Supplementary Online Content

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Associations between baseline participant characteristics and week 12 Estrone concentration

Characteristic	N (%)[a]	Week 12 Estrone (pg/mL): Geometric Mean (SD)	p-value[b]
Treatment group			0.78
Vaginal estrogen	88 (51)	11.8 (5.9)	
Placebo	86 (49)	11.5 (6.3)	
Study pill adherence, %			0.21
< 80	22 (13)	10.2 (4.7)	
≥ 80	152 (87)	11.9 (6.3)	
Age, years			0.29
< 60	72 (41)	12.3 (5.9)	
≥ 60	102 (59)	11.2 (6.2)	
Time since menopause, years			0.12
< 5	25 (14)	13.5 (7.5)	
≥ 5	147 (85)	11.3 (5.8)	
Body Mass Index, kg/m^2			0.001
< 25	82 (47)	10.2 (5.6)	
≥ 25	92 (53)	13.1 (6.1)	
Estradiol concentration			0.002
< median (3.5 pg/mL)	87 (50)	10.3 (5.3)	
≥ median	87 (50)	13.2 (6.6)	
Estrone concentration			<0.001
< median (10.5 pg/mL)	87 (50)	8.8 (4.1)	
≥ median	87 (50)	15.5 (6.3)	
SHBG concentration			0.27
< median (59.3 nmol/L)	87 (50)	12.2 (5.9)	
≥ median	87 (50)	11.2 (6.2)	
Race			0.01
African American	8 (5)	20.0 (8.5)	
Other or unknown	14 (8)	10.9 (5.0)	
White	152 (87)	11.4 (5.9)	
Sexually Active			0.79
Yes	143 (82)	11.7 (6.0)	
No	30 (17)	11.4 (6.4)	
Most Bothersome Symptom			0.26
Pain with vaginal penetration	97 (56)	11.3 (5.4)	
Vaginal dryness	38 (22)	11.5 (6.8)	
Vulvar / vaginal itching	15 (9)	15.2 (6.3)	
Vulvar / vaginal irritation	13 (8)	11.7 (6.6)	
Vulvar / vaginal soreness	7 (4)	14.3 (8.1)	
pH			0.20
≤ 5	22 (13)	13.3 (6.6)	
> 5	151 (87)	11.4 (6.0)	
Vaginal Maturation Index, % superficial cells			0.08
≤ 5	143 (82)	11.5 (6.1)	
> 5	11 (6)	15.4 (8.9)	

MENQOL total				0.27
< median (< 3.16)	83 (48)	11.1 (5.3)		
≥ median	84 (48)	12.1 (6.9)		
PHQ-8 depression				0.27
No symptoms (< 5)	121 (70)	11.3 (5.4)		
Mild or worse symptoms	52 (30)	12.5 (7.7)		
GAD-7 anxiety				0.30
No symptoms (< 5)	108 (62)	11.3 (5.6)		
Mild or worse symptoms	65 (37)	12.3 (7.0)		
ISI insomnia				0.18
No symptoms (< 8)	92 (53)	11.1 (5.2)		
Mild or worse symptoms	81 (47)	12.4 (7.1)		

[a]Percentages may not add up to 100% due to missing characteristic data

[b]p-value from an ANOVA model of log-transformed week 12 Estrone by category of participant characteristic

