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Gross morphological and scanning electron microscopic study of rumen of sheep (*Ovis aries*) during pre-natal life

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Abstract
A study was designed to elucidate the gross morphological development of rumen of sheep during pre-natal life. For this study 12 foeti were divided into three groups i.e. Group I (0-50 days of pregnancy), Group II (51-100 days of pregnancy) and Group III (101-150 days of pregnancy) with 04 foeti in each group. The developing rumen appeared to have a left and a right ruminal bud together assuming the quadrilateral shape at the age between 32 and 44 days of gestation. The whole rumen appeared as a sac like structure with a slight caudo-ventral projection at 63 days of gestation. Between 63 and 94 days of gestation, the rumen was seen to be attached with septum transversarium dorsally. During this period the parietal surface was related to the developing pancreas and left adrenal gland. The visceral surface was related to the coils of the intestine. At 150 days of gestation, the visceral surface of dorsal sac was adjacent to the developing abomasum. The developing rumen was noted to extend from 8th rib to 1st lumbar at 150 days of gestation. The cranial and caudal transverse grooves appeared at 32 and 48 days of gestation respectively. The right and left longitudinal grooves appeared at 48 and 94 days of gestation respectively. The internal surface of rumen revealed cranial and caudal pillars at 32 and 63 days of gestation respectively. By 116 days of gestation, small conical papillae were grossly seen to project into the lumen giving the internal surface a rough appearance. The scanning electron microscopic observations revealed appearance of ruminal papillae of very small size at 44 days of gestation. These papillae gradually became tall and appeared as distinct projections from 63 days of gestation and onwards.

Keywords: Sheep, rumen, pre-natal, ruminal papilla

1. Introduction
Livestock sector has provided structural support to the rural economy, at par with the Agriculture or a little beyond on date [1]. On account of favourable socio-economic factors such as change in eating habits and high affordability of people, urbanization, increasing health consciousness towards protein rich diet, there has been increase in demand for livestock products and the sector has gained momentum in terms of its contribution to income, employment and foreign exchange earnings [2]. Among livestock, sheep plays an important role in rural economy by providing wool, meat, milk, skin and manure. The common saying, "poor man's cow" for the rural farmer essentially signifies both sheep and goat. The sheep can withstand the most adverse husbandry conditions and yet grow up with profits. The Indian cuisine get aristocratic when served with mutton, thus sheep has the potential to contribute significantly to the livelihood of a large section of small and marginal farmers, nomads and landless labourers, above all the weakest section of our society. The gastrointestinal-hepato-portal system functions for metabolic turnover of assimilated food into flesh yield. The gastric-apparatus prepares the building blocks from pasture by way of enzymatic and microbial digestion. The ruminant stomach has four separate compartments i.e. rumen, reticulum, omasum and abomasum; whereas the first three chambers are regarded as the fore-stomach or proventricular stomach. Out of these three chambers, the largest is the rumen which engages the microbial digestion of cellulose and other carbohydrate ferment the end products to volatile fatty acids and convert nitrogenous substances to ammonia and protein. The microbes additionally synthesize essential amino acids and water soluble B-vitamins. Therefore development, maturation and function of the ruminal structural components are believed to evoke appropriate physiological responses which change with age and diet. It is well understood that aging brings about changes in the structure and function of organ systems and

the sheep stomach is no way exception to this phenomenon [3]. As different structures of rumen appear, consolidate and mature at particular age of gestation during the intra-uterine life, documentation of normal embryonic and foetal developments is necessary to understand the consequences of harmful influences at various stages of gestation [2]. Among the different components of rumen, ruminal papillae are the vital part which increase surface area and led to increase absorption upto ten times. The concentration of volatile fatty acids, ammonia, pH, and osmotic pressure in rumen along with internal factors such as glucose and insulin concentration affected the size and number of papillae [5]. The appearance and structural details of ruminal papillae during the prenatal developmental period can be well observed with the help of scanning electron microscopy. But reports on scanning electron microscopic study as well as gross anatomical development of rumen of the pre-natal sheep are gravely scanty. The sporadic reports on rumen of goat [6], on rumen of red deer [7] and on ontogeny of fore-stomach of Merino sheep [8], in the current literature, do not provide a fully formed foundation to understand the age related morphogenesis of the ovine rumen. Therefore the present investigation was undertaken to record the gross morphological developmental changes of rumen of pre-natal sheep along with scanning electron microscopic study.

