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Published online: 21 Mar 2019.

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REVIEW

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Pharmacotherapy for the treatment of vaginal atrophy

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ABSTRACT

Introduction: Despite its frequency, recognition and therapy of vulvovaginal atrophy (VVA) remain suboptimal. Wet mount microscopy, or vaginal pH as a proxy, allows VVA diagnosis in menopause, but also in young contraception users, after breast cancer, or postpartum. Intravaginal low dose estrogen product is the main therapy. Ultra-low-dose vaginal estriol is safe and sufficient in most cases, even in breast cancer patients, while hyaluronic acid can help women who cannot or do not want to use hormones.

Areas covered: The authors provide an overview of the current pharmaceutical treatment for vulvovaginal atrophy and provide their expert opinions on its future treatment.

Expert opinion: The basis of good treatment is a correct and complete diagnosis, using a microscope to study the maturity index of the vaginal fluid. Minimal dose of estriol intravaginally with or without lactobacilli is elegant, cheap and can safely be used after breast cancer and history of thromboembolic disease. Laser therapy requires validation and safety data, as is can potentially cause vaginal fibrosis and stenosis, and safer and cheaper alternatives are available.

1. Introduction

Vulvovaginal atrophy (VVA) is a chronic condition that commonly occurs mostly in postmenopausal women, but sometimes in reproductive age patients under the influence of natural or induced decrease or activity of female sex hormones. External factors like smoking or alcohol abuse can also trigger this condition [1,2].

Taking into consideration that hypoestrogenic condition, combined with a decrease of other sex hormones, results in a broad array of both urinary tract and vulvovaginal symptoms [3,4], it was suggested by International Society for the Study of Women's Sexual Health and The North American Society to change 'vulvovaginal atrophy' into 'genitourinary syndrome of menopause' (GSM) [5–8].

Although we agree that the term 'atrophic vaginitis' is not always accurate in describing all aspects of the clinical condition (e.g. due to the fact that there is not always a concomitant inflammation present) we also have to admit the newly proposed syndrome might make the diagnosis vague and increase the risk to underdiagnose important specific vulvar and vaginal diseases such as lichen sclerosus, lichen ruber, vulvar dysplasia, aerobic vaginitis, etc. Hence, for the purpose of clarity in this chapter, we will use the term 'vulvovaginal atrophy (VVA)' in the following paragraphs.

2. Pathogenesis

The vagina is composed of four layers – the epithelium, the lamina propria, the muscular layer, and the adventitia. All layers have receptors for ovarian sex steroid hormones. Shortage of sex hormones in the postmenopausal period affects all layers of the vagina and leads to dramatic anatomical and physiological changes, such as shortening and narrowing of the vagina, loss of rugae, and flattening and keratinization of the surface [9].

2.1. Changes in the epithelium

2.1.1. Estrogen receptor stimulation

The epithelium is a superficial non-keratinized layer of vagina. Stimulated estrogen receptors (ER) increase epithelial proliferation and stratification. Supra(Para)basal cells undergo differentiation, move up through to the superficial layers of the epithelium, while undergoing cornification [10]. Nuclear αER stimulation occurs in both the epithelial and stromal layers, as stimulation of only epithelial or stroma is insufficient to observe expression of cytokeratin. Alfa-ER stimulation differentiates the vaginal epithelium through Wisp2 (WNT1 inducible signaling pathway protein 2), inducing stratification of vaginal stoma cells [11]. Due to the decrease of estrogen, epithelial cells became smaller, with less cytoplasmic content. The vaginal epithelium loses intermediate epithelial cells, leading to a severely reduced intermediate cell layer [12].

2.1.2. Progesterone

Except if administered together with estrogen, progesterone did not restore the morphological changes of the vagina in oophorectomized mice, although the epithelial proliferation

was less pronounced than after estrogen-only stimulation [12]. So progesterone may be less important for epithelial restoration, but helps to maintain epithelial integrity after restoration [12].

2.1.3. Androgens

Androgen receptors, located at the proximal ends of the nerves, are present in both the epithelial layer, lamina propria and muscular layer [13]. Data about the androgenic effect on the epithelial layer are conflicting.

Systemic estrogen replacement, followed by higher levels of circulating sex hormone binding globulin (SHBG) and lower free testosterone lead to lower densities of adrenal receptors in postmenopausal women [14]. In postmenopausal women, lack of testosterone is held responsible for decreased lubrication, vaginal maturation and sexual dysfunction, which was reversed by high doses of intravaginal testosterone through its conversion to estrogen, mediated by 5α-reductases [14,15]. Topical application of testosterone was also observed to decrease keratinization, and increase mucification [12,13]. However, before considering it as a potential treatment option for vaginal atrophy, more research is necessary to determine its dose and a safe cut-off level of serum testosterone for follow-up.

2.1.4. Dehydroepiandrosterone (DHEA)

The positive mechanism of action of DHEA (prasterone) on the vagina and sexual health [16] is likely due to the local aromatization of androstenedione and testosterone to estrone (E1) and estradiol (E2) [17,18]. After menopause, the secretion of estrogen by ovaries is stopped and its serum level remains in biologically inactive concentrations, whereby DHEA becomes the only source of estrogens and androgens, converted in peripheral tissues. There is also no feedback control mechanism which allows to increase the level of DHEA when its serum level decreases [19], making supply of exogenous DHEA the only way to correct for this deficiency.

2.1.5. Vitamin D and E influence on vaginal epithelium

Vitamin D [1,25-dihydroxyvitamin D3 1,25(OH) 2D3] is a promoter of keratinocyte differentiation and proliferation in the epidermis [20]. It stimulates the proliferation of the vaginal epithelium by activating the vitamin D receptor (VDR)/p-RhoA/p-Ezrin pathway, upregulating cell-to-cell junction. This opens an interesting nonhormonal alternative for the treatment of VVA, but clinical studies are necessary to support this theory.

Being a fat-soluble vitamin with strong antioxidant properties, vitamin E is involved in the metabolism of all cells and prevents the tissue damage caused by oxidants. It keeps the arteries flexible and facilitates blood circulation, which consequently increases the metabolism of vaginal connective tissues and enhances the moisture and flexibility of vaginal walls [21]

2.1.6. Oxytocin

Oxytocin stimulates oxytocin receptors co-located with caveolin enriched micro-domains (a subset of lipid rafts containing the scaffolding protein caveolin-1) [22]. This stimulation is important for epithelial proliferation [23]. The effect is dose and time dependent, and increases the number of layers in the epithelium [24]. As not all cells have oxytocine receptors, the effect is moderate [23].