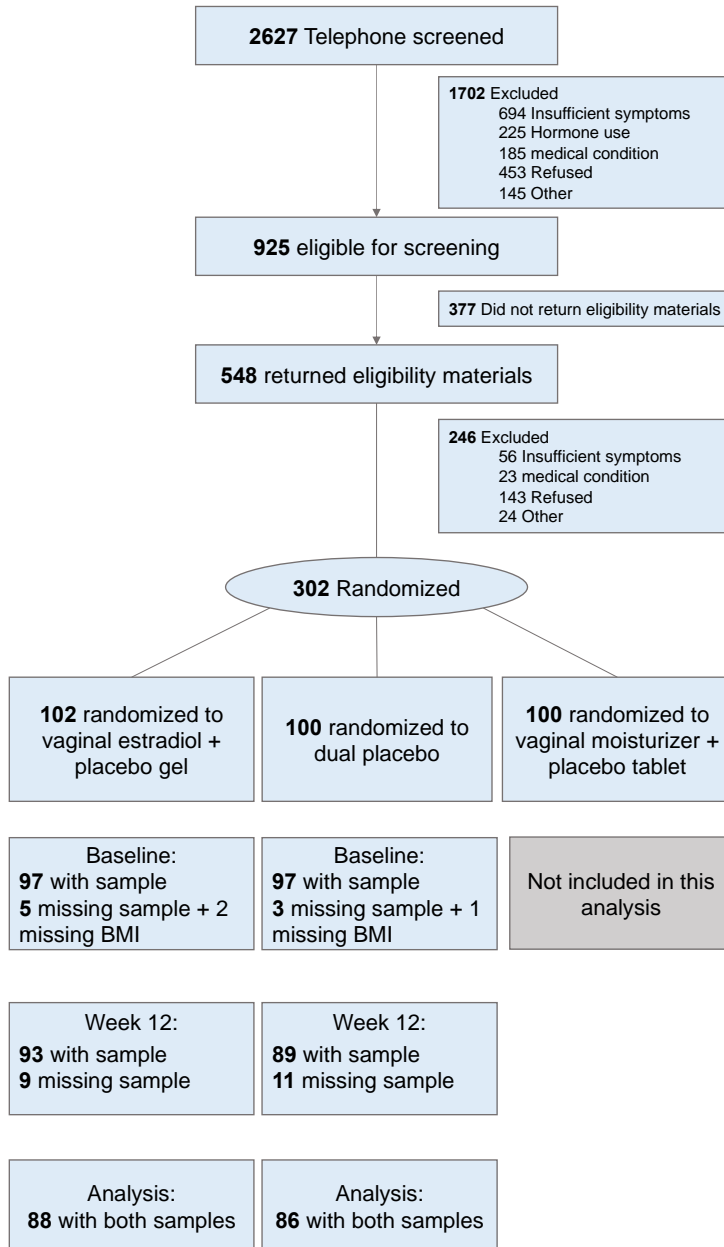**eTable 2**. Associations between baseline participant characteristics and week 12 SHBG concentration

Characteristic	N (%)[a]	Week 12 SHBG (nmol/L): Geometric Mean (SD)	p-value[b]
Treatment group			0.48
Vaginal estrogen	88 (51)	56.7 (26.8)	
Placebo	86 (49)	52.9 (42.1)	
Study pill adherence, %			0.93
< 80	22 (13)	54.2 (36.7)	
≥ 80	152 (87)	54.9 (35.7)	
Age, years			0.03
< 60	72 (41)	62.4 (25.8)	
≥ 60	102 (59)	50.0 (38.3)	
Time since menopause, years			0.41
< 5	25 (14)	60.4 (19.1)	
≥ 5	147 (85)	53.7 (37.3)	
Body Mass Index, kg/m^2			<0.001
< 25	82 (47)	68.5 (25.1)	
≥ 25	92 (53)	45.0 (34.9)	
Estradiol concentration			0.18
< median (3.5 pg/mL)	87 (50)	58.5 (45.4)	
≥ median	87 (50)	51.3 (25.4)	
Estrone concentration			0.72
< median (10.5 pg/mL)	87 (50)	55.8 (44.9)	
≥ median	87 (50)	53.8 (24.5)	
SHBG concentration			<0.001
< median (59.3 nmol/L)	87 (50)	37.8 (26.9)	
≥ median	87 (50)	79.5 (20.7)	
Race			0.83
African American	8 (5)	62.2 (27.3)	
Other or unknown	14 (8)	56.7 (18.5)	
White	152 (87)	54.3 (37.1)	
Sexually Active			0.36
Yes	143 (82)	55.9 (25.8)	
No	30 (17)	49.6 (60.4)	
Most Bothersome Symptom			0.33
Pain with vaginal penetration	97 (56)	55.1 (24.0)	
Vaginal dryness	38 (22)	59.9 (26.4)	
Vulvar / vaginal itching	15 (9)	49.1 (28.9)	
Vulvar / vaginal irritation	13 (8)	39.6 (71.7)	
Vulvar / vaginal soreness	7 (4)	63.3 (27.3)	
pH			0.91
≤ 5	22 (13)	55.5 (27.6)	
> 5	151 (87)	54.6 (36.8)	
Vaginal Maturation Index, % superficial cells			0.65
≤ 5	143 (82)	54.0 (37.3)	
> 5	11 (6)	49.0 (26.9)	

