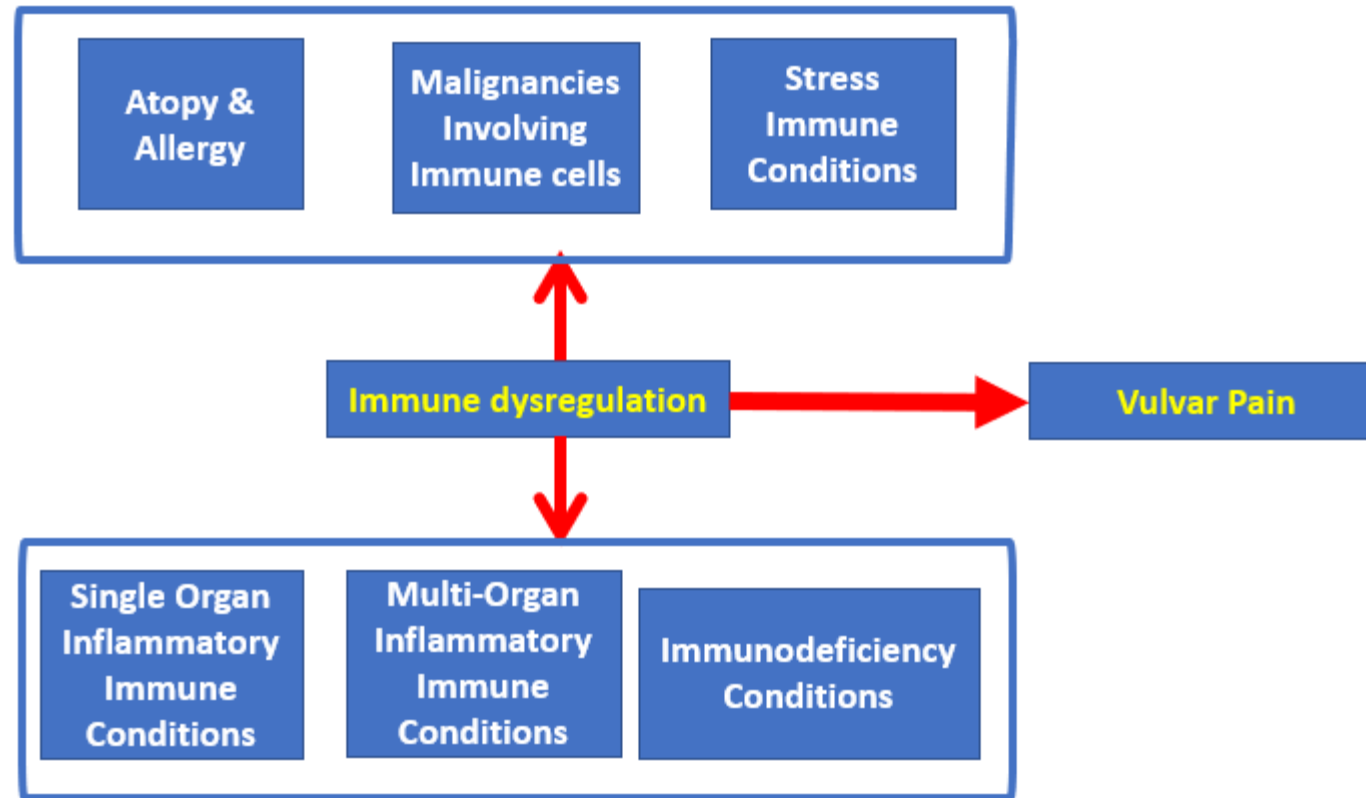
Swedish National Registry Study *Current Etiological Hypothesis*



Maternal/Fetal Exposures	Crude OR (95%CI)	Adjusted ^{&} OR (95%CI)
Delivered by Cesarean Section	1.1 (1.0-1.2)	1.1 (1.0-1.2)
Moderate/Severe SGA	1.1 (1.0-1.2)	1.1 (1.0-1.2)
Preterm Birth (<37 weeks)	1.2 (1.1-1.3)	1.2 (1.1-1.4)
Low Birthweight (<2500grams)	1.3 (1.1-1.5)	1.3 (1.2-1.5)
^{&} Adjusted for Birth Year, Parity, Education, Region of Residency		

Harlow et al, J Pain 2023

Swedish National Registry Study *Current Etiological Hypothesis*



Swedish Registry Study Methods

Immune-related Categories

- Immunodeficiencies
 - Antibody Defects
 - Combined Immunodeficiencies
- Single Organ Autoimmune disorders
 - Iodine deficiency Thyroid disorders
 - Adrenal gland deficiencies
 - Crohn's and other GI autoimmune disease
- Multi-Organ Autoimmune disorders
 - Polyglandular dysfunction
 - Hemorrhagic Conditions
 - Arthritic conditions
- Immune Cell Malignancies
 - Hodgkin's Lymphoma
 - Mast cell neoplasms
- Allergy/Atopy Conditions
 - Vasomotor allergenic conditions
 - Urticaria
 - Dermatitis conditions
 - Asthma
 - Food/drug allergies
- Stress Immune Conditions
 - Depression; Anxiety; Behavioral Disorders