2.2. The lamina propria changes

Collagen and elastan are two important players in the dynamic structural changes of the lamina propria: the first provides rigidity, the second elasticity to the tissue [25]. The balance between matrix synthesis in fibroblasts and degradation is a continuous process. Matrix metalloproteases (MMT 2 and 9) and cathepsins are responsible for the degradation of collagen in post-menopausal women [25,26]. Increasing weakness of fibroblast function is one explanation of the vaginal atrophy process, while estrogen substitution was shown to restore collagen production and inhibits MMT expression [27]. The fibroblast dysfunction leads to loss of rigidity and increases elastan density, which was more obvious in post-menopausal women with prolapse compared to controls [25]. Histological evidence of increased collagen degradation and replacement by new collagen was found during local estrogen replacement therapy, however without clinically significant improvement of stress incontinence for urine [26]. After treatment with DHEA, a precursor for estrogen and testosterone, a higher density of collagen was observed in oophorectomized rats [13]. So the lamina propria is thickening due to estrogen shortage, which can be reverted by estrogen replacement by its dual effect on the balance between collagen synthesis and on destruction of it.

2.3. The muscular layer changes

Under the lamina propria is an inner circular and outer longitudinal smooth muscle layer, composed of smooth muscle cells embedded in collagen. Smooth muscle loss is observed in post-menopausal vaginas, especially in women with prolapse [12,28]. In women with vaginal prolapse, replacement of collagen I to collagen III, re-differentiation of smooth muscles into myofibroblasts and muscular architectural changes were observed in the anterior vaginal wall [29]. So the loss of collagen is related both to hypoestrogenism and to genetic factors.

2.4. Changes in vessels' endothelium

Large blood vessels, accompanied with nerve bundles with ganglion cells, are found in the adventitia, while smaller nerve fibers and vessels pierce the muscular layer, even to the lamina propria up to the basal membrane [12].

2.4.1. Endothelial Nitric oxide synthase (NOS)

NOS is important for the endothelium of the vaginal blood vessels. It modulates both vaginal blood supply and vaginal smooth musculature [12,30]. Endothelial nitric oxide synthase (NOS) secretion was decreased in the hypoestrogenical status of oophorectomized mice and restored after estrogen replacement therapy [30]. Estrogen replacement in oophorectomized rabbits significantly increased genital blood flow and lubrication [31].

2.4.2. Androgen receptors

Androgen receptors were found in the vascular endothelium as well [14]. However, systemic treatment with testosterone did not increase blood flow, nor lubrification [31]. Topical application of testosterone, on the other hand, was observed to stimulate mucus secretion [12]. In rabbits, the NOS and arginase secretion are regulated differently in proximal and distal parts of vagina. Testosterone increases enzyme activity and secretion only in the distal part of vagina [32].

2.5. Nerve fibers

Researchers found that cyclic variation of adrenergic innervation was most associated with muscular tissue [12,33] where mostly tyrosine hydroxylase (TH)-positive nerve fibers were found. Increased sympathetic innervation during menopause possibly has an influence on vasoconstriction, as well as on the hypersensitivity of nociceptors [34]. Furthermore, apoptosis in vaginal nerves, regulated by NOS, was higher in oophorectomized mice than in controls [30]. Estrogen replacement therapy downregulates these mechanisms [34], reducing neuro-fiber density [35].

However, the results are conflicting. Pessina et al. did not find decrease of fibers under estrogen administration, while testosterone administration increased length and density of TH-positive nerve fibers [12], the functional meaning of which is still unclear.

Acetylcholinesterase positive fibers are responsible for contraction of the proximal and relaxation of the distal part of the vagina [36]. This vaginal physiological reaction was reported

while studying sexual response during arousal [37]. This response is most pronounced in the subepithelial plexus. In atrophic vaginas, Li et al. noticed a significant decrease of the number of neuro-fibers and microvessels [38].

Androgens have a regulative effect on the nerve fiber network, as testosterone is associated with neurogenic relaxation of these non-vascular smooth muscles [32]. Testosterone administration was found to increase the protein gene product 9.5 (PGP 9.5) in oophorectomized animals, thereby thickening these fibers [12], as well as a DHEA-derived androgenic effect [13]. According to some authors, estrogen has no effect on in PGP 9.5 dependent nerve thickening [12], while according to another it has [34].

3. Diagnosis

3.1. Signs and symptoms of VVA/GSM

According to the REVIVE survey [39] of 3,046 postmenopausal women, experiencing VVA symptoms, the most common are vaginal dryness (55%), pain during intercourse/dyspareunia (44%) and vaginal irritation (37%). Itching, pain during exercise, tenderness, and bleeding related to intercourse can also be present [40]. The main urinary tract symptoms can be urgency, dysuria, recurrent urinary tract infections, urge incontinence, stress incontinence, and involuntary voiding. All these symptoms could have a significant impact on the quality of life [1,39–48].

3.2. Clinical history and questionnaires

There is no precise consensus at this moment about the number of symptoms, neither the intensity needed to diagnose VVA [49,50]. Most often the diagnosis is based on patient interview, sometimes by precisely describing vulvovaginal or urinary tract symptoms, but often only by registering vague complaints about the decreased quality of life [1,43,51,52]. A recent European Vulvovaginal Epidemiological Survey (EVES) has shown that severe VVA symptoms had a direct association with worse quality of life in postmenopausal women and should be promoted for appropriate clinical assessment and early therapeutic intervention [48,53].

Vaginal health index (VHI) as a five-score system developed by Bachmann et al. [54] can be a tool of diagnosis which allows to assess objectively and accurate the aging changes occurring in a patient's urogenital tissue. It includes five parameters: overall elasticity, fluid secretion type and consistency, vaginal Ph, epithelial mucosa, and moisture. The lower scores correspond to greater urogenital atrophy.

