Effects of vaginal prolapse surgery and ageing on vaginal vascularization
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CHAPTER 3

Vaginal microcirculation: non-invasive anatomical examination of the micro-vessel architecture, tortuosity and capillary density


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ABSTRACT

Aim: To describe the vaginal microcirculatory architecture and capillary density parameters using sidestream dark-field imaging (SDFI), and determine feasibility and reliability of this method.

Methods: In nine healthy female volunteers SDFI measurements were performed at two different time points in the luteal phase of the menstrual cycle. Non-invasive tissue micro-angioarchitecture and vaginal capillary density measurements were assessed independently by two observers. Agreement was expressed with mean differences between the measurements of both observers and the limits of agreement. Inter- and intra-observer agreement was quantified with the intra-class correlation coefficient (ICC).

Results: Vaginal microcirculatory assessment with the SDFI device was easy in use, painless and well accepted by the participants. Morphologically, the vaginal microcirculation revealed an array of single hairpin-shaped capillary loops distributed homogeneously across an imaged tissue segment. The intra-observer assessment of the capillary density measurements (comparing two measurement time points of one observer) showed good agreement with an ICC ranging from 0.62 to 0.85. The inter-observer assessments of the capillary density measurements (comparing assessments of two observers at one time point) revealed very good agreement, with small differences between observers and an ICC of more than 0.9.

Conclusions: This is the first report on both microcirculatory architecture and quantitative microcirculatory parameters of the vagina with the use of SDFI. Micro-vessels of the vagina show a recognizable pattern in our study population of young, healthy women. SDFI gives a reproducible assessment of the vaginal microcirculation offering the researcher a wide field of applications.
INTRODUCTION
Vascularization of the vagina is supplied by the vaginal artery, the vaginal branch of the uterine artery, the internal pudendal artery, and vaginal branches of the middle rectal artery (1). The microcirculation comprises all vessels with a diameter smaller than 100 μm including arterioles, capillaries and venules and is the site from which hormones, gases (like oxygen and carbon dioxide), immune support, nutrients, water, and waste products between the blood and tissue cells are exchanged (2). In critical care medicine, the microcirculatory compartment has proven to be of importance in septic patients (3, 4). The density of the microcirculation in septic patients was significantly reduced, and this reduction was most significant in non-survivors (5). Given the importance of the microcirculation in septic patients and the numerous clinical investigations on microcirculatory alterations in different conditions including the process of wound healing (6), diabetes (7), hypertension, and cardiovascular disease (8), we hypothesize that the vaginal microcirculation plays an important role in the proper metabolic support and health of pelvic floor organs and their function (i.e., micturition, defecation, and sexual functioning). Vaginal surgery may have an impact on vaginal microcirculation as a result of dissection and traction applied to the tissues, in particular, the vaginal epithelium and its direct microenvironment. If and to what extent the vaginal microcirculation is damaged during surgery is at present unknown; it is also unknown whether possible damage to the microcirculation of the vagina is reversible.

The ability to inspect and measure vaginal microcirculation is a necessary first step to be able to study the effects of vaginal reconstructive surgery on vaginal microcirculation and its relation to micturition, defecation and sexual functioning.

In the past, several indirect methods have been developed to evaluate vaginal blood flow, such as photoplethysmography, Doppler ultrasound and laser Doppler flowmetry (9-11), however, these techniques were unable to provide direct visualization of the microcirculation’s anatomy and functionality. The introduction of bedside optical spectroscopic-based imaging techniques such as orthogonal polarization spectral imaging (OPSNI) (12, 13) and the more recent sidestream dark-field imaging (SDFI) (14) have enabled direct visualization of the human microcirculation in solid organs and mucous membranes. SDFI has been validated in the human nailfold microcirculation by
comparison to OPSI and showed superior imaging capabilities with ease in quantifying vessel density (14, 15).

To our knowledge there has been only one study on vaginal microcirculation describing the effect of two consecutive anaesthetic interventions using OPSI (16). No information was given concerning the basal morphological properties of the vaginal microcirculation.

In this study, we used SDFI trans-vaginally to study the vaginal microcirculation in healthy volunteers. We assessed the ability to obtain microcirculatory images suitable for offline analysis of both quantitative microcirculatory parameters and microcirculatory architecture and propose a vaginal microcirculatory architecture score for rapid recognition of sub-epithelial vascular patterns. In addition, we quantified the intra- and inter-observer reproducibility of SDFI-derived vaginal microcirculation.

