INVESTIGATING THE ROLE OF GUT AND REPRODUCTIVE TRACT MICROBIOMES IN HUMAN INFERTILITY

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Under the Supervision and Guidance of

Dr. Alok Kumar Mathur

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TO WHOM SO EVER IT MAY CONCERN

This is to certify that **Dr. Megha Gupta**, student pursuing MBA (Hospital and Health Management), IIHMR University, Jaipur has successfully completed her Dissertation title "**Investigating the Role of Gut and Reproductive Microbiomes in Human Infertility**" in her internship at the National Institute of Health and Family Welfare, New Delhi during 17th March to 6th June, 2025 under the guidance of Dr. S. Ramachandra Rao, Reader, Department of RBM.

Her conduct during the Summer Training Programme was good and I wish her a bright career ahead.

(Dr. Manish Chaturvedi)
Dean, Additional charge

IIHMR UNIVERSITY

CERTIFICATE

This is to certify that dissertation titled **"Investigating the Role of Gut and Reproductive Tract Microbiomes in Human Infertility"** is a record of the research work undertaken by **Dr. Megha Gupta** in partial fulfilment of the requirements for the award of the degree of MBA Hospital and Health Management from the IIHMR University, Jaipur under my guidance and supervision and her approach to the research study has been sincere, scientific, and analytical.

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DECLARATION BY STUDENT

I hereby declare that this dissertation titled "**Investigating the Role of Gut and Reproductive Tract Microbiomes in Human Infertility**" is the bona fide record of my original research work. I declare that it has not been submitted to any other university or institution for the award of any degree or diploma. Information derived from the published or unpublished work of others has been duly acknowledged in the text.

(Signature)

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Date- 18/06/2025

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June 2025

Dr. Megha Gupta

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
WHO	World Health Organization
PCOS	Polycystic Ovary Syndrome
STIs	Sexually Transmitted Infections
SDI	Socio-Demographic Index
ASPR	Age-Specific Prevalence Rate
SRHR	Sexual and Reproductive Health and Rights
CSTs	Community State Types
GIT	Gastrointestinal Tract
SCFA	Short-Chain Fatty Acids
TMAO	Trimethylamine-N-Oxide
LPS	Lipopolysaccharide / Lipopolysaccharides
TLR4	Toll-Like Receptor 4
NAFLD	Non-Alcoholic Fatty Liver Disease
CVD	Cardiovascular Disease
IBD	Inflammatory Bowel Disease
UC	Ulcerative Colitis
MAPK/NF-κB	Mitogen-Activated Protein Kinase / Nuclear Factor kappa-light-chain-enhancer of activated B cells
CNS	Central Nervous System
GPCRs	G-Protein-Coupled Receptors
FFA2 / FFA3	Free Fatty Acid Receptor 2 / 3
GPR109a	G Protein-Coupled Receptor 109a
Olfr78	Olfactory Receptor 78
MCTs	Monocarboxylate Transporters
HDACs	Histone Deacetylases
MGB Axis	Microbiota-Gut-Brain Axis
GLP-1	Glucagon-Like Peptide-1
PYY	Peptide YY
GABA	Gamma-Aminobutyric Acid
BBB	Blood-Brain Barrier
CSF	Cerebrospinal Fluid
BDNF	Brain-Derived Neurotrophic Factor

NGF	Nerve Growth Factor
GDNF	Glial Cell Line-Derived Neurotrophic Factor
HPA Axis	Hypothalamic-Pituitary-Adrenal Axis
BAs	Bile Acids
FXR	Farnesoid X Receptor
TGR5	Takeda G Protein-Coupled Receptor 5
FGF19	Fibroblast Growth Factor 19
CRH	Corticotropin-Releasing Hormone
HSL	Hormone-Sensitive Lipase
StAR	Steroidogenic Acute Regulatory Protein
NMDA	N-Methyl-D-Aspartate (Receptor)
BCAAs	Branched-Chain Amino Acids
mTOR	Mammalian Target of Rapamycin
ENS	Enteric Nervous System
HIV	Human Immunodeficiency Virus
E. coli	Escherichia coli
IL / IL-6 / IL-10	Interleukin / Interleukin 6 / Interleukin 10
MS	Multiple Sclerosis
SLE	Systemic Lupus Erythematosus
COPD	Chronic Obstructive Pulmonary Disease
TB	Tuberculosis
GDM	Gestational Diabetes Mellitus
GPR41 / GPR43	G-Protein-Coupled Receptors 41 and 43
T2DM	Type 2 Diabetes Mellitus
ASD	Autism Spectrum Disorders
V1–V9	Hypervariable Regions 1 to 9 (of the 16S rRNA gene)
rRNA	Ribosomal Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
tRNA	Transfer Ribonucleic Acid
AHR	Aryl Hydrocarbon Receptor
IPA	Indole propionic Acid
Treg	T-Regulatory Cells
SFB	Segmented Filamentous Bacteria
CFB	Cellulomonas Flava Bacteria
T1DM	Type 1 Diabetes Mellitus

PM2.5	Particulate Matter (≤ 2.5 micrometres)
NO_2	Nitrogen Dioxide
SO_2	Sulphur Dioxide
O_3	Ozone
CO	Carbon Monoxide
ICPD	International Conference on Population and Development
FGM	Female Genital Mutilation
PID	Pelvic Inflammatory Disease
SDG	Sustainable Development Goals
GBD	Global Burden of Disease
IHME	Institute for Health Metrics and Evaluation
ART	Assisted Reproductive Technology
PHGG	Partially Hydrolysed Guar Gum
GnRH	Gonadotropin-Releasing Hormone
FSH	Follicle-Stimulating Hormone
LH	Luteinizing Hormone
DHT	Dihydrotestosterone
HPG Axis	Hypothalamic-Pituitary-Gonadal Axis
MAMPs	Microbial-Associated Molecular Patterns
XOD	Xanthine Oxidase
FMT	Faecal Microbiota Transplantation
ND	Normal Diet
HFD	High-Fat Diet
OAT	Oligoasthenoteratozoospermia
IVF	In Vitro Fertilization
MR	Mendelian Randomization
GWAS	Genome-Wide Association Study
IVW	Inverse Variance Weighted
MR-Egger	Mendelian Randomization Egger Regression
MR-RAPS	Mendelian Randomization Robust Adjusted Profile Score
MR-PRESSO	Mendelian Randomization Pleiotropy Residual Sum and Outlier Test
IUGR	Intrauterine Growth Restriction
β-glucuronidase	Beta-Glucuronidase (enzyme)
β-glucosidase	Beta-Glucosidase (enzyme)
Erβ	Oestrogen Receptor Beta

IAP	Intestinal Alkaline Phosphatase
HA-PCOS	Hyperandrogenic Polycystic Ovary Syndrome
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
ROS	Reactive Oxygen Species
SHBG	Sex Hormone Binding Globulin
TNF-α	Tumour Necrosis Factor alpha
BTB	Blood-Testis Barrier

ABSTRACT

Title Investigating the Role of Gut and Reproductive Tract Microbiomes in Human Infertility

Background

Infertility is a significant and growing global public health concern, affecting approximately 17.5% of individuals during their reproductive years. In India alone, nearly 27.5 million couples are estimated to experience infertility, often leading to emotional, social, and financial distress. While conventional causes of infertility include hormonal imbalances, structural abnormalities, or lifestyle factors, recent scientific advancements have drawn attention to a new area of interest: the human microbiome.

The gut microbiome, a complex community of trillions of microorganisms, plays a critical role in digestion, immune function, hormonal regulation, and the control of inflammation. Likewise, the reproductive tract microbiome, particularly the vaginal microbiota in females and seminal microbiota in males, contributes to maintaining reproductive health by preventing infections, supporting fertilization, and ensuring a favorable environment for implantation and embryo development.

An imbalance in these microbial communities, termed dysbiosis, has been linked to several infertility-related conditions such as Polycystic Ovary Syndrome (PCOS), endometriosis, implantation failure, and reduced sperm quality. Despite the growing body of evidence connecting microbiome health to fertility, mainstream reproductive health initiatives and government programs have not yet integrated microbiome-focused diagnostics or treatments into standard practice.

This literature review explores the emerging role of gut and reproductive tract microbiomes in the development and management of infertility. By understanding these relationships, there is a potential to improve fertility

outcomes through non-invasive, cost-effective, and personalized interventions such as probiotics, dietary modifications, and lifestyle changes—thereby contributing to better public health outcomes and aligning with Sustainable Development Goal 3, which emphasizes universal access to reproductive healthcare.

General Objective:

To investigate the role of gut and reproductive tract microbiomes in the development, progression, and management of human infertility, with a focus on understanding microbial influence on reproductive health in both males and females.

Specific Objectives:

1. To review the structure and functions of the gut microbiomes.
2. To review reproductive health and infertility and its current trends in India.
3. To understand the mechanism and factors which causes dysbiosis
4. To compare the gut microbiome and reproductive tract microbiome, highlighting similarities and differences.
5. To identify the association between gut microbiome dysbiosis and infertility.
6. To analyse the impact of gut microbiome composition and dysbiosis on female reproductive disorders, including PCOS, endometriosis, and implantation failure.
7. To analyse the impact of gut microbiome composition and dysbiosis on male reproductive disorders, such as low sperm count, motility issues, and testicular dysfunction.
8. To explore current and emerging strategies for improving infertility through gut microbiome modulation, including probiotics, dietary changes, and lifestyle interventions.

Methodology

This study is a literature review-based analysis of peer-reviewed journals, clinical reports, and scientific publications focused on microbiome research and reproductive health. It synthesizes evidence from microbiology, endocrinology, and reproductive medicine using structured thematic exploration across key areas such as microbiome composition, dysbiosis mechanisms, sex-specific reproductive impacts, and emerging therapeutic interventions.

Results

The review found that dysbiosis—imbalances in microbial composition—is linked to multiple reproductive disorders. In females, it is associated with PCOS, endometriosis, implantation failure, and hormonal imbalances. In males, it correlates with low sperm motility, testicular inflammation, and sperm quality deterioration. Factors contributing to dysbiosis include poor diet, lifestyle, pollution, antibiotic misuse, infections, and genetic predisposition. Improvement in gut microbiota through probiotics, diet modulation, and lifestyle changes shows promising effects in restoring fertility.

Conclusion

The gut and reproductive microbiomes play an essential yet under-recognized role in human fertility. Integrating microbiome-focused approaches into reproductive health frameworks offers a promising avenue for non-invasive, cost-effective fertility management. This review advocates for the inclusion of microbiome health in national infertility programs and public health policies to improve reproductive outcomes and meet Sustainable Development Goals.

Keywords Gut microbiome, reproductive microbiome, infertility, dysbiosis, probiotics, PCOS, male infertility, microbial diversity, reproductive health, SDG 3.

CHAPTER 1

INTRODUCTION

INTRODUCTION

The human body comprises a vast and diverse number of microorganisms essential for the human body's normal functioning. The “human microbiome” is referred to as the genomic content of organisms that live in and on the human body. It includes bacteria, viruses, fungi, and protozoa. These microorganisms reside at various human body sites, such as the skin, Gastrointestinal tract, respiratory tract, reproductive tract, urinary tract, and mammary glands. They play crucial roles in maintaining health and help in disease prevention. (Turnbaugh et al., 2007) From birth, a steady interaction existed between the microbiome and the human body and they form a symbiotic relationship that can be commensal, mutualistic, or pathogenic. Over the period, these microbes adapt in the body and fit themselves into the unique environment of the human body showed in Figure 1.

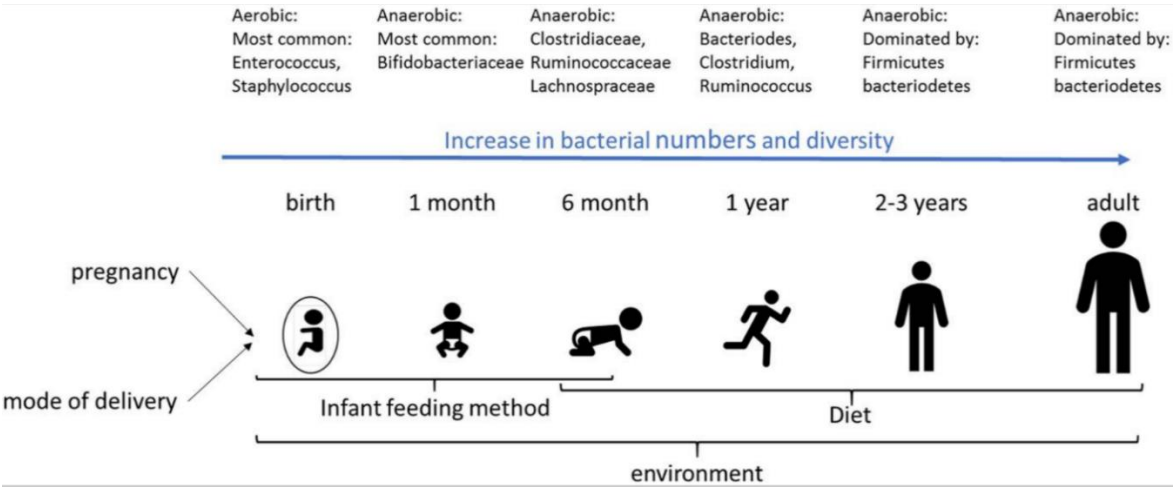


Figure 1: Infant gut microbiome development and its influencing factors. (Adapted from Mohajeri et al., 2018)

They evolve consistently with age, nutrition, lifestyle, genes, and underlying disease, as result of this, these organisms are identified as part of the human body and influence physiological changes from birth to death. The gut contains the highest number of microbiomes and supports the human body the most (Ogunrinola et al., 2020). The gut consists of more than 1000 different species of bacteria and approximately 150 times the

number of genes in the human genome. Microbiomes are biologically significant from an early stage of life. Postnatal contributes to the development of the neonatal immune system and later on helps in various physiological processes like maintaining homeostasis, immune regulation, Central Nervous System (CN) and Enteric Nervous System (ENS) modulation. Gut Dysbiosis refers to an imbalance or disruption within the gut microbiome, the community of microorganisms living in digestive tract. This imbalance can lead to various health issues, as the gut microbiome plays a crucial role in digestion, immunity, and overall well-being. In other words, it is a decrease in microbiome numbers and diversity or the absence of beneficial microbiomes (Shen et al., 2025). Any changes in the composition of the microbiome (Dysbiosis) lead to a life-threatening condition. And associated with multiple health conditions like cancers, inflammatory bowel diseases, cardiovascular diseases, etc. (Ogunrinola et al., 2020).

Microbiomes composition in various parts of the body

Gastrointestinal Tract

The Gastrointestinal Tract (GIT) consist of mouth, pharynx, oesophagus, stomach, small intestine, large intestine and ends at anus. Each site of GIT has different types of microbial load, and its diversity increases from proximal to distal end (Figure 2). Further, the lists of harmful and beneficial gut bacteria present in various organs are shown in Figure 3.

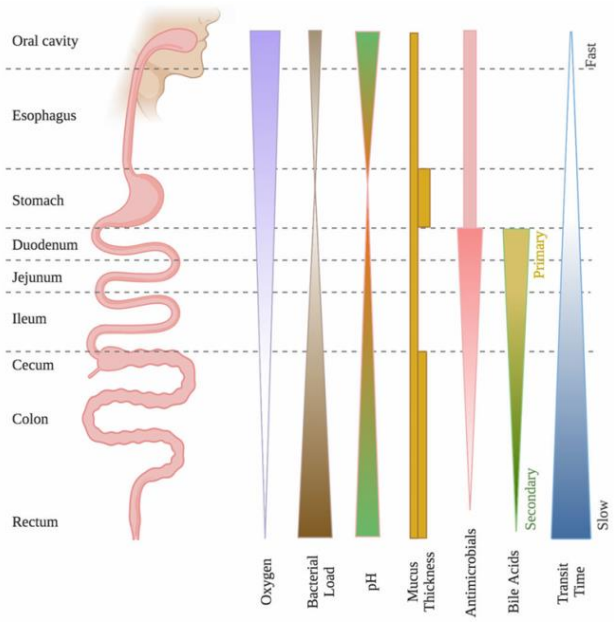
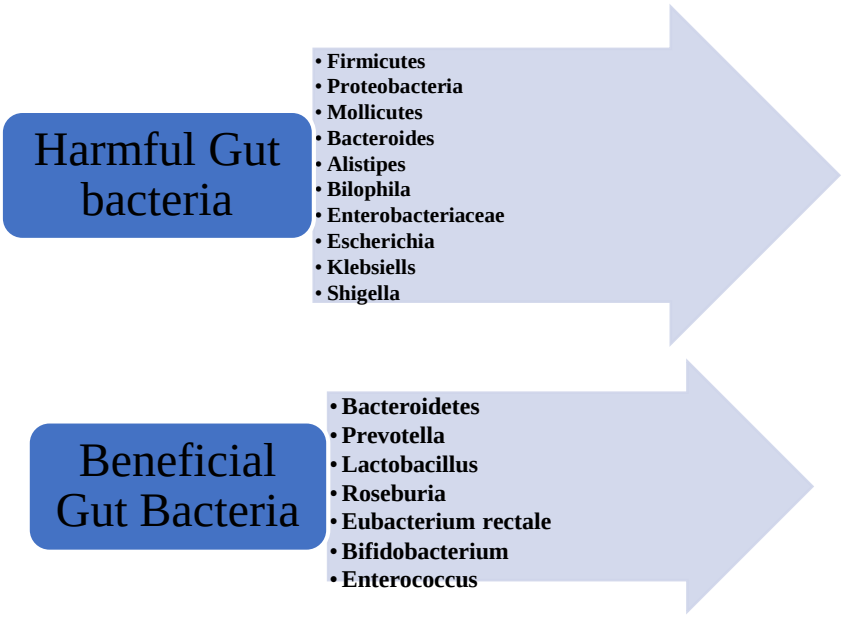


Figure 2: Gut loads from Proximal to Distal These change along the gut and create different environments that determine which microbes can live in each section. (Adapted from Kennedy & Chang, 2020)

Figure 3: List of Gut bacteria



In the small intestine, microbes are *Lactobacillus, Veillonella, Leptotrichia, and Helicobacter*. The large intestine has the most microbial load and diversity in the GIT, such as *Oscillibacter*, *Ruminiclostridium*, *Ruminococcaceae*, and *Lachnospiraceae*. These microbiomes help in performing multiple functions: digestion, absorption, defence, metabolism, immune, neurological, and host signalling. The oral cavity predominantly contains 700 bacterial species, as well as fungi, viruses, archaea, and protozoa. Some of

them are *Streptococcus (dominant genus), Haemophilus, Veillonella, Rothia, Neisseria, Actinomyces, Fusobacterium, Granulicatella, Capnocytophaga, Porphyromonas, and Prevotella.* In the stomach, major microbes are *Treptococcus, Prevotella, Veillonella, and Rothia*. The small intestine has less diversity and density of microbiome as in comparison to large intestine some of them are *lactobacillus, clostridium, Streptococcus, E. coli, and others.* (Kennedy & Chang, 2020). It was reported that differences in gut microbiome in men and women, which is showed in Figure 4.

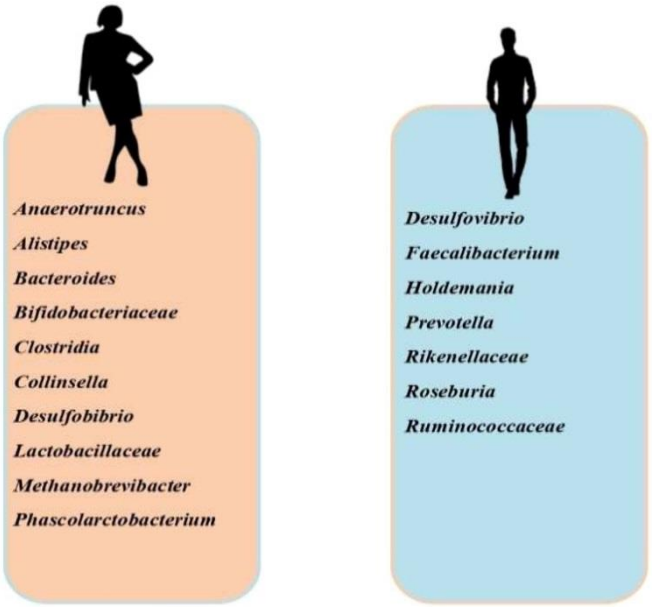


Figure 4: Sex selective differences in Gut Microbiome (Adapted from Ashonibare et al., 2024)

Gut is having largest load of microbiomes and it interact with every organ with the formation of axis such as gut brain axis, gut skin axis, gut genitourinary axis, gut liver axis and others. when gut microbiome alters this leads to a reduction in Short chain fatty acids (SCFA) and increased toxin-producing bacteria in the body which will leads to disrupts intestine lining and leads to inflammation, a visual presentation of gut organ axis is depicted in Figure 5.

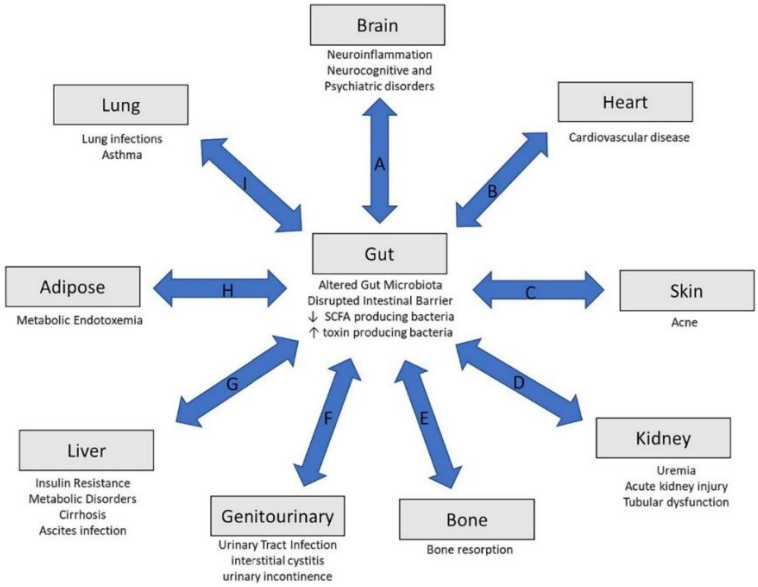


Figure 5: Gut Organ axis (Adapted from Alagiakrishnan et al., 2024)

Skin

Skin is the largest organ of the human body, serving as the protective covering of the body. Axilla is a moist area in which *Corynebacterium* is present, on the face, thoracic area, and back *Propionibacterium* is present, while *Staphylococcus* is present at moist and folded areas and at sebaceous sites, *Malassezia* and Propionibacterium are present.

