

Pelvic Ultrasonography and Urogenital Nerve Ultrastructure in Fur Animals

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Abstract—The authors have studied the organs of the pelvic cavity by ultrasonography as well as the ultrastructure of the hypogastric and pelvic nerves in representatives of the order of the carnivorous canine (the silver-black fox) and marten (the American mink). It was established that the topography of the bladder changes depending on its filling. The ultrasound examination allows diagnosing diseases accompanied by the accumulation of concrements and blood clots in the bladder cavity. The structure of the hypogastric and pelvic nerves is combined, their internal structure is universal. Fibers containing different amount of myelin in all nerves are identical in their structure; differences in the structure are due to the number and content of fibers in the bundles. The results of the study can be useful to fur animal breeders, veterinary physiologists and morphologists, practicing veterinarians.

Keywords—*fur animals, fox, mink, reproductive organs, ultrasonography, ultrastructure, nerves.*

I. INTRODUCTION

The use of real-time ultrasound scanners to study some organs of body cavities in modern veterinary medicine makes it possible to obtain high-resolution images. The use of doppler ultrasonography and color mapping, which allow the evaluation of the morphofunctional aspects of the object under study, expands significantly research possibilities. Therefore, ultrasonographic research methods are used in almost all branches of medicine [1], including veterinary medicine [2, 3].

In most cases, in small domestic animals, some organs of the pelvic cavity can be visualized with the use of the ultrasound. Much research has been done on ultrasound examination of the urinary organs in cats and dogs with and without pathology. Very few works can be found on ultrasonography of the organs of body cavities of fur animals. These works are of a fragmented applied character [4] and cannot cover the whole range of tasks facing clinical veterinary medicine.

When studying the structures of the nervous system, ultramicroscopic research methods are of great importance. Electron microscopy gives a relatively complete understanding of the structure of the peripheral nervous system and, in particular, its vegetal section. Over the past decades the research has been done on the ultrastructure of neural tissue which expanded our understanding of the structure of its fibrous and cellular components [5, 6].

In mammals, the general ideas of the ultramicroscopic organization of nerves have been studied almost in full. The fine structure of both the cellular and extracellular components of different nerves has been studied [6]. Interstitial and neuroglial (between neuritis and neurolemmocytes) relationships, as well as a neurovascular relationship have been determined [7]. However, the specialized literature does not contain data on the ultrastructure of the nerves of the urogenital organs, including the pelvic and hypogastric nerves of some fur animals.

Thus, we have defined the following tasks:

- 1) to give an ultrasonographic characterization of organs located in the body cavities of fur animals;
- 2) to determine the information content of different modes of ultrasonographic examination of the organs of the pelvic cavity using sensors of different frequencies;
- 3) to describe the general principles of the ultrastructural organization of the hypogastric and pelvic nerves.

II. RESEARCH METHODOLOGY

The objects of our study are 10 male and 10 female clinically healthy silver-black foxes and American minks.

The ultrasonography was carried out in the real-time “B” mode using the SHIMADZU SDU-500 ultrasonic apparatus with electronic linear sensors (frequency 3.5; 5.0 MHz), as well as the ETS-DMU-02 diagnostic ultrasound apparatus with a mechanical sector sensor (frequency 3.0 MHz).

When preparing nerves for ultrastructural studies, their fragments were exposed in the mixture of the following composition: 1% glutaraldehyde solution, 4% formaldehyde solution, 5% sucrose solution and 0.1M phosphate buffer solution (pH 7.4). The materials were exposed in this mixture at room temperature for two hours. Then, the nerve pieces were dissected into fragments and exposed in the similar mixture for another two hours at a temperature of + 4 ° C. After that, the materials were washed in phosphate buffer solution for an hour and exposed in the 1% solution of osmium tetroxide. Then, the materials were dehydrated in an alcohol solution with increasing concentration. The prepared cylinders were embedded in the *Araldite-Epon* mixture. The 70-100 nm thick transverse sections were prepared on the Reichert-Jung U1-tracut-E ultra-microtome. The sections were placed on a grid without a substrate and were contrasted with uranyl acetate and lead citrate. The sections were

visualized and photographed using a Hitachi-600H electron microscope.

III. RESEARCH RESULTS AND DISCUSSION

The findings have revealed that in the "B" mode of ultrasonographic examination, only parts of some studied organs are visualized in the fox and mink. In the cranial part of the abdomen during transverse and longitudinal scanning by linear sensors, the liver is seen in the form of a rounded formation on the line located between the costal arches. Being of increased echogenicity, sulci, which divide the lobes of the liver, are very well visible. Behind the liver, the dorsal part of the stomach, which has smooth contours, is also visible.

