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NOTE

Pathology

Unilateral luteoma of the ovary in a pregnant Risso's dolphin (*Grampus griseus*)

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ABSTRACT. A white, lobular mass was found in the right ovary of a pregnant Risso's dolphin (*Grampus griseus*) at necropsy. The mass was unilateral and occupied most of the pre-existing ovarian tissue. Histologically, the mass was composed of diffuse sheets of polyhedral cells with abundant eosinophilic cytoplasm and oval nuclei, separated by fibrous connective tissue. Only a few ovarian follicles were observed at the periphery of the mass. Immunohistochemically, the large eosinophilic cells were positive for vimentin and negative for pan-cytokeratins. Based on the histopathological features, the present case was diagnosed as luteoma. In human medicine, luteoma of pregnancy, a tumor-like proliferative lesion occurring in pregnant women, is well described. In veterinary medicine, luteoma associated with pregnancy has never been described. The present study would provide useful information for understanding the characteristics of luteoma in animals.

KEY WORDS: dolphin, luteoma, ovary, pregnancy, sex cord-stromal tumor

In veterinary literature, ovarian tumors are divided into 3 broad categories: tumors of the surface celomic epithelium, tumors of the sex cord and gonadal stroma, and tumors of germ cells [10]. Sex-cord stromal tumors include granulosa cell tumor, thecoma and luteoma (interstitial cell tumor). Granulosa cell tumors, including granulosa-theca cell tumors, are the most common ovarian tumors in most animals, such as cows and mares [6, 10]. Thecomas and luteomas are rare [10]. In bitches, luteomas account for less than 5% of primary ovarian tumors [9, 11]. In a study of 22 cats with primary ovarian tumors, 5 cats had luteoma [2]. To the best of our knowledge, ovarian tumors are rarely reported in marine mammals [8].

An adult female Risso's dolphin (*Grampus griseus*) in late pregnancy was presented with anorexia in a zoo. Increased levels of serum sodium (187 mEq/*l*; reference range, 153–158 mEq/*l*), chloride (156 mEq/*l*; reference range, 113–125 mEq/*l*) and potassium (4.3 mEq/*l*; reference range, 3.2–4.2 mEq/*l*) were detected. Serum β-lipoprotein level (778 mg/d*l*) increased compared with that in healthy condition of this animal (170 mg/d*l*).

After 10 days, the dolphin died, and necropsy was performed. At necropsy, a 4 × 4 × 3 cm, whitish mass was observed in the right ovary, and a fetus was seen on the same (ipsilateral) side of the uterine horn. The cut surface of the mass was lobulated and well encapsulated, and the pre-existing ovarian tissue was almost replaced (Fig. 1). Contralateral ovary was normal in shape and 4 × 1 × 1 cm in size. Pulmonary edema, accumulation of foamy fluid within the trachea and extensive abrasion in the head skin were also observed.

The tissues were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were cut at 5 μm and stained with hematoxylin and eosin (HE). Immunohistochemistry (IHC) was performed with mouse monoclonal antibodies specific for proliferating cell nuclear antigen (PCNA; clone PC10, 1 in 500; Dako, Glostrup, Denmark), pan-cytokeratin (clone AE1/AE3, ready to use; Dako), vimentin (clone V9, ready to use; Dako) and inhibin-α (clone R1, 1 in 500; Serotec, Oxford, U.K.). After dewaxing, sections for vimentin, pan-cytokeratin and PCNA were pretreated by microwaving for 20 min in citrate buffer (pH 6.0), while those for inhibin-α were pretreated by microwaving for 20 min in Tris-EDTA (pH 9.0) for antigen retrieval. All sections were treated with 5% skimmed milk in phosphate buffered saline (PBS) for 15 min and incubated with each primary antibody for 1 hr at room temperature. The sections were then incubated with 3% H_2O_2 in PBS for 15 min to quench endogenous peroxidase, followed by 1-hr incubation with horseradish peroxidase-conjugated secondary antibody (Histofine Simple Stain MAX PO, Nichirei, Tokyo, Japan). Positive reactions were detected with 3,3′-diaminobenzidine (DAB Substrate Kit, Nichirei). Sections were counterstained lightly with hematoxylin. Contralateral ovary served as an internal control for immunohistochemistry.

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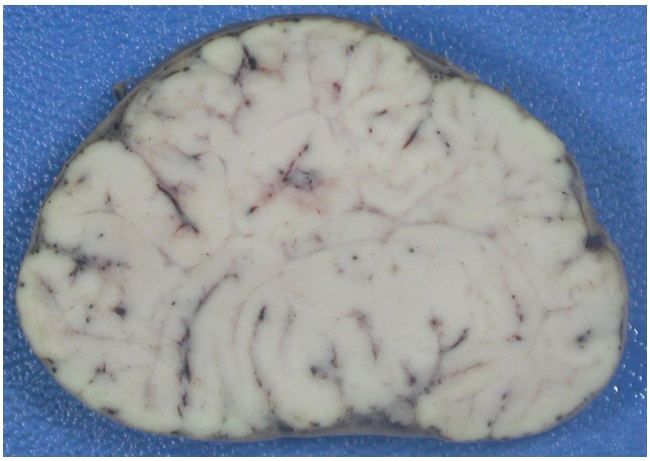

Fig. 1. Cut surface of the ovarian mass after formalin fixation. The mass is whitish to grey, solid and lobulated, encapsulated by fibrous tissue.

