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ORIGINAL ARTICLE

Fetal growth of the anal sinus and sphincters, especially in relation to anal anomalies

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Abstract
Purpose and methods The anal sinuses, small furrows above the pectinate line, sometimes form perianal abscesses in adults. We examined the pattern of fetal growth of the anal sinus and sphincters using 22 mid-term (8–18 weeks) and 6 late-stage (30–38 weeks) fetuses.
Results In mid-term fetuses, the external and internal sphincters gradually increased in thickness, depending on specimen size (from 0.2 to 1.5 mm), whereas the anteroposterior diameter of the anal canal at the epithelial junction was relatively stable (0.5–1.0 mm) irrespective of specimen size. Anal canal diameter increased less than twofold between mid-term and late-stage fetuses, from 0.5–1.0 to almost 2 mm, whereas sphincter thickness increased over tenfold, from 0.2–1.5 to almost 3.5 mm. The anal sinus often showed balloon-like enlargement when the sphincter muscle bundles were tightly packed in mid-term, but not in late-stage fetuses.
Conclusions Large concentric mechanical stress from the sphincters in late-stage fetuses apparently prevented the anal sinus from expanding in a balloon-like manner. Conversely, to avoid anal stenosis, the growing sinuses maintained a luminal space of the anal canal in response to stress from rapidly growing sphincters. The inferiorly extending sinus usually provided temporal double canals separated by a thick column. In the presence of double lumens, anal canal duplication is likely to develop without any abnormalities of the anal epithelium and sphincters.

Keywords Anal sinus · Anal sphincter · Anal transitional zone · Anal stenosis · Anal canal duplication · Human fetus

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Introduction

The anal columns, consisting of six to ten vertical epithelial folds in the lower anal canal, are lined by non-keratinized stratified squamous epithelium and are much more evident in children than in adults. The lower ends of the columns are linked by small crescentic mucous folds, the anal valves, above each of which is a small recess, i.e., the anal sinus [1]. The sinuses or crypts of Morgagni, deepest in the posterior wall, may retain fecal matter and become infected, leading to abscess formation inside and outside the anal canal wall [2–5]. In those mucosal structures, anal sinuses first appear in mid-term fetuses, of gestational age 8–14 weeks [6–9]. Although corresponding to a site immediately above the pectinate line in adults, the non-keratinized stratified squamous epithelium from which the anal sinuses originate has been called the anal transitional zone [10] and the intermediate epithelium [8]; we describe it as the transitional epithelium.

Although many studies have examined the early development of the fetal anus (e.g., van der Putte [11]; Kluth et al. [12]; Nievelstein et al. [13]; Fritsch et al. [14]; Yamaguchi et al. [15]; Zhang et al. [16]), few have described the manner of growth of fetal anal sinuses. Since a supero-inferior extension of the developing anal sinus corresponds to an extension of the external anal sphincter [6], there is likely a positive or negative correlation in the growth of the anal sinus and sphincter. In infants with anal canal duplication, a rare anomaly in the posterior midline without any anomaly in the sphincters and transient epithelium, the additional canal is histologically similar to the developing anal sinus [17–19]. Similarly, evaluation of a 22-week specimen found that the developing anal sinus provides a longitudinal mucosal septum separating the anal canal into two lumens [20]. Therefore, we hypothesized that the developing anal sinus contributes to both the normal and abnormal morphogenesis of the anal canal. Consequently, this study was designed to examine the manner of growth of the fetal anal sinus and sphincter.

Materials and methods

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). Sagittal or frontal horizontal paraffin sections stained with hematoxylin and eosin (5–10 μm in thickness) were obtained from the sacral and lower lumbar region of 28 fetuses: 5 of crown-rump length (CRL) 35–41 mm (approximately 8 weeks), 4 of CRL 45–52 mm (10 weeks), 7 of CRL 61–80 mm (12 weeks), 4 of CRL 105–118 mm (15 weeks), 2 of CRL 155 and 160 mm (18 weeks), and 6 of CRL 240–310 mm (30–37 weeks). Sections from the mid-term fetuses (8–15 weeks) were serial, while those from the late-stage fetuses were prepared at 0.2–0.5-mm

