Cells
Tissues
Organs

Cells Tissues Organs , DOI: 10.1159/000545751
Received: December 26, 2024
Accepted: April 2, 2025
Published online: April 8, 2025

Superior mesenteric artery during intestinal loop formation and its positional changes from the extracoelom to the abdominal cavity

Takakuwa T, Kakeya M, Ishida N, Kanahashi T, Fujii S, Männer J, Yamada S

ISSN: 1422-6405 (Print), eISSN: 1422-6421 (Online)
https://www.karger.com/CTO
Cells Tissues Organs

Superior mesenteric artery during intestinal loop formation and its positional changes from the extracoelom to the abdominal cavity

Tetsuya Takakuwa[1], Maki Kakeya[1], Nanase Ishida[1], Toru Kanahashi[1], Sena Fujii[1], Joerg Männer[2], Shigehito Yamada[1,3]

[1]Human Health Science, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

[2] Institute of Anatomy and Cell biology, UMG, Georg-August-University of Göttingen, Kreuzbergring 36, 37075 Göttingen, Germany

[3]Congenital Anomaly Research Center, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

Short Title: Superior mesenteric artery in human embryo and fetus

Corresponding author: Dr. Tetsuya Takakuwa
E-mail: tez@hs.med.kyoto-u.ac.jp

Abstract
Introduction: Features of the superior mesenteric artery (SMA) and its intestinal branches during the embryonic and early fetal periods have not been fully described. We aimed to comprehensively elucidate the characteristics of intestinal branch artery formation in the SMA.
Methods: Serial tissue sections of seven early fetal specimens belonging to the Blechschmidt Collection were digitalized and used for segmentation and reconstruction of the intestinal loop, SMA trunk, intestinal branch arteries, and mesentery for further analysis.
Results: The intestinal branch arteries fed the intestinal tract from the oral side to the anal side, according to the order of their origin from the root to the periphery of the SMA trunk. SMA and intestinal branches were not as strongly conserved in their morphology as indicated in previous research but varied between specimens. Most intestinal branch arteries exhibited frequent branching with small intervals at the periphery, whereas the proximal branch exhibited few branches. Only a few peripheral branches made contact with the neighboring intestinal branch arteries. The fetal intestinal branch artery architecture differed greatly from that of adults. There were considerable inter- and intra-specimen variations in the intestinal tract length per feeding intestinal branch artery. The SMA branching arteries did not always supply each tertiary loop individually, and not every loop is connected to one branching artery.
Conclusion: This study elucidates the characteristics of forming the SMA intestinal branch arteries. Specifically, the findings suggest that the SMA is similar to other arteries in that its branches show a level of variability in feeding tissues.

Keywords: superior mesenteric artery, intestinal branch, feeding, tertiary intestinal loop, extraembryonic coelom, descriptive embryology, human embryology

INTRODUCTION

Anatomical features of the superior mesenteric artery (SMA), which is distributed throughout the small intestine and the oral half of the colon, have been described in a limited number of studies, even in adults [1,2]. Soffers et al. [3] described the features of the intestinal branches of the SMA in human embryonic and early fetal stages. However, the primary objective of their study was not to provide a detailed account of the intestinal branches of the SMA but rather to examine the formation and positional alteration of the intestinal tracts during the herniated and return phases. To identify valuable markers of the long intestine with complex loop formation, the distribution of the intestinal branch arteries and the mesentery were used as landmarks. This approach proved fruitful in analyzing hierarchical loop formations, including primary, secondary, and tertiary loop formations, in the four regions, as observed in their study. Kakeya et al. [4] visualized the SMA trunk and all intestinal branches until they entered the intestines using histological sections. This visualization is valuable for anticipating the course of the intestinal tract and confirming a safe and continuous blood supply via the SMA to the intestine. This study's primary objective was to investigate the formation and positional alterations in the intestinal tract during the herniation and return phases. The features of the intestinal branch arteries, the relationship between their distributions, and feeding intestinal tracts are yet to be fully elucidated.

