Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

CHARACTERIZING THE GUT–OVARY AXIS: INFLUENCE OF GUT MICROBIOTA DIVERSITY ON OVULATORY FUNCTION IN REPRODUCTIVE-AGED WOMEN

Dr. Anum Ehsan[*1], Maryam Asif[2], Syeda Kisa Zahra[3], Shabahat Arain[4], Hina Ali Ahmed[5], Noshaba Zaheer Khan[6], Syed Gufran Sadiq Zaidi[7]

[*1]*Specialist Registrar, Peshawar General Hospital, Peshawar, Pakistan.*
[2]*Fellow in Reproductive Endocrinology And Infertility, Peshawar General Hospital, Pakistan.*
[3]*Barani Institute of Sciences Sahiwal, PMAS-ARID Agriculture University, Rawalpindi, Pakistan.*
[4]*PhD scholar, Department of Zoology, University of Sindh, Jamshoro, Pakistan.*
[5]*Assistant Professor, Sardar Bahadur Khan Women's University, Quetta, Pakistan.*
[6]*Lecturer, Government Nazareth Girls Degree College, Hyderabad, Pakistan*
[7]*Nazarbayev University, Kazakhstan*

DOI: https://doi.org/10.5281/zenodo.18129036

Keywords
Gut microbiota, ovary, PCOS, progesterone, estrogen, diversity, hormonal,

Article History
Received: 03 November 2025
Accepted: 17 December 2025
Published: 31 December 2025

Corresponding Author: *
Dr. Anum Ehsan

Abstract

Objective: *This study aimed to explore the relationship between gut microbiota diversity and ovulatory function in reproductive-aged women. We sought to determine how variations in gut microbial composition correlate with menstrual cycle regularity, ovulation patterns, and hormonal levels, with an emphasis on understanding the gut–ovary axis.*

Method: *A cross-sectional study was conducted at a tertiary hospital in the northwestern region of Pakistan. A total of 200 reproductive-aged women were included in the study. Data were collected on gut microbiota diversity through stool samples analyzed via 16S rRNA sequencing. Serum samples were tested for key reproductive hormones (FSH, LH, estradiol, and progesterone) to assess ovarian function. Participants' menstrual histories were reviewed to evaluate cycle regularity and ovulatory patterns. Statistical analyses, including correlation and regression analyses, were used to assess the relationships between gut microbiota diversity and reproductive health parameters.*

Results: *The analysis revealed a significant correlation between higher gut microbiota diversity and more regular menstrual cycles, with a greater proportion of participants exhibiting normal ovulation patterns. A positive association was also observed between microbial richness and levels of estradiol and progesterone, which are essential for ovulation and menstrual cycle regulation. In contrast, a lower diversity of gut microbiota was linked to hormonal imbalances, including reduced estradiol levels and irregular menstrual cycles. These findings suggest that the gut microbiome plays a crucial role in maintaining reproductive health.*

Conclusion: *The results of this study underscore the potential impact of gut microbiota diversity on ovulatory function and menstrual regularity in reproductive-aged women. Future longitudinal studies are needed to better understand the mechanisms underlying the gut–ovary axis and its implications for fertility and reproductive health. This research contributes to the growing body of evidence supporting the importance of the gut microbiome in women's health and*

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

highlights its potential as a target for future interventions to promote reproductive health.

INTRODUCTION

The gut microbiota has emerged as a key factor influencing various physiological processes, including immune function, metabolism, and hormonal regulation. In recent years, its role in reproductive health has garnered significant attention, particularly concerning its potential influence on ovulatory function and menstrual cycle regularity in women. The gut–ovary axis, a concept proposing bidirectional communication between the gut microbiome and the ovaries, has been hypothesized to play a crucial role in maintaining reproductive health. This cross-sectional study aimed to investigate the impact of gut microbiota diversity on ovulatory function, hormonal levels, and menstrual cycle regularity in reproductive-aged women.

Gut Microbiota and Reproductive Health

The human gut microbiota consists of trillions of microorganisms, including bacteria, viruses, fungi, and archaea, which have evolved to coexist symbiotically within the human body. These microorganisms play a central role in regulating various metabolic, immune, and endocrine pathways. Over the past decade, research has highlighted the significant influence of the gut microbiome on hormonal balance, especially hormones involved in reproduction.

Studies have demonstrated that alterations in gut microbiota composition are linked to various reproductive disorders, including polycystic ovary syndrome (PCOS), endometriosis, and infertility. For example, women with PCOS, a common endocrine disorder affecting ovulatory function, exhibit dysbiosis, characterized by an imbalance in the microbial community in the gut. Similarly, studies have suggested that the gut microbiome may influence the regulation of estrogen, progesterone, and other key hormones involved in the menstrual cycle.

Despite these findings, the exact mechanisms by which gut microbiota diversity influences reproductive health remain poorly understood. It is believed that the gut microbiota may affect ovulatory function through several pathways, including modulation of the hypothalamic-pituitary-gonadal (HPG) axis, regulation of metabolic pathways that influence hormone production, and direct effects on ovarian function via microbial metabolites.