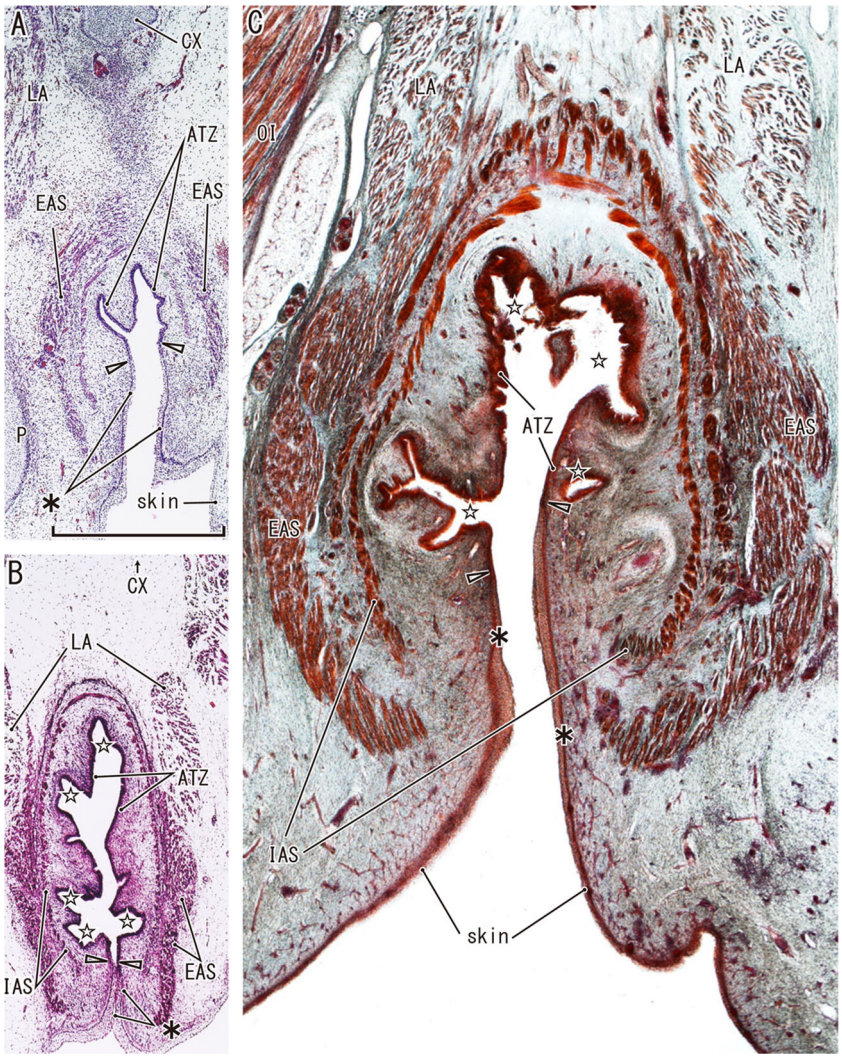

Fig. 1 Frontal sections of the anal canal of three mid-term specimens, one each at approximately 8, 10, and 18 weeks, stained with HE. All panels were prepared at the same magnification (*scale bar* in (**a**), 1 mm). **a** A fetus of CRL 38 mm (8 weeks); **b** a fetus of CRL 52 mm (10 weeks); **c** a fetus of CRL 150 mm (18 weeks). The anal sinus (*stars*) originated from the transitional epithelium (anal transitional zone, ATZ). *Arrowheads* indicate the skin-transitional epithelial junction. The transitional epithelium is thick and may contain mucus cells, as in (**c**). The keratinized squamous epithelium covering the lowermost part of the anal canal (*asterisk*) is thin in (**a**), but thick in (**b**) and (**c**). In contrast to the anal canal width, the external and internal anal sphincters (EAS, IAS) showed increases in thickness, from 0.2 mm (8 weeks) to 1.0 mm (18 weeks), at the epithelial junction level. The difference in numbers of anal sinuses (*stars*) among panels depended on the sectional plane. Other abbreviations, see the common abbreviations

intervals. Sagittal sections were prepared from 16 mid-term and two late-stage fetuses, whereas frontal sections were prepared from six mid-term and four late-stage fetuses.

Ten of the mid-term specimens (nine for sagittal sections and one for frontal sections) belonged to the Blechschmidt collection at the Medical Museum of Georg-August-Universität Gőttingen [21]. We did not need approval of the university ethics committee to examine this material. The other 12 mid-term fetuses had been donated by families to the Department of Anatomy, Chonbuk National University, South Korea, and their use for research was approved by the university ethics committee. These fetuses had been obtained by induced abortion, after which the mother was orally informed by an obstetrician (at a single hospital) about the possibility of donation for research; no attempt was made to actively encourage the donation. If the mother subsequently agreed, the fetus was given an assigned specimen number and stored in 10 % *w*/*w* formalin solution for more than 3 months. Because of specimen number randomization, there was no possibility of contacting the family at a later date.

The six late-stage fetuses were part of the large collection kept at the Embryology Institute, Universitad Complutense, Madrid, and were products of miscarriages managed by the Department of Obstetrics at the university. The study protocol was approved by our university ethics committee (No. B08/374).

In addition to routine staining methods, such as hematoxylin and eosin (HE) staining, paraffin sections from 5 of the 22 mid-term fetuses and from 2 of the 6 late-stage fetuses were

