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## Two case studies of 129/Sv testicular teratoma

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## Δύο ενδιαφέροντα περιστατικά ορχικού τερατώματος σε 129/Sv μύες

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**ABSTRACT.** Testicular teratoma is classified among testicular germ cells tumors. While its incidence is relatively low among animals, in 129/Sv mice is presented in relatively high prevalence. It has been revealed that murine teratoma arises from genetically altered germ cells that undergo malignant transformation during embryogenesis. A point mutation on the 129/Sv genome has been incriminated for failing to regulate normal pathways in germ cell development leading in high teratocarcinogenic risk. For a neoplasm to be characterized as teratoma, derivatives from at least two germ cell layers have to be detected. In the present study, two cases exhibiting all three layers (mesoderm, endoderm, ectoderm) are presented while the tissues observed were of epithelial, connective, muscular, and neural origin. Findings include an almost fully shaped large intestine, a structure closely resembling the heart (blood, endocardium, myocardium) and endochondral ossification.

**Keywords:** Teratoma, mouse, 129/Sv strain, histogenesis

**Περίληψη.** Στους όγκους των γεννητικών κυττάρων του όρχεως συμπεριλαμβάνεται και το ορχικό τεράτωμα. Είναι μια σχετικά σπάνια εμφανιζόμενη νεοπλασία των θηλαστικών, η οποία όμως παρουσιάζεται με σχετικά υψηλότερη συχνότητα στο στέλεχος 129/Sv του μύδς. Φαίνεται ότι το τεράτωμα του μύδς προέρχεται από γεννητικά τροποποιημένα κύτταρα τα οποία υφίστανται κακοήθη εξάλλαγή κατά τη διάρκεια της εμβρυογένεσης. Για το φαινόμενο αυτό έχει ενοχοποιηθεί μια

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Οι συγγραφείς Σίσκος Ν. και Λελόβας Π. συνεισέφεραν ισότιμα στην παρούσα μελέτη

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σημειακή μετάλλαξη στο γονιδίωμα του στελέχους 129/Sv στην οποία οφείλεται η αποτυχία να ρυθμιστεί η φυσιολογική ανάπτυξη των βλαστικών κυττάρων και αυτό έχει σαν συνέπεια αυξημένο κίνδυνο καρκινογένεσης. Για το χαρακτηρισμό ενός νεοπλασματος ως τερατώματος είναι απαραίτητη η παρουσία τουλάχιστον δύο από τις τρεις βλαστικές στοιβάδες. Στη παρούσα μελέτη, παρουσιάζονται δύο περιστατικά ορχικού τερατώματος που εκδήλωσαν και τις τρεις βλαστικές στοιβάδες (ενδόδεσμα, μεσόδεσμα, και εκτόδεσμα). Επιπλέον οι ιστοί που παρατηρήθηκαν ήταν επιθηλιακής, συνδετικής, μυϊκής και νευρικής προέλευσης. Στα ευρήματα περιλαμβάνονται ένα σχεδόν πλήρες ανεπτυγμένο παχύ έντερο, μια δομή που προσομοίαζε εξαιρετικά σε καρδιά (παρατηρήθηκαν αίμα, ενδοκάρδιο μυοκάρδιο) όπως επίσης και εστίες ενδοχόνδριας οστεοποίησης.

**Λέξεις ευρητηρίασης:** Τεράτωμα, μυς, 129/Sv στέλεχος, ιστογένεση.

## INTRODUCTION

**T**esticular malignancies can be classified into three broad categories: germinal, non germinal and metastatic (Amato, 2002). Non germinal tumors include Leydig cell and Sertoli cell tumors (among others) and arise from cells whose role is rather subsidiary in gametogenesis. Germinal testicular neoplasms (or Testicular Germ Cell Tumors - TGCTs) originate from cells directly involved in spermatogenesis. TGCTs can be further classified into seminomas (classic and spermatocytic), embryonal carcinomas, yolk sac tumors, trophoblastic tumors and teratomata.

