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PATHOMORPHOLOGICAL FEATURES OF TESTICULAR TUMORS IN DOGS

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Abstract. *The research was conducted to study the morphological, clinical, and macroscopic characteristics of the different histological types of canine testicular tumors. The material was obtained during a routine surgery for the removal of testicular tumors in dogs in clinics of veterinary medicine in Kyiv. Tumors were divided into sex cord-stromal tumors and germ cell tumors. Among the sex-cord stromal tumors recorded Leydig (interstitial) and Sertoli cell tumors. Macroscopic and histologic characteristics of canine interstitial (Leydig) cell tumor are described. It comprises 12% of all testicular tumors. Interstitial (Leydig) cell tumor has a slower growth compared to other testicular tumors, metastases are not observed. Half of the animals had no clinical signs. Most interstitial (Leydig) cell tumor go together with the pathology of adnexal structures. This is because Leydig cell tumor leads to increased levels of androgens. This fact may cause the pathology of adnexal structures. Neoplasms are recorded both on the right and the left testis. Leydig cell tumor was not related to the cryptorchid testicles. Mostly, tumors are regular oval, brown or gray color, different texture, and size with no signs of necrosis or ulceration. Macroscopic and histologic characteristics of malignant tumors of the testicle are described.*

A macroscopic picture was different and did not have clear meaning with the histological type of tumors. Microscopically a seminoma was characterized by the presence of layers of oval cells with the expressed grainy cytoplasm, surrounded by connective tissue, in which there was significant lymphoid infiltration. Leydig cell tumor is composed of cells that resemble Leydig cells and that are arranged into islands or tubular structures. The polygonal eosinophilic cells with granular or vacuolated, which contains lipids, were detected. Nuclei were a round mesh of chromatin and distinct nucleolus. Sometimes the large cells with 1, 2, or more nucleoli were noted. In Sertoli cell, tumor marked the various spectrum of cages from polygonal to prismatic sustenocytes.

Keywords: *non-germ cell tumors, genital stromal tumors, benign tumors, Leydig cell tumor, sertolioma, seminoma, testicular neoplasms, dogs*

Introduction

Risk factors for cancer in dogs are generally similar in humans, although many questions remain unclear in this regard. The important role of genetic factors is evidenced by the data on the unequal frequency of neoplasms in dogs of different breeds, as well as the fact that tumors of dogs are often observed in purebred animals (about 75%) compared with crossbreed or mixed breed ones (Moulton, 2002; Dobson & Lascelles, 2003). This assumption is not always confirmed, some authors believe that the high incidence of tumors in mongrel dogs is associated with poor living conditions. It has been shown that among breeds of various groups, the highest numbers of sick animals are registered among service (50–70%) and hunting (25–37%) breeds, and the lowest – in Toy breeds (8–28%) (Bernshtein, 2000; Sereda, 2009; Henry & Higginbotham, 2010). It is possible, however, that a greater percentage of service and hunting dogs breeds in the structure of morbidity, particularly tumors, is associated with a more attentive attitude towards them by owners and more frequent visits to veterinary hospitals, given that these dogs are more valuable in terms of economic considerations of their use.

Analysis of recent research and publications

An important endogenous risk factor for tumors is the age of animals: morbidity increases with age and is a well-known fact. According to the age range of diagnosis, tumors of dogs registering throughout their life, even in the first year, however, the largest part of sick animals is observed at the age of 7–12 years. The average age of dogs with testicular cancer is 10 years. Extremely rare neoplasms of the testes are found under the age of 6 years.

One of the factors that increase the risk of testicular tumors is cryptorchidism with an inguinal or abdominal location. Most authors note that the testicles, which have not descended, sertoliomas and seminomas develop more often (Dobson & Lascelles, 2003; Villalobos, 2010). Higher number of tumors register in the right testicles is explained by the fact that they do not descend more often.

The breed predisposition to this pathology is also described. Most often, testicular tumors are recorded in dogs of such breeds as Siberian Laika, Fox Terrier, Afghan Hound, and Sheltie (Bernshtein, 2000).Clinical signs vary and depend on several factors, first of all on the location of the testicles (in the scrotum, groin, or abdominal cavity), the presence of metastasis, and the development of paraneoplastic syndromes. At the same time, testicular neoplasms are found accidentally very often (North & Banks, 2009). The most common clinical manifestation of testicular tumors is feminization syndrome. The development of feminization syndrome depends on the localization of the neoplasm, localization in the scrotum – 16% of cases, in the groin – 50%, and in intra-abdominal location – 70% (Bernshtein, 2000). Moreover, bone marrow hypoplasia with thrombocytopenia, bleeding with anemia and granulocytopenia, gynecomastia and galactorrhea, attractiveness for males, atrophy of the opposite testis, atrophy of the penis with preputial sagging, bilateral symmetrical alopecia in the genital area, inner thighs, abdomen, and chest are also observed (Sereda, 2009).

