

## Variations in Laminar Arrangements of the Mesocolon and Retropancreatic Fascia: a Histological Study Using Human Fetuses Near Term

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**Objective:** The embryonic mesentery of the ascending and descending colons as well as the pancreas disappears due to peritoneal fusion, but there might be no or few photographic demonstrations of the intermediate morphologies during the process. The aims of this study were to characterize the morphological relationship of the interface between the renal fascia and peritoneum.

**Methods:** Fourteen late-stage fetuses with crown rump lengths (CRLs) of 250–325 mm (gestational age: 30–38 weeks) were histologically examined.

**Results:** The renal fascia, a thick or thin layer consisting of densely-distributed abundant fibers, was consistently separated from the renal capsule by a perirenal space containing fat. The transverse colon carried a typical mesocolon histologically different from the renal fascia. The ascending and descending mesocolons were irregularly divided into multiple laminae and the colic external longitudinal muscle appeared to directly contact the renal fascia. There was a spectrum of variations from multiple laminae to a single thick fascia between the pancreatic body and the left kidney or adrenal.

**Conclusions:** A fascial development after retroperitoneal fusion of the mesentery showed great individual and site-dependent differences in proportion of 1) a complete fusion with the renal fascia and 2) a multilaminar structure including the remnant peritoneum. These variations masked the likely stage-dependent change.

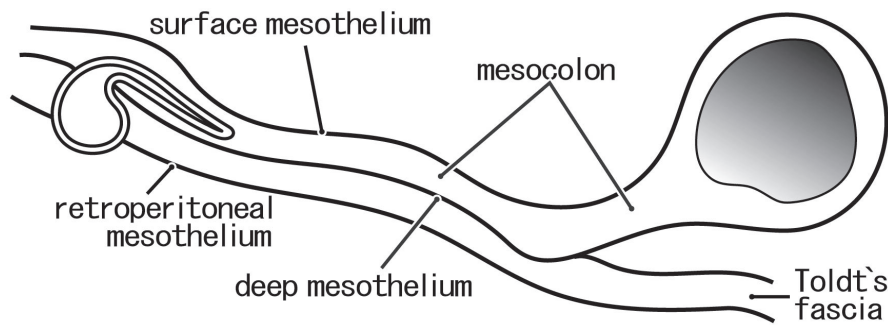
**Key words:** Toldt's fascia, fusion fascia of Treitz, peritoneum, mesocolon, pancreas, renal fascia, human fetus

### INTRODUCTION

The embryonic mesenteries of the ascending and descending colons (AC, DC), as well as of most parts of the duodenum and pancreas “disappear” because of the peritoneal fusion after repackaging from physiological umbilical herniation of the abdominal alimentary canal [1, 2]. As a retroperitoneal fascial configuration after disappearance, anatomists and surgeons hypothesize that remnants of the peritoneum cover the embryonic mesentery, that is, Toldt's fascia behind the AC and DC [3–5] and the fusion fascia of Treitz behind the duodenum and pancreas [6–8]. Culligan *et al.* [5] defined Toldt's fascia as a loose tissue layer sandwiched by two membranous structures (Fig. 1): 1) a “deep mesothelium” derived from the posterior lamina of the covering peritoneum of the initial mesentery in fetuses and; 2) a “retroperitoneal mesothelium” derived from the initial parietal peritoneum behind the fetal mesocolon. Thus, Toldt's fascia is not a membrane, but a tissue layer with some thickness. However, they only

presented low magnification histology images.

There might be no or few photographic demonstrations of the fetal development of Toldt's fascia behind the AC and DC, as well as the fusion fascia of Treitz. To our knowledge, limited studies using human fetuses might be conducted by our groups: 1) Cho *et al.* [9] demonstrated an initial anatomy of the mesocolon, duodenum, and pancreas and its topographical variation at a repackaging process of physiological herniation; 2) Cho *et al.* [10] demonstrated that an initial peritoneum covering the posterior aspect of the pancreatic head disappears at and until midterm; 3) Likewise, using midterm and late-stage fetuses, Jeong *et al.* [11] and Suzuki *et al.* [12] exhibited irregularly interrupted fasciae behind the transverse colon in the late stage, despite the fact that the posterior lamina of the covering peritoneum of the mesocolon once disappeared at and until midterm. Based on these previous studies, we suspected the presence of “deep and retroperitoneal mesothelia” (see above, the first paragraph) behind the colon and pancreas.



**Fig. 1** Anatomy of Toldt's fascia or a fusion fascia behind the mesocolon. Three layers of the mesothelium (peritoneum) were hypothesized along the mesocolon. Thus, Toldt's fascia, sandwiched by two mesothelia, is not plane, but a structure with a certain thickness. The renal fascia seemed to not be taken into consideration.

According to Matsubara *et al.* [13], the perirenal fat or adipose capsule of the kidney develops inside a sac made by the renal fascia (i.e., in the perirenal space) during the late stage of fetal development. Therefore, the expanding perirenal fat is usually adjacent to the internal aspect of the renal fascia. Fixation of the AC and DC to the renal fascia is believed to occur late during fetal development. Therefore, near-term fetal specimens will likely provide better histological evidence of Toldt's fascia and the fusion fascia of Treitz, including their intermediate morphologies during the fixation process. The aims of this study were to characterize the morphological relationship of the interface between the renal fascia and peritoneum, and to examine individual and site-dependent differences. Finally, we will compare the disappearing mesocolon with a fascia between the pancreatic body and the left kidney or adrenal gland. A midgut containing the pancreas seemed to attach to the kidney and adrenal gland much earlier than the colon.

