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Histological aspects of the esophagus at Chinchilla (*Chinchilla lanigera*)

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Summary

From three clinically healthy Chinchilla males slaughtered by the owner for their fur, were collected esophagus fragments belonging to the three different areas: cervical, thoracic and abdominal. The tissue pieces were processed by the paraffin inclusion method in order to perform histological investigation. In all three different segments, the esophageal mucosa is represented by a stratified squamous epithelium with a granular layer twice as developed compared with the spinosum layer and with a medium degree of surface keratinization. Muscularis of the mucosa is well represented and present in all three segments, with a discreet thickening tendency from the cervical to the abdominal segment. It is disposed on a single layer and is formed from smooth muscle cells with longitudinal orientation. Regarding the submucosa tunica in all the three investigated constituent segments there were no esophageal glands to put on evidence.
The muscularis externa is developed and consists of striated muscle cells, disposed on two layers, one internal with predominantly circular and partially longitudinal arrangement and the other external with longitudinal orientation. The inner layer is the most developed and relatively uniform in thickness in all three segments, while the longitudinal muscular layer increase in thickness from the cervical to the abdominal segment of the esophagus.

Introduction

The esophagus is a cavitary organ that connects the pharynx with the stomach. It presents 3 topographical regions: cervical, thoracic and post-diaphragmatic (Hudson & Cummings, 1985).
The wall of the esophagus comprises four layers, or tunics.These are, from within outward: (1) the tunica mucosa, (2) the tunica submucosa, (3) the tunica muscularis, and (4) tunica adventitia in the pre-diaphragmatic segments, which is replaced by the tunica serosa in the post-diaphragmatic segment (Gal & Miclaus, 2013).
The innermost layer of esophagus consists to most species from a non- keratinized stratified squamous epithelium, partially keratinized in rodents, keratinized in ruminants (Miclaus et al., 2017) and intensely keratinized in some birds (Rus et al., 2009).In some species, the corion layer and tunica submucosa of the esophagus presents exocrine glands. (Koak & Winslet, 2002). These secretory cells form mucous or serum glands (Aughey & Frye, 2001; Rus et al., 2016) and are well represented in dogs and pigs (Shiina et al., 2005) and highly developed in snakes (Aldesberg et al., 1955). In rabbits and guinea pigs the esophageal glands are not present (Mohammadpour, 2015).
In the majority of the animals muscularis tunica of esophagus is generally arranged in two layers (Ingelfinger, 1958; Guber,1968; Guber, 1978; Samarasinghe, 1972) disposed the deeper in a circular arrangement and the

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more superficial muscle layer assumes a longitudinal arrangement (Whitmore 1982) while Francis (1974) describes four muscular layers at guinea pigs.
Many species of animals (mice, rats, guinea pigs, rabbits, bats, dogs, sheep, horses, cows, elephants and giraffes) have the muscular layer composed on almost the whole length of the esophagus from striated muscles cells (Ingelfinger, 1958; Thomas &Trounce, 1960, Lawn, 1964, Code & Schlegel, 1968, Samarasinghe, 1972, Floyd, 1973, Francis, 1974, Floyd & Morrison, 1975, Weisbrodt, 1976, Asmussen & Gaunitz, 1978).
In primates, marsupials, cats and humans the transition from striated muscular cells to smooth muscular cells is done in the third-middle of the esophagus (Ingelfinger 1958; Code & Schlegel 1968; Schofield, 1968; Floyd & Morrison 1975; Weisbrodt 1976; Enzmann et al 1977; Dodds et al., 1978). Some authors claims that in humans only 5% of the proximal third contains striated muscle cells, followed by a short area where there are striated muscular cells mixed with smooth muscular cells, so that in the distal two thirds there are only smooth muscular cells (Raica et al 2004).
In the scientific literature, information regarding the structure of the digestive system at Chinchilla Lanigera is not very comprehensive and most of them refer to the macroscopic aspects (Stan 2013, 2014), which is why we considered it appropriate to carry out microscopic investigations on this species.

