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Macroscopic and Microscopic Development of the Stomach in Hamdani Crossbred Sheep Fetuses (*Ovis aries*)

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Abstract

The ruminant stomach is a critical digestive department in terms of converting low-quality feed into high nutrient-value substances. In these animals, there is a compound stomach structure with 4 compartments. Understanding the developmental structure of the stomach at macroscopic and microscopic levels is especially important in terms of nutrition. Türkiye can be considered one of the leading countries in the world in terms of sheep breeding. Our study aimed to investigate macroscopically and microscopically the developmental structures of the stomach of Hamdani crossbred sheep fetuses collected at different gestation periods. For this purpose, a total of 15 fetuses, 5 fetuses each from 3 different periods of pregnancy, were included in the study. The stomachs of the fetuses were dissected simultaneously and their topographic and macroscopic features were determined. After routine histological procedures, the stomachs separated from the body cavities were stained with Hematoxylin & Eosin and Masson Trichrome stains. As a result of the study, macroscopic and microscopic information about fetal development of the stomach was obtained. It was revealed macroscopically and microscopically that the rumen was the largest part of the stomach until the middle of the 2nd period of gestation, and that the abomasum grew rapidly at the end of this period and showed similar characteristics to the adult sheep stomach. The data of the study will contribute to macroscopic, microscopic, and ultrasonographic studies on the stomach in ruminant animals.

Keywords: Fetus, Hamdani sheep, histology, morphology, stomach

Introduction

The ruminant stomach is a complex structure that guarantees microbial digestion with the ability to convert low-quality feed, especially cellulose, into products with high nutritional value.(1,2) The stomach is shaped into four separate compartments rumen, reticulum, omasum and abomasum. There are differences in terms of the location of each compartment in the body, the area it occupies and its functional functions.(3) Before reaching the abomasum, which is the equivalent of the monogastric glandular stomach, food is digested in three other compartments known as the forestomach.(4) These act as fermentation chambers, while peristaltic movements as well as contractions and separation of liquids from solids are also observed in them.(5) In ruminants, especially the abomasum is developed during the fetal period and is similar to that of non-ruminant animals.(6) Morphological and histological changes occur in the pre-and postnatal periods for all stomach parts of an adult ruminant to fulfil their functional tasks.(7) Ruminant stomachs are structures that should be well known for the development of feeding strategies(8) to increase productivity.

Various macroscopic, microscopic and ultrasonographic studies have been conducted in cattle, sheep and goats.(2,9,10) However, more research is needed to fully understand the developmental structure of the stomach. Sheep

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are animals under the ruminant subgroup. These animals stand out with their role in organic production in meeting sustainability demands.(11) Hamdani sheep breed has an oily tail structure and is a successful breed in milk and fleece yield.(12) Especially in the southeastern regions of Türkiye, Hamdani sheep are crossbred to increase their productivity characteristics.(13)

The histological structure of the stomach wall consists of four layers: mucosa, submucosa, tunica muscularis and serosa. In the mucosa, it is possible to distinguish stratified squamous epithelium, lamina propria and muscle layer. The muscle layer is not present in the rumen mucosa.(14)

In recent years, fetal tissues have been frequently used in prenatal treatments such as stem cell transplantation and gene therapy. It has been stated that the available information about the fetal gastrointestinal system in diagnosis and treatment is limited.(10) Thus, the importance of studies in fetal tissues increases considerably. Our study aimed to determine macroscopically and microscopically the changes that occur in the stomach sections during fetal growth in sheep.

Material and Methods

Hamdani crossbred sheep fetuses were collected from pregnant animals slaughtered in Siirt slaughterhouse. Fetuses were removed from the collected pregnant uteruses and amniotic fluid was cleaned. Having a singleton pregnancy uterus tissues were included in the study, while twin pregnancies were excluded. Crown-rump length (CRL) was measured according to the formula described by Harvey.(15) Pregnancy period was determined using the formula X=2.1(Y+17) (X=gestation period in days, Y=CRL).(16,17) A total of 15 fetal stomachs were analyzed: 5 fetuses from the 1st trimester (46-48 days), 5 fetuses from the 2nd trimester (64-96 days), and 5 fetuses from the 3rd trimester (105-118 days). Fetal weights were calculated using a weighing scale. Simultaneous dissection was performed to determine the location and topographic position of the stomach parts. Macroscopic changes were noted. Their weights were determined with a precision balance.