**METHODS**

We performed a prospective, observational study in the Department of Obstetrics and Gynaecology of the Academic Medical Center of the University of Amsterdam. The Medical Ethics Committee of the Academic Medical Center of the University of Amsterdam approved this study. Healthy volunteers enrolled in this study received a full explanation of the study guidelines and procedures and written informed consent was obtained from each participant.

*Setting and participants*

Female students were recruited from the medical faculty of the Academic Medical Center. Recruitment was done by online advertisement on the student Facebook page of the University of Amsterdam. Nine healthy female volunteers participated in this study. A medical history was obtained and individuals with cardiovascular (e.g., angina pectoris, hypertension), inflammatory (e.g., rheumatoid arthritis, eczema), other systemic illness (e.g., (non-) insulin dependent diabetes mellitus) or those taking medications (e.g., anticoagulants, anti-inflammatory, or immunosuppressive agents) that could influence the microcirculation were excluded from participation. Additionally, volunteers were excluded from participation if they had delivered vaginally or had a previous history of...
vaginal surgery. Because the goal of this study was not to investigate hormonal effects on
the vaginal microcirculation and most participants used oral contraceptives, all
participants underwent measurements in the same phase of their menstrual cycle (the
luteal phase) at two different time points.

Tolerability of the measurement was discussed verbally with the participant after
every measurement and documented.

**Microcirculatory examination and data acquisition procedures**

All participants were accommodated in a gynaecological examination chair in a stable 45°
semi-reclined position in the same room kept at a constant temperature of 21±1°C. The
SDFI device, covered with a sterile disposable cap, was gently placed into contact with the
vaginal wall at exactly 3 cm above the hymen and adjusted for optimal focus and
contrast. Pressure of the device on the vaginal wall was avoided to prevent pressure
artefacts like disturbance of capillary flow. To determine and obtain a general
measurement of the distribution and quantity of vaginal microcirculation, the anterior,
posterior and lateral vaginal walls were the four target locations and were measured
starting from the anterior wall in a clockwise direction (anterior: 3 cm above the hymen in
the midline on the anterior vaginal wall, left lateral: 3 cm above the hymen on the left
lateral vaginal wall, posterior: 3 cm above the hymen in the midline on the posterior
vaginal wall, right lateral: 3 cm above the hymen on the right lateral vaginal wall). To
ensure measurements were performed exactly 3 cm above the hymen the Microscan
disposable cap was marked at 3 cm from the tip. All microcirculation data acquisition and
measurements were performed by one investigator (MAW). Each measurement was
recorded for 2 minutes, stored on DVI tapes on a Sony DSR-11 DVCAM™ recorder (Sony,
Shinagawa-ku, Tokyo, Japan), and viewed on a 19-inch Samsung SyncMaster 932MV LCD
monitor (Samsung, Seoul, South Korea) with a 1440×900-screen resolution.

**Microcirculation imaging**

Vaginal microcirculation assessments were performed using sidestream dark-field
imaging (SDFI) technology (Microscan Video Microscope System, MicroVision Medical,
Amsterdam, the Netherlands); technical details on this system have been described
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before (14). Briefly, the SDFI technique is built into an easy to use commercially available hand-held apparatus and employs spectroscopic-based imaging principles via light-emitting diodes that emit green light (530 nm wavelength) by epiilluminating the tissue of interest where it is absorbed by haemoglobin in red blood cells, allowing detailed observations of sub-epithelial (imaging depth range 100-500 µm) flowing erythrocytes in the microcirculatory beds (14).

The female investigator performing all the measurements in this study was trained by a researcher with extensive clinical experience using SDFI (DMJM) for obtaining images of adequate quality for offline analysis.

We determined the microcirculatory architecture (A), the capillary tortuosity (B) and the capillary density (C), described in more detail below.

A. Tissue microcirculatory architecture

After systematically examining the available frames, three types of sub-epithelial vascular patterns could be recognized. Microvascular architecture from each measurement site was classified with a score of 1, 2, or 3: appearance of an array of capillary loops (score 1), appearance of both capillary loops and vascular network (score 2), and appearance of vascular network without capillary loops (score 3). This scoring method was devised to provide rapid recognition of sub-epithelial vascular patterns. As this is a newly developed scoring method we assessed the inter-rater variability, each of the two observers (MAW and DMJM) scored the microcirculatory architecture in each frame independently.

