

Malignant Sertoli cell tumor in the retained abdominal testis of a unilaterally cryptorchid horse

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- ▶ Testicular neoplasms in stallions are infrequently reported, and Sertoli cell tumors are considered rare.
- ▶ Sertoli cell tumors may develop in descended or retained testes; atrophy of the contralateral testis and metastasis to other tissues may also develop.

A 13-year-old Morgan gelding was referred to the Veterinary Medical Teaching Hospital for evaluation of a mass in the caudal region of the abdomen. The horse had a history of fever, weight loss, lethargy, cough, and nasal discharge. Previously, the referring veterinarian had prescribed a 5-day course of tetracycline for presumptive *Ehrlichia equi* infection; treatment had temporarily reduced the fever but did not otherwise improve the horse's clinical condition. Several days prior to referral, the horse had been lifting its left hind limb intermittently and appeared to be straining to urinate. The referring veterinarian detected a mass via transrectal palpation in the caudal aspect of the abdomen. Results of a serum biochemical panel indicated high activities of alkaline phosphatase (393 U/L; reference range, 50 to 136 U/L) and aspartate aminotransferase (287 U/L; reference range, 100 to 190 U/L). On physical examination, the gelding was quiet and thin (body condition score, 2.5/9); rectal temperature was 37.8°C (100.0°F), heart rate was 32 beats/min, and respiratory rate was 20 breaths/min. Mucous membranes were tacky and slightly congested, with a capillary refill time of 3 seconds. The horse had a mild intermittent cough and bilateral mucoid nasal discharge. Thoracic auscultation did not reveal any abnormal lung sounds, but a systolic murmur (grade 2/5) was audible over the base of the left heart. Fecal material was dry, and gastrointestinal tract motility was reduced. The horse did not appear to be lame when walking but lifted the left hind limb intermittently and held it off the ground for ≤ 1 minute. Normal urination was observed.

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Transrectal palpation revealed a firm lobulated mobile mass (approx 6 × 8 cm) on the right side of the caudal portion of the abdomen. Multiple soft, smooth, round masses (approx 4 cm in diameter) arranged in chains bilaterally were also palpated in the caudal portion of the abdomen and were presumed to be enlarged lymph nodes. Transrectal ultrasonography revealed a large multilobulated mass; the smaller, round masses had an echogenic pattern consistent with that of lymph nodes. At the time of admission, results of a CBC and serum biochemical analyses indicated mild neutrophilia (8,626 neutrophils/ μ L; reference range, 2,260 to 8,580 neutrophils/ μ L), hyperfibrinogenemia (500 mg/dL; reference range, 100 to 400 mg/dL), hyperproteinemia (9.0 g/dL; reference range, 5.8 to 7.7 g/dL) because of marked hyperglobulinemia (7.5 g/dL; reference range, 1.7 to 4.7 g/dL), decreased BUN (9.0 mg/dL; reference range, 12 to 27 mg/dL), and mildly high activities of γ -glutamyltransferase (54 U/L; reference range, 8 to 22 U/L) and alkaline phosphatase (403 U/L; reference range, 86 to 285 U/L). Abdominocentesis yielded a hazy yellow modified transudate with a mildly high protein concentration (3.4 g/dL; reference limit, < 2.5 g/dL) and a nucleated cell concentration of 4,800 cells/ μ L, of which 86% were neutrophils. No organisms were seen on microscopic examination of stained smears of this fluid, and bacteriologic culture failed to yield growth.

