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Gross Morphological and Scanning Electron Microscopic Study of Abomasum of Sheep (*Ovis aries*) during Pre-natal Life

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ABSTRACT

Background: The enzymatic digestion occurs in the abomasum of sheep. A systematic comprehensive study on the embryogenesis of abomasums of sheep is lacking due to availability of sporadic information in current literature. So the current research was designed to explain the detail gross ontogenic occurrences along with scanning electron microscopic study of abomasum in sheep.

Methods: From the local slaughter house, 12 healthy sheep foeti of non-descript breed were gathered and grouped into Group I (early pregnancy), Group II (mid pregnancy) and Group III (late pregnancy) having 04 foeti in every group. The abdomen of the foetus was incised and opened. The stomach was exposed and the gross observations were recorded. Then tissue samples from the abomasum were processed for scanning electron microscopic study.

Result: The abomasums assumed comma shape at the gestational age of 32 to 48 days. At 67 days of foetus, the external constriction between fundus and pylorus of the abomasum was observed distinctly. In foeti of 32 to 48 days age, it was present between 13th rib and 1st lumbar vertebra. This topography changed to 8th rib and 3rd lumbar vertebra by 121 days of foetal age. The outline of the abomasal folds were noted first at 32 days of age. The height of the abomasal folds in fundus was larger than pylorus. The appearance of gastric pits on the mucosal surface was first observed at 63 days of gestation through scanning electron microscope.

Key words: Abomasum, Foetus, Scanning electron microscope, Sheep.

INTRODUCTION

At present situation in India, agriculture along with livestock sectors is the supporting framework of strengthening the economic status of rural people (Anonymous, 2012). Livestock provides income and employment to the rural households. It provides food in form of milk, meat and egg; wool, hair and hides; farmyard manure, bio-gas *etc*. Thus farmers in India adopt a balanced husbandry system *i.e*. a mixed form of farming crop as well as livestock where the byproduct of one farm becomes the input of the other farm, thereby decreasing the invest cost (Gupta *et al*., 2017). Among all the domesticated animals of India, sheep devote largely to the agricultural wealth, particularly in the dry, droughty and mountain regions where neither crop farming nor dairy farming is suitable. Both wool and sheep skin are being utilized as basic material in various leather industries. The manure of sheep is a valuable contributor in enhancing soil fertility. Sheep rearing is commonly done for wool and meat. The sheep in India are mostly managed on common pasture lands, fallow lands and wastelands, tree toppings and stubbles of cultivated crops. The digestive system works for metabolic conversion of assimilated food into meat, wool and milk yield. The stomach of sheep has four compartments *viz*. reticulum, rumen, omasum and abomasum. Out of four, the non-glandular compartments are reticulum, rumen and omasum where occur only the mechanical digestion and the abomasum is the glandular or true stomach where occurs the enzymatic digestion. The mucosa of abomasum contains gastric glands which release hydrochloric acid and other enzymes needed to break down

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the carbohydrates, proteins and fats into building blocks such as glucose, amino acid, fatty acid *etc*. These nutrients ultimately get utilized to form the highly nutritious quality mutton. It is similar to the simple stomach of monogastric animals. Hence growth, differentiation and physiology of the abomasal architectural elements are supposed to induce pertinent physiological reactions that get tuned in accordance to type of food habit and age. It is inevitable that progress of age accomplishes maturation with respect to morphology as well as physiology of body systems and the sheep abomasum is not at all an exception to this occurrence (Noden and Lahunta, 1985). As different features of abomasum take place, integrate and mature at a definite gestational age in the course of the foetal life inside the mother's uterus, authentication of normal sequence and phenomenon of developments during this period is mandatory to acknowledge the outcome of detrimental effects at different phases of pregnancy (Gao *et al*., 2008). Reports on gross ontogenesis of stomach of sheep during fetal period are meagerly available. The isolated reports available in the current literature on ontogeny of abomasum of goat (Gupta *et al*., 2016), abomasum of buffalo (Panchamuki, 1975; Singh *et al*., 2012) and on embryogenesis of compound stomach of Merino sheep (Franco *et al*., 2017), are only helpful to provide a semilunar idea to figure out the embryogenesis of the ovine glandular stomach in relation to intra-uterine age. So the present research work was designed to document the gross anatomical ontogenic sequential events of abomasum of sheep during pre-natal life together with study of structural entities through scanning electron microscopy.

