

Architecture of structures in the urogenital triangle of young adult males; comparison with females

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Abstract

The fibro-muscular architecture of the urogenital triangle remains contentious. Reasons are small size of the constituting structures and poor visibility with most imaging methods. We reinvestigated the area in serial sections of three males (21–38 years old) of the American and Chinese Visible Human Projects and two 26-week-old male fetuses, and compared the findings with earlier observations in females. The mass of the levator ani muscle was approximately twofold smaller and its funnel shape steeper in males than females. In the levator hiatus, a strand of the smooth longitudinal muscle layer of the rectum, the ‘rectourethral (RU) muscle’, extended anteriorly from the anorectal bend to the penile bulb. Fibrous tissue that formed in the inferior reach of the fetal RU muscle identified the location of the developing perineal body (PB) and divided the muscle into posterior ‘rectoperineal’ and anterior ‘deep perineal’ portions. In males, the PB remained small and bipartite, so that the RU muscle presented as an undivided midline structure. The well-developed female PB, instead, intertwined with the deep perineal muscle and both structures passed the vagina bilaterally to form the perineal membrane in the posterior portion of the urogenital triangle. The urethral rhabdosphincter extended in the anterior portion of the urogenital triangle between the penile bulb inferiorly and the bladder neck superiorly, and consisted of a well-developed circular ‘membranous’ portion with bilateral posteroinferior ‘wings’ and a thinner ‘prostatic’ portion on the prostate anterior side. In men, muscles occupy the urogenital triangle, but additional tightening of the locally fibrous adipose tissue by the superficial transverse perineal muscle appears necessary to generate functional support in women. An interactive 3D pdf file with these anatomical details (available online) should allow more accurate interpretation of ultrasound, computed tomography and magnetic resonance images.

Key words: deep perineal muscle; levator ani muscle; perineal body; rectoperineal muscle; rectourethral muscle; sexual dimorphism; urethral sphincter.

Introduction

The pelvic floor is one of the regions in the body that continue to generate disputes with respect to its topographic

anatomy. An example is the issue whether the urogenital triangle represents a (fibro-)muscular diaphragm. A common criticism of this concept is that its superior fascia does not exist because the striated urethral sphincter extends superiorly up to the bladder neck in both men and women (Oelrich, 1980, 1983; Myers et al. 1987; Tichy, 1989; Strasser et al. 1996; Dorschner et al. 1999; Sebe et al. 2005a,c; Wu et al. 2017). In addition, the existence of the deep transverse perineal muscle as the putative posterior part of the muscular urogenital diaphragm was questioned (Dorschner et al. 1999; Mirilas & Skandalakis, 2004). The existence of an inferior fascia of the urogenital diaphragm is, on the other hand, generally acknowledged and is now known as the ‘perineal membrane’ (Oelrich, 1980, 1983). The perineal membrane reportedly consists of a posterior portion with

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bilateral fibrous bands between the perineal body (and vaginal wall) and the ischiopubic rami, and an anterior portion that encompasses the compressor and urethrovaginal portions of the urethral sphincter (Stein & DeLancey, 2008; Brandon et al. 2009). Although many studies used histology to assess the architecture of the tissues, it is often difficult to appraise the underlying topography, as the areas studied histologically were limited and their position hard to locate.

We have reinvestigated the topographic anatomy of the urogenital triangle and its adjacent regions with the sectional approach that is the basis of the Visible Human Projects (Spitzer et al. 1996; Zhang et al. 2003; Dai et al. 2012). This approach reveals structures by serially removing a thin layer ('section' of 0.1–1.0 mm) from the surface of a frozen body before recording a new image and is in our view the technique of choice in areas with poor accessibility and small size of some of the relevant structures. The limitation of the approach is that the structures need to be delineated (segmented) based on their natural color and surrounding connective tissue. Furthermore, they have to be reconstructed to restore their 3D appearance. Using this approach, we previously reported the precise topographic anatomy of pelvic structures in the anterior and middle compartments of four young adult and one postmenopausal women (Wu et al. 2017). In that study we identified the 'deep perineal muscle' as a smooth muscle that intertwined with the fibrous anterior extensions of the perineal body, and that passed the vagina laterally towards the inferior fascia of the urethral sphincter and puborectal muscle. In the present study we describe the topography of pelvic structures in the anterior compartment of three young adult males and compare these findings with our earlier findings in similarly aged females (Wu et al. 2017). In addition, we studied serial histological sections of two 26-week-old male fetuses. The findings are presented as 3D-PDFs that allow the reader to inspect a topographic model of the young male pelvic floor and to evaluate and interpret its spatial anatomy in an interactive fashion.

Materials and methods

The true pelvis of two male specimens of the Chinese Visible Human [CVH1 (35 years) and CVH3 (21years)] dataset (Zhang et al. 2003), and the single male specimen (38 years) of the VHP dataset (Spitzer et al. 1996) were studied. The medical history of the VHP donor is known in detail (Spitzer et al. 1996) but that of the Chinese specimens is not. However, we have observed no pathologically changed structures. Some biometric details of the bodies are shown in Table 1. For the present study, it should be kept in mind that the VHP subject practiced body building and had correspondingly hypertrophied (pelvic floor) muscles.

Structures of interest were segmented manually as described, using (thin) fibrous tissue fasciae and differences in tissue color and texture as criteria (Wu et al. 2015, 2017). Before deciding on the boundaries of a complex or delicate structure, we always inspected the corresponding sections in the other specimens. Furthermore, we always identified and delineated the best visible (parts of a)

structure first and then proceeded to less clear-cut structures and portions. Using this iterative approach, we have identified and delineated 45 structures in the male pelvis (Table 2). These structures in the CVH1 specimen were used to assemble a detailed 3D reconstruction using the AMIRA software package (<http://www.amiravis.com>) that can be visualized with the interactive 3D-PDF file provided online (Supporting Information Fig. S1). In our description, we use superior, inferior, anterior, and posterior for the description of topographical relationships.

To establish histological details of the urethral sphincter and surrounding pelvic floor, we also studied two male fetuses (both ~ 20 cm crown-rump-length, or ~ 26 weeks post-fertilization) from the historical collection of the Department of Anatomy & Embryology, Leiden University Medical Center, Leiden, The Netherlands. One specimen (S2289) was sectioned transversally and stained with Azan, the other (S2600) sagittally and stained with trichrome. Both fetal specimens contained only the anterior compartment of a hemi-pelvis. The S2289 specimen was visualized with Amira 3D-reconstruction and Cinema 4D-remodeling software as described (Hikspoors et al. 2017; Supporting Information Fig. S2)

Results

The levator ani muscle complex in males

The levator ani muscle (LAM) forms the superior layer of the structures in the urogenital triangle and embraces the pelvic organs laterally and posteriorly. In males, it was funnel-shaped with steep, near-vertical walls laterally and posteriorly (Fig. 1A–C). The volumes of the LAM of CVH1, CVH3, and VHP were 15, 26, and 35 cm³, respectively. Superiorly, the LAM attached to the pubic bone anteriorly, the internal obturator muscle laterally, the coccygeal muscle posteriorly, and the fascia surrounding the rectococcygeal muscle posteromedially. The tendinous arch of the LAM was a transversely oriented fibrous structure. Based on perimysia and orientation of the muscle fibers, the LAM had puborectal and pubovisceral divisions. These partitions were present posteriorly and inferiorly but formed a single thick belly where the LAM attached to the pubic bone. The LAM is, therefore, a bicipital muscle with a common anterior

Table 1 Biometric details of the CVH and VHP males.

