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HISTOLOGICAL AND MORPHOMETRICAL STUDY OF THE OESOFAGUS IN ADULT DOMESTIC RABBIT (*ORYCTOLAGUS CUNICULUS*)

STUDIU HISTOLOGIC ȘI MORFOMETRIC AL ESOFAGULUI LA IEPURELE DOMESTIC ADULT (*ORYCTOLAGUS CUNICULUS*)

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ABSTRACT | REZUMAT

The rabbit oesophagus is a target organ used in neonatological surgery simulations, which cannot be reproduced by artificial simulators. The biological material used in this study was represented by five common breeds, 14 months old domestic rabbits. The rabbits come from an authorized breeder who slaughtered them for their consumption and on this occasion fragments from the three oesophageal segments were collected for histological investigations. The samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at a 5 µm thickness and stained by Goldner's trichrome method. Histologically the wall of the oesophagus is composed as in all domestic animals of the mucosa, submucosa, muscularis externa and adventitia (in the cervical and thoracic segments) or serosa (in the abdominal segment). The oesophageal wall has a thickness that differs between certain limits, being the thickest in the abdominal portion. Structurally, the mucosa consists of the epithelium, lamina propria, and muscularis mucosae. The epithelium is stratified squamous with a tendency of keratinization, and the muscularis mucosae is composed of a discontinuous layer of smooth muscle fibers arranged longitudinally, with a thickness that increases progressively in an anteroposterior sense. No glands were identified in the mucosa and oesophageal submucosa of rabbits. The muscularis externa is composed of striated muscle fibers arranged in three layers, along the entire length of the organ. The inner muscular layer is arranged longitudinally, the middle circular layer, and the outer longitudinal layer. The thickness of the muscularis differs in the three segments, the thickest being in the abdominal segment, which seems to be a necessity related to the progression of the food bowl in the last part of the oesophagus. Adventitia and serosa are made up of loose connective tissue. The particular aspects reported in the components of the rabbit oesophagus are adaptive structures that appeared in response to the specifics of the diet.

Keywords: rabbit, oesophagus, histological

Esofagul de iepure este un organ țintă utilizat în simulări de chirurgie neonatologică care nu pot fi reproduse prin simulatoare artificiale. Materialul biologic utilizat în acest studiu a fost reprezentat de cinci iepuri domestici de rasa comună, în vârstă de 14 luni. Iepurii provin de la un crescător autorizat care i-a sacrificat pentru consum propriu și cu această ocazie au fost recoltate fragmente din cele trei segmente esofagiene, pentru efectuare de investigații histologice. Probele au fost fixate în formol 10%, incluse în parafină, secționate la 5 µm și colorate prin metoda Trichrom Goldner. Histologic peretele esofagului este compus ca la toate animalele domestice din mucoasă, submucoasă, musculară și adventiție (în segmentele cervical și toracal) sau seroasă (în segmentul abdominal). Peretele esofagian are o grosime care diferă între anumite limite, fiind cel mai gros în porțiunea abdominală. Structural, mucoasa este formată din epiteliu, corion și musculara mucoasei. Epiteliul este stratificat pavimentos cu tendință de cheratinizare, iar musculara mucoasei este compusă dintr-un strat discontinuu de fibre musculare netede dispuse longitudinal, cu o grosime care crește progresiv anteroposterior. Nu s-au identificat glande la nivelul mucoasei și submucoasei esofagiene a iepurilor. Musculara este compusă din fibre musculare striate dispuse în trei straturi, pe toată lungimea organului. Stratul muscular intern este dispus longitudinal, stratul mijlociu circular și cel extern longitudinal. Grosimea musculării diferă în cele trei segmente, cea mai groasă fiind în segmentul abdominal, aspect ce pare să fie o necesitate legată de progresia bolului alimentar pe ultima porțiune a esofagului. Adventiția și seroasa sunt formate din țesut conjunctiv. Aspectele particulare semnalate la componentele esofagului de iepure sunt structuri adaptative apărute ca răspuns la specificul alimentației.