Finally, it should be mentioned that testicular malignancies might contain more than one of the aforementioned tumors thus designated as mixed tumors (Woodward *et al.*, 2004).

Teratomata are encapsulated tumors with tissue or organ components that can be traced to derivatives of the three primordial germ cell layers (endoderm, mesoderm, ectoderm) (Ozolek and Castro, 2011). Based upon the maturity level of tissues contained, teratomata may be classified either as mature or as immature (more or less well-differentiated respectively). World Health Organization expands the aforementioned definition in order to include dermoid cysts (mature teratomata that contain predominantly one or more keratinized squamous epithelium lined cysts), monodermal teratomata (consisting of tissues that derive only from one germ-cell layer) and teratomata combined with other somatic-type malignancies (Woodward *et al.*, 2004).

Among laboratory animals, 129/Sv mice present relatively high spontaneous teratoma formation incidence (Stevens, 1984). Thus, they have been used as spontaneous animal models devoted to the study of teratocarcinogenesis. Moreover, there have been introduced induced teratoma animal models shedding light in this strictly defined and elaborate process (Sundstorm *et al.*, 1999).

## CASE DESCRIPTION

### Animals

Mice presented in this study were kept at the Animal Unit of the replace:Center of Clinical Experimental Surgery and Translational Research of the Biomedical Research Foundation of the Academy of Athens. Mice were housed at individually ventilated cages (IVC, 1284L, Tecniplast), at a room temperature of 24±2 °C, relative humidity of 55-60%, 12 h:12 h light/dark cycle (0700/1900). All animals in the facility are screened regularly according to the Federation of European Laboratory Animal Science Associations' (FELASA) recommendations and were free from the respective pathogens. Animals were fed with irradiated vacuum packed industrial pellet (Teklad 2918, Envigo, Italy) and had ad libitum access to food and water.

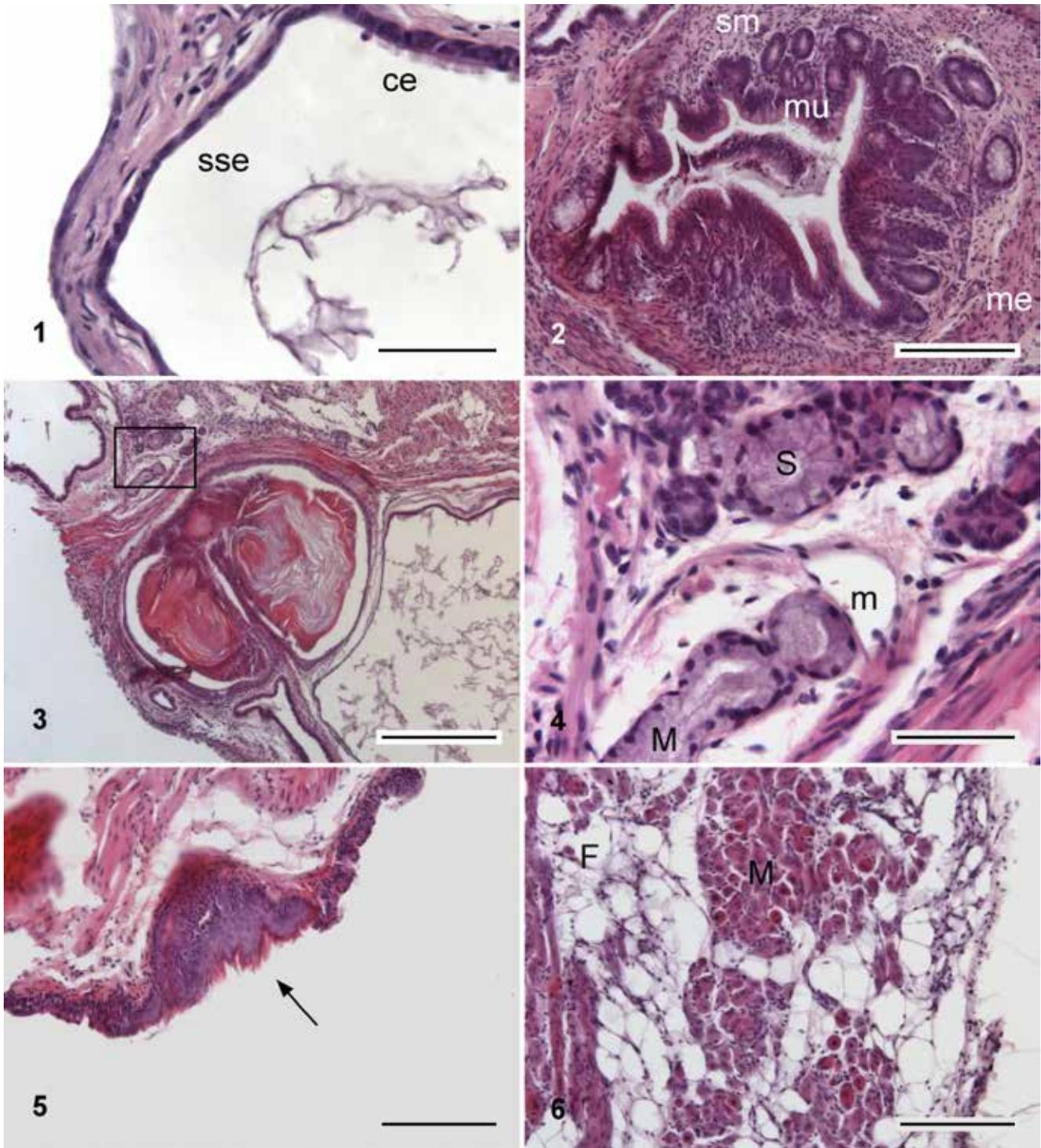
Two spontaneous cases, with a significant testicular asymmetry were presented at the Animal Unit of the replace:Center of Clinical Experimental Surgery and Translational Research of Biomedical Research Foundation of the Academy of Athens. All cases were reported during everyday health inspection by the animal caretaker. The animals were euthanised for humane reasons by an overdose (200 mg/kg) of sodium thiopental administered intraperitoneally.