When the animal is on its back and the sensor is located over the cranial part of the abdomen behind the chest, the following ultrasound images are seen: the fascia is hyperechoic, but the abdominal fat, on the contrary, is anechoic. This phenomenon makes it possible to view the boundaries of organs.

When the bladder is filled in animals of both sexes, a rounded anechoic region is observed in the caudal region of the abdomen, which does not give dorsal enhancement. With insufficient filling, the contours of the bladder are vague. Therefore, it is not possible for us to detail the structure of the wall of the bladder.

The real-time ultrasonographic examination of the abdominal and pelvic organs in the fox and mink makes it possible to visualize parts of the stomach, the liver and the cranial section of a filled bladder with a high degree of reliability. Visualization of other organs without special preparation of the equipment and animals is not possible, since the accumulation of intestinal gases impedes the passage of the ultrasonic wave. Using sensors with a frequency of 5.0 MHz, one can determine the histogram. When examining the cranial part of the abdominal cavity using sensors with a frequency of 3.0 MHz and more, one can see the visualized stomach and liver as echogenic rounded formations with clearly defined contours.

When examining the organs of the pelvic cavity in the fox, it was found out that the bladder filled with urine up to 10 ml is visualized in the form of a circular anechogenic formation without detailing the wall. The rectum is visible in the form of a round hyperechoic formation, the visualization becomes possible when the bladder is filled up to 20 ml. The other organs of the pelvic cavity are not possible to visualize in ultrasound examination. For example, the prostate gland is surrounded on all sides by bones that shield an ultrasound wave.

In the animals studied, the bladder can only be visualized if it is filled up enough. In the fox, the bladder should be filled up to 10 ml, whereas in the mink, it should be filled up to 2-4 ml. Therefore, in foxes and minks, ultrasonographic examination allows diagnosing diseases which are accompanied by the accumulation of concretions and blood clots in the bladder cavity.

Literature review and analysis of our findings show that if the bladder is filled up, its topography changes. According to F. Barr (1999), the bladder, being a superficial organ, is easier to examine and diagnose when it is filled up [8]. The empty bladder is located in the pelvic cavity, when it is filled, it extends into the abdominal cavity [3,9]. In some predatory animals, in particular in cats and dogs, during ultrasound examination the filled bladder has clear contours. In this case, the thin wall of the bladder is visualized, and if you do not use a high-frequency ultrasonic sensor, then its structure is difficult to differentiate. According to N.D. Bru, I.G. Reil (1996), the image of some organs (kidneys, the bladder, and the womb) can be of good quality when using a sensor at 5 MHz [10]. Also, many researchers state that the ultrasound method reveals the bladder as an anechoic structure with a thin echogenic wall of a round or pear-shaped shape. Since the wall uniformity and thickness vary with bladder expansion, the latter must be expanded in full for a proper examination.

According to ultrasonographic studies, in animals of both species, the body and the neck of the empty bladder are visualized in the pelvic cavity, and its apex extends beyond the cranial border of the pubic bones. When the bladder is filled, its physiological volume in the fox is about 20 ml, and in the mink, it is 1.5-4.2 ml. However, only the top of the fox's bladder is visible in ultrasound examination, since most of the bladder is hidden by the pubic bones. We have the same problem with dogs and cats [9]. Though in the mink, the top and body of the bladder can be visualized.

For visualization of adjacent organs, a filled bladder can be used as an "echogenic window" for ultrasound examination of the womb and the rectum in female species, and the rectum - in male species. According to N.D. Bru, I.G. Reil (1996), an ultrasonographic image of a non-pregnant womb in predators usually depicts the body of the womb. It is visualized mainly before estrus and during it in the form of a hypoechoic tubular structure.

The electron microscopic study of the hypogastric and pelvic nerves of the fox and mink has produced the following findings: in most cases they have one or two-beam structure. Each bundle contains its own sheath - the perineurium, and it is surrounded by the epineuria along the periphery. The epineurium is the most massive sheath that integrates bundles in the nerve. It consists of loose connective tissue, which contains collagen and elastic multidirectional fibers, assembled into groups according to the type of bundles. There is accumulation of adipocytes, labrocytes and fibroblasts in the epineuria. Blood capillaries are closely adjacent to each nerve trunk.