Transabdominal ultrasonography revealed multiple circular, well-circumscribed, hypoechoic masses throughout the splenic parenchyma (Fig 1). The largest of these

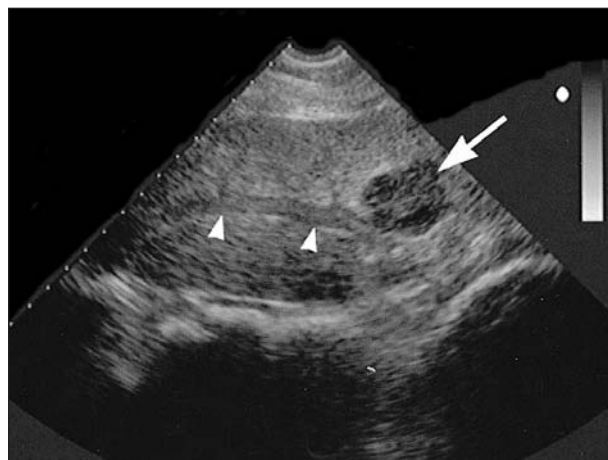


Figure 1—Ultrasonographic view (via the left eleventh intercostal space, with a 3.5 MHz sector transducer at a scanning depth of 16 cm) of the spleen of a cryptorchid horse with malignant Sertoli cell tumor. Notice a mass (arrow) adjacent to the splenic vein (arrowheads), which is representative of numerous nodular masses throughout the splenic parenchyma. Dorsal is to the right.

splenic masses measured 4.0×5.9 cm; several of the masses had echogenic to hyperechoic central foci, and 1 cast a sonographic shadow that was suggestive of mineralization. The right and left liver lobes were markedly enlarged with irregular surfaces and rounded margins. Liver parenchyma appeared diffusely abnormal. Inter-costal ultrasonographic views revealed heterogeneous and mottled echogenicity (Fig 2), and no normal liver parenchyma was observed. Thoracic radiography revealed numerous discrete, well-demarcated nodular densities (2 to 12 mm in diameter) scattered throughout all lung lobes. In addition, mild pleural effusion and generalized cardiomegaly were noted.

On the basis of these findings, metastatic neoplasia was considered to be the most likely diagnosis, and biopsy specimens of the liver were obtained to further characterize the lesions. Results of histologic examination of frozen sections of hepatic tissue were inconclusive, but examination of formalin-fixed specimens revealed tightly packed neoplastic cells that infiltrated and compressed adjacent hepatocytes. The origin of the neoplastic population could not be discerned; however, a presumptive diagnosis of metastatic seminoma was made on the basis of cell morphology and lack of reactivity to immunohistochemical stains (vimentin, pancytokeratin, melan-A, leukocyte markers [CD3, CD79a], and chromogranin-A). The prognosis for recovery was grave, and the decision was made to euthanize the horse.

At necropsy, a retained testis was identified in the right retroperitoneal space. The testis was multilobular and measured $3.0 \times 3.4 \times 2.3$ cm (Fig 3). On gross examination of a cut surface, the testicular architecture was effaced by diffusely tan, firm lobules separated by bands of fibrous tissue. Two masses of similar character were identified in the spermatic cord.

The liver was markedly enlarged, and numerous multifocal, coalescing nodules (approx 0.5 to 4.0 cm in diameter) elevated the capsule surface. Greater than 90% of the hepatic parenchyma appeared to be effaced. In sectional view, the hepatic nodules were firm, tan,

and frequently subdivided into lobules by fibrous bands of various widths. The spleen also was enlarged and contained numerous similar masses that were diffusely distributed throughout the parenchyma; these masses ranged in size from $0.5 \times 0.7 \times 0.2$ cm to $7.0 \times 5.0 \times 2.0$ cm (Fig 4). In addition, the right and left lungs contained innumerable firm nodules distributed diffusely throughout the lung parenchyma, with the greatest concentration in the caudodorsal regions. Most of these nodules ranged from 0.1 to 0.5 cm in diameter; infrequently, larger nodules (2.0 to 2.5 cm in diameter) were observed. In all of these organs, the nodules were characterized by a prominent fibrous component and frequently had a central area of necrosis. Additional necropsy findings included an approximately $6.7 \times 3.7 \times 3.7$ -cm, multinodular mass that was adhered to the right atrium. The mass was firm, and the cut surface was diffusely tan; the mass did not appear to invade the adjacent myocardium. Sublumbar lymph nodes were also enlarged (approx $14.0 \times 4.0 \times 4.0$ cm), multinodular, and gray to tan in sectional view.

