Vascular architecture of the human uterine cervix, as assessed in light- and scanning electron microscopy

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Background: The aim of this study was to visualise and describe the vasculature of the human uterine cervix.

Material and methods: The material for this study was obtained from women (age between 20 to 45 years) during autopsy. The material was collected not later than 24 h post-mortem. This study was performed using uteri from cadavers of menstruating nulliparas (33 uteri) and menstruating multiparas (27 uteri). Collected uteri were perfused via the afferent vessels with Mercox resin (for corrosion-casting and SEM assessment) or acrylic paint solution (light microscopy assessment). The research protocol was approved by the Jagiellonian University Ethics Committee (registry KBET/121/8/2007).

Results: In all cases bilateral cervical branches (1–4), originating from the uterine artery, were found. Both in the vaginal and supravaginal parts of the cervix, four distinct vascular zones were found. In the pericanalar zone ran small veins, responsible for draining the mucosal capillaries. Both in the muscular layer, as well as in the pericanalar zone, arterioles, and venules passed close to each other, often adjoining.

Conclusions: This study does not confirm the existence of a single “cervicovaginal” artery, but shows that the vascular supply of the cervix comes from several vessels. It also introduces the idea of two systems, responsible for draining blood from the mucosal capillaries. Neither assessment in light microscopy nor in SEM revealed any differences between multiparas and nulliparas, regarding the vascular architecture of the cervix. (Folia Morphol 2012; 71, 3: 142–147)

Key words: uterine cervix, vasculature, light microscopy

INTRODUCTION

The uterine cervix receives most of its blood supply from the uterine artery (UA) [15]. The classic approach divides the course of the UA into three parts — descending, transverse, and ascending. Lateral branches originate mostly from the transverse and ascending parts. Among those branches there are also vessels supplying the uterine cervix [15].

Due to its unique remodelling capabilities, associated with the menstrual cycle, implantation, and pregnancy [13], the vascular structure of the uterus has been the target of many studies. The earliest of them date back to the 19th century [11]. The microvascular structure of the uterus has also been the subject of extensive studies [2, 5, 19], but most of them dealt with the microvascular structure of the uterine corpus. In contrast to the abundance of studies dealing with the microvasculature of the uterine corpus, studies on the subject of uterine cervix microvasculature are scarce [17] and concentrate most-
ly on the pathological vasculature of cervical cancer or dysplastic changes [6, 7].

The vasculature of the female reproductive organ is especially interesting for clinicians. This is because of surgical interventions undertaken to stop bleeding that may occur in the course of cervical cancer, ectopic pregnancies, or leiomyomata.

The aim of this study was to visualise and describe the vasculature of the human uterine cervix using stereoscopic light microscopy supplemented by haematoxylin and eosin and immunohistochemistry staining as well as by corrosion casting together with scanning electron microscopy (SEM).

MATERIAL AND METHODS
The material for this study was obtained from women (age 20 to 45 years) during autopsies performed at the Department of Forensic Medicine, Jagiellonian University Medical College. Women who died due to disorders of the reproductive system were not included into the study. The material was collected not later than 24 h post-mortem. This study was performed using uteri from cadavers of menstruating nulliparas (33 uteri) and menstruating multiparas (27 uteri).

The research protocol was approved by the Jagiellonian University Ethics Committee (registry KBET/121/8/2007).

Each uterus together with the ovaries and the cervical portion of the vagina was removed in such a way that relatively long fragments of uterine and ovarian vessels (both arteries and veins) were retained.

Preparing material after autopsy
Immediately after removal, the uteri were perfused via the afferent arteries with pre-warmed (37°C), heparinised saline (Heparin, Polfa, Poland, 12 IU/mL) containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine, Polfa), until the fluid outflowing via the veins was completely transparent (5–10 min).

Preparing material for stereoscopic light microscopy assessment
For uteri (n = 58) selected for stereoscopic light microscopy assessment, after post-autopsy preparations, perfusion (via arteries and veins) was continued using acrylic paint emulsion (Liquitex R, Binney and Smith, USA) [21]. When the solution started to flow out of the open vessels, they were closed using haemostatic forceps. Injection was continued until the smallest visible vessels of the perimetrium were filled with acrylic paint emulsion. After vessel closure the uteri were stored in a 10% solution of formaldehyde for two weeks. Next the specimens were sectioned (slice 0.5–1 cm thick) along the sagittal, transverse, or coronal planes and dehydrated in increasing concentrations of ethyl alcohol. Then the slices were placed in mixtures of 96% ethyl alcohol and methyl salicylate (with increasing concentrations of the latter). Finally, the slices were placed in pure methyl salicylate for 2–3 weeks to enable tissue transparency. The obtained slides were assessed using a stereoscopic light microscope (PZO MST-130), magnification 5–80×.

Preparing histological slides
From uteri, with vessels filled with acrylic paint emulsion, tissue samples were acquired. These samples were dehydrated, embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin. The slides were assessed using magnifications of 5–40×.

Part of the samples underwent immunohistochemistry staining for the von Willebrandt factor (present in vessel epithelial cells), using primary anti Human von Willebrandt factor (Dako) and the EnVision + HRP/MO kit (Dako). The slides were assessed using magnifications of 20–330×.