The perineurium consists of cells of a flattened form, which alternate with collagen fibers layer by layer. The flattened fibers and cells are located inside the perineurium, they divide the internal structure of the nerve by the septa that form the endoneuria. The endoneural cells are connected with each other by nerve processes. The loose bundles of collagen fibrils fill the interstitial space between the fibers. The mass accumulation of collagen fibers is found around the capillaries of the nerve and under the perineurium (Fig. 1).

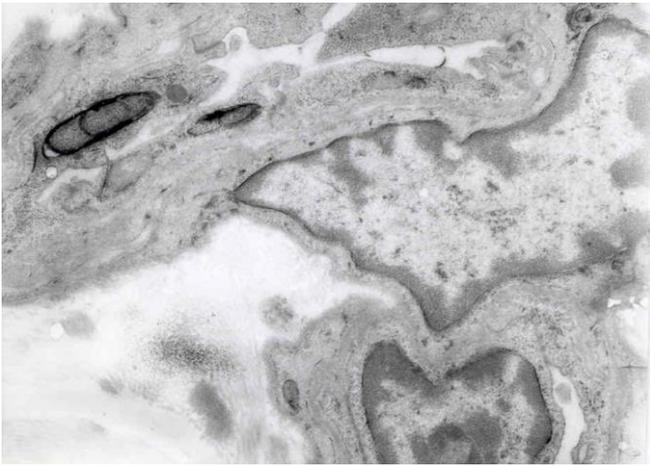


Fig. 1. The accumulation of collagen fibers around the capillaries of the nerve and under the perineurium. Silver-black fox pelvic nerve. A magnification of x 16000.

In the studied nerves of the fox and mink, we determined myelinated, low- myelinated and amyelinic nerve fibers that are closely connected with neurolemmocytes (Fig. 2).

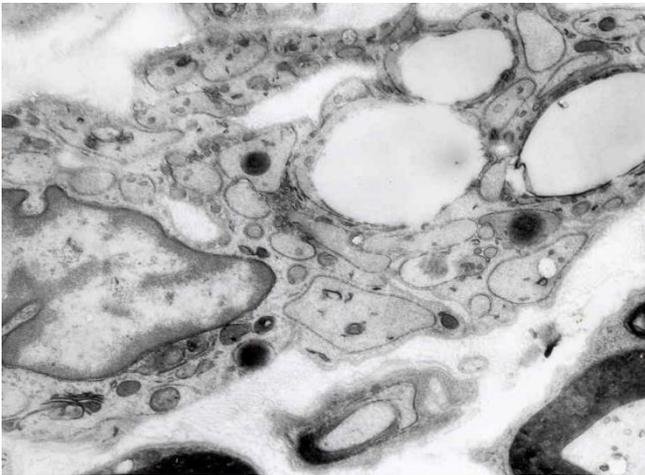


Fig. 2. The neurolemmocyte with vacuoles and amyelinic processes. Silver-black fox pelvic nerve. A magnification of x 16000.

Neurolemmocytes are closely adjacent to the outer surface of the myelin sheath (Fig. 3). The latter is characterized by a rather high degree of osmiophilia and has a layered structure.

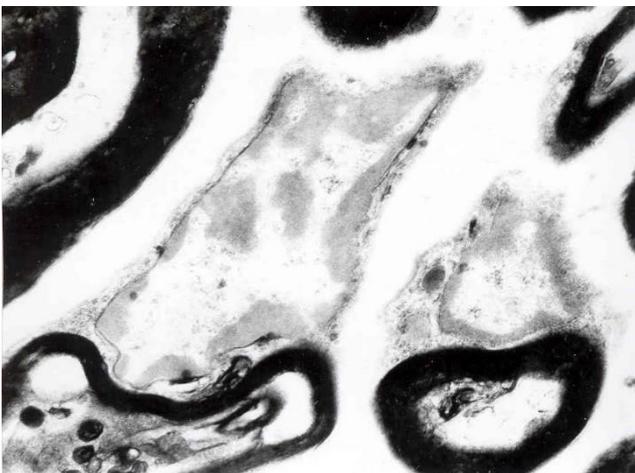


Fig. 3. Neurolemmocytes are closely adjacent to the outer surface of the myelin sheath. Silver-black fox pelvic nerve. A magnification of x 18000.

In the hypogastric nerve of the fox, the thickness of the myelin sheath in the nerve fibers varies significantly (300 - 650 nm), and, in the mink, it is 300-500 nm. In the pelvic nerves of the fox, the myelin sheath is 320–500 nm thick and, in the mink, it is 260–400 nm thick. The direct proportional dependence of the neurite diameter on the thickness of the myelin sheath has not been identified. It is noted that a combination of the thick myelin sheath and the thin neuritis, as well as the inversion of this combination, can often be observed (Fig. 4).

