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RESEARCH ARTICLE

Histogenesis of rumen in one-humped camel (*Camelus dromedarius*)

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ABSTRACT

The aim of this study was to follow several sequence histological changes that occur during the histogenesis of the rumen in one-humped camel (*Camelus dromedarius*). Histogenesis study was carried out on 66 fetuses of camel from 50th day of gestation until birth (390 days), according to the most relevant histo-differentiation characteristics of the rumen in fetuses, these were divided into four groups: group I (5-24 cm crown-rump length (C-RL); 50-140 days); group II (24-30 cm C-RL; 140-160 days); group III (30-60 cm C-RL; 160-250 days); group IV (60-108 cm C-RL; 250-390 days). At 50 days, the rumen consisted of four layers: the epithelial layer, propria-submucosa, tunica muscularis and serosa. The epithelium glandular region was pseudostratified and in non-glandular region was stratified. The muscularis mucosa was observed incompletely from 140 days between lamina propria and submucosa in glandular region of the rumen to the birth day. The primary lymphatic nodules appeared in lamina propria of glandular region of the rumen at 160 days of gestation. The epithelium of the glandular region in rumen was formed by a simple columnar layer at 250 days. In all groups, the tunica muscularis layer of rumen was increased with ruminal development, gradually. The non-glandular region of rumen was formed by a stratified epithelium and number of these cells increased with ruminal development. The lymphatic nodules and muscularis mucosa in non-glandular region did not observe in all groups. The study observations revealed that non-glandular region of the rumen in the fetuses of camel are less precocious than the rumen of the domestic ruminants.

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INTRODUCTION

The dromedary stomach consists of three compartments (rumen, reticulum and abomasum) (Dougbag and Berg, 1980, 1981; Singh *et al*., 1996). The rumen is the first and largest compartment and presents two regions that are glandular and non-glandular. The glandular portion has two glandular sacs on its visceral surface. The glandular sac region is formed by a simple columnar epithelium (Hoshino, 1985). Previous studies reported that the glandular sac region consisted of "water cell", however; these studies have been disproved (Naghani *et al.*, 2010). It has been confirmed that the glandular sac region of rumen formed by a simple columnar cells, similar to the cardiac gland region of the pig (Naghani *et al*., 2010). On the other hand, Amasaki *et al.* (1992) described that the glandular regions of rumen secrete pepsinogen/pepsin. The most of mucosal layer of the rumen in dromedary camel comprises of stratified squamous epithelium which don't have any glands and are similar to the rumen in ruminants (Abdel-Magied and Taha, 2003). The prenatal development of the rumen in one-humped camel has not been studied. Therefore, the present study was conducted with routine histological method to investigate the construction of the rumen in dromedary camel during prenatal development and to acquire the information of the histogenesis rumen in the species.

MATERIALS AND METHODS

The present study was carried out using 66 fetuses of the one-humped camel. The fetuses of the camel were collected from the slaughterhouses in Yazd province, Iran. After measuring the crown-rump length (C-RL) for determining the age of fetuses (ranged from 5-108 cm

C-RL, approximately 50-390 days of gestation), the rumen samples for histological study were collected and fixed in 10% formalin for 1-2 week(s). The rumens were separated and small pieces of the tissue were dissected from the non-glandular and glandular regions of the rumen of each fetus. All specimens of the regions were dehydrated in ethanol, cleared in methyl benzoate, and embedded in paraffin. Tissue sections were cut at 4-5µm thickness and mounted on glass slides and stained with hematoxylin and eosin (Pousty and Adibmoradi, 2006).

RESULTS AND DISCUSSION

The rumen was histologically differentiated into four stages during prenatal development of the camel, thereby the fetuses of the camel were divided in four groups according to histo-differentiation of the rumen in the fetuses.

Group I (5-24 cm C-RL; 50-140 days of gestation): At this stage of rumen's development, the wall of the glandular region was consisted of four well differentiated layers: the epithelial layer, propria-submucosa, tunica muscularis and serosa. The epithelium layer was of a non-ciliary, pseudostratified, cylindrical type and the wall of the non-glandular region was stratified (Fig. 1, 2). Its cells displayed a round nucleus localized in the middle and basal portions of the epithelial layer, leaving a band clear cytoplasm peripherally. The propria-submucosa (pluripotential blastemic tissue) was subepithelial and had a mesenchymatous nature, forming the largest part of the organic wall. It was formed from stellate cells and immersed in an abundant ground substance. The other authors described this layer in other animals as a submucosa from the first fetal stages and a distinct layer that will be derived from the pluripotential blastemic tissue (El-Bab *et al*., 1983; Franco *et al*., 1992, 2004). The tunica muscularis was composed of smooth muscles in two layers (internal and external) of fusiform myoblastic cells: an internal (circular) layer and external (longitudinal) layer. The internal layer wasn't complete in this group. The serosa was formed by a subserosa that covered by a mesothelium.

