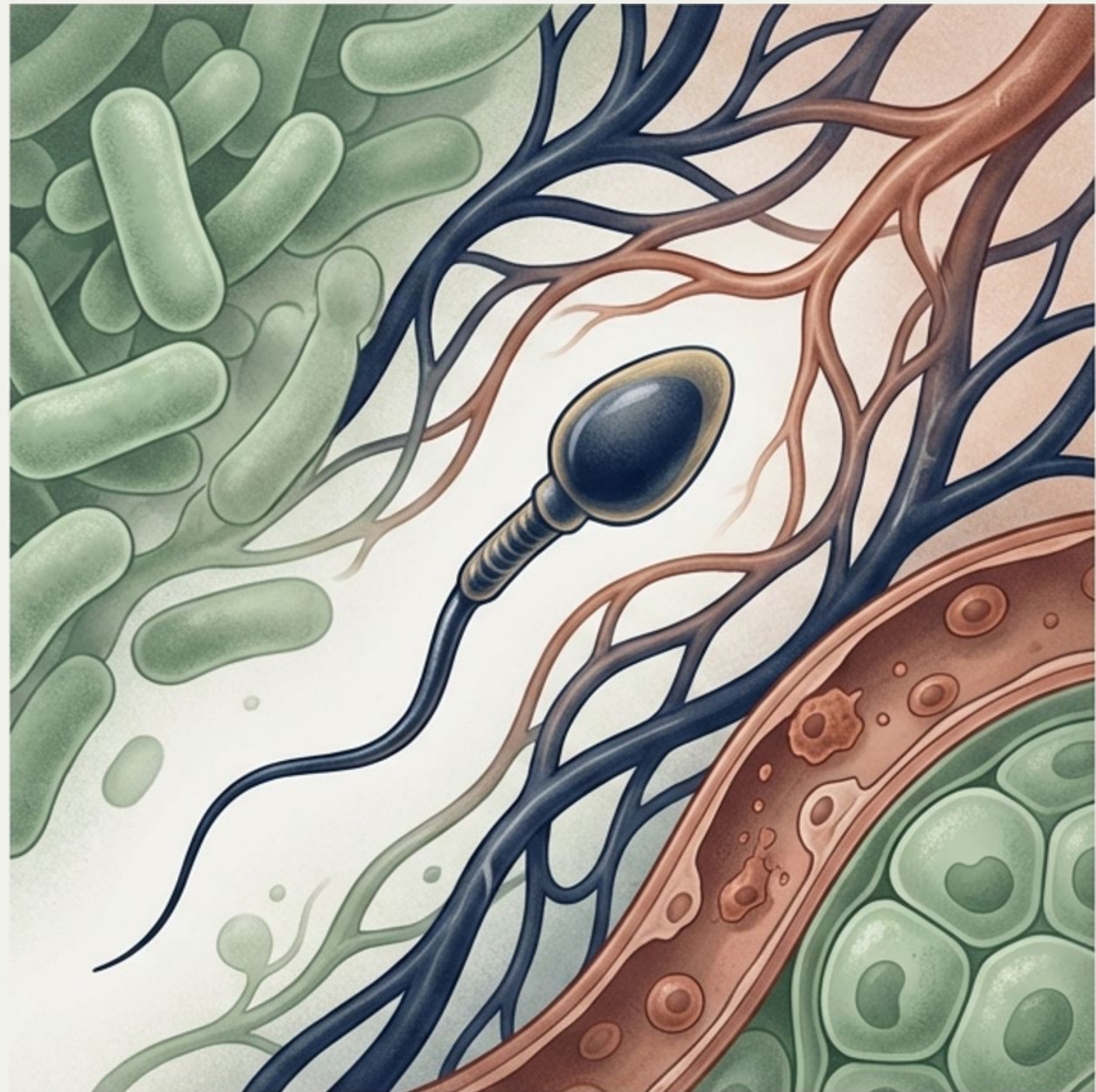


# The Systems Biology of Male Fertility

How the Microbiome,  
Microbiota, Cellular  
Inflammation, Leaky  
Gut Syndrome,  
Metagenome Shape Male  
Reproductive Health

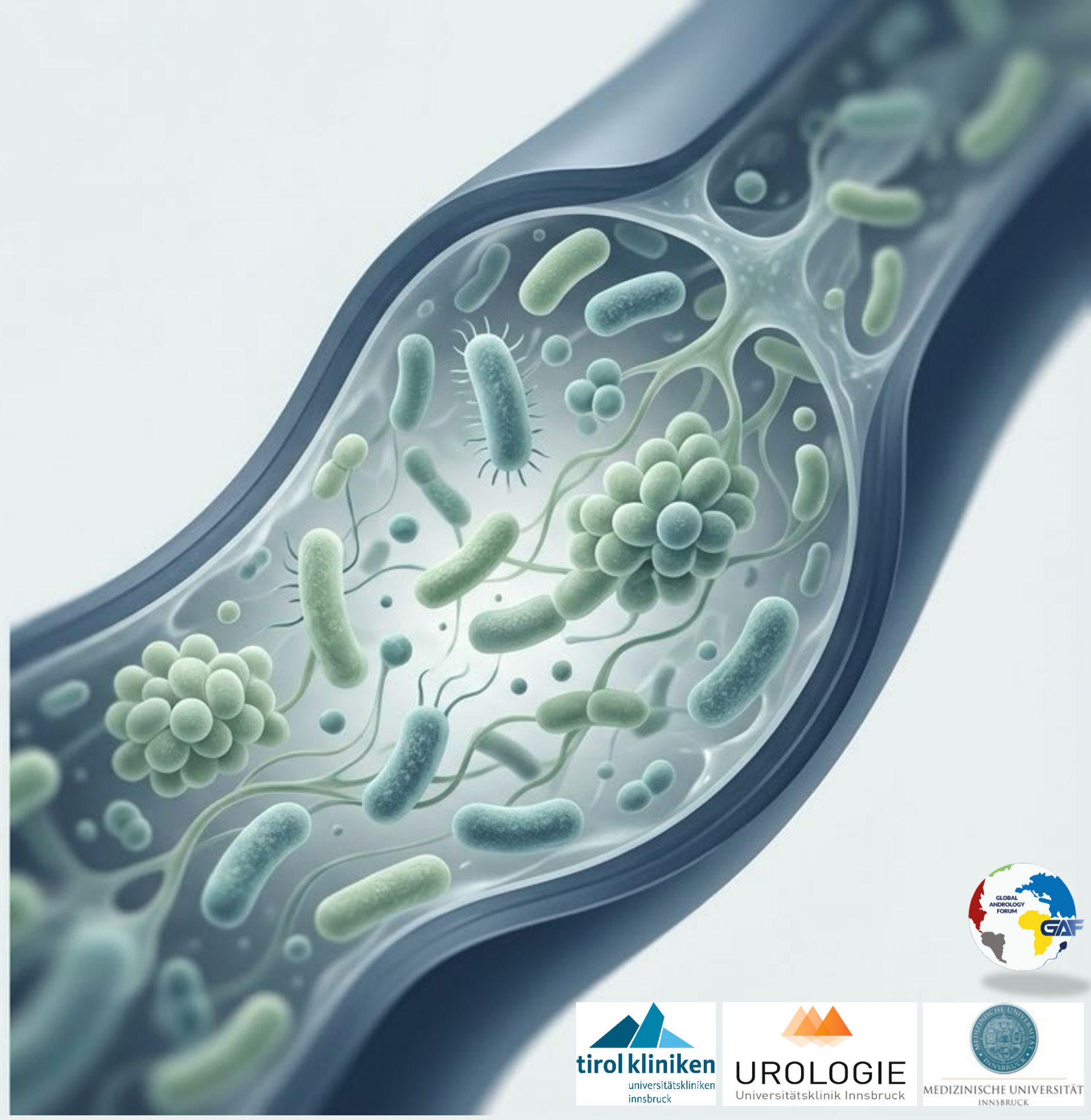


# Rethinking Male Reproductive Health

## The Genitourinary Microbiome

Integrating microbiome profiling and targeted therapies into future infertility management.

Pinggera, Germar-M., Dr.med., PLL.M., FECSM, Univ.Prof.hc  
Dept. Of Urology, Medical University Innsbruck, Austria

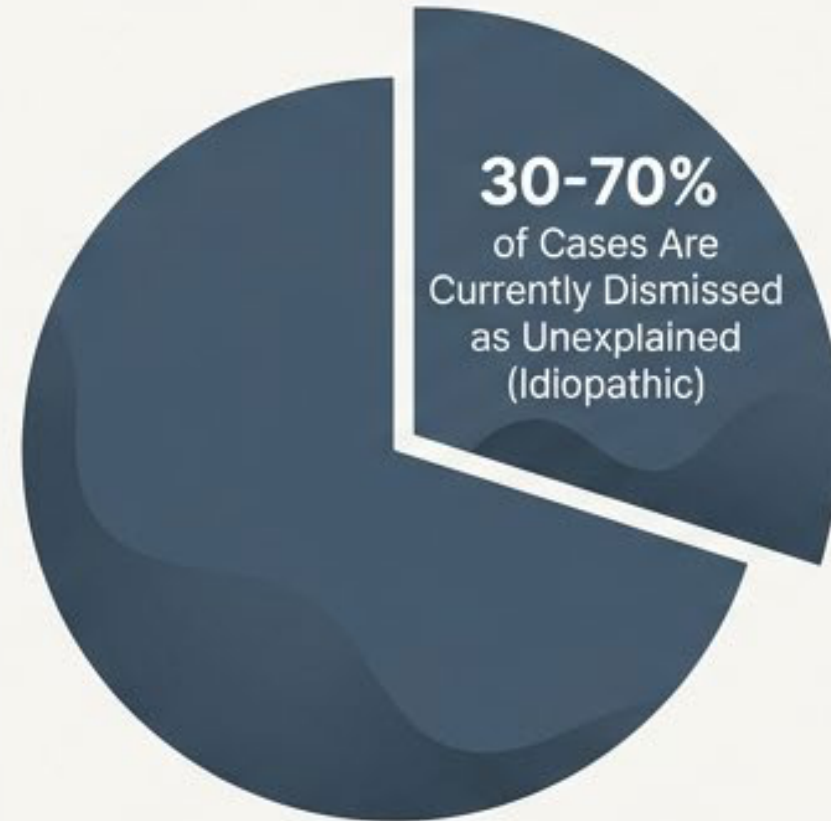


# Moving Beyond ‘Idiopathic’ Diagnoses in the Male Fertility Crisis

**+23%-(0-55%) Rise in Male Infertility Over 30 Years**



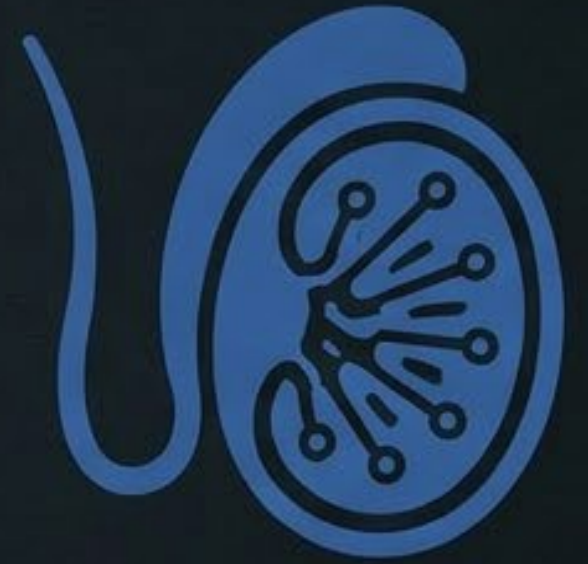
**56 Million Men Affected Globally**



- Historically, male infertility has been viewed through a narrow lens of genetics and direct endocrine failure.
- However, emerging evidence points to oxidative stress, systemic infections, and microbial dysbiosis as silent, primary drivers.

- The New Paradigm: The Environment-Gut-Testis Triad.

We must reframe male fertility as a **highly reactive system vulnerable to everyday lifestyle and systemic inflammatory factors.**

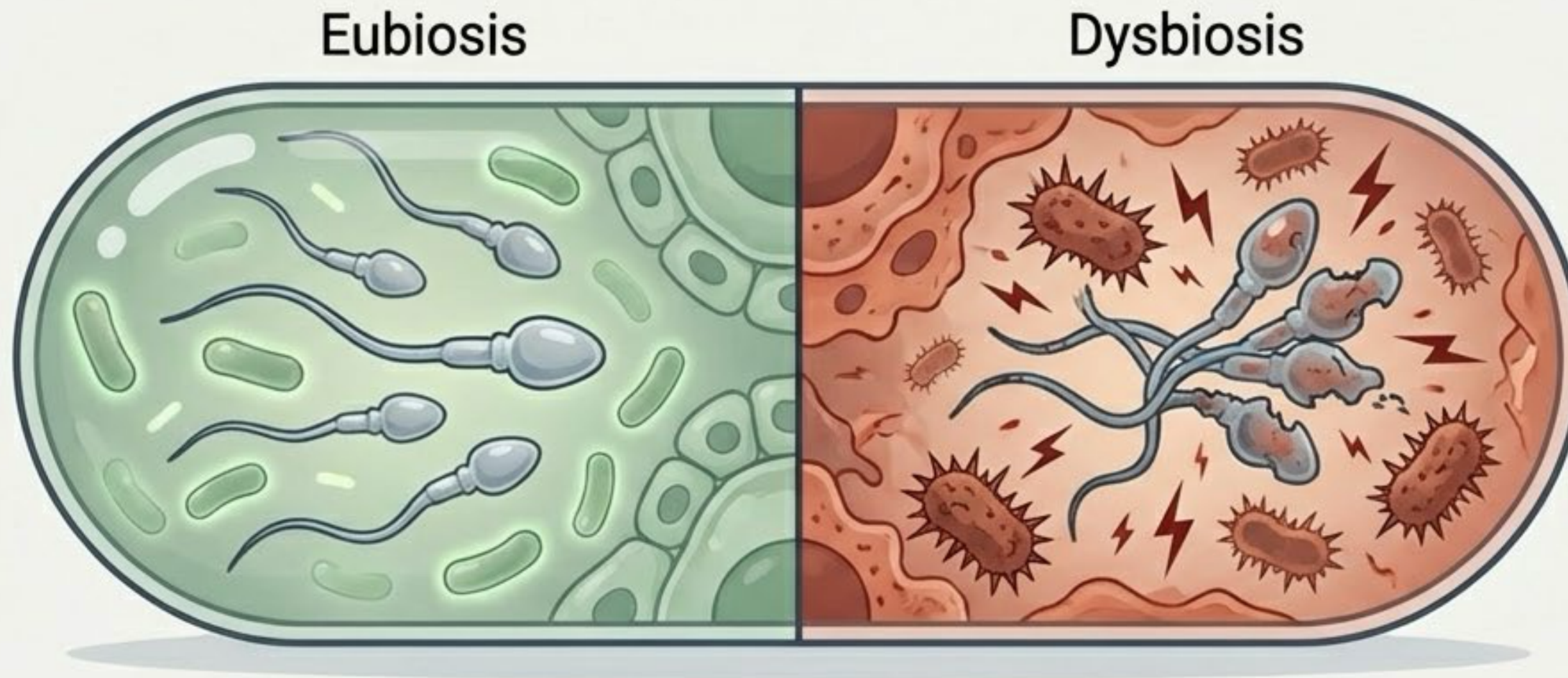


# Can gut dysbiosis impair male fertility?

Testosterone • Sperm quality • HPG axis

Leaky gut • LPS • Hormonal disruption

# The Seminal Microbiome and the Threat of Reactive Oxygen Species



- Semen contains its own complex microbial ecosystem that directly dictates sperm quality and IVF success.
- **Eubiosis:** *Lactobacillus*-dominant semen is associated with the highest sperm motility, antioxidant defenses, and ART outcomes.
- **Dysbiosis:** Pathogens like *Ureaplasma parvum* adhere to spermatozoa, causing agglutination and summoning leukocyte infiltration.
- This immune response releases high levels of **Reactive Oxygen Species (ROS)**, causing catastrophic lipid peroxidation and sperm DNA fragmentation.

Unlike somatic cells, sperm lack robust antioxidant defenses, making them exceptionally vulnerable to microbiome-induced oxidative stress.

