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To cite this article: A. Kolokythas, C. Betschart, D. Wunder, H. Janka & P. Stute (02 Jan 2024): Impact of vaginal estriol on serum hormone levels: a systematic review, *Climacteric*, DOI: [10.1080/13697137.2023.2287624](https://doi.org/10.1080/13697137.2023.2287624)

To link to this article: <https://doi.org/10.1080/13697137.2023.2287624>



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Impact of vaginal estriol on serum hormone levels: a systematic review

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ABSTRACT

The genitourinary syndrome of menopause (GSM) affects up to 84% of postmenopausal women and may significantly reduce the quality of life in some. For symptom relief, there are several non-hormonal and hormonal vaginal products available. In Europe, vaginal estriol (E3) is the most frequently chosen estrogen for GSM treatment. The aim of this systematic review was to assess the impact of vaginal E3 on serum sex hormone levels, an outcome that has been previously used to assess safety in similar products. In our review, we did not find any alterations in serum estrone, estradiol, testosterone, progesterone and sex hormone binding globulin levels after vaginal E3 application. In contrast, some studies showed a minimal and transient decrease in serum gonadotropin levels, which however remained within the postmenopausal range. Similarly, only a few studies reported a minimal and transient increase of serum E3 levels, with the rest reporting no changes. The lack of clinically relevant long-term changes in serum sex hormone levels supports the current literature providing evidence about the safety of vaginal E3 products.

ARTICLE HISTORY

Received 1 July 2023
Revised 7 October 2023
Accepted 14 November 2023
Published online 2 January 2024

KEYWORDS

Vaginal estriol; serum hormone level; menopause; safety; genitourinary syndrome of menopause

Introduction

The term genitourinary syndrome of menopause (GSM) was introduced as an all-encompassing term for vulvovaginal and bladder atrophy by the International Society for the Study of Women's Sexual Health (ISSWSH) and North American Menopause Society (NAMS) in 2014 [1] and describes the hypoestrogenic changes to the vulvovaginal and bladder-urethral areas that occur in menopausal women [2]. GSM symptoms may comprise genital, sexual and urinary symptoms [3]. Symptoms associated with GSM are highly prevalent, affecting up to 84% of postmenopausal women [4], and may have a negative impact on quality of life [3]. Even among menopausal women using systemic menopausal hormone therapy, about 45% still experience GSM symptoms [2].

First-line therapy for GSM symptoms is non-hormonal products [5]. If not sufficient, vaginal estrogens are recommended [5]. According to current guidelines, this may also apply to women suffering from, for example, hormone-sensitive cancers [5]. Approved vaginal estrogens comprise conjugated equine estrogens (CEE), estradiol (E2) and estriol (E3). Yet approval status is not the same internationally. For example, vaginal E3 has not been approved by the US Food and Drug Administration (FDA) for GSM treatment. Hence, it is not listed in the NAMS guideline on GSM management [5]. In Europe, on the contrary, vaginal E3 is the most frequently chosen vaginal estrogen for GSM management (data based on EFFIK's internal analysis). Therefore, the aim of this comprehensive systematic review was to assess the impact of vaginal E3 on sex hormone serum levels, GSM signs and

symptoms, and breast safety. Here, we address the first topic – the other two will follow in separate publications.



Methods and materials


Protocol, information sources and search strategy

A protocol with a detailed methodological strategy was developed in a collaboration of all authors and this protocol was prospectively submitted for registration in the PROSPERO database (acknowledgment of receipt: 296147). However, due to the COVID-19 pandemic, all systematic reviews not studying COVID-19 were automatically deemed out of scope by PROSPERO and therefore not accepted for registration. The protocol was hence kept in the University of Bern archives and the exact same protocol was later registered with INPLASY (INPLASY202320023).

Complex literature searches were designed on the topic and executed for the following information sources to identify all potentially relevant documents on the topics:

- MEDLINE (Ovid) (including epub ahead of print, in-process and other non-indexed citations, MEDLINE Daily and Ovid MEDLINE versions) (1946–30 March 2021)
- CINAHL (1937–30 March 2021)
- Embase (Ovid) (1974–30 March 2021)
- Cochrane Library (Wiley) (1996–30 March 2021)
- Web of Science (Clarivate) (1900–30 March 2021)
- ClinicalTrials.gov (NLM)

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/13697137.2023.2287624>.

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Initial search strategies in MEDLINE/Ovid were drafted by a medical information specialist (H.J.) and tested against a list of core references to see whether they were included in the search results. After refinement and consultation, complex search strategies were set up for each information source based on database-specific controlled vocabulary (thesaurus terms/subject headings) and textwords. Synonyms, acronyms and similar terms were included in the textword search. No limits have been applied in any database considering study types, languages, publication years or any other formal criteria.

The searches were run in the medical bibliographic databases MEDLINE and Embase, the Cochrane Library, CINAHL, Web of Science and ClinicalTrials.gov, an international clinical trials registry. The initial searches were conducted on 31 March 2021, whereas an update using the exact same algorithm was conducted prior to publication, on 26 January 2023.

The search concepts used were 'postmenopausal women', 'genitourinary syndrome of menopause '(GSM)', 'vaginal estriol (E3)' and 'serum levels' (estriol, estradiol, follicle stimulating hormone [FSH]).

In addition to electronic database searching, reference lists and bibliographies from relevant publications were checked for relevant studies. The final detailed search strategies are presented in Appendix 1.

Included were only controlled trials comparing intravaginal application of E3 and their effect on serum hormone levels to other local estrogens, non-hormonal local treatments or E3 in different doses in postmenopausal women (natural or iatrogenic) as well as single-arm pre–post interventional studies [6] studying administration of vaginal E3 monotherapy, systematic reviews and meta-analyses. The serum hormones of interest were mainly E2, E3, and FSH, but estrone (E1), luteinizing hormone (LH), sex hormone binding globulin (SHBG), prolactin (PRL), testosterone and progesterone were also included in the study. Excluded were studies not studying the outcomes of interest or studying local treatments other than vaginal E3 monotherapy or other routes of E3 administration as well as all other study types not falling under the inclusion criteria.

Study selection process

All identified citations were imported into EndNote and duplicates were removed. The remaining citations were imported into the Rayyan Screening tool, and titles and abstracts were independently screened by two authors (A.K. and P.S.) and tested against the inclusion criteria. The full-text screening and the data extraction followed and were conducted by the same team with the same standards. Any discrepancies throughout the process were resolved after discussion. The same process was followed after the updated search before publication.

Results

After the initial search, 489 articles were identified and after deduplication 285 articles remained to be screened. A total of 232 articles were deemed out of scope after the initial title and abstract screening and were excluded. The remaining 53 articles were screened in full text. A total of 23 articles were excluded as duplicates, study protocols or conference abstracts, five articles as not systematically conducted literature reviews and one article as a cross-sectional/no intervention study. Two further articles were excluded for not studying our outcome of interest (hormonal

serum concentrations) and one article for studying a combined E3/progesterone vaginal product (not monotherapy), whereas for three articles no full-text access could be obtained even after requests to the corresponding authors and as such they had to be excluded. The 18 remaining articles met the inclusion criteria and data were extracted. The updated search returned 49 new articles, none of which fulfilled the inclusion criteria (Figure 1).

Of the 18 included articles, seven were controlled trials (two placebo-controlled trials, two head-to-head comparisons [E3 vs. CEE], two dose-comparisons, and one placebo-controlled and head-to-head comparison study [E3 vs. E2 vs. placebo]), eight were pre–post interventional studies (six single-arm and two multi-arm), two were systematic reviews and one was a meta-analysis [6–8].

The following serum hormones and protein levels were reported, respectively: E2 by 14 studies [9–23], E3 by 15 studies [9–13,15,18–27], gonadotropins (FSH and/or LH) by 13 studies [9–11,15–17,19–26], E1 by 11 studies [9–11,13,15–21,24], SHBG by 11 studies [9,14–16,19–25], PRL by four studies [9,15,20,24], testosterone by two studies [14,16] and progesterone by one study [22]. Within the two systematic reviews there were seven additional studies that were not picked up by our algorithm and are reported as part of those two reviews [28–34].

