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The macro-anatomy of the vaginal outlet wall differs from the vaginal wall: A prospective, cadaveric case series study

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August 25, 2022

Abstract

Objectives To describe and document the anatomical and histological architecture of the vaginal outlet wall. Design A prospective observational case series study. Setting International centers. Population Twelve fresh human female cadavers. Methods Female corpses were subjected to stratum-by-stratum, macro-, and micro-anatomical dissections in sagittal, transverse, and coronal planes. Multiple excisional biopsies were obtained from the vaginal outlet wall at 12, 3, 6, and 9 o'clock locations for histological examination. Digital photograms documented the anatomical and histological features. Primary Outcome measures gross, topographic anatomy and characteristic histologic features of the vaginal outlet wall. Results The vaginal outlet wall (VOW) was positioned in the vertical orientation and fused with horizontally located the vaginal wall at the hymeneal plate level. The hymeneal membrane and ring were not attached directly to the vaginal wall. VOW has three identifiable anatomical-histological layers a) the hymeneal membrane, b) the hymeneal ring (cartilage) with different anatomy and histology than the vaginal wall, and c) the hymeneal plate (the deep level of VOW), which connects to the vaginal wall. Conclusions The vaginal outlet wall is an anatomical structure consisting of the hymeneal membrane, hymeneal ring (cartilage), and hymeneal plate. This structure is in vertical topographic orientation. Anatomically and histologically, it differs from the vaginal wall, which fuses with the deep layer of the hymeneal plate.

The macro-anatomy of the vaginal outlet wall differs from the vaginal wall: A prospective, cadaveric case series study

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Running Head: The vaginal outlet wall anatomy

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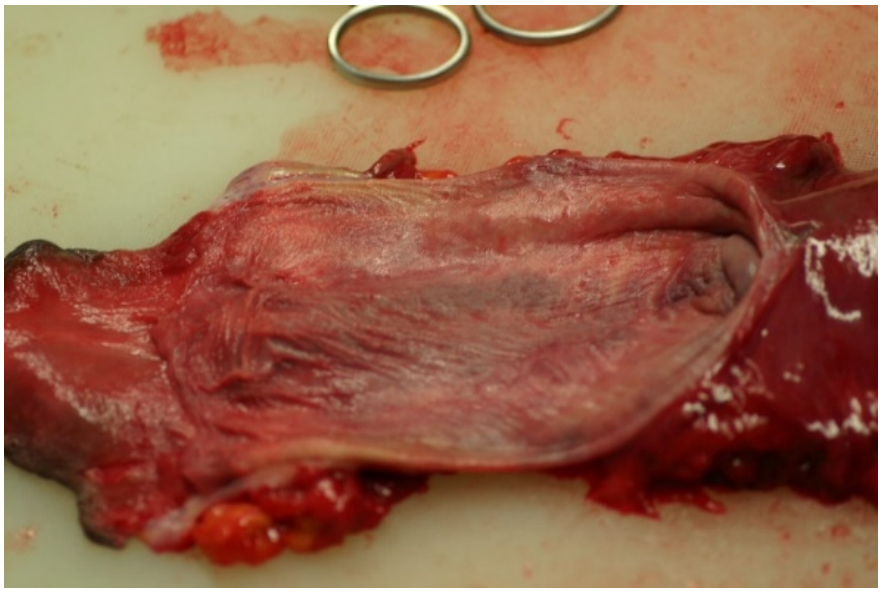
Graphical Abstract