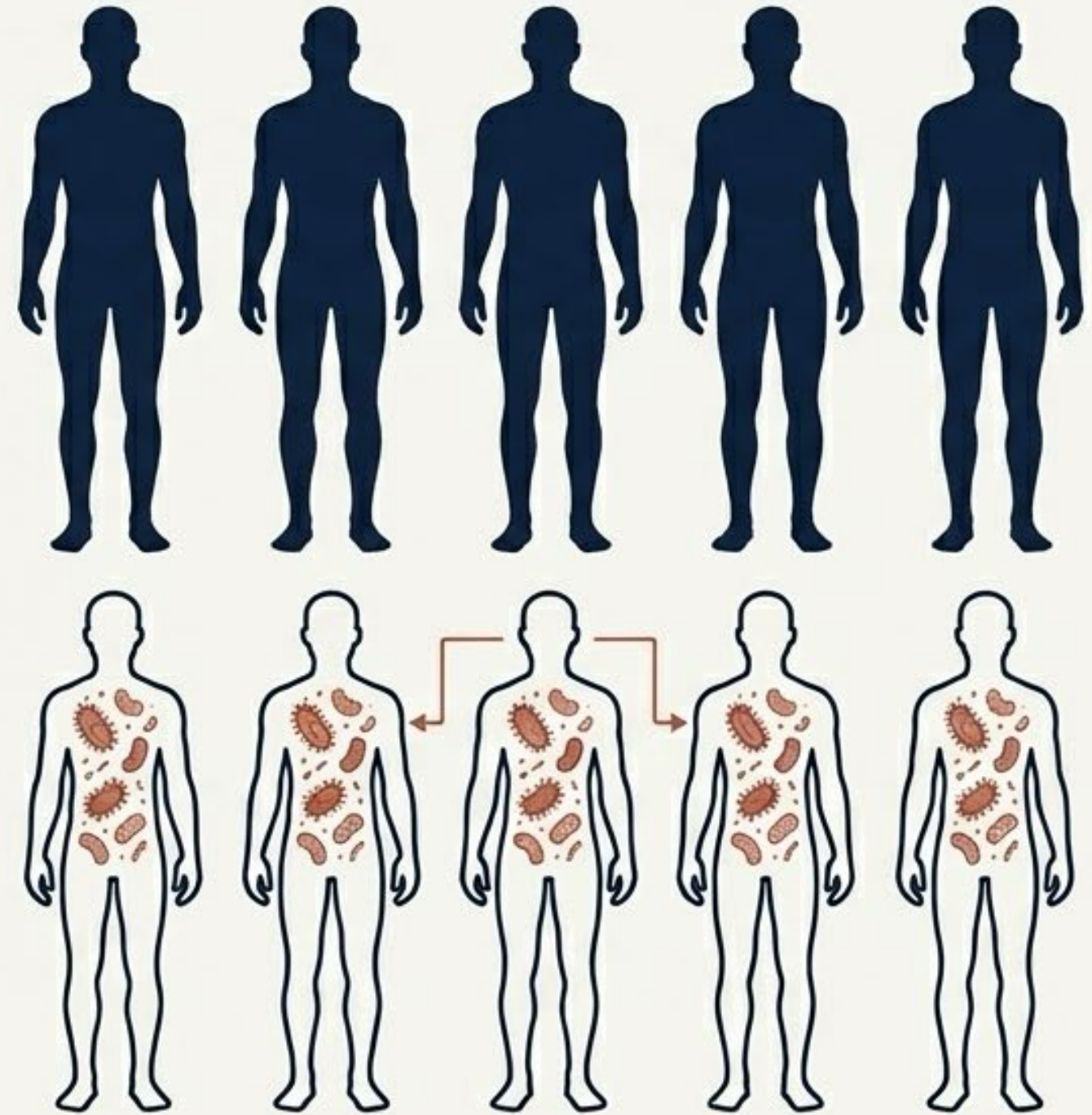
# Idiopathic infertility accounts for up to half of all clinical cases

## The Missing Link

# 30% - 50%

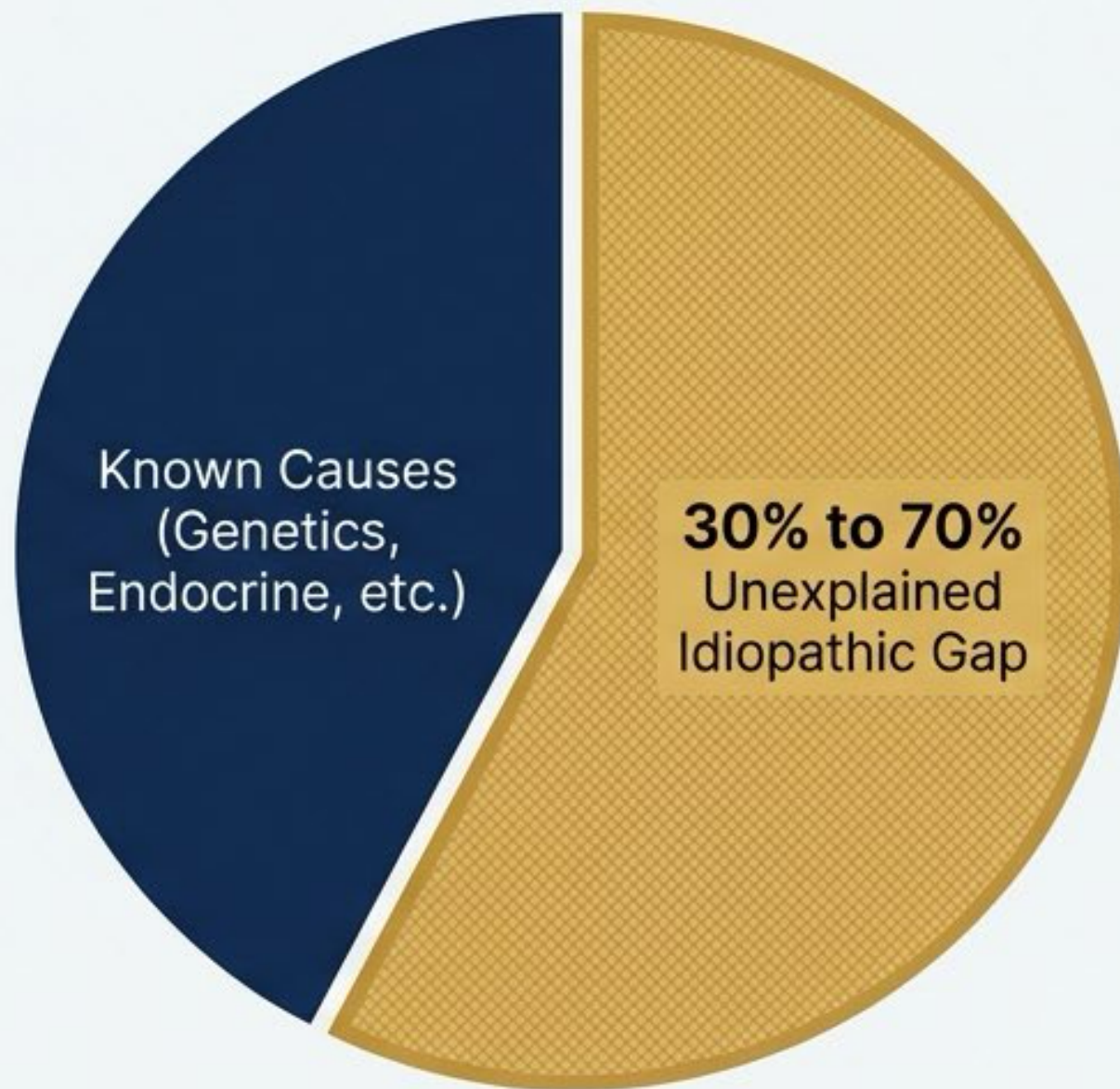
For decades, up to half of all male infertility cases have been labeled “idiopathic.”

Disruptions in microbiota homeostasis—termed **dysbiosis**—alter microbial composition, functional capacity, and metabolic activity, directly reducing reproductive outcomes.



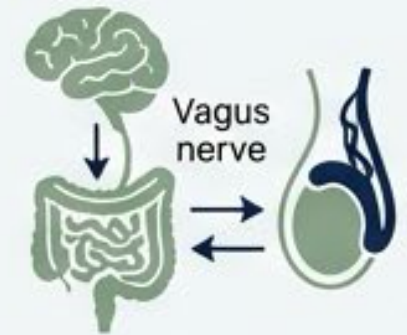
# The Idiopathic Gap in Modern Andrology

Over 56 million men globally face infertility. Despite decades of research into genetics and endocrine failures, 30% to 70% of these cases are classified as entirely idiopathic.

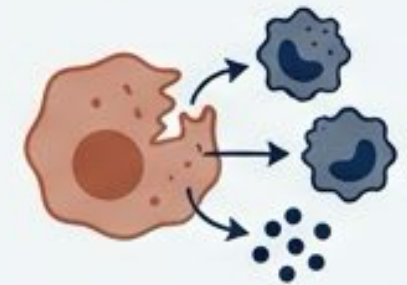


A new triad of influencers has emerged to explain the unexplained:

- The Gut-Testis Axis



- Cellular Inflammation



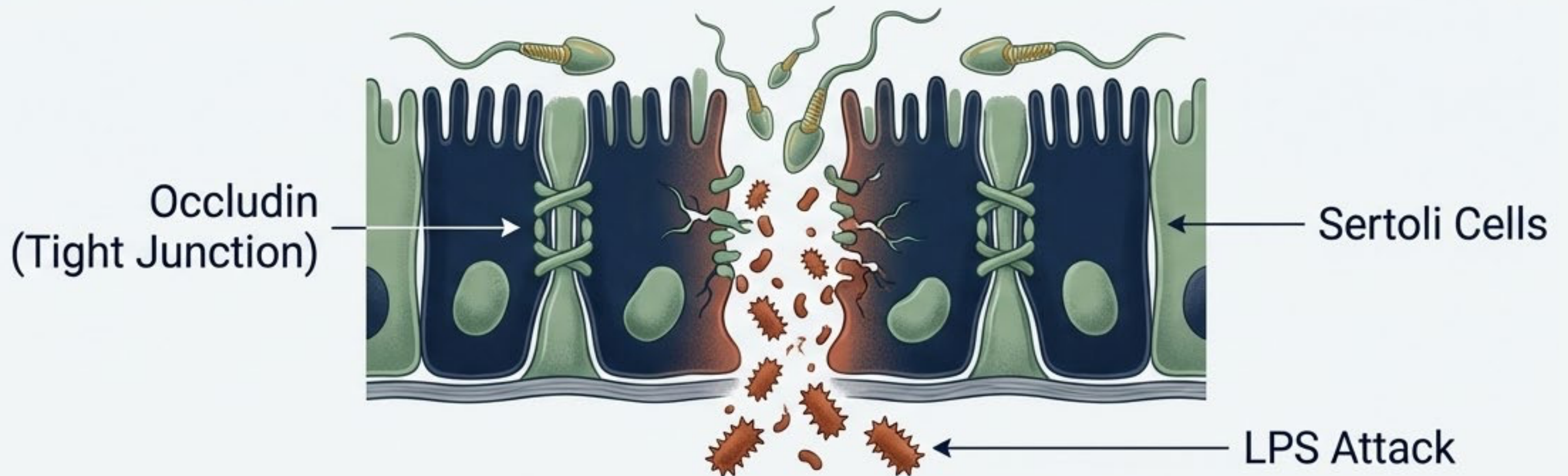
- Acute Nutritional Intake



# The Frontline: The Blood-Testis Barrier

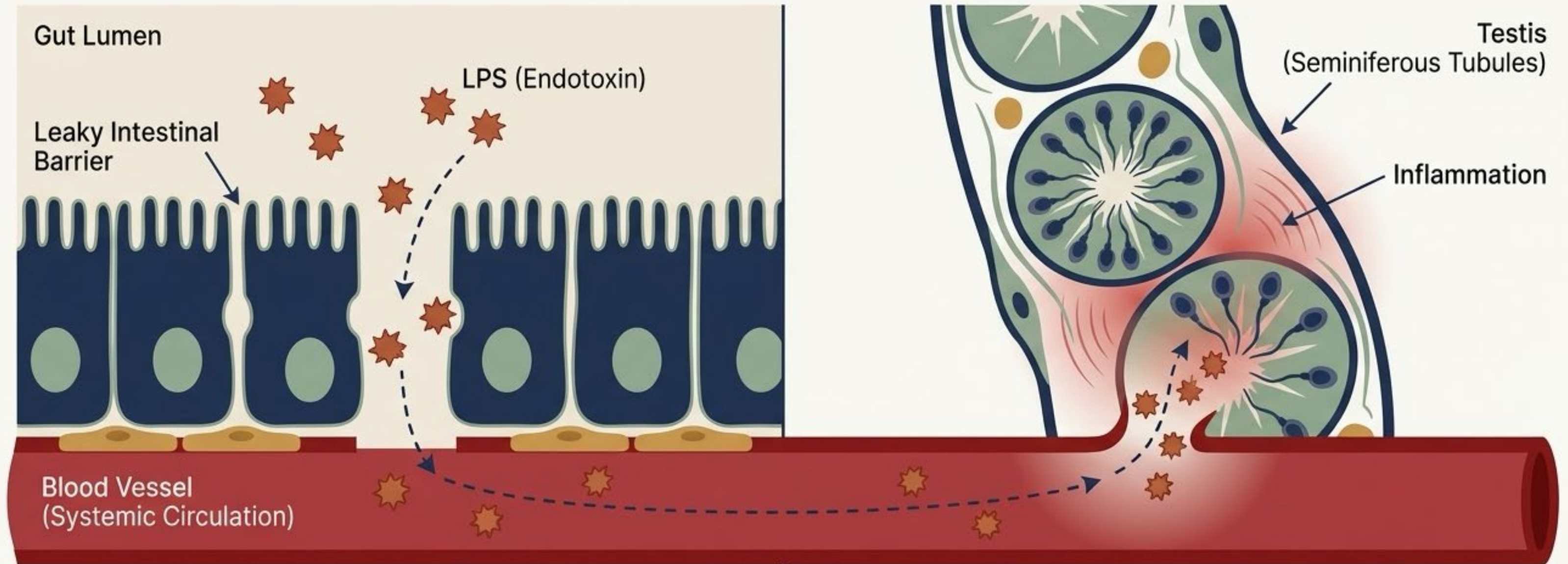
The BTB creates a local tolerogenic microenvironment, providing critical immune privilege for developing sperm.

Lipopolysaccharide (LPS) endotoxins are the primary antagonist. LPS triggers acute inflammation, disorganizes actin filaments, and violently degrades the tight junction protein occludin, destroying the barrier.



# Where Does the Inflammation Come From?

Systemic endotoxemia originates in the gut. Intestinal dysbiosis compromises the intestinal barrier, allowing bacterial LPS to enter the bloodstream. This "**Gut-Testis Axis**" transmits chronic, low-grade inflammation directly to the **reproductive tract**, suppressing the HPG axis and actively damaging the BTB.

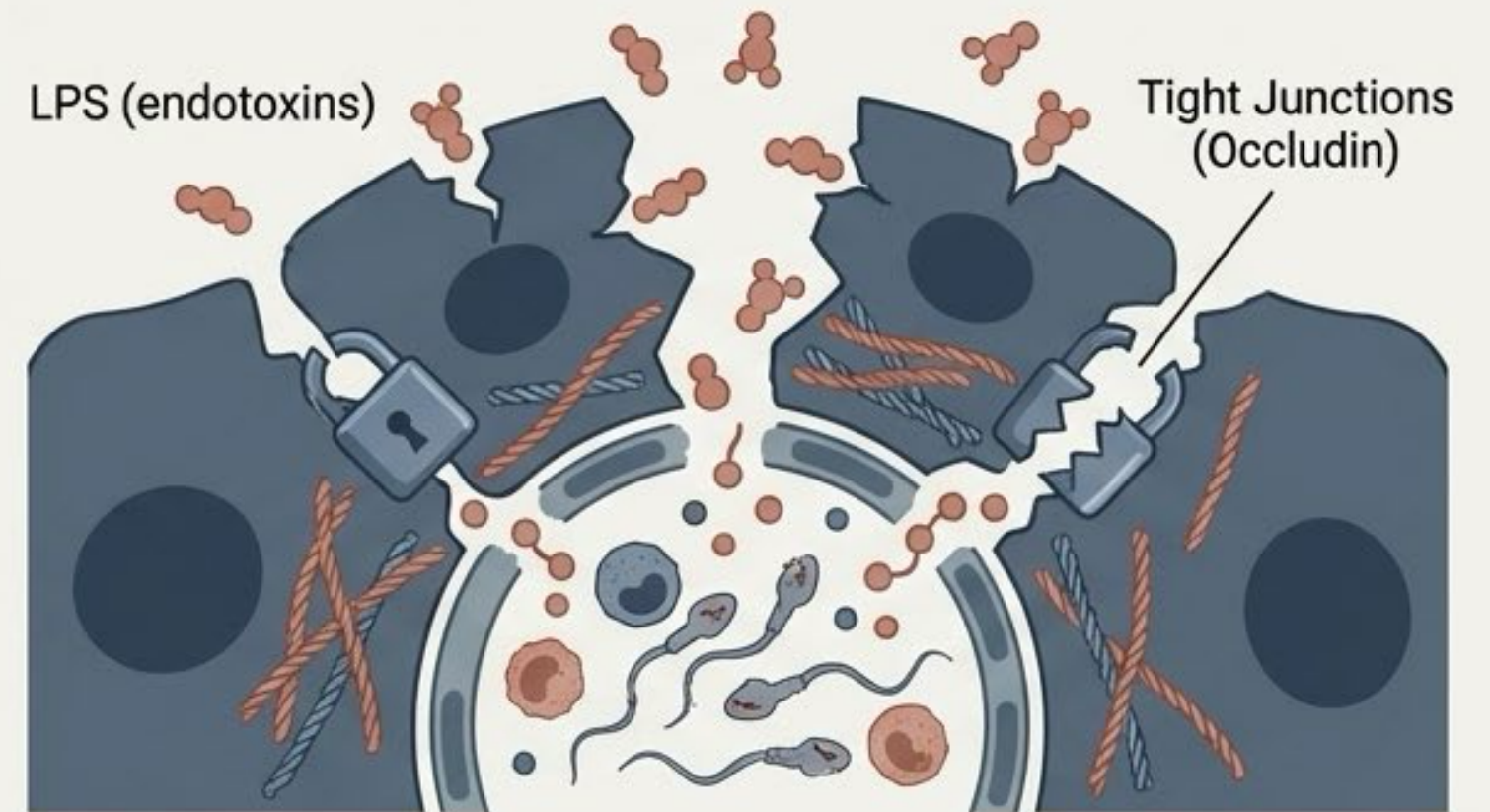


# The Micro Level: The Blood-Testis Barrier Under Siege

**Before: Intact BTB**



**After: BTB Collapse & Inflammation**



- To protect developing sperm from auto-immune attacks and systemic pathogens, Sertoli cells form the Blood-Testis Barrier (BTB).

- The structural integrity of this fortress relies heavily on tight junction (TJ) integral membrane proteins, notably occludin.

- When LPS (endotoxins from leaky gut) enter the testes, they trigger acute inflammation.

- This inflammation physically disorganizes the actin filament bundles and destroys occludin, causing the fortress walls to fragment and collapse.

Systemic inflammation physically dismantles the microscopic architecture required for safe spermatogenesis.

# The Seminal Microbiome and Eubiosis

The male reproductive tract harbors its own distinct microbiome. *Lactobacillus* dominance ensures high motility and acts as a shield against oxidative stress.

Overgrowth of opportunistic pathogens like *Ureaplasma parvum* drives leukocyte infiltration and massive Reactive Oxygen Species (ROS) release—a primary cause of sperm DNA fragmentation.