Group II (24-30 cm C-RL; 140-160 days): In this group, the ruminal wall in glandular region was composed of four layers: mucosa, submucosa, tunica muscularis and serosa. The epithelium of mucosal layer was pseudostratified. Lamina propria was separated from the submucosa by the muscularis mucosa from 140 days to the birth day. In both layers was formed by connective tissue with mesenchymal and a large amount of fibroblasts (Fig. 3). In the cardiac region of the third compartment in camel (Naghani, 2010) described its similarity to this study at 140 days of prenatal development. The muscularis mucosa wasn't observed completely and consisted of two, three and more cells of smooth muscle protruding into the greater folds. The submucosa was formed by a loose connective tissue with abundant ground substance. The tunica muscularis in both region consisted of two layers: internal (circular) and external (longitudinal) layers. Intense vascularization was observed in serosa of the both region. In non-glandular region was showed that epithelium of this region was stratified, as in previous stage, consisted of two or three visible layers in contact with a basal cells layer. The basal cells layer undergoes a sharp mitosis in this region.

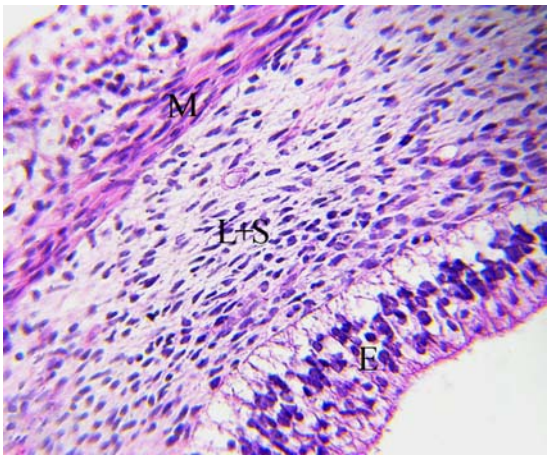

Fig.1: Photomicrograph of transverse section of the glandular region wall at 5 cm C-Rl. Epithelium (E), Lamina propria+submucosa (L+S), tunica muscularis (M). H&E; X180.

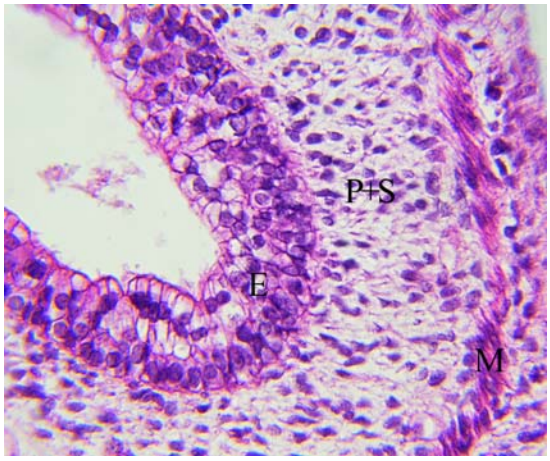

Fig.2: Photomicrograph of transverse section of the non-glandular region wall at 5 cm C-RL. Epithelium (E), propria+submucosa (P+S), tunica muscularis (M). H&E; X180.

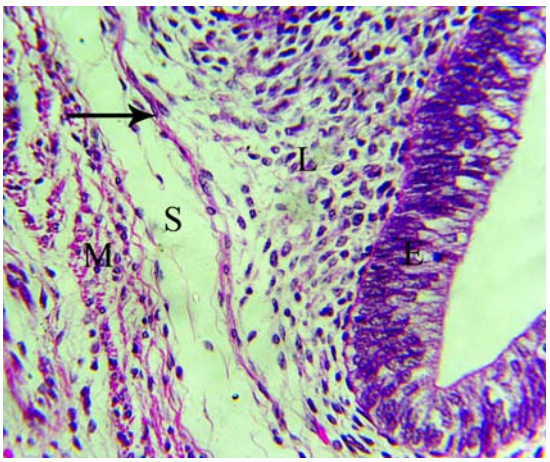

Fig. 3: Photomicrograph of transverse section of the glandular region wall at 24 cm C-RL. Epithelium (E), Lamina propria (L), submucosa (S), tunica muscularis (M), muscularis mucosa (arrow). H&E; X110.