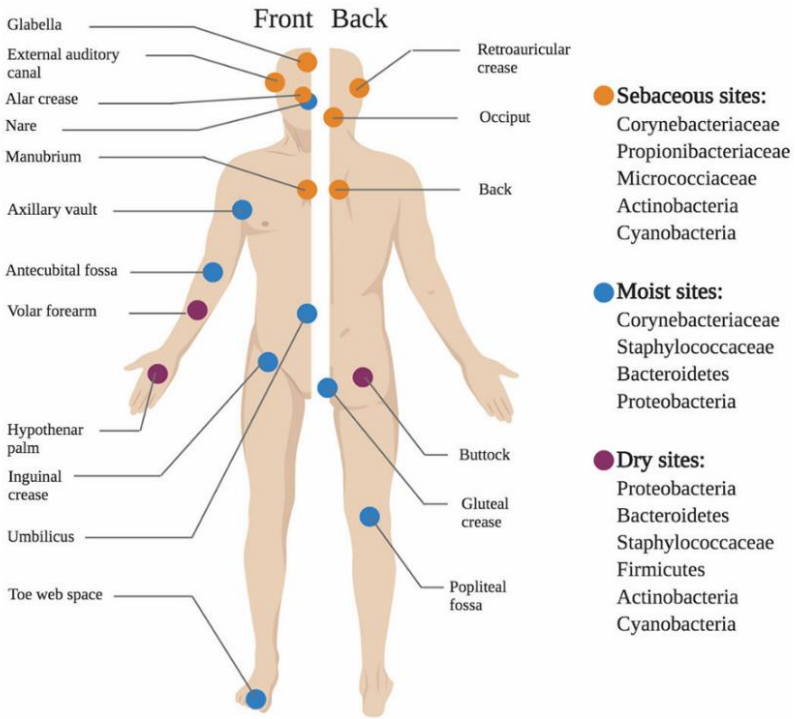


Figure 6: Skin microbiomes with its region (Adapted from (Kennedy & Chang, 2020)

Female reproductive tract

Vagina is a part of female reproductive organ it is elastic and muscular having pH (3.5-4.5). It connects the cervix to the outside of the body serves as a birth canal during delivery and passage of menstrual fluid. Vaginal Microbiota first discovered in the year 1892 by German gynaecologist Albert Doderlein which name this bacterium Doderlein bacillus. And later it was renamed as Lactobacillus. Thus, bacilli having an antagonistic effect on *Staphylococcus* growth and their absence are associated with perpetual fever. (Kwon & Lee, 2022) Vaginal microbiome composition is dominantly having *lactobacillus (crispatus, gasseri, jensenii, iners)* this bacillus having various beneficial properties which contributing in the acidic environment, it is preventing overgrowth of pathogenic microorganisms and promoting sperm survival (O'Mahony & Comizzoli, 2023) In healthy women, there are five types of Community State types (CSTs) as shown in Figure 4. CST 1,2,3,5 are related to lactobacillus, and CST-4 has (*Atopobium, Corynebacterium, Anaerococcus, Peptoniphilus, Prevotella, Gardnerella).* Lactic acid produced in vagina by lactobacillus which protect from pathogens like chlamydia trachomatis, Neisseria gonorrhoea and HIV. It also exhibits immunomodulatory properties, produces antimicrobial bacteriocins that fight against Klebsiella, E. coli, and others. (Kennedy & Chang, 2020) Vaginal Microbiome can be altered by lifestyle factors such as diet, antibiotic use, smoking, stress obesity, sexual activity, hygienic practices, use of contraceptives and many. As shown in Figure 7, Vaginal mucosa is covered with stratified multilayered squamous epithelium, oestrogen stimulation leads to glycogen storage in the epithelial cells. Humans have a higher vaginal glycogen concentration than other mammals. *Lactobacillus* levels depend on glycogen in vaginal cells, which rises with oestrogen. Therefore, prepubertal girls and postmenopausal women have lower *Lactobacillus* abundance due to reduced glycogen. Lactobacillus doesn't use glycogen directly, Human alpha amylase breaks down glycogen into

smaller carbohydrates (maltose, malt triose, maltopentaose, maltodextrins). Lactobacillus use these sugars for energy, producing lactic acid which reduces vaginal ph. (Kwon & Lee, 2022)

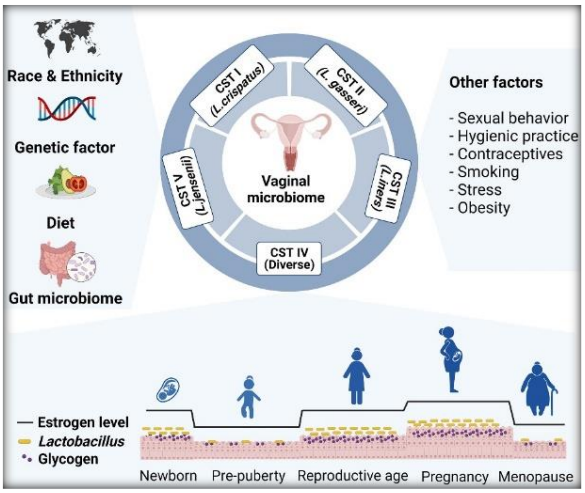


Figure 7: Vaginal Microbiome and factors affecting them, and level of oestrogen, lactobacillus and glycogen with age (adapted from Kwon & Lee, 2022)

Hormonal changes in women from birth to post menopause as seen in Table 1. The vaginal microbiome stability fluctuates during menses and pregnancy. During pregnancy microbiome is more stable, and an increase of lactobacillus is seen. At a later stage of life, when glycogen levels drops, reduction in the lactobacillus level occurs that can be restored by hormonal replacement therapy (Kwon & Lee, 2022). (Table 2)

Table 1: Impact of hormonal changes across life stages

Life stages	**Hormonal/Glycogen status**	**Vaginal microbiome composition**
Newborns (early days)	High maternal oestrogen- High glycogen	Adult-like glycogen; Lactobacillus not yet dominant
Prepubertal Girls	Low oestrogen and glycogen	Dominated by *Staphylococcus epidermidis*, *E. coli*, *Pepto coccus*
Reproductive age women	High oestrogen and glycogen	Dominated by Lactobacillus species (healthy CSTs)
Postmenopausal women	Decreased oestrogen and glycogen	Low Lactobacillus abundance, high microbial diversity (often CST-IVA)

Table 2: Microbiome and hormonal changes during pregnancy and its effects

Pregnancy stage	Microbiome changes	Hormonal changes	Functional effects
First trimester	Similar to non-pregnant women	Rising oestrogen, progesterone	Baseline metabolism and immune modulation
Second trimester	Increased Proteobacteria, Actinobacteria; decreased diversity	High oestrogen and progesterone	weight gain, insulin resistance, inflammation (adaptive)
Third trimester	Gradual return to pre-pregnancy state	Low oestrogen and progesterone	Microbiota resembles the non-pregnant state

Male reproductive tract

The male reproductive organs function to produce, nourish, and deliver sperm. They are divided into external and internal organs: The external male reproductive organs consist of the penis and the scrotum. Penis delivers sperm into the female reproductive tract during intercourse and urine excretion. The scrotum, a pouch of skin that covers the testes, is essential for regulating the temperature necessary for sperm production, maintaining it slightly cooler than the body temperature. The internal male reproductive organs include the testes, epididymis, vas deferens, seminal vesicles, prostate gland, bulbourethral glands (Cowper's glands), and the urethra. The testes are responsible for producing sperm through a process called spermatogenesis and secreting a male hormone, testosterone. After sperm are produced, they are stored and mature within the epididymis, a coiled tube located on the back of each testis. During ejaculation, sperm travel through the vas deferens, a muscular tube that connects the epididymis to the urethra. The seminal vesicles contribute a significant portion of the semen, producing a fluid rich in sugars that nourish sperm. The prostate gland adds an alkaline fluid to the semen, which helps increase sperm survival within the female reproductive tract. The bulbourethral glands produce a clear mucus before ejaculation that lubricates the urethra and neutralizes its acidity, creating a safer passage for sperm. Finally, the urethra serves a dual

purpose, conducting both urine from the bladder and semen from the reproductive organs to the outside through the penis (Cleveland Clinic, 2023). Microbiome present in the male reproductive tract is showed in Figure 8. Bacterial genera present in testes, including *Blautia, Cellulosibacter, Clostridium groups (XIVa, XIVb, XVIII), Collinsella, Prevotella, Prolixibacter, Robinsoniella, and Wandonia.* Semen contains genera including *Staphylococcus, Enterococcus, Escherichia, Ureaplasma, Lactobacillus, Corynebacterium, Streptococcus, Prevotella, Gardnerella, Veillonella, Neisseria, Burkholderia,* and *Haemophilus*. The prostate hosts bacteria such as *Burkholderia, Escherichia coli, Klebsiella, Proteus, Pseudomonas aeruginosa, Enterococcus,* and *Staphylococcus aureus.* On the penile surface and in the urethra, microbes like *Enhydrobacter, Brevibacterium, Ruminococcaceae, Campylobacter, Rothia, Staphylococcus, Enterococcus, Escherichia,* and *Ureaplasma* are commonly present (Zuber et al., 2023).

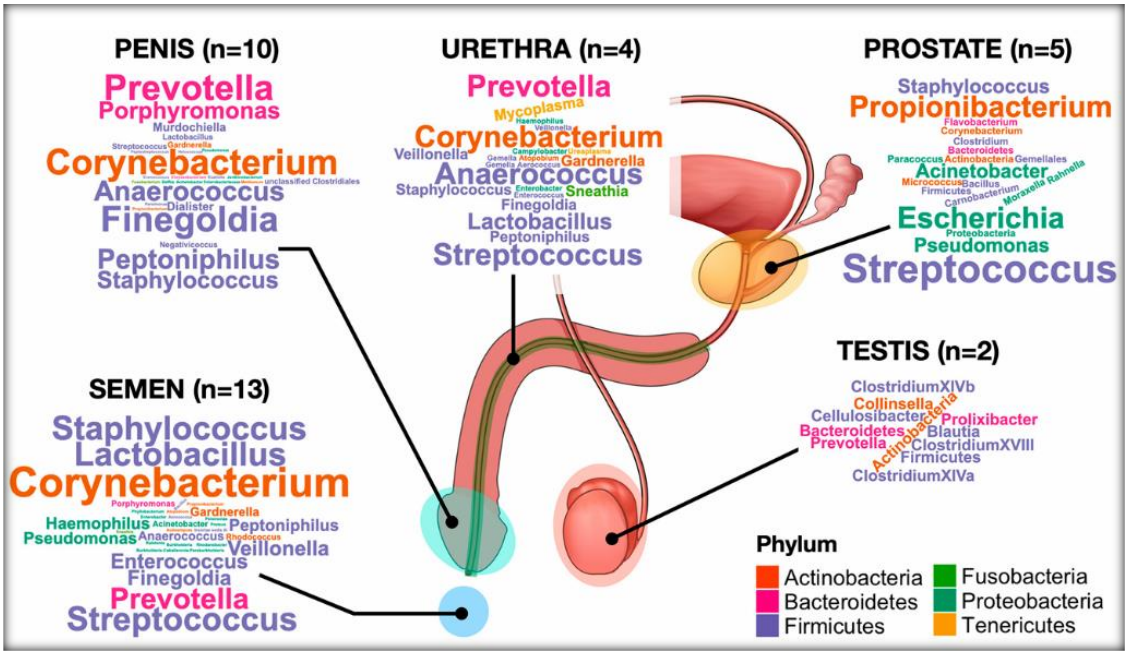


Figure 8: Microbiome of the male reproductive tract (Adapted from Zuber et al., 2023)

Sex-specific disparity in the prevalence of gut microbiota

Females have an abundance of Anaerotruncus, Alistipes, Bacteroides, Bifidobacteriaceae, Clostridia, Collinsella, Desulfobibrio, Lactobacillaceae, Methanobrevibacter, and Phascolarctobacterium. While males have Desulfovibrio, Faecalibacterium, Holdemania, Prevotella, Rikenellaceae, Roseburia, and Ruminococcaceae are more abundant (Ashonibare et al., 2024).

Respiratory tract

The respiratory tract is divided into two parts, upper and lower. The upper respiratory tract includes the nasal cavity, paranasal sinuses, pharynx, and larynx above the vocal cords. The lower tract consists larynx, the lower vocal cord, the trachea, lungs, bronchi, bronchioles, and alveoli. Its primary function is of exchange of oxygen and carbon dioxide and maintaining gas exchange. The microbiomes of the respiratory tract are distinct from each other according to their location in the lower tract, having diverse and lower biomass microbiomes, and it is nutrient-poor, aerobic, and immunologically active in the environment, as in comparison with the upper tract. Upper respiratory tract microbiomes at the Nasal vestibule are cooler and drier, and are dominated by *Corynebacterium*, *Staphylococcus*, and *Propionibacterium.* Deeper nasal mucosa is warmer and moister, dominated by *Actinobacteria* (e.g., *Corynebacterium*) and *Proteobacteria* (e.g., *Moraxella*), alongside *Streptococcus* and *Dolosigranulum.* The nasopharynx has Haemophilus, Streptococcus, and Moraxella. The lower respiratory tract forms two functional zones- conducting airways (trachea through bronchioles) respiratory portion (alveoli) for gas exchange. It is dominated by bacteria such as *Prevotella*, *Veillonella*, *Streptococcus*, *Moraxella*, *Haemophilus*, and *Staphylococcus.* Viruses from the *Anellovirus family*, and fungi, such as *Malassezia.* (Kennedy & Chang,

2020) Dysbiosis is a decrease in microbiome numbers and diversity or the absence of beneficial microbiomes (Shen et al., 2025) Changes in the composition of the microbiome (Dysbiosis) lead to a life-threatening condition (Ogunrinola et al., 2020). Although its pathogenesis is unclear but various factors contribute to dysbiosis, such as diet, lifestyle, antibiotics, medications, infections, inflammation, host genetic predisposition, demography, and others. Dysbiosis is associated with various diseases shown in Table 3. (Shen et al., 2025).

Table 3: Diseases associated with Dysbiosis

Disorder	Associated Disease
Gastrointestinal disorder	• Irritable bowel Syndrome, Inflammatory bowel disease, Colorectal cancer, Alcoholic hepatitis, etc
Neurological diseases	Alzheimer's disease, Parkinson's disease, autism spectrum disorder, depression, anxiety, etc.
Immune-related disorders	Autoimmune diseases include: • Multiple sclerosis (MS) • Rheumatoid arthritis • Systemic lupus erythematosus (SLE) • Allergic disease mediated by IgE • Chronic spontaneous urticaria
Cardiovascular diseases	• Hypertension, atherosclerosis, heart failure, obesity, diabetes, thrombosis
Metabolic diseases	• Obesity • Type 2 diabetes mellitus • NAFLD- Non-Alcoholic Fatty Liver Disease
Skin disorders	• Atopic dermatitis, Psoriasis, Acne, Rosacea, Alopecia areata, and Hidradenitis suppurativa
Respiratory diseases	• Allergic asthma • Chronic obstructive pulmonary disease (COPD) • Pulmonary fibrosis • Tuberculosis (TB) • Ulcerative colitis (linked via IL-33 signalling and gut–lung immune crosstalk) • Lung cancer (impacting immunotherapy response)
Placental origin disease	• Foetal growth restriction • Preeclampsia • Preterm birth • Placental oxidative damage and mitochondrial dysfunction • Placental cell apoptosis • Recurrent miscarriages • Maternal malnutrition-related placental dysfunction

	• Neurodevelopmental disorders in offspring • Allergic diseases in offspring • Pregnancy complications related to altered immune responses • Gestational diabetes mellitus (GDM)

Source: (Shen et al., 2025)

RATIONALE

Infertility is an escalating public health challenge in India, impacting an estimated 27.5 million couples, according to the ICMR. Despite various diagnostic and therapeutic advancements, there is a large burden of infertility cases. Scientific evidence suggests that the gut and reproductive tract microbiomes, the diverse communities of microorganisms residing in the human body, may play various important roles in determining reproductive health. The gut microbiome performs various body functions such as immune modulation, inflammation control, hormonal homeostasis, etc. which affects reproductive function. Dysbiosis can lead to chronic inflammation, insulin resistance, thyroid dysfunction, and oestrogen dominance, all of which are associated with conditions like PCOS, endometriosis, and implantation failure, low sperm quality, disturbed pH balance, risk of reproductive tract infections and others. Despite these insights, the role of microbiomes is not yet formally integrated into government initiatives Programs like the National Reproductive and Child Health (RCH) Programme, Ayushman Bharat (Health & Wellness Centres), and National Health Mission (NHM) focus largely on maternal care, contraceptive access, and infertility treatments, but overlook the preventive and diagnostic potential of microbiomes and their impact in the body. There is a critical need to align reproductive health policies to research on microbiota, especially as they offer cost-effective, non-invasive, and personalized treatment for managing fertility disorders.

This literature review aims to critically examine the role of gut and reproductive microbiome impact on infertility. This review will give new insights that will help in preventing infertility by managing microbes. This not only enhances treatment outcomes but also supports many couples to achieve parenthood and governments to achieve Sustainable Development Goal 3 by 2030, which includes targets as universal access to reproductive healthcare and well-being.

CHAPTER 2

OBJECTIVES

OBJECTIVES

General Objective:

To investigate the role of gut and reproductive tract microbiomes in the development, progression, and management of human infertility, with a focus on understanding microbial influence on reproductive health in both males and females.

Specific Objectives:

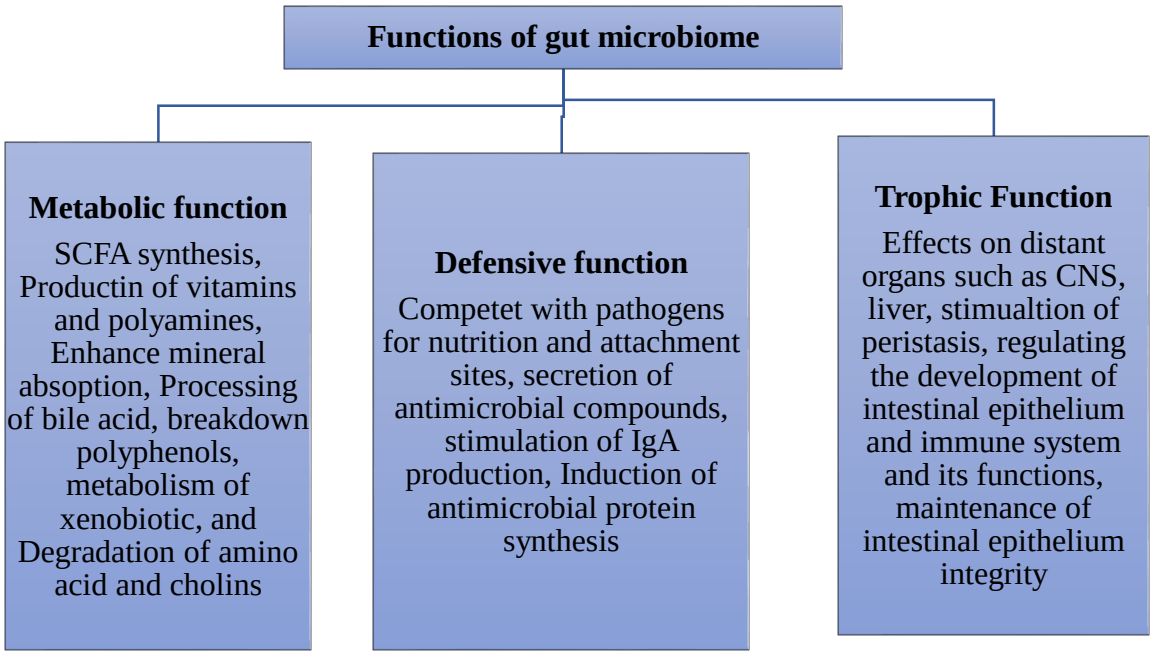
9. To review the structure and functions of the gut microbiomes.
10. To review reproductive health and infertility and its current trends in India.
11. To understand the mechanism and factors which causes dysbiosis
12. To compare the gut microbiome and reproductive tract microbiome, highlighting similarities and differences.
13. To identify the association between gut microbiome dysbiosis and infertility.
14. To analyse the impact of gut microbiome composition and dysbiosis on female reproductive disorders, including PCOS, endometriosis, and implantation failure.
15. To analyse the impact of gut microbiome composition and dysbiosis on male reproductive disorders, such as low sperm count, motility issues, and testicular dysfunction.
16. To explore current and emerging strategies for improving infertility through gut microbiome modulation, including probiotics, dietary changes, and lifestyle interventions.

CHAPTER 3

FUNCTIONS OF THE GUT MICROBIOME & FACTORS CAUSING DYSBIOSIS

FUNCTIONS OF THE GUT MICROBIOME

The gut microbiome helps in performing various functions of the body, such as digestion, immunity, cognitive, metabolic, and other functions:



Gut microbiota functions are classified into metabolic, defensive, and trophic functions.

- **Metabolic function**: Gut microbiota performs various metabolic functions such as the Synthesis of SCFA, Vitamin B12, K synthesis, facilitation of calcium, magnesium, and iron absorption, breakdown of polyphenols and choline and amino acids, metabolism of bile acid and drugs, production of polyamines. Short-chain fatty acids are synthesised by gut microbiota. When gut bacteria break down fibres, primarily acetate, propionate, and butyrate are produced. (den Besten et al., 2013) In the ratio of 3:1:1 to 10:2:1, and plays various roles in health. Acetate is most abundant and supports bacterial growth and participates in cholesterol metabolism and regulates paetitte through a central mechanism. (Rowland et al., 2018). Butyrate is the primary source of energy for colonocytes, approximately. 70%, they have anti-cancer properties and activate intestinal gluconeogenesis, which contributes to glucose and energy homeostasis. (Pant et al., 2023) Propionate plays a vital function in human metabolism and appetite

regulation. It uses intestinal epithelial cells and transports it to the liver, where it serves as a substrate of gluconeogenesis and helps in glucose production. Additionally, this influences satiety by activating G-protein-coupled receptors GPR41 and GPR43 on enteroendocrine L cells in the gut. This activation stimulates the release of appetite-regulating hormones such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which promote feelings of fullness and reduce food intake. Also helps in maintaining energy balance and obesity prevention. (X. Qin et al., 2025) SCFA performs various functions, such as maintaining glucose levels, regulating the immune system, lubricating and fuelling intestinal cells, and reducing inflammation. (Koh et al., 2016a) (Morrison & Preston, 2016). Vitamin K is produced by certain gut bacteria, Vitamin K is an insoluble vitamin essential for blood clotting, bone health, and vascular function. (Rowland et al., 2018) Vitamin B group is also synthesized by gut microbiota, several water-soluble B vitamins, including Thiamine (B1), Riboflavin (B2), Niacin (B3), Pantothenic acid (B5), Pyridoxine (B6), Biotin (B7), Folate (B9), and Cobalamin (B12) (Tarracchini et al., 2024). Gut microbiota significantly contributes to the vitamin pools. **Indolepropionic acid-** it is an antioxidant produced by gut bacteria that helps in the protection of the nervous system. (Alena Pribyl, 2023). Gamma aminobutyric acid GABA is a neurotransmitter produced by the body and some amount by gut bacteria. This helps in transmitting the signal to the brain when their level is low, which is associated with anxiety and depression. (Möhler, 2012) **Lipopolysaccharides (LPS)-** It is a cell wall component of Gram-negative bacteria. When bacteria die, they release it into the gut and promote inflammation inside the gut. This is high in people having colon cancer, Crohn's disease, insulin resistance, also high BP, T2DM, NAFLD and obesity its amount is in excess additionally, a high-fat diet contributes to this (Manco et al., 2010; Alena Pribyl, 2023).