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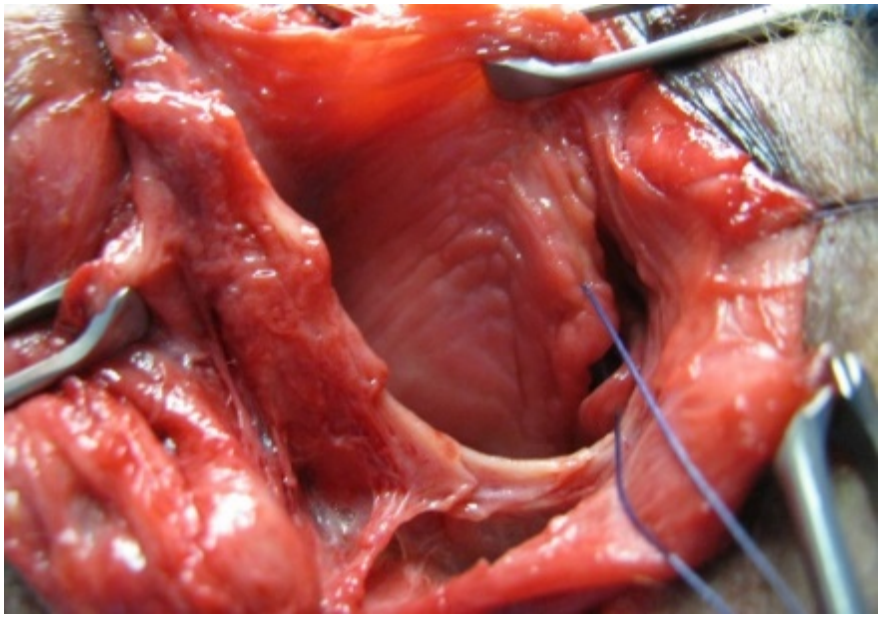

The macro-anatomy of the vaginal outlet wall differs from the vaginal wall: A prospective, cadaveric case series study

Tweetable abstract

The discovery of the vaginal outlet wall anatomy allows a better understanding

the vagina as a whole structure.

Introduction

Dickinson pioneered wax cylinders for vaginal wall impression, and later, Morgan introduced the material that dentists use.[1, 2] These three-dimensional vaginal impression molds demonstrate gross-topographical anatomy and its potential aberrations. Pendergrass verified these techniques and findings.[3] When analyzing these vaginal molds, one can conclude there is a separate anatomical structure in vertical orientation that

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fused with the vaginal wall being in the horizontal orientation.[1, 2] The vaginal casting method findings were confirmed by magnetic resonance imaging (MRI) with gel contrast filling the vaginal canal.[4] MRI technology provides more detailed information about the shape and strata of the vaginal walls with three identifiable zones a) the "distal vaginal sphincteric zone" (the vaginal outlet wall); b) the "transition zone"; c) the "forniceal zone." [4] Also, the dynamic ultrasonographic investigation showed the sphincteric zone within the distal vaginal wall.[5] Furthermore, Hart documented that the hymen wall was always present in the female newborn at term without demonstrating the hymeneal anatomical wall layers.[6]

The current variable terminology used for the vaginal entrance wall did not reflect the vaginal outlet wall. Often terms are used such as vaginal introitus (introitus vaginae), the vaginal vestibule (vestibulum vaginalis), the vaginal opening, and the vaginal orifice.[2-5] These terminologies do not reflect a specific anatomical architecture of the vaginal outlet. The analysis of the current nomenclatures reveals that a) the "vaginal introitus" is a vaginal atrium located in front of the hymen; b) "the vaginal vestibule" is located between the posterior surface of the hymen and the anterior border of the posterior perineum; anteriorly, it runs from the posterior surface hymen to the inferior edge of the clitoral frenulum; laterally, it extends from the inferior hymen to base of the labia minora, and there is a small crease between them[6, 7]; c) The "vaginal orifice "or vaginal opening" is a space and isn't an anatomical structure. These terminologies do not represent the entry to the vaginal canal. The electronic and manual medical literature searches failed to identify any scientific-clinical article describing the vaginal outlet wall. The new terminology suggested by the author adequately encompasses this part of the vaginal ingress or egress, and the author suggests the term "the vaginal outlet wall." Therefore, the present study is the first anatomical and H/E histological description of the vaginal outlet wall.

