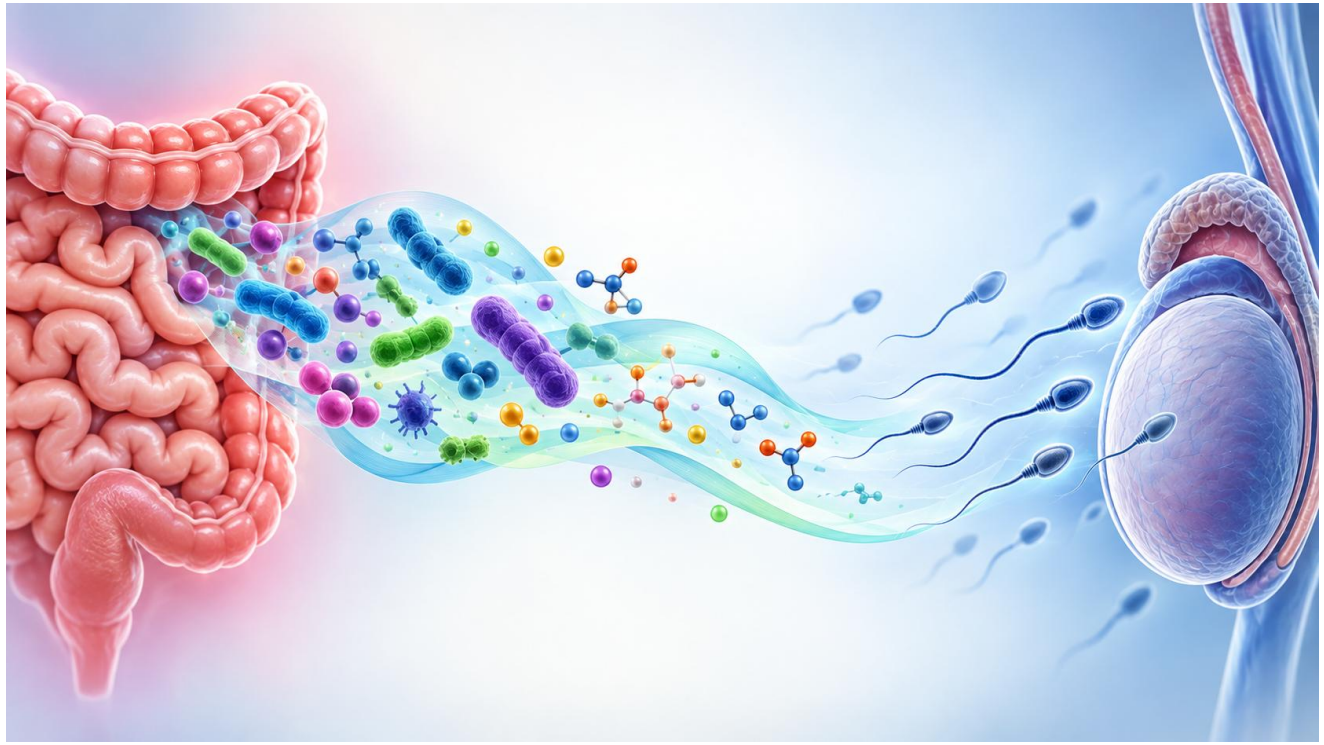
UNIVERSITÀ DI PADOVA
Dipartimento di Medicina

REGIONE DEL VENETO
Azienda Ospedale Università Padova

DIDAS MEDICINA DEI SISTEMI
UOC ANDROLOGIA E MEDICINA DELLA RIPRODUZIONE
Direttore: Prof. Alberto FERLIN

ANDROLOGIA E MEDICINA DELLA RIPRODUZIONE

MICROBIOTA AND PROBIOTICS: IMPACT ON TESTICULAR FUNCTION, SEMEN QUALITY AND MALE FERTILITY



GIUSEPPE GRANDE



AGENDA

1. Microbiota/microbiome, gut dysbiosis and diseases
2. Dysbiosis and endocrine testicular function
3. Male tract and semen «normal» microbiota
4. Seminal microbiota and infertility
5. Microbiota in clinical practice: beyond semen parameters
6. Male tract infections and microbiota
7. Male and female microbiota: 1+1 is not 2!
8. Vaginal microbiota and sperm function

Image generated with AI



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MICROBES, MICROBIOTA & MICROBIOME



Micro-organism

A single organism living in an environment



Microbiota

A community of microorganisms within a specific environment



Microbiome

The collective genetic material of the microbiota

Image generated with AI

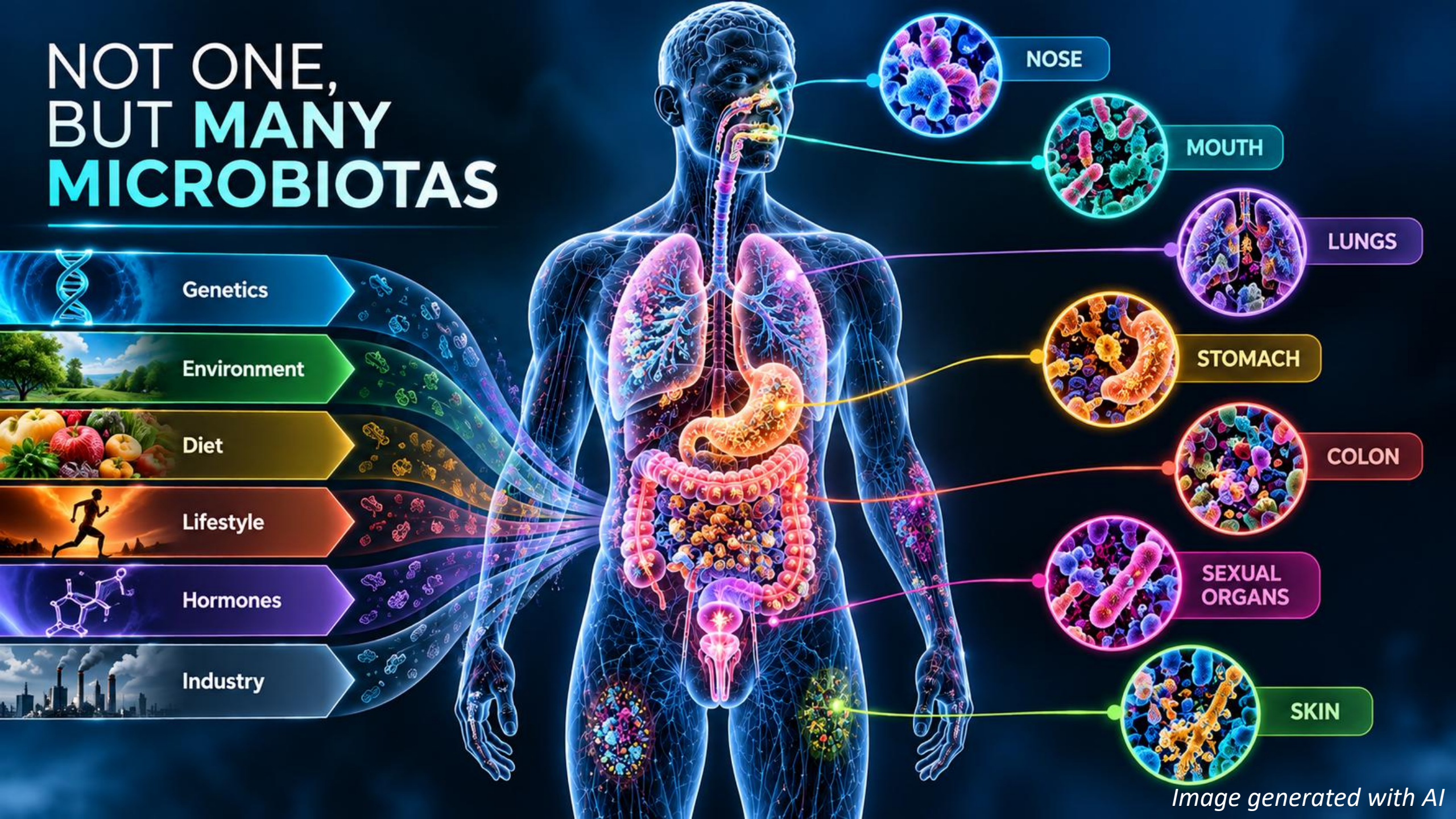
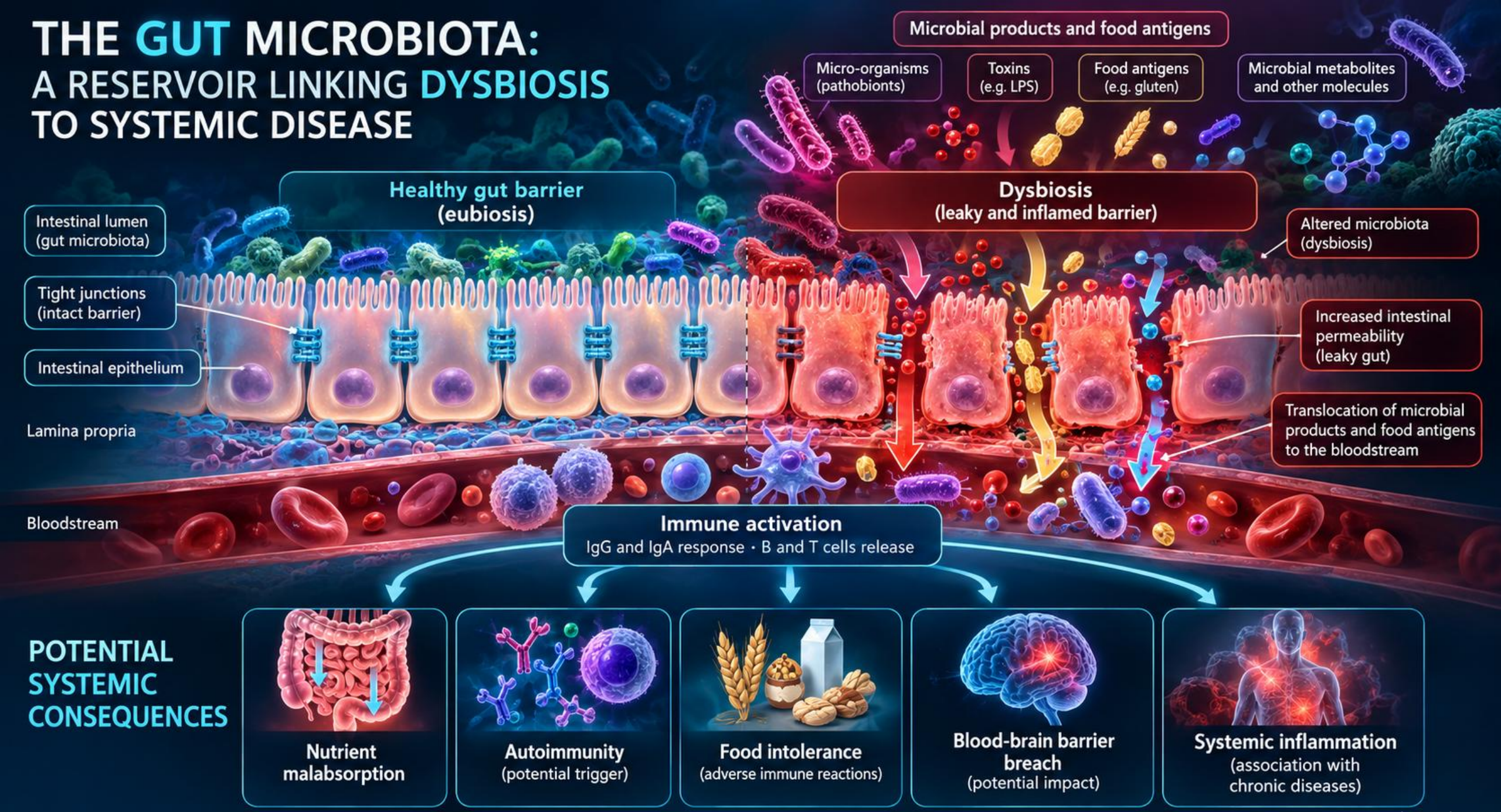


Image generated with AI

THE GUT MICROBIOTA:
A RESERVOIR LINKING DYSBIOSIS TO SYSTEMIC DISEASE



POTENTIAL SYSTEMIC CONSEQUENCES

- **Nutrient malabsorption**
- **Autoimmunity** (potential trigger)
- **Food intolerance** (adverse immune reactions)
- **Blood-brain barrier breach** (potential impact)
- **Systemic inflammation** (association with chronic diseases)

Image generated with AI



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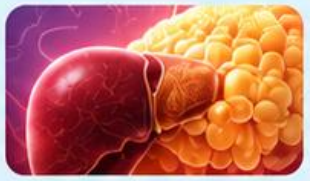
FUNCTIONAL HYPOGONADISM: OBESITY AND METABOLIC DYSFUNCTION



Obesity-related hypogonadism



Age-associated hypogonadism



Metabolic hypogonadism



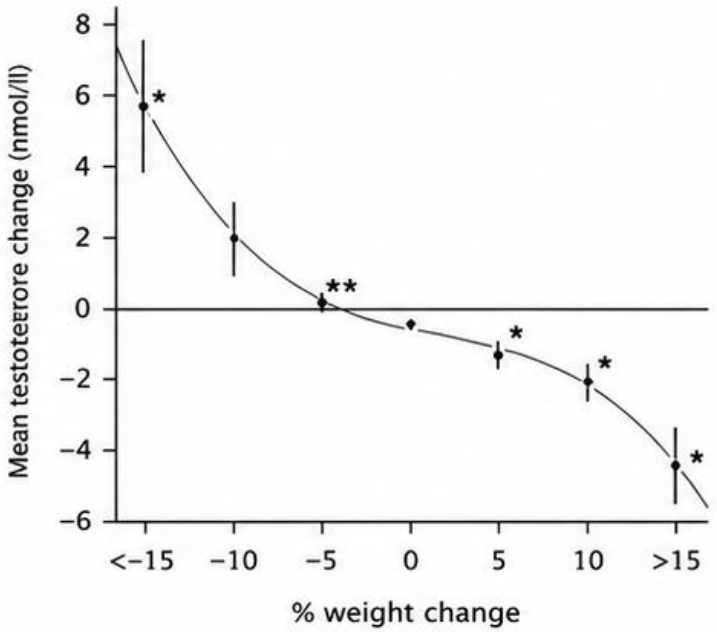
Functional hypogonadism

European Journal of Endocrinology (2013) **168** 445–455

CLINICAL STUDY

Age-associated changes in hypothalamic–pituitary–testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European Male Ageing Study

I M Camacho,[1] T T Huhtaniemi,[1] T W O'Neill,[2] J D Finn, S R Pye[3], D M Lee[2], A Tajar[2,4], P P Casanueva[2,5], G Forti[6], Casaeemvaa[2,5], G Forti[6], A Giwerman[7,8], T S Han[10], K Kula[2,5], B Keevil[1], M E Lee[11,3], D N Pendleton[2,4], M Punab[13], D Vanderschueren[6,14], F C W Wu and the EMAS Group[1]

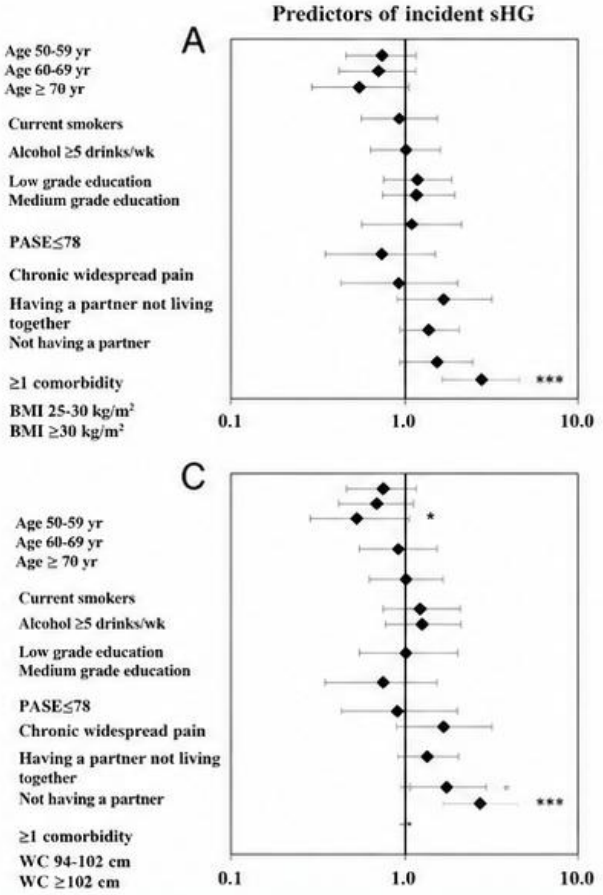


Increasing body weight is associated with a progressive and significant decline in testosterone levels.