The prognosis of the disease depends on many factors. The significance of the size of the primary tumor, its location, the presence of regional and distant metastases, the histological type and level of

differentiation of tumor cells, infiltration into the surrounding tissues, and the clinical stage of the disease are considered. However, the opinions about determining the prognostic value of certain characteristics of tumors differ among the researchers. Thus, most authors believe that a tumor size less than 3 cm in diameter in case of absence of metastases is associated with better prognosis (Henry & Higginbotham, 2010; Dobson & Lascelles, 2003), and the presence of metastasis is associated with a poor further prognosis. At the same time, there are opposite opinions: some authors believe that metastases to regional lymph nodes cannot be considered a prognostic factor (Villalobos, 2010).

However, in the literature data, the issues on the frequency of tumors occurrence in dogs, factors of anamnesis, as well as the peculiarities of tumor micro- and macrocharacteristics, which a veterinarian encounters, are not sufficiently highlighted. The studying of the pathomorphological features of testicular tumors is extremely important for an accurate diagnosis.

Purpose. The aim of the work was to study the frequency of diagnosis, clinical morphological, and macroscopic features of various histological types of canine testicular tumors. According to the goal, it was planned to investigate: the pathomorphological features of various histological types of tumors, their clinical manifestation, and the relationships between testicular tumors emerging and anamnestic data.

Materials and methods of research

Experimental materials were obtained during elective operations for testicular tumors removal in dogs at the veterinary clinic "Animal health".

The pathological material was processed according to generally accepted histological methods.

For histological studies, a tumor or part of it was sampled, along with the capsule and surrounding tissues, and placed into a special glass or plastic container with a tightly ground lid. They were fixed in a 10% neutral aqueous solution of formalin according to Lilly's prescription, dehydrated in ethanol of increasing strength, and after chloroform embedded in paraffin. Using a sled microtome, sections of 6–8 μm were obtained. The histological structure of organs and tissues was studied by sections staining with Carazzi's hematoxylin and eosin. The histological slides were examined and pictures were made using an Olympus BX-41 microscope (Goralskij, Radzihovskij, & Zayika, 2018).

Results of the research and their discussion

The priority, when a testicular tumor suspected, is a thorough history taking, which must include data on the dog's behavior, the time of the disease emerging, the rate of tumor development, and visual changes along this process. It is important to find out if the treatment was provided, what kind of treatment, and when it was carried out. During the clinical examination, a general examination of the animal is carried out and considerable attention is paid to the area of the pathological process. The general condition of the animal depends on the type of tumor and its stage, but in most cases, it changed insignificantly. Decreased appetite and apathy are observed in 18% of dogs. Testicular tumors are accompanied by pain in 16%.

Palpation was used to determine the location of the testicles. Their size, densi-

ty, shape, consistency, and mobility were determined. Examining regional lymph nodes, attention was paid to their size, density, mobility, and pain. Additionally, a rectal examination was performed to determine the condition of the prostate.

Morphological studies are the main diagnostic procedure to establish the nature of the tumor and its histological type. These data in combination with other methods allow determining the stage of the disease and choosing the most effective method of treatment. We used two types of morphological studies: cytological and histological.

Testicular neoplasms are easily amenable to surgical removal followed by histological examination. Tumors up to 1 cm in diameter were examined completely. For a homogeneous tumor, 3 samples were taken along a line passing through the center of the tumor. In the case of macroscopic inhomogeneity, samples were taken from all areas, as well as along the median line. In all cases, it is important to take a sample at the border of the healthy and affected tissue. Together with the tumor tissues, a histological examination of regional lymph nodes was carried out, which provided a full characterization of the level of the pathological process development. Dogs with poorly differentiated tumors had a poor prognosis compared to animals with tumors made up of highly differentiated cells.