There is no description of the vaginal outlet wall in anatomy educational textbooks or atlases of human anatomy.[8-13]To the best of this author's knowledge, there is no anatomical description of the vaginal outlet in the scientific-clinical articles.

The present study question is, *"does the vaginal outlet wall have different anatomical and histological architectures than the vaginal wall?"* The study's objectives are to describe the vaginal outlet wall gross, topographic, and microscopic anatomy and document findings by digital photograms. The primary outcome measures gross, topographic anatomy and characteristic histologic features of the vavinal outlet wall.

Methods

Twelve fresh human adult female corpses were examined in this experimental anatomical investigation of the vaginal outlet wall. The author executed all dissections at the Department of Forensic Medicine. Due to the Department of Forensic Medicine services, no medical records of the deceased women were available. The University Bioethical Committee approved this experimental research (WUM-AKBE 146/12).

Inclusion/exclusion criteria

Women who expired within twenty-four hours and did not undergo the body preservation process were incorporated into the current research. For legal reasons, subjects who were victims of incest or rape were excluded from the investigation. Also, those corps presented with anatomical deformity due to surgery or trauma to the vagina or external genital anatomical disfigurement of the urogenital tract, the vulvar skin, or transitional vulvar skin lesions, identifiable tumor, enlarged lymphatic nodes, and those who grossly demonstrated contiguous disease were excluded.

Anatomical dissection

The corpses were placed on the examining table in the supine position with the lower extremities separated laterally and bent in the knees-hip joints and the upper limbs along the body side. To stabilize the body in this position, a medical bandage was placed between the ankle and wrist to hold them together, and the knees were kept laterally by fixing them to IV poles. Dissection planes were in various directions a) vertical plane from front to back that splits the vaginal outlet wall into visible layers; b) coronal-vertical

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planes, which allow visualizing the front and back of the segments of the vaginal outlet wall and its variations among the subjects The author executed all the anatomical macro- and micro-dissection.

All macro- and micro-dissections were performed with a 3.5-4.0x magnification loupe. The labium minus was despised laterally from the midline, and traction 2-0 size sutures were used to secure the labia to the vulvar skin—this maneuver allowed access to the vaginal outlet. The vaginal outlet was marked with a dermo-marker at 12, 6, 3, and 9 o'clock for the future orientation of taking biopsies for histological examinations.

In the sulcus between the labia minora minora, the fossa navicularis, and under the inferior membraneous urethral sphincter, the incision was made to expose the outer surface of the vaginal outlet wall. To visualize the inner surface of the vaginal outlet wall, the incision was made at the junction of the vaginal wall and the vaginal outlet inner surface. The Bartholin's duct orifice and membranous urethral sphincter were outside the incisions.

The subsequent step was to make the vertical incision on the posterior-midline hymeneal membrane with the number 15-blade of the surgical scalpel to dissect the soft tissue from the hymeneal ring to demonstrate a well-defined white color structure, resembling a cartilage appearance that fuses the inferiorly with the hymeneal plate and superiorly with the hymeneal membrane. The dissection continued until the entire hymeneal plate was visualized. Consequently, the three anatomical layers of the vaginal outlet wall are identified. The vertical position vaginal outlet wall fuses with the vaginal wall that is in a horizontal position.

Biopsy

Multiple cadaveric vaginal outlet wall biopsies were obtained in the V-shape from the superior to the inferior edge of the vaginal outlet wall. The excisional V-shape biopsies were carried out from the sulcus between the posterior-distal vaginal wall and the vaginal outlet wall and continued to the sulcus between the vaginal outlet wall and the labia minora. Neither the vaginal wall nor the labia minora was included in the excision specimen—the excisional biopsies between 7 and 8 o'clock were used for the histological examinations in the present study.