Soffers et al. [3] observed a consistent branching pattern in the SMA in herniated phase specimens (n=5). The primary findings can be summarized as follows: 1) the SMA has 10–13 intestinal branches, which originate from the caudal side of the artery, and 1–3 smaller colonic branches, which originate from the cranial side. 2) The seventh to eighth intestinal branch arteries of intra-abdominal origin supply the first and second secondary loops of the intestinal tract. 3) The 10–12th intestinal branch arteries that arise in the extraembryonic coelom (extra-abdominal branches) feed the third secondary loop, while the terminal branches feed the fourth secondary loop of the small intestine. 4) The portion of the SMA passing through the umbilical ring does not give rise to any intestinal branch arteries. 5) A colic branch consistently originates on the cranial side of the SMA between the seventh and ninth caudal branches. The regions of the intestinal tract where the eighth and ninth intestinal branch arteries feed have not been described. Given the absence of prior studies examining the formation of the intestinal branch arteries during the herniated and return phases, except for the studies by Soffers et al. [3] and Kakeya et al. [4], it is prudent to further confirm and analyze the features of the intestinal branches of the SMA. Soffers et al. [3] further indicated that the mesenteric folds associated with the secondary loops remained discernible at the herniated phase and throughout the subsequent developmental stages, and these landmarks remained useful for the longitudinal observation of intestinal tract formation, including during the early fetal period. Ishida et al. [5] employed three-dimensional (3D) reconstruction of the mesentery to analyze intestinal loop formation during the herniated phase of the embryonic period, in which complex tertiary loops were successfully discerned as each tertiary intestinal loop.

The present study aimed to elucidate the formation characteristics of the intestinal branches of the SMA by a comprehensive analysis of the morphology of the intestinal branch arteries, tertiary intestinal loop formation, and mesenteric folds. Concerning the intestinal tract, the precise number and location of the tertiary intestinal loops were identified in the transition and return phase specimens and applied to determine their relationship with intestinal branch artery distribution.

MATERIALS AND METHODS

Human embryo specimens

The Ethics Committee of Kyoto University Faculty and Graduate School of Medicine approved this study, which used human embryos and fetal specimens (approval no. R0316).

The Blechschmidt Collection, which is famous for its reconstructed models and fine histological slices, is stored at the Center of Anatomy, University Medical Centre, Göttingen [6]. The collection contains over 120 embryos from the first trimester of pregnancy, including some specimens of earlier fetuses, and over 200 organs or body parts of fetuses from all phases of pregnancy. The specimens were obtained from therapeutic interventions (e.g. surgical removal of a tubal pregnancy, hysterectomy due to malignant tumors) that were carried out in German hospitals during the 1950s and early 1960s. During this period, it was neither necessary nor customary to obtain informed consent from patients for the use of therapeutically removed tissues or organs in biomedical research and teaching.

In this study, serial tissue sections from the early fetal phase (n=7; crown-rump length [CRL] range: 30–50 mm) belonging to the Blechschmidt Collection were used. Each specimen was categorized into the herniation (n=1, CRL: 32 mm), transition (n=5, CRL: 30–41 mm), and return (n=1, CRL: 50 mm) phases (Table 1). Most of these specimens have been subjected to a previous study [4]. No information about the staging and gestational age was provided. Based on the degree of the ossification in the femur, four specimens (CRL, 30–35 mm) were estimated as Carnegie stage 23, while the remaining three specimens belong to the fetal period.

Digitization of serial tissue sections and histological observation

Serial tissue sections of the fetuses from the Blechschmidt Collection were digitized using a flatbed scanner (CanoScan 9000F; Canon, Tokyo, Japan) [7]. The sections were scanned at 4800 pixels per inch and saved in BMP and JPG formats.

Three-dimensional reconstruction of the digestive tracts and SMA

The acquired two-dimensional images were cropped and stacked to sequence the images using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Data were processed using the Amira software (ver. 5.5.0; Visage Imaging, Berlin, Germany). Segmentation of the intestinal loop (duodenum, small intestine, and large intestine), SMA trunk, intestinal branch arteries, and mesentery was performed manually, and 3D images were computationally reconstructed. The AmiraSkel software module was used to determine the centerline of the intestine and its length.

The oral end of the duodenum was defined based on its 3D morphology, specifically, the constricted portion at the anal end of the pyloric antrum [8]. The intestinal tract extends from the oral end of the duodenum to the inflection point of the colon, which represents the midgut's caudal boundary following the intestinal return [3,5]. The intestinal branch arteries were numbered in the order of their origin from the root to the periphery of the SMA, as indicated in the rainbow color scheme (Fig. 1Ai and 1Aii). The small intestine between the oral end of the duodenum and the ileum was divided into 11–13 regions according to the feeding arteries branching from the SMA. In addition, the regions of the intestinal tract had the same rainbow colors as the intestinal branch arteries (Fig. 1Aiii). The mesentery was reconstructed (Fig. 1Aiv) and divided into four segments (S1–S4) based on the three narrowing points (Nps) and the cecum, as described in previous studies [3,5] (Figs. 1Av and 1B). S1 and S2–S4 corresponded to the duodenum and jejunum-ileum, respectively. Using 3D reconstruction of the course of the intestinal tracts and mesenteric folds, each tertiary intestinal loop was discerned [5] (Fig. 1C). One tertiary intestinal loop is recognized by the presence of a two-fold mesentery.

