Neoplasia of the Sertoli cells in wild carp, Cyprinus carpio: Optical, immunohistochemical and ultrastructural study

ARTICLE *in* BULLETIN- EUROPEAN ASSOCIATION OF FISH PATHOLOGISTS · JANUARY 2013

Impact Factor: 0.37

8 AUTHORS, INCLUDING:

Ricardo Vieira Rodrigues

Universidade Federal do Rio Grande (FURG)

25 PUBLICATIONS **136** CITATIONS

SEE PROFILE

Luís André Sampaio

Universidade Federal do Rio Grande (FURG)

98 PUBLICATIONS **469** CITATIONS

SEE PROFILE

Marcelo Borges Tesser

Universidade Federal do Rio Grande (FURG)

47 PUBLICATIONS **191** CITATIONS

SEE PROFILE

Neoplasia of the Sertoli cells in wild carp, *Cyprinus carpio*: optical, immunohistochemical and ultrastructural study

L. A. Romano[1]*, M. Klosterhoff[2], F. Fuhr, R. V. Rodrigues[2], E. Pereira Gusmao[2], M. A. R. Garrido-Pereira[2], L. A. Sampaio[3] and M. B. Tesser[3]

[1]Instituto de Oceanografia, Universidade Federal do Rio Grande Laboratório de Imunologia e Patologia de Oraganismos Marinos; [2]Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande, Laboratório dePiscicultura Estuarina e Marinha, CEP 96201-900, Rio Grande, RS, Brazil; [3]Instituto de Oceanografia, Universidade Federal do Rio Grande, Laboratório de Piscicultura Estuarina e Marinha, CEP 96210-030, Rio Grande, RS, Brazil

Abstract

Sertoli cell tumours are primary neoplasms of the testis which arise from the supporting cells within the seminiferous tubules. This report describes a case of tumour of the Sertoli cells in carp. The diagnosis of Sertoli cell tumour is supported by the histopathological features, the characteristic presence of Charcot-Böttcher crystals visualised in immature Sertoli cells by electron microscopy and the immunohistochemical positive reaction for neuronspecific enolase (NSE) and inhibin α.

Introduction

Teleost fish constitute the largest and most diverse class of vertebrates, with over 20,000 known species. Their diversity and placement in the phylogenetic tree make them ideal subjects for comparative neoplasia studies. Fish share a common histiogenesis of various neoplasms of mammals and humans, allowing a better understanding of the basic mechanisms of carcinogenesis, compared with studies limited to mammalian models only. (Anders, 1984; Schmale, 1986; Baumann et al., 1991; Webe et al., 2002; Devi, 2010).

Previously such studies have dealt largely with tumours in the higher vertebrates, mammals and birds; much less attention has been paid to the lower vertebrates, fish, amphibians and reptiles and the classification of fish neoplasia is consequently largely based on mammalian criteria (Romano, 1987; Masahito, 1998).

All the major varieties of neoplasias that occur in mammals and birds, have been recorded in fish. These include tumours of epithelial tissue, mesenchymal tissues, lymphoid tissue, nervous tissues, haematomas and melanomas (Romano, 2004 ; Romano, 2010)

Sertoli cells, in fish, as in mammals, provide a

* Corresponding author's e-mail: dcluis@yahoo.com

number of supportive functions in regard to the spermatogenic maturation process, certain changes and proliferation of these cells as an been described (Blazer, 2002). Sertoli cell neoplasia is a primary neoplasm of the testis arising from the supporting cells within the seminiferous tubules. It is common in dogs, especially in cryptorchid testicles, and has also been reported in the stallion, ram, cat, and bull (MacLachlan and Kennedy, 2002). Normal and neoplastic Sertoli cells are reported to be the only cells of the testes that stain immunohistochemically with antibody to neuron-specific enolase (NSE) (Owston and Ramos-Vara, 2007; Saegusa et al., 2011). They also stain with vimentin, and there are varied reports of staining with other antibodies including S100, melan-A, and inhibin α. Cytokeratin stains were negative (Uetsuka et al., 2012). The inhibin α is reported only in Sertoli cell neoplasia in canine and human and is considered the most specific marker for this neoplasm (Kuroda et al., 2004)