	CVH1	CVH3	VHP male
Age (years)	35	21	38
Height (mm)	1700	1820	1860
Weight (kg)	65	66	90
Section thickness (mm)	0.5, 1.0	0.1	1.0
Pixel size (μm)	150 × 150	122 × 122	330 × 330
Image resolution	3072 × 2048	4064 × 2704	2048 × 1216
LAM (cm ³)	15	26	35
EAS (cm ³)	6.0	5.3	10.6
Perineal body (cm ³)	0.64	1.17	1.08
Rhabdosphincter (cm ³)	0.96	2.93	3.44

Table 2 List of structures identified and reconstructed in the male pelvis.

1.	Internal obturator muscle
2.	Coccygeal muscle
3.	Pubovisceral muscle (internal layer)
4.	Pubovisceral muscle (external layer)
5.	Puborectal muscle (deep part)
6.	Puborectal muscle (superficial part)
7.	Rectal lumen
8.	Smooth muscle of rectum
9.	Mesorectum
10.	Rectococcygeal muscle
11.	Rectoperineal muscle
12.	Internal anal sphincter
13.	Conjoint longitudinal muscle of rectum
14.	External anal sphincter
15.	Anal intermuscular septum
16.	Anococcygeal ligament
17.	Testicle
18.	Epididymis
19.	Deferent duct
20.	Prostate
21.	Seminal vesicle
22.	Denonvillier's fascia
23.	Perineal body
24.	Deep perineal muscle
25.	Superficial transverse perineal muscle
26.	Bladder wall
27.	Ureter
28.	Pubovesical ligament
29.	Bladder and urethral lumen
30.	Urethral sphincter proper (rhabdosphincter)
31.	Posterolateral wings of rhabdosphincter
32.	Smooth urethral sphincter (lissosphincter)
33.	Submucous layer of urethra
34.	Ischiocavernosus muscle
35.	Bulbospongiosus muscle
36.	Cowper's gland
37.	Spongiosus body of penis
38.	Cavernous body of penis
39.	Peritoneum
40.	Fat tissue
41.	Arteries
42.	Veins
43.	Pelvic bone
44.	Sacral and coccygeal bone
45.	Skin

head. We could not distinguish pubo- or ilio-coccygeal divisions in the LAM.

The puborectal muscle formed a muscle loop around the rectum at the level of the recto-anal junction. The inferior fibers of the puborectal muscle also attached to the posterolateral side of the perineal body. This 'deep' portion of the puborectal muscle is identical to the deep portion of the external anal sphincter (EAS) in Anatomical Terminology (FCAT, 1998), whereas a smaller, more superficial portion of the puborectal muscle that was only present lateral

and posterior to the anal canal, is identical to the superficial portion of the EAS (Fig. 1C, Table 3; Fritsch et al. 2002; Wu et al. 2015). Near their attachment to the perineal body, the superficial and deep portions of the puborectal muscle were hard to separate.

The pubovisceral muscle had internal (medial) and external (lateral) layers (Fig. 1A,B). The much larger external layer was mainly present anteriorly and superiorly: it extended from the pubic bone, the tendinous arch, and the ischial spine superiorly towards the fasciae of the rectococcygeal muscle posteriorly and the deep puborectal muscle inferiorly. Fiber orientation was mostly antero-posterior. The smaller internal layer was present posterolaterally as an internal 'patch' on the external layer and extended downwards to continue into the conjoint longitudinal muscle.

The architecture of the muscles of the pelvic floor in males resembled that in females, including the presence of a rectal diaphragm consisting of the rectoperineal, rectococcygeal, and bilaterally the internal layers of the pubovisceral muscle (Wu et al. 2015). The differences between our description and that present in Anatomical Terminology (FCAT, 1998) are summarized in Table 3.

The anal sphincter complex in males

Although fascial tissue between the different parts of the external anal sphincter (EAS) was not as well developed in males as in females (Wu et al. 2015), it was possible to distinguish its three components. Fascial tissue was least developed in the VHP specimen and best in CVH-1. The subcutaneous portion of the EAS proper (Table 3) surrounded the anal canal in its entirety, was markedly oblong, and differed from that in females in having well-developed anterior and posterior spurs that extended beyond the perineal body in the subcutaneous space and to the anococcygeal ligament, respectively. The anterior spur also attached to midline-crossing fibers of the superficial transverse perineal (STP) and the bulbospongiosus muscles. The volumes of the subcutaneous portion of the EAS of the CVH1, CVH3 and VHP males were 6.0, 5.3 and 10.6 cm³, respectively.

The distal end of the smooth muscles of the rectum

The internal anal sphincter is the thick inferior end of the circular smooth muscle layer of the rectum. Its superior boundary coincided with the anorectal junction and its inferior end with the 'white line of Hilton'. Its length in the CVH1, CVH3, and VHP males was 27, 35, and 44 mm, respectively, on all sides, and its thickness was ~ 3 mm. The topography of the inferior end of the longitudinal smooth muscle layer of the rectum was complex. Posteriorly, a smooth-muscle strand detached from the rectal wall in the concave portion of the anorectal junction and ascended to attach to the anococcygeal ligament near the coccygeal bone