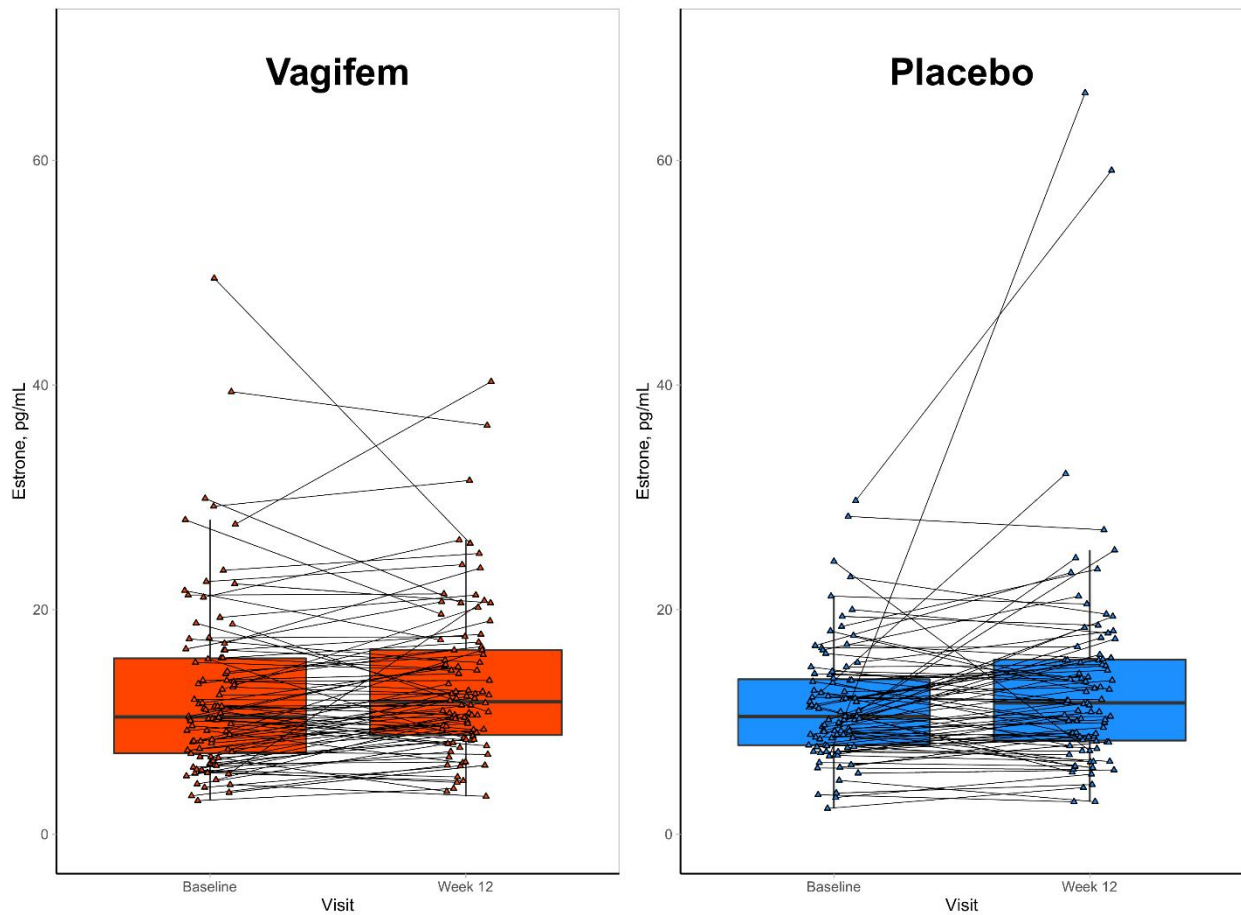|				
MENQOL total				0.18
< median (< 3.16)	83 (48)	51.6 (41.6)		
≥ median	84 (48)	59.0 (25.4)		
PHQ-8 depression				0.85
No symptoms (< 5)	121 (70)	55.1 (26.1)		
Mild or worse symptoms	52 (30)	54.0 (51.6)		
GAD-7 anxiety				0.87
No symptoms (< 5)	108 (62)	55.1 (27.1)		
Mild or worse symptoms	65 (37)	54.2 (46.8)		
ISI insomnia				0.82
No symptoms (< 8)	92 (53)	55.4 (26.6)		
Mild or worse symptoms	81 (47)	54.1 (43.8)		

