ABSTRACT: A case of an ovarian tumor is reported in an 8-yr-old South American sea lion (*Otaria flavescens*) kept in a marine park in Malta (35.57° N, 14.25° E). The neoplasm was a solid mass of dense sheets and nests of round to polygonal cells with abundant, finely vacuolated cytoplasm. The nuclei were uniformly small and round to oval. The supporting stroma contained thecal cells. The tumor cells were positive for positive inhibin and vimentin and locally positive for cytokeratin by immunohistochemistry. The neoplasm was diagnosed as an ovarian sex cord–stromal tumor, specifically an interstitial cell tumor.

Key words: Interstitial cell tumor, marine mammals, *Otaria flavescens*, ovary, South American sea lion.

The literature documenting marine mammal neoplasia is gradually expanding. Among marine mammals, the majority of neoplasms reported concern California sea lions (*Zalophus californianus*) and St. Lawrence beluga whales (*Delphinapterus leucas*; Newman and Smith, 2006). Overall, the reported incidence of neoplasia in marine mammals appears to be low (Newman and Smith, 2006). This may be due to an unknown death rate in the wild population, to lack of available tissues for histopathology at necropsy due to an advanced state of autolysis, or to lack of necropsy examinations performed on stranded animals (Newman and Smith, 2006). A wide variety of tumors in many different organ systems have been reported in California sea lions, but to our knowledge this is the first report of an ovarian tumor in a South American sea lion.

This South American sea lion was part of a pinniped collection in Malta (35.57° N, 14.25° E) and was housed in an outdoor pool with three other South American sea lions. Veterinary care was routinely provided, as dictated by facility protocol. The only significant clinical history was chronic gastrointestinal disease with diarrhea observed for the previous 10 mo with intermittent frequency. Routine fecal examinations for gastrointestinal parasites were negative. Blood was collected from this animal monthly as part of a preventive medicine protocol followed by the hosting facility, and values were consistently within normal parameters. The animal presented with acute pain on 8 September and was treated with enrofloxacin (5 mg/kg PO, twice a day [BID]) for 10 days, and carprofen (4 mg/kg PO, BID) for 5 days and total probiotics (3 g PO, once a day [SID]) for a month. Although the animal maintained a normal appetite and attitude throughout the treatment period, no clinical improvement was seen. Because of the poor clinical response, gastroscopy and colonoscopy were attempted to investigate the cause of the diarrhea further. Forty minutes into the procedure the animal went into cardiac arrest. Resuscitation was attempted, but the animal never regained consciousness and died 30 min later. A necropsy was immediately performed.

Postmortem examination of the abdominal cavity revealed mild to severe enteritis and gastritis. The uterus and left ovary were grossly normal. The right ovary was larger than the left and had an active follicle present. Within the parenchyma of the right ovary, a 3×3×4-cm neoplasm was observed (Fig. 1). The cut surface of the neoplasm was yellow with small areas of hemorrhage.

Tissue samples of the tumor and major organs were fixed in a 10% buffered...
formalin solution, followed by paraffin wax embedding, sectioning, and staining with hematoxylin and eosin (H&E). Immunohistochemical staining for pancytokeratin, vimentin, and inhibin was also performed. The formalin-fixed, paraffin-embedded sections (3–4 μm) were dewaxed in xylene for 5 min, followed by rehydration through graded alcohols (100%, 90%, and 70%) and water. Antigen retrieval was done on the slides by placing them in a bath of 10 mmol/l citric acid (pH 5–6) and boiling for 16 min in an 800-W microwave oven (2,450 MHz; Panasonic NN-6453BBPQ; John Lewis, Watford, United Kingdom). The slides were dried at room temperature and then washed with running tap water. A peroxidase block was performed and slides were then incubated with each of the following primary antibodies: anti-pancytokeratin monoclonal antibody (mAb; Clone AE1/AE3; Dako, Glostrup, Denmark), anti-inhibin mAb (Clone R1; Serotec, Inc., Raleigh, NC, USA), and antivimentin mAb (Clone V9, Dako). Each antibody was diluted 1:50 in a buffer solution prior to incubation. Antibody binding was detected with the use of an ABC-peroxidase kit (Vector Laboratories, Inc., Burlingame, CA, USA) with a 1:200 dilution of biotinylated goat antiamouse immunoglobulin (Ig) as a secondary antibody (AO433; Dako), which was applied to the sections for 45 min at room temperature. The enzymatic reaction was developed with the use of 3-1-diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, Missouri, USA) as a substrate. Stained slides were subsequently counterstained in hematoxylin for 30 sec, followed by a wash in tap water, dehydration in graded alcohols (70%, 90%, and 100%) and clearance with xylene. Sections were then mounted in DPX (08600E; Surgipath Europe Ltd., Bretton, Peterborough, United Kingdom). Normal California sea lion skin and canine ovaries with corpora lutea were used as positive controls for pan-cytokeratins, vimentin, and inhibin. A negative control for each antibody used in this study consisted of the substitution of the primary antibody for an isotypic antibody control at the same protein concentration.