Cuvinte cheie: iepure, esofag, histologic

The oesophagus is a muscular tube that ensures the passage of food from the oral cavity to the stomach, without intervening in any way in the process of digestion. The oesophageal wall delimits a wide central lumen and is made of several layers: mucosa, submucosa, muscularis externa, and adventitia (in the

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cervical segment) respectively, serosa (in the thoracic and abdominal segment). The mucosa consists of the epithelium, lamina propria, and muscularis mucosa. The mucosa of the oesophagus is lined by a squamous stratified epithelium, which can be non-keratinized or keratinized depending on the species (7, 12). The epithelium shows keratinization degree of different intensities in species such as ruminants (9), some birds (15), chinchilla (16), etc. while in other species it is non-keratinized, such as in humans (17), the giant African rat (1), etc. The degree of keratinization depends on the specific diet in each species (11). In mice and rats, the submucosa and muscularis mucosae are not present in the anterior portion of the oesophagus (17). The domestic rabbit is one of the most preferred species for biomedical research, being a medium-sized animal, gentle, easy to handle and is phylogenetically closer to humans than most species used in scientific research (8).

In experimental medicine, the rabbit oesophagus can be used in advanced neonatological surgical simulations, that cannot be reproduced through artificial simulators. The major advantage is that the oesophagus of rabbits is close in size to that of new-born babies. Also, rabbit oesophagus is often used as an experimental research model for reflux esophagitis (5, 6).

The aim of the study was to capture the histological and morphometric characteristics of the oesophagus of the domestic rabbit, to serve as fundamental information for future experimental procedures or in the research of pathologies affecting this organ.

MATERIALS AND METHODS

The biological material used in this study was five adult domestic rabbits, 14 months old common breed. The animals came from a licensed breeder who slaughtered them for their own consumption. With this occasion, we harvested fragments from the three segments of the oesophagus: cervical, thoracic and abdominal for performing histological investigations. The samples were fixed in 10% buffered formalin for five days, a time in which the solution was changed twice. After the ending of the fixation period, the pieces were dehydrated by successively passing them in three baths with ethyl alcohol (70°, 96° and absolute), with a passing time of one hour in each bath. The next step was the clarification that was made by successively passing through three baths with butyl alcohol (1-butanol), the time in each bath being one hour. The pieces were then passed successively through three baths of melted paraffin, with a passage time of one hour in each bath, in a drying stove set at 56°C. This was followed by pouring the blocks into aluminium trays filled with melted paraffin, where the pieces were placed piece by piece at a distance between them to allow easy individualization of the blocks after the paraffin has hardened. Cross-sections with a thickness of 5 µm thickness were performed on a Leica Rm2125 RT microtome. After displaying the slides, the sections were stained by Goldner's trichrome method. To examine the histological samples, we used an Olympus BX41 microscope, equipped with an Olympus E-330 digital camera, to capture images. Microscopic photographs were taken from the three oesophageal segments. For morphometric investigations, measurements were performed with the AmScope v4. 8.15934 digital microscopy program.

RESULTS AND DISCUSSIONS

The oesophagus in rabbits is a tubular structure with comparable thickness in the three segments, cervical, thoracic, and abdominal. Throughout its length, the wall of the oesophagus consists of four tunics that from the inside to the outside are: mucosa, submucosa, muscularis externa, and adventitia (in the cervical and thoracic segments), respectively serosa (in the abdominal segment), as described in other mammals (7). Along the entire length of the oesophagus, the mucosa and submucosa form longitudinal folds in rest, which gives the lumen a particular, star-like appearance. During the passage of the food bowl, the lumen of the oesophagus dilates so that these folds reduce. The average thickness of the oesophageal wall differs between certain limits in the three oesophageal segments of the rabbit. Thus, in the cervical segment, the average wall thickness is 1530 µm, slightly lower in the thoracic segment where it registers 1486 µm so that in the abdominal segment the highest thickness is registered, respectively 1907 µm.

The mucosa consists of the epithelium, the lamina propria, and the muscularis mucosa. The mucosal epithelium is squamous stratified with a tendency to keratinization, but not typical keratinization. Regarding the degree of keratinization, there are small differences between the three segments in the sense that the keratinization tendency is more obvious in the thoracic segment than in the other two segments. The epithelium has an unequal thickness generally varying between 90-140 µm. In the cervical segment, the average thickness of the epithelium is 103 µm, in the thoracic segment it reaches an average thickness of 124 µm and in the abdominal segment, it has a value close to the thoracic one, respectively 118 µm. The lamina propria is composed of connective tissue which is relatively dense towards the epithelium and obviously with a loose appearance towards the muscularis mucosa and contains many small and medium-sized blood vessels. The muscularis mucosae is present but does not form a continuous muscular layer. It is made of discontinuous bundles of smooth muscle