Literature search

The medical literature was searched electronically and manually for the gross and topographic anatomy of the vaginal introitus, vaginal vestibule, vaginal orifice, and vaginal outlet. In addition, anatomy articles, conference proceedings, and specializing websites were also included. The Medical Subject Headings (MeSH) were applied. The following keywords or phrases were used: the human vagina, vaginal outlet gross human vaginal outlet topographic anatomy, human vaginal introitus gross anatomy, human vaginal topographic anatomy, human vaginal orifice gross anatomy, human vaginal orifice topographic anatomy, perineal body location, vaginal posterior-distal laceration, vaginal outlet (vaginal introitus, and vaginal orifice) histology, posterior vaginal colporrhaphy.

Results *Anatomy*

The vaginal outlet wall was in the anatomical region of the external genitalia between the membranous urethral sphincter anteriorly, the fossa navicularis posteriorly, and laterally. It was connected to the labia minora. The deep segment (the hymeneal plate) of the vaginal outlet wall fused directly with the vaginal wall and was topographically positioned in the vertical orientation in the direction of a) superiorly towards the vaginal canal; b) inferiorly to the fossa navicularis; c) anteriorly towards the posterior perineal urethra; d) laterally to the surface of the inner labia, Fig. 1B.

Grossly, the vaginal outlet wall was the distal ending of the vaginal wall and showed a different anatomical structure than the vaginal wall. Furthermore, it was the narrowest part of the vagina. The vaginal outlet wall was naturally slightly projecting outward, and it appeared as a "belt-like" structure surrounding all vaginal walls (the anterior, posterior, and lateral), Fig. 1B. The crease separated the vaginal outlet wall a) anteriorly by the inferior membranous urethral sphincter; b) posteriorly by the fossa navicularis; c) laterally by the labia minora. The vaginal outlet wall was a monolithic structure composed of three layers, Fig. 1,

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Fig. 2. The vaginal outlet ring layer demonstrated much less elasticity to palpations than the vaginal walls and the hymeneal plate. After dissecting the soft tissue of the hymen, the hymeneal ring looked like smooth, white tissue and macroscopically resembled cartilage. Superiorly, this elastic cartilage was fused with the soft tissues of the hymeneal membrane and inferiorly with the hymal plate. Such a subdivision of the vaginal outlet wall into the layers was somewhat arbitrary but valuable for comprehension. The hymeneal ring rested on the hymeneal plate, and both structures created a protuberance. The hymeneal plate was a mixture of soft to palpation, elastic, muscular, connective tissues, and reddish, and had more stretchable tissues than the hymeneal ring, Fig. 1B.

Histology

Microscopic characteristic features showed three layers of the vaginal outlet: superficial, intermediate, and deep, Fig. 2. The superficial layer (the hymeneal membrane) of the vaginal outlet structure was the mucosal type of tissue, Fig. 2. The epithelium that covers the hymen membrane is a nonkeratinized striated squamous cell arranged in roses of epithelial cells, on the average of three roses. The elastic lamina propria consisted of a dense connective fibers layer projecting papillae into the overlying epithelium with the capillary blood vessel network and nerve endings, Fig. 2. Beneath the lamina propria was a strip of the columnar epithelial cells. In addition, the parabasal, basal cells, and basal lamina were identifiable. The intermediate-hymeneal ring was composed of dense connective and muscle fibers, Fig. 2. The deep layer of the vaginal outlet wall (the hymeneal plate) was composed of longitudinal smooth muscles and connective tissues, Fig. 2. This stratum was connected to the outer longitudinal vaginal wall muscles.

Discussion

Main findings

Grossly, the vaginal outlet wall resembles a "belt-like" structure in the vertical orientation when compared to the horizontal position of the vaginal wall, Fig. 3. The vaginal walls' distal edges fuse with the vaginal outlet wall a) anteriorly — the inferior border of the membraneous urethral sphincter; b) posteriorly — the fossa navicularis; c) laterally — the perineal body extension and the labia minora. The H/E histology examination confirmed these anatomical findings. The vaginal hymeneal crease is visible and distinguishes those two parts. The crease between the labia minora and the vaginal outlet wall separates them and the fossa navicularis.

