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Original Research Article

Estradiol-associated shifts in vaginal microbiota: Insights from menopausal and reproductive women

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Abstract

Introduction: Estradiol plays a key role in maintaining vaginal microbial balance, particularly by supporting Lactobacillus dominance. Hormonal shifts during menopause may disrupt this balance, increasing susceptibility to dysbiosis and related symptoms.
Aim and Objective: This study aims to analyze the correlation between estradiol levels and vaginal microbiota patterns in menopausal and reproductive women.
Materials and Methods: This cross-sectional study involved 59 women (29 menopausal and 30 reproductive-aged). Blood samples were collected to measure serum estradiol using ELISA, and vaginal swabs were analyzed via 16S rRNA gene sequencing. Correlations between estradiol levels and microbiota patterns were assessed using IBM SPSS Statistics Version 30.0.0.0.
Result: Menopausal women showed significantly lower estradiol levels (4.0 vs 100.84 ng/mL, $p<0.001$) and distinct microbiota profiles, including greater diversity (Shannon Index 0.77 vs 0.25, $p=0.031$) and higher dysbiosis prevalence (62.1% vs 10%, $p=0.015$). Anaerobes (Veillonella, Dialister) were more abundant in menopause ($p<0.05$). Estradiol correlated positively with Lactobacillus percentage (Rho=0.334, $p=0.010$) and negatively with diversity (Rho=-0.310, $p=0.017$), though subgroup analyses were non-significant.
Conclusion: Lower estradiol levels are significantly associated with altered vaginal microbiota, including reduced Lactobacillus dominance and increased microbial diversity. While these changes are most evident during the menopausal transition, our findings suggest that menopausal status further modifies this relationship, potentially amplifying the hormonal effects on microbial composition. Together, these results highlight that both declining estradiol levels and menopausal transition collectively contribute to vaginal ecological changes, emphasizing the need for comprehensive hormonal and microbial assessment in management of menopausal problems.

Keywords: Estradiol, Dysbiosis, Menopause, Microbiota.

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1. Introduction

The vaginal microbiome represents a dynamic ecosystem that plays a crucial role in women's reproductive health across the lifespan. Dominated primarily by Lactobacillus species in healthy reproductive-age women, this microbial community maintains vaginal homeostasis through acid production, antimicrobial compound secretion, and immune modulation.[1] This understanding originates with Döderlein's pioneering 1892 identification of vaginal bacilli and their antagonistic effects against pathogens, a discovery that first revealed the protective role of vaginal microbiota.[2] The characteristic low vaginal pH results from Lactobacillus metabolism of epithelial glycogen into lactic acid, creating a barrier against pathogen colonization. Emerging evidence reveals that such host-microbe interactions extend beyond local protection, as microbial-endocrine-immune crosstalk has been implicated in systemic health outcomes, with microbiome-metabolome interactions linked to inflammatory, neoplastic, and even neuropsychiatric conditions.[3] Community state typing

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analyses have identified five distinct vaginal microbiota profiles, four of which show Lactobacillus dominance (*L. crispatus, L. gasseri, L. iners,* or *L. jensenii*), while another (CST-IV) exhibits diverse anaerobic species.[4,5]

The vaginal ecosystem undergoes dynamic microbial colonization beginning within hours after birth and persists throughout a woman's lifespan. During reproductive years, the vaginal environment typically contains 1-4 mL of fluid with a dense bacterial population (10^8-10^9 cells/mL), dominated by glycogen-metabolizing Lactobacillus species under the effect of estrogen hormone.[6] This ecological balance, however, undergoes profound transformation during menopausal transition. Menopause, defined clinically as 12 months of amenorrhea, marks the end of ovarian follicular activity. The circulating estradiol levels drop from reproductive-phase concentrations (19-410 pg/mL) to postmenopausal levels (<35 pg/mL),[7] with estron becoming the predominant estrogen through peripheral aromatization production.[8] This hormonal shift triggers genitourinary syndrome of menopause, characterized by vaginal atrophy, decreased glycogen stores, and rising pH, changes which fundamentally alter the microbial habitat.[9] Studies demonstrate that only 20-30% of postmenopausal women maintain Lactobacillus-dominant microbiota, with most developing diverse anaerobic communities resembling skin flora.[10]

