Multiple primary testicular tumours in the dog – a case report

Stanisław Dzimira[1], Małgorzata Kandefer-Gola[1], Dominika Kubiak-Nowak[2]

University of Environmental and Life Sciences, Faculty of Veterinary Medicine,
[1]Department of Pathology, [2]Department of Surgery, Wrocław, Poland

Abstract

A 13-year old male Poodle dog was presented with a considerable disparity in the size of testes. Palpation demonstrated enlargement of the right testicle; smooth surface without any sign of nodular hyperplasia was detected. Both testes were removed, the enlarged one was sent to a histopathology laboratory. Microscopic examination revealed massive neoplastic proliferation of the testicular germ cell tumour (seminoma) and an accompanying smaller tumour originating from the interstitial Leydig cells (Leydigoma), which was confirmed by immunohistochemistry. Simultaneous occurrence of two different types of tumours in testes is possible, representing a multiple primary malignancy case, which is a rare phenomenon in veterinary practice.

Canine, testis, multiple primary malignancies, seminoma, interstitial cell tumour, Leydig cell tumour

Testicular tumours are second in terms of frequency of occurrence in dogs and constitute a serious oncological problem in this species. Dogs are most frequently affected among domestic animals. A longer life span of dogs and less frequent castration in comparison with other species significantly affect the high incidence of the tumours in this species. Testicular tumours occur in older dogs, usually over 10 years of age (7–12 years), mostly unilaterally, though a bilateral location is not unusual (Kennedy et al. 1998). The aetiology, as in almost every type of a spontaneous tumour, is not unequivocally established, but the off-scrotum positioning of testes certainly has a major effect on tumour growth (Peters and Sluijs 1996; Thonneau et al. 2003; Maiolino et al. 2004; Reuter 2005). It is estimated that the risk of neoplasia in the retained testes increases several times (Romagnoli 1991; Thonneau et al. 2003). Isolated cases of testicular tumours are also described in dogs with sex development disorders (Dzimira et al. 2015). In humans it was proved that the increased risk of testicular tumour development is observed in syndromes associated with testicular dysgenesis, such as testicular feminization syndrome, or Klinefelter's syndrome in men (Oosterhuis and Looijenga 2005).

There are a number of histological types that behave differently, the type of tumour depending on specific histological structures. Morphological differences influence the rate of growth of tumours and any potential metastases, and thus also their malignancy and the observed clinical symptoms. The latter are related not only to the size and location of the tumour but also to its potential hormonal activity.

Primary testicular tumours are most often derived from seminiferous epithelial cells (testicular seminomas - seminomata)*,* Sertoli supporting cells and Leydig interstitial cells. Testicular germinal cell tumours develop from primitive cells that can further differentiate into gonads, or can convert into the populations of pluripotent cells. Pluripotent cells can remain at the stage of undifferentiated cells (embryonal carcinoma - carcinoma embryonale), differentiate into extra-embryonic tissues (endodermal sinus tumour - yolk sac tumour, malignant chorionic epithelioma - choriocarcinoma) or somatic tissues (teratoma).

Address for correspondence:
Dr Stanisław Dzimira
Department of Pathology, Faculty of Veterinary Medicine
University of Environmental and Life Sciences
Norwida 31, 50-375 Wrocław, Poland

Phone: +48 71 320 52 56
E-mail: stanislaw.dzimira@upwr.edu.pl
http://actavet.vfu.cz/

According to most authors metastatic testicular tumours occur very rarely in dogs. Dissemination of seminomas occurs primarily through the lymphatic vessels and the metastases are localized mostly in the retroperitoneal lymph nodes. Seminomas metastasize by both the lymphatic vessels and blood vessels primarily to the lungs, liver, bone, and brain. Less common forms of malignant tumour of supporting and interstitial cells metastasize mainly to the inguinal and lumbar lymph nodes (Restucci et al. 2003). It should be noted that hormonally active tumour metastases may also produce hormones (Kennedy et al. 1998; Grieco et al. 2008).

In the initial stage of the disease, testicular tumours manifest themselves most often by enlargement of the organ or a small nodule. Pain usually accompanies rapid growth, bleeding into the nodule or cryptorchidism. Azoospermia and/or oligospermia are often observed. Infertility and dermatitis (acanthosis, seborrhea) are often reported as well. Sertoli supporting cell tumours and seminomas may secrete androgens or oestrogens (Patnaik and Mostofi 1993).