Other tools have been developed more recently, such as the -to-Day Impact of Vaginal Aging (DIVA) questionnaire. It is a new multidimensional structured, self-administered measure designed to facilitate the evaluation of the impact of vaginal symptoms on women's activities of daily living, emotional well-being, sexual functioning, and self-concept and body image [55].

Diagnosing only based on patient-reported symptoms, however, can be misleading and more serious alternative diagnoses should be ruled out. Besides hypo-estrogenemia-

related, other types of dyspareunia or vulvodynia may occur [56,57]. Vulvovaginal symptoms can indicate infections such as bacterial vaginosis (BV) or aerobic vaginitis (AV) [58]. Also, several important dermatoses or high-grade squamous intraepithelial or neoplastic lesions of the vulva could be missed, unless proper examination with or without biopsy is performed [49,59].

3.3. Inspection

During the inspection, a pale and fragile mucosa can be seen in 91% and 72% of women with VVA, respectively. In half of the affected women, petechiae can be seen and in 92% of women suffering from VVA, thinning or disappearance of the vaginal rugae can be detected [40]. While performing the vulvovaginal inspection, a vaginal smear can be taken for pH measurement and fresh mount microscopy reading, which will provide a treasure of precise information.

3.4. Microscopy

As a result of declining ovarian oestrogen production, fewer vaginal epithelial cells mature to superficial cells (Figure 1(a)), resulting in a decrease of glycogen content in the epithelial wall and appearance of desquamated intermediate mature cells from the middle layer becoming visible during microscopy of the vaginal smear (Figure 1(b)). Upon further thinning of the oestrogen deprived vaginal mucosa, in a later phase, parabasal (Figure 1(c)) and even basal cells (Figure 1(d)) may appear in the smear. The relative proportion of these cell types compose a 'Vaginal Epithelial Cell Maturation Index (VCMI)', which directly relates to the severity of the VVA. This index indicates the degree of maturation of vaginal epithelial cells by showing the percentage of parabasal cells, intermediate

cells, and superficial epithelial cells. Therefore, ideally, 100 epithelial cells should be counted using a phase contrast, 400 x magnification microscope, determining the percentages of each type of epithelial cells [60,61]. A shift to the left indicates oestrogen deprivation, while a shift to the right indicates oestrogen stimulation.

The glycogen derived from the vaginal epithelial cells comprises the main nutrition base for the vaginal microflora, mainly composed of lactobacilli [43]. As a result of declining levels of oestrogen and glycogen, there will be a concomitant decrease in the number of lactobacilli, and a rise in vaginal pH. As a result, as Tucker et al. demonstrated, vaginal pH can be a simple but effective proxy method to assess vaginal morphology. They in their study a significant correlation between vaginal pH and changes in microscopic (superficial and parabasal cell counts), clinical (changes of color, integrity, thickness, and secretion of the vaginal wall) and the symptoms of vaginal dryness and pain during or when attempting sexual intercourse [62]. Also in another study, vaginal pH correlated with vaginal atrophy symptoms [63].

However, one must be aware that the use pH as the only substitute diagnostic marker to diagnose VVA is insufficient, as increased pH may also indicate the presence of infections like BV, AV or *Trichomonas* vaginitis, or non-infectious causes like presence of blood, intravaginal products used, or sperm. During this microscopy reading, the presence of lactobacillae (lactobacillary grades) should be evaluated [64]. Also the presence of aerobic and/or anaerobic bacteriae, in relation to the amount of lactobacilli, could be scored, leading to more specific therapeutic actions if required [65].

Therefore, taking a vaginal smear for wet mount microscopy is the most important and indispensable tool to confirm the diagnosis of VVA. It is easy to learn [66] and to perform at the office (e.g. while your patients get dressed) and the costs are negligible.
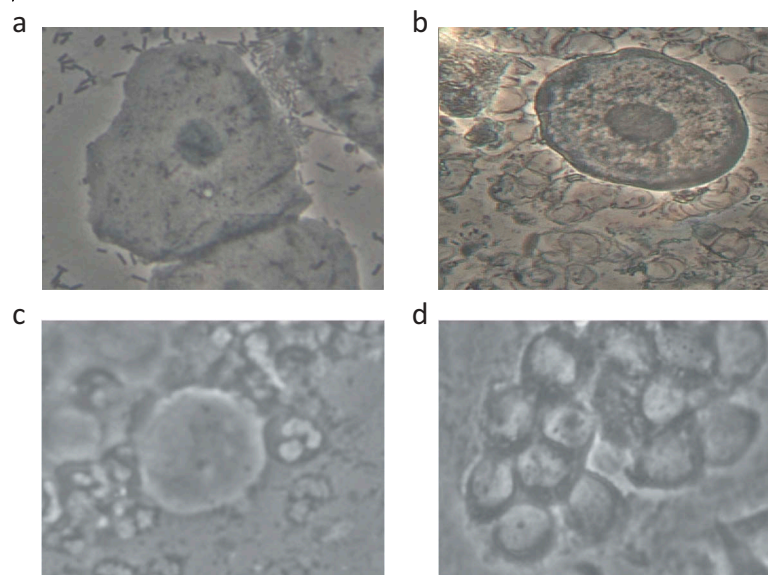

Figure 1. Depiction of different vaginal epithelial cell types composing the maturity index, and implied in the origin and diagnosis of vulvovaginal atrophy (VVA). Picture copyright of Femicare vzw (Prof G Donders).

4. Treatment

VVA can have several etiologies, and hence demands an individual treatment approach based on its specific pathophysiology. VVA in young women on contraception pills (aerobic vaginitis, atrophic type), menopausal women suffering from hypo-oestrogenemia, lactating women, women using anti-estrogenic medications (e.g. breast cancer) or after surgical castration, all need an adapted approach. Furthermore, some asymptomatic patients may also benefit from estrogen therapy, e.g. as a preoperative preparation before vaginal surgery or as a prevention of vaginal abrasions from pessaries in patients with pelvic organ prolapse.