Development of and Recovery from Secondary Hypogonadism in Aging Men: Prospective Results from the EMAS

Giulia Rastrelli, Emma L. Carter, Tomas Ahern, Joseph D. Finn, Leen Antonio, Terence W. O'Neill, Gyorgy Bartfai, Felipe F. Casanueva, Gianni Forti, Brian Keevil, Mario Maggi, Aleksander Giwercman, Thang S. Han, Ilpo T. Huhtaniemi, Krzysztof Kula, Michael E. J. Lean, Neil Pendleton, Manjore Pinab, Dirk Vanderschueren, Frederick C. W. Wu, and the EMAS Study Group*

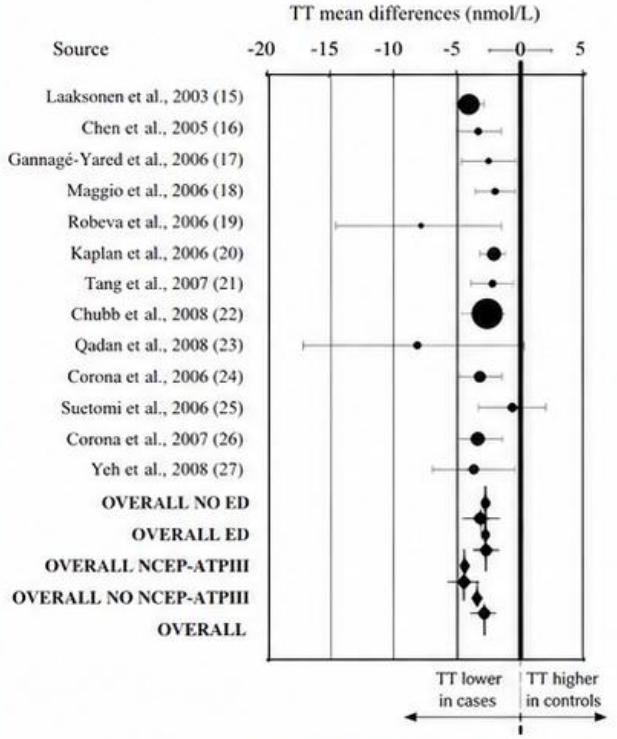


JOURNAL OF SEXUAL MEDICINE



Metabolic Syndrome (MetS) and Testosterone: A Meta-Analysis Study

Giovanni Corona, MD,† Mario Maggi, MD,‡ and Emmanuele A. Jannini, MD, PhD§



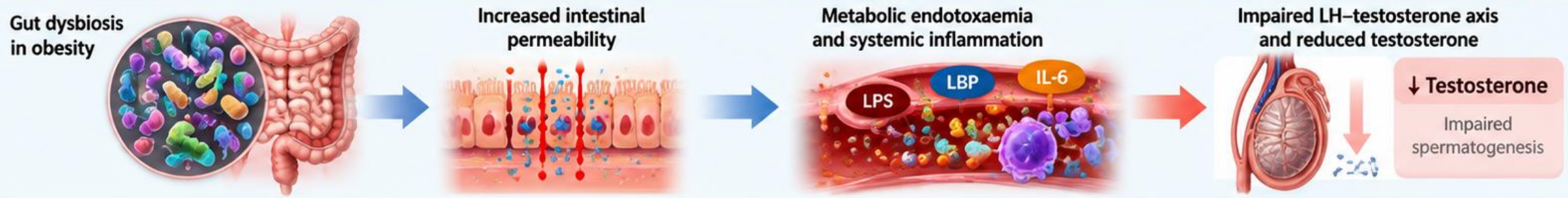
Corona G et al. J Sex Med 2011

MetS patients have significantly lower T plasma levels.



Image generated with AI

GUT DYSBIOSIS, INTESTINAL PERMEABILITY AND FUNCTIONAL HYPOGONADISM



Increased gut permeability correlates with LH and testosterone levels

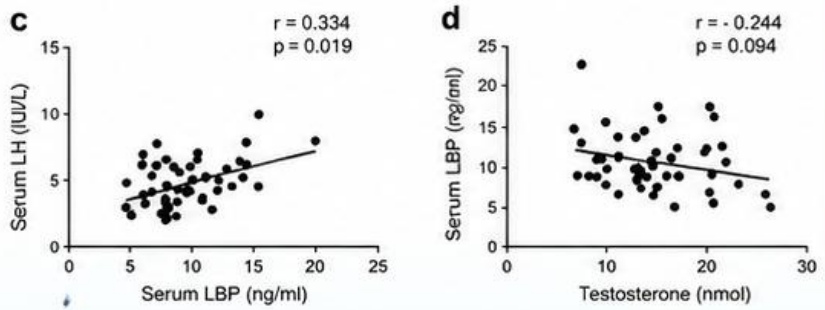
Tremellen Basic and Clinical Andrology (2018) 8:7
DOI 10.1186/s12610-018-00847

Basic and Clinical Andrology

COMMENTARY Open Access

Gut Endotoxin Leading to a Decline IN Gonadal function (GELDING) - a novel theory for the development of late onset hypogonadism in obese men

Kelton Tremellen



Higher serum LBP (marker of endotoxaemia) correlates with higher LH and lower testosterone levels.

Tremellen K. Basic Clin Androl 2018

Gut endotoxin impairs testicular function in obesity

Tremellen Basic and Clinical Andrology (2018) 8:7
DOI 10.1186/s12610-018-00847

Basic and Clinical Andrology

COMMENTARY Open Access

Gut Endotoxin Leading to a Decline IN Gonadal function (GELDING) - a novel theory for the development of late onset hypogonadism in obese men

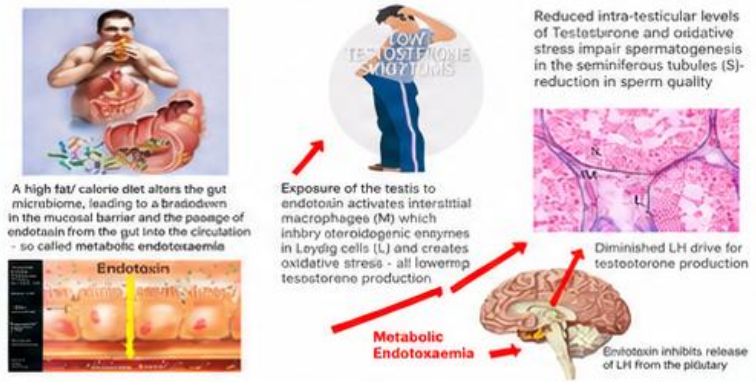


Fig. 1 An overview of the GELDING theory of obesity related male hypogonadism

Tremellen K. Basic Clin Androl 2018

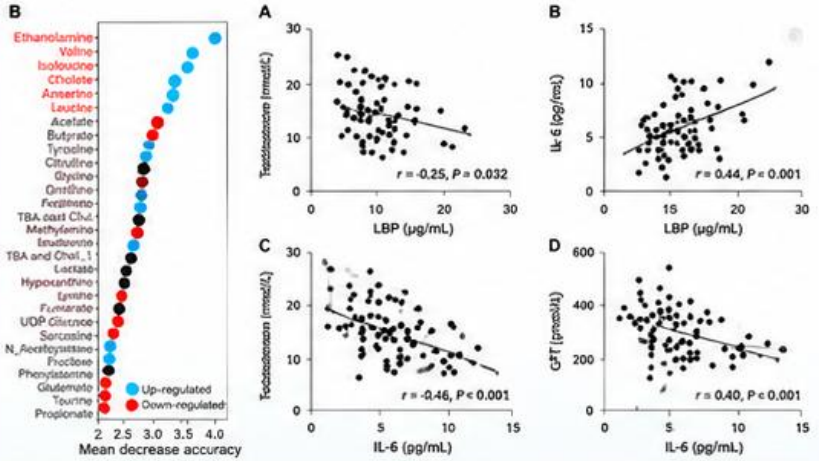
Altered gut permeability and inflammation in obesity correlate with lower testosterone

OPEN endocrinology

OPEN ACCESS

A mechanism by which gut microbiota elevates permeability and inflammation in obese/diabetic mice and human gut

Sidharth P Mishra [1,2] Bo Wang,[3] Shalini Jain,[1,2] Jingzhong Ding,[4] Jared Rejeski,[4] Cristina M Furdui,[4] Dalane W Kitzman,[4,5] Subhash Tarapüder,[6] Christian Brechot,[7] Ambuj Kumar,[7] Hariom Yadav [1,2]



Increased gut permeability and higher IL-6 levels are associated with lower testosterone levels.

Mishra S.P. et al. Gut Microbiota 2023

Image generated with AI

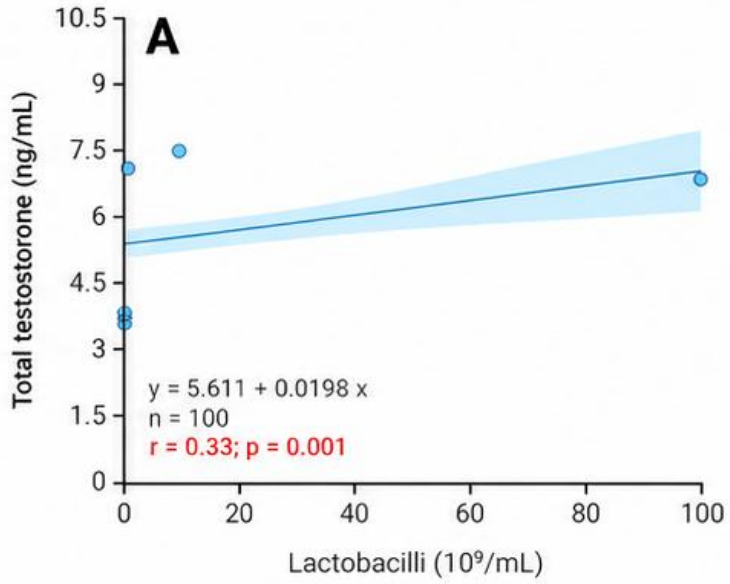
EUBIOSIS IS ASSOCIATED WITH NORMAL TESTICULAR FUNCTION

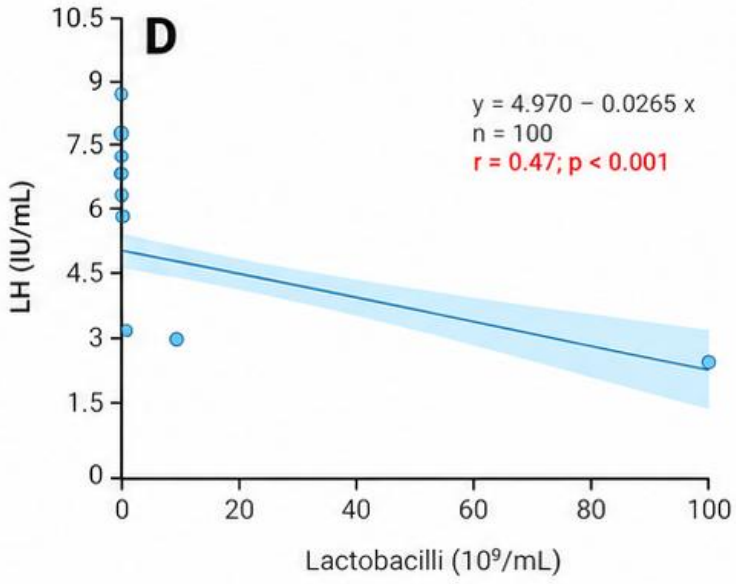
Journal of Assisted Reproduction and Genetics (2021) 42:893–907
https://doi.org/10.1007/s10815-020-02027-y

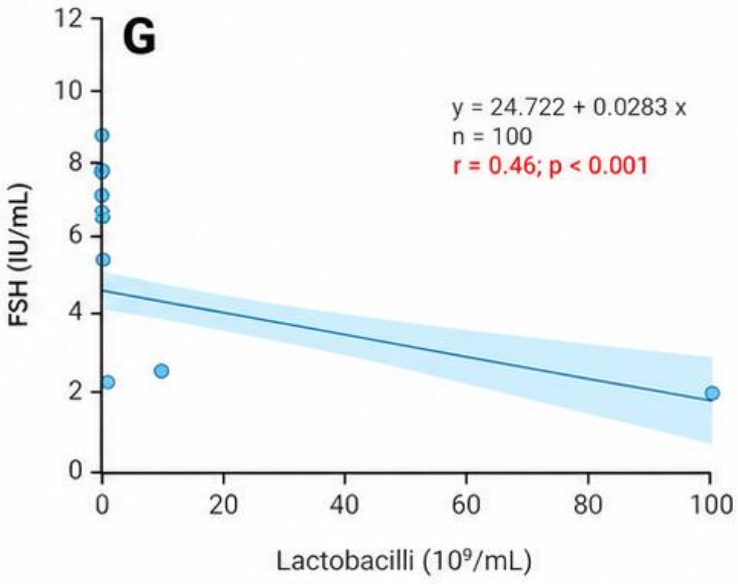
REPRODUCTIVE PHYSIOLOGY AND DISEASE

The influence of seminal microbiota on human testicular steroidogenesis: a prospective study

Rossella Cannarella[1] · Roberto Conte[1] · Rosita A. Condorelli[1] · Agnese Gallo[1] · Antonio Aversa[2] · Aldo E. Calogero[1] · Sandro La Vignera[1]