### Histological analysis

At necropsy, testicles were harvested, dissected in the middle and fixed in neutral 4% formalin solution for one day. Overnight immersion in 70% ethanol solution followed, tissues were further processed automatically and embedded in paraffin. Blocks were sectioned approximately at 3µm on a microtome and stained with haematoxylin & eosin.

### Case No.1

First case is a four-month-old male 129/Sv mouse presenting testicular asymmetry. Left testicle gross



**Figure 1.** Case No.1; H&E. Cyst lined by simple squamous epithelium (sse) transformed from cuboidal ciliated (ce). Scale bar: 50µm

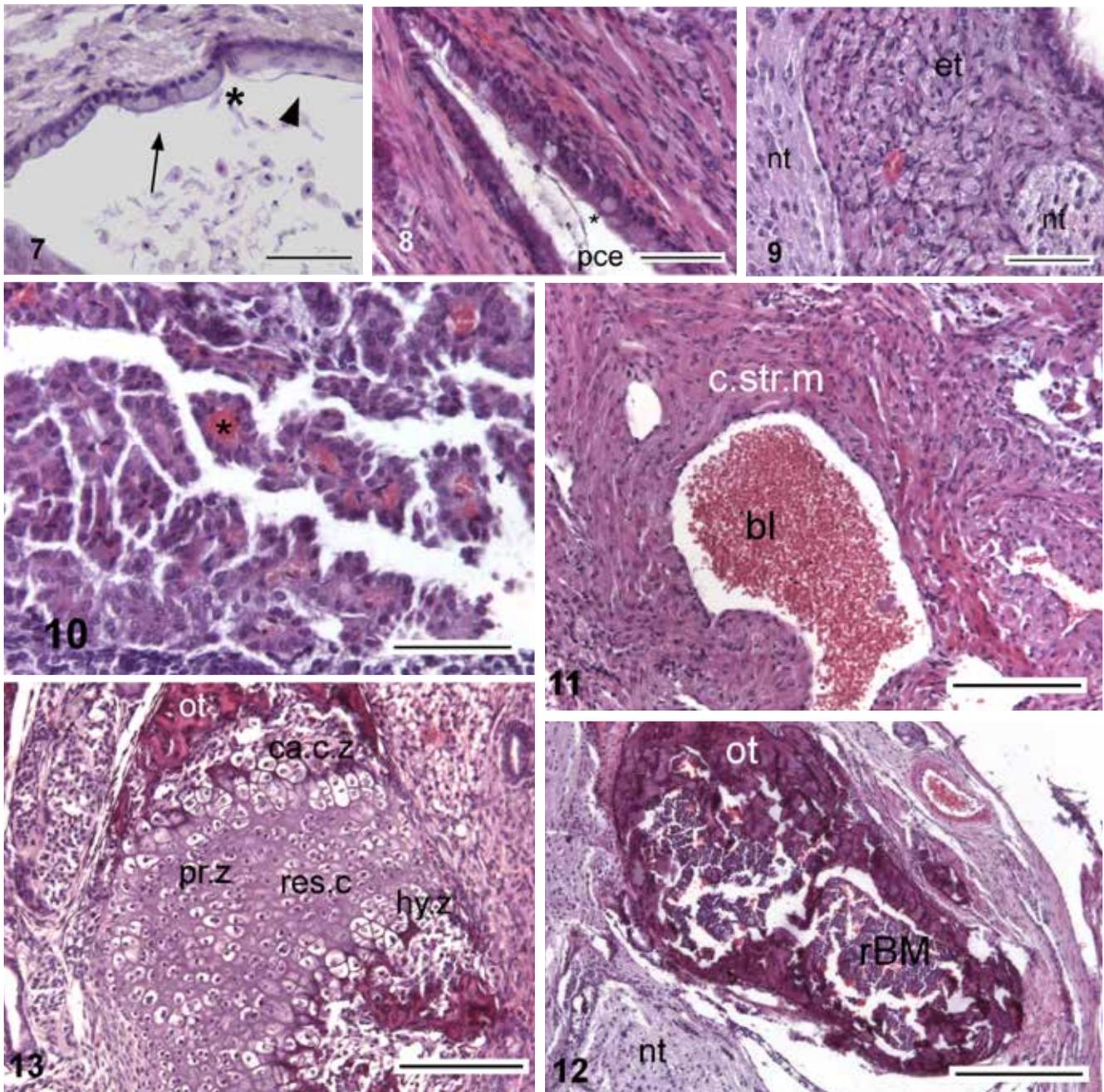
**Figure 2.** Case No.1; H&E. Structure lined by simple, tall, non-ciliated, mucous producing epithelium (mu: mucosa). Lumen lining epithelium forms crypts that extend into underlying connective tissue stroma (sm: submucosa). At least two smooth muscle layers (me: muscularis externa) circumscribe mucosa, while whole structure resembles large intestine. Scale bar: 150µm

**Figure 3.** Case No.1; H&E. Low power microphotograph depicting many “empty” cysts, a large “double-chambered” dermoid cyst, adipose cells, muscular tissue and a group of exocrine glands (small rectangle). Scale bar: 300µm

**Figure 4.