Serum estriol

The majority of studies did not observe a change in serum E3 levels after vaginal E3 application [9–13,15,18,20,22,25,27] (Table 1). Four studies reported significant changes in serum E3 levels after vaginal E3 application [19,21,24,26]. The first assessed serum E3 levels before and 2 weeks after daily vaginal application of 0.5 mg E3, either as cream or a suppository [24]. Compared to baseline, conjugated serum E3 levels were significantly increased at the end of treatment regardless of application type (from 0.28 ± 0.01 nmol/l to 1.48 ± 0.2 nmol/l for the vaginal cream and from 0.33 ± 0.05 nmol/l to 1.57 ± 0.2 nmol/l for the vaginal suppositories); unconjugated serum E3 levels were only increased after vaginal E3 cream treatment (from <0.05 nmol/l to 0.23 ± 0.08 nmol/l). The second study assessed the pharmacokinetics of daily vaginal application of 0.03 mg E3 for 4 weeks, followed by a maintenance therapy three times weekly for 8 weeks [21]. As the serum E3 peak level was significantly lower and somewhat delayed at week 4 compared to baseline (from a maximum concentration of 168 pg/ml at 3 h post administration to a maximum of 43.7 pg/ml at 8 h post administration), a minimal and transient systemic E3 absorption was suspected. Another pharmacokinetics study found a dose-proportional increase of serum E3 after single application of 0.002% and 0.005% E3 vaginal gel but a less than dose-proportional increase for the 0.1% E3 vaginal cream [26]. The same study concluded that the systemic exposure is reduced after chronic use, as after daily treatment with the aforementioned doses for 21 days the mean serum E3 was lower in each group to a statistically significant degree for the two lower concentration products (no arithmetic results available). A systematic review of 22 studies included three additional studies that reported significant but transient serum E3 level increases after vaginal E3 application [19]. Normalization of serum E3 levels was found 8 h (vaginal 0.5 mg E3 ovula – from a baseline of 0.15 nmol/l to an increase to 0.4 nmol/l at 4 h and normalization to <0.10 nmol/l at 8 h) [32], 24 h (unconjugated serum E3 levels: vaginal 0.5 mg E3 suppository and cream – from a baseline of zero for both to 0.3 nmol/l at 30 min for both, a further increase to 0.51 nmol/l for the suppositories and 0.61 nmol/l for the cream at 1 h and

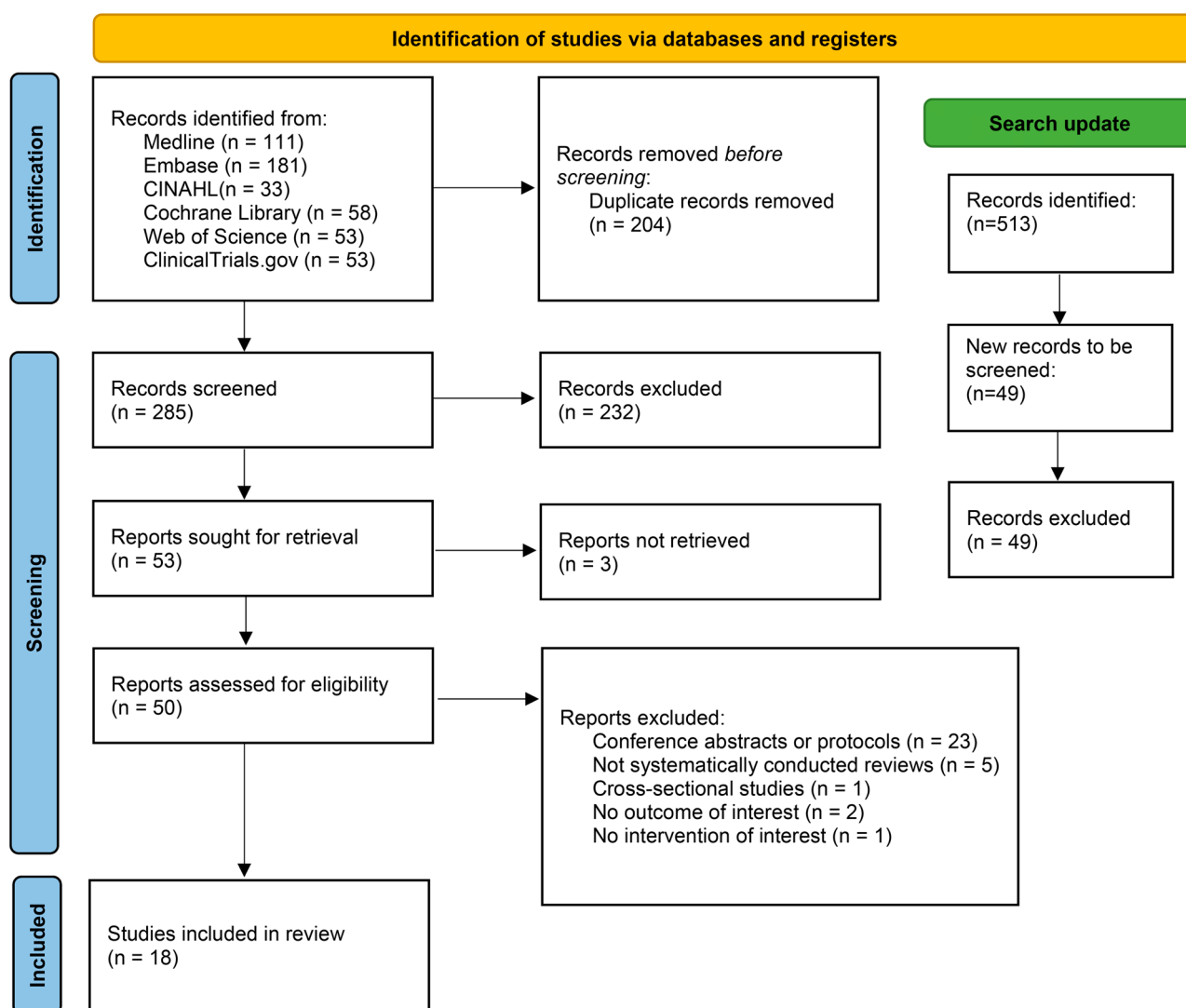


Figure 1. Flow diagram of the selection and assessment of the studies.

normalization after 24 h for both) and 48 h (conjugated serum E3 levels: vaginal 0.5 mg E3 suppository and cream – from a baseline of 0.3 nmol/l for both to 1.43 nmol/l at 8 h for the suppositories and 1.33 nmol/l at 4 h for the cream to normalization after 48 h for both) [33] after singular application [32], or after a 14-day application respectively [33]. The third study concluded that vaginal absorption of E3 (measured by unconjugated serum E3 levels – area under the curve 9133 on day 0 to 6743 on day 21) declined with time after daily treatment with vaginal E3 suppositories at 1 mg for 21 days [30].

Serum estradiol and estrone levels

None of the identified studies reported an increase of serum E2 [9–22] or E1 [9–11,13,15–21,24] levels after vaginal E3 application (Table 2 and Supplemental Table 1). This indeed is no surprise as the enzymatic metabolism from E2 to E1 to E3 is a 'one-way street' [35]. In contrast, one study using vaginal CEE at 1.25 mg per day as a comparator found a significant increase in serum E2 and E1 levels [9]. However, if vaginal CEE was applied at a lower dose (0.625 mg) and frequency (daily for 2 weeks and then once weekly for 2 years), serum E2 levels remained unchanged after 2 years [14]. Similarly, studies using vaginal E2 as a comparator

did not report significant long-term serum E2 [16,32,34] or serum E1 [16,32,34] level changes, respectively.

