Supplementary Online Content

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eTable 1. Associations Between Baseline Participant Characteristics and Week 12 Estrone Concentration

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eFigure 1. CONSORT Diagram of How Original Trial Participants Were Enrolled and How This Subset Was Selected

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

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This supplementary material has been provided by the authors to give readers additional information about their work.
**Table 1.** Associations between baseline participant characteristics and week 12 Estrone concentration

| Characteristic                              | N (%)<sup>a</sup> | Week 12 Estrone (pg/mL): | p-value<sup>b</sup> |
|--------------------------------------------|-------------------|--------------------------|---------------------|
| **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<sup>2</sup>**      |                   |                          | 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)               |                     |
| Characteristic       | Less Than Median | Greater Than or Equal to Median | p-value |
|---------------------|------------------|---------------------------------|---------|
| 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       | 52 (30)          | 12.5 (7.7)                      |         |
| GAD-7 anxiety       |                  |                                 | 0.30    |
| No symptoms (< 5)   | 108 (62)         | 11.3 (5.6)                      |         |
| Mild or worse       | 65 (37)          | 12.3 (7.0)                      |         |
| ISI insomnia        |                  |                                 | 0.18    |
| No symptoms (< 8)   | 92 (53)          | 11.1 (5.2)                      |         |
| Mild or worse       | 81 (47)          | 12.4 (7.1)                      |         |

*aPercentages may not add up to 100% due to missing characteristic data

bp-value from an ANOVA model of log-transformed week 12 Estrone by category of participant characteristic
**Table 2.** Associations between baseline participant characteristics and week 12 SHBG concentration

| Characteristic                                      | N (%) | Week 12 SHBG (nmol/L): | p-value |
|-----------------------------------------------------|-------|------------------------|---------|
|                                                     |       | Geometric Mean (SD)     |         |
| 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/m²                               |       |                        | <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)            |         |
| Characteristic | N (%) | Mean (SD) |
|---------------|-------|-----------|
| 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) |

aPercentages may not add up to 100% due to missing characteristic data
bP-value from an ANOVA model of log-transformed week 12 SHBG by category of participant characteristic
Figure 1: CONSORT diagram of how original trial participants were enrolled and how this subset was selected.

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

- 102 randomized to vaginal estradiol + placebo gel
  - Baseline: 97 with sample
    - 5 missing sample
    - 2 missing BMI

- 100 randomized to dual placebo
  - Baseline: 97 with sample
    - 3 missing sample
    - 1 missing BMI

- 100 randomized to vaginal moisturizer + placebo tablet
  - Not included in this analysis

- Week 12:
  - 93 with sample
    - 9 missing sample

- Week 12:
  - 89 with sample
    - 11 missing sample

Analysis:
- 88 with both samples
- 86 with both samples

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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.