# Histotopographic study of the pubovaginalis muscle

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*Abstract:* The pubovaginalis muscle (PVM) is one of the described components of the pubococcygeus muscle. The aim of the study was to investigate its topography and histological characteristics. After in situ formalin fixation, the pelvic viscera were removed from 16 female cadavers (range of age: 54-72 years). Serial macrosections of the pelvic viscera and pelvic floor complex, cut in horizontal (8 cases) and coronal (8 cases) planes, underwent histological and immunohistochemical study. PVM was identified in 13/16 (81%) specimens. In both coronal and transverse sections it appears as a layer of muscular tissue at the passage of the inferior and middle thirds of the vagina, along the lateral vaginal walls. In coronal sections, it appeared as a fan-shaped layer of muscular tissue, arising from the pubococcygeus muscle, running with an oblique course towards the lateral vaginal walls. The mean (± SD) thickness of the PVM was 1.8 (± 1.25) mm. In the transverse sections, a bundle of muscle fibres with oblique course splits from the medial margin of the pubococcygeus muscle towards the lateral walls of the vagina, mingling with the outer longitudinal fibers of the muscular layer of the vagina. Immunohistochemical stainings showed that it consisted predominantly of striated muscle fibers. The PVM could represent anatomical evidence of a functional connection between the vagina and the muscular system of the pelvic floor.

*Key words:* Female pelvis; Dissection; Levator ani muscle.

## INTRODUCTION

The levator ani muscle is considered the most important supportive system of the pelvic floor and has been divided into many portions, according to their attachments or physiological functions. Standring et al.[1] subdivide the levator ani muscle into the ischiococcygeus, iliococcygeus and pubococcygeus portions. The pubococcygeus muscle is often subdivided into separate parts according to the pelvic viscera to which they relate, i.e. pubourethralis and puborectalis in the male, pubovaginalis (PVM) and puborectalis in the female. At the level of the vagina and the rectum, the muscle bundles of the pubococcygeus muscle are continuous with those controlateral, forming a sling (pubovaginalis and puborectalis). From the functional point of view, Hanzal et al.[2] and Ashton-Miller and De Lancey[3] describe three regions of the levator ani muscle: the iliococcygeal portion (that is flat and relatively horizontal and spans the potential gap from one pelvic sidewalls to the other), the pubovisceral muscle (the portion of the levator ani that arises from the pubic bone on either side attaching to the walls of the pelvic organs and the perineal body), and the puborectal muscle. The pubovisceral muscle consists of three subdivisions: the puboperineus, the PVM and the puboanalis. Shafik[4-5] suggests that the levator ani muscle consists essentially of the pubococcygeus, the iliococcygeus being rudimentary in humans; the puborectalis muscle does not belong to the levator ani muscle, having different origin, innervation and function (the former being a constrictor, the latter a dilator of the intrahiatal organs).

Kearney et al.[6] found sixteen terms used for the different portions of the levator ani muscle, differences that may be in consequence of the preponderance of studies conducted on male subjects. The difference of opinions concerning the anatomy of the levator ani[7] reflects also on the description and terminology of the PVM. Lawson[8] called the muscular fibers that join the vaginal wall to the pubic bone as the 'pubovaginalis/pubourethralis', whereas the same structure has been called as the 'pubococcygeus' by Curtis et al.[9] and Roberts et al.,[10] 'puborectalis' by Courtney,[11] 'pelvic fibers of anterior layer' by Ayoub[12] and 'superficial perineal layer of anterior fibers' by Bustami.[13] Furthermore, Smith[14] states that these muscular fibers arising from the pubis just run adjacent but do not insert into the wall of the vagina. The

"Terminologia Anatomica"[15] mentions the PVM, referring to those bundles of the pubococcygeus which surround the vagina, intermingling with the controlateral ones.

The microscopic anatomy of the PVM is poorly described. DeLancey and Starr[16] studied the histology of the connection of the vagina with the medial portion of the levator ani muscles, in the region of the proximal urethra. Thus, the term 'pubovaginalis' has also been used for the 'pubourethralis' muscle, defined as the portion of the levator ani muscle that is attached to the urethral supports. A damage of this part of the levator ani muscle might affect urethral support.[6]

The aim of the present study was to investigate the histological structure, the characteristics and topography of the PVM in order to evaluate its role in static and dynamic of the pelvic floor.