## MATERIALS AND METHODS

The study was performed in accordance with the provisions of the Declaration of Helsinki of 1995 (as revised in 2013), and the study protocol was approved by the Ethics Committee of the Universidad Complutense (B08/374). We used 14 late-stage fetuses with crown rump lengths (CRLs) of 250–325 mm (gestational age: 30–38 weeks). All specimens were part of a large collection kept at the Embryology Institute of the Universidad Complutense, Madrid, and were from miscarriages and ectopic pregnancies at the Department of Obstetrics. All fetuses were fixed by immersion in a 10% neutral formalin solution and stored in a 60–70% ethanol solution.

First, we observed the entire intestine during removal of most parts of the liver, stomach, jejunum, and ileum. During dissection, photographs were taken using a Pentax K-1 camera (Ricoh imaging, Tokyo, Japan) that had a 50–100 mm zoom lens. To avoid artificial separation of the colon and kidney, special attention was paid to maintain the colon at its original location. Second, after routine histology using paraffin-embedded samples, the abdomen was horizontally sectioned at 50–100  $\mu$ m intervals using a rotary microtome (Leica RM2125RT), and then stained with hematoxylin and eosin (HE). Thus, the paraffin block contained the de-

scending and ascending colons, left and right marginal parts of the transverse colon, and the kidneys and other retroperitoneal structures (including the aorta and parts of the posterior body wall muscles). Histological samples were examined with notice of four points of view: 1) whether or not the typical mesocolon (i.e., the transverse mesocolon) will be histologically discriminated from the renal fascia; 2) identification of a thick part of the mesocolon of the AC and DC near the contained vessels and lymph nodes; and 3) how the covering peritoneum changes or disappears at a site distant from the vessels and nodes. Most photographs were taken using a Nikon Eclipse 80. Photographs at an ultra-low magnification (objective lens lower than  $\times 1$ ) were taken with a high-grade flat scanner using translucent illumination (Epson scanner GTX970).

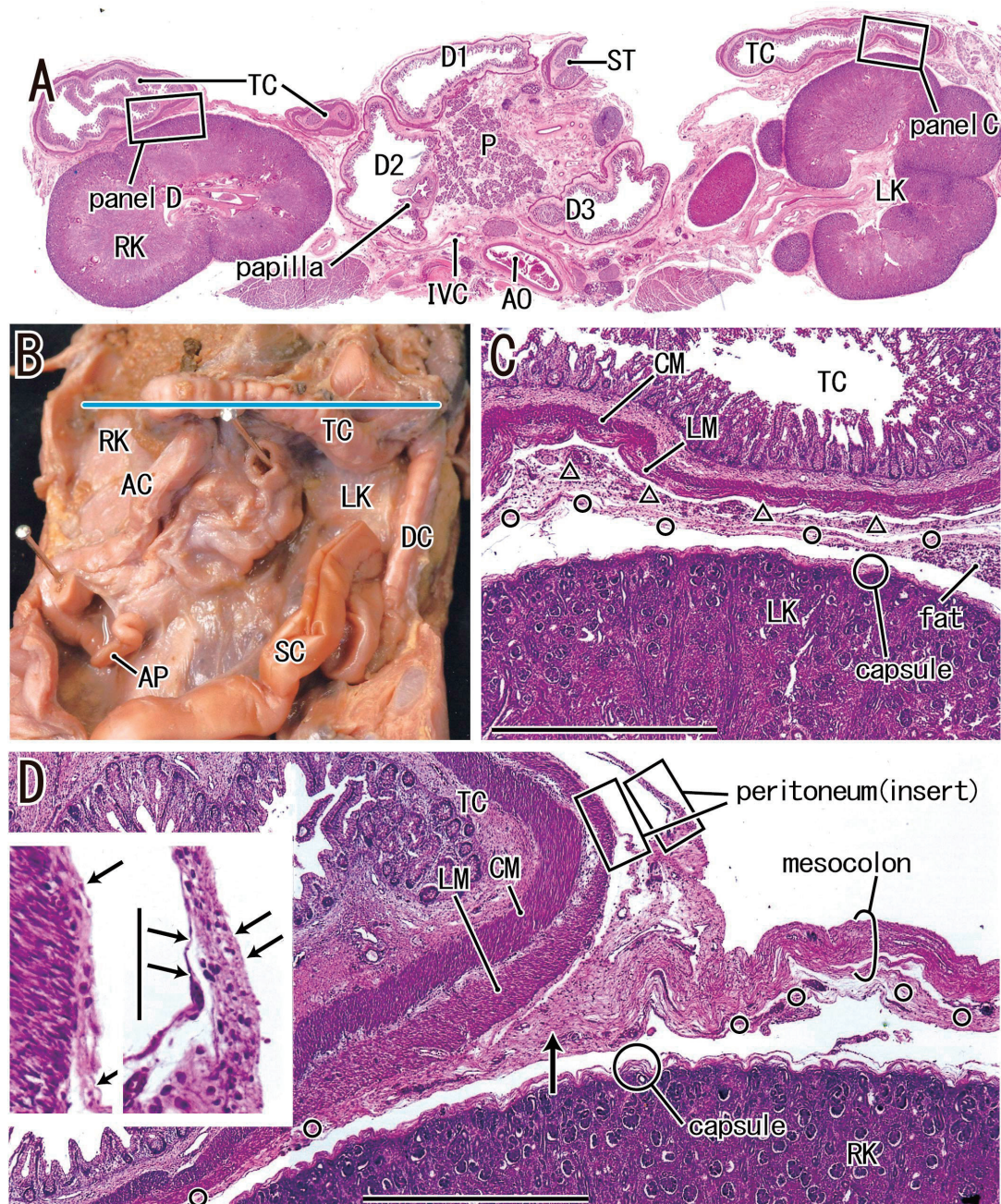
## RESULTS

### Mesocolon, renal fascia and peritoneum: their typical histology

The interface morphology between the mesocolon and renal fascia differed between individuals, among sites along colons, and even between nearby sections. Figs. 2 and 3 show a difference between nearby sections with or without a “free” transverse mesocolon that was separated from the renal fascia by a narrow space (Figs. 2CD and 3A). Without the free transverse mesocolon, a thin layer of the visceral peritoneum appeared to fuse with the renal fascia, and these tissues could not be distinguished (Figs. 2D and 3BC). Thus, the external muscle layer of the transverse colon was likely to directly contact with the renal fascia, without evidence of an interposed visceral peritoneum.