The loss of Lactobacillus-mediated protection, particularly hydrogen peroxide-producing species, correlates with increased vulnerability to bacterial vaginosis (BV), characterized by overgrowth of *Gardnerella vaginalis*, *Prevotella spp*., *Mycoplasma hominis*, and other anaerobes such as *Peptostreptococcus* and *Mobiluncus spp*.[5,11] Estrogen decline drives dysbiosis dominated by *L. iners*, *Anaerococcus*, *Peptoniphilus*, and *Streptococcus*, exacerbating risks of recurrent BV and vulvovaginal candidiasis.[12,13] Furthermore, menopausal dysbiosis associates with elevated proinflammatory cytokines and heightened risks of urinary infections, HIV acquisition, and cervical neoplasia. These microbial alterations also further modulated by lifestyle factors, including dietary patterns, sexual behavior, hygiene practices, contraceptive use, smoking, and obesity as recognized determinants of vaginal microbiota composition.[3,14] While hormonal therapy can partially restore the premenopausal microbiota, recent work by Clabaut et al. (2021) suggests that simple pH correction without microbial recolonization provides incomplete protection, highlighting the complexity of host-microbe-hormone interactions.[1]

Current understanding of these relationships remains limited by traditional culture-based methods that fail to capture over 80% of vaginal microbes. Modern sequencing approaches like 16S rRNA profiling now enable comprehensive characterization of microbial communities.[5,15,16] However, critical gaps persist regarding how circulating hormone levels during menopausal transition influence this microbial pattern. This study therefore aims to explore the relationship between serum estradiol levels and vaginal microbiota composition across reproductive stages.

2. Materials and Methods

2.1. Study design and population

This cross-sectional analytic observational study was conducted in primary health centers in Makassar, Indonesia. The study commenced in November 2023-July 2024 with population comprised married women aged 18-65 years, divided into two groups: menopausal women aged 45-65 years with at least one year of amenorrhea, and reproductive-age women aged 20-50 years with regular menstrual cycles and no current pregnancy. Exclusion criteria included use of hormonal contraceptives or antibiotics within the previous three months, immunosuppressive conditions such as HIV or diabetes mellitus, congenital ovarian anomalies, and current hormone replacement therapy. Demographic characteristics including age, socioeconomic status, education level, parity, body mass index (BMI), and the presence of vulvovaginal symptoms were recorded for all participants. The primary outcomes of the study were the composition of the vaginal microbiota and serum estradiol levels. All laboratory analyses were performed at the Clinical Microbiology Laboratory (HUM-RC) of Hasanuddin University Hospital.

2.2. Vaginal microbiota sampling and assessment

Vaginal specimens were collected by trained obstetricians using sterile polyester-tipped swabs with Amies transport medium. Participants were placed in dorsal lithotomy position and swabs were collected from the posterior fornix while avoiding contact with vaginal walls to minimize contamination. Specimens were immediately placed in transport medium, stored at -20°C until further processing. For DNA extraction, we employed a modified phenol-chloroform protocol with proteinase K digestion. The V3-V4 regions of bacterial 16S rRNA genes were amplified using barcoded primers (16S Barcoding Kit SQK-16S114.24, Oxford Nanopore) and sequenced on MinION/GridION platforms targeting 50,000 reads/sample. Bioinformatic analysis used EPI2ME (v2023.12.1) with NCBI 16S rRNA reference database for taxonomic classification. Microbial parameters included relative abundances (phylum to genus levels), alpha diversity indices (Shannon Index, Richness), and dysbiosis classification (non-Lactobacillus dominance).[5,15-18]

2.3. Estradiol measurement

Venous blood samples (3 mL) were collected via cubital fossa venipuncture using sterile techniques. After clotting, samples were centrifuged at 3000 rpm for 10 minutes, with serum aliquots stored at -80°C until analysis. Serum estradiol levels were quantified using competitive ELISA (Hitachi 7050 analyzer) following manufacturer protocols.