- **Defensive function-** The human gut microbiota plays a crucial defensive role by preventing the colonisation and overgrowth of pathogenic organisms, such as *Candida* species and *Clostridioides difficile*. This defence is achieved through mechanisms like competitive exclusion, where bacteria occupy attachment sites and consume available nutrients, thereby limiting resources for potential pathogens. Additionally, resident microbes produce antimicrobial substances, including bacteriocins, which inhibit the growth of competing harmful microorganisms. These combined actions help maintain a balanced gut ecosystem and protect against infections (Ramirez et al., 2020).
- **Trophic functions-** Producing metabolites such as short-chain fatty acids (SCFAs), including butyrate, the microbiota supports the growth and differentiation of epithelial cells, which are essential for maintaining the integrity of the gut lining. Additionally, these metabolites stimulate intestinal motility and influence neuroendocrine pathways. Another function is the Development and regulation of the immune system. It helps in the maturation of lymphoid structures and the differentiation of T- and B-cells, which are vital components of the adaptive immune response (Ramirez et al., 2020). Gut microbiota communicates with the central nervous system through the gut-brain axis. This bidirectional signalling pathway involves various mechanisms, including neural, endocrine, immune, and metabolic routes. Through this axis, the gut microbiota can influence brain function and behaviour (Rusch et al., 2023).
- **16S rRNA:** The 16S rRNA (16S ribosomal RNA) is a vital component of the 30S small subunit of prokaryotic ribosomes, which play a central role in protein synthesis in bacteria (Janda & Abbott, 2007; Woese & Fox, 1977). Encoded by the 16S rRNA gene, this molecule is highly conserved among all bacteria and archaea, yet it contains specific hypervariable regions (V1–V9) that differ between species(Chakravorty et al., 2007; Johnson et al., 2019). This structural combination makes it an ideal molecular

marker for bacterial identification and taxonomic classification (Janda & Abbott, 2007; Johnson et al., 2019).

In the context of the gut microbiome, 16S rRNA gene sequencing is extensively used to identify bacterial species present in fecal samples, assess microbial diversity, and investigate the relationship between the gut microbiota and various clinical conditions such as inflammatory bowel disease (IBD), obesity, type 2 diabetes, depression, autism spectrum disorders (ASD), and neurodegenerative diseases, infertility and others (Gilbert et al., 2018; Johnson et al., 2019; J. Qin et al., 2010). Functionally, the 16S rRNA molecule helps maintain ribosomal structure by correctly positioning ribosomal proteins, facilitates the binding of messenger RNA (mRNA) and transfer RNA (tRNA) during translation. Clinically, 16S rRNA gene sequencing has become a powerful diagnostic and research tool in microbiology, especially for detecting bacterial pathogens in cases where traditional culture methods fail (Janda & Abbott, 2007; Johnson et al., 2019). The complex ecosystem of the human gut, which harbours hundreds of bacterial species, 16S rRNA gene sequencing offers a non-invasive, sensitive, and specific approach to investigate the gut microbiome.

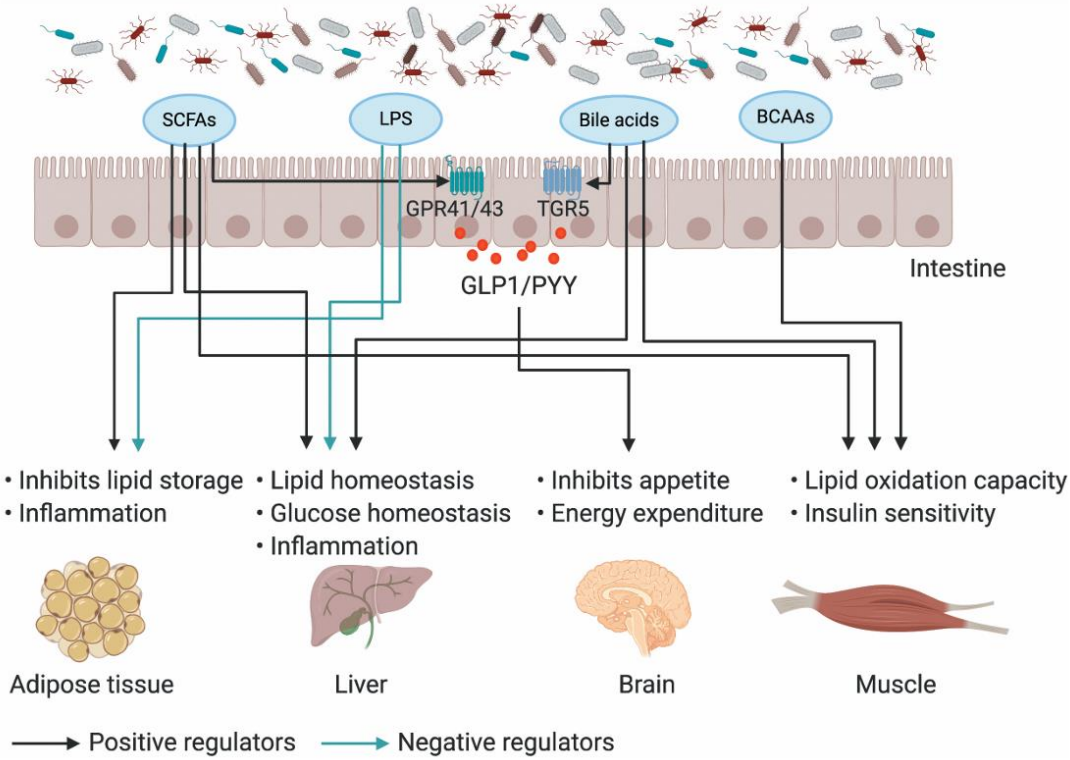


Figure 9: The impact of gut microbiota and its metabolites on organ (Adapted from Qi et al., 2021a).

Microbial metabolites:

The impact of gut microbiota and its metabolites on various organs has been shown in Figure 9.

Short-chain fatty acids SCFAs are small molecules (mainly acetate, propionate, and butyrate) produced by gut bacteria fermenting fibres in the large intestine. These SCFAs are absorbed by colon cells and serve as an energy source for both colon and liver cells. Beyond energy, SCFAs act as signalling molecules by binding to specific receptors (like FFA2, FFA3, GPR109a) found on immune, gut, and nervous system cells. This signalling supports communication between the gut, immune system, and brain (neuro-immuno-endocrine regulation). SCFAs promote gut health by strengthening the intestinal barrier, enhancing mucus production, reducing inflammation, and modulating immune cells. They also regulate

gene expression through epigenetic mechanisms (inhibiting histone deacetylase) (Rusch et al., 2023) Koh et al., 2016b).

Bile Acids (BAs) Bile acids are cholesterol-derived molecules made in the liver and chemically modified by gut bacteria. They help digest fats but also act as hormones, activating receptors like FXR and TGR5 found in many tissues, including the gut and brain. BAs regulate gut motility, barrier function, inflammation, and metabolism (lipid and glucose). They also influence the gut-brain axis through signalling pathways, including the production of hormones like GLP-1 (Rusch et al., 2023; Ridlon et al., 2016).

Branched-Chain Amino Acids (BCAAs) (leucine, isoleucine, valine) are essential amino acids involved in energy production, protein synthesis, insulin secretion, and immunity. The gut microbiota produces specific BCAAs that influence gut health. BCAAs activate the mTOR signalling pathway, a key regulator of cell growth. (Rusch et al., 2023)

MECHANISMS AND FACTORS CAUSE DYSBIOSIS

Dysbiosis of gut microbiota not only leads to changes in microbial composition but also disturbs its metabolites. These disturbances lead to affecting the body's normal functioning and become a contributing factor for disease onset and progression.

Mechanism of gut dysbiosis leads to the onset of disease, and progression occurs mainly in 3 stages is shown in Figure 10. How the dysbiosis leads to onset and progression of diseases is shown in Flow Chart (Figure 11)

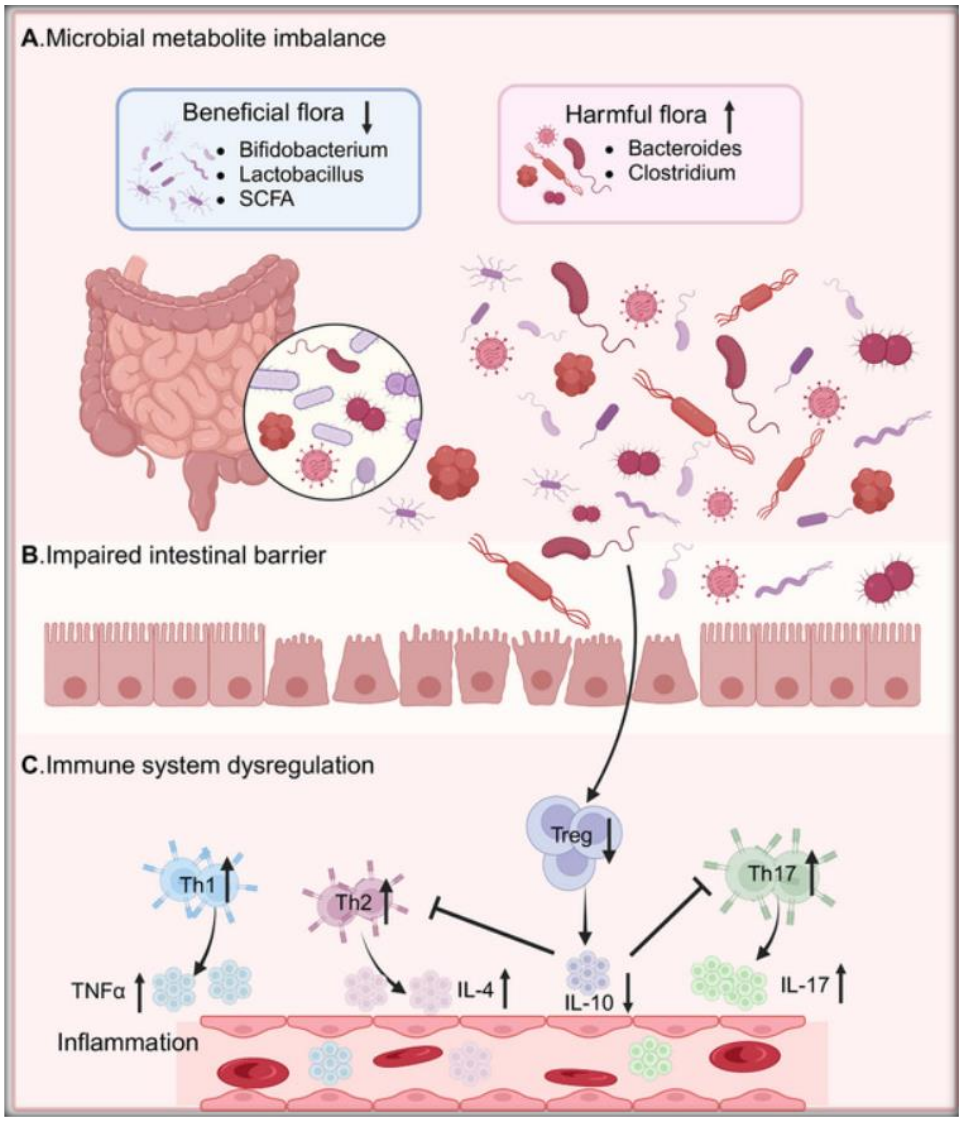


Figure 10: Gut Microbiome dysbiosis leads to the onset and progression of disease (Adapted from Shen et al., 2025)

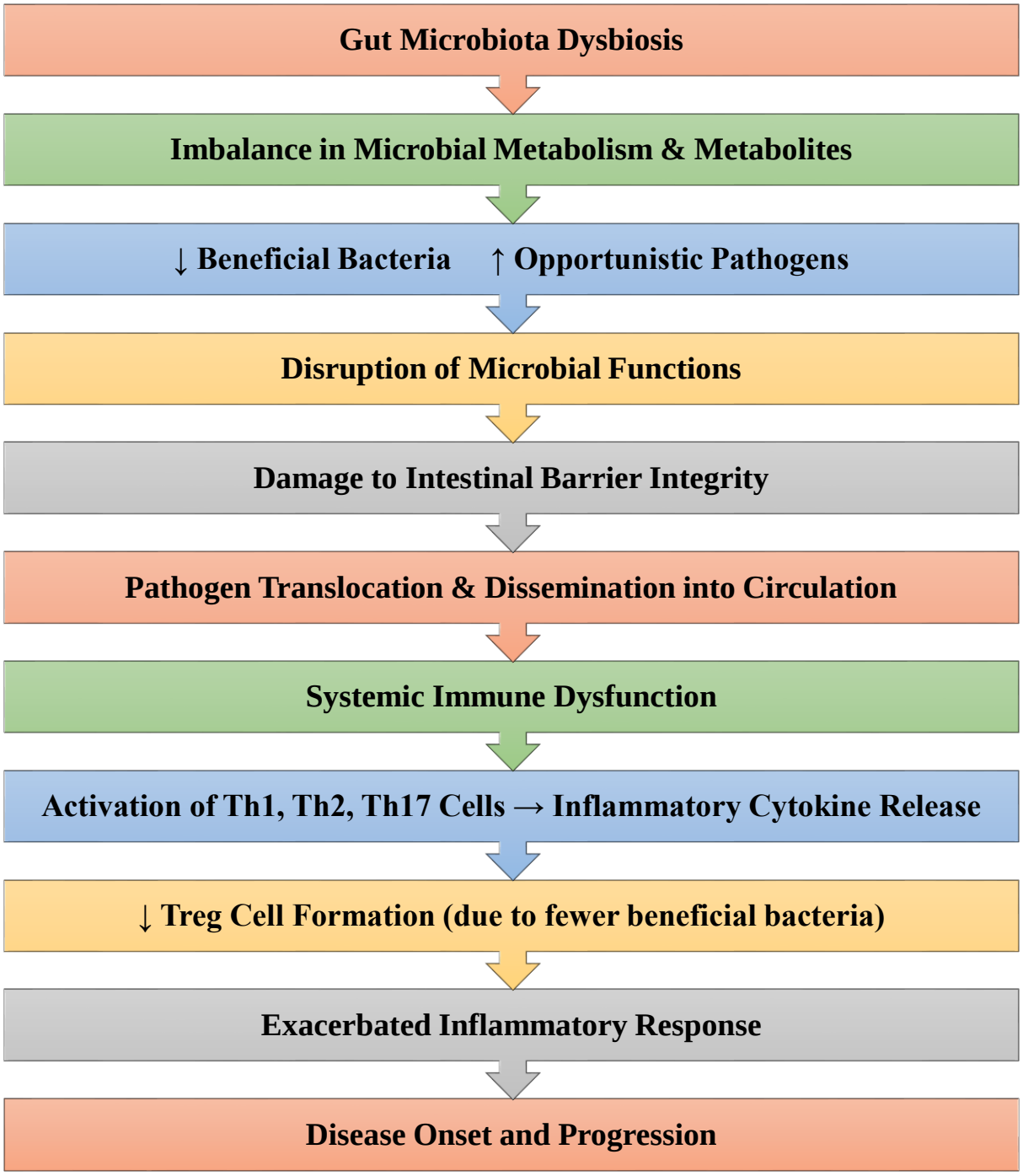


Figure 11: A Flow Chart shows how dysbiosis leads to the onset and progression of diseases.

1. **Microbial metabolite imbalance**- The microbial metabolite imbalance is shown in Figure 12. Due to a hot and humid environment, *Lactobacillus murinus* is reduced, and an increase in secondary bile acids, which increase brain inflammation and increase the chances of anxiety disorders. In the gut, various microbes are present, out of which the Genus *Akkermansia* is key

for the production of SCFA. Due to gut dysbiosis, there is a reduction in *Akkermansia* and SCFAS, which increases the chance of CVD disease and hypertension. If this dysregulation continues, then it will promote the onset of neurological disease. Reduction of butyrate and SCFA increases brain inflammation and inhibits microglial maturation, which contributes to the development of Alzheimer's disease. Additionally, a Deficiency of propionate reduces the survival of dopaminergic cells and the growth of neurites. Further progression of inflammation leads to Parkinson's disease. Tryptophan is one of the important molecules that comes through food and is processed in the gut, but when dysbiosis occurs, tryptophan activates the AHR (Aryl Hydrocarbon Receptor) signalling pathway. This activation leads to an increased chance of CVD and Chronic kidney disease. A by-product of tryptophan is Indolepropionic acid (IPA), which helps in immune system regulation in the kidneys and especially in hypertensive people. But when gut dysbiosis occurs, IPA is reduced, and an increase in the number of Th17 cells occurs, which causes inflammation. The number of T- T-regulatory cells (Treg) decreased, which reduces inflammation. This imbalance in the metabolites leads to Hypertension. Low levels of Vitamin D3 also contribute to this (Shen et al., 2025; Banfi et al., 2021; Loh et al., 2024).

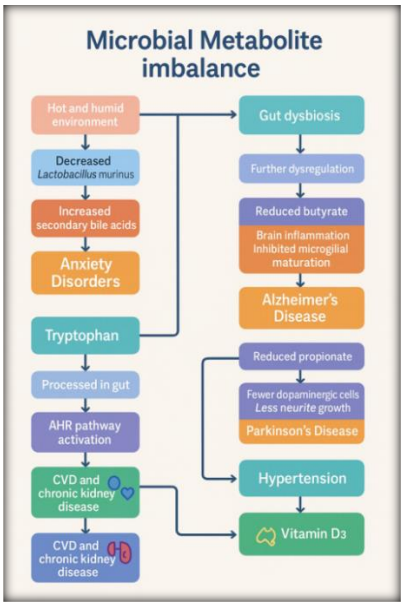


Figure 12: Flow chart showing Microbial metabolic imbalance

2. Impaired Intestinal Barrier Function: A healthy gut lining has a certain degree of permeability, which allows nutrients to pass and prevents harmful substances from passing through the gut lining and spreading throughout the body. When gut dysbiosis occurs, gut lining permeability increases, and this leads to "leaky gut" or intestinal permeability syndrome. Due to leaky gut, SCFAS decrease, which will increase the chances of irritable bowel diseases IBD and Crohn's disease. Further increase intestinal inflammation when it reaches to gut-brain axis, this will give rise to neurogenerative disorders like Parkinson's, multiple sclerosis, anxiety disorders. In addition to these inflammatory responses occur in mesenteric lymph nodes which will give rise to liver inflammation and further progression, increasing chances of liver fibrosis. Gut lining disruption is also associated with metabolic disorders like obesity, Type 2 diabetes mellitus and insulin resistance (Shen et al., 2025; Hrncir, 2022). The leaky gut and its effects have been depicted in Figure 13.

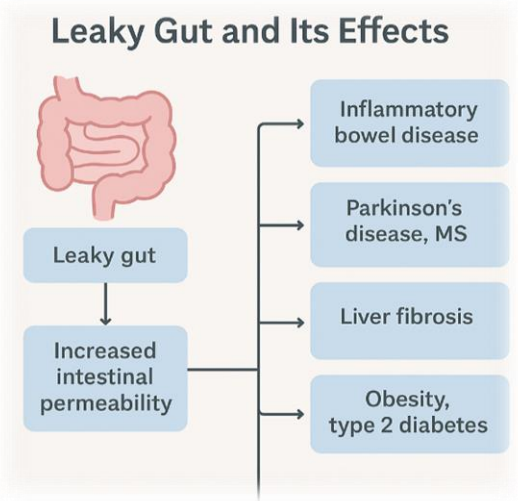


Figure 13: Impaired intestinal functions (adapted from Shen et al. 2025)

3. Immune System Dysregulation: The intestinal lining disruption due to dysbiosis affects the intestinal microenvironment's immune response. This allows harmful bacterial growth and increase chances of autoimmune diseases. When increase in segmented filamentous bacteria (SFB) trigger the elevation of Th17 cells and production of autoantibodies this will give rise to arthritis. Additionally, affected Th17/Treg balance due to *Cellulomonas flava* (CFB) causes

immune haemostasis disruption and contribute to various autoimmune responses. Microbial dysbiosis leads to bacterial growth that produce acetate and propionate in the gut environment, which impair neutrophil function s which later on leads to T1DM. Additionally chances of multiple sclerosis increased due to increase Th1/Th17 which will leads to increase inflammation in the brain and potentially contribute to MS development (Shen et al., 2025; Toor et al., 2019).

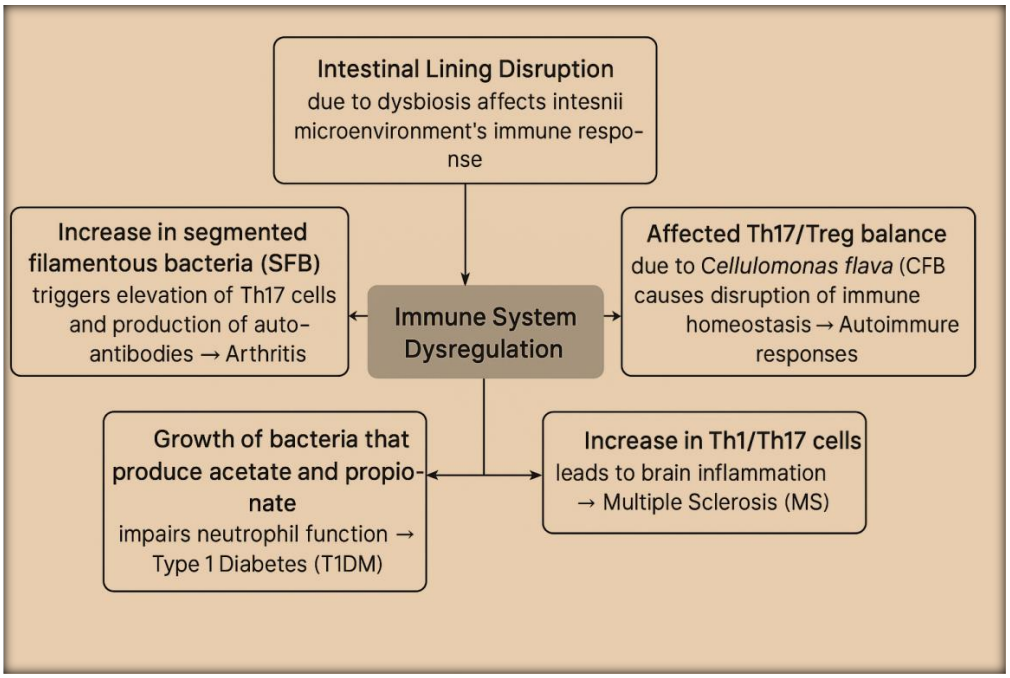


Figure 14: Immune system dysregulation and its effects (Adapted from Shen et al., 2025; Toor et al., 2019).

Factors causing Dysbiosis

There are various factors contributing to dysbiosis, such as diet, lifestyle, antibiotics, other medications, infections and inflammations, genes, Differences in Intestinal Flora Among Different Populations, mode of delivery, feeding methods and others (Figure 15).