Case description

During the physical examination of a 13-year-old male Poodle dog, a considerable disparity in the size of testes was identified, which suggested a tumour growth. Palpation demonstrated enlargement of the right testis and reduction of the left one. The surface of both testicles was smooth, without any signs of nodular hyperplasia. The examination also identified no adhesions or fluid within the scrotum. Blood test showed no abnormality. Ultrasound examination was not performed. Both testicles were removed. Only the enlarged right testicle was sent to the histopathological analysis.

Histopathological examination

The specimens of canine testis were fixed in 7% buffered formalin (Chempur, Piekary Slaskie, Poland) for 24 h, embedded in paraffin and cut into 4 μm thick sections. Histopathological evaluation of haematoxylin (Sigma-Aldrich, Saint Louis, MO, USA) and eosin (Chempur) stained microscopic slides were based on the WHO classification of testicular tumours (Kennedy et al. 1998).

Immunohistochemical examination

The immunohistochemical analyses (IHC) were conducted on 4 μm thick paraffin sections mounted on glass slides (Dako, Glostrup, Denmark), deparaffinized in xylene (Stanlab, Lublin, Poland) and rehydrated in alcohol gradients. The slides were heated in citrate buffer (pH 6.0, 97 °C, 20 min) to retrieve the antigens. Then the slides were double washed with phosphate buffered saline (PBS), blocked in 10% normal serum with 1% Bovine Serum Antigen (BSA) in PBS. Afterwards, respective primary antibodies: monoclonal mouse anti-human calretinin (clone DAK-Calret 1, 1:100, Dako), monoclonal mouse anti-human E-Cadherin (clone NCH-38, 1:50, Dako), monoclonal mouse anti-human inhibin-α (clone R1, 1:100, Dako), and rabbit monoclonal anti-human MCM3 (minichromosome maintenance 3, clone EP202, 1:50, Novocastra) were applied, and the slides were incubated at room temperature for 20 min. Subsequently, the slides were washed in PBS. After rinsing, endogenous peroxidase was blocked in 3% solution of hydrogen peroxide for 10 min. The immunohistochemical reactions were developed with 3,3-diaminobenzidine tetrahydrochloride (DAB). Finally, the slides were counterstained with haematoxylin. Either a positive or a negative control was performed for each marker studied. The specificity of the immunolabelling was verified by incubation with PBS instead of the specific primary antibody, and the sections of the canine skin were used as positive controls. The slides were subjected to computer-aided image analysis with a computer

connected to Olympus CX41 optic microscope (Olympus, Tokyo, Japan) equipped with Olympus camera DP-20 (Olympus, Tokyo, Japan).

Staining evaluation

The immunoexpression of E-cadherin, inhibin-α, and calretinin was scored according to the modified semi-quantitative IRS scale proposed by Remmele and Stegner (1987). The method takes into account both the proportion of positively stained cells and the intensity of the colour reaction. The score constitutes the product of multiplication of both indicators and ranges from 0 to 12 points [no reaction = 0 points (–); weak reaction = 1–2 points (+), moderate reaction = 3–5 points (++), intense reaction = 6–12 points (+++)]. Expression of MCM3 was evaluated quantitatively, based on the percentage of positively-stained cells [0–5% = no reaction (–), 6–25% = weak reaction (+), 26–50% = moderate reaction (++), above 50% = intense reaction (+++)]. Evaluation of the expression of the markers was performed by three observers with relevant experience in analyzing immunohistochemical reactions.

Results

On macroscopic examination of the testis, less than 5% constituted two light brown fragments of normal testicle parenchyma. The vast majority constituted an abundant light greyish parenchyma of the tumour tissue covering approximately 90% of the of the testis surface, and a small nodule of about 1 cm in diameter, in orange-yellowish colour, haemorrhaged, bulging above the cross-sectional area, occupying no more than 5–7% of the organ (Plate I, Fig. 1).

The microscopic examination of the slices stained with haematoxylin and eosin identified a dominant tumour proliferation of the disseminated seminoma type. It consisted of polygonal cells with round or oval nuclei, fairly scant cytoplasm, and a high mitotic index. Tumour cells completely blurred the tubular structure of the testicle, in many places interrupted the basal membrane of the tubules and invaded the stroma forming a common tumour weaving of a relatively high mitotic index. The tumour cell proliferation was accompanied by the infiltrates of lymphocytes on the perimeter and in the centre.