2.4. Statistical analysis

All statistical analyses were performed using SPSS version 25.0 for Mac (IBM Corp., Armonk, NY, USA). Data normality was assessed with Shapiro-Wilk tests, with non-parametric variables reported as medians (IQR) and analyzed using Mann-Whitney U tests. Categorical data were compared via Fisher's exact tests or Chi-square tests as appropriate. Spearman's rank correlation coefficients evaluated relationships between estradiol levels and microbiota parameters. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Study population characteristics

A total of 59 participants were included in the final analysis, consisting of 30 reproductive-age women (median age 38 years, IQR 33-46) and 29 menopausal women (median age 56 years, IQR 54-59; $p < 0.001$). Most participants in both groups had low socioeconomic status and were multiparous, with no significant differences observed. Lower education levels were more frequent in the menopausal group, but the difference was not statistically significant. Among menopausal participants, the median duration of menopause was 6 years (IQR 2–21). The prevalence of vulvovaginal symptoms and median BMI did not differ significantly between groups (**Table 1**).

Table 1: Baseline characteristics of study participants

Subject Characteristics	Groups		P value
	Menopause (n=29)	Reproductive (n=30)	
Age (year)	56 (54-59)	38 (33-46)	**<0.001[a]**
Socioeconomic Status			
Low SES	23 (79.3)	20 (66.7)	0.382[b]
Middle-High SES	6 (20.7)	10 (33.3)	
Education			
Low education level	18 (62.1)	12 (40.0)	0.120[b]
High education level	11 (37.9)	18 (60.0)	
Parity			
Multiparous	25 (86.2)	26 (86.7)	0.367[c]
Primiparous	1 (3.4)	3 (10.0)	
Nulliparous	3 (10.3)	1 (3.3)	
Duration of Menopause (year)	6 (2-21)	-	-
Vulvovaginal symptoms			
Present	10 (34.5)	8 (26.7)	0.580[b]
Absent	19 (65.5)	22 (73.3)	
BMI (kg/m^2)	23.9 (21.2-26.5)	25.3 (22.5-29.1)	0.087[a]

Mann–Whitney U test for non-parametric numerical data,[b] Fisher's exact test for categorical data,[c] Chi-square test for categorical data. SES: Socioeconomic Status; BMI: Body Mass Index.

Table 2: Comparison of vaginal microbiota composition, alpha diversity, and dysbiosis between menopausal and reproductive women

Vaginal Microbiota Profile	Groups		P value
	Menopause (n=29)	Reproductive (n=30)	
Microbiota Abundance in Phylum Level (5 highest)			
Bacillota	8558 (3317-17279)	9143 (3290-17589)	0,885[a]
Pseudomonadota	60 (7–496)	20 (2.75–88)	0.133[a]
Bacteroidota	18.50 (0.001-144)	2.50 (0.001-98)	0.291[a]
Actinomycetota	3 (0.001-11)	0.001 (0.001-2.25)	0.054[a]
Mycoplasmatota	2.5 (0.001-33)	1 (0.001-4.5)	0.233[a]
Microbiota Abundance in Genus Level (5 highest)			
Lactobacillus	3761 (81-18440)	8293 (3112-17375)	0.172[a]
Dialister	45 (4-624)	3 (0-218)	0.052[a]
Streptococcus	33 (1-141)	1.5 (0.001-19.5)	0.065[a]
Veillonella	9 (1-185.5)	1 (0.001-9.5)	**0.034**[a]
Campylobacter	1 (0.001-40)	0.001 (0-11)	0.207[a]
Alpha Diversity			
Shannon Index	0.77 (0.50-1.82)	0.25 (0.23-1.14)	**0.031**[a]

Richness	30 (21.50-51.00)	20.5 (12.00-26.5)	**0.006**[a]
Vaginal Dysbiosis Analysis			
Lactobacillus (%)	73.12 (3.37-87.03)	78.17 (62.36-94.85)	**0.019**[a]
Dysbiosis, n (%)	11 (62.1)	3 (10)	**0.015**[b]
Normal, n (%)	18 (37.9)	27 (90)	

[a]Mann–Whitney U test for non-parametric numerical data, [b]Fisher's exact test for categorical data.