To evaluate the possibility that the tumor secreted steroids, a retrospective study was performed to evaluate serum progesterone levels. Five serum samples collected between October 2005 and September 2006 were analyzed by RIA method with petroleum ether extraction (Biancani, 2008), and results ranged between 2.55 and 64.06 ng/ml (41.18 ± 23.87 ng/ml [mean ± SD]). Because of the small amount of serum available, it was not possible to evaluate more steroids.

Based on histopathologic and immunohistochemistry findings, an ovarian interstitial cell tumor was diagnosed. Microscopically the right ovarian neoplasm was a solid mass of dense sheets and nests of round to polyhedral irregularly shaped cells with abundant pale and finely vacuolated cytoplasm (Fig. 2). The nuclei were uniformly small and round to oval. Mitotic figures were infrequent and nuclear atypia was modest. The supporting stroma contained thecal cells. Tumor cells stained positive for inhibin (Fig. 3) and vimentin (Fig. 4) by immunohistochemistry; inhibin was expressed along the cell membrane. Staining for cytokeratin was focally positive and random cells had a perinuclear
staining pattern. Other microscopic changes included mild dilatation of the hepatic centrilobular veins, mild atrophy of centrilobular hepatocytes, Kupffer cell hemosiderosis, and moderate hypertrophy of the mammary glands. The intestine had diffuse lymphoplasmacytic infiltrates.

Comparing the progesterone levels observed in the present study to levels reported for California sea lions by Greig et al. (2007), it appears that progesterone levels in this animal were not abnormally elevated. This may imply that the tumor in this case was not inducing abnormal progesterone production or that species-specific differences among different species of sea lions are present. Further studies on normal and abnormal progesterone production, as well as other steroid production in Patagonia sea lions, are warranted.

Sex cord–stromal tumors are the most commonly reported ovarian neoplasm in all animal species. This group of tumors includes granulosa cell tumors, thecomas, and interstitial cell tumors (luteomas, lipid cell tumors, and steroid cell tumors; Kennedy et al., 1998). The terms lipid cell tumor and steroid cell tumor have been proposed for ovarian neoplasms composed of large rounded or polyhedral cells that resemble luteal, interstitial, or adrenal cortical cells, but cannot be identified as any one of those (Yamini et al., 1997).

In our case, in addition to the observed morphology, a diagnosis of the tumor was made by immunohistochemical evidence of strong α-inhibin expression in almost all neoplastic cells. The diagnostic role of anti-inhibin antibodies in the evaluation of tumors of the female genital tract has been extensively studied (Costa et al., 1997; Pelkey et al., 1998; McCluggage, 2001). Inhibins are heterodimeric hormones within the transforming growth factor superfamily and are primarily of gonadal origin. These hormones regulate pituitary follicle-stimulating hormone secretion via feedback inhibition (Vale et al., 1988; Zheng et al., 1997). The distribution of these hormones, particularly the α-subunit

![Figure 2. Ovarian tumor. Adrenal-like cells (arrow), with abundant vacuolated clear cytoplasm. Hematoxylin and eosin; bar=100 μm.](image2)

![Figure 3. Ovarian tumor. Note the strong positivity of neoplastic cells for inhibin. Immunohistochemistry. Bar=25 μm.](image3)

![Figure 4. Ovarian tumor. Weak and diffuse positivity of neoplastic cells for vimentin. Immunohistochemistry. Bar=25 μm.](image4)
(α-inhibin), has been shown to include normal gonadal (Cuevas et al., 1987), adrenal cortex (Munro et al., 1999) and pituitary tissue (Ucella et al., 2000). Neoplasms shown to express inhibin include gonadal neoplasms, predominantly sex cord–stromal tumors (McCluggage et al., 1997; Rishi et al., 1997; Iczkowski et al., 1998; Zheng et al., 2000), neoplasms of the adrenal cortex (Arola et al., 2000) and pituitary adenomas (Ucella et al., 2000).

The etiology of the ovarian tumor described in the present study remains obscure. In the California sea lion, a significant association between urogenital tumors and an endemic novel gammaherpesvirus, otarine herpesvirus-1 (OtHV-1) infection has been described (King et al., 2002). Recently, polychlorinated biphenyls have been suggested as a possible cofactor (Gulland et al., 1996). Even though for the present study it was not possible to investigate further the association between viral infection and the presence of the tumor, this association should be taken into consideration in future cases of urogenital neoplasia in this species.

LITERATURE CITED


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