Materials and methods

A mass of 23 g was obtained from the abdominal cavity of a carp from the river Salado, Buenos Aires, Argentina. The fish had a weight of 1367 g and a total length of 620 mm. Necropsy showed a tumour of 22 cm diameter found in the abdominal cavity. The tumour was whitish and solid lobed with haemorrhagic areas (Figure 1). Fragments of the tumour were fixed in 10% buffered formalin, embedded in paraffin and stained with haematoxylin and eosin, PAS and oil red O (for lipid staining) on frozen sections. Histological sections were stained with immunohistochemical procedures, according to a modified avidin-biotinperoxidase complex technique (Hsu, 1981). Tissue slides were deparaffinized rinsing them with xylol and then rehydrated with. The endogenous peroxidase activity was blocked by incubating the slides for 20 min in 0.3% H_2O_2 in a 5% methanol solution. After washing the slides in water and PBS/0.05%-Tween 20 solution, they were incubated in normal 1/100 serum (Vectastain Universal Elite, BC Kit, Vector), in a 10% PBS bovine serum albumin (BSA) solution for 30 min at 22 °C in a humid chamber. After incubation, the primary anti- cytokeratin, inhibin α, and neuron-specific enolase (NSE) antibody (Dako, Argentina) and the slides were incubated overnight at 41°C in a humid chamber. The slides were then rinsed in PBS and incubated for 7 min in a 50 mL 30.3-diaminobenzidine (DAB, Sigma- Aldrich) solution containing 1% PBS-BSA in 50 mL H_2O_2. Counterstaining was then performed with haematoxylin.

For electron microscopy small fragments of the tumour were cut into 1 mm blocks and immediately fixed in phosphate buffered glutaraldehyde (pH 6.9 at 4 °C), washed in Millonig's solution and post-fixed in 1% osmium tetroxide; the tissue blocks were then dehydrated in a graded series of ethanol-acetone, immersed in propylene oxide and embedded in Durcupan ACNI (Fluka Chemie A.G., Switzerland). Ultrathin sections were cut with an LKH ultramicrotome and doublestained with uranyl acetate and lead citrate before examination in a Jeol JEM-8T electron microscope (Jeol, Tokyo, Japan).

Results

Neoplasic cells were oval or round contained round nuclei and abundant vacuolated cytoplasm, and was arranged in nested and cord-like patterns surrounded by basal membrane with lymphocytic infiltrate (Figure 2). Mitotic

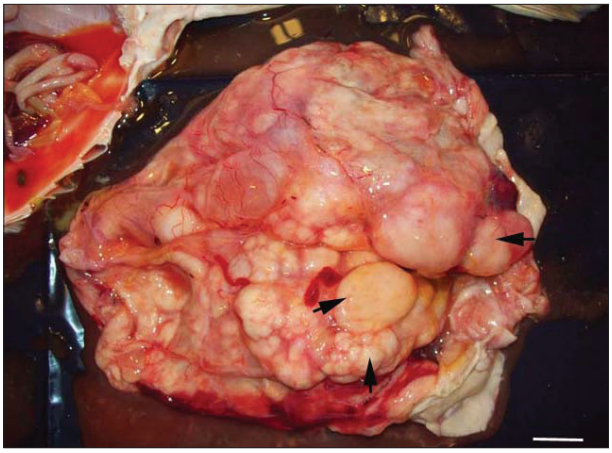

Figure 1. Gross appearance of the abdominal tumor with multiple masses of various sizes. (arrow), Bar= 2 cm.

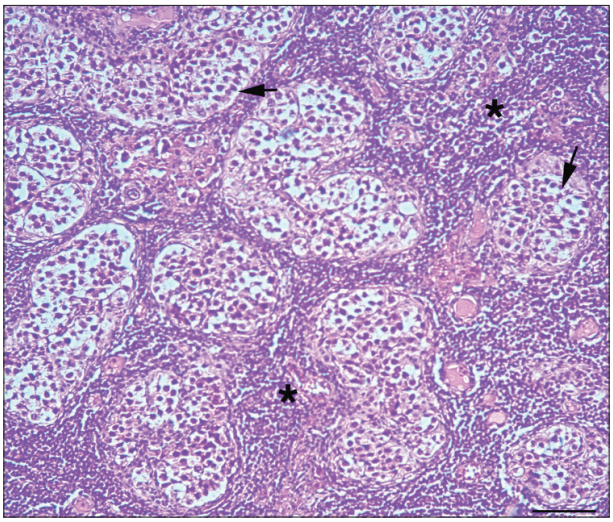

Figure 2. Neoplastic cells are arranged in tubules irregularly distributed tending to position perpendicular to the basement membrane (arrow). Between the tubules are observed lymphocyte infiltration (*). H&E, Bar = 100 μm.

