## **Supplemental Table 1**: Randomized, Controlled Trials (n = 20) of Endometrial Histology with Vaginal Estrogen Use in Menopausal Women

| **Reference** | **Treatment(s)** | **Study design and population** | **Endometrial hyperplasia or cancer** |
| --- | --- | --- | --- |
| Constantine et al, 201720  Duration: 12 weeks | * Vaginal E2 softgel capsule of either   + 25 µg (n = 190), 10 µg (n = 191), or 4 µg (n = 191) [Imvexxy®, TherapeuticsMD, Boca Raton, FL]   + Placebo (n = 192) * Treatments were inserted daily for 2 weeks, then twice weekly for 10 weeks | * Randomized, double-blind, placebo-controlled, 12-week study (89 centers) in the US and Canada * Postmenopausal women (40–75 years) with moderate-to-severe dyspareunia * Endometrial biopsies taken at baseline, week 12 or end of treatment | * No endometrial hyperplasia or malignancy observed in any group at week 12 |
| Rahn et al, 201410  Duration: 4 to 8 weeks (required before surgery) | * Vaginal CEE cream 0.625 mg/1g (n = 8) [Premarin®, Pfizer, Philadelphia, PA] or placebo cream (n = 12) * Cream was inserted daily for 2 weeks, then twice weekly until surgery | * Randomized, double-blind, placebo-controlled trial conducted at a single center in the US * Postmenopausal women (40–70 years) with symptomatic uterine and/or anterior vaginal wall prolapse of stage ≥2 desiring surgical repair or total hysterectomy * Endometrial biopsy taken at time of surgery | * No cases of endometrial hyperplasia or cancer reported |
| Bachmann et al, 20095  Duration: 52 weeks | * CEE 0.3 mg vaginal cream   + Once daily for 21 days and off for 7 days (n = 143; 21/7) or 21/7 placebo (n = 72), or   + Twice weekly (n = 140; 2X/week) or 2X/week placebo (n = 68) for 12 weeks * After 12 weeks, 21/7 placebo converted to 21/7 CEE cream and 2X/week placebo converted to 2X/week CEE cream; other treatments continued as previously, all were administered for up to 52 weeks (open-label study) | * Randomized, double-blind, placebo-controlled, multicenter trial conducted in the US and Canada * Healthy postmenopausal women (45–80 years) with symptoms of moderate-to-severe vaginal atrophy and an intact uterus * Endometrial biopsies taken at baseline and week 52 | * 155 women had evaluable biopsies at week 52 (n = 82, 21/7 group and n = 73, 2X/week group, respectively) * No endometrial hyperplasia or carcinoma observed * Incidence rate of hyperplasia for CEE cream 0.3 mg 21/7 was 0/82 * Incidence rate for CEE cream 0.3 mg 2X/week was 0/73 |
| Freedman et al, 20099  Duration: 12 weeks | * SCE-A vaginal cream for 12 weeksa   + 0.625 mg (1 g; n = 150)   + 1.25 mg (2 g; n = 161)   + 1 g placebo (n = 155)   + 2 g placebo (n = 156) * Daily for the first 7 days, and then twice weekly * Placebo was given in a double dummy fashion based on different cream volumes | * Randomized, double-blind, placebo-controlled trial conducted at 88 sites in the US * Postmenopausal women (30–80 years) with symptoms of VVA * Endometrial biopsy taken at baseline and at study end | * No cases of endometrial hyperplasia or carcinoma observed |
| Simon et al, 200819  and  Simon et al, 201018  Study VAG-2195  Duration: 52 weeks | * Estradiol (E2) vaginal tablet 10 µg (n = 205) [Vagifem®, Novo Nordisk Inc, Bagsvaerd, Denmark] or placebo (n = 104) daily for 2 weeks, and then twice weekly * Additional details reported in Simon et al 201018 | * Randomized, double-blind, placebo-controlled trial conducted at 45 sites in the US and 4 in Canada * Non-hysterectomized, postmenopausal women (≥45 years) with at least 3 urogenital symptoms (one moderate to severe) * Endometrial thickness <4 mm at study entry (by transvaginal ultrasound) * Endometrial biopsies taken at screening and at study end | * 2/386 with a 0.52% [two-sided 95% CI, 0.06%–1.86%] incidence rate per year for endometrial hyperplasia and carcinoma in the combined E2 vaginal tablet 10 µg group (evaluable biopsies = 386)18   + One case of a grade 2, stage 1B endometrioid adenocarcinoma (woman was treated for 324 days; screening biopsy revealed no tissue and was not repeated)   + A case of complex endometrial hyperplasia without atypia (reported in Simon et al, 2010); woman discontinued after 9 days of study exposure * No reports of hyperplasia or cancer in the placebo group |