[a]Percentages may not add up to 100% due to missing characteristic data

[b]p-value from an ANOVA model of log-transformed week 12 SHBG by category of participant characteristic

eFigure 1: CONSORT diagram of how original trial participants were enrolled and how this subset was selected.


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                    ┌─────────────────────────────┐
                    │ 2627 Telephone screened     │
                    └─────────────────────────────┘
                                   │
                                   │    ┌──────────────────────────────┐
                                   │    │ 1702 Excluded                │
                                   │    │   694 Insufficient symptoms  │
                                   ├───▶│   225 Hormone use            │
                                   │    │   185 medical condition      │
                                   │    │   453 Refused                │
                                   │    │   145 Other                  │
                                   │    └──────────────────────────────┘
                                   ▼
                    ┌─────────────────────────────┐
                    │ 925 eligible for screening  │
                    └─────────────────────────────┘
                                   │
                                   │    ┌─────────────────────────────────────────┐
                                   ├───▶│ 377 Did not return eligibility materials │
                                   │    └─────────────────────────────────────────┘
                                   ▼
                    ┌──────────────────────────────────┐
                    │ 548 returned eligibility materials│
                    └──────────────────────────────────┘
                                   │
                                   │    ┌──────────────────────────────┐
                                   │    │ 246 Excluded                 │
                                   │    │   56 Insufficient symptoms   │
                                   ├───▶│   23 medical condition       │
                                   │    │   143 Refused                │
                                   │    │   24 Other                   │
                                   │    └──────────────────────────────┘
                                   ▼
                        (  302 Randomized  )
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- **102** randomized to vaginal estradiol + placebo gel
- **100** randomized to dual placebo
- **100** randomized to vaginal moisturizer + placebo tablet

Baseline:	Baseline:	Not included in this analysis
97 with sample	**97** with sample	
5 missing sample + 2 missing BMI	**3** missing sample + 1 missing BMI	

Week 12:	Week 12:
93 with sample	**89** with sample
9 missing sample	**11** missing sample

Analysis:	Analysis:
88 with both samples	**86** with both samples

eFigure 2: Serum concentrations of estrone at enrollment and 12 weeks for participants in the MsFLASH Vaginal Health randomized trial assigned to the vaginal 10 µg estradiol tablet + placebo gel (n = 88) or dual placebo (n = 86) arms. Each triangle represents a measurement from an individual participant, and the lines connect the pre-treatment and week 12 values for each participant.

eFigure 3: Serum concentrations of sex hormone binding globulin (SHBG) at enrollment and 12 weeks for participants in the MsFLASH Vaginal Health randomized trial assigned to the vaginal 10 μg estradiol tablet + placebo gel (n = 88) or dual placebo (n = 86) arms. Each triangle represents a measurement from an individual participant, and the lines connect the pre-treatment and week 12 values for each participant.