Serum gonadotropin levels

Six [9–11,21,22,24,26] out of 13 identified studies [9–11,15–17,19–22,24–26] reported significant changes in serum FSH levels, two of which also found significant changes in serum LH levels [9,22] (Table 3). Vaginal E3 at 0.5 mg daily for 2 weeks followed by 0.5 mg twice weekly for 6 weeks significantly decreased serum FSH levels without affecting LH (from a baseline of 14.2 µg/l to 13.5 µg/l at 2 weeks for the cream and from 14.9 µg/l to 13.7 µg/l for the suppositories) [24]. Similarly, high-dose vaginal E3 cream at 0.1% daily for 3 weeks significantly decreased serum FSH levels (from 79.1 IU/l on day 1 to 68.25 IU/l on day 21), which was not found for lower doses (0.005%, 0.002%) [26]. Indeed, the dose and frequency of vaginal E3 application are relevant factors. Daily application of vaginal E3 at 0.5 mg for 2 weeks [22], at 0.05 mg for 3 weeks [10,11] or at 0.03 mg for 4 weeks [21], respectively, significantly decreased serum FSH levels (from 75.7 mU/ml on day 1 to 66.0 mU/ml on day 14, from a baseline of 54.6 mIU/ml to 47.6 mIU/ml on day 7, and from a baseline of 107.88 mU/ml to 98.94 mU/ml on day 28, respectively). Serum FSH levels returned

Table 1. Effects of vaginal estriol (E3) on serum E3 levels.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison, measurement methodology	E3 (A)	E3 (B)	E3 (C)
Mattsson and Cullberg (1983) [24]	Multi-arm pre-post interventional studies	Total: 30 patients (3 withdrawn) A: 14 B: 13	A: mean age 59.5 years B: mean age 58.0 years	A: 0.5 mg E3/day for 14 days + 2x/week for 6 weeks (Ovestin [®] vaginal cream) B: 0.5 mg E3/day for 14 days + 2x/week for 6 weeks (Ovestin [®] vaginal suppositories)	Pre vs. post comparison (mean ± SE) 0 weeks, 2 weeks (20 h after last E3) Radioimmunoassay	0 weeks (unconjugated): <0.05 nmol/l 2 weeks (unconjugated): 0.11 ± 0.02 nmol/l 0 weeks (conjugated): 0.33 ± 0.05 nmol/l 2 weeks (conjugated): 1.57 ± 0.2 nmol/l, $p < 0.001$	0 weeks (unconjugated): <0.05 nmol/l 2 weeks (unconjugated): 0.11 ± 0.02 nmol/l 0 weeks (conjugated): 0.33 ± 0.05 nmol/l 2 weeks (conjugated): 1.57 ± 0.2 nmol/l, $p < 0.001$	E3 (C)
Luisi et al. (1980) [9]	Controlled trial	Total: 14 patients A: 7 B: 7	Participants between 53 and 65 years No further data available	A: 0.5 mg E3/day for 3 weeks (Ovestin [®] vaginal cream) B: 1.25 mg CEE/day for 3 weeks (Premarin [®] vaginal cream)	A vs. B (mean) 0 days, 8 days, 15 days, 21 days (morning) Radioimmunoassay	0 days: <12 pg/ml 8 days: 15 days: 21 days: 23.4 pg/ml	0 days: <12 pg/ml 8 days: 15 days: 21 days: 23.9 pg/ml	
Sánchez-Rovira et al. (2020) [10] / Hirschberg et al. (2020) [11]	Controlled trial	Total: 61 patients A: 50 B: 11	A: mean age 58.9 years B: mean age 61.4 years	A: 50 µg E3/day for 3 weeks + 2x/week for 9 weeks (Blissel [®] , Gelistrol [®] , Gélisse [®] vaginal gel) B: moisturizer 1x/day for 0 weeks, 1 week, 3 weeks, 8 weeks, 12 weeks + 2x/week for 9 weeks (Ainara [®] vaginal gel)	Pre vs. post comparison (median + Δ): physiological variability vs. treatment variability) 0 weeks, 1 week, 3 weeks, 8 weeks, 12 weeks Liquid chromatography with tandem mass spectrometry	0 weeks: <0.5 pg/ml (below limit of quantitation) 1 week: 3.9 pg/ml (median) 3 weeks: 1.9 pg/ml (median) 8 weeks: <0.5 pg/ml (below limit of quantitation) 12 weeks: <0.5 pg/ml (below limit of quantitation)	0 weeks: <0.5 pg/ml (below limit of quantitation) 1 week: <0.5 pg/ml (below limit of quantitation) 3 weeks: <0.5 pg/ml (below limit of quantitation) 8 weeks: <0.5 pg/ml (below limit of quantitation) 12 weeks: <0.5 pg/ml (below limit of quantitation)	
Wesel et al. (1981) [12]	Single-arm pre-post interventional study	Total: 50 patients A: only 15 for the outcome of interest	A: mean age 61.76 years	A: 0.5 mg E3/day for 3 weeks + 2x/week for 4 weeks (Ortho-Gynest [®] vaginal pessaries)	Blood samples: 0 weeks, 1 week, 3 weeks, 5 weeks, 7 weeks Radioimmunoassay	Values individually given for each participant (calculated means/median) 0 days: mean <25.4 pg/ml median: <25 pg/ml 7 days: mean <40.4 pg/ml median: 26 pg/ml 21 days: mean <37.5 pg/ml median: <25 pg/ml 35 days: mean <27.0 pg/ml median: <25 pg/ml 49 days: mean <27.7 pg/ml median: <25 pg/ml		

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Table 1. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison, measurement methodology	E3 (A)	E3 (B)	E3 (C)
Te West et al. (2020) [13]	Multi-arm pre-post interventional studies	Total: 76 patients (90 recruited) A: 27 (40 recruited, 13 withdrew) a: 10, b: 10, c: 20 B: 49 (50 recruited, 1 excluded)	A: mean age 66.6 years (A=new users) B: median age 75.5 years (B=chronic users, >3 months)	Aa: 0.5 mg E3/day for 3 weeks + 2x/week for 9 weeks (Ovestin® vaginal cream with applicator) Ab: 0.5 mg E3/day for 3 weeks + 3x/week for 9 weeks (Ovestin® vaginal cream, digitally, measured) Ac: 0.5 mg E3/day for 3 weeks + 3x/week for 9 weeks (Ovestin® vaginal cream, digitally, free) B: 0.5 mg E3 2-3x/week for 9 weeks (Ovestin® vaginal cream)	Median (IQR) – Aa, Ab, Ac analyzed as one group Blood samples: 0 weeks, 2 weeks (12 h after last E3) Liquid chromatography with tandem mass spectrometry	0 weeks: <5.0 pmol/l 2 weeks: <22.8 pmol/l (9.2–108.5)	2 weeks: 15.1 pmol/l (2.7–33.9)	
Haspels et al. (1981) [15]	Single-arm pre-post interventional study	Total: 11 patients A: 11	A: between 54 and 70 years (no further information provided)	A: 0.5 mg E3/day for 3 weeks + 2x/week for 5 weeks (Ovestin® vaginal cream)	Pre vs. post (mean ± SD) Blood samples: 0 days, 21 days, 35 days, 56 days (ca. 10–12 h after application) Radioimmunoassay	0 days: <35.0 pmol/l 21 days: 86.8 ± 8.1 pmol/l 35 days: 68.2 ± 6.3 pmol/l 56 days: 60.4 ± 10.1 pmol/l E3 rose from undetectable to a mean baseline of 86.8 at day 21		
de Oliveira Filho et al. (2019) [25]	Controlled trial	Total: 31 patients A: 10 B: 10 C: 11	A: mean age 56.1 years B: mean age 55.9 years C: mean age 55.5 years	A: 0.125 mg E3/day for 21 days (single 100 mg vaginal ring) B: 0.250 mg E3/day for 21 days (single 300 mg vaginal ring) C: 0.500 mg E3/day for 21 days (single 600 mg vaginal ring)	Pharmacokinetic/dynamics Liquid chromatography with tandem mass spectrometry	$C_{day\ 21}$: 7 pg/ml C_{max} : 68 pg/ml @ $t=2$ h C_{9h} : 25 pg/ml Clear dose dependency – C_{max} increase with dose was less than proportional	$C_{day\ 21}$: 6 pg/ml C_{max} : 97 pg/ml @ $t=5$ h C_{9h} : 38 pg/ml Clear dose dependency – C_{max} increase with dose was less than proportional	$C_{day\ 21}$: 10 pg/ml C_{max} : 155 pg/ml @ $t=9$ h C_{9h} : 59 pg/ml Clear dose dependency – C_{max} increase with dose was less than proportional C 1 day vs. 21 days: not statistically significant change 1 day A/B vs. C: statistically significant (higher), $p \leq 0.0005$ 21 days A/B vs. C: statistically significant (higher), $p = 0.0004$
Delgado et al. (2016) [26]	Controlled trial	Total: 43 patients A: 13 B: 12 C: 12 D: 6	A: mean age 57.4 years B: mean age 59.6 years C: mean age 57.8 years D: mean age 62.7 years	A: 0.002% E3/day for 21 days (vaginal gel) B: 0.005% E3/day for 21 days (vaginal gel) C: 0.1% E3/day for 21 days (Ovestin® vaginal cream) D: placebo for 21 days (vaginal gel)	Pre vs. post and among groups (E3 mean, FSH/LH median) Blood samples: 1 days, 21 days Liquid chromatography with tandem mass spectrometry	A 1 day vs. 21 days: statistically significant (lower), $p = 0.0006$ 1 day A vs. B: statistically significant different, $p = 0.0043$	B 1 day vs. 21 days: statistically significant (lower), $p = 0.0004$ 21 days A vs. B: no statistically significant difference	