It was possible to segment from the initial originating point from the SMA main trunk (B0) to the first (B1), second (B2), and third branches (B3) in all branches in all specimens; several further peripheral branches were also segmented (Fig. 1D). The entry points (EPs) of the artery were defined at the locations where peripheral branches enter the intestine, which can be well detected at the boundary between the mesentery and intestinal tract. The lengths between B0 and B1, B1 and B2, B2 and B3, and B3 and the EPs were calculated. Fusion to the artery from the next branch was observed in only one or two rows. Thus, the boundary of the intestinal tract by each intestinal branch artery feed could be determined.

RESULTS

Features of the intestinal branch arteries arising from the SMA main trunk

There were 11–13 intestinal branch arteries. All intestinal branch arteries originate from the main trunk of the SMA, irrespective of their position relative to the umbilical ring. The distribution of the intestinal branch arteries to the four segments of the intestine varied across the seven specimens analyzed (Fig. 2A). The numerous intestinal branch arteries that originated in the abdominal cavity dispersed to S2 and S3 of the intestine situated in the extraembryonic coelom by traversing the umbilical ring (asterisk in Fig. 2A).

The interval of the points of origin of the intestinal branch arteries on the main trunk of the SMA demonstrated inter- and intra-specimen variability (Fig. 2B). The colic arteries were irregular and did not correlate with the positions of the intestinal branch arteries (arrowheads in Fig. 2B).

Each intestinal branch artery was excised and aligned (Fig. 3A). This "vascular broom" revealed unique features based on the feeding segments. The majority of the arteries exhibited frequent branching with small intervals at the periphery, which was close to the EPs of the intestine in the majority of the intestinal branch arteries. These

features were commonly observed in all specimens, irrespective of the phase (herniated, transition, and return) (Supplementary Figs. 1–3).
The length of each intestinal branch artery and the proportions of the proximal and distal parts were calculated. The length at the proximal end, between B0 and B3, which may be considered analogous to the handle of a broom, changed according to the order of the intestinal branch arteries arising from the main trunk of the SMA. The length of the proximal region affected the total length of the intestinal branch arteries (Fig. 3. B). The fourth intestinal branch artery exhibited the greatest length and subsequently decreased in length, following the order of the remaining intestinal branch arteries. The mean total length was greater in the intestinal branch arteries distributed in S2 than in the other three segments (S1, S3, and S4).

Reconstruction of the intestinal tract accompanying the mesentery
The application of mesentery reconstruction facilitated the identification of the intestinal tract trajectory and delineation of its four segments (Fig. 4). Furthermore, the intricate tertiary loops appeared distinct, showing variation in number and position (Fig. 4, 5).
Notably, the mesentery changed in form in conjunction with a positional change in the intestinal tract from the extraembryonic coelom to the abdominal cavity (Fig. 4). During the herniated phase (Hn1) and early transition phases (Ts1, Ts2, and Ts3), the mesentery was long in the dorsal-ventral direction and narrow in the transverse direction. The mesentery in the cranio-caudal direction in the extraembryonic coelom was either equal in length to or longer than that in the abdominal cavity (see lateral view of the 3D reconstructions of the mesentery from Hn1, Ts1, Ts2, and Ts3 in Fig. 4). In the late transition phases (Ts4 and Ts6), the proximal part of the mesentery in the abdominal cavity broadened in all directions but shortened in the dorsoventral direction.

Relation of the feeding intestinal branch arteries and Intestinal tract and loops
There was considerable variation in the length of the intestinal tract per feeding intestinal branch artery between the inter- and intra-specimens (Fig. 6). The mean intestinal length per feeding intestinal branch artery was between 4.4 and 6.0 mm in the specimens in the herniated (Hn1) and early transition (Ts1, Ts2, and Ts3) phases. The mean intestinal length per feeding intestinal branch artery exhibited a gradual increase in the late transition phases (Ts4 and Ts6), reaching a value of 12.5 mm in the return phase (Rt2).
Generally, the intestinal branch arteries fed the intestinal tract from the oral side to the anal side, according to the order of their origin from the root to the periphery of the SMA trunk (Fig. 5). The SMA branching arteries supplying the tertiary loops did not always have a one-to-one relationship. In over half of the cases, a single intestinal branch artery supplied a single tertiary intestinal loop. In the other cases, two or three branches supplied a single loop. Conversely, one intestinal branch artery could feed one to five tertiary intestinal loops, with two loops in the mode.