Swedish Registry Study Methods

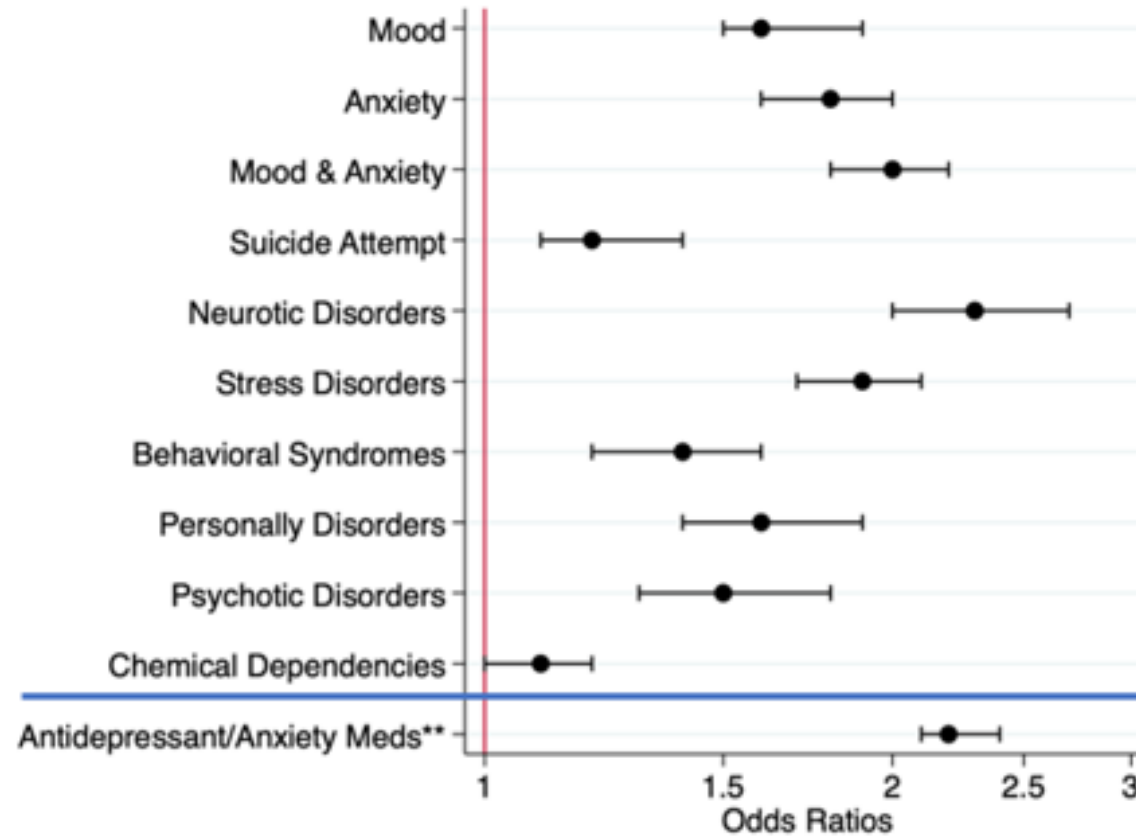
Immune-related Categories

Immune Related Conditions	All Women <u>aOR^{&}</u> (95%CI)	No MFM Conditions <u>aOR^{&}</u> (95%CI)
Immunodeficiency Diagnoses	2.2 (1.4-3.4)	2.5 (1.5-1.1)
Single Organ Immune conditions	1.6 (1.5-1.7)	1.6 (1.5-1.7)
Multi-organ immune conditions	1.5 (1.4-1.6)	1.6 (1.5-1.7)
Immune Cell Malignancies	1.5 (0.9-2.5)	1.6 (1.0-2.7)
Allergy/Atopy Conditions	1.7 (1.6-1.8)	1.7 (1.6-1.8)
Stress Immune - Depression	2.0 (1.8-2.2)	2.2 (2.0-2.3)
Stress Immune - Anxiety	2.1 (2.0-2.3)	2.0 (1.9-2.2)
^{&} Adjusted for Birth Year, Parity, Education, Region of Residency		

MFM=Maternal-Fetal Conditions

Harlow et al, J Pain 2023

Figure 1. Forest plot of Odds Ratios* and 95% confidence intervals for the association between psychiatric disorders or pharmaceutical prescriptions of antidepressants or anxiolytics and vulvar pain.



* Adjusted for Birth Year, Parity, Education, Birth region of Residency, c-section delivery, preterm birth, low birthweight

**Includes codes beginning in 2005

Swedish Registry Study Methods

Immune-related Categories

Multiple Unique ICD coded Immune Conditions	Crude OR (95%CI)	Adjusted ^{&} OR (95%CI)
None	1.0	1.0
1 Immune condition code only	1.4 (1.4-1.6)	1.6 (1.5-1.7)
2 Immune condition codes	2.0 (1.8-2.1)	2.1 (2.0-2.3)
3 or more Immune condition codes	2.9 (2.5-3.4)	3.3 (2.8-3.9)

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Certain birth events associated with 10-30% greater likelihood of vulvar pain

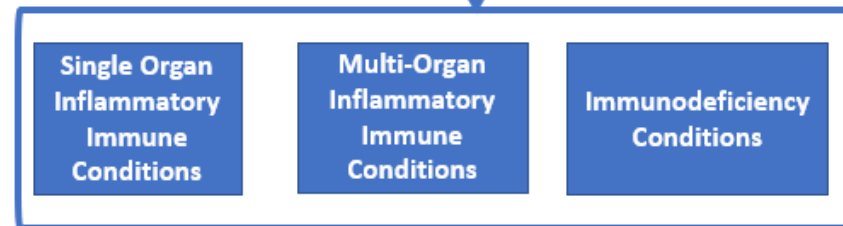


Immune dysregulation

Immune exposures associated with 50-100% greater likelihood of having vulvar pain