** Case No.1; H&E. Higher magnification of the small rectangular area of Figure 3. Serous (S), mucous (M) and mixed (m) type acini can be observed. Scale bar: 50 µm

**Figure 5.** Case No.1; H&E. Structure resembling to skin (arrow: keratinized epithelium). Scale bar: 150µm

**Figure 6.** Case No.1; H&E. Transverse sections of striated muscle bundles (M) and white adipose tissue (F). Scale bar: 150µm



**Figure 7.** Case No.2; H&E. Cyst lined by simple, columnar mucous epithelium (arrow) transformed into multiple squamous (arrow head). Detached squamous cells can be seen inside cyst lumen. Asterisk marks transition area between the epithelial types. Scale bar: 50µm

**Figure 8.** Case No.2; H&E. Respiratory (pseudostratified, ciliated with goblet cells) epithelium (pce) surrounded by smooth muscular tissue. Asterisk marks goblet cell; notice the cytoplasmic clearance that distinguishes the goblet cell from its neighbors. Scale bar: 75µm

**Figure 9.** Case No.2; H&E. Epithelioid tissue (et) surrounded by nervous tissue (nt).Featuring large cells with abundant cytoplasm, a spherical euchromatinic nucleus with a prominent nucleus, this area is consistent with steroid producing cells. Scale bar: 75µm.

