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Effects of topical estrogen therapy on the vaginal microcirculation in women with vulvovaginal atrophy

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Abstract

Aims: This study aims to assess vaginal wall angioarchitecture and function in women with vulvovaginal atrophy (VVA) and determine the effect of topical estrogen on the vaginal microcirculation.

Materials and Methods: In this prospective observational study, incident dark field imaging was used to assess the vaginal microcirculation. In patients with VVA, measurements were performed before and after treatment with topical estrogen and compared to measurements performed in women without VVA. Vaginal angioarchitecture was studied by assessing microcirculatory architecture and capillary tortuosity scores at four regions of the vaginal wall. In addition, the capillary density and microvascular flow index (MFI) were obtained.

Results: Seventeen women were included in this study. Of these, eight women were diagnosed with VVA and nine women were considered healthy controls. Significant differences were observed between groups with regard to microcirculatory architecture scores. The architecture of the microvasculature in women with VVA was characterized by the appearance of a vascular network without capillary loops, whereas an array of capillary loops was predominantly seen in women without VVA. After estrogen treatment, no difference in architecture scores between patients and healthy controls was observed. Capillary tortuosity, capillary density, and MFI were similar in both groups before and after estrogen treatment.

Conclusions: The architecture of vaginal microvasculature is altered in patients with VVA. In case of similar vascular architecture, capillary tortuosity and density seem to be comparable. Treatment with topical estrogen results in restoration of the angioarchitecture.

KEYWORDS

estrogen, imaging, microcirculation, vulvovaginal atrophy

1 | INTRODUCTION

Vulvovaginal atrophy (VVA) is a common condition in postmenopausal women. The prevalence of VVA dramatically increases during the menopausal transition, whereas most postmenopausal women suffer from their associated symptoms.[1] As a consequence of vaginal discomfort and pain, VVA has a significant impact on daily activity, sexual function, and overall quality of life.[2] VVA is characterized by dryness, erythema, and petechiae of the vaginal wall and is associated with a thin and pale epithelium resulting from low estrogen levels specific for menopause.[3]

It is well known that vaginal estrogen therapy is efficacious in treating symptoms of VVA.[4] Therapy with estrogen thickens the epithelium, decreases vaginal dryness, and seems to optimize vaginal blood flow.[5] Since sufficient estrogen levels support the proliferation of vascular and nonvascular smooth muscle cells in the subepithelial layers of the vagina, estrogen is considered to affect vaginal wall perfusion and oxygenation.[6] The actual perfusion of vaginal tissue and the exchange of nutrients and gases (ie, O_2 and CO_2) between the intravascular space and the vaginal tissue occurs at the level of the microcirculation (ie, in the capillaries). Therefore, the capillary vessel bed should be considered essential for a healthy vaginal environment.[7]

To what extent VVA affects the vaginal microcirculation is not well objectified, and it is unknown whether treatment with vaginal estrogen results in changes to the microcirculation. Therefore, assessment of the vaginal microcirculation before and after estrogen treatment can improve understanding of the working mechanism of vaginal estrogen therapy.

With the use of handheld microscopy imaging, human microcirculation can be assessed in great detail.[8] This imaging technique has been used to assess the microcirculatory status of different human organ surfaces.[9-11] Incident dark field (IDF) imaging was used to assess the vaginal microcirculation in healthy subjects[12] and in patients with prolapse.[13] IDF imaging uses high-brightness green LEDs. The emitted green light is absorbed by hemoglobin in the red blood cell, allowing visualization of the microcirculation.[8] In another study, the same authors demonstrated that topical estrogen therapy results in thickening of the epithelial layer and that it could be assessed noninvasively with IDF imaging.[14]

It is hypothesized that topical estrogen therapy has an effect on the vaginal microcirculation in women with VVA: therapy possibly changes the angioarchitecture and quantity of capillaries, thereby improving the diffusion and convection of oxygen to the vaginal tissue. To test these hypotheses, this study compares the microcirculation between women with and without VVA and objectifies the effects of topical estrogen on the vaginal microcirculation.

2 | MATERIALS AND METHODS

2.1 | Participants and setting

We performed a prospective, single-center observational study at the outpatient clinic of the Department of Obstetrics and Gynecology of a University Medical Center. Women enrolled in this study received a full explanation of the study guidelines and procedures. Written informed consent was obtained from each participant.

