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Original article

The effect of hormone therapy on vaginal microbiota in women with genitourinary syndrome of menopause: A double-blind, randomized, placebo-controlled trial

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ABSTRACT

This double-blind, randomized, placebo-controlled trial investigated the effects of oral combined menopausal hormone therapy (MHT) on vaginal microbiota and clinical outcomes in postmenopausal women with genitourinary syndrome of menopause (GSM). Thirty-four healthy postmenopausal women aged 40–60 years with moderate to severe GSM were randomized to receive either oral estradiol 1 mg/dydrogesterone 5 mg (Femoston conti®) or placebo daily for 12 weeks. Vaginal microbiota was assessed at baseline and week 12 using 16S rDNA gene sequencing. Clinical outcomes, including the most bothersome symptom (MBS), vaginal atrophy score (VAS), vaginal pH, vaginal health index (VHI) and vaginal maturation value (VMV), were evaluated at baseline, week 6, and week 12. The MHT group demonstrated significant more improvement in all clinical measures compared with placebo: MBS ($p = 0.008$), VAS ($p < 0.001$), vaginal pH ($p = 0.001$), VHI ($p < 0.001$) and VMV ($p < 0.001$). However, no significant differences were observed in microbial composition or diversity between groups after treatment. These findings suggest that while systemic hormone therapy provides meaningful symptom relief and improves vaginal tissue health, these clinical benefits can occur without concurrent, statistically significant changes in the vaginal microbiota. This supports the clinical value of early systemic MHT in managing GSM, even in the absence of microbiome restoration.

1. Introduction

Genitourinary syndrome of menopause (GSM) is a common yet often overlooked condition affecting many postmenopausal women [1,2]. Studies indicate that up to 87.2% of postmenopause in Thailand experience symptoms associated with GSM, with the prevalence increasing with age [3]. Despite its high incidence, GSM remains underrecognized, as many women perceive these symptoms as a natural part of aging that does not require treatment [4]. Additionally, healthcare providers may fail to fully appreciate the significant impact GSM has on a woman's physical and emotional well-being. Symptoms such as vaginal dryness, urinary incontinence, dyspareunia, and increased vulnerability to infections severely affect a woman's quality of life and can cause relationship and emotional distress. [5,6].

The pathophysiology of GSM is closely linked to estrogen deficiency following the cessation of ovarian function. This hormonal decline results in thinning of the vaginal epithelium, reduced moisture, and changes to the vaginal microbiota, particularly a decrease in Lactobacillus species. [7,8]. This imbalance increases the risk of bacterial vaginosis and other infections. While effective treatments exist, many women do not receive appropriate care due to a lack of awareness and the stigmatization of menopause-related symptoms [9,10].

Several clinical guidelines suggest various approaches for the management of GSM, including the use of moisturizers, lubricants, and local

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estrogen therapies to restore vaginal moisture and elasticity [1,5,11,12]. Systemic menopause hormone therapy (MHT) can also improve GSM symptoms but is generally reserved for women who experience other menopause-related issues, such as hot flashes [1].

While the symptomatic benefits of vaginal estrogen on GSM are well established, much less is known about how systemic hormone therapy affects the vaginal microbiome in postmenopausal women. To date, there is a notable lack of clinical trials specifically examining whether giving oral estrogen and progesterone therapy can modulate the composition of the vaginal microbiota using molecular-based techniques in women with GSM. Most research on the vaginal environment in menopause has focused on local treatments [13], other type of estrogens [14–16] or observational comparisons of women on hormone therapy versus none [17–20]. While local estrogen therapy has been shown to improve the vaginal microbiota, the effects of systemic hormone therapy have not been sufficiently studied [21]. Understanding whether systemic estrogen and progesterone therapy influences the vaginal microbiota could help refine GSM treatments.

This study aims to investigate the effects of systemic hormone replacement therapy on the vaginal microbiota in postmenopausal women using high-throughput DNA sequencing. By analyzing the microbial composition before and after systemic hormone therapy, this research will provide insights into how hormone therapy may alter the vaginal environment and affect GSM symptoms.

The findings may contribute to a better understanding of how systemic hormone replacement therapy influences the vaginal microbiota, potentially leading to more targeted and effective treatments for GSM, ultimately improving the quality of life for postmenopausal women.

2. Materials and methods

2.1. Study design and ethical consideration

This study was a prospective, double-blind, randomized, placebo-controlled trial conducted at the Gender Health Clinic, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. This study received ethical approval from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA No.0167/2025, IRB No.804/63), and the Human Research Ethics Committee of the Faculty of Medicine, Thammasat University (COA No.092/2568). All research procedures complied with internationally recognized ethical guidelines for human research, including the Declaration of Helsinki, the Belmont Report, the CIOMS International Ethical Guidelines, and the ICH-GCP standards.

2.2. Study participants

Eligible participants were healthy postmenopausal women aged 40–60 years, defined by the absence of menstruation for at least 12 consecutive months and serum follicle-stimulating hormone levels ≥40 IU/L. Participants were required to have at least one GSM symptom (vaginal dryness, itching/irritation, soreness, or dyspareunia) and moderate to severe vasomotor symptoms. All participants provided written informed consent prior to enrollment.

Exclusion criteria included prior use of systemic or local hormone therapy, vaginal lubricants or moisturizers within the last 3 months; recent vaginal procedures or surgery; active vaginal infection; history of hormone-dependent malignancy, thromboembolic disorders, liver or kidney disease; and use of antibiotics or probiotics within one month prior to the study.

2.3. Study protocol

2.3.1. Randomization and blinding

Participants were randomized in block of four with a 1:1 ratio to receive either the study drug or placebo using permuted block randomization. Both participants and investigators were blinded to group allocation. The placebo was matched in size, shape, and color to the active drug to ensure blinding.

2.4. Intervention

The intervention group received a daily oral tablet containing 17β-estradiol (1 mg) and dydrogesterone (5 mg) (Femoston conti®) for 12 weeks. The control group received a placebo tablet identical in appearance. Participants were instructed to take the tablet once daily at bedtime.