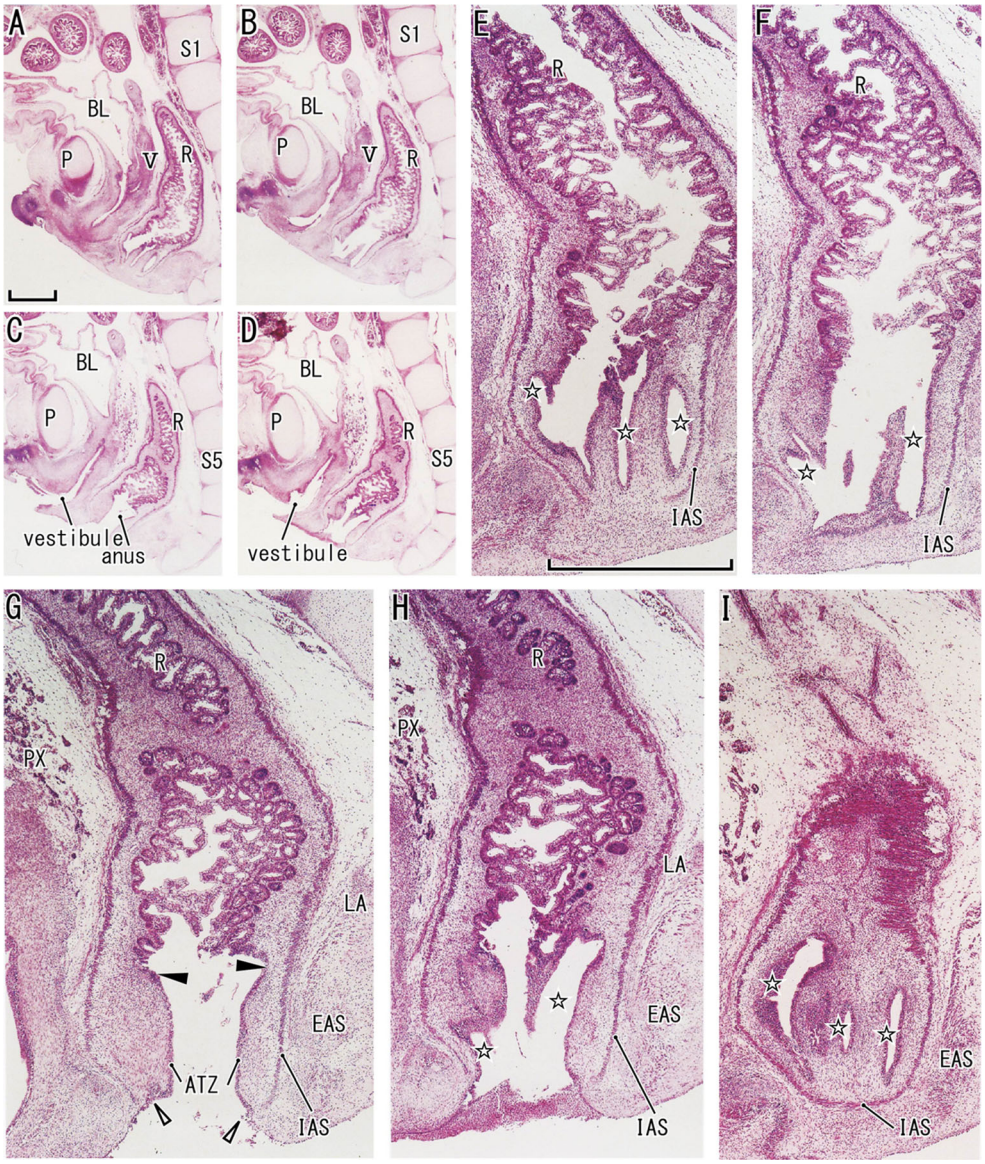

Fig. 2 Sagittal sections of the anal canal in a fetus of CRL 36 mm (approximately 8 weeks). HE staining. **a**–**d** Topographical anatomy at and around the anal canal at the lower magnification (*scale bar* in (**a**), 1 mm). The vagina (*V*) has not fully descended. Intervals between panels were 0.1 mm. **e**–**h** Higher-magnification views (*scale bar* in (**e**), 1 mm) of the anal canal shown in (**a**)–(**d**). **i** A site 0.1 mm lateral to (**h**). **e**, **f**, **h**, **i** The anal sinuses (*stars*) make slit-like cavities. **g** The epithelium between the *filled* and *open arrowheads* corresponds to the anal transitional zone (*ATZ*). *Filled arrowheads* indicate the transitional-columnar epithelial junction, while *open arrowheads* indicate the skin-transitional epithelial junction. The internal anal sphincter (*IAS*) is not differentiated but appears as a lower continuation of the circular muscle layer of the rectum. The external anal sphincter (EAS) contains sparsely distributed striated muscle fibers. Other abbreviations: See the common abbreviations

assessed immunohistochemically for proteins present in smooth muscles and in epithelia. The primary antibodies used in these experiments were (1) mouse monoclonal anti-human alpha smooth muscle actin (SMA; 1:100; Dako, Glostrup, Denmark; #M0760), a marker of the internal anal sphincter; and (2) mouse monoclonal anti-human cytokeratin-19 (CK19 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA; #sc-6278), a marker for transitional epithelium. When testing for CK19 expression, antigen retrieval was performed by microwave treatment (500 W, 15 min, pH 6). The samples were washed and incubated with secondary antibody (1:1000; Histofine Simple Stain Max-PO, Nichirei, Tokyo) labeled with horseradish peroxidase (HRP) for 30 min, and antigen–antibody reactions were detected by the HRP-catalyzed reaction with diaminobenzidine (incubation for 3–5 min; Histofine Simple Stain DAB). All samples were counterstained with hematoxylin. Negative controls consisted of samples without primary antibody.

Results

Figure 1 shows a summary of anal canal growth in mid-term fetuses. The increase in size of the external anal sphincter was greater than that of the anal canal lumen, including the anal sinus. Sagittal sections (Figs. 2, 3, 4, and 6) showed the entire topographical anatomy around the anal canal and rectum, while frontal sections (Figs. 1, 5, and 7) showed both the