Microscopic characteristic features showed three strata of the vaginal outlet wall: superficial (the hymeneal membrane), intermediate (the hymeneal ring), and deep (the hymeneal plate), Fig. 2. These findings verify the anatomical discovery of the vaginal outlet wall in the present study, Fig. 1B, and Fig. 2. The *superficial layer* of the vaginal outlet wall structure is the thin mucosal lining—the hymeneal membrane, which consists of striated non-keratinizing squamous epithelial cells arranged, on the average, in three roses. The basal stratum is a single layer of columnar cells with scant cytoplasm surrounding the oval and hyperchromatic nuclei. Additionally, there are two roses of cuboid cells with centrally located, round, and hyperchromatic nuclei. Directly beneath the squamous epithelium were well-organized lamina propria with projecting papillae into the overlying squamous epithelium and with connective fibers, the capillary blood vessels network, and nerve endings. In addition, the parabasal, basal cells, and basal lamina are present, Fig. 2.

The Intermediate layer — the hymeneal ring — of the vaginal outlet wall is composed of dense connective avascular and aneural tissue fibers with laguna cell aggregates and muscle fibers, Fig. 2. The hymeneal ring presents itself grossly as anatomical cartilage, which provides biomechanical tensile strength, tensile resistance strength, and elasticity. This cartilage tissue within the hymeneal ring plays a significant role in clinical obstetrics (tissues resistant during the second stage of vaginal delivery) and gynecology (superficial dyspareunia or entry dyspareunia, vaginal outlet stenosis, vulvar vestibulitis syndrome.[18-20]

Finally, *the hymeneal plate's deep layer* is the stratum of the vaginal outlet wall composed of dense elastic, connective, and smooth muscle fibers, Fig. 2. Longitudinal smooth muscles of the hymeneal plate fuse with the vaginal wall muscular layer.

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The MRI study showed the presence of the vaginal outlet wall and termed it "the vaginal sphincteric zone." [4] The authors of this MRI clinical investigation published their findings before the current anatomical study. MRI description of the vaginal sphincteric zone documented the existence of the vaginal outlet wall.

Strengths and limitations

The discovery and photographic documentation of the vaginal outlet wall are the significant strength of this study. The vavinal outlet wall provides anatomical stabilization to the vaginal opening and is one of the structures that prevent the vaginal orifice from gaping. Reviewing the medical literature failed to identify any scientific-clinical article about the vaginal outlet wall; therefore, the present investigation is a groundbreaking discovery that provides scientific data about this structure. Additionally, this discovery offers new knowledge about the vaginal outlet wall within the female external genital anatomy that has the potential for practical clinical implementation.

An anatomical study has inherent limitations because a single researcher's interpretation of the anatomical findings can be considered a limitation, which sets up an expectancy and confirmation bias. The doubt that the solo investigation purposively dissected toward the expected outcome could not be dispelled. Moreover, the study's weakness on fresh cadavers is the occurrence of gross and topographic postmortem distortions due to vaporization and tissue shrinkage; therefore, any cadaveric investigation will not represent the actual size and, to the same degree, altered shape. Also, the absence of accepted terms influences a descriptive meaning. Additionally, the research on the corpses can not establish the functional anatomy of the vaginal outlet wall; however, it can be used as a practical understanding of these anatomical structures.

Interpretation Anatomy

The present study findings show that the vaginal outlet wall and its tensile resistance *are not* directly connected to the posterior perineum; therefore, clinicians need to know that cutting the posterior perineum in traditional episiotomies is questionable. The current investigation shows that the hymeneal ring (cartilage) is the tissue responsible for the resistance during fetal vaginal delivery. Furthermore, there are several reports in the medical literature about gynecological entities that are specifically related to the vaginal outlet, such as "vulvar outlet stenosis" (vaginal stenosis), which is recognized as causing superficial dyspareunia.[6-8]Those authors postulated that the vulvar outlet participates in the pelvic organ prolapse; therefore, it should be evaluated when the posterior-distal vaginal wall relaxation is present. However, they did not present the gross, topographic, or surgical anatomy.[6-8]

Histology

Histology of the excised vaginal outlet biopsy showed no perineal skeletal muscle present in the excised specimens and verified three distinctive layers: the hymeneal membrane, ring, and plate, Fig. 1C. The absence of the perineal muscle within the excised V-shape tissue from the vaginal outlet wall helps understand the mechanism of perineal postpartum pain and later, occurrences of superficial dyspareunia. Therefore, the present study shows that the posterior perineal musculature is unnecessary to cut to widen the vaginal outlet during fetal delivery.