Tissue samples were taken from each stomach compartment in three separate trimesters. Samples were fixed in 10% neutral formalin, during 48h. The tissues were embedded in paraffin wax after routine tissue follow-up and 5 µm thick sections were taken. The sections were stained with Hematoxylin & Eosin(18-20) and Masson Trichrome(21,22 stains. The slides were examined under a light microscope (DM750, Leica) equipped with a digital camera (MC170, Leica).

With the ethics committee report numbered 2024/03/10, the Siirt University Experimental Animals Application and Research Center approved the procedures used in our investigation.

Results

Macroscopic Findings

When the abdominal cavity was opened all parts were in the form of a small bulb (Figure 1A-B). In first trimester of fetuses, the liver was observed to occupy a large area on both the right and left sides (Figure 1C). When the liver was removed, it was determined that the stomach was completely formed. Since the stomach was small, it was located behind the diaphragm and between the ribs. The abomasum was seen as a bulb protruding ventrally caudal to the rumen (Figure 1D). The rumen was observed in front of the stomach and the reticulum was observed to the left of the rumen. No clear cleft was observed on the rumen. The average fetal weight was 18.50 g and the average stomach weight was 0.246 g.

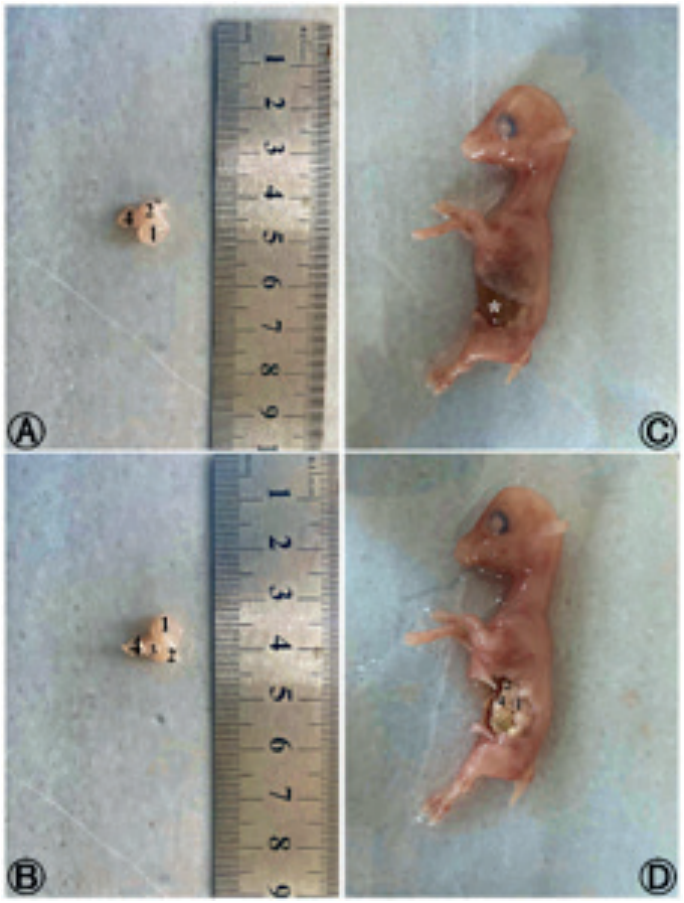

Figure 1. View of the stomach in sheep fetus at first-trimester gestation; A,C,D: left view; B: right view. 1: Rumen; 2: Reticulum; 3: Omasum; 4: Abomasum; *: liver.

In the second trimester of fetuses, it was observed that the size of the rumen started to increase with growth and the clefts on it became prominent (Figure 2A). Starting from

the caudal 11th rib, the rumen was positioned towards the abdominal cavity on the left side. The spleen was located dorsally between the abdominal wall and the rumen. The growing rumen also pushed the reticulum to the right and the abomasum ventrally. The omasum was located on the right side under the reticulum (Figure 2B). The abomasum was completely covered by the liver (Figure 2C). When the liver was removed, the abomasum was seen (Figure 2D). The mean fetal weight and mean stomach weight were 700.57 g and 9.36 g, respectively.