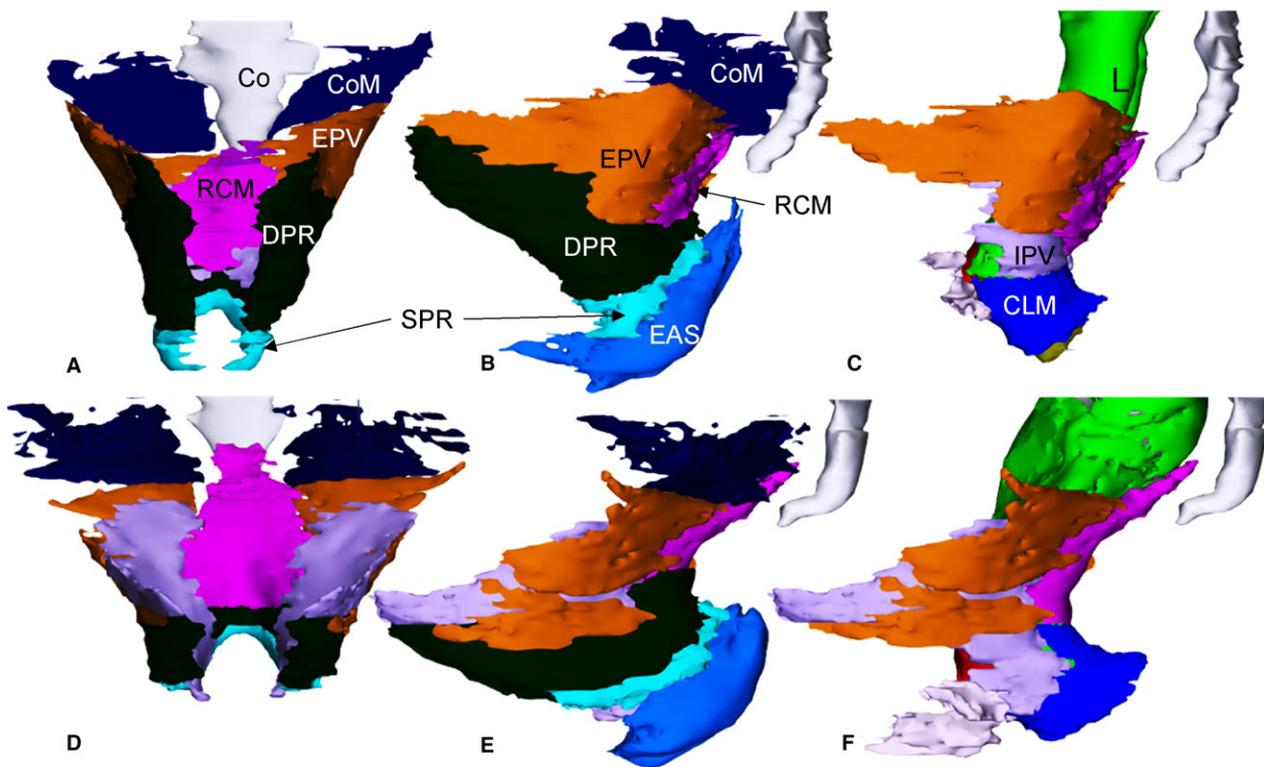


Fig. 1 Reconstructions of the male and female pelvic floor. (A,D) Anterior views and (B,C,E,F) left lateral views of the pelvic floor muscles of a male (A–C; CVH1) and a female (D–F; CVH5). (A,D) The deep (DPR) and superficial (SPR) puborectal muscles, the internal (IPV) and external (EPV) pubovisceral muscles, the rectococcygeal (RCM) and coccygeal (CoM) muscles, and the coccygeal bone (Co). Note that the male pelvic floor has a much steeper and narrower funnel shape than the female. (B,E) The same structures in a left-lateral view. The subcutaneous external anal sphincter proper (EAS) is added. Note the more elongated shape of the EAS in males than in females, with long anterior and posterior spurs. (C,F) Some muscles are removed, and the smooth muscle layer of the rectum (LSR), the conjoint longitudinal muscle (CLM), and the perineal body (PB) are added. Please note that the color code is the same in all panels.

(Fig. 1A–C). The puborectal slings passed the rectococcygeal muscle posteriorly. The longitudinal smooth-muscle layer and the medial layer of the pubovisceral muscle merged to descend as the smooth (Kim et al. 2015) conjoint longitudinal muscle between the internal and external anal sphincters (Fig. 1B). The fibers of the conjoint longitudinal muscle ended distally by radiating into the external anal sphincter proper.

In the convex, anterior portion of the anorectal junction, a strand of the longitudinal smooth muscle detached from the rectal wall and extended anteriorly towards the bulbourethral gland, spongious body, and lissosphincter, and

inferiorly towards the bulbospongious muscle and perineal body (Figs 2–4). The anterior portion of the muscle strand is known as the deep transverse perineal (Oelrich, 1980; Zhai et al. 2011) or rectourethral muscle (Brooks et al. 2002; Porzionato et al. 2005; Gil-Vernet et al. 2016), and the posterior portion of the muscle strand is known as the rectoperineal muscle (Sebe et al. 2005b) (for our preferred nomenclature, see Table 4 and the Discussion). Smooth-muscle strands intermingled with fibrous stands of the perineal body and were markedly hypertrophic in the VHP male (cf. Brooks et al. 2002). The length of the entire smooth muscle was 17.6, 18.2, and 18.0 mm in CVH1, CVH3,

Table 3 Terminology of muscles in present study compared with that in Anatomical Terminology (FCAT, 1998).

Present study	LAM (TA)	EAS (TA)
Pubovisceral	Pubococcygeal + iliococcygeal	–
Levator ani	(pubo- and iliococcygeal) + puborectal	–
Puborectal (deep portion)	Puborectal	Deep portion
Puborectal (superficial portion)	–	Superficial portion
External anal sphincter	–	Subcutaneous portion

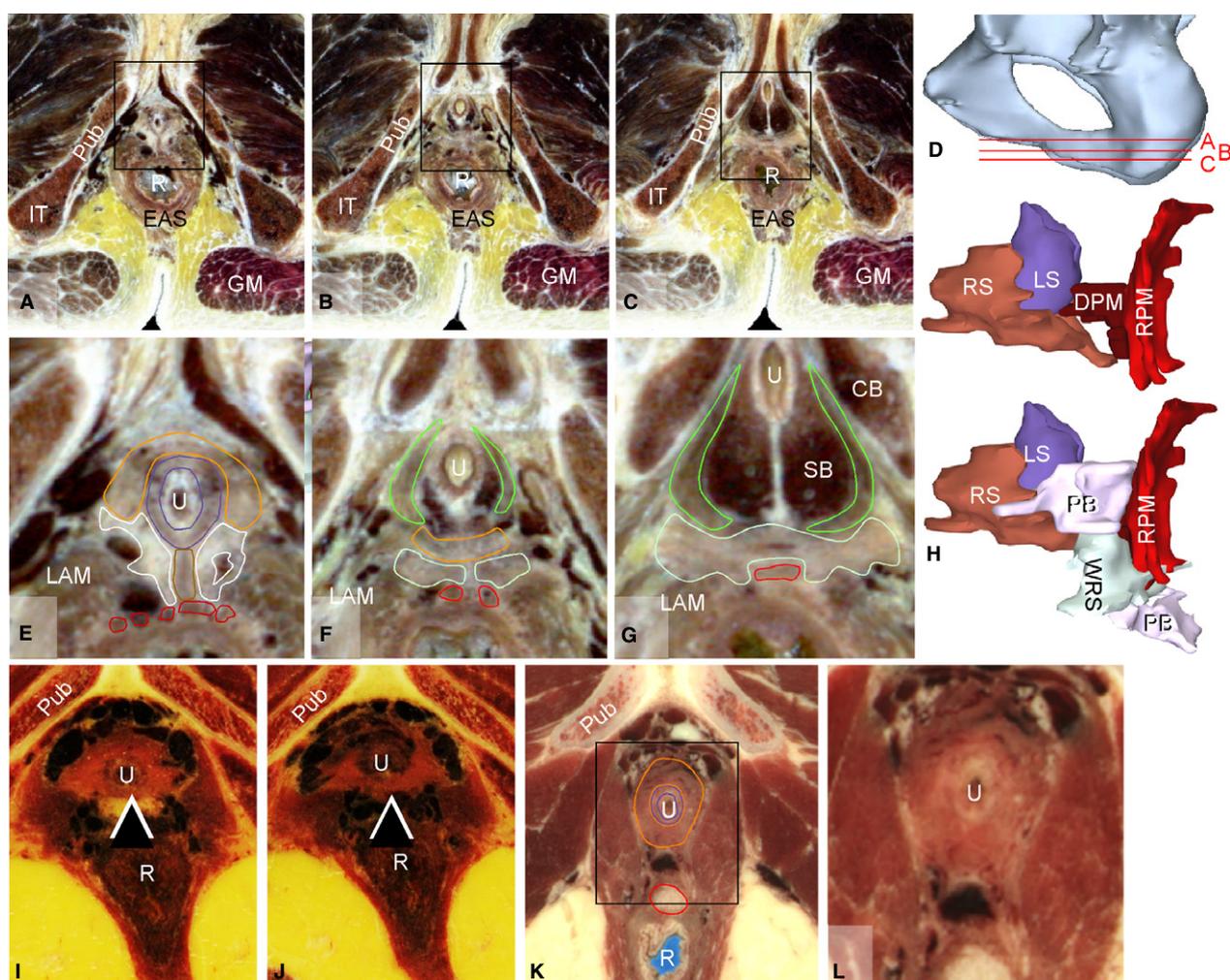


Fig. 2 The urethral sphincter and its adjacent structures. (A–C) Sections of the perineal area of CVH1. The position of these sections in the pelvic floor is shown in (D). (E–G) Magnifications of the boxes in (A–C), respectively. The line colors are the same as shown in (H), except that the bulbospongiosus muscle is also delineated (light green). (H) A left-lateral view of a reconstruction of the lissosphincter (LS), the rhabdosphincter (RS), the wings of the rhabdosphincter (WRS), the perineal body (PB), deep perineal muscle (DPM), and rectoperineal muscle (RPM) portions of the rectourethral muscle. In the upper subpanel, the perineal body and the wings of the rhabdosphincter were removed. (I, J) Serial sections of the perineal area of the CVH3 male with arrowheads indicating the perimembranous portion of the rhabdosphincter. (K) Section of the perineal area of the VHP male, with a magnification of the box in (L). Please note that the color codes are the same as those used in Fig. 1 and that the rhabdosphincter is circular in all three specimens.

and VHP, and its total volume was 0.25, 0.48, and 1.19 cm³, respectively. Attachments of the puborectal muscles to the perineal body marked the lateral boundaries of this fibromuscular complex.