Microscopic examination of the retained testis revealed replacement of the normal tissue architecture

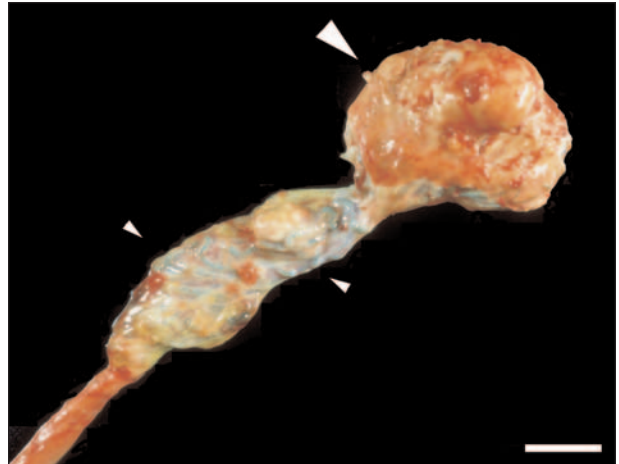


Figure 3—Photograph of the retained right testis in a horse with malignant Sertoli cell tumor. Notice that the testis (large arrowhead) is expanded by multiple lobules. Multiple metastases are seen in the spermatic cord (small arrowheads). Bar = 2.0 cm.

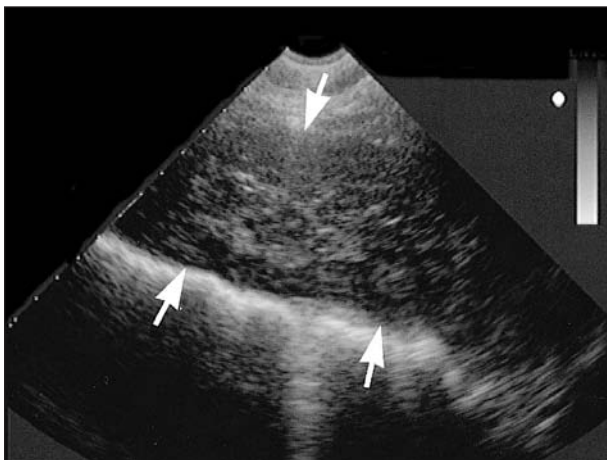


Figure 2—Ultrasonographic view (via the twelfth intercostal space, with a 3.5 MHz sector probe at a scanning depth of 14 cm) of the right liver lobe of a cryptorchid horse with malignant Sertoli cell tumor. Notice diffusely heterogeneous echogenicity of the liver and lack of normal vascularity; the liver capsule (arrows) has a nodular appearance. Dorsal is to the right.

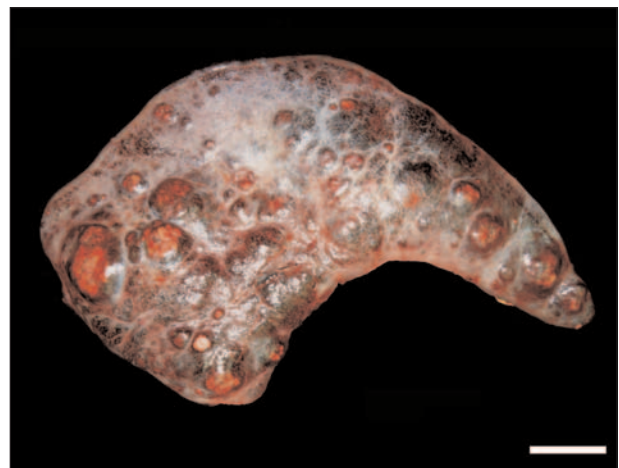


Figure 4—Photograph of the spleen of a cryptorchid horse with malignant Sertoli cell tumor. Notice that $\geq 90\%$ of splenic parenchyma is effaced by tumor metastases. Bar = 8.0 cm.