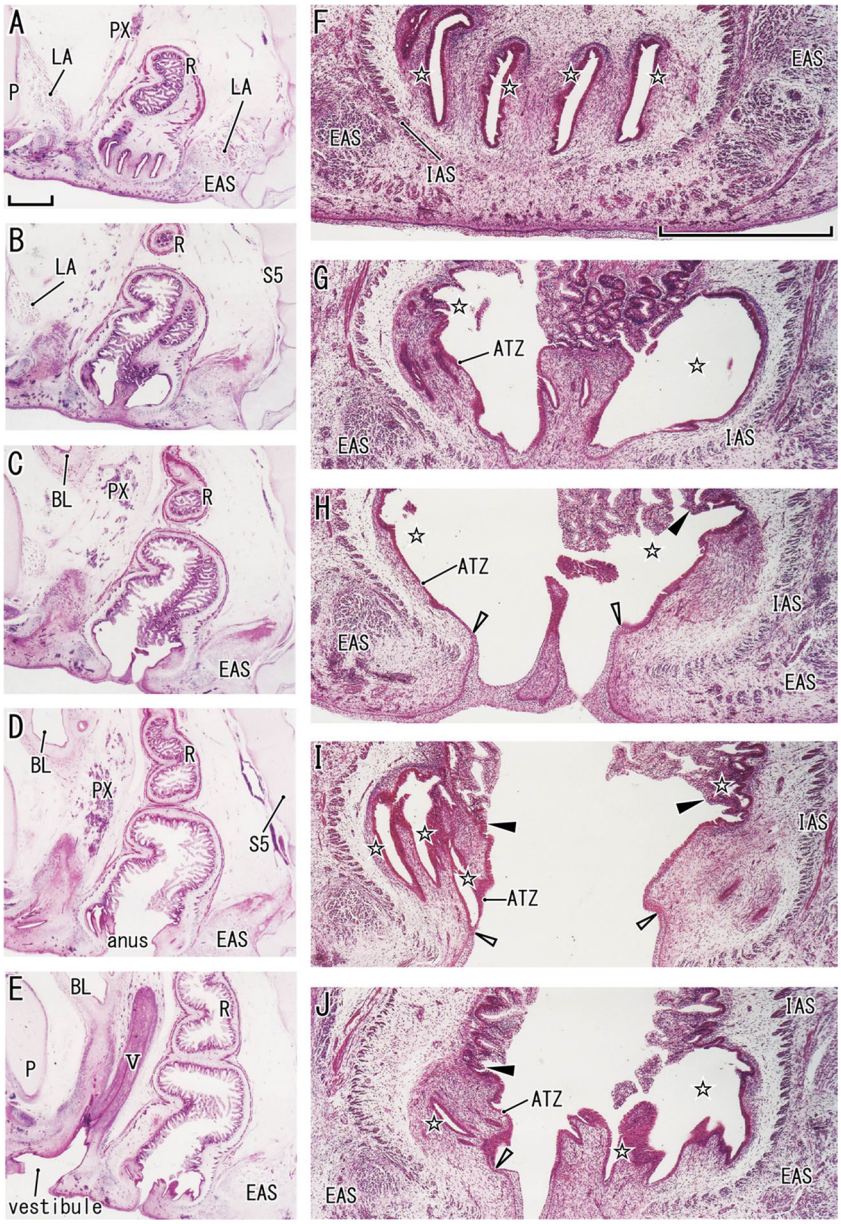

Fig. 3 Sagittal sections of the anal canal in a fetus of CRL 61 mm (approximately 12 weeks). HE staining. **a**–**e** Topographical anatomy at and around the anal canal at lower magnification (*scale bar* in (**a**), 1 mm). Intervals between panels were 0.5 mm (**a**–**b**), 0.2 mm (**b**–**c**), 0.4 mm (**c**–**d**), and 0.9 mm (**d**–**e**). **f**–**j** Higher-magnification views (*scale bar* in (**f**), 1 mm) of the anal canal shown in (**a**)–(**e**). The anal sinuses (*stars*) make slit-like cavities in the lateral site but show a balloon-like appearance on the medial site (**g** and **h**). *Filled arrowheads* indicate the transitional-columnar epithelial junction, while *open arrowheads* indicate the skin-transitional epithelium junction. The internal anal sphincter (*IAS*) is developing, while the external anal sphincter (*EAS*) increases in thickness. Other abbreviations: see the common abbreviations