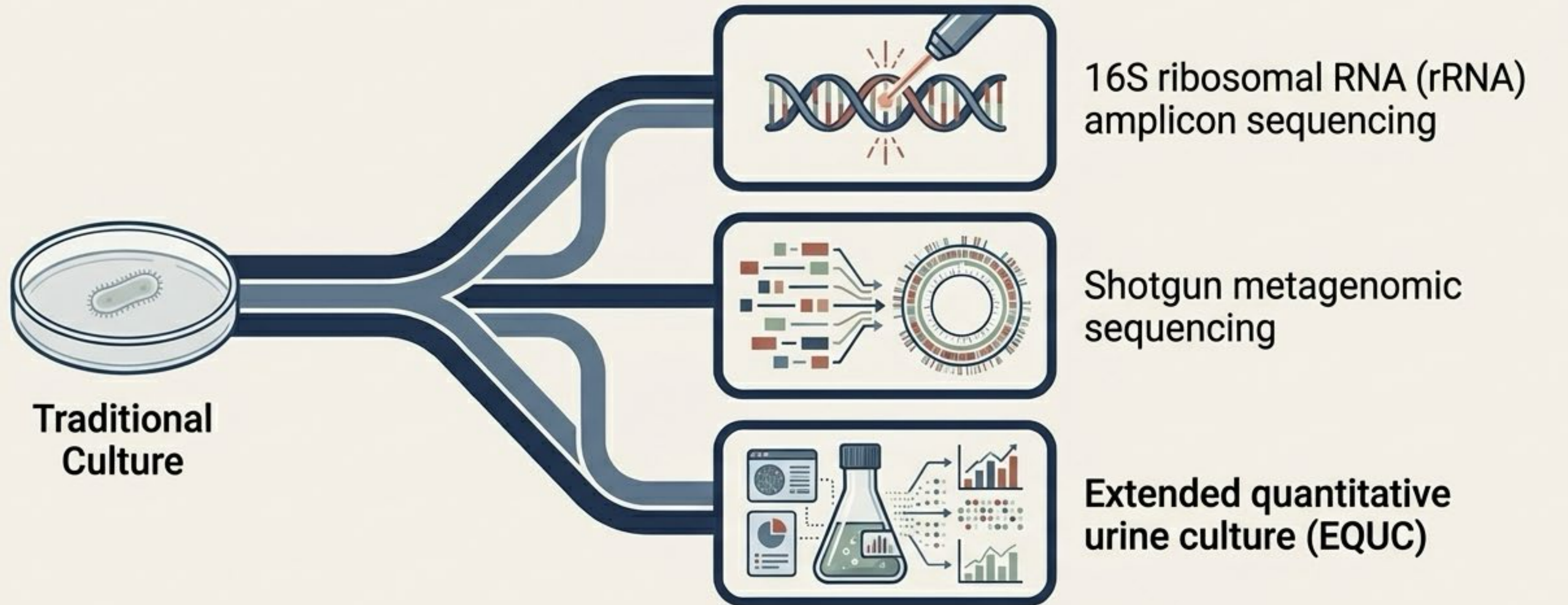
Healthy Microbiome (Eubiosis)



Dysbiosis & Inflammation



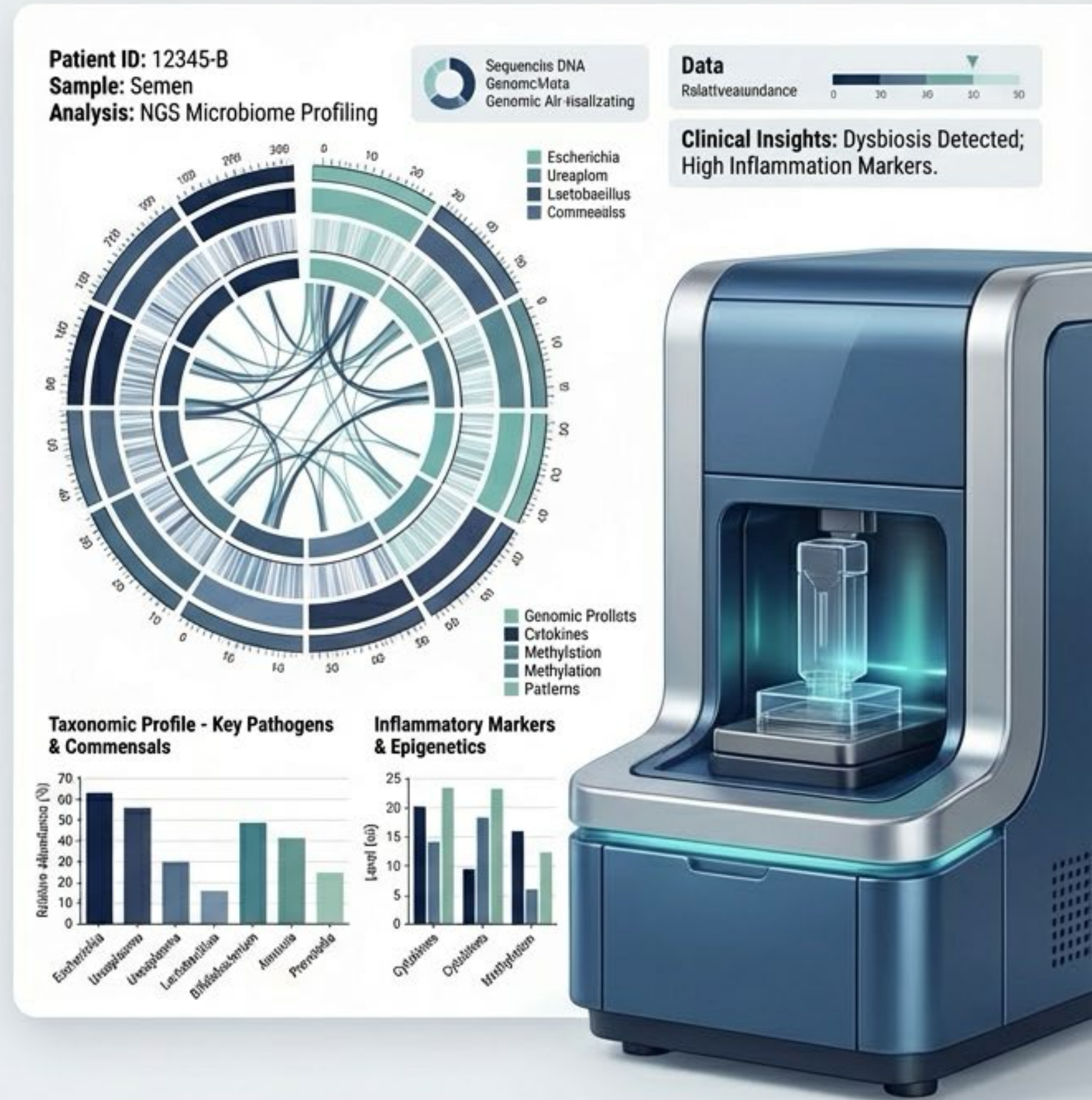
# High-resolution molecular analysis reveals the hidden ecosystem



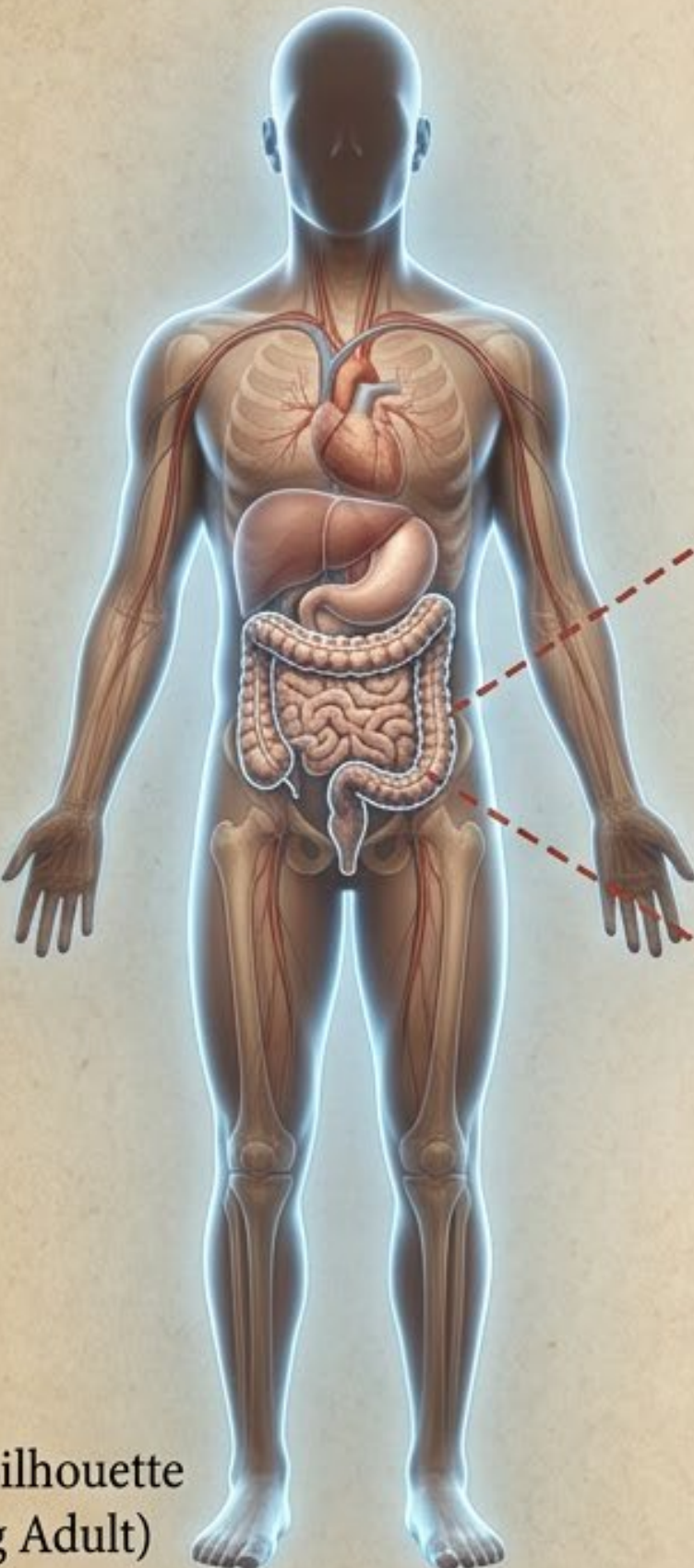
These technologies bypassed the limitations of traditional cultures, mapping complex metabolic pathways previously invisible to science.

# Next-Generation Sequencing profiles the modifiable microbiome

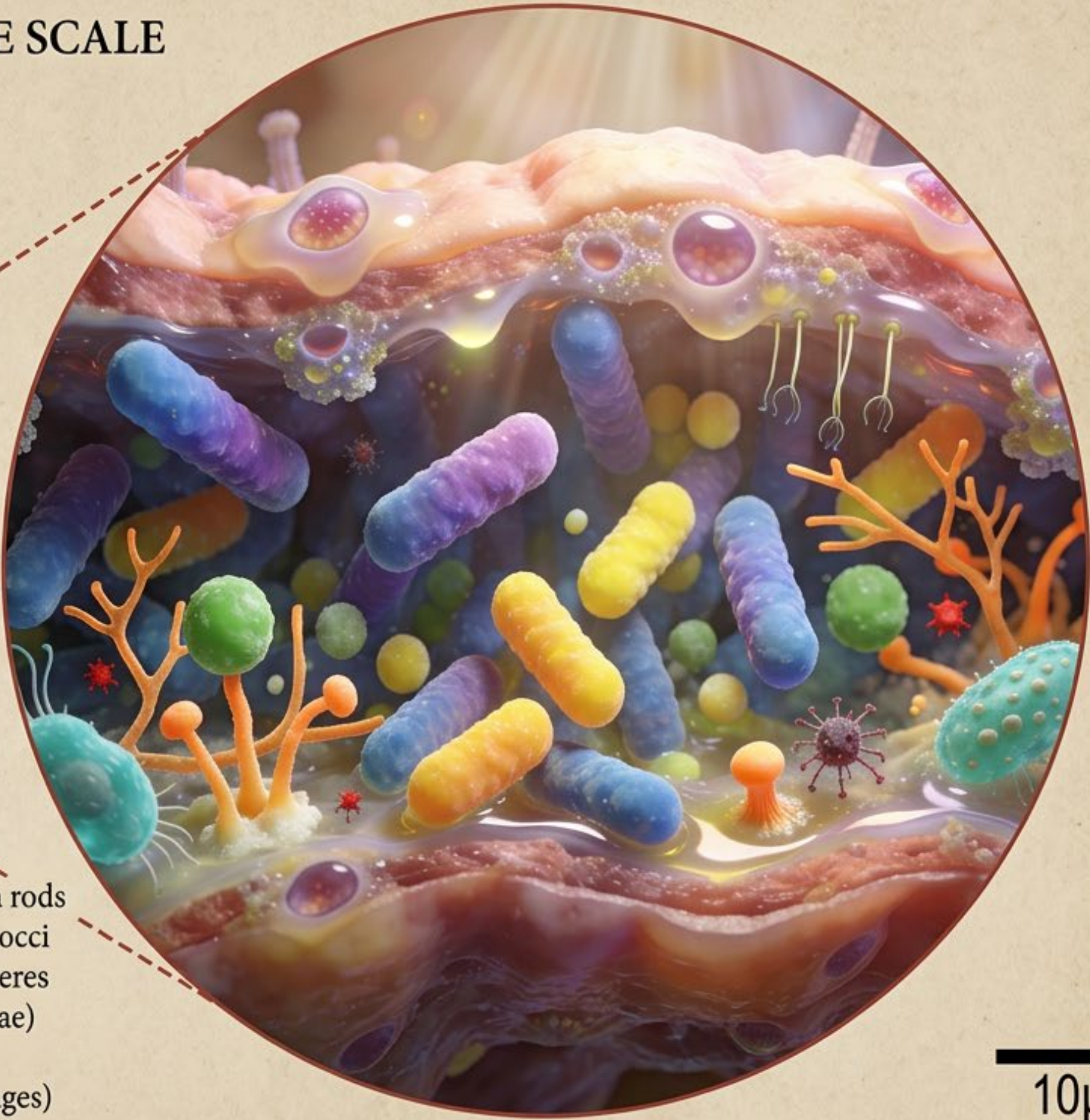
- High-throughput genetic techniques (NGS) act as a clinical game-changer.
- Identifies exact bacterial taxa associated with male factor infertility, unexplained infertility, and recurrent pregnancy loss.
- Augments standard semen analysis by offering deep insights into inflammatory processes, oxidative stress, and epigenetic modifications.



# FIGURE 1: HUMAN GUT MICROBIOME SCALE



Male Silhouette  
(70kg Adult)



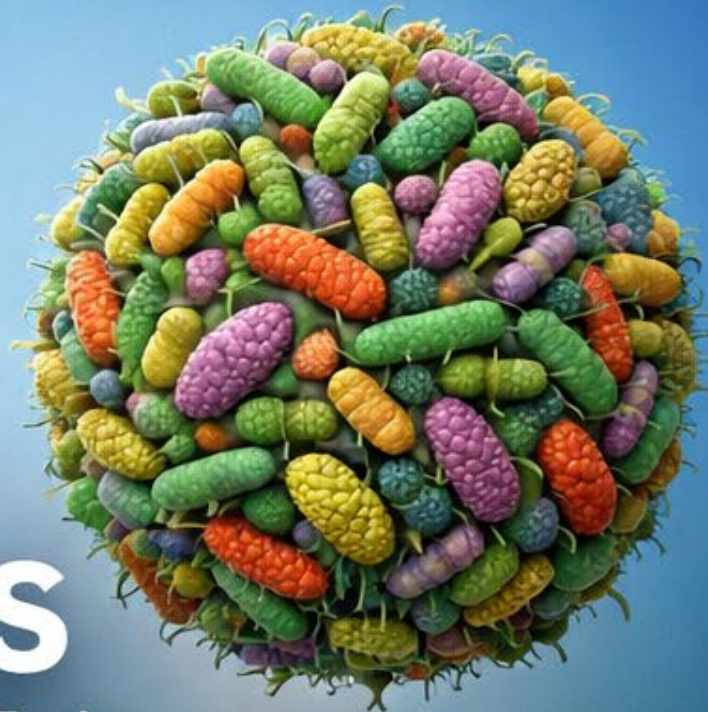
- Bacteroidota rods
- Firmicutes cocci
- Archaea spheres
- Fungi (hyphae)
- Protists
- Viruses (phages)