Histopathological examination of the smaller nodule revealed round or polygonal cells, with small dark nuclei, containing lipid droplets of different sizes in abundant cytoplasm. The figures of mitotic divisions were rare. In this case, Leydig cell tumour was identified (Plate I, Fig. 2A). The fragment of normal tissue of the testicle showed characteristics of atrophy. The seminiferous tubules contained only supporting cells of Sertoli with no spermatogonia and sperm.

The expression of the proteins measured in two concomitant tumour types is shown in Table 1 and Fig. 2 (Plate II, B, C, Plate III, D, E). The reaction with the cell division capacity marker, i.e. MCM-3 was intensive (+++), positive in more than 75% of the seminoma cells, which indicates a high proliferative capacity of the tumour. However, in leydigioma it was weak positive (+) with about 10% cells reacting positively. The examined sample material demonstrated the response on calretinin staining - the strongest in seminoma (+++) and far weaker (+) in the Leydig tumour cells. It is believed that a strong expression of calretinin in steroid hormone producing cells may be indicative of a secretory activity of the tumour (Radi and Miller 2005). Both of the tumour types failed to react with E-cadherin, which is a bad signal. It is a protein involved in mutual adhesion of cells, having a strong relationship with the metastatic potential of tumour cells. The results obtained by using E-cadherin tracing antibodies allow inferring about the strength of the connections between the tumour cells. With a decrease in the expression of E-cadherin, the tumour cells are more easily released from the primary tumour, which results in an increased risk of metastasis (Ciaputa et al. 2014). A strong cytoplasmic

Table 1. Cell markers expression in canine testicular tumours.

Tissue	E-cadherin	Calretinin	Inhibin-α	MCM3*	Mitotic index
Seminoma	+	+++	-	+++	3
Leydigioma	+	+	+++	+	1
Testis tissue	++	-	-	-	0

*MCM3 - minichromosome maintenance - 3

reaction to inhibin-α was observed in the leydigioma, which indicates a slow growth rate of tumour; and a negative one in the seminoma, which shows the rapid lesion development (Taniyama et al. 2001; Ciaputa et al. 2014).

To sum up, based on the histopathological and immunohistochemical examination, the bigger tumour was identified as seminoma, which shows the secretory activity, rapid growth rate, high proliferative capacity and increased risk of metastasis. Due to the malignant phenotype of seminoma, the prognosis is unfavourable, contrary to the smaller lesion, diagnosed as a leydigioma, characterized by the secretory activity, slow development, low proliferative rate and increased risk of metastasis.

Discussion

The described case can be considered as an example of a multiple primary malignancy syndrome (MPM), which is a rare phenomenon. It is defined as the occurrence of two or more tumours in the same or different organs of the patient. The syndrome was described for the first time in the second half of the nineteenth century by Theodor Billroth (Jiao et al. 2014).

The contemporary definition of multiple primary malignancies, given by the International Agency for Research on Cancer IARC in 1991, assumes that the following conditions must be met for diagnosing a second primary tumour:
- histopathological examination confirming the malignant nature of both identified tumours: the primary tumour (index tumour) and a second primary metastatic neoplasm (second primary tumour, SPT);
- a separate location of both tumours, and if located in the neighbourhood separated with at least 2 cm thick healthy tissue; if the SPT develops in the same organ, it must be at least five years after the diagnosis of the first tumour;
- histopathological features should exclude the possibility that the second tumour is a metastasis of the primary tumour spot.

Depending on the diagnosis time of each tumour, there are two types of MPM distinguished: synchronous - tumours occurring at the same time or within six months; and metachronous – tumours occurring successively at an interval of over six months (Jiao et al. 2014). Though uncommon, the phenomenon has been increasingly reported and can affect many systems and organs. Based on a literature review on malignancies in humans, Demandante et al. (2003) have shown that the prevalence of such cases varies from 0.73 to 11.7%, and they most commonly involve respiratory, gastrointestinal and genitourinary systems. The incidence of MPM increases with age, affecting significantly more often males. It is estimated that e.g. in human patients with clear-cell renal carcinoma the risk of developing another primary malignancy ranges from 30 to 42% and it can include the cancer of lungs, prostate, colon, bladder and blood, as well as non-Hodgkin lymphoma. The potential causes include environmental pollution, population aging, genetic predisposition and obesity. The incidence rate in men vs. women was 1.44 (223/154)