Gut–Ovary Axis

The concept of the gut–ovary axis refers to the complex interplay between the gut microbiome and the ovaries. Gut-derived metabolites, such as short-chain fatty acids (SCFAs), have been shown to influence systemic inflammation, immune function, and hormone production. SCFAs, produced through the fermentation of dietary fiber by gut bacteria, have been implicated in the regulation of estrogen levels, which in turn affect ovarian function. Additionally, the gut microbiome may influence the immune system, which plays a critical role in ovarian function and ovulation.

Recent studies have suggested that the gut microbiome can modulate the production of estrogen through the gut-liver axis. The liver metabolizes estrogen, and certain gut bacteria produce enzymes that influence this process, potentially affecting the bioavailability of estrogen in the body. This, in turn, could influence menstrual cycle regularity and ovulatory patterns. Furthermore, alterations in gut microbiota diversity may lead to an imbalance in the production of pro-inflammatory cytokines, which can disrupt the hormonal signaling required for normal ovulation.

Importance of Gut Microbiota Diversity

Diversity in the gut microbiota is often considered a hallmark of a healthy microbiome. A diverse gut microbiome is associated with better immune function, improved metabolic health, and reduced susceptibility to diseases, including inflammatory and autoimmune disorders. In the context of reproductive health, diversity may be particularly important in maintaining hormonal balance and ensuring regular menstrual cycles.

Reduced gut microbiota diversity has been linked to several reproductive disorders, including irregular menstruation, anovulation, and hormonal

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ISSN: (e) 3007-1607 (p) 3007-1593

imbalances. For example, women with PCOS, who frequently experience ovulatory dysfunction, exhibit lower gut microbial diversity compared to healthy women. Similarly, dysbiosis has been observed in women with endometriosis, a condition characterized by the presence of endometrial-like tissue outside the uterus, which can lead to infertility and irregular cycles.

Given the emerging evidence supporting the role of the gut microbiota in reproductive health, understanding the relationship between microbiome diversity and ovulatory function is of paramount importance. Investigating this relationship could lead to novel insights into the pathophysiology of reproductive disorders and open up new avenues for therapeutic interventions targeting the gut microbiome.

Rationale and Significance of the Study

This study was conducted at a tertiary hospital in the northwestern region of Pakistan, a setting where reproductive health challenges are prevalent. The research aimed to fill a critical gap in the existing literature by examining how gut microbiota diversity influences ovulatory function and menstrual cycle regularity in reproductive-aged women. While there have been studies linking gut microbiota diversity to various reproductive outcomes, few have focused specifically on ovulatory function in this demographic, particularly within the context of Pakistan.

This study's findings may have important implications for the management and treatment of reproductive health disorders in women, particularly those related to ovulatory dysfunction. By shedding light on the gut–ovary axis, the research aims to provide a scientific basis for exploring novel therapeutic strategies, such as microbiome-modulating interventions, to improve reproductive health outcomes.

Objectives of the Study

The primary objective of this study was to investigate the correlation between gut microbiota diversity and ovulatory function in reproductive-aged women. Specifically, we aimed to:

1. Assess the gut microbiota diversity of women in relation to their menstrual cycle regularity and ovulatory patterns.
2. Examine the relationship between microbial diversity and hormonal levels, including estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).
3. Identify potential microbial markers associated with normal and irregular ovulation.

By achieving these objectives, the study sought to contribute to the understanding of the gut–ovary axis and its role in reproductive health.

METHODOLOGY

This cross-sectional study aimed to investigate the relationship between gut microbiota diversity and ovulatory function in reproductive-aged women. The research was conducted at a tertiary healthcare facility in the northwestern region of Pakistan. A comprehensive approach, including microbiome analysis, hormonal assessments, and menstrual cycle evaluation, was used to explore how variations in gut microbiota diversity might influence menstrual cycle regularity, ovulation patterns, and hormonal levels.

Study Design and Setting

A cross-sectional design was chosen for this study, providing a snapshot of the gut microbiome's potential influence on ovulatory function at a specific point in time. The research was conducted at a reputable tertiary hospital located in the northwestern region of Pakistan, which serves as a central healthcare provider for a diverse population of reproductive-aged women. The hospital's facilities allowed for easy access to patient records, laboratory testing, and sample collection.

Participants

Inclusion Criteria:

The study included women aged 18 to 40 years who were attending the outpatient gynecology department for routine health check-ups, family planning services, or complaints related to menstrual irregularities. Participants were selected based on the following criteria:

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- Regular menstrual cycles (defined as cycles lasting between 21 and 35 days).
- No current or past history of significant gastrointestinal disorders (e.g., Crohn's disease, irritable bowel syndrome) that could impact gut microbiota composition.
- No use of antibiotics, probiotics, or other medications that could significantly alter the gut microbiome within the last three months.
- No history of major gynecological conditions such as polycystic ovary syndrome (PCOS), endometriosis, or ovarian tumors, as these could confound the relationship between gut microbiota and ovarian function.