Postmenopausal women that were meant to receive treatment with topical estrogen for VVA were recruited. Synapause-E3 ovules 0.5 mg were self-administered at home daily for 2 weeks followed by two times per week for at least 4 consecutive weeks. Medical history was obtained and women with cardiovascular (eg, angina pectoris, uncontrolled hypertension), inflammatory (eg, rheumatoid arthritis, eczema), other systemic illness (eg, inadequately controlled [non-] insulin-dependent diabetes mellitus) or those taking medication (eg, anticoagulants, anti-inflammatory, or immunosuppressive agents) that could affect the microcirculation were excluded from participation. Women with a history of vaginal surgery were also excluded, because of its uncertainty to what extent vaginal surgery affects the vaginal microcirculation.

VVA was diagnosed based on the following three characteristics: (1) the presence of a most bothersome symptom involving at least one of the following symptoms: vaginal dryness, vaginal itching or irritation, dyspareunia;[15] (2) the presence of one or more of the following signs at physical examination: vaginal wall pallor and petechiae, friability of the vaginal wall (defined as any bleeding occurring during examination), conization (markedly decreased elasticity), or the absence of rugae;[16] and (3) a vaginal pH of at least 5.5.[17] pH was measured using a pH strip with a pH range of 3.8 to 5.5 with intervals of 0.3 (Dosatest; Prolabo; VWR International, Leuven, Belgium).

The control group consisted of women aged 40 years or older with no signs or symptoms of VVA, attending the outpatient clinic because of bothersome pelvic floor symptoms and no previous history of vaginal surgery or use of topical estrogen.

2.2 | Microcirculation imaging

The vaginal microcirculatory assessment was performed using IDF imaging (Cytocam; Braedius Medical, Huizen, the Netherlands). This technique has been described in

detail previously.[8] In summary, IDF imaging contains high-brightness LEDs with a very short illumination pulse time of 2 ms. Greenlight is absorbed by hemoglobin in the red blood cell, allowing visualization of the microcirculation. The device is a lightweight (120 g) and pen-like instrument (length 220 mm, diameter 23 mm). The combination of factor 4 optical magnification and a large sensor image area provides a field of view of 1.55×1.16 mm, which equates 1.80 mm^2. The optical system provides an optical resolution of more than 300 lines/mm. The camera is connected to a device controller on a computer that is used for image storage and off-line analysis.

2.3 | Microcirculatory examination and data acquisition procedure

Participants were examined in a gynecological chair in a stable 45°° semi-reclined position in a room kept at a constant temperature. An experienced researcher, skilled in measuring the vaginal microcirculation, conducted all measurements. The CytoCam was placed into contact with the vaginal wall at exactly 3 cm above the hymen. The pressure of the device on the vaginal wall was avoided to prevent pressure-induced flow-artifacts, and the CytoCam was adjusted for optimal focus and contrast. The anterior, posterior, and lateral vaginal walls were measured to calculate a general vaginal measurement. All measurements were stored as video clips which were directly saved as digital AVI-DV files to a hard drive. Videos were blinded and anonymized so that the observer was not aware of the intervention and time point. Image assessment was performed in a random fashion.

2.4 | Quality score assessment

Each video clip was assessed through a scoring system based on six parameters (illumination, focus, content, stability, pressure, duration); failure to meet a parameter disqualified a video clip from the analysis.[18]

2.5 | Tissue angioarchitecture

This scoring system allows for classification of subepithelial vascular patterns.[12,13] Microvascular architecture scores from each measurement site were examined and systematically classified with a score of 1, 2, or 3. Score 1, defines the appearance of an array of capillary loops; in score 2, both capillary loops and vascular network are visible, and score 3, is defined by the appearance of a vascular network with a complete absence of capillary loops. The overall microcirculatory architecture score per anatomical region per subject was determined by selecting the most frequently observed architecture score in the available frames.

2.6 | Capillary tortuosity score

Additional description of the microvasculature is the capillary tortuosity (number of twists per capillary). Capillary tortuosity is a microvascular feature which has previously been associated with chronic disease.[19] A frame was judged suitable for assessment if capillary loops and the presence or absence of twists could be clearly identified. Capillary tortuosity was classified as score 0: no twists (or pinhead capillaries) to 4: four or more twists.[12] The overall tortuosity score per anatomical region per subject was determined by selecting the most frequently observed tortuosity score in the available frames.

2.7 | Assessment of capillary density

Assessment of the capillary density was performed by counting the number of capillary loops per visual field from the video clips from each of the four anatomical regions. The capillary density obtained from one isolated image frame expressed as the median number of capillary loops per square millimeter (cpll/mm^2), was used to quantify the vaginal microcirculation at each of the associated vaginal wall regions. Each image frame provides a field of view of 1.55×1.16 mm, which equates 1.80 mm^2. Capillary density was assessed in each frame where capillary loops could be clearly identified and calculated by counting the number of capillary loops per field of view area and divided by 1.80 to obtain a unit cpll/mm^2.

