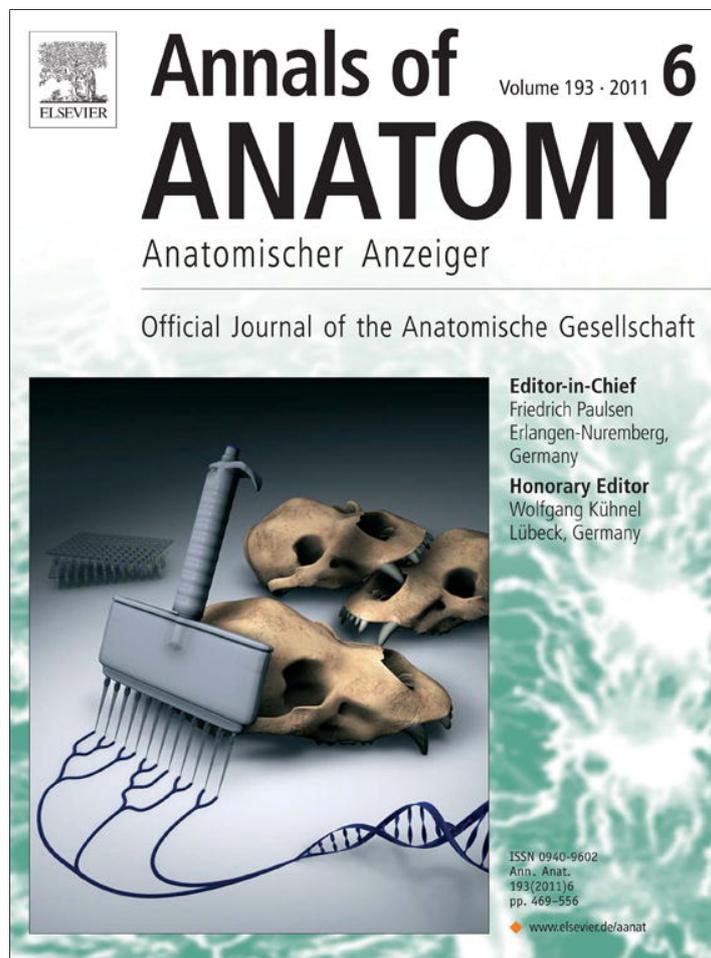


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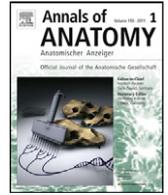
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Research article

Fetal topographical anatomy of the female urethra and descending vagina: A histological study of the early human fetal urethra

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ABSTRACT

Background: Which parts of the male urethra correspond to the female urethra? To resolve this question, we need to understand fetal topographical changes in the urethra, its external sphincter and vagina. The vagina joins the mid-course of the primitive urethra and, later “descends” to the vaginal vestibulum.**Methods:** We examined histological sections of 14 female and 4 male mid-term fetuses.**Results:** The inferior end of the vagina was consistently embedded in the posterior wall of the urethra at 9–12 weeks. The supero-inferior level of the vaginal merging was lower in larger fetuses. Thus, the sequential variation in levels appeared to reflect the process of vaginal descent. However, in spite of penetration of the vaginal end into the posterior urethral wall, we found no sign of destruction of the urethral wall after vaginal descent in the low-merging types. Before vaginal descent, the female external sphincter extended posterolaterally around the urethra.**Conclusion:** The vaginal descent is classically regarded as a relative topographical change, but it is likely to be a result of elongation of the proximal urethra in the superior side of the vaginal merging. Conversely, the distal urethra is likely to be incorporated into the vaginal vestibulum by 15 weeks. During these processes, most of the female external sphincter seems to be expelled from the original anterior position into the vestibular wall as the urethrovaginal sphincter. The adult female urethra seems to correspond to the male prostatic urethra superior to the prostatic colliculus.

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1. Introduction

Our basic question at the beginning of this study was “which parts of the male urethra correspond to the female urethra?” The female urethra is much shorter than that in males but accompanies the external urethral sphincter in its anterior and lateral aspects (Strasser et al., 2000; Kurihara et al., 2004). Although previous reports have denied the existence in females (e.g., Dorschner et al., 1999; Mirilas and Skandalakis, 2004), our group demonstrated that the deep transverse perineal muscle is present in both genders (Nakajima et al., 2007). Moreover, the female pelvis carries the distinct, elastic fiber-made perineal membrane (Kato et al., 2008a). Thus, the membranous part is likely to be present in the female urethra. Therefore, the female urethra may correspond to the pro-

static and membranous parts of the male urethra (Fig. 1). Do the embryological findings agree with this hypothesis?

The vagina is considered to be a Müllerian duct derivative that includes some contribution of cells from the urogenital sinus or primitive urethra (Ulfelder and Robboy, 1976; O’Rahilly and Müller, 1996; Shapiro et al., 2000). The inferior end of the developing vagina, which is associated with Gartner’s ducts (Wolffian duct remnants), is known as the Müllerian–Wolffian duct complex (van der Putte, 2005), the Müllerian tubercle (Oelrich, 1983; Cai, 2009), or the sinovaginal bulb (Shapiro et al., 2000) at the CRL 40–70 mm stage or approximately 9–12 weeks of gestation. At the interface between the vagina and primitive urethra, an evagination of the urethral epithelium has been described (Shapiro et al., 2000; Fig. 2). In contrast, in males, both the Müllerian duct remnant (prostatic utricle) and the Wolffian duct derivative (ejaculatory ducts) merge with the urethra at the prostatic colliculus (Arey, 1974; Hamilton and Mossman, 1978; Moore and Persaud, 1998), although later, the urethra-derived cells seem to replace the Müllerian duct-derived utricle (Shapiro et al., 2004). Thus, the vaginal merging with the female urethra seems to correspond to the male colliculus. However, the vaginal merging site descends with advancing

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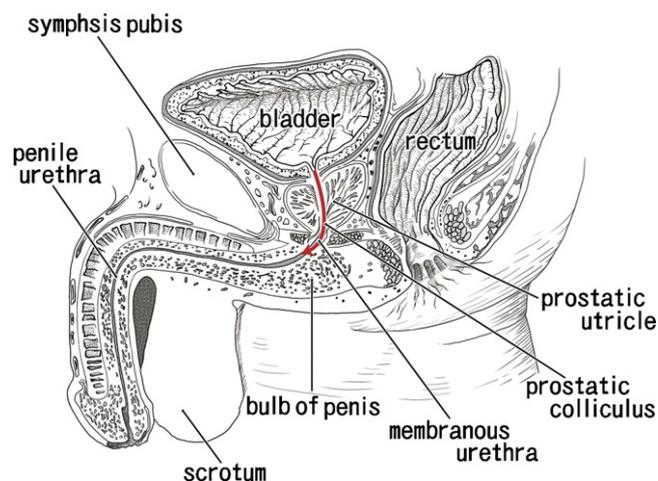