by a multilobular, infiltrative mass of densely cellular islands and tubules separated by prominent fibrous septa of various widths (Fig 5). The neoplastic cell population consisted of pleomorphic polygonal to columnar cells, each with a moderate amount of amphophilic cytoplasm, indistinct cell borders, and a single large, round to oval nucleus that contained finely stippled chromatin and multiple prominent nucleoli. Tumor cells frequently contained multiple large, cytoplasmic vacuoles. There was marked anisocytosis and anisokaryosis among the cells; mitotic rate was regionally variable (mean number of mitotic figures per high power field [4,000X], 3 to 5), with atypical mitotic figures observed frequently. Within the tumor lobules, neoplastic cells frequently aligned perpendicularly along the fibrovascular stroma to form disorganized tubules. Anaplastic regions were present in abundance in which the cells were more polygonal and arranged into nests and densely packed tubules. Also, large areas of necrosis were observed in the central region of the mass.

Poorly differentiated neoplastic cells, similar to those in the testis, infiltrated the liver, spleen, lungs, and sublumbar lymph nodes. Tubule formation and prominent fibrous stroma characterized these metastases. Also, tumor emboli were observed in the pulmonary vasculature.

Tissue sections of the retained testis and metastases were evaluated immunohistochemically. In contrast to the biopsy specimen, the neoplastic cells in samples obtained at necropsy were lightly and multifocally immunoreactive for vimentin. Immunohistochemical staining for pancytokeratin and melan-A performed on necropsy samples yielded negative results, as with the biopsy specimen. Additional immunostaining was performed using simple epithelium markers (AE1, CAM 5.2, and cytokeratin 07) and S100. The neoplastic cells were nonreactive to all of these markers with the exception of a small area of cells within the testis that was immunoreactive to AE1. This area was considered to be rete testes.¹ A diagnosis of malignant Sertoli cell tumor was made on the basis of the abundant fibrous stromal component, cellular organiza-

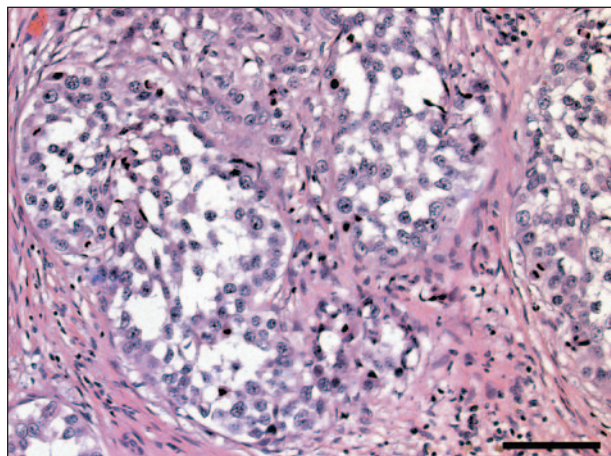


Figure 5—Photomicrograph of a section of the retained right testis of a horse with malignant Sertoli cell tumor. Notice that neoplastic Sertoli cells are arranged into tubules and surrounded by prominent fibrous stroma that replaces the normal testicular architecture. H&E stain; bar = 100 μ m.

tion and morphology of the more well-differentiated regions of tumor, and vimentin immunoreactivity.²