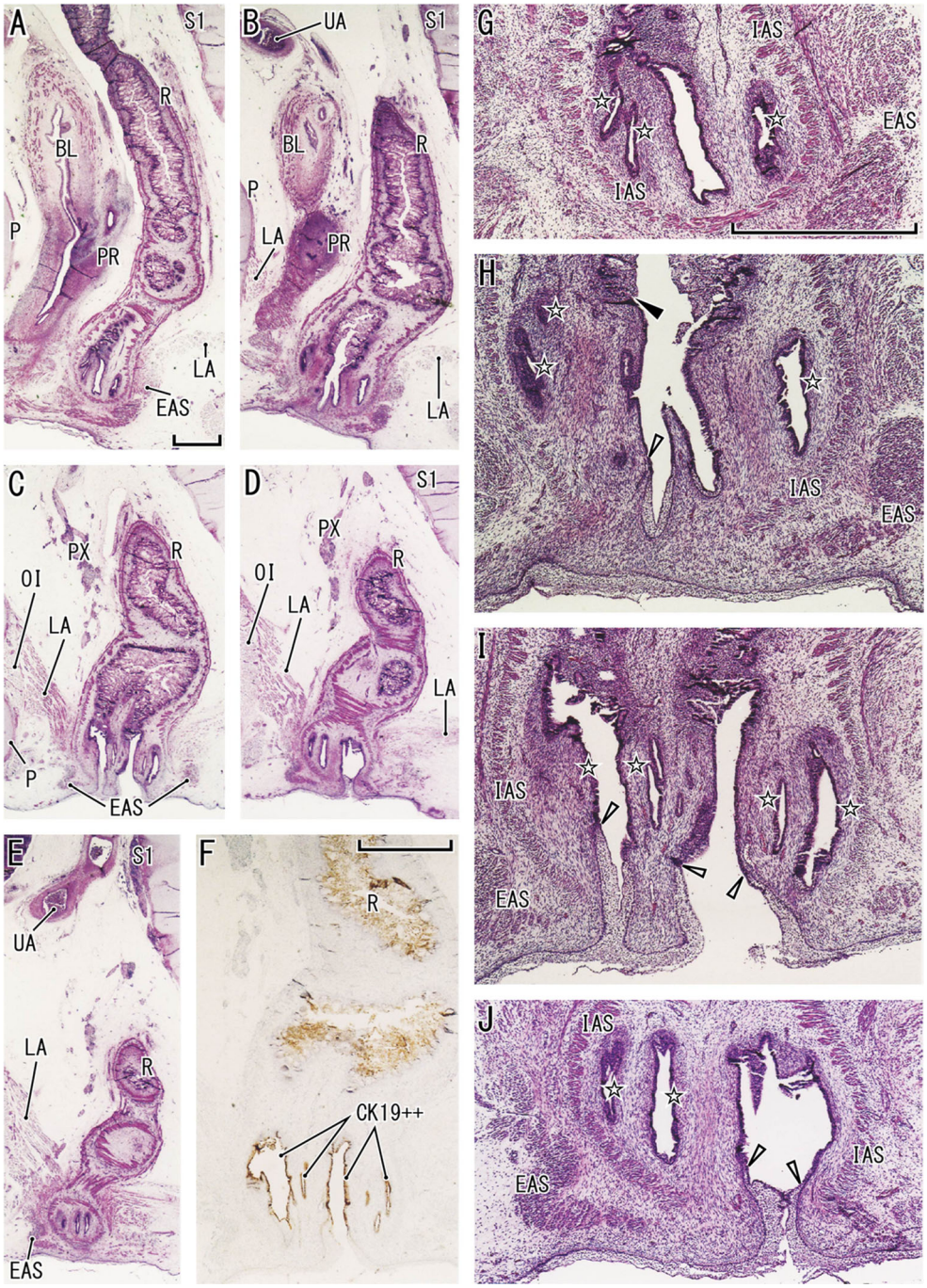

Fig. 4 Sagittal sections of the anal canal in a fetus of CRL 115 mm (approximately 15 weeks). HE staining. **a**–**e** Topographical anatomy at and around the anal canal at lower magnification (*scale bar* in (**a**), 1 mm). Intervals between panels were 0.8 mm (**a**–**b**, **b**–**c**), 0.5 mm (**c**–**d**), and 0.3 mm (**d**–**e**). **f** A section near (**c**), showing strong reactivity of the anal sinus to antibody against cytokeratin-19 (*CK19++*) (*scale bar*, 1 mm). **g**–**j** Higher-magnification views (*scale bar* in (**g**), 1 mm) of the anal canal shown in (**a**)–(**d**), respectively. The anal sinuses (*stars*) make slit-like cavities. The *filled arrowhead* in (**h**) indicates the transitional-columnar epithelial junction. *Open arrowheads* indicate the skin-transitional epithelial junction. Both the external and internal anal sphincters (*EAS*, *IAS*) are well developed. Other abbreviations: see the common abbreviations

coccyx and anus because of the curving of the vertebral column from the sacral to the coccygeal segments.

In the mid-term fetuses, the anal transitional epithelium was stained dark violet, whereas the squamous epithelium continuous with the anal skin showed pale staining (Figs. 1, 2, 3, 4, 5, and 6). The columnar epithelium could be distinguished from the transitional epithelium by the presence of abundant goblet cells with palely stained, abundant cytoplasm. The morphology of late-stage fetuses was similar to that of adults, with the transitional epithelium becoming column-like, although the actual columnar epithelium contained abundant tall goblet cells (Figs. 6 and 7). The internal anal sphincter in 8- and 10-week fetuses was thin and appeared as a lower continuation of the circular smooth muscle layer of the rectum (Fig. 2e–h), becoming thicker than the rectal circular muscle layer at 12–18 weeks (Figs. 3h, 4j, and 5c). Despite the muscle layer being much thicker in the internal sphincter, the striated muscle bundles of the external sphincter were often

