

**Immunohistochemical Study on the Ruminal Wall of Adult Baladi Goats (*Capra hircus*)**

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**Abstract**

A total number of eight Baladi goats (*Capra hircus*) of both sexes aged between 11-18 months-old were used to describe the histological structure of the ruminal wall in addition to investigate the immunohistochemical localization and distribution of ki-67, caspase 3 and vimentin. The results revealed no sex differences in the ruminal wall histology or immunohistochemistry. Histologically, the ruminal wall consisted of mucosa-submucosa, muscularis and serosa. The mucosa of the rumen was thrown into ruminal papillae that were covered by keratinized epithelium. The submucosa was consisted of loose connective tissue lacking glands. Circular and longitudinal smooth muscle layers were the components of muscularis however, the rumen was surrounded externally by typical serosa. The immunohistochemical study was done using the avidin-biotin immunoperoxidase method. Ki-67 immunostaining was limited to nuclei of basal cells layer in the ruminal epithelium which explained the importance of ki-67 in epithelial cells proliferation as well as keratin biosynthesis. Caspase 3 immunostaining was localized in cytoplasm and nuclei of some epithelial cells that were going to apoptosis. The vimentin immunostaining was widely spread in epithelial cells as well as, fibroblasts in propria and submucosa, and endothelia of blood vessels. This showed the importance of vimentin as an intermediate filament protein. Detection of vimentin in the glial cells of enteric plexuses indicated its supportive role in the nervous control of the rumen. Overall, the histological and immunohistochemical findings of this study explained the structure-function relationship of the rumen of Baladi goats.

**Keywords:** Caspase 3, Histology, Ki-67, Rumen, Vimentin.

**Received:** September 4, 2018

**Accepted:** September 26, 2018

**Published:** October 1, 2018

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**Citation:** Emam and Abugherin, Immunohistochemical Study on the Ruminal wall of Adult Baladi Goats (*Capra hircus*). SVU-IJVS 2018, 1 (2): 25-32.

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**Competing interest:** The authors have declared that no competing interest exists.

## Introduction

Baladi goat (*Capra hircus*) as a ruminant animal is considered as an intermediate feeder which feeds on both concentrate and roughage (Hofmann, 1989). El-Gendyet al. (2010) mentioned that goats are highly adapted to grazing over a wide range of vegetation. Moreover Gihad et al. (1980) considered the goats as the best user of poor roughage among ruminants. Digestion is important trait for industry of animal production. Rumen is the first compartment of the forestomach which is responsible for digestion in ruminants. Rumen is a home to anaerobic microorganisms that ferment plant cells into carbohydrates and produce volatile short chain fatty acids (Parish et al., 2009).

The interest in the ruminal absorption has incited a series of studies pointed to the microscopic structure of the rumen in ruminants. Most available literatures on histology of rumen were discussing its prenatal development (Franco et al., 1992; Franco et al., 2004; Masot et al., 2007 and Garcia et al., 2012). Recently, few literatures pointed to the immunohistochemistry of rumen in adult goats but Garcia et al. (2012 and 2014a) investigated prenatal expressions of synaptophysin, non-neuronal enolase, glial fibrillary acidic protein, neuropeptide Y, and vasoactive intestinal peptide in the goat forestomach.

Therefore, as a new investigation, the present study was carried out to detect immunohistochemical localization and distribution of the ki-67, caspase 3 and vimentin in the rumen in adult Baladi goat. The detection of these substances helps to evaluate their roles in cell proliferation, cell apoptosis, and cytoskeleton formation, respectively.

## Materials and methods

### *I- Specimen collection and processing*

Rumen of eight Baladi goats (*Capra hircus*) of both sexes (4 males and 4 females), aged between 11-18 months-old were collected from Benha abattoir in Kalubya Governorate, Egypt. Small specimens from the rumen were taken and washed with physiological saline then fixed in 10% neutral buffered formalin for 48h at 4 °C. Tissue specimens were dehydrated in alcohol, cleared in xylene, embedded in paraffin.

### *II- Histological examination*

Sections of 5 µm thick were taken and stained with hematoxylin and eosin for general structure, and Periodic acid Schiff technique for neutral mucopolysaccharides as outlined by Bancroft and Gamble (2007). Crossmon's trichrome stain was used as a specific stain for collagen fibers according to (Crossman, 1937).