2.8 | Microvascular flow index

Microvascular flow characteristics were assessed using the microvascular flow index (MFI). This score was previously developed and tested for reproducibility by Boerma et al[20] and is based on the determination of the predominant type of flow in four quadrants. Flow is characterized as absent (0), intermittent (1), sluggish (2), or continuous (normal) (3). The MFI score per region was calculated by averaging the MFI scores over four quadrants per frame per region.

2.9 | Statistical analysis

The aim of the analysis was (1) to compare microcirculatory parameters between women with and without VVA, and (2) to compare microcirculatory parameters in women before and after treatment with topical estrogen. Significant differences were found in a previous study on this topic using a small sample size,[14] therefore, this study used the

same sample size. Descriptive statistics were used to present the demographic variables. Non-normally distributed data are presented as medians with interquartile ranges. Normally distributed data are presented as means and standard deviations. For differences between the two groups in tissue microcirculatory architecture and capillary tortuosity scores, the χ^2 test was used. Capillary density was compared between groups using the Mann–Whitney U test for non-normal distribution. A two-sided $P < 0.05$ was considered statistically significant. All data were analyzed using SPSS, version 23.0 for Windows (IBM Corp, Armonk, NY).

2.10 | Ethical approval

This study was approved by the Medical Ethics Committee of the Amsterdam UMC.

3 | RESULTS

3.1 | Participants

A total of 17 women were included in this study. Of these women, 8 women were diagnosed with VVA (median age 65 years; interquartile range [IQR] 56-74 years) and 9 women were considered healthy controls (median age 49 years; IQR 45-59). Women in both groups were comparable with regard to parity (VVA median 2 [range 1-3] vs without VVA 2 [range 1-3]). In the control group, women had no signs or symptoms of VVA and visited the clinic because of an abnormal pap smear (n = 2), urinary incontinence (n = 4), recurrent urinary tract infections (n = 1), and/or pelvic organ prolapse (n = 4). A few patients did not undergo the second measurement in the VVA group (n = 1) and control group (n = 2).

3.2 | Physical examination

Before treatment, there was a statistically significant difference between the median vaginal pH in the VVA and healthy control group (5.5 vs 5.1, respectively; $P < 0.01$). pH of the VVA group did change from 5.5 to 5.0, however, this results was not significant ($P = 0.07$). No significant difference was found in the control group over time (5.1 vs 5.2; $P = 0.32$). Clinical signs of VVA (eg, vaginal wall pallor and petechiae, friability of the vaginal wall, conization, or the absence of rugae) improved in six out of seven patients in the VVA group.

3.3 | Tissue angioarchitecture

Vaginal angioarchitecture differed significantly between women with and without VVA (Table 1). In women with

TABLE 1 Microcirculatory architecture scores of patients and controls at both time points

Microcirculatory architecture score at baseline ($t = 0$)				Microcirculatory architecture score, 2nd measurement ($t = 1$)		
	Patients (n = 8)	Control (n = 9)	P*	Patients (n = 7)	Control (n = 7)	P*
Anterior			**0.02**			0.30
Score 1	2 (25)	8 (89)		6 (86)	7 (100)	
Score 2	4 (50)	0 (0)		1 (14)	0 (0)	
Score 3	2 (25)	1 (11)		0 (0)	0 (0)	
Left			**0.02**			0.84
Score 1	2 (25)	8 (89)		4 (57)	3 (43)	
Score 2	2 (25)	1 (11)		2 (29)	3 (43)	
Score 3	4 (50)	0 (0)		1 (14)	1 (14)	
Posterior			0.07			0.58
Score 1	2 (25)	7 (78)		5 (71)	4 (57)	
Score 2	4 (50)	2 (22)		2 (29)	3 (43)	
Score 3	2 (25)	0 (0)		0 (0)	0 (0)	
Right			0.08			0.08
Score 1	2 (25)	6 (67)		2 (33)**	6 (86)	
Score 2	3 (37.5)	3 (33)		3 (50)	0 (0)	
Score 3	3 (37.5)	0 (0)		1 (17)	1 (14)	

Score 1: appearance of an array of capillary loops; Score 2: both capillary loops and vascular network are visible, and Score 3: appearance of a vascular network, absence of capillary loops. Values are number (percentage).

Bold values <0.05 are considered statistically significant.

*The χ^2 test.