According to the principles underlying the classification of testicular tumors of dogs (Meuten, 2002), tumors that occurred in the studied random sample were divided into sex cord-stromal tumors and germ cell tumors. Three types of testicular tumors were most often found in small domestic animals: a tumor from Leydig cells – leydigioma, a tumor from Sertoli cells – sertolioma, and a tumor of the spermatogenic epithelium of the seminiferous tubules – seminoma.

An important prognostic factor is the histological structure of the tumor. Thus, an animal diagnosed with sertolioma or leydigioma has a more favorable prognosis than animals diagnosed with seminoma. Some authors also consider unfavorable factors such as the degree of vascular and tissue invasion, lymphoid infiltration, the size of the zones of necrosis.

Leydigioma is an interstitial cell tumor that develops from Leydig cells. Leydig cells are hormone-producing cells located between the seminiferous tubules in the testes. They produce the hormone testosterone. Also, in small amounts, they synthesize female sex hormones estrogens and progestins. Leydig cells are stimulated by luteinizing hormone, which is produced by the hypothalamic-pituitary system. Leydigioma can secrete testosterone, an excess of which leads to prostatic hyperplasia and the formation of adenomas of the hepatoid glands.

The tumor belongs to non-germinogenic ones that can cause feminization syndrome. In males observing libido reduction, mastopathy development, prepuce tissue swelling, testes size decreasing, becoming flabby, but spermatogenesis is preserved.

Changes in the skin and the hairline are usually started as the shedding period increasing. The coat becomes dull and brittle, alopecia develops, which are mainly localized on the sides. In most cases, the tumor is benign, very rarely metastasizes.

Leydigiomas most often are not accompanied by an increase in testes volume, but they can be detected with scrotum ultrasound examination. Leydigiomas made up 12% of all testicular neoplasms and ranked third after seminomas and sertoliomas. Leydigiomas

occur, as a rule, in the prolapsed testes, and in 15% of cases both testes can be affected. If only one testis is affected, then the other one atrophies.

The neoplasms were mainly represented by one node, but there were also cases when several nodes form were registered in one testicle.

Tumor consistency is soft, gray in color with no signs of ulcers or necrosis.

Microscopically tumor consists of atypical cells that are very similar to their "normal cell analogues". Tumor cells are large, round, or polygonal, sometimes hexagonal, have eosinophilic granular cytoplasm, as well as a small or medium nucleus located in the center. Chromatin reticular, nucleoli are well-defined. Cell boundaries are often not visible. Sometimes large cells with one, two, or several nucleoli were noted. In some cases, spindle-shaped cells with small nuclei and eosinophilic granular or vacuolated cytoplasm were observed. Pronounces cell pleomorphism and mitotic activity was noted.

Cytological diagnosis of testicular tumors is well developed and has a high sensitivity for the diagnosis of each individual type (Fig. 1).

Sertolioma is a tumor of Sertoli scaffold cells (sustenocytes), which perform a trophic function and produce proteins that bind androgens. Most sertoliomas are benign, although morphologically benign and malignant forms do not differ. The malignancy of the process is considered by the presence of metastases. Sertoli cells have testosterone and estradiol receptors and are involved in the regulation of spermatogenesis.

Macroscopically, the tumor is presented in the form of a node with clear contours (Fig. 2). Very often the tumor is surrounded by a connective tissue pseudocapsule.

The color of the tumor is predominantly whitish or whitish-yellow.