| Bachmann et al, 200812  Duration: 52 weeks | * Vaginal E2 tablets of either   + 25 µg (n = 91) or 10 µg (n = 92) [Vagifem], or   + Placebo (n = 47) for 12 weeks. * Treatments were inserted daily for 14 days, then once twice weekly * After 12 weeks, all women took E2 25 µg (n = 102; 38 previously on 25 µg, 46 previously on 10 µg, and 18 previously on placebo) administered twice-weekly for up to 52 weeks (open-label study) | * Randomized, double-blind, placebo-controlled, 12-week study (9 centers), followed by a 52-week, open-label extension (8 centers) in the US * Postmenopausal women (≥45 years) with moderate-to-severe vaginal dryness and soreness * Endometrial thickness of ≤5 mm (sonogram) * Endometrial biopsies taken at weeks 12 and 52 | * Endometrial biopsies were performed on 86 women   + 1/32 case of simple hyperplasia without atypia was observed at week 12 with 25 µg * Endometrial biopsies were taken from 42 women who completed 52 weeks   + No endometrial hyperplasia or malignancy observed in any group at week 52 |
| Vesna and Neli, 200611  Duration: 2 weeks | * CEE vaginal cream 0.625 mg/day (1g, n = 150) or transdermal E2 50 mg/day (n = 119) [FemSeven®, Teva Pharmaceuticals, Netherlands] * Daily cream treatment 14 days prior to hysterectomy or daily transdermal treatment for 14 days prior and 14 days after to vaginal hysterectomy | * Randomized trial conducted at 1 site in Macedonia between 1998 and 2003 * Postmenopausal women with genital prolapse requiring vaginal hysterectomy * Women with endometrial thickness >4 mm at baseline were further assessed by curettage * Endometrium was histologically assessed at hysterectomy | * No complex or atypical hyperplasia or endometrial cancer reported * 2/150 in the vaginal cream group had simple hyperplasia * 2/119 in the transdermal group had simple hyperplasia |
| Weisberg et al, 200516  Duration: 48 weeks | * Micronized E2 vaginal ring releasing 8 µg/day of E2 (n = 126) [Estring®, Pfizer, New York, NY], or vaginal E2 tablets 25 µg (n = 59) [Vagifem] * Ring kept in place for 12 weeks at a time; one tablet inserted daily for 2 weeks, and then twice weekly | * Prospective, randomized, open-label, comparative study at 4 sites in Australia * Non-hysterectomized postmenopausal women with signs and symptoms of urogenital atrophy * Women had to have an endometrium thickness ≤5 mm, and could not have significant uterine prolapse or vaginal bleeding of unknown origin | * Endometrial biopsies were taken in women with endometrial thickness >7 mm (n = 5; three with Estring and two with Vagifem) * No endometrial hyperplasia or carcinoma reported |
| Pinkerton et al, 200323  Duration: 6 months | * All women received (as open label) E2vaginal ring releasing 7.5 µg/day of E2 [Estring] and were randomized to daily oral raloxifene 60 mg (n = 46) or placebo (n = 45) * Vaginal ring kept in place for 3 months at a time | * Randomized, double-blind, placebo-controlled, parallel-group trial * Healthy postmenopausal women (42–80 years) with an intact uterus and at least 2 signs of vaginal atrophy * Endometrial biopsy at baseline, and 3 and 6 months | * No endometrial hyperplasia or carcinoma found in either group |