2.5. Sample collection and DNA extraction

Vaginal swabs were collected at baseline and 12 weeks post-treatment using sterile cotton swabs from the upper lateral vaginal wall and placed in 1 mL of NAPseq (BioEntist, Thailand). Total DNA was extracted using ZymoBIOMICS™ DNA Miniprep Kit (ZYMO Research, USA) following the manufacturer's protocol. The DNA was stored at −20 °C until further processing.

2.6. 16S rDNA sequencing based on Oxford Nanopore Technologies

The bacteria full-length 16S rDNA (V1-V9 regions) was amplified using the primers from a previous study (https://doi.org/10.1038/s41598-024-53880-w). The PCR reaction contained 50–100 ng of DNA template, 10 μM of each forward and reverse primer, 1× KOD OneTM PCR master Mix (Toyobo Biotech, Japan), and nuclease-free water. The thermal condition for PCR was 98 °C for 2 min, 30 cycles of 98 °C for 10 s, 60 °C for 10 s, 68 °C for 10 s, and a final extension at 68 °C for 5 min. The PCR products were subsequently amplified by 5 cycles of barcode PCR (98 °C for 10 s, 60 °C for 10 s, 68 °C for 10 s) with PCR Barcoding Expansion 1–96 (EXPPBC096) kit (Oxford Nanopore Technologies, UK). The products were observed by 1% agarose gel electrophoresis and were purified using the HiYield™ Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience, Taiwan). The products were measured for quantity using a Qubit 4 fluorometer with a QubitTM dsDNA HS Assay Kit (Thermo Fisher Scientific, USA). Then, DNA library was equimolarly pooled (1 μg in the total volume of 48 μL) and size-selected using and purified using 0.5× Agencourt AMPure XP beads (Beckman Coulter, USA). After that, the purified DNA library was end-repaired and adaptor-ligated using a Ligation Sequencing Kit (SQK-LSK114) and sequenced on the R10.4.1 flow cell using the MinION Mk1B platform (Oxford Nanopore Technologies, UK).

2.7. Data processing of vaginal microbiota

Raw reads (FAST5) were basecalled using Guppy v6.5.7 (Oxford Nanopore Technologies, UK) with supper-accuracy basecalling model (SUP) and quality score threshold of >15 (https://doi.org/10.1186/s13059-019-1727-y). The FASTQ reads were demultiplexed and adaptor-trimmed using Porechop v0.2.4 (https://github.com/rrwick/Porechop). Filtered reads underwent clustering, polishing, and taxonomic identification using NanoCLUST65 pipeline, targeting the V1–V9 regions of 16S rDNA gene sequences from the Ribosomal Database Project (RDP) (https://doi.org/10.1093/nar/gkg039). The data were then converted into QIIME2 format v2021.266 to generate a collapsed taxa table. Relative abundance analysis, alpha diversity (Chao1 and Shannon indices), beta diversity were performed using MicrobiomeAnalyst (https://doi.org/10.1038/s41596-019-0264-1).

In addition to microbiota profiling, several clinical outcomes were assessed to evaluate the effect of treatment. These included the participant's most bothersome symptoms (MBS) [22], vaginal pH [23], vaginal atrophy score (VAS) [24], vaginal maturation value (VMV) [25], and Vaginal Health Index (VHI) [26]. These parameters were measured at three time points: baseline, 6 weeks, and 12 weeks post-treatment. All

vaginal samples were collected by a single researcher. Cytological and microbial assessments were evaluated by a single pathologist and microbiome analyst to reduce inter-observer bias. Further methodological details are available in the supplementary material file.

2.8. Statistical analysis

Baseline characteristics were summarized using descriptive statistics. Continuous variables were summarized as mean ± standard deviation and median (25th and 75th percentiles) for normally distributed and non-normally distributed continuous variables, and as the frequency and percentages for categorical variables. Chi-squared test and Fisher's exact test were used to compare categorical variables, while the Student's *t*-test and Mann–Whitney *U* test were used to assess continuous variables with normal and skewed distributions, respectively.

Longitudinal changes in clinical outcomes (MBS, VHI, VMV, vaginal pH, and VAS) and microbiota diversity indices were assessed using a linear mixed-effects model with an unstructured correlation matrix to account for repeated measures over time. Due to the absence of significant differences in microbial community composition between groups, further differential abundance analysis was not performed. Adverse events were analyzed descriptively. Statistical analyses were performed in Stata 17.0 software (StataCorp, College Station, TX, USA). *P*-value 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

A total of 34 postmenopausal women were enrolled and randomized equally into the oral MHT (Femoston conti®) group and the placebo group. Each group consisted of 17 participants. The baseline demographic and clinical characteristics were generally well-balanced between the two groups, as shown in Table 1. The mean age was 55.59 ± 2.96 years in the MHT group and 54.29 ± 4.12 years in the placebo group ($p = 0.301$). The median time since menopause was comparable, and there were no significant differences in BMI, parity, marital status, or history of underlying diseases. However, two symptoms were reported at significantly different rates between groups at baseline. Vaginal irritation was more prevalent in the oral MHT group (52.9% vs. 17.6%, $p = 0.031$), whereas dyspareunia was more common in the placebo group (47.1% vs. 5.9%, $p = 0.017$). Other GSM-related symptoms such as vaginal dryness and soreness were not significantly different at baseline.

3.2. Primary outcome: vaginal microbiota composition

No statistically significant differences were observed in the overall composition or diversity of the vaginal microbiota between the MHT and placebo groups following 12 weeks of treatment shown in Fig. 1 and Fig. 2. Analysis of alpha diversity metrics, including Shannon and Chao1 indices, showed no significant changes in microbial richness or evenness within or between groups. Similarly, beta diversity assessment using Bray–Curtis dissimilarity showed no clear separation in microbial community structure between treatment arms at 12-week endpoint (Fig. 3).

In addition, no significant changes in the relative abundance of dominant bacterial taxa, including Lactobacillus spp., Gardnerella, Atopobium, or Prevotella, were observed following treatment in either group. These findings suggest that 12 weeks of oral estrogen–progestin therapy did not significantly alter the vaginal microbial community structure in this cohort of postmenopausal women.