Preparing material for scanning electron microscopy assessment
For uteri (n = 2, from multiparous cadavers) selected for SEM assessment, after post-autopsy preparations, perfusion was continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde (Sigma) in 0.1 M cacodylate buffer (pH 7.4) supplemented with 0.2% lidocaine. Finally, the vascular system was injected with 60–80 mL of Mercox CL-2R resin (Vilene Comp. Ltd., Japan) containing 0.0625 mg/mL methyl methacrylate with the polymerisation initiator (Vilene Comp. Ltd.). The uteri were left in a warm water bath (56°C) for 12 h to allow polymerisation and tempering of the resin [18]. After polymerisation completion, the uterine tissues were macerated for 5–6 days by repeated soaking in 10% potassium hydroxide at 37°C, followed by washing with warm (50–55°C) running tap water. The obtained vascular casts were washed in several changes of distilled water, cleaned in 5% trichloroacetic acid for 1–2 days, washed again in distilled water for 2–3 days, and freeze dried in a lyophiliser (Liovag G2, Aqua Fina, Germany). Parts of the freeze-
-dried casts corresponding to the uterine cervix and a small fragment of the uterine body were excised and examined macroscopically. In order to facilitate sectioning of the casts, they were then embedded at 55°C in a mixture of polyethylene glycols (PEG 2000/PEG 600, 20:1), cooled to room temperature to solidify PEG [20], and gently dissected longitudinally in the plane of the endocervical canal to expose the vasculature of the cervix wall. The sectioned fragments were washed with stirred distilled water to remove PEG and stored in an exsiccator containing phosphorus pentoxide until microscopic examination. The fragments were then mounted onto copper plates using colloidal silver and “conductive bridges” [9] and coated with gold. The casts were examined in a JEOL SEM 35-CF SAM at 20–25 kV. Casts of arteries and arterioles were distinguished from those of veins and venules on the basis of different imprints of endothelial cell nuclei [12].

RESULTS

Macroscopic assessment and assessment using stereoscopic light microscopy

In the majority of cases the UA was an independent vessel. The following branching variations were found: in 51 cases the UA branched off the stem of the anterior internal iliac artery (in 6 cases the UA was accompanied by the inferior vesical artery); in 41 cases the UA branched off the internal iliac artery at the anterior and posterior trunk division point; and in 8 cases the UA branched off directly from the internal iliac artery, before its division.

In all cases bilateral cervical branches, originating from the UA, were found. These vessels branched off from the transverse and ascending UA parts in the parametrium, partially penetrating the paracervix (Fig. 1). Usually there were one to four larger trunks branching off the ascending part of the UA, which, after a short course, gave off a variable number of smaller branches that supplied the cervix and the upper portion of the vagina.

Specimen transverse sections showed that in the region of the cervical internal os, the upper part of the cervix is supplied by vessels running perpendicularly to the long axis of the cervical canal. The arteries produced a thick vascular network, with numerous anastomoses between the smaller vascular branches (Fig. 2).

Coronal sections of the supravaginal part of the cervix showed arteries (60–70 μm in diameter) that branched off as spiral vessels, but their distal parts ran a straight course. The arteries were accompanied by veins (100–200 μm in diameter). In the mid portion of the cervix the spiral arteries became thicker but still ran a radial course towards the light of the cervical canal (Fig. 3). Moving further towards the external os, the vessels became less numerous and in deeper layers, near the cervical canal, ran a parallel course to the long axis of the canal (Fig. 4).

When performing macroscopic inspection and assessment using light microscopy, no differences between multiparas and nulliparas, as to the vascular architecture of the cervix, were noted.

When assessing histological slides (haematoxylin and eosin supplied with von Willebrandt factor immunohistochemical staining) numerous empty (not filled with acrylic paint emulsion) vessels were noted, especially in the pericanalar zone of the cervix (Fig. 5).

Assessment using scanning electron microscopy

At the junction between the supravaginal part of the cervix and the corpus of the uterus, four distinct vascular zones could be seen (Fig. 6). Going from lateral to medial (towards the cervical canal) the zones were identified as the:

— outer zone containing large arteries and veins;
— zone of arterioles and venules of the muscular layer, characterised by loose and irregular texture;
— zone of endocervical mucosal capillaries, characterised by dense texture;
— pericanalar zone containing small veins and capillaries.

In the subepithelial region of the cervical canal ran veins (80–150 μm in diameter), which drained the mucosal capillaries. The veins ran parallel to the long axis of the cervical canal and were joined by capillaries (12–20 μm in diameter), which ran perpendicular to their course. The capillaries covered the veins, forming a sort of plexus around the larger vessels (Fig. 7). Both in the muscular layer, as well as in the pericanalar zone, numerous places could be seen in which the arterioles and venules passed close to each other, often adjoining.

The vessel arrangement in the vaginal part of the cervix was similar to that described in the supravaginal part. However, the arteries and veins of the outer zone were of significantly smaller calibre, and the veins of the pericanalar zone were much more exposed.