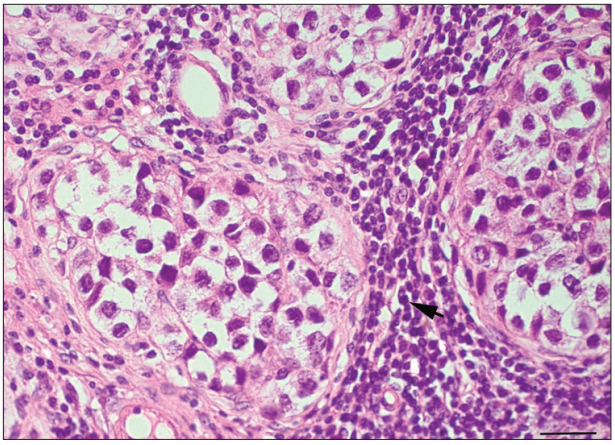

Figure 3. The tumour cells were oval or round contained round nuclei and abundant vacuolated cytoplasm, and was arranged in nested and cord-like patterns surrounded by basal membrane. Lymphocyte infiltrates are observed (arrow) H&E-staining, Bar = 50 μm.

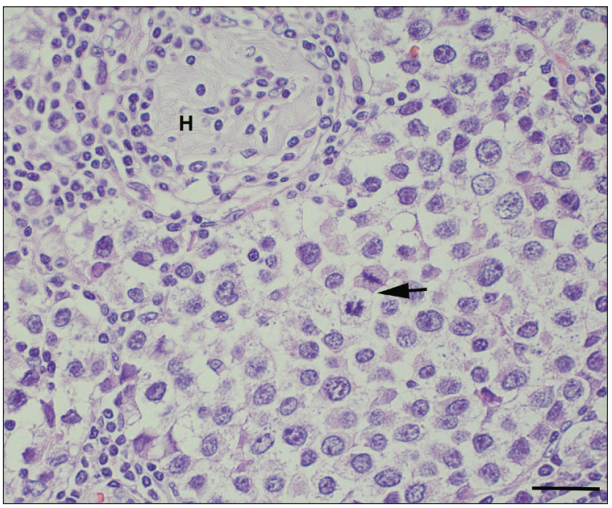

Figure 4. Tumour mass with hyaline areas (H) and cells with mitotic figures (arrow). H&E-staining, Bar = 50 μm.

figures were scarcely visible at a frequency of 0–3 cells/high-power field (Figure 3, 4). Some tumour cells contained pigments which were stained red by oil red O staining, indicating that the pigments contained fatty material (Figure 5). Immunohistochemical examination revealed that the tumour cells were positive for NSE and inhibin α antibodies while negative for cytokeratin antibody. (Figure 6, 7).

Ultrastructural examination revealed a pseudostratified disposition of the nuclei. In the supranuclear cytoplasm, a well developed Golgi complex, small vesicles, and parallel cisternae of rough endoplasmic reticulum were the most prominant features (Figure 8). In the most immature cells was observed in the cytoplasm moderate development of both rough and smooth endoplasmic reticulum, as well

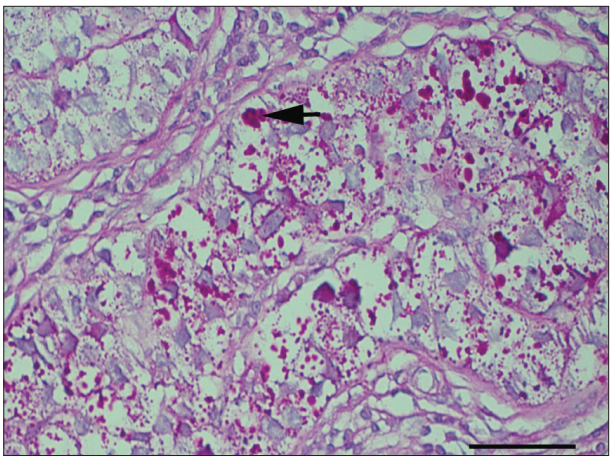

Figure 5. Tumour cells with pigments were stained red by oil red O staining, indicating that the pigments contained fatty material. Oil red O staining, Bar = 50 μm.