**Figure 10.** Case No.2; H&E. Choroid plexus. Notice the polygonal structures lined by simple cuboidal to short columnar epithelium. Beneath the epithelium lies loose connective tissue (resembling to pia matter) or blood vessels (asterisk marked). Scale bar: 70µm.

**Figure 11.** Case No.2; H&E. Luminous structure featuring bundles of cardiac striated muscle (c.str.m), lined by endothelium and containing red blood cells. Finding consistent with presence of heart-like tissue. Scale bar: 150µm.

**Figure 12.** Case No.2; H&E. Cancellous bone (ot: osseous tissue) containing red bone marrow (rBM). Neural elements (nt: nervous tissue) can be noticed in its periphery. Scale bar: 300µm.

**Figure 13.** Case No.2; H&E. Hyaline cartilage undergoing endochondral ossification on its margins. Zones of reserve cartilage (res.c), proliferation (pr.z), hypertrophy (hy.z) and calcified cartilage (ca.c.z) as well as newly formed bone (ot: osseous tissue) can be seen. Scale bar: 150µm.

appearance was normal. Right testicle grossly appeared enlarged containing a relatively large quantity of fluid. Hematoxylin and eosin (H&E) histological evaluation revealed well differentiated teratoma.

Simple columnar mucous-producing epithelia reminding of goblet cells, and ciliated epithelia, possessing longer or shorter cilia (Fig 1, ce: and ciliated with and ciliated), were often observed lining cystic structures. All three types of epithelial glandular structures (Fig 4; mucous, (M) serous (S) and mixed (m) type), could be noticed. Further endodermal derivatives included a structure lined by gastrointestinal-like epithelium (Fig 2).

The latter can be described as a tubule consisting of at least mucosa (Fig 2; mu), submucosa (Fig 2; sm) and muscularis externa (Fig 2; me). Its mucosa was lined by simple tall columnar epithelium resting on loose connective tissue stroma. Deep straight tubular structures extended through its whole thickness resembling enteric crypts (aka crypts of Lieberkühn). Muscularis mucosa was present but did not seem to extend all the way around mucosa. Finally, muscularis externa seemed to consist of one longitudinal and one circular smooth muscle layers. According to these observations, this structure resembled large intestine. Ectodermal derivatives such as a large “double-chambered” dermoid cyst (Figure 3) and a separate stratified squamous keratinized epithelium indicating skin (Fig 5; marked with arrow). Mesoderm most characteristic representatives were muscular tissue bands (Figure 6; marked as M), adipose tissue (Fig 6; marked as F) and bone fragments.

## Case No.2

Second case was a three months old 129/Sv male mouse that presented testicular asymmetry. Left testicle gross and histological evaluation did not reveal any pathology. Right testicle grossly appeared enlarged. Routine (H&E) histological examination verified presence of mature (well differentiated) teratoma. In accordance with Case 1, nearly all kinds of epithelia were present. There were also cystic structures lined by multiple epithelial types (Fig 7; arrow: single columnar epithelium, arrowhead: multiple squamous epithelium). Cyst lumen appeared to contain products related to mucous epithelium secretions. Ciliated, tall columnar, pseudostratified epithelia (Fig 8; pce: pseudostratified ciliated epithelium) with many goblet cells (Fig 8; asterisk) resembling respiratory track were also frequently observed. Another striking feature was the focal presence of relatively large, polyhedral cells with euchromatinic nuclei (usually with prominent nucleoli – not shown in picture), which was