Fig. 1. Which parts of the male urethra correspond to the female urethra? A diagram showing the midsagittal section of the male urethra. The ejaculatory duct (not shown) opens to the prostatic urethra in the lateral side of the prostatic utricle. The female urethra is likely to be compatible with the proximal parts of the male urethra (red-colored zone), i.e., (1) the prostatic urethra and (2) the membranous urethra because, as in males, the female urethra should pass through the external urethral sphincter at the levator histus. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

gestational age, and finally the site corresponding to the male colliculus becomes located at the vestibulum or vaginal vestibulum (Fig. 2). In this context, the female urethra seems to correspond to the male urethra superior or proximal to the prostatic colliculus, i.e., the upper part of the prostatic urethra. However, this conclusion might appear illogical because it would not take into account the presence of the female membranous urethra.

In attempting to resolve this issue, however, there is little information about the descent of the vaginal merging site (or simply the “vaginal descent”) along the primitive urethra. During this descent, topographical changes in the external urethral sphincter would likely occur. A comprehensive review by Yucel and Baskin (2004) provided an excellent comparison between genders. However, it is based on observations of fetuses at 36 weeks in which the vagina was fully elongated. Thus, they did not include suspected morphological changes of the sphincter during the process of the vaginal descent. A detailed topographical study of the levator ani muscle and developing urethra by Wallner et al. (2009),

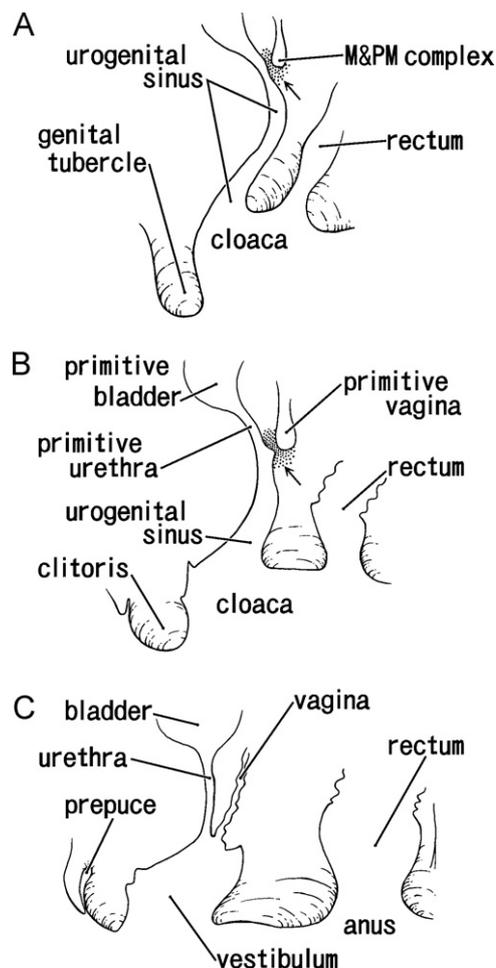


Fig. 2. Usual diagrams showing fetal development of the female urethra and vagina. Panel A (CRL 40 mm-stage), panel B (CRL 70 mm-stage) and panel C (CRL 125 mm-stage) are schematic representations based on midsagittal sections. Most researchers consider that the Müllerian and Wolffian ducts-complex (M&W complex; the future vagina and Gartner's ducts) merges the urogenital sinus (the future urethra) at its superior part (panel A). At the merging, there is a plate or cluster of sinus-derived endodermal cells (the sinovaginal bulb or vaginal plate; arrow in panels A and B). After decent or sliding of the merging site along the urethra (panel B), both the urethra and vagina open at the vestibulum (panel C). In figures below (Figs. 5–8), the M&W complex is simply labeled by “vagina” (VAG; Müllerian duct derivative) with or without “Gartner's ducts” (GD; Wolffian duct remnants). BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

Table 1
Stages and sites of the vaginal merging with the urethra.

CRL (weeks)	Levels of the vaginal merging with the urethra or urogenital sinus			
	Near vestibulum	Between pubic arch	Behind symphysis pubis	
36 mm (9)				+
48 mm (9)			+	(Fig. 5)
48 mm (9)		+		(Fig. 6)
62 mm (10)			+	(Fig. 7)
76 mm (12)				+
78 mm (12)				+
84 mm (12)				+
84 mm (12)			+	
92 mm (14)		+		
95 mm (14)			+	
110 mm (15)		+		
110 mm (15)	+			
120 mm (15)		+		
125 mm (15)	+			

the female external sphincter is horseshoe-shaped with the posterolateral extension before the descent at 12 weeks. However, the authors did not describe a process of changing itself. Along the urethra, O'Rahilly and Müller (1996) proposed sliding of the vagina, while Cai (2009) hypothesized elongation of the vagina. However, they also made no comment regarding the female external urethral sphincter and other perineal structures. Consequently, the aim of the present study was to examine the early fetal topographical anatomy of the human female urethra in order to reconsider the established concept of the intergender difference in the urethra.