Because of the extent of anaplasia within the primary tumor and metastases, an alternative means was pursued to provide evidence to support the diagnosis of Sertoli cell tumor. Müllerian inhibiting substance (MIS) is a gonadal peptide hormone of the transforming growth factor β superfamily that is responsible for regression of Müllerian ducts during mammalian embryogenesis and sexual differentiation.³ Expression of MIS is limited to Sertoli cells of the testis and granulosa cells of the ovary post natum.³ Furthermore, MIS expression is greatest during testicular development and Müllerian duct regression, with marked decline in postpubescent males in multiple species.³ A commercially available polyclonal antibody^a directed against the highly conserved carboxy terminus⁴ of human MIS and a kit^b with the 3-amino-9-ethcarbazole peroxidase substrate^c were used for immunohistochemical detection of MIS. A fetal and a mature equine testis were used as positive and negative control specimens, respectively. Immunoreactivity of the MIS antibody with the fetal testis was intense and localized to Sertoli cells. The Sertoli cells of the mature testis yielded negative results, which was consistent with the postpubescent decline in MIS expression observed in other animals. In well-differentiated regions of the tumor, occasional clusters of neoplastic cells had light cytoplasmic immunoreactivity, suggesting low-grade expression of MIS in these cells (Fig 6). The anaplastic regions of the primary tumor and metastases yielded negative results; therefore, the possibility of a mixed cell-type tumor could not be completely ruled out. However, expression of a Sertoli cell-specific hormone within the primary tumor supported a diagnosis of malignant Sertoli cell tumor.

In the horse of this report, a malignant Sertoli cell tumor developed in the retained right testis and subse-

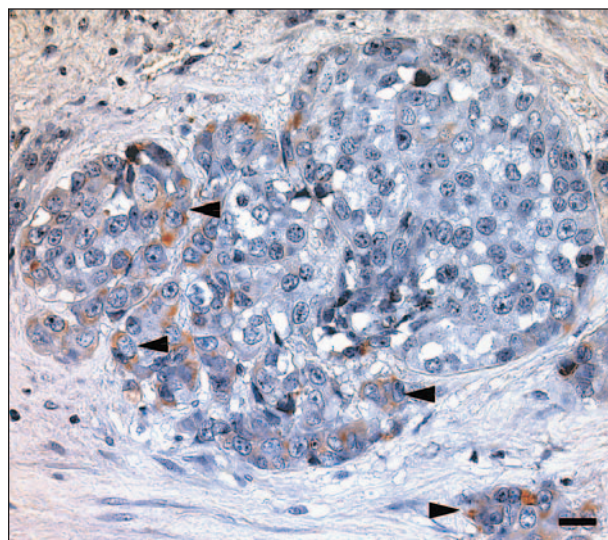


Figure 6—Photomicrograph of a section of primary tumor from the retained right testis of a cryptorchid horse with malignant Sertoli cell tumor. The section has been stained immunohistochemically to detect expression of Müllerian inhibiting substance (MIS). Neoplastic cells (arrowheads) that reacted with the MIS-specific stain are distributed multifocally throughout the tumor. Bar = 10 μ m.

quently metastasized to the liver, spleen, lungs, mediastinum, and sublumbar lymph nodes. Testicular neoplasms in horses have been reported infrequently and are considered rare.⁵⁻¹⁵ Results of a study⁸ conducted in an abattoir indicate that testicular tumors comprise approximately 1% of equine tumors. The actual incidence of testicular tumors is difficult to determine because of the common practice of early castration of stallions and infrequent histologic examination of removed testes.

Four types of testicular neoplasia have been reported in stallions. The most common testicular neoplasm of adult horses is seminoma, which occurs in inguinally retained or descended testes, causes marked testicular enlargement¹⁰ and may metastasize.^{11,12} Teratoma is the most common testicular tumor in young horses but occurs rarely in other species. Teratomas contain a variety of tissues representing multiple germ layers; these tumors are usually benign and most frequently found in retained testes.^{5,13} Interstitial (Leydig) cell testicular tumors are rarely reported in horses¹⁰ but may develop unilaterally or bilaterally; they are usually found in retained testes and grow slowly.^{5,14} Sertoli (sustentacular) cell tumors are found commonly in dogs but are considered rare in stallions.^{6,7,13}