surrounded by an extensive cytoplasmic clearance. These cells were considered to be morphologically approximating steroid producing cells, since they exhibit many common characteristics (large polyhedral cells, spherical nuclei, extensive smooth-surfaced endoplasmic reticulum and multiple Golgi complexes; the latter remained unstained during routine specimen preparation, attributing them their cytoplasmic-clearance appearance) (Ross *et al.*, 1995). Thus, they were classified as epithelioid tissue (Fig 9; et: epithelioid tissue). Connective tissue was present in different forms. Dense irregular connective tissue, adipose tissue, bone and cartilage were its main representatives. Hyaline cartilage and osseous tissue could be seen in two different areas: A large structure of cancellous bone (Fig 12; ot: osseous tissue) surrounding red bone marrow (Fig 12; rBM: red bone marrow), and bone tissue together with cartilage (Fig 13). Reserve cartilage (Fig 13; res.c), proliferation (Fig 13; pr.z), hypertrophy (Fig 13; hy.z) and calcified cartilage (Fig 13; ca.c.z) zones could also be clearly seen. Muscular tissue was also evident in the form of striated and smooth muscle. As far as striated muscle is concerned, both skeletal and cardiac muscle cells could be distinguished. Cardiac muscle bundles (Fig 11; c.str.m) were orientated in such a manner, forming a thick luminal structure (Fig 11). The latter was lined by endothelium, while red blood cells resided in its lumen (Fig 11; bl). The following findings were considered consistent with the presence of a heart-like structure.

Finally, nervous tissue in different stages of maturation seemed to dominate over this case (Fig 9; nt: nervous tissue). Cuboidal structures resembling to choroid plexuses were noticed. In detail, they were lined by a single row of cuboidal cells, while blood vessels (Fig 10; asterisk) containing erythrocytes resided beneath their epithelium with their epithelium (Fig 10).

## DISCUSSION

The origin of 129/Sv mice dates back to 1950s. Among stem cells, germ cells (male or female) are the only pluripotent cell population known to be able to develop into a complete organism while undergoing a differentiation process. Through an extensive analysis of serial sectioned teratoma specimens derived from mice in different ages, it became clear that testicular teratomata originate within seminiferous tubules. It is reported that teratomatous tumors in newborn mice are often associated with malignant cells spilling out of seminiferous tubules (Stevens, 1984). Consequently, it was hypothesized that rapidly proliferating embryonic stem cells

inside the tubular lumen resulted in rupturing the tubule itself. Further evidence came from the observation that there was direct continuity between seminiferous and neoplastic epithelium. As long as the identity of malignant cells is concerned, engrafted embryonal genital ridges to adult mice testicles, provided evidence that they originate from germ cells prior to their differentiation to spermatogonia (Bustamante-Marin *et al.*, 2013).

In humans germ cell tumors (GCTs) are classified into five categories (Oosterhuis and Looijenga, 2005). According to this classification testicular teratomata can be either prepubertal or postpubertal. Apart from the age they occur however, post- and prepubertal teratomata differ also in their histogenesis. Human testicular prepubertal teratoma arises in a fashion same as in mice. Contrary, postpubertal teratoma histogenesis is completely different (Sundstorm *et al.*, 1999; Bustamante-Marin *et al.*, 2013).

Commonly observed structures in human teratomata are cartilage, smooth and skeletal muscle, neuroglia, enteric type glands, squamous epithelial islands and cysts, respiratory epithelium and urothelial islands. Bone and pigmented choroidal epithelium are less commonly seen, while choroid plexus is reported as very rare (Ulbright and Emerson 2014). Although teratomata are considered a relatively common entity for 129/Sv mice, there is an evident lack of reporting about the tissues or organs from which they consist. In a study conducted from Stevens and Little common tissues observed were nervous and cysts lined with cuboidal epithelium (Steven and Little 1954). According to our knowledge there is no reference indicating the presence of structure resembling

heart or choroid plexus in murine teratomata.

