Early Organogenesis of the Caprine Stomach

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ABSTRACT. Early organogenesis of the caprine stomach was studied in a series of 11 embryos ranging from 6.5 mm neck-rump length (NRL) to 13.3 mm crown-rump length (CRL). In embryos with 6.5–6.7 mm NRL, a part of the primordial proper esophagus extended to the dorsal side of the primordial stomach. The primordial proper esophagus and its extension were lined with a simple epithelium and stained dark brown with Con A III, while the primordial stomach was weakly stained. In embryo with 7.3 mm NRL, the esophageal extension was separated from the proper esophagus by constriction, and became a primordial forestomach situated in an area outside the omental sac. In embryos with 8.3 mm NRL-10.7 mm CRL, primordial forestomach and primordial stomach were united and formed a spindle shaped primordial ruminant stomach with foregut rotation. The primordial ruminant stomach was similar to the primordial simple stomach except that it was more flattened laterally with the convex at the area of ‘lesser curvature’. Primordial rumen, omasum and abomasum appeared from the spindle shaped primordial ruminant stomach in an embryo with 12.9 mm CRL. In an embryo with 13.3 mm CRL, primordial reticulum originated from an area between the primordial rumen and omasum.—KEY words: caprine embryo, esophageal extension, primordial forestomach, primordial stomach.


The ruminant stomach consists of four compartments: rumen, reticulum, omasum and abomasum. The first three parts form the forestomach with stratified squamous epithelium, while the abomasum is a glandular stomach with simple columnar epithelium. Owing to the similarity of epithelium, it was thought that the forestomach was formed by the esophageal succulation [24, 26, 29]. However, organogenetic studies by Pernkopf [23], Lambert [15] and Warner [32] have shown that the four compartments of the ruminant stomach develop from a simple spindle-shaped gastric primordium similar to the simple stomach primordium in man. Many studies and veterinary textbooks have adapted this view [1–11, 13, 20, 22, 27, 31]. On the other hand, glands in the submucosa and muscle layer of the reticular groove (sheep) and the omasal base (goat), were found similar to histological characteristics of the esophageal gland [8, 14, 18, 19, 30, 33]. Furthermore the area containing glands which resemble esophageal one may be closely related to the esophagus in the organogenesis of the forestomach.

In this study, we examined the early histogenesis and organogenesis of the ruminant stomach in caprine embryos by histological procedures and techniques of three-dimensional reconstruction.

MATERIALS AND METHODS

Eleven caprine embryos, ranging from 6.5 to 13.3 mm neck-rump length (NRL) or crown-rump length (CRL), were collected from the Saanen goats that were bred by farmers in Aomori Prefecture (Table 1). Specimens were fixed in 10% formalin and embedded in toto in paraffin using routine
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Table 1. Materials examined

<table>
<thead>
<tr>
<th>Embryo Number</th>
<th>Length (mm)</th>
<th>somites</th>
<th>Thickness of foregut (μm)</th>
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<tr>
<td>1</td>
<td>NRL 6.5</td>
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<td>330</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td>34</td>
<td>330</td>
</tr>
<tr>
<td>3</td>
<td>7.3</td>
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<td>4</td>
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</tr>
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<td>5</td>
<td>8.6</td>
<td>44</td>
<td>560</td>
</tr>
<tr>
<td>6</td>
<td>CRL 10.3</td>
<td></td>
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<td>10</td>
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<td></td>
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<td>11</td>
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</table>

The thickness of foregut was measured from dorsal to ventral edge of reconstructional styrene models which were traced along the serous membrane of the foregut.

procedures. Serial sections were cut at 5 or 10 μm and every second or fifth section was stained with hematoxylin and eosin (H-E). The other sections were stained with H-E or Concanavaline A paradox type III (Con A III) [12, 28]. Along the line of lumen, basement membrane of epithelium and serous membrane, the outline of the foregut were traced on transparent polypropylene sheets. Three-dimensional images of the ruminant stomach of caprine embryos were formed by the superposition of these drawings consecutively, or processed with a three-dimensional analyzer (Cosmozone 98, Nikon). The reconstruction models of the ruminant stomach were made by overlaid styrene sheets.