To the authors' knowledge, this is the first report of a malignant Sertoli cell tumor in a retained testis in a horse. In 2 other horses, Sertoli cell tumors were identified in descended testes^{7,9}; 1 of the 2 horses was cryptorchid,⁷ but the tumor was located in the descended testis. Malignancy of the tumor was confirmed in 1 horse.⁹ In the horse in which both testes were descended, atrophy of the contralateral descended testis was evident. In the horse of this report, the descended left (contralateral) testis had been removed at castration; therefore, physical examination of that testis was not possible. In dogs, Sertoli cell tumors have been associated with retained testes.⁵ In a retrospective study¹⁵ of 350 horses with cryptorchidism, 121 abdominally retained testes were examined histologically. Six testes had abnormalities; in 1 testis, Sertoli cell adenoma was identified. Although Sertoli cell tumors may occur in descended and abdominally retained testes, there have been too few reports from which to draw conclusions regarding an association of development of this tumor type with cryptorchidism.

Typical biological behavior of Sertoli cell tumors in horses remains unknown, although metastasis to multiple organs was evident in the horse of this report and in the 19-year-old stallion described by Duncan.⁹ In the report of Rahalay et al,⁷ the horse was euthanatized shortly after removal of the Sertoli cell tumor; therefore, further examination for metastases was not performed. Metastasis of Sertoli cell tumors in humans is rare¹⁰ but has been reported in several dogs.⁵ Although metastatic lesions were found in the liver, diaphragm, lung, and kidney of the horse described by Duncan,⁹ regional lymph nodes were not involved, and there was no invasion of lymphatic vessels in the spermatic cord. In contrast, metastases were found in the spermatic cord and sublumbar lymph nodes as well as in the liver, spleen, lungs, and mediastinum in the horse of our report, which suggested local lymphatic invasion

and hematogenous dissemination of tumor cells. Disseminated peritoneal metastasis has been reported¹⁶ in horses with more commonly encountered tumor types such as gastric squamous cell carcinoma, lymphosarcoma, and mesothelioma. At necropsy, there was no gross evidence of peritoneal metastasis in the horse of this report, and the nucleated cell concentration in the peritoneal fluid sample obtained previously was less than expected if metastasis to this region had occurred. The abdominal fluid was classified as a modified transudate on the basis of mildly high protein concentration and predominance of neutrophils among cell types; this is consistent with neoplasia of parenchymal organs but can also be seen in the peritoneal fluid of horses with internal encapsulated abscesses.¹⁶

Neoplasia involving a retained testis should be considered in the differential diagnosis of horses, including apparent geldings, that have 1 or more masses palpable transrectally in the caudal region of the abdomen or have signs consistent with abdominal neoplasia. Transrectal and transabdominal ultrasonographic examinations are useful for characterization of palpable masses and evaluation of the extent and location of metastases. Although a mass was palpable in the horse of this report, transabdominal ultrasonography should be considered for evaluation of all horses that have clinical signs compatible with neoplasia. Interestingly, in the horse of this report, the sonographic appearance of hepatic and splenic metastases of the same Sertoli cell tumor were different; this supports 1 author's (MBW) experience that sonographic appearance of neoplasms in abdominal organs cannot be used reliably to differentiate tumor type. Sampling of abnormal tissue should be performed by use of ultrasound-guided biopsy techniques to provide specimens for histologic evaluation and definitive diagnosis of tumor type.

To our knowledge, use of immunohistochemical detection of MIS to identify cells of origin in poorly differentiated or mixed cell-type testicular tumors of domestic animals has not been reported. In the horse of this report, results of MIS-specific immunohistochemical evaluation supported the diagnosis of malignant Sertoli cell tumor. Further investigation of MIS expression in descended and retained testes, as well as in sex-cord stromal tumors, is required to validate the use of this immunohistochemical stain as an adjunctive diagnostic technique.

^aPolyclonal antibody against carboxy terminus of human MIS, Santa Cruz Biotechnology Inc, Santa Cruz, Calif.

^bVectastain Elite ABC kit, Vector Laboratories, Burlingame, Calif.

^cAEC kit, Vector Laboratories, Burlingame, Calif.

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