Materials and methods

The biological material was represented by three clinically healthy Chinchilla males, aged 1 year and 6 months, belonging to a breeder from Salaj County. The slaughtering of the animals was performed by the owner for the purpose of harvesting the fur, and then where collected fragments demanded for the histological examinations from the three segments of the esophagus: cervical, thoracic and abdominal. The collected tissues were fixed for 24 hours in a Stieve solution, then dehydrated with increasing ethanol (700 , 950 and absolute), after that clarified in three N-butanol baths each last for 24 hours and then paraffin included. Then five micrometers thick sections were cut and stained with the Hematoxylin-Eosin solution.
Histological slides were examined using a Olympus BX41 microscope, equipped with a digital camera for image capturing. Image processing was performed using the Adobe Photoshop CS2 image editor.

Results and discussions

The cervical esophagus wall appears at Chinchilla organized on the four characteristic tunics encountered in the digestive system: mucosa, submucosa, muscularis and adventitia. The tunica mucosa does not present any particularities regarding the constituent elements, being composed from epithelium, corion and the muscularis layer of the mucosa.

The mucosa is a stratified squamous epithelium, comprising the dedicated layers found in such epithelial tissues, but with some particularities related to their thickness and especially the proportion of them (Figure 1).

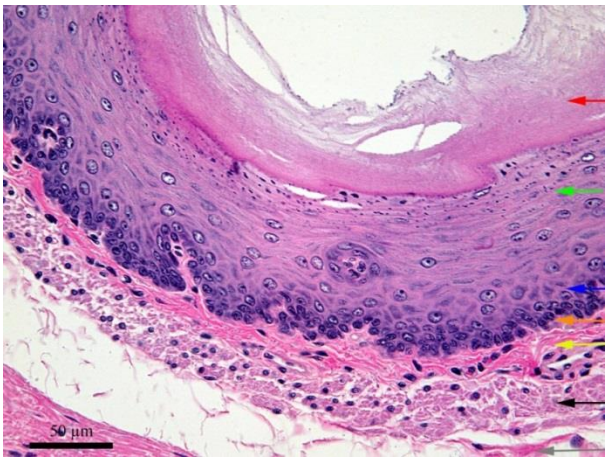

Fig.1. Esophagus-cervical region – mucosa and submucoasa (hematoxylin & eosin stain) orange arrow - basale layer; blue arrow - spinosum layer; green arrow - granulosum layer; red arrow - corneum layer; yellow arrow – corion layer; black arrow - muscularis of musosa layer; gray arrow – tunica submucosa.

The basale stratum is formed, like in all layered epithelial tissues, from a single row of cells. Instead the spinosum layer

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appears relatively thin containing no more than 4-5 rows of cells.

Due to these observations, we notice that the spinosum layer is not the most developed stratum, as it usually appears in the keratinized stratified squamous epithelium. The granulosum layer is much more pronounced, being at least twice as thick compared to the spinosum layer.

It appears to consist of 10-12 layers of cells that become more and more flattened as they approach the corneum layer. The granular cells contain keratin precursors (eleidin) in all the layers, the intracytoplasmic eleidin granules being small in the first layers, then progressively increasing, so that in the last 5-6 layers there are both small granules but also granules of larger size, which seem to be formed by the fusion to the small ones.

The size of the nucleus found in the granule cells is significantly bigger than the nucleus of the cells found in the spinosum layer and they have vacuolar aspect. The nucleus are few, with differences from one area to another, but related to the number of cells that do not have nucleus examined on the cross section and it is at most 1:1.

If we corroborate the vacuolar aspect of nucleus that appear hypertrophic suggesting even a discrete intranuclear edema, with the small number of nucleus, we can allege that some of the cell nucleus disintegrate still from the granulosum layer.

It is noteworthy that these histological aspects are present in the entire thickness of the granulosum layer, even though it is amplified as we approach to the corneum layer. These particularities appear as an exception from the common rule of these epithelial tissues, where the cells lose their nucleus just in the immediate vicinity of the corneum layer and not in the entire thickness of these layers. The corneum stratum is very well represented, with typical keratinization aspects and high density in the deep half of the layer and with tendency to exfoliate from

The corion layer is thin but dense and is composed of relatively thick collagen fibers, most of them with circular or oblique arrangement.