2. Materials and methods

The present study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). We examined the paraffin-embedded histology of 18 mid-

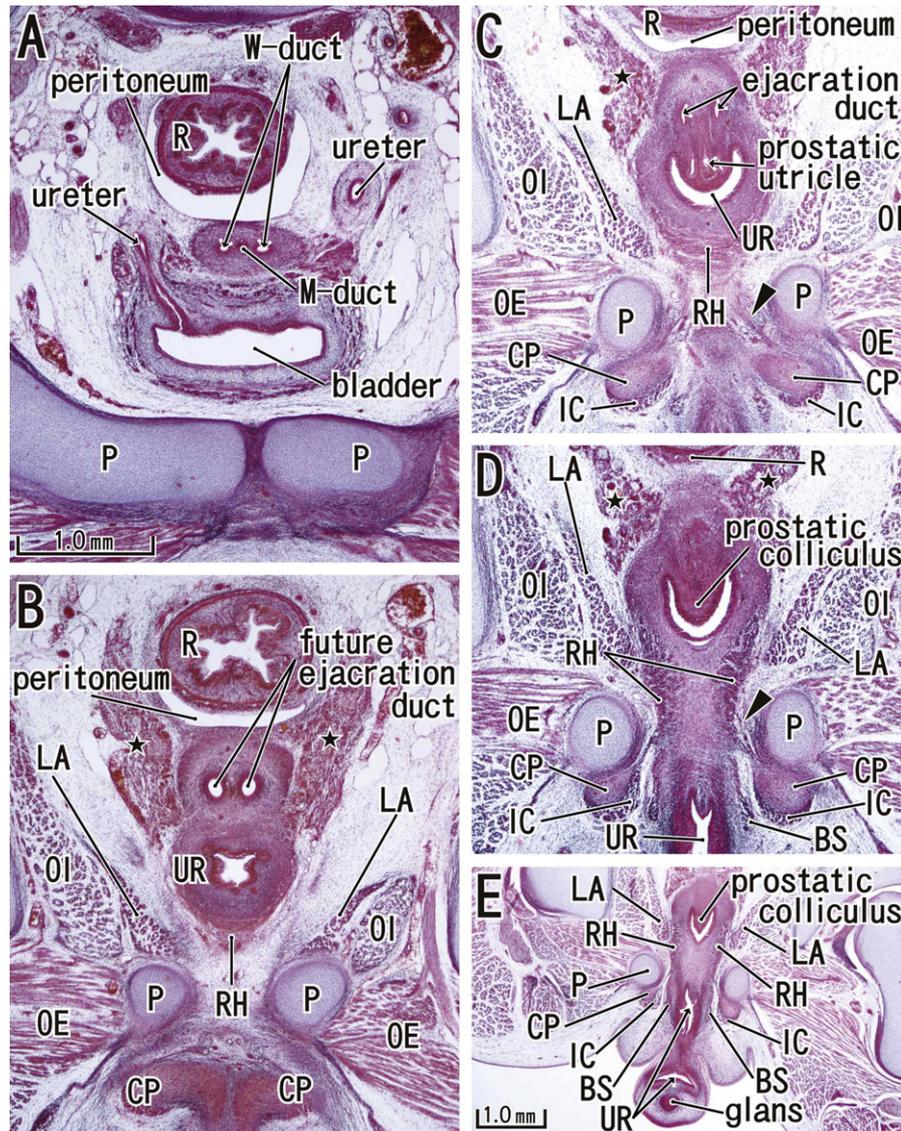


Fig. 3. Early stage of the male urethra and future prostatic colliculus (CRL 52 mm). Horizontal sections. Panel A (or E) is the most superior (or inferior) level in the figure. Panels A–D are prepared at the same magnification (scale bar in panel A), while panel E at the lower magnification (scale bar in panel E). Intervals between panels are 1.1 mm (A and B), 0.2 mm (B and C), 0.2 mm (C and D) and 0.1 mm (D and E). Panels A–C show the inferiormost part of the peritoneal cavity (peritoneum) in front of the rectum (R), while panels D and E depict the future penile urethra (UR in panels D and E). Notably, the future prostatic colliculus is seen at the level including the penile urethra and glans under the pubic arch. In panel A at the level of the urethra opening to the bladder, the bilateral Wolffian ducts (W-duct) as well as a single Müllerian duct after fusion is clearly identified. In panel C, the future ejaculatory ducts (Wolffian duct derivative) merge with the urethra. The external urethral sphincter (rhabdosphincter, RH) is not circularly arranged but attaches to the anterior (panels B and C) or lateral (panels D and E) aspects of the urethra. Arrowheads in panels C and D indicate a fascia connecting the crus penis (CP) and external sphincter. Stars indicate the pelvic nerve plexus. Other abbreviations, see common abbreviation. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

term fetuses (4 males and 14 females). The cranio-rump length (CRL) of the male specimens was 52 mm and 60 mm (approximately 10 weeks of ovulation age) and 165 mm and 170 mm (approximately 20 weeks). The female specimens varied in size and stage, being 36–125 mm CRL or approximately 9–15 weeks of ovulation age (Table 1). All specimens were part of the large collection kept at the Embryology Institute of the Universidad Complutense, Madrid, and were the products of miscarriages and ectopic pregnancies at the Department of Obstetrics of the University. Approval for the study was granted by the university ethics committee. Because of the origin of the specimens, we were unable to rule out the possibility that some may have contained pathology, but no pathology was evident in the developing umbilical vessels, liver, intestine, adrenal or kidney in the specimens examined. Identification of gender was based on observations of the urogenital fold including the primi-

tive uterus, in accordance with our previous study (Niikura et al., 2008).

After routine procedures for paraffin-embedded histology, all the specimens were cut horizontally at a thickness of 5 μm and at intervals of 50 (or 100) μm in females (or in males). Depending on the size of the specimen, approximately 30–200 sections including the entire urethra were examined. All sections were stained with hematoxylin and eosin.

3. Results

3.1. Observations of male fetuses

At an ovulation age of approximately 10 weeks (2 fetuses), a set of genital tracts (2 Wolffian ducts and a Müllerian duct) was clearly