| Manonai et al, 20018  Duration: 12 weeks | * Vaginal micronized E2 tablet 25 µg (n = 27), or CEE cream 0.625 mg (1 g, n = 26) * Daily for 2 weeks, then twice weekly for 10 weeks | * Prospective, randomized, open-label, comparative study in Thailand * Healthy postmenopausal women (45–70 years) with urogenital symptoms | * Biopsies were only taken from 2 women * No endometrial hyperplasia or carcinoma reported |
| Cardozo et al, 200117  Duration: 12 weeks | * Vaginal E2 tablet 25 µg (n = 56) [Vagifem], or placebo (n = 54) inserted daily | * Randomized, double-blind, placebo-controlled trial * Postmenopausal women (45–83 years) with urinary tract symptoms of urgency and frequency for <3 years * Endometrial biopsies taken at end of study | * No endometrial hyperplasia or carcinoma reported |
| Rioux et al, 20004  Duration: 24 weeks | * Vaginal E2 tablets 25 µg (n = 80), or CEE vaginal cream 2 g (1.25 mg) (n = 79) * Tablets were inserted once daily for 2 weeks and then twice weekly thereafter; CEE cream applied daily for 21 days, then withheld for 7 days, and repeated | * Randomized, open-label, parallel-group, multicenter study conducted at 6 sites in Canada * Postmenopausal women (42–85 years) with intact uteri and moderate-to-severe vaginal symptoms * Endometrial biopsies taken at screening and week 24 | * No cases of endometrial cancer reported * 1/28 using vaginal cream had simple endometrial hyperplasia * 1/28 using vaginal cream had complex endometrial hyperplasia without atypia |
| Barentsen et al, 199721  Duration: 6 months | * E2vaginal ring, 7.5 µg/day (n = 83; ring‑cream sequence) [Estring], or estriol vaginal cream 0.5 mg (n = 82; cream‑ring sequence) [Synapause®, Aspen Pharma Trading Limited, Dublin, Ireland] daily for first 2 weeks followed by 3 times weekly up to 3 months * 3 months of one regimen, then crossed over to the second regimen for another 3 months (total 6 months; no washout specified) | * Randomized, open-label, cross-over study conducted in The Netherlands * Postmenopausal women with symptomatic atrophic vaginitis (including vaginal dryness) | * Endometrial hyperplasia was reported as an adverse event without histologic confirmation   + One case (1/82) of endometrial hyperplasia at day 50 (method of diagnosis not reported); treated with estriol vaginal cream   + Two cases (2/82) of endometrial hyperplasia with atypia in cream-ring sequence (one on cream, one on ring); diagnosed after curettage due to bleeding during treatment |
| Henriksson et al, 199622  Duration: 48 weeks | * Vaginal ring releasing micronized E2 6.5–9.5 µg/day (n = 96) [Estring], or estriol vaginal pessaries (n = 39) * Rings and pessaries were kept in place for 12 weeks * Initial study was 12 weeks. Women in ring group could continue treatment up to 12 months. Women in pessary group could convert to ring (n = 39) for 36 weeks (12-month total treatment) after washout if VVA symptoms continued. | * Randomized, open-label, parallel-group trial conducted in Sweden, Denmark, and Finland * Postmenopausal women with atrophic vaginitis and atrophic vaginal mucosa | * No cases of endometrial hyperplasia or malignancy reported |
| Nachtigall 19957  Duration: 12 weeks | * Vaginal ring releasing E2 7.5 µg/day (n = 129), or CEE vaginal cream 1.25 mg (2 g) 3 times per week (n = 67) * Women received study medication for 12 weeks followed by a 3-week period of no medication | * Randomized, open-label, parallel-group trial in the US * Postmenopausal women (≥55 years) with signs and symptoms of atrophic vaginitis * Women had to have an atrophic or inactive endometrium at baseline (based on pelvic sonography) * Biopsies taken (pipelle) after treatment (exact timing not specified) | * No cases of endometrial cancer or hyperplasia reported * After 12 weeks of treatment, one case (n = 129) of hyperplasia in an endometrial polyp was reported in the ring group |