Tunica muscularis of the mucosa is continuous and is well developed, being about twice as thick compare with the corion layer. It consists of smooth muscle cells with longitudinal alignment.

The submucosa tunica is well represented also, consisting of lax connective tissue and is very well vascularized. No glands were identified in either the thickness of the tunica mucosa or in the tunica submucosa.

The muscularis externa tunica is formed in this region from skeletal muscle cells, disposed on two layers (Figure 2). The inner layer is the most developed, being predominantly composed from circularly arranged striated muscle cells. In the inner half of this layer there are few areas where the skeletal muscle cells have longitudinal arrangement.

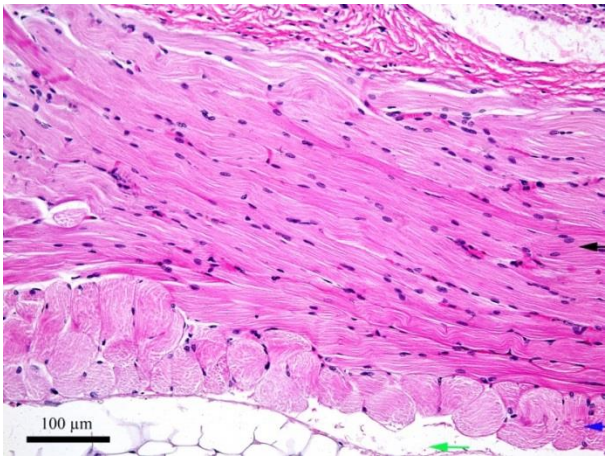

Fig. 2. Esophagus-cervical region – muscularis and adventitia (hematoxylin & eosin stain) black arrow - internal circular layer; blue arrow - external longitudinal layer; green arrow – adventitia.

These histological aspects entitle us to conclude that the orientation of the muscle cells in the inner layer is predominantly circular and partly longitudinal. The external muscular layer is thinner compared with the internal muscular layer and does not have the same thickness over the entire circumference. The arrangement of the muscle cells in this layer is entirely longitudinal.

Regarding the adventitia tunica we didn't find any particular histological aspect, having a common structure with other adventitia, composed from vascularized lax tissue and adipocyte deposits.

The structure of the esophagus wall found in thoracic segment is mostly similar to that

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we found in the cervical segment, with minimal differences and present only in some components. One particular aspect to highlight is that the muscularis of the mucosa is three times thicker, with the corion layer (Figure 3) and the outer muscular layer appears more developed here (Figure 4).

The abdominal segment of the esophagus has a very similar structure compared to the anterior segment (Figure 5), the only difference to consider is that the outer layer of this digestive organ wall is represented by the tunica serosa (Figure 6).

The Chinchilla esophagus is a digestive organ adapted to the specificity of this animals diet in order to ensure that the ingested food is transferred from the mouth to the stomach in optimum conditions. The presence of well-developed striated muscles cells provide to the esophageal wall a special mobility that can be controlled voluntary by the animal. This voluntary muscularis externa tunica is doubled by the muscularis tunica of the mucosa also well developed and present throughout the esophagus length.

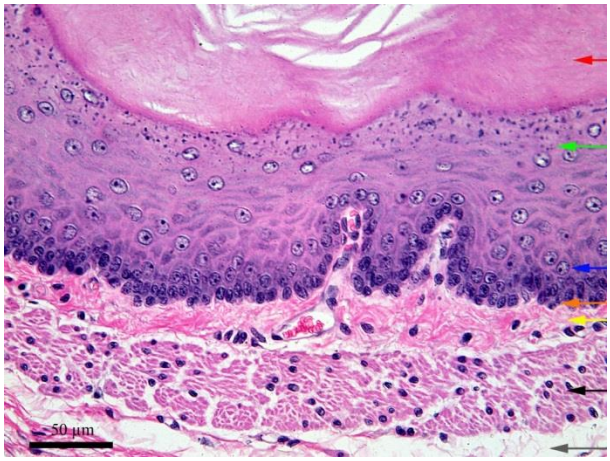

Fig. 3. Esophagus-thoracic region – mucosa and submucoasa (hematoxylin & eosin stain) orange arrow - basale layer; blue arrow - spinosum layer; green arrow - granulosum layer; red arrow - corneum layer; yellow arrow – corion layer; black arrow - muscularis of musosa layer; gray arrow – tunica submucosa.