There are two main approaches to treatment of vaginal atrophy: nonhormonal and hormonal treatment (Figure 2). Estrogen therapy is the most consistently effective treatment used for VVA, dryness, and dyspareunia in women with estrogen deficiency. But on the other hand, oestrogen treatment is known for its increased risk of thromboembolism and stroke [67] and caution is necessary for survivors of hormone-sensitive cancers as systemic absorption of estrogen can stimulate the growth of breast cancer cells. So following safety issues and continued subject requests for treatment, it is a challenge for clinicians to identify the lowest effective dose of the safest estrogen, while research is going on to develop and test an even safer estrogen than those currently used. Although systemic estrogen therapy also improves symptoms of VVA, doses are in general higher than used for local application, and should only be used if broader menopausal symptoms require treatment. In this paper, we mainly discuss local therapy, in the absence of such general menopausal complaints. One novel hormonal product, specifically tested for treating VVA, estetrol, was briefly mentioned.

4.1. Hormonal treatment, local application

As vaginally applied estrogens may cause increase serum estradiol, estron, and estriol levels, the aim is to use the minimal dose that is effective to treat VVA. Type, dose, and duration of treatment should be individualized according to the severity of symptoms and findings. Due to the absence of hepatic metabolism and the rapid vaginal response, vaginal estrogens are in general more effective to relieve urogenital symptoms than oral preparations [68].

Promestriene (estradiol 3-propyl 17β-methyl diether) is a synthetic steroidal estrogen used in 1% topical cream formulations. After almost 40 years of market experience use of such low dose, local estrogens have failed to show any systemic estrogenic side effects, such as stroke or venous thromboembolism, while being effective in resolving symptoms of VVA [69–71].

4.1.1. Estrogens and estrogen receptor modulators (SERMs)

4.1.1.1. Estradiol (E2). Estradiol can be used locally in a form of cream, tablet insert or vaginal ring.

A Cochrane review of 30 randomized controlled trials including 6235 women showed that there was no difference in efficacy in symptom relief between these forms treatment [72]. None of the included studies reported data on the endometrial thickness or serum E2 levels.

In a pilot study, the effect of 50 mcg vaginal E2 in a cream was effective to relieve symptoms of VVA, without raise in systemic hormone levels: serum E2 at 8 and 16 weeks of treatment was similar to baseline levels [73].

A multicenter, double-blind, placebo-controlled, phase 3 study evaluating the pharmacokinetics of 4, 10, and 25 µg of vaginal estradiol softgel capsules for the treatment of post-menopausal VVA likewise found very low systemic absorption of estradiol in all treatment groups [74]. All three doses showed a robust positive effect on the vaginal physiology and reduced severity of dyspareunia [75].

4.1.1.2. Estriol (E3). After being discovered in the human placenta, E3 concentrations were found to be related to pregnancy outcome [76]. Being specific for humans, E3 stimulates proliferation and maturation of the vaginal epithelium, local glycogen release, converted into lactic acid by lactobacilli, and resulting in low vaginal pH. Due to a short contact duration with its vaginal receptor, systemic effects are minimal, making topical administration the preferred route, being more adequate than oral one [68].

After single-dose oral or vaginal applications of E3 in usual doses (oral: 2–10 mg daily; vaginal: 0.5 mg or less) in contrast to E2, there is no or only weak proliferative effects on the endometrium [77–79]. In both a systematic review and a meta-analysis it was concluded that intravaginal E3 in a dose of 0.5 mg or less is effective in treating VVA without endometrial hyperplasia [80,81].

Over time, local E3 doses as low as 0.2 mg and 0.03 mg were tested, with still a profound effect on the VVA parameters in both formulations [80].

4.1.1.3. Ultra-low-dose of estriol and probiotic lactobacilli. A synergistic action of estrogen and lactobacilli supports the rationale of vaginal use of lactobacilli and estrogen combination in treatment of a disturbed vaginal flora and VVA [82]. Gynoflor® contains 10^8 viable lyophilized *Lactobacillus acidophilus* KS400 bacteria and 0.03 mg E3, which is a 16–32 times lower dose than in conventional E3 vaginal preparations (0.5–1 mg). This product has been proven to be safe and

NONHORMONAL	HORMONAL
Moisturizers / lubricants	**Local action:**
Hialuronic acid	- Estrogens: E2, E3
Phyto-estrogens	- DHEA: Prasteron
	- Testosterone
Mechanical (sexual activity / vaginal dilatators)	
Vaginal Vit E / Vit D	**Systemic action:**
Oral / vaginal probiotics	- Estrogen: E2, E3, E4
	- SERM: Ospemifen
Laser	

Figure 2. Nonhormonal and hormonal treatment possibilities of vulvovaginal atrophy.

efficacious in restoration of disturbed vaginal flora [83,84] and in the treatment of postmenopausal vaginal atrophy [85,86]. The synergy of both products is elegantly demonstrated in a study wherein combination therapy of pelvic floor rehabilitation (pelvic floor muscle training and electrical stimulation) plus 0.03 mg E3 was compared with the same therapy plus 0.03 mg E3 with *L. acidophilus* KS400. It was found that in a group of women using the E3/lactobacilli combination, all symptoms of the genitourinary syndrome were significantly more improved than in the arm of women using only E3 [87].

A small Phase I pharmacokinetic study assessing circulating estrogens in breast cancer (BC) patients on non-steroidal aromatase inhibitors (nsAI) with severe vaginal atrophy after using vaginal 0.03 mg E3/*L.acidophilus KS400* showed a small and transient increase in serum E3 in 50% of the patients, but not of E1 or E2. After one month of treatment, the E3 rise was even less, indicating the increased thickness of the vaginal mucosa prevented absorption. Furthermore, the vaginal atrophy and microflora abnormalities resolved or improved in all women [88], leading to resumed sexual activity and increased the quality of life [89]. The product was well tolerated, and discontinuation of therapy was not observed. With the safer product profile than E2, also in in vitro studies [90], this locally applied ultra-low-dose of E3, together with lactobacilli, can therefore be considered as safe and efficacious for treatment of atrophic vaginitis in BC patients taking AIs [91]. Still, due to the low number, more elaborate, randomized studies to confirm the efficacy and safety would be welcome.

4.1.1.4. Conjugated estrogens.

Conjugated equine estrogens (CE) is a mixture of the sodium salts of estrogen conjugates found in horses – estrone sulfate and equilin sulfate. They can be manufactured from the urine of pregnant mares or synthetically replicated. CEs are widely used as replacement hormonal therapy in menopausal women in the USA, but less popular in Europe where bioidentical E2 and E3 (not marketed in the USA) are more preferred. CEs are usually taken orally, but for the treatment of VVA also cream is available.