(Continued)

Table 1. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison, measurement methodology	E3 (A)	E3 (B)	E3 (C)
Te West et al. (2021) [18]	Single-arm pre-post interventional study	Total: 10 patients A: 10	A: mean age 70.0 years Chronic users	A: chronic usage omitted for ≥ 36 h prior to the study, then 0.5 mg E3 once (Ovestin [®] vaginal cream)	Pre vs. post (mean (SE)) Blood samples: 0 h, 1 h, 2 h, 3 h, 5 h, 8 h, 10 h, 12 h, 24 h Liquid chromatography with tandem mass spectrometry	0 h: 5 pmol/l (1.7) 1 h: 317 pmol/l (65.4) 2 h: 505 pmol/l (81.9) 3 h: 455 pmol/l (78.4) 5 h: 296 pmol/l (62.5) 8 h: 69 pmol/l (19.7) 10 h: 36 pmol/l (11.8) 12 h: 19 pmol/l (7.0) 24 h: 7 pmol/l (3.5) C_{max} (mean): 546 pmol/l (SD 310) @ $t = 2.3$ h	E3 was absorbed rapidly and the decline in serum concentrations was rapid At 24 h all women had E3 levels < 30 pmol/l	
Rueda et al. (2017) [19]	Systematic review	Total: 22 articles – Studying E3: 20 – Studying E3 and hormones: 11 studies (4 already included [14,19,23]) – 1 excluded based on our criteria since combination E3/P4 studied – 6 not separately included) a) Batra and Iosif (1987) [28] b) Keller et al. (1981) [29] c) Heimer and Englund (1984) [30] d) Punnonen et al. (1980) [32] e) Mattsson and Cullberg (1983) [33] f) Heimer and Englund (1986) [31]	a: (Controlled trial) 17 patients (10 E3 vs. 7 no treatment) b: (Pre-post interventional study) 3 patients c: (Pre-post interventional study) 6 patients d: (Controlled trial) 10 patients (5 E3) e: (Controlled trial) 8 patients f: (Pre-post interventional study) 9 patients	a: 1 mg E3 ovula 14 h pre and 1 h post-OP for uterine prolapse b: 0.5 mg E3 for 10 days (ovula) c: 1 mg E3 for 21 days (ovula) d: 2.4 mg CEE vs. 2 mg E2 vs. 0.5 mg E3 for 24 h e: 0.5 mg E3 cream or ovula for 14 days (time comparison) f: 0.5 mg E3 for 21 days	Radioimmunoassay – most studies	a – pre: 10 pg/ml, post: 50 mg/ml b – 0 h: 40 pg/ml 1 h: 1290 pg/ml 2 days: 123 pg/ml 10 days: 293 pg/ml c – 0 days: 9133 AUC 21 days: 6743 AUC, $p = 0.05$ d – 0 h: 0.15 nmol/l 2 h: 0.4 nmol/l 8 h: < 0.10 nmol/l, 12 h: 0.09 nmol/l 24 h: < 0.05 nmol/l, $p = 0.05$ e – cream and unconjugated E3, 0 h: 0 30 months: 0.3 nmol/l ($p = 0.05$) 2 h: 0.61 \pm 0.11 nmol/l ($p = 0.01$) 24 h: baseline levels – cream and conjugated E3, 0 h: 0.3 nmol/l 4 h: 1.33 \pm 0.17 nmol/l ($p < 0.01$) 48 h: baseline levels – ovula and unconjugated E3, 0 h: 0 30 months: 0.3 nmol/l ($p = 0.05$) 1 h: 0.51 \pm 0.24 nmol/l ($p = 0.01$) 24 h: baseline levels – ovula and conjugated E3, 0 h: 0.29 nmol/l 8 h: 1.43 \pm 0.33 nmol/l ($p < 0.01$) 48 h: baseline levels f – AUC 0–12 h: morning 5943 evening 4713.5 AUC 0–24 h: morning 9941 evening 8722.5		

(Continued)

Table 1. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison, measurement methodology	E3 (A)	E3 (B)	E3 (C)
Kicovic et al. (1980) [20]	Controlled trial	Total: 74 patients (32 for serum studies) A: 23 (17 serum studies) B: 30 (10 serum studies) C: 21 (5 serum studies)	Ages between 44 and 82 years (no further information provided)	A: 1 mg E3/day for 3 weeks + subgroup further 2x/week for 13 weeks (Ovestin® vaginal cream) B: 0.5 mg E3/day for 3 weeks + subgroup further 2x/week for 13 weeks (Ovestin® vaginal cream) C: 0.5 mg E3/day for 2 weeks (Ovestin® vaginal suppositories)	Blood samples: 1 day (0 h, 1 h, 2 h, 3 h, 4 h, 6 h) and 21 days (A and B) or 15 days (C) – 12 h after last dose (=0 h and 1 h, 2 h, 3 h, 4 h, 6 h) Radioimmunoassay	1 day, 0 h (unconjugated): <12 pg/ml 1 day, 1 h: 123.6 pg/ml (mean peak value) 1 day, 1–5 h: gradual decline 21 days, 0 h: 26 pg/ml 21 days, 1 h: 104.9 pg/ml (mean peak value) 21 days, 1–5 h: gradual decline	1 day, 0 h (unconjugated): <12 pg/ml 1 day, 1 h: 110.8 pg/ml (mean peak value) 1 day, 1–5 h: gradual decline 21 days, 0 h: 26 pg/ml 21 days, 1 h: 95.0 pg/ml (mean peak value) 21 days, 1–5 h: gradual decline	1 day, 0 h (unconjugated): <12 pg/ml 1 day, 2 h: 92.6 (mean peak value) 1 day, 2–5 h: gradual decline 15 days, 0 h: 30.8 pg/ml 15 days, 2 h: 92.8 pg/ml (mean peak value) 15 days, 2–5 h: gradual decline Mean E3 values 0 days: 0.00 (0) 14 days: 5.11 pg/ml (14.01) 28 days: 0.91 pg/ml (3.63) 56 days: 0.00 (0) 84 days: 0.00 (0)
Donders et al. (2014) [21]	Single-arm pre-post interventional study	Total: 16 patients A: 16	A: mean age 57.0 years	A: 0.03 mg E3/day for 4 weeks + 3x/week for 8 weeks (Gynoflor® vaginal tablets)	Pre vs. post (mean (SD)) Blood samples: 0 days and 28 days; 0 h, 0.5 h, 1 h, 3 h, 2 h, 4 h, 6 h, 8 h, 24 h Gas chromatography–mass spectrometry	Day 0 (A Group) [LLOQ set 10.00 pg/ml] 0 days, 0 h: LLOQ (0) 0 days, 0.5 h: 3.83 pg/ml (5.74) 0 days, 1 h: 11.04 pg/ml (6.66) 0 days, 2 h: 72.73 pg/ml (49.11) 0 days, 3 h: 84.35 pg/ml (43.77) 0 days, 4 h: 28.29 pg/ml (18.64) 0 days, 6 h: LLOQ (0) 0 days, 8 h: LLOQ (0) 0 days, 24 h: LLOQ (0) 0 days C_{max}: 168 pg/ml @ t=3 h 0 days mean C _{max} : 104.5 pg/ml (40.9), 2.5 h	Day 28 (A Group) 28 days, 0 h: 0.91 pg/ml (3.51) 28 days, 0.5 h: 1.58 pg/ml (4.05) 28 days, 1 h: 1.55 pg/ml (4.16) 28 days, 2 h: 1.96 pg/ml (5.20) 28 days, 3 h: 2.48 pg/ml (7.35) 28 days, 4 h: 3.12 pg/ml (9.40) 28 days, 6 h: 7.33 pg/ml (12.26) 28 days, 8 h: 10.23 pg/ml (12.33) 28 days, 24 h: LLOQ (0) 28 days C_{max}: 43.7 pg/ml @ t=8 h, p < 0.0001 28 days mean C _{max} : 15.8 pg/ml (13.9), 7.2 h	
Pfeiler et al. (2011) [22]	Single-arm pre-post interventional study	Total: 10 patients A: 10	A: median age 65.0 years	A: 0.5 mg E3/day for 2 weeks (Ovestin® vaginal tablets)	Pre vs. post comparison (mean) 0 weeks, 2 weeks (8–12 h after E3) Gas chromatography–mass spectrometry	0 weeks: <0.2 ng/ml 2 weeks: <0.2 ng/ml		