2. Materials and Methods

2.1 Experimental animal

For this investigation 12 apparently healthy and normal embryos/foeti of either sex of non-descript breed of sheep were collected from the local slaughter house situated at Jadupur and Laxmisagar, Bhubaneswar. The adhering amniotic fluid from the body of the foeti was wiped by wet cotton. The crown rump length (CRL) for each foetus was measured in centimetres (cm) with the help of non-stretchable nylon thread and graduated scale and the CRL was placed on the standard CRL-Gestation Age Curve [3] to estimate the approximate age of foeti in days.

2.2 Experimental design

The experiment was designed to record the changes in the gross morphology of the rumen of the sheep foeti during pregnancy at three phases i.e. early pregnancy (0 to 50 days of gestation, Group-I), mid-pregnancy (51 to 100 days of gestation, Group-II) and late pregnancy (101 to 150 days of gestation, Group-III). Group-I constituted of 4 foeti with gestation age of 32, 37, 44 and 48 days; foeti with gestation age of 63, 67, 75 and 94 days in Group-II; and foeti with gestation age of 111, 116, 121 and 150 days were studied under Group-III. After collecting the embryo/foeti from the gravid uterus, their abdomen was opened to explore the ruminal compartment for record of its gross morphological and scanning electron microscopic observations.

2.3 Gross morphological study

The abdominal cavity of the foetus was cut-open by giving a longitudinal ventro-median incision. The stomach of the foetus was then exposed by carefully reflecting the lobes of the liver and then *in-situ* relationship of the ruminal compartment with adjacent abdominal viscera and topography of this chamber in relation to ribs and vertebrae were recorded. Its shape and colour were noted. The rumen was then cut open to expose its interior by incising the greater/lesser curvatures for record of internal observations. Thereafter, tissue pieces from the rumen was excised for Scanning Electron Microscopic (SEM) study at Central Lab, Odisha University of Agriculture & Technology, Bhubaneswar-751003 (Scanning Electron Microscope, Make: Hitachi and Model: S-3400N) and the electron-photomicrographs were recorded.

3. Results

The developing rumen appeared to have a left ruminal bud and a right ruminal bud and together they assumed the quadrilateral shape at the age between 32 and 44 days of gestation. The demarcation of both these buds was indistinct during this period. The right and left ruminal buds were distinctly visible at 48 days of gestation. The small left ruminal bud was quadrilateral in shape and the large right ruminal bud was pear shaped at this age of foetal life. The whole rumen appeared as a sac like structure with a slight caudo-ventral projection at 63 days of gestation (Fig. 1). This shape of the developing rumen was observed to remain static till terminal stage of gestation, and its size grew large with the advancement of age.

The rumen was seen on the left side of the median plane and was completely covered by the left lobe of the developing liver at 32 days of gestation. It was in contact with the septum transversarium anteriorly and with the mesonephros dorsally. The antero-dorsal part of the developing rumen was related to the developing spleen and the postero-dorsal part was related to developing gonads at 44 days of gestation. The developing rumen was observed to gradually change its position from 48 days of gestation. At this age the dorsal part of the rumen was related to the anterior end of mesonephros and mesonephric duct and its ventral part was related to the developing gonads. The caudo-ventral blind sac was in contact with intestinal coils at 63 days of gestation. Between 63 and 94 days of gestation, the rumen was seen to be attached with septum transversarium dorsally. During this period the parietal surface of the rumen was related to the developing pancreas and left adrenal gland. The visceral surface of the rumen was related to the coils of the intestine. The cranial 2/3rd part of dorsal sac of the rumen was in contact with the developing spleen. By 111 days of gestation, the anterior part of the caudo-ventral blind sac was related to 1/3rd part of the developing abomasum and its posterior part was in contact with the intestinal coils. At 150 days of gestation, the visceral surface of dorsal sac was adjacent to the developing abomasum.

The developing rumen was noted to extend from 11th to 13th rib on 32 days of gestation. This topographic position was maintained by the rumen up to 63 days of gestation. The rumen was observed to change its position to the new location between 9th rib and 2nd lumbar vertebra at 67 days of gestation. This topographic position of the rumen continued up to 121 days of foetal age. At 121 days of gestation, the rumen was observed to extend between 8th rib and 1st lumbar vertebra. Its dorsal sac extended up to 1st lumbar vertebra and the ventral sac up to anterior border of 12th rib. This topographic location of rumen continued up to terminal stage of the gestation.