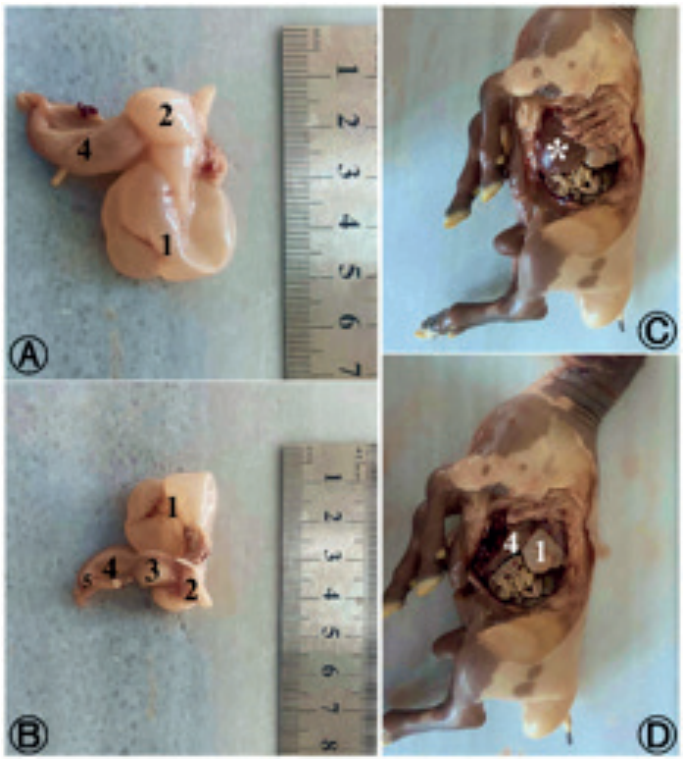

Figure 2. View of the stomach in sheep fetus at second-trimester gestation; A,C,D: left view; B: right view. 1: Rumen; 2: Reticulum; 3: Omasum; 4: Abomasum; 5: pylorus; *: liver.

In the third trimester fetuses, it was determined that the rumen started from a region close to the 8th intercostal space on the left side with growth. While clefts were observed on the organ, sac structures were also formed (Figure 3A). While the reticulum was located in the anterior part of the stomach, the omasum was also observed on the right and overgrown (Figure 3B). In this period, the rumen and part of the abomasum were observed on the left side during abdominal cavity dissection (Figure 3C-D). Abomasum was detected to be located close to the midline. At the end of this period, the organ assumed an oblique position (Figure 3D). The pylorus section extended cranially in an upright position. Macroscopic examination showed that the abomasum was the largest and the omasum was the smallest part of the stomach. The wall structures of the stomach were very thin in the first trimester of fetuses. The wall thickness started to increase in the middle period and reached maximum thickness in the third period. The average fetal weight and stomach weights were determined as 2.338 g and 34.01 g in this trimester.