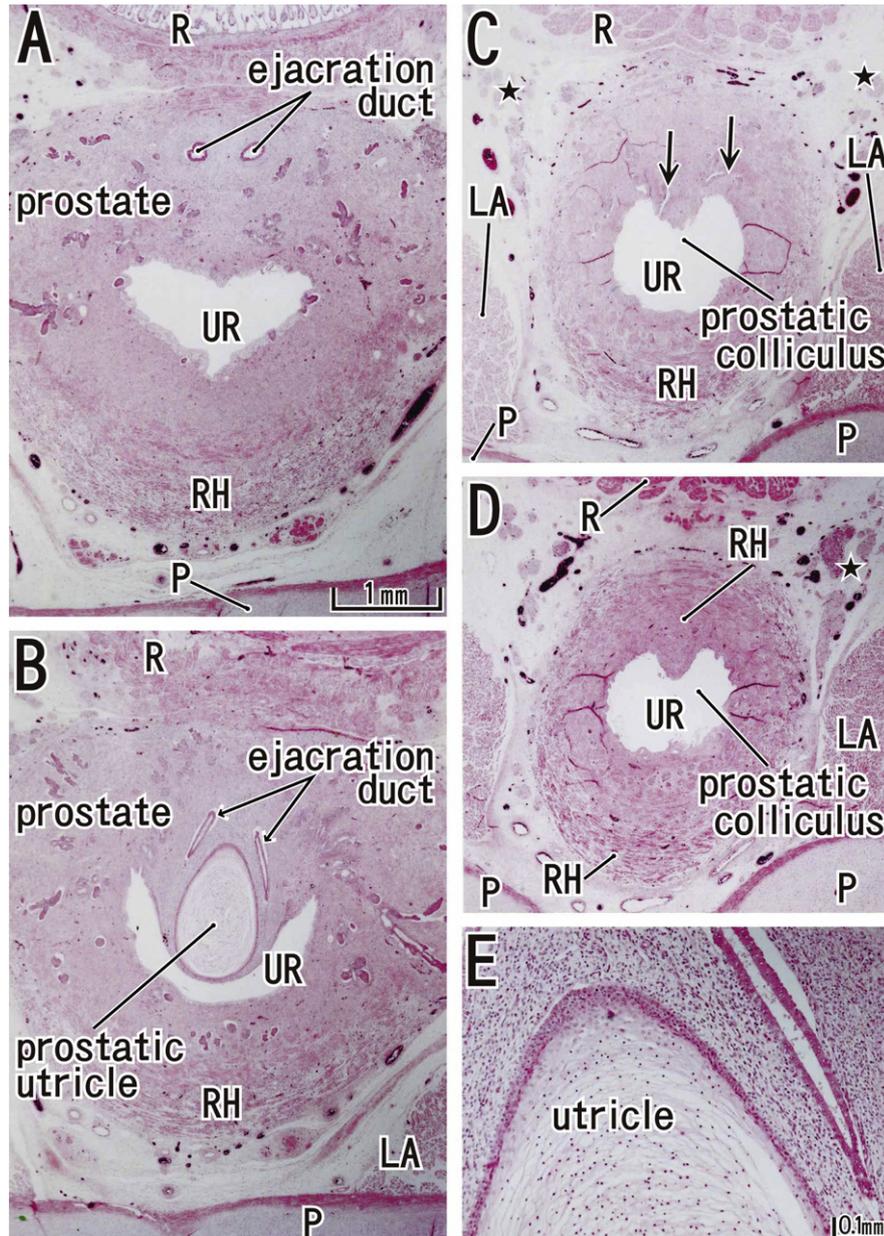


Fig. 4. Prostatic utricle and colliculus in the late stage male fetus (CRL 170 mm). Horizontal sections. Panel A (D) is the most superior (inferior) level in the figure. Panels A–D are prepared at the same magnification (scale bar in panel A), while panel E is a higher magnification view of the central part of panel B. The intervals between the panels are 0.8 mm (A and B), 1.6 mm (B and C) and 0.4 mm (C and D). The prostatic colliculus is located behind the symphysis pubis (panels B–D). Ducts of the developing prostate (arrows in panel C) appear to open to the urethra (UR) independently of the ejaculatory ducts. The external urethral sphincter (rhabdosphincter, RH) is circularly arranged around the urethra in the inferior side of the colliculus (panel D). The utricle, a possible Müllerian duct remnant, is composed of loose mesenchymal tissues (panel E). Stars indicates the pelvic nerve plexus. Other abbreviations, see common abbreviation. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

identified behind the ureteric openings to the bladder (Fig. 3). The bilateral Müllerian ducts had already fused to form a single duct at this stage. The ejaculatory ducts, Wolffian duct derivatives, merged with the urethra or urogenital sinus at the level of the pubic arch (Fig. 3C). The future prostatic colliculus, containing the ejaculatory ducts and future prostatic utricle, was identified as a large protrusion covering the urethral epithelium (Fig. 3C–E). More strictly, a tight tissue mass or solid epithelial cord containing Müllerian and Wolffian ducts was embedded in the posterior wall of the urethra, and absolutely interrupted the urethral wall architecture. It did not simply attach but penetrated the posterior urethral wall. The early utricle carried no epithelial lining, in contrast to the ejaculatory ducts.

At 10 weeks, the external urethral sphincter was attached to the anterior and/or lateral aspects of the urethra depending on the supero-inferior level. However, at 20 weeks, the prostatic colliculus was located behind the symphysis pubis (Fig. 4): thus, the location in the pelvis was superior to that at the early stage. The colliculus contained a large utricle that was filled with loose mesenchymal tissues (Fig. 4E). The utricle was lined by an epithelium that was possibly derived from the urethra or sinus, as reported by Shapiro et al. (2004). Below the developing prostate, the completely circular sphincter was evident between the bilateral levator ani slings (Fig. 4D). The prostatic ducts appeared to open independently to the urethra at the colliculus (Fig. 4C). A tight tissue mass containing the ejaculatory ducts and utricle was embedded in the developing

prostate. Conversely, no absolute interruption or gap of the urethral wall architecture was clear at this stage.

3.2. Observations of female fetuses

The vagina, accompanied by the bilateral Gartner's ducts, was deeply embedded in the posterior wall of the urethra and completely interrupted the urethral wall at 9–12 weeks (Figs. 5–8); thus, the inferior end of the vagina occupied in a "gap" in the urethral wall. This morphology was similar to the early stage in males, but different from the images of a loose attachment shown in sagittal sections by Shapiro et al. (2000). At the site of vaginal merging, we found no interface structure such as the vaginal plate or sinovaginal bulb (Fig. 2; see Section 1). Instead, possibly due to differences in stage from those in previous studies, the Müllerian–Wolffian duct-derived tissue mass crossed the posterior wall of the urethra and pushed or protruded into the urethral lumen. Therefore, the inferior end of the vagina, containing both Müllerian and Wolffian duct derivatives, did not simply attach but penetrated the posterior urethral wall.