Vulvar Pain



3 or more Immune exposures associated with 2-3x greater likelihood of having vulvar pain

ICD-10 Codes - Overall Global Categories of Diseases (Excluding all primary immune-related conditions)	No Vulvar Pain (N=15,434)	Vulvodynia, Vaginismus, or both (N=4787)	Percent Difference
	N (%)	N (%)	%
N80-N99.9 (Non-inflammatory disorders of female genital tract)	5,698 (36.9)	4,846 (62.8)	25.9
L00-L99 (Skin conditions)	3,253 (21.1)	2,677 (34.7)	13.6
A00-B99 (Infectious and parasitic diseases)	9,674 (62.7)	5,797 (75.1)	12.4
F00-F99 (Mental health and behavioral disorders)	4034 (26.1)	2866 (37.1)	11.0
K20-K99 (Digestive diseases, Appendix, hernias, liver)	3,301 (21.4)	2,504 (32.4)	11.0
Z00-Z99 (Health care screenings and counselling)	9,678 (62.7)	5,648 (73.2)	10.5
M00-M99 (Arthropathies, orthopedic, bone conditions)	4,491 (29.1)	2,966 (38.4)	9.3
N00-N39.9 (Kidney and bladder conditions)	2,029 (13.1)	1,519 (19.7)	6.6
H00-H99 (Disorders of the eye and ear)	3,667 (23.8)	2,344 (30.4)	6.6
G00-G99 (CNS Inflammatory disorders)	2,120 (13.7)	1,552 (20.1)	6.4
V00-X99 (Collisions, falls, accidents, assaults)	2,156 (14.0)	1,452 (18.8)	4.8
C00-D49.9 (Benign/malignant neoplasms)	4,574 (29.6)	2,630 (34.1)	4.5
J00-J99 (Respiratory conditions)	3,193 (20.7)	1,885 (24.4)	3.7
S00-T99 (Injuries, trauma, poisonings, maltreatment)	7,758 (50.3)	4,132 (53.5)	3.2
N60-N64.9 (Benign breast related conditions)	623 (4.0)	470 (6.1)	2.1
E00-E99 (Endocrine organ disorders)	2270 (14.7)	1288 (16.7)	2.0
I00-I99 (CVD disorders and diseases)	982 (6.4)	636 (8.2)	1.8
K00-K19.9 (Oral cavity, salivary glands, mouth and jaw)	737 (4.8)	503 (6.5)	1.7
D50-D99 (Anemias, Spleen, Blood disorders)	571 (3.7)	341 (4.4)	0.7

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**Immune
Related??**

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**Health-care
Seeking??**

Pathways to be discussed

Is there evidence that immune dysfunction plays a role in the etiology of sexual pain disorders?



But wait! There's more!

➤ J Low Genit Tract Dis. 2021 Oct 1;25(4):296-302. doi: 10.1097/LGT.0000000000000620.

Characterizing Differences in Thymic Function in Women With and Without Vulvodynia: A Community-Based Study

Sydney K Willis ¹, Allison E Aiello ², Devavani Chatterjea ³, Julie A Nelson ⁴, Patricia L Hibberd ⁵, Bernard L Harlow ¹

- Thymus produces T-cells - orchestrates immune response
- Signal joint T-cell receptor excision circles (sjTREC) measured from DNA
- sjTREC serves as marker of T-cell production
- Lower sjTREC → reduced functional immunity
- Assessed in women with and without vulvodynia