The current anatomical research demonstrated that the vaginal outlet wall was not the extension of the vaginal walls but a separate anatomical structure with characteristic histological features connected to the vaginal wall, Fig. 1, B, 2. Furthermore, the vaginal outlet gets thinner, progressing from the thick posterior to the thin anterior vaginal wall's edges. These anatomical findings help determine where to place an incision when an obstetrical episiotomy procedure is performed or how to reconstruct the defective vaginal outlet wall. The vaginal outlet wall is the narrowest part of the vaginal canal. This finding may play a significant role during the fetal head vaginal delivery because it creates tissue resistance by the hymeneal ring cartilage on passing through a fetal head, Fig. 3B.

Comparison

The vaginal wall anatomical and histological descriptions are well-studied, and the findings are

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noncontroversial.[8-17] Therefore, this type of study on the vaginal wall was not performed in the current experimental investigation. However, the medical literature does not describe the vaginal outlet wall anatomy and histology. The vaginal introitus, vaginal vestibule, and vaginal orifice are often used for the vaginal outlet. The present cadaveric gross-topographic anatomy investigation shows that the vaginal outlet wall is a different structure than the vaginal wall, vaginal introitus, vaginal vestibule, or vaginal orifice because the hymeneal ring layer (intermedial stratum) of the vaginal outlet wall consists of a white cartilage structure Fig. 1B. The gross vaginal anatomy consists of four walls that create a canal (the anterior, posterior, and two lateral walls). The vaginal outlet comprises only one circular wall surrounding the vaginal wall's distal edges. The anterior vaginal wall fuses with the posterior urethral wall and the base of the urinary bladder, the vaginal outlet anterior-superior part fuses with the inferior border of the membraneous urethral sphincter, and the free margin of the vaginal outlet wall hangs over the vaginal lumen. The topographical position of the vaginal wall is in a horizontal orientation, and the vaginal outlet is in a vertical position. The vaginal sagittal dissection reveals three vaginal layers: the mucosa, muscular, and adventitia (surgical fascia). The sagittal dissections of the vaginal outlet present different layers: the membraneous soft tissue — the thin mucosa (hymeneal membrane), flexible but rigid tissue — hymeneal ring (hymeneal cartilage), soft, muscular, and elastic tissue — hymeneal plate.

The educational textbooks of histology and atlas also provide an uncontroversial description of the vaginal wall mucosal epithelium, histologically described as a stratified squamous epithelium with a small degree of keratinization. The vaginal outlet wall membrane layer consists of a stratified non-keratinized squamous epithelium. In the vaginal wall below the epithelium is a thick and loosely organized layer of dense connective tissue containing blood vessels and nerves ending. The vaginal mucosa is thicker (up to 10-roses of the epithelium squamous cells) than the vaginal outlet wall (3-rose). There is no corresponding vaginal histology to the cartilage of the hymeneal ring. *Documentation*

The digital photographic documentation of the anatomy and histology of the vaginal outlet wall proves that this structure is fused with the vaginal walls; however, it is not an identical anatomical extension of the vaginal walls. Moreover, the present study's findings can assist obstetricians, general practitioners, midwives, and surgeons in better understand this vital anatomical structure. Finally, this study confirmed the accuracy of the existing clinical classification of vaginal introitus.[9] *Future Research*

The present study laid the foundation for basic and clinical research. Determining the cartilage, type of collagens, and elastin distribution within the vaginal outlet wall was beyond the scope of the present investigation; therefore, it creates an additional essential medical study option.