3.3. Secondary outcomes

In contrast to the primary outcome, which showed no significant

Table 1
Baseline characteristics.

Characteristics	Oral MHT (*N* = 17)		Placebo (N = 17)		p-Value	
Age (years)	55.70 ± 2.76		54.42 ± 3.99		0.250	a
Age of menopause (years)	50.20 ± 3.81		50.21 ± 3.14		0.993	a
Years since menopause (years)	5	(2.5–6.5)	2	(2–6)	0.270	b
Body weight (kilogram)	62.99 ± 10.14		62.63 ± 8.93		0.909	a
Height (centimeter)	157.73 ± 6.00		154.71 ± 4.91		0.095	a
Body mass index (kg/m^2)	25.26 ± 3.25		26.28 ± 4.53		0.419	a
Normal (18.5–22.9 kg/m^2)	6	(30.0)	5	(26.3)	1.000	d
Overweight (23.0–24.9 kg/m^2)	4	(20.0)	3	(15.8)		
Obesity I (25.0–29.9 kg/m^2)	8	(40.0)	9	(47.4)		
Obesity II (≥30.0 kg/m^2)	2	(10.0)	2	(10.5)		
Marital status						
Married	17	(85.0)	15	(78.9)	0.955	d
Single	2	(10.0)	1	(5.3)		
Widowed	1	(5.0)	2	(10.5)		
Divorce	0	(0.0)	1	(5.3)		
Parity						
Nulliparous	5	(25.0)	5	(26.3)	1.000	d
Multiparous	15	(75.0)	14	(73.7)		
Underlying disease	14	(70.0)	15	(78.9)	0.716	d
Diabetes	4	(20.0)	5	(26.3)	0.716	d
Hypertension	8	(40.0)	5	(26.3)	0.365	c
Dyslipidemia	5	(25.0)	6	(31.6)	0.648	c
Allegic rhinitis	2	(10.0)	0	(0.0)	0.487	d
Thyroid disease	1	(5.0)	1	(5.3)	1.000	d
Rheumatoid arthritis	0	(0.0)	1	(5.3)	0.487	d
Asthma	1	(5.0)	0	(0.0)	1.000	d
Others	1	(5.0)	4	(21.1)	0.182	d
Medication	10	(50.0)	14	(73.7)	0.129	c
Active sexual activity						
Last sexual activity, (*n* = 33)						
<1 Week	2	(11.8)	3	(18.8)	0.731	d
1 Week	4	(23.5)	2	(12.5)		
>1 Week - 1 Month	3	(17.6)	3	(18.8)		
>1 Month - 1 Year	4	(23.5)	2	(12.5)		
>1 Year - 10 Years	2	(11.8)	5	(31.3)		
>10 Years	2	(11.8)	1	(6.3)		
Frequency of sexual activity, (*n* = 28)						
3–4 Time per month	2	(13.3)	3	(23.1)	0.778	d
2–3 Times per month	3	(20.0)	2	(15.4)		
1–2 Times per month	4	(26.7)	2	(15.4)		
Once a month	4	(26.7)	3	(23.1)		
Once a year	2	(13.3)	1	(7.7)		
Less than once a year	0	(0.0)	2	(15.4)		
Vulvovaginal symptoms and signs at baseline						
The most bothersome symptoms	9.00 ± 2.36		9.11 ± 1.97		0.881	a
Vaginal pH	6.50 ± 0.61		6.32 ± 0.56		0.331	a
Vaginal atrophy score	11.75 ± 1.71		12.11 ± 2.05		0.560	a
Vaginal health index	7.70 ± 1.87		7.95 ± 1.99		0.691	a
Vaginal maturation values	59.63 ± 27.18		54.15 ± 34.46		0.610	a
Dryness	18	(90.0)	18	(94.7)	1.000	d
Soreness pain	8	(40.0)	9	(47.4)	0.751	c
Irritation	10	(50.0)	3	(15.8)	**0.023**	c
Dyspareunia	2	(10.0)	9	(47.4)	**0.010**	c

SD = Standard deviation, **%** = Percentage, N = Sample size, BMI = Body mass index
IQR = Interquartile range, pH = Potential of hydrogen.
Data are presented as number (%), mean ± standard deviation or median (interquartile range).
P-value corresponds to [a]Independent samples t-test, [b]Mann-Whitney U test, [c]Chi-square test or [d]Fisher's exact test.