The dorsal and ventral sacs of the developing rumen were partially demarcated by a faint cranial transverse groove at 32 days of the foetal age. The cranial transverse groove became distinct and the faint caudal transverse groove appeared at 48 days of gestation. The dorsal and ventral sacs were observed distinctly with deep cranial and caudal transverse grooves at

63 days of gestation (Fig. 1). The dorsal sac was large in size and the ventral sac appeared to be small in size. The right longitudinal groove on the wall of the rumen was first seen at 48 days of gestation. It became distinct with advancement of age (Fig. 2). The left longitudinal groove was first identified at 94 days of gestation. It became prominent at 111 days of gestation. The left and right caudo-ventral coronary grooves were observed as very faint demarcation at 44 days of foetal age and became prominent at 63 days of gestation revealing the caudo-ventral and caudo-dorsal blind sacs. All the grooves were conspicuously prominent at 150 days of gestation.

The internal surface of developing rumen revealed folds i.e. the ruminal pillars. They were the representatives of the respective grooves present on the external surface. The cranial and caudal pillars were observed to project into the anterior and posterior ends of lumen of the rumen at 32 and 63 days of gestation respectively. The dorsal and ventral sacs appeared to be continuous in the region between cranial and caudal pillars. The right longitudinal fold was first documented at 44 days of gestation and gradually became prominent with advancement of age. The left longitudinal fold was first noticed at 94 days of gestation (Fig. 3) and gradually became distinct towards terminal age of the gestation. The right and left ventral coronary folds were first observed at 37 days of gestation and were seen to be well developed at 44 days of foetal life. The junction between the rumen and the reticulum was demarcated by the rumino-reticular fold.

The gross appearance of the internal surface of the rumen revealed a soft and smooth texture with a yellowish white colour from the age of 32 days to 111 days of gestation. By 116 days of gestation, small conical papillae were seen to project into the lumen giving the internal surface a rough appearance. They were prominently visible at 121 days of gestation and were observed with large height at 150 days of gestation (Fig. 4). The scanning electron microscopic observations revealed appearance of ruminal papillae of very small size at 44 days of gestation (Fig. 5). These papillae gradually became tall and appeared as distinct projections from 63 days of gestation (Fig. 6) and onwards. The ruminal papillae of large heights were observed at 150 days of gestation (Fig. 7).

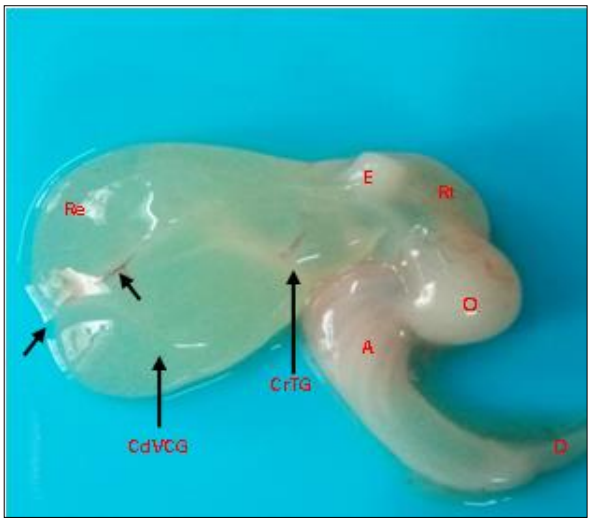

Fig 1: Photomicrograph showing the growing compartments of the stomach i.e. rumen (Re), reticulum (Rt), omasum (O), abomasum (A), esophagus (E) and duodenum (D) of sheep foetus aged 63 days. Note the cranial and caudal transverse grooves (CrTG, CdTG) with blood vessels (arrow), caudo-ventral coronary groove (CdVCG) of rumen.

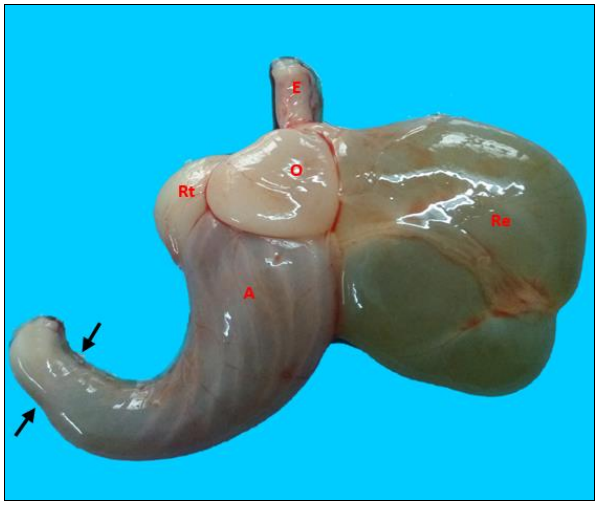

Fig 2: Photomicrograph showing the growing stomach of sheep foetus aged 94 days. Note the light brown to grey colour of the rumen (Re) and abomasum (A) and a whitish colour of reticulum (Rt) and omasum (O).