MATERIALS AND METHODS

Experimental animal

Twelve normal healthy embryos or foeti of nondescript sheep breed were collected from nearby slaughter houses in Jadupur and Laxmisagar, Bhubaneswar. Wet cotton was used to clean the amniotic fluid that adhered to the surface of the foetal body. Every foetus' CRL (crown rump length) was observed in cm (centimetres) with a non-stretchy nylon fiber and advanced scale. The measured CRL was plotted against the standard CRL-Gestation Age Curve to calculate the foetial (tentative) age in days (Noden and DeLahunta, 1985).

Design of the experiment

In order to document the sequential changes in the gross anatomy of the ovine abomasum, the three stages of pregnancy were taken into account *i.e*.: Group-I: early pregnancy (0-50 days of gestation), Group-II: mid pregnancy (51-100 days of gestation,) and Group-III: late pregnancy (101-150 days of gestation). The Group-I included four foeti aged 32, 37, 44 and 48 days of gestation; Group-II included four foeti aged 63, 67, 75 and 94 days and Group-III included four foeti aged 111, 116, 121 and 150 days. After removing the embryos/foeti from the expectant uterus, abdomen was cut with a longitudinal ventro-median incision to explore the abomasal compartment for gross anatomical features and abomasum samples were also processed in order to document the scanning electron microscopic findings.

Gross morphological study

Each foetus' abdomen was opened and by delicately reflecting the lobes of the liver, all compartments of the foetal stomach were exposed. Afterward, the in-situ association of the abomasum with adjoining abdominal viscera and its topographic presentation were noted with respect to vertebrae as well as ribs. Its size and colour were also noted. The abomasum was opened by making incisions at the greater/lesser curvatures to observe its internal features.

The tissue samples from the abomasum were collected and processed for Scanning Electron Microscopic analysis (Make and Model: Hitachi; S-3400N) at the Central Instrumentation facility, Odisha University of Agriculture and Technology, Bhubaneswar for electron-photomicrographs.

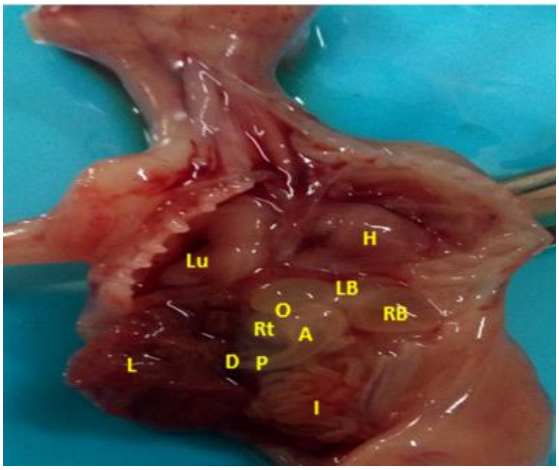
RESULTS AND DISCUSSION

In all the foeti of 32 to 48 days of gestation, the abomasum appeared to be comma shaped. The foeti aged 63 to 150 days of gestation revealed an elongated shape with wide anterior end and narrow posterior end. The abomasum was totally concealed by the left lobe of liver between 32 to 63 days of gestation. During this period the developing abomasum was located on the left side of median plane and its ventral aspect was associated to the developing intestine (Fig 1). The greater curvature was ventral and the lesser curvature was dorsal in position. The external constriction between the fundus and pylorus of the abomasum was observed at 67 days of gestation. The fundic part was observed to be wide and the pyloric part was narrow. At this age, the greater curvature of the fundus was in contact with the developing gonads and the caudal part of pylorus was in contact with the developing intestine. During 63 to 150 days of gestation the fundus was present on the left side of the median plane and pylorus was situated on the right side of the median plane. The greater curvature of abomasum was related anteriorly to the developing pancreas and posteriorly to intestinal coils at 75 days of gestation. These relations of developing abomasum with the surrounding structures remained constant till terminal stage of gestation. In congruence with the present findings, Roy (2009) also reported the displacement of the abomasum in bovine to right side of the median plane due to change in position of the rumen. Hejazi and Frik-aghaji (2013) also noted an oblique orientation of the abomasum from left to right side in full term sheep foetus. In goat foetus, Gupta *et al*. (2016) noted the location of fundic and pyloric parts on the left side and right side of the median plane respectively.