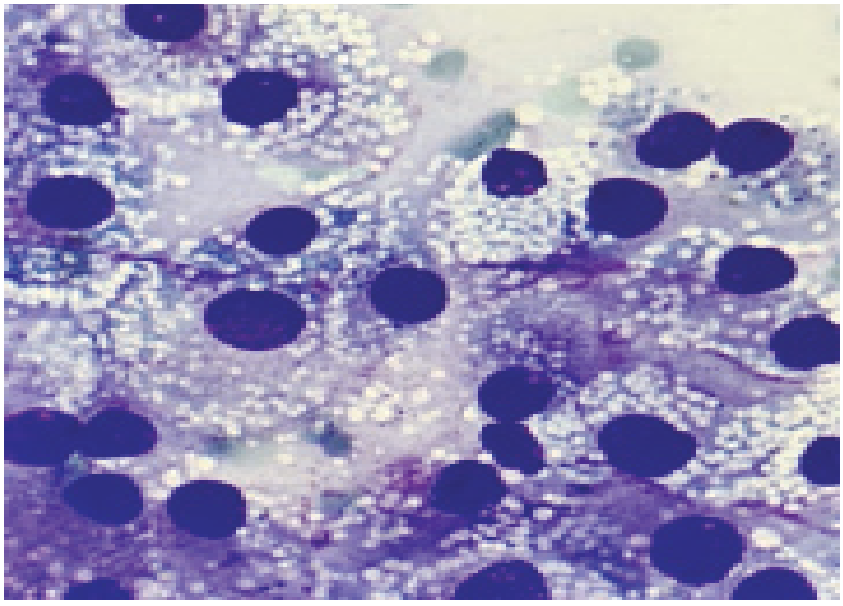

Fig. 1. Cytological structure of leydigioma. Tumor cells with large nuclei and vacuolated cytoplasm

Microscopically the proliferation of tumor cells in the interstitial tissue is detected. Sertolioma cells form sheets and cords. The tumor is mitotically active. Dystrophic changes were observed in the epithelium of the seminiferous tubules. The dysfunction of spermatogenesis is considered by a change in the level of maturity and shape of sperm. The tumor is accompanied by secondary inflammation very often. Tumor tissue vascularization is predominantly moderate.

The tumor has a tubular structure (small tubes separated by thin fibrous connective tissue layers). Glandular structures are visualized in it, which have two types of cell structure (cells with optically empty cytoplasm and cells with hyperchromic nuclei and mitosis presence).

The histological structure of sertolioma was different. A wide range of cells from epithelioid to spindle-shaped resembling fibroma prevailed. Most of the tumors consisted of sustenocytes of various shapes: from polygonal to high prismatic, which formed tubules or solid areas. The tumor cells were eosinophilic, with vacuolated cytoplasm, a rounded or oval nucleus with a delicate chromatin mesh, and a small nucleolus (Fig. 3).

Tumor cells have a clearly distinguishing nuclear structure and a pronounced rim of the cytoplasm. The nuclei are large, characterized by moderate polymorphism, nucleoli are visible in them. In the structure of the tumor tissue, the cells are located chaotically, sometime they lie freely in tubules lumen as small groups. Signs of cellular atypism were observed in the form of nuclear and cellular polymorphism.

The tumor stroma is represented by fibrous connective tissue layers, in which the slit-like, often collapsed vessels are located.

Seminoma is a highly malignant tumor, most often found in cryptorchids. In advanced cases, the tumor occupies a large volume of the abdominal cavity, is adherent to surrounding tissues, and metastasizes to regional lymph nodes. It