(Continued)

Table 1. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison, measurement methodology	E3 (A)	E3 (B)	E3 (C)
Buhling et al. (2012) [27]	Single-arm pre-post interventional study	Total: 19 patients A: 19	A: mean age 62.7 years	A: 0.03 mg E3/day for 21 days (OekoIp [®] vaginal pessaries)	Pre vs. post (mean) Blood samples: 1 day (after first administration); 6 days, 11 days, 16 days (predose); 21 days (after last administration) Liquid chromatography with tandem mass spectrometry	1 day, 1 h: 42.1 pg/ml (max mean plasma concentration) 1 day, 12 h: <5.0 pg/ml (LLOQ) in all patients Repeated administration did not result in an accumulation of estradiol 21 days, 2 h: 11.9 pg/ml (max mean plasma concentration) Very low systemic bioavailability, which decreased even more after multiple dosing confirming a favorable safety profile of low dose pessaries administered daily over 21 days		

The meta-analysis of Pavlović et al. [23] is not included in the table since the results of all included studies are separately presented. AUC, area under the curve; CEE, conjugated equine estrogens; FSH, follicle stimulating hormone; IQR, interquartile range; LH, luteinizing hormone; LLOQ, lower limit of quantitation. A, B, C refer to the groups within each study and the respective treatment and outcomes for each. The statistically significant results ($p < 0.05$) appear in bold.

to baseline levels when the application frequency of vaginal E3 at 0.03 mg was reduced to three times weekly for 8 weeks (103.55 mIU/ml) [21] and the frequency of vaginal E3 at 0.05 mg to two times weekly for 9 weeks (51.4 mIU/ml) [10,11]. When comparing vaginal E3 to other vaginal estrogen therapies, Luisi et al. showed that the daily use of 1.25 mg vaginal CEE for 3 weeks decreased both FSH and LH more than daily use of 0.5 mg vaginal E3 throughout the treatment (days 8, 15 and 21), at a statistically significant level (FSH from a baseline of 75.2 mIU/ml to 70.5 mIU/ml on day 21 for the vaginal E3 group and from a baseline of 71.0 mIU/ml to 55.6 mIU/ml on day 21 for the CEE group) [9].

Serum SHBG levels

None of the included studies induced significant changes in serum SHBG levels after vaginal E3 application [9,14–16,19–22,24] (Supplemental Table 1). In contrast, one study using vaginal CEE at 1.25 mg per day as a comparator found a significant increase of serum SHBG levels [9].

Serum prolactin, testosterone and progesterone levels

All but one study [24] reported no impact of vaginal E3 application on serum PRL levels (Supplement Table 1). The latter reported a significant increase in serum PRL levels after application of vaginal E3 cream at 0.5 mg daily for 2 weeks but not for vaginal E3 suppositories at the same dose and frequency of application [24]. Serum testosterone [14,16] and progesterone [22] levels remained unchanged after vaginal E3 application, which again is no surprise as E3 cannot be enzymatically metabolized 'back' to E2, testosterone and progesterone.

Discussion

This systematic review did not find any clinically relevant long-term serum hormone or protein level changes. Serum levels are often used as a proxy when studying the safety of vaginal estrogenic products [10,11,17,19–21,23], although that is not a prerequisite posed by authorities' guidelines (either the FDA or European Medicines Agency [EMA]) [36,37]. Due to the enzymes' characteristics in sex hormone synthesis, an increase of serum E1, E2, testosterone and progesterone levels after E3 application is simply impossible [35]. The reported increase in serum E3 levels by some studies can be explained as a direct effect of vaginal absorption. Indeed, several other studies on the effect of vaginal E2 on serum levels of estrogens only showed a small increase in serum E2 levels, which was the estrogen that was directly applied and absorbed in that case [38,39] and failed [38,40] or only found a small increase [39] in the levels of E1. This increase in E1 could be explained by the potential conversion of E2 to E1 through 17 β -hydroxysteroid dehydrogenase, a step that is not even feasible after application of E3, as there is no known physiological pathway for the conversion of E3 to a different type of estrogen. In theory, vaginal application of E2 could also lead to an increase in serum E3, but we were not able to find any studies on that topic.

Only one study reported an increase in serum PRL levels [24], which can probably be attributed to the known fluctuation of PRL itself. Furthermore, there were no serum PRL level changes if the same vaginal E3 dose was applied in another galenic form [24]. Also, the reported increased serum PRL level was within the normal range and thus clinically irrelevant.