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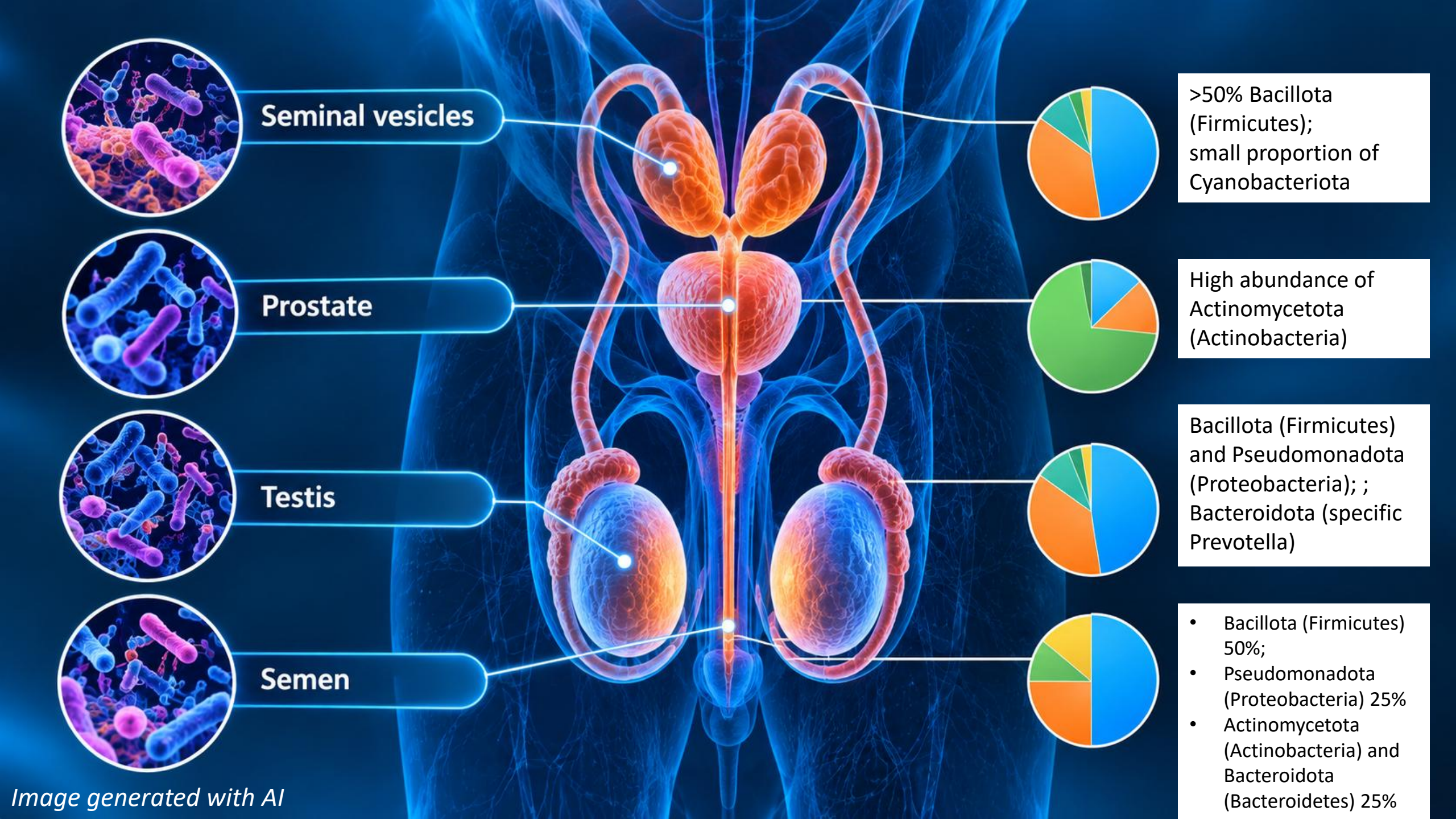

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NORMAL SEMEN MICROBIOTA

frontiers | Frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 23 June 2021
doi: 10.3389/fcimb.2021.690692

Seminal Microbiota of Idiopathic Infertile Patients and Its Relationship With Sperm DNA Integrity

*Claudia Papaleo[1,2], Elisa De Seta[1], Luca Vitale[1,3], Simona Rapino[1], Lucia Cozzolino[1], Paola Gallo[1], Nicola D'Antuono[1], Letizia Lafirenza[1], Loris Alfonsetti[1,2] and Maria Conti Fusta[1,2]**

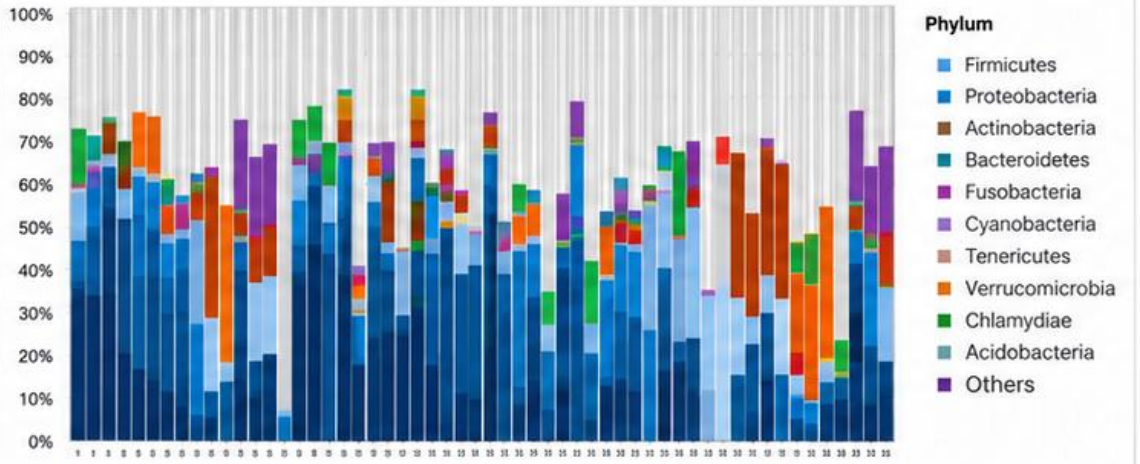

FIGURE 3 | Relative abundance of bacteria at phylum level in semen samples. Each column represents one sample. The x-axis shows the sample identification number. The y-axis indicates the relative abundance (%) of different phyla. Only phyla with a relative abundance >0.5% in at least one sample are shown.

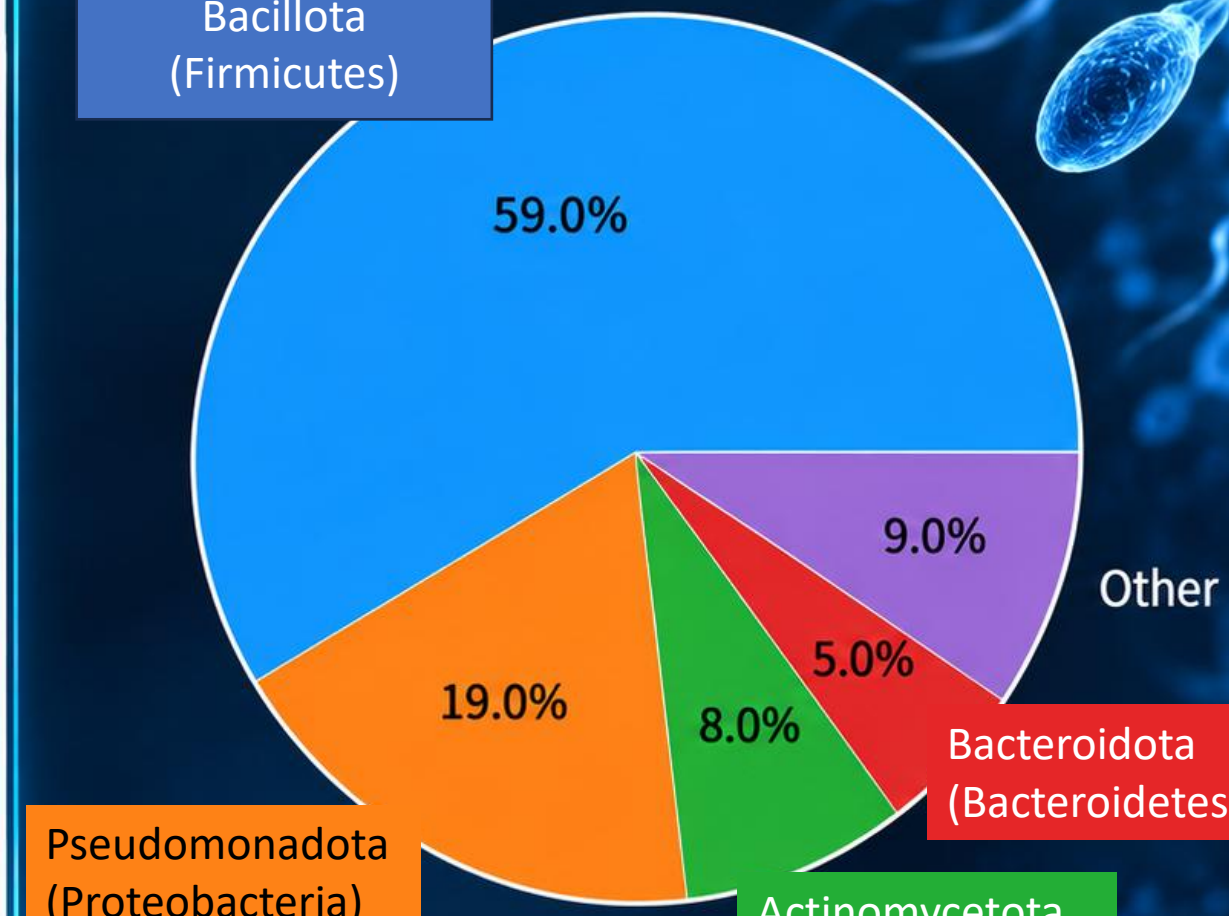

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Image generated with AI

PREVOTELLA-DOMINANT SEMINAL MICROBIOTA IS ASSOCIATED WITH ALTERED TESTICULAR FUNCTION AND POOR SEMEN PARAMETERS

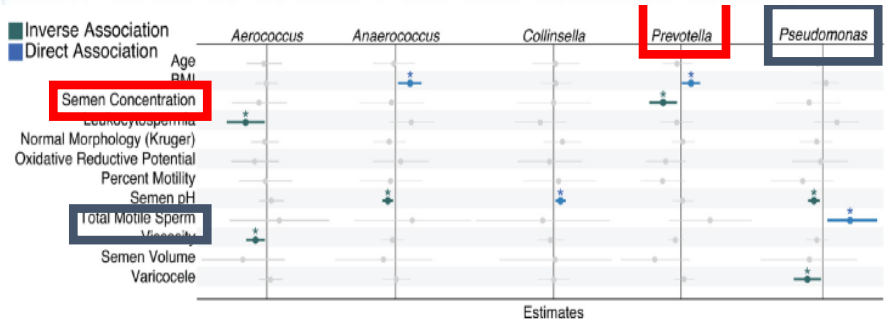
TWO SEMINAL MICROBIOTA CLUSTERS ARE ASSOCIATED WITH DIFFERENT SEMEN PARAMETERS

European Association of Urology

Platinum Priority – Sexual Medicine – Editor's Choice

Functional and Taxonomic Dysbiosis of the Gut, Urine, and Semen Microbiomes in Male Infertility

Scott D. Lundy, Jureeporn Sugrue, Joel V. Turgeon, Moises Gomez Porner Solano, Sachi Gondim Deus Maciel Rojan, Kaan Ramasamy, Ahmadi Saad, Aaseenan, Eduviad S. Sohanaph, Somah C. W., Curtis Frey



PREVOTELLA DOMINANCE IS ASSOCIATED WITH LOW-QUALITY SEMEN AND SPERMATOGENESIS DEFECTS

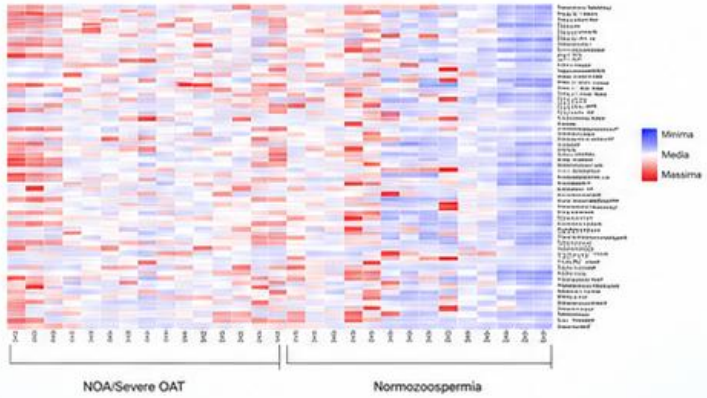
RBMO REPRODUCTIVE BIOMEDICINE ONLINE

ELSEVIER



Assessing the testicular sperm microbiome: a low-biomass site with abundant contamination

Fedra M. Di Stasi, Camilla Rizzato, Giorgia Ricci, Anna Giulia Galia, Elena Ablondi, Elisabetta Marchiani, Elisabetta Baldi, Costantino Krausz, Laura Guidi, Silvia De Francia



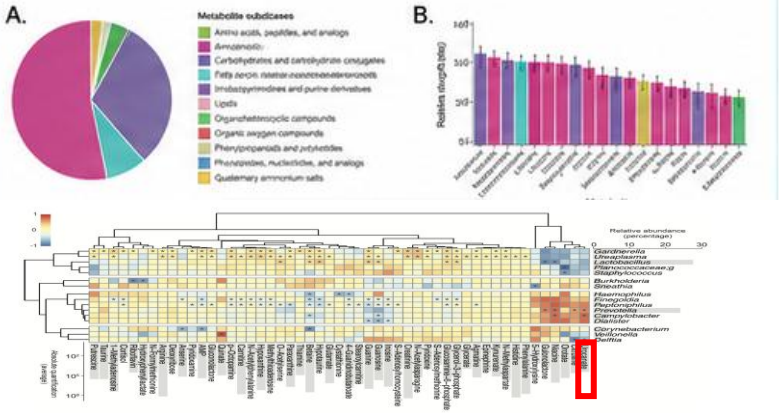
PREVOTELLA-DOMINANT PROFILE AND ALTERED METABOLIC PATHWAYS

American Society for Microbiology | Microbiology Spectrum

Open Peer Review | Open Microbial Innovations Research Article

Impact of semen microbiota on the composition of seminal plasma

O. Roletz, A. Balercia, A. Peeri, R. Manfrini, N. Vuillemin, M. Baniomar



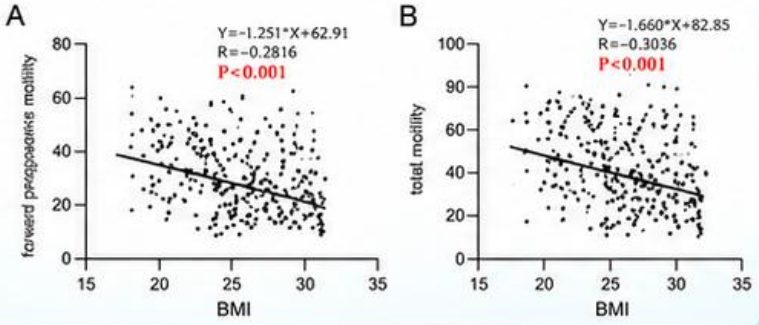
PREVOTELLA ABUNDANCE CORRELATES WITH OLIGOZOOSPERMIA AND SECRETORY AZOOSPERMIA

scientific reports

OPEN

Enterotypes in asthenospermia patients with obesity

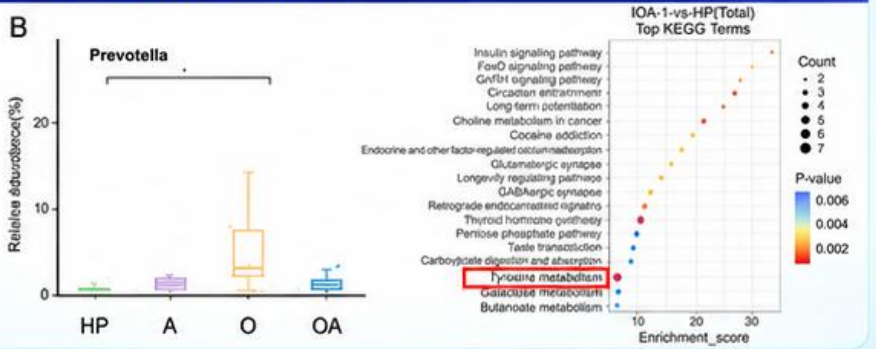
Jieqiu Jieg, Pengze Xiu, Xiaobin Wang, Zrr Xing, Shinya Deng, Guang Li, Xinyu Yan, Rantao Guo, Tao Feng, Weuho Yang, Baochen Pan, Xunbo Zhu, Xiaxia Ma