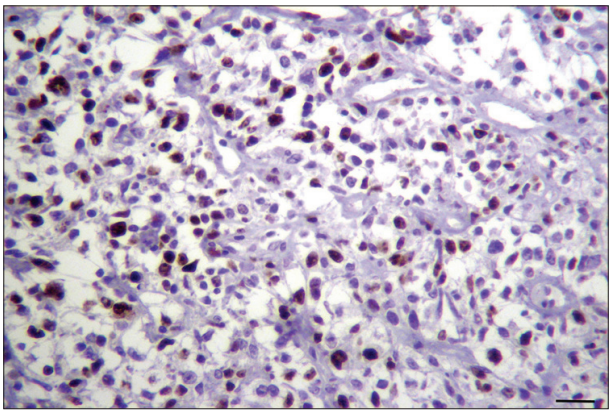

Figure 6. Immunohistochemical of the Sertoli cells. The tumour cells show cytoplasmic and nuclear-positive staining for NSE. Anti-NSE staining, Bar = 50 μm.

as of the Golgi complex, and the presence of free ribosomes and abundant microfilament. In some Sertoli cells, typical Charcot-Böttcher crystals were also present (Figure 9) and lipid inclusions were present in most neoplastic cells examined. The nucleus generally showed deep infolding. These cells were observed in isolation and are similar to human Type C or nearly mature Sertoli cell found in the Del Castillo's syndrome (Paniagua, et al., 1987). The nucleolus was more prominent than in mature Sertoli cells (Figure 10).

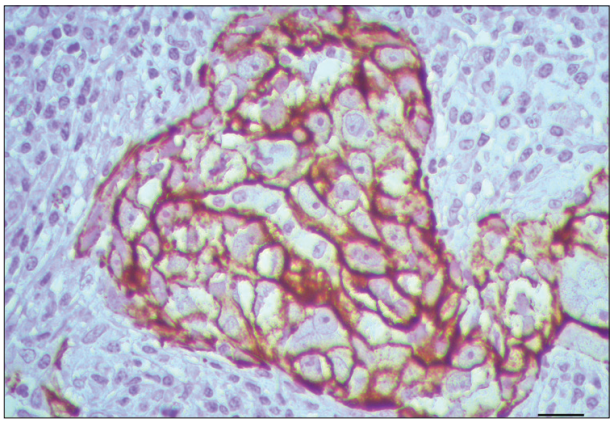

Figure 7. Immunohistochemical of the Sertoli cells. The tumour cells show cytoplasmic membrane positive staining for inhibin α. Anti- inhibin α staining, Bar = 50 μm.