The current study findings showed that the vaginal outlet wall could play a significant role in developing a new episiotomy procedure (vaginal outlectomy) and, by doing so, eliminating the incisions of the posterior perineal musculature postpartum posterior perineal pain. Clinical-scientific research will answer that traditional episiotomy techniques should be changed to a new episiotomy (vaginal outlectomy). Future studies on a new episiotomy can revolutionize our current obstetrical practice.

The present study suggests that superficial dyspareunia can be caused by vaginal outlet wall stenosis or other aberrations. Additionally, vulvar vestibulitis syndrome (vulvodynia) can be associated with the abnormality of the vaginal outlet wall because the vaginal entry severe pain is dominant in vulvodynia. This characteristic-induced pain suggests that the vaginal outlet can play a significant role in vulvodynia and superficial dyspareunia, and vaginal wall prolapse, Fig. 3A and Fig. 3C. **Conclusion**

The vaginal outlet wall is an anatomical structure that consists of the hymeneal membrane, hymeneal ring (cartilage), and hymeneal plate. This structure is in vertical topographic orientation. Anatomically and histologically, it differs from the vaginal wall, which fuses with the deep layer-hymeneal plate.

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protocol approval from the local Bioethics Committee. The author also wishes to express gratitude to Zbigniew Antosz, M. D. from Diagnostics Consilatione, Poland, who assisted in histologic staining. Finally, the author wishes to thank those families who donated bodies to science.

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Contribution to authorship: it is solo scientific research, and the author developed the concept and protocol of this investigation and also carried out the analysis and writing up of the manuscript.

Details of patient's consent: The family of the deceased subjects granted permission for the research and publication of the findings.

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Details of ethics approval: The University Bioethical Committee approved this experimental research (WUM-AKBE 146/12).**References**

1. Dickinson RL. Atlas of human sex anatomy. Baltimore, Md, USA, Williams and Wilkins, Publishing Company, 1949.
2. Morgan KF Jr. Casts of the vagina as a means of evaluating structural changes and treatment. Calif Med 1961(Jan);94(1):30-2.
3. Pendergrass PB, Reeves CA, Belovicz MW, Molter DJ, White JH. The shape and dimensions of the human vagina as seen in three-dimensional vinyl polysiloxane casts. Gynecol Obstet Invest 1996;42(3):178-82.
4. Appelbaum AH, Zuber JK, Levi-D'Ancona R, Cohen HL. Vaginal Anatomy on MRI: New Information Obtained Using Distention. South Med J 2018 Nov;111(11):691-697.
5. Jung SA, Pretorius DH, Padda BS, Weinstein MM, Nager CW, den Boer DJ, Mittal RK. Vaginal high-pressure zone assessed by dynamic 3-dimensional ultrasound images of the pelvic floor. Am J Obstet Gynecol. 2007 Jul;197(1):52.e1-7.
6. Hart DB. Preliminary note on the development of the clitoris, vagina, and hymen. Trans Edinb Soc, 1896;21:106-116.
7. Haylen BT, Vu D, Wong A. Surgical anatomy of the vaginal introitus. Neurourol Urodyn 2022; Pg. 1-8.
8. Keith L. Moore, Arther F. Dalley, Anne M.R. Agur. Clinically Oriented Anatomy. Eighth edition. Baltimore, Md: Lippincott Williams & Wilkins; 2018.
9. Anne Gilroy, Brian R. MacPherson, Lawrence M. Ross. Anatomical atlas: Thieme Atlas of Anatomy. Third Edition. New York, Thieme Medical Publishers; 2016.
10. Netter FH. The Ciba Collection of Medical Illustrations. Reproductive System.Vol. 2. Ciba-Geigy Corporation, West Caldwell NJ, USA; 1984.
11. Tank PW. Grant's Dissector. Sixteenth edition. Baltimore Md, USA, Lippincott Williams & Wilkins; 2008.
12. Clemente CD. Gray's Anatomy. 30th edition. Lea & Febiger, Philadelphia, PA, USA. Pg. 1579; 1985.
13. Dunglison RJ. Gray's Anatomy. Anatomy of the Human Body. First American Edition. Blanchard and Lea, Philadelphia, PA, USA; 1859.
14. Robby SJ, Cunha GR, Kurita T, Strickland KC. Vagina. In: Mills SE (ed). Histology for pathologists. 5th Ed. Wolters Kluwer, Philadelphia, PA, USA; 2019, Pg. 1047-1106.
15. Fawcett DW, Ronald P. Jensh RP. Bloom and Fawcett: Concise histology. 2nd Ed. Hodder Education Publishers, Bunbury, England; 2002.
16. Kurman RJ. Blaustein's Pathology of the Female genital tract. 5th Ed. Springer-Verlag, New York, USA 2002, Pg.133-136.
17. Robboy SJ, Prade M, Cunha G. Vagina. In Sternberg SS(ed) Histology for pathologists, Chapter 45, Pg. 881-892. Raven Press, New York, USA; 1992.
18. Broso P, Buffetti G, Sacco A. Vulvar vestibulitis. Minerva Ginecol 1994 Mar;46(3):109-114.

