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Short Communication

Malignant Mixed Sex Cord-stromal Tumour in a Stallion

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Contents

A 30-year-old Standardbred stallion was examined for unilateral scrotal swelling. Physical and ultrasound examinations revealed a painless enlarged left testis with a non-homogeneous echogenicity, when compared with the controlateral testis. The stallion underwent left unilateral orchiectomy. Grossly, the excised testis was irregularly enlarged (12 × 9 × 9 cm; weight: 530 g) and firm. The sections showed that testicular parenchyma was replaced by a lobulated, greyish-white mass, which involved the epididymal head. At microscopy, a dual Leydig and Sertoli cell tumour component could be seen. Neoplastic Sertoli cells were prevalent and presented pleomorphic cells, mitotic figures and occasional vascular invasion. Tumour patterns showed tubular and solid areas, cord-like or diffuse in appearance, among which newly formed Leydig cell nests and low-density fibrillar bundles were interposed. Immunohistochemically, a weak to moderate immunostaining for vimentin, AE_1/AE_3 cytokeratin, α-1-antitrypsin and CD99 antigens was found in the growing Sertoli cells, whose nuclear MIB-1 labelling index scored 13 ± 2%. The Leydig tumour cells, on the other hand, displayed a moderate to strong positivity for α-inhibin, vimentin, AE_1/AE_3 cytokeratin, neurone-specific enolase and CD99. On the basis of these findings, a diagnosis of malignant mixed sex cord-stromal tumour was made.

Introduction

Testicular neoplasms are uncommon in horses as most of them as a rule, are castrated at an early age unless destined to become breeding stock (Schumacher and Varner 1993). Seminomas and teratomas are the most frequent testicular neoplasms (McEntee 1990; Schumacher and Varner 1993; Brinsko 1998), while Leydig cell tumours are infrequent (Gelberg and McEntee 1987; Ladds 1993; Brinsko 1998; May et al. 1999) and Sertoli cell tumours are very rare (Stickle and Fessler 1978; Rahaley et al. 1983; Brinsko 1998; Duncan 1998). So far, three sets of mixed testicular tumours have been reported in the stallion: a teratocarcinoma, a mixed germ cell/sex cord-stromal tumour and a carcinoma *in situ*/seminoma (Shaw and Roth 1986; Cullen et al. 1987; Veeramachaneni and Sawyer 1998). Cryptorchidism does not increase the risk of neoplastic growth in the horse, as tumours have been reported with the same prevalence in both descended and retained testes (McEntee 1990; Brinsko 1998). Clinically, the stallion with a neoplasm in a descended testis is generally characterized by painless and warm enlargement of the affected gonad (Schumacher and Varner 1993). Ultrasound examination may aid in finding small nodules when a loss of the normal testicular echogenicity occurs (Brinsko 1998). A new case of a malignant mixed sex cord-stromal tumour is described here, focusing on some diagnostic approaches to such tumours.

Case History

A 30-year-old Standardbred stallion was examined for a unilateral scrotal swelling. In previous reproductive seasons the animal had been successfully used for breeding a large number of mares but, in the past season, presented limping and was unable to breed. General health conditions were good. The left testis was enlarged and firm, no heat or pain was detected on palpation. The right testis was of normal size and consistence. No involvement of the inguinal rings and iliac lymph nodes was evident on transrectal physical examination. Longitudinal and transversal ultrasonographs, using a 6.5 MHz convex transducer, showed loss of parenchyma homogeneity. The testicular parenchyma appeared to be replaced by a solid tissue with thin hyperechoic bundles (Fig. 1). A testicular neoplasm was suspected, but the owner expressed interest in maintaining the stallion as a breeder. Unilateral orchiectomy in the standing animal was then recommended. Food was withheld from the horse for 24 h prior to surgery to allow the colon to empty. Water was allowed *ad libitum*. Tetanus prophylaxis, flunixin meglumine (0.5 mg/kg, i.v.) and procaine penicillin G (22 000 IU/kg, i.m.) were administered. The horse was sedated with xylazine (0.5 mg/kg, i.v.) allied to butorphanol (0.05 mg/kg, i.v.). The region was prepared for aseptic surgery and the incision was carried out through the skin, tunica dartos, and external vaginalis so that the testis was exposed, revealing an unaffected spermatic cord. The testis was then removed by emasculator. Antimicrobical and analgesic therapy was given for 36 h after surgery.