A randomized, parallel-design study compared the pharmacokinetic profile of CE vaginal cream (0.625 mg CE-A/g) administered at intervals (1 g once daily for 7 days, then twice weekly) with 0.3 mg SCE-A oral tablets once daily for 27 days. of the SCE-A vaginal cream, Serum estrogen levels were lower after intravaginal than after oral administration. Absorption of equilin, estradiol, and estrone further decreased with twice-weekly use of cream [92]. As discussed, however, with the arrival of monoclonal estrogens such as E2 and E3, the use of horse-derived estrogens becomes more and more obsolete, and is being progressively abandoned, especially in Europe. To our surprise, the American College of Gynecologists (ACOG) still tolerates use of such high doses of intravaginal CE for women with BC suffering from vaginal atrophy [93], despite elegant and safe alternatives exist (see above).

4.1.1.5. Ospemifen.

Ospemifen is a selective estrogen receptor modulator (SERM) that acts as an estrogen agonist in the vagina while having no proliferative effect on the endometrium or breast. Ospemifene is a triphenylethylene, a chemical structure with similarities to tamoxifen and toremifene, two SERMS used as anti-hormonal therapy in breast cancer.

Ospemifen showed a strong estrogen agonist effect on the vaginal epithelium while remaining neutral on the endometrium, making it suitable for the treatment of VVA [94,95]. One month intake of ospemifene increased the VCMI, and established an increased expression of the vaginal alfa estrogen receptor [94].

A phase III study of postmenopausal women receiving 30 or 60 mg ospemifene or placebo for 12 weeks showed a significant improvement in the maturation index by treatment week 4 for both ospemifene groups compared with placebo (p < 0.001). The use of 60 mg ospemifene significantly reduced dyspareunia by 12 weeks, and 30 or 60 mg significantly decreased vaginal dryness. During the same period, the mean change of endometrial thickness from baseline was 0.4, 0.7, and −0 .02 mm for ospemifene 30 mg, ospemifene 60 mg, and placebo, respectively [95]. Therefore, the potential proliferation of endometrium, although theoretically absent, needs to be further evaluated and monitored. Like other SERMs, also the potential risk of thromboembolism, increased risk of breast cancer, and/or appearance of hot flashes should be investigated further, although, unlike with tamoxifene, to our knowledge, there are no reports of thromboembolism associated with ospemifene use yet. A post-hoc analysis of five randomized, placebo-controlled trials suggest that ospemifene has no adverse effect on lipid profiles: in 2,166 postmenopausal women exposed to ospemifene, HDL increased, and LDL decreased after 3, 6, and 12 months of treatment [96].

Animal and preclinical studies demonstrated a neutral or inhibitory effect of ospemifene on carcinogenesis in the breast [97–99]. In an *in situ* mouse model, ospemifene reduced breast cell proliferation in animals with ductal carcinoma [97]. Despite this, the Endocrine Society Clinical Practice Guideline does not yet recommend ospemifene for use in women with a history of breast cancer [100].

A recent retrospective analysis demonstrated that 39 symptomatic menopausal patients receiving 60 mg ospemifene daily for 6 months had a lower rate of recurrent urinary tract infections (UTIs) [101].

In vitro data suggest that ospemifene mediates a positive effect on osteoblasts, resulting in a reduction of mineral bone loss in ovariectomized rats, with an activity similar to raloxifene [102]. Clinical data from a randomized, double-blind study in which 118 healthy postmenopausal women receiving 30, 60, or 90 mg ospemifene or 60 mg raloxifene for 3 months showed a superior response in the 90 mg ospemifene versus the 60 mg raloxifene group [103].

4.1.2. Androgens

4.1.2.1. Testosterone.

Intravaginal testosterone has emerged as a potential treatment for vulvovaginal atrophy through its local aromatization into estrogens. A randomized, placebo-controlled trial of 60 postmenopausal women VVA demonstrated laboratory and endometrial safety when compared with placebo after 12 weeks (3 times a week) of treatment with topical testosterone or estrogen [104]. However, a recent systematic review indicated doubts on its effect on sexual function. Also, safety remains uncertain because of the small number

of women exposed, short study durations, and inconsistent and incomplete outcome reporting for sex steroid levels [105].

4.1.2.2. Dehydroepiandrosterone (DHEA).

The mechanism of action of DHEA (prasterone) on the vagina is likely due to the local aromatization of androstenedione and testosterone to estrone (E1) and E2. In a prospective, randomized, double-blind, and placebo-controlled phase III clinical trial, the effect of daily intravaginal 0.50% DHEA (6.5 mg) was found to be more effective than placebo on vaginal pH, cell maturation, and dyspareunia [106], while serum steroid levels stayed within the normal postmenopausal levels. In another study, 6.5 mg of 0.50% prasterone cream showed similar efficacy on VVA symptoms than 0.3 mg CE or 10 µg E_2 applied locally [107].

Compared to low-dose vaginal estrogen, DHEA appears to cause a lower increase in serum E2 [106]. However, it has to be applied daily, as twice-weekly dosing appeared suboptimal to treat VVA [108].

4.2. Experimental hormonal (systemic) treatment

4.2.1. Estetrol (E4)

E4 is a natural estrogen exclusively produced by 15- and 16 alpha-hydroxylases in the human fetal liver [109]. Its estrogenic steroid structure with four hydroxyl groups explains the acronym E4. Maternal plasma and urine levels increase during pregnancy to high concentrations towards the end of gestation (≥1 ng/mL). After birth, the neonatal liver rapidly loses its capacity to synthesize E4 because these two enzymes are no longer expressed [110]. Based on its unique pharmacokinetic, pharmacological and safety profiles in human studies, E4 has a potential in applications such as HRT and contraception [109].

Its impact on vaginal cytology was assessed after intake of 2 or 10 mg of E4 versus 2 mg of E2 on a vaginal smear before and after 28 days of treatment. The left shift VCMI (see above) in the 2 mg E2V group was between that observed in the 2 mg E4 and the 10 mg E4 groups. This demonstrates that E4, besides its contraceptive and hot flush reducing capacity, is able to restore vaginal maturation and functionality [111]. Further studies are underway, but currently, this drug is not available for clinical use yet.