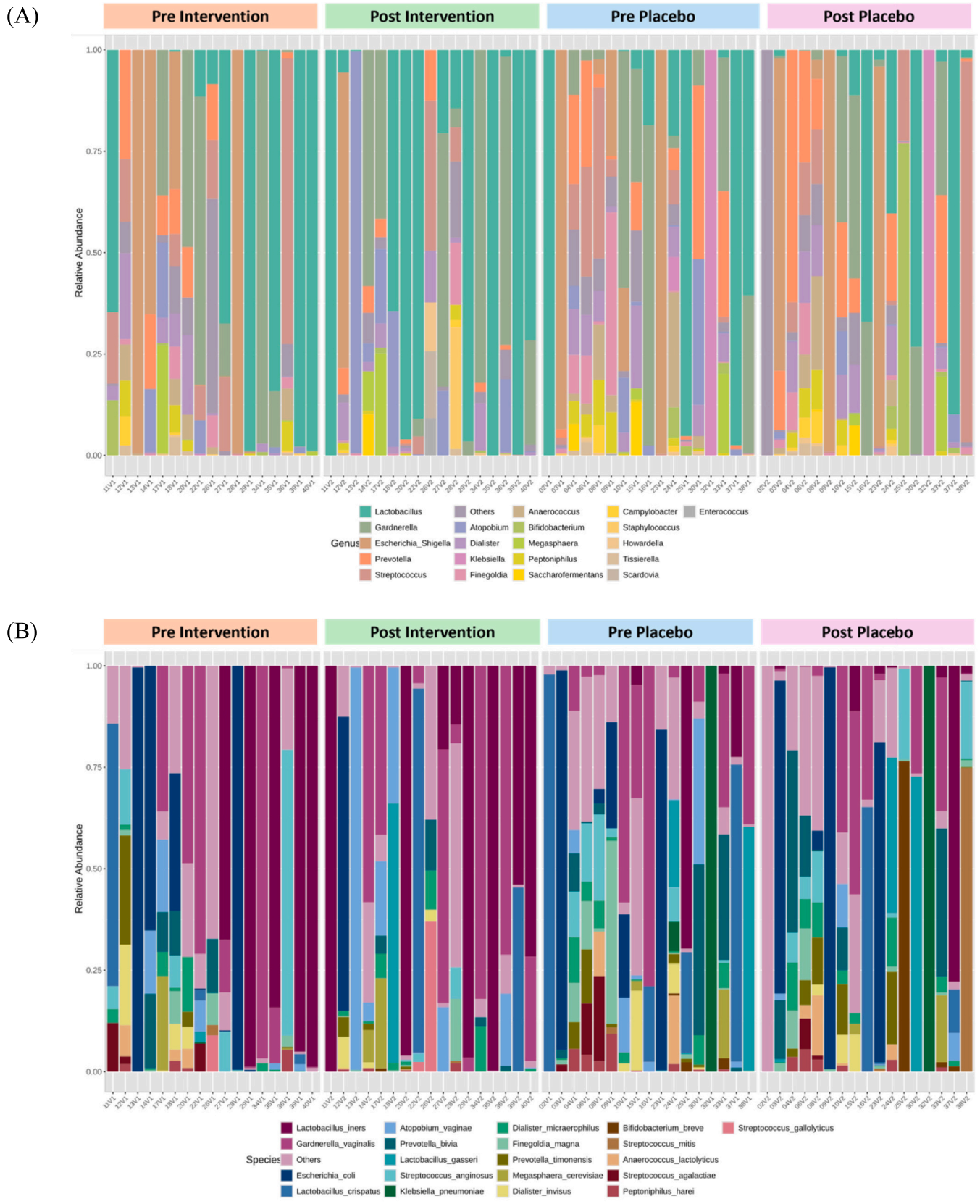

Fig. 1. Relative abundance of the vaginal microbiota displayed at the (A) Genus level and (B) Species level.

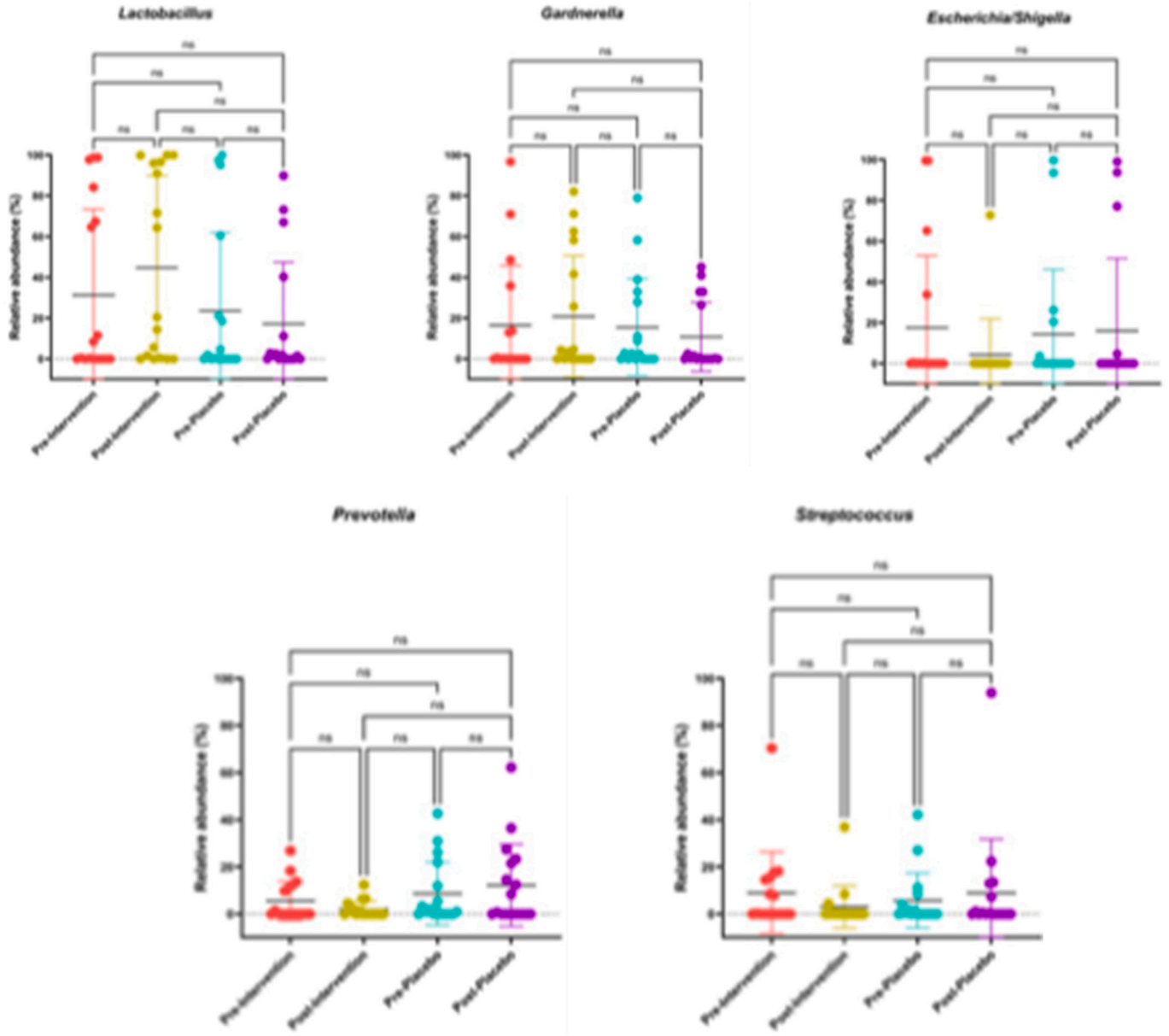

Fig. 2. Comparison of the Relative Abundance of the Top 5 most abundant bacterial genera in the vaginal microbiota.

difference in vaginal microbiota composition, the secondary outcomes revealed statistically significant and clinically meaningful benefits from systemic hormone therapy, shown in Table 2 and Fig. 4. Participants who received oral MHT reported significantly greater improvements in GSM over the 12-week period. At study completion, the MBS score in the intervention group decreased to 0.35 ± 0.86, compared to 2.12 ± 2.67 in the placebo group (mean difference − 1.77, 95% CI: −3.08 to −0.45; $p = 0.008$). Similarly, the VAS dropped dramatically in the MHT group (2.29 ± 2.20) relative to placebo (10.29 ± 3.69), reflecting an 8-point mean difference, 95% CI: −10.03 to −5.97; $p < 0.001$). These results highlight the strong symptomatic improvement perceived by participants receiving hormone therapy.