Microscopic Findings

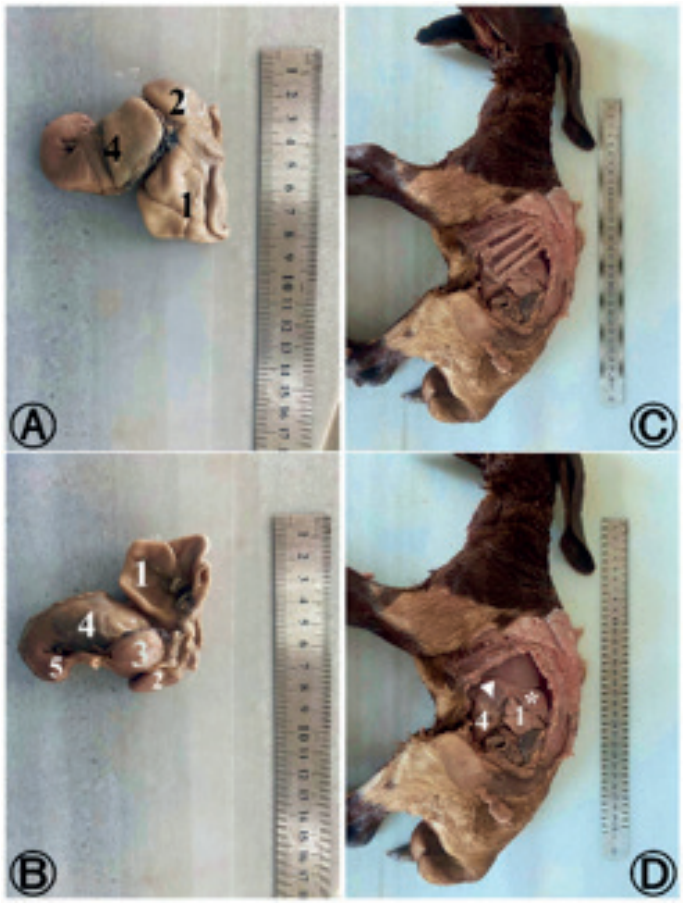

Figure 3. View of the stomach in sheep fetus at third-trimester gestation; A,C,D: left view; B: right view. 1: Rumen; 2: Reticulum; 3: Omasum; 4: Abomasum; 5: pylorus; *: spleen; arrowhead: diaphragm.

The changes in the tunica mucosa were similar in the rumen and reticulum. The lamina epithelialis layer was in the form of multilayered squamous epithelium in all three trimesters and keratinization was not observed. The thinnest epithelial layer was observed in the first trimester and the thickest epithelial layer was found in the last trimester (Figure 4A-C). It was observed that the lamina propria was invaginated between the epithelial cells due to epithelial thickening in the rumen and tissue in the last trimester. In the rumen, the lamina propria and tunica submucosa were a thick connective tissue layer that could be separated by Masson trichrome staining in the last trimester (Figure 4C). The tunica muscularis layer was observed as a cellular distribution which was difficult to distinguish in the first trimester, but in the second trimester, it was observed as circular on the inside and longitudinal on the outside with equal thickness. In the last trimester, it was seen as two layers with a thick circular arrangement on the inside and a thinner longitudinal arrangement on the outside.

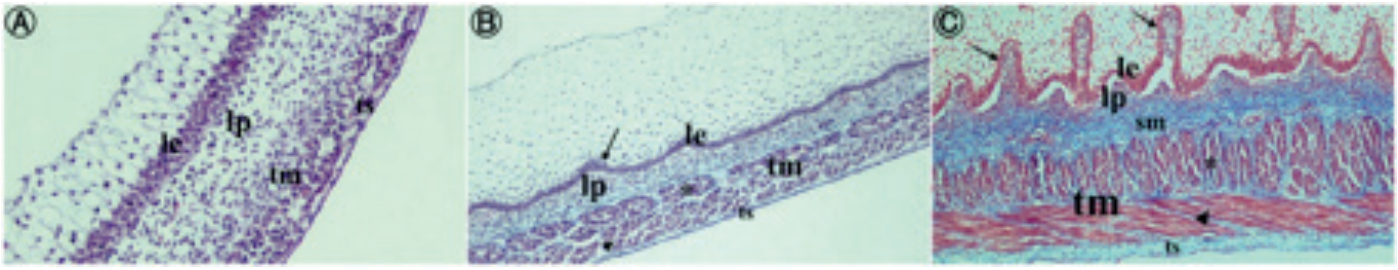

Figure 4. Transversal sections of Rumen. A: 1st trimester X20; B: 2nd trimester X10; C: 3rd trimester X20. le: lamina epithelialis; lp: lamina propria; tm: tunica muscularis; sm: submucosa; ts: tunica serosa; *: circular muscle; arrowhead: longitudinal muscle.