The perineal body

The perineal body was an irregular fibrous structure (Figs 2–4) at the inferior end of Denonvillier's fascia. The perineal body was relatively well-developed and had deep and more superficial portions in the CVH1 and CVH-3 males, whereas only the superficial part was present in the VHP male (see also Brooks et al. 1998). The volume of perineal bodies of the CVH1, CVH3, and VHP males was 0.64, 1.17,

and 1.08 cm³, respectively. The fibers of the deep portion intertwined with the smooth-muscle cells of the rectourethral muscle, whereas the superficial and deep portions sandwiched the wings of the rhabdosphincter (see section on urethral sphincter). In addition, both portions of the puborectal muscle attached laterally, and the superficial transverse perineal and bulbospongiosus muscles attached inferiorly to the perineal body. The external anal sphincter was not in direct contact with the perineal body.

Denonvillier's fascia

Denonvillier's fascia is the dense connective tissue layer between the prostate and the adjacent part of the rectum

(Fig. 3D,E,J), and forms the anteroinferior continuation of the mesorectal fascia of the rectum (Kraima et al. 2015). Its superior boundary was arbitrarily taken as the location of the inferior boundary of the anterior mesorectal space, and its lateral boundaries corresponded with the width of the rectum. Inferiorly, the fascia ended on fibrous tissue of the perineal body.

The muscles in the urogenital triangle

The superficial transverse perineal, ischiocavernosus, and bulbospongiosus muscles form a superficial layer of striated muscles in the urogenital triangle (Fig. 5). In the VHP male, these muscles were markedly hypertrophic, suggesting they support the anterior part of the levator hiatus (Fig. 5). Medial fibers of the superficial transverse perineal muscle mingled with those of the bulbospongiosus muscle anteriorly and the anterior spur of the external anal sphincter

inferiorly. Its lateral fibers did not reach ischial tuberosity, but inserted, as in the female (Wu et al. 2017), on the connective tissue septa of the fat in the ischioanal fossa. The ischiocavernosus muscles attached to the anterolateral side of the penile crus, whereas fibers of the bulbospongiosus muscle encircled the penal root posteriorly.

Urethral sphincter

The inner layer of the urethral sphincter consisted of smooth muscle (lissosphincter) and the outer layer of striated muscle (rhabdosphincter). The urethral sphincter complex surrounded the membranous urethra as a complete cuff between the penile bulb and bulbospongiosus muscle inferiorly and the base of the prostate superiorly in all three specimens (Figs 2, 3, and 5). Anteriorly, the prostatic part of the rhabdosphincter extended upward towards the base of the bladder as a thinning muscle sheet, but was absent

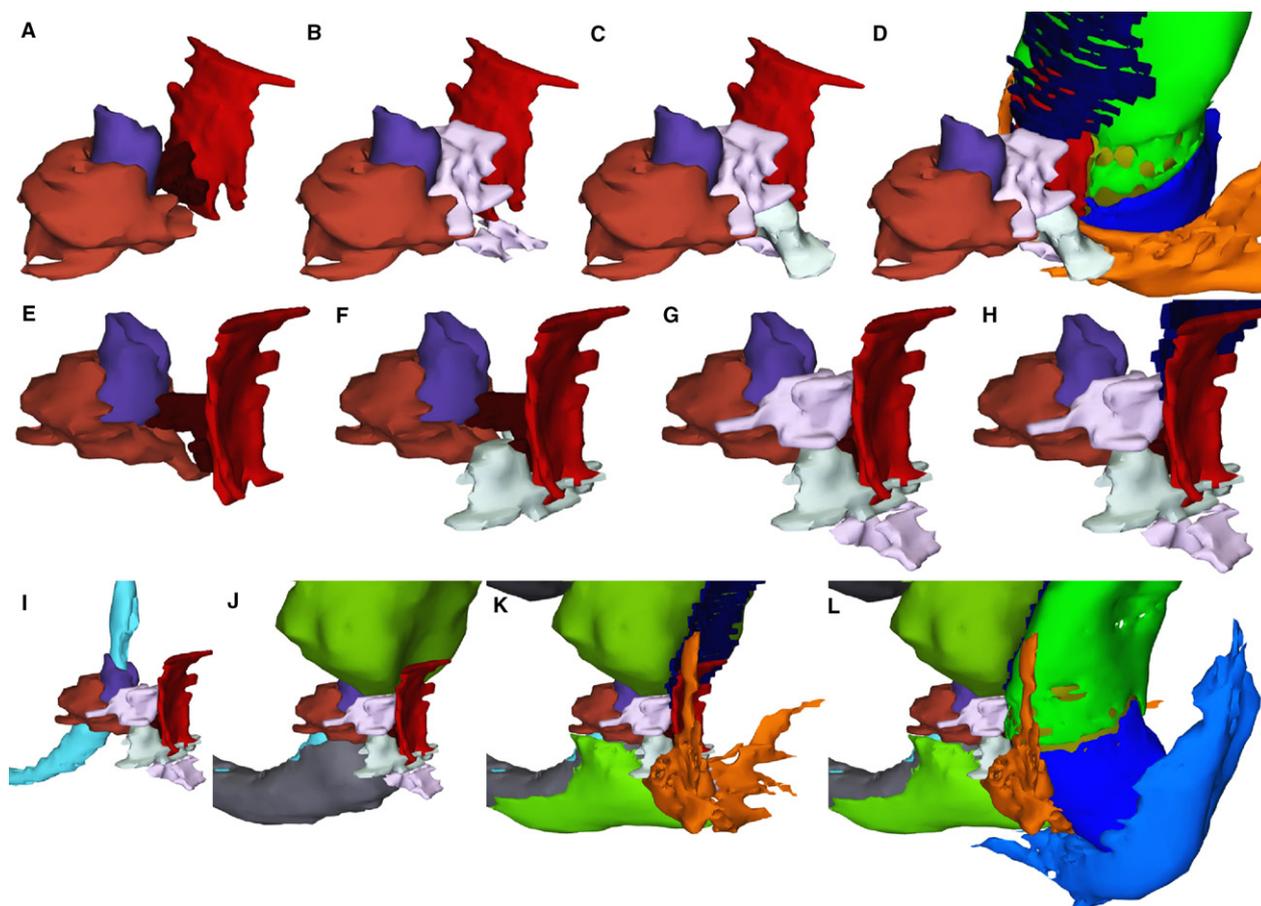


Fig. 3 Reconstruction and topography of the urethral sphincter and rectourethral muscle with its rectoperineal, deep-perineal muscle, and perineal-body portions. The reconstructions are seen from right anterior (A–D), left posterior (E–H), and left lateral (I–L). Color code: rhabdosphincter: brown; its wings: metal green; lissosphincter: purple; deep perineal and rectoperineal portions of the rectourethral muscle: dark brown and dark red, respectively; perineal body: white; submucous portion of urethra: bright blue; prostate: green; bladder wall: dark gray; spongiosus body: gray; overlying bulbospongiosus muscle: bright green; Denonvillier's rectogenital fascia: deep blue; superficial transverse perineal muscle: orange; smooth muscle of rectum: bright green; conjoint longitudinal muscle: dark blue; external anal sphincter: blue.