10µm



**VS**

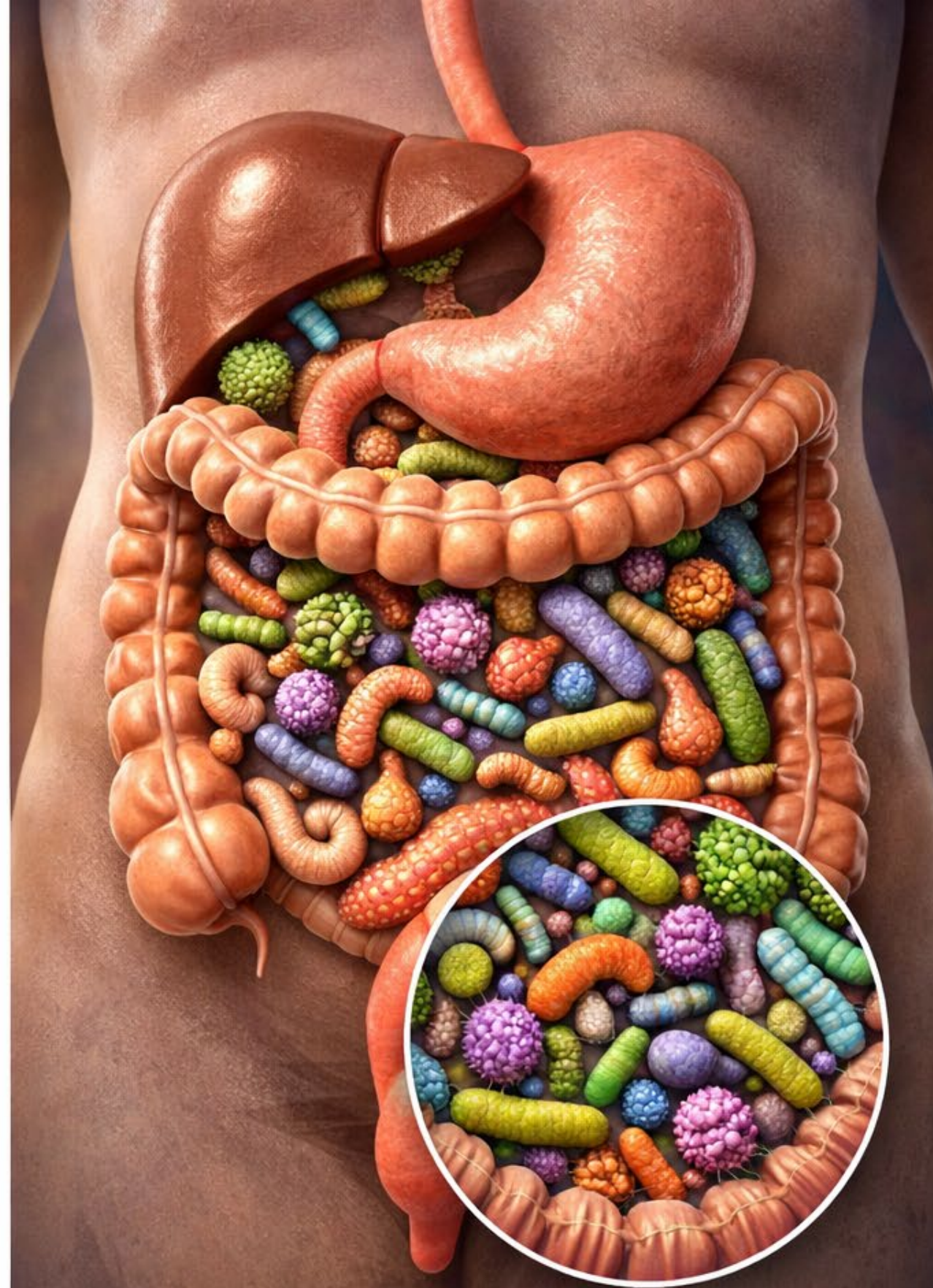
1:1.3 Ratio



**Human Cells**  
~30 Trillion



**Microbial Cells**  
~39 Trillion



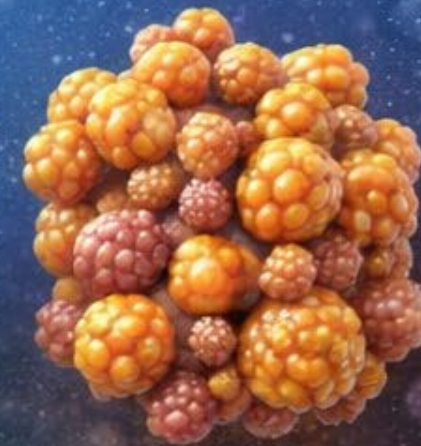
# Human Microbiota: ~ 38 trillion cells



**Bacteria:**  
~38 trillion cells



**Archaea:**  
~ $10^{11}$  cells,  
highly variable.



**Fungi:**  
~ $10^9$ ~ $10^{10}$   
approximate.

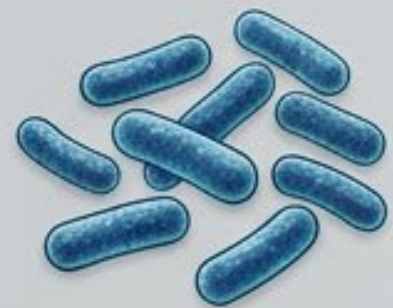
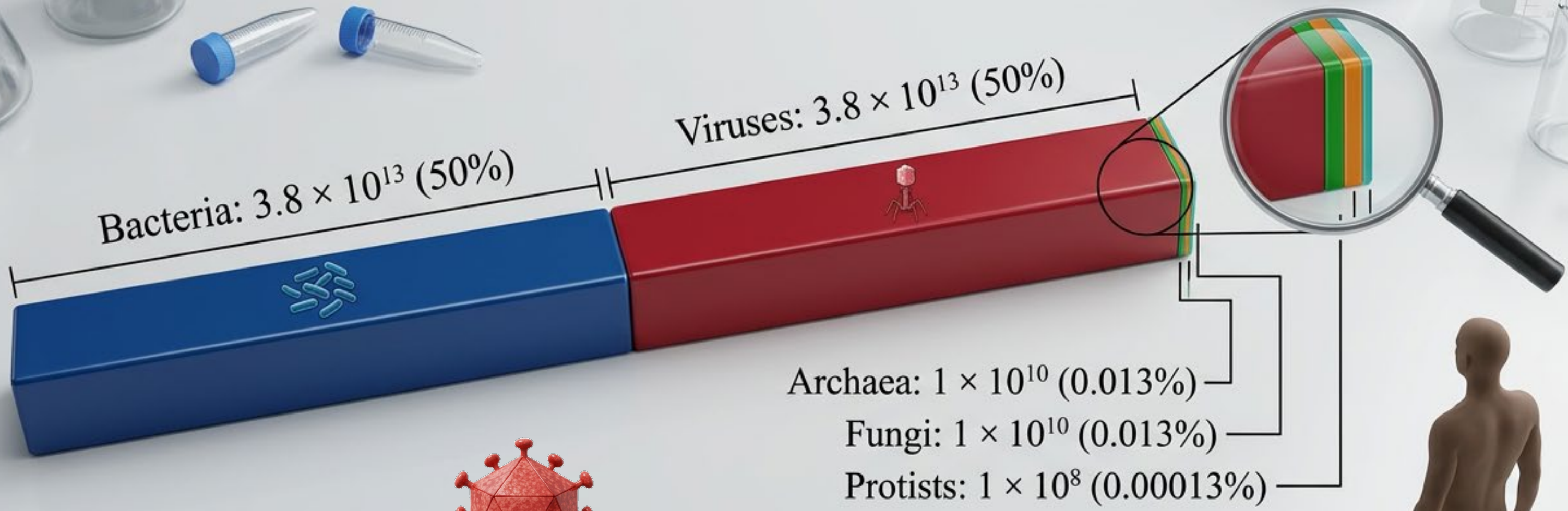


**Viruses:**  
no agreed :  
whole-body  
total; gut alone :  
contains ~ $10^9$ ~  
- $10^{10}$  particles per :  
gram of feces:

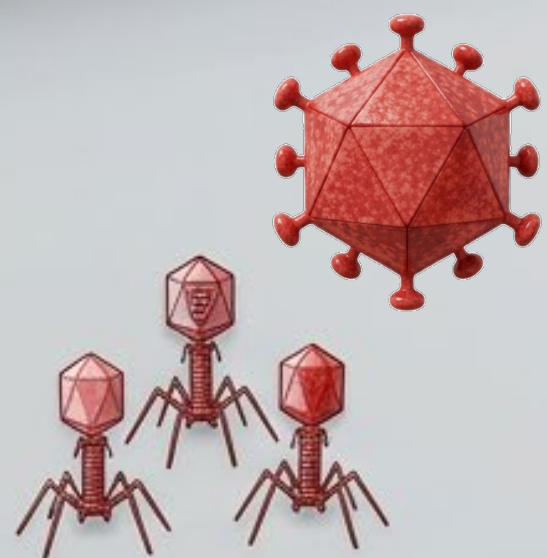


**Other**  
microeucaryotes  
/protists:  
present variably;  
no accepted  
universal total

# Human Microbiota Cell Composition



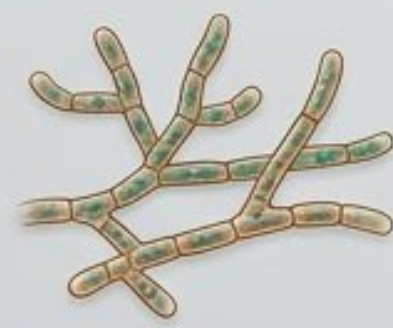
Bacteria



Viruses



Archaea

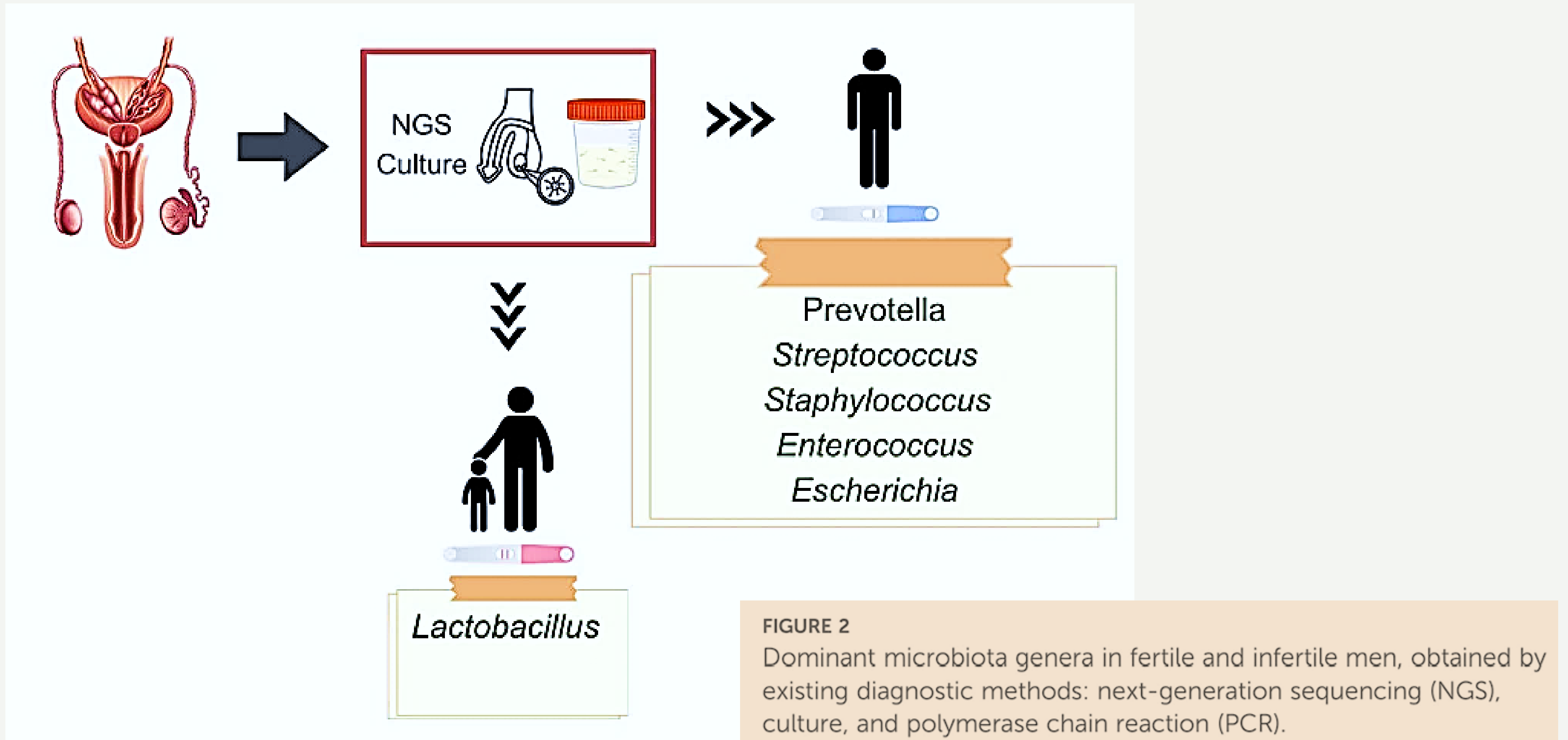


Fungi



Protists

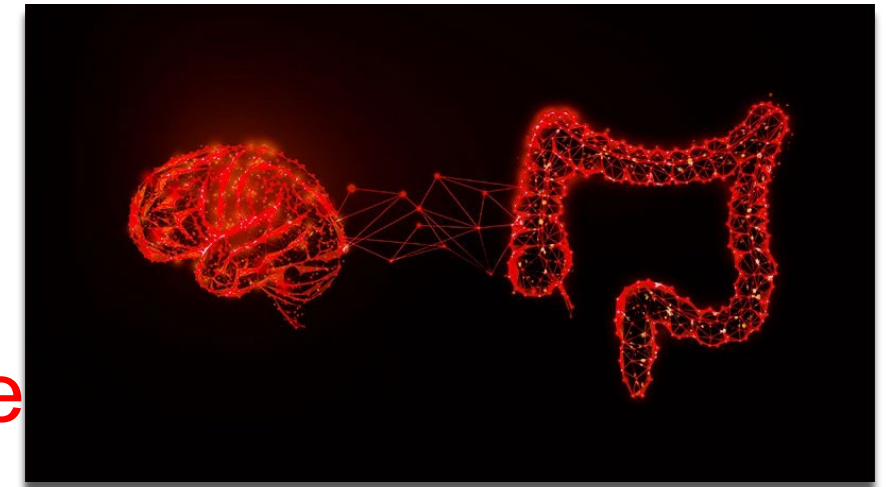




# GUT MICROBIOM OVERVIEW

- 3 million genes versus 23,000 genes in the human genome
- The gut microbiota in humans mainly include *Firmicutes*, *Bacteroides*, *Proteus*, *Actinomyces*, *Fusobacteria* & *Verrucomicrobia*
- A plethora of proteins, enzymes or other bioactive compounds are „produced“ by the intestinal microbiom
- These can be classified as<sup>1</sup>:
  1. Metabolites directly produced from the diet, (short-chain fatty acids (SCFAs), polyunsaturated fatty acids (PUFAs), and amino acid derivatives;
  2. de novo produced metabolites, such as lipopolysaccharide (LPS) and K vitamins;
  3. metabolites produced by the host and modified by gut microbiota, such as secondary bile acids and hydroxysteroid dehydrogenase (HSDH)

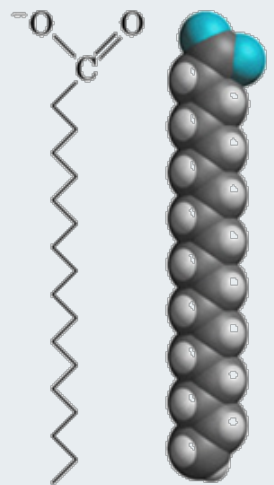
(Liu et al., 2022).



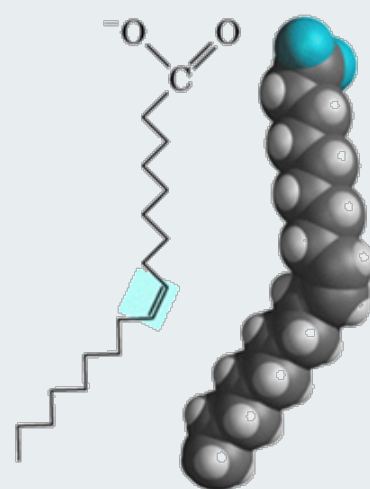
# GUT MICROBIOTA AND SPERM & UNSATURATED FATTY ACIDS (UFAS):

- I. **Mono-unsaturated** fatty acids (MUFAs): no problematic for PUFA, serve as source
- II. **PUFAs**: essential FA, ( $\alpha$ -linoleic acid, arachidonic acid, docosahexaenoic acid ...)

1. **anti-inflammatory** effects by interfering via TLR4/MyD88 pathway & GPR120/NF- $\kappa$ B pathway.
2. **Antioxidative** effects via higher catalase and superoxide dismutase (SOD) =>reduction ROS (*Korbecki et al., 2019*).
3. **increased stability** and fluidity of lipid rafts (*Kotlyarov and Kotlyarova, 2021*) for sperm activity (*Castellini et al., 2022*).



**Saturated**

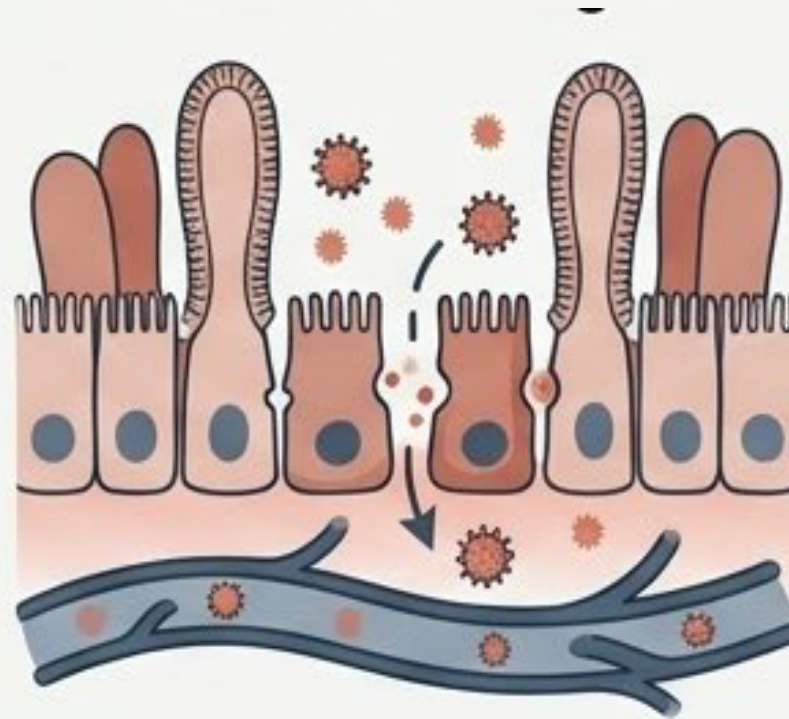


**Unsaturated**

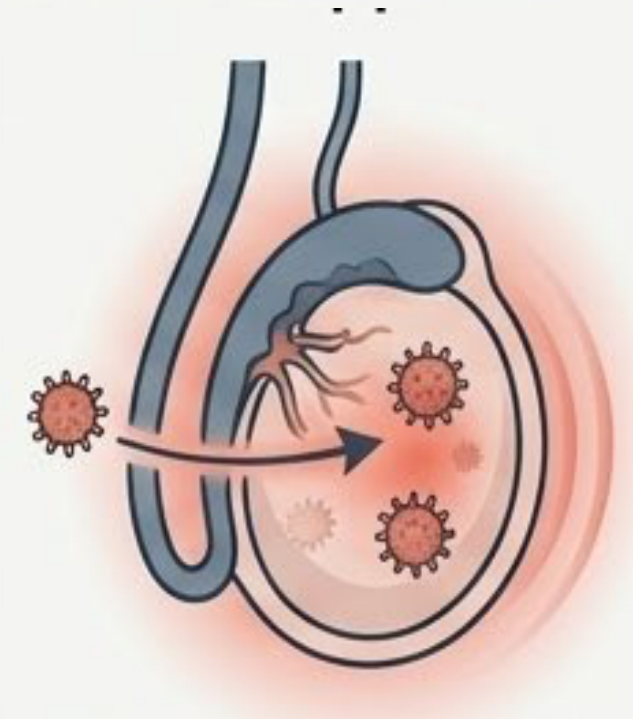
# The Gut-Testis Axis: Where Diet Becomes Pathology



High Fat / Sugar Diet



Intestinal Permeability  
& LPS Leakage

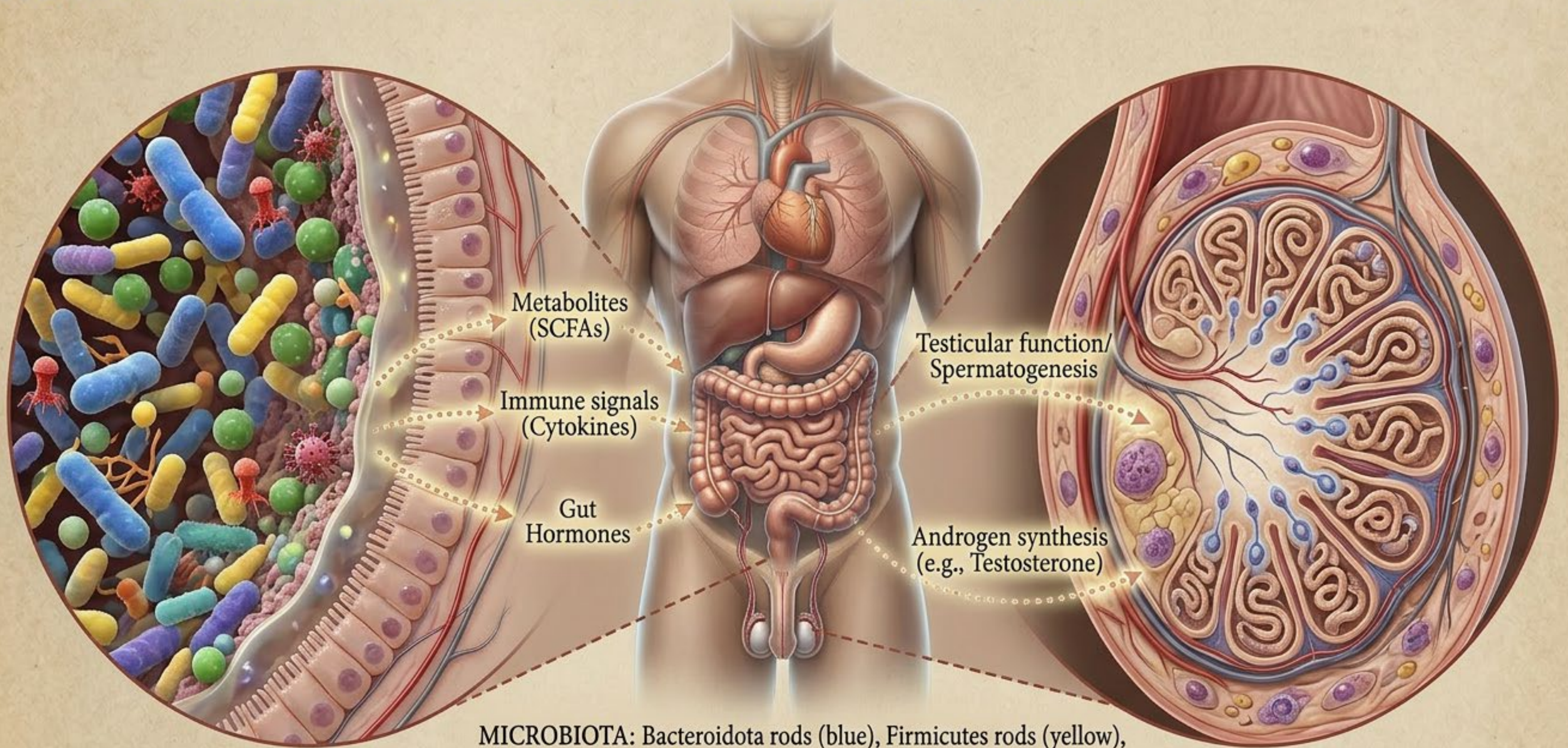


1. Testicular Inflammation  
2. & HPG Suppression

- The relationship between diet and hypogonadism is mediated by the microbiome.
- High-fat, low-fiber diets induce intestinal barrier dysfunction.
- This 'leaky gut' allows bacterial endotoxins (LPS) to escape into systemic circulation (Metabolic Endotoxemia).
- Chronic low-grade endotoxemia suppresses LH release and triggers localized testicular inflammation, directly impairing spermatogenesis.