DISCUSSION

In this study we used digital reconstruction and segmentation to study the characteristics of intestinal branch artery formation in the SMA. In general, the distribution of the intestinal branches of the SMA may be useful for marking the region of the intestinal tract because of the consistency between the organization of the intestinal tract from the oral to the anal side and that of the proximal to peripheral intestinal branch arteries feeding them. In addition, the application of mesentery reconstruction and detection of the vitelline duct, as demonstrated in the previous study by Soffers et al. [3], facilitated the identification of the intestinal tract trajectory and delineation of its four segments. Recently, our studies analyzing positional changes in the intestinal tract have confirmed and utilized these features [4,5].
Soffers et al. [3] observed a consistent branching pattern in the SMA in herniated phase specimens (n=5). Several discrepancies were observed in this study. The relationship between the intestinal branch arteries and corresponding segments to which the tertiary intestinal loops belong was not constant and instead varied among specimens. Soffers et al. [3] reported that seven to eight intestinal branch arteries of intra-abdominal origin supplied S1 and S2. However, in our study, several intestinal branch arteries with an intra-abdominal origin ran through the umbilical cord and supplied S3.
Soffers et al. [3] also described that branching at the umbilical ring had several features: the SMA passing the umbilical ring did not give rise to any intestinal branch arteries, a colic branch arose between the seventh and ninth intestinal branch arteries around the umbilical ring in the herniated stage, and the eighth and ninth

intestinal branch arteries may not reach the intestinal tract. In our study, no such feature was observed in any of the specimens, including those in the herniated and early transition phases. All the intestinal branch arteries reached the small intestine, and no obvious gaps in arising points from the intestinal branch arteries were noted in our specimens. Soffers et al. [3] reported that only one peripheral intestinal branch artery and ileocolic artery fed S4; however, in this study, several peripheral branches fed S4. The condition of the specimens might have caused this discrepancy. Our specimens were exclusively derived from the Blechschmidt collection, whereas Soffers et al. [3] obtained specimens from different sources, such as LUMC, AMC, and Carnegie collections. Moreover, the specimens were sectioned differently. The sagittal and horizontal sections were used in our present study, while their study used the transverse sections. Given the absence of prior studies examining the formation of intestinal branch arteries during the herniated and return phases, except for Soffers et al.'s study [3], it is prudent to confirm and analyze this issue further.

Soffers et al. [3] observed that positional changes in the three segments occurred by sliding along the short course rather than taking a roundabout route and proposed a "slide-and-stack" model. They hypothesized that the midgut in the extraembryonic coelom returns to the abdominal cavity, with three segments by the secondary loops moving as an independent unit. Kakeya et al. [4] visualized the SMA trunk and all intestinal branches until they entered the intestines using histological sections. They demonstrated that each SMA branching artery proceeded with positional change in sequence in an orderly and structured manner. Each SMA branching artery immediately reached its final position in the abdominal cavity after traversing the umbilical ring. However, no rotation event was recognized, supporting the "slide-stack" model. Kakeya et al.'s observation [4] revised Soffer et al.'s hypothesis [3] in that the positional changes proceeded per smaller unit consisting of each SMA branch and the feeding intestinal tract. This revised model is essential for continuous blood supply via the SMA to the intestine during transition and for safe intestinal return. Both studies excluded the "en-block rotation" of the mid-gut during the herniated and return phases as merely a descriptive concept. The en-bloc model overlooked the movement of the SMA branching arteries and mesentery accompanying the intestinal tract, and safe blood supply appeared not to be guaranteed.

Ginzel et al. [9] observed analogous alterations in a rat model. In rats, three SMA branching arteries supply the intestinal tract herniated into the extraembryonic coelom, which may be homologous with the three segments determined by Soffers et al. [3]. They observed that the three branching arteries and related segments in the intestinal tract returned per each artery in a highly organized fashion, orderly into the abdominal cavity. However, the return units were not concluded to be dependent on the branching artery or intestinal tract segment because both definitions became the same units. The length of the intestine in humans exceeds that in rats, and more SMA branching arteries (11–13 arteries in the present study) are necessary in humans. Nevertheless, intestinal tract returns are surely proceeded by the units of each SMA branch and feeding intestinal tract [4].