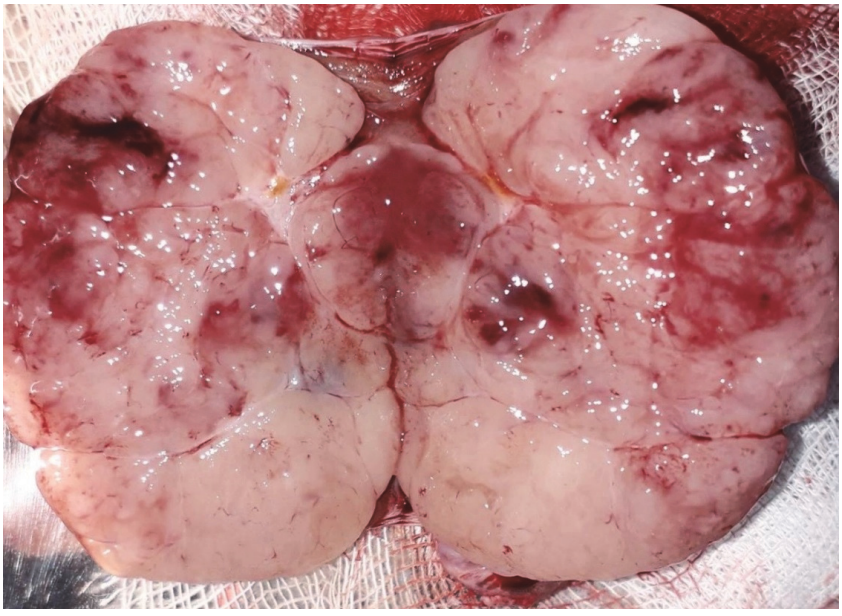

Fig. 2. Sertolioma. Macropreparation

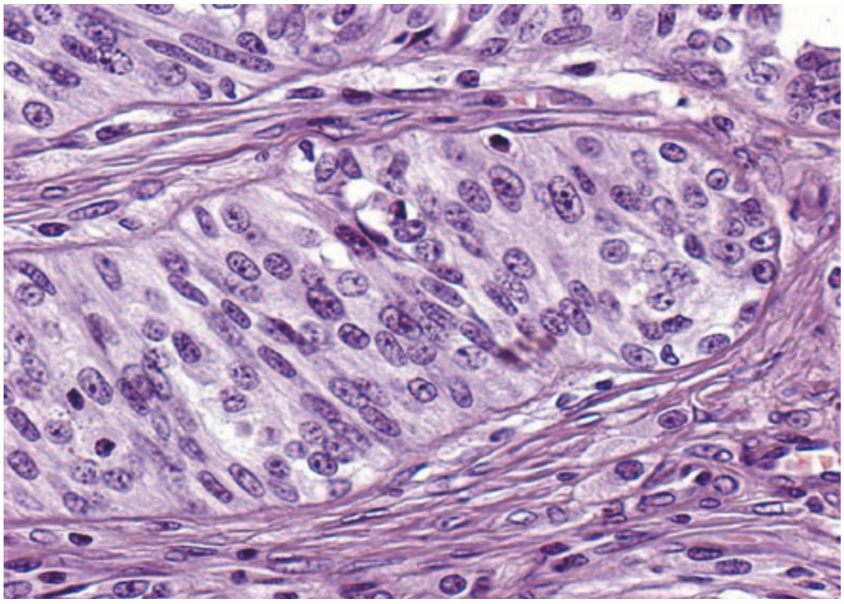

Fig. 3. Histological structure of sertolioma: hyperplasia of spindle cells with hyperchromic nuclei. 400 x.

is possible to observe the bilateral affection or affection of one of the testicles. To suspect seminoma is possible by the appearance of the affected organ. A palpating node or nodes are located in the testicular tissue under the outer shell or inside the organ. The tumor is mostly painful, accompanied by secondary inflammation, and characterized by limited growth in the form of a node or diffuse growth with complete organ affection.

The initial localization of the neoplasm is the seminiferous tubules, but in the case of testicular stromal invasion, it acquires a diffuse form.

Macroscopically, the testicle is enlarged, the tumor has no clear contours, the surface is bumpy, thin-walled cysts were palpated under the capsule. The consistency depends on the presence and number of necrosis foci. Section showed that the neoplasms were loose, swollen, looked like multiple cavities filled with viscous grayish-white pus on the periphery, and in the central part – with dark red bloody fluid. The central part had significant areas of hemorrhage and necrosis. Tissue vascularization was poor. Stroma swelling (from moderate to pronounced) with areas of hemorrhage was observed. Spermatogenesis in the tubules was significantly weakened or completely absent in partly preserved tissue near the site of tumor localization.

In the tumor growth zone atypical, polymorphic cells of epithelioid or polygonal form with rounded often large nuclei with various figures of mitosis, including atypical ones, are detected. In some fields of vision multiple nucleoli in the cells are visible (Fig.4).

In the other fields of vision, sometimes in the capsule, sometimes in places outside of it, there are fields and groups of monomorphic cells with light cytoplasm and clear boundaries of the cell wall. The cytoplasm is vacuolated or optically transparent. Vacuolization of the cytoplasm indicates the presence of