PREVOTELLA DOMINANCE IS ASSOCIATED WITH REDUCED SEMINAL PARAMETERS AND ALTERED METABOLIC PATHWAYS

Comprehensive microbiome and metabolome analysis revealed the changes of semen microbial characteristics and metabolic phenotypes in patients with idiopathic oligoasthenozoospermia

Shixuan Lu, Zhiyu Wu, Yipeng Zhao, Tang Tang, Ye Dong, Meilin Wu, Peihai Zhang, and Ziyang Ma



PSEUDOMONADOTA-DOMINANT SEMINAL MICROBIOTA IS ASSOCIATED WITH POORER SEMEN PARAMETERS AND ALTERED HOST METABOLISM

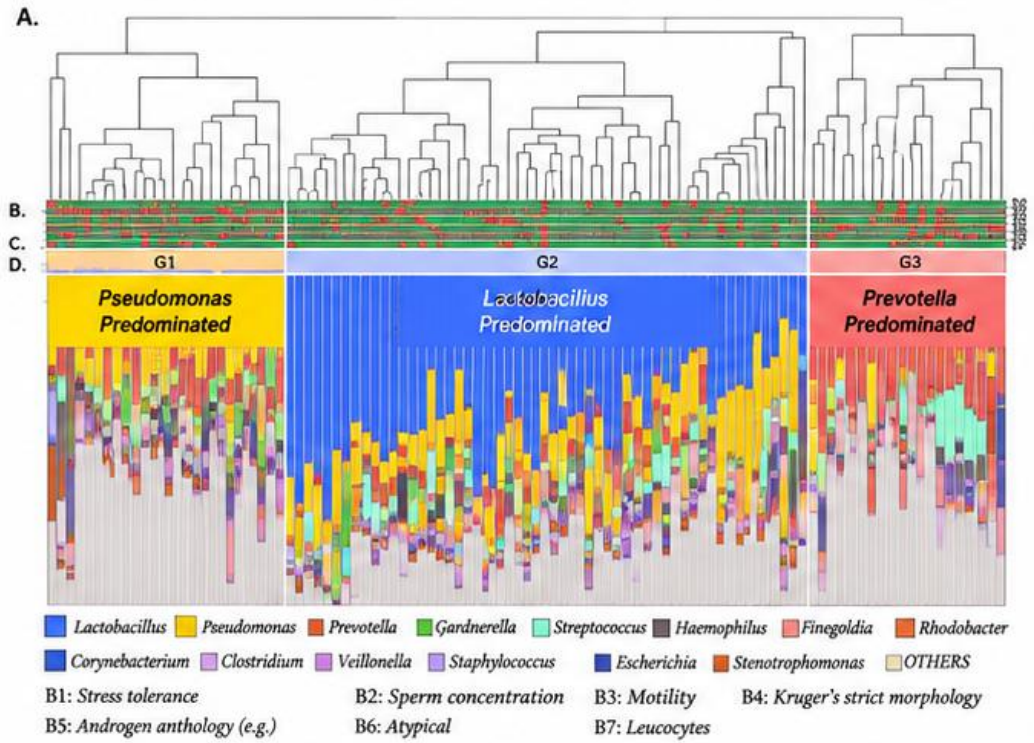
THREE SEMINAL MICROBIOTA CLUSTERS

OPEN ACCESS Freely available online

PLOS ONE

Bacterial Communities in Semen from Men of Infertile Couples: Metagenomic Sequencing Reveals Relationships of Seminal Microbiota to Semen Quality

Shun-Long Weng[1,2,3,4,5], Chih-Min Chiu[2], Feng-Miao Lin[1], Wei-Chih Huang[1], Chao Liang[1], Ting Yang[1], Tzu-Lung Yang[1], Chia-Yu Liu[1], Wei-Tun Wu[1], Yi-Ran Chang[1,2], Tzu-Hao Chang[1,2], Hsien-Oa Huang[1,3,4,5,*]

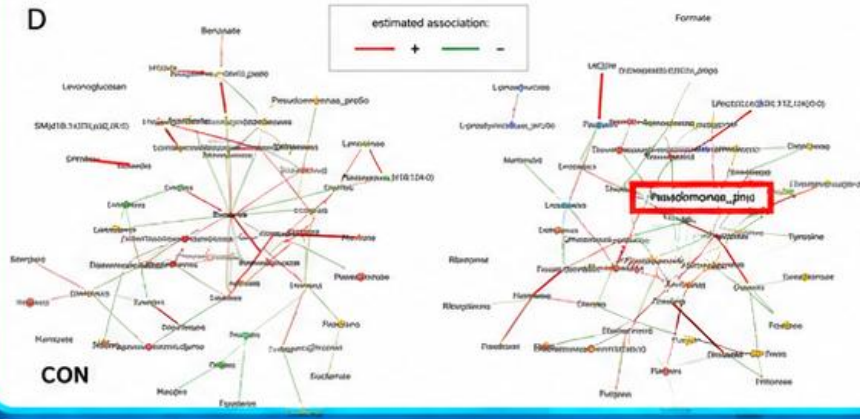


PSEUDOMONADOTA-DOMINANT PROFILE

American Society for Microbiology | Microbiology Spectrum

Interaction between Host and Microbes in the Semen of Patients with Idiopathic Nonobstructive Azoospermia

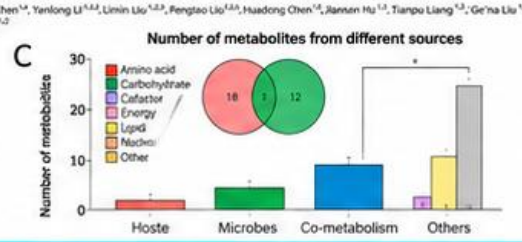
Qingxing Chen[1,4], Yanlong Li[1,2,3], Limin Liu[1,2,3], Pengtao Liu[1,2,4], Huadong Chen[1,2], Jiannan Hu[1,2], Tianpu Liang[1,2], Ge'na Liu[1,2], Qing Yang[1,2]

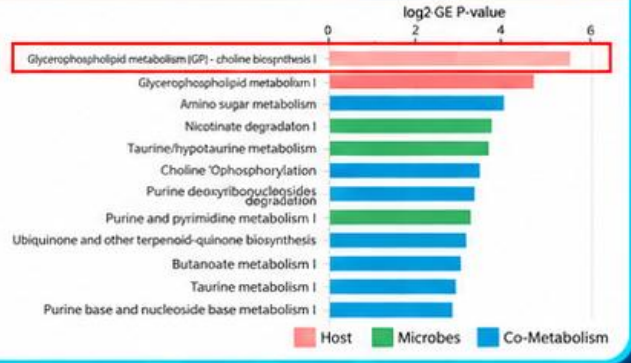


ALTERED HOST–MICROBE METABOLISM

American Society for Microbiology | Microbiology Spectrum

Interaction between Host and Microbes in the Semen of Patients with Idiopathic Nonobstructive Azoospermia





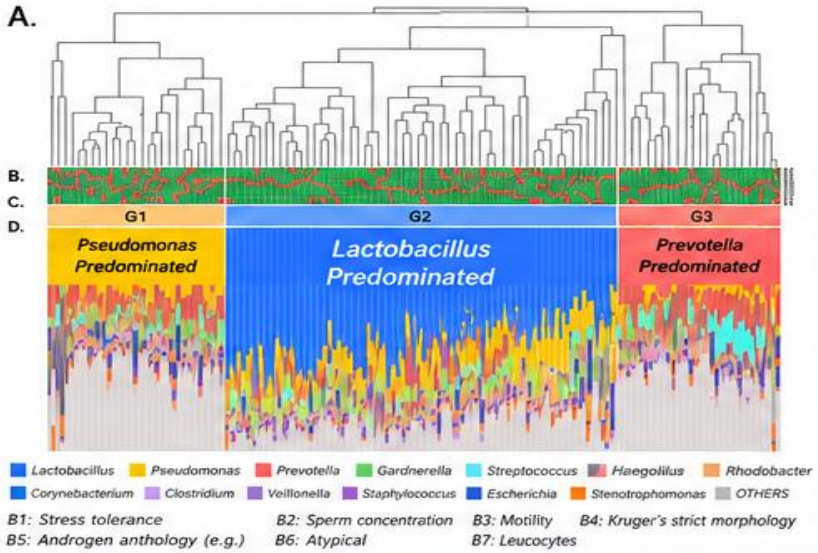
LACTOBACILLUS-DOMINANT SEMINAL MICROBIOTA IS ASSOCIATED WITH BETTER SEMEN PARAMETERS, BUT NOT *ALL LACTOBACILLI* ARE THE SAME

LACTOBACILLUS DOMINANCE CHARACTERIZES >80% OF NORMOZOOSPERMIC SUBJECTS

OPEN Access Freely available online — PLOS ONE

Bacterial Communities in Semen from Men of Infertile Couples: Metagenomic Sequencing Reveals Relationships of Seminal Microbiota to Semen Quality

Shun-Long Weng[1,2,3,4], Chih-Min Chiu[2,3], Feng-Miao Lin[2], Wei-Chih Huang[1], Chao Liang[1], Ting Yang[1], Tzu-Lung Yang[1,3], Chia-Yu Liu[1,3], Wei-Tun Wu[2,3], Yi-Ran Chang[2,3], Tzu-Hao Chang[3], Hsien-Oa Huang[1,2,4,5]*

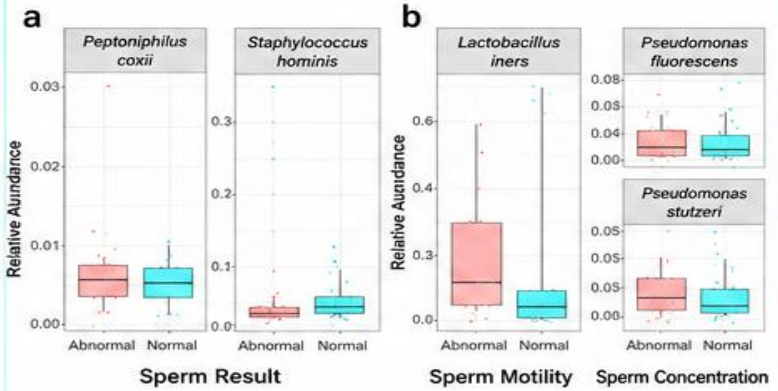


LACTOBACILLUS INERS IS ASSOCIATED WITH REDUCED SPERM MOTILITY

scientific reports

OPEN **Semen microbiota are dramatically altered in men with abnormal sperm parameters**

Yajiao Zhang[1,2], Juda Amanzholov[2], Boyu Guan[3], Lei Wang[3], Ling Lai[3], Jiani Li[3], Bin Yang[3], Ruiyuan Zhang[3], Yenshan Wang[3], Lijiao Wang[3], Xin Jiang[3], Lina Wang[3], Zhijie Sun[3,2], Chengcui Long[1,2,3]*

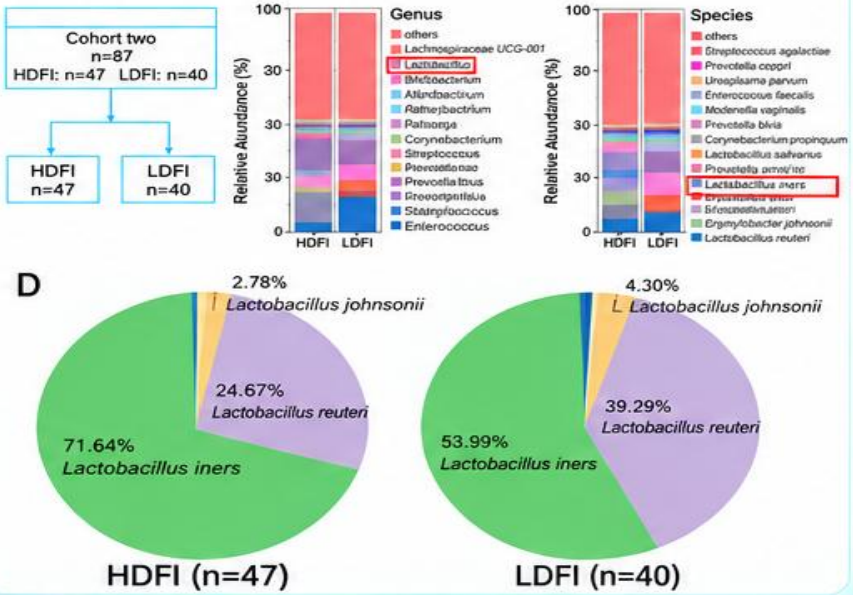


LACTOBACILLUS INERS IS ASSOCIATED WITH INCREASED SPERM DNA FRAGMENTATION

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum — RESEARCH ARTICLE

Association between semen microbiome disorder and sperm DNA damage

Jianbin Ma[1,2,3,4], Mengkai Ma[1,2,3,4], Xuebin Wang[1,2,3,4], Yanan Guo[1,2,3,4], Hangbu Guo Q40[1,2,3,4], Hantong Chen[1,2,3,4], Yongge Yang[1,2,3,4], Peijie Chen[1,2,3,4], Qing Yang[1,2]

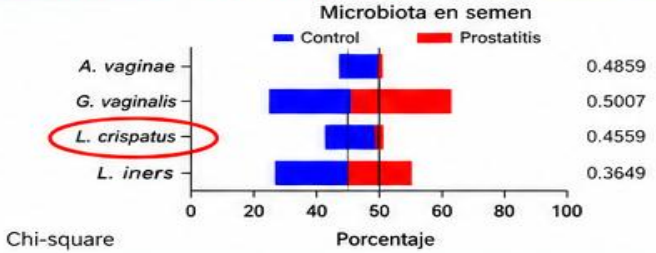


LACTOBACILLUS CRISPATUS IS ASSOCIATED WITH ABSENCE OF PROSTATITIS AND (PRELIMINARY EVIDENCE OF) BETTER SEMEN PARAMETERS

ORIGINAL RESEARCH — REV INT ANDROL Revista Internacional de Andrología

The microbiota of sexual intercourse and its effect prostatitis

Yurani Marllely Saldarriaga López[1,1], Valeria Santacruz Restrepo[1,1], Walter Darío Cardona Maya[2], Jennifer Puerta Suárez[1,*]



- *Lactobacillus crispatus* is more frequently detected in controls compared to men with prostatitis (preliminary evidence).
- Suggests a potential protective role of *L. crispatus* on male genital tract health and possibly on semen parameters.