B. Capillary tortuosity score

A morphologic characteristic that has been described in the assessment of microvascular architecture is the capillary tortuosity (number of twists per capillary). Although this morphologic characteristic has been associated with diabetes, its biological explanation is currently unknown. To assess the capillary tortuosity score, the number of twists per capillary in the majority of capillaries was evaluated in each selected frame and classified as score 0: no twists (or pinhead capillaries) to 4: four or more twists as described by Djaberi and co-workers (7). Subsequently, the overall tortuosity score per anatomical region per subject was determined by selecting the most frequent tortuosity score. As
this is a previously described scoring method which showed good intra- and inter-
observer agreement of measurements of the lip (7), we did not assess the inter-rater
variability. The capillary tortuosity score in each frame was assessed by one observer
(MAW).

C. Capillary density
All SDFI-derived data were stored on digital video imaging tapes and converted into audio
video interleave (.avi) files off-line using Adobe Premier Pro 1.5 (Adobe Systems Inc., San
Jose, California, USA). At off-line analysis five video frames were randomly captured in all
four measured regions of the vaginal wall, in total 20 frames per patient. The area of each
SDFI frame was 0.75 mm².

A frame was judged suitable for subsequent assessment of capillary density if vaginal
discharge was not so excessive that it caused blurring of the image and if capillary loops
were identifiable. We determined the capillary density by counting the number of
capillary loops per visual field for each of the randomly captured video frames from the
four anatomical regions on a 19-inch Samsung SyncMaster 932-mv LCD monitor with a
1440x900 screen resolution. The mean capillary density obtained from the five isolated
image frames, expressed as the mean number of capillary loops per square millimetres
(cpll/mm²) was used to quantify vaginal microcirculation at each of the associated vaginal
wall regions (17). Two investigators (MAW [Observer 1] and DMJM [Observer 2]) analysed
all images independently according the method presented above.

Statistical analysis
The inter-observer agreement of the capillary density assessment was expressed as the
mean difference between the measurements of the two investigators with the standard
deviation of this difference. The 95% limits of agreement were calculated, defined as the
mean difference between the investigators ± 1.96 x SD of this difference (Bland and
Altman plot (18)).

The Intra-class Correlation Coefficient (ICC) was used to assess the reliability of the
capillary density measurements and the tissue angioarchitecture score (19): the ICC can
theoretically range from 0 to 1, with a value of 1 indicating all observed variation is true
variation and a value of 0 meaning all observed variation is due to error. Differences between the first and second measurements of capillary density performed by Observer 1 were described as medians and ranges, $P$-values were calculated using the paired Wilcoxon signed rank test. The intra-observer reproducibility was determined per anatomical region for Observer 1 by calculating the ICC between each first and second measurement. Capillary tortuosity scores of the first and second measurement performed by Observer 1 were compared using a Wilcoxon signed rank test for paired data. Differences of tissue microcirculatory architecture and tortuosity scores between anatomical regions were compared using the Chi-square test. A $P$-value of $<0.05$ was considered to be statistically significant. All analyses were performed using the statistical software SPSS version number 20.0.

RESULTS
Median age of the nine volunteers was 22 years [range 19-23]. Six volunteers used oral contraception. Median body mass index (BMI) was 21 kg/m$^2$ [range 17-25]. None of them had relevant co-morbidity.

Vaginal microcirculation assessments with the use of the SDFI-device were painless and well tolerated by the participants.

*Tissue microcirculatory architecture*

Figure 1 illustrates the microcirculatory architecture. There were 346 frames available, 170 from the first measurement and 176 from the second measurement. In 323 (93%) frames there was complete agreement; a scoring difference of -1/+1 was found in 23 (7%) frames (Table 1). The ICC between Observer 1 and Observer 2 was 0.78.

Assessment of the microcirculatory architecture showed in the majority of frames an array of single hairpin-shaped capillary loops (score 1) distributed homogeneously across a tissue segment. We did not observe a characteristic microcirculatory distribution for one of the measured anatomical regions. Scores did not differ significantly between the four regions (Chi-square test $P=0.07$)
Vaginal microcirculation

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Figure 1 Sidestream dark-field images of the vaginal microcirculation
A & B: score 1: appearance of an array of capillary loops
C & D: score 2: appearance of both capillary loops and vascular network
E & F: score 3: appearance of vascular network without capillary loops
Table 1 Inter-observer agreement for microcirculatory architecture score