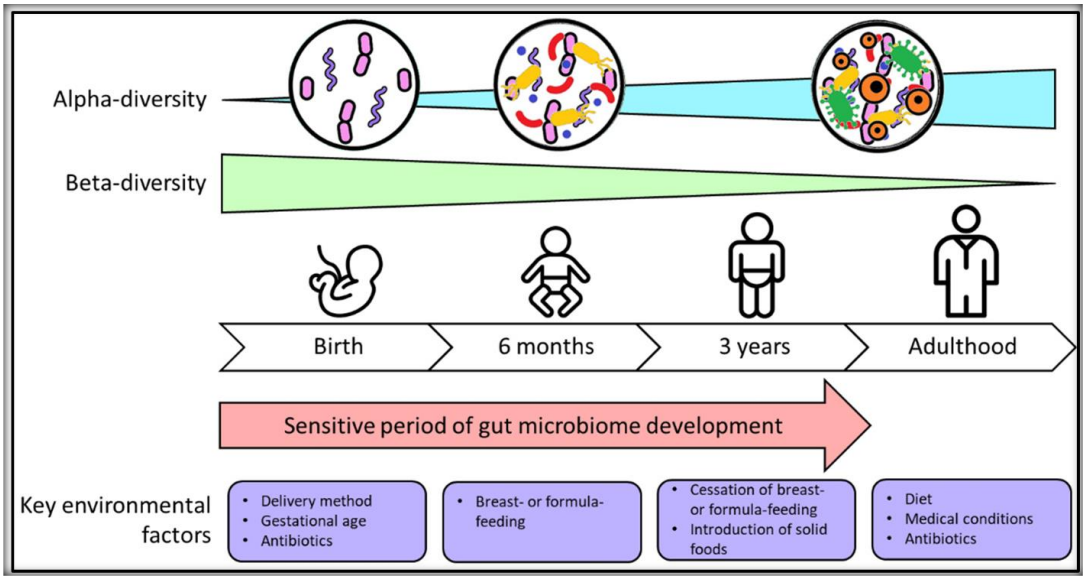


Figure 15: Factors causing dysbiosis from birth to adulthood (Adapted from (Parkin et al., 2021)

Table 4: Human gut microbiome composition throughout life in healthy conditions and its modifying factors (Adapted from (D'Argenio & Salvatore, 2015)**)**

Age	Microbiome composition	Modifying factors
Infant up to 2-3 years	Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes	Vaginal vs caesarean delivery − Gestational age − Infant hospitalization − Breast vs formula fed – Age at solid food introduction − Malnutrition − Antibiotic treatments
Adult	Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria	− Diet − Hormonal cycles − Travel − Therapies − Illness
Elderly above 70 years	Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria	− Lifestyle changes − Nutritional changes − Increased susceptibility to infections and inflammatory diseases − Use of more medications

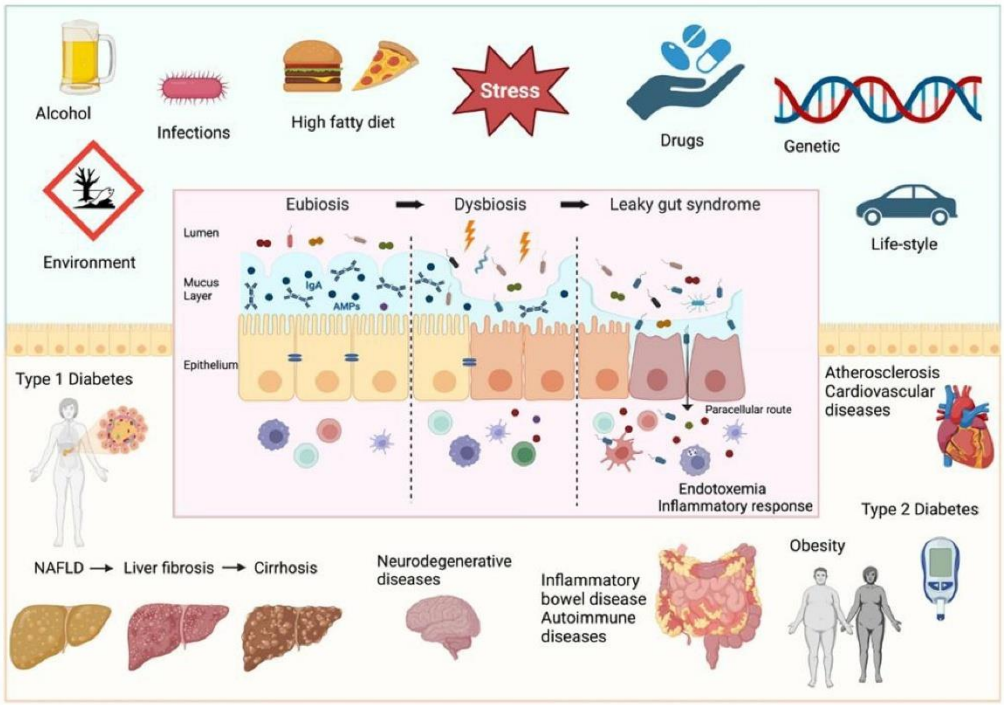


Figure 16: Factors determining intestinal barrier impairment and consequent systemic diseases (Adapted from Di Vincenzo et al., 2024)

1. **Mode of delivery- (vaginal or caesarean C-section)** first microbiome interaction starts from day of conception. Caesarean deliveries negatively impact the infant gut microbiome, alter the immune system, and reduce the diversity of microbes. Risk of various immune and allergic diseases are higher in them. During the first week after birth child having lower level of beneficial gut bacteria like *Bacteroides, Lactobacillus,* and *Bifidobacterium* and a higher level of skin associated microbes such as *staphylococcus, Streptococcus, and Propionibacterium.* After first week reduction in the ***Bifidobacterium*** and **increase** in bacteria like *Klebsiella*, *Haemophilus*, and *Villanella*. Till the time of end of first month mainly difference in vaginal and caesarean microbiomes fades though gut some of the variation in *Lactobacillus, Bacteroides,* and *Bifidobacterium* are there. At six-month microbes of both delivery mode looks similar however **C-section infants** have **higher levels** of *Clostridium* species. And *Bacteroides* and *Parabacteroides* remain **more abundant** in vaginally born infants.(Parkin et al., 2021; Stewart et al., 2017).

2. **Population differences-** Gut microbiome composition differs significantly across populations from the U.S., Europe, Africa, and Asia due to variations in diet, genetics, and environment. These regional and age-related differences impact microbiome diversity and are linked to chronic conditions like obesity and diabetes.(Porras et al., 2021; Shen et al., 2025).
3. **Exposure to pet or siblings-** This helps to protect children from developing allergies and more diverse gut microbes develop due to this exposure. Babies exposed to pets before and after birth had more of certain helpful gut bacteria (*Ruminococcus* and *Oscillopria*), which are linked to lower risks of obesity and allergies. (Parkin et al., 2021; Tun et al., 2017).
4. **Diet:** Changes in nutrition can influence the microbial composition. some food like meat, fish and fibre having long term effect on microbial composition. High calorie, high fat and low fibre diet increase the amount of proteus species. Food additive intake impairs glucose level in blood and induced the proteus proliferation. Obesity is linked with gut microbiome imbalance. Mediterrian food reduces the chances of unhealthy aging. A low fat and high fibre diet helps in the ulcerative colitis, increase number of bactericides and decrease inn actinobacteria in the UC patients' faeces also increase in Faecal bacterium prausnitzii and other microbes led to an anti-inflammatory shift in the microbiome it reduces inflammation marker in the patients' faeces and reduce dysbiosis (Shen et al., 2025; Simó & García-Cañas, 2020; Dasriya et al., 2024). (Figure 17).

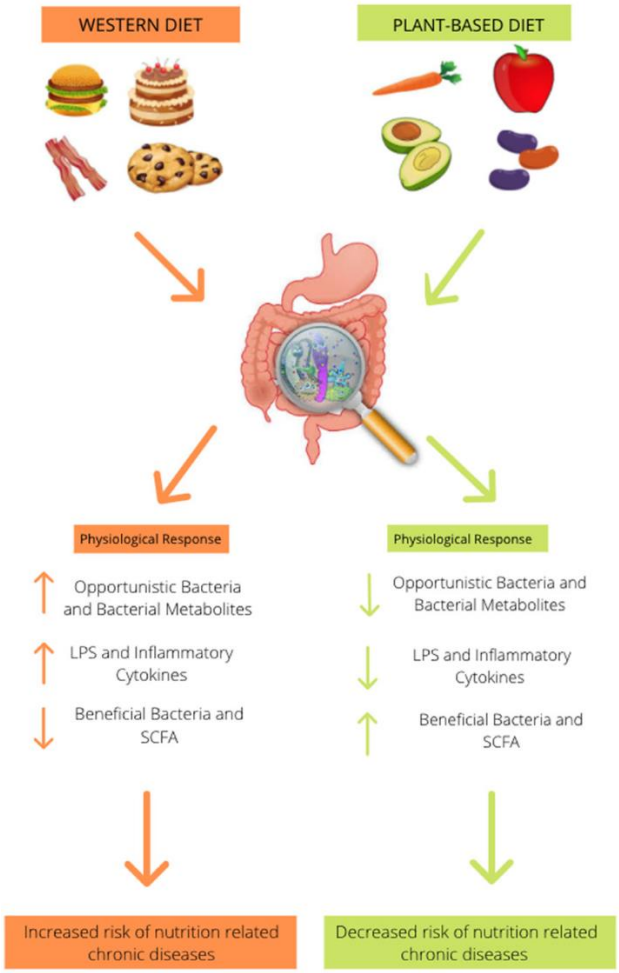


Figure 17: Dietary intake and its association with risk of nutrition related diseases (Adapted from Beam et al., 2021)

- As seen in Figure 18, Western diet rich in fat and processed carbohydrates but low in fibre, which disturbs gut microbiome balance by encouraging harmful bacteria such as bacteroides, shigella, Enterobacteriaceae, etc and beneficial bacteria such as Bacteroidetes, lactobacillus, enterococcus, etc. This imbalance decreases SCFA production, which is essential for gut health, while increasing toxic metabolites such as lipopolysaccharides and TMAO, as well as pro-inflammatory cytokines. These alterations increase the risk of chronic diseases. **Western Diet: ↑ Harmful bacteria + inflammation → ↑ Chronic disease risk** (Figure 18).

- A plant-based diet, which includes whole grains, fruits, and vegetables, reverses these impacts. It suppresses opportunistic infections, decreases LPS/TMAO levels, and reduces inflammation. It also enriches SCFA-producing bacteria, which improves metabolic health and reduce the risk of chronic diseases.

  Plant-Based Diet: ↑ Beneficial bacteria + SCFAs → ↓ Inflammation + disease risk. (Beam et al., 2021)

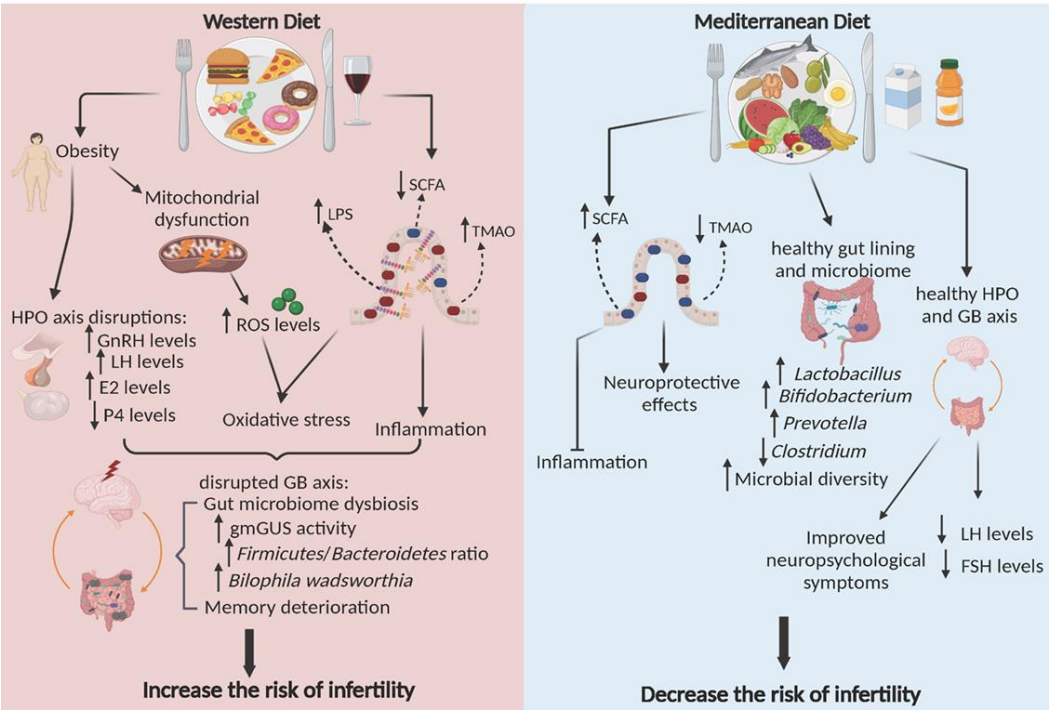


Figure 18: Effect of consumption of western and mediterranean diet on infertility (Adapted from Ahmad et al., 2025)

5. **Antibiotic use-** Antibiotics can disturb the gut microbiome by decreasing its diversity and encouraging the overgrowth of harmful bacteria such as *Proteobacteria*, leading to inflammation and greater antibiotic resistance (Shen et al., 2025). Antibiotics are prescribed mostly to fight against infections to infants as well as adults but their use is increasing the risk of various diseases. They are also linked with short term and long-term negative effects. In short term effects such as reduction in variety of microbes like *Bifidobacterium* increases the harmful bacteria vulnerability and risk of antibiotic-

resistant infections it is associated with *Clostridium difficile* infections—commonly after use of antibiotics, causing diarrhoea and colitis. In long term effects depends upon antibiotic class, route of administration, dose, duration, and spectrum of activity (Parkin et al., 2021). Long lasting effects on immunity, metabolism and hormonal functions and it takes longer time for gut to recover from antibiotic disruption and sometimes permanent changes can be seen. It also effects genetic expressions and other cellular processes.(Shen et al., 2025; (Francino, 2016; (Ubeda & Pamer, 2012).

6. **Lifestyle-** Lifestyle choices plays a major roles in gut microbiome and its effects on reproductive health specially by affecting systematic changes and inflammation control as shown in Figure 19 A shows Healthy lifestyle like regular physical activity and high fibre diets supports the gut environment by maintaining homeostasis, gut barrier integrity and increased production of metabolites (Figure 19B) shows unhealthy lifestyle and its negative effects habits like physical inactivity and high fat diet disrupts the gut microbiome and leads to dysbiosis (Xiao et al., 2024).

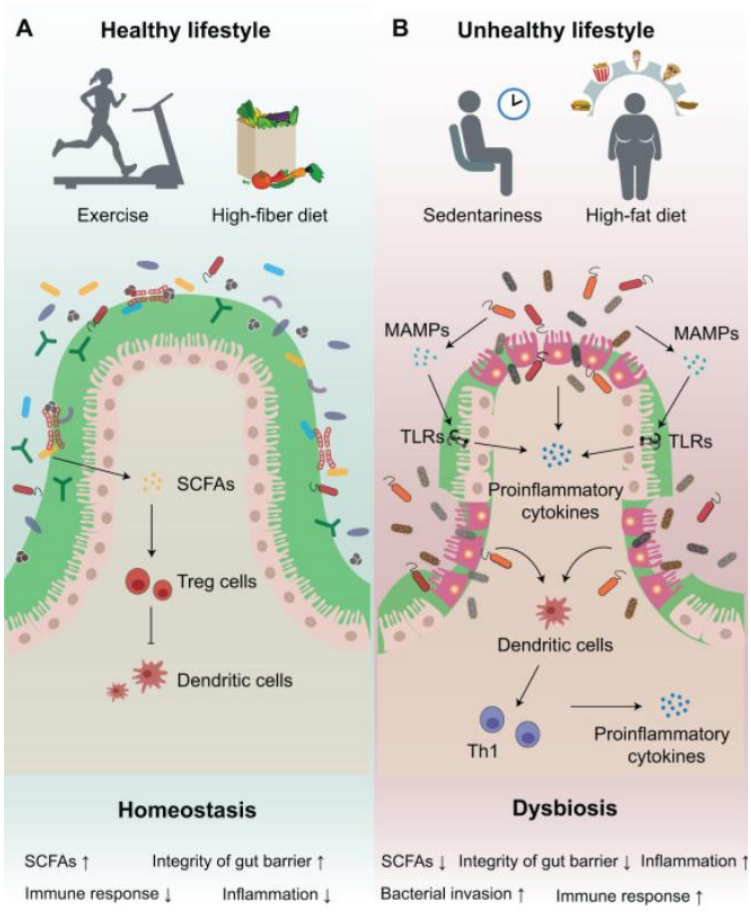


Figure 19: Lifestyle effects on gut microbiome (Adapted from (Xiao et al., 2024)

7. **Inflammation and infection-** Inflammation is a natural defence mechanism of the body against infections and injury. It increases intestinal permeability and contributes to microbial imbalance. Infections from viruses and bacteria trigger the inflammation and disturbance of beneficial microorganisms. During the state of haemostasis, gut microbiota maintains beneficial microbiomes to maintain the immune response. But during dysbiosis, this bacterial composition is disturbed, which is associated with various diseases such as IBD and MS. Intestinal immunity normally triggers inflammation to protect the body from harmful pathogens. But when T-helper (Th) cells become overactive, they can worsen intestinal inflammation. Infections from foreign microbes can disrupt the balance of gut bacteria, leading to an increase in harmful

bacteria and the release of toxins. This creates a proinflammatory environment and weakens the gut barrier (J. Wang et al., 2020; Shen et al., 2025).

8. **Host genetic-** Besides environmental factors, host genetic factors affect the gut composition, and some genetic variations make the host susceptible to dysbiosis. Various diseases linked with genetics, such as T2DM, IBD, and Neurological diseases, and various body alterations are also associated with this, such as BMI, BO, Antibiotic resistance, and microbial protein secretion. Specific genes, like *SLC22A5*, *GPR35*, and *GPR65*, have been associated with a higher risk of inflammatory bowel disease (IBD) and altered host–microbe interaction. In addition to this, in some cases, genetic changes may lead to disease and then alter the gut microbiome; in other cases, the genes might directly shift the microbiome, which then contributes to disease (Shen et al., 2025; Goodrich et al., 2014).

9. **Pollution-** Exposure to environmental pollution significantly alters the composition and function of the gut microbiome. Individuals living in highly polluted areas have gut microbiome compositions such as *Actinomycetota*, *Pseudomonadota*, and *Bifidobacterium*. At the same time, beneficial bacteria like *Faecalibacterium spp.*, known for their anti-inflammatory properties, are reduced due to pollution (De Filippis et al., 2024). The short-term and long-term exposure to particulate matter, pollutants like PM2.5, NO_2, SO_2, O_3, and CO changed the composition of the gut microbiome. Mainly air pollution (Kish et al., 2013) and mainly affects children, pregnant women, elderly, obese and schizophrenia (Filardo et al., 2022) And chronic exposure of PM 2.5 leads to dysbiosis and contribute in the development of abnormality in glucose metabolism (Wang et al., 2018). Additionally long exposure of particulate matter

increase risk of diabetes (Liu et al., 2019) (Figure 20).

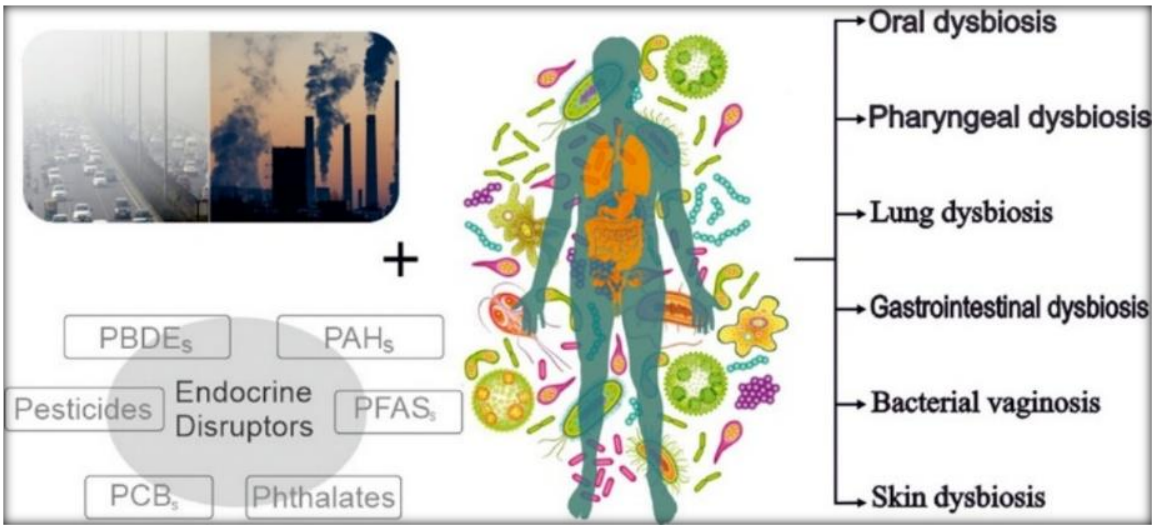


Figure 20: Combination of air Pollution + endocrine disruptors leads to Dysbiosis (Adapted from Mousavi et al., 2022)

CHAPTER 4

REPRODUCTIVE HEALTH & INFERTILITY

REPRODUCTIVE HEALTH AND INFERTILITY

Reproductive health, in the year 1995, 4th conference on women was held in Cairo, Egypt sponsored by United Nations, in which International conference on population and development (IPCD) adopted as definition which says "Reproductive health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system and its functions and processes" this concept includes various elements such as satisfying and safe sex life, capability to reproduce, freedom to decide when and how, Key components of reproductive health include access to family planning services, safe pregnancy and childbirth care, prevention and treatment of sexually transmitted infections (STIs), infertility services, promotion of healthy sexuality, and, where legal, safe abortion services. Reproductive health also involves access to education, adolescent health services, and the protection of reproductive rights such as bodily autonomy and informed decision-making. (Glasier et al., 2006; Report of the International Conference on Population and Development: Cairo, 5-13 September 1994, 1995) After technical consultation WHO in the year 2002 defines sexual health as a state of physical, emotional, mental, and social well-being about sexuality; it is not merely the absence of disease, dysfunction, or infirmity. It includes a positive and respectful approach to sexuality and relationships, the possibility of having pleasurable and safe sexual experiences, free from coercion, discrimination, and violence. Respect, protection, and satisfaction of sexual rights (Glasier et al., 2006) (Report of the International Conference on Population and Development : Cairo, 5-13 September 1994, 1995). The broader aspects of reproductive health cover family planning, maternal and newborn health, sexual health, prevention and treatment of reproductive tract infections, adolescent sexual and reproductive health, and the prevention and response to gender-based violence, including harmful practices like female genital mutilation (FGM).