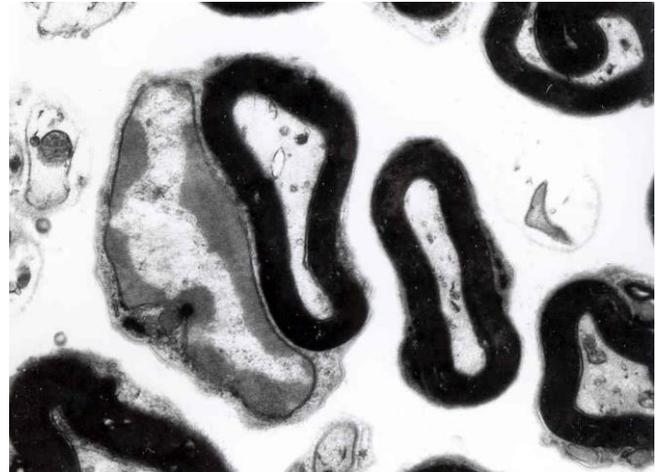


Fig. 4. The myelin neuritis in a neurolemmocyte, amyelinic neuritis. Silver-black fox hypogastric nerve. A magnification of x 14000.

In the hypogastric and pelvic nerves of the animals under study, the neurite cytoskeleton is the network of neurofilaments that is 8-11 nm thick and microtubules with a diameter of 20 nm. They come into contact with each other, as well as with the membrane of the neuritis and organoids. It was established that, the distance between the neurofilaments increases with the increase of the nerve fiber diameter (Fig. 5).

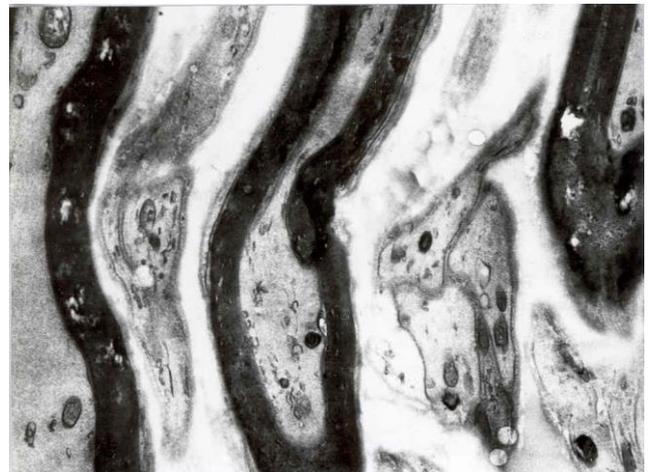


Fig. 5. The neurite cytoskeleton is the network of neurofilaments. American minks hypogastric nerve. A magnification of x 18000.

In addition to neurofilaments, various vacuoles and microbubbles, mitochondria and multilayer membrane formations are also detected in the neurites, which is common to all types of neurites (Fig. 6).

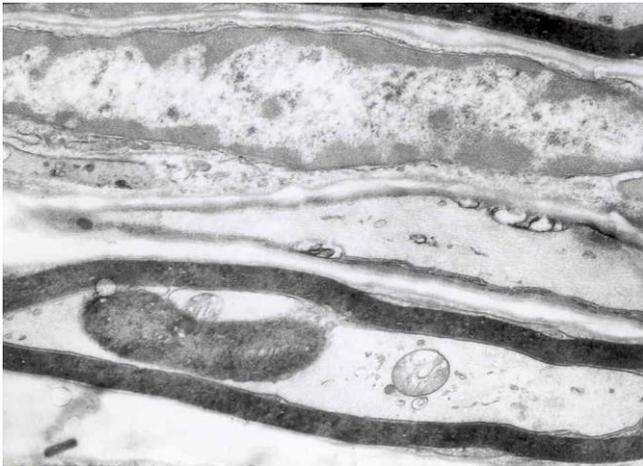


Fig. 6. The vacuoles and microbubbles, mitochondria and multilayer membrane formations in the neurites. American minks hypogastric nerve. A magnification of x 18000.

The neurolemmocytes have a large nucleus of an irregular shape with a size of 2.0x4.3 microns. At the periphery, the nucleus contains a large amount of condensed chromatin. The cytoplasm of neurolemmocyte forming the myelin sheath is a narrow rim that contains ribosomes and mitochondria. The neurolemmocytes that do not form the myelin sheath have large vacuoles in the cytoplasm. On the neurolemmocyte periphery, there are bundles of collagen fibers going in the longitudinal and transverse directions. The endoplasmic reticulum of neurolemmocytes is poorly developed (Fig. 7).