These subjective improvements were corroborated by objective findings. The VHI, which evaluates tissue elasticity, moisture, epithelial integrity, and pH, increased markedly in the MHT group. By week 12, the VHI score had risen to 20.24 ± 3.17, compared to only 8.65 ± 3.60 in the placebo group (mean difference 11.59, 95% CI: 9.09 to 14.09; $p <$ 0.001). This represents a clinically significant restoration of vaginal tissue condition. Additionally, vaginal pH declined in the MHT group to 5.03 ± 0.37, whereas it remained elevated in the placebo group at 6.24 ± 1.64, 95% CI: −1.92 to −0.49; $p = 0.001$), indicating a re-acidification of the vaginal environment likely attributable to estrogenic effects. While the microbiota composition remained largely unchanged, this biochemical shift may contribute to a more favorable vaginal milieu.

Further supporting these findings, the VMV, a cytological indicator of estrogenic effect on the vaginal epithelium, marked improved in the MHT group up to 93.49+ 7.45 whereas the value decreased in the placebo group to 43.24 + 38.60, 95% CI: 32.26 to 68.24; $p < 0.001$). This cytological response reinforces the physiological impact of systemic estrogen on vaginal tissue renewal and differentiation. Altogether, these results demonstrate that while oral MHT may not significantly alter the vaginal microbiome within a 12-week period, it produces substantial clinical and histological benefits, confirming its therapeutic role in the management of GSM.

3.4. Adverse events

Adverse events were more commonly reported in the MHT group as

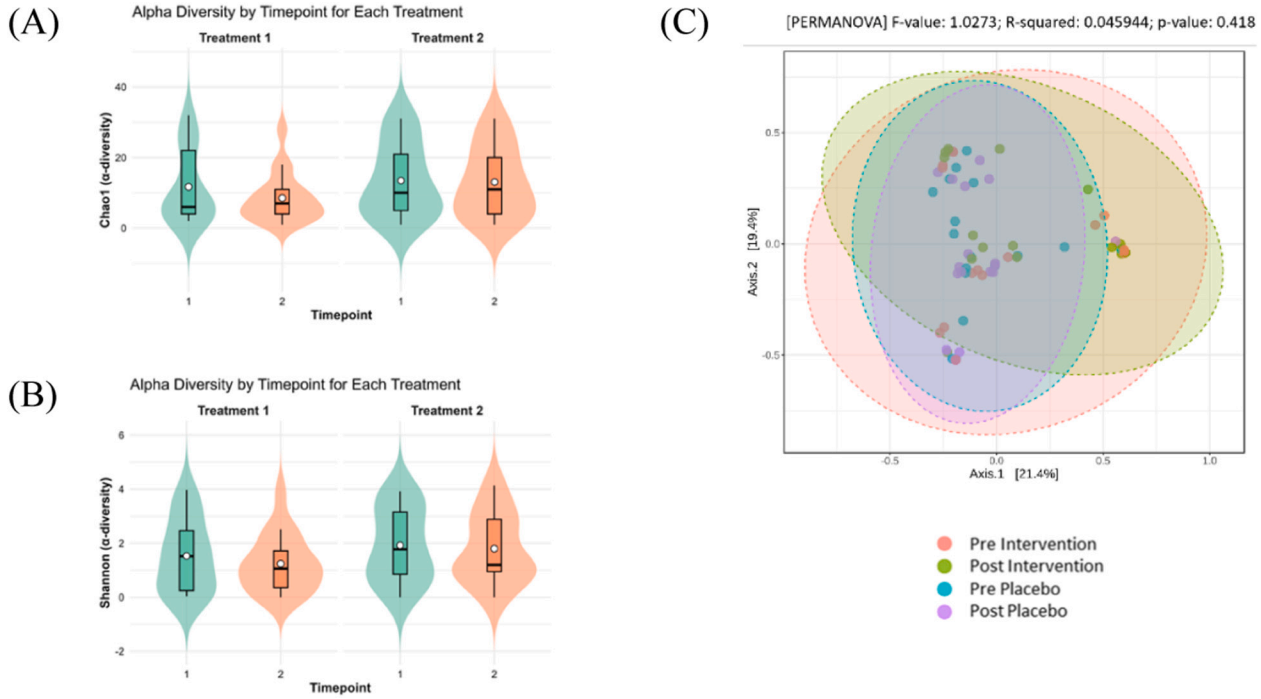

Fig. 3. Microbial diversity analysis, including (A) Chao1 and (B) Shannon Alpha-diversity indices analyzed by Linear Mixed Model, and (C) Beta-diversity index based on Bray-Curtis's dissimilarity with PERMANOVA.

Table 2
Secondary outcome.