| Vartiainen et al, 199324  Duration: 6 months (duration of estrogen exposure 2–4 months) | * Vaginal ring releasing  100–400 μg/day of E2, or placebo ring (n = 26 total)b * The controlled-release system of the ring initially gives 0.4 mg E2/day, then decreases to 0.2 mg/day after 20 days, and stabilizes to ~0.1 mg/day after 50 days * Rings kept in place for 2 months at a time * After 2 months, women switched treatments (no washout reported) * Women could choose treatment after 4 months | * Double-blind, placebo-controlled, cross-over study (with blind allocation to treatment) * Postmenopausal women (47–56 years) with vasomotor symptoms (hot flushes/sweating) * Endometrial sampling by curettage at baseline and at 4 and 6 months | * No endometrial hyperplasia or carcinoma reported |
| Felding et al, 199213  Duration: 3 weeks | * Vaginal E2 pessary containing 25 µg tablets (n = 22) [Vagifem] or placebo (n = 23) inserted daily | * Randomized, double-blind, controlled trial * Postmenopausal women (44–82 years) scheduled for vaginal operation because of genital prolapse * Endometrial biopsy by curettage at time of surgery | * No endometrial carcinoma reported * 2/22 (9%) women in the E2 treated group had a report of simple hyperplastic endometrium without epithelial atypia with 3 weeks of treatment (63 and 67 years old) * At curettage 1 month later, both cases had normal atrophic endometrium |
| Mettler and Olsen, 199115  Duration: 1 year (2 years for 9 women) | * Vaginal E2 tablets of 25 µg [Vagifem] daily for 2 weeks then randomized to either 1X/week (n = 17) or 2X/week (n = 34) for an additional 50 weeks * 9 women completed 2 years of treatment twice weekly | * Randomized, controlled, open-label study * Healthy, postmenopausal, non-hysterectomized women (≤70 years) with estrogen-deficiency-related vaginal symptoms * Endometrial biopsies taken at baseline and years 1 and 2 | * No cases (0/45) of endometrial cancer or hyperplasia found after 1 year |
| Mattsson et al, 198914  Duration: 18 weeks | * Vaginal pessaries releasing daily doses of micronized E2 of 25 µg or 50 µg [Vagifem] were used daily for 3 weeks, and then twice weekly for 6 weeks (n = 20) * Women had a 4-week, wash-out period, followed by the cross-over with 9-week treatment of the other dose | * Randomized, double-blind, controlled, cross-over study * Healthy, postmenopausal women (56–69 years) with VVA-related symptoms * Endometrial biopsies at baseline, and 3 and 9 weeks | * 58 endometrial samples were retrieved from 18 women * No endometrial hyperplasia or carcinoma reported |
| Luisi et al, 19806  Duration: 3 weeks (biopsies at day 15) | * Daily vaginal CEE cream 1.25 mg (2 g) (n = 7) [Premarin] or estriol cream 0.5 mg (0.5 g) (n = 7) [Ovestin®, Aspen Bad Oldesloe GmbH, Germany] for 3 weeks | * Randomized, open-label, comparative study at a single center in Italy * Postmenopausal women (53–65 years) with vaginal atrophy and related symptoms * Endometrial biopsies taken at baseline and day 15 | * Endometrial biopsies were obtained from only 2 women in each group * No cases of endometrial hyperplasia or cancer reported |

CEE, conjugated estrogens; US, United States; SCE-A, synthetic conjugated estrogens-A; VVA, vulvar and vaginal atrophy; E2, 17β-estradiol; CI, confidence interval; LOCF, last observation carried forward.

aWomen with uterus received 300 mg daily micronized progesterone [Prometrium] or 10 mg MPA daily for 14 days at study end9

bAll women received 10 mg/day MPA for 10 days after study discontinuation (at 6 months [timing relative to biopsy not specified])24