Table 3: Estradiol levels in menopausal and reproductive women

Group	Estradiol (ng/mL)	P value
Menopause (n=29)	4.0 (3.00 – 17.48)	< **0.001**[a]
Reproductive (n=30)	100.84 (53.05 – 226.38)	

[a]Mann–Whitney U test for non-parametric numerical data

Table 4: Estradiol concentrations according to vaginal microbiota status in menopausal and reproductive women

Groups	Estradiol (ng/mL)		P value
	Disbiosis	Normal	
Overall (n=59)	6.49 (2.75 – 19.92)	73.95 (9.47 – 197.32)	0,004[a]
Menopause (n=29)	5.0 (2.00 – 9.46)	4.0 (3.0 – 24.65)	0,465[a]
Reproductive (n=30)	29.43 (23.08 – 128.03)	104.79 (72.07 – 219.80)	0,283 [a]

[a]Mann–Whitney U test for non-parametric numerical data

Table 5: Correlation between serum estradiol levels and vaginal microbiota parameters in menopausal and reproductive groups

Vaginal Microbiota Analysis	Estradiol					
	Overall (n=59)		Menopause (n=29)		Reproductive (n=30)	
	Spearman Correlation (Rho)	P value	Spearman Correlation (Rho)	P value	Spearman Correlation (Rho)	P value
Dysbiosis Rate	0.311	0,017*	0,225	0,240	0,174	0,357
Shannon Index	-0,310	0,017*	-0,193	0,317	-0,234	0,213
Richness	-0,294	0,024*	-0.066	0,735	-0,336	0,070
Lactobacillus %	0,334	0,010*	0,246	0,198	0,234	0,214

3.2. Vaginal microbiota profiles in menopausal vs. reproductive-age women

Distinct vaginal microbiome configurations were observed between reproductive-age and menopausal women. Reproductive-age women maintained a homogeneous ecosystem characterized by pronounced Lactobacillus dominance within the Bacillota phylum (87.3% median relative abundance). In contrast, menopausal women exhibited significantly greater compositional variability (**Figure 1Figure 1**A, **Table 2**), maintaining Bacillota and Lactobacillus as predominant taxa (77.8% and 3,761 copies/µL, respectively) but with emerging clusters of facultative anaerobes.

At the genus level, menopausal participants had significantly higher abundance of Veillonella (9 vs. 1 copies/µL, p = 0.034) and Streptococcus (33 vs. 1.5 copies/µL, p = 0.065), along with borderline increases in Dialister (45 vs. 3 copies/µL, p = 0.052) (**Figure 1**C,**Table 2**). Phylum-level analysis revealed parallel shifts, though Bacillota dominated both groups (87.3% reproductive vs. 77.8% menopause), menopausal women showed expansion of Pseudomonadota (2.13% vs. 0.51%) and Campylobacterota (3.18% vs. 0.14%), phyla containing many anaerobic pathogens (**Figure 1**B, **Table 2**).

These changes corresponded to measurable ecological disruption. Menopausal microbiota displayed threefold higher Shannon diversity (0.77 vs. 0.25, p = 0.031) and 50% greater Richness (30 vs. 20.5 taxa, p = 0.006). The prevalence of dysbiosis (Lactobacillus <50% dominance) was sixfold higher in menopause (62.1% vs. 10.0%, p = 0.015), with most cases showing co-dominance of multiple anaerobic genera (**Table 2**).

3.3. Vaginal microbiota variations linked to vulvovaginal symptoms

Among participants reporting vulvovaginal symptoms (n=12/59), microbial analysis revealed distinct genus-level variations while maintaining overall Lactobacillus predominance in most cases. Women presenting with pruritus (n=8) showed elevated levels of Veillonella, Dialister, and Streptococcus. The two participants reporting abnormal discharge demonstrated increased Streptococcus and Campylobacter. Single cases of vaginal dryness and dyspareunia were characterized by moderate increases in Streptococcus and Dialister respectively, without displacement of Lactobacillus as the primary genus.