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7 Male and female microbiota: 1+1 is not 2!

8 Vaginal microbiota and sperm function

Image generated with AI

DIAGNOSTIC FLOW-CHART FOR MALE FACTOR INFERTILITY

FROM DIAGNOSIS TO PERSONALIZED MANAGEMENT

Couple infertility

Both partners should be evaluated according to current guidelines

Male partner

Female partner

Refer to dedicated guidelines

Scrotal US and CDUS (4.4.1)

Semen analysis (3.2)

- ASA in selected cases (4.1.2)
- Sperm DNA integrity in very selected cases (4.1.3)

General physical and genital examination (3.1)

Familiar and personal history (3.1)

Family history of chromosomal aberrations or recurrent abortions

Suspect MAGI (4.2)

- Sperm culture
  Test for *Chlamydia trachomatis* and Mycoplasma
  Extend the microbiological assessment to the female partner
- MAGI confirmed

Suspect duct obstruction/ abnormalities or retrograde ejaculation

- TRUS (4.4.2)
- Urine post ejaculation (4.1.1)

Idiopathic asthenozoospermia

- HPV DNA test (4.2)

CAVD

- CFTR gene mutations (5.2)

Oligozoospermia/ asthenozoospermia and azoospermia

- LH, FSH, total testostorene and SHBG (4.3)
  Inhibin B in selected cases (4.3)
  Consider general health (cardiometabolic and bone) and regular follow up (6)
- Suspect HH
  - Prolactin (4.3) and HH gene panel (5.2)
- Androgen resistance
  - *AR* gene mutations (5.2)
- Genetic counselling (5.2)

Azoospermia

- Testicular FNAC (5.1)

Testicular damage

- Primary testicular damage
- Sperm concentration
  - ≤5 mill/ml
    - Yq microdeletions (5.2)
  - ≤10 mill/ml
    - Karyotype (5.2)

Semen analysis is the initial step, not the final diagnosis.

Further investigations are guided by clinical findings, to identify the specific cause and treat accordingly.

Ferlin A, et al., J Endocrinol Invest 2022

Traditional classification

BASED ON ANATOMICAL/ETIOLOGICAL LEVEL



PRE-TESTICULAR

- Hypothalamic disorders
- Pituitary disorders
- Systemic and environmental factors



TESTICULAR

- Primary testicular failure



POST-TESTICULAR

- Epididymal disorders
- Ductal obstruction
- Ejaculatory disorders

- Does not fully capture pathophysiology
- Limited guidance for targeted therapy

Clinical history

Physical examination

Semen analysis

Hormonal assessment

Scrotal ultrasound

Microbiological assessment

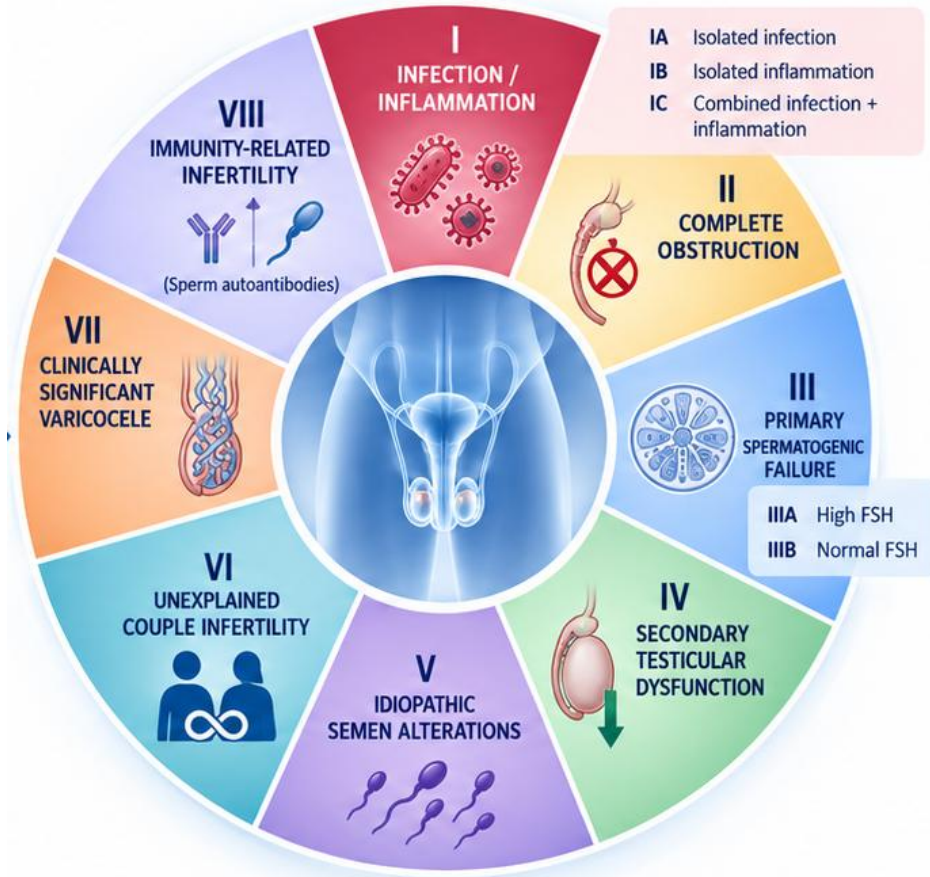
Genetic testing

COMPREHENSIVE WORK-UP

PATHOPHYSIOLOGICAL PHENOTYPING

New pathophysiological classification

BASED ON MECHANISMS AND THERAPEUTIC IMPLICATIONS



The Journal of Clinical Endocrinology & Metabolism, 2026, 00, 1–10
https://doi.org/10.1210/clinem/dgag263
Published: 4 July 2026
Clinical Research Article

ENDOCRINE SOCIETY OXFORD

Pathophysiology-based classification of male infertility: evidence from an 800-patient prospective cohort

Giuseppe Grande[1*], Andrea Graziani[1,2*], Nicola Caretta[1], Antonella Di Mambro[1], Andrea Garolla [1,2], Luis Lopez-Fando Lavalle[3,4], Maurizio Merico[1], Giulia Masi[2], Pierfrancesco Palego[1], Maria Santa Rocca[1], Alberto Scala[2], Riccardo Selice[1], Cinzia Vinanzi[1], Luca De Toni[2], and Alberto Ferlin [1,2]

The Journal of Clinical Endocrinology & Metabolism, 2026, 00, 1–10
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Table 3 Diagnostic classification and putative causes within a cohort of 800 male partners of infertile couples

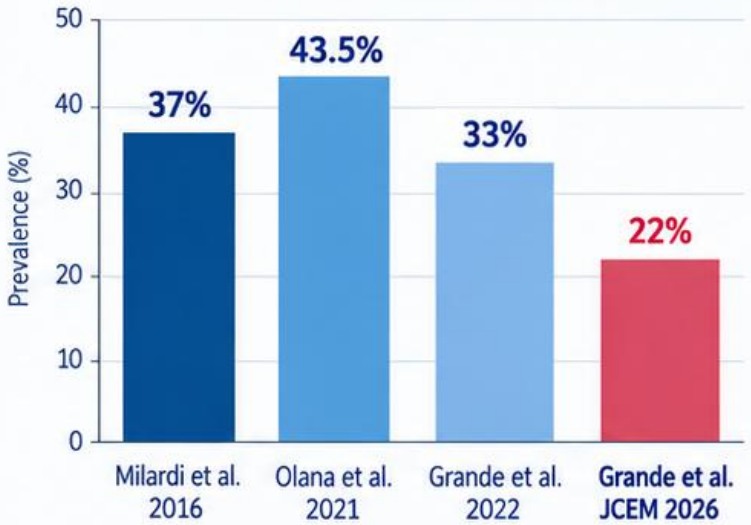
Category	Subcategory	Associated etiology	N (%)	
I (infection/inflammation)	IA	Isolated infection	35/800 (4.4%)	179/800 (22.4%)
	IB	Isolated inflammation	64/800 (8.0%)	
	IC	Combined infection and inflammation	80/800 (10.0%)	
II (congenital/acquired total obstruction)		CBAVD (with CFTR gene mutations)	21/800 (2.6%)	37/800 (4.6%)
		CBAVD (without CFTR gene mutations)	3/800 (0.4%)	
		Acquired/iatrogenic obstruction	8/800 (1.0%)	
		Sexual dysfunctions, including retrograde ejaculation	5/800 (0.6%)	
III (primary spermatogenic failure)	IIIA		305/800 (38.1%)	448/800 (56.0%)
	IIIB		143/800 (17.9%)	
		Karyotype alterations, including Klinefelter syndrome	55/800 (6.9%)	
		Y chromosome microdeletions	41/800 (5.1%)	
		Androgen receptor mutations	5/800 (0.6%)	
		Postsurgery/infection/trauma	25/800 (3.1%)	
		Previous testicular cancer	21/800 (2.6%)	
		Previous oncological therapy	22/800 (2.8%)	
		Cryptorchidism	90/800 (11.3%)	
		Idiopathic primary spermatogenic failure	189/800 (23.6%)	
IV (secondary testicular dysfunction)		Congenital hypogonadotropic hypogonadism.	6/800 (0.7%)	68/800 (8.5%)
		Acquired—organic hypogonadotropic hypogonadism	7/800 (0.9%)	
		Acquired—functional hypogonadotropic hypogonadism	55/800 (6.9%)	
V (idiopathic semen alterations)				15/800 (1.9%)
VI (idiopathic couple infertility)				27/800 (3.4%)
VII (varicocele)				21/800 (2.6%)
VIII (immunity-related semen conditions)				5/800 (0.6%)

Male Genital Tract Infections and Male Infertility

PREVALENCE, MECHANISMS OF DAMAGE AND MOLECULAR EVIDENCE

FROM MICROORGANISMS
TO ALTERED SEMINAL FUNCTION
A CLINICAL AND MOLECULAR PERSPECTIVE

1 Prevalence in infertile populations



n = 454 (single tertiary care center)	n = 930 (symptomatic GTI)	n = 302 (retrospective multicentric)	n = 800 (prospective cohort)

Male genital tract infection/inflammation is a frequent finding in infertile men, affecting approximately **20–40%** of patients, with a prevalence of **22%** in a recent large prospective cohort.

2 Mechanisms of damage

Secretory dysfunction

- Altered function of accessory glands
- Changes in seminal plasma composition (e.g. zinc, citrate, seminal proteins)

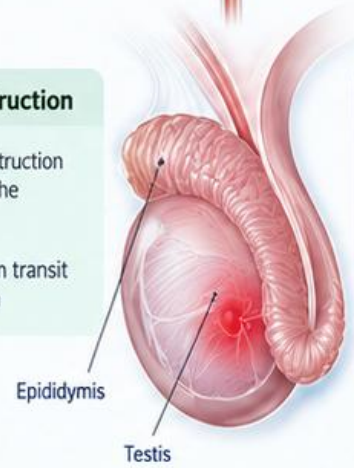


Inflammatory microenvironment

- Release of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α)
- Increased reactive oxygen species (ROS)
- Leukocyte activation

Excretory obstruction

- Anatomical obstruction at any level of the seminal tract
- Impaired sperm transit and maturation



Other mechanisms

- Reduced zinc concentration (chronic prostatitis)
- Sperm DNA fragmentation
- Development of anti-sperm antibodies
- Altered seminal fluid viscosity and liquefaction

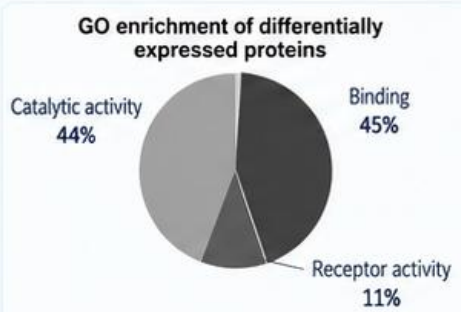
3 Molecular evidence: *Enterococcus faecalis* infection

Semen proteomics reveals the impact of *Enterococcus faecalis* on male fertility

Grande G. et al. Protein Peptide Lett. 2018; 25: 472-477.



Enterococcus faecalis (25–40% of isolates)



Altered proteins in infected patients

Proteins under-expressed

- CD177 antigen (CD177)
- EF-hand domain-containing protein D2 (EFHD2)
- 2-oxoglutarate dehydrogenase (ODO1)
- Zinc finger protein 735 (ZN735)
- Pro-epidermal growth factor (EGF)
- Kinesin heavy chain isoform 5C (KIF5C)
- CXXC-type zinc finger protein 1 (CXXC1)
- Usherin (USH2A)

Proteins over-expressed

- Metalloproteinase inhibitor 1 (TIMP1)
- WAP four-disulfide core domain protein 2 (WFDC2)
- Carboxypeptidase E (CBPE)

***Enterococcus faecalis* infection is associated with significant changes in the seminal proteome, particularly in proteins involved in the secretory function of the prostate and seminal vesicles.**

Male Genital Tract Infections:
From Antibiotics to Dysbiosis

1 CURRENT GUIDELINES (ANTIBIOTIC THERAPY)

European Association of Urology

Urological Infections Guideline (2025)

Fluoroquinolones
First-line therapy for chronic bacterial prostatitis (CBP)

Doxycycline (or macrolides)
Recommended for identified intracellular/atypical pathogens (e.g. *C. trachomatis*, *Mycoplasma* spp.)

Culture-guided therapy and local resistance patterns should guide treatment.