(Babacan et al. 2012). Patel et al. (1990) described synchronous and metachronous bilateral testicular cancer, and Forbes et al. (2013) described a case of a three primary testicular tumours in a 19-year-old man. Veterinary literature offers descriptions of MPM in animals. Rebhun and Thamm (2010) reported only 53 such cases among 1722 dogs whose tissues were examined at a veterinary centre at the Colorado State University over 21 months (from 01.04.2006 to 31.12.2007) in relation to suspected malignancy.

The case described in our work of simultaneous occurrence of different types of testicular tumours in dogs is rare in veterinary practice. Despite the inability to satisfy the IARC's recommendations needed to classify the discussed case as MPM (immediate vicinity of the two tumours with no 2 cm barrier of healthy tissue and inability to determine the time of their emergence), we decided it was worth describing. Although it does not meet all the criteria, we believe that the presented case falls within the definition of MPM. The unusual location of both tumours inspired the authors to present the exact histological and clinical picture in an attempt to clarify the aetiopathogenesis and to facilitate the differential diagnosis of testicular tumours in dogs.

To conclude, it can be assumed that simultaneous occurrence and coexistence of two different types of tumours in testes is possible. In such cases, the application of markers specific to diverse histological types of testicular tumours is of great diagnostic importance. Malignancies occurring jointly have the morphological characteristics which are typical of their equivalents occurring individually. In animals, it is not possible to determine in each occurrence of multiple primary malignancies their inception, in spite of their different origin and reported malignant nature.

References

Babacan NA, Aksoy S, Cetin B, Ozdemir NY, Benekli M, Uyeturk U, Ali Kaplan M, Kos T, Karaca H, Oksuzoglu B, Zengin N, Buyukberber S 2012: Multiple primary malignant neoplasms: multi-center results from Turkey. J BUON **17**: 770-775

Ciaputa R, Nowak M, Madej JA, Poradowski D, Janus I, Dziegiel P, Gorzynska E, Kandefer-Gola M 2014: Inhibin-a, E-cadherin, calretinin and Ki-67 antigen in the immunohistochemical evaluation of canine and human testicular neoplasms. Folia Histochem Cytobiol **52**: 326-34

Demandante CG, Troyer DA, Miles TP 2003: Multiple primary malignant neoplasms: case report and a comprehensive review of the literature. Am J Clin Oncol **26**: 79-83

Dzimira S, Nizanski W, Ochota M, Madej JA 2015: Histopathological pattern of gonads in cases of sex abnormalities in dogs: an attempt of morphological evaluation involving potential for neoplasia. Pathol Res Pract **211**: 772-775

Forbes CM, Metcalfe C, Murray N, Black PC 2013: Three primary testicular tumours: Trials and tribulations of testicular preservation. Can Urol Assoc J **7**: 630-633

Grieco V, Riccardi E, Greppi GF, Teruzzi F, Iermano V, Finazzi M 2008: Canine testicular tumours: a study on 232 Dogs. J Comp Path **138**: 86-89

Jiao F, Yao LJ, Zhou J, Hu H, Wang LW 2014: Clinical features of multiple primary malignancies: a retrospective analysis of 72 Chinese patients. Asian Pac J Cancer Prev **15**: 331-334

Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, Nielsen S 1998: Histological classification of the tumours of the genital system of domestic animals. In 2nd series, vol. 4, Armed Force Institute of Pathology American Registry of Pathology, Washington, 12-20 p.

Maiolino P, Restucci B, Paparella S, Paciello O, De Vico G 2004: Correlation of nuclear morphometric features with Animal and Human World Health Organization International histological classification of canine spontaneous seminomas. Vet Pathol **41**: 608-611

Oosterhuis JW, Looijenga LHJ 2005: Testicular germ-cell tumours in a broader perspective. Nat Rev Cancer **5**: 210-222

Patel SR, Richardson RL, Kvols L 1990: Synchronous and metachronous bilateral testicular tumors. Mayo Clinic Experience. Cancer **65**: 1-4

Patnaik AK, Mostofi FK 1993: A clinicopathologic, histologic and immunohistochemical study of mixed germ cell-stromal tumors of the testis in 16 dogs. Vet Pathol **30**: 287-295