Table 1

Morphometrical data in the oesophagus segments

Anatomic region	Average thickness of the oesophageal wall	Average thickness of the epithelium	Average thickness of the muscular component	(%) of the muscular component reported from the wall
Cervical segment	1530 µm	103 µm	931 µm	60.8 %
Thoracic segment	1486 µm	124 µm	750 µm	50.5 %
Abdominal segment	1907 µm	118 µm	1373 µm	72.0 %

cells with longitudinal orientation, arranged at a certain distance from each other. The number and density of muscle bundles differ from one oesophageal segment to another. The fewest muscle bundles are present in the cervical segment of the oesophagus, but their number increases visibly from one segment to another. This makes the number and density of muscle bundles in the thoracic segment double compared to the cervical segment, whereas in the abdominal region it doubles compared to the thoracic segment.

The submucosa consists of a very well vascularized loose connective tissue, which ensures the sliding of the mucosa on the muscularis externa during significant variations in volume that the oesophagus presents from the state of rest to the passage of the food bowl. There are no significant differences in the structure and density of the submucosa between the three oesophageal segments. It should be noted that in this species we did not identify oesophageal glands in the submucosa, in any of the segments, as they were reported in other animals such as dogs (13) or stork (15). The muscularis externa of the rabbit oesophagus consists, along its entire length, of three layers of striated muscle fibers: an external longitudinal one, a medium circular layer, and an internal longitudinal layer. The external longitudinal muscle layer is the thinnest in the cervical and thoracic segments, so that in the abdominal segment it thickens significantly, reaching a thickness somewhat comparable to the other two. After measuring the thickness of the oesophageal muscularis externa, differences were observed between the oesophageal segments. Thus, in the cervical segment, the average thickness of the muscular coat was 931 µm, slightly lower in the thoracic segment where it recorded 750 µm, so that in the abdominal segment to increase significantly reaching 1373 µm (Table 1). If we report the muscle thickness to the total wall thickness, we find differences in the three oesophageal segments, it representing 60.8% in the cervical segment, 50.5% in the thoracic segment, and in the abdominal segment increases significantly reaching 72.0%. Adventitia and respectively serosa covers the oesophagus on the outside and consists of loose well-vascularized connective tissue. It has a variable thickness generally between 80 and 180 µm, but in some areas it can be even thicker.

If we compare the results obtained by us with those in the literature, we find differences both compared to those reported in rabbits by other authors and compared to other mammal species. Compared to the tendency of keratinization of the oesophageal mucosal epi-thelium observed by us in the rabbits studied, some authors reported in rabbits an increased level of keratinization (11), stating that the degree of keratinization is directly influenced by the specifics of the diet. A keratinized squamous stratified epithelium has been reported in the oesophagus of rodent species such as chinchilla (16) and guinea pig (14). Also, in some species, the epithelium of the oesophageal mucosa is a stratified non-keratinized type, as it is in cats and humans (10). The muscularis mucosae is also disposed in a thin layer and consist of smooth muscle cells oriented longitudinally as in other rodent species such as chinchilla (16), guinea pig (14).

Regarding the muscular component, there are some similarities regarding the number of layers and their disposition in rabbits with the situation related to ruminants and dogs, where the muscularis externa is also arranged on three layers of striated muscle fibers: one external with longitudinally arranged fibers, a middle circular layer and an internal longitudinal one. Four layers of striated muscle cells in areas of the abdominal segment of the oesophagus have been reported in some species, as is the case in guinea pigs (14). The number of muscle layers is different from the rabbit in the oesophagus of cats, marsupials, primates, and humans, where there are two muscle layers. Furthermore, in the cat and horse, the oesophageal muscle consists of striated muscles only in the first two-thirds of the oesophagus, and the last third contains smooth muscle fibers (7). Most authors claim that the transition between the two types of muscles takes place in the thoracic segment of the oesophagus in cats, horses, and pigs and progressively after the first 60 mm in humans (2-4), but some authors claim that the portion with smooth muscle fibers is significantly larger than the portion with striated muscle fibers in some species (18). Thus Raica et al. (2004) state that in humans, there is striated muscle only in approximately 5% of the first third, then follows a

short transition zone, and the two distal thirds contain only smooth muscles organized rigorously on two planes: an internal circular one and a longitudinal external one (10).