For males, reproductive health involves fertility awareness, STI prevention, and conditions like male infertility, prostate disorders, erectile dysfunction, and testicular cancer. For females, it includes menstrual and hormonal health, maternal care, and conditions such as PCOS, endometriosis, uterine fibroids, cervical cancer, PID, and pregnancy-related complications. Shared concerns for both genders include HIV/AIDS, infertility, sexual dysfunction, and the psychosocial impacts of reproductive illness and rights violations. Reproductive health is also closely associated with gender equality, women's empowerment, and the elimination of violence and discrimination based on gender. Poor reproductive health also contributes significantly to the global burden of disease through maternal mortality, unsafe abortions, unintended pregnancies, and STIs. Therefore, ensuring universal access to comprehensive reproductive health services is essential for individual well-being, public health, and sustainable development (Glasier et al., 2006; Report of the International Conference on Population and Development: Cairo, 5-13 September 1994, 1995). Infertility is one component of reproductive health in which the desire to become a parent is a timeless and universal dream, shared by people across cultures and throughout history. It is a journey that brings immense joy and fulfilment, allowing individuals to experience the profound roles of motherhood and fatherhood. However, this journey begins with healthy fertility. Still, many people are facing issues or are sometimes unable to achieve this great happiness due to infertility or conditions that make conception difficult for couples. It is a persistent global reproductive health problem that is affecting mental, physical, and societal health. Infertility is demographically defined as a lack of live birth in couples who are together for at least 5 years and actively try to conceive without using a contraceptive but fail (Liang et al., 2025a). According to the WHO, infertility affects millions of people and has a significant impact on families and communities. Overall, approximately one out of every six individuals worldwide face infertility in their lifetime. It is a disease of reproductive system in which continuous

failure to achieve pregnancy despite of having regular unprotected sexual intercourse for more than 1 year, this can be happened in males and females, Infertility is mainly of two types primary and secondary, primary fertility is when person is unable to achieve pregnancy in their lifetime, secondary infertility is one first pregnancy happens in past but now unable to achieve pregnancy. Both types of infertility occur due to multiple causes, both unknown and known. Additionally, some of the causes are preventable, and treatment is available, while some of them are unpreventable. In males, infertility causes are sperm morphology changes, low sperm motility, low level of sperm production, low level of testosterone, ejaculation problems, duct blockage, testicular cancer, and others. In females, infertility causes are abnormality of ovaries, fallopian tubes, uterus, hormonal imbalances, STIS (sexually transmitted infections), endometriosis, fibroids, PCOS (polycystic ovarian syndrome), follicular disorders, pituitary cancer, hypopituitarism, postpartum sepsis, post-surgical complications, and others. Some of the factors also affect decreasing levels of fertility, such as eating habits, hydration level, smoking, alcohol consumption, obesity, stress level, and others. All these factors contribute to and increase the chances of infertility (WHO, 2024). In 37% of infertile couples, female infertility contributes 35%, and male and female common causes contribute 8%. The most common factors of female infertility are Ovulatory disorders account for 25%, Endometriosis, 15%, Pelvic adhesions, 12%, Tubal blockage, 11%, Other tubal/uterine abnormalities, 11%, and Hyperprolactinemia, 7% Walker; and Tobler., 2022). Whereas in males, common causes are hypogonadism 2-5%, vasectomy 5%, testicular defects 65-80%, and unknown 10-20%.(Stephen W. Leslie; Taylor L. Soon-Sutton; Moien AB Khan, 2024) Addressing infertility is an important aspect of sexual and reproductive health and rights, but this is neglected in the SRHR agenda. This leads to various issues like social stigma, gender-based violence, financial difficulties, effects on mental health, and others. Addressing infertility is an important aspect as it aligns with SDG-3, Ensure healthy lives and promote well-being for all

at all ages, and SDG 5 – Achieve gender equality and empower all women and girls to achieve Sustainable Development Goals by 2030. Every goal needed to be focused and achieved (Infertility Prevalence Estimates, n.d.-a). Human beings have a right to achieve the highest attainable standards of health (physical as well as mental), couples having freedom of family planning (number of children, timing, spacing between children, and others). After attaining legal age regardless of their gender, race, nationality, or religion, people have the right to marry and start their families. Infertility must be addressed as it is an integral part of these fundamental human rights (Shah & Gher, 2023). But females experience infertility earlier than males due to multiple causes. The primary cause is that females have a fixed number of oocytes since birth, and these oocytes quality and quantity rapidly decrease over time, starting at the age of 32 years and more rapidly declining after 37 years. Secondary causes include increased pregnancy complication with age, miscarriages, declining in reproductive health, exposures to environmental toxins, aneuploidy (germ cell- egg or sperm having incorrect number of chrosomes) increases risk 10 times after the age of 40 it contributes 65-75% of early pregnancy failure and almost 35% miscarriages (Amy Owen; Karen Carlson; Paul B. Sparzak., 2024). According to the WHO report, global infertility prevalence estimates from 1990 to 2021 suggest that lifetime prevalence contributes approximately 17.5% globally have experienced infertility. At the same time, period prevalence was estimated at 12.6%, for example, in Europe.

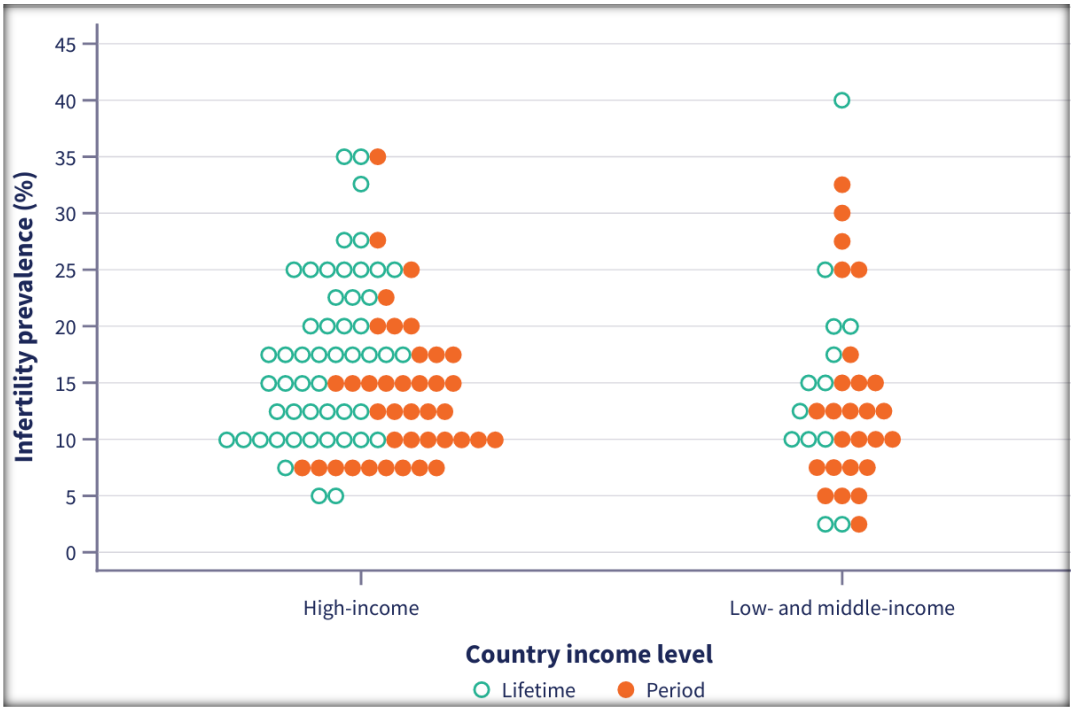


Figure 21: Lifetime and period infertility prevalence by country income level (Adapted from Infertility Prevalence Estimates, n.d.-b)

On the comparison of income levels, high-income countries have a greater lifetime prevalence than low- and middle-income countries. But period prevalence is equal. These estimates help to understand that there are disparities that exist according to regional differences and income levels of the countries. In addition to this estimated lifetime prevalence of infertility is highest in the Western Pacific regions and lowest in the Eastern Mediterranean region. But the Estimated period prevalence of infertility is lowest in eastern Mediterranean regions and highest in the African areas (Infertility Prevalence Estimates, n.d.-b) (Figure 21).

In the year 2021, 55 million males (1.8% of the male population) and 110 million females (3.7% of the female population) globally were living with infertility. Global prevalence of infertility among the reproductive age group from 1990-2021 was approximately twice as high in women as compared to men. The highest infertile age group was 35- 39 years. Age

specific prevalence rate of male infertility decreases with increasing in socio demographic index levels (SDI is calculated as geometric mean of three key components that reflect a population's level of development: income per capita, average educational attainment among individuals aged 15 years and older, and the total fertility rate under the age of 25). And this trend is similar for women. As the infertility burden increases, male infertility rates will be projected to grow more rapidly than female rates from 2022 to 2040.(Liang et al., 2025b).

According to IHME, the global burden of disease 2021 fertility infographic shows that by 2100, fertility levels are expected to fall below replacement levels (2.1) in 97% of the countries and territories. Additionally, a major increase in population will be expected in low-income regions, while in high-income regions, fertility rates will fall rapidly (Figure 22). The findings highlight the urgent need for policies that support family planning, promote gender equality, and help caregivers. These steps are important to tackle problems caused by falling birth rates and shrinking populations. Careful planning is needed to keep populations stable and societies strong in the future. (Highlights Most Countries Will Experience Below-Replacement * Levels of Fertility by Mid-Century, n.d.).

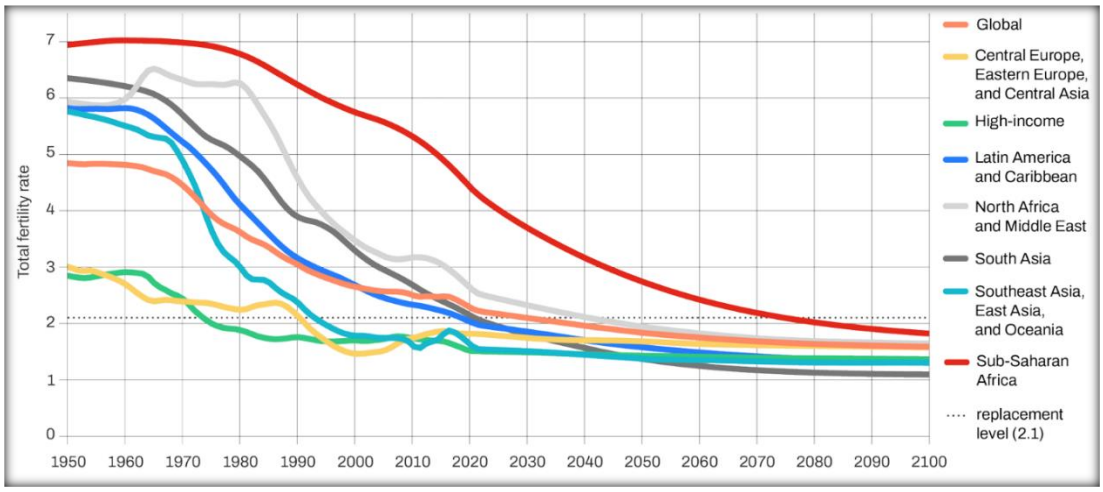


Figure 22: Total fertility rate, 1950–2100, by GBD super-region and for the globe (Adapted from(Highlights Most Countries Will Experience Below-Replacement * Levels of Fertility by Mid-Century, n.d.)

CHAPTER 5

GUT MICROBIOME Vs REPRODUCTIVE MICROBIOME

GUT MICROBIOME VS REPRODUCTIVE MICROBIOME

Gut and reproductive microbiomes have various similarities but there are various significant differences between them like their location, diversity, dominating phyla, functions , factors affecting them, gender differences, clinical relevance and implications, etc. gut having more diversity in comparison with reproductive microbiome gut is dominated by hundreds to thousands of species such as firmicutes, Bacteroidetes, actinobacteria and proteobacteria whereas reproductive tract is mainly dominated by mainly lactobacillus species. Gut microbiome performs variety of functions defensive, metabolic and immune additionally helps in digestion absorption, hormonal regulation and others. But reproductive microbiome performs local defensive and protection functions, maintenance of pH, sperm maturations and helps in conception and fertility. Other than lactobacillus some other bacteria are present such as Gardnerella, prevotella, streptococcus and pseudomonas. Gut id dominated by genera such as clostridium, bacteriodes, prevotella, akkermansia, ruminococcus, bifidobacterim and others. There are various factors whuch inluences gut microbiome such as diet, age, genetics, environment, antibiotic useage and others, whereas reproductive microbiome influenecs by pH, hormones, genetics, antibiotic use, sexual activity, pregnancy, menstrual cycle and others. Reproductive microbiome is not remained stable they are dynamic and varies with reproductive cycle, and [pregnancy in females and in males by sexual activity, infections and inflammation. But gut microbiome are remains stable until dysbiosis occurs. Gut microbiomes are remains similar in both sexes but reproductive microbiome is having sexual differences male are having testicular microbiome, females having lactobacillus more. If we talk about dysbiosis gut dysbiosis linked with various metabolic diseases such as diabetes, obesity, cancers, etc. It also affects fertility by manipulating hormonal regulation and impact on fertility. But reproductive microbiome dysbiosis is directly linked with various diseases such as PCOS, endometriosis, prostitis, preterm birth, impair sperm morphology and functions and others. Gut can influence

the reproductive tract by gut-reproductive axis. Additionally, gut is having higher diversity, mainly roles in systematic metabolism but reproductive mainly roles in defensive and locally, depends mainly on hormones. Major taxa differences in them is Lactobacillus I dominated in reproductive tract, Akkermansia, Bacteiocidales, and clostridiales are abundant present in gut (Pahirah et al., 2024; Shterzer et al., 2024; Valdés-Bango et al., 2024).

CHAPTER 6

GUT MICROBIOME RELATIONS WITH INFERTILITY

GUT MICROBIOME RELATIONS WITH INFERTILITY

A healthy gut microbiome is essential for maintaining hormonal balance, which is key for successful conception. The gut microbiota interacts with the body's endocrine system, helping to regulate the production and metabolism of hormones such as oestrogen and progesterone. Imbalances in these hormones can disrupt the menstrual cycle and make it difficult to conceive. The gut microbiome also plays a role in nutrient absorption. Without proper nutrient absorption, the body may lack the essential vitamins and minerals needed for reproductive health. This can impact the quality of eggs and sperm, making it more challenging to conceive. To nurture gut health and enhance fertility, it's important to focus on nourishing the gut microbiome. This can be achieved through a gut-friendly diet that includes specific nutrients and beneficial bacteria. The intricate relationship between the gut and hormone balance is fascinating. The gut microbiota produces enzymes that metabolise hormones in the body, ensuring that hormone levels remain balanced. When the gut microbiome is disrupted, this delicate balance is disturbed, leading to hormonal imbalances. High levels of particular bacteria in the gut can cause an increase in the production of enzymes called β-glucuronidases. These enzymes can interfere with the metabolism of oestrogen, leading to increased oestrogen recycling and an excess of this hormone in the body. Elevated levels of oestrogen can disrupt the menstrual cycle and make it more challenging to conceive. Our microbiome also plays a role in the balance of androgens, particularly in PCOS. Restoring microbiome balance has been shown to reduce circulating androgens, helping to maintain a balanced hormonal environment. Nurturing gut health can support the proper metabolism and balance of hormones for better periods, healthier sperm, and conception. The Impact of Inflammation on Fertility. Inflammation is a natural response by the body to injury or infection. However, chronic inflammation can have detrimental effects on fertility. When the gut microbiome is imbalanced, it can lead to increased intestinal permeability, commonly known as "leaky gut."

This can allow toxins and harmful bacteria to enter the bloodstream, triggering an inflammatory response in the body. Chronic inflammation can interfere with the delicate balance of hormones and disrupt the reproductive system. It can also promote conditions such as polycystic ovary syndrome (PCOS) and endometriosis, which are known to affect fertility. To reduce inflammation and support fertility, focus on whole, nutrient-dense foods that are rich in antioxidants and anti-inflammatory compounds. (The Gut-Fertility Connection: Expert Insights on How to Eat for Succes-Fertile Gut Https://Www.Fertile-Gut.Com/Blogs/News/the-Gut-Fertility-Connection-Expert-Insights-on-How-to-Eat-for-Successful-Conception?Srsltid=AfmBOorUcu3… The Gut-Fertility Connection: Expert Insights on How to Eat for Successful Conception, n.d.)

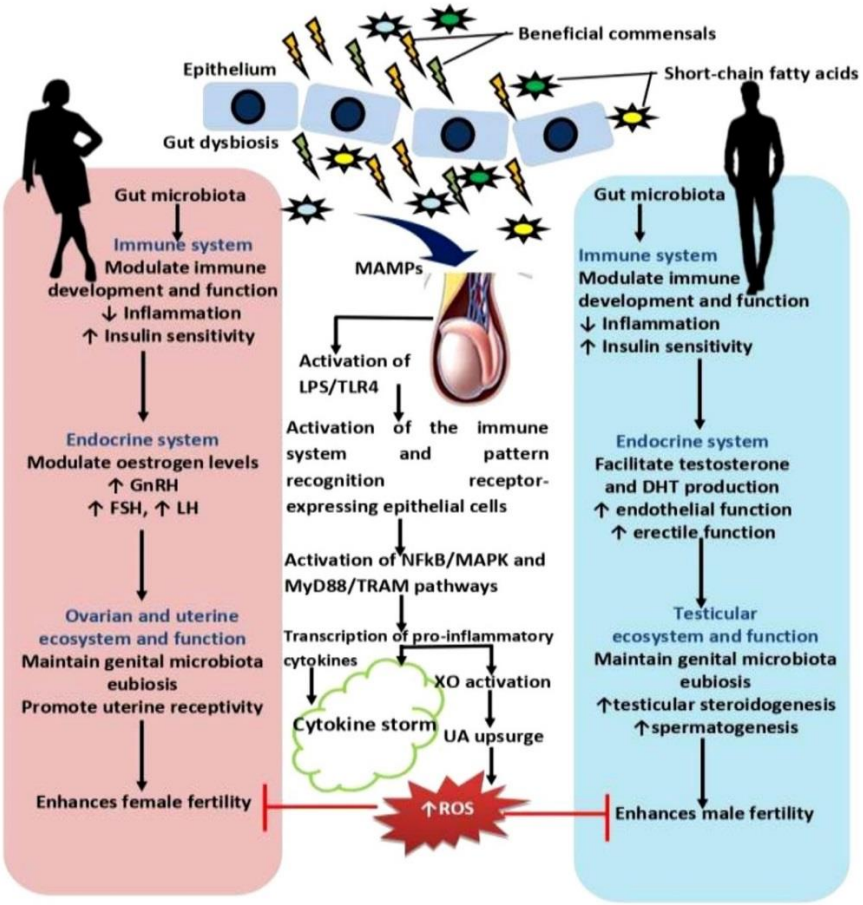


Figure 23: Effect of gut microbiome and dysbiosis on male and female reproductive function (Adapted from (Ashonibare et al., 2024).

As shown in the Figure 23, a Healthy gut supports fertility by

A. Immune system regulation by helping the development and regulation of innate and acquired immune responses that will control inflammation.
B. Insulin sensitivity improvement which will helps in maintenance of balance between oxidant and antioxidants
C. Activation of hormonal axis this supports the functioning of HPG axis and helps in hormonal balance s, hormones such as GnRH, FSH, LH.
D. Steroidogenesis in female leads to healthy oestrogen production and in males it ensures testosterone and DHT levels and manage sperm production.

All these helps in improvement of reproductive function in male and female and enhances fertility. But when dysbiosis happens gut intestinal lining weakens and harmful substances like MAMPs (Microbial associated molecular patterns) – such as LPS, peptidoglycans and lipoproteins leaks into the bloodstream by lymphatic cystic system and hepatic portal vein, this leakage of MAMPs activate the immune system and generate a hyperimmune response in ovaries and testis by various pathways, these pathways in response triggers inflammation and tissue damage in testis and ovaries, This leads to activation of an enzyme named as Xanthine oxidase, this is responsible for uric acid production and leads to oxidative stress which will impair fertility by injuring ovaries and testis (Ashonibare et al., 2024). The researchers also identified 6 bacterial taxa that may be involved in male infertility. Among these 5, including the Bacteroides, Enterobacteriales may have a protective role against male infertility; instead, Allisonella was associated with a high risk of infertility in men. The team also identified 11 bacterial taxa that may be involved in female infertility Faecal bacterium was associated with a high risk of infertility in women, whereas the other 10, including the Ruminococcin, Desulfovibrio, Bifidobacterial have a protective role against female infertility. (Li et al., 2023). Sexual activity also alters the male and female genital microbiomes, bacterial transfer during

intercourse from vaginal microbiome to male partners leads to inflammation in males, additionally couples having positive bacterial cultures having lower pregnancy rates and higher chances to miscarriages during IVF. (Magill & MacDonald, 2023) To identify the relationship between the microbiome and infertility, various study was conducted based on 16S rRNA by using different sample methods mentioned in table 5.

Table 5: 16S rRNA technique used to identify the relationship in gut microbiome and reproductive microbiomes dysbiosis leads to changes in fertility

SAMPLE/ METHOD	MAIN FINDINGS	MICROBIAL CHANGES	KEY MICROBES	REFERENCES
36 women total: 18 infertile and 18 age-matched fertile controls. Faecal samples analysed via 16S rRNA (V3–V4 regions) sequencing,12 infertile women received ART + PHGG Intervention	Gut microbiota composition was significantly different in infertile women. Higher abundance of Verrucomicrobia,58.3% pregnancy rate (7/12) with PHGG + ART	Infertile women had altered β diversity, PHGG may help restore microbial balance, Positive trends in microbial profile linked to pregnancy success	Increase in Infertile Women: Verrucomicrobia, Phascolarctobacterium, unclassified Barnesiellaceae Decreased: Roseburia, Streptococcus, Stenotrophomonas Associated with Pregnancy Success: Higher Bifidobacterium, Lower Paraprevotella & Blautia	(Komiya et al., 2020)
Bidirectional two-sample Mendelian Randomization (MR) analysis, GWAS data from MiBioGen consortium (gut microbiota), GWAS data on male infertility and abnormal spermatozoa, Methods used: IVW, MR-Egger, weighted median, Steiger filtering	Causal links found between specific gut microbes and male infertility and abnormal spermatozoa, no evidence of pleiotropy, indicating robust causal inference	Increased risk: *Eubacterium oxidoreducens* group (infertility), *Streptococcaceae* family (abnormal spermatozoa) - Decreased risk: *Bacteroidaceae*, *Porphyromonadaceae* families	Risk Factors: *Eubacterium oxidoreducens* group, *Streptococcaceae* family - Protective Factors: *Bacteroidaceae*, *Porphyromonadaceae* families	(Han et al., 2024)

Two-sample Mendelian Randomization (MR) study, Data from MiBioGen consortium (18,340 individuals), Methods: IVW, MR-Egger, MR-RAPS, MR-PRESSO	Certain gut microbes are causally linked to infertility, some microbes show protective effects while others increase infertility risk	Protective: Family XIII AD3011 group, Ruminococcaceae NK4A214 group, Bacteroidaceae, Bacteroides -Risk factors: Betaproteobacteria, Burkholderiales, etc.	Protective: - Ruminococcaceae NK4A214 group - Bacteroidaceae - Bacteroides - Family XIII AD3011 group Risk-associated: - Betaproteobacteria - Burkholderiales - Candidatus Soleaferrea - Lentisphaerae - Anaerotruncus	(Zhang et al., 2023)
- Animal Study: Male mice fed high-fat diet (HFD) or normal diet (ND) - FMT: Transplantation of gut microbes from HFD-fed mice to ND-fed mice - Assessments: Sperm analysis, RNA sequencing of testes, cytokine profiling, and inflammation markers - Human Study: Stool, blood, and semen analysis from human subjects	- HFD-induced gut dysbiosis caused reduced sperm count and motility - Increased inflammatory responses in the epididymis - Downregulation of genes involved in spermatogenesis and mitochondrial function - In humans, poor sperm motility was associated with higher levels of gut-derived endotoxins	- Increased abundance of pro-inflammatory gut bacteria - Shift in microbial composition leading to systemic inflammation and testicular dysfunction	*Bacteroides* - *Prevotella* - Notably, *Prevotella copri* linked to poor sperm quality and increased endotoxemia	(Ding et al., 2020a)
36 infertile couples undergoing IVF Samples: Seminal fluid and vaginal swabs, Technique: 16S rRNA gene sequencing (V4 region, Illumina MiSeq)	Semen: Lower bacterial concentration, higher diversity - Azoospermic men had more *Mycoplasma* and *Ureaplasma* - *Lactobacillus*	Semen (Positive IVF): ↑ *L. jensenii*, *Faecalibacterium* Semen (Negative IVF): ↑ *Proteobacteria*, *Prevotella*, *Bacteroides*	Positive IVF: *Lactobacillus jensenii*, *Faecalibacterium*, *Lactobacillus gasseri* Negative IVF: *Proteobacteria*, *Prevotella*, *Bacteroides*, *L. iners*	(Okwelogu et al., 2021)

	dominant in both sexes	Vagina (Positive IVF): ↑ *L. gasseri* Vagina (Negative IVF): ↑ *L. iners*, *Bacteroides*		
Semen 16S RNA sequencing pooled (89 mens with infertility conditions and 29 healthy controls)	Seminal viscosity associated with distinct microbiota, OAT cases show increased pathogens and reduced beneficial bacteria, infertile men's having reduced number of microbial diversity and increased inflammation risk	Increased proteobacteria, gram negative bacteria and pathogens Decreased lactobacillus and diversity	Increased pseudomonas klebsiella, Neisseria and prevotella Decreased lactobacillus	(Monteiro et al., 2018)
Human clinical study of infertile men and fertile controls - Biological samples: gut (stool), urine, and semen - Microbial analysis using 16S rRNA gene sequencing and shotgun metagenomics	Infertile men exhibited significant dysbiosis across all three microbiomes - Functional changes linked to oxidative stress, immune disruption, and poor sperm quality - Strong associations with reduced sperm motility, concentration, and increased DNA fragmentation	Loss of beneficial commensals and overrepresentation of pathogenic/pro-inflammatory microbes - Altered pathways: LPS biosynthesis, bacterial secretion, oxidative phosphorylation	Decreased: *Lactobacillus*, *Bifidobacterium*, *Faecal bacterium* - Increased: *Prevotella*, *Escherichia-Shigella*, *Gardnerella*, and altered Firmicutes/Bacteroidetes ratio	(Lundy et al., 2021)

As we are aware of the fact that the gut microbiome has the largest composition of microbes in the body, and it also influences the hormonal regulations in the body, 16S rRNA sequencing techniques used to figure out the relation between gut microbiome and infertility there are few evidences in the table that shows a change in gut microbiome composition or gut dysbiosis leads to infertility.