Testicular function is directly tethered to intestinal barrier integrity.

FIGURE 2: MECHANISTIC SCHEMATIC OF THE GUT-TESTIS AXIS



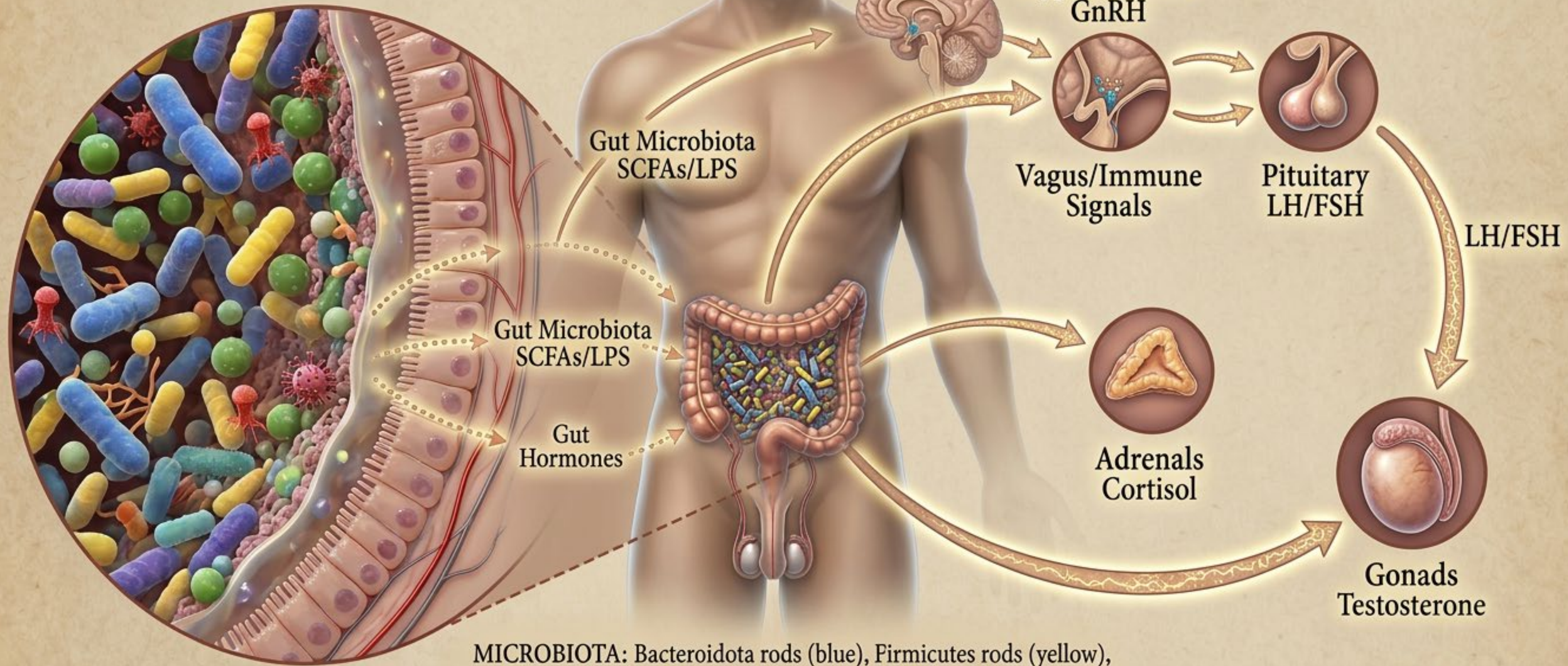
MICROBIOTA: Bacteroidota rods (blue), Firmicutes rods (yellow), Archaea spheres (green), Viruses/Phages (red).

PATHWAYS:  $\cdots \blacktriangleright$  Metabolites (SCFAs), Immune signals (Cytokines), Gut Hormones

ANATOMY: Vasculature (solid red), Nerves (solid grey)

10μm

# EXCLUSIVE AND DIRECT LINK



MICROBIOTA: Bacteroidota rods (blue), Firmicutes rods (yellow), Archaea spheres (green), Viruses/Phages (red).

Glowing Bidirectional Paths PATHWAYS:  $\cdots \blacktriangleright$  Metabolites (SCFAs),  $\blacktriangleright$  LPS  $\blacktriangleright$  Immune signals (Cytokines), Gut Hormones

ANATOMY: Vasculature (solid red), Nerves (solid grey)

10 $\mu$ m

## MICROBIOM AND INFERTILITY: DOUBLE-EDGED SWORD.

- Gut microbiota & metabolites activate abnormal immune signals via LPS, binding to TLR-24 complexes, increasing the production of proinflammatory cytokines and ROS/RNS ([Wiest and Garcia-Tsao, 2005](#)),
- are involved in inflammation-induced damage to testicular structures

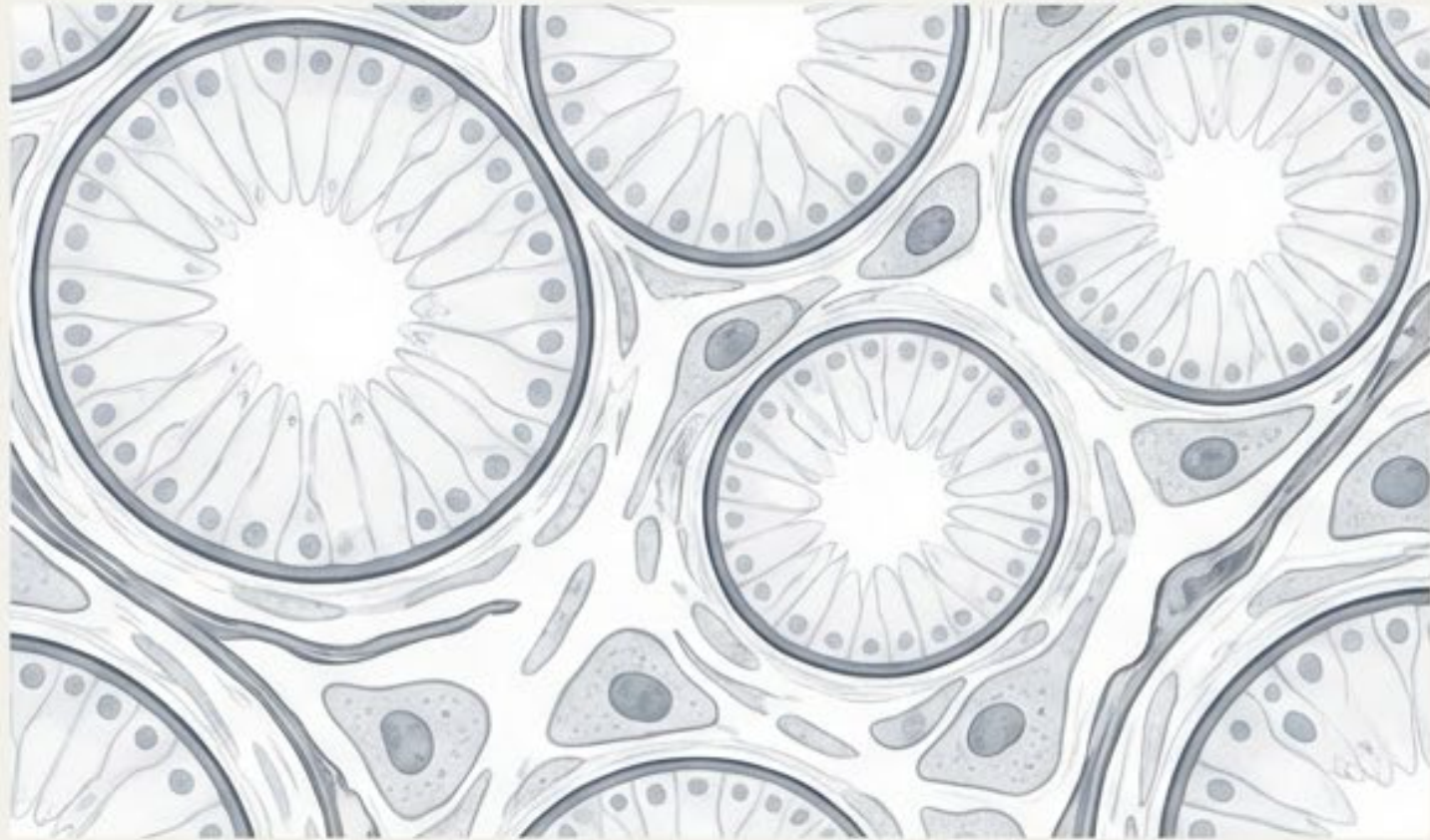


mucolytic *Bacteroides caccae* and *Akkermansia muciniphila* increase the activity of mucin-degrading enzymes for survive, but leading to the erosion of the colonic mucus barrier ([Desai et al., 2016](#)). Structural damage of this barrier leads to greater LPS translocation and reduced production of SCFAs ([Li et al., 2022c](#)), causing low-grade inflammation, metabolic disorders, endocrine disorders, and insulin resistance, **all of which affect spermatogenesis**

# Shattering the dogma of testicular sterility

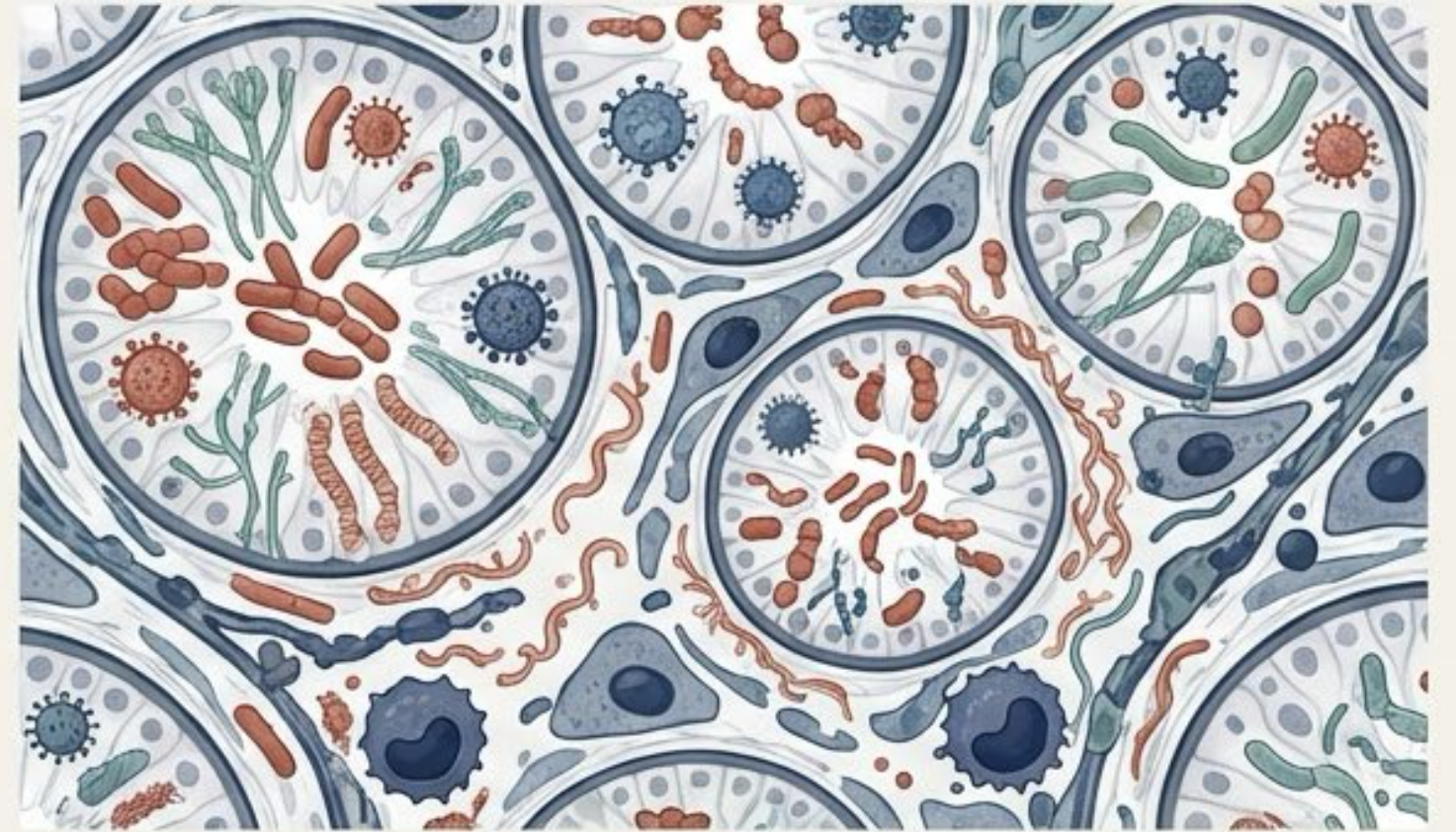
## Historical Dogma

Testicular tissue and seminal fluids were physiologically sterile.



## Modern Reality

The male genitourinary tract harbors a diverse, dynamic microbial ecosystem.



## Key Evidence

Alfano et al. provided the pioneering evidence of bacterial presence in testicular specimens, permanently altering the scientific consensus.

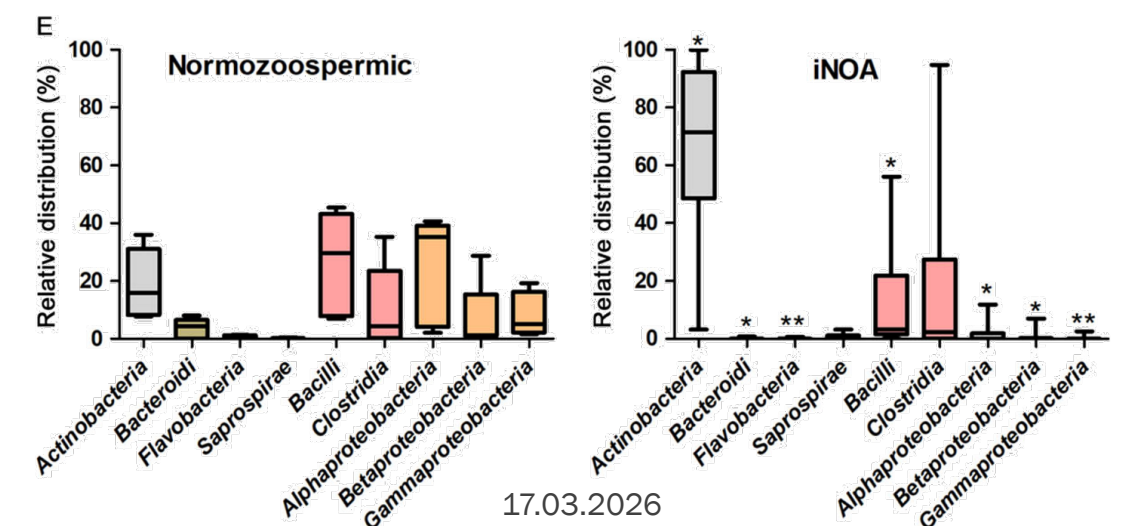
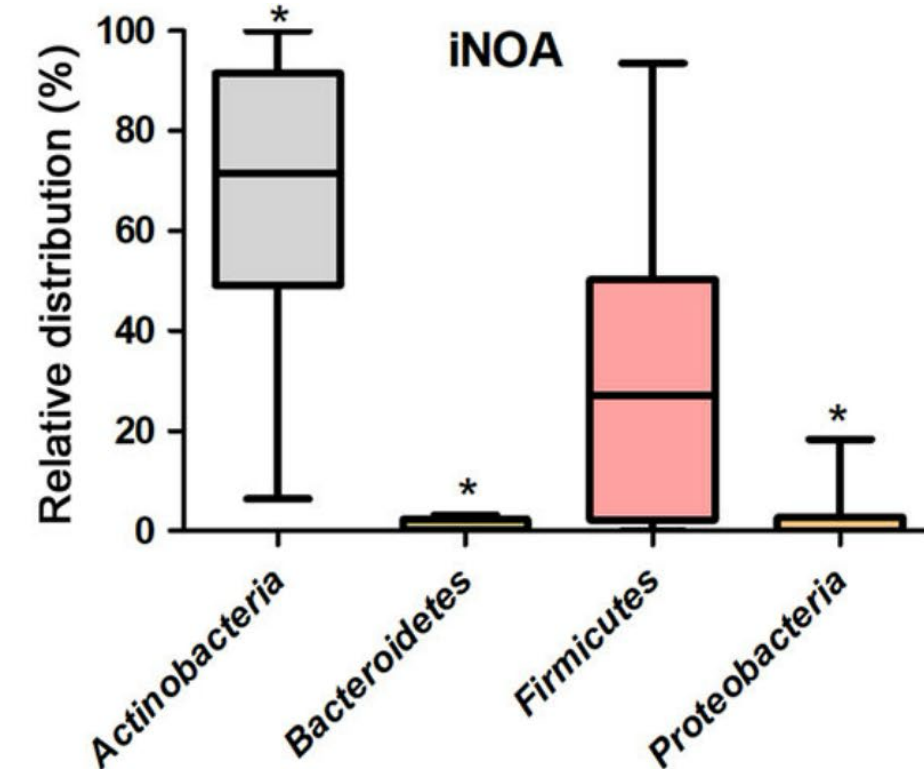
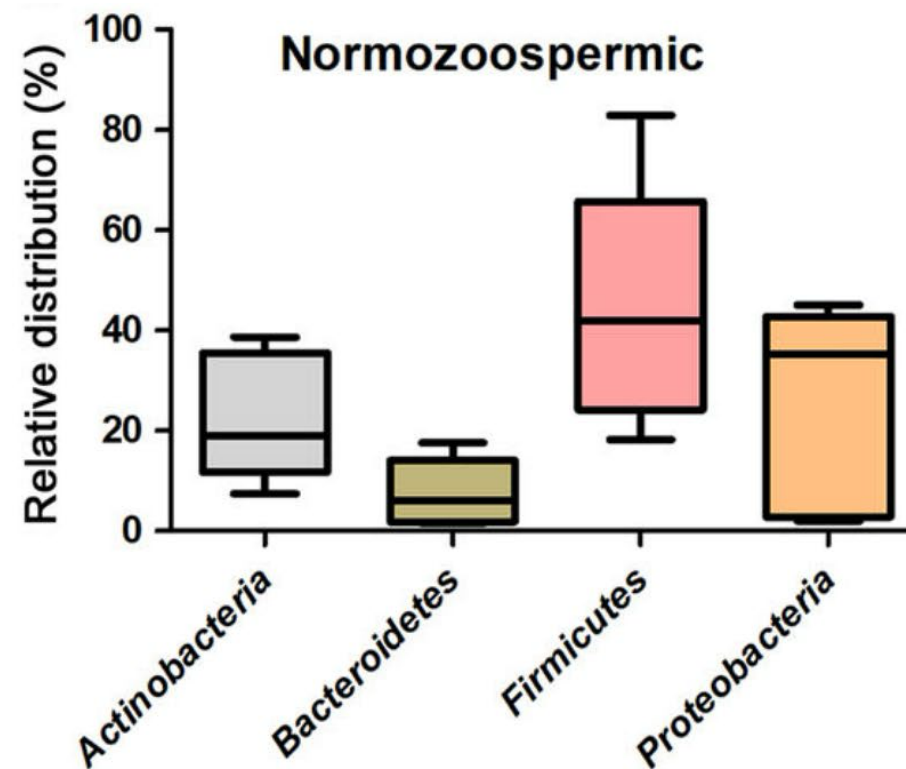
# NOVELTY:

# TESTICULAR TISSUE IS NOT SERILE

- Tissues with normal germline maturation showed detectable amounts of 16 S DNA (median; 3.4; IQR; 1.7-7 copies/ng of loaded DNA).
- *Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria* were phyla associated with a normal germline.