Fig. 1. The cervical segment of the rabbit oesophagus: orange arrows-mucosa, yellow arrows-epithelium, black arrow-chorion; red arrow-muscularis mucosa, pink arrows-submucosa, green arrows-the layers of the muscularis externa, blue arrow-adventitia, Goldner's trichrome staining, objective 4x

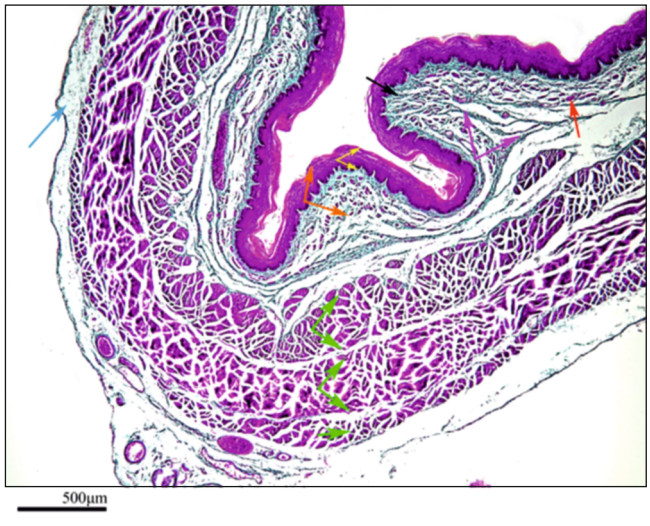

Fig. 2. The thoracic segment of the rabbit oesophagus: orange arrows-mucosa, yellow arrows-epithelium, black arrow-chorion; red arrow-muscularis mucosa, pink arrows-submucosa, green arrows-the layers of the muscularis externa, blue arrow-serosa, Goldner's trichrome staining, objective 4x

Our study brings information regarding the thickness of the oesophageal wall and its various components in the domestic rabbit. The results obtained by us show that some components of the oesophagus represent adaptive structures to mechanical stress, such as epithelium, which in the case of granular feed as in our case, is not very demanding so it does not keratinize in the true sense of the word, but shows only a tendency to keratinization. Also, the muscle, although it has the same disposition in the three oesophageal segments, is different in thickness being the thickest in the abdominal segment to ensure the progression of the food bowl on the last portion of the oesophagus.

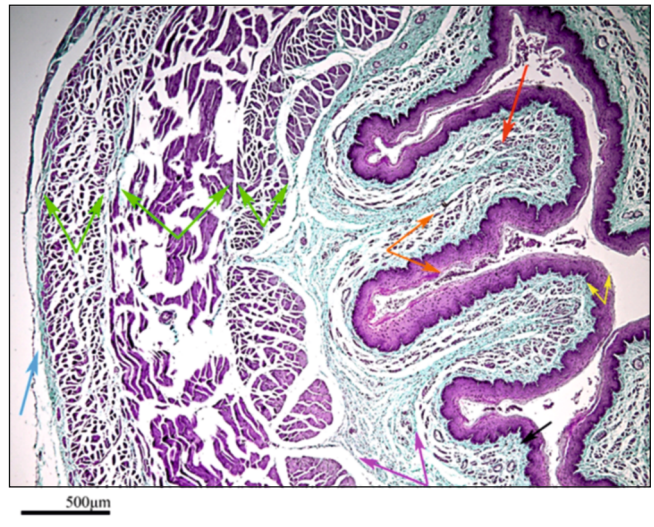

Fig. 3. The abdominal segment of the rabbit oesophagus: orange arrows-mucosa, yellow arrows-epithelium, black arrow-chorion; red arrow-muscularis mucosa, pink arrows-submucosa, green arrows-the layers of the muscularis externa, blue arrow-serosa, Goldner's trichrome staining, objective 4x

CONCLUSIONS

The oesophageal mucosa presents in the domestic rabbit a squamous stratified epithelium with a discreet tendency of keratinization. The muscularis mucosae is made by smooth longitudinally oriented muscle bundles, which increase in number gradually, up to the abdominal segment. In the domestic rabbit, the oesophageal muscle consists of striated muscle cells arranged in three layers, internal longitudinal, middle circular, and external longitudinal. The external layer is thinner in the first two segments, but has a comparable thickness in the abdominal segment. The total thickness of the oesophageal wall differs from one segment to another, being the largest in the abdominal segment, especially due to the muscular coat which is the thickest there and seems to help the progression of the food bowl on the last part of the oesophagus.

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