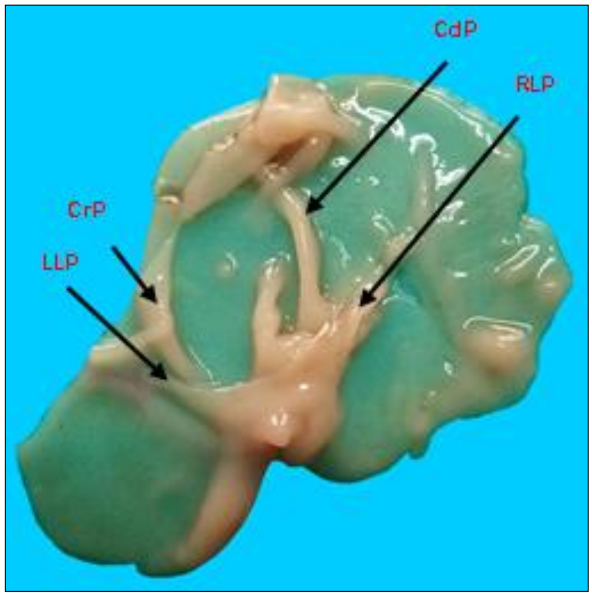

Fig 3: Photomicrograph showing the smooth internal surface of the rumen of sheep foetus aged 94 days. Note the cranial and caudal pillars (CrP, CdP) and left and right longitudinal pillars (LLP, RLP)

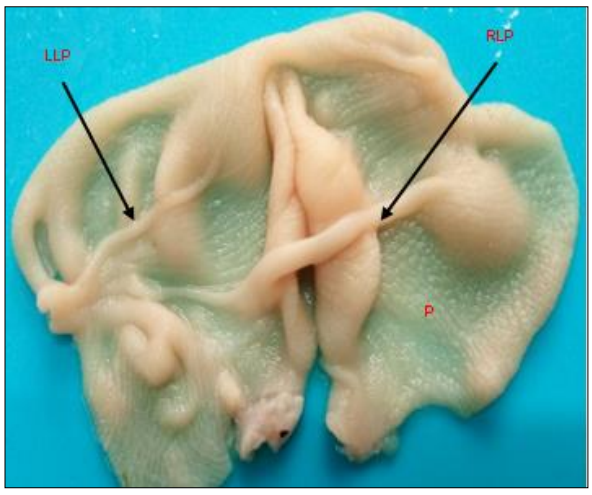

Fig 4: Photomicrograph showing the appearance of ruminal papillae (P) as tiny mucosal projections on the internal surface of rumen of sheep foetus aged 150 days. Note the left and right longitudinal pillars (LLP, RLP)

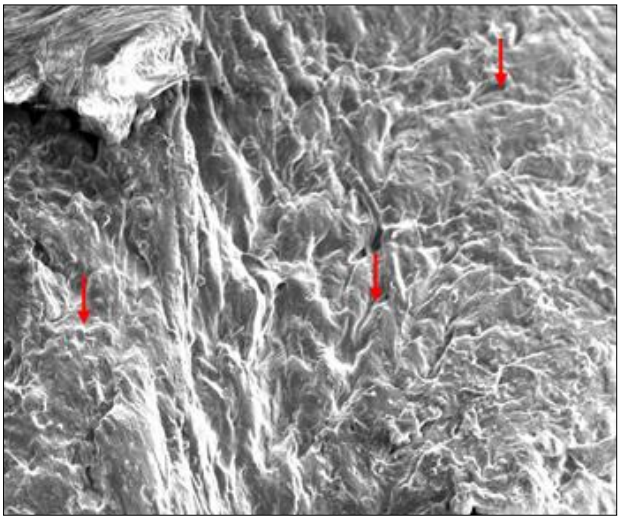

Fig 5: Photomicrograph showing the morphology of the mucosal surface of the rumen of sheep foetus aged 48 days. Note the minute ruminal papillae at initial stage of formation (arrow). Scanning electron micrograph × 5.00kv