## MATERIALS AND METHODS

### Sampling of pelvic viscera

Specimens were obtained from 16 female cadavers (age range: 54-72 years), with anamnesis negative for pelvic pathology. All the subjects were postmenopausal. The pelvic viscera and pelvic floor were sampled according to a protocol previously described.[17-19]

### Histology

Twelve specimens were fixed in 10% formalin for 15 days and then 5-mm thick slices were cut in the transverse (8 cases) plane. Four thick transverse slices of the vagina were sampled. Two slices, one cranial and one caudal, were collected at the level of the middle third of the vagina, and two slices, one cranial and one caudal, were sampled at the level of the inferior third of the vagina (levels II and III respectively, according to DeLancey.[20] Moreover 8 cases were cut on coronal plane. The slices were embedded in paraffin and then cut into 10-µm thick sections, which were stained with hematoxylin and eosin (H.E.), azan-Mallory and Weigert's Van Gieson stain for elastic fibres. In the histological sections, the course and characteristics of the PVM were analysed. Topographical relationships with the vagina, rectum, and aponeurotic structures of the perineum were also evaluated. Morphometric evaluation was carried out with the help of image analysis software (Qwin Leica Imaging System,

Cambridge, UK). Immunohistochemistry used monoclonal anti-human alpha-smooth muscle actin (mouse IgG2a, kappa, Dako-Smooth muscle actin 1A4, Code No. M151, 1:50 solution in phosphate-buffered saline) and monoclonal anti-rabbit sarcomeric actin (mouse IgM, kappa, Dako-Sarcomeric actin, Alpha-Sr-1, Code No. M874, 1:50 solution in PBS) (Dako A/S, Glostrup, Denmark).[21-23] The distribution of smooth and/or striated muscle fibers within the PVM was evaluated in the immunostained sections.

## RESULTS

In coronal sections, stained with H.E. and a-M., the PVM was identifiable in 7/8 specimens (87.5%). It appeared as a fan-shaped layer of muscular tissue, located at the passage between the inferior (cranial level III) and middle third (caudal level II) of the vagina. Muscles fibres arise from the pubococcygeus muscle, run with an oblique course towards the lateral vaginal walls, where they mingle with the outer longitudinal fibers of the muscular layer of the vagina. From their origin the muscle fibres are progressively separated by loose connective tissue, forming a fan, with the apex corresponding to their origin from the pubococcygeus muscle and the base corresponding to the lateral walls of the vagina. At the level of the junction of the muscles fibres of the PVM and muscular layer of the vagina the mean thickness of the PVM is 1.8 ± 1.25 mm.

In the transverse sections, the PVM was identifiable in 6/8 specimens (75%). When the pubococcygeus muscle runs lateral to the vagina, a bundle of muscle fibres with oblique course splits from the medial margin of the pubococcygeus muscle towards the lateral walls of the vagina, mingling with the outer longitudinal fibers of the muscular layer of the vagina (Fig. 1). The mean thickness of the bundle of muscular fibres is 872 ± 56 micron. Other muscle fibers run towards the posterior vaginal wall, mingling with the longitudinal fibres of the vagina at the level of the lateral thirds of posterior vaginal wall. In 3/8 cases (37.5%) some muscle fibers were recognizable along the midline, between the posterior vaginal wall and the rectovaginal septum.

Immunohistochemical staining showed that the PVM consisted predominantly of striated muscle fibers. At the level of the midline, between the posterior vaginal wall and the rectovaginal septum, sparcely smooth muscle fibers were
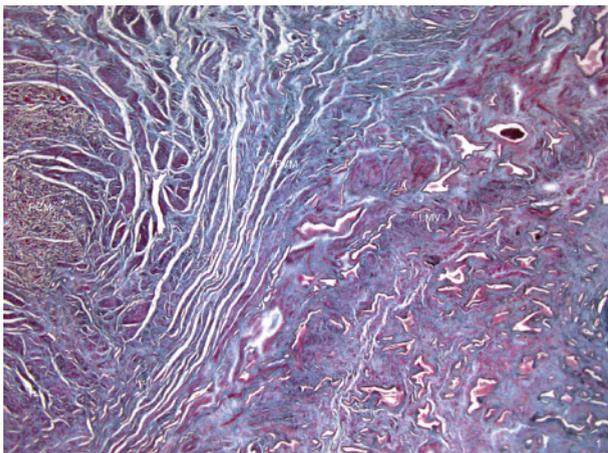


Fig. 1. – Magnification of a transverse section of a female pelvic block showing the pubococcygeus muscle (PCM) and the lateral wall of the vagina. A bundle of muscle fibres of the pubovaginal muscle (PVM) run towards the outer longitudinal fibers of the muscular layer of the vagina (LMV). Note the longitudinal and transverse course of the muscular fibres of the PCM (azan Mallory staining, original magnification X 1.25).

recognisable. At the boundary between the PVM and the vagina, obliquely running muscle fibres were recognizable, connecting the PVM with the outer longitudinal muscular layer of the vagina.