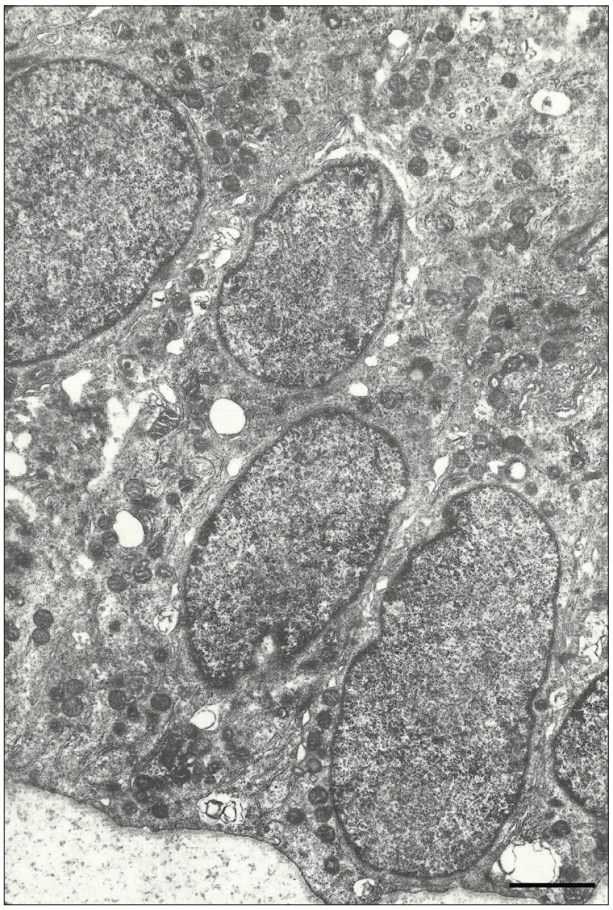

Figure 8. Neoplastic Sertoli cells. The nuclei are round to eleongated and regular in outline and show a pseudostratified disposition. Bar = 2 μm.

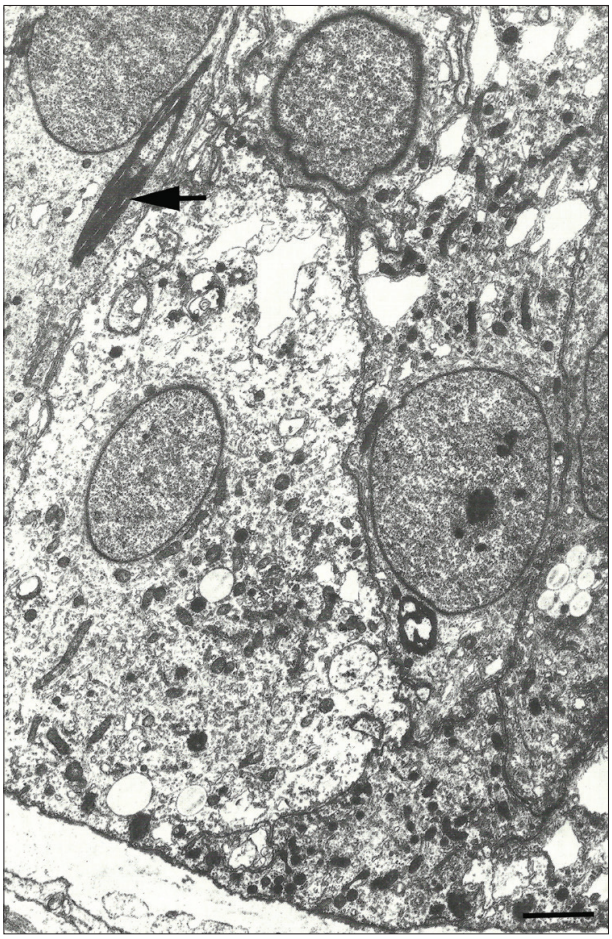

Figure 9. Immature Sertoli cells. The cytoplasm shows moderate development of the endoplasmic reticulum, numerous microfilaments Charcot-Böttcher crystals (arrow). Bar = 2 μm.

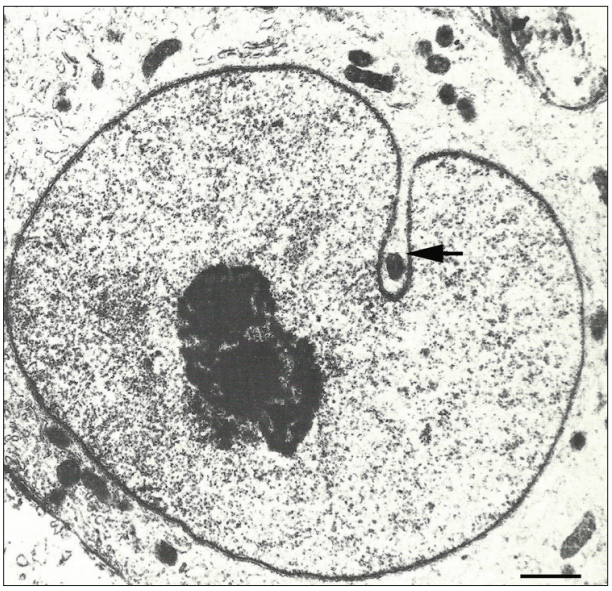

Figure 10. Immature Sertoli cell. The nucleus shows a deep infolding (arrow). The nucleolus is more prominent than in mature Sertoli cells. Bar = 0.1 μm.