### *III- Immunohistochemical examination*

After dewaxing, rehydration and reducing of endogenous peroxidase with 3% hydrogen peroxide for 10 minutes, sections were treated with citrate buffer pH 6 in steamer for 40 min, to induce antigen retrieval. Sections were then incubated for 1 h at room temperature (RT) with the primary antibodies which were purchased from Santa Cruz Biotechnology, CA, USA (mouse anti- ki-67, at 1:200; mouse anti-caspase 3, sc-7272, at 1:150; mouse anti-vimentin, at 1:300). Sections were subsequently incubated with secondary antibody for 30 min at RT then the reaction products were visualized using the ready to use Vectastain®Elite ABC reagent for 30 min at RT. Sections were counterstained with haematoxylin. For the negative

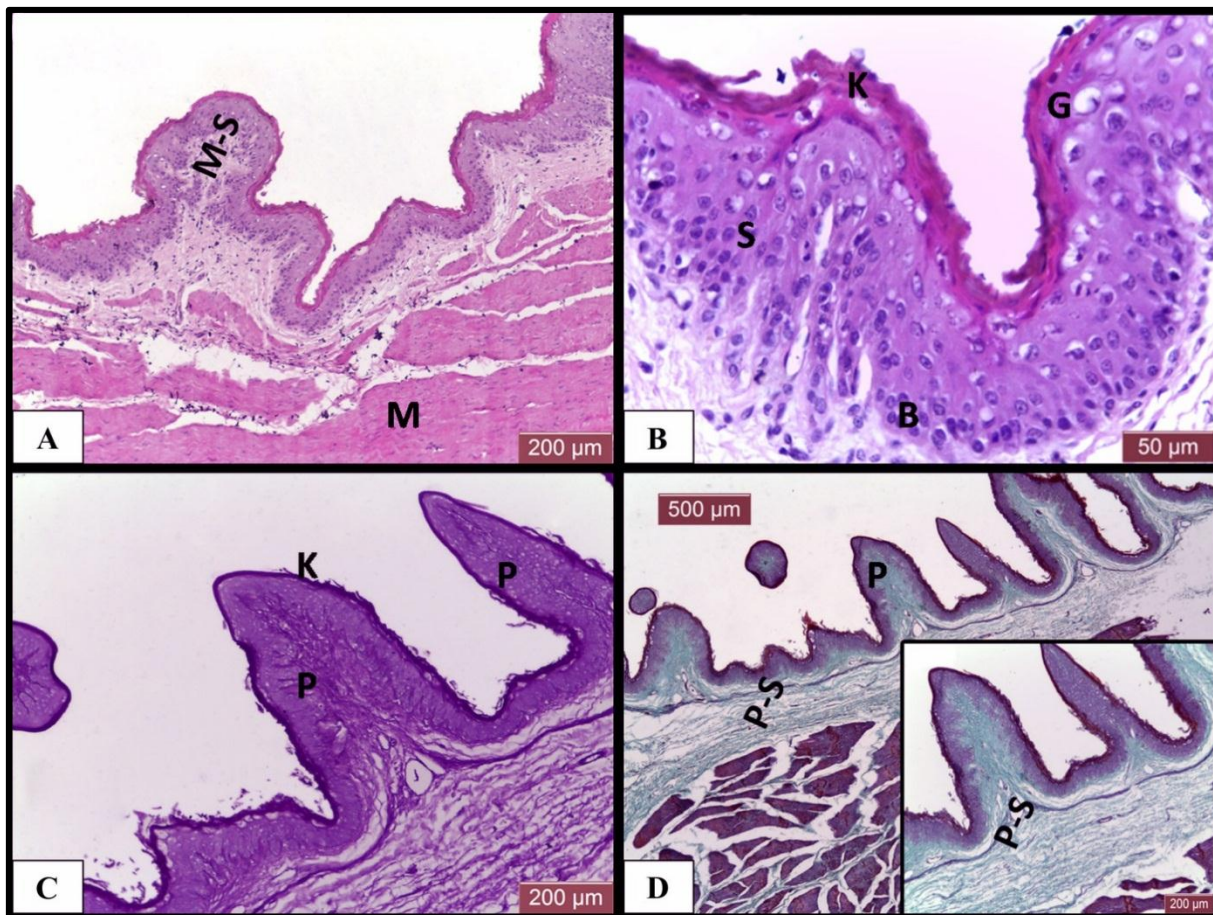
controls, the primary antibodies were substituted with normal mouse IgG. The specificity of the immunoreactivities was confirmed by the absence of immunostainings.