## DISCUSSION

The levator ani muscle plays a critical role in supporting the pelvic organs.[24-26] Standring et al.[1] subdivide the levator ani muscle into the ischiococcygeus, iliococcygeus and pubococcygeus portions. The pubococcygeus muscle is often subdivided into separate parts according to the pelvic viscera to which they relate, i.e. pubourethralis and puborectalis in the male, pubovaginalis and puborectalis in the female. At the level of the vagina, the muscle bundles of the pubococcygeus muscle, are continuous with those controlateral, forming a sling (pubovaginalis and puborectalis).[1, 15] Testut and Jacob[27] reported that at this level a dense and compact connective tissue is interposed between the vagina and the levator ani muscle, that links each other. Cruveilhier[28] described that small fibres of the levator ani muscle penetrate into the vaginal wall. More recently, Guo and Dawei[29] in their radiological study of the pelvic floor, describe the PVM, located 3 mm below the puborectalis plane, indicating it in the axial section of MR imaging – PDW turbo SE sequences – in the component of the pubococcygeus muscle in proximity of the vagina. Our findings show that in the transverse sections the PVM is a dependence of the pubococcygeus muscle, from which splits at the level of the vagina. The muscle fibers show an oblique course and connect to the longitudinal fibres of the outer muscular layer of the vagina by oblique decussating fascicule at the level of the lateral vaginal walls and the lateral thirds of the posterior vaginal wall. So rather than a sling, the PVM is closely connected to the vagina, closing it on the lateral and posterior aspects.

As regards muscle characteristics, the PVM origins from the striated muscular fibres of the levator ani muscle. DeLancey and Starr[16] describe the presence of smooth muscle, collagen and elastic fibers of the vaginal wall and paraurethral tissues that directly interdigitate with the muscle fibers of the most medial portion of the levator ani. Our study shows that the PVM consists predominantly of striated muscle fibers, mainly located at the level the lateral vaginal walls and the lateral thirds of the posterior vaginal wall; these muscle fibers origin directly from the striated levator ani muscle. On the other hand, sparce smooth muscle fibers have been recognisable, located at the level of the midline, between the posterior vaginal wall and the rectovaginal septum. These fibres could ascribed to the component of smooth muscle fibers recognisable at the level of the rectovaginal septum, that is located in an oblique coronal plane, close to the posterior vaginal wall, and is formed of a network of collagen, elastic fibres, smooth muscle cells with nerve fibres, emerging from the autonomic inferior hypogastric plexus, and variable numbers of small vessels.[30-31] We must also be considered that the age group in all the studied cadavers were 54-72 years old. Thus, the histological structure, the characteristics and topography of the PVM in younger women, especially nulliparous, may be different.

From the functional point of view the PVM plays a role in the static and dynamic of the pelvic floor. In rectocele, failure of support of the rectum and perineum by the puborectalis and pubovaginalis muscles contributes to the prolapse by allowing descent of the posterior perineum during straining.[1] With particular reference to the role on the vagina, the contraction of PVM approaches the posterior vaginal wall to the anterior one[27] and elevates the vagina in the region of the mid-urethra.[15] Shafik[4, 32] attributed to the contraction of

the levator ani the modification of the shape of the vagina, transformed from a cone into a flat shape. It becomes elevated and laterally retracted, and pulling on the hiatal ligament which is attached to the vagina at the lateral fornices. These are pulled up and opened, resulting in elongation, narrowing and partial straightening of the vaginal tube, as well as elongation of the uterus.[33] Our study shows that the fibres of the PVM are recognisable on the passage between the inferior and middle thirds of the vagina, mingling with the longitudinal fibres of the muscular layer of the vagina. It could be hypothesized that the fibres of the PVM represent an intermediate course of bridging muscle bundles going reciprocally from the striated pubococcygeous muscle to the smooth fibres of the longitudinal layer of the vagina and viceversa. Thus, the PVM could represent anatomical evidence of a functional connection between the vagina and the muscular system of the pelvic floor.

## ACKNOWLEDGEMENTS

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