Outcomes	Oral MHT ($N = 17$) Mean ± SD	Placebo (N = 17) Mean ± SD	Difference between groups (95%CI)		p-Value
The most bothersome symptom (MBS)					
Baseline	8.88 ± 2.45	9.29 ± 1.99	−0.41	(−1.78, 0.95)	0.555
6 weeks	0.65 ± 0.86	1.88 ± 2.29	−1.24	(−2.57, 0.10)	0.070
12 weeks	0.35 ± 0.86	2.12 ± 2.67	−1.77	(−3.08, −0.45)	0.008
Vaginal atrophy scores (VAS)					
Baseline	11.75 ± 1.71	12.11 ± 2.05	−0.36	(−1.62, 0.91)	0.581
6 weeks	2.89 ± 1.88	11.06 ± 2.86	−7.53	(−9.22, −5.84)	<0.001
12 weeks	2.29 ± 2.20	10.29 ± 3.69	−6.83	(−9.08, −4.58)	<0.001
Vaginal pH					
Baseline	6.50 ± 0.61	6.32 ± 0.56	0.18	(−0.17, 0.54)	0.304
6 weeks	4.94 ± 0.34	6.56 ± 0.77	−1.49	(−2.00, −0.99)	<0.001
12 weeks	5.03 ± 0.37	6.24 ± 1.64	−1.06	(−1.78, −0.34)	0.004
Vaginal health index (VHI)					
Baseline	7.70 ± 1.87	7.95 ± 1.99	−0.25	(−2.36, 1.87)	0.819
6 weeks	17.89 ± 5.19	8.82 ± 4.03	8.27	(5.68, 10.85)	<0.001
12 weeks	20.24 ± 3.17	8.65 ± 3.60	9.99	(7.06, 12.92)	<0.001
Vaginal maturation value (VMV)					
Baseline	59.63 ± 27.18	54.15 ± 34.46	267	(−15.53, 20.88)	0.773
6 weeks	93.16 ± 7.93	43.90 ± 29.43	35.47	(16.06, 54.88)	<0.001
12 weeks	93.49 ± 7.45	43.24 ± 38.60	33.79	(10.69, 56.88)	0.004

CI = confident interval; SD = standard deviation.
Analyses were conducted with the use of a linear mixed-effects model with an unstructured correlation matrix, and multiple imputation with chained equation (MICE) for imputation of missing data values.

shown in Table 3. The most notable was abnormal vaginal bleeding, which occurred in 41.2% of women receiving Femoston conti®, compared to none in the placebo group ($p = 0.007$). Other reported side effects in the MHT group included increased vaginal discharge (29.4% vs. 5.9%) and mastalgia (17.7% vs. 5.9%), although these did not reach statistical significance. No serious adverse events or treatment discontinuations due to adverse effects were reported.

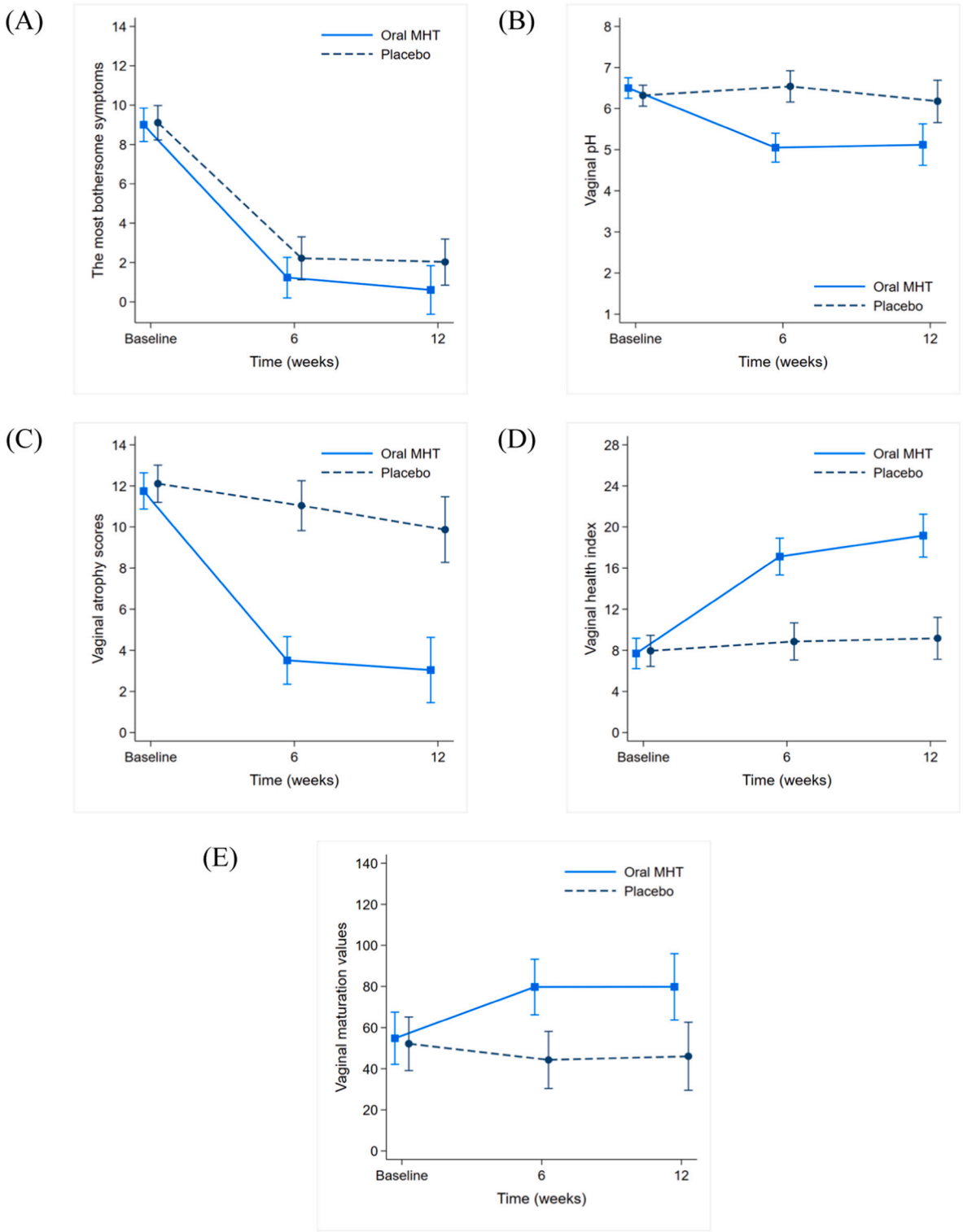

Fig. 4. Secondary outcomes (A) The most bothersome symptoms (B) Vaginal pH (C) Vaginal atrophy score (D) Vaginal Health Index (E) Vaginal maturation value.