CHAPTER 7

GUT MICROBIOME RELATIONS WITH UNDERLYING FEMALE REPRODUCTIVE DISORDERS

GUT MICROBIOME RELATIONS WITH UNDERLYING FEMALE REPRODUCTIVE DISORDERS

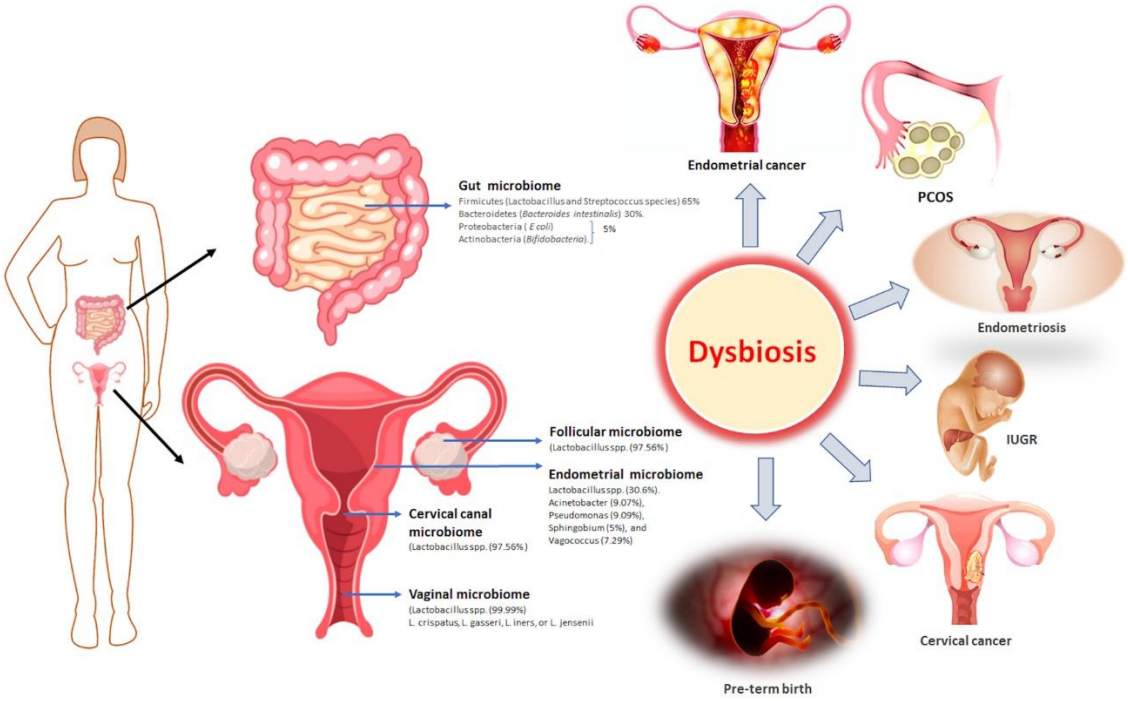


Figure 24: Reproductive microbiome composition and associated diseases (adapted from (Chadchan et al., 2022a)

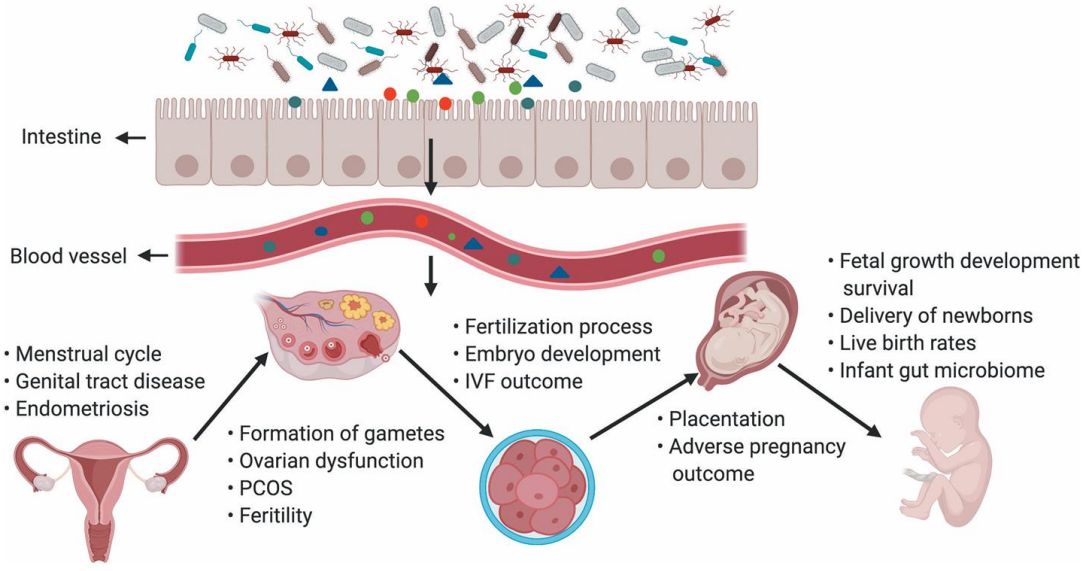


Figure 25: The gut microbiota and its impact on the female reproductive tract, embryo development, and pregnancy. (Adapted from Qi et al., 2021a)

The reproductive microbiome in females' composition in the Figure 24 shows that follicular microbiome (*Lactobacillus* 97.56%), endometrial microbiome (*Lactobacillus* 30.6%), cervical

canal microbiome (Lactobacillus 97.56%) and vaginal microbiome (Lactobacillus 99.99%) their dysbiosis leads to various disease like PCOS, endometrial cancer, endometriosis, cervical cancer, IUGR and pre term birth. As Figure 24 shows gut microbiome impact on tract, embryo development and pregnancy, Gut microbiota products transported through the circulation and influence the female reproductive tract (e.g., may cause disruptions in the menstrual cycle, genital tract disease, and endometriosis), ovarian function, embryonic development, and the health of the mother and foetus. The gut can affect the formation of gametes, embryo development, and fertilisation processes, and alteration of intestinal flora can lead to ovarian dysfunction, PCOS, infertility, and adverse IVF outcomes. In pregnant women, the gut microbiota affects placentation, pregnancy outcomes, the delivery of newborn babies, the infant microbiome, the live birth rate, and foetal growth, development, and survival. This all relation of gut dysbiosis with infertility and female reproductive tract disorders will be explained with evidence in the table 6. Before that it is important to understand the gut microbiome and hormones relations and how they affect each other.

Oestrogen and gut microbiome—Estrobolome is a microbial gene that metabolises oestrogen. With the help of β-glucuronidase and β-glucosidase (Kumari et al., 2024) As seen in Figure 25. The gut microbiome secretes β-glucuronidase, which converts conjugated oestrogen into its active, free form, and enables the reabsorption. This deconjugated oestrogen is reabsorbed into the bloodstream and delivered to the uterus, where it regulates uterine functions & facilitates binding to oestrogen receptors (Chadchan et al., 2022a) Facilitation binding to oestrogen receptors as seen in figure 22 after reabsorption of oestrogen related substances in the blood stream then it travels to other distinct areas like vaginal lining where these substances binds to oestrogen receptors and activated, they triggers and activated various processes in the body like Change in DNA expression, cell communication and genetic activities. And when dysbiosis occurs this whole process gets disturbed and leads to reduced

oestrogen activities in the body. A high level of microbial diversity enhances the anabolic activity and increases oestrogen levels in the body (Baker et al., 2017). Oestrogens help in the formation of gut microbiome by promoting SCFA-producing bacteria such as Lactobacillus, *Ruminococcaceae*, upregulate intestinal alkaline phosphatases (IAP), preventing chronic inflammation, and oestrogens modulate *oestrogen receptor β (Erβ)* activity, which maintains microbial diversity and suppresses pathogens like *Prevotellaceae.* (Wu et al., 2024) due to dysbiosis.

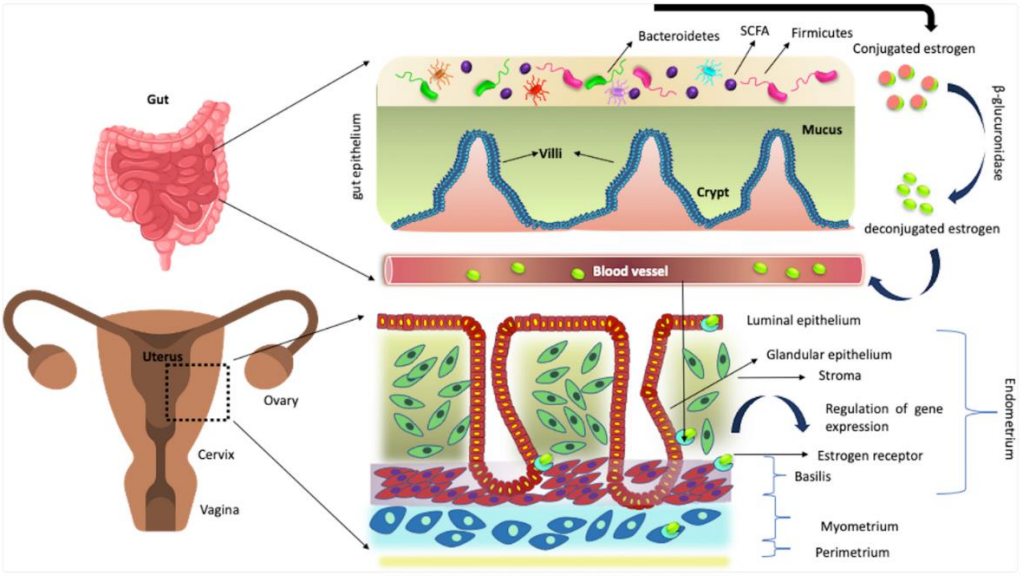


Figure 26: Gut microbiome secretes β-glucuronidase, converting conjugated oestrogen and its impact on uterine function when absorbed in blood stream (adapted from Chadchan et al., 2022a).

These activities and the level of oestrogen in the body get disturbed. In hypoestrogenic states, active oestrogen levels are reduced, contributing to the development of obesity, metabolic syndromes, insulin resistance, cognitive decline, cancers, and postmenopausal conditions like osteoporosis and increased abdominal fat. In hypoestrogenic state, oestrogen level increases which contributes in the development of Endometriosis, breast cancer, and endometrial

hyperplasia (Baker et al., 2017). Additionally in post-menopausal women's oestrogen deficiency leads to non-alcoholic fatty liver disease (Chadchan et al., 2022a).

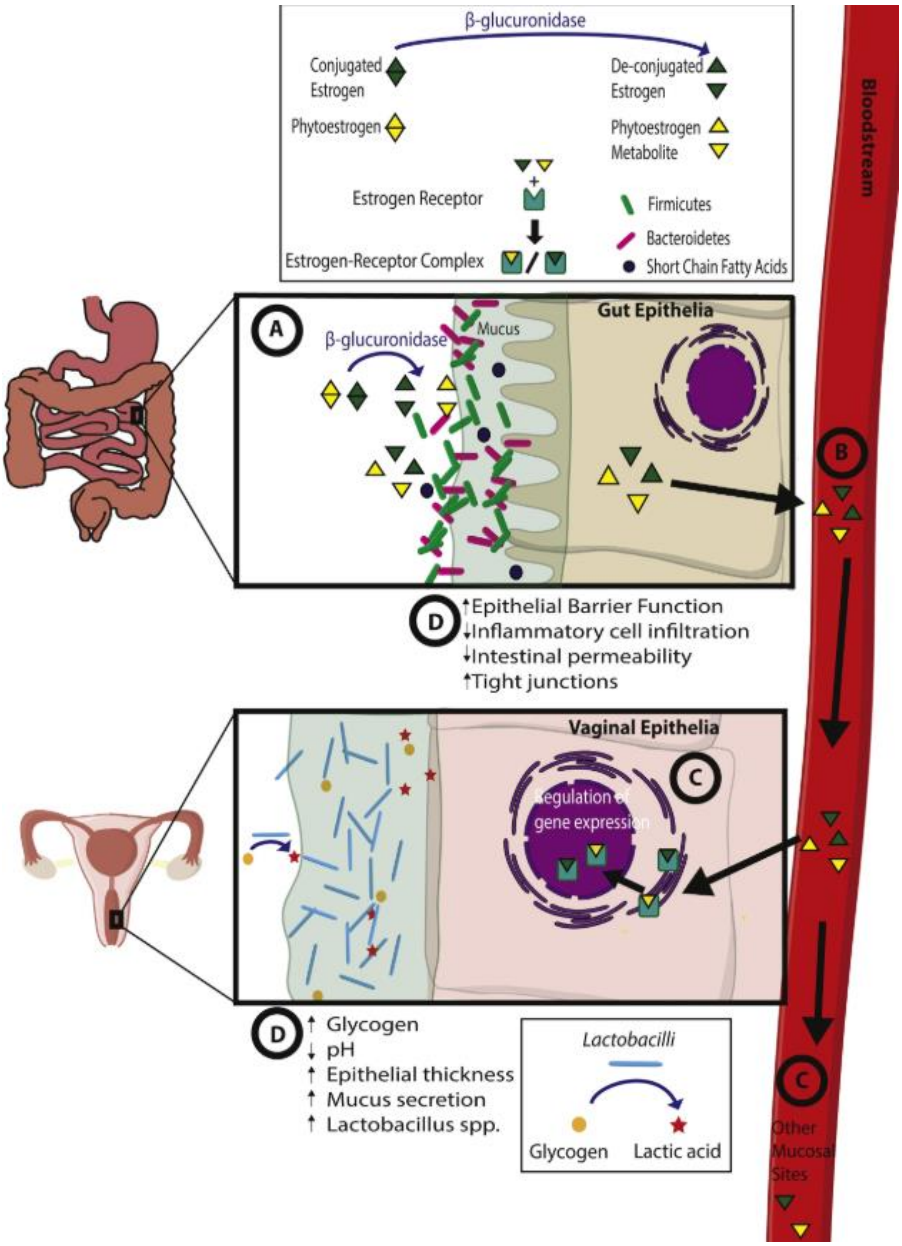


Figure 27: Gut microbiome and oestrogen: Regulation and maintenance of systemic and reproductive health (Adapted from (Baker et al., 2017).

Progesterone and gut microbiome: Progesterone is an essential hormone of the female reproductive organs. It is essential for fertility and pregnancy. It performs various functions such as preparing the endometrium for conception, supporting early pregnancy, preventing

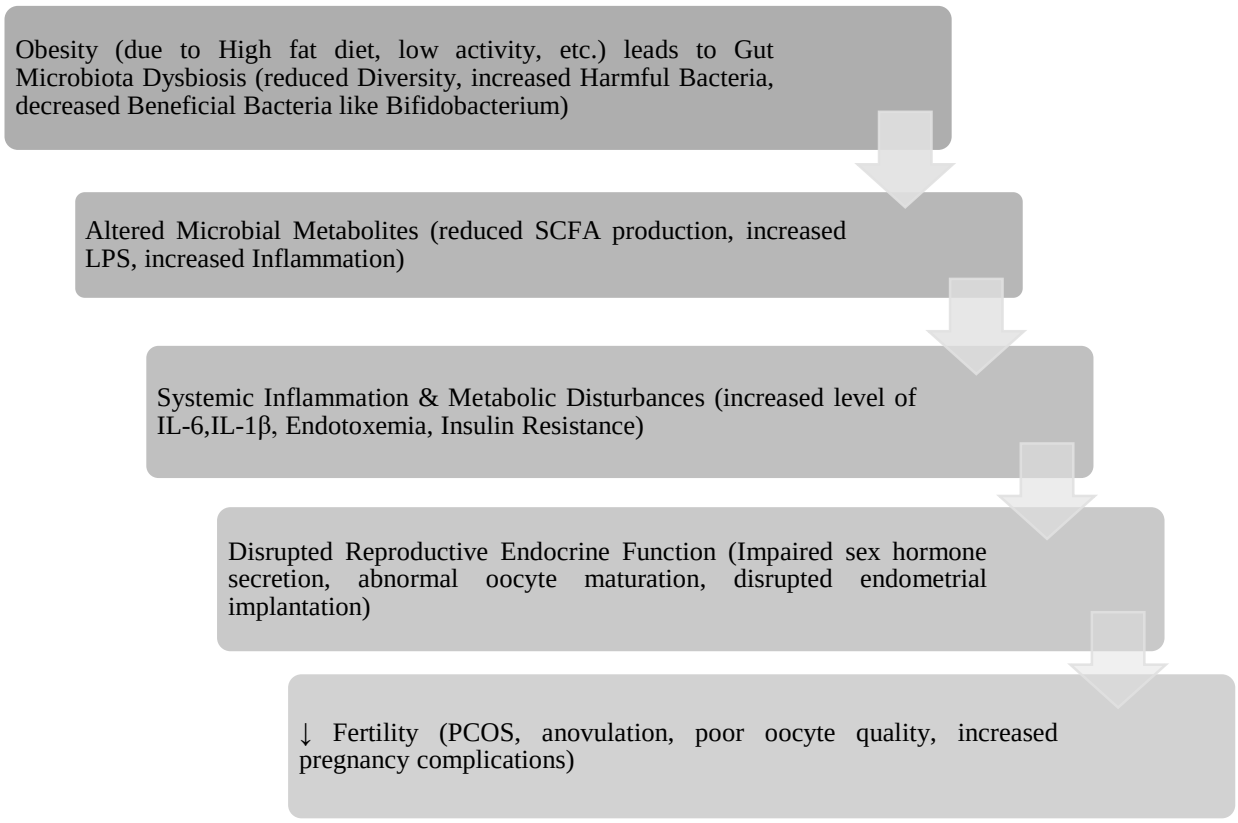
uterine contractions during pregnancy to prevent preterm labour, and others. Gut microbiome produces SCFAs like butyrate, propionate, and acetate through dietary fibre fermentation. Butyrate regulates progesterone and oestradiol secretion in porcine granulosa cells via the cAMP pathway (Lu et al., 2017). Low concentration of butyrate stimulates progesterone secretions, but when the concentration is high, it inhibits progesterone secretions and disrupts normal cellular functioning. For optimal functioning, it is essential that balance is required. (Chadchan et al., 2022a). Due to gut dysbiosis, SCFAs production is hampered, which affects progesterone levels in the body. This creates a negative impact on infertility, disturbed level of progesterone secretion is also seen in various conditions such as PCOS and endometriosis. (Qi et al., 2021b)

Androgen and gut microbiome- Hyperandrogenaemia (excess of androgens), it's like testosterone, and this is closely linked with changes in gut microbes' composition and variety. Gut flora and hyperandrogenaemia are in a complex relationship, and they affect metabolic as well as reproductive health. (Qi et al., 2021a). Gut microbes influence androgen levels which are closely associated with PCOS, Gut microbes' express enzymes that metabolizes androgens such as *Clostridium scindens* can convert glucocorticoids into androgens, potentially contributing to elevated androgen levels, dysbiosis in HA-PCOS is impacting the various metabolic activities and reproductive health such as increased body weight, insulin resistance, chronic inflammation, affected CVS (Sun et al., 2023a) Women having PCOS and High levels of androgens reduce gut microbes' diversity, and their dysbiosis includes a reduction of various beneficial bacteria such as *Akkermansia*, *Bacteroides,* and *Lactobacillus.* Specific genera-including *Bifidobacterium, Enterobacteriaceae,*

unclassified, Streptococcus, Saccharimonadaceae, Enterococcus, and Eubacterium nodatum group, are more abundant in hyperandrogenic PCOS patients. This also alters the SCFA

production, which worsens insulin resistance and inflammation. (M. Li et al., 2024). Additionally, Vitamin D3 deficiency is associated with HA-PCOS.

Maternal obesity's impact on fertility:



----------Transgenerational impact----------

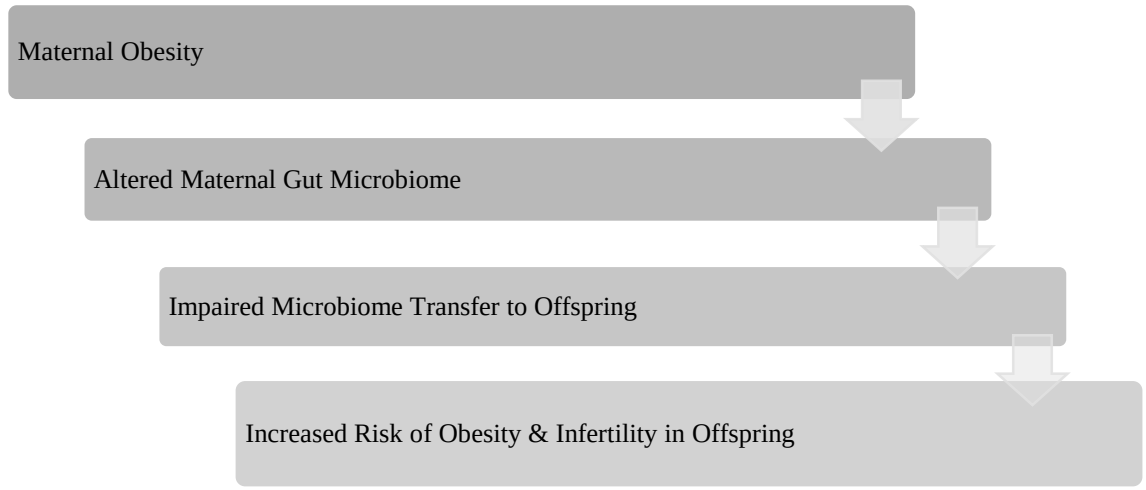


Figure 28: Flow chart shows maternal obesity impact on infertility

Female Reproductive disorders and Gut Dysbiosis:

Various pieces of evidence are mentioned in the table, which shows a significant change due to dysbiosis causes or improves the risk of reproductive disorders in females and contributes to infertility. This is explained with evidence in the Table 6. Additionally, in figure 23 it shows how PCOS impacts intestinal hormones, gut microbiome dysbiosis, and metabolites disturbances, leads to increased inflammation and increased androgens in the body.