The peritoneum (a surface mesothelium with a thin subperitoneal tissue layer) was present around the colon and pancreas (Figs. 2D insert and 3E). The kidney and adrenal gland were covered by the capsule, which was tightly attached to the renal or adrenal parenchyma, and consisted of multiple wavy fibers. The capsule was separated from the renal fascia by a perirenal space containing perirenal fat in all specimens. Perirenal fat, adjacent to the internal aspect of the renal fascia (see Introduction), emphasized the presence of the fascia (Figs. 2C, 3A, 4D, and 5A). The renal fascia was as thick as the typically free mesocolon (i.e., transverse mesocolon) at a site distant from the vessels and lymph nodes contained (Fig. 3A), but the compos-





**Fig. 2** Transverse colon attaching to the left and right kidney in a specimen of a 256 mm CRL.

Panel A displays a plane represented by a blue line in panel B. Panels C and D are higher magnification views of squares in panel A, respectively. The upper (or lower) needle in panel B indicates the jejunum (or the ileum) that was cut when most of the intestines were removed. In panels C and D, the renal capsule (capsule) is tightly attached to the parenchyma of the left and right kidney (LK, RK). In panel C, the renal fascia (circles) is separated from the mesocolon (triangles). In panel D, the mesocolon is fused with the renal fascia (circles) at sites near the colon (arrow). Inserts on the left-hand side of panel D exhibit the peritoneum at a higher magnification. Arrows indicate the mesothelium. Scale bars: 1 mm in panels C and D; 0.1 mm in inserts in panel D.

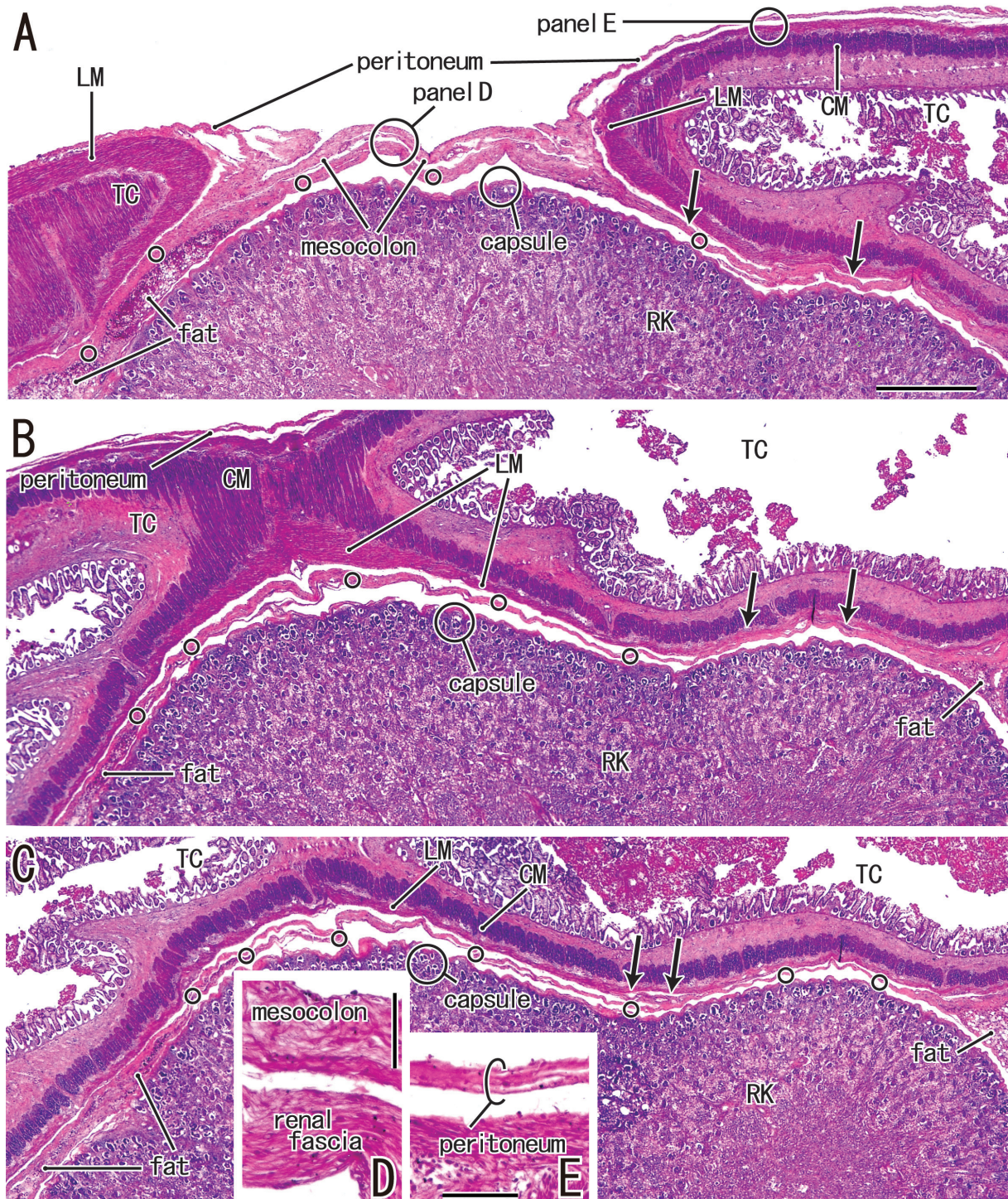
AC, ascending colon; AO, abdominal aorta; AP, appendix vermiformis; CM, circular muscle layer of the colon; D1, D2, or D3, superior, descending, or horizontal portion of the duodenum; DC, descending colon; fat, perirenal fat or adipose capsule of the kidney; IVC, inferior vena cava; LM, longitudinal muscle layer of the colon; P, pancreas; SC, sigmoid colon; ST, stomach; TC, transverse colon.

ite fibers were denser than those of the mesocolon (Fig. 3D). The renal or adrenal capsule was detached from the parenchyma in some specimens due to limitations of the present specimens (Figs. 4D and 5DE; see also **Discussion**).