Peters MA, Van Sluijs FJ 1996: Testicular tumors in dogs: a literature review. Tijdschr Diergenneeskd **121**: 36-38

Radi ZA, Miller DL 2005: Immunohistochemical expression of calretinin in canine testicular tumours and normal canine testicular tissue. Res Vet Sci **79**: 125-129

Rebhun RB, Thamm DH 2010: Multiple distinct malignancies in dogs; 53 cases. J Am Anim Hosp Assoc **46**: 20-30

Remmele W, Stegner HE 1987: Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe **8**: 138-140

Restucci B, Maiolino P, Paciello O, Martano M, De Vico G, Papparella S 2003: Evaluation of angiogenesis in canine seminomas by quantitative immunohistochemistry. J Comp Pathol **128**: 252-259

Reuter VE 2005: Origins and molecular biology of testicular germ cell tumors. Modern Pathology **18**: 51-60

Romagnoli SE 1991: Canine cryptorchidism. Vet Clin North Am **21**: 533-544

Taniyama H, Hirayama K, Nakada K, Numagami K, Yaosaka N, Kagawa Y, Izumisawa Y, Nakade T, Tanaka Y, Watanabe G, Taya K 2001: Immunohistochemical detection of inhibin-α,-β B and –β A chains and 3β-hydroxysteroid dehydrogenase in canine testicular tumors and normal testes. Vet Pathol **38**: 661-666

Thonneau PF, Candia P, Mieusset R 2003: Cryptorchidism: incidence, risk factors and potential role of environment. J Androl **24**: 155-162

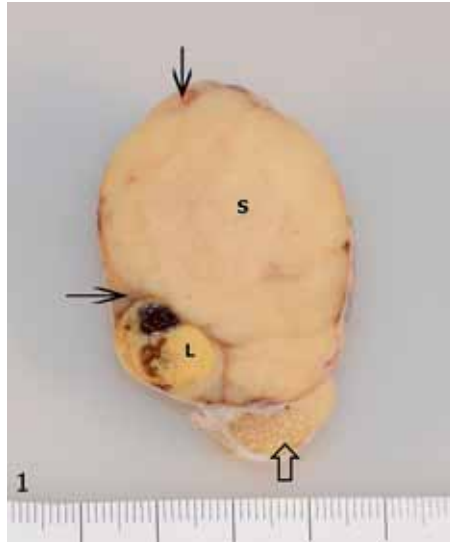Plate I
Dzimira S. et al.: Multiple primary ... pp. 373-378