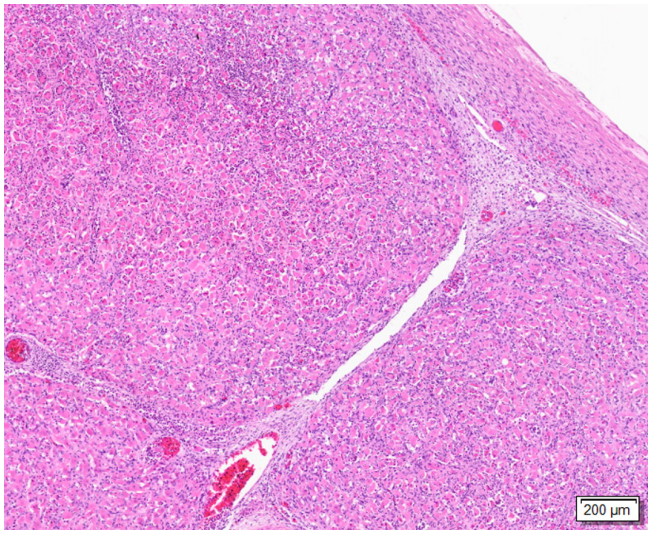

Fig.2. The ovarian mass is separated into lobules by fibrovascular tissue. The lobules are composed of diffuse sheets of large eosinophilic cells. HE. Bar: 200 μm.

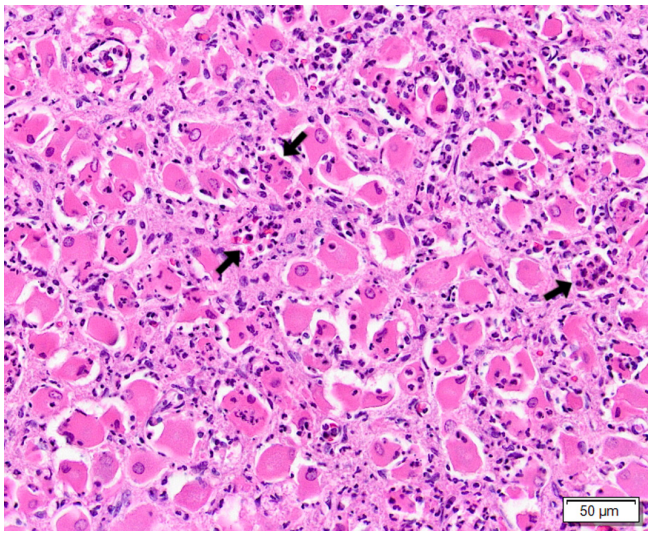

Fig. 3. The large eosinophilic cells are oval to polyhedral in shape and have abundant eosinophilic cytoplasm and oval nuclei. Infiltration of neutrophils within the large eosinophilic cells is remarkable (arrows). HE. Bar: 50 μm.

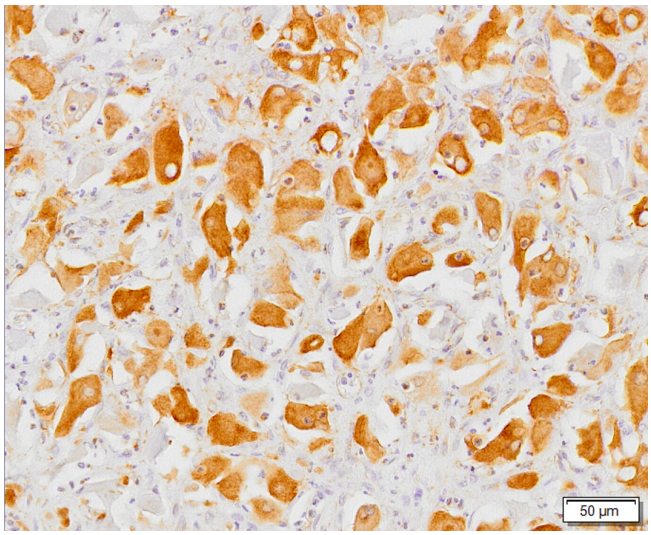

Fig. 4. The large eosinophilic cells are positive for vimentin. IHC for vimentin. Bar: 50 μm.