#### Ascending and descending mesocolon with or without vessels and nodes

A thick mesocolon of the AC and DC contained vessels and lymph nodes (Figs. 4 and 5), and it was separated from the renal fascia by a narrow space (Figs. 4CD and 5B). However, the loose tissue of the mesocolon was likely to continue to the renal fascia without a clear delimitation (Figs. 4D and 5B). Thus, we usually



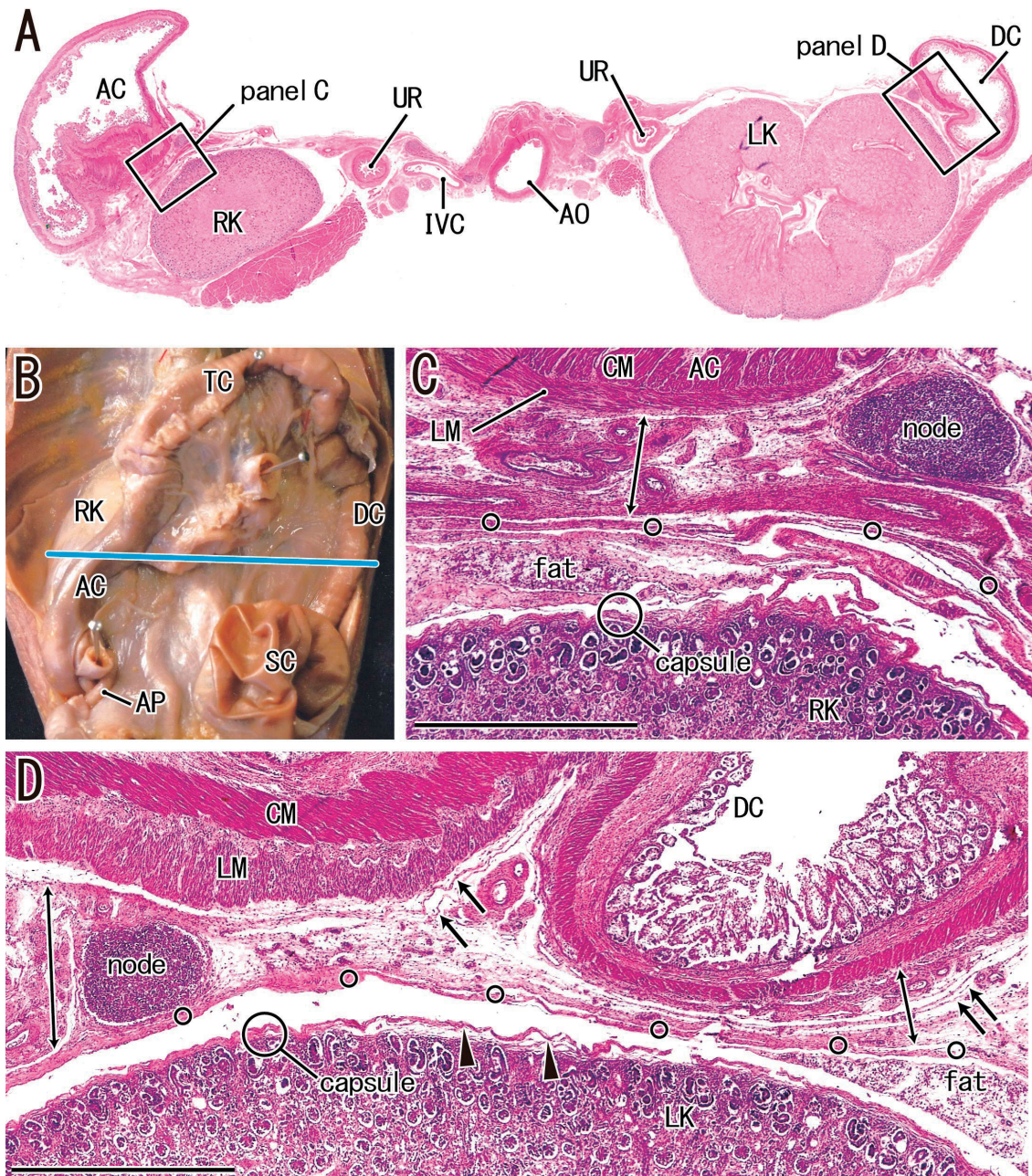


**Fig. 3** Transverse colon extending along the right kidney in a specimen of CRL 272 mm. All panels were prepared at the same magnification (scale bar in panel A, 1 mm). Panel A (or C) displays the lowest (uppermost) plane in the figure. The renal capsule (capsule) is tightly attached to the parenchyma of the right kidney (RK) in all panels. The mesocolon is clearly separated from the renal fascia (circles) at the center of panel A, but at other sites (arrows in panels A–C), it appears to be fused with the renal fascia. The adipose capsule of the kidney (fat) is inside the renal fascia. Panel D (mesocolon and renal fascia) and E (visceral peritoneum) are higher magnification views of circles in panel A, respectively, (scale bars, 0.1 mm). CM, circular muscle layer of the colon; LM, longitudinal muscle layer of the colon; TC, transverse colon.