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19. Paniel BJ, Haddad B, el Medjadji M (1995) Orifice dyspareunia: the surgeon's point of view. Contracept Fertil Sex;23(4):271-274.
20. Haylen BT, Fischer G, Vu D, Tse K. The vaginal vestibule: assessing the case for an anterior and posterior division. Neurourol. Urodynamics., 2017, 36: 979-983.

Figure Legends

Figure 1. The gross and topographic anatomy of the vaginal outlet**A.** The specimen of the 10-year-old subject depicts the vaginal outlet wall (the yellow arrow). The horizontal location of the vaginal wall and the vertical position of the vaginal outlet are noticeable.

B. Three anatomical architecture and orientation of the vaginal outlet in the vertical position are presented: a) the hymeneal membrane is the superficial soft tissue layer-the black arrow; b) the green arrow shows the hymeneal ring-cartilage; c) the yellow arrow indicates the hymeneal plate and the visible groove between the hymeneal ring and the hymeneal plate structures.

C. The V-shape excised fragment of the vaginal outlet anatomical structures. The black arrow indicates the soft tissue superficial layer (the hymeneal membrane) of the vaginal outlet; the green arrow represents the middle layer - the hymeneal ring (cartilage); the yellow arrow depicts the deep layer of the hymeneal plate.

Figure 2. Microscopic characteristic features of the vaginal outlet wall

The microscopic architecture of the vaginal outlet wall consists of three layers – superficial, intermediate, and deep. The superficial layer (the hymeneal membrane) is the mucosal type of tissue. The epithelium that covers the hymen membrane is a nonkeratinized striated squamous cell arranged in roses of epithelial cells, on an average of three roses. The elastic lamina propria consisted of a dense connective fibers layer projecting papillae into the overlying epithelium with the capillary blood vessel network and nerve endings. Beneath the lamina propria was a strip of columnar epithelial cells. In addition, the parabasal, basal cells, and basal lamina were identifiable. The intermediate-hymeneal ring was composed of dense connective fibers with a network of arterial blood vessels, veins, nerve endings, and muscle fibers. The deep layer of the vaginal outlet wall (the hymeneal plate) was composed of predominantly longitudinal smooth muscles and connective tissues. This stratum was connected to the longitudinal vaginal wall muscles.

Figure 3. The clinical function of the vaginal outlet wall selected examples**A.** Resistance force of the hymeneal outlet wall structure on the anterior vaginal wall prolapse (prolapse cystocele),**B.** The vaginal outlet wall resists the fetal head passing during vaginal delivery,**C.** The defective vaginal outlet wall causes the posterior-distal vaginal wall to descend.

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