Microscopically, the mass was composed of diffuse sheets of large eosinophilic cells separated by fibrovascular connective tissue (Fig. 2). The proliferating cells were oval to polyhedral with abundant eosinophilic cytoplasm and oval nuclei (Fig. 3). A few lipid vacuoles were seen in the proliferating cells. No mitotic figures were observed in 10 high-power fields (HPF). Diffuse infiltration of inflammatory cells, mainly neutrophils, were observed within the mass (Fig. 3). There are some degenerative large eosinophilic cells infiltrated by neutrophils (Fig. 3, arrows). Only a few ovarian follicles were observed at the periphery of the mass. Immunohistochemically, the proliferating cells were positive for vimentin (Fig. 4) and were negative for cytokeratin AE1/AE3. As an internal control, surface epithelia of the contralateral ovary stained positive for cytokeratin AE1/AE3. Vascular endothelial cells and stromal cell of the contralateral ovary stained positive for vimentin. Four percent of proliferating cells were positive for PCNA, while twenty-seven percent of colon crypt epithelia, used as a positive control, were positive for PCNA. The clone R1 inhibin-α antibody did not react with dolphin tissue as no staining was detected in the contralateral ovary. Corpus luteum of pregnancy was not observed in the both ovaries, suggesting that it was replaced by the mass. In other organs, congestive edema in the lungs and congestion in the liver were observed. Pulmonary edema with decreased body condition due to pregnancy was considered as a cause of death.

Based on the histopathological findings, the ovarian lesion was diagnosed as luteoma. Other sex-cord stromal tumors, such as granulosa cell tumor and granulosa-theca cell tumor, should be considered in the differential diagnosis. Granulosa cell tumors have various histological patterns including follicular, island, trabecular, nest and solid arrangements, resembling granulosa cells of the ovarian follicle [1, 6]. The tumors often include proliferation of spindle cells resembling theca cells; they are then classified as granulosa-theca cell tumor. In the present case, there were no cells showing the morphology of granulosa or theca cells, and the mass was composed of a uniform proliferation of large polyhedral eosinophilic cells.

In cetaceans, the basic biology and morphology of corpus luteum of pregnancy are the same as those in terrestrial mammals [4]. In striped dolphin (*Stenella coeruleoalba*), the corpus luteum rapidly increases its size up to approximately 25 mm in diameter after initiation of pregnancy; the size is kept throughout the pregnancy. Histologically, corpus luteum of pregnancy is composed of two types of luteal cells: the theca and granulosa cells, as seen in other terrestrial animals. Although the body size of Risso's dolphin is larger than that of striped dolphin, the size of the ovarian mass in the present case may be more than the normal range of pregnant corpus luteum. Moreover, the mass is composed of a single population of luteal-like cells, with compression of the adjacent tissue. Thus, the present case was considered as the proliferative disease of corpus luteum of pregnancy.

In humans, luteomas are classified in benign steroid cell tumors, derived from the ovarian stroma [13]. They are usually unilateral and well-encapsulated. Microscopically, the lesion is characterized by a nodule of lutenized cells with abundant eosinophilic cytoplasm. Additionally, luteoma of pregnancy, a rare tumor-like condition in pregnant women, is described in WHO classification of ovarian tumors [7, 13]. Luteoma of pregnancy was first described in 1966 [12], and approximately 200 cases have been reported to date [15]. Most patients are asymptomatic; the lesion is unilateral or bilateral, and usually found incidentally during caesarean section or postpartum tubal ligation. Elevated levels of plasma testosterone and other androgens may be observed. Microscopically, the lesion is characterized by a diffuse proliferation of polygonal cells with abundant eosinophilic cytoplasm and little or no lipids. Luteoma of pregnancy is considered to be dependent on β-human chorionic gonadotropin as it regresses after the conclusion of the pregnancy. Steroid cell tumors occurring during pregnancy may present a difficult differential diagnosis; however, the typical clinical sign of luteoma of pregnancy would be unusual for steroid cell tumor [13]. Besides, steroid cell tumor may have higher mitotic rate than luteoma of pregnancy. In veterinary medicine, luteoma related to pregnancy has never been reported.

In the present case, the proliferative activity of the large eosinophilic cells was relatively low. Additionally, the lesion contained some degenerative changes of the large eosinophilic cells with diffuse infiltration of neutrophils. These findings may indicate a reactive proliferative change rather than true tumor. However, there was no clear evidence of hormonal alteration in this case; the mass was single and unilateral. Therefore, the present case was diagnosed according to the histological classifications in Domestic Animals [1, 6].

There was no discernible evidence of infection in the systemic organs of this animal, and necrosis was not prominent within the mass. Additionally, neutrophil infiltration was diffusely seen throughout the mass, but not apparent in other organs including the contralateral ovary. These findings raise the possibility that neutrophil may be recruited by some factors produced by the luteoma. In the cow, a large number of neutrophils migrate in the developing corpus luteum, partially due to increased production of interleukin-8, a major neutrophil chemoattractant [5, 14].

Increased levels of serum electrolytes indicate severe dehydration in this animal. Increased level of serum β-lipoprotein indicates dyslipidemia. These abnormalities may be associated with deterioration of general conditions, rather than the luteoma itself.

Reported ovarian tumors in cetaceans are granulosa cell tumors, epithelial tumors and a dysgerminoma [3, 8]. In pinnipeds, granulosa cell tumors and epithelial tumors are reported [8]. Luteoma has never been reported in marine mammals. Further accumulation of cases is required to understand the characteristics of luteoma in dolphins.

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