Pathology

Grossly, the removed testis (12 × 9 × 9 cm, weight: 530 g) was of irregular shape and enlarged, firm and with prominent vascularization (Fig. 2). On the cut surface, a solid neoplasm, greyish-white in colour with a finely lobular architecture was observed, extensively replacing the testicular parenchyma (Fig. 3). The latter, confined to the cranial extremity of the organ, was softer than normal and brown in colour, and included four small nodules (Ø < 6 mm), similar to the tissue described above. The epididymis was atrophic and the

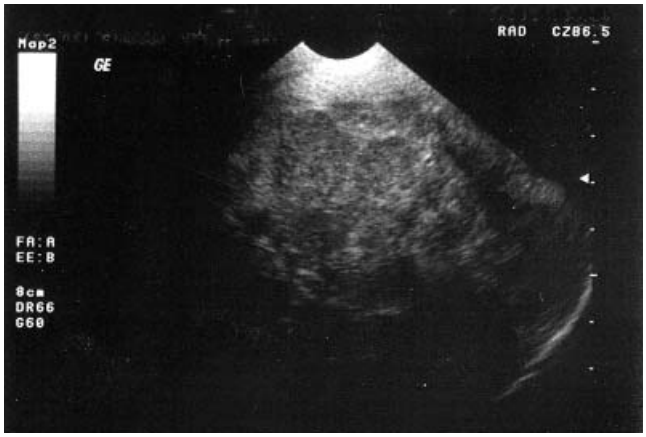

Fig. 1. Sonogram of the left testis showing thin hyperechoic bundles

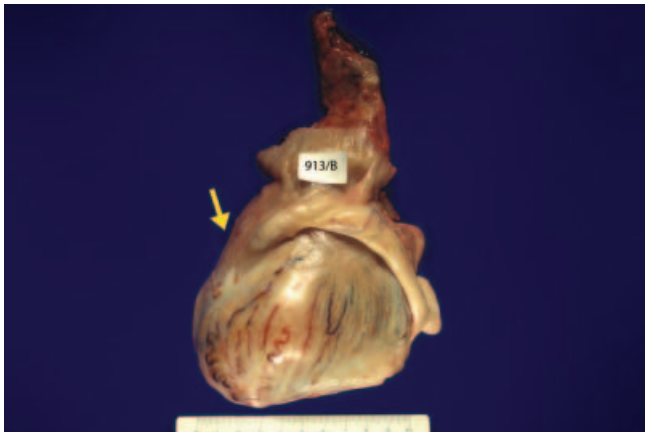

Fig. 2. The testicular neoplastic lesion involving the epididymal head (arrow)

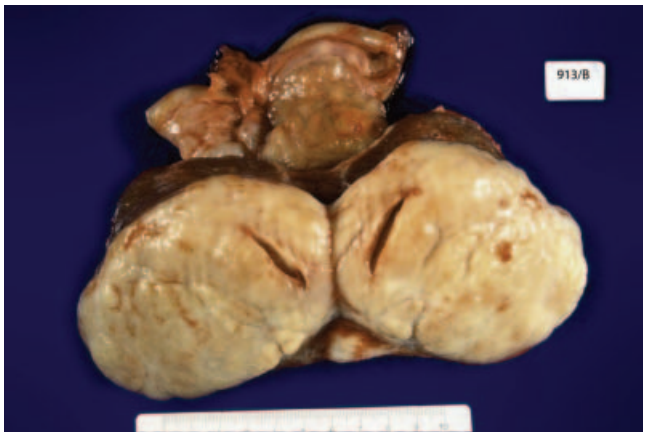

Fig. 3. On the cut surface the testicular parenchyma is recognizable along the cranial margin of the tumour