2 THE CLINICAL CHALLENGE
RESISTANCE AND PERSISTENCE



ANTIMICROBIAL RESISTANCE
Limits treatment efficacy

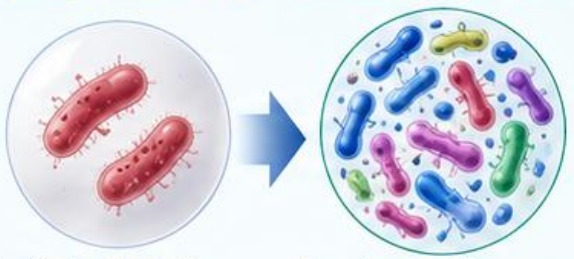
40–50%
Recurrent/persistent infection after treatment

- Repeated antibiotic exposure
- Selection of resistant strains
- Incomplete eradication and relapse

3 A NEW PARADIGM: DYSBIOSIS
FROM PATHOGENS TO A MICROBIAL ECOSYSTEM

FROM: pathogen-centered view

TO: microbiota-centered view



Single pathogen(s)

A complex microbial ecosystem

A CLINICAL APPROACH

1. Characterize the male tract microbiota
2. Recognize dysbiosis
3. Personalize diagnosis & treatment

cells MDPI

Review

Male Tract Microbiota and Male Infertility

Grande G, Graziani A, De Toni L, Garolla A, Ferlin A.
Cells. 2024;13:1275.

Narrative review: semen microbiota, infertility, infections and HPV.

EAU Guidelines on

Urological Infections

Prostatic calcifications are associated with a more severe symptom burden in men with type II chronic bacterial prostatitis

Konstantinos Stamatiou [1], Vittorio Magri [2], Gianpaolo Perletti [3], Alberto Trinchieri [4], Richard Lacroix [1], Nektaria Rekleiti [1], Hippocrates Moschouris [1]

Images generated with AI

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2 Dysbiosis and endocrine testicular function

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7 Male and female microbiota: 1+1 is not 2!

8 Vaginal microbiota and sperm function

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INFECTIONS AND SEMINAL MICROBIOTA

ALTERED COMPOSITION IN MALE TRACT INFECTIONS

1 SEMINAL DYSBIOSIS IN CHRONIC PROSTATITIS
ALTERED MICROBIAL COMPOSITION

UROLOGY
Volume 153, March 2021, Pages 183-192
Original Article: Clinical Investigation

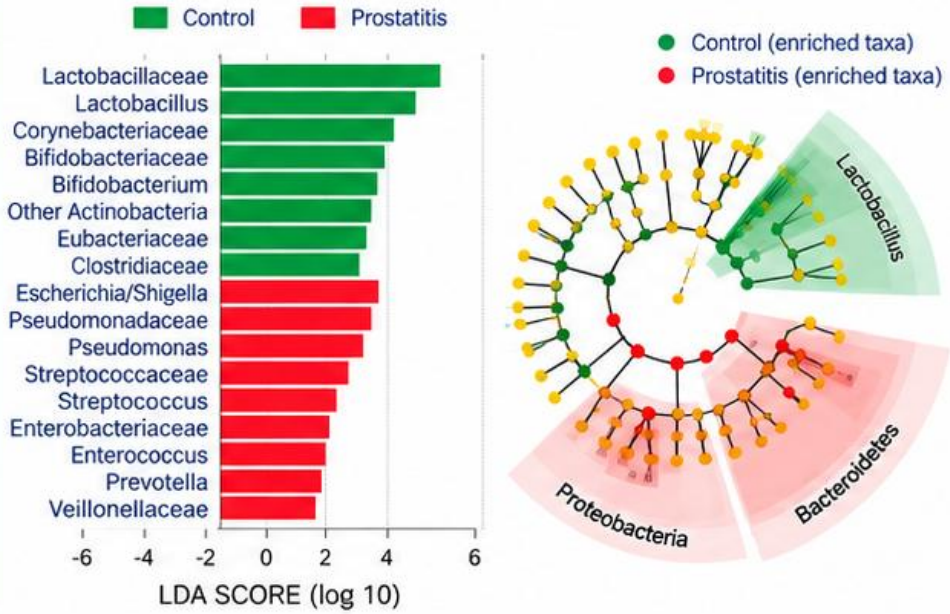
Seminal microbiome in men with and without prostatitis

Brenes Hemudet*, Ine Drungeboot*, Lecneorpheroebrd, Ia. Ae. Greptoeand, the Enteroeched.*, Lbda Gerecaeraes, Yeta, Eeig, Ea.j, Oa. Ceneegrwebeb, Ronomoo c Geenbgr, Yum Cleungrebd, Rierlogeic, Oa. Ceoeeia, Ia., Ittat Oa, Geeteenge Eneo Geis, Oaeoo Oa, Ia Ia, Oeac Ceiech, Ieo, Eotarbed.

- Firmicutes: 41.7%
- Proteobacteria: 19.5%
- Actinobacteria: 9.7%
- Other phyla: 29.1%

Reduction in Bacillota (Firmicutes) (severe reduction in Lactobacilli)

Increase in Pseudomonadota (Proteobacteria)

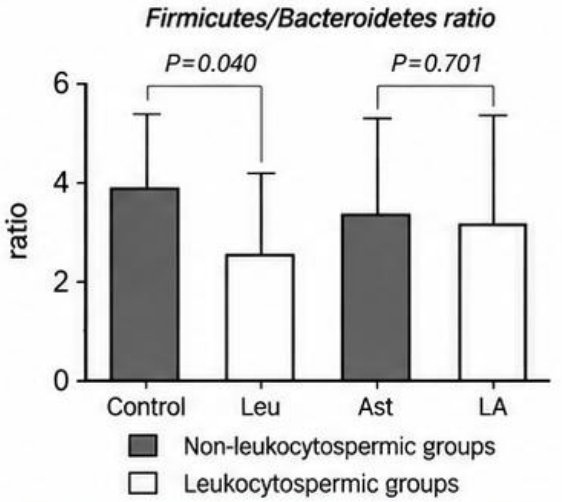


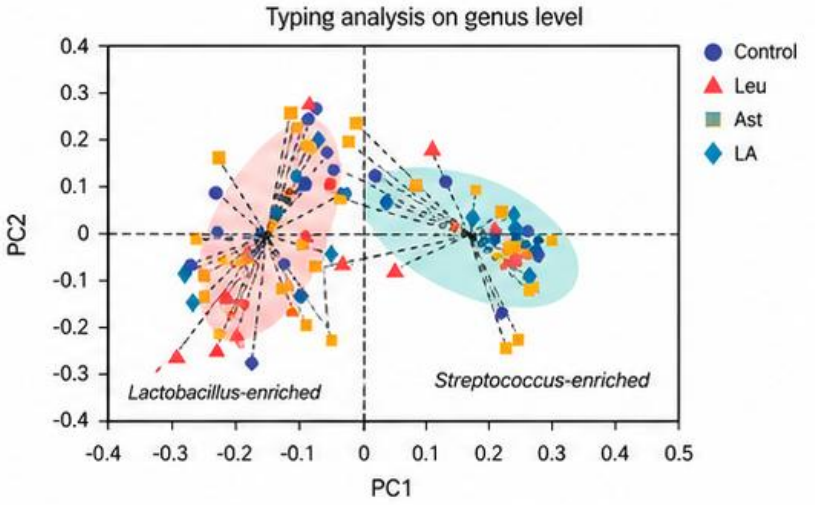
2 LEUKOCYTOSPERMIA AND SEMINAL MICROBIOTA
ALTERED BACTERIAL PROFILE

ORIGINAL ARTICLE
Andrology, 2021, 9, 390–402

Semen microbiota in normal and leukocytospermic males

Tao Zhen Liu, Tao Peiquan Dai, Yunfeng Du, Nengchi hu, Haóngpi Chen, Haidong Chen, Beraba, Eaiocoa, Ia, Gaoo Oaimeay.





Type	Control	Ast	Non-leukocytospermia groups	Leu	LA	Leukocytospermia groups
Type 1	8 (40.0%)	6 (46.2%)	14 (42.4%)	12 (54.5%)	16 (50.0%)	28 (51.9%)
Type 2	12 (60.0%)	7 (53.9%)	19 (57.6%)	10 (45.5%)	16 (50.0%)	26 (48.1%)

MAIN ALTERATIONS OF SEMINAL MICROBIOTA IN MALE TRACT INFECTIONS

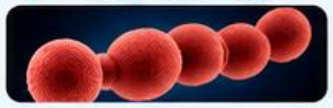
Increase in Prevotella



Reduction in Lactobacilli



Increase in Streptococcus



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GUT DYSBIOSIS IN PATIENTS WITH RECURRENT MALE TRACT INFECTION

STUDY DESIGN AND PATIENT SELECTION

First evaluation for MFI
First level evaluations

↓

Second evaluation for MFI
Selection of patients with semen infections

Exclusion criteria:
- presence of genetic disease
- testicular disease
- hormonal alterations

↓

Third evaluation for MFI
Selection of patients with persistent semen infections (same bacteria)

Enrollment criteria:
- persistent semen infections
- signature of informed consent

↓

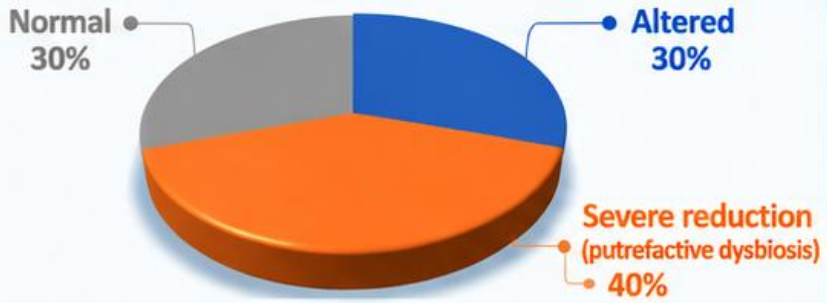
Fourth evaluation for MFI
Discussion of gut microbiota analysis

ISOLATED PATHOGEN IN PATIENTS WITH RECURRENT INFECTIONS

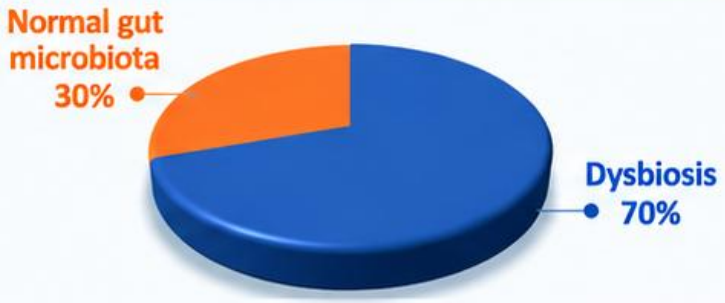
Isolated pathogen	Prevalence (n, %)
Ureaplasma urealyticum	7/20 (35%)
Escherichia coli	5/20 (25%)
Enterococcus faecalis	5/20 (25%)
Citrobacter freundii	2/20 (10%)
Staphylococcus	1/20 (5%)

GUT MICROBIOTA COMPOSITION

BACILLOTA/BACTEROIDOTA (FIRMICUTES/BACTEROIDETES) RATIO



PREVALENCE OF GUT DYSBIOSIS IN PATIENTS WITH RECURRENT MFI



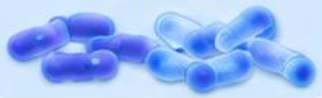
KEY FINDINGS

- Gut dysbiosis is present in 70% of patients with recurrent male tract infections.
- Severe reduction of Bacillota/Bacteroidota (Firmicutes/Bacteroidetes) ratio (putrefactive dysbiosis) is observed in 40% of cases.
- The two most frequent enterotypes are:

Prevotella-dominant



Bacteroidetes-dominant



life

Article

Gut Dysbiosis in Infertile Patients with Persistent Male Accessory Gland Infection

Giuseppe Grande [1,*], Andrea Graziani [2], Raffaele Scafa [2], Luca De Toni [2], Andrea Garolla [1,2] and Alberto Ferlin [1,2]

MDPI

PROBIOTIC SUPPLEMENTATION IMPROVES ERADICATION RATES IN MEN WITH ACCESSORY GLAND INFECTIONS



STUDY DESIGN AND PROBIOTIC SUPPLEMENTATION

Original Article

Association of Probiotic Treatment With Antibiotics in Male Accessory Gland Infections

Giuseppe Grande[1,2,3], Giuseppina Pompa[1,2], Anna Laura Astorri[1,2], Alfredo Pontecorvi[1,2], and Domenico Milardi[1,2]

American Journal of Men's Health
September-October 1-7
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DOI: 10.1177/1557988832119064
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SAGE

Antibiotic treatment

+

Probiotic supplementation

Saccharomyces boulardii during antibiotic treatment

- **Lactobacillus rhamnosus LR32**
- **Lactobacillus plantarum LP115**
- **Lactobacillus acidophilus LA14**

after antibiotic treatment

ISOLATED PATHOGENS IN OUR STUDY POPULATION

(N = 104)

Type of germ	No. of patients (N = 104)	%
Enterococcus faecalis	57	54.98
Escherichia coli	24	22.64
Klebsiella pneumoniae	10	9.7
Citrobacter freundii	10	9.7
Morganella morganii	3	3.23
Proteus mirabilis	3	3.23
Pseudomonas aeruginosa	3	3.23

ERADICATION RATE

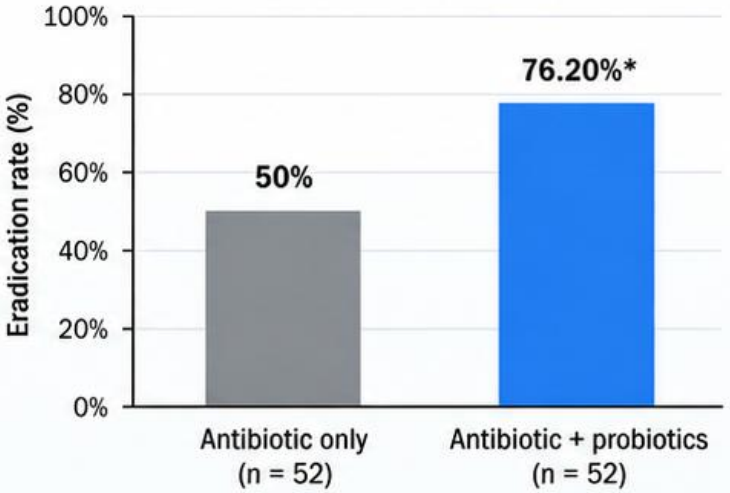


Figure 1. Eradication Rate in Patients Treated With Antibiotics and With Antibiotics Associated With Probiotics. (*p < .005).

KEY TAKEAWAYS

Probiotic supplementation added to antibiotic therapy significantly **improves eradication rates (76.2% vs 50%, p < 0.005)**.

The most frequently isolated pathogens were ***Enterococcus faecalis* (55%)** and ***Escherichia coli* (23%)**.

A combination of *Saccharomyces boulardii* and specific *Lactobacillus* strains (*L. rhamnosus LR32*, *L. plantarum LP115*, *L. acidophilus LA14*) may support the **restoration of healthy seminal microbiota** during and after antibiotic treatment.

Preliminary study (2020) | Published in 2022

Grande G, Pompa G, Astorri AL, Pontecorvi A, Milardi D. Association of Probiotic Treatment With Antibiotics in Male Accessory Gland Infections. *Am J Men's Health*. 2022. doi: 10.1177/1557988322119064.