The abomasum was present at the most caudal part of stomach in foeti aged 32 to 48 days of gestation (Fig 1). The groove between the developing omasum and abomasum was faint. The abomasum appeared to be continuous with the omasum. In foeti aged 32 to 48 days, it

was located between 13th rib and 1st lumbar vertebra. The topographic position of abomasum was 10th rib to 4th lumbar vertebra between 63 and 75 days of gestation. This location changed to 9th rib and 2nd lumbar vertebra at 94 days of gestation, to 9th rib and 3rd lumbar vertebra at 111 days of pregnancy and to 8th rib and 3rd lumbar vertebra at 121 days of pregnancy. This topography of abomasum remained constant till terminal stage of the gestation. It indicated that the growth of abomasum occurred in a cranio-caudal direction. These findings on the external parameters of abomasum of sheep are in partial agreement with the findings in goat foetus (Gupta *et al*., 2016). In contradiction to the findings of the present study, the constriction between fundus and pylorus was observed at 41 days of gestation in bovine (Panchamukhi *et al*., 1975). It is therefore likely that the differentiation of abomasum into fundic and pyloric parts was earlier in bovine than in non-descriptive breed of the sheep used in the present study. At all stages of gestation, the fundic part of abomasum was found to be longer than the pyloric part. Similar finding was also recorded by Gupta *et al*. (2017) in goat foetus. This refers that the development of fundic region is faster than the pyloric part.

The internal surface of abomasum revealed a slightly rough texture and was semi-transparent to pale in colour during 32 and 44 days of pregnancy. The surface of the mucosa revealed a number of small undulations, representing the outline of the abomasal folds at 32 days of gestation (Fig 2). These folds became clearly demarcable



Mark the transparency of the compartments, omasum (O), abomasum (A), pylorus (P), duodenum (D), intestine (I), liver (L), heart (H), lungs (Lu), reticulum (Rt), rumen divided into a small left bud (LB) and a large right bud (RB).

Fig 1: Photo revealing the *in-situ* disposition of the stomach-compartments in the abdominal cavity of sheep embryo of 32 days age.

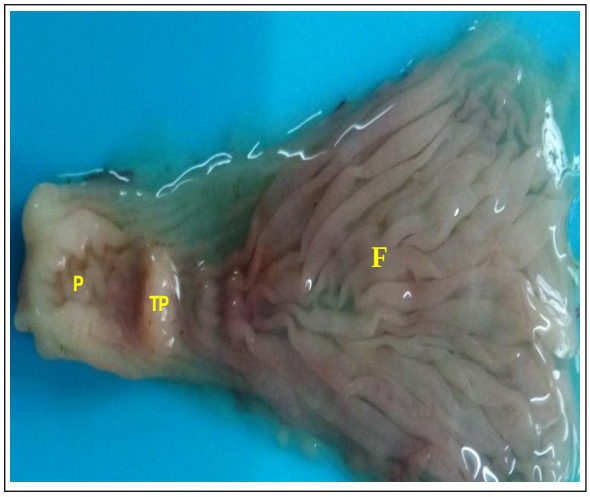
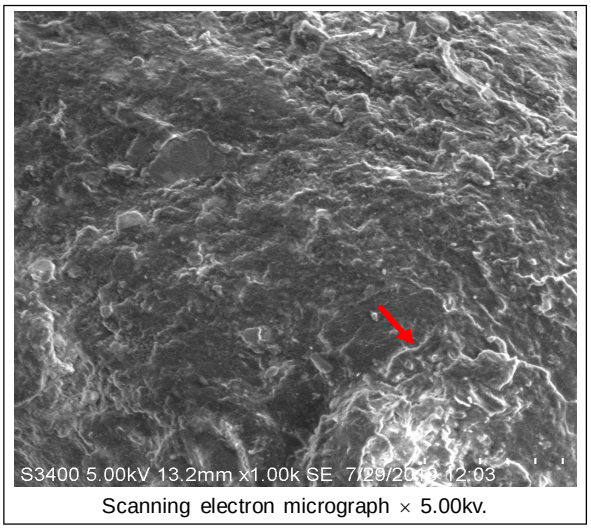