Propria-submucosa, adjacent to the basal cells layer, was formed by morphologically stellate cells and a large quantity of ground substance. In the cow (Vivo *et al*., 1990), this has previously been reported at earlier stages of development (around 18% of gestation). This region in its other layers didn't show significant variation with respect to the glandular region in this group.

Group III (30-60 cm C-RL; 160-250 days): In this stage of development, the cells of mucosa layer was pseudostratified at 160 days and it formed simple columnar cells at 250 days that the nucleuses were arranged in basal or middle row of the cells (Fig. 4). The lamina propria was thinner than previous groups and highly vascularized and protruded into the folds with muscularis mucosa. At 160 days, the lamina propria had the primary lymphatic nodules which contained many lymphocytes. The lymphatic nodules were observed at 160 days up to the birth time in the lamina propria of glandular region in the rumen (Fig. 5). Similar result has been reported for the abomasum of camel's fetuses that lymphatic nodules in the cardiac region of abomasum formed at 160 days of gestation of camel (Naghani, 2010). These anatomical peculiarities are absent in the stomach of the ruminant (Alzola *et al*., 2004) and cause increase immunity level of mucosal layer in the rumen of camel in contact with microorganisms.

The wall of the non-glandular region of this group was contained of: epithelial layer, propria-submucosa, tunica muscularis and serosa. The epithelium of this region was stratified that increased in number as well as its development. It was formed by two clearly differentiated zones. The first; basal or stratum germinativum, made up one or two layers of morphologically oval cells, with a large central nucleus occupying most of the cell and a basophilic cytoplasm. The second was apical of two or three layers of polyhedral cells, with a small nucleus and clear-colored cytoplasm, referred to as the stratum granulosum. Various researchers have described its earlier in ruminants, in sheep (Franco *et al*., 1992, 2011), in goat (Molinari and Jorquera, 1988) and in cow (Vivo *et al*., 1990; Masot *et al*., 2007; Scala *et al*., 2011). The lamina propria-submucosa was formed by a connective tissue that the connective tissue, located under the epithelium, was dense type with low quantity of ground substance and the connective tissue, adjacent to the tunica muscularis, was loose type. The dense connective tissue plays an important role in protecting the other layers from mechanical damage. The tunica muscularis in both region consisted of two layers that

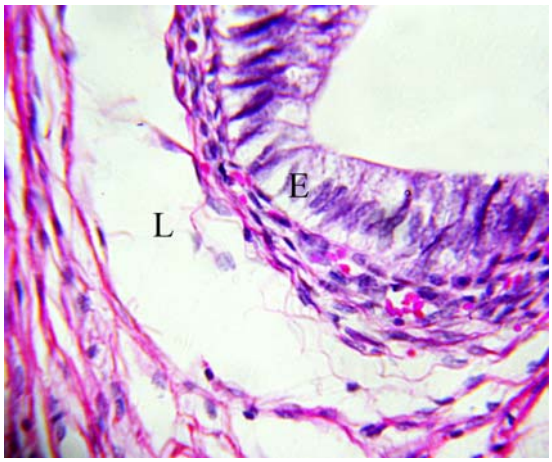

Fig. 4: Photomicrograph of transvers section of the glandular region wall at 60 cm C-RL. Epithelium (E), Lamina propria (L). H&E; X 440.

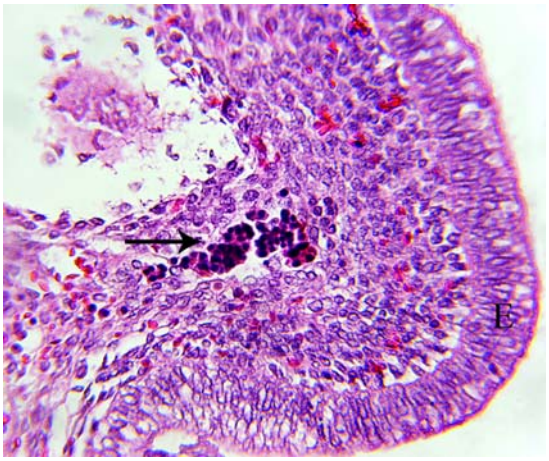

Fig. 5: Photomicrograph of transvers section of the glandular region wall at 30 cm C-RL. Epithelium (E), primary lymphatic nodule (arrow). H&E; X180.