The feature of the SMA branching may adapt and contribute to a safe return. It can be hypothesized that as the intestinal length and number of tertiary loops per branching artery increases, uncoiling and passing through the umbilical ring may become laborious and time-consuming. The present study demonstrated that SMA branching arteries provided the intestinal tract with an average length of 4.4–6.0 mm, occasionally exceeding 10 mm, and one to five tertiary loops, with a mean of one loop. These lengths and numbers might be within the range to change in position safely. In addition, the present study demonstrated that the interval of the branching artery varies but has no significant gaps; moreover, the length of the branching artery gradually decreases. Ginzel et al. [9] had previously pointed out that the similarity in length of the SMA branches was a prerequisite for this return sequence because otherwise, a disordered shift of random midgut loops could occur. The intestinal tract is uncoiled and runs across the umbilical ring, with SMA branches unit by unit. This positional change proceeds in a well-prepared, orderly, and highly organized manner [4]. These features might facilitate the sequential progression of SMA branches through the umbilical ring smoothly and safely. The absence of fusion to the artery from the subsequent branch, which differs from that observed in adults [1,2], may be advantageous because each branching artery could move independently. However, this may have disadvantages regarding the collateral flow preventing ischemic changes from occurring as anticipated.

The SMA supplies the midgut, the basal parts of the branching artery supply the oral side, and the peripheral parts of the branching artery supply the anal side of the small intestine, in this order. The colic artery, which supplies the colon branching, arises within the order of the intestinal branches, supplying the midgut part of the colon. Although SMA and intestinal branches were not as well-preserved in their morphology as indicated in a previous study [3], this ordering rule appeared to be a minimum but essential requirement when the uncoiled small intestine ran through the umbilical ring smoothly and the colic part of the midgut synchronically during

transition and return. Ginzel et al. [9] pointed out that in rats, the sequence of return for the three segments was attributable to the arrangement of supplying vessels to the midgut loops.
The risk for accidents during the transition from the extraembryonic coelom to the final position in the abdominal cavity must be minimal. The obstruction of arteries has the potential to result in ischemic changes to the intestinal tract, which can lead to intestinal rupture and occlusion. Even minor surface injuries to the mesentery and intestinal tract can potentially lead to adhesions, resulting in unanticipated maldevelopment, including the inappropriate position of the intestinal tracts, incarceration, and torsion. The "slide-stack" model offers a compelling explanation for the continuous blood supply via the SMA branching artery to the intestine during the transition, ensuring safe intestinal return. This model facilitates direct and uninterrupted transit to the destination, avoiding detours. Furthermore, the unique characteristics of the SMA and the branching arteries may adapt and contribute to a safe return.
The present study has some limitations. First, errors may have been introduced in the quantitative histological analysis of the formalin-fixed paraffin-embedded materials. Furthermore, there was an additional potential for error during the 3D reconstruction of the digitized images. Second, the anal end of the pyloric antrum was defined as the oral end of the intestinal tract. Notably, the duodenum, which originates from the foregut, may also be included as the superior part of the intestinal tract in this definition. This definition can be refined by referencing additional anatomical landmarks, such as the pancreatic ducts and inferior vena cava, to facilitate more precise measurements. Third, each intestinal branch artery was pursued as far as possible peripherally. The EPs in the intestine were identified with a high degree of certainty, although complete identification of the peripheral branching still needs to be achieved. Consequently, quantitative analysis of the peripheral aspects of each intestinal branch artery was not conducted. Finally, only seven specimens were included in this study.
The present study elucidates the characteristics of the formation of the SMA intestinal branch arteries, suggesting that the SMA is similar to other arteries in that its branches show differences in feeding the tissues to some extent. The literature has not fully described the features of the SMA and its intestinal branches during the embryonic and early fetal periods. Our study makes a significant contribution to this field.

Statements

Funding Sources
This study was supported by Grant No. 21K07772 from the Japan Society for the Promotion of Science.

Statement of Ethics
The ethics committee of the Kyoto University Faculty and Graduate School of Medicine approved this study, which used human embryos and fetal specimens (R0316).

Consent to Participate
The specimens were obtained from therapeutic interventions (e.g. surgical removal of a tubal pregnancy, hysterectomy due to malignant tumors) that were carried out in German hospitals during the 1950s and early 1960s. During this time period, it was neither necessary nor customary to obtain informed consent from patients for the use of therapeutically removed tissues or organs in biomedical research and teaching.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.
Professor Shigehito Yamada was a member of the journal’s Editorial Board at the time of submission.

Author Contributions
T.T.: concept/design, critical revision of the manuscript.
M.K, N.I.: data analysis/interpretation
T.K., S. F.: data analysis/interpretation
J.M. S.Y.: acquisition of data.