The supero-inferior level of the vaginal merging with the urethra varied between specimens (Table 1), but tended to be lower in larger specimens. Thus, the present 14 female fetuses, as a whole, appeared to exhibit the process of vaginal descent. We found a point of high merging behind the symphysis pubis (Fig. 8), a point of intermediate merging at the level of the pubic arch (Figs. 5 and 7), and a point of low merging near the vestibulum or perineum (Fig. 6). The vaginal descent appeared to be complete at and around 15 weeks, or CRL 110–120 mm, although we did not examine any age groups later than 15 weeks. However, even at the early stage (CRL 48 mm or 9 weeks), a point of low merging was seen, in which the external urethral sphincter was located anterior to, and along the lateral aspects of the urethra (Fig. 6). In specimens with intermediate-level merging, the external urethral sphincter was attached to the anterior half of the urethra (Figs. 5B, C and 7A, B). The posterolaterally extending sphincter, which was similar to the male morphology, was evident at the level of the pubic arch in the specimens showing intermediate and high merging (Figs. 7 and 8D and E). Nevertheless, among specimens with various levels of vaginal merging, any difference was limited to 1–2 mm: e.g., the distance from the merging site to the vestibulum was 2.2 mm in the high-level merging type (Fig. 8) and 1.6 mm in the intermediate-level merging type (Fig. 7).

Along the female urethra at 9–12 weeks, a colliculus-like structure (an epithelial fold or protrusion) was seen at the level of the pubic arch or behind the symphysis pubis (6 specimens; Figs. 5B and 7A, B). At the colliculus-like structure, the opening of Gartner's ducts to the urethra was seen in 3 specimens (Fig. 8B). In a single specimen, the midline epithelial seam in the colliculus-like structure suggested an opening of the vaginal lumen into the urethra (Fig. 7B). In specimens with low merging (4 specimens), the vaginal lumen opened widely to the urethra near the vestibulum (Fig. 6D). However, on the superior side of the vaginal opening (Fig. 6B), there was no evident remnant of the urethral wall gap suggesting down-sliding of the vagina: we found only a belt-like protrusion of the vaginal tissue into the vaginal lumen. Likewise, we did not find either macrophage-like large cells or pyknotic or fragmented cells suggestive of cell death along and around the developing urethra and vaginal vestibulum.

3.3. Perineal structures in both genders

The topographical anatomy of the perineal structures, i.e., the bulbospongiosus muscle, the ischiocavernosus muscles and the crus of the clitoris or penis, were very similar between males and females in the early stages (Figs. 3 and 6D and E). However, the

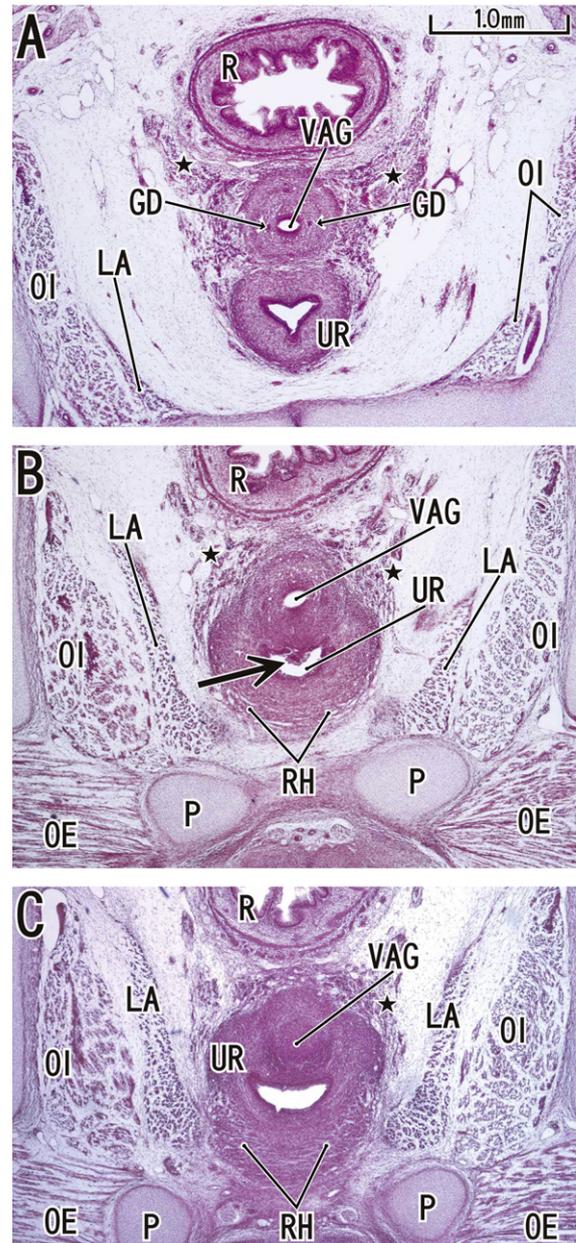


Fig. 5. Vagina ends at the urethra in the level immediately below the symphysis pubis (CRL 48 mm; middle-level merging of the vagina). Horizontal sections. Panel A (C) is the most superior (inferior) level in the figure. All panels are prepared at the same magnification (scale bar in panel A). Intervals between panels are 0.9 mm (A and B) and 0.4 mm (B and C). In panel A, the vagina (VAG) accompanies Gartner's ducts (GD; Wolffian duct remnants). In panels B and C, the vagina is deeply embedded in and interrupts the posterior wall of the urethra. A male colliculus-like structure is seen in panel B (arrow). The external urethral sphincter (rhabdosphincter, RH) attaches to the anterior half of the urethra (panels B and C). Stars indicate the pelvic nerve plexus. Other abbreviations, see common abbreviation. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

bulbospongiosus muscle was located alongside the vestibulum in females (Figs. 6–8), whereas it was alongside the urethra in males (Fig. 3). The male external urethral sphincter was connected with the crus penis by a distinct fascia. However, as we have described previously (Kato et al., 2008b), the female external urethral sphincter was located adjacent to the bulbospongiosus muscle because of