Figure 1a. TREC values per 100,000 stratified by case-control status by age

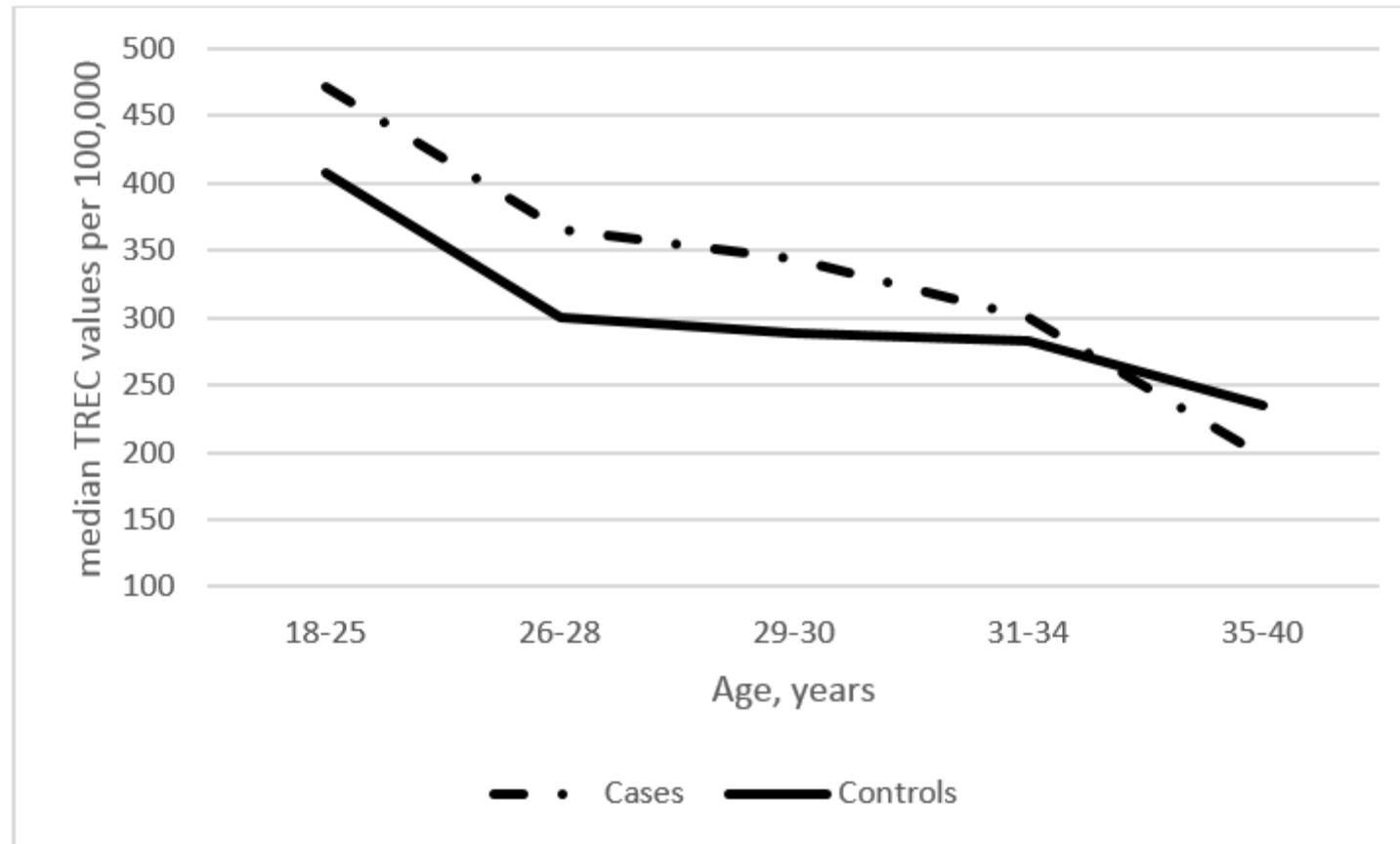