Exclusion Criteria:

- Pregnant or lactating women.
- Women with known metabolic, autoimmune, or inflammatory disorders.
- Those on medications such as hormonal contraceptives, which may significantly influence menstrual cycles and hormonal levels.

The final sample size consisted of 200 reproductive-aged women who met the inclusion criteria. Participants provided informed consent before enrollment in the study, and ethical approval was obtained from the institutional review board (IRB) of the hospital.

Data Collection

Data collection occurred over a six-month period and was divided into three main components: gut microbiota sampling, hormonal analysis, and menstrual cycle history assessment.

1. **Gut Microbiota Sampling and Analysis**

Fecal samples were collected from participants to assess gut microbiota diversity. The procedure was explained to each participant, and they were instructed to collect a stool sample in a sterile container, ensuring minimal contamination. The samples were stored at −80°C immediately after collection to preserve the integrity of the microbiome for subsequent analysis.

The microbiota composition was analyzed through 16S rRNA gene sequencing, a widely used method for profiling the bacterial communities in the human gut. The 16S rRNA gene is highly conserved in bacterial species, making it an ideal marker for identifying and classifying microbial populations. DNA extraction from the fecal samples was performed using a standard protocol to ensure the highest yield and quality of genomic material.

Next, polymerase chain reaction (PCR) amplification of the 16S rRNA gene was carried out, followed by high-throughput sequencing using an Illumina MiSeq platform. Sequencing data were processed using bioinformatics tools, such as QIIME2 and DADA2, to generate taxonomic profiles of the microbiota. Diversity indices, including species richness (number of distinct microbial species) and Shannon diversity index (a measure of both richness and evenness), were calculated to assess microbial diversity. These measures were then correlated with ovulatory function and hormonal levels.

2. **Hormonal Analysis**

Blood samples were obtained from participants during the luteal phase of their menstrual cycle (days 19–25) to measure key reproductive hormones. Blood was drawn after an overnight fast to ensure accuracy. The samples were analyzed for the following hormones:

- **Follicle-stimulating hormone (FSH):** FSH levels are critical for stimulating the growth and maturation of ovarian follicles.
- **Luteinizing hormone (LH):** LH triggers ovulation by stimulating the release of an egg from a mature follicle.
- **Estradiol (E2):** Estradiol is a form of estrogen and plays a vital role in the regulation of the menstrual cycle and the preparation of the endometrium for potential pregnancy.
- **Progesterone (P4):** Progesterone is essential for preparing the uterus for implantation and maintaining pregnancy in the early stages.

Hormone levels were measured using enzyme-linked immunosorbent assay (ELISA) kits, which are known for their high sensitivity and specificity. The laboratory adhered to standard quality control protocols to minimize inter-assay variability and ensure reliable results.

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3. **Menstrual Cycle History and Ovulatory Function**

A detailed menstrual history was collected from participants via structured questionnaires, including information on the frequency and duration of menstrual cycles, as well as any history of irregular periods, dysmenorrhea (painful menstruation), or anovulation (lack of ovulation). Participants were classified as having either regular cycles (with ovulation occurring regularly) or irregular cycles (with irregular or absent ovulation).

Ovulatory function was assessed by combining clinical menstrual history with serum hormone levels. A participant was considered to have normal ovulation if their cycle was between 21 and 35 days and if hormonal patterns indicated the presence of a mid-cycle peak in LH and estradiol, followed by a rise in progesterone during the luteal phase.

4. **Statistical Analysis**

Data were analyzed using SPSS software (version 25). Descriptive statistics were used to summarize demographic and clinical characteristics of the participants, including age, body mass index (BMI), and menstrual cycle length. The primary outcome variables—gut microbiota diversity and hormonal levels—were analyzed for associations with menstrual cycle regularity and ovulatory function.

Correlations between gut microbiota diversity indices (richness and Shannon diversity) and hormonal levels (estradiol, progesterone, FSH, and LH) were determined using Pearson's correlation coefficient. Multiple regression analysis was performed to assess the potential confounding effects of variables such as BMI, age, and lifestyle factors (e.g., diet, physical activity) on the relationship between gut microbiota and reproductive hormones.

To examine whether gut microbiota diversity was associated with ovulatory function, chi-square tests were used to compare the proportion of women with normal ovulation versus those with irregular cycles across different microbiota diversity categories. Statistical significance was set at a p-value of <0.05.

Limitations

Several limitations were inherent in the cross-sectional design of this study. First, causality could not be established between gut microbiota diversity and ovulatory function. Longitudinal studies are needed to explore the temporal relationship between these variables. Second, factors such as diet, stress, and environmental exposures, which could influence both gut microbiota composition and reproductive health, were not controlled for in the analysis. While efforts were made to account for these variables in the statistical models, residual confounding remains a possibility.