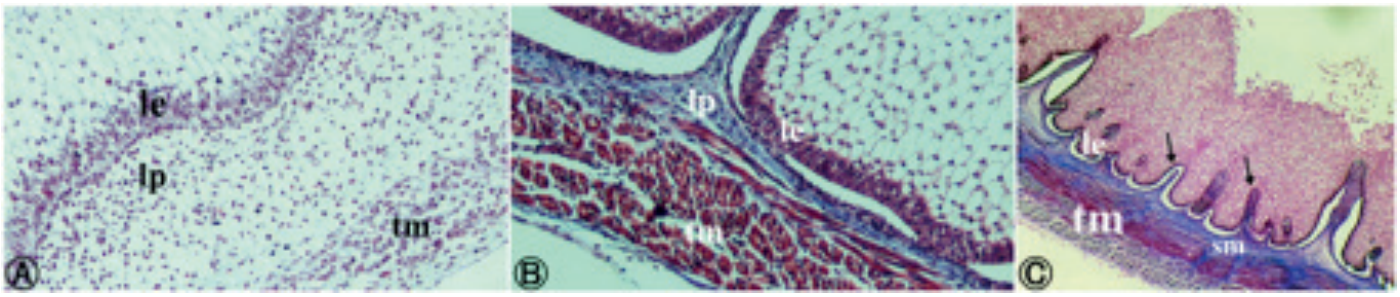

Figure 5. Transversal sections of the reticulum. Masson trichrome staining. A: 1st trimester X20; B: 2nd trimester X20; C: 3rd trimester X10. le: lamina epithelialis; lp: lamina propria; tm: tunica muscularis; sm: submucosa; arrow: crista reticuli

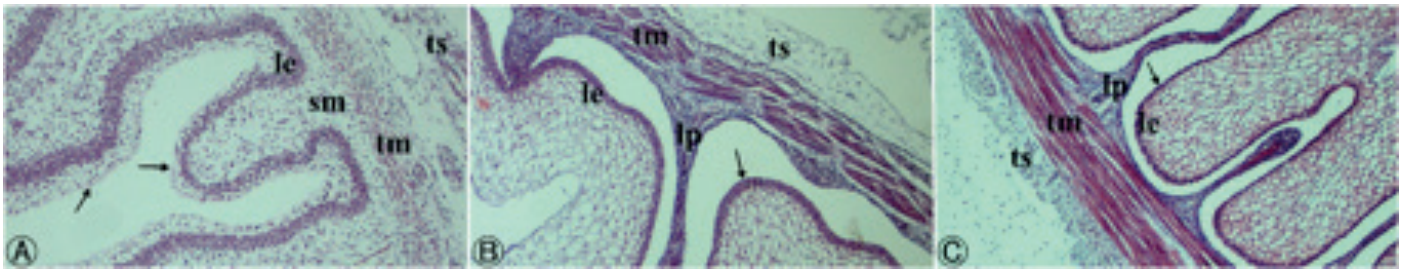

Figure 6. Transversal sections of omasum. Masson trichrome staining. A: 1st trimester X10; B: 2nd trimester X10; C: 3rd trimester X10. le: lamina epithelialis; lp: lamina propria; tm: tunica muscularis; sm: submucosa; ts: tunica serosa; arrow: lamina omasi

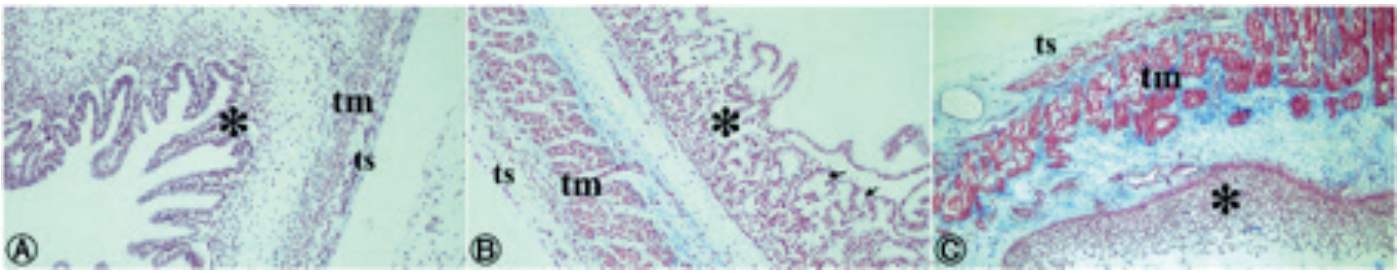

Figure 7. Transversal sections of the abomasum. Masson trichrome staining. A: 1st trimester X10; B: 2nd trimester X10; C: 3rd trimester X4. tm: tunica muscularis; ts: tunica serosa; arrow: glandular formations; *: tunica mucosa

Masson trichrome staining of the reticulum showed an increase in the amount of collagen fibers in the lamina submucosa in the last trimester (Figure 5C). The tunica muscularis layer was defined as cellular distributions that could not be selected in the first trimester (Figure 5A), whereas in the second trimester, two layers were observed as an inner circular thin layer and an outer thick longitudinal layer (Figure 5B). In the last trimester, it was examined as circular on the inside and longitudinal on the outside, similar to the mature histological appearance.