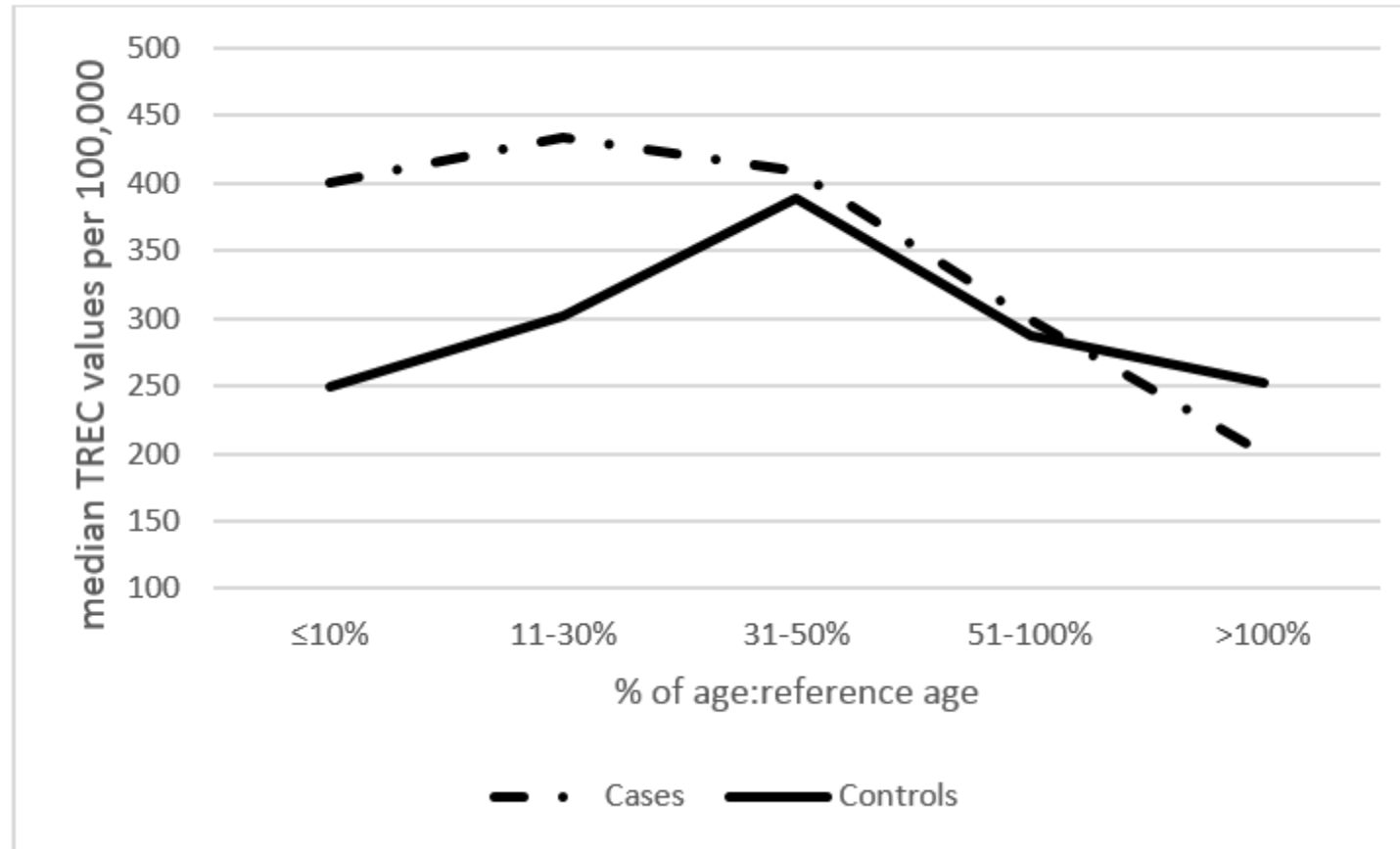
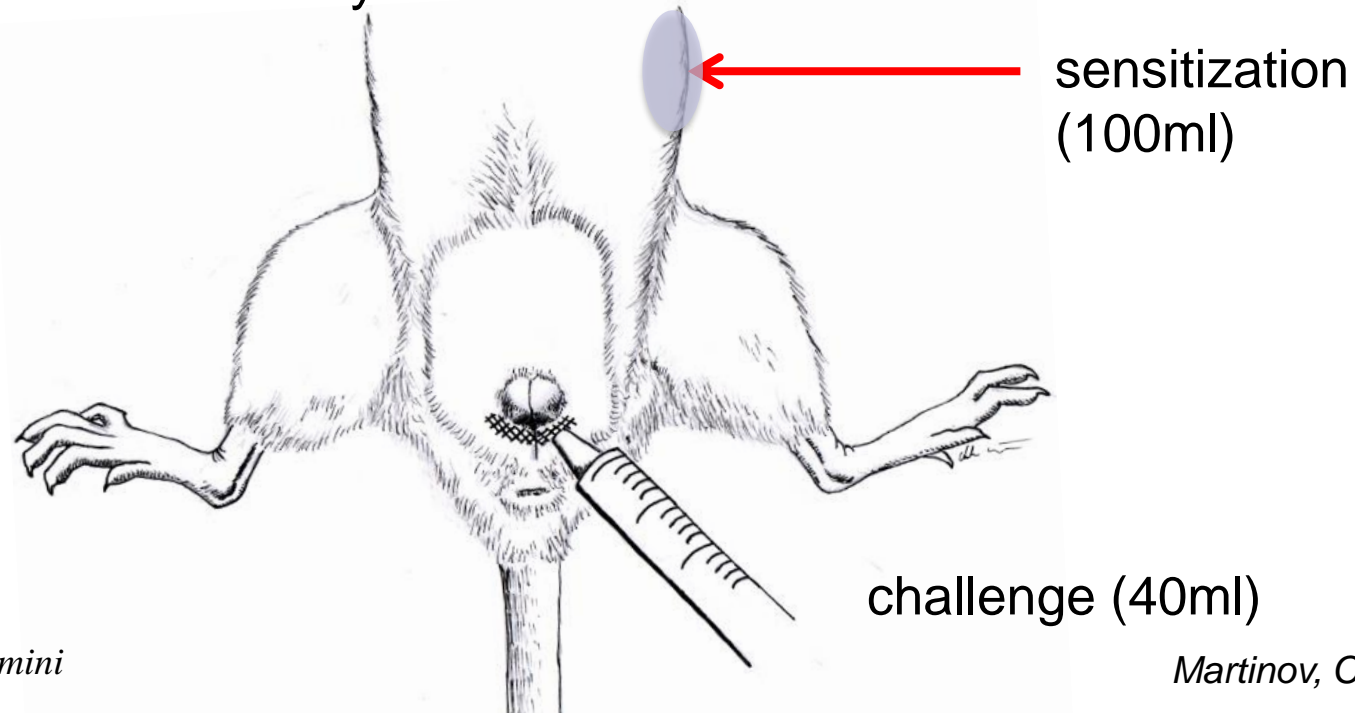