<table>
<thead>
<tr>
<th>Observer 2</th>
<th>Array of capillary loops (1)</th>
<th>Capillary loops and vascular network (2)</th>
<th>Vascular network without capillary loops (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Array of capillary loops (1)</td>
<td>288</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Capillary loops and vascular network (2)</td>
<td>1</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Vascular network without capillary loops (3)</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>346</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Capillary tortuosity*

At the first measurement, 170 frames were selected for offline analysis, 116 out of the 170 frames (68.2%) were suitable for subsequent assessment of capillary tortuosity. At the second measurement 176 frames were selected of which 121 (68.8%) were suitable for capillary tortuosity assessment. Based on the presence of excessive vaginal discharge 82 out of 346 frames (24%) were rejected, based on hardly identifiable capillary loops 27 out of 346 frames were rejected (8%). At least two frames were assessable per measurement per patient.

Overall tortuosity scores per region are shown in Table 2. The most frequent scores for all four anatomical regions were score 1 (1 twist) and 2 (2 twists). Scores did not differ significantly between the first and second measurement (see Table 2). There was a consistency in tortuosity scores per individual.
Vaginal microcirculation

Table 2 Comparison of capillary tortuosity scores between first and second measurement assessed by Observer 1

<table>
<thead>
<tr>
<th>Score</th>
<th>Measurement 1st</th>
<th>Measurement 2nd</th>
<th>Measurement 1st</th>
<th>Measurement 2nd</th>
<th>Measurement 1st</th>
<th>Measurement 2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: no twists</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1: 1 twist</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2: 2 twists</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3: 3 twists</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4: 4 or more twists</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>p-value</td>
<td>0.16</td>
<td>1.00</td>
<td>0.56</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assessment of capillary density

At the first measurement, 147 out of the selected 170 frames (87%) were suitable for assessment of capillary density. At the second measurement 156 out of the 176 frames (89%) were suitable for capillary density measurements. Reasons for rejecting any frames from the analysis were based on the presence of excessive vaginal discharge, which caused blurring of the image (16 out of 346 frames (4.6%)) or hardly identifiable capillary loops with mainly the appearance of the underlying vascular network (27 out of 346 frames (7.8%)). More frames were suitable for capillary density measurements compared to assessment of tortuosity scores due to less clarity needed for counting the loops compared to counting the number of twists.

Table 3 presents the results of the inter-observer agreement (comparing assessments of two observers at one time point) and intra-observer agreement (comparing assessments of one observer at two time points). The mean differences in capillary density scores between the observers in all measured locations were small. The inter-observer agreement was high with ICC values of more than 0.98 (P-values <0.001). The intra-observer agreement measured by the ICC ranged from 0.62 to 0.85 (P-values <0.05).
Table 3 Intra- and inter-observer agreement between capillary density measurements

<table>
<thead>
<tr>
<th></th>
<th>Inter-observer agreement</th>
<th>Intra-observer agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observer 1 Mean (SD)</td>
<td>Observer 2 Mean (SD)</td>
</tr>
<tr>
<td>Anterior</td>
<td>33.5 (10.7)</td>
<td>33.8 (10.7)</td>
</tr>
<tr>
<td>Left</td>
<td>31.0 (7.6)</td>
<td>31.3 (6.9)</td>
</tr>
<tr>
<td>Posterior</td>
<td>32.7 (10.4)</td>
<td>33.3 (10.1)</td>
</tr>
<tr>
<td>Right</td>
<td>29.7 (7.6)</td>
<td>30.2 (7.4)</td>
</tr>
<tr>
<td>Total</td>
<td>32.0 (8.2)</td>
<td>32.3 (7.9)</td>
</tr>
</tbody>
</table>

* 95% limits of agreement: the mean difference between the observers ± 1.96 x SD of this difference

Figure 2 shows the Bland and Altman plot for the scores of Observer 1 and Observer 2 for the overall capillary density of the nine participants. The difference in capillary density scores between Observer 1 and Observer 2 is given as a function of the average density measured by Observer 1 and Observer 2. Here the mean difference was 0.39 cpll/mm² and did not differ significantly from zero (95% confidence interval [CI]; [-0.06-0.84]), indicating no systematic difference between the two observers. As shown in table 3 the limits of agreement were small (-0.76–1.54 cpll/mm²). There did not appear to be a relation between the difference and the size of the capillary density, the measurement differences were equally distributed across the range of measured values. There was one outlier. In this subject, capillary density was difficult to assess for both observers, multiple frames had to be excluded for this subject due to the absence of capillary loops with only the appearance of the underlying vascular network. Excluding this patient from the calculations, as has been suggested as an option by Bland and Altman (18) produced a mean difference of 0.23 cpll/mm² (95% CI: [-0.06-0.51]).