In the 1st trimester, lamina omasi-like extensions formed by the mucosa and submucosa extending towards the lumen were remarkable, while the presence of muscle trabeculae was observed from the 2nd trimester (Figure 6A-B). Thickening of the lamina epithelialis layer as the trimester progressed and papillary invagination of the lamina propria in this stomach part within the epithelial tissue due to epithelial thickening in the last trimester were observed (Figure 6C). The tunica muscularis layer was observed as a thin cellular distribution which could not be seen in the first trimester, whereas it was observed as 2-layered, circular on the inner side, and longitudinal on the outer side from the 2nd trimester onwards.

In the first trimester, it was observed that the tunica muco-

sa in the abomasum was in the form of villous structures consisting of epithelium and lamina propria (Figure 7A). In the 2nd trimester, the villous structures were shortened and glandular formations similar to mature histological appearance were determined between them (Figure 7B). In the last trimester, the epithelium in the tunica mucosa layer had the appearance of a simple stomach with a glandular structure (Figure 7C). The tunica muscularis layer was detected as a single layer in the first trimester. In the 2nd trimester, this layer consisted of a thick longitudinal layer and a thin circular layer, whereas in the last trimester, it was observed in 3 layers oblique in the innermost, circular in the middle, and longitudinal in the outermost. In general, there was no significant change in the tunica serosa layer in the stomach parts between trimesters.

Discussion

There are studies on the stomach, which is an important part of the digestive system in ruminants. However, studies on fetal periods are limited. In our study, it was aimed to give detailed information about the macroscopic and microscopic development of the stomach in Hamdani crossbred sheep fetuses in all three trimesters of pregnancy.

In a study conducted in sheep fetuses, it was reported that the stomach started to expand and curl on the 17th day,(23) and the primitive stomach became prominent on the 18th day of embryonic life and there was a spindle-shaped expansion in the anterior part of the intestine. On the 19th day after fertilization, a simple tube-shaped structure, on the 23rd day, an elongated, twisted, and herniated structure towards the umbilical cord, and the first coiling starting on the 27th day and continuing until the 34th day was recorded.(24) Hejazi and Erik-aghaji(25) reported that the stomach was tubular and originated from the digestive tract on day 38 of gestation. The structure of the stomach was determined as four consecutive extensions in the goat embryo at day 38 in a visible position. The first extension on the left is the rumen and the second extension caudoventral to the rumen is the reticulum. The third extension visible along the minor curvature is the omasum. The abomasum is located along the ventral midline of the stomach and is located in the pyloric part.(26) In present study, the stomach parts of the fetuses at day 46 consisted of 4 small bulbs with no clear demarcation.

In a study on 50 deer embryos, it was reported that the rumen could be seen at 60 days.(27) In this study, that at the beginning of the sixth week of pregnancy, the stomach fundus expands in the craniodorsal position and turns to the left side to form the primary rumen, and in the seventh week, the rumen moves from the craniodorsal position to the caudodorsal position to form the reticulum.(25) In Merino sheep fetuses, it was mentioned that the rumen was visible on the 33rd day.(28) In the goat, the dorsal and ventral sacs of the rumen are in the same position at the beginning of the second trimester, while the visceral surface of the dorsal sacs is adjacent to the developing abomasum throughout pregnancy.(7) In bovine fetuses, two clefts were observed on the rumen on days 40-44 of gestation, while cranial and caudal clefts started to form on days 45-50. In addition, coronary clefts were formed from the 55-60th days and the sacs became clear.(2) In our study, the stomach parts were not separated from each other with a clear boundary in the first trimester of pregnancy.