**Only six measurements, clips from one patient were extracted because of a low-quality score.

VVA, a score 3 was predominantly seen (Figure 1A), whereas a score 1 architecture was predominantly seen in healthy controls (Figure 1B). After treatment with estrogen, a significant change in architecture score was observed, resulting in angioarchitecture that was comparable to the angioarchitecture y seen in the control group (Table 1).

3.4 | Capillary tortuosity score

Capillary tortuosity score was assessed in all frames except for frames with hardly identifiable capillary loops and mainly the appearance of the underlying vascular network (architecture score 3). Capillary tortuosity scores were similar in both groups before and after treatment with estrogen. Score 1 and 2, illustrating one or two capillary loop twists subsequently, were most frequently found (Tables S1 and S2).

3.5 | Assessment of capillary density

Assessment of capillary density was performed on all frames including an array of capillary looks, architecture score 1. Capillary density was similar during the first measurement for all anatomical regions (anterior vaginal wall VVA 16.2 cpll/mm^2 [13.2-17.3] vs control 12.8 cpll/mm^2 [11.4-15.1]; $P = 0.20$). Also after treatment with estrogen, no significant differences were seen in capillary density between the two sites for all anatomical regions (anterior vaginal wall VVA 12.4 cpll/mm^2 [11.4-15.8] vs control 13.3cpll/mm^2 [12.1-17.2]; $P = 0.93$) (Table S1).

3.6 | Microvascular flow index

All patients and controls had at both time point an MFI score of 3.0 [confidence interval, 3.0 to 3.0] corresponding to continuous flow.

4 | DISCUSSION

This is the first study to report on the effects of VVA and topical estrogen therapy on the architecture of vaginal microcirculation using an in vivo noninvasive technique. Our results demonstrate that in women with VVA, angioarchitecture in the subepithelial layer of the vagina is altered. Treatment with estrogen has a distinct effect on the architecture of the vaginal microcirculation and has the potential to reverse the vascular alterations observed in VVA to normal angioarchitecture as observed in women without VVA.

Four layers characterize vaginal tissue: the epithelium, the lamina propria, a muscularis layer, and the adventitia.[21] The epithelial layer is not directly vascularized but receives nutrients and oxygen via capillary loops and diffusion from the vascular network beneath, located in the lamina propria. In this case, IDF imaging shows a thick epithelial layer and a lamina propria with a pattern of capillary loops (architecture score 1), without a visible background of the vascular network. However, in women with VVA, a significantly higher degree of architecture scores 2 and 3 were observed at baseline: a vascular network without capillary loops. After treatment with estrogen, a score 1 architecture was also predominant in the VVA group. This is graphically illustrated in Figure 2.

It is unknown why a hypoestrogenic state alters vaginal vasculature. The altered angioarchitecture could be an effect of a thinner epithelium and loss of crypts, for which delivery of oxygen and nutrients through capillary loops is no longer necessary. However, since estrogen regulates the growth and function of vascular and nonvascular smooth muscle cells in the subepithelial layers in the vagina,[22] another explanation could be that the lack of estrogen has a direct negative effect on the vaginal vasculature. The absence of capillary loops in the vaginal microcirculation can have clinical consequences. Capillary loops are also present in the skin, where this form of vascularization is considered to be better resistant against trauma, and protects the larger vessel bed beneath and facilitates thermal regulation by constriction and dilation of the capillary loops.[23] Similar features can possibly be attributed to the vaginal epithelium with capillary loops. Among other functions, capillary loops could play a role in vaginal lubrication under the mediation of endothelial nitric oxide synthases and a

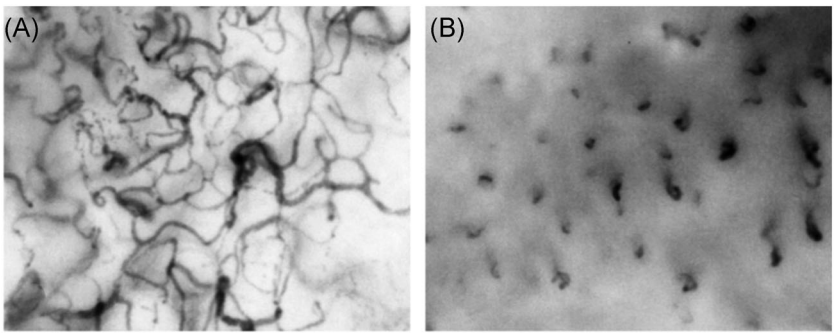

FIGURE 1 Incident dark-field illumination images of the vaginal microcirculation. A, Architecture score 3 (appearance of the vascular network without capillary loops) seen in vulvovaginal atrophy. B, Architecture score 1 (appearance of an array of capillary loops) seen in healthy controls