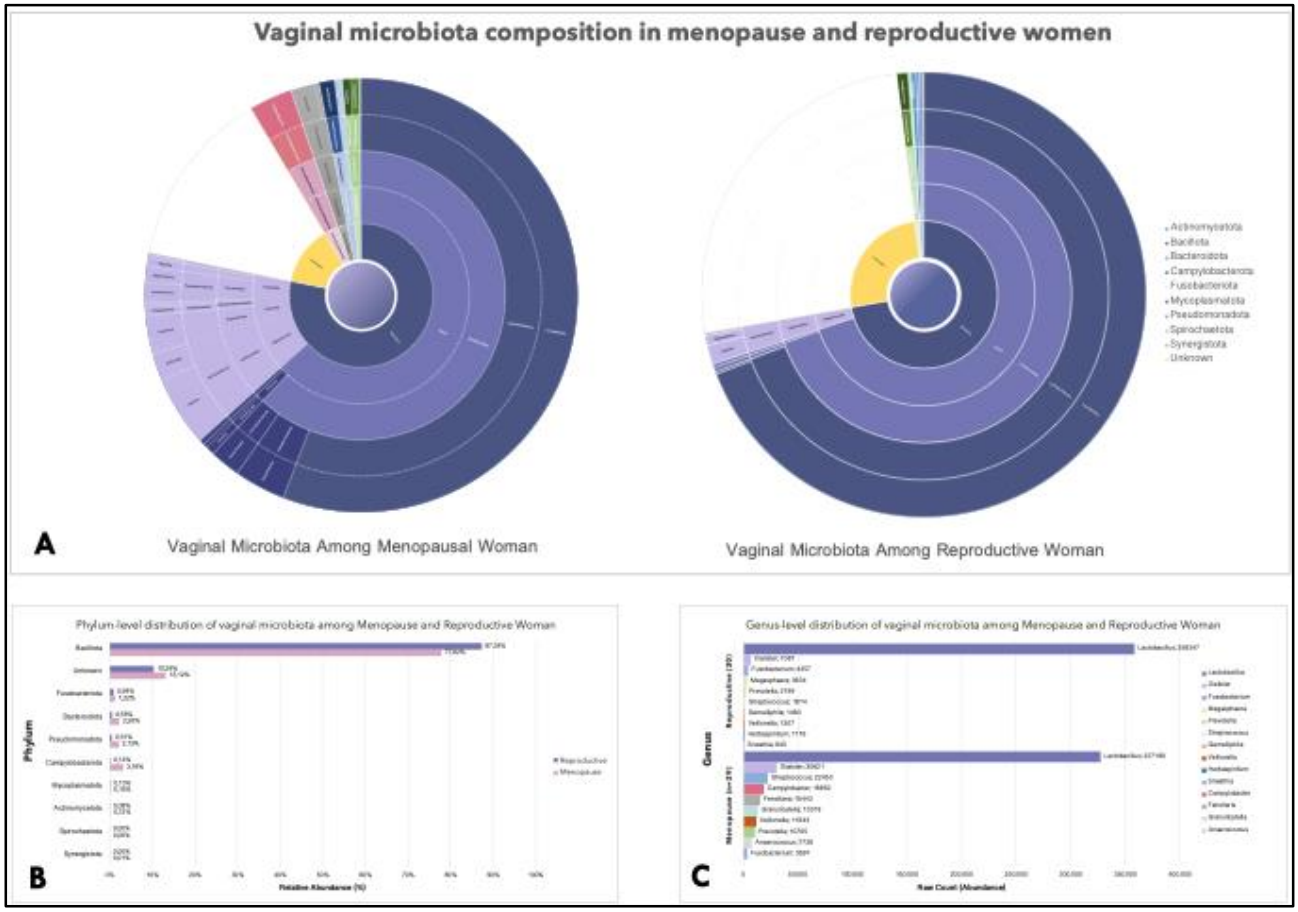

Figure 1: Vaginal microbiota composition in reproductive and menopausal women; **A:** Comparison of taxonomic profile (sunburst diagram); **B:** Phylum-level relative abundance; **C:** Genus-level relative abundance.

3.4. Serum estradiol levels across menopausal status

A notable difference in circulating estradiol levels was observed between groups. Menopausal women exhibited significantly lower median serum estradiol concentrations (4.0 ng/mL, IQR 3.00-17.48) compared to reproductive-age participants (100.84 ng/mL, IQR 53.05-226.38; $p < 0.001$). This represented a 25-fold difference in median values between groups (**Table 3**). The distribution pattern revealed complete separation between populations, with no overlap in the interquartile ranges.