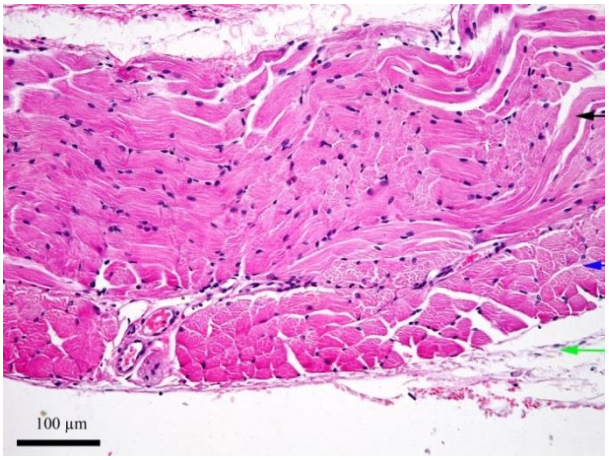

Fig. 4. Esophagus-thoracic region – muscularis and adventitia (hematoxylin & eosin stain) black arrow - internal circular layer; blue arrow - external longitudinal layer; green arrow – adventitia.

Although some researchers have described two layers of smooth muscle cells present in the muscularis of the mucosa in Chinchilla esophagus (Calamar et al., 2014), we identified in our study just a single continuous and thick layer of smooth muscle cells with longitudinally arranged pattern. A single longitudinally oriented smooth muscle cells layer was also described in humans (Raica et al 2004).

The muscularis of the mucosa is composed from smooth muscle tissue with involuntary (vegetative) action, acting independently of the voluntary one. This does not mean that they do not cooperate to ensure the optimal muscular activity demanded for normal organ function. Even if the muscularis of the mucosa is highly involved in the formation and removal of mucosa folds, it also appears to be involved in the progression of the nutrients during the entire esophagus length.

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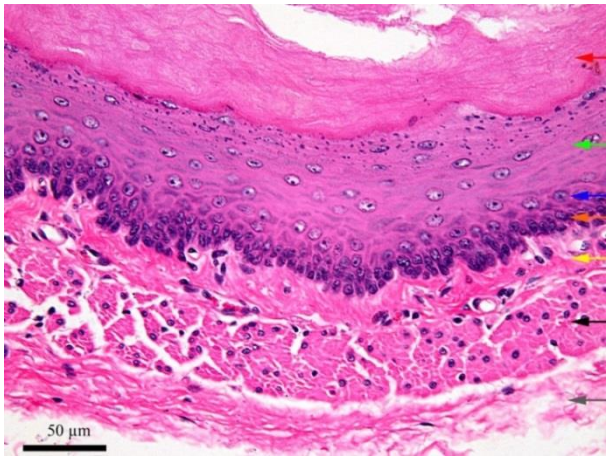

Fig. 5. Esophagus-abdominal region – mucosa and submucoasa (hematoxylin & eosin stain) orange arrow - basale layer; blue arrow - spinosum layer; green arrow - granulosum layer; red arrow - corneum layer; yellow arrow – corion layer; black arrow - muscularis of musosa layer; gray arrow – tunica submucosa.