- An increased amount of 16 S DNA was found within testicular specimens from iNOA men (12.1; 5-15)

- *Actinobacteria and Firmicutes*



# Dysbiosis distinctly characterizes Non-Obstructive Azoospermia



## Normozoospermia (Healthy Control)

Higher bacterial richness and taxonomic diversity.

Dominant phyla: Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria.



## Non-Obstructive Azoospermia (NOA)

Higher overall bacterial concentration but diminished diversity (divergent beta diversity).

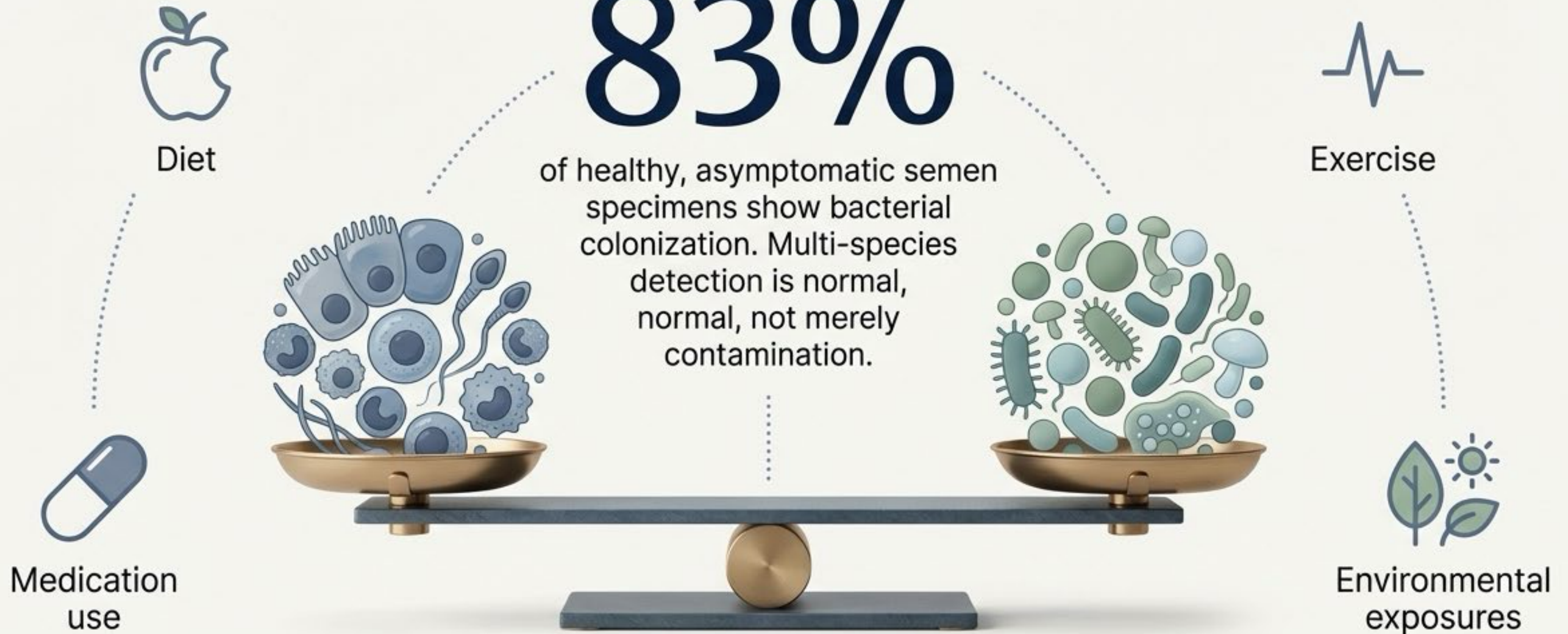
Higher abundance of Actinobacteria and Firmicutes. Notable absence of Bacteroidetes and Proteobacteria.

**mTESE Note:** Negative sperm retrieval rates are linked to lower Firmicutes/Clostridia and a complete absence of *Peptoniphilus asaccharolyticus*.

# The healthy male genitourinary tract requires a balanced ecosystem

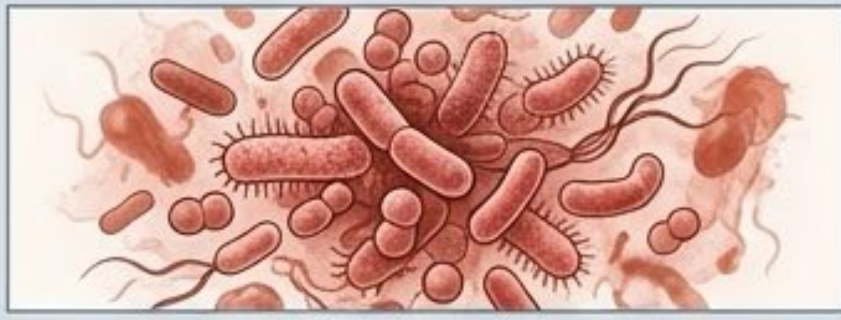
# 83%

of healthy, asymptomatic semen specimens show bacterial colonization. Multi-species detection is normal, normal, not merely contamination.



The ecosystem is highly dynamic and individualized.

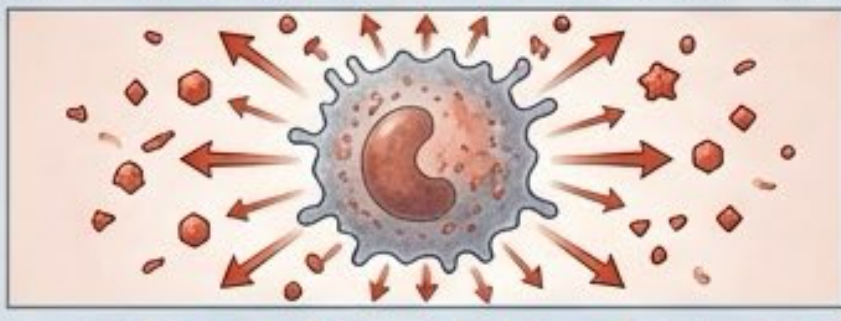
# Dysbiosis triggers a localized inflammatory cascade



Step 1: Dysbiosis / Bacteriospermia ( $>10^3$  CFU/mL)



Step 2: Immune Activation  
(Infiltration of Macrophages, T lymphocytes, Mast cells)



Step 3: Cytokine Release  
(Secretion of IL-1, IL-6, TNF- $\alpha$ , and chemokines)



Step 4: Oxidative Stress  
(Massive increase in Reactive Oxygen Species [ROS])



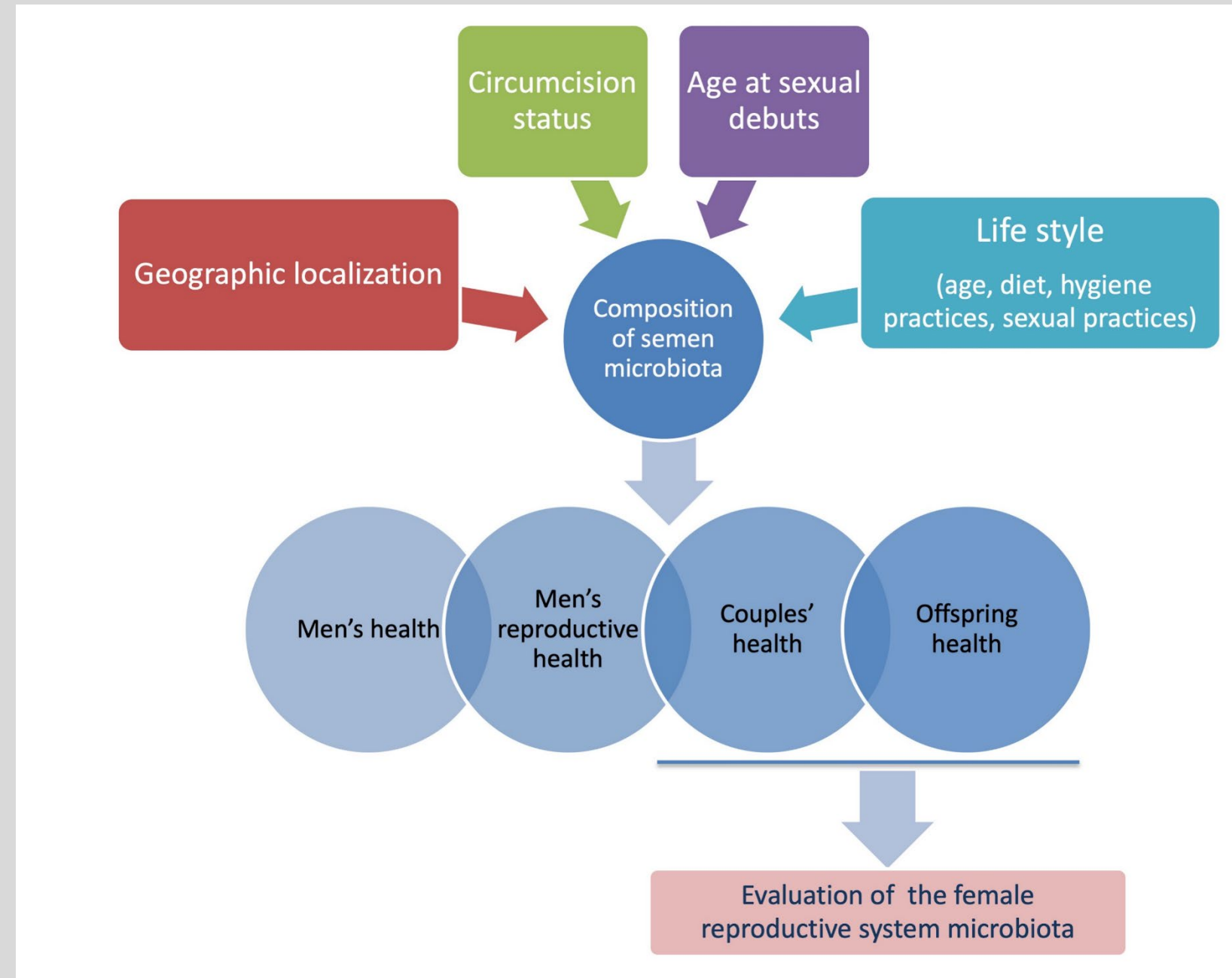
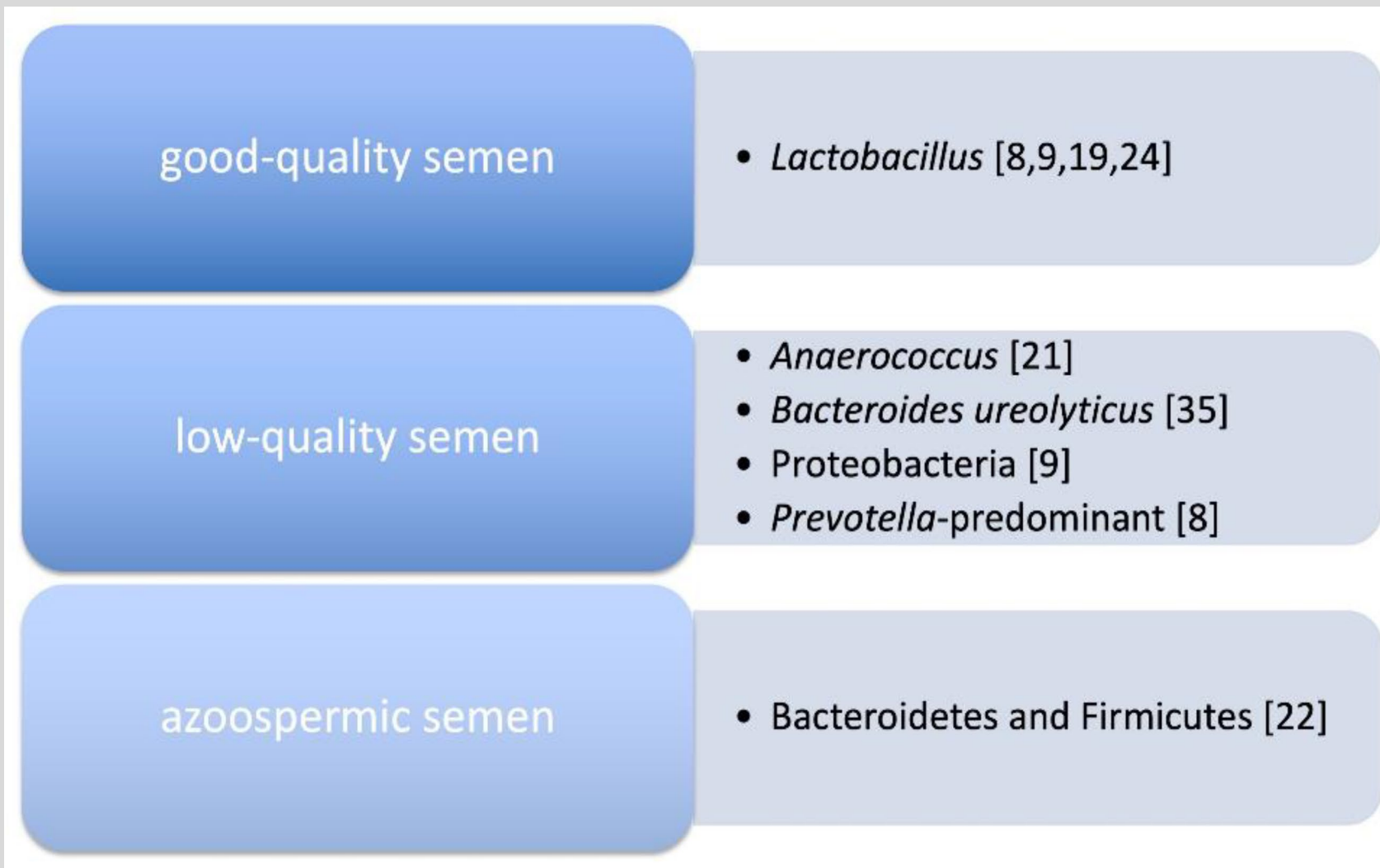
Step 5: Sperm Damage  
(DNA fragmentation [DFI], lipid/protein damage, impaired mitochondrial function, disrupted blood-testis barrier)

# Microbiota and Human Reproduction: The Case of Male Infertility

Rossella Tomaiuolo<sup>1,2,3</sup>, Iolanda Veneruso<sup>2,3</sup>, Federica Cariati<sup>1,3</sup> and Valeria D'Argenio<sup>3,4,\*</sup>

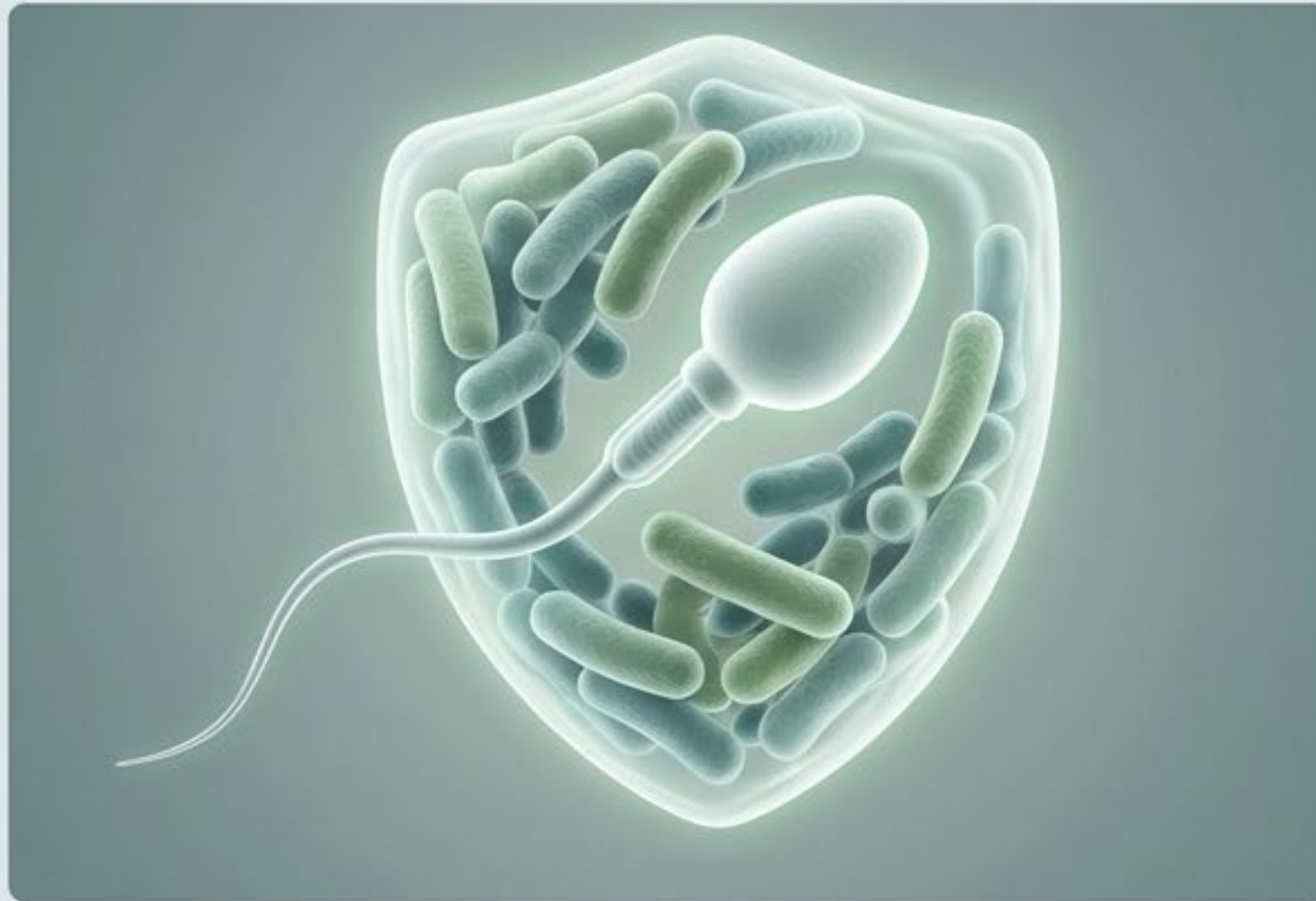
High-Throughput 2020, 9(2), 10; <https://doi.org/10.3390/ht9020010>

PubMed articles published 2010 and 2020 with keywords: “male infertility and microbiota”, “male infertility and metagenomics”, “male reproductive system microbiome”, and “semen microbiome”.