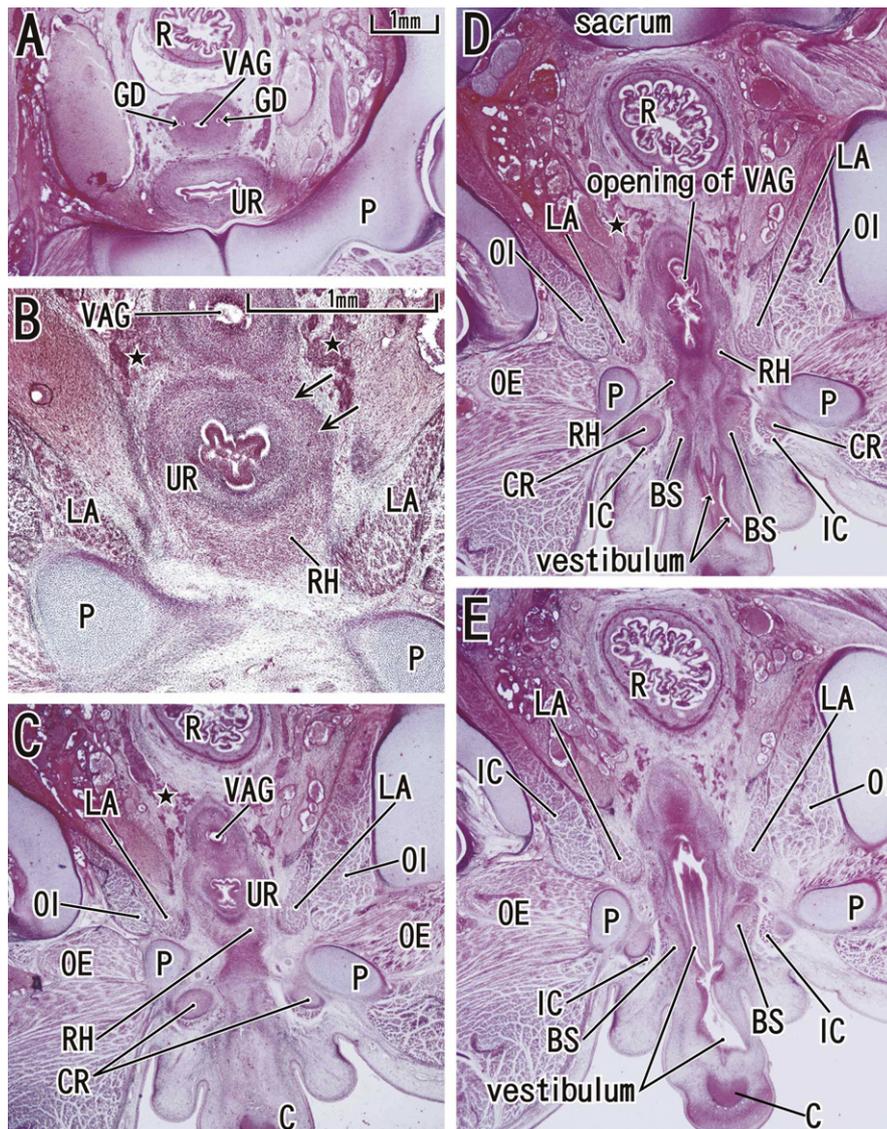


Fig. 6. Vagina merges with the urethra near the future vestibulum under the pubic arch (CRL 48 mm; low merging of the vagina). Horizontal sections. Panel A (E) is the most superior (inferior) level in the figure. Panels A, C–E are prepared at the same magnification, while panel B is different (scale bar in panels A and B). Intervals between panels are 1.5 mm (A and B), 0.5 mm (B and C), 0.6 mm (C and D) and 0.1 mm (D and E). Panel A displays the vagina (VAG) accompanying Gartner's ducts (GD; Wolffian duct remnants). In panel B, the vagina gives off a tissue band (arrows) toward the urethra (UR) but it does not suggest a destruction of the urethral posterior wall. The vagina becomes embedded into the urethral wall in panel C. In panel D, the vaginal lumen opens to the urethra in the level including the crus of the clitoris (CR). The external urethral sphincter (rhabdosphincter, RH) is located in the anterior side of the urethra (panels B and C) or alongside the urethra (panel D). Stars indicate the pelvic nerve plexus. Other abbreviations, see common abbreviation. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

absence of the fascia. This fascia was different from another fascial interface between the sphincter and inferomedial margin of the levator ani. On the dorsal side of the vestibulum, the deep transverse perineal muscle was seen connecting the bilateral Bartholin's glands (Fig. 7E). Neither the perineal membrane nor the vestibular bulb had developed in the specimens examined.

When the vagina merged with the urethra at a low level including the vaginal vestibulum, the topographical anatomy of the perineal muscles was very similar to that in males, e.g., Fig. 3D (male) and Fig. 6C (female). In these specimens at and around CRL 50 mm (approximately 9–10 weeks of gestation), both the external urethral sphincter and bulbospongiosus muscle were arranged along the lateral walls of the future membranous urethra (male) or vaginal vestibulum (female).

4. Discussion

The present study demonstrated differences between specimens in the supero-inferior levels at which the vagina and Gartner's ducts (Wolffian–Müllerian duct complex or Müllerian tubercle) merge with the developing urethra. Because the level became lower in larger specimens (Table 1), the sequence from a high merging morphology to low merging may represent the process of vaginal descent along the urethra. Previous German researchers seem to consider this sequential variation as a process of the vaginal descent itself (e.g., Nagel, 1896; Felix, 1911). Due to shortening of the urethra in the low-merging specimens, the distal urethra appeared to be incorporated in the vestibular wall or the future minor labium. However, we do not simply consider the lower vaginal merging is