Table 2. Effects of vaginal estriol (E3) on serum estradiol (E2) levels.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison	E2 (A)	E2 (B)	E2 (C)
Luisi et al. (1980) [9]	Controlled trial	Total: 14 patients A: 7 B: 7	Participants between 53 and 65 years No further data available	A: 0.5 mg E3/day for 3 weeks (Ovestin® vaginal cream) B: 1.25 mg CEE/day for 3 weeks (Premarin® vaginal cream)	A vs. B (mean) 0 days, 8 days, 15 days, 21 days (morning)	0 days: ? 8 days: ? (no effect) 15 days: ? (no effect) 21 days: ? (no effect)	0 days: 26.3 pg/ml 8 days: ?, $p < 0.05$ (A vs. B) 15 days: ?, $p < 0.05$ (A vs. B) 21 days: 139.2 pg/ml $p < 0.05$ (A vs. B)	
Sánchez-Rovira et al. (2020) [10] / Hirschberg et al. (2020) [11]	Controlled trial	Total: 61 patients A: 50 B: 11	A: mean age 58.9 years B: mean age 61.4 years	A: 50 µg E3/day for 3 weeks + 2x/week for 9 weeks (Blissel®, Gelistrol®, Gélisse® vaginal gel) B: moisturizer 1x/day for 3 weeks + 2x/week for 9 weeks (Ainara® vaginal gel)	Pre vs. post comparison (median + Δ): physiological variability vs. treatment variability) 0 weeks, 1 week, 3 weeks, 8 weeks, 12 weeks	0 weeks: <1.5 pg/ml (below limit of quantitation) 1 week: <1.5 pg/ml (below limit of quantitation) 3 weeks: <1.5 pg/ml (below limit of quantitation) 8 weeks: <1.5 pg/ml (below limit of quantitation) 12 weeks: <1.5 pg/ml (below limit of quantitation)	0 weeks: <1.5 pg/ml (below limit of quantitation) 1 week: <1.5 pg/ml (below limit of quantitation) 3 weeks: <1.5 pg/ml (below limit of quantitation) 8 weeks: <1.5 pg/ml (below limit of quantitation) 12 weeks: <1.5 pg/ml (below limit of quantitation)	
Wesel et al. (1981) [12]	Single-arm pre-post interventional study	Total: 50 patients A: only 15 for the outcome of interest	A: mean age 61.76 years	A: 0.5 mg E3/day for 3 weeks + 2x/week for 4 weeks (Ortho-Gynest® vaginal pessaries)	Blood samples: 0 weeks, 1 week, 3 weeks, 5 weeks, 7 weeks	No change, always remained <30 pg/ml for the majority of patients		
Te West et al. (2020) [13]	Multi-arm pre-post interventional studies	Total: 76 patients (90 recruited) A: 27 (40 recruited, 13 withdrew) a: 10, b: 10, c: 20 B: 49 (50 recruited, 1 excluded)	A: mean age 66.6 years (A = new users) B: median age 75.5 years (B = chronic users, >3 months)	Aa: 0.5 mg E3/day for 3 weeks + 2x/week for 9 weeks (Ovestin® vaginal cream with applicator) Ab: 0.5 mg E3/day for 3 weeks + 3x/week for 9 weeks (Ovestin® vaginal cream, digitally, measured) Ac: 0.5 mg E3/day for 3 weeks + 3x/week for 9 weeks (Ovestin® vaginal cream, digitally, free) B: 0.5 mg E3 2-3x/week for 9 weeks (Ovestin® vaginal cream)	Median (IQR) – Aa, Ab, Ac analyzed as one group Blood samples: 0 weeks, 2 weeks (12h after last E3)	0 weeks: 17.0 pmol/l (9.7–26.0) 2 weeks: 16.4 pmol/l (8.3–26.0)	2 weeks: 12.8 pmol/l (6.7–18.1)	

(Continued)

Table 2. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison	E2 (A)	E2 (B)	E2 (C)
Hoz and Orozco-Gallego (2018) [14]	Controlled trial	Total: 387 patients A: 192 B: 195	A: mean age 59.0 years B: mean age 58.0 years	A: 1 mg E3/day for 2 weeks + 1×/week for total 2 years (vaginal cream) B: 0.625 mg CEE/day for 2 weeks + 1×/week for total 2 years (vaginal cream)	A vs. B (mean ± SD) 0 months, 6 months, 12 months, 18 months, 24 months	0 months: 41.4 ± 11.4 pg/ml 24 months: 52.8 ± 13.5 pg/ml	0 months: 43.2 ± 13.5 pg/ml 24 months: 54.6 ± 11.7 pg/ml	
Haspels et al. (1981) [15]	Single-arm pre-post interventional study	Total: 11 patients A: 11	A: between 54 and 70 years (no further information provided)	A: 0.5 mg E3/day for 3 weeks + 2×/week for 5 weeks (Ovestin® vaginal cream)	Pre vs. post (mean ± SD) Blood samples: 0 days, 21 days, 35 days, 56 days (ca. 10–12 h after application)	0 days: 46.2 ± 9.1 pmol/l 21 days: 49.9 ± 11.7 pmol/l 35 days: 51.0 ± 11.1 pmol/l 56 days: 52.1 ± 12.3 pmol/l Treatment did not induce changes in E2 levels		
Biglia et al. (2010) [16]	Controlled trial	Total: 26 patients A: 10 B: 8 C: 8	A+B: mean age 54.1 years C: mean age 46.1 years	A: 0.25 mg E3 2×/week for 12 weeks (Colpogyn® vaginal cream) B: 12.5 µg E2 2×/week for 12 weeks (Vagifem® vaginal tablets) C: 2.5 g moisturizer 2×/week for 12 weeks (Replens® vaginal cream)	A vs. B and pre vs. post for E3 (mean or score change ± SD) A+B vs. C (excluded, E2/E3 combined)	0 weeks: 14.4 ± 5.3 pg/ml 12 weeks: +3.5 ± 5.1 pg/ml	0 weeks: 19.4 ± 10.0 pg/ml 12 weeks: +2.7 ± 10.6 pg/ml	0 weeks: 27.5 ± 19.4 pg/ml
Crandall et al. (2020) [17]	Systematic review	Total: 63 articles – studying E3: 2 – serum hormones: 1 not separately included a) van Haafden et al. (1989) [34] – controlled trial	a: 29 patients (A: 9, B: 8, C: 12)	A: 0.05 mg E2/day for 3 weeks (vaginal ovula) B: 0.5 mg E3/day for 3 weeks (vaginal ovula) C: control (untreated)	Pre vs. post 3 weeks (mean)	Specific values not provided Levels were unchanged by either of the active treatments No results of statistical comparisons were presented	Specific values not provided Levels were unchanged by either of the active treatments No results of statistical comparisons were presented	Mean: 29 pmol/l Levels were unchanged by either of the active treatments No results of statistical comparisons were presented
Te West et al. (2021) [18]	Single-arm pre-post interventional study	Total: 10 patients A: 10	A: mean age 70.0 years Chronic users	A: chronic usage omitted for ≥36 h prior to the study, then 0.5 mg E3 once (Ovestin® vaginal cream)	Pre vs. post (mean (SE)) Blood samples: 0 h, 1 h, 2 h, 3 h, 5 h, 8 h, 10 h, 12 h, 24 h	No evidence of a change in E2 concentrations following application of E3		

(Continued)

Table 2. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison	E2 (A)	E2 (B)	E2 (C)
Rueda et al. (2017) [19]	Systematic review	Total: 22 articles – studying E3: 20 – studying E3 and serum hormones: 11 studies (4 already included [14,19,23] – 1 excluded based on our criteria since combination E3/P4 studied – 6 not separately included)	a: (Controlled trial) 17 patients (10 E3 vs. 7 no treatment) b: (Pre–post interventional study) 3 patients c: (Pre–post interventional study) 6 patients d: (Controlled trial) 10 patients (5 E3) e: (Controlled trial) 8 patients f: (Pre–post interventional study) 9 patients	a: 1 mg E3 ovula 14 h pre and 1 h post-OP for uterine prolapse b: 0.5 mg E3 for 10 days (ovula) c: 1 mg E3 for 21 days (ovula) d: 2.4 mg CEE vs. 2 mg E2 vs. 0.5 mg E3 for 24 h e: 0.5 mg E3 cream or ovula for 14 days (time comparison) f: 0.5 mg E3 for 21 days	b: no change d: no change			
Kivovic et al. (1980) [20]	Controlled trial	Total: 74 patients (32 for serum studies) A: 23 (17 serum studies) B: 30 (10 serum studies) C: 21 (5 serum studies)	Ages between 44 and 82 years (no further information provided)	A: 1 mg E3/day for 3 weeks + subgroup further 2x/week for 13 weeks (Ovestin® vaginal cream) B: 0.5 mg E3/day for 3 weeks + subgroup further 2x/week for 13 weeks (Ovestin® vaginal cream) C: 0.5 mg E3/day for 2 weeks (Ovestin® vaginal suppositories)	Blood samples: 1 day (0 h, 1 h, 2 h, 3 h, 4 h, 6 h) and 21 days (A and B) or 15 days (C) – 12 h after last dose (=0 h and 1 h, 2 h, 3 h, 4 h, 6 h)	1 day: ? 21 days: unaffected	1 day: ? 21 days: unaffected	1 day: ? 15 days: unaffected
Donders et al. (2014) [21]	Single-arm pre–post interventional study	Total: 16 patients A: 16	A: mean age 57.0 years	A: 0.03 mg E3/day for 4 weeks + 3x/week for 8 weeks (Gynoflor® vaginal tablets)	Pre vs. post (mean (SD)) Blood samples: 0 days and 28 days; 0 h, 0.5 h, 1 h, 3 h, 2 h, 4 h, 6 h, 8 h, 24 h	No increase, always under LLOQ (1.00 pg/ml) both 0 days and 28 days		
Pfeiler et al. (2011) [22]	Single-arm pre–post interventional study	Total: 10 patients A: 10	A: median age 65.0 years	A: 0.5 mg E3/day for 2 weeks (Ovestin® vaginal tablets)	Pre vs. post comparison (mean) 0 weeks: 2 weeks (8–12 h after E3)	0 weeks: <10 pg/ml 2 weeks: <10 pg/ml		

The meta-analysis of Pavlović et al. [23] is not included in the table since the results of all included studies are separately presented. CEE, conjugated equine estrogens; IQR, interquartile range; LLOQ, lower limit of quantitation. A, B, C refer to the groups within each study and the respective treatment and outcomes for each. The statistically significant results ($p < 0.05$) appear in bold.