identified the renal fascia as a posterior margin of the mesocolon of the AC and DC. Even in the same fetus, a site distant from vessels and nodes exhibited a rather simple morphology, different from the thick mesocolon with vessels and nodes (Fig. 5A vs. Fig. 5D). At the former sites, interrupted laminae (Fig. 5DE)

and/or a bundle of multiple laminae (Fig. 5C) were seen between the external longitudinal muscle layer of the colon and the renal fascia. Some of these laminae merged with and ended at the external muscle layer (Fig. 5D). Moreover, the external muscle layer of the AC and DC was likely to directly contact with the renal





**Fig. 4** Ascending and descending colons attaching to the kidney in a specimen of a 322 mm CRL.

Panel A displays a plane represented by a blue line in panel B. Panels C and D are higher magnification views of squares in panel A, respectively. The upper (or lower) needle in panel B indicates the jejunum (or the ileum) that was cut when most of the intestines were removed. In panels C and D, the renal capsule (capsule) is tightly attached to the parenchyma of the left and right kidney (LK, RK), but it is partly detached (arrowheads in panel D). A mesocolon is thick (double-headed arrow) and, along almost all parts, it is separated from the renal fascia (circles). The mesocolon contains membrane-like or laminar structures (small arrows in panel D). The adipose capsule of the kidney (fat) is inside the renal fascia. Scale bar in panels C and D, 1 mm.

AC, ascending colon; AO, abdominal aorta; AP, appendix vermiformis; CM, circular muscle layer of the colon; DC, descending colon; fat, perirenal fat or adipose capsule of the kidney; IVC, inferior vena cava; LM, longitudinal muscle layer of the colon; node, lymph node; SC, sigmoid colon; TC, transverse colon; UR, ureter.

fascia (Fig. 5DE).

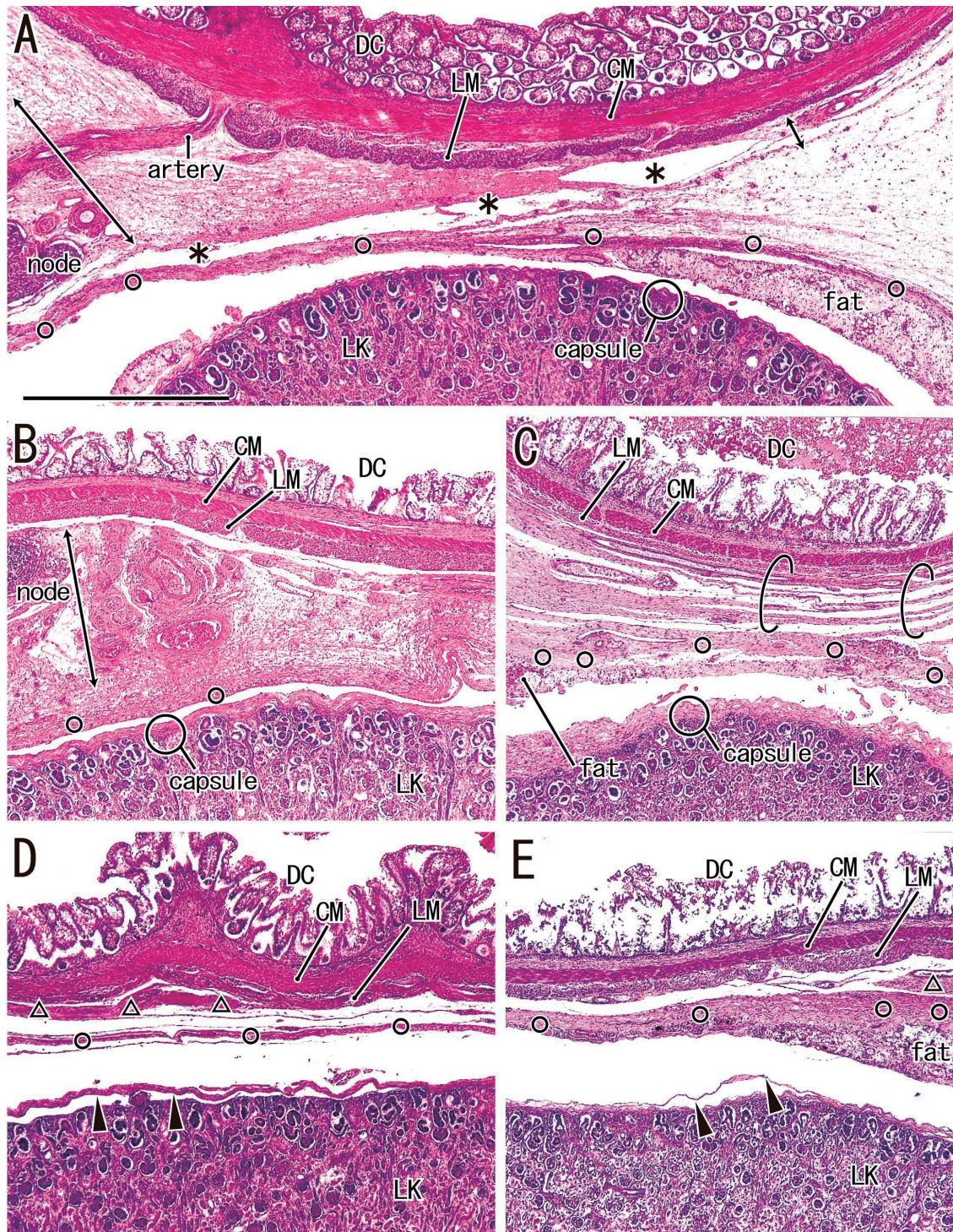
Consequently, the monolayered mesothelium was not evident behind the colon, but we found multilaminar and/or interrupted fascial structures. Both the interrupted lamina and the suggested direct contact of the colon were found in a series of sections of all 14 fetuses examined. In contrast, the multilaminar structure between the colon and kidney was observed in 6 of the 14 fetuses (Table): CRL of 262 mm and 282

mm for the AC; CRL of 250, 262, 264, 282, 310, and 322 mm for the DC. Fig. 6 summarizes the variations at the interface between the colon and kidney.