Macroscopically, the abomasum was seen as a bulb protruding ventrally caudal to the rumen. The rumen was observed in the front of these parts and the reticulum was noted to the left of the rumen. During the second trimester, it was determined that the reticulum moved forward due to the movement of the rumen to the caudal side following the literature. On the 46th day, clefts on the rumen started to be noticed, but in the second trimester, on the 74th day, the clefts had almost completed their development. In addition, rumen papillae were observed to have completed their development to a great extent on the 105th day of gestation. Our findings are consistent with the results reported in goats.(14,29)

In the first trimester, the reticulum was smooth and appeared as a small bulb. When compared between trimesters, we agree with studies that suggest that the reticulum grows on the dorsoventral axis. In addition, the growing rumen has pushed the reticulum cranioventrally.(26) While the first cristae structures started to be observed in the second trimester, the honeycomb appearance of the inner surface was completed in the third trimester. It was also reported that cellula reticuli structures were visible from the outside at 80-100 days of age.(25)

It has been reported that the omasum is the smallest stomach part of small ruminants and can be distinguished as a bulb in front of the reticulum at about 30 days of age in sheep fetuses. The omasum also has a vertical axis of growth.(25,26) Following the literature, the omasum is located under the reticulum and on the right side as of the second trimester of pregnancy. The organ, which had a flat surface in the first period, started to show protrusions on its mucosa in the second trimester and lamina omasi structures were seen in the last gestational period.30 In the approximately 107-day-old fetus, conical papillae protru-

sions of the laminae were observed.(26)

The abomasum was a bulb extending ventrally in the caudoventral part of the rumen in the approximately 48-day-old fetus. During the second gestation period, the abomasum which was longitudinal started to curl. Following the literature, the abomasum was completely covered by the liver in the first trimester.(26,31,32) In a study conducted in bovine fetuses, it was reported that spiral folds of the abomasum were seen on average at 45-50 days.(2) In present study, the spiral folds, which started to be observed in sheep fetuses at approximately 64 days of age, were determined to have almost completed their development at 111 days of age.

In studies conducted on goats, it was mentioned that the abomasum is the largest stomach in all periods.(26,32) In our study, following Hejazi and Erik-aghaji(25)the rumen was noted as the largest stomach part until the middle of the second period. By the end of second trimester and the beginning of third trimester, the abomasum was in a position larger than the rumen. This supports the view that it was the largest stomach part during parturition. This development of the abomasum may be related to the fact that it needs to function during parturition. The other stomach parts should develop more in the postnatal period to function.

In goat fetuses, a muscle layer consisting of two myoblast layers, an inner circular layer, and an outer longitudinal layer, was observed in the rumen on day 50. In the present study, the tunica muscularis layer was observed in the first trimester as a cellular distribution that was difficult to distinguish, while in the second trimester, it was observed as an inner circular layer and an outer longitudinal layer with equal thickness. In the last trimester, it was seen as two layers with a thick circular arrangement on the inside and a thinner longitudinal arrangement on the outside. The invagination of the lamina propria between the epithelial cells, especially in the last trimester, and the lamina propria and tunica submucosa layer as a thick connective tissue layer with clear separation in the last trimester were consistent with the literature.(14,29,33,34) Reticulum part on day 59 of goat gestation, primary crista reticuli can be seen as protrusions from the basal epithelial layer in the fetal reticulum.(14,359 In our study, it was observed that crista reticuli structures started to form in the second period of pregnancy. The increase in the amount of collagen fibers in the lamina submucosa in the last trimester was consistent with the literature.(34) It has been reported that secondary omasal laminae appear between the primary laminae on the 50th day of fetal life and tunica muscularis is observed in the omasal lamina after the 100th day of fetal life.(33,35,36) In our study, lamina omasi extensions formed by mucosa and submucosa extending towards the lumen were observed in the omasum in the first trimester. From the second trimester, the presence of muscle trabeculae was observed.

Conclusion

In conclusion, macroscopic and microscopic changes occurring in the stomachs of Hamdani crossbred sheep fetuses in different periods were presented. It was revealed macroscopically and microscopically that the rumen was the largest stomach part until the middle of the 2nd period of pregnancy, and the abomasum grew rapidly at the end of this period and showed similar characteristics to the adult sheep stomach. The data of the study will contribute to macroscopic, microscopic, and ultrasonographic studies on the stomach in ruminants. In addition, the data have an important place in determining nutritional strategies.

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