RESULTS

In the embryo with 6.5 mm NRL, 31 somites, the reconstructional (RC) image along the serous membrane of the foregut showed an ovoid contour with a dorsoventral thickness of 0.33 mm. The cavity of the omental sac spread from the dorsomedial side to the right side of the foregut. The mesothelium of the dorsal mesogastrium, which separated the cavity of the omental sac from the peritoneal cavity, consisted of high columnar cells closely arranged on the dorsal surface of the foregut. The part of the foregut surrounded by omental sac was considered a primordial stomach. It was lined with simple columnar epithelium. The image along the basement membrane of the epithelium of the primordial stomach gave a pear-like contour as seen in lateral view. There were a pair of lung buds with an indistinct boundary groove between the left and right lobes under the primordial esophagus and rostral to the primordial stomach.

In this specimen, an extension of primordial esophagus formed a flat and long pouch continuous to the primordial proper esophagus. The extension was situated dorso-dextrally on the primordial stomach, and dorsocranially to the omental sac. Lumen of the extended primordial esophagus were connected with dorsodextral part of the primordial stomach by a long narrow orifice. The primordial proper esophagus, esophageal extension and primordial stomach were lined with simple columnar epithelium. The epithelium of the primordial proper esophagus and esophageal extension were stained dark brown with Con A III, while epithelium of the primordial stomach was stained yellow (Figs. 1A-C, 7A).

In the embryo with 6.7 mm NRL, 34 somites, the RC image along the serous membrane of the foregut showed a longer contour with a dorsoventral thickness of 0.33 mm. The RC image along the basement membrane of primordial stomach was somewhat flattened on the lateral surface. The esophageal extension was situated on the dorsal side of the primordial stomach from a right-cranial to left-caudal position with a 30° rotation to the left around the long axis. In the midsagittal section of the foregut the esophageal extension had an oval lumen.
The epithelium of the primordial proper esophagus and dorsal part of its extension were lined with simple columnar epithelium and were stained dark brown with Con A III. Epithelium of the primordial stomach was pseudostratified and stained yellowish brown with Con A III. The esophageal extension was situated in an area outside the omental sac. The boundary groove of lung buds was more distinct in this specimen (Figs. 2A-C, 7A).

In the embryo with 7.3 mm NRL, 38 somites, the omental sac cavity extended dorso-dextrally to ventro-medially on the right side of the primordial stomach. A left rotation of the foregut on its long axis was indicated by the oblique position of the lumen in relation to the mid-sagittal body plane. The rotation was approximately 45° in this embryo. A constriction appeared between the primordial esophagus and esophageal extension in the RC image along the basement membrane of the foregut epithelium. The primordial stomach surrounded by the omental sac was shaped like a spindle. The esophageal extension not surrounded by the omental sac was situated on the dorsocranial side of the primordial stomach. The esophageal extension developed into a primordial forestomach. A wide junction between primordial forestomach and stomach formed a hook-like lumen at the midsagittal section of the foregut. The simple columnar and pseudostratified epithelium of primordial forestomach was stained dark brown with Con A III. The pseudostratified epithelium of primordial stomach was stained brown with
Con A III (Figs. 3A-D, 7B).

In embryos with 8.3 mm NRL, 42 somites and 8.6 mm NRL, 44 somites, the RC image along the basement membrane of the foregut epithelium showed that the primordial esophagus became longer and slender, and that the primordial forestomach united with the stomach primordium to form a primordial ruminant stomach. A shallow groove ran obliquely from a right cranial to left caudal direction on the dorsal surface of the primordial stomach. This groove evidenced a union of primordial forestomach with the stomach primordium. The posterior part of this groove was situated in the area surrounded by the omental sac, while the anterior part was outside the sac. The simple columnar epithelium appeared only on a small area of the anterior part of the groove. The other part of the primordial ruminant stomach was lined with the pseudostratified epithelium (Figs. 4A-C, 7C).

In embryos with 10.3 mm and 10.7 mm CRL, the RC image along the basement membrane of epithelium of the primordial ruminant stomach showed a complete fusion of primordial forestomach and primordial stomach. They were lined with pseudostratified epithelium, except for the dorso-cranial region which was lined with simple columnar epithelium. The primordial ruminant stomach was spindle-shaped, flattened laterally with a convexed right side, and had a lumen which was similar profile to the itself (Figs. 4D, 7C).