3.5. Associations between vaginal microbiota and circulating estradiol

Analysis of the overall cohort revealed significant relationships between vaginal microbiota composition and serum estradiol levels. Women exhibiting dysbiosis demonstrated markedly lower median estradiol concentrations (6.49 ng/mL, IQR 2.75-19.92) compared to those with normal microbiota (73.95 ng/mL, IQR 9.47-197.32; p=0.004) (**Table 4**). This pattern was particularly evident in Spearman correlation analyses, which showed positive associations between estradiol levels and both Lactobacillus percentage (Rho=0.334, p=0.010) and normal microbiota status (Rho=0.311, p=0.017), alongside inverse correlations with microbial diversity metrics (Shannon Index: Rho=-0.310, p=0.017; Richness: Rho=-0.294, p=0.024). However, when examined separately by menopausal status, these associations were not observed, with neither the menopausal nor reproductive-age subgroups showing statistically significant relationships between estradiol and microbiota parameters (all $p>0.05$). Notably, among reproductive-age women with dysbiosis, median estradiol levels were numerically lower (29.43 vs 104.79 ng/mL in normal microbiota) though this difference did not reach statistical significance (p=0.283). Similarly, in menopausal women, estradiol levels showed minimal variation between dysbiotic and normal microbiota groups (5.0 vs 4.0 ng/mL, p=0.465) (**Table 5**).

4. Discussion

The vaginal microbiome's dependence on estrogenic support has long been hypothesized, yet direct comparisons of circulating estradiol-microbiota relationships across reproductive stages remain limited. Our study of 59 women (29 menopausal, 30 reproductive) bridges this gap by demonstrating that the dramatic estradiol decline in menopause coincides with a destabilization of the vaginal

ecosystem characterized by reduced Lactobacillus dominance and a sixfold higher dysbiosis prevalence. While both groups retained Bacillota as the dominant phylum, menopausal participants exhibited significant expansions of facultative anaerobes (Veillonella, Dialister) and threefold higher microbial diversity. Notably, while overall correlations linked estradiol to Lactobacillus abundance and diversity, subgroup analyses revealed no significant associations, suggesting that the abrupt hormonal shift of menopause itself, rather than incremental estradiol fluctuations, may be the critical disruptor of vaginal microbial hemoeostasis.

The observed microbial transitions in menopause reflect a confluence of biological and social determinants. While the typical menopausal age (median 56 years) and duration (6 years) in our cohort align with global patterns the high prevalence of low socioeconomic status (~70% overall) and limited education likely compound dysbiosis risk by restricting access to preventive care and health literacy, a disparity exacerbated in resource-limited settings.[19,20] Multiparity in this study (86% of participants) may confer structural and immunological changes that subtly prime the vaginal microenvironment for instability, as nulliparity has been linked to resilient *Lactobacillus crispatus* dominance.[21] The marginally higher BMI in reproductive women (25.3 vs. 23.9 kg/m²) hints at adipose tissue's role in modulating residual estrogen synthesis via aromatase activity,[22] though this did not reach significance.

Our study confirmed significant differences in serum estradiol levels between menopausal (median 4.5 ng/mL) and reproductive-aged women (median 100.84 ng/mL; $p < 0.001$), consistent with previous reports.[23] This profound estrogen decline during menopause triggers systemic changes including altered lipid metabolism, glucose intolerance, and vascular endothelial dysfunction, while locally contributing to vaginal epithelial thinning, reduced glycogen production, and subsequent microbiota alterations.[23] This highlights estradiol's central role in maintaining genital tract homeostasis. Interestingly, while reproductive women experience natural estradiol fluctuations throughout the menstrual cycle, these variations do not typically result in dysbiosis, suggesting the existence of protective threshold mechanisms that maintain microbial stability despite hormonal cycling.

Our results demonstrate significant vaginal microbiota alterations during menopausal transition, showing reduced Lactobacillus dominance (77.8% vs 87.3% in reproductive women) and increased dysbiosis prevalence (62.1% vs 10%). These changes were accompanied by elevated microbial diversity and emergence of anaerobic genera (Veillonella, Dialister), consistent with some reports of menopausal microbial restructuring.[24] The pathogenesis of these shifts involves multiple interrelated mechanisms, where estrogen withdrawal reduces epithelial glycogen deposition, limiting substrate for Lactobacillus fermentation while elevating vaginal pH above the optimal range for lactobacilli survival.[25] This pH shift creates an ecological niche favoring anaerobes through both direct competition[26] and indirect effects on host immunity, particularly reduced secretory IgA and altered defensin profiles that normally suppress pathogen growth. The result imbalance creating opportunities for opportunistic pathogen growth and process to trigger chronic inflammation that underlies various vulvovaginal complaints including abnormal discharge, pruritus, erythema, pain, or dysuria.[27]