## Results

### *I- Histological results*

There were no sex differences in the ruminal histology. Tunica mucosa-submucosa and muscularis were clearly obvious in the wall of rumen (Fig. 1A). The mucosa of rumen was lined with highly keratinized stratified squamous epithelium which consisted of 4 layers; basal, spinous,

granular, and keratin (Fig. 1B). Many intercellular spaces among the spinous and basal layers were identified in epithelia of rumen (Fig. 1B). Ruminal papillae were the characteristics of ruminal mucosa (Fig. 1A, 1C-D). The keratin layer revealed strong PAS reaction (Fig. 1C). The propria-submucosa showed loosely arranged collagenous tissue that had no glands and lamina muscularis mucosae (Fig. 1D). Tunica muscularis of rumen consisted of thick inner circular and outer longitudinal smooth muscle layers (Figs. 1A, 1D) that were surrounded by collagenous tissue sheath (Fig. 1D).



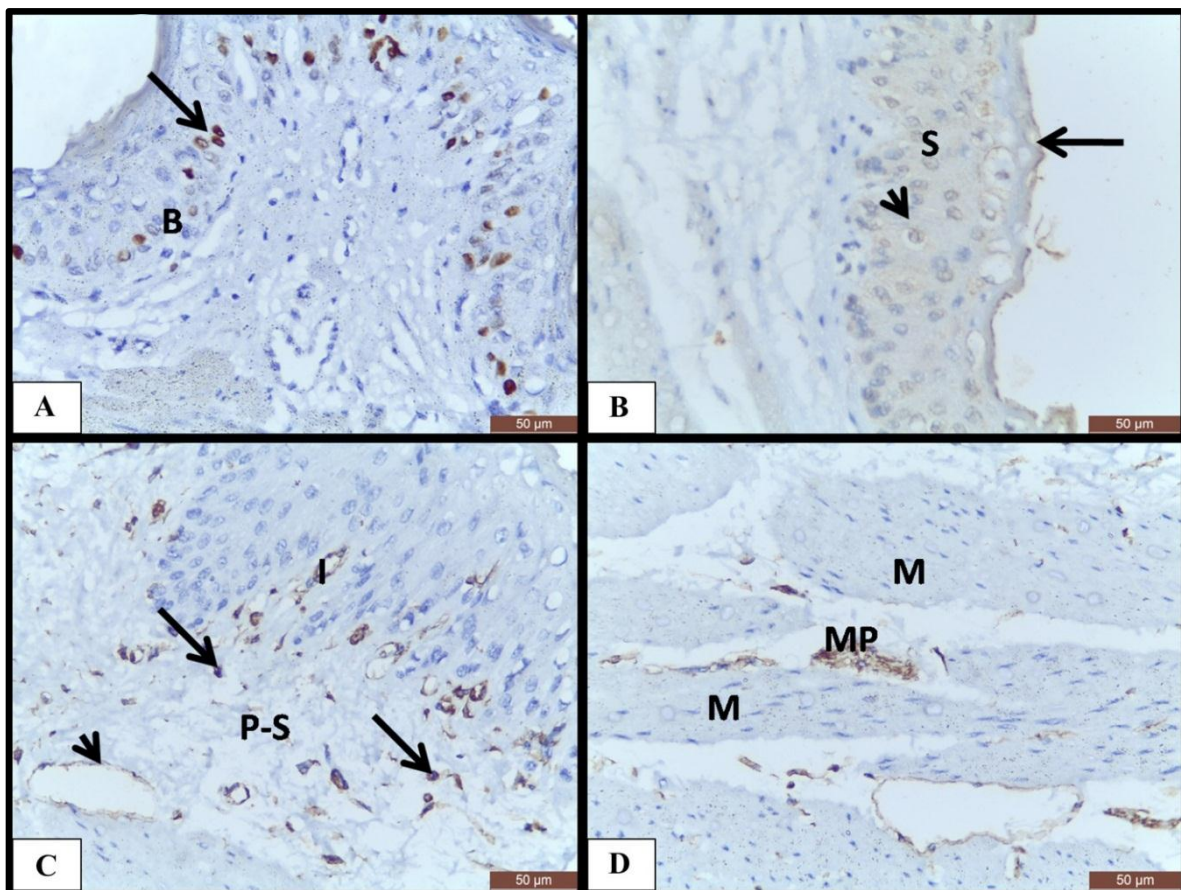
**Fig. 1.** Photomicrographs show cross sections in the wall of the rumen of Baladi goats. **A.** Showing ruminal wall consisted of tunica mucosa-submucosa (M-S) and tunica muscularis (M), H&E stain. **B.** A higher magnification of A. shows the ruminal epithelium, consisted of 4 layers; basal (B), spinous (S), granular (G), and keratin (K). Notice the intercellular spaces, H&E stain. **C.** Shows strong PAS positive reaction keratinized cells (K). Notice the ruminal papillae (P), PAS technique. **D.** Shows

loosely arranged collagenous tissue in the propria-submucosa (P-S). Notice the ruminal papillae (P). Higher magnification square shows no glands and shows lamina muscularis mucosae, Crossman's method. Scale bars = 200 $\mu$ m (A, C), 50 $\mu$ m (B) and 500 $\mu$ m (D).