We observed no significant differences between the first and second measurements performed by Observer 1 (see Table 4).
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Figure 2 Bland and Altman plot for scores of Observer 1 (MW) and Observer 2 (DM) for the total capillary density (CD) in 9 participants.

Table 4 First and second measurement of median capillary density of the four different locations and total capillary density in healthy volunteers (n=9)

<table>
<thead>
<tr>
<th>Location</th>
<th>1st measurement Obs1</th>
<th>2nd measurement Obs1</th>
<th>p-value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>30.0 [22.0-49.8]</td>
<td>31.2 [17.3-50.2]</td>
<td>0.78</td>
</tr>
<tr>
<td>Left</td>
<td>29.4 [23.0-44.6]</td>
<td>31.8 [18.5-42.6]</td>
<td>0.68</td>
</tr>
<tr>
<td>Posterior</td>
<td>31.4 [16.0-53.4]</td>
<td>30.8 [19.0-54.2]</td>
<td>0.86</td>
</tr>
<tr>
<td>Right</td>
<td>29.8 [18.5-41.8]</td>
<td>27.8 [21.0-43.8]</td>
<td>0.86</td>
</tr>
<tr>
<td>Total</td>
<td>31.1 [23.3-47.4]</td>
<td>30.0 [22.9-47.6]</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Values are median [range]
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DISCUSSION

This is the first study reporting on both quantitative microcirculatory parameters and microcirculatory architecture of the vaginal epithelium using in vivo microscopy. In order to better understand the physiology of the vagina, the ability to measure vaginal microcirculation is a necessary first step.

Morphologically, the vaginal microcirculation consistently revealed an array of single hairpin-shaped capillary loops distributed homogeneously across an imaged tissue segment. Classifying the microcirculatory architecture with a score ranging from an appearance of capillary loops to an appearance of the underlying vascular network without capillary loops (score 1 to 3) allowed a rapid recognition of sub-epithelial vascular patterns. The mean vaginal capillary density in young and healthy female volunteers was 32 cpl/mm². Vaginal microcirculatory assessment with the use of the SDFI device was easy to apply in the vagina, not experienced as painful and well tolerated by the healthy volunteers. The inter-observer assessments of the vaginal microcirculatory architecture and capillary density measurements revealed very good agreement between the two observers.

The microcirculatory morphology observed in this study is comparable to what has been seen from the tongue (20), in gingival mucosa (17, 21) and the nailfold capillary bed (14).

In previous studies, several scoring systems have been used to quantify the microcirculation. Besides the assessment of capillary density as described in the study of Milstein and co-workers (17), we chose the tortuosity score as described by Djaberi and co-workers (7) for assessment of the number of twists per capillary. We developed a new microcirculatory architecture score based on examination of the vascular patterns in the available frames, which showed a high agreement between observers. In our study score 1 (appearance of an array of capillary loops) was the most prevalent score. In 16 out of 346 frames (5%), the provided contrast of the vaginal epithelium was insufficient to obtain clearly visible loops for capillary density measurements due to excessive vaginal discharge. More frames (24%) had to be rejected in the assessment of tortuosity score, because even more clarity of the images is necessary to count the number of twists. This might indicate that assessment of the capillary tortuosity in younger (premenopausal)
women is more difficult to interpret considering the presence of more vaginal discharge in premenopausal compared to postmenopausal women. In 27 frames (8%), there was no excessive discharge; however, capillary loops could not be clearly identified and mainly the underlying vascular network was visible. It could be that, in these women, the vaginal epithelium is slightly thinner compared to the women with clearly visible capillary loops, revealing the underlying vascular network (score 2 and 3). The same pattern was seen in the follow-up measurement in these patients.

The inter-observer assessments of the capillary density measurements and microcirculatory architecture score revealed very good agreement between the two observers, which indicates that the method of multiple frames for assessment of these measurements is reliable. We found the lowest ICC for the measurement on the left vaginal wall. It could be that placement of the probe in a straight angle to the left vaginal wall is more difficult for a right-handed investigator. In our study, the limits of agreement in all measured locations are small indicating a small measurement error between observers. How far apart measurements can be without being clinically relevant will be a question of judgment and needs further research.