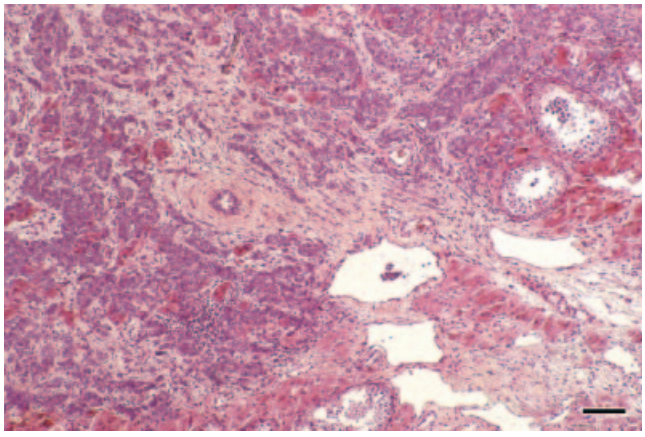

Fig. 4. At low magnification, the tumour shows prevalent tubular and cord-like patterns. Haematoxylin and eosin (bar = 93 μm)

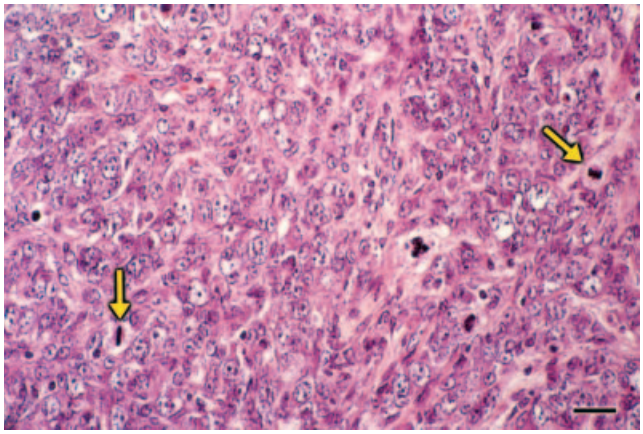

Fig. 5. Among the malignant Sertoli cells, nuclear pleomorphism and mitotic figures (arrows) can be seen. Haematoxylin and eosin (bar = 25 μm)

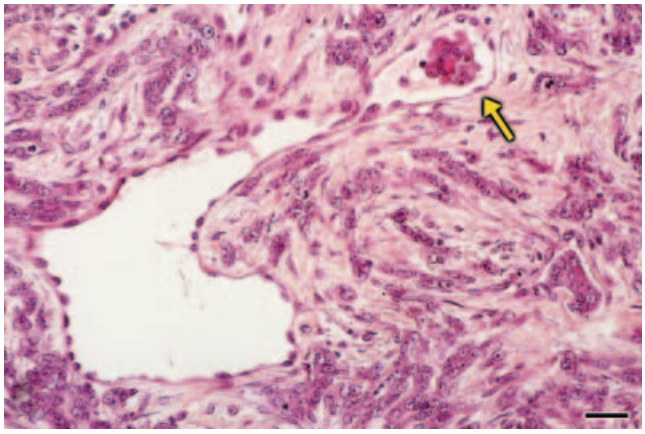

Fig. 6. Neoplastic cells inside a lymphatic vessel (arrow). Haematoxylin and eosin (bar = 30 μm)

head was not recognizable. At microscopy, a dual Sertoli cell and Leydig cell tumour component was evident. Neoplastic Sertoli cells were prevalent, including some pleomorphic cells and frequent atypical mitotic figures (Fig. 5). The tumour patterns displayed tubular, cord-like and solid areas, and occasional vascular invasion (Figs 4 and 6). The interstitial connective tissue was slightly fibrillar and contained neoplastic Leydig cells, with a lower mitotic index and grade of atypia. Granulosa-like cell elements arranged in follicle structures were also detected. Immunohistochemistry, performed using the avidin-biotin peroxidase complex