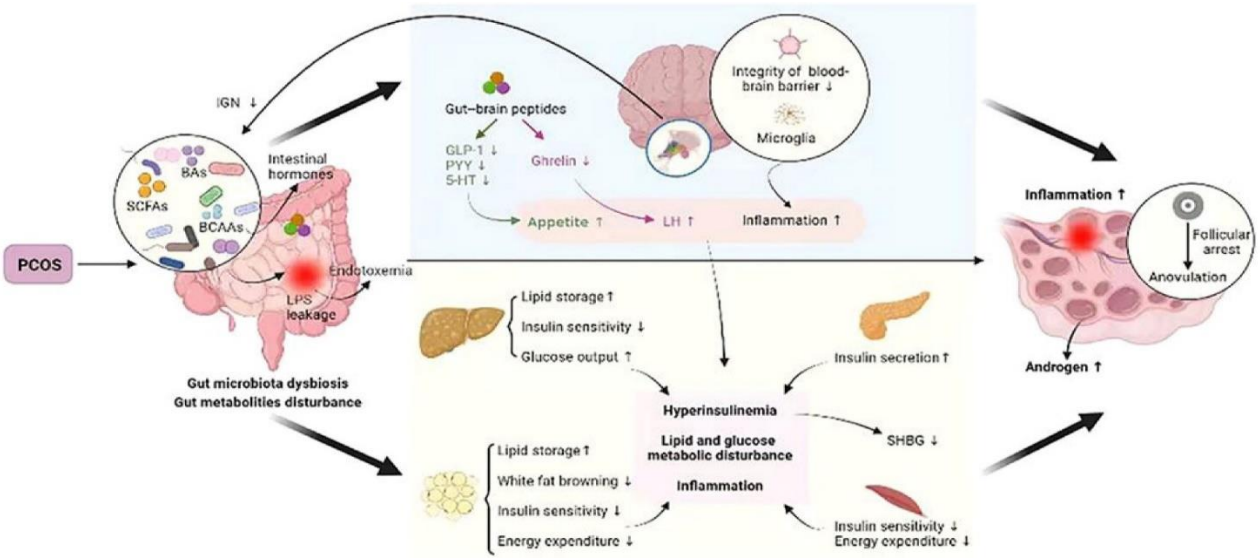


Figure 29: Crosstalk between PCOS and gut metabolites (Adapted from Di Vincenzo et al., 2024)

Table 6: Gut microbiome dysbiosis association with female reproductive diseases:

Disease name	Clinical Finding	Hormonal Change	Microbial Change	Key Microbes (↑/↓)	Reference
PCOS	Hirsutism, acne	↑ Androgens (testosterone)	↓ Alpha diversity, ↑ LPS-producers	↑ *Bacteroides, Escherichia/Shigella,* ↓ *Akkermansia, Ruminococcaceae*	(Sun et al., 2023b) (R. Liu et al., 2017)17a)
PCOS	Anovulation, infertility	↑ LH/FSH ratio, ↑ androgens	↓ *Lactobacillus, Bifidobacterium*	↑ *Firmicutes,* ↓ *Lactobacillus, Bifidobacterium*	(Sun et al., 2023b) (R. Liu et al., 2017)

PCOS	Insulin resistance, obesity	↑ Insulin, ↓ gut-brain peptides	↑ Gram-negative bacteria, ↓ beneficial bacteria	↑ *Bacteroides, Proteobacteria, ↓ Akkermansia*	(Sun et al., 2023b) (R. Liu et al., 2017)
PCOS	Infertility, hyperandrogenism, oligo/amenorrhea, polycystic ovaries	↑ Androgens, ↑ LH/FSH ratio, insulin resistance	↓ Diversity, ↑ Firmicutes/Bacteroidetes ratio, ↑ Gram-negative bacteria	↑ Bacteroides, ↑ Escherichia/Shigella, ↑ Streptococcus, ↓ Lactobacillus, ↓ Akkermansia, ↓ Ruminococcaceae	(Chadchan et al., 2022b)
PCOS	Chronic inflammation	↑ Pro-inflammatory cytokines	↑ LPS, altered SCFA, and bile acid metabolism	↑ *Bacteroides*, ↓ *Lactobacillus, Bifidobacterium*	(Sun et al., 2023b) (Corrie et al., 2023)
Endometriosis	Chronic pelvic pain, infertility	↑ Oestrogen (oestradiol), ↑ Erβ	↓ Lactobacillus, ↑ β-glucuronidase producers	↑ Escherichia/Shigella, Bacteroides, Clostridia, Ruminococcaceae, Blautia, Dorea, ↓ Lactobacillus	(Ata et al., 2019; Chantalat et al., 2020; Guo & Zhang, 2024; Uzuner et al., 2023)
Endometriosis	Inflammation, peritoneal lesions	↑ Pro-inflammatory cytokines	↑ Gram-negative bacteria, altered Firmicutes/Bacteroidetes ratio	↑ Escherichia/Shigella, Streptococcus, Gardnerella, Ureoplasma	(Ata et al., 2019; Uzuner et al., 2023)
Endometriosis	Chronic pelvic pain, infertility, endometriotic lesions, inflammation	Altered oestrogen metabolism, increasing local oestrogen	↓ Diversity, ↑ Pro-inflammatory bacteria, altered gut and reproductive tract microbiota	↑ Escherichia, ↑ Shigella, ↑ Streptococcus, ↓ Lactobacillus, ↓ Ruminococcaceae	(Chadchan et al., 2022b)

Endometriosis	Cyclic bleeding of lesions	↑ Circulating oestrogen via the oestrobolome	↑ β-glucuronidase activity, ↑ oestrogen reabsorption	↑ β-glucuronidase (from Bacteroides, Clostridia, Escherichia)	(Guo & Zhang, 2024; Uzuner et al., 2023)
Endometrial cancer	Abnormal uterine bleeding, postmenopausal bleeding	↑ Oestrogen (unopposed), altered oestrogen metabolism	↑ β-glucuronidase-producing bacteria, dysbiosis, ↑ Pro-inflammatory bacteria	↑ Clostridium, ↑ Bacteroides, ↑ Escherichia, Atopobium, ↑ Porphyromonas, ↓ Lactobacillus	(Chadchan et al., 2022b)
Pregnancy Complications	Preeclampsia, gestational diabetes, preterm birth	Altered progesterone, oestrogen, and insulin	↓ Diversity, ↑ Pathobionts, altered SCFA producers	↑ Prevotella, ↑ Streptococcus, ↑ Escherichia, ↓ Lactobacillus, ↓ Bifidobacterium	(Chadchan et al., 2022b)
Recurrent Vaginal Infections	Vaginal discharge, irritation, 64lebsiella infection risk	Decreased oestrogen, altered vaginal pH	↓ Lactobacillus dominance, ↑ Anaerobes	↓ Lactobacillus, ↑ Gardnerella, ↑ Atopobium, ↑ Mobiluncus	(Chadchan et al., 2022b)
Female genital tract disease	Bacterial vaginosis	Often linked to menstrual/hormonal fluctuations (↓ oestradiol, ↑ pH)	↓ *Lactobacillus*, ↑ diversity, ↑ anaerobes	↑ *Gardnerella, Atopobium, Mobiluncus, Prevotella, Bacteroides, Sneathia, Leptotrichia, Clostridia*; ↓ *Lactobacillus*	(*Bv*, n.d.; Prof. Ina Schuppe Koistinen, 2025; Song et al., 2020; Vinerbi et al., 2025)
Female genital tract disease	Pelvic inflammatory disease	Not mentioned	↑ pathogenic bacteria	↑ *Gardnerella, Atopobium, Mobiluncus, Prevotella, Bacteroides, Sneathia, Leptotrichia, Clostridia, ↓ Lactobacillus*	(*Bv*, n.d.)

Female genital tract disease	Tubal factor infertility, early abortion	Not mentioned	↑ BV-associated bacteria	↑ *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella*, *Bacteroides*, *Sneathia*, *Leptotrichia*, *Clostridia*; ↓ *Lactobacillus*	-
Female genital tract disease	Increased STI risk	Not mentioned	↓ *Lactobacillus*, ↑ pathogens	↑ *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella*, *Bacteroides*, *Sneathia*, *Leptotrichia*, *Clostridia*; ↓ *Lactobacillus*	(*Bv*, n.d.)

This table links various evidence of female infertility to gut and genital tract dysbiosis and associated microbial alteration with various female reproductive tract disorders, such as PCOS, endometriosis, endometrial cancer, pregnancy complications and recurrent vaginal infection.

CHAPTER 8

GUT MICROBIOME RELATIONS WITH UNDERLYING MALE REPRODUCTIVE DISORDERS

GUT MICROBIOME RELATIONS WITH UNDERLYING MALE REPRODUCTIVE DISORDERS

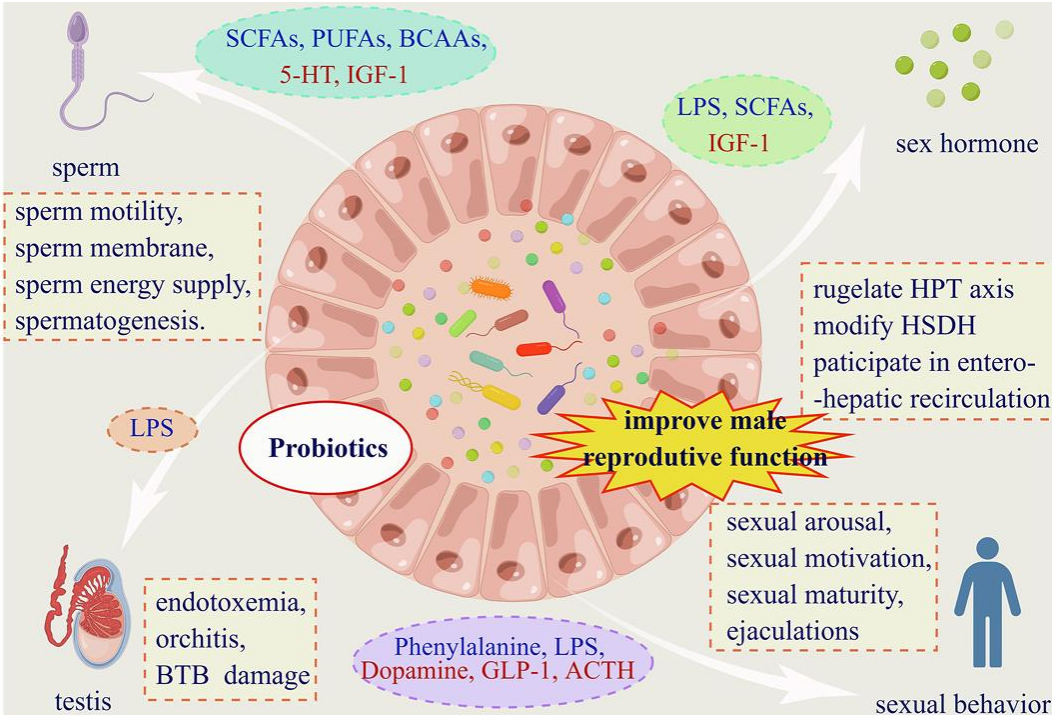


Figure 30 Gut microbiome affects male reproductive functions (Adapted from Lv et al., 2024)

There are number of interrelated pathways, the gut microbiota plays a critical role in controlling male reproductive function (Figure 30).

a. **Hypothalamus–Pituitary–Testis (HPT) axis**, which is in charge of spermatogenesis and testosterone production, thereby influencing hormonal regulation.

b. **Immune function and inflammation** gut microbes dysbiosis can cause systemic inflammation, which can harm the Blood-Testis Barrier (BTB) and compromise testicular health.

c. **Generating and regulating important metabolites** like SCFA, PUFAs, BCAAS the gut microbiota supports metabolic regulation. These metabolites support hormone production, energy metabolism, and testicular function.

d. Influenced by gut microbes through **neurotransmitters** like serotonin and peptides like adrenocorticotropic hormone (ACTH) and glucagon-like peptide (GLP), thus indirectly impacting reproductive health. These various but connected pathways demonstrate how important the gut microbiota is to preserving male fertility (Lv et al., 2024)

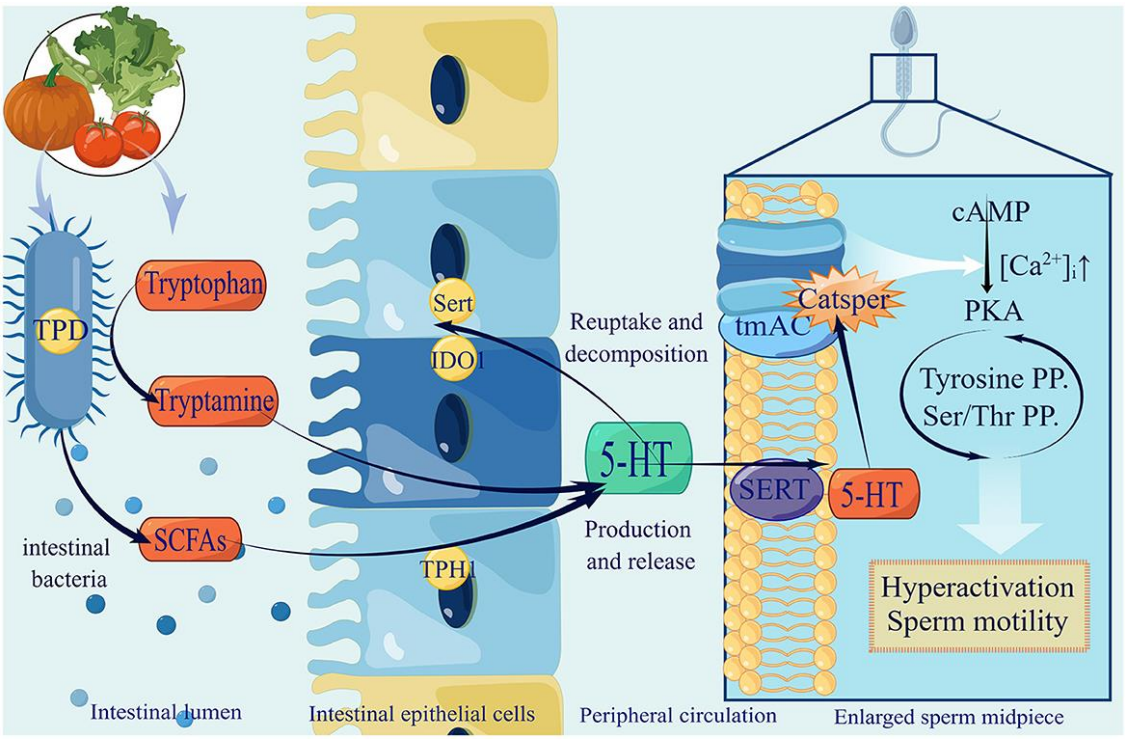


Figure 31 The Production of gut microbiome derived 5-HT in spermatozoa (Adapted from (Lv et al., 2024)

Gut microbiome and its metabolites having positive and negative effects male reproduction functions such as: Positive effects it improves spermatogenesis, sperm motility, semen quality. Negative effects: dysbiosis leads to inflammatory response and impact fertility negatively. Gut dysbiosis leads to disruption of intestinal barrier which results in increased inflammation and metabolic disorders that affects sperm production.

Gut metabolites improve sperm motility as shown in Figure 31, Tryptophan it's an essential amino acid this plays a strong role in diet, it undergoes 3 metabolic pathways such as Indole derivatives, Kynurenine pathways and 5- Hydroxy tryptamine (5-HT) pathways.

Gut microbiome plays an important role in fertility Because it affects important metabolic pathways. Gut bacteria control the enzyme IDO1, which degrades tryptophan, affecting hormone balance and immunological function through the kynurenine pathway. Gut microbes such as Clostridium ramosum and Blautia increase serotonin production in the colon through the serotonin pathway. Sperm contain serotonin, which activates calcium channels (tmAC and CatSper) to improve sperm motility, fertilization, and protein phosphorylation, all of which are essential for successful reproduction. Furthermore, gut bacteria regulate polyunsaturated fatty acids (PUFAs), including DHA. These fats enhance sperm membrane stability, motility, and general male fertility while lowering inflammation and guarding against oxidative stress. These pathways collectively demonstrate how a healthy gut microbiota promotes reproductive health by controlling sperm function, metabolism, and immunity (Lv et al., 2024) Explained below in flow chart

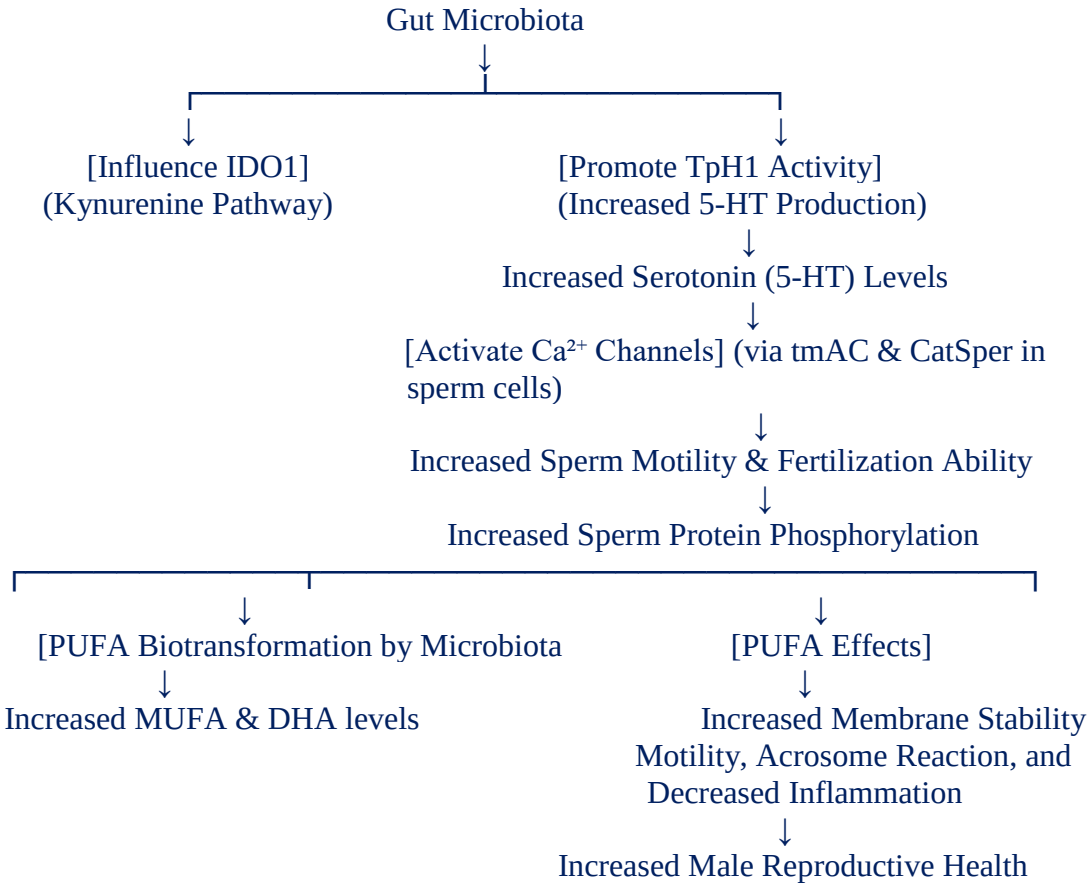


Figure 32 Flow chart shows how gut microbiome impact Male Reproductive Health

Gut microbiome improves spem quality by producing various metabolites whuch contributes in various functions such as maintenance of sperm membrane integrity, Promotion of calcium influx to sperm, support sperm energy levels a s improves process of spermatogenesis (Lv et al., 2024)

Gut microbiome promotes sperm energy supply Through glycolysis, Sertoli cells in the testes turn glucose (through GLUT transporters) into lactate, which developing sperm use (through MCT2) to produce ATP, which is essential for capacitation. This glycolysis is enhanced by gut-derived metabolites such as SCFAs and tryptophan derivatives. Lactate production and sperm motility are also improved by leucine supplementation and melatonin, which are both regulated by gut serotonin (5-HT). The spermatid energy metabolism is supported by bacteria such as Streptococcaceae and Bacteroides.(Lv et al., 2024)

By stimulating TPH1 in enterochromaffin cells and expressing tryptophan decarboxylase, gut microbes also aid in the production of serotonin. Serotonin increases sperm hyperactivation and motility by entering sperm through SERT, activating tmAC, opening CatSper channels, causing Ca2+ influx, and activating PKA. Reduced sperm production may result from impaired Sertoli and Leydig cell development caused by serotonin deficiency. Additionally, the gut microbiota supports spermatogenesis by controlling insulin-like growth factor 1 (IGF-1) and blood glucose (through bile acids). All together, gut microbes alter metabolic, hormonal, and mitochondrial processes that are critical to the fertility and quality of sperm (Lv et al., 2024)

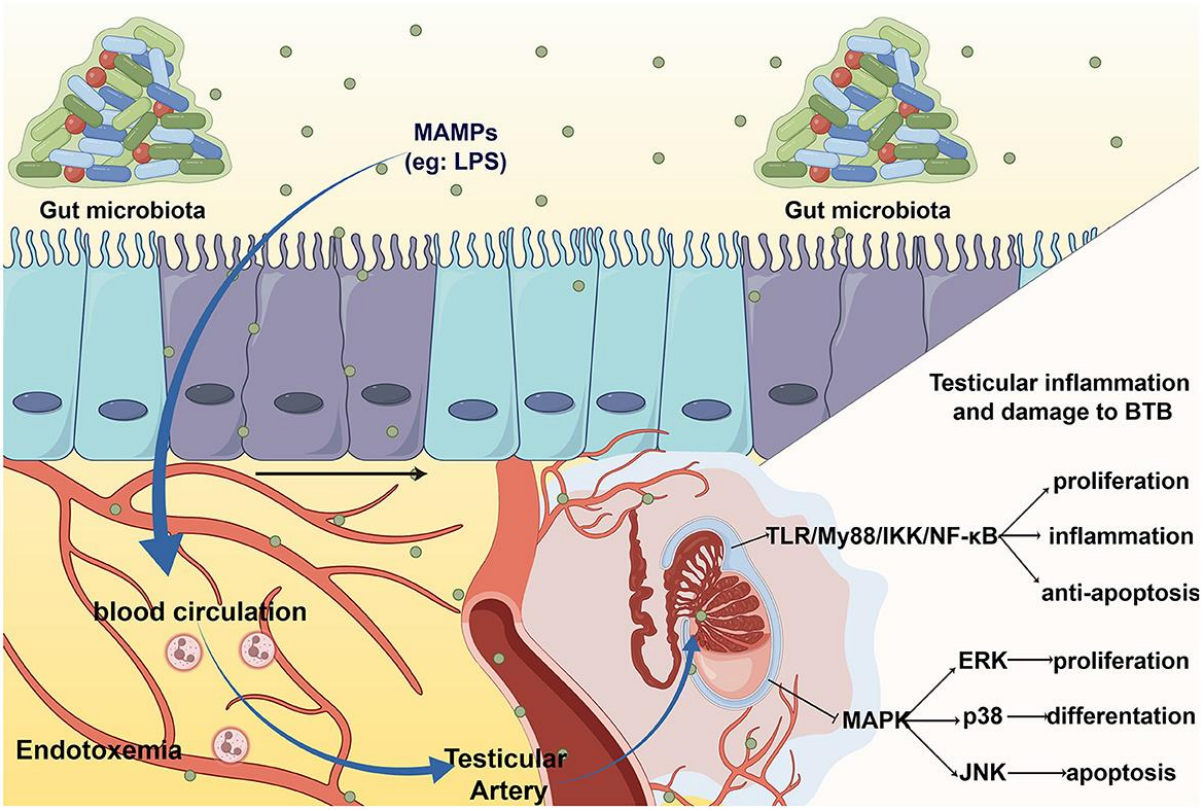


Figure 33 Entry of Lipopolysaccharides into blood circulation leads to BTB damage (Adapted from Lv et al., 2024) **.**