4. Discussion

This randomized controlled trial represents the first investigation using 16S rDNA gene sequencing techniques to examine the effects of oral combined hormone therapy on vaginal microbiota composition in Thai postmenopausal women with GSM. Our findings reveal a paradoxical but clinically important outcome: while 12 weeks of oral estradiol-dydrogesterone therapy produced substantial symptomatic and physiological improvements in GSM, it did not significantly alter the overall composition or diversity of the vaginal microbiome.

The absence of measurable microbiome changes following systemic hormone therapy challenges a prevailing assumption about the mechanistic basis of GSM treatment. Despite robust clinical improvements, including dramatic reductions in the MBS, VAS, and significant improvements in VHI, the fundamental bacterial community structure remained largely unchanged. This dissociation between clinical efficacy

Table 3
Adverse events.

Adverse events	Oral MHT (N = 17)		Placebo (N = 17)		p-Value	
Abnormal vaginal bleeding	7	(41.2)	0	(0.0)	0.007	d
Irritation	2	(10.0)	0	(0.0)	0.487	d
Pain	0	(0.0)	0	(0.0)	NA	
Ulcer	0	(0.0)	0	(0.0)	NA	
Vaginal discharge	6	(30.0)	1	(5.3)	0.053	d
Mastalgia	3	(17.7)	1	(5.9)	0.601	d

NA = data not applicable.
Data are presented as number (%), mean ± standard deviation or median (interquartile range).
P-value corresponds to [a]Independent samples t-test, [b]Mann-Whitney U test, [c]Chi-square test or [d]Fisher's exact test.

and microbiome composition suggests that the therapeutic benefits of systemic hormone therapy in GSM may operate through mechanisms beyond simple restoration of lactobacilli-dominated communities [27,28].

Several explanations may account for this unexpected finding, most notably the timeline required for ecological succession versus tissue regeneration. Systemic estrogen rapidly acts on host estrogen receptors to stimulate vaginal epithelial cell maturation, increase glycogen production, and improve clinical symptoms—often within 4 to 8 weeks [13,29,30]. However, the restructuring of the vaginal microbiome is a much more complex, secondary ecological process. The postmenopausal vaginal microbiome, having adapted to a low-estrogen, depleted environment over years, likely possesses a resilience that resists rapid ecological remodeling [31]. Our findings suggest a distinct chronological hierarchy in the treatment response: host cytological responses (such as improvements in VMV and VHI) occur rapidly, while the subsequent selection, colonization, and stabilization of a Lactobacillus-dominant ecosystem is a delayed process. Therefore, while a 12-week treatment duration is clearly adequate to observe robust clinical and tissue-level improvements, it is likely insufficient to capture the slower, stable ecological changes necessary for complete microbiome restoration [32].

Second, the route of hormone administration likely influences microbiome effects. Local vaginal estrogen therapy delivers concentrated hormonal exposure directly to vaginal tissues, potentially creating a more conducive environment for lactobacilli proliferation than systemic administration. Studies investigating vaginal estrogen preparations have consistently demonstrated microbiome improvements, often with increases in Lactobacilli abundance and decreases in pathogenic bacteria [14,15,17]. Our findings suggest that while systemic therapy effectively addresses many GSM symptoms through improved tissue integrity, moisture, and pH reduction, it may not achieve the localized hormonal concentrations necessary for substantial microbial community restructuring.

The observed clinical improvements without microbiome changes illuminate the multifaceted pathophysiology of GSM. The significant reduction in vaginal pH from 6.41 to 5.03 in the treatment group represents a meaningful shift toward the acidic environment traditionally associated with vaginal health, yet this change was observed without a concurrent, statistically significant increase in lactobacilli abundance. This suggests that systemic estrogen therapy may influence vaginal pH through direct effects on epithelial cell metabolism, glycogen production, or other biochemical pathways rather than solely through lactobacilli-mediated lactic acid production [33].

Likewise, the marked increase in VHI and improved VMV affirm the trophic effects of systemic estrogen on the urogenital tract [34]. These changes likely account for the alleviation of GSM symptoms, even in the absence of microbiome modulation. Such findings align with the concept that hormonal effects on tissue integrity may precede or even supersede microbial restoration in the treatment cascade.

Our findings contrast with several observational studies that have reported associations between hormone therapy use and altered vaginal microbiome profiles [17–20]. However, these cross-sectional comparisons between hormone users and non-users may reflect selection bias, confounding variables, or cumulative effects of longer treatment durations rather than direct causal relationships. The controlled nature of our study design provides more definitive evidence regarding the short-term microbiome effects of systemic hormone therapy initiation.

The differential effects observed between systemic and local hormone therapy align with emerging evidence suggesting that route of administration fundamentally influences therapeutic mechanisms. While local estrogen therapy consistently demonstrates microbiome benefits, systemic therapy appears to primarily exert its effects through tissue-level changes in epithelial function, moisture production, and pH regulation [13,29,30].

Shifting to clinical safety and tolerability, the 41.2% incidence of abnormal vaginal bleeding in the treatment group warrants careful clinical consideration. Although unscheduled bleeding is a known side effect of initiating continuous combined MHT in early postmenopausal women, a rate this high poses significant real-world challenges. Such unpredictable bleeding can severely impact patient acceptability and drive treatment discontinuation. Consequently, comprehensive upfront counseling regarding bleeding expectations is mandatory when prescribing this regimen to optimize long-term compliance.

4.1. Strength

This study's key strengths include its randomized, placebo-controlled design and integrated evaluation of both microbiological and clinical endpoints. The application of high-throughput 16S rDNA gene sequencing offers greater sensitivity and resolution than traditional culture-based techniques, enabling detailed characterization of vaginal microbiota. Comprehensive clinical assessments, including validated measures of symptom severity, tissue integrity, and physiological parameters, provides a holistic evaluation of treatment effects.

The single-center setting, with uniform specimen collection and standardized laboratory protocols, minimized technical variation and potential analytic bias. Incorporating both subjective and objective measures permitted meaningful correlation between clinical outcomes and biological changes. The consistent alignment between symptom relief and objective improvement in the hormone group supports the internal validity of our findings. Moreover, strict participant screening and minimal attrition further reinforce the study's methodological rigor.