Table 3. Effects of vaginal estriol (E3) on serum gonadotropin (FSH and LH) levels.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison	FSH (A)	FSH (B)	FSH (C)	LH (A)	LH (B)	LH (C)
Mattsson and Cullberg (1983) [24]	Multi-arm pre-post interventional studies	Total: 30 patients (3 withdrawn) A: 14 B: 13	A: mean age 59.5 years B: mean age 58.0 years	A: 0.5 mg E3/day for 14 days + 2x/week for 6 weeks (Ovestin® vaginal cream) B: 0.5 mg E3/day for 14 days + 2x/week for 6 weeks (Ovestin® vaginal suppositories)	Pre vs. post comparison (mean ± SE) (20h after last E3)	0 weeks: 14.2 ± 0.4 µg/l 2 weeks: 13.5 ± 0.4 µg/l, p < 0.01	0 weeks: 14.9 ± 0.7 µg/l 2 weeks: 13.7 ± 0.6 µg/l, p < 0.05	0 weeks: 9.2 ± 0.5 µg/l 2 weeks: 8.0 ± 0.6 µg/l	0 weeks: 10.3 ± 0.9 µg/l 2 weeks: 11.6 ± 1.0 µg/l		
Luisi et al. (1980) [9]	Controlled trial	Total: 14 patients A: 7 B: 7	Participants aged between 53 and 65 years No further data available	A: 0.5 mg E3/day for 3 weeks (Ovestin® vaginal cream) B: 1.25 mg CEE/day for 3 weeks (Premarin® vaginal cream)	A vs. B (mean) 0 days, 8 days, 15 days, 21 days (morning)	0 days: 75.2 mIU/ml 8 days: ? 15 days: ? 21 days: 70.5 mIU/ml	0 days: 71.0 mIU/ml 8 days: ?, p < 0.05 (A vs. B) 15 days: ?, p < 0.05 (A vs. B) 21 days: 55.6 mIU/ml, p < 0.05 (A vs. B)	0 days: 59.3 mIU/ml 8 days: ? 15 days: 53.1 mIU/ml 21 days: ?	0 days: 57.6 mIU/ml 8 days: ?, p < 0.05 (A vs. B) 15 days: ?, p < 0.05 (A vs. B) 21 days: 44.4 mIU/ml, p < 0.05 (A vs. B)		
Sánchez-Rovira et al. (2020) [10] / Hirschberg et al. (2020) [11]	Controlled trial	Total: 61 patients A: 50 B: 11	A: mean age 58.9 years B: mean age 61.4 years	A: 50 µg E3/day for 3 weeks + 2x/week for 9 weeks (Blisse®, Gellistrol®, Gellisse® vaginal gel) B: moisturizer 1x/day for 3 weeks + 2x/week for 9 weeks (Ainara® vaginal gel)	Pre vs. post comparison (median + Δ; physiological variability vs. treatment variability)	0 weeks: 54.6 mIU/ml 1 week: 47.6 mIU/ml, Δ = -4.8, p < 0.05 3 weeks: 48.7 mIU/ml, Δ = -4.2, p < 0.05 8 weeks: 51.4 mIU/ml, Δ = -2.6 12 weeks: 51 mIU/ml, Δ = -2.8	0 weeks: 47.6 mIU/ml 1 week: 53.8 mIU/ml, Δ = +4.2 3 weeks: 65.1 mIU/ml, Δ = -0.9 8 weeks: 55.7 mIU/ml, Δ = +5.2 12 weeks: 62.1 mIU/ml, Δ = +1.4	0 weeks: 19.8 mIU/ml 1 week: 18.2 mIU/ml, Δ = -0.6 3 weeks: 19.6 mIU/ml, Δ = -0.5 8 weeks: 17.7 mIU/ml, Δ = -0.3 12 weeks: 19.3 mIU/ml, Δ = -0.8	0 weeks: 25.3 mIU/ml 1 week: 30.1 mIU/ml, Δ = +0.2 3 weeks: 28.7 mIU/ml, Δ = +0.3 8 weeks: 27.8 mIU/ml, Δ = -1.3 12 weeks: 28.9 mIU/ml, Δ = +1.3		
Haspels et al. (1981) [15]	Single-arm pre-post interventional study	Total: 11 patients A: 11	A: between 54 and 70 years (no further information provided)	A: 0.5 mg E3/day for 3 weeks + 2x/week for 5 weeks (Ovestin® vaginal cream)	Pre vs. post (mean ± SD) Blood samples: 0 days, 21 days, 35 days, 56 days (ca. 10–12 h after application)	0 days: 66.0 ± 13.8 U/l 21 days: 62.8 ± 12.7 U/l 35 days: 64.9 ± 11.5 U/l 56 days: 65.8 ± 14.2 U/l FSH had a slight suppression on day 21	0 days: 48.7 ± 7.4 U/l 21 days: 45.1 ± 6.2 U/l 35 days: 46.5 ± 7.3 U/l 56 days: 47.0 ± 8.7 U/l LH had a slight suppression on day 21				

(Continued)