Figure 1c. TREC values per 100,000 stratified by case-control status by ratio of age:reference age

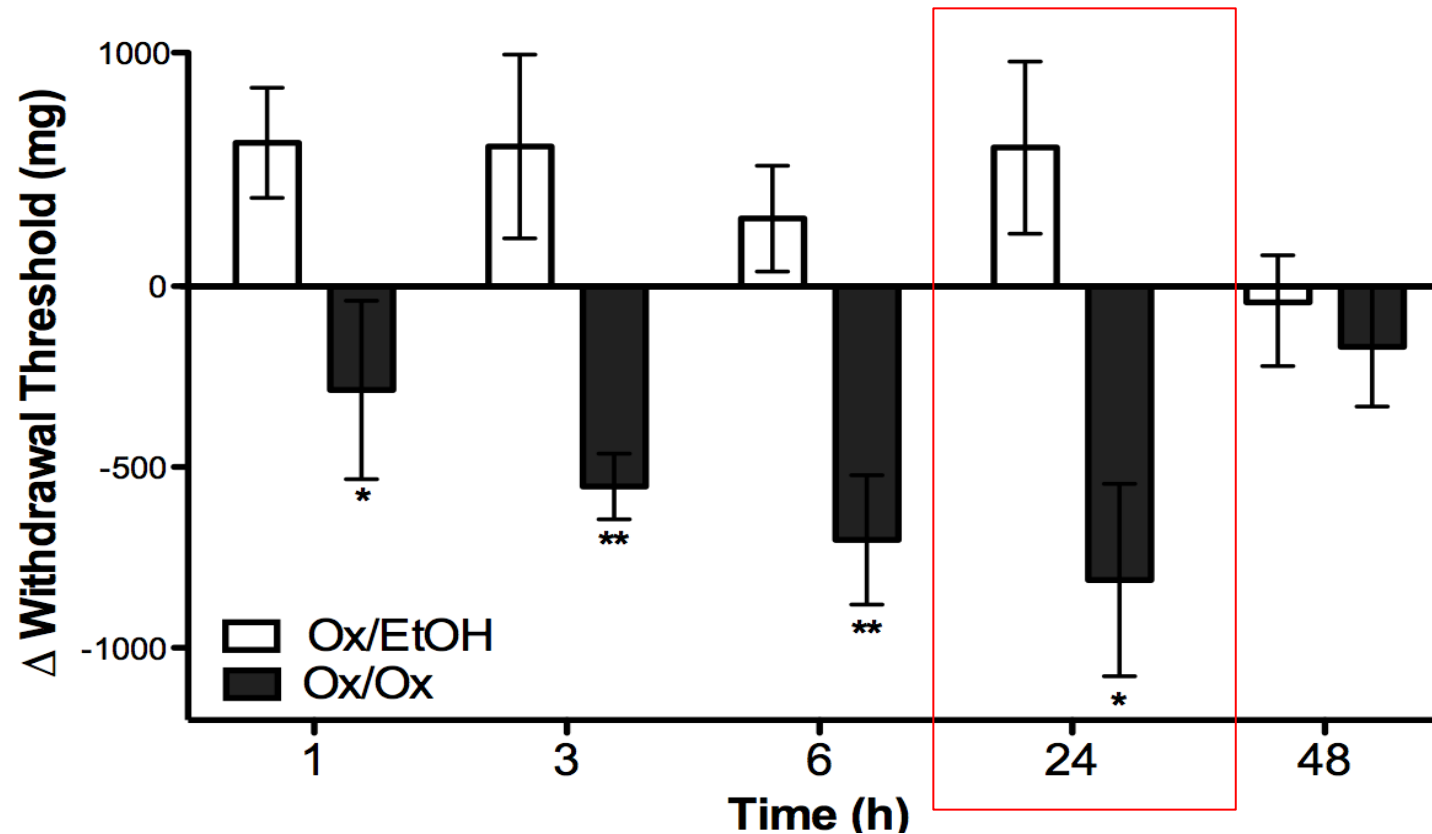


Oxazolone-Contact hypersensitivity to female ND4 Swiss mice

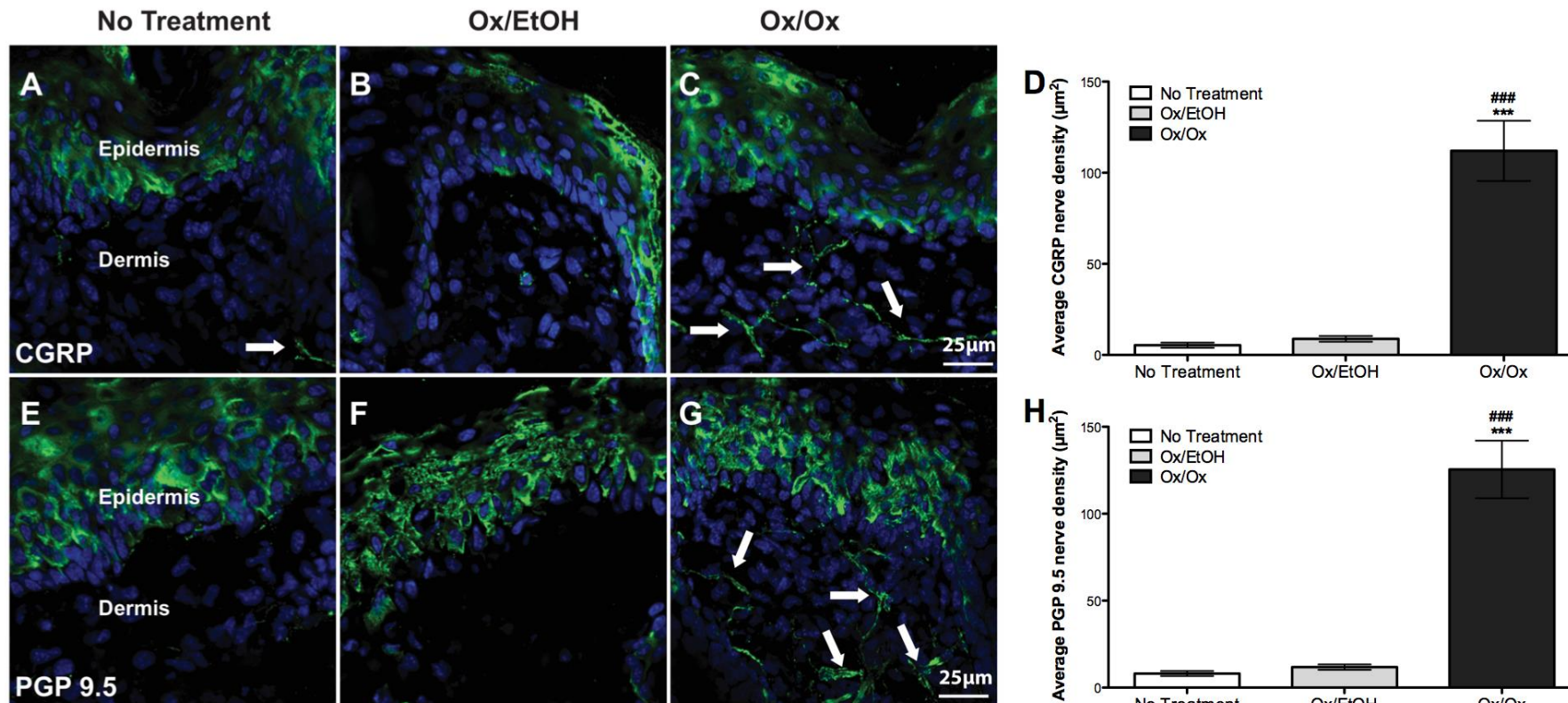
- 2% Oxazolone (chemical allergen) sensitization on shaved flank on day 1
- 1 % Ox (or ethanol vehicle) challenge on labia on day 5
- Measure change in vulvar mechanical sensitivity
- Measure inflammatory outcomes in labiar tissue