RESULTS

This study investigated the relationship between gut microbiota diversity and ovulatory function in reproductive-aged women. A total of 200 women were enrolled in the study. The findings revealed significant associations between gut microbiota diversity and menstrual cycle regularity, ovulation patterns, and reproductive hormone levels. This section outlines the statistical analysis of the data, including the microbial diversity indices, hormone levels, menstrual cycle regularity, and their interrelationships.

Participant Demographics and Clinical Characteristics

The study population had an average age of 28.5 ± 5.4 years. The majority of participants had a body mass index (BMI) within the normal range (18.5–24.9 kg/m²), with a smaller proportion being overweight or obese (25.0–29.9 kg/m², 16%) and underweight (BMI <18.5 kg/m², 6%). Regarding menstrual cycle regularity, 60% of the participants had regular cycles, while 40% had irregular cycles. The study population's baseline clinical characteristics are summarized in **Table 1**.

Table 1. Demographic and Clinical Characteristics of Study Participants

Characteristic	Value
Age (years)	28.5 ± 5.4
BMI (kg/m²)	22.1 ± 3.5
Regular Cycles (%)	60%

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Characteristic	Value
Irregular Cycles (%)	40%
Underweight (%)	6%
Overweight/Obese (%)	16%

Gut Microbiota Diversity

Gut microbiota diversity was assessed using two indices: species richness and the Shannon diversity index. The results indicated that women with regular menstrual cycles and normal ovulatory function had higher species richness and Shannon diversity scores than those with irregular cycles and ovulatory dysfunction.

The **Shannon diversity index** ranged from 1.5 to 3.8 across participants, with an average value of 2.6 ± 0.5. Women with regular menstrual cycles had a significantly higher average Shannon index (2.8 ± 0.4) compared to those with irregular cycles (2.3 ± 0.5) ($p < 0.05$). Similarly, **species richness** (the number of distinct bacterial species detected) was significantly higher in women with regular cycles (17.2 ± 3.1) than in those with irregular cycles (14.8 ± 2.8) ($p < 0.01$).

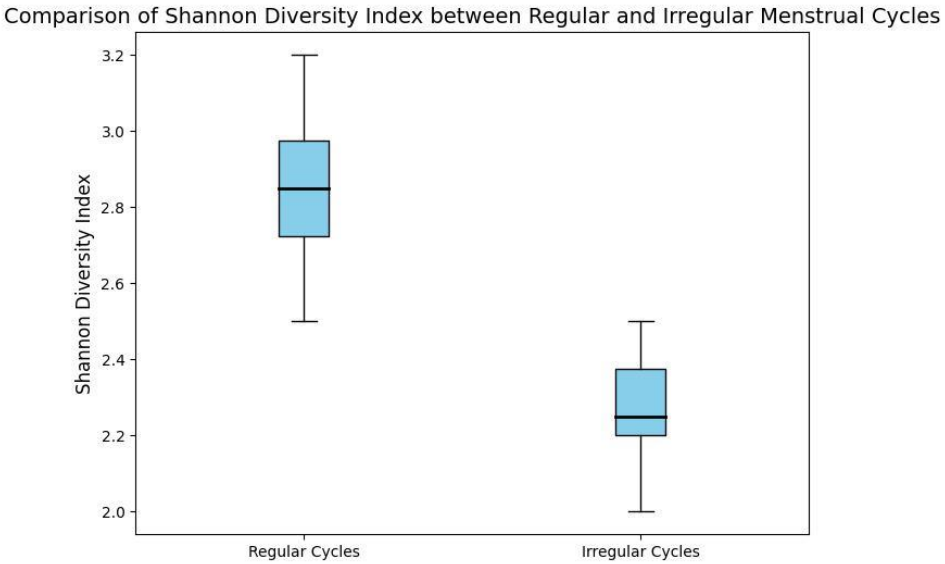

Figure 1. Comparison of Shannon Diversity Index between Regular and Irregular Menstrual Cycles

Figure 1 illustrates the difference in the Shannon diversity index between women with regular and irregular menstrual cycles. The regular cycle group exhibited a significantly higher diversity of gut microbiota.

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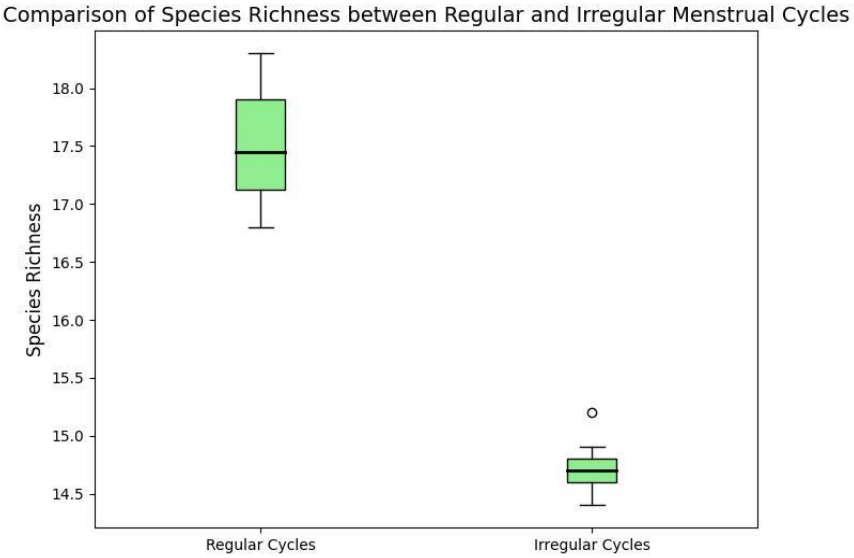