The persistence of Lactobacillus as the predominant genus despite dysbiosis suggests a transitional "dysbiotic adaptation" phase, where residual estrogen activity may delay complete ecological collapse.[28] This contrasts with studies reporting near-total Lactobacillus depletion potentially reflecting population-specific differences in mucosal resilience or alternative estrogen sources such as adipose tissue aromatization. Clinically in our study, the association of specific anaerobes with symptoms (Streptococcus with discharge; Dialister with dyspareunia) exemplifies how microbial changes propagate genitourinary syndrome pathogenesis, where anaerobes activate TLR2/4-mediated inflammation, increase proinflammatory cytokines, and degrade protective mucins leading to specific local symptoms.[27] These findings underscore menopause as a multisystem transition where hormonal, microbial, and immunological changes collectively drive symptomatic presentations, suggesting future therapies should target all three axes simultaneously.

The correlation analyses revealed several key relationships: estradiol levels showed a positive association with Lactobacillus abundance (Rho=0.334, p=0.010) and negative associations with both dysbiosis (Rho=0.311, p=0.017) and microbial diversity (Shannon Index Rho=-0.310, p=0.017). These findings are supported by Clabaut et al. (2021) who demonstrated that 17β-estradiol enhances *Lactobacillus crispatus* adhesion to vaginal epithelium through specific membrane receptors.[1] However, the maintenance of normal microbiota in 37.9% of our menopausal participants despite low estradiol levels indicates additional regulatory factors, likely including estrone, the predominant estrogen in menopause that was not measured in our study.[29] This observation is consistent with clinical evidence that estrogen therapy can restore Lactobacillus dominance in postmenopausal women, though individual responses vary considerably.

Notably, subgroup analyses revealed no significant estradiol-microbiota correlations within either group (all $p > 0.05$). The absence of significancy suggests complex, non-linear relationships between estrogen levels and microbial composition. In menopause, this may reflect the limited dynamic range of estradiol levels below a critical threshold needed to influence microbial populations. The potential role of estrone as the dominant estrogen in menopause,[29] along

with unmeasured factors such as duration of estrogen deficiency or individual differences in mucosal estrogen sensitivity, likely contribute to this variability. While reproductive women showed a non-significant positive trend (Lactobacillus vs estradiol: Rho = 0.319, p = 0.086), hormonal therapy studies demonstrate that estrogen restoration can recover Lactobacillus dominance suggesting menopause status itself may be the primary dysbiosis driver rather than absolute estradiol levels.

This study has several strengths. It is novel in being the first to compare the relationship between serum estradiol levels and vaginal microbiota composition in both menopausal and reproductive-age women. The use of 16S rRNA gene sequencing also allowed for a comprehensive characterization of the vaginal microbiota, enhancing the depth and accuracy of microbial analysis. However, the study has some limitations. The cross-sectional design limits the ability to observe longitudinal changes or infer causal relationships between estradiol decline and dysbiosis. Additionally, only serum estradiol was measured, without assessing other estrogenic forms such as estrone and estriol, which may also influence the vaginal microbiome particularly in menopausal women. Future studies should investigate the underlying mechanisms linking estrogen decline to microbiota shifts, including the roles of immune modulation, glycogen metabolism, and interactions with pathogenic microbes. Larger sample sizes, longitudinal designs, and the inclusion of vaginal pH and broader estrogen profiling will be essential for understanding hormonal-microbial dynamics.

5. Conclusion

Our findings highlight a significant association between hormonal status and vaginal microbial ecosystems. Lower estradiol levels were linked to reduced Lactobacillus dominance and greater microbial diversity, particularly during the menopausal transition. While these patterns were more pronounced when analyzing all participants, they became less distinct when reproductive-age and menopausal women were evaluated separately, suggesting menopausal status may be the primary driver of microbial shifts, rather than estradiol levels alone. The persistence of Lactobacillus in symptomatic reproductive-age women contrasts with its depletion in menopause, underscoring the role of reproductive phase in shaping the vaginal microenvironment. These results emphasize the importance of considering both hormonal context and local microbial ecology in maintaining vaginal health and highlight the relevance of menopausal status in clinical assessments, while acknowledging the potential influence of additional factors.

6. Ethical Committee Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of Hasanuddin University (No. 988/UN4.6.4.5.3L/PP36/2023).

7. Conflict of Interest

The authors declare no conflict of interest

8. Source of Funding

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