#### **Retropancreatic fascial configuration: a comparison with the mesocolon**

The pancreatic body was attached to the left kidney and/or adrenal gland. However, a capsule of the pancreas (membrane attached tightly to the pancreatic





**Fig. 5** Individual and site-dependent variations in attachment morphologies between the descending colon and left kidney.

Panel A, CRL of 258 mm; panel B, 282 mm; panel C, 264 mm; panel D, 258 mm; panel E, 264 mm. In panels A–C, the renal capsule (capsule) is tightly attached to the parenchyma of the left kidney (LK), while the capsule is detached in panels D and E (arrowheads). In panels A, C, and E, the adipose capsule of the kidney (fat) is inside the renal fascia (circles). Panels A and B display a relatively thick mesocolon (double-headed arrows), containing a lymph node (node), arteries, and nerves. However, the renal fascia in panel B is not well discriminated from the fibrous contents of the mesocolon. Asterisks in panel A indicate artifact spaces made during the histological procedure. Panel C contains multiple laminae from the mesocolon (semicircle). In panels D and E, a mesocolon (triangles) appears to be interrupted. All panels were prepared at the same magnification (scale bar in panel A, 1 mm). CM, circular muscle layer of the colon; DC, descending colon; fat, perirenal fat or adipose capsule of the kidney; LM, longitudinal muscle layer of the colon; node, lymph node.



**Table** Fetus size and the multilaminar retroperitoneal fascial lamination

CRL mm	Multilaminar fascia			Figures (site)
	AC	DC	Pancreas	
256	—	+	—	not shown
258 (a)	—	—	—	Fig. 2 (colon)
258 (b)	—	—	+	Figs. 5A & 7A (colon, pancreas)
260	—	—	—	Fig. 5D (colon)
262	+	+	—	Fig. 7D (pancreas)
264 (a)	—	+	—	Fig. 5C (colon)
264 (b)	—	—	—	Fig. 5B (colon)
272 (a)	—	—	—	Fig. 3 (colon)
272 (b)	—	—	—	Fig. 7C (pancreas)
276	—	—	+	Fig. 7B (pancreas)
278	—	—	—	not shown
282	+	+	—	Fig. 5B (colon)
301	—	+	—	not shown
322	—	+	—	Fig. 4 (colon)

parenchyma) was difficult to identify, in contrast to the distinct renal capsule. On the anterior aspect of the pancreas, a surface peritoneum or a peritoneum-derived structure was fused with the transverse colon. A fascial structure between the pancreatic body and the left kidney varied widely among specimens, with some having sparsely distributed multiple laminae, and others having a single thick fibrous structure (Fig. 7). In the former, one lamina of the multilaminar structure likely corresponded to the renal fascia (2 specimens; Fig. 7AB); in the latter, the single thick layer was most likely the renal fascia because of its topographical relationship with perirenal fat (5 specimens; Fig. 7D). Intermediate morphologies (7 specimens; Fig. 7C) had variants of the renal fascia, rather than independent retropancreatic fascia. Depending on the site, the renal fascia was tightly attached to the pancreatic parenchyma (Fig. 7CD). Overall, most specimens had a renal fascia that appeared to be a limited structure behind the pancreatic body.

Consequently, being similar to an interface between the mesocolon and renal fascia, the retropancreatic fascia was likely to show either the multilaminar configuration or a single thick fascia possible due to a fusion between the peritoneum remnant and the renal fascia. The former tended to be seen more frequently behind the descending colon than behind the pancreas (Table). However, in both of the colon and pancreas, a fetus with a larger size did not usually carry multilaminar structures. The ascending colon exhibited a low incidence of the multilaminae as behind the pancreas (Table).