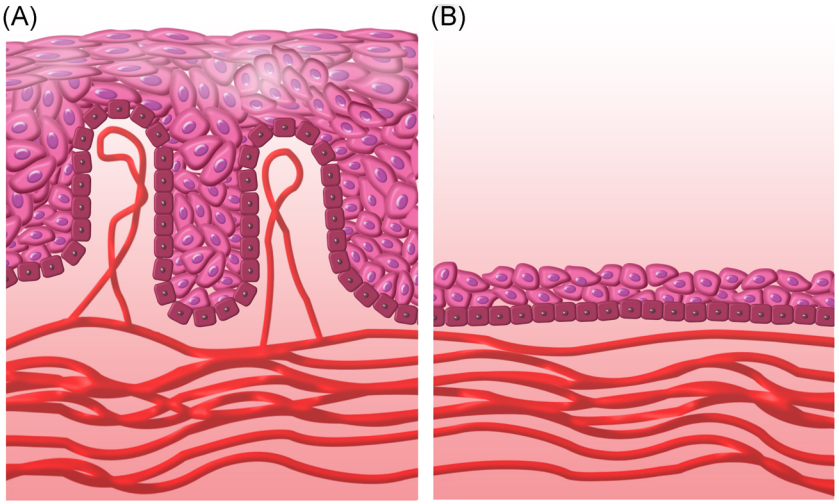

FIGURE 2 A, Illustration of healthy vaginal tissue with a dense epithelial layer including crypts and capillary loops originating from the vascular network (architecture score 1). B, Illustration of atrophic vaginal tissue, a thin layer of epithelium with loss of crypts and capillary loops, residual vascular network visible (architecture score 3)

hypoestrogenic state.[24] Hypothetically, a microvasculature without capillary loops can also cause vaginal dryness with itching or irritation as result.

Analysis of the capillary density and microvascular flow showed no statistically significant differences between patients and controls, neither before nor after treatment. Capillary density and flow are both parameters for tissue perfusion, which depends on the number and distribution of the capillaries in combination with blood viscosity and pressure. The two main hemodynamic principles that determine oxygen supply to tissue are convection and diffusion. Convection is quantified by flow, whilst diffusion is normally quantified by the density of the perfused microvessels. However, it can be argued if this also applies to the vagina, where angioarchitecture might be a more important factor facilitating oxygen diffusion rather than a high density of the microvessels. In addition, we analyzed capillary tortuosity. This morphologic characteristic has been associated with pathology such as diabetes, but its biological explanation is currently unknown.[19] We observed no differences in tortuosity scores between groups, and these scores were comparable to nondiabetic patients. Future research should be focused on the relationship between the qualitative aspect of microvessels and their ability to deliver oxygen to the cells.

Potential limitations of this study need to be addressed. First of all, although the CytoCam has the largest field of view among existing handheld video microscopes, its field of view is still relatively small (1.55×1.16 mm). Therefore, it can be debated if the imaged area is an adequate representation of the entire vaginal wall or vaginal canal. In a future study, it could be interesting to more extensively map microvascular changes throughout the vagina in patients with VVA. A thorough mapping could also provide information on the course of VVA and how and when it affects the microcirculation in different parts of the vagina. Secondly, the assessment of the vaginal vessel density is challenging with analysis programs that are currently available (eg, automated vascular analysis). Such programs can calculate total vessel densities, but not when capillary loops are present. Therefore, it is not possible to use these programs for analysis of vaginal images, and it is impossible to compare density parameters between different architecture scores. In this study, we calculated capillary density by manually counting loops per field of view in architecture score 1 and 2 dominant tissue (as was validated by Weber et al[12]). Unfortunately, with this method, capillary density cannot be calculated in architecture score 2 and 3 tissue, since capillary loops were limited to absent. This limits the report on vaginal capillary density. Thirdly, since this is the first study on this topic, no data from previous studies were available. Consequently, we could not perform a power calculation. Therefore, we should be cautious to generalize these findings, which were observed in a relatively small patient population.

5 | CONCLUSIONS

The architecture of the vaginal microvasculature is altered in women with VVA. In case of similar vascular architecture, capillary tortuosity, and density seem to be comparable. Treatment with topical estrogen results in restoration of the vaginal angioarchitecture. From other studies we have learned that vaginal estrogen therapy improves the quality of the vaginal epithelium, and apparently, qualitative aspects of the microcirculation play an important role as well. Future research should be focused on how to preserve the quality of the microcirculation in women who are exposed to events like aging, vaginal delivery, and pelvic floor surgery.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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