Discussion

Neoplasia processes are ubiquitous among vertebrates, and comparison has shown that neoplasias in cold-blooded animals are essentially identical in regard to their structure and behavior with the corresponding neoplasias of warm-blooded animals. Neoplastic disease in carp is well represented in the literature, particularly affecting goldfish. An important factor in this group of fish is their longevity, especially in koi. Goldfish are however also well adapted to environments that are suboptimal to other species of fish. A consequence of this adaptation is the ability to detoxify and repair damage caused by toxins that may be carcinogenic (Stoskopf, 1993). In studies of hybrid carp. from a eutrophic lake of Spain, gonadal tumors were found only in males (Granado-Lorencio, et al., 1987). The most prevalent type was dysgerminoma; other tumors, of lower prevalences, included seminomas, leiomyomas, Sertoli cell tumors and spermatocytic seminomas

Two types of testicular neoplasia were evident in cyprinid fishes collected from Lake Ontario. Both types of neoplasias appeared to be of Sertoli cell origin. One contained predominantly Sertoli cells with little or no lipid content and germinal cells in only small numbers. The second contained large numbers of spermatogonia together with Sertoli cells that contained large amounts of lipid and smooth endoplasmic reticulum. Small, apparently inactive Leydig cells were present in both types of neoplasias and also in testes of fish not bearing gonadal

neoplasias (Leatherland and Sonstegard, 1978).

It is well known that the Sertoli cell is the most resistant cell of the seminiferous epithelium. (Clermont and Perey, 1957; Heller et al., 1968), In addition, it has been shown that this cell does not undergo mitotic division in the human adult testis (Steinberger and Steinberger, 1976). Because of this stability, the use of the Sertoli cell as a reference cell or constant compensating factor for the quantitation of the germinal epithelium under different physiologic or pathologic conditions has been proposed (Nistal et al., 1982).

The distinctive histologic and immunohistochemical features of the testicular neoplasias and the lack of any continuity between them tend to eliminate a mixed germ cell–sex cord stromal neoplasia from the differential diagnosis. The immunohistochemical expression of the Sertoli cell neoplasia is similar to that reported for NSE in normal and neoplastic Sertoli cells in human (Nistal et al., 1990). The inhibin α is reported only in Sertoli cell neoplasia in canine and human and is considered the most specific marker for this neoplasm (Kuroda et al., 2004). The diagnosis of Sertoli cell neoplasia in the present case is supported by the morphological and staining characteristics, ultrastructure and reactivity to NSE and inhibin α antibodies.

References

Anders F, Schartl M, Barnekow A and Anders A (1984). *Xiphophorus* as an in vivomodel for studies on normal and defective control of oncogenes. *Advances Cancer Researcher* **42**, 191-275.

Baumann PC, Mac MJ, Smith SB and Harshbarger JC (1991). Tumor frequencies in walleye (*Stizostedion vitreum*) and brown bullhead (*Ictalurus nebulosus*) and sediment contaminants in tributaries of the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **48**,1804-1810

Blazer VS (2002). Histopathological assessment of gonadal tissue in wild fishes *Fish Physiology and Biochemistry* **26**, 85–101,

Clermont Y and Perey B (1957). Quantitative study of the cell population of the seminiferous tubules in immature rats. *American Journal of Anatomy* **100**, 241-268.

Devi PU (2010). Basics of carcinogenesis. *Health Administrator* **17**, 16-24.

Granado-Lorencio C, Francisco Garcia-Novo F and Lopez-Campos J (1987). Testicular tumors in carp-funa hybrid: Annual cycle and effect on a wild population. *Journal of Wildlife Diseases* **23,** 422-427

Heller GV, O'Keefe KB and Heller I C (1968). Effects of follicle stimulating hormone (FSH) on Sertoli cells in the hypophysectomized rat. *Clinical Research* **98**, 113-123.

Hsu SM, Raine L and Fanger H (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC an unlabeled antibody (PAP) procedures. *Journal of Histochemistry and Cytochemistry* **29,** 577-585.