In embryos with 11.6 mm and 11.9 mm CRL, the RC image along the basement
Fig. 3. A. Sagittal section of the foregut in the caprine embryo with 7.3mm NRL, H-E stain, ×20. B. Adjacent section of Fig. 3A stained with Con A III. The primordial forestomach (F) was stained dark brown, while the primordial stomach (S) was stained brown. The lung but (A) and omental sac (O) are observed. ×35. C. Dorsal view of the reconstructional styrene model along the basement membrane of the foregut epithelium. The primordial forestomach is situated on the dorsal side of the primordial stomach. ×22.5. D. Consecutively piled tracing sheets. The relation of the primordial forestomach (F), primordial stomach (S) and omental sac (O) is shown. Arrow(↓) indicates constriction between primordial forestomach and primordial stomach. ×40.

membrane of epithelium of the primordial ruminant stomach showed a spindle profile in the lateral view, and could be divided into dorsal and ventral parts. The dorsal part was flat in shape and extended to the left, while the ventral part was shaped like a slender spindle. In the mid-part of the cross section, the lumen showed an inverted L profile. The epithelium were pseudostratified except in a small dorso-cranial area with simple columnar epithelium (Figs. 4D, 7D).

In the RC image along the serous membrane of the 12.9 mm CRL embryo, the primordial rumen, omasum and abomasum could be distinguished by shallow grooves running on the dorsal surface of the primordial ruminant stomach. The primordial rumen extended left cranially to the primordial omasum occupying the right area to almost the middle of the primordial ruminant stomach. The primordial abomasum was situated left caudally. The greater omentum was attached to the dorsal surface of the primordial rumen and abomasum. The lesser omentum was found on the ventral surface of the primordial omasum and pylorus. The RC image along the basement membrane of this embryo showed that each compartment of the primordial ruminant stomach, except the reticulum, became more distinct. The cranial area of the primordial rumen was lined with simple columnar epithelium, but other regions of the primordial ruminant stomach were lined
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with pseudostratified epithelium. The muscle layer appeared in the primordial esophagus and in a cranial part of the primordial rumen (Figs. 5A, 7E).

The primordial ruminant stomach in the embryo with 13.3 mm CRL was longer than that in the previous stage, but the epithelium and muscle layers were similar to those in the previous stage. Additionally, in the RC image along the basement membrane of epithelial cells, the primordial reticulum appeared ventrally and between the primordial rumen and omasum (Figs. 5D, 6A-D and 7E).

DISCUSSION

Pernkopf [21] and Lambert [15] showed that the ruminant stomach, like the simple stomach, developed from a simple spindle-shaped gastric primordium. Warner [30] emphasized that the esophagus remained a tube of uniformly small diameter with no expansions during development in any kind of ruminant stomach, and that there was no esophageal contribution to any parts of the ruminant forestomach. Many previous authors supported this view [1–11, 13, 16, 17, 20, 27, 31]. In the present study, however, it was found that a part of the esophagus clearly extended toward dorso-dextrally of the primordial stomach and formed the primordial forestomach, during development of ruminant stomach of caprine early embryos below 7.3 mm NRL. In this view, the flat and long pouch situated dorso-dextrally on the primordial stomach is not considered a part of the primordial stomach but an
extended part of the primordial esophagus because this pouch was situated in the area outside of the omental sac surrounding the primordial stomach. As the foregut rotates during embryonic development, the esophageal extension is united with the primordial stomach and becomes the spindle-shaped primordial ruminant stomach.

Fath El-Bab et al. [5] showed that the epithelium of the stomach in sheep embryo with 13 mm CRL consisted of pseudostratified. Warner [32] stated that the esophageal and gastric epithelium of bovine embryos of 19 mm or less appeared to consist of 2 to 4 layers of cells and that was difficult to determine whether true stratification or pseudostratification existed. Warner [32] observed that the area of simple columnar epithelium in the abomasum, appeared first in 50 mm CRL embryos. In addition, Asari et al. [1] reported that the epithelium of the abomasum was already pseudostratified in 23 mm CRL bovine embryos, and that simple columnar epithelium was seen throughout the abomasum in 13–14 cm CRL embryos. In the present study, all of the primordial esophagus, its extension and the primordial stomach were lined with simple columnar epithelium in the embryo with 6.5 mm NRL. In the embryo with 6.7 mm NRL, epithelium of the primordial stomach became pseudostratified histologically, but simple columnar epithelium remained in a dorsal part of the primordial forestomach in the embryo with 7.3 mm NRL. Areas of simple epithelium were present in a dorsal
part of the primordial forestomach or associated area of the primordial forestomach in 8.3 mm NLR-11.9 mm CRL caprine embryos. The existence of the simple epithelium may be useful in tracing derivation of the primordial forestomach. The area of simple epithelium moved cranially, and spread with the cranial expansion of the primordial rumen in embryos with 12.9 and 13.3 mm CRL.