From probiotic supplementation towards a probiotic precision medicine

life

MDPI

Article

Associations of Adjunctive Multistrain Probiotic/Prebiotic Supplementation with Microbiological Eradication and Semen Parameters in Infertile Men with Male Tract Infections: A Retrospective Cohort Study

Giuseppe Grande [1,*,†], Andrea Graziani [1,2,†], Rossella Mazzilli [3], Davide Abate [4], Luca De Toni [2] and Alberto Ferlin [1,2]

Published: 9 October 2026

Integrated therapy in male genital tract infections

ANTIBIOTICS PLUS PROBIOTICS IMPROVE MICROBIOLOGICAL ERADICATION

FROM EMPIRICAL TREATMENT TO EVIDENCE-BASED MICROBIOME-ORIENTED STRATEGIES

1 Rationale

Why combine antibiotics and probiotics?



High rate of recurrence and persistence after antibiotic therapy (40–50%)



Infections are associated with genital and gut dysbiosis



Probiotics may help to restore microbial balance, improve eradication and sperm parameters

A combined antibiotic–probiotic approach could enhance treatment efficacy and reduce recurrence.

2 Study design

Retrospective observational cohort

Potentially eligible patients
with microbiologically confirmed MTIs
(n = 120)

↓

Excluded before final cohort
Other antibiotic regimens due to contraindications or resistance
(n = 20)

↓

Final analytic cohort
Doxycycline-susceptible MTIs; adherence verified
(n = 100)

↓

Cases
Antibiotic + multi-strain probiotic/prebiotic
(n = 50)
Doxycycline + probiotics/prebiotics
Standardized counseling

Controls
Antibiotic alone
(n = 50)
Doxycycline regimen only
Standardized counselling

↓

Follow-up assessments
Microbiological evaluation: 30 ± 5 days
Semen analysis: 90 ± 10 days

Probiotic and prebiotic supplementation

Probiotic used:



OMNi-BiOTiC® 10 AAD

During antibiotic treatment

- *Lactob. acidophilus* **W55**
- *Lactob. acidophilus* **W37**
- *Lactiplantib. plantarum* **W1**
- *Bifidob. animalis ssp. lactis* **W51**
- *Enteroc. lactis* **W54**
- *Lacticaseib. paracasei* **W20**
- *Lacticaseib. rhamnosus* **W71**
- *Ligilactob. salivarius* **W24**
- *Lactiplantib. plantarum* **W62**
- *Bifidob. bifidum* **W23**

Probiotic used:

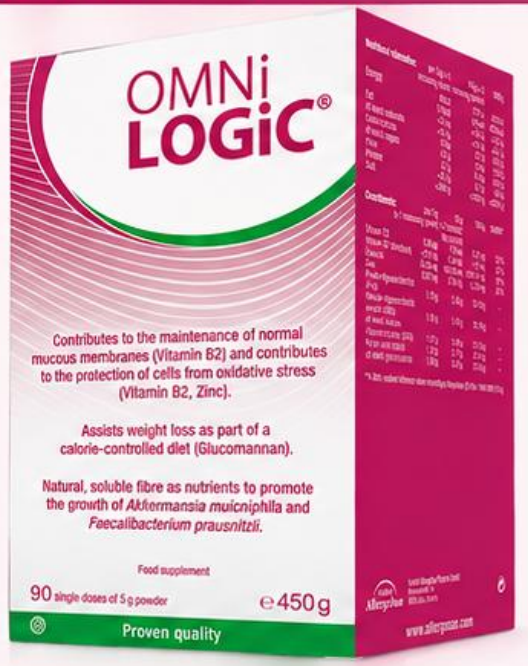


OMNi-BiOTiC® Flora Plus

For 30 days after antibiotic treatment

- *Lactob. crispatus* **LBV88**
- *Lacticaseib. rhamnosus* **LBV96**
- *Lactob. gasseri* **LBV150N**
- *Lactob. jensenii* **LBV11**

Prebiotic used:



OMNi-LOGiC® Plus

For 30 days after antibiotic treatment

- FOS
- GOS
- Glucomannan

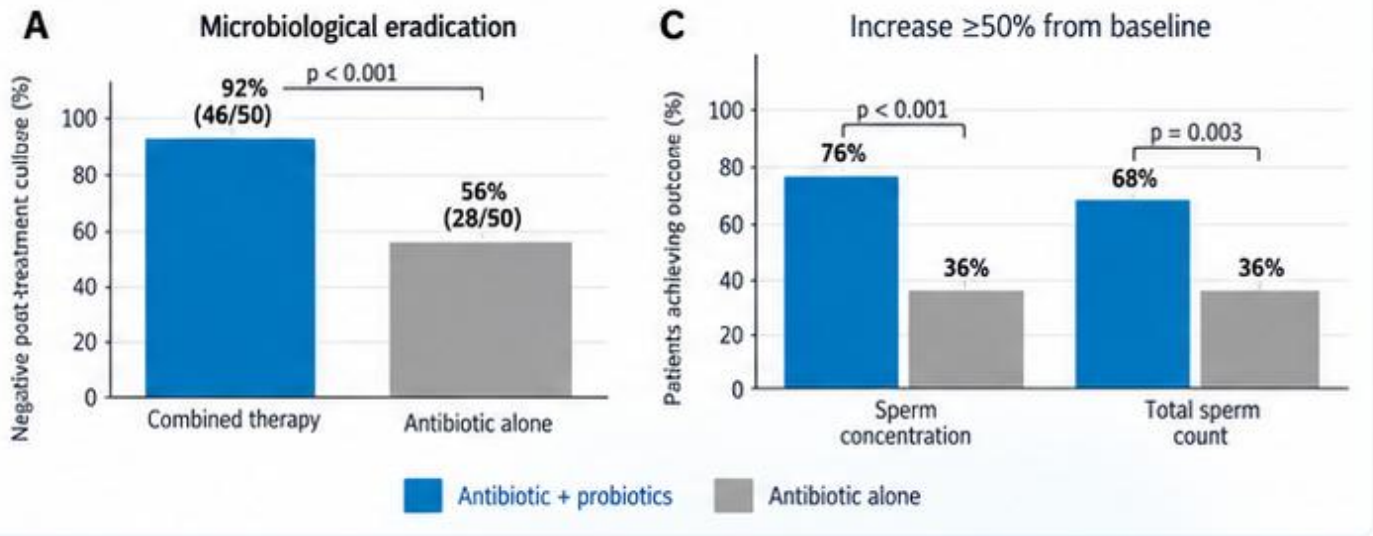
Integrated therapy in male genital tract infections

ANTIBIOTICS PLUS PROBIOTICS IMPROVE MICROBIOLOGICAL ERADICATION

FROM EMPIRICAL TREATMENT TO EVIDENCE-BASED MICROBIOME-ORIENTED STRATEGIES

3 Results

Outcome	Cases n/N (%)	Controls n/N (%)	Risk difference % (95% CI)	OR (95% CI)	p-value
Microbiological eradication	**46/50 (92.0)**	**28/50 (56.0)**	**36.0 (20.3 to 51.7)**	**9.04 (2.82–28.95)**	**<0.001**
Sperm concentration > baseline	47/50 (94.0)	30/50 (60.0)	34.0 (18.9 to 49.1)	10.44 (2.85–38.21)	<0.001
Sperm concentration ≥1.5-fold baseline	38/50 (76.0)	18/50 (36.0)	40.0 (22.2 to 57.8)	5.63 (2.36–13.42)	<0.001
Total sperm count > baseline	48/50 (96.0)	28/50 (56.0)	40.0 (25.2 to 54.8)	18.86 (4.12–86.28)	<0.001



Antibiotic–probiotic therapy significantly improves microbiological eradication and seminal parameters in men with male genital tract infections.

These findings support a transition

- from empirical antibiotic treatment
- towards an integrated, microbiome-oriented strategy
- and the development of probiotic precision medicine in male reproductive infections.

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2 Dysbiosis and endocrine testicular function

3 Male tract and semen «normal» microbiota

4 Seminal microbiota and infertility

5 Microbiota in clinical practice: beyond semen parameters

6 Male tract infections and microbiota

7 Male and female microbiota: 1+1 is not 2!

8 Vaginal microbiota and sperm function

Image generated with AI

From genital to couple microbiota

1 Human genital tracts microbiota: dysbiosis crucial for infertility

Journal of Endocrinological Investigation (2022) 45:1151–1160
https://doi.org/10.1007/s40618-022-01752-3

SHORT REVIEW

Human genital tracts microbiota: dysbiosis crucial for infertility

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Received: 13 December 2021 / Accepted: 18 January 2022 / Published online: 3 February 2022
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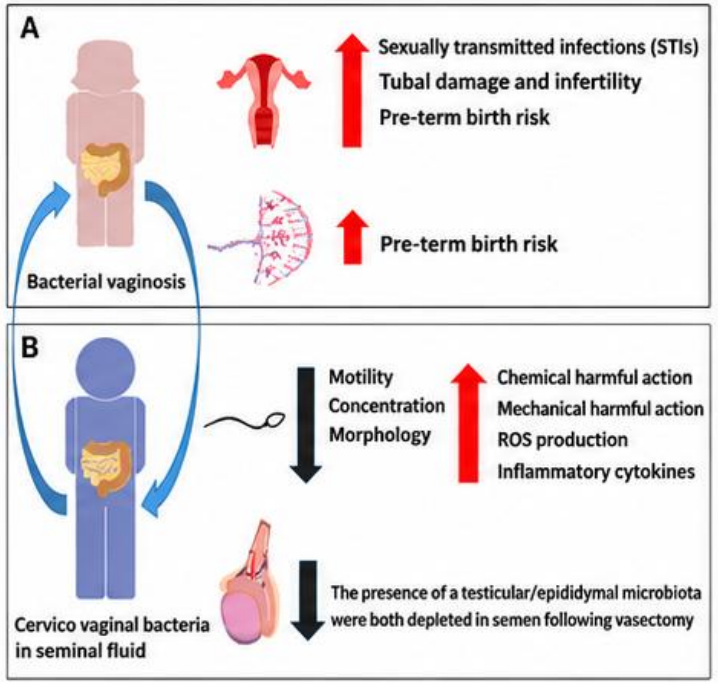

2 *Lactobacillus crispatus*-dominated vaginal microbiome and *Acinetobacter*-dominated seminal microbiome support beneficial ART outcome

Received: 28 December 2022 | Revised: 6 March 2023 | Accepted: 23 March 2023
DOI: 10.1111/aogs.14598

ORIGINAL RESEARCH ARTICLE

Lactobacillus crispatus-dominated vaginal microbiome and *Acinetobacter*-dominated seminal microbiome support beneficial ART outcome

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Community type		Healthy group, n (%)	ART group, n (%)	ART success rate, n (%)[a]
Vaginal communities				
I	*Lactobacillus iners*	4 (33.3)	31 (31.9)	8 (25.8)
II	*Lactobacillus crispatus*	4 (33.3)	25 (25.8)	9 (36.0)
III	BV community	0	19 (19.6)	5 (26.3)
IV	Two lactobacilli (*L. iners* + *L. crispatus*)	2 (16.7)	9 (9.3)	1 (11.1)
V	*Lactobacillus jensenii*	1 (8.3)	3 (3.1)	1 (33.3)
VI	*Lactobacillus gasseri*	0	4 (4.1)	0
VII A	Diverse community A (*Lactobacillus*+*Bifidobacterium*)	0	4 (4.1)	3 (75.0)
VII B	Diverse community B (*Lactobacillus*+*Streptococcus*)	1 (8.3)	2 (2.1)	1 (50.0)
Women with community types with higher success rate (II, V, VII), mean age 34.6 ± SD 4.3				14 (41.2)**
Women with community types with lower success rate (I, III, IV, VI), mean age 34.0 ± SD 4.9				14 (22.2)**
Semen communities				
I	*Acinetobacter* (plus *L. iners*, *Corynebacterium*, *Prevotella*)	7 (58.3)	48 (49.5)	17 (35.4)*
II	Gram-negative anaerobic/ microaerophilic *Porphyromonas*, *Dialister* or *Campylobacter*)	1 (8.3)	14 (14.4)	1 (7.1)*
III	Gram-negative + Gram-positive (*Prevotella*, *Acinetobacter*, *Porphyromonas*, *Dialister*, *Campylobacter*, *L. iners*, *L. crispatus*, *Corynebacterium*, *Gardnerella vaginalis*, *Finegoldia*, *Fenollaria*)	1 (8.3)	14 (14.4)	4 (28.6)
IV	*L. iners* + vaginal bacteria (*L. crispatus*, *G. vaginalis*, *Sneathia*, *Corynebacterium*)	2 (16.7)	13 (13.4)	2 (15.4)
V	*Corynebacterium*	1 (8.3)	4 (4.1)	2 (50)
VI	*L. iners*	0	2 (2.1)	1 (50)
	Veillonella-predominated community	0	1 (1.0)	1 (7.1)
	Haemophilus parainfluenzae- predominated community	0	1 (1.0)	0
Men with community type with higher success rate (I), mean age 38.6 ± SD 7.6				17 (35.4)
Men with community types with lower success rate or scarce types (II–VI), mean age 36.4 ± SD 5.8				11 (22.4)

3 Genital microbiota in infertile couples

RBMO

ARTICLE

Genital microbiota in infertile couples



BIOGRAPHY
Miloš Stojanov is a Senior Lecturer at the University Hospital of Lausanne, Switzerland. He leads multiple research initiatives in the field of genital microbiota. Notably, he is leading several projects on the characterization of the male genital tract microbiota and investigates the implications of sexual transmission on genital microbiota composition.