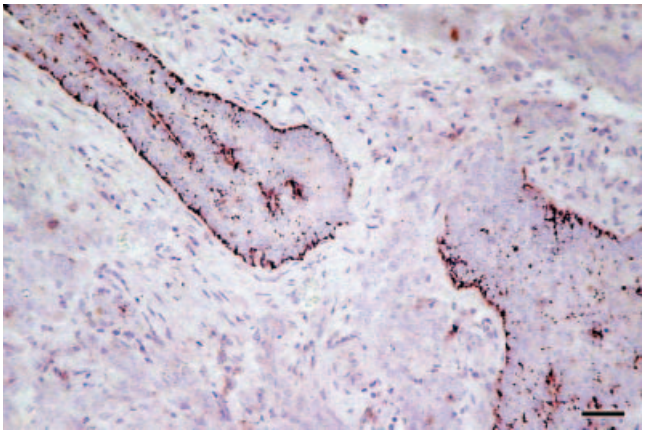

Fig. 7. Focal and moderate immunostaining for α-1-antitrypsin in neoplastic Sertoli cells. ABC method – Mayer's haematoxylin counterstain (bar = 37 μm)

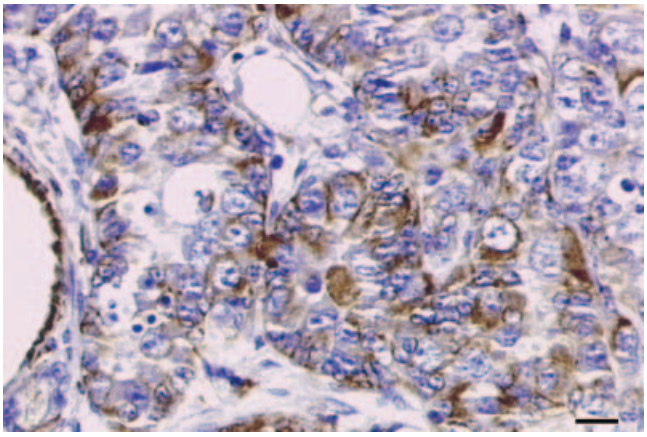

Fig. 8. Moderate immunostaining for vimentin in neoplastic Sertoli cells. ABC method – Mayer's haematoxylin counterstain (bar = 12 μm)

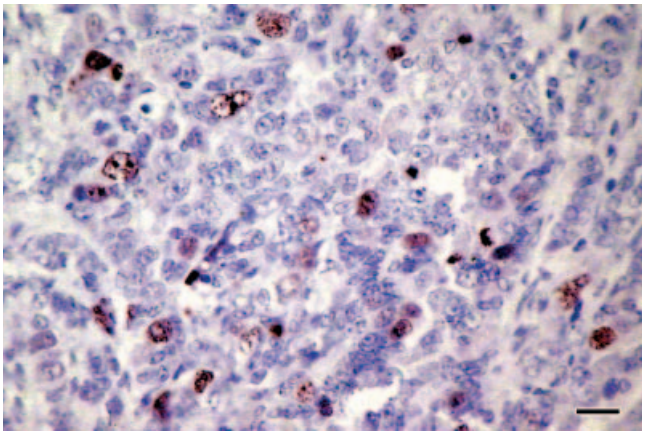

Fig. 9. Pleomorphic Sertoli cells display strong MIB-1 nuclear immunostaining. ABC method – Mayer's haematoxylin counterstain (bar = 17 μm)

method (ABC Vectastain Kit elite, Vector Lab, Burlingame, CA, USA), showed weak to moderate immunostaining for vimentin (Novocastra Laboratories Ltd,