Table 3. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison	FSH (A)	FSH (B)	FSH (C)	LH (A)	LH (B)	LH (C)
Biglia et al. (2010) [16]	Controlled trial	Total: 26 patients A: 10 B: 8 C: 8	A + B: mean age 54.1 years C: mean age 46.1 years	A: 0.25 mg E3 2x/week for 12 weeks (Colpogyn* vaginal cream) B: 12.5 µg E2 2x/week for 12 weeks (Vagifem* vaginal tablets) C: 2.5 g moisturizer 2x/week for 12 weeks (Replens* vaginal cream)	A vs. B and pre vs. post for E3 (mean or score change ± SD) A + B vs. C (excluded, E2/E3 combined)	0 weeks: 34.5 ± 20.6 mU/ml 12 weeks: -4.02 ± 6.9 mU/ml	0 weeks: 62.0 ± 40.9 mU/ml 12 weeks: -2.64 ± 9.8 mU/ml	0 weeks: 23.3 ± 24.2 mU/ml	0 weeks: 25.3 ± 19.5 mU/ml 12 weeks: -6.4 ± 13.8 mU/ml	0 weeks: 30.5 ± 18.3 mU/ml 12 weeks: -10.6 ± 19.5 mU/ml	0 weeks: 10.5 ± 19.4 mU/ml
de Oliveira Filho et al. (2019) [25]	Controlled trial	Total: 31 patients A: 10 B: 10 C: 11	A: mean age 56.1 years B: mean age 55.9 years C: mean age 55.5 years	A: 0.125 mg E3/day for 21 days (single vaginal ring) B: 0.250 mg E3/day for 21 days (single 300 mg vaginal ring) C: 0.500 mg E3/day for 21 days (single 600 mg vaginal ring)	Pharmacokinetic/dynamics	0 days: 57.35 mU/ml Clear dose dependency in mean maximum decrease of FSH and rate of increase afterwards Back to baseline within second half of the treatment (only group)	0 days: 76.27 mU/ml Clear dose dependency in mean maximum decrease of FSH and rate of increase afterwards	0 days: 74.05 mU/ml Clear dose dependency in mean maximum decrease of FSH and rate of increase afterwards	0 days: 31.72 mU/ml Clear dose dependency in mean maximum decrease of LH Back to baseline within 7 days	0 days: 48.46 mU/ml Clear dose dependency in mean maximum decrease of LH Back to baseline within 7 days	0 days: 44.19 mU/ml Clear dose dependency in mean maximum decrease of LH Back to baseline within 7 days
Delgado et al. (2016) [26]	Controlled trial	Total: 43 patients A: 13 B: 12 C: 12 D: 6	A: mean age 57.4 years B: mean age 59.6 years C: mean age 57.8 years D: mean age 62.7 years	A: 0.002% E3/day for 21 days (vaginal gel) B: 0.005% E3/day for 21 days (vaginal gel) C: 0.1% E3/day for 21 days (Ovestinon* vaginal cream) D: placebo for 21 days (vaginal gel)	Pre vs. post and among groups (E3 mean, FSH/LH median) Blood samples: 1 day, 21 days	1 day: 60.8 IU/l 21 days: 64.2 IU/l	1 day: 51.2 IU/l 21 days: 63.4 IU/l	1 day: 79.1 IU/l 21 days: 68.25 IU/l p = 0.045 also p = 0.03 when comparing day 0-day 21 change among groups A, B, C	1 day: 28.05 IU/l 21 days: 30.05 IU/l	1 day: 26.9 IU/l 21 days: 30.8 IU/l	1 day: 35.1 IU/l 21 days: 40.4 IU/l
Crandall et al. (2020) [17]	Systematic review	Total: 63 articles – studying E3: 2 – studying E3 and serum hormones: 1 not separately included a) Van Haften et al. (1989) [34] – controlled trial	a: 29 patients (A: 9, B: 8, C: 12)	A: 0.05 mg E2/day for 3 weeks (vaginal ovula) B: 0.5 mg E3/day for 3 weeks (vaginal ovula) C: control (untreated)	Pre vs. post 3 weeks (mean)	3 weeks mean: 38.44 IU/l No further details provided, nor between-group comparisons No results of statistical comparisons presented	3 weeks mean: 31.25 IU/l No further details provided, nor between-group comparisons No results of statistical comparisons presented	3 weeks mean: 29.00 IU/l No further details provided, nor between-group comparisons No results of statistical comparisons presented	3 weeks mean: 35.11 IU/l No further details provided, nor between-group comparisons No results of statistical comparisons presented	3 weeks mean: 43.50 IU/l No further details provided, nor between-group comparisons No results of statistical comparisons presented	3 weeks mean: 27.92 IU/l No further details provided, nor between-group comparisons No results of statistical comparisons presented

(Continued)

Table 3. Continued.

Study (authors and year)	Study design	Study cohort (n)	Cohort characteristics	Intervention/control (dose, form, duration)	Type of comparison	FSH (A)	FSH (B)	FSH (C)	LH (A)	LH (B)	LH (C)
Donders et al. (2014) [21]	Single-arm pre-post interventional study	Total: 16 patients A: 16	A: mean age 57.0 years	A: 0.03 mg E3/day for 4 weeks + 3x/week for 8 weeks (Gynoflor® vaginal tablets)	Pre vs. post (mean (SD)) Blood samples: 0 days and 28 days; 0h, 0.5h, 1h, 3h, 2h, 4h, 6h, 8h, 24h	0 days: 107.88 mU/ml (48.62) 14 days: 103.71 mU/ml (39.76) 28 days: 98.94 mU/ml (34.95), p < 0.025 56 days: 103.55 mU/ml (37.53) 84 days: 105.93 mU/ml (43.69) 0 weeks: 75.7 mU/ml 2 weeks: 66.0 mU/ml, p = 0.01	0 days: 36.46 mU/ml (11.05) 14 days: 34.52 mU/ml (12.18) 28 days: 34.02 mU/ml (12.68) 56 days: 34.2 mU/ml (49.87) 84 days: 35.13 mU/ml (10.34) No statistically significant changes 0 weeks: 32.4 mU/ml 2 weeks: 28.9 mU/ml, p = 0.02				
Pfeiler et al. (2011) [22]	Single-arm pre-post interventional study	Total: 10 patients A: 10	A: median age 65.0 years	A: 0.5 mg E3/day for 2 weeks (Ovestin® vaginal tablets)	Pre vs. post comparison (mean) 0 weeks, 2 weeks (8–12 h after E3)	0 weeks: 75.7 mU/ml 2 weeks: 66.0 mU/ml, p = 0.01					

The meta-analysis of Pavlović et al. [23] is not included in the table since the results of all included studies are separately presented. CEE, conjugated equine estrogens; FSH, follicle stimulating hormone; LH, luteinizing hormone. A, B, C refer to the groups within each study and the respective treatment and outcomes for each. The statistically significant results ($p < 0.05$) appear in bold.

Although some studies found significant alterations in serum gonadotropin levels [9–11,21,22,24,26], they always remained within the postmenopausal range and are thus clinically irrelevant. When looking only into data from studies that used lower-dose E3 vaginal preparations (<0.5 mg), two studies [10,11,26] still reported a statistically significant decrease in FSH with a 0.03 mg and a 0.05 mg preparation, yet as mentioned those decreases were transient and clinically insignificant with the lowest reported FSH to be at 68.25 IU/l (decreased from a baseline of 79.1 IU/l).

Thus, the only clinically relevant serum hormone level change would be that of E3. The majority of studies did not report any changes in serum E3 levels after vaginal E3 application [9–13,15,18,20,22,25,27]. The remainder only found transient increases of serum E3 levels [19,21,24,26], and importantly no significant differences when compared to vaginal E2, CEE or placebo application [9–11,19,32]. If we focus again on studies using low-dose E3 preparations (<0.5 mg), there are still two to report statistically significant increases in serum E3 levels [21,26]. This increase was less prominent for those preparations when compared to the 0.5 mg preparations, while as mentioned it seemed to be less prominent with chronic use of the products. Of note, the method used to measure the serum E3 did not appear to have significant effect on the results. All included studies used either radioimmunoassay, gas chromatography–mass spectrometry or liquid chromatography with tandem mass spectrometry, yet all three methods were used both in studies reporting no changes as well as in those reporting a statistically significant increase.

There are also some limitations to this systematic review. First, some studies were conducted for pharmacokinetic purposes allowing no extrapolation to long-term use. Secondly, sample sizes were quite small, which is common for pharmacokinetic studies. Third, comparisons between studies were difficult due to different doses, galenic forms and application frequencies.

Overall, the results of our systematic review support previous reviews that vaginal E3 application in postmenopausal women does not cause any clinically significant serum hormone level changes [17,19]. Furthermore, from a biochemical point of view, E3 products have a lower potential to alter the rest of serum estrogens when compared with E2 products, as there is no known pathway for the conversion of the less potent E3 to the more potent E2. However, in order to assess the long-term safety of vaginal E3 products and be able to draw definite conclusions, larger observational and randomized trials would be necessary with additional endpoints other than serum hormone levels, such as estriol conjugates in the breast, endometrial or myometrial tissue.

Conclusions

Vaginal E3 for GSM treatment does not exert clinically relevant effects on serum sex hormone levels. Thus, based on the current literature and considering serum hormones' alterations as a proxy for safety, vaginal E3 products can be considered as safe as other vaginal estrogens.

Acknowledgements

The authors would like to thank Dr Marc von Gernler from the University Library of Bern for his valuable help in the literature searches, especially the update prior to publication.

Potential conflict of interest This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. P. Stute, C. Betschart and D. Wunder have been part of an interdisciplinary expert board funded by EFFIK SA. A. Kolokythas reports no conflict of interests. The authors alone are responsible for the content and writing of the article.

Source of funding Nil.

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