# Beneficial strains protect against oxidative stress and morphological damage

## Taxonomy Scorecard



### The Protectors

- *Lactobacillus*
- *Clostridium butyricum*

### Mechanisms

Associated with improved sperm morphology.

### Evidence

*Clostridium butyricum* has been shown (in mouse models) to successfully counteract the harmful cellular effects of *Pseudomonas* infections.

**Key takeaway:** Bacteria in semen are not inherently deleterious; reproductive success depends on the ecological balance.

# Specific bacterial taxa drive negative reproductive outcomes

## Taxonomy Scorecard

### Drivers of Inflammation & ROS

*Ureaplasma urealyticum*

*Mycoplasma hominis*

*Mycoplasma genitalium*

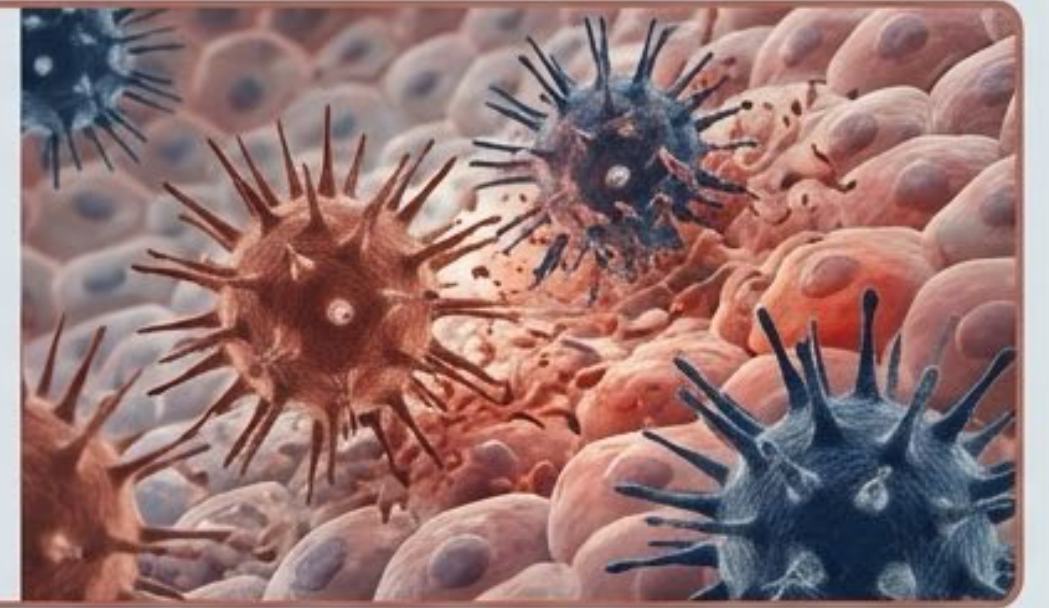
*Prevotella*

*Pseudomonas*

*Enterococcus faecalis*

*Aerococcus*

*Corynebacterium*



### Drivers of Sperm DNA Fragmentation

*Moraxella*

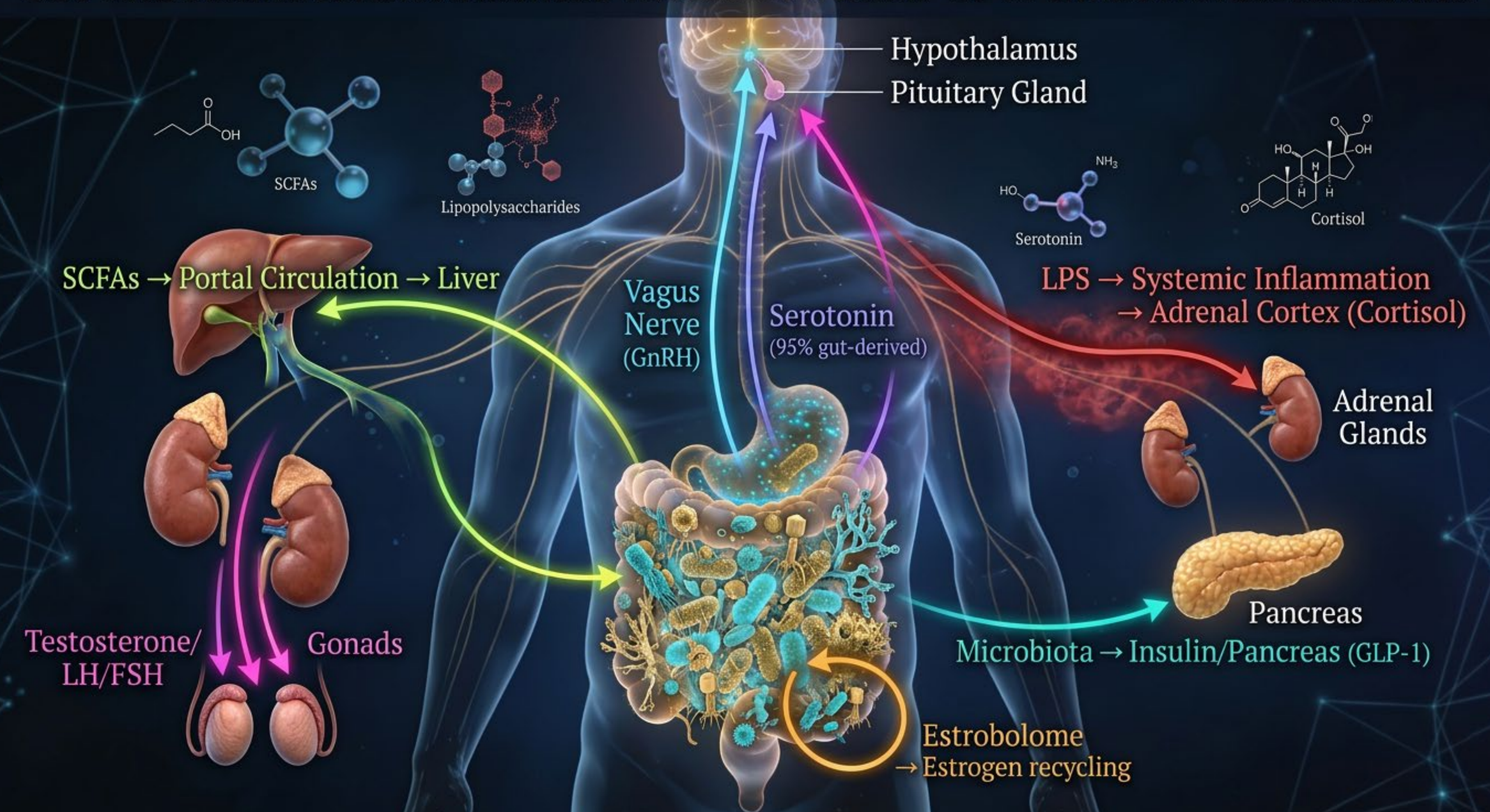
*Brevundimonas*

*Flavobacterium*

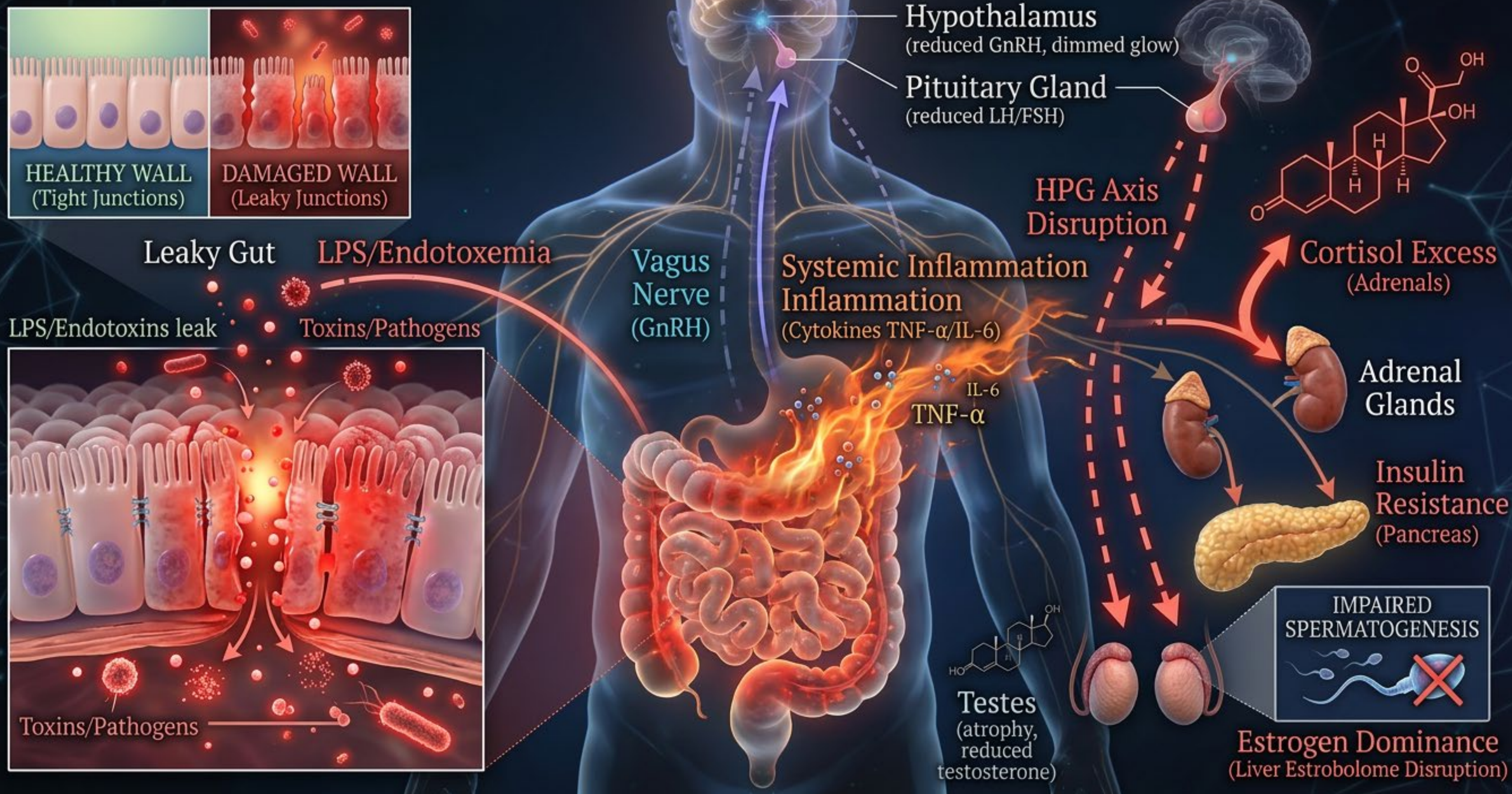


**Key takeaway:** Relative overabundance of these taxa directly correlates with poorer semen quality.

# GUT-ENDOCRINE AXIS: A DETAILED INTERACTIVE MAP OF BI-DIRECTIONAL PATHWAYS

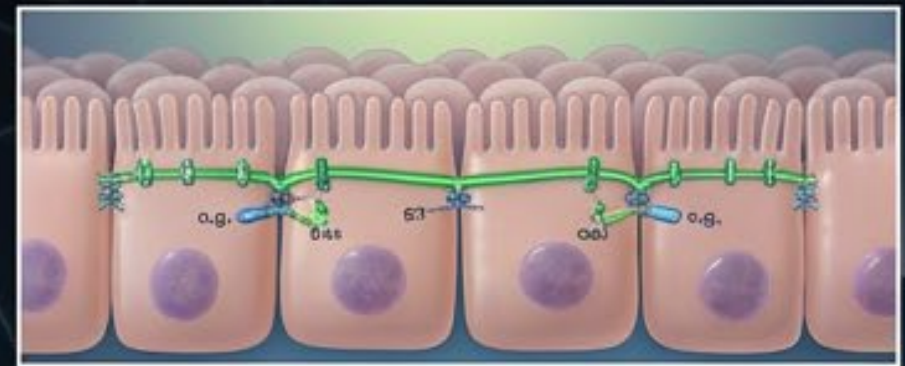


# IMPACT OF LEAKY GUT SYNDROME ON MALE ENDOCRINE BALANCE & FERTILITY



# COMPARATIVE ANALYSIS: LEAKY GUT SYNDROME DISRUPTING MALE ENDOCRINE AXIS & FERTILITY

## HEALTHY MALE AXIS

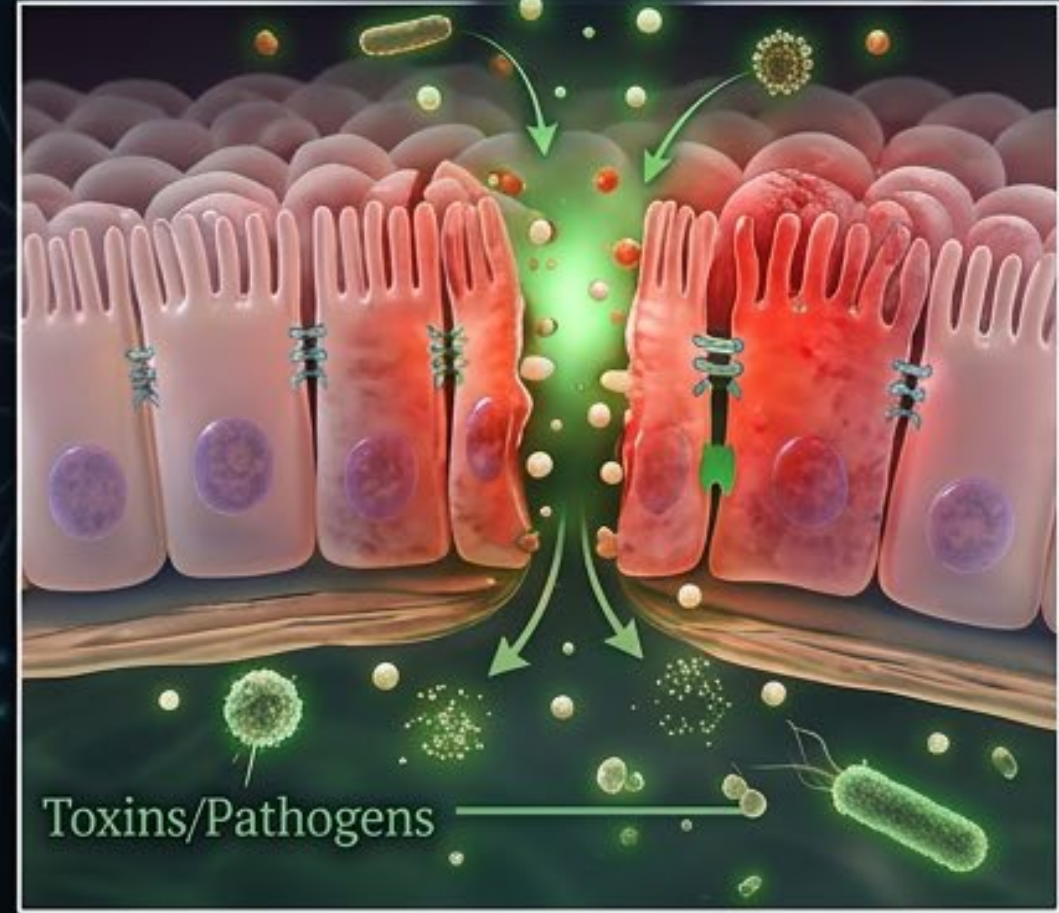


Intact Epithelium & Tight Junctions

Normal Cortisol

Balanced Microbiota

Endotoxemia



Toxins/Pathogens

Normal Cortisol

Normal Spermatogenesis

Hypothalamus (reduced GnRH, dimmed glow)

Pituitary Gland (reduced LH/FSH)

## DISRUPTION

HPG Axis Suppression

Systemic Inflammation  
Inflammation (Cytokines TNF- $\alpha$ /IL-6)