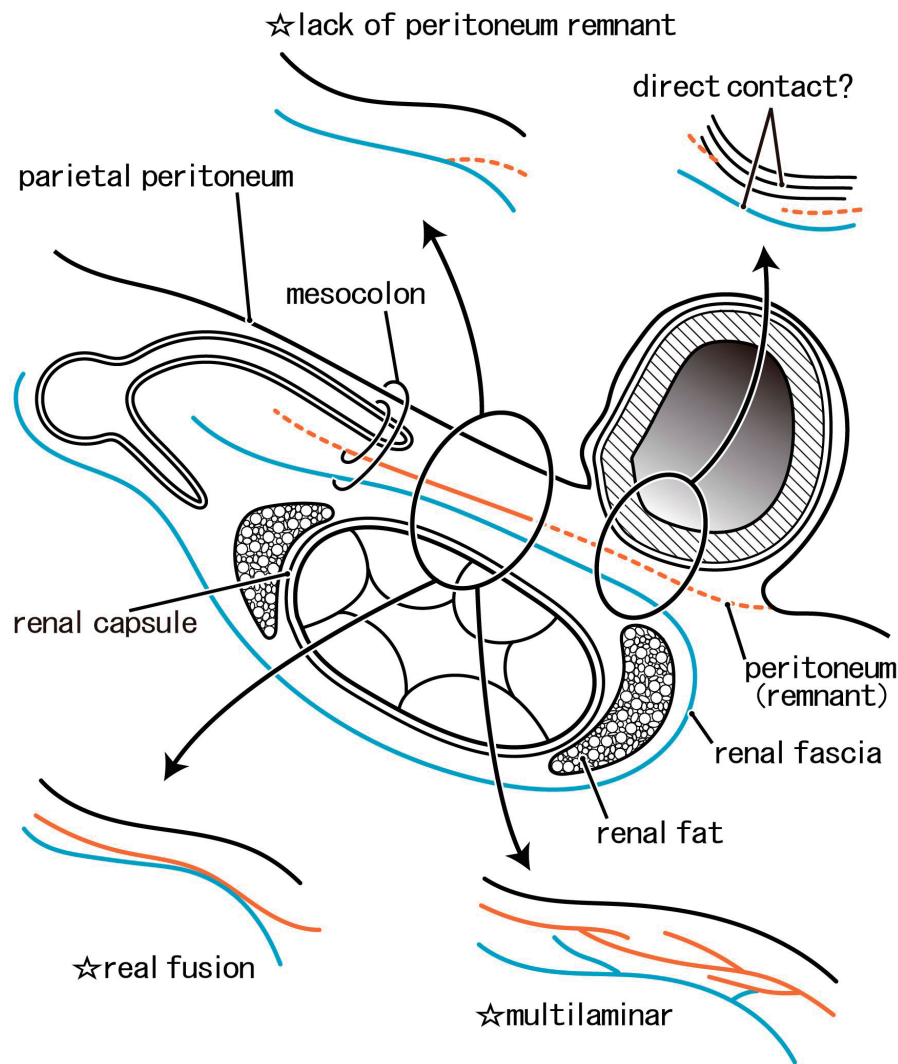
## DISCUSSION

In the present study, we demonstrated a fascial configuration behind the colon in near-term fetuses and compared it to a site behind the pancreatic body that is considered to fix much earlier than the colon. Since we had reported the disappearance of the initial peritoneum behind the colon and pancreas at and until midterm, we hypothesized that 1) the peritoneum of the initial mesentery, as well as the parietal peritoneum attached to the mesentery, disappears once at midterm, and 2) new fasciae develop secondarily at the later stage (see the Introduction). Indeed, the present results

were unable to deny the possibility that the fasciae contained “deep and retroperitoneal mesothelia” (Fig. 1) [5]. However, we successfully demonstrated that areas or layers termed Toldt’s fascia and the fusion fascia of Treitz often contain multilaminar structures. Therefore, in fetuses near term, Toldt’s fascia and the fusion fascia of Treitz might not be a simple fascia sandwiched between two mesothelial remnants, but a thick or thin retroperitoneal tissue layer containing variable fasciae and attaching to the renal fascia. We believe that, to a greater extent, this configuration will be maintained in adults. In the endoscopic surgical experience of two of the present authors (ZWJ and GM), not two laminae (remnant peritoneum), but multilaminar structures, were identified between the colon and renal fascia.

In the present study, we first observed the typical free mesocolon (i.e., parts of the transverse mesocolon) histologically different from the renal fascia. Second, the mesocolon of the AC and DC was, when identified according to vessels and lymph nodes contained, delineated by the renal fascia at the posterior margin. Third, at sites distant from mesocolic vessels and nodes, we found that 1) the mesocolon contained interrupted fibers or multiple laminae; 2) the external longitudinal muscle layer of the colon appeared to directly contact with the renal fascia without evidence of an interposed visceral peritoneum. Finally, a spectrum of variations from a multilaminar structure to a single thick fascia was evident at the interface between the pancreatic body and the left kidney or adrenal gland. Behind the colon, we also found a multilaminar structure, as well as a thick renal fascia attached to the colon. Moreover, the incidence of the multilaminar or single-thick configuration did not correlate with size of fetuses. Therefore, a fascial development after retroperitoneal fusion of the mesentery seemed to advance in two ways: 1) a complete fusion of the original peritoneum with the renal fascia; 2) the development of multilaminar structures, including both the remnant peritoneum and the renal fascia. Individual and site-dependent differences in the proportion of these two manners might determine a retroperitoneal fascial configuration at birth or in children. After birth, these fascial configurations might not change into the so-called Toldt’s fascia or fusion fascia of Treitz, which





**Fig. 6** Summary of variations in fascial structures behind the colon

Although the histology was different from the typical mesothelium, a remnant peritoneum was likely to present behind the mesocolon (orange line). However, the morphology was different between individuals and between sites: 1) a remnant peritoneum fused with the renal fascia; 2) multiple laminae; and 3) no fascial structure, except for the renal fascia. Likewise, the colon external muscle layer was likely to be in direct contact with the renal fascia. Therefore, a definite structure for providing a posterior margin to the mesocolon is the renal fascia.