Single Ox challenge provokes vulvar pain



Labial cutaneous nerve density increases ~10-fold at site of Ox challenge



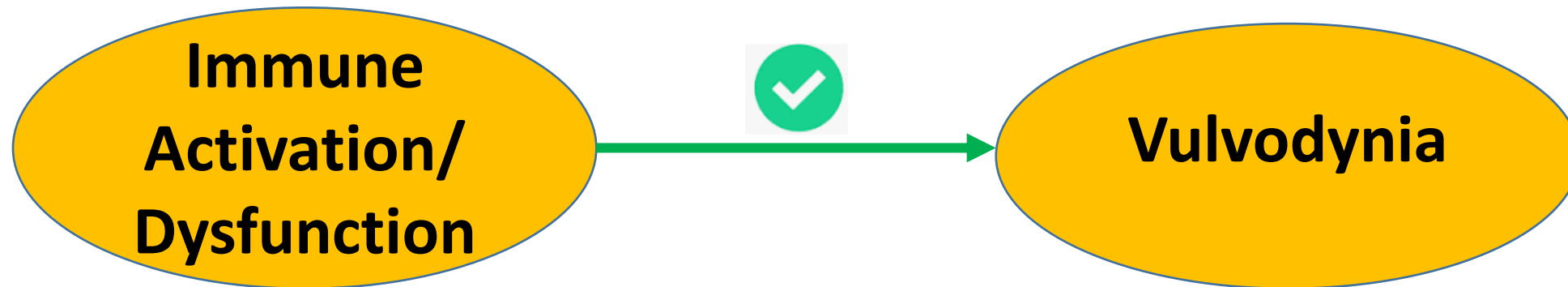
48h

Immunohistochemistry; n=3/group

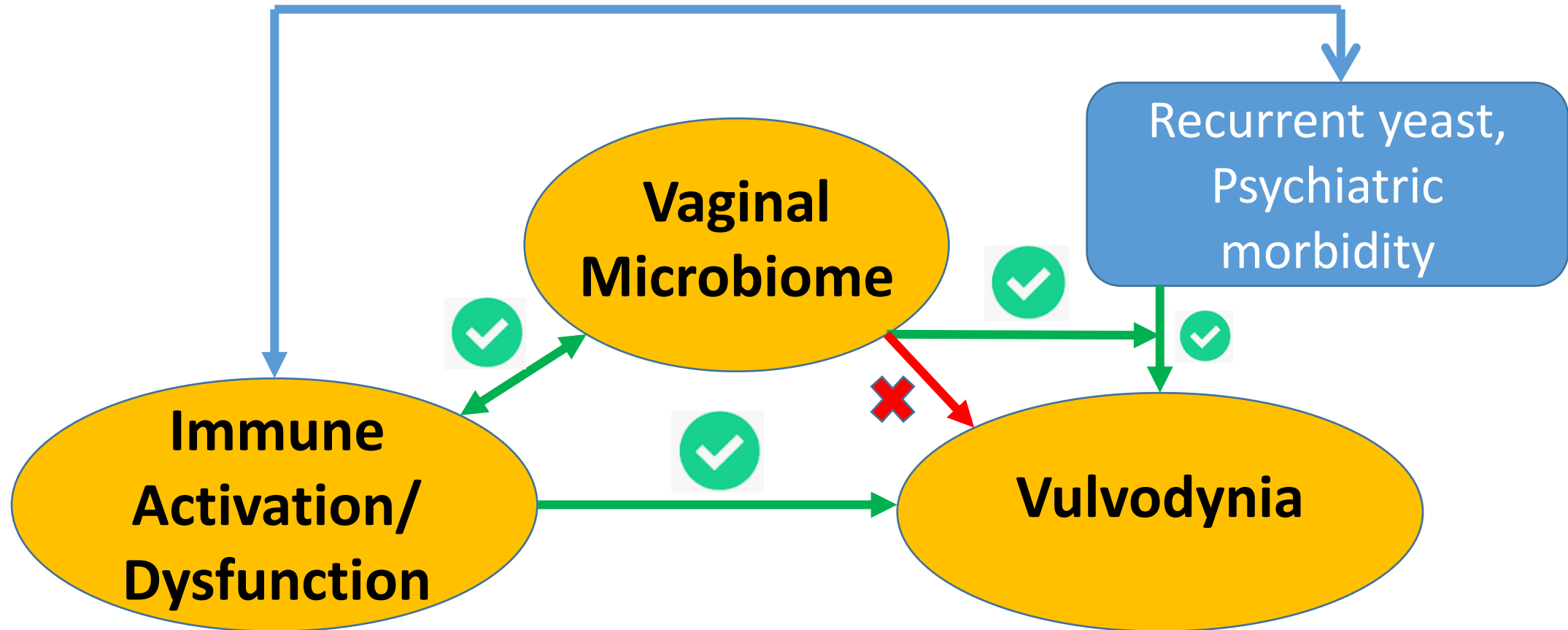
Martinov, Chatterjea, PLoS ONE, 2013

Pathways to be discussed

Is there evidence that immune dysfunction plays a role in the etiology of sexual pain disorders?