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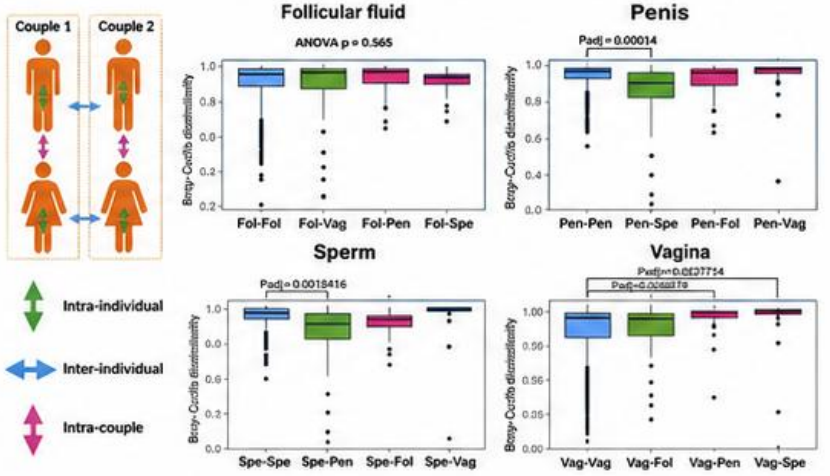

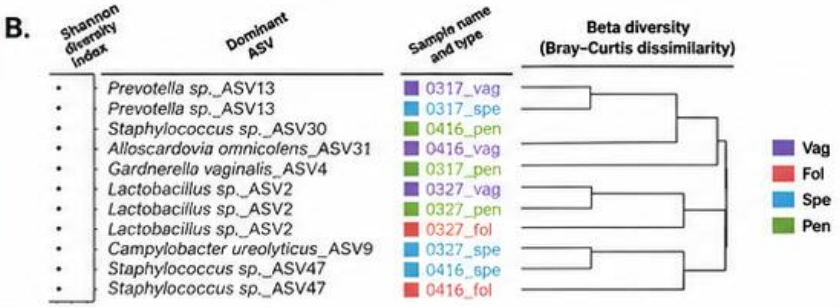

FROM GENITAL TO COUPLE MICROBIOTA

frontiers in Medicine

TYPE Mini Review
PUBLISHED 2 May 2026
DOI 10.3389/fmed.2026.1784130

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The overlooked role of the seminovaginal microbiota in infertility: a narrative mini review

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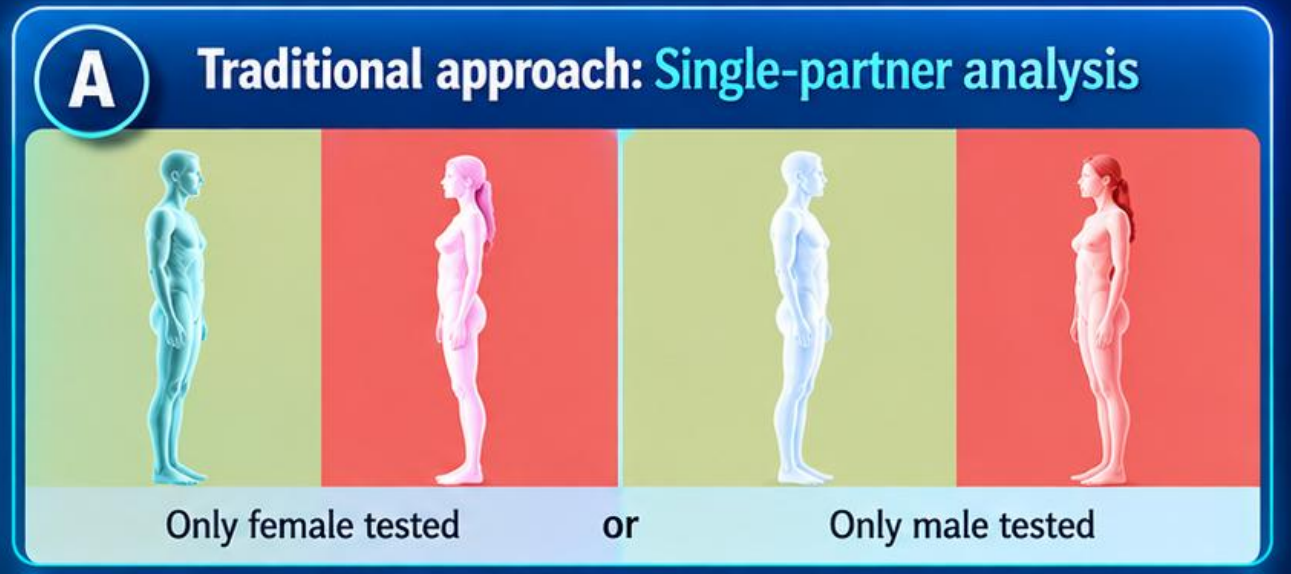

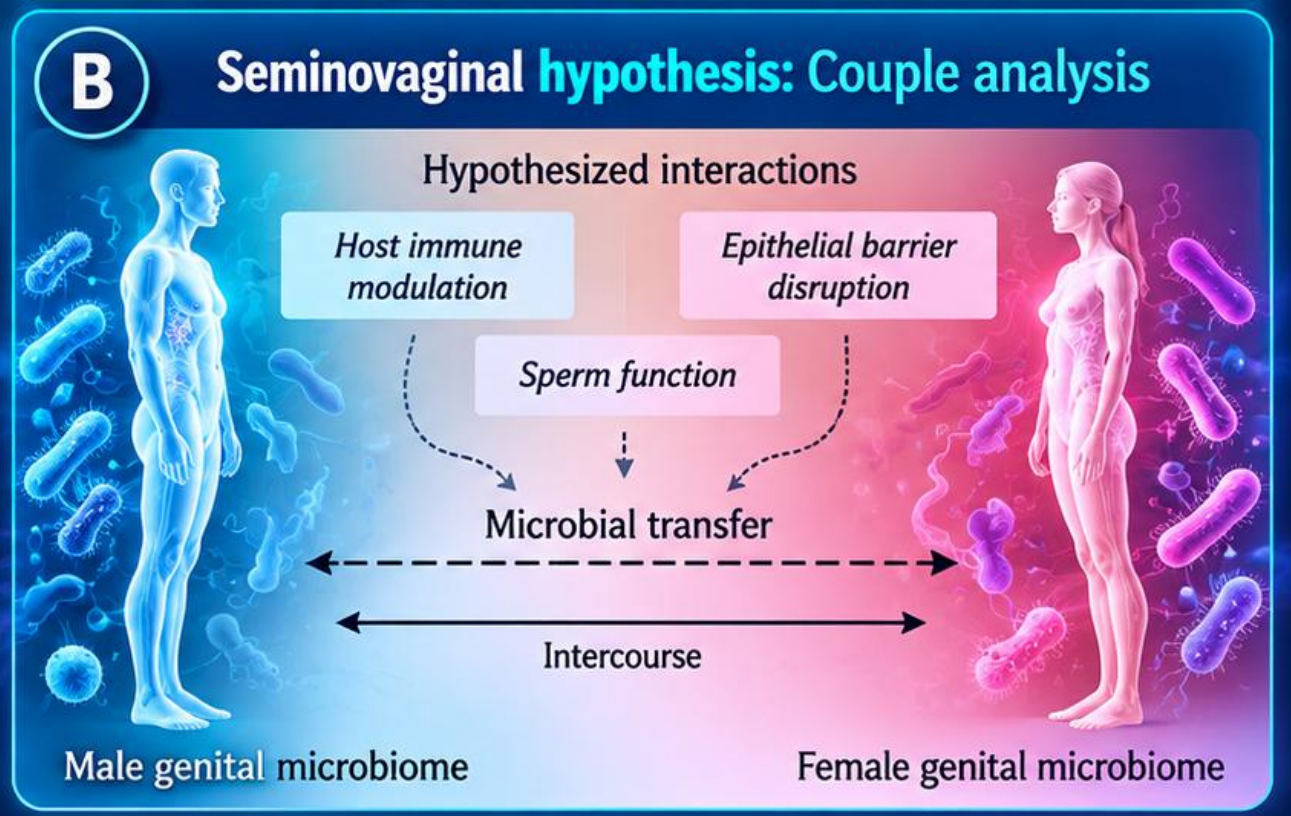

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Ruolo dei prodotti del metabolismo del microbiota vaginale sull'induzione dell'attivazione della motilità dello spermatozoo attraverso stimolazione dei recettori olfattori

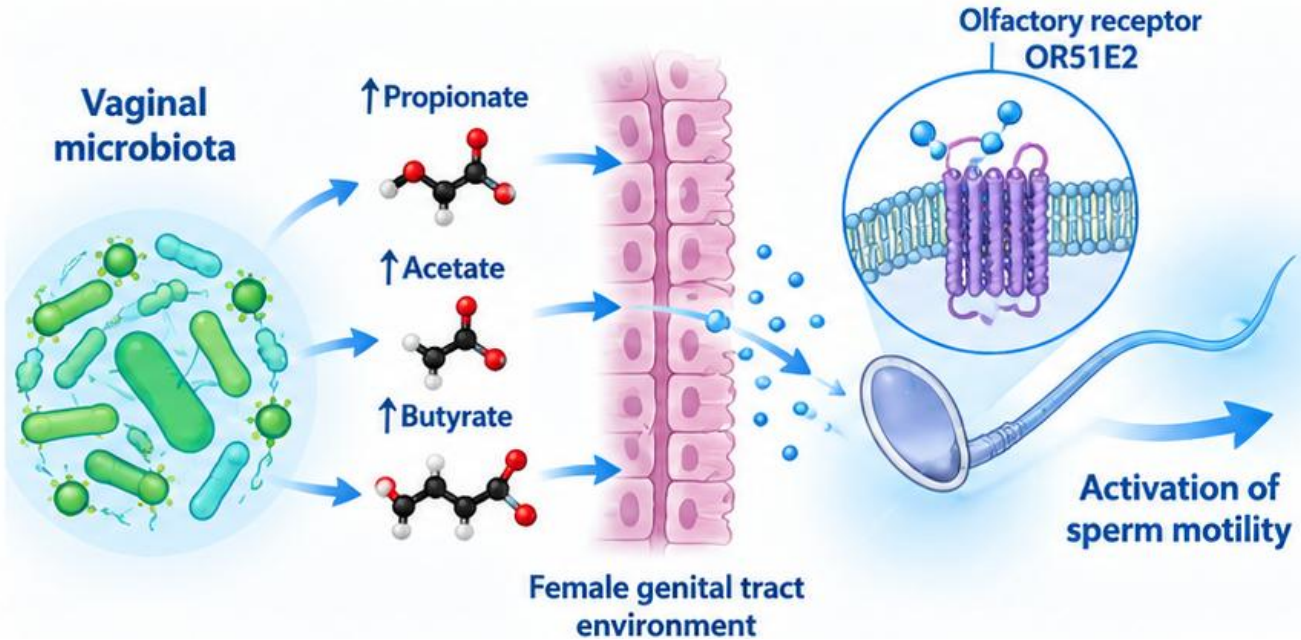
International Journal of *Molecular Sciences*

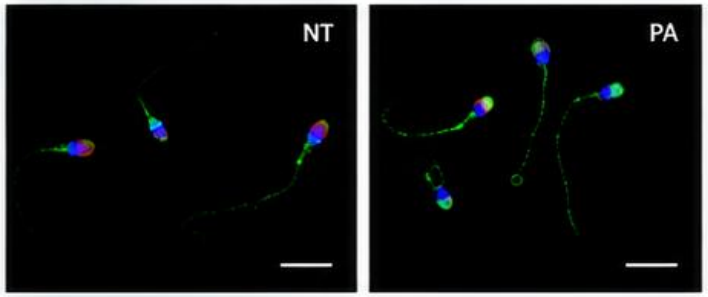
MDPI

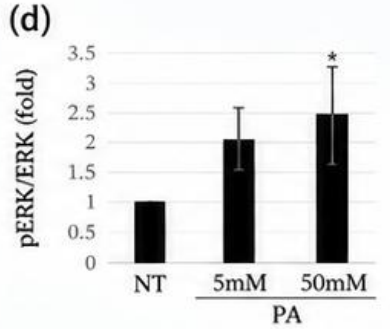
Article

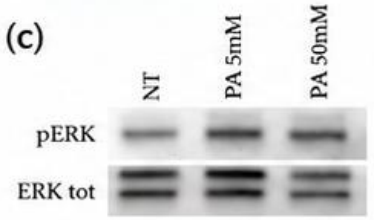
Short-Chain Fatty Acids Modulate Sperm Migration through Olfactory Receptor 51E2 Activity

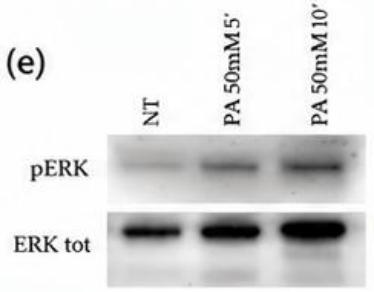
Emanuela Teveroni [1,†], Fiorella Di Nicuolo [1,†], Edoardo Vergani [2], Carmine Bruno [2], Giuseppe Maulucci [3,4], Giada Bianchetti [3,4], Anna Laura Astorri [2], Giuseppe Grande [5], Jacopo Gervasoni [6], Lavinia Santucci [7], Marco De Spirito [3,4,‡], Andrea Urbani [6,‡], Alfredo Pontecorvi [1,2,*], Francesca Mancini [1,†] and Domenico Milardi [2,†]

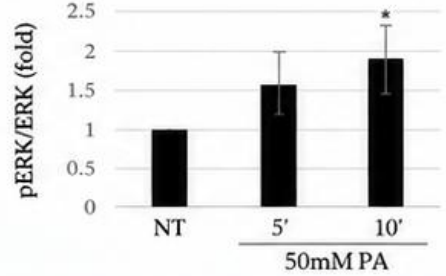


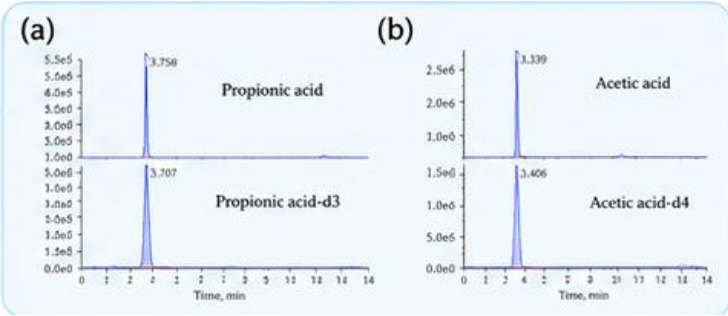












cells

MDPI

Review

Male Tract Microbiota and Male Infertility

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Abstract: In recent years, several studies have analyzed the composition of the male genital tract microbiota and its changes in infertility or in different situations associated with infertility. The aim of this narrative review is to obtain more insight on this topic; in particular, to describe actual evidence about changes in the semen microbiota in patients with infertility, male tract infections, or HPV infections. In semen, an increase in semen *Prevotella* spp. is associated with oligozoospermia and with obesity-associated asthenozoospermia; an increase in *Pseudomonas* is more frequently associated with asthenozoospermia and oligozoospermia; a reduction in *Lactobacilli* spp. (namely in *Lactobacillus crispatus*) may represent a marker of low semen quality. However, an increase in *Lactobacillus iners* is considered a risk factor for a reduced sperm concentration. In patients with prostatitis, there is a reduction in *Lactobacillus* spp. and an increase in *Streptococcus* spp., opening important perspectives about the role of probiotic treatments in these patients. Finally, an increase in *Fusobacteria* spp. was observed in patients with an HPV infection. In the conclusion, we underline the interactions between the seminal and vaginal microbiota, so that further studies should focus on the "couple genital microbiota".

Keywords: microbiome; microbiota; male infertility; gut; prostate; prostatitis

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REVIEW ARTICLE · Articles in Press, 100128, October 09, 2026

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The Seminal Microbiome in Male Reproductive Health: A Systematic Review of Current Evidence, Biological Mechanisms, and Clinical Implications

Beatrice Crestani, MD [1,2] · Rossana Di Paola, MD [1] · Stefano Uccella, MD, PhD [1] · Filippo Alberto Ferrari, MD [3] · Mauro Cozzolino, MD, PhD [4,5] · Francesco Gebbia, MD [2] · Giuseppe Grande, MD, PhD [6] · Alberto Ferlin, MD, PhD [6,7] · Danilo Cimadomo, MD, PhD [8,9] · Gemma Fabozzi, MD, PhD [9,10] · Rossella Mazzilli, MD, PhD [2,9,11] Show less

Regional Specialized Center for Klinefelter Syndrome

Regional Specialized Center for Male Gamete Cryopreservation

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