Kuroda N, Senzaki T, Yamasaki Y, Miyazaki E, Hayashi Y, Toi M, Hiroi M, Enzan H (2004). Sertoli cell tumor of the testis (not otherwise specified) with the expression of neuroendocrine markers and without the expression of inhibin-α. *Pathology International* **54**, 719–724.

Leatherland JF and Sonstegard RA (1978). Structure of normal testis and testicular tumors in cyprinids from Lake Ontario. *Cancer Research* **38**, 3164-3173.

MacLachlan N J and Kennedy P C (2002). Tumors of the genital systems. *In*: **Tumors in domestic animals 4th ed** (D. J. Meuten, Ed.). Iowa State Press, Ames, Iowa pp. 547–573. ISBN: 978-0-8138-2652-3.

Masahito P, Ishikawa T and Sugano H (1998).

Fish tumors and their importance in cancer research. *Japanese Journal of Cancer Research* **79**, 545-555.

Nistal M, Pauiagua R, Abaurrea A and Santamarfa L (1982). Hyperplasia and the immature appearance of Sertoli cells in primary testicular disorders. *Human Pathology* **13**, 3- 12.

Nistal M, Jimenez F and Paniagua R (1990). Sertoli cell types in the Sertoli-cell-only syndrome: relationships between Sertoli cell morphology and aetiology. *Histopathology* **16**,173–80.

Owston MA and Ramos-Vara JA (2007). Histologic and immunohistochemical characterization of a testicular mixed germ cell sex cord-stromal tumor and a leydig cell tumor in a dog. *Veterinary Pathology* **44**, 936–943.

Paniagua RM, Rodriguez MC, Nistal M, Fraile B and Amat P (1987). Changes in the lipid inclusion/Sertoli cell cytoplasm area ratio during the cycle of the human seminiferous epithelium. *Journal of Reproduction & Fertility***.** 80, 335 - 341.

Romano LA and Cueva F O (1987). Producción experimental de melanomas cutáneos en el pez espada *Xiphophorus helleri* (Heckel) y el pez platis. *Xiphophorus maculatus* (Gunther) (Pisces Poecilidae). *Revista de la Asociación de Ciencias Naturales del Litoral* **18,** 185, 1987.

Romano LA and Marozzi VA (2004). Epithelioreticular cell thymoma in carp *Cyprinus carpio* L: A case with ultrastructural study. *Journal of Fish Diseases* **27**, 369-373.

Romano LA, Aiko Tabata Y and Alexandrino AC(2010). Histopathological and immunohistochemical diagnostic of carcinosarcoma of mandible in a rainbow trout (*Onchorhyincus mykiss*). *Acta Scientiae Veterinariae* **38**, 319-322.

Schmale MC, Hensley GT and Udey LR (1986). Neurofibromatosis in the bicolor damselfish (*Poacemtrus partitus*) as a model for von Recklinghausen neurofibromatosis. *Annals of the New York Academy of Sciences* **486,** 386-402.

Saegusa Y, Hayashi H, Taniai E, Imaoka M, Ohishi T, Wang L, Mitsumori K and Shibutani M (2011). Spermatocytic seminoma with neuroectodermal differentiation and Sertoli cell tumor in a dog. V*eterinary Pathology* 48, 1024 - 1028.

Steinberger A and Steinberger L (1976), Secretion of an FSH-inhibitory factor by cultured Sertoli cells. *Endocrinology* **99**, 918-92.

Stoskopf MK (1993). Golfish, koi, and carp neoplasia. *In*: **Fish Medicine** (M.K. Stoskopf, ed.) Saunders Press, London, pp. 490- 491. ISBN 0-7216-2629-7.

Uetsuka K, Suzuki T, Doi K and Nunoya T (2012). Malignant Sertoli cell tumor in a goose (*Anser cygnoides domesticus*). *Avian Diseases* **56**, 781-785.

Weber LP, Kiparissis Y, Gap S, Hwang GS, Arthur J, Niimi AJ, Janz DM and Metcalfe CD (2002). Increased cellular apoptosis after chronic aqueous exposure to nonylphenol and quercetin in adult medaka (*Oryzias latipes*). *Comparative Biochemistry and Physiology Part C* **131**, 51-59.