As far as we know, there has only been one study that investigated the vaginal microcirculation with the use of OPSI (16). Van den Oever and co-workers examined the microcirculation of the vaginal wall in nine anesthetized patients during two consecutive anesthetic interventions, however they did not standardize their measurement methods or systematically evaluated qualitative and quantitative microcirculatory parameters, which makes their results not ready for use in other studies. Our results show a promising basis for future research. The group of young healthy volunteers in the present study could represent a reference for further evaluation in other groups.

Several indirect methods have been developed to evaluate the vaginal blood flow. Vaginal photoplethysmography has been the most commonly used method to evaluate vaginal vasocongestion (11). It allows measurement of phasic changes in vaginal vasocongestion in the peripheral vessels with every heartbeat (VPA). Other methods to assess genital blood flow are duplex Doppler ultrasound (gray scale or color), and laser Doppler flowmetry (9, 11). Doppler ultrasound uses standard ultrasound methods to provide information about the speed and direction of blood flow through the vessel being
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evaluated. Laser Doppler flowmetry is a non-invasive method of assessing microcirculatory blood flow, it can detect flow in capillaries as small as 11 µm and as deep as 2 mm below the skin surface (22). However, these techniques do not provide direct visualization of the microcirculation and often flow is expressed in arbitrary units. Dynamic MRI provides excellent visualization of anatomic detail through serial images, however, MRI is expensive, time consuming, and machine availability can be a problem.

We hypothesize that SDFI of the vaginal wall is a tool that could enhance our understanding of the effects that local conditions or vaginal surgery have on vaginal vascularization. We also want to study the relationship between microcirculation and pelvic floor function; however, much larger studies are needed to perform such analysis. Considering the correlation of a reduced density of the microcirculation with death in septic patients (5), it is very well possible that the vaginal microcirculation, hence, the representative of oxygenation of the vaginal tissue, plays an important role in the functionality of the tissue and its surrounding organs. Vaginal SDFI forms a method to get an impression of the oxygenation and therefore the functionality of the vaginal tissue through inspection of the microcirculation. The non-invasive and fast SDFI technique enables patient compliance without the need for lengthy appointments or the use of contrast materials. It therefore also forms a method that could be of help in evaluations prior to surgical reconstruction, optimizing design of surgical incision approach or post-operatively to monitor repair and regeneration during the healing phase.

Potential limitations of this study need to be addressed. One could argue that all microcirculatory measurements were performed by one researcher and only the off-line frames were interpreted and analyzed by two researchers. However, after training and performing several test measurements, we quickly discovered the ease of use of the equipment and images of adequate quality could be obtained. We therefore decided to focus on the reliability analysis of the off-line image assessment for which interpretation is required. Other quantitative parameters such as capillary blood flow velocity or assessment of vessel diameters would have made the characterization of the vaginal microcirculation more complete. With SDFI and the available software modalities this is currently a time consuming process requiring a lot of skill to obtain images fit for these kinds of analyses. The introduction of a new generation of hand-held microscopes
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(CytoCam, Braedius Medical BV) with increased spatial and temporal resolution in combination with a sensor attached to a powerful computer, might provide the needed hardware requirements to allow instant online automatic analysis in future research (3).

One could criticize that we did not assess microvascular flow using the microvascular flow index (MFI) as has been described by Boerma and co-workers (23), however in this group of young and healthy volunteers we did not observe differences in the spectrum of microvascular flow, ranging from no flow (MFI score 0) to normal flow (MFI score 3) during the assessment of microcirculatory architecture and capillary density and we therefore decided to focus on the morphologic assessment.

One could question how representative the group of young and healthy volunteers is. We chose this particular study group, free of comorbidities that could influence the microcirculation, for validation of the measurement technique. We are planning to compare the data of this group of young and healthy volunteers to a group of postmenopausal women in future studies.

In conclusion, this is the first report on both quantitative microcirculatory parameters and microcirculatory architecture of the vagina using SDFI. Micro-vessels of the vagina show a typical and recognizable pattern in our study population of young, healthy women. SDFI provides an immediate and reproducible assessment of the vaginal microcirculation offering the researcher a wide field of applications. The ability to examine and measure vaginal microcirculation provides an opportunity for future clinical research aimed at examining the possible associations between vaginal microcirculation, pelvic floor disorders, and the effects of vaginal reconstructive surgery.
Chapter 3

References