Fig. 1. The cross-section of the dog testis (sample material fixed in formalin). Visible small fragments of normal parenchyma (thin arrows), massive tumour growth of the seminoma type (S), a significantly smaller tumour growth of the leydigioma type (L) and the unaffected epididymis (thick arrow).
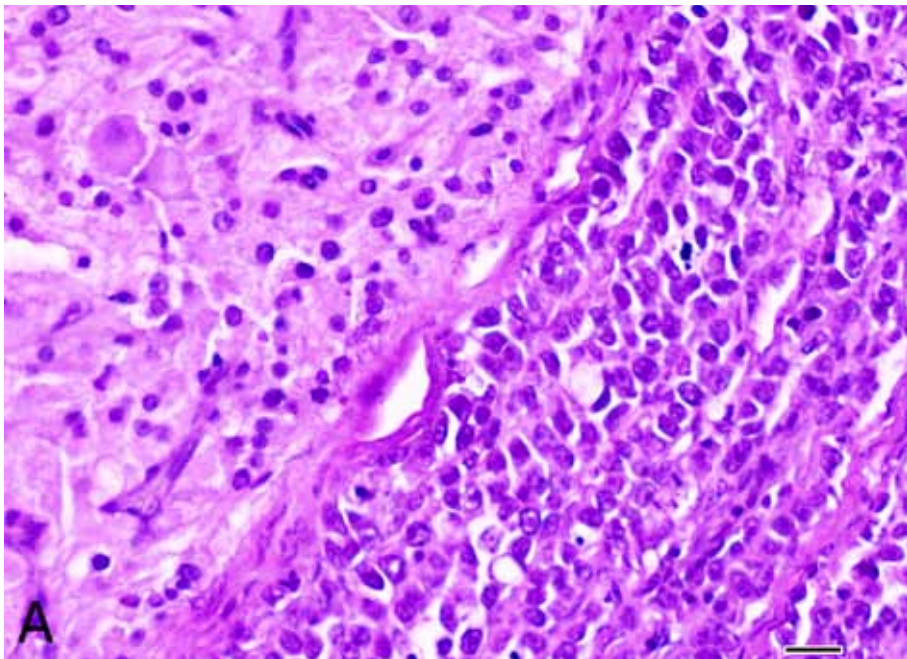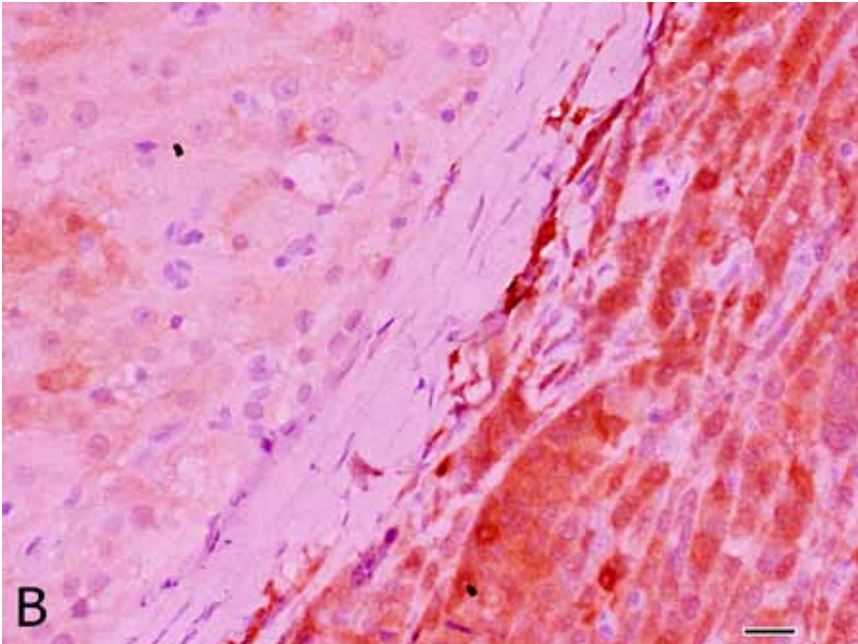

Fig. 2. Histological picture of the border area of both tumours (leydigoma on the left, seminoma on the right): A – haematoxlin and eosin (HE) staining. Magnified × 200

Plate II

Fig. 2. Histological picture of the border area of both tumours (leydigoma on the left, seminoma on the right): B - expression of calretinin, magnified × 200.
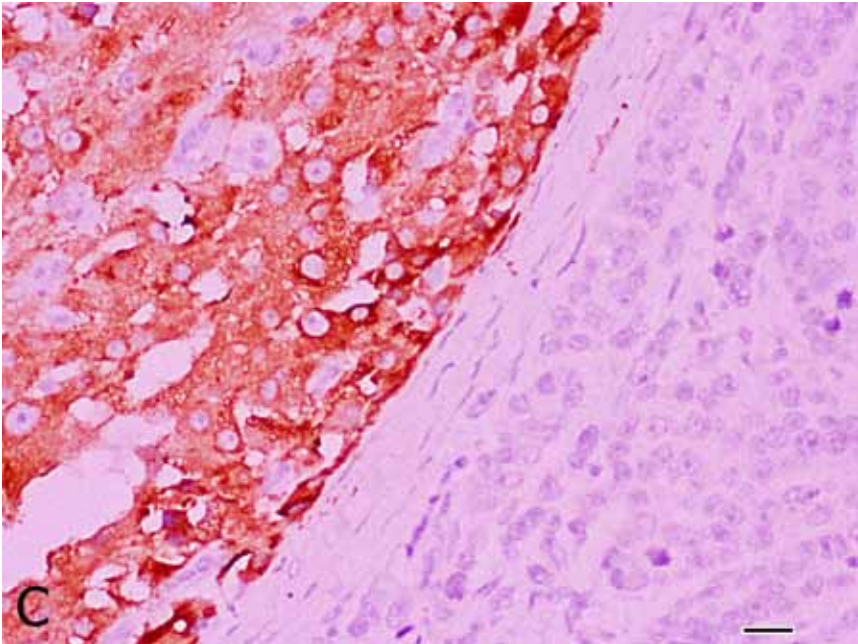