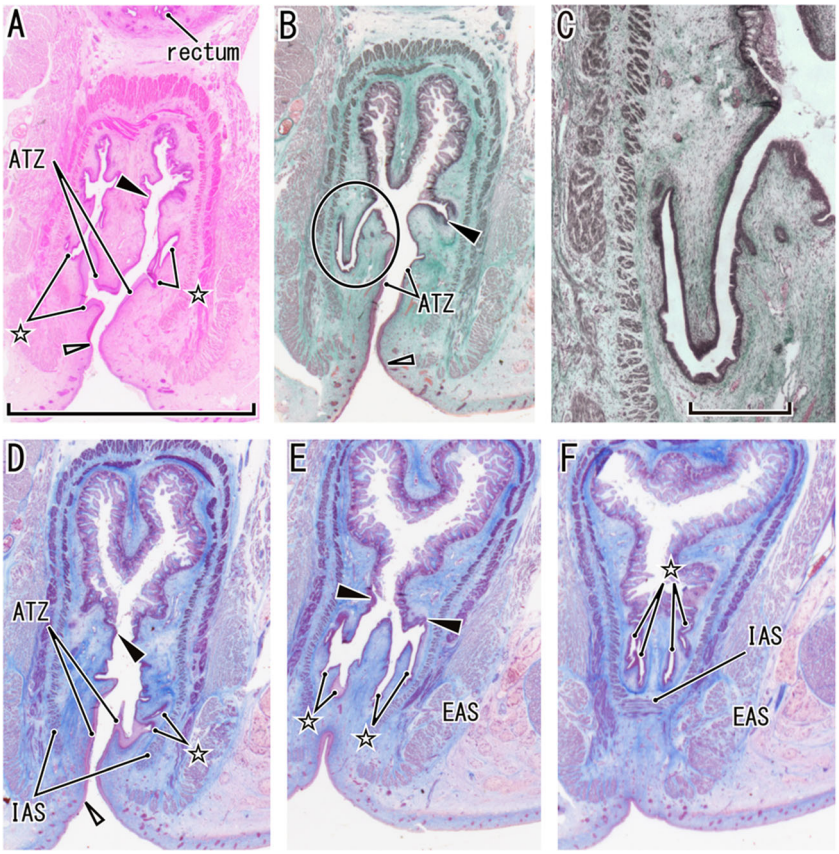

Fig. 5 Frontal sections of the anal canal in a fetus of CRL 160 mm (approximately 18 weeks). HE staining (**a**); Masson trichrome staining (**b** and **c**); azan staining (**d**–**f**). **a**, **f** The most anterior and posterior sides of the figure, respectively. **c** A higher-magnification view of the circle in (**b**). Intervals between panels were 0.1 mm (**a**–**b**, **b**–**d**, **d**–**e**, **e**–**f**). The anal sinuses (*stars*) formed slit-like cavities. A curved long protrusion of the anal sinus was present in the anal mucosa (**b** and **c**). *Filled arrowheads* indicate the transitional-columnar epithelial junction. *Open arrowheads* indicate the skin-transitional epithelial junction. **a**, **b** and **d**–**f** Preparations at the same magnification (*scale bar*: 10 mm in (**a**); 1 mm in (**c**)). Abbreviations: see the common abbreviations

sparsely packed along the external aspects of the internal sphincter at 8–12 weeks (Figs. 2g and 3h and Table 1). At 15 weeks (Fig. 4a–c), the external sphincter became tight and thick, and a continuation with the levator ani muscle became evident.

Along the entire circumferential wall, the anal sinus at 8 weeks had already started to develop from the transitional epithelium (Figs. 1a and 2e, i). All mid-term specimens showed three to four sinuses along each of the lateral aspects of the anal canal and one to two each from the anterior and posterior aspects. Anal sinuses appeared as vertical slit-like shapes at 8 and 10 weeks, becoming enlarged and showing a balloon-like appearance, especially in the lateral aspects, at 12 and 15 weeks (Fig. 3a and Table 1). However, balloon-like sinuses were not observed in any of the 18-week specimens and late-stage fetuses. One or two of the primitive anal columns between the developing sinuses usually reached the primitive anal skin, dividing the anus into two or three openings (Figs. 2f, 3h, 4i, and 6c, e). The anal sinuses were lined by transitional epithelium that showed strong expression of CK19 (Figs. 4f and 6f), but the covering epithelial cells became tall in the late-stage fetuses. We did not observe extension of the anal gland from the anal sinus to insert into the internal anal sphincter or a more external site. However, the anal sinus sometimes showed a long curved protrusion into the anal mucosa; this morphology was similar to a pathological fistula (Figs. 5b and 6b, e).

The diameter of the anal canal lumen was consistently greater along the anteroposterior than the left–right axis (e.g., Fig. 1a vs. Fig. 2g). The anteroposterior diameter of the anal lumen, when measured in sagittal sections of 16 mid-term fetuses at the level of the skin-transitional epithelial junction, varied between 0.5–1.0 mm. Notably, anal canals of larger diameter were not usually present in the larger specimens (Table 1), indicating that anal canal diameter and fetal size were not correlated (Fig. 1). In contrast, at the same supero-inferior level of the anal canal, the thickness of the external and internal sphincters increased gradually from 0.2 to 1.5 mm (Fig. 1 and Table 1), with sphincter thickness reaching 4.5 mm in late-stage fetuses. Although sagittal sections were prepared from only two of the six late-stage fetuses, the difference in anal canal diameter between mid-term and late-stage fetuses was less than twofold, from 0.5–1.0 to almost 2 mm. In contrast, sphincter thickness differed markedly, from 0.2–1.5 to 3.0–4.5 mm. In the late-stage fetuses, immunohistochemistry of smooth muscle markers demonstrated that the rectal muscularis mucosae extended to the upper part of the anal sinus (Figs. 6g, h and 7d). In addition, the conjoint longitudinal muscle was usually well developed in these late stage fetuses (Figs. 6a, b and 7b).