The study of murine teratoma has fundamentally contributed into understanding the molecular and the genetic procedures involved in the development and tissue lineage commitment (Ozolek and Castro, 2011). Although significant differences between mouse and human teratoma exist, mouse has significantly contributed into gaining insights of the pathogenesis of the human counterpart. Furthermore, according to Oosterhuis and Looijenga, the murine spontaneous model could serve as an appropriate one for the study of pediatric TGCTs or type I GCTs (Oosterhuis and Looijenga, 2005). Moreover, Tzuckerman *et al.* proposed the use of teratomata as an intermediate and valid model between *in vitro* and murine *in vivo* testing and clinical trials for therapeutic agents used against cancer (Tzuckerman *et al.*, 2003).

### Concluding remarks

Scrotal enlargement in mice may be due to hernias, inflammation or neoplasia. Although in 129/Sv strain such an enlargement is very suggestive of teratoma, humane sacrifice of the animals must always be accompanied by harvesting of the affected tissue and histological analysis and reporting. Studying and reporting of murine teratoma may provide answers to questions that have not currently been addressed. Such a diligent approach, addresses both the societal demand for ethical use of laboratory animals as well as scientific need on deeper insights about the pathogenesis, phenotypic expression and possible correlation to chromosomal and genetic anomalies. ■

## REFERENCES

- Amato R (2002) Testicular Cancer. In: (ed.: Bertino JR) Encyclopedia of Cancer 2nd edn. New York: Academic Press, pp. 363-375.
- Bustamante-Marin X, Garness JA, Capel B (2013) Testicular teratomas: an intersection of pluripotency, differentiation and cancer biology. *Int J Dev Biol* 57:201-210.
- Oosterhuis JW, Looijenga LH (2005) Testicular germ-cell tumours in a broader perspective. *Nat Rev Cancer* 5:210-222.
- Ozolek JA, Castro CA (2011) Teratomas Derived from Embryonic Stem Cells as Models for Embryonic Development, Disease, and Tumorigenesis. In: (ed.: Kallos M) Embryonic Stem Cells - Basic Biology to Bioengineering, Intech, pp. 231-262.
- Ross MH, Romrell LJ, Kaye GI (1995) Endocrine Organs In: (eds.: Ross MH, Romrell LJ, Kaye GI) *Histology: A text and atlas*, 3rd edn, Williams & Wilkins, Baltimore, Maryland, USA, pp. 596-635
- Stevens LC (1984) Spontaneous and experimentally induced testicular teratomas in mice. *Cell Differentiation* 15:69-74.
- Stevens LC, Little CC (1954) Spontaneous Testicular Teratomas in an Inbred Strain of Mice. *Proc Natl Acad Sci USA* 40:1080-1087.
- Sundstrom J, Pelliniemi LJ, Kuopio T, Verajankorva E, Frojzman K, Harley V, Salminen E, Pöllänen P (1999) Characterization of the model for experimental testicular teratoma in 129/SvJ-mice. *Br J Cancer* 80:149-160.
- Tzuckerman, M., T. Rosenberg, Y. Ravel, I. Reiter, R. Coleman, K. Skorecki (2003) An experimental platform for studying growth and invasiveness of tumor cells within teratomas derived from human embryonic stem cells. *Proc Natl Acad Sci U S A* 100:13507-13512.
- Ulbright TM, Emerson RE (2014) Neoplasm of the testis In: (eds.: Bostwick DG and Liang C) *Urologic Surgical Pathology* 2nd edn, Elsevier Saunders Philadelphia, pp. 757-862.
- Woodward PJ, Mostofi FK, Talerma A, Heidenreich A, Haillemariam S, Kaplan GW, Looijenga LHJ, Parkinson MC, Ulbright TM, Oosterhuis JW, Grigor K, Sesterhenn IA, McLeod DG, True L, Rushton HG, Möller H, Jacobsen GK, Michael H, Manivel JC, Oliver TD, Reuter VE (2004) Germ cell tumors. In: (eds.: Eble JN, Sauter G, Epstein JI, Sesterhenn IA) *Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs*. IARC Press, Lyon, pp. 221-249.