Gut microbiome disrupts testicular structure as shown in figure LPS enters into blood circulation through intestinal barriers and causes systemic endotoxins. LPS reaches to testicular artery and activates TLR MAPK which causes endotoxemia, orchitis and damage to BTB.(Lv et al., 2024). The male reproductive microbiome also having diversity such as Desulfovibrio, Faecal bacterium, Holdemania, Prevotella, Rikenellaceae, Roseburia, and Ruminococcaceae and others. Just like females' gut microbiota regulates male reproductive system through hormones, insulin sensitivity, the immune system, and testicular microbiota Prevotella having a positive association with testosterone. Some bacteria such as Clostridium Scindens and ruminococcus gnavus can convert steroid precursors into testosterone and dihydrotestosterines in the gut, But the link between gut microbiomes and testosterone is complex, testosterone remodels gut microbiome, gut microbiome also regulate testosterone biosynthesis. For the development of blood testis barrier presence of gut microbiomes plays important roles. Blood

testis barrier protects sperms from immune attacks, Gut Dysbiosis, increase gut permeability ("leaky gut"), allowing harmful components like LPS to enter the bloodstream and reach the testis, causing inflammation and disrupting the blood-testis barrier. This damages Leydig and Sertoli cells, impairing spermatogenesis. Dysbiosis also affects insulin sensitivity, leading to insulin resistance, hormonal imbalance (LH, FSH, testosterone), and chronic inflammation, all of which contribute to reduced male fertility (Ashonibare et al., 2024). According to Magil & Macdonald in their study male microbiome shows similarity but gut microbiome but this is distinctively difference are there specially in upper genital tract microbiome, semen contains various bacterial diversity either in healthy as well as in infertile men's but bacterial presence doesn't mean that semen quality is poor, the composition of semen and male reproductive organs mainly depends on sexual hygiene and activity, personal hygiene, sexual partner and diets. Those men are sexually inactive their microbiome or bacterial diversity and concentrations is low. Additionally seminal or gut dysbiosis is one of the major causes of chronic prostitis where prostate is inflamed and this is also associated with Inflammatory bowel syndrome, prostitis in men's having lower lactobacillus diversity this means that protection is compromised in those males due to low lactobacillus. Additionally, where the cause of infertility is unknown at this situation idiopathic male infertility is having association with oxidative stress, and some factors like alcohol can increase this oxidative stress which damage the sperm their functions and leads to inflammations. Various bacteria's like Ureaplasma urealyticum, aerococus, mycoplasma is associated with poorer semen quality, Bacteroidetes, firmicutes are linked with azoospermia, and some are associated with DNA damage in sperm quality and it reduces the fertility potential (Magill & MacDonald, 2023) There are various studies done to figure out the association of gut microbiome and male reproductive tract disorders which will contribute to infertility

Table 7: Gut microbiome dysbiosis association with male reproductive tract disorders

Disease name	**Clinical Finding**	**Hormonal Change**	**Microbial Change**	**Key Microbes (↑/↓)**	**Reference**
Erectile dysfunction	Reduced erectile function, vascular 73klebsiella73s, and endothelial injury	↓ Testosterone (due to low Alistipes), ↑ TNF-α	↑ Clostridium XVIII, ↓ Alistipes	↑ *Clostridium XVIII*, ↓ *Alistipes*	(Hsu et al., 2015)
Insulin Resistance & testicular damage	Impaired spermatogenesis, testicular damage, metabolic syndrome, hyperinsulinemia	↓ LH/FSH, ↓ Testosterone, ↑ Insulin (disrupts SHBG and free testosterone balance)	↓ Diversity, ↑ LPS-producing bacteria, ↑ pro-inflammatory taxa	↑ *Bacteroides*, ↑ *Prevotella*, ↓ *Lactobacillus*, ↓ *Bifidobacterium*	(Ding et al., 2020b) (Pedersen et al., 2016)
nonobstructive azoospermia	Idiopathic infertility, impaired BTB development, delayed spermatogenesis	↓ Testosterone (Leydig cell dysfunction)	↓ Diversity in testicular microbiota, ↓ Bacteroides/Proteobacteria	↓ *Bacteroides*, ↓ *Proteobacteria*, ↑ *Actinobacteria*, ↑ *Firmicutes*	(Alfano et al., 2018; Molina et al., 2021)
Spermatogenesis Impairment	Reduced sperm motility, DNA fragmentation, oxidative stress	↓ Testosterone, ↑ ROS (oxidative stress)	Altered gut-testis axis, ↑ LPS, ↓ SCFA producers	↓ *Lactobacillus*, ↓ *Bifidobacterium*, ↑ *Escherichia/Shigella*, ↑ *Streptococcus*	(Valcarce et al., 2017) (Ding et al., 2020b)
Gut-Testis Axis Inflammation	Chronic testicular inflammation, Blood testis barrier leakage, Leydig/Ser	↓ Anti-inflammatory factors (e.g., IL-10), ↑ Pro-inflammatory	↑ LPS-producing bacteria, ↑ TLR4/NF-κB signalling	↑ *Clostridium*, ↑ *Bacteroides*, ↑ *Prevotella*, ↓ *Lactobacillus*	(Ding et al., 2020b)

	toli cell damage	cytokines (TNF-α, IL-6)			
Male Infertility (linked to impaired spermatogenesis and reduced sperm motility)	Reduced sperm count and motility, Epididymal 74klebsiella74s (↑ T cells, macrophages) - ↑ Blood endotoxin levels (endotoxemia)	No significant systemic sex hormone changes reported; local testicular dysfunction via altered gene expression	Gut dysbiosis due to high-fat diet (HFD) - ↑ *Bacteroides*, ↑ *Prevotella* - Microbial profile linked to inflammation and endotoxin	↑ *Bacteroides* ↑ *Prevotella* ↓ (Implied loss of beneficial microbial diversity)	(Ding et al., 2020a)
Male Infertility	Men with infertility showed significant alterations in the gut, urine, and semen 74klebsiella74s compared to fertile controls. - Presence of sperm DNA fragmentation and impaired semen quality.	Not mentioned in the study	Reduced microbial diversity in gut, urine, and semen samples of infertile men. - Increased pro-inflammatory bacteria and decreased beneficial commensals	↑ Bacteroides, ↑ Streptococcus, ↑ Prevotella - ↓ Lactobacillus, ↓ Faecal bacterium, ↓ Ruminococcaceae	(Lundy et al., 2021)
Seminal hyper viscosity	Increased semen viscosity and impaired sperm motility	Not mention	Increased proteobacteria and altered microbial diversity	Increased pseudomonas and 74klebsiella	(Monteiro et al., 2018)

Oligoasthenoteratozoospermia (OAT)	Low sperm count, disturbed morphology and motility	Not mentioned	Increased gram-negative pathogens and reduced lactobacillus	Increased Neisseria, klebsiella, pseudomonas and decreased lactobacillus	(Monteiro et al., 2018)
Male infertility	Reduced sperm quality and DNA damage		Reduced diversity and increased pathogens	Increased Neisseria, Prevotella, Anaerococcus and decreased lactobacillus	(Monteiro et al., 2018)

This table links various evidence of male infertility to gut dysbiosis and associated microbial alteration with various male reproductive disorders, such as erectile dysfunctions, insulin resistance leading to testicular damage, nonobstructive azoospermia, idiopathic infertility, and spermatogenesis impairment. seminal hyper viscosity, OAT, and others, these conditions occur due to dysbiosis, which are increased and contributes to infertility with increased negative bacteria and reduced beneficial bacteria.

CHAPTER 9

MANAGEMENT OF INFERTILITY THROUGH GUT MICROBIOME IMPROVEENT

MANAGEMENT OF INFERTILITY THROUGH GUT MICROBIOME IMPROVEMENT

There are various management can be done to improve gut health it includes diet an important measure to improve and manage gut health, lifestyle, Probiotics and others. Additionally, various factors which can be avoided for management of dysbiosis, such as Alcohol consumption, Tobacco consumption in any form, smoking, chewing etc. Fast food consumption, ultra-processed foods, stress, regular antibiotic usage**,** Preservatives and food additives, microwave-cooked food and others these all helps in the management of dysbiosis.

Gut Microbiome improvement by adopting various management Strategies

- **Dietary changes:** Various dietary changes can be done for the management of dysbiosis by consuming Fruits and Vegetables High in Fibre diet, which helps maintain regular bowel movements and encourages a varied gut flora. Try to eat a range of colourful produce, including cruciferous vegetables (like broccoli and cauliflower), leafy greens (like spinach and kale), and berries. These foods promote general reproductive health because they are high in vitamins, minerals, and antioxidants. (The Gut-Fertility Connection: Expert Insights on How to Eat for Succes-Fertile Gut.) . This is also seen that when plant-based food is taken into diet good bacteria in gut improves and reduction in inflammatory and opportunistic bacteria's which helps in the management of dysbiosis (Alagiakrishnan et al., 2024). Vegetarian, vegan diet, which is rich in plant-based fibres, is strongly associated with increased prevotella and food which is low fibre, high/fat sugar mainly western diets is strongly associated with Bacteroides which enrich the gut microbiomes. Fibre rich and plant-based diet increase

microbial diversity and improve SCFA production which will improve gut health and reduces disease risks, and reduce chances of infertility (Beam et al., 2021).

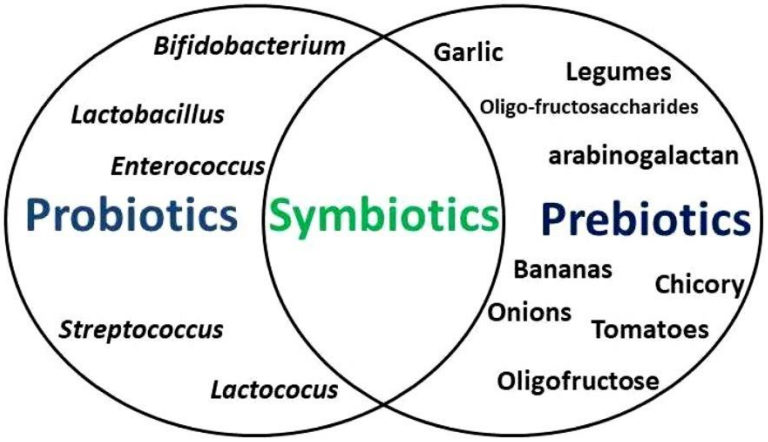


Figure 34: Symbiotic relationship of probiotics and prebiotics (Adapted from Ashonibare et al., 2024).

- **Probiotics:** Probiotics are various bacteria and yeasts helps in health improvement when taken as food supplements or with some foods in right quantity. Probiotic rich foods are Yogurt, fermented milk, cheese, panner and others. (Hill et al., 2014) Prebiotics are foods that is rich in fibre such as garlic, bananas, chicory, onions, tomatoes, legumes and others and they help in the growth of gut flora. When Probiotics and prebiotics are consuming together, they become symbiotic. This combination is having several health benefits. Probiotics helps in development of normal gut flora and maintain the functions of bacteria. Also, it restores gut microbiome after drug therapy. Whereas prebiotics modifies the gut flora according to the need of the body and helps in the growth of normal gut flora. Natural sources of probiotic are Yogurt, Kefir, Kimchi, etc. Adding these foods help and maintain the gut health and environment (Ashonibare et al., 2024).
- **Omega-3 Fatty Acids**- Omega-3 fatty acids have strong anti-inflammatory properties that support gut and reproductive health. Include these omega-3-rich foods such as Fatty fish (salmon, mackerel, sardines), Chia seeds, and Flaxseeds.

They help in hormone regulation and improvement of egg quality, which will maintain the functioning of the body and reduce chances of various lifestyle-related disorders which will leads to infertility. Additionally, to this Omega 3 reduces gut inflammation by increasing beneficial bacteria such as lactobacillus, bifidobacterium and others and these microbes helps in maintenance of gut barrier integrity, which will help in gut health maintenance and in case of dysbiosis omega 3 help in management and reducing the chances of infertility (Janićijević et al., 2022). In case of PCOS Omega 3 improves the insulin, sensitivity and reduce androgens levels which will directly enhances the oocytes quality by traducing the oxidative stress, it also regulates hormones such as FSH, Lhand estrogen which will manage the regularity of menstruation cycle and improve reproductive health in females (Fu et al., 2021; Trop-Steinberg et al., 2024). Long chain Omegha-3 fatty acids are beneficial for female fertility (Panth et al., 2018).

- **Wholegrains-** Wholegrains are unrefined grain foods rich in fibre, vitamins, minerals, and antioxidants. They support a balanced gut microbiome and provide slow-releasing energy, they increase gut microbial diversity and manage the level of beneficial bacteria's and firmicutes/Bacteroidetes ratio which will reduce inflammation and improves glucose metabolism (Martínez et al., 2013). Wholegrains are Oats, Quinoa, Brown and wild rice, Buckwheat, Barley, Millet, Wholegrain bread, and Popcorn.
- **Gluten-free wholegrains:** Various foods such as quinoa, millet, buckwheat, amaranth, teff, corn, and brown rice. These foods alter gut microbes and increase beneficial genera like Rosebury and Faecal bacterium which will reduces inflammation and improves gut integrity (Caio et al., 2020). Gluten free wholegrains are better alternatives for gut health in sensitive individuals (San

Mauro Martín et al., 2024). A diet high in wholegrains in the 12 months prior to conception is associated with increased live birth rates in women undergoing fertility treatments like IVF (Gaskins et al., 2016).

- **Plant-Based Proteins**- There are various foods available that are rich in protein and sourced from plants such as chickpeas, black beans, kidney beans, soyabeans, tofu, soymilk, Quinoa, Brown rice, oats, Almonds, chia seed, walnut, sunflower seed, green peas and others. Plant based proteins and healthy fats like mediterranean diet are linked to the reducing risk of infertility in females (Panth et al., 2018) Eating plant-based meals regularly will helps increase fibre intake and while reducing saturated fats, which will support gut health and balance hormones. A plant-rich diet enhances the growth of beneficial bacteria, which produce beneficial compounds such as SCFA and others that helps in Strengthen the intestinal barrier, reduce inflammation, regulating hormones, and Support metabolism (Tomova et al., 2019).
- **Dairy products**: There are various dairy products such as milk, cheese, yogurt, kefir, chaach and others are all improves gut health because they are rich in probiotics and associated with the abundance of beneficial gut bacteria like lactobacillus and bifidobacterium species. These product consumptions promote the growth of SCFA producing bacteria's that will help in maintain the gut health and which will directly linked with maintenance hormonal regulation nd reproductive health (Aslam et al., 2020).
- **Animal-based proteins:** Meat is a good source of animal proteins; its consumption helps in the composition of gut microbes; white meat is rich in protein and associated with reduced serum lipopolysaccharide-binding protein (marker of inflammation); it also contains Lactobacillus. Chicken protein consumption

increases the level of A muciniphila and lactobacillus, it improves metabolic health and immune functions (Lee et al., 2023).

- **Physical activity:** Regular exercise has multiple positive effects on health. In case of gut health, regular physical activity creates a great and positive impact on gut health It is highly associated with gut microbial diversity and higher levels of beneficial bacteria such as Akkermansia, Bifidobacteria, Etc. Regular aerobic exercise with resistance training at moderate intensity is beneficial and increases SCFA production, metabolic, and immune functions. They help in the maintenance of the gut environment and reduce the chances of infertility (Pedroza Matute & Iyavoo, 2023).
- **Stress Management:** Stress is related directly with gut brain axis, chronic mental stress leads to gut microbiome disruption and reduction of beneficial bacteria's which make gut environment susceptible for harmful microbes which will leads to increase gut inflammation, permeability and long run Give rise to mood disorders. To manage stress various activities can be done such as meditation, yoga, music, sound therapy and many more there reduce the stress hormones such as cortisol and improve mental as well as gut health, reduction chance of infertility (Pedroza Matute & Iyavoo, 2023).
- **Faecal microbiota transplantation (FMT)**: Transfer of faecal bacteria of from a healthy donor to recipients it is used in the patients which are having *Clostridioides difficile* infection, to overcome and maintain gut environment this technique is used. For the prevention of recurrence Fidaxomicin is used. FMT helps those patients who are antibiotic dependent due to recurrent infection (Alagiakrishnan et al., 2024).

CHAPTER 10

CONCLUSION, RECOMMENDATIONS & LIMITATIONS

CONCLUSION

The complex interactions between the microbiomes of the gut and reproductive tract, as well as their combined roles in the genesis, development, and treatment of human infertility, were thoroughly examined in this thesis. After a thorough analysis of the composition and diverse roles of the gut microbiome, it became clear that these microbial communities play a crucial role in the regulation of hormones, the immune system, and metabolism.

- In addition to influencing the host's systemic inflammation and endocrine signalling, the gut microbiota plays a major role in the synthesis of short-chain fatty acids (SCFAs), vitamins, and neurotransmitters factors that are directly related to reproductive health. With a focus on the growing prevalence of infertility, which affects almost 27.5 million couples, the thesis also looked at current trends in reproductive health in India. Infertility is still not sufficiently addressed in public health frameworks despite medical advancements, and the role of the microbiome
- The mechanisms of gut dysbiosis were then explained, emphasizing how microbial imbalance affects metabolite production, increases intestinal permeability, and impairs immune function. Systemic diseases that are known to contribute to infertility, such as neurological, autoimmune, and metabolic disorders, have been associated with dysbiosis.
- A thorough analysis of the reproductive and gut microbiomes showed both similar and different microbial populations and physiological functions. The reproductive tract, particularly in females, is dominated by Lactobacillus species, which are essential for preserving vaginal pH, immunological balance, and pathogen resistance, even though the gut contains a variety of microbial taxa, including Firmicutes, Bacteroidetes, and Actinobacteria.

- Crucially, the study found a strong correlation between infertility and dysbiosis of the gut microbiota. Mechanistically, dysbiosis causes chronic inflammation, damages gut barrier function, and interferes with hormone metabolism (e.g., oestrogen recycling via β-glucuronidase), all of which lead to reproductive dysfunction in both sexes.
- By changing insulin sensitivity, elevating oxidative stress, and modifying immune responses, dysbiosis has been demonstrated to impact the pathophysiology of diseases such as PCOS, endometriosis, and implantation failure in females. Likewise, in males, low sperm count, decreased motility, poor sperm morphology, and testicular inflammation which is primarily caused by increased endotoxins and oxidative damage were all associated with gut dysbiosis.
- Lastly, the thesis looked at both established and new approaches to interventions based on the microbiome. Prebiotics, probiotics, dietary changes, and lifestyle adjustments like more exercise have been shown to improve reproductive outcomes, lower inflammation, and restore microbial balance. Personalized microbial therapies and faecal microbiota transplantation (FMT) are also showing promise as additional treatments for infertility.

In conclusion, this study provides a solid scientific justification for incorporating research on the microbiome into reproductive health plans. A non-invasive, economical, and preventative strategy for addressing infertility through the lens of microbial health supports Sustainable Development Goal 3: ensuring healthy lives and promoting well-being for all by 2030. Microbiome modulation must be given top priority in future clinical frameworks and public health policies as a crucial aspect of reproductive healthcare.

LIMITATIONS

1. Absence of Primary Data: This study relied on existing literature, scientific publications, and secondary data sources. It is difficult to prove a direct link between gut microbiota and infertility in the Indian population due to the lack of primary clinical or microbiome sequencing data.
2. Limited Regional Studies: There was little data from India specifically pertaining to any one region; the majority of the reviewed studies were carried out in Western or developed nations. Findings might not accurately represent the Indian population because microbiome composition varies greatly by location, diet, and lifestyle.
3. Study design heterogeneity: It was challenging to compare or extrapolate findings from the reviewed studies due to their varied approaches (e.g., sample size, sequencing technologies like 16S rRNA, shotgun metagenomics).
4. The science of the microbiome is still developing. Some findings are preliminary, and many microbial interactions are still unknown. Current hypotheses require validation through clinical trials and longitudinal studies.
5. Absence of Standardized Microbiome Protocols: Particularly in reproductive contexts, there is no widely recognized protocol for identifying a "healthy" microbiome. Because of this, standardizing diagnosis and treatment based on microbial profiles is difficult.
6. Confounding variables: Although they can affect both microbiome health and fertility, factors like the use of antibiotics, stress, environmental pollutants, body mass index, and co-existing medical conditions were not taken into account in the majority of literature studies.
7. The evidence for the intervention is preliminary. Despite the potential of probiotics, diet, and lifestyle changes, there is not enough extensive clinical data to support the use of standardized microbiome-based treatments for infertility.

RECOMMENDATIONS

The following suggestions are put forth in light of the study's findings to improve clinical practice, public health policy, and future research on microbiome health and infertility management:

1. Including microbiome screening in fertility clinics infertility workups should routinely evaluate the microbiome profiles of the gut and reproductive tract, especially in cases of infertility that cannot be explained. Microbial imbalances can be detected using techniques such as 16s rRNA sequencing.
2. Campaigns for public health awareness to inform the general public and medical professionals about the importance of gut health for reproductive health, awareness campaigns should be created. Fertility and microbiome balance can be supported by minor dietary and lifestyle adjustments.
3. Participation in national initiatives microbiome research on reproductive health should be integrated into national health initiatives such as Ayushman Bharat and the RCH programme.
4. Clinical trials and research in India more clinical trials and longitudinal studies conducted in India are required to confirm the link between gut dysbiosis and infertility and to create treatment plans that are specific to the country.
5. Creation of treatments based on the microbiome in order to improve fertility, support pharmaceutical and nutraceutical research into microbiome-modulating treatments like faecal microbiota transplantation, probiotics, prebiotics, and symbiotic.
6. Multidisciplinary cooperation encourages cooperation between public health specialists, gynaecologists, andrologists, microbiologists, and nutritionists in order to develop comprehensive fertility treatment models that incorporate microbial health.

7. Counselling on diet and lifestyle in fertility programs in order to improve reproductive outcomes, fertility counselling should include individualized dietary and lifestyle recommendations targeted at reestablishing the balance of gut microbes.
8. Policy assistance for innovation in the microbiome as part of the larger endeavours to accomplish SDG-3: "good health and well-being" by 2030, promote financing and policy support for advancements in microbiome diagnostics and treatments.

CHAPTER 11

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