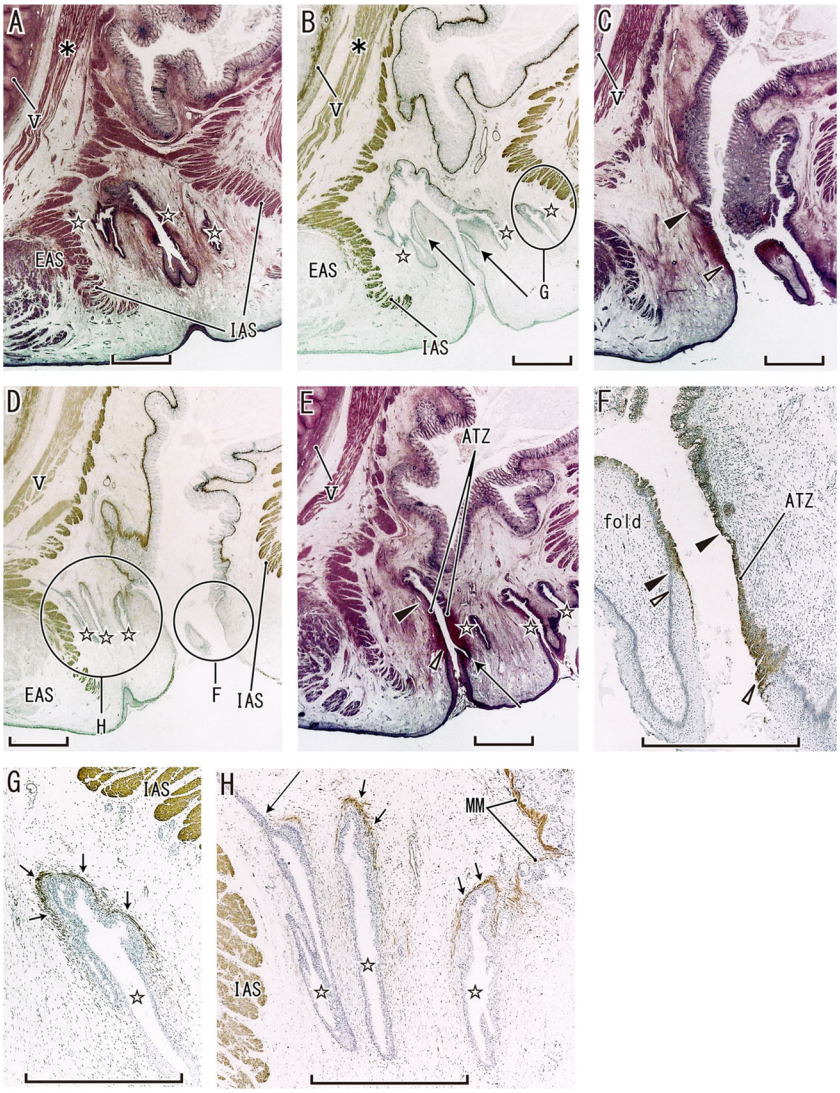

Fig. 6 Sagittal sections of the anal canal in a fetus of CRL 250 mm (approximately 30 weeks). The left-hand side of each panel corresponds to the anterior side of the pelvis. **a**, **c**, **e** HE staining and **b**, **d** immunohistochemistry of smooth muscles showing slit-like cavities of the anal sinus (*stars*). The external and internal anal sphincters (*EAS*, *IAS*) are very thick. Intervals between panels are 0.1 mm (**a**–**b**), 0.3 mm (**b**–**c**), 0.1 mm (**c**–**d**), and 0.3 mm (**d**–**e**). **b** Two protrusions of the anal sinus (*long arrows*). Note the multiple sinuses on the posterior side of the true anal canal in (**e**). **f** Immunohistochemistry of cytokeratin-19, corresponding to the circle labeled *F* in (**d**), showing high expression of cytokeratin at the transitional epithelium (*ATZ*). The transitional zone at the surface of the mucosal fold was very small (*filled arrowheads*, the transitional-columnar epithelial junction; *open arrowheads*, the skin-transitional epithelial junction). **g**, **h** (immunohistochemistry of smooth muscles) Higher-magnification views of the anal sinus corresponding to circles in (**b**) and (**d**), respectively. The muscularis mucosa (*MM*) extends to the upper part of the anal sinus (*short arrows*). The conjoint longitudinal muscle on the anterior side of the anal canal was well developed (*asterisks* in (**a**) and (**b**)). **a**–**e** (**f**–**h**) were prepared at the same magnifications (*scale bar*: 1 mm). Other abbreviations, see the common abbreviations

Discussion

Relatively little is known about the embryologic development of the anal sinus, primarily because of its very slow growth in mid-term fetuses. In contrast, we observed a gradual but marked increase in anal sphincter size especially of the external striated sphincter. To maintain a narrow lumen in mid-term fetuses, the potentially growing anal sinus seemed to resist concentric stress from the thicker sphincter. Great concentric stress from the sphincters in late-stage fetuses apparently prevented the anal sinus expanding in a balloon-like manner, despite the thickness of the anal canal. Even in late-stage fetuses, in which the prostate or vagina occupied a large space on the anterior side of the anal canal, the anal sinuses were equally deep along the entire circumferential wall. Although the change appeared not to be evident, the anal sinus seemed to maintain the anal luminal space despite concentric mechanical stress from the rapidly growing

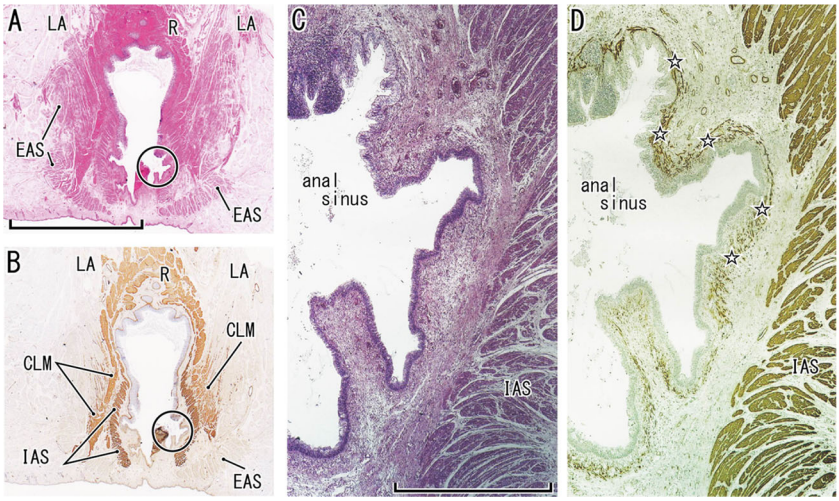

Fig. 7 Frontal sections of the anal canal in a fetus of CRL 310 mm (approximately 37 weeks). **a** HE staining and **b** smooth muscle immunohistochemistry display adjacent sections at the same magnification (*scale bar* in (**a**), 10 mm). The external and internal anal sphincters (*EAS*, *IAS*) are very thick. The levator ani muscle (*LA*) approaches the longitudinal smooth muscle layer of the rectum to provide the conjoint longitudinal muscle (*CLM*). **c**, **d** Higher magnifications (*scale bar* in (**c**), 1 mm) of a circle in (**a**) and (**b**), respectively. The anal sinus is lined by the muscularis mucosae (*stars* in (**d**)). Most parts of the anal sinus are still covered by the transitional epithelium. Other abbreviations: see the common abbreviations