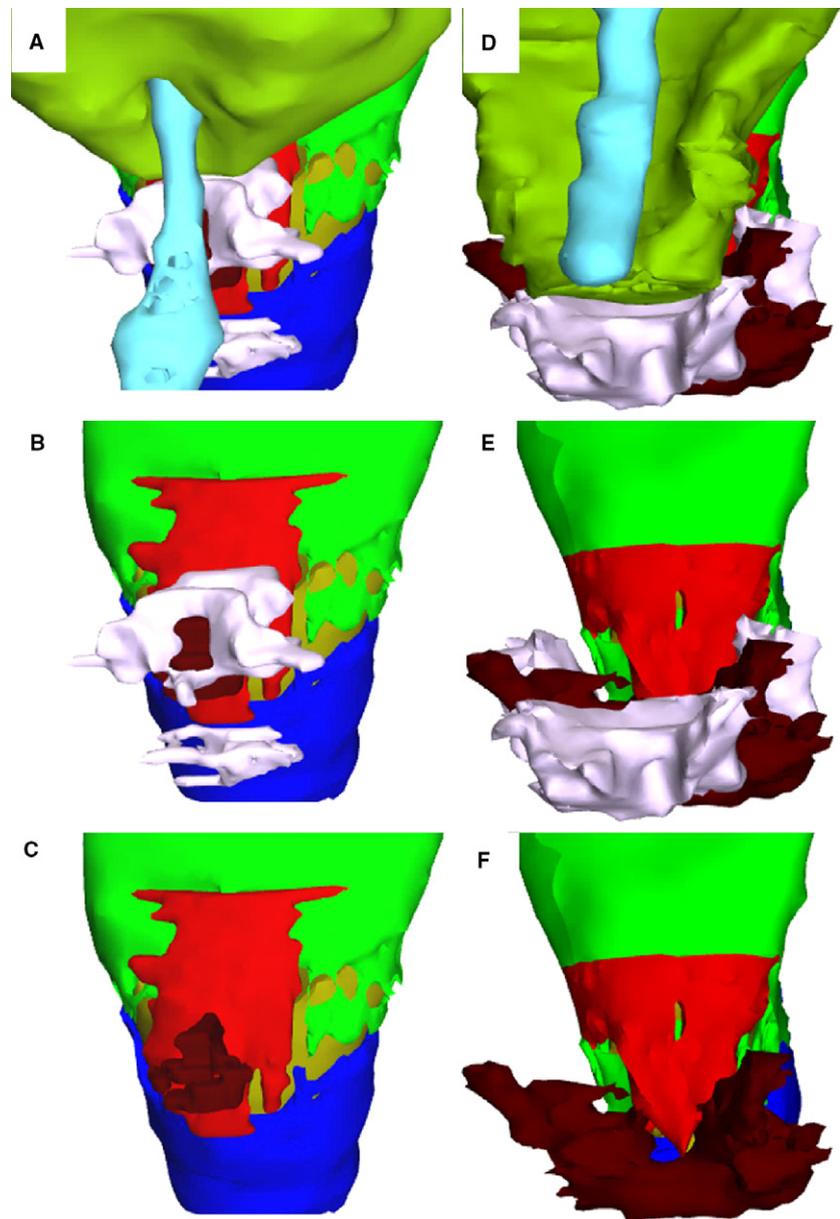


Fig. 4 Compared to the other Figures, this Figure is huge. For us, it could cover one column. Topographic relationship of deep perineal and rectoperineal muscles, and perineal body to urethra and prostate or vagina. The panels show left anterior views of CVH1 (A–C) and CVH5 (D–F). The perineal body (white) and deep perineal muscle (brown) bifurcate near the urethra, but both structures are larger and the bifurcation is wider and longer in the female (around vagina) than in the male (near urethra; light blue). In the female the complex of the deep perineal muscle and perineal body forms the ‘perineal membrane’ (4E). Note that the male deep perineal muscle is a relatively small, median structure. The rectoperineal muscle (red) is similar in size in the males and female. Further shown are the longitudinal smooth muscle of the rectum (green) and the conjoint longitudinal muscle (blue).

from the posterior half of the prostate. The prostatic extension of the rhabdosphincter was prominent in the CVH3 and VHP males (cf. Brooks et al. 1998) but was only small in the CVH1 male. The rhabdosphincter had two posteroinferior wings that passed the rectourethral muscle laterally and were sandwiched between the upper and lower parts of the perineal body. The volume of urethral sphincter of the CVH1, CVH3, and VHP males was 0.96, 2.93, and 3.44 cm³, respectively.

Venous plexus in male pelvis

A very well-developed venous plexus was present between the prostate and the levator ani muscle. Both sides of the venous plexus were continuous across the midline

anteriorly, just behind the symphysis, and extended posteriorly to the lateral boundaries of the mesorectum.

The topographic anatomy of the male fetal pelvis

We confirmed the anatomy of the male urogenital triangle at the histological level in two male fetuses of 26 weeks of development (Figs 6 and 7). In both cases, the specimen only contained the right anterior compartment. A detailed 3D topographic model of the S2289 fetus was prepared and can be visualized with the interactive 3D-PDF file that is provided online (Fig. S2). In these fetuses the rhabdosphincter was a well-developed muscular cuff without posterior fibrous raphe between the penile bulb inferiorly and the bladder neck anterosuperiorly and the seminal colliculus

Table 4 Arrangement of the rectourethral muscle in males and females. For details, see main text.

Anterior	Rectourethral muscle			Posterior
	Deep perineal muscle	Perineal body	Rectoperineal muscle	

posterosuperiorly. Posteroinferiorly, the rhabdosphincter had lateral 'wings' consisting of a loose network of thin striated muscle fibers. The wings covered the bulbourethral glands. The nerves to the rhabdosphincter entered medially and just superiorly to the wings. The prostatic portion of the rhabdosphincter was present on the anterior side of the prostate only. Smooth muscle covered the anterior and lateral sides of the prostate, surrounded the submucous portion of the membranous urethra, and ended as a dorsal strip underneath the symphysis.

A thick strip of smooth muscle from the longitudinal muscle layer of the rectum extended anteriorly between both puborectal muscles towards the penile bulb. Posteriorly, its fibers had a predominantly sagittal orientation (Fig. 7D–F), whereas anteriorly the fiber orientation was mostly transverse (Fig. 7E). The abundant connective tissue in the inferior reach of this smooth muscle (Fig. 7E) represented the developing perineal body. The fibrous tissue of the perineal body incompletely separated the posterior rectoperineal from the anterior deep perineal portion of the muscle. The smooth-muscle fibers of the deep perineal muscle were connected to the rhabdosphincter via relatively well-developed fibrous tissue and contacted the lissosphincter in between both bulbourethral glands and just superior to perineal body.