SEM assessment did not reveal any differences between multiparas and nulliparas, regarding the vascular architecture of the cervix.
Figure 1. Light microscopy. Specimen injected through arteries with acrylic paint emulsion. Cervix coronal section — supravaginal part. Spiral arteries (1) branching off the uterine artery (2) and penetrating the paracervix. Bar = 1 cm.

Figure 2. Light microscopy. Specimen injected through arteries with acrylic paint emulsion. Cervix transverse section — supravaginal part. The figure presents the cervical branch of the uterine artery (1) and the light of the cervical canal (2). Bar = 1 mm.

Figure 3. Light microscopy. Specimen injected through arteries with acrylic paint emulsion. Cervix transverse section — mid part (1 cm above the external os). The figure presents arteries (2) running towards the cervical canal (3) and peripheral arteries (1). Bar = 1 mm.

Figure 4. Light microscopy. Specimen injected through arteries with acrylic paint emulsion. Cervix sagittal section. Deeper lying arteries (1) running approximately on a parallel course to the long axis of the cervical canal (2). Bar = 1 cm.

Figure 5. Human uterine cervix histological slide seen in light microscopy. Haematoxylin and eosin supplied with von Willebrand factor immunohistochemical staining. Specimen injected through veins with acrylic paint emulsion. The asterisks mark filled vessels, while the arrows point to the vessels that did not fill with acrylic paint emulsion. Bar = 100 μm.

Figure 6. Corrosion casting and scanning electron microscopy. Cervix sagittal section — supravaginal part. Vertical interrupted lines divide the vasculature into four zones. The outermost zone can be seen on the far right of the figure. The straight arrows point to the places at which perivascular connective tissue was located. The triangles mark the vessels running transversely and supplying the pericanalar vessels. “Serpent” arrows point to the cervical canal. Bar = 1000 μm.
DISCUSSION

This study describes the vascular architecture of the human uterine cervix, as assessed in light microscopy and SEM. Although assessing vasculature by injecting the vessels with acrylic paint emulsion is not considered an up-to-date method when used alone, this study becomes state-of-the-art when supplemented with corrosion casting and assessment in SEM. Corrosion casting combined with SEM assessment is currently the best available technique to visualise vascular architecture [8].

When assessing blood supply to the cervix, we did not observe a “cervicovaginal” artery, which was reported to exist by Chen et al. [3]. This further confirms the findings of Pilarczyk et al. [15] and Palascios-Jaraquemada et al. [14], whose reports are consistent with ours. The observations reported in this study show that the cervix is supplied by several vessels originating from the UA on different levels. These vessels supply both the left and right parts of the cervix and divide into a series of smaller branches, which penetrate the paracervix. The smaller branches from the left and right side communicate by a series of anastomoses. Such findings have been reported earlier [3, 10].

Radiological studies point to the existence of a centrally orientated (medial plane), poorly vascularised zone in the cervix [15]. The supposed existence of this zone is not modified by gynaecological history. The specimens injected with acrylic paint emulsion were not suitable for conducting a search to find such a zone, due to the poor penetration of the emulsion. However, SEM assessment excluded beyond doubt the existence of such a layer. It is very likely that this zone is an artefact, created when relatively large contrast medium molecules fail to pass further through the small blood vessels. The other reason why such an artefact might develop is the fact that, in vivo, microclots might exist in the vessels, which block the passage of the contrast medium. This problem does not exist when applying resin, because of the thorough study material preparation.

Small subepithelial veins running along the endocervical canal have not been described in previous studies that dealt with cervical vasculature. In our observations they seem to be a frequent finding in the human cervix. This might suggest the existence of two drainage systems of the mucosal capillary plexus. One using venules and veins of the middle and peripheral zones, the other based on small pericanalar veins. The veins of the second drainage system might contribute to the formation of pregnancy-induced cervical varices, which may lead to thrombosis [16].

In this study we have described the arrangement of cervical blood vessels, which are divided into four zones. The existence of these zones has been demonstrated in magnetic resonance imaging by deSouza et al. [4]. The zones of the cervix are a continuation of the uterine body wall layers. The zone of larger blood vessels corresponds to the vascular layer of the myometrium, while the other three zones belong to the mucosal layer.

This study has the following two limitations. Injecting vessels with acrylic paint emulsion proved to be unsuitable when it came to filling small calibre arteries and veins (Fig. 5). The problem with filling the fragile veins with acrylic paint emulsion might have been caused by vessel rupture or the existence of vein valves that have been proven to exist in uterine vessels [1]. The second limitation, pertaining to corrosion casting, was that during the corrosion step, the fragile microvasculature of the ectocervical mucosa was lost. This, however, did not influence the outcome of this study as the vascular architecture of the ectocervical mucosa has already been described by other authors [17].

CONCLUSIONS

This study describes the vascular architecture of the human uterine cervix. It does not confirm the existence of a single “cervicovaginal” artery but shows that the vascular supply of the cervix comes from several vessels. It also introduces the idea of
two systems responsible for draining blood from the
mucosal capillaries.

Neither assessment in light microscopy nor in
SEM revealed any differences between multiparas
and nulliparas, regarding the vascular architecture
of the cervix.

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