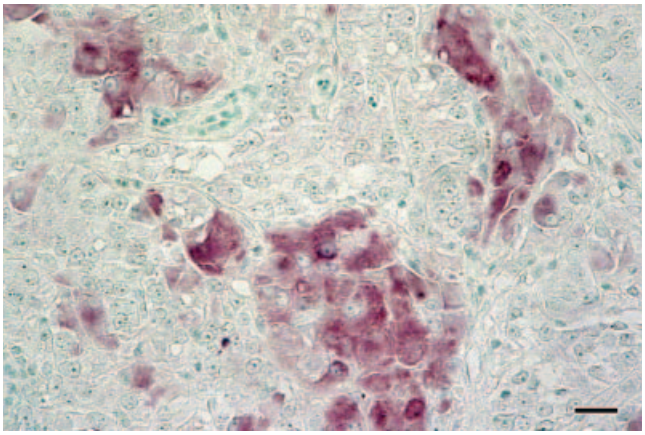

Fig. 10. Immunostaining for α-inhibin occurring in the neoplastic Leydig cell component. ABC method – Methyl green counterstain (bar = 25 μm)

Benton Lane, UK; dilution 1 : 100), AE_1/AE_3 cytokeratin (Novocastra Laboratories Ltd; dilution 1 : 50), α-1-antitrypsin (Novocastra Laboratories Ltd; dilution 1 : 400) and CD99 (Novocastra Laboratories Ltd; dilution 1 : 50) of the neoplastic Sertoli cells (Figs 7 and 8). The Leydig cell component presented a moderate to strong immunoreaction for α-inhibin (Serotec, Kidlington, Oxon, UK; dilution 1 : 100), vimentin, AE_1/AE_3 cytokeratin, neurone-specific enolase (Novocastra Laboratories Ltd; dilution 1 : 200) and CD99 (Fig. 10). The MIB-1 labelling index (Novocastra Laboratories Ltd; dilution 1 : 100) was 13 ± 2%, evaluated by image analysis (Immagini & Computer Snc, Milano, Italy) for neoplastic Sertoli cells (Fig. 9). On the basis of these findings, a malignant mixed sex cord-stromal tumour was diagnosed.

Follow-up

Follow-up information obtained from the owner and from further clinical examinations indicated normal post-operative healing. The stallion died from acute colic 1 year after surgery. Necroscopy was performed but no metastases were found.

Discussion

The clinical features presented were consistent with a primary testicular neoplasia in a scrotal testis. The painless scrotal swelling was the only physical sign of the disorder. Despite the origin of the neoplasm, no endocrinological disturbances were recorded, either before or after surgery, but aggressive behaviour as a result of the tumour has been reported only for a few equine Leydig cell tumours (Gelberg and McEntee 1987). No testicular feminization syndrome related to Sertoli cell tumour has been described in the horse, possibly because oestrogen production takes place in Leydig cells rather than in Sertoli cells (Zanghì et al. 1993; Hess and Roser 2004). Ultrasound examination would be expected to be a powerful diagnostic tool but a large number of cases are necessary to demonstrate this. The diagnosis of malignant mixed sex cord-stromal

tumour was made on the basis of histology. In humans, the term sex cord-stromal tumour refers to neoplasms containing epithelial elements of a sex-cord origin (Sertoli and granulosa cells) mixed with mesenchymal-derived cells (Leydig, theca and lutein cells), in respective proportions and differentiations (Ulbright 1997). The mixed neoplasm we describe here can be ascribed to the aforementioned kind of tumours and has never been reported in horses. The tumour size, the cell pleomorphism, the MIB-1 labelling index and the vascular invasion of the Sertoli cell component predicted malignant behaviour. Although rare in horses, other Sertoli cell tumours with a malignant metastasizing behaviour or high proliferation indices have been reported (Rahaley et al. 1983; Duncan 1998). This could be a distinctive feature, which differentiates them from the canine and human counterparts (McEntee 1990; Ulbright 1997). Immunohistochemistry of testicular tumours has to be considered experimental in the horse, as only a small number of studies have been made. In our report some immunohistochemical markers of stromal differentiation, employed in humans, are proposed to distinguish such tumours from those of germ cell origin (Gordon et al. 1998; Kommoss et al. 2000; Henley et al. 2002). Moreover, the MIB-1 labelling index is regarded as a reliable predictor of malignancy for gonadal and extragonadal tumours in domestic animals (Benazzi et al. 1995; Nicòtina et al. 2002).

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