Discussion

Sexual dimorphism of the pelvic-floor muscles

The architecture of the muscles of the pelvic floor in the male resembled that in the female. However, quantitative differences were observed. The (subcutaneous) external anal sphincter in the male differed, with its long anterior and posterior spurs (cf. Fig. 1B,E), markedly from that in the female. Another unexpected feature was the approximately twofold smaller volume of the LAM in males than females (Wu et al. 2015), even though overall body muscle mass is, after correction for size, ~25% greater in males than in females (Janssen et al. 2000). Since hypertrophy of the VHP LAM correlated with his bodybuilding practices, the thicker female pelvic floor muscles probably point to a higher working load of the LAM in females than in males. Part of this higher working load appears to arise from the shallower funnel shape of the female (laterally ~30° relative to the axial plane) than the male LAM (~20°; cf. Fig. 1A,D).

Architecture of the male urethral sphincter

In the embryo the rhabdosphincter is first identifiable in the anterior wall of the urethra (Oelrich, 1980; Tichy, 1989; Sebe et al. 2005c), and then extends posteriorly around the urethra and superiorly towards the bladder neck (Figs 6 and 7; Oelrich, 1980; Tichy, 1989; Strasser et al. 1996; Ludwikowski et al. 2001; Sebe et al. 2005c). The male rhabdosphincter is best developed inferiorly, where it surrounds the membranous urethra (Figs 2 and 3; Oelrich, 1980; Burnett & Mostwin, 1998). Concomitant with the growth of the prostate, the superior portion of the rhabdosphincter becomes thinner (cf. Oelrich, 1980; Elbadawi et al. 1997; Burnett & Mostwin, 1998; Koraitim, 2008) and can even be visually absent (Fig. 3).

Another debate centers on whether the adult male rhabdosphincter is a circular (Brooks et al. 1998; Karam et al. 2005; Gil-Vernet et al. 2016) or an omega-shaped structure with posteriorly a raphe that anchors the rhabdosphincter to the perineal body (Oelrich, 1980; Strasser et al. 1996; Burnett & Mostwin, 1998; Dorschner et al. 1999; Ludwikowski et al. 2001; Yucel & Baskin, 2004; Sebe et al. 2005c; Koraitim, 2008). In fact, some describe the rhabdosphincter as evolving from a circular structure before birth into an omega-shaped configuration after birth (Tichy, 1989; Kououa et al. 1993; Strasser et al. 1996; Karam et al. 2005; Gil-Vernet et al. 2016), whereas others found an omega-shaped structure *ab initio* (Oelrich, 1980; Ludwikowski et al. 2001; Sebe et al. 2005c). In our specimens, the rhabdosphincter was circular in fetuses (Fig. 6) and adults (Fig. 2), and had posterolateral wings that consisted of a very loose web of striated muscle fibers. Such wings or 'tails' were previously described (Nakajima et al. 2007; Gil-Vernet et al. 2016) and may be identical to the 'tails' of the omega-shaped rhabdosphincter (Strasser et al. 1996; Ludwikowski et al. 2001). The posterior side of the circular rhabdosphincter was connected by fibrous tissue to the anterior surface of the deep perineal muscle and perineal body (Fig. 3). Whether a dorsal raphe is identified in the male rhabdosphincter may well depend on the degree of development of this connective tissue and the use of oblique sections that make the connective tissue appear to extend forward towards the urethra (Fig. 6A).

Sexual dimorphism of the urethral sphincter

The Bauplan of the rhabdosphincter is similar in both sexes: inferiorly, the sphincter is circular, whereas more superiorly, striated muscle only covers the anterior and lateral portions of the urethra. In the male, the prostate determines the appearance of this portion of the sphincter. In the female, the middle (compressor) portion of the sphincter is U-shaped where it passes the vagina laterally, whereas the superior portion, which reaches the bladder neck, is pseudo-circular due to the presence of a dorsal connective-

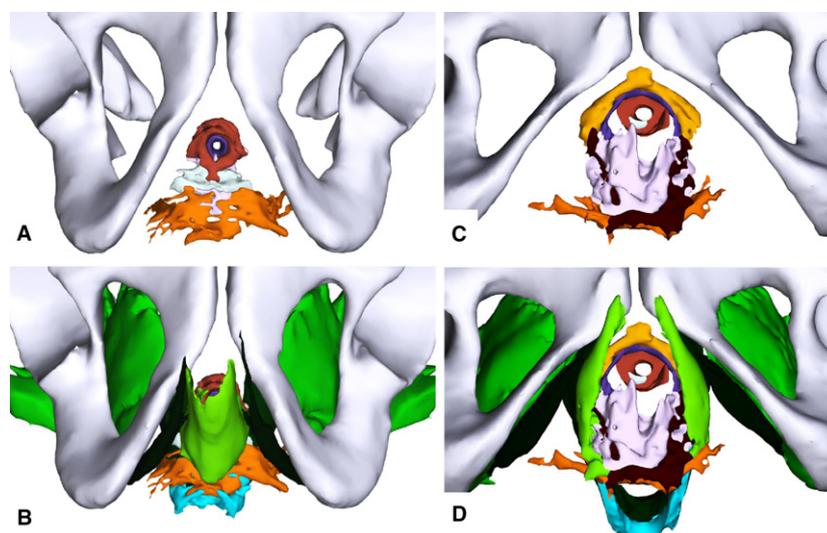


Fig. 5 The urogenital trigone in the male and female. The panels show an anteroinferior frontal view of the urogenital trigone of the CVH1 male (A,B) and CVH5 female (C,D). (A,C) The pelvic bones, the male lisso- (purple) and rhabdosphincter (brown) with its wings (metal green), the female urethral sphincter (brown), urethral compressor (yellow), and urethrovaginal sphincter (purple), the perineal body (white), the deep perineal muscle (dark brown), and the superficial transverse perineal muscle (orange). (B,D) The same view with the internal obturator (green), ischiocavernosus (dark green), bulbospongiosus (bright green), and deep and superficial puborectal muscles (deep green and cyan, respectively), added. The color code is identical to that in Figs 1–3. The wings of the rhabdosphincter hide the deep perineal portion of the rectourethral muscle in the male. Note that the muscular structures mentioned completely fill the urogenital triangle in the male but leave bilaterally a large area unguarded in the female.

tissue raphe (Wu et al. 2017). The transition between the inferior circular and superior U-shaped portion of the urethral sphincter is just inferior to the entrance of the deferent ducts in the male and at the entrance of the vagina into the vestibule in the female. These positions correspond to the entrances of the Wolffian and Müllerian ducts into the embryonic urogenital sinus, respectively. Sexual dimorphism results from differential development of the respective portions of the sphincter: the inferior, circular ‘membranous’ portion is best developed in males (Oelrich, 1980; Burnett & Mostwin, 1998; Koraitim, 2008), whereas the middle, U-shaped compressor portion is best developed in females (Oelrich, 1983; Sebe et al. 2005a; Wallner et al. 2009; Wu et al. 2017).

The smooth muscles of the ‘middle’ compartment

The 26-week-old male fetuses unambiguously show that the distal portion of the longitudinal smooth-muscle layer of the rectum extends anteriorly as a well-defined entity towards the bulbourethral glands and the penile bulb, and that this muscle forms the posterior muscular component of the urogenital triangle. The muscle fiber orientation in the posterior part of this muscle slip is predominantly sagittal and that in the anterior part transverse. The perineal body originates in between these components in the inferior reach of this muscle (Figs 6 and 7). This anterior extension of the longitudinal smooth muscle of the rectum in males is

variously known as the deep transverse perineal muscle (Oelrich, 1980), the rectourethral muscle (Brooks et al. 2002; Porzionato et al. 2005; Uchimoto et al. 2007; Gil-Vernet et al. 2016) or the rectoperineal muscle (Aigner et al. 2004; Sebe et al. 2005b). In females (Oelrich, 1983; Aigner et al. 2004), but according to some (Dorschner et al. 1999) also in males, the deep transverse perineal muscle is inconspicuous or absent. Based on these arguments the term ‘deep transverse perineal’ muscle was removed from the official Anatomical Terminology (FCAT, 1998) and replaced by ‘rectoperineal’ and ‘rectourethral’ muscles.