sphincters. Conversely, without the developing anal sinus, the anal canal could experience stenosis. Although anal stenosis is considered a rare anomaly [22], our findings suggested that, depending on the magnitude of unbalanced growth between the anal sinus and external sphincter, various types of stenosis likely occur in infants.

Table 1 Morphologies of the anal sinus and sphincters in 18 specimens for sagittal sections

Specimen (CRL)	Anal canal lumen[a]	Sphincters[b]	Anal sinus[c]
2-6-49 (35 mm)	0.5 mm	0.2 mm, loose	Slit-like
K36 (36 mm), Fig. 2	0.5 mm	0.3 mm, loose	Slit-like
8-8-50 (39 mm)	0.5 mm	0.3 mm, loose	Slit-like
17-12-48 (41 mm)	0.6 mm	0.5 mm, tight	Balloon-like
23-4-50 (45 mm)	0.5 mm	0.5 mm, tight	Slit-like
8-1-59 (45 mm)	0.5 mm	0.6 mm, tight	Slit-like
8-6-49 (51 mm)	0.8 mm	0.5 mm, loose	Slit-like
B61 (61 mm), Fig. 3	1.0 mm	0.7 mm, loose	Balloon-like
24-4-50 (64 mm)	0.6 mm	0.8 mm, tight	Balloon-like
7-7-50 (65 mm)	0.8 mm	0.8 mm, loose	Slit-like
13-12-51 (76 mm)	0.5 mm	1.0 mm, tight	Balloon-like
L80 (80 mm)	0.5 mm	0.8 mm, tight	Balloon-like
12-14P (105 mm)	1.0 mm	0.9 mm, tight	Balloon-like
N 110 (110 mm)	0.5 mm	1.2 mm, tight	Balloon-like
12-15P (115 mm), Fig. 4	0.6 mm	1.0 mm, tight	Slit-like
12-16P (118 mm)	0.5 mm	1.5 mm, tight	Slit-like
250 F (250 mm)	1.5 mm	3.0 mm, tight	Slit-like
300 F (300 mm), Fig. 6	2.4 mm	4.5 mm, tight	Slit-like

Above the dotted line, 16 mid-term fetuses

[a] The anteroposterior length of the anal canal lumen was measured immediately below the anal sinus or at the skin-transitional epithelial junction

[b] The thickness of the external and internal sphincters was measured in the level of the skin-transitional epithelial junction in the posterior side of the canal lumen. tight or loose: Muscle bundles of the external anal sphincter were packed tightly or loosely in the posterior side of the internal sphincter

[c] Shape of the anal sinus

The external outgrowth of the anal sinus might occur after birth to provide the anal gland. Perianal abscess in adults, an infection of the anal gland, is a major target of anal surgeons. Although its incidence is much lower in children than in adults, its frequency is higher in infants than in children [23]. One of the striking features observed in the present study was the curved long protrusions from the anal sinus. These long protrusions into the anal mucosa may connect with the future anal gland via a complicated anatomic course and with many ramifications. Therefore, inter-individual differences in anal abscesses in adults may be due to the individual fetal morphology of the anal sinus. In late stage fetuses, the conjoint longitudinal muscle coat [24] was usually well developed. Along with the internal anal sphincter, this thick smooth muscle layer may prevent outgrowth of the anal gland.

Another striking feature in the present study was found in the anal double lumens divided by a thick vertical column: One was a real anal lumen, while the other was an anal sinus. Anal canal duplication is a rare anomaly [18], which may be due to (1) an abnormal (duplicated) cloaca [11] or (2) a failure of recanalization [13]. An abnormal cloaca is most likely to be combined with severe abnormalities of the vertebrae and urogenital sinus. However, such combined abnormalities have not been reported in patients with anal duplication [18]. Recanalization has been suggested (e.g., Fritsch et al. [14]) but may not occur [10]. Despite the epithelia being attached to each other, we did not observe recanalization in any of these specimens. Anal canal duplication has been associated with normal sphincters and epithelia [17, 19]. We hypothesized that an inferiorly extending anal sinus may result in an additional lumen for anal canal duplication. In addition, some of the anomalous ano-urogenital communications in imperforate anuses are apparently similar to those of developing anal sinuses [25]. Indeed, the fetal anal sinus developed behind the great mass of the developing vagina.

The term “skin-transitional epithelial junction” may be unfamiliar in comparison with the term “squamous-columnar epithelial junction” (i.e., the dentate line) in adults. Actually, many embryologists have ignored the presence of the transitional epithelium (e.g., Fritsch et al. [14]). However, the transitional epithelium plays a critical role in anal development because it differentiates into the anal sinus. Thus, we used the original term, anal transitional zone (ATZ [11]), for the present figure caption.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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