Fig. 2. Histological picture of the border area of both tumours (leydigoma on the left, seminoma on the right): C - expression of inhibin-α, magnified × 200.
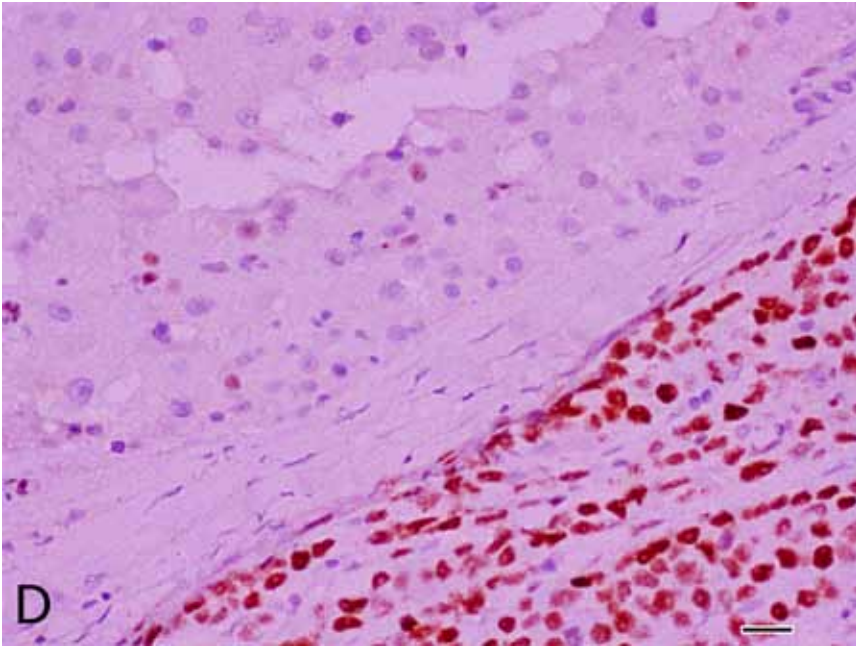
Plate III

Fig. 2. Histological picture of the border area of both tumours (leydigoma on the left, seminoma on the right): D - expression of MCM3 (minichromosome maintenance 3), magnified × 200.
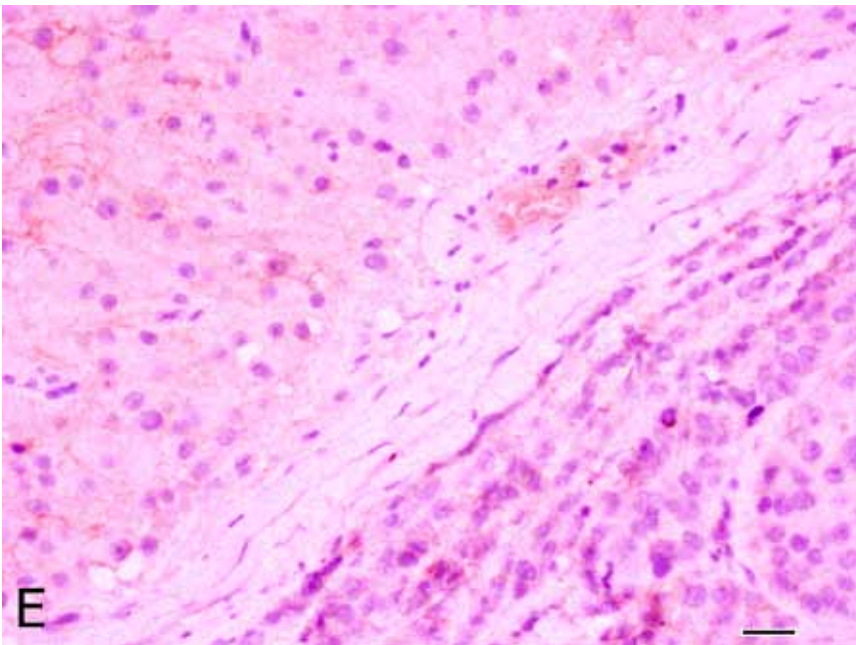

Fig. 2. Histological picture of the border area of both tumours (leydigoma on the left, seminoma on the right): E - expression of E-cadherin, magnified × 200.