Data availability statement:

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author [T.T.; tez@hs.med.kyoto-u.ac.jp] upon reasonable request.

REFERENCES

1. Beaton, L. E., & Anson, B. J. (1942). The arterial supply of the small intestine. *Quarterly bulletin of the Northwestern University Medical School*, 16, 114–122.
2. Conley, D., Hurst, P. R., & Stringer, M. D. (2010). An investigation of human jejunal and ileal arteries. *Anatomical Science International*, 85, 23–30.
3. Soffers, J. H., Hikspoors, J. P., Mekonen, H. K., Koehler, S. E., & Lamers, W. H. (2015). The growth pattern of the human intestine and its mesentery. *BMC Developmental Biology*, 15, 31.
4. Kakeya, M., Matsubayashi, J., Kanahashi, T., Männer, J., Yamada, S. & Takakuwa, T. (2022). The return process of physiological umbilical herniation in human fetuses: the possible role of the vascular tree and umbilical ring. *Journal of Anatomy*, 241, 846–859.
5. Ishida, N., Ueda, Y., Kanahashi, T., Matsubayashi, J., Imai, H., Yamada, S., & Takakuwa, T. (2025). Hierarchical loop formation in human midgut during physiological umbilical herniation. *J Anatomy*, in press (DOI: 10.1111/joa.14228)
6. Brand-Saberi, B., Wingender, E., Rienhoff, O., Viebahn, C. (2012). Presenting human embryology in an international open access reference Centre (HERC). In S. Yamada, & T. Takakuwa (Eds.), *The human embryo* (pp. 21–34), Dermatol intech.
7. Miyazaki, R., Makishima, H., Männer, J., Sydow, H. G., Uwabe, C., Takakuwa, T., Viebahn, C., Yamada, S. (2018). Blechschmidt Collection: Revisiting specimens from a historical collection of serially sectioned. human embryos and fetuses using modern imaging techniques. *Congenital Anomalies*, 58, 152–157.
8. O'Rahilly, R., & Müller, F. (1987). *Developmental Stages in Human Embryos: Including a Revision of Streeter's Horizons and a Survey of the Carnegie Collection*. Carnegie Institution of Washington, 306pp.
9. Ginzel, M., Martynov, I., Haak, R., Lacher, M., & Kluth, D. (2021). Midgut development in rat embryos using microcomputed tomography. *Communications Biology*, 4, 190.

FIGURE LEGENDS

Fig. 1. Illustrations indicating methods used in this study
A) Three-dimensional reconstructions of the intestinal tract, mesentery, and intestinal branches of the SMA (right-lateral view)
i) Reconstruction of the SMA main trunk and intestinal branches with the intestinal tract
Each intestinal branch artery is represented by a colored rainbow, and the intestinal tract is indicated by green translucency.
ii) Image after removing the intestinal tract from Figure 1. A_i
Each intestinal branch artery is colored with a rainbow. The SMA branch arteries are traced to the periphery so that the line-up of the entry points can indicate the course of the intestinal tract.
iii) The centerline of the intestinal tract consists of colored rainbows corresponding to the feeding intestinal branch arteries. This is accomplished by comparing the distribution of the intestinal branch arteries and the course of the intestinal tract.
iv) The centerlines of the intestinal tract and mesentery are visualized simultaneously so that the tertiary intestinal loops could be recognized by the mesenteric folds.
v) The mesentery is colored with the corresponding segments of the intestinal tract. S1, red; S2, yellow; S3, green; S4, blue.
B) Diagram of the three mesenteric narrowing parts (Np1, Np2, and Np3), and the ileocecum (purple dot) divided into four segments (S1, S2, S3, and S4)
a, anal side; o, oral side
+ The oral end of the duodenum in the present study corresponds to the end of the pyloric antrum (thick black). Note that the point is defined based on morphological observations and that the oral part includes the duodenum of foregut origin.
C) Recognition of the tertiary intestinal loop with the two successive mesenteric folds
D) An illustration of the intestinal branch artery originating from the main trunk of the superior mesenteric artery (red).
The intestinal branch artery is traced to the entry points (EPs) of the intestinal tract (vasa recta).
SMA, superior mesenteric artery; B0, branching point from the SMA main trunk; B1, first branching point; B2, second branching point; B3, third branching point