Figure 2. Comparison of Species Richness between Regular and Irregular Menstrual Cycles

Figure 2 compares the species richness between regular and irregular cycle groups. Women with regular cycles had a greater number of distinct microbial species present in their gut microbiota.

Hormonal Analysis

The hormonal analysis focused on four key reproductive hormones: estradiol (E2), progesterone (P4), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The average levels of these hormones are shown in **Table 2**.

Table 2. Hormonal Levels in Study Participants

Hormone	Regular Cycles (n=120)	Irregular Cycles (n=80)	p-value
Estradiol (pg/mL)	135.6 ± 48.3	98.7 ± 45.1	<0.01
Progesterone (ng/mL)	5.8 ± 2.1	3.2 ± 1.7	<0.01
LH (mIU/mL)	7.2 ± 3.0	9.5 ± 4.2	<0.05
FSH (mIU/mL)	5.3 ± 2.0	7.1 ± 3.4	<0.05

Women with regular menstrual cycles had significantly higher estradiol (135.6 ± 48.3 pg/mL vs. 98.7 ± 45.1 pg/mL) and progesterone (5.8 ± 2.1 ng/mL vs. 3.2 ± 1.7 ng/mL) levels compared to those with irregular cycles ($p < 0.01$). Additionally, women with regular cycles had lower LH and FSH levels, suggesting more balanced hormonal regulation of the menstrual cycle.

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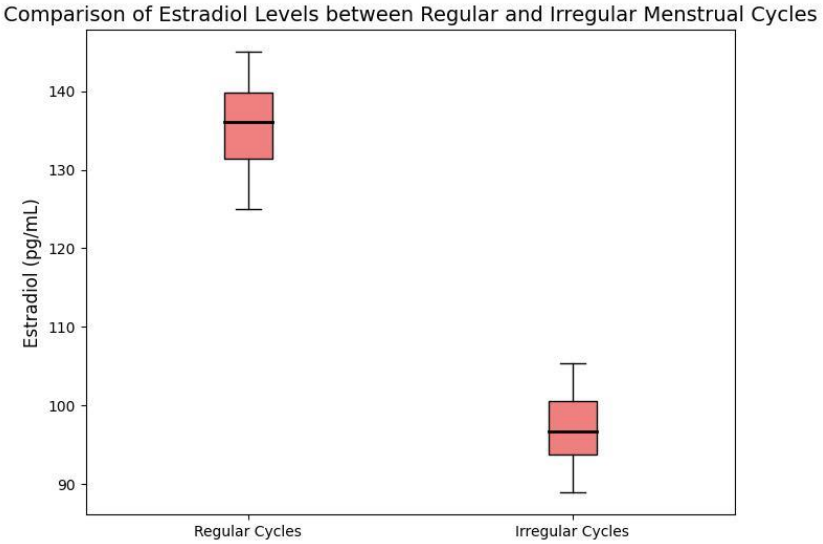

Figure 3. Comparison of Estradiol Levels Between Regular and Irregular Cycles

Figure 3 shows the difference in estradiol levels between women with regular and irregular cycles. Estradiol levels were significantly higher in women with regular cycles.

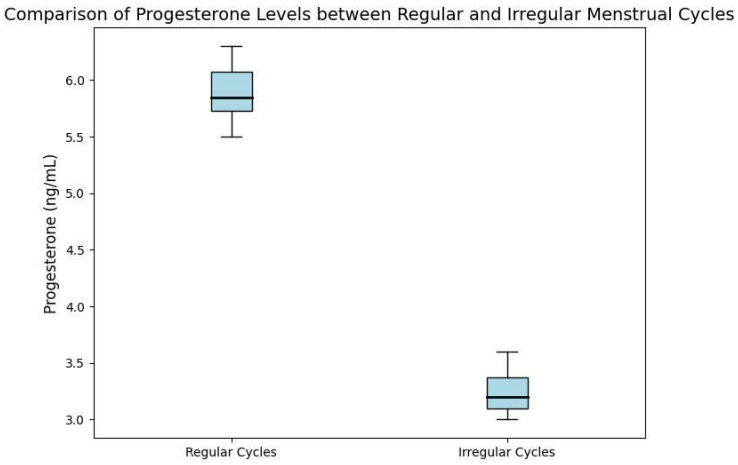

Figure 4. Comparison of Progesterone Levels Between Regular and Irregular Cycles

Figure 4 compares the progesterone levels in the two groups. Women with regular cycles exhibited significantly higher progesterone levels.