4.3. Nonhormonal treatments

4.3.1. Moisturizers/lubricants

Moisturizers and lubricants are effective in relieving discomfort and pain during intercourse for women with mild to moderate VVA, but despite a clinical advantage, they do not improve the vaginal tropism and have no pathogenesis-based treatment action.

There are a variety of moisturizers and lubricants commercially available, making it challenging to choose the best, depending on the treatment purpose. Vaginal moisturizers are intended to rehydrate dry mucosal tissue. Most of them contain water, which is absorbed into the skin, while the gel is adhering to the vaginal lining to aim long term effect. Vaginal lubricants, on the other hand, only provide short-term relief of vaginal dryness and pain during sex. They are particularly beneficial for women whose vaginal dryness is a concern only or mainly during sex.

4.3.2. Hyaluronic acid

Hyaluronic acid plays an important role in maintenance of function and reepithelization of the epiderm. It serves as an integral part of the extracellular matrix of basal keratinocytes, but also has an ability to form an extracellular water film in the vaginal epithelium, thereby maintaining the water balance [112].

Some claim hyaluronic acid is as effective as estrogen. Relief of vaginal dryness ($p < 0.05$) and urinary incontinence were improved in the hyaluronic acid ($p < 0.05$), but not in the CE cream group. Also, the improvement of VCMI ($P < 0.05$) and of the composite score of vaginal symptoms ($P < 0.001$) was more pronounced in the hyaluronic acid than in the CE group [113].

In a multicenter, randomized, controlled, open-label, parallel-group trial it was shown that hyaluronic acid vaginal gel was not inferior compared to E3 vaginal cream in women with vaginal dryness due to various causes [114]. However, E3 in a higher dose, but still as low as 50 µg/g gel, appeared to be significantly more effective than hyaluronic acid. Similar findings were reported by Serati. et al. [115]

4.3.3. Colostrum gel

Colostrum is the first form of milk produced from mammary gland of mammals during the first few hours postpartum. It is known that colostrum not only contains immunoglobulins, lactoferrin and cytokines, which help to modulate the immune response and promote antibacterial action in the neonate, but it also contains a transforming growth factor and epidermal growth factor, which promote normal cell growth and proliferation [116].

Taking into consideration that the vaginal mucosa in postmenopausal period is exposed to epithelial damage and alterations of local defense factors, there is some logic to presume that colostrum might help in promoting the mucosal restoration. Local treatment with a gel product based on purified bovine colostrum showed improves vaginal hemodynamics and thickness of vaginal epithelium in ovariectomized rats [117]. Vaginal gel applied twice daily for 8 weeks significantly improved both Vaginal Health Index (VHI) and maturation index in the vagina of postmenopausal women [118]

4.3.4. Phytoestrogens

Despite being popular among postmenopausal women who have a contra-indication or do not want to use hormones, alternative therapies for hot flushes based on phytoestrogens derived from plants (herbal supplements, soy, chasteberry, etc.) cannot be recommended as a treatment for VVA, as they have not been properly tested for this indication.

In a systematic review of 71 trials, involving over 20,000 women reporting on urogenital atrophy outcomes, high efficacy against placebo was demonstrated for vaginal estrogens (-0.44 (95% CI: -0.65 to -0.23; 12 trials, n = 3,419), systemic estrogens (-0.36 (95% CI: -0.35 to -0.26; 14 trials, n = 5,141), and ospemifen (-0.75 (95% CI: -1.05 to -0.45; 3 trials, n = 1,889),

but for isoflavones and other non-prescription agents low or insufficient effectiveness was found [119].

The use of vaginal soy-derived isoflavons showed more encouraging results in one study, but these investigations should be considered preliminary and need to be verified in larger, prospective studies [120,121].

4.3.5. Mechanical (sexual activity/vaginal dilators)

The idea that sexual activity can positively influence the vaginal epithelium and prevent vaginal atrophy in a 'natural way' looks attractive. It was shown that sexually active women had less vaginal atrophy than inactive women, and had higher levels of androgens and gonadotropins [122]. However, microscopic or cytologic evaluation was not performed in this study making it impossible to differentiate what was 'the chicken or the egg': was it sexual activity preventing vaginal atrophy or was it symptomatic vaginal atrophy being the reason to reduce sexually activity. This question was answered in a recent prospective cohort study SWAN study [123]. Questionnaire and biomarker data from 2,435 participants from baseline and 13 approximately annual visits over 17 years (1996–2013) showed that both vaginal dryness and lubricant use were associated with pain during intercourse, but not with a decline of intercourse frequency. In fact, sexual function improved most when pain upon intercourse was reduced [124]. Therefore, it does not seem plausible that intercourse improves sexual function as much as sexual function is less impaired if VVA and pain is absent.

4.3.6. Vaginal Vit D/Vit E

Vitamin D is frequently used during menopause due to its positive effect on bone health. On a vaginal cell line and human vaginal tissue samples, the vitamin D induced expression of RhoA and Ezrin proteins in vaginal tissue leads to increased vaginal re-epithelization, comparable to what was obtained after estrogen use [125]. However, the study was based on cell models only and the number of tissue samples of premenopausal and postmenopausal women was relatively small. In one double-blind, placebo-controlled study, the use of vaginal vitamin D improved the VCMI and decreased pH and dryness in women suffering from VVA [126].

Despite some trials claiming that Vitamin E vaginal suppositories may be an alternative to vaginal estrogen use to relieve postmenopausal VVA symptoms [127,128], more clinical trials are needed in to study the clinical outcomes and VCMI before and after treatment.

4.3.7. Probiotics

There is an inverse correlation between the absence of *Lactobacillus* (especially *L. iners* and *L. crispatus*) and increased bacterial diversity and moderate to severe vaginal dryness [129]. So, oral and vaginal probiotics hold great promise, but additional trials are required to determine the efficacy of bacterial therapeutics to modulate or restore vaginal homeostasis.