Recently, however, Li and colleagues re-introduced the deep transverse perineal muscle (Zhai et al. 2011). They reported that the medial fibers of the anterior extension of the longitudinal smooth muscle of the rectum ended at the perineal body, forming the rectoperineal muscle, whereas more lateral bundles (‘their’ rectourethral muscle) bypassed the perineal body to end at the posterior connective tissue of the penile bulb. They then described the deep transverse perineal muscle as a third, yet more lateral muscle bundle (Zhai et al. 2011). Our present data show that the rectoperineal and rectourethral bundles as defined by Li and colleagues (Zhai et al. 2011) derive from the anterior extension of the longitudinal smooth muscle (Fig. 2A,E; cf. Fig. 4 in Gil-Vernet et al. 2016 for very convincing histological sections). The most lateral muscle bundle between the rectourethral and puborectal muscles of Li and colleagues (Zhai et al. 2011) most likely corresponds with the loose

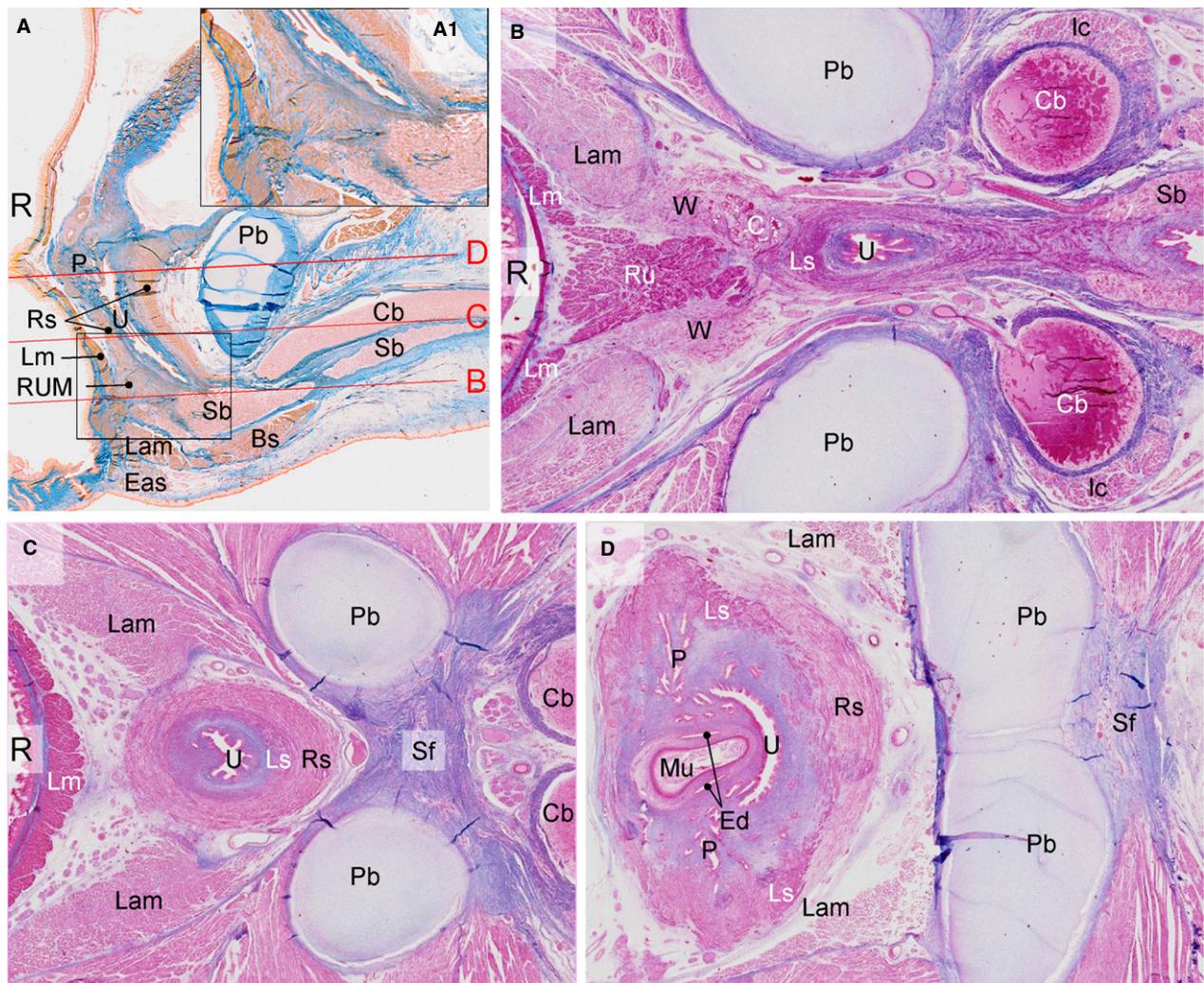


Fig. 6 The urethral sphincter and rectourethral muscle complex in the 22-week male fetus. (A) A midsagittal trichrome-stained section of specimen S2600 and (B–D) transverse azan-stained sections of specimen S2289. (A1) A magnification of the box in (A), whereas the near horizontal lines in (A) show the level of the transverse sections in (B–D). Note the circular structure of the rhabdosphincter and position of the rectourethral muscle complex on the posteroinferior side of the rhabdosphincter. Bs, bulbospongiosus muscle; Cb, cavernous body; EAS, external anal sphincter; Ed, ejaculatory duct; LAM, levator ani muscle; Lm, longitudinal smooth muscle of rectum; Ls, lissosphincter; Mu, male urethra; P, prostate; Pb, pubic bone; R, rectum; Rs, rhabdosphincter; Ru, rectourethral muscle complex; Sb, spongiosus body; Sf, subpubic fascia; U, urethra; W, wing-like extension of rhabdosphincter.

posterolateral muscular wing of the rhabdosphincter that we describe at this location and histologically identified as striated muscle.

Li and colleagues' (Zhai et al. 2011) and our studies show that the entire anterior extension of the longitudinal smooth muscle of the rectum develops as a single, well-defined smooth-muscle mass (rectourethral muscle) that becomes subdivided by the developing perineal body into posterior (rectoperineal muscle between rectal wall and perineal body) and anterior portions (deep perineal muscle between perineal body and penile bulb; Fig. 3, Table 4). This description complies with earlier descriptions (Brooks et al. 2002; Porzionato et al. 2005; Sebe et al. 2005b; Zhai et al. 2011; Gil-Vernet et al. 2016) but emphasizes that the

smooth muscles in the urogenital triangle develop from a single precursor and clarifies their topographical relation with the perineal body. The various small muscles in the levator hiatus (e.g. 'prerectal' fibers of the pubococcygeal and puborectal muscles (Roberts et al. 1988) and pubovaginal, -prostatic, -perineal, and -anal subdivisions of the pubococcygeal muscle (FCAT, 1998, Kearney et al. 2004) are, therefore, parts of the rectourethral muscle. The reinstatement of the deep perineal muscle as an anatomical structure also facilitates the understanding of sexual dimorphism of perineal anatomy. In females the muscle extends around the wall of the vagina (Fig. 4D–F) to attach anteriorly to the fascia covering Bartholin's glands and the urethral compressor (Fig. 5C,D), whereas in males it follows