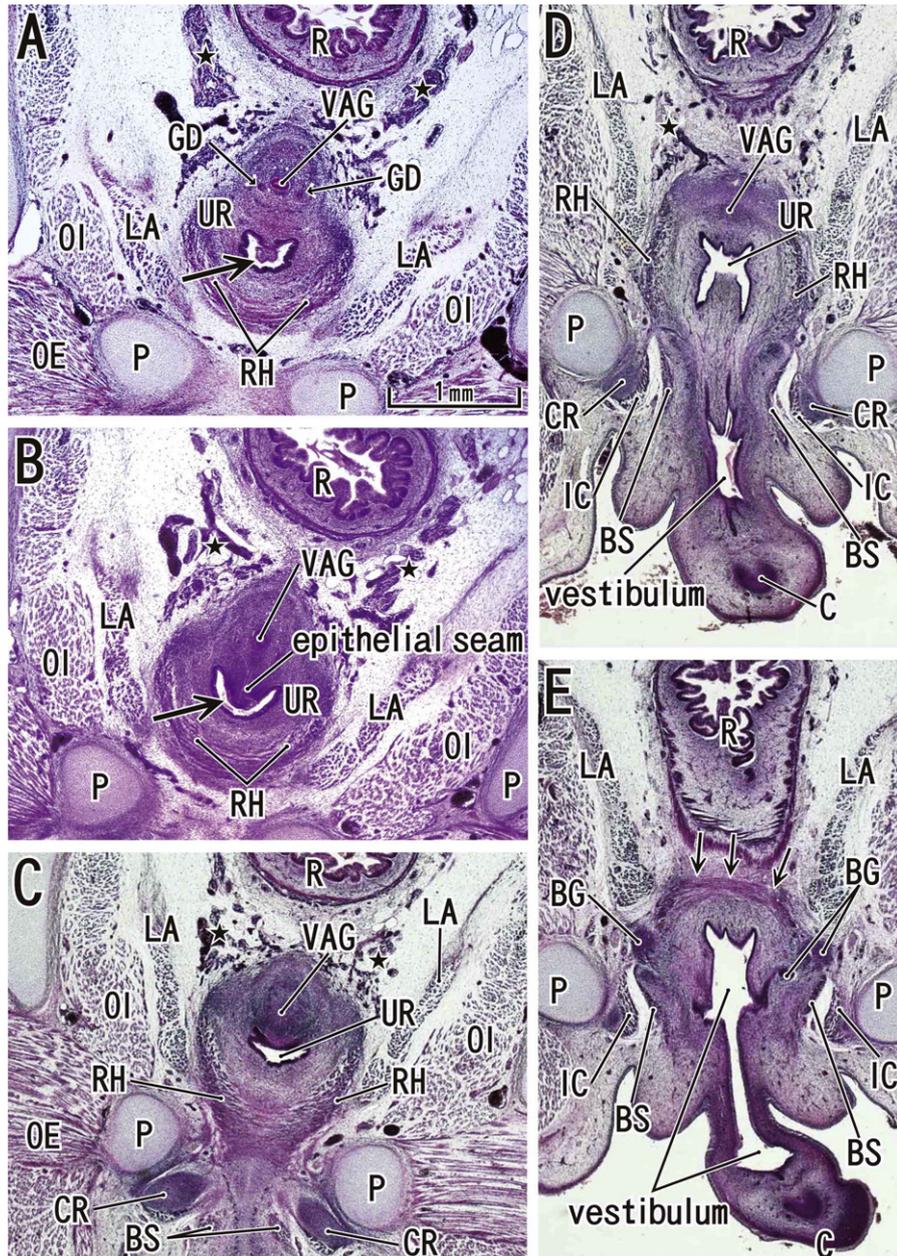


Fig. 7. Vagina ends at the urethra at the level immediately inferior to the symphysis pubis (CRL 62 mm; middle-level merging of the vagina). Horizontal sections. Panel A (or E) is the most superior (inferior) level in the figure. All panels are prepared at the same magnification (scale bar in panel A). Intervals between panels are 0.1 mm (A and B), 0.5 mm (B and C), 1.1 mm (C and D) and 0.5 mm (D and E). In panel A, the vagina (VAG) accompanies the Wolffian duct remnants, i.e., Gartner's duct (GD). The male colliculus-like structure is seen in panels A and B (arrows). In panel B, a midline epithelial seam is seen running to the urethra (UR). The vagina is deeply embedded in and interrupts the posterior wall of the urethra (panels B–D). The external urethral sphincter (rhabdosphincter, RH) attaches to the anterior half of the urethra (panels A–C) or the lateral aspects of the urethra (panel D). The deep transverse perineal muscle (arrows) extends between bilateral Bartholin's glands (BG) and along the posterior aspect of the vestibulum in panel E. Stars indicates the pelvic nerve plexus. Other abbreviations, see common abbreviation. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

a result of (1) sliding of the vagina along the urethra (O'Rahilly and Müller, 1996) or (2) elongation of the vagina (Cai, 2009).

One of the striking findings of this study appeared to be that, at the vaginal merging with the urethra, irrespective of the difference in supero-inferior levels, the inferior end of the vagina was very similar to the prostatic colliculus: in both genders, a tight tissue mass or solid epithelial cord was deeply embedded in the posterior wall of the urethra. Thus, a "simple attachment" of the genital tracts to the urethra seemed unlikely, as shown in Fig. 2. Previous researchers hypothesized that interfacial tissue had a lubrication role to assist the smooth sliding of the vagina (O'Rahilly and Müller,

1996). Even molecular guidance along the urethra has been proposed, such as a superior–inferior gradient of BMP4 expression (reviewed by Cai, 2009). However, the vaginal inferior end completely interrupted the urethral wall architecture. Therefore, sliding of the vagina along a "gap" in the urethral wall seems to be unlikely, as marked epithelial cell death and subsequent recovery would probably be required to accomplish this. In specimens showing low merging of the vagina, we did not find any evidence suggesting destruction of the urethral wall architecture during the vaginal descent. Likewise, it would seem difficult for openings of Gartner's ducts to slide along the urethral wall. Normal development would