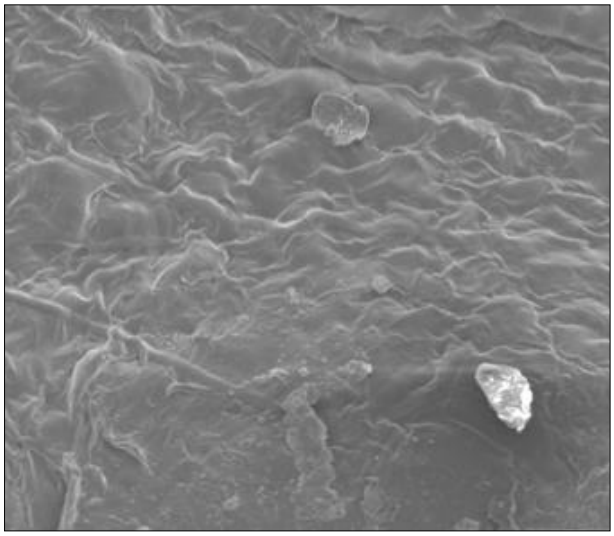

Fig 6: Photomicrograph showing the development of ruminal papillae on the mucosal surface of rumen in sheep foetus aged 63 days. Note the wavy mucosal projections of the papillae (arrow). Scanning electron micrograph × 5.00kv

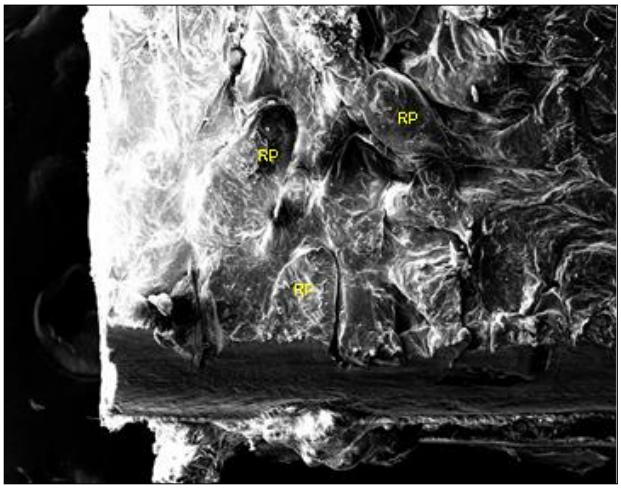

Fig 7: Photomicrograph showing the morphology of the ruminal papillae (RP) on the mucosal surface of rumen of sheep foetus aged 150 days. Note the tongue-shape of the papillae. Scanning electron micrograph × 10.00kv

4. Discussion

The developing rumen revealed the indistinctly demarcated left and right ruminal buds and together resumed a quadrilateral shape at the age between 32 and 44 days of gestation. Both these buds became distinct by 48 days of gestation wherein the left ruminal bud was smaller and quadrilateral in shape while the right ruminal bud was larger and pear shaped at this 48 days of foetal age. The rumen then grew in size with advancement of age and the whole rumen appeared as a sac like structure with a slight caudo-ventral projection between 63 and 150 days of gestation. In partial consonance to the observations of present findings Hejazi and Farhoudi [9] reported that the rumen was quadrilateral in shape in initial stage of pregnancy and then became rectangular during late stage of pregnancy. Gupta *et al.* [10] reported that the shape of the developing caprine rumen was roughly quadrilateral throughout the study period which became caudo-laterally pointed at 120 days of foetal age.

By the first age of observation i.e. 32 days, the rumen of the sheep foetus was seen to be completely covered by the left lobe of the developing liver. Similar findings were also observed in buffalo [11] and in goat foeti [10]. The rumen was in contact with the septum transversarium cranially and with mesonephros dorsally. The antero-dorsal part of the developing rumen was related to the developing spleen and its postero-dorsal part was related to developing gonads at 44 days of gestation of sheep foetus. With ageing the developing rumen changed its position i.e. between 63 and 94 days of gestation, the parietal surface of the rumen was related to the developing pancreas and left adrenal gland while its visceral surface was related to the coils of the intestine. At 150 days of gestation, the visceral surface of the dorsal sac remained adjacent to the developing abomasum.