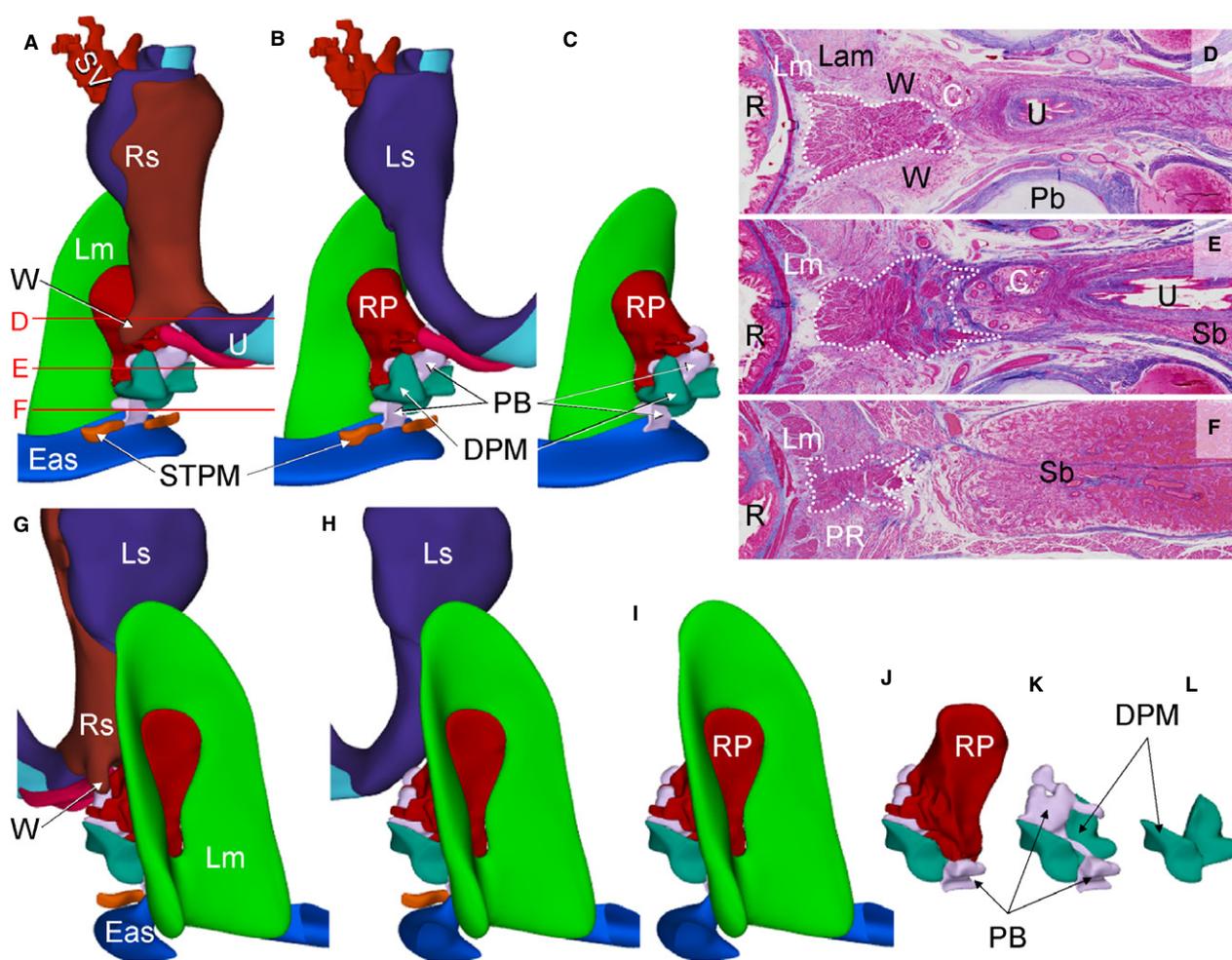


Fig. 7 3D representations of the rhabdosphincter and rectourethral muscle complex in the 22-week-old male fetus. (A–C) Right anterior views and (G–L) left posterior views. (B) differs from (A) in that the rhabdosphincter was removed and (C) from (B) in that the lissosphincter and Cowper's glands were removed. (H) differs from (G) in that the rhabdosphincter and Cowper's glands were removed; (I) differs from (H) in that the lissosphincter and superficial transverse perineal muscles were removed, and (J) differs from (I) in that the external anal sphincter and longitudinal muscle of the rectum were removed. (J) Three components of the rectourethral muscle (cf. Table 4). In (K) the rectoperineal muscle is removed and in (L) the developing perineal body. (D–F) Histological sections of the rectourethral muscle complex (boundaries indicated by white interrupted line) at the level of the red lines in (A). The rectourethral muscle complex is an anterior extension of the longitudinal smooth muscle of the rectum towards the spongious body and Cowper's glands, with mostly sagittally oriented fibers in its rectoperineal and transversely oriented fibers in its deep perineal muscle portion. The fibrous perineal body (violet strands between purple muscle fibers) develops in its anteroinferior portion. For abbreviations, see Fig. 6.

the same course, but, due to the absence of the vagina, remains a mostly single midline structure with only a small bifurcation near Cowper's glands and the penile bulb (Figs 2A,E, 4A–C, and 5A,B).

The urethral rhabdosphincter and the rectourethral smooth-muscle complex with their fasciae were previously known as the urogenital 'diaphragm' (for review, see Mirilas & Skandalakis, 2004). The 3D shape of the rhabdosphincter and the absence of a perimysium on its prostatic surface are, however, incompatible with these structures forming the anterior part of a sheet-like striated muscular 'urogenital diaphragm' (Mirilas & Skandalakis, 2004), as still depicted in many textbooks. Instead, smooth-muscle fibers of the

deep perineal muscle interlace with the radiating fibrous strands of the perineal body. This fibromuscular complex is known as the 'perineal membrane' in females (Fig. 4D–F; Oelrich, 1980; Dorschner et al. 1999; Hudson et al. 2002; Mirilas & Skandalakis, 2004; Stein & DeLancey, 2008; Brandon et al. 2009), but is also present, albeit less well-developed, in males (Figs 4A–C and 5A). We, therefore, advocate that a middle compartment is acknowledged in both females and males.

The perineal body was still relatively small in fetuses and remained about twofold smaller in males than in age-matched females (Wu et al. 2015; cf. Oh & Kark, 1973). All male bodies contained the superficial portion of the

perineal body, but both CVH specimens had in addition a deep portion. The locations of the superficial and deep portions of the perineal body were described earlier (Oh & Kark, 1973; Aigner et al. 2004). The developmental increase in connective tissue in the pelvic floor, particularly in females, suggests that the perineal body develops as a regenerative response to wear and tear during life (cf. Magnusson et al. 2007; Nygaard & Shaw, 2016).

Striated muscles completely cover the anterior portion of the urogenital trigone in males but leave a large area unguarded in females (Fig. 5). These 'empty' areas are occupied by an 'adipose cushion pillow' (Tansatit et al. 2013) that is interlaced with well-developed fibrous septa (De Blok, 1982), which can be tightened by the superficial transverse perineal muscle (Wu et al. 2015).

Conclusion

The architecture of the anterior and middle portions of the pelvic floor is similar in males and females. Sexual dimorphism arises from differential growth of different parts of the male and female urethral sphincter and the much stronger development of connective tissue in the female pelvic floor.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Interactive 3D rendering of the topographic anatomy of the CVH1 pelvic floor.

Fig. S2. Interactive 3D rendering of the topographic anatomy of the VHF pelvic floor.