## II- Immunohistochemical results

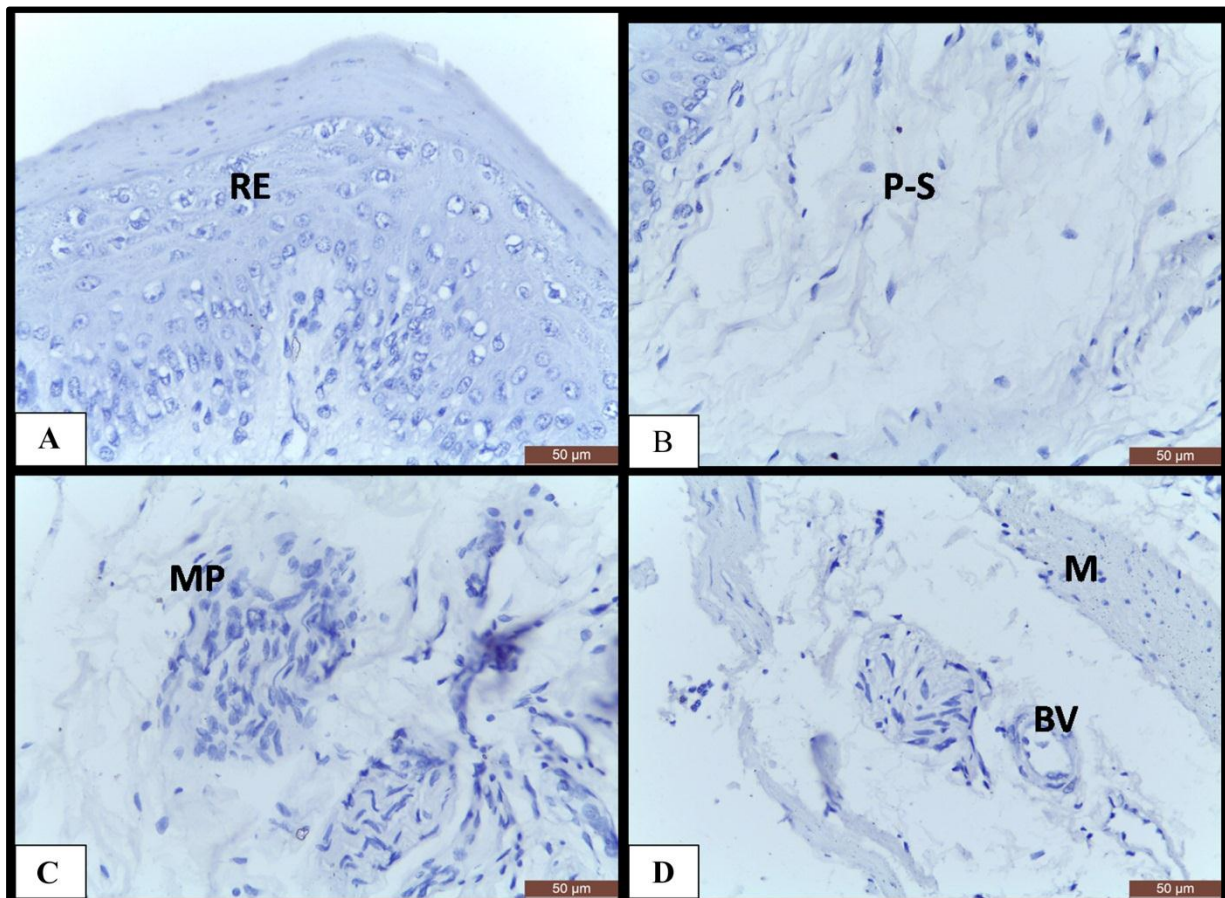
Immunostainings for ki-67, caspase3, and vimentin were detected in the different compartments of ruminal wall. There were no immunostainings for any antibody in the negative control sections (Figs. 3A-D). Ki-67 immunostaining was detected in the ruminal epithelium. Nuclear ki-67 was localized only in the basal cell layers (Fig. 2A). Immunostaining for caspase3 was localized in cytoplasm and nuclei of some

epithelial cells especially cells of spinous and cornified layers (Fig. 2B). Immunostaining for vimentin was localized in the cytoplasm of the immuno-positive cells. The vimentin immunostaining was widespread throughout the different layers of the rumen. It was seen in intercellular spaces of the epithelium, fibroblasts in propria-submucosa, endothelia of blood vessels and glial cells of myenteric plexus (Fig. 2C, D).