Fig. 2. SMA and its intestinal branches
A) Distribution of the intestinal branch arteries to four segments of the intestinal tract.
The rainbow colors with numbers correspond to the intestinal branch artery order.
S1, pink; S2, yellow; S3, green; S4, blue. The grey panel of the specimens indicates no corresponding branches.
* indicates branches running through the umbilical ring.
Several intestinal branch arteries feeding segment three run through the umbilical ring in Hn1, Ts1, Ts2, and Ts3.
** Distribution of the intestinal branch arteries to four segments of the intestinal tracts according to Soffers et al. [3]. The distribution of the eighth and ninth intestinal branch arteries is ambiguously described. The eighth and ninth intestinal branch arteries are indicated in grey in the Figure in Soffer's study.
Hn, herniated phase; Ts, transition phase; Rt, return phase
B) Pictorial graph indicating the interval of the intestinal branches of the SMA
The main trunk (vertical line) is divided into four segments so that the intestinal branch arteries could be categorized according to the feeding intestinal segments (S1, pink; S2, yellow; S3, green; S4, blue).
Rainbow-colored horizontal lines from the vertical SMA trunk indicate the intestinal branch arteries originating from the main trunk. The colors correspond to the intestinal branch artery numbering, as shown in Figure 2. A.
The intestinal branch arteries originating from the SMA trunk (black vertical line) run and reach the intestinal tract in the extraembryonic coelom.
The blue double-line indicates the position of the umbilical ring.
The black arrowhead indicates the branching points of the colic branch arteries.
SMA, superior mesenteric artery; Hn, herniated phase; Ts, transition phase; Rt, return phase

Fig. 3. Morphology and morphometry of the intestinal branch arteries

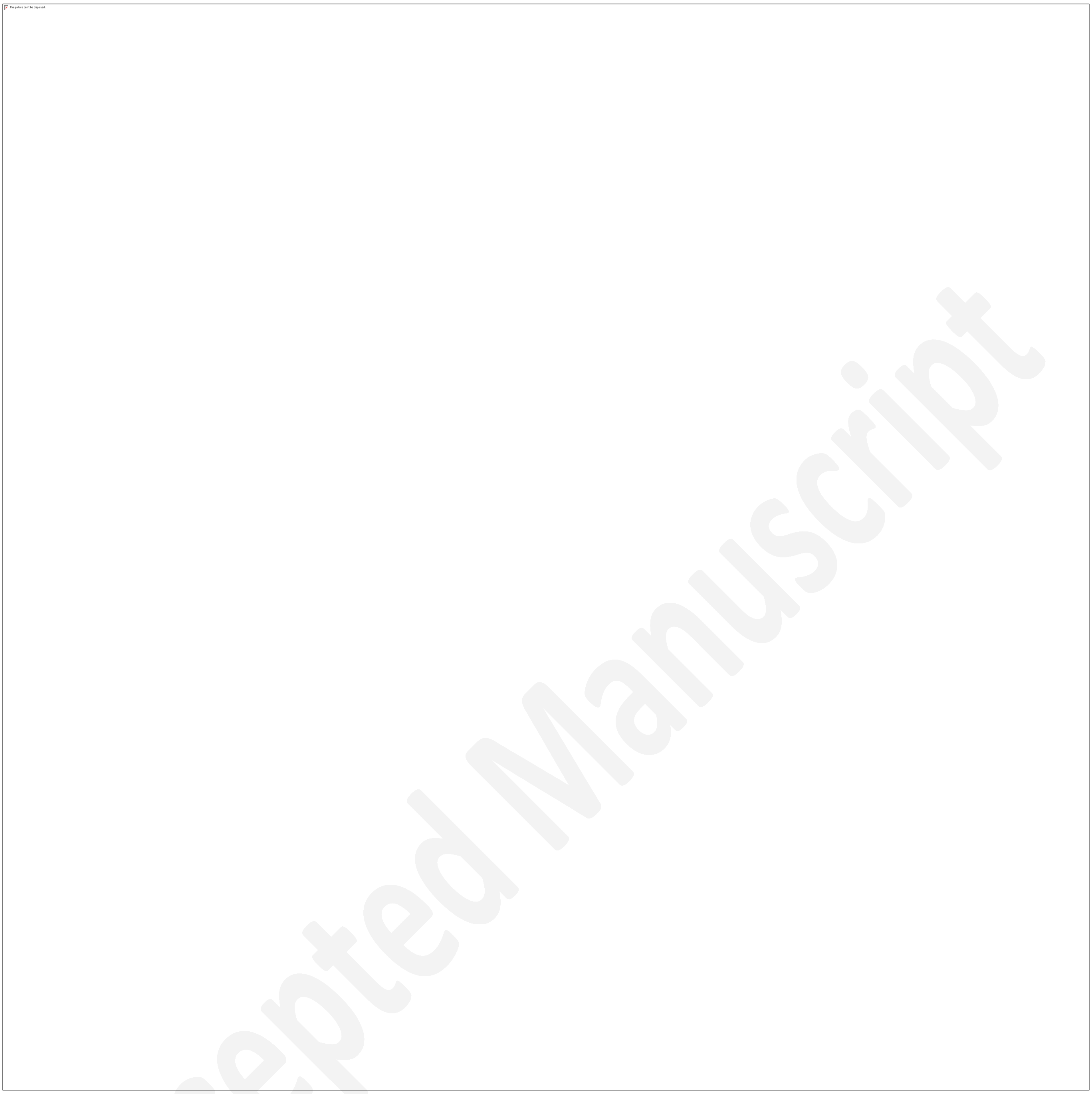
A) Morphology of the intestinal branch arteries in representative specimens at the transition phase (Ts6, CRL 39 mm)
Each intestinal branch artery is followed from its branching point to the entry point of the intestinal tract (vasa recta).
They are lined up according to the feeding intestinal segments.
The morphology of the intestinal branch arteries in the three other specimens at different phases is shown in Supplementary Figures 1, 2, and 3.
B) Length of each intestinal branch artery
The bar graph indicates the distances between B0 and B1 (L0-1, black), B1 and B2 (L1-2, gray), B2 and B3 (L2-3, light gray), and B3 and EP (L3-EP, blue).
The definition of the branching points (B0, B1, B2, and EP) is described in the Materials and Methods section and indicated in Figure 1D.
SMA, superior mesenteric artery; Ts, transition phase; CRL, crown-rump length