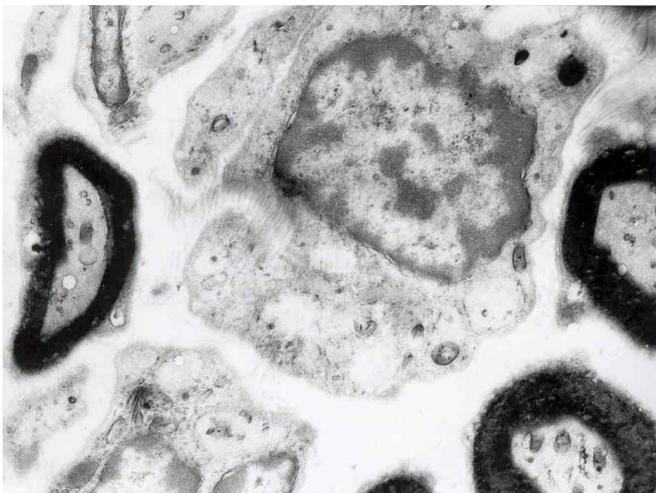


Fig. 7. The neurolemmocyte and the myelin neurites containing neurofilaments and microtubules. Silver-black fox pelvic nerve. A magnification of x 16000.

The examination of longitudinal sections of myelin fibers has revealed nodal interceptions and incisions of myelin (Fig. 8).

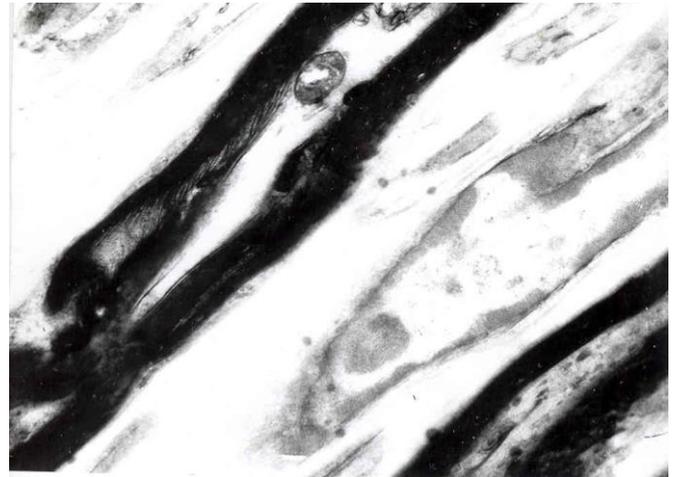


Fig. 8. The longitudinal section of the neurite and the neurolemmocyte. The myelin incisions. Silver-black fox hypogastric nerve. A magnification of x 15000.

The incisions look like microscopic islets of the cytoplasm. They are included in the area of cleavage of dense myelin lines near nodal interceptions. In the endoneurium we determined myelinated, low- myelinated and amyelinic nerve fibers. The perineurium consists of plate cases, in which the epithelial and fibrous parts are visible. The perineural septa separate bundles of nerve fibres. The connective-tissue inclusions separate from each other of the epithelial layers of perineurium.

IV. CONCLUSION

The ultrasonographic examination in real time using sensors of different frequencies (from 3 to 5.0 MHz) of the pelvic organs and abdominal cavities in foxes and minks, we have made of the possibility to visualize part of the stomach, of the liver and moderately filled bladder.

In female minks and foxes, it was not possible to identify with a high degree of reliability a non-pregnant womb through a filled bladder. In males, visualization of organs located in the pelvic cavity is not possible due to the shielding of ultrasound rays by the bones of the pelvis. In "B" mode, an ultrasonographic examination of the pelvic organs can only be used to determine the depth of the organ location.

In ultramicroscopic studies of the fox and the mink, we did not reveal any significant differences in the structure of the hypogastric and pelvic nerves. The internal structure of the studied nerves is universal, which is true about the peripheral nerves of the mixed structure of other mammals. In all studied nerves, myelinated, low-myelinated and amyelinic nerve fibers have a similar structure. The differences are noted only in their relative content in the nerve bundles and in their absolute number. The light-optical study of the ultrastructure of the hypogastric and pelvic nerves in fur animals has revealed that the majority of the fibers in the pelvic nerves are myelin, they are of medium size. In the hypogastric nerves, there is a higher content of low-myelinated and amyelinic fibers.

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