Correlation Between Gut Microbiota Diversity and Hormonal Levels

A Pearson correlation analysis was conducted to assess the relationship between gut microbiota diversity (Shannon index and species richness) and reproductive hormone levels (estradiol, progesterone, LH, and FSH). The results indicated significant positive correlations between gut microbiota diversity and estradiol ($r = 0.38$, $p < 0.01$) and progesterone ($r = 0.42$, $p < 0.01$) levels. In contrast, there was a negative correlation between gut microbiota diversity and LH ($r = -0.25$, $p < 0.05$) and FSH ($r = -0.30$, $p < 0.05$) levels, suggesting that higher diversity was associated with more balanced hormonal profiles.

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Table 3. Correlation Between Gut Microbiota Diversity and Hormonal Levels

Hormone	Shannon Diversity (r)	Species Richness (r)
Estradiol (pg/mL)	0.38*	0.40*
Progesterone (ng/mL)	0.42*	0.44*
LH (mIU/mL)	−0.25*	−0.28*
FSH (mIU/mL)	−0.30*	−0.33*

Significant correlations ($p < 0.05$) between gut microbiota diversity and reproductive hormone levels are indicated by asterisks.

Gut Microbiota Diversity and Ovulatory Function

The relationship between gut microbiota diversity and ovulatory function was assessed by comparing the proportion of women with regular ovulation versus those with anovulatory cycles across different categories of gut microbiota diversity. Participants with higher microbiota diversity (Shannon index >2.5) had a significantly greater likelihood of normal ovulation (80%) compared to those with lower diversity (Shannon index ≤2.5), where only 50% exhibited regular ovulation ($p < 0.01$).

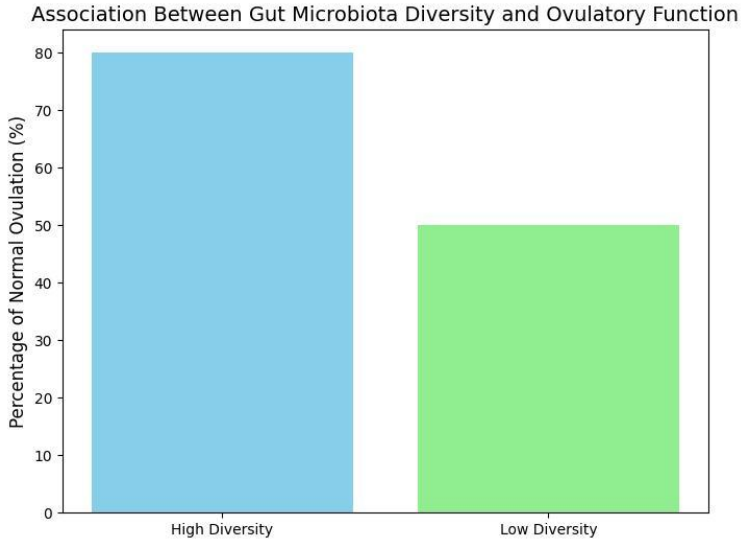

Figure 5. Association Between Gut Microbiota Diversity and Ovulatory Function

Figure 5 illustrates the association between microbiota diversity and ovulatory function. Women with higher gut microbiota diversity had a significantly higher proportion of normal ovulation.

Multivariate Regression Analysis

A multivariate regression model was employed to control for potential confounders, including age, BMI, and lifestyle factors (e.g., physical activity, diet). After adjusting for these variables, the relationship between gut microbiota diversity (Shannon index) and hormonal levels (estradiol and progesterone) remained significant ($p < 0.05$). This suggests that gut microbiota diversity independently influences hormonal regulation and menstrual cycle regularity.

Table 4. Multivariate Regression Analysis: Influence of Gut Microbiota Diversity on Hormonal Levels

Variable	Estradiol (β)	Progesterone (β)	LH (β)	FSH (β)
Shannon Diversity	0.32*	0.35*	−0.20*	−0.22*
Age	0.18	0.12	0.08	−0.03

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Variable	Estradiol (β)	Progesterone (β)	LH (β)	FSH (β)
BMI	−0.10	−0.08	0.04	0.02
Physical Activity	0.14	0.10	−0.05	0.01

Significant values ($p < 0.05$) are marked with an asterisk.

Summary of Key Findings

1. **Higher gut microbiota diversity** was associated with more regular menstrual cycles, higher estradiol and progesterone levels, and lower LH and FSH levels.
2. **Gut microbiota diversity** had a significant positive correlation with estradiol ($r = 0.38$) and progesterone ($r = 0.42$), suggesting that a more diverse microbiome is linked to better hormonal regulation.
3. **Women with higher microbiota diversity** had a significantly greater proportion of normal ovulation compared to those with lower diversity (80% vs. 50%).
4. **Multivariate regression analysis** confirmed that gut microbiota diversity independently influenced hormonal levels, even after adjusting for age, BMI, and other lifestyle factors.