4.2. Limitations

Several limitations warrant consideration in interpreting these results. A primary limitation of this study is the absence of a microbiome-specific power calculation. The sample size of 17 participants per arm was primarily powered for clinical outcomes and is likely insufficient to detect subtle or highly variable shifts in the vaginal microbiome.

The 12-week treatment duration may be insufficient to detect microbiome changes that require longer periods to manifest, as extended follow-up studies (6–12 months) might reveal delayed effects not captured in this timeframe.

Our study population consisted of Thai postmenopausal women aged 40–60 years (mean 55.6 years treatment, 54.3 years placebo) with a median time since menopause of 2–5 years. This relatively younger postmenopausal cohort may not have developed the profound microbiome dysbiosis typically observed in older women with longer duration of estrogen deficiency. However, this demographic enhances the clinical relevance of our findings, as it closely aligns with current clinical guidelines that advocate initiating systemic hormone therapy within the window of opportunity [1,5,11]. Accordingly, our results support early intervention with systemic hormone therapy in appropriately selected women experiencing GSM symptoms during the early postmenopausal

period.

The focus exclusively on Thai women may limit generalizability to other ethnic groups with different baseline microbiome profiles, dietary patterns, and genetic variations. Another limitation is the presence of clinically relevant baseline imbalances in specific symptoms between the study arms; notably, vaginal irritation was more prevalent in the MHT group, whereas dyspareunia was more common in the placebo group. While linear mixed-effects models help account for these baseline variations statistically, these specific symptom imbalances could still introduce confounding trajectories. For instance, a group starting with significantly worse dyspareunia may possess a fundamentally different biological or microbial baseline environment that the statistical model cannot fully erase, potentially influencing the observed outcomes.

Finally, while 16S rDNA sequencing provides comprehensive bacterial analysis, it does not capture other important vaginal microbiome components including fungi, viruses, and metabolomic profiles that may also be influenced by hormone therapy [35].

4.3. Implications and future research

Our findings contribute to evolving understanding of GSM as a multifactorial condition with distinct but interconnected pathophysiological components. While microbiome dysbiosis represents an important aspect of GSM, our exploratory findings suggest that clinically meaningful symptom relief can be achieved alongside improvements in vaginal tissue health, even without concurrent statistically significant microbiome restoration. This mechanistic insight suggests that GSM may be more accurately conceptualized as a tissue-level disorder with secondary microbiome consequences, rather than primarily a microbiome-driven condition, with important implications for treatment approach and outcome assessment in clinical practice. The demonstration that clinically meaningful improvements in validated outcome measures can occur without microbiome changes challenges the notion that microbiome restoration is necessary for successful GSM treatment, potentially explaining why some women achieve excellent symptom control with treatments that do not directly target the microbiome.

These findings open several avenues for future investigation. Long-term studies examining microbiome changes over 6–12 months of systemic hormone therapy could reveal delayed effects not apparent in shorter timeframes, while comparative studies directly contrasting systemic versus local hormone therapy effects on both clinical outcomes and microbiome composition would provide valuable insights into optimal treatment selection. Mechanistic studies investigating the specific pathways through which systemic hormone therapy influences vaginal pH, tissue integrity, and symptom relief—even in the absence of major taxonomic microbiome shifts—could identify novel therapeutic targets. Furthermore, metabolomic analyses examining changes in vaginal metabolite profiles following hormone therapy may reveal functional alterations not reflected in taxonomic composition alone. Investigation of combination therapies, such as systemic hormone therapy paired with targeted probiotics or prebiotic supplementation, could determine whether synergistic approaches provide superior clinical outcomes compared to hormone therapy alone, while studies in diverse populations with varying baseline microbiome profiles would enhance understanding of how demographic factors, genetics, and environmental influences modulate treatment responses.

5. Conclusion

This randomized controlled trial provides exploratory evidence that a 12-week course of oral combined hormone therapy produces significant clinical, cytological, and biochemical improvements in GSM. However, this 12-week timeframe is likely insufficient to induce stable ecological remodeling of the vaginal microbiota. These findings suggest that the rapid symptomatic relief provided by systemic hormone therapy is driven primarily by direct tissue-level estrogenic effects, which clinically precede any long-term microbial shifts.

The results support the continued use of systemic hormone therapy for GSM management while highlighting the complexity of vaginal ecosystem responses to hormonal interventions. Future research should focus on optimizing treatment duration, exploring combination approaches, and further elucidating the mechanisms underlying hormone therapy's clinical benefits in the absence of microbiome changes.

Contributors

Krasean Panyakhamlerd conceived and designed the study, conducted experiments, and performed the analysis and interpretation of results.

Tassawan Rungruxsirivorn conceived and designed the study, conducted experiments, and performed the analysis and interpretation of results.

Punika Panichaya reviewed the literature, contributed to the analysis and interpretation of results, and helped draft and revise the manuscript.

Panachai Nimitpanya conducted experiments, collected data, and performed data analysis.

Sunchai Payungporn contributed to the study conceptualization.

Chai Ariyasriwatana performed the cytological assessment.

Ammarin Suwan analyzed and interpreted the results.

Suthida Visedthorn performed formal analysis, data curation, and visualization.

Pakorn Ruengket contributed to the methodology and data curation.

Chamnan Tanprasertkul provided supervision and acted as the senior consultant.

All authors saw and approved the final version and no other person made a substantial contribution to the paper.

Ethical approval

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki, the Belmont Report, the CIOMS International Ethical Guidelines, and the ICH-GCP standards.

The protocol was registered in the Thai Clinical Trials Registry and approved on October 14, 2023 (TCTR20241216001).

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, approved the study protocol (COA No.0167/2025, IRB No.804/63).

The Human Research Ethics Committee of the Faculty of Medicine, Thammasat University (COA No.092/2568, Project No. MTU-EC-ES-1-017/68).

All participants provided written informed consent prior to enrollment.

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Data sharing and collaboration

The datasets generated from the next-generation sequencing in this study are available from the NCBI Sequence Read Archive (SRA) repository, Bioproject ID: PRJNA128615. The authors declare that the data supporting the findings of this study are available in the article and

from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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