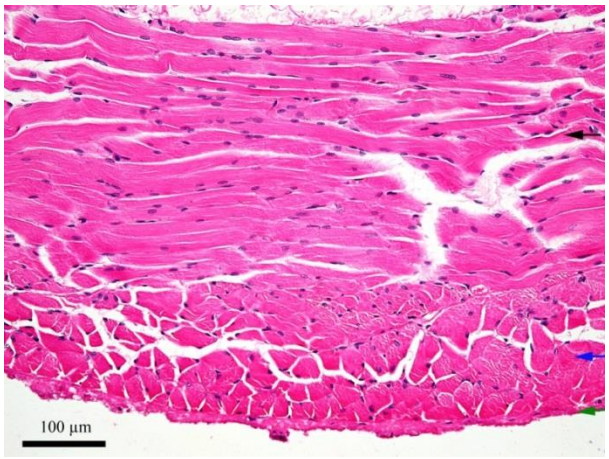

Fig. 6. Esophagus-abdominal region – muscularis and serosa (hematoxylin & eosin stain) black arrow - internal circular layer; blue arrow - external longitudinal layer; green arrow – serosa.

This appearance is suggested by the thickness of the muscularis layer of the mucosa, by is presence over the entire length of the esophagus, and especially by the longitudinal arrangement of the smooth muscle cells.

The aspect of the surface of the esophageal epithelium with a thick corneum stratum suggests that the nutrients consumed by the Chinchilla also contains some content with increased hardness and the keratinization of the epithelium tissue appears here as an adaptive structure related to food behavior.

Despite of some authors scientific research that in Chinchilla the esophageal epithelium is covered by a thick layer of mucus (Calamar et al., 2014), we did not confirmed this particular findings, instead we find keratins at the surface of this tissue.

The frequent presence of the tissue fragments that have a tendency to exfoliate from the esophagus epithelium suggests that ingested nutrients have some mechanical action given by their consistency and they are only soaked by the secretion of salivary glands and passes as such , without their passage being facilitated by esophagial glands secretions as usually happens in other species.

Regarding Chinchilla, there are some researchers who reported the existence of mucus secretory glands with a large lumen in the tunica submucosa (Calamar et al., 2014).

The apparent tendency to exfoliate from the epithelial tissue suggest that the removal of the keratinocytes at the end of the cell cycle occurs at a faster rate compare to similar epithelial tissues present in other areas of the body.

Due to these circumstances, it seems that the exchange rate of keratinocytes existing in the esophageal epithelial tissue differs from that encountered in the epidermis.

In other words, it seems that the cell cycle of the keratinocytes found in epithelial tissues from the Chinchilla esophagus is shorter than similar cycle which is found in the epidermis.

This pattern seems to be induced due to frequent mechanical stress during the passage of the food bowl. At the same time it can be greatly influenced by the fact that this epithelium tissue is permanently moistened on the surface by the salivary glands secretions, found either in the composition of the food bowl or by the swallowed saliva.

Thus, this permanently moistened epithelium tissue is naturally adapted to these conditions, in order to preserve its structure and functionality, related to local internal demands.

It is also possible that the aspects of the deep layers, especially of the granulosum one, which appear with absolutely atypical thickness, that does not occur in epithelial tissue of the same kind, do not contribute to this particularity.

In addition, also the small number of cell layers found in the spinosum stratum may suggest that from the time of keratinocytes

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formation through the division of the cells in the basal layer to the beginning of the keratinization processes in the first cells of the granulosum layer, not too long of time passes. This is of course, only a logical deduction based on the morphological aspects highlighted, to which other aspects could be added to simplify or to complicate the present scientific topic.

Conclusions

The epithelium of the esophagus mucosa is a relatively thick stratified squamous epithelium with a granulosum layer twice as developed compared to the spinosum layer and with a medium degree of surface keratinization.

The muscularis of tunica mucosa is present throughout the esophagus length, appear thick and compose from longitudinally oriented smooth muscle cells with a tendency to thicken from the thoracic to the abdominal segment of the esophagus.

The Chinchilla Lanigera tunica submucosa of the esophagus contains no glands in any of the three segments: cervical, thoracic and abdominal.

Tunica muscularis externa of the esophagus is composed from striated muscle cells, disposed on two layers, one deeper, thick and relatively homogeneous with predominantly circular and partially longitudinal orientated fibers and the outer longitudinal muscular layer, thinner and progressive thickening tendencies from the thoracic to the abdominal segment.

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