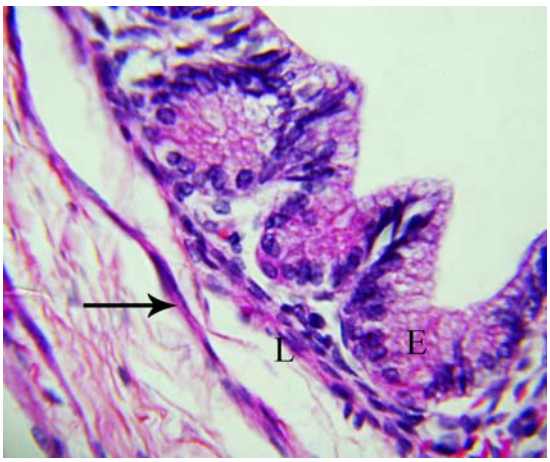

Fig. 6: Photomicrograph of transvers section of the glandular region wall at 108 cm C-RL. Epithelium (E), Lamina propria (L), muscularis mucosa (arrow). H&E; X160.

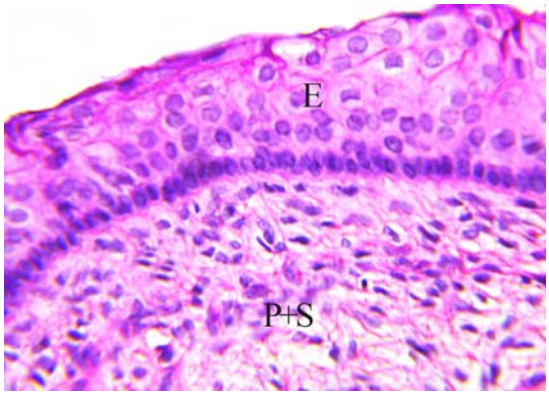

Fig. 7: Photomicrograph of transvers section of the non-glandular region wall at 108 cm C-RL. Epithelium (E), propria+submucosa (P+S). H&E; X165.

increased in thickness as well as differentiation. The serosa, meanwhile, showed continuity in growth the same as the previous group.

Group IV (60-108 cm C-RL; 250-390 days): In this group, the ruminal folds of glandular region in comparison with the previous groups showed a remarkable increase in size and number. In this region, surface mucosal cells were simple columnar epithelium cells with ovoid nuclei in basal region of cells. Cell divisions were observed in the upper gland cells. The glands of this region were simple tubular gland that existed in lamina propria (Fig. 6). The glandular sacs secretions play important roles in protecting the underlying epithelium from chemical, enzymatic and mechanical damage (Dellman and Brown, 1987).

The lamina propria was separated from submucosa by a thin muscularis mucosa that was incomplete and protruding into the folds of this region. The tunica muscularis in both regions were showed by clearly defined bundles arranged in the manner commonly found in the digestive system as a whole and the serosa in comparison with previous groups observed intense vascularization. In the non-glandular region of rumen in this group was formed by a stratified epithelium that was contained of three strata at 390 days (Fig. 7). The stratum germinativum, situated in the basal zone displayed staining of the basophils. The stratum granulosum was formed by many polyhedral cells. The appearance of a transition zone external of stratum granulosum represented the morphological expression of the stratum lucidum. Other authors described it in other ruminants like in the sheep (Franco *et al*., 2011) and goat (Molinari and Jorquera, 1988). Franco *et al.* (2004) and Scala *et al*. (2011) reported to the appearance of this stratum in sheep and buffalo at 28.5 cm, respectively. In the cow it is described in stage of development close to birth (Vivo *et al*., 1990; Masot *et al*., 2007; Scala *et al*., 2011). In this region wasn't observed muscularis mucosa in between lamina propria and submucosa. This region in other layers was similar to glandular region.

This study revealed that the prenatal development of glandular epithelium in rumen was slow as compared to abomasum's epithelium in the cardiac region of dromedary camel as described by Naghani (2010) who also showed that the lymphatic nodules located in lamina propria in this region that cause increase immunity level in the rumen of camel.

The study results revealed that the non-glandular region of rumen in the fetuses of camel is less precocious than rumen of the ruminants. From observations of this study, we can conclude that the rumen in one-humped camel during prenatal development undergoes changes that the fetuses will be prepared for postnatal life. Further investigations also needed to indicate the anatomical and histological structures of prenatal development of the other organs in camel.

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