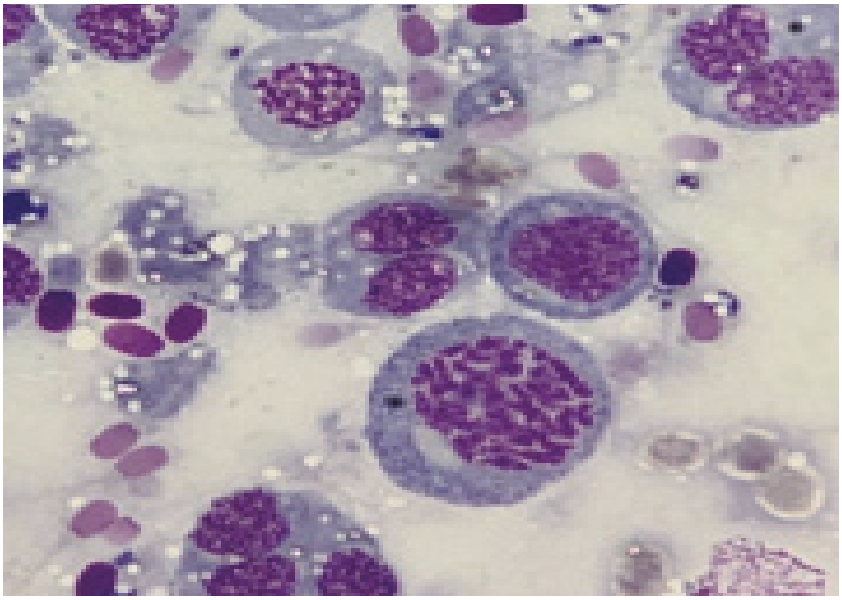

Fig. 4. The cytological structure of the seminoma

degenerative changes in the cells. The cells are located diffusely or in the form of small complexes bounded by fibrous tissue, in which a large number of mature lymphocytes and small foci of necrosis are determined.

In the process of progressive tumor tissue proliferation, violations of the stromal-parenchymal ratio observed. Lymphocytic accumulations were shown in the tumor stroma.

As a result of the seminoma development, the adjacent areas of the organ undergo changes as well. As a result of pressure from the tumor on healthy tissue, circulatory disorders, edema develop followed by disruption of the structure of the spermatogenic epithelium and loss of its functional activity take place. In some cases, the tumor tissue invasion into the capsule was also noted. In this case, the invasion of tumor elements into the skin of the scrotum is observed, the tumor becomes immobile, areas of inflammation and ulcers are traced in the skin.

Conclusions

1. The ratio between benign and malignant neoplasms of the canine testicles was 1:4.2.
2. The prognosis of the disease and the appointment of postoperative chemotherapy depend on the histological verification of the tumor.
3. The largest proportion among all tumors of canine testicles in the studied samples were germ cell tumors, namely seminomas, which were characterized by malignant course prevalence. Among benign neoplasms, Leydigiomas and sertoliomas were observed.
4. The right testis is more often affected. Half of the cases were recorded in the testicle that did not descend with an abdominal location.
5. No dependence on the consistency or shape of tumors on their histological type was found.
6. Carrying out the cytological studies, it is important to remember that in 40%

of dogs, multiple tumors develop in the testicles both of the same type and different (seminoma and sertolioma, seminoma and Leydigioma, etc.). In this regard, a biopsy of all testicular tumors found in the animal by palpation or ultrasound is required.

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Анотація. *Проведені дослідження з вивчення частоти діагностування, морфологічних, клінічних та макроскопічних особливостей різних гістологічних типів новоутворень сім`яників собак. Матеріал для досліджень був отриманий під час планових операцій із приводу видалення новоутворень сім'яників у собак у різних клініках ветеринарної медицини міста Києва. Пухлини були розподілені на пухлини строми статевого тяжу та герміногенні пухлини. Серед пухлин строми статевого тяжу реєстрували лейдигому та сертоліому. Описано макроскопічну та гістологічну характеристики лейдигоми у собак, яка становить 12 % від усіх новоутворень сім'яників. Лейдигома має дещо повільніший ріст у порівнянні з іншими пухлинами сім'яників, метастазів не відмічають. Більшість лейдигом супроводжуються патологією парааанальних залоз. Новоутворення реєструють, як на правому, так і на лівому сім'яниках. Переважно пухлини правильної овальної форми, бурого або сірого кольору, різної консистенції та розміру без ознак виразок чи некрозу.*

Описано макроскопічну та гістологічну характеристики злоякісних пухлин сім›яника собаки. Макроскопічна картина була різною та не мала чіткого зв›язку з гістологічним типом новоутворень. Мікроскопічно семінома характеризувалась наявністю пластів мономорфних овальних клітин із вираженою зернистою цитоплазмою, що були оточені сполучною тканиною, серед якої знаходили значну лімфоїдну інфільтрацію. Мікроскопічно лейдигома складається із гландулоцитів різного ступеня зрілості, які розташовані у вигляді тяжів, пучків, пластів. Спостерігають полігональні клітини з еозинофільною зернистою або вакуолізованою цитоплазмою, що містить ліпіди. У сертоліомі відмічали різноманітний спектр клітин від полігональних до призматичних сустеноцитів.

Ключові слова: *недрібноклітинні пухлини, генітальні стромальні пухлини, доброякісні пухлини, лейдиго-клітинна пухлина, сертоліома, семінома, новоутворення яєчок, собаки*