Pathways to be discussed



And one more thing.....!

Self-reported Urogenital Infections Prior to Onset of vulvar pain or comparable age in controls

Variable	BOSTON Odds Ratio (95% CI)*	MINNESOTA Adjusted OR (95% CI)*
Genital Warts	3.0 (1.3 – 8.8)	3.1 (0.6 – 17.3)
Genital Herpes	1.2 (0.4 – 4.1)	1.1 (0.2 – 5.9)
Chlamydia	1.2 (0.2 – 3.3)	1.5 (0.5 – 4.2)
Gonorrhea	1.5 (0.3 – 6.8)	1.5 (0.2 – 10.1)
Trichomoniasis	5.7 (1.1 – 2.9)	3.1 (0.4 – 21.7)
Bacterial Vaginosis	3.7 (1.2 – 12)	2.3 (1.1 – 5.0)
Urinary Tract Infection	2.0 (1.3 – 3.1)	2.1 (1.2 – 3.7)
Yeast Infection	2.1 (1.3 – 3.3)	1.4 (0.8 – 2.6)

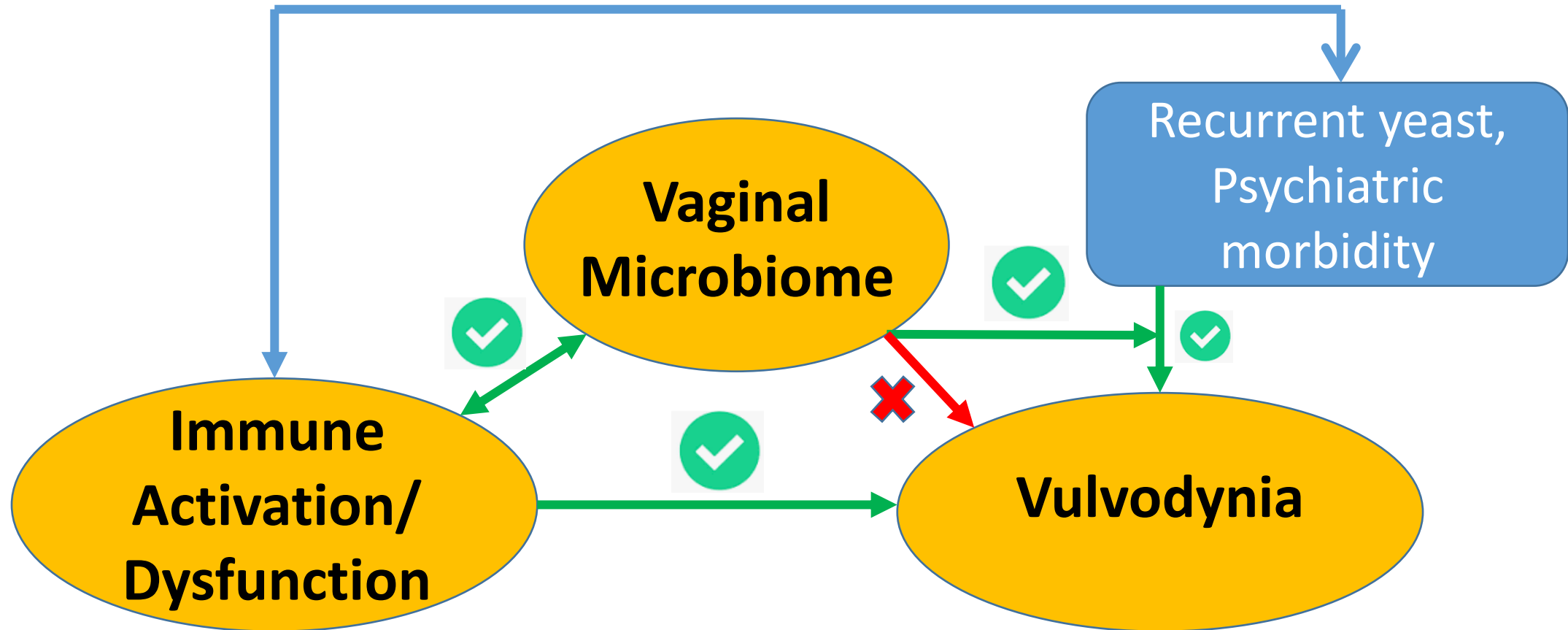
*Adjusted for age, **age at first intercourse**, race, **number of sex partners** around reference age, and **coital frequency** around reference age..

Multiple Urogenital Infections Prior to Onset of vulvar pain or comparable age in controls - Boston

Yeast Infections	Urinary Tract Infections	Other (BV, Trich, Warts)	N	Odds Ratios (95% CI)*
+	—	—	87	1.3 (0.7 – 2.5)
—	+	—	31	1.3 (0.5 – 3.1)
—	—	+	4	1.7 (0.2 – 15)
+	+	—	115	2.4 (1.3 – 4.5)
+	—	+	12	6.6 (1.4 – 30)
+/—	+	+	28	9.9 (2.9 – 34)

*Adjusted for matching variables (town, age), race, number of sex partners, and coital frequency. Reference category included women without any of these infections (n=85).

Pathways to be discussed



Immune dysregulation, the vaginal microbiome, and sexual pain disorders