Fig. 4. Three-dimensional reconstructions of the tertiary intestinal loops and accompanying mesentery
For large-scale reconstructions, a right-lateral view at the same magnification is provided for all specimens. Additionally, a ventral view is provided for one specimen (Rt2). The centerline of the intestinal tract is indicated by rainbow colors corresponding to the feeding intestinal branch arteries. The mesentery is indicated in grey. The blue lines indicate the umbilical rings. The scale bar indicates 1000 µm.
For small-scale reconstructions, the mesentery is colored according to the corresponding segments. S1, red; S2, yellow; S3, green; S4, blue.
The number of intestinal branch arteries and tertiary loops and the percentage of the intestinal tract in the extraembryonic coelom to the total intestinal length are determined.
Rt2, return phase

Fig. 5. Distribution of the intestinal branch arteries to the tertiary intestinal loops
A) The bar graph indicates the intestinal length and feeding intestinal branch arteries per each tertiary intestinal loop
The rainbow colors correspond to the intestinal branch arteries and the SMA main trunk.
The black bar along the X-axis indicates the region where the tertiary intestinal loops are in the abdominal cavity.
The number of tertiary intestinal loops per segment and the total number of tertiary intestinal loops are shown.
B) Three-dimensional reconstruction of the intestinal tract (right-lateral view)
The centerline of the intestinal tract is indicated by rainbow colors corresponding to the feeding intestinal branch arteries.
For Ts6 and Rt2, the intestinal branch arteries of the SMA with rainbow colors are used (ventral-cranial view).
The total and mean intestinal lengths per tertiary loop are provided.
SMA, superior mesenteric artery; Ts, transition phase; Rt2, return phase

Fig. 6. Intestinal length per the feeding intestinal branch artery
The rainbow colors correspond to the intestinal branch and ileocolic arteries.
The total and mean intestinal length per intestinal branch artery are provided.

The picture can't be displayed.


The picture can't be displayed.

The picture can't be displayed.

The picture can't be displayed.

The picture can't be displayed.

The picture can't be displayed.

Table 1 Specimens of the Blechschmidt Collection used in the present study

Stage*	Phase	ID	CRL (mm)	Histological sections		
				Orientation	Thickness (μm)	Staining
CS23 (n=4)	Herniation	Hn1	32	Horizontal	10	HE, Azan
	Transition	Ts1	35	Sagittal	10,11	HE, MT
	Transition	Ts2	31	Coronal	10	HE, MT
	Transition	Ts3	30	Sagittal	10	HE, Azan
fetus (n=3)	Transition	Ts4	41	Sagittal	10	HE
	Transition	Ts6	39	Horizontal	25	HE, Azan
	Return	Rt2	50	Horizontal	10	HE, Azan

CRL, crown-rump length; CS, Carnegie stage; HE, hematoxylin-eosin; MT, Masson trichrome

*Stage was estimated by the degree of the ossification in the Femur