4.3.8. Laser treatment and radiofrequency

Intravaginal laser therapy (based on both CO2 laser (SmartXide2 V2LR, Monalisa Touch, DEKA, Florence, Italy), and E:rYAG laser (Fotona SmoothTM XS, Fotona, Ljubljana Slovenia) technology) was introduced to treat vaginal atrophy since 2013 [130–132]. The principle of its treatment action is based on destructing parts of the lamina propria by drilling small holes by use of fractional CO_2 or YAG laser energy, with the purpose to improve the blood supply, the vaginal epithelium morphology, and subsequently relieve symptoms of atrophic vaginitis in the repair process. Short term data suggest that three weekly treatment sessions lead to improvement in both subjective symptoms (dryness, burning, dyspareunia) and clinical signs ($p < 0.01$). Ninety percent of patients was satisfied with the procedure, and reported a significant improvement in quality of life. No adverse events were recorded throughout the study period [133], but caution is warranted as long term data are scarce and complications start to appear [134,135].

In the first systematic Cochrane review on the efficacy and safety of intravaginal laser therapy, none of the 165 articles identified in the search was a randomized controlled trial. Only 14 studies comprising 524 women were eligible for inclusion in the review. Of those, 12 studies were retrospective before-and after comparisons, while only two compared the results with alternative therapy (local estriol cream) in a prospective way [136]. All studies tested the effect of three weekly laser sessions. In two studies the VAS score 0–10 was not used for assessing dyspareunia and dryness and were therefore excluded in the meta-analysis of these outcomes. There was a significant improvement of all primary outcomes after 1 month in all studies included in the meta-analysis. There was also a decrease of urinary incontinence rate at 1-month follow-up, which was maintained for up to 6 months. However, the analysis of the quality of the body of evidence for dryness, dyspareunia, and incontinence was rated as 'low', and for itching, burning and dysuria even 'very low'.

In subgroup-analysis of CO2-laser the pooled mean difference of dryness and dyspareunia was -5.5 (95% CI: -6.6 to -4.4; $p < 0.00001$, I2: 0%; n = 255) and -5.5 (95% CI: -6.6 to -4.4; $p < 0.00001$; I2:0%; n = 229), respectively. The VAS 0–10 mean difference of dryness and dyspareunia remained significant after 3-month follow-up. While subgroup-analysis of Er: YAG-laser group could not be performed due to low numbers of data, a significant improvement of dryness and dyspareunia seemed to maintain up to 18-month follow-up. In the E3-group the significant improvement maintained up to 6 months after the last application. In questionnaires probing for sexual health, global health and in histological examinations before and after laser therapy, significant improvement was noted in most studies.

Side effects were scarce, as 90% of women said to be 'happy' with the technique. However, a large answering bias is introduced if people are offered for free a new, expensive and innovative technique. Indeed, in daily practice, a certain minority of women admit to have suffered intense pain during the treatment, and 10% stopped the therapy after the first session due to excruciating pain starting 2 days after a session, lasting up to 2 days [136]. One has to realize that these complications rarely or not show up in the data presented, and may be more frequent than admitted.

Even more importantly, as low dose local estrogen is the standard treatment for vulvovaginal atrophy, properly

randomized studies comparing this standard treatment with the new laser techniques have to be awaited before this new technique can be recommended as an alternative routine practice. Also, very crucial will be the proper registration of short term, but even more importantly, long term complications, that most likely will arise due to the increased appearance of fibroblasts, which can lead to fibrosis, stenosis, and scarring. As these complications are not known in estrogen-treated patients, assessment of the burden of such complications is crucial before any official recommendation can be made.

Also the argument that laser techniques would be the only alternative treatment for vulvovaginal atrophy in women with contra-indications to hormonal therapy, such as patients with a history of thromboembolism and hormone-sensitive breast cancer, is not valid, as several efficacious alternatives such as hyaluronic acid and ultra-low-dose of local estriol have been demonstrated to be safe for use in such patients [88,113,114].

Finally, the limitations of the number and duration of performing repeat sessions of this technique will have to be determined. While the use of estrogens can safely be tailored to the need of the patient (e.g. by using microscopy) [88], also on a long term base, this is still unknown and insecure for a new technique based on tissue destruction.

Another innovative treatment with a new low-energy dynamic quadripolar radiofrequency (DQRF) device also became available on the market. This energy-based technology is designed to trigger anatomical remodeling in vulvar tissues. Women with objective evidence of vaginal dryness and/or moderate/severe dyspareunia were exposed to four sessions of 10-min lasting DQRF thermal treatment sessions every 10 ± 1 days [137]. The results were evaluated with the help of Vaginal Laxity Questionnaire, a modified Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire, and a Sexual Satisfaction Questionnaire. Significant improvements in a self-perceived sensation of looseness and symptoms of dysuria/urinary incontinence and sexual function were demonstrated during the study and after 12 months of follow-up [137,138]

No adverse effects, nor thermal burns or injuries, were reported during or after the treatment. Randomized controlled studies using objective outcome parameters are needed to confirm these preliminary data.

As a summary, despite the collected data suggest that laser therapy may become a valuable alternative nonhormonal therapeutic modality in the management of VVA, higher quality of evidence from randomized controlled trials is required to establish the efficacy and long term safety of laser treatment in the management of this pathology [139]. Hence, this device has not yet been approved by the US Food and Administration for the treatment of vulvovaginal atrophy.

5. Conclusion

Several safe and efficient therapies are optional for the treatment of vulvovaginal atrophy, both hormonal and nonhormonal. After trying nonhormonal moistening products, a first-line therapy, intravaginal application of a low dose estrogen product is recommended, unless more generalized menopausal symptoms demand for systemic treatment (Figure 3). For women with contra-indications for estrogen therapy, nonhormonal therapy such as hyaluronic acid or laser therapy is possible, but also ultra-low-dose of estriol, with or without combined probiotic lactobacilli, seems safe and very efficacious. Laser therapy cannot be recommended until randomized studies prove its efficacy and safety, especially on the long term.