Suganuma et al. [28] observed Con A III reactivity in esophageal glands of the frog, snake and man, and in esophageal mucous cells of the frog and skink. They also found Con A III reactivity only in mucous neck cells among the gastric gland cells. There was no reaction in surface epithelial cells. Ohta et al. [21] reported that Con A III reactive cells were distributed in the esophagus of the tadpole at stage 50, and appeared in the stomach at stage 60. In the present study, the epithelium of the primordial esophagus and its extension in 6.5, 6.7 and 7.3 m NRL embryos reacted more intensely to Con A III than epithelium of the primordial stomach. These reactions were resemble those in the tadpole.

The forestomach of the domestic ruminant is lined with stratified squamous epithelium like the esophagus. The striated muscle bundle extends longitudinally from the esophagus into the external layer of the reticular groove. In addition, the presence of the glands closely resembling esophageal glands were found in the submucosa of lips of reticular groove in sheep and in omasal
The primordial esophagus consists of the proper esophagus (Ep) and its extension (Ex) in the CRL 6.5–6.7 mm embryos. Sp: Primordial stomach. L: Primordial lung sac. B. The esophageal extension was separated from the primordial proper esophagus (Ep) by constriction (arrow), and became a primordial forestomach (Fp) in the CRL 7.3 mm embryo. Sp: Primordial stomach. L: Primordial lung sac. C. D. E: The primordial forestomach and primordial stomach were united, and formed a spindle shaped primordial ruminant stomach (RSp) in the CRL 8.3–11.9 mm embryos. Ep: Primordial proper esophagus. L: Primordial lung sac. F: The primordial rumen (Rp), Omasum (Op) and abomasum (Ap) appeared from the primordial ruminant stomach in the CRL 12.9–13.3 mm embryos.

base in goats [8, 14, 25, 30, 33]. Furthermore, the glands of the forestomach had myoepithelial cells like those in esophageal glands [18, 19].

Pernkopf [23] showed that during the developmental process of the early ruminant stomach, the esophageal extension was situated on the dorsal surface of the stomach, and reported that it disappeared during fetal life. Habel [7] described the similarity in gastric nerve distribution between species with simple or ruminant stomachs and, like Warner [32], reported no esophageal contribution to any part of the compartmental stomach. McGeady & Sack [17] also found no esophageal contribution in an embryological study of the bovine vagal development. They reported that a branch of the dorsal vagal trunk was found in the stomach of bovine embryo at the 11 mm CRL stage with the first appearance of the ruminoreticular primordium, and that each subsequent compartment derived from the ruminoreticular primordium received branches from the vagal trunk. Findings of the present study, however, suggest that at the stage of embryo development examined by Warner [32] and McGeady & Sack [17], the primordial forestomach (i.e., esophageal extension) and primordial stomach were already united. As noted previously, Warner [32] also reported no esophageal contribution to the ruminant forestomach development but did report that certain areas of the stomach, especially the esophageal groove, showed changes similar to those in the esophagus in early bovine embryos by making layers of the stratified epithelium.

The present study found that the ruminant stomach of caprine embryos is formed by the union of the esophageal extension and the proper primordial stomach. The esophageal extension becomes the primordial forestomach before the primordial spindle shaped stomach is formed. In caprine embryo of this study, primordial rumen, omasum and abomasum were derived from the spindle shaped primordial ruminant stomach at 12.9 mm CRL, and the primordial reticulum was developed at 13.3 mm CRL.
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REFERENCES

要約

ヤギの胃の初期形態形成：武藤顕一郎・和栗秀一（北里大学獣医畜産学部獣医解剖学教室）——頭殻長6.5mmから頭殻長13.3mmのヤギ胎子11例における胃の初期形態形成について、光学顕微鏡および三次元復雑法的に検討した。頭殻長6.5mmないし6.7mm胎子において、食道原基の一部が紡錘形で網観腔に開まれた胃原基の右背方に小囊状に伸張し、その上皮は単層円柱状でConA III型染色に強く反応した。頭殻長7.3mm胎子において食道の伸張部と食道管葉部の間に括れが生じ、前者は前胃原基となって胃原基を構成する網観腔の前背側に位置するようになり、その上皮は単層円柱状で、ConA III型染色において胃原基粘膜上皮より強く反応した。頭殻長8.3ないし10.7mm胎子において前胃原基と胃原基両者の組織学的構造を比較すると全体的に扁平で、その小葉部に相当する部は透明な中を形成する以外はほとんどそれと類似する紡錘型の複胃原基となっていた。複胃原基の全ての上皮はConA III型染色に強く反応した。この複胃原基から、頭殻長12.9mm胎子において第一胃、第三胃、第四胃の各原基が生じ、第二胃原基は頭殻長13.3mm胎子において第一胃と第三胃原基の間に認められた。