IL-6  
TNF- $\alpha$

Cortisol

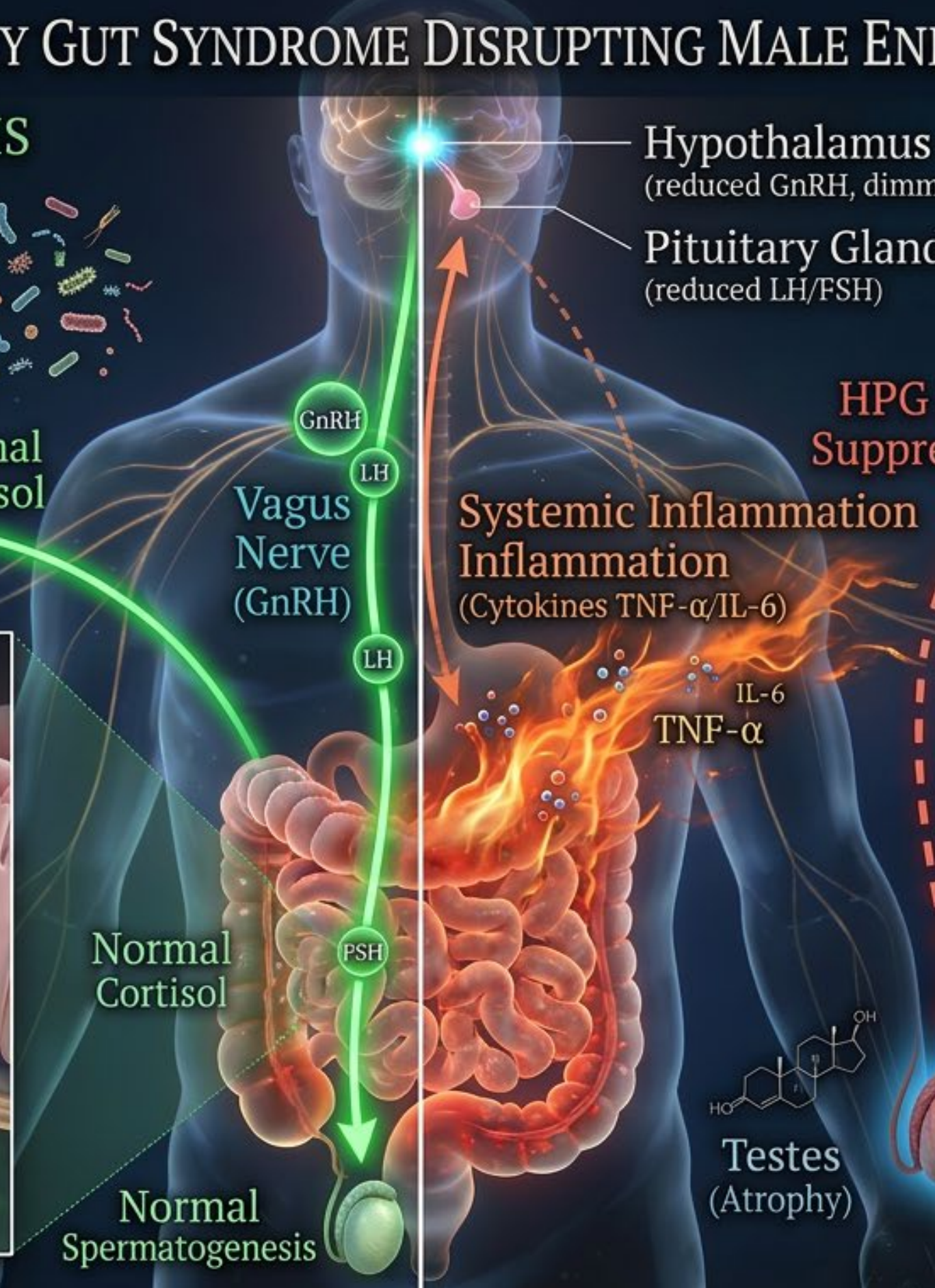
Adrenals ( $\uparrow$  Cortisol)

Liver ( $\uparrow$  Estrogen)

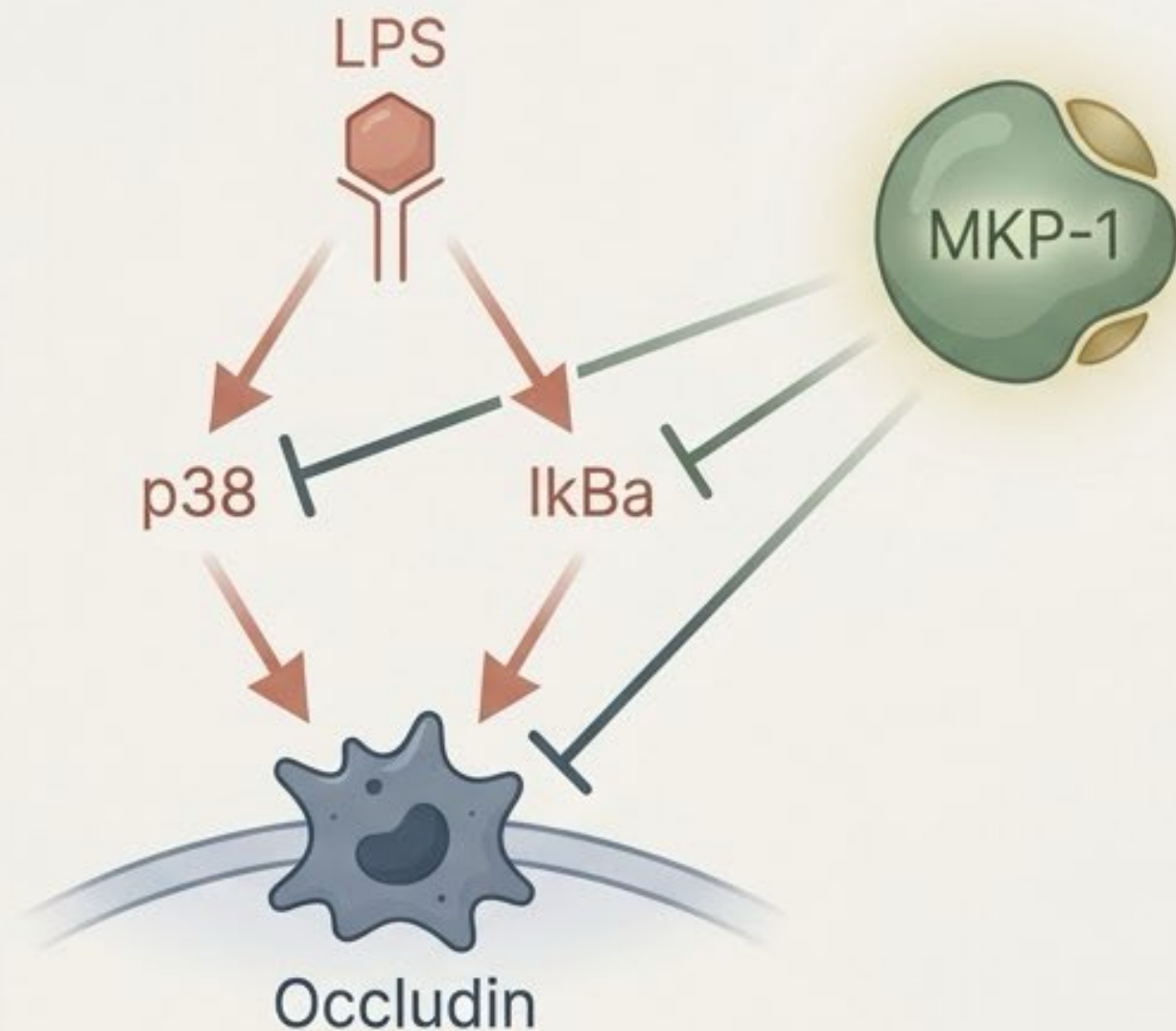
Testes (Atrophy)

IMPAIRED SPERMATOGENESIS

Low T, Poor Sperm Quality  
Oligo-/Azoospermia



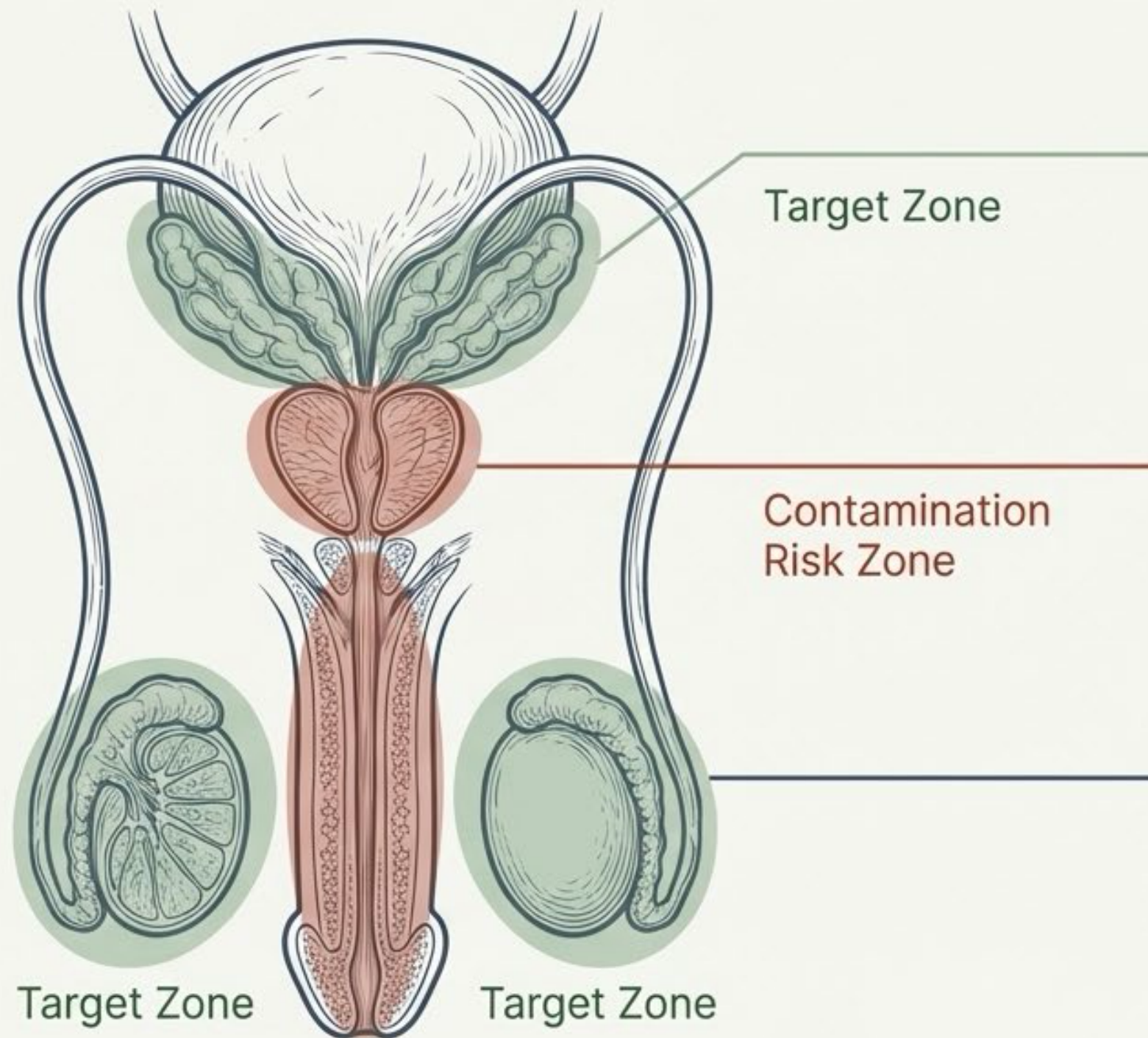
# MKP-1: The Endogenous Defender of Testicular Immune Privilege



- The testis has a localized defense mechanism to prevent permanent sterility during acute inflammation.
- MAP phosphatase-1 (MKP-1) acts as a crucial negative regulator.
- During an LPS attack, MKP-1 expression strongly up-regulates in the nuclei and tight junctions of Sertoli cells within 60-90 minutes.
- MKP-1 neutralizes the inflammatory cascade by dephosphorylating the p38 MAP kinase and IκBa pathways.

MKP-1 serves as the crucial biological “brake” that stops the innate immune system from destroying the Blood-Testis Barrier.

# Non-invasive sampling remains a critical diagnostic hurdle



## **The primary unresolved task:**

Collecting non-invasive cultures from testicular tissue or seminal glands.

## **The Contamination Risk:**

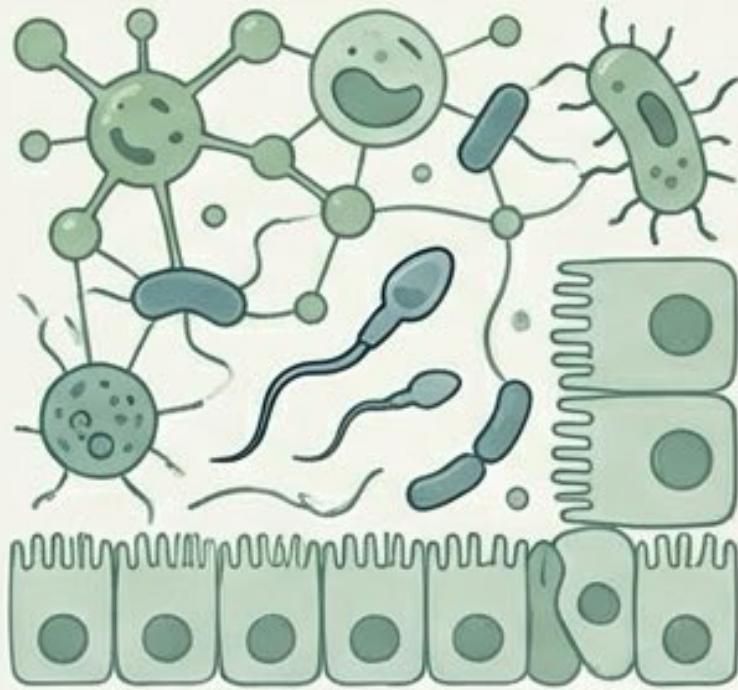
Samples must avoid prostatic or urethral bacterial sources.

## **Clinical Necessity:**

True, uncontaminated testicular sampling is required to accurately address spermatogenesis and specific maturation steps affecting fertility.

# Microbial communities are active contributors to male fertility

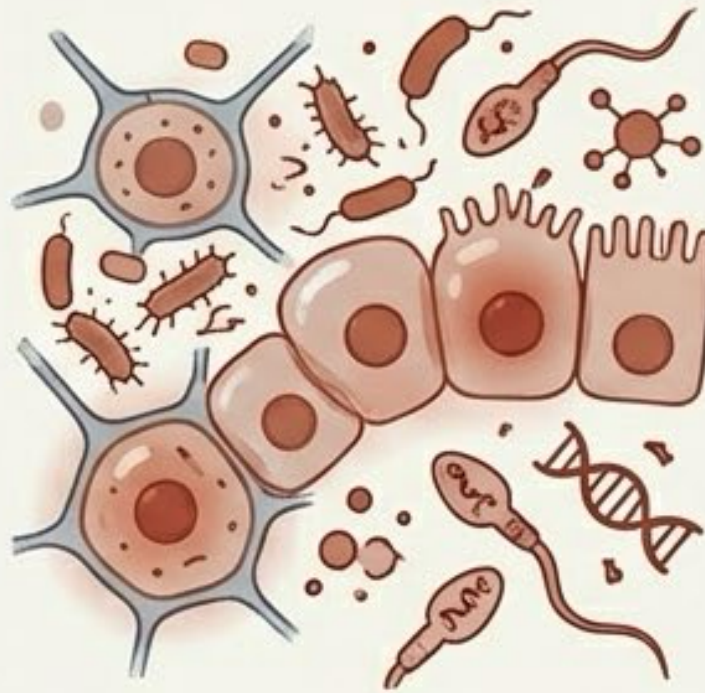
## The Ecosystem



## The Ecosystem

Semen and testes are not passive or sterile; they host a dynamic microbiome that directly influences sperm function.

## The Pathology



## The Pathology

Pathogenic dysbiosis and bacteriospermia drive local inflammation, oxidative stress, and genomic damage in sperm.

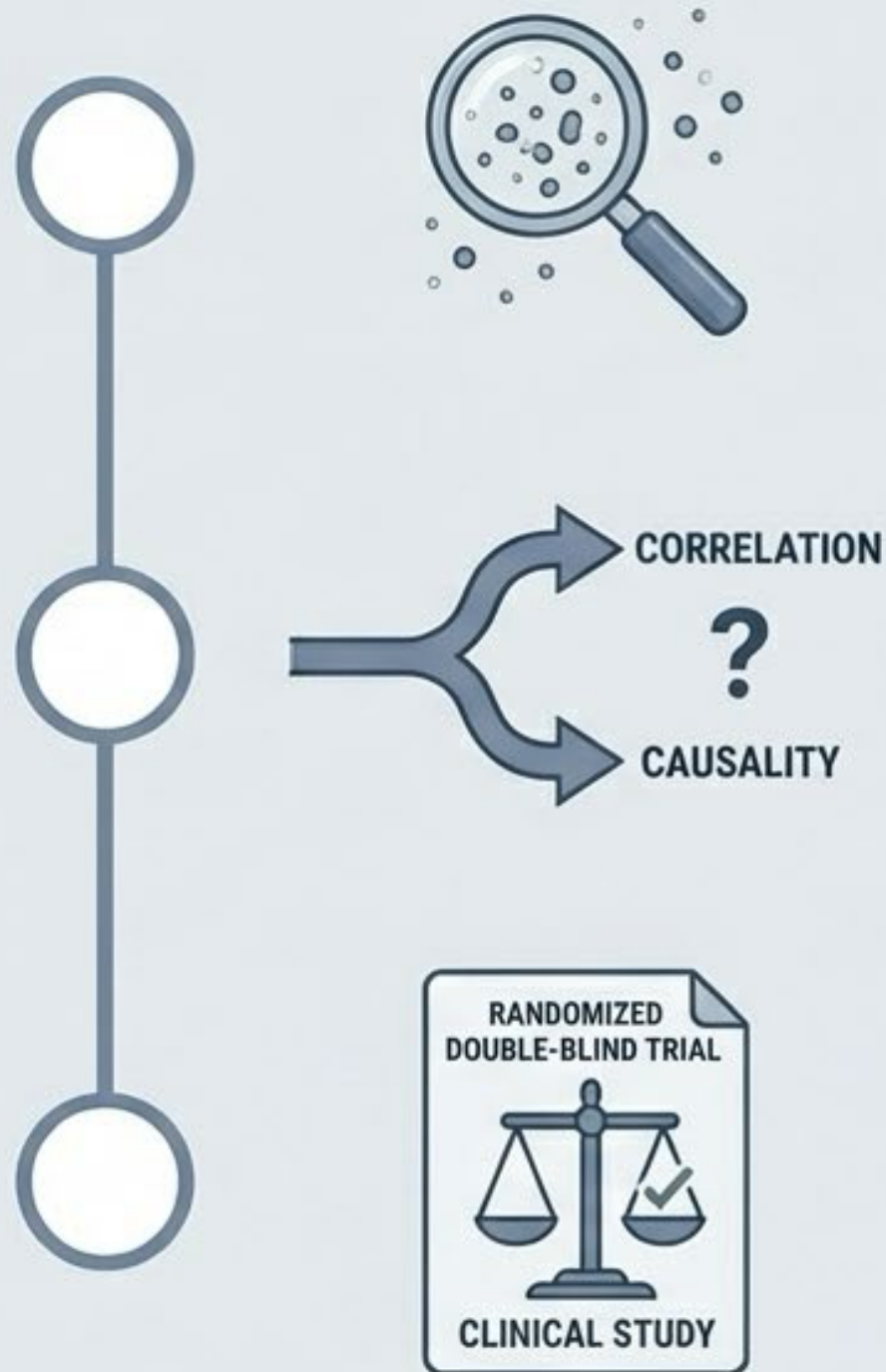
## The Future



## The Future

Microbiome profiling (NGS) and targeted modifiable therapies (probiotics) represent the next critical frontier in male infertility management.

# Routine clinical implementation requires longitudinal validation



## Current Limitations

Existing human studies feature small sample sizes.

## Correlation vs. Causality

Many approaches derive from gut-microbiome improvements; precise causal relationships in the genitourinary axis require clarification.

## Future Needs

High-quality, randomized interventional studies are mandatory to establish long-term safety and efficacy before routine clinical adoption.