Fig 3: Photograph showing the abomasal folds in sheep foetus aged 75 days with prominent abomasal folds in fundus. Note the torus pyloricus (TP) and the pylorus (P).

Fig 2: Photograph showing the abomasal folds at cardiac end (C) and fundus of the abomasum of a foetus aged 32 days. Note the abomasal folds are diminishing towards the pylorus (P).

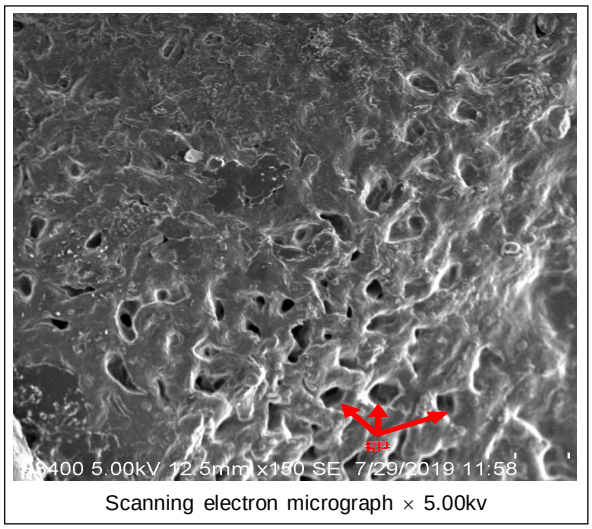
Fig 4: Photograph manifesting the whitish appearance of mucosal surface and distinct folds of abomasum (arrow) in sheep foetus aged 121 days.

at 63 days of gestation and gradually their height and number became more with advancement of age (Fig 3). The surface appeared white in colour from 48 days of gestation and onwards (Fig 4). The height of the abomasal folds in the fundus was large and in the pylorus it was small. At the junction between the fundus and the pylorus, few transverse folds were observed. The scanning electron microscopic observations also revealed abomasal folds by 32 days of gestation and onwards (Fig 5) along with the appearance of very small size gastric pits on the mucosal surface at 63 days of gestation (Fig 6). These pits were observed to have large dimensions from 67 days of gestations and onwards (Fig 7). In contrast to the findings of the present study, Masot *et al*. (2007) in red deer foetus recorded the appearance of abomasal folds at 67 days of pregnancy. (Fig 8). Gupta *et al*. (2016) reported a smooth internal surface of abomasum without abomasal folds up to 48 days of gestation. This variation in the time of appearance of abomasal folds may be attributed to the species variations. Also due to lack of information on embryonic development in the gross morphology of abomasum of sheep, a vivid discussion could not be done.