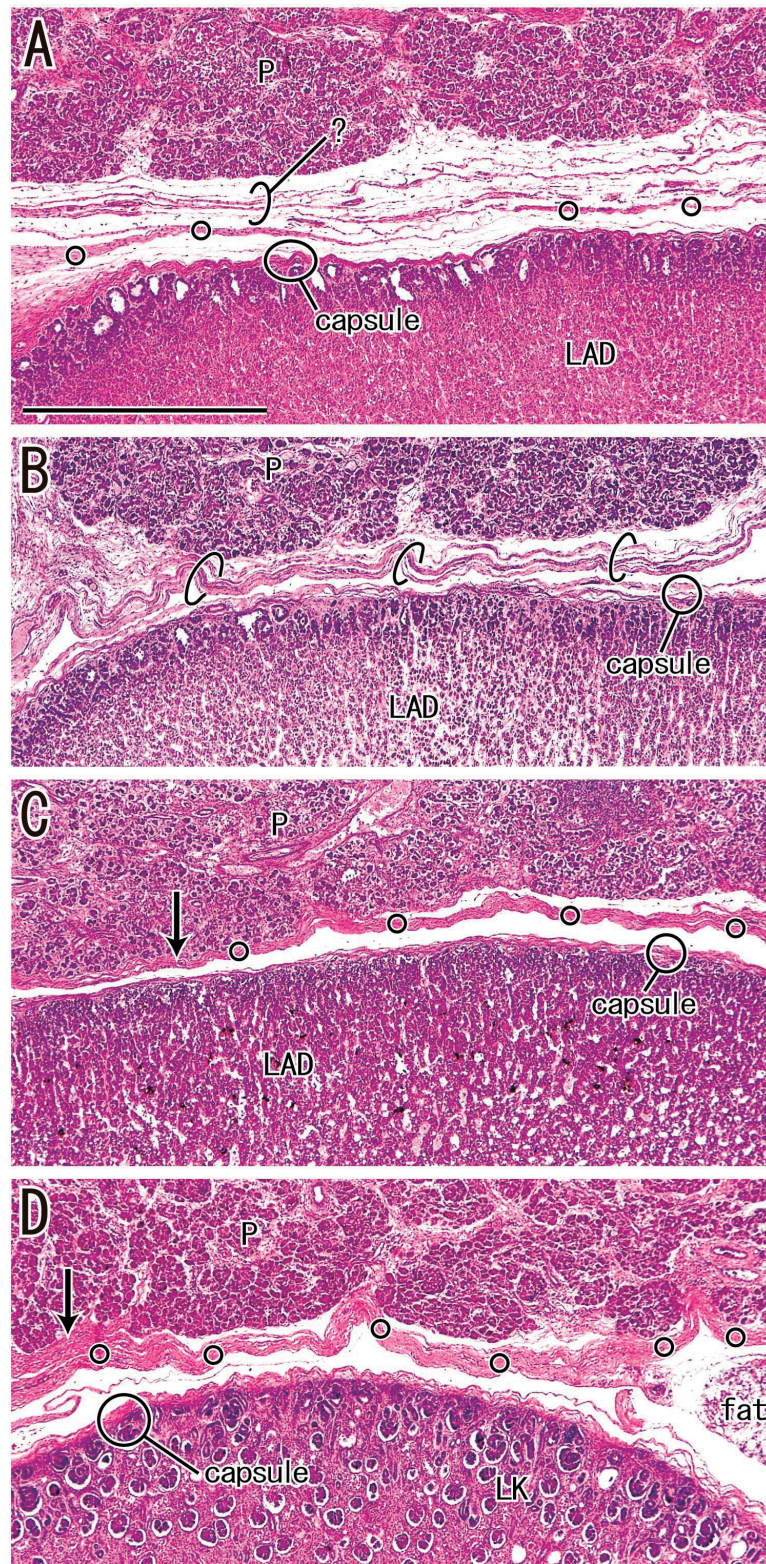
should be sandwiched between two sheets of remnant peritoneum.

We ensured that the renal fascia was an actual histological structure and rejected the hypothesis that the renal fascia is no more than a surgical artifact, due to the concentration of collagen fiber bundles, as a consequence of surgical maneuvers [14]. The most striking feature for us was the absence of a definite border or a specific marginal membrane at the interface between the mesocolon and renal fascia. Likewise, the pancreatic body usually has direct contact with the renal fascia. Conversely, the renal fascia seemed to be a limited structure that provides a posterior border to the mesocolon or pancreatic body. Much to our regret, histological specimens used in previous reports by surgeons, such as those used by Culligan *et al.* [5], usually exclude the renal fascia since, during gastrointestinal surgery, the kidney enclosed by the fascia should be intact. Finally, we cannot exclude the possibility that the tightly attached colon and renal fascia are widely separated in elderly adults because of their increased retroperitoneal fat. Likewise, pathologies such as

inflammation and/or peritoneal effusion, can also modify the fascia in adults. Otherwise, during an abdominal approach, surgeons may be able to divide a thick renal fascia into two or more different sheets: one is part of the renal fascia and the other is an artefactual “retropancreatic fascia.”

The fact that not a large number of specimens were examined was a great limitation of this study, but it was very difficult to collect sections of human fetuses near term. We used histology in an attempt to discriminate an actual narrow space from a space that is an artifact of histological procedures, but there may have been some confusion because of the ongoing retroperitoneal fixation of the colon. Likewise, the renal capsule was detached from the renal parenchyma, possibly due to an excess dehydration during the long preservation of fetuses (Figs. 4D and 5DE). We successfully used a higher magnification to demonstrate the presence of a peritoneum with the subperitoneal tissue (Figs. 2D inserts and 3D). However, as with pathological diagnoses, more definitive evidence for a mesothelium requires data from immunohistochemistry and/or transmission





**Fig. 7** Individual variation in retropancreatic fasciae.

Panel A, CRL of 258 mm; panel B, 276 mm; panel C, 272 mm; panel D, 262 mm. In all 4 specimens, the capsule was tightly attached to the parenchyma of the left adrenal gland or kidney (LAD, LK). Panel A displays a multilaminar structure between the LAD and the pancreatic body (P). One of the laminae (circles) is most likely to correspond to the renal fascia, but the other, anterior laminae (semicircle with ?) may originate from a remnant of the peritoneum. Panel B shows a multilaminar renal fascia (semicircles). Panels C and D contain a thick renal fascia (circles), parts of which appear to tightly attach to the pancreatic parenchyma (arrow). The adipose capsule of the kidney (fat) is inside the renal fascia. All panels were prepared at the same magnification (scale bar in panel A, 1 mm).

electron microscopy studies, using fresh specimens treated with fixatives soon after death [15–17]. Even without immunostaining, a dense content of elastic fibers in the subperitoneal tissue layer would allow discrimination of a visceral peritoneum from other fascial structures [18, 19].

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