The rumen was observed to extend from 11^{th} to 13^{th} rib between 32 and 63 days of gestation thus it covered two intercostal spaces, from 9^{th} rib to 2^{nd} lumbar vertebra between 67 and 121 days indicating enlargement of ruminal size and from 8^{th} rib to 1^{st} lumbar vertebra suggesting a cranial displacement of the rumen at 150 days of gestation. The dorsal sac extended up to 1^{st} lumbar vertebra and ventral sac up to anterior border of 12^{th} rib at this term end. Therefore the dorsal sac was larger than the ventral sac. In agreement to the present findings Gupta *et al.* [10] also found the placement of the developing rumen against 11^{th}-13^{th} rib between 0 to 50 days of gestation, 9^{th} rib to 2^{nd} lumbar vertebra between 51 to 100 days of gestation and 8^{th} rib to 1^{st} lumbar vertebra between 101 and 150 days of gestation in goat foetus. There is paucity of literature specifically on the topography of developing rumen of pre natal sheep for a vivid discussion. However in adults, the rumen was seen to extend from 7^{th} or 8^{th} intercostal space and pelvic inlet [12].

A faint cranial transverse groove appeared on the developing rumen by 32 days of gestation in sheep foetus, demarcating the dorsal and ventral sacs. The faint caudal transverse groove appeared by 48 days of gestation and simultaneously the cranial transverse groove became distinct. The caudal transverse groove became distinct by 63 days of gestation. The right and left longitudinal grooves on the wall of rumen first appeared at 48 and 94 days of gestation respectively. Gradually with advancement of age, these grooves became distinct and all the grooves became conspicuous at 150 days of gestation. These findings are in partial agreement with the findings of Gupta *et al.* [10] in goat foetus. Panchamukhi *et al.* [13] observed the presence of transverse grooves at 3.2 cm

CRL and left and right longitudinal grooves at 5 cm CRL in buffalo foetus. In buffalo foeti, these grooves became prominent at 10 cm CRL and above [14]. The differences noted on the age of appearance of these grooves may be endorsed to the species character of the animal used in these studies. The grooves present on the external surface of the developing rumen represented muscular pillars on its internal surface of the rumen. The cranial and caudal pillars were observed to project in to the anterior and posterior ends of ruminal lumen at 32 and 63 days of gestation respectively. The right longitudinal fold was first observed at 44 days of gestation and the left longitudinal fold at 94 days of gestation. Both became distinct with advancement of age. Panchamukhi *et al*. [13] observed the internal pillars in buffalo foetus at 2.2 cm CRL. The internal surface of the rumen o non-descriptive sheep foetus appeared yellowish white in colour with soft and smooth texture from the age of 32 days to 111 days of gestation. By 116 days of gestation, small conical papillae were noted to project in to the lumen giving the internal surface a rough appearance as per visual estimate. However with scanning electron microscopy, these papillae were noted prominently at an early age of 63 days. The ruminal papillae became prominent at 121 days of gestation and were of larger height at 150 days of gestation. Similar findings were observed by Garcia *et al*. [6] who observed that the internal surface was smooth and soft due to absence of prominent ruminal papillae at 75 days of gestation in goat and ruminal papillae became clearly visible at 113 days of gestation. In contradiction to the findings of the present study, Gupta *et al*. [10] observed a slight roughness of mucosal surface of rumen at 90 days and the papillae became sharp at 145 days.

5. Conclusion

The developing quadrilateral shaped rumen revealed the indistinct left and right ruminal buds at the age between 32 and 44 days of gestation which became distinct by 48 days of gestation. By this age the left ruminal bud was smaller and quadrilateral in shape and the right ruminal bud was larger and pear shaped. At term end the whole rumen appeared as a sac like structure. The rumen was in contact with the septum transversarium cranially and with mesonephros dorsally. The antero-dorsal part of the developing rumen was related to the developing spleen and its postero-dorsal part was related to developing gonads at 44 days of gestation. Between 63 and 94 days of gestation, the parietal surface of the rumen was related to the developing pancreas and left adrenal gland while its visceral surface was related to the coils of the intestine. At 150 days of gestation, the visceral surface of the dorsal sac remained adjacent to the developing abomasum. The rumen was observed to extend from 11th to 13th rib between 32 and 63 days of gestation, from 9th rib to 2nd lumbar vertebra between 67 and 121 days of pre-natal life and from 8th rib to 1st lumbar vertebra by 150 days of gestation. A faint cranial and caudal transverse grooves appeared first by 32 and 48 days of gestation respectively. The right and left longitudinal grooves on the wall of rumen first appeared at 48 and 94 days of gestation respectively. The internal surface of the rumen appeared yellowish white in colour with soft and smooth texture upto 111 days of gestation. By 116 days of gestation, small conical papillae were noted to project into the lumen as per visual estimate. With scanning electron microscopy, these papillae were noted at an early age of 44 days.

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