**Fig. 2. Photomicrographs of immunohistochemical staining for ki-67, caspase 3, and vimentin in the rumen of Baladi goats.** A. Shows ki-67 immunostaining reaction (arrows) only in basal cell layer (B) of the ruminal epithelium. B. Shows caspase 3 immunostaining reaction in some epithelial cells (arrowhead) of spinous layer (S) as well as in the cornified epithelial cells (Arrow). C. Shows vimentin immunostainings reaction in intercellular spaces of the epithelium (I), fibroblasts (Arrows) in propria-submucosa (P-S), and endothelia of blood vessels (arrowhead). D. Shows vimentin

immunostaining in glial cells of the myenteric plexus (MP) among the smooth muscles of tunica muscularis (M). Scale bars = 50  $\mu$ m (A-D).



**Fig. 3.** Photomicrograph showing negative control sections for ki-67, caspase3 and VIM in rumen of goat. No positive reaction was seen in the cells of the ruminal epithelium (RE) as shown in (3A). No positive reaction was seen in the fibroblasts of the probria-submucosa (P-S) as shown in (3B). No positive reaction was seen in the glial cells of the myenteric plexuses (MP) as shown in (3C). No positive reaction was seen neither in the endothelial cells of the blood vessels nor of tunica muscularis (M) as shown in (3D). Scale bars = 50  $\mu$ m (A-D).

## Discussion

Ruminal mucosa and its papillae of the Baladi goats (*Capra hircus*) in the current study were covered with keratinized stratified squamous epithelium which was formed of basal, spinous, granular, and keratin layers that was in consistence with Garcia et al. (2012) in goat and Dilda et al.(2012) in cow. This multi-cellular and multi-layer structure of the ruminal epithelium prevents the translocation of

toxic compounds into blood (Plaizier et al., 2012). Intercellular spaces in the spinous and basal layers were seen in the ruminal epithelium that was similar to the finding of Scala et al. (2011) in both reticulum and omasum. The other histological structure of ruminal wall of Baladi goat was in accordance with previous reports of Garcia et al. (2012).

Our immunohistochemical observations revealed detection of ki-67 immunostainings in the ruminal epithelium only that was in accordance with Blättler et al. (2001) in calves. The limitation of Ki-67 immunostaining to the basal cells layer of the epithelium refers to starting of keratin biosynthesis in the basal cells. Moreover, ki-67 in basal cells helps in permanent renewal of the epithelial cells that was supported by Bjerknes et al. (2005) and Conto et al. (2010).

On the other side, the identification of caspase3 immunostainings in the cells of spinous layer indicates the process of apoptosis that will substituted from the proliferation of basal cell layer. Also, our finding revealed localization of caspase3 in the cornified epithelial cells that referred to the continuous tearing of the keratin layer of ruminal epithelium. This was similar to findings of Gu et al. (2016) and Xu et al. (2018) who studied the apoptosis of ruminal epithelium in goat and sheep respectively. Both reported morphological, cell proliferation and apoptosis adaptation of rumen epithelium in relation to diet.

In the current study, vimentin immunostainings were identified in boundaries of the intercellular spaces and some spinous cells in the ruminal epithelium. On contrary, vimentin was not seen during prenatal life in stomach of goat (Garcia et al., 2014a and b). Moreover, our results revealed vimentin in endothelia of blood vessels in rumen that did not be recorded in the previous studies during prenatal life in goat (Garcia et al., 2014a and b). our finding indicates that the action of vimentin as cytoskeleton protein may be associated with postnatal life to support the cells of rumen to support the organelles in cytosol of cells (Katsumoto et al., 1990)., Fibroblasts in lamina propria-submucosa of rumen of Baladi goat showed vimentin immunoreactivity that was similar to

findings of Ikemizu et al. (1994) in the bovine rumen.

The visible vimentin immunostaining in glial cells of myenteric plexuses of rumen that was in accordance with findings of Garcia et al. (2014a and b) in goat. Also, Teixeira et al. (1998), in bovine reticulum, described immunoreactivity for glial cells in the reticular folds. The identification of glial cells in rumen of Baladi goat supports the critical role of glial cells as non-neuronal elements of the enteric plexuses in controlling gastrointestinal functions and protecting enteric neurons (Abdo et al., 2010).

Overall, the present study revealed expressions of ki-67, caspase3 and vimentin which gave a picture about epithelial cells proliferation, apoptosis, and cytoskeleton formation respectively in the rumen of Baladi goat.

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