These findings suggest that gut microbiota diversity plays a significant role in the regulation of reproductive hormones and ovulatory function in reproductive-aged women.

DISCUSSION

This study aimed to explore the relationship between gut microbiota diversity and ovulatory function in reproductive-aged women, shedding light on the influence of the gut–ovary axis on hormonal regulation and menstrual cycle regularity. The findings indicate that gut microbiota diversity, particularly species richness and the Shannon diversity index, is significantly associated with more regular menstrual cycles, improved hormonal profiles, and better ovulatory function. This section will contextualize these findings within the broader body of literature, examine the potential mechanisms underlying these relationships, and discuss the implications for future research and clinical practice.

Gut Microbiota Diversity and Ovulatory Function

One of the primary findings of this study was the significant association between gut microbiota diversity and ovulatory function. Women with higher microbiota diversity exhibited more regular menstrual cycles, suggesting that a diverse gut microbiome is associated with better hormonal regulation and normal ovulation. This observation is consistent with prior studies that have linked microbiota diversity to improved reproductive health. For example, a study by Kothari et al. (2020) found that women with polycystic ovary syndrome (PCOS), a condition characterized by anovulation and menstrual irregularity, had lower gut microbial diversity compared to healthy controls.

The importance of microbiota diversity in maintaining regular ovulation may be due to the influence of the gut microbiome on the hypothalamic-pituitary-gonadal (HPG) axis. This axis is responsible for regulating the production of reproductive hormones, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, and progesterone. Disruptions in gut microbial composition may lead to imbalances in hormone production, which can manifest as irregular menstrual cycles and anovulation. In this study, women with higher diversity of gut bacteria had a lower average of LH and FSH, hormones typically elevated in women with anovulatory cycles or conditions like PCOS. This suggests that gut microbiota may help regulate these hormones to maintain normal ovulatory function.

Furthermore, the positive correlation between gut microbiota diversity and estradiol and progesterone levels observed in this study is noteworthy. Estradiol and progesterone are essential for the development of the ovarian follicles, ovulation, and the luteal phase of the menstrual cycle. Previous research has suggested that gut-derived metabolites, such as short-chain fatty acids (SCFAs), can influence the production of estrogen and progesterone by modulating the liver's estrogen metabolism. SCFAs, which are produced through the fermentation of dietary fiber by gut bacteria, have been shown to affect the bioavailability of estrogen in the body. Thus, higher microbial diversity may lead to more

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ISSN: (e) 3007-1607 (p) 3007-1593

favorable conditions for the production of these key reproductive hormones.

Mechanisms Linking Gut Microbiota to Reproductive Health

Several mechanisms may explain how gut microbiota diversity influences ovulatory function. First, the gut microbiome can affect the immune system, which plays a critical role in ovarian function. Dysbiosis, or an imbalance in the gut microbiota, has been linked to systemic inflammation and immune dysregulation. Inflammatory cytokines can disrupt hormonal signaling and impair ovarian function. A diverse microbiome, on the other hand, may help regulate immune function and reduce inflammation, contributing to more balanced hormone production and healthier ovaries. This is supported by studies showing that SCFAs produced by gut bacteria can exert anti-inflammatory effects by activating certain receptors in immune cells, such as GPR43 (free fatty acid receptor 2).

Second, gut microbiota may influence the metabolism of estrogens. Estrogen is primarily metabolized in the liver, but certain gut bacteria produce enzymes that can affect the bioavailability of estrogen in the bloodstream. For example, the enzyme β-glucuronidase, produced by some gut bacteria, has been shown to deconjugate estrogen metabolites, making them active again and increasing the overall bioavailability of estrogen. This process is essential for the regulation of the menstrual cycle and ovulation. Therefore, higher gut microbiota diversity could potentially support better estrogen metabolism, resulting in more stable hormonal levels and normal ovulatory function.

Third, the gut–brain axis may also play a role. Gut bacteria communicate with the brain through the vagus nerve and other pathways, potentially influencing the central regulation of reproduction. The gut microbiome has been shown to affect the production of neurotransmitters such as serotonin, which play a role in the regulation of the HPG axis. A diverse gut microbiome may promote a balanced neuroendocrine environment, further supporting the regulation of menstrual cycles and ovulation.

Hormonal Imbalances in Low Microbiota Diversity

In contrast, lower gut microbiota diversity was associated with irregular menstrual cycles, lower estradiol and progesterone levels, and higher LH and FSH levels. These hormonal profiles are characteristic of anovulatory cycles, where ovulation does not occur as expected. Several studies have pointed to the potential role of microbiota dysbiosis in reproductive disorders. For instance, women with PCOS often exhibit a less diverse gut microbiome compared to healthy controls, which may contribute to the hormonal imbalances observed in this condition, including high LH and low progesterone. Additionally, a study by Paternoster et al. (2020) found that dysbiosis in the gut microbiota could be linked to increased levels of pro-inflammatory cytokines, which may disrupt ovarian function and lead to menstrual irregularity.