6. Expert opinion

Vulvovaginal atrophy is still insufficiently addressed, underdiagnosed and too often left untreated, while a myriad of answers is possible to help almost every single woman with this problem. The newest term 'Genito-Urinary Syndrome of Menopause' which despite the vow of originality was published in eight different Journals (1–8), encompasses the entity 'vulvovaginal atrophy (VVA)'. In contrary to the designers of
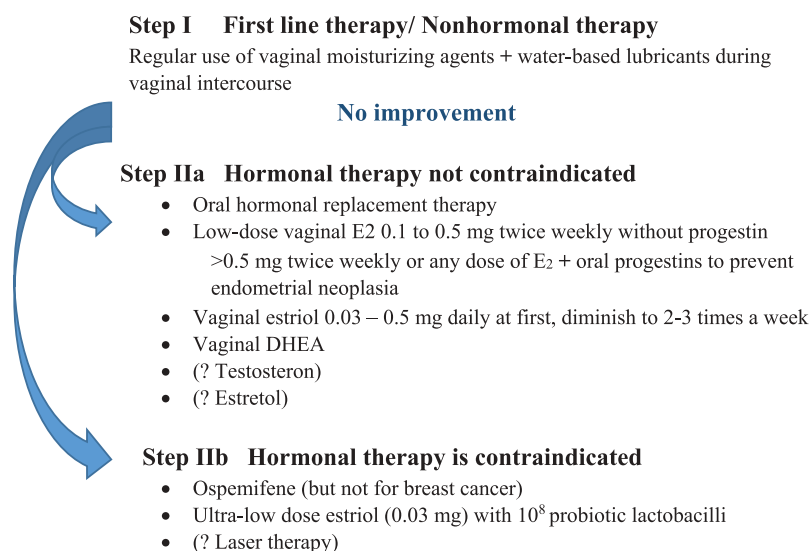
Step I   First line therapy/ Nonhormonal therapy
Regular use of vaginal moisturizing agents + water-based lubricants during vaginal intercourse

No improvement

Step IIa   Hormonal therapy not contraindicated
- Oral hormonal replacement therapy
- Low-dose vaginal E2 0.1 to 0.5 mg twice weekly without progestin
  >0.5 mg twice weekly or any dose of E_2 + oral progestins to prevent endometrial neoplasia
- Vaginal estriol 0.03 − 0.5 mg daily at first, diminish to 2-3 times a week
- Vaginal DHEA
- (? Testosteron)
- (? Estretol)

Step IIb   Hormonal therapy is contraindicated
- Ospemifene (but not for breast cancer)
- Ultra-low dose estriol (0.03 mg) with 10^8 probiotic lactobacilli
- (? Laser therapy)

Figure 3. Algorithm of treatment for vulvovaginal atrophy.

that new term, we do not think of menopause as a 'syndrome', nor do not we think our patients are ashamed of the words 'vulva' or 'vagina'. Therefore, a term that describes the diagnosis more precisely, such as VVA, is still adequate and can readily be used.

The basis of good treatment is a correct and complete diagnosis. Most physicians rely on the medical history and (at best) on clinical examination. In our opinion, however, wet mount microscopy should be performed on all women with such complaints, in order to assess the proportion of superficial, intermediate and parabasal, or in severest cases, even basal epithelial cell types to assess the vaginal maturation index. This examination also allows to exclude infections, and assess the lactobacillary grades and the presence of inflammation. Importantly, it allows to make the diagnosis of VVA not only in menopausal women, but also in young women (aerobic vaginitis), women with breast cancer, during the postpartum period and in contraception users, in all of whom VVA can be detected to some extent. At the same time, it helps avoid claiming VVA to be the cause of the complaints, as these can be caused by a large number of differential diagnoses, such as lichen planus, vulvar (pre-)neoplastic lesions, lichen slerosus, psoriasis, vulvar narrowing due to scarring, and infections as described above.

Add-back therapy with estrogens remains the cornerstone of the therapy, especially in menopause. To our opinion, the only microscopy can guide which therapy, and which dose, frequency, and application type (oral, transdermal, intravaginal) is most appropriate. Unless there is other menopausal symptoms or menopause-associated long term health risks, when systemic estrogens are indicated, the general rule is to use the lowest dose of the safest local therapy in the form of intravaginal tables, ovules or creams. In our opinion, for this indication, ultra-low-dose estriol (e.g. with lactobacilli at 0.03 g three times a week), intravaginally is safe a sufficient therapy in most cases, even in breast cancer patients on aromatase inhibitors with nasty VVA, and even in women with a history of venous thromboembolic complications.

Newer methods like using the hormones DHEA and estretol (E4) and laser therapy are recently been introduced in the treatment arsenal of VVA. However, all of these treatments require more research before they should be used in routine clinical practice. In our opinion, especially the laser therapy cannot be used in clinical routine as it is a potentially dangerous device, which can cause vaginal fibrosis and stenosis, and there is still no proper, randomized, long term studies to prove its safety, nor its efficacy as compared to standard estrogen treatment. Given the high safety profile of intravaginally applied, low dose sex hormones, even in high-risk patients, it is hard to believe that any destructive method would benefit a badly harmed and atrophic vagina more than a carefully chosen locally active estrogen. For ospemifem, estretol and DHEA we would also welcome more safety data for patients with hormone-dependent breast cancer and/or at risk for thromboembolic events.

To our opinion, only in seldom cases, nonhormonal therapy should be tried as only therapy. Although some papers show good results with hyaluronic acid gel, or other lubricants,

most women in our experience are not so happy using it, with the exception as an adjuvant tool during sex. It requires time, expertise and training to explain affected women the details of the safety and advantages of low dose and safe intravaginal hormonal methods, in order to avoid suboptimal treatment and disappointing experiences. If hormonal therapy failed to obtain painless sexual intercourse, some women may require a small surgical widening plasty of the posterior vulval vestibulum, certainly if the condition of atrophy and pain has been longstanding without having been taken care of. Putting a laser in such women's vagina is, besides expensive, often very painful and in our experience can even worsen the condition. Also in this context, it is not enough to encourage such women to have sex in order to improve the painful condition, driving these women crazy as they are considered lunatics who do not want to have sex, while it is simply not possible, because too painful. Elderly women are more likely to have sex if they have no atrophy, and the atrophy is a result of estrogen deficiency, not of refraining sexual intercourse.

More research to find the lowest efficient doses of existing nonhormonal and hormonal products are needed, using hard and comparable outcomes such as epithelial cell maturity index. Also newer products deserve more attention and research, especially to ensure their safety profiles. The place of laser therapy has to be determined after properly designed and performed randomized studies have proven its efficacy compared to standard therapy and the full spectrum of its side effects, especially on the long term.

Funding

Declaration of interest

Reviewer disclosures

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