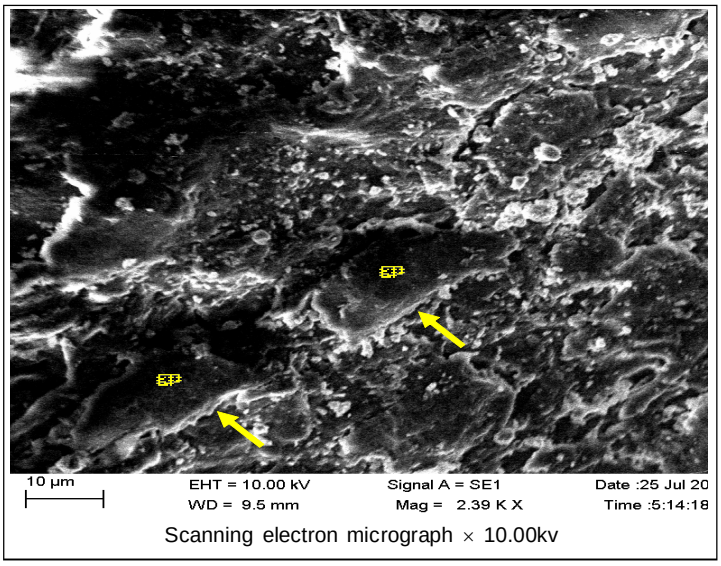
Scanning electron micrograph × 5.00kv.

Fig 5: SE microphotograph manifesting the small spiral orientation of the folds of abomasum (arrow) in sheep foetus aged 48 days.



Scanning electron micrograph × 5.00kv

Fig 6: SE microphotograph exhibiting the anatomical features of internal side of abomasum of sheep foetus at 63 days of gestation. Note the gastric pits (arrow).



Scanning electron micrograph × 10.00kv

Fig 7: SE microphotograph revealing the structural features of mucosal surface of abomasum at 94 days of gestation of sheep foetus. Note the openings of gastric pit (gp) surrounded by the mucosal folds.

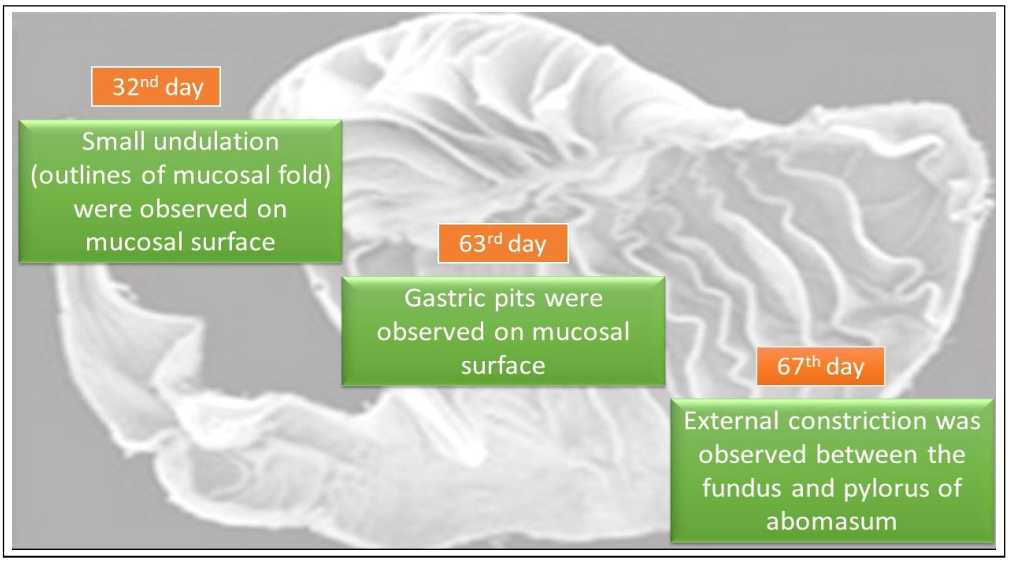


Fig 8: Schematic presentation showing the particular ages of the sheep foetus when the appearance of important morphological events was observed.

CONCLUSION

From the observations of the present study, the conclusion can be drawn that whether it is early or mid or late pregnancy, all the stages of embryogenesis are vital because important developments like abomasal fold development, gastric pit development, outer demarcation between fundus and pylorus *etc*. are occurring in this period. These anatomical developments are physiologically important for effective digestive procedure in the next phase of life *i.e*. post-natal period. So the duration of pregnancy is crucial in the life of the ewe.

Conflict of interest: None.

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