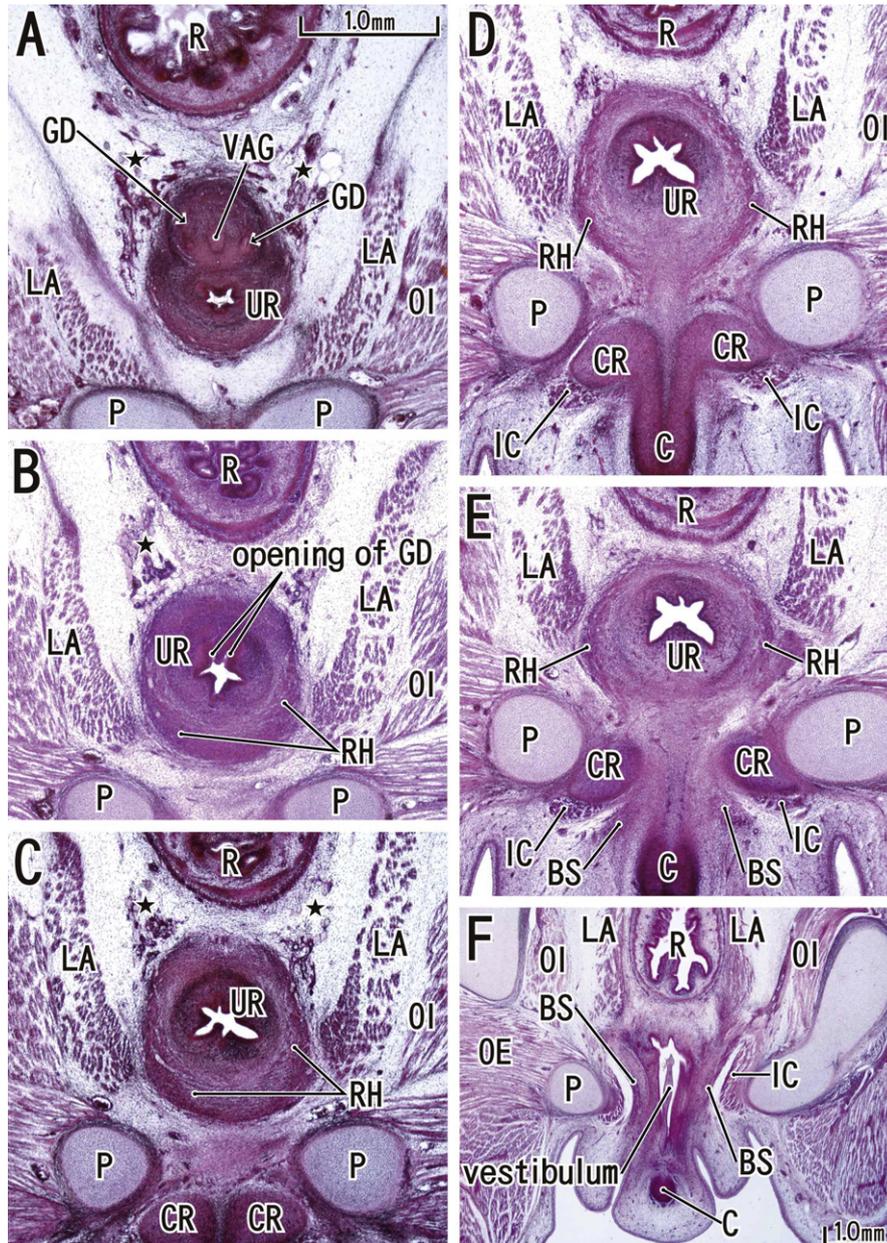


Fig. 8. Vagina ends at the urethra in the level behind the symphysis pubis (CRL 78 mm; high merging of the vagina). Horizontal sections. Panel A (F) is the most superior (inferior) level in the figure. Panels A–E are prepared at the same magnification (scale bar in panel A), while panel F at the lower magnification (scale bar in panel F). Intervals between panels are 0.6 mm (A and B), 0.2 mm (B and C), 0.4 mm (C and D), 0.2 mm (D and E) and 0.8 mm (E and F). The vagina (VAG) accompanies Gartner's ducts (GD) in panel A and they open to the urethra (UR) in panel B. The external urethral sphincter (rhabdosphincter, RH) attaches to the anterior half of the urethra in panels B and C, while it shifts posterolaterally in panels D and E. The bulbospongiosus muscle (BS) along the vestibulum faces to the ischiocavernosus muscle (IC). Stars indicates the pelvic nerve plexus. Other abbreviations, see common abbreviation. BS, bulbospongiosus muscle; C, clitoris; CR, crus of clitoris; GD, Gartner's duct (Wolffian duct remnant); IC, ischiocavernosus muscle; LA, levator ani muscle; OE, obturator externus muscle; OI, obturator internus muscle; P, pubic arch or symphysis pubis; R, rectum; RH, urethral external sphincter (rhabdosphincter); UR, urethra (or urogenital sinus); VAG, vagina; stars, pelvic nerve plexus.

be different from an experimental condition in which the Wolffian duct is able to descend into the penile bulb (Drews, 2007).

Because of suspected difficulty in changing site of the vaginal inferior end penetrating through the urethral wall (see above), we hypothesize that individual variations of levels (Table 1) occur in the “initial site of attachment” of the vagina to the urethra. However, in the variations, the difference in distance between the high and low merge cases was restricted to within 1–2 mm. Along the primitive urethra, we found no specific structure to “catch” or introduce the vagina and connect it with the urethra. Thus, the variation between 1 and 2 mm seems to be likely in the developmental dynamics. The growth rate of the urethral epithelium is lower than

that of the vagina (Boutin and Cunha, 1996, 1997): this data may be true. However, it seems to be more reasonable that, in accordance with the vaginal elongation, the urethra also elongates in the proximal side of the “initial attachment” of the vagina. Therefore, we consider the initial attachment between the vagina and urethra is fixed and stable during the relative descent (or elongation or sliding) of the vagina.

The topographical relationships among the urethra, Müllerian duct and Wolffian duct appear to be common between genders until the start of vaginal descent or prostate development (see the final paragraph of Section 3). However, in female adults, the urethrovaginal sphincter (Oelrich, 1983), originating from the

superficial and inferior parts of the external urethral sphincter and extending through a layer between the vestibular bulb and vaginal wall to reach Bartholin's gland, is thicker than the anteriorly restricted external sphincter (Kurihara et al., 2004; Kato et al., 2008a). Using 3D reconstruction, the developing urethrovaginal sphincter is well described by Wallner et al. (2009) as the "large muscular wings". To create the female specific topographical anatomy, in addition to elongation of the vagina and proximal urethra, the distal urethra is likely to be incorporated or absorbed into the vestibular wall. Therefore, as the minor or upper parts of the male external urethral sphincter are incorporated into the prostatic capsule (Elbadawi et al., 1997), the major or lower parts of the female external urethral sphincter in fetuses seem to be expelled from the original anterior position into the future minor labium.

As Wallner et al. (2009) suggested, the female urethra and its surrounding muscles develop under the strong influence of the relative descent of the vagina. In the present study, we dissected the "influence" into 3 factors: (1) individual variations in the site of initial attachment of the Wolffian–Müllerian duct complex to the urogenital sinus; (2) elongation of the proximal urethra in the superior side of the initial attachment and; (3) incorporation of the distal urethra into the vaginal vestibulum. These factors may cause considerable individual variations of the adult perineal membrane: the thick and wide case of the membrane takes the high superolateral margin in contrast to the thin and narrow case along the vaginal vestibulum (Kato et al., 2008a; Hirata et al., 2010). According to Kato et al. (2008a), the external urethral sphincter is located in the superior side of the elastic fiber-made perineal membrane, while the urethrovaginal sphincter and compressor urethra (Oelrich, 1983) are embedded in the membrane. Thus, the female distalmost urethra shows a morphology somewhat similar to the male membranous urethra. Nevertheless, according to the present hypothesis that the initial attachment of the vagina is fixed and stable, a part corresponding to the male membranous urethra seems to be absent in females. Consequently, the answer to our original question is that the female urethra seems to correspond to the male prostatic urethra superior to the prostatic colliculus.

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