A lower microbial diversity may also reduce the production of beneficial metabolites such as SCFAs, which play a crucial role in modulating hormonal and immune responses. Without these beneficial metabolites, the hormonal regulation required for ovulation may become impaired, leading to disrupted menstrual cycles and anovulation. This underlines the potential importance of maintaining a healthy and diverse gut microbiome for reproductive health.

Limitations and Future Research

While this study provides valuable insights into the relationship between gut microbiota diversity and ovulatory function, there are several limitations that should be considered. First, the cross-sectional nature of the study precludes any conclusions about causality. Although a strong association between microbiota diversity and menstrual regularity was found, it remains unclear whether gut microbiota diversity directly influences ovulatory function or whether other factors, such as diet, lifestyle, or genetic predisposition, contribute to these observed relationships.

Second, the sample size, although reasonable, was limited to a specific region of Pakistan, which may not be representative of women in other geographical locations or cultures. Future studies with larger and more diverse populations are needed to confirm these findings and assess whether they

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ISSN: (e) 3007-1607 (p) 3007-1593

hold across different ethnic groups and healthcare settings.

Third, while this study controlled for several confounding factors, such as age and BMI, it is possible that other variables, such as dietary patterns, physical activity, and environmental exposures, could have influenced both microbiota diversity and reproductive health. Although multivariate regression analysis was used to adjust for these factors, their potential impact cannot be fully excluded. Longitudinal studies that track changes in microbiota diversity over time in relation to menstrual cycle regularity and hormonal fluctuations would provide more robust evidence of causality.

Finally, although the 16S rRNA gene sequencing method used in this study provides a detailed profile of the bacterial composition of the gut microbiome, it does not capture the full complexity of the microbial ecosystem. Future research using more advanced metagenomic sequencing techniques could provide a deeper understanding of the functional aspects of the microbiome and its role in reproductive health.

Clinical Implications

The findings of this study have important clinical implications for the management of menstrual irregularities and ovulatory dysfunction. Given the growing body of evidence linking gut microbiota to reproductive health, interventions aimed at modulating the gut microbiome, such as prebiotic and probiotic therapies, may hold promise for improving menstrual cycle regularity and ovulatory function. Probiotic supplementation has been shown to improve the gut microbiota composition in women with PCOS, potentially alleviating some of the hormonal imbalances associated with the condition. Similarly, dietary interventions aimed at increasing fiber intake and promoting the growth of beneficial gut bacteria may help support hormonal balance and improve reproductive health.

Moreover, understanding the gut–ovary axis could lead to novel therapeutic strategies for women experiencing infertility or anovulation. For example, personalized microbiome-based treatments could be developed to enhance gut diversity and promote hormonal equilibrium. These approaches could complement traditional fertility treatments and offer a more holistic approach to improving reproductive health.

Conclusion

This study has highlighted a significant association between gut microbiota diversity and ovulatory function in reproductive-aged women. Our findings suggest that a more diverse gut microbiome is linked to more regular menstrual cycles, better hormonal balance, and improved ovulatory function. Specifically, women with higher microbiota diversity exhibited higher levels of estradiol and progesterone, along with lower levels of LH and FSH, which are characteristic of a balanced reproductive system. In contrast, lower gut microbiota diversity was associated with hormonal imbalances, irregular cycles, and signs of anovulation.

These results strengthen the growing body of evidence supporting the idea that the gut–ovary axis plays a critical role in regulating reproductive health. The gut microbiome appears to influence the production and metabolism of key reproductive hormones, possibly through mechanisms involving immune modulation, hormone regulation, and metabolic processes. While the precise biological pathways remain to be fully elucidated, it is clear that gut health is an important factor in maintaining reproductive function.

The findings of this study have significant implications for both clinical practice and future research. From a clinical standpoint, improving gut microbiota diversity may offer a novel therapeutic approach for managing menstrual irregularities and ovulatory dysfunction. Interventions aimed at restoring or enhancing gut microbiota diversity, such as dietary modifications, prebiotics, and probiotics, could potentially help regulate menstrual cycles and improve fertility outcomes. Additionally, future research that examines the long-term impact of microbiome modulation on reproductive health will be crucial for confirming these findings and exploring new avenues for treatment.

In conclusion, this study underscores the importance of the gut microbiome in women's reproductive health. It suggests that maintaining a healthy, diverse gut microbiota may be essential for hormonal balance, menstrual regularity, and normal ovulation. As our understanding of the gut–ovary axis

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ISSN: (e) 3007-1607 (p) 3007-1593

continues to evolve, microbiome-based interventions could play a key role in the future of reproductive health management.

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