

## BRIEF COMMUNICATIONS and CASE REPORTS

### Fetus in Fetus in a Harbor Seal (*Phoca vitulina richardi*): Histopathologic, Genetic, and Toxicologic Analysis

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**Abstract.** A young harbor seal (*Phoca vitulina richardi*), stranded on the coast of California, was found to have a 20-cm-diameter cranial cervical mass. Surgical excision revealed the subcutaneous mass to be covered in haired skin with multiple glabrous areas and structures resembling a jaw with tooth buds, eyelids, and a tail. The mass deformed the host pup's skull. Histologic examination revealed a complete vertebra in the tail, teeth in the jaw, and areas resembling tongue and larynx. Class I MHC sequences amplified by polymerase chain reaction from the mass and the host twin were identical. The mass was diagnosed as a fetus in fetus, a rare congenital anomaly in which 1 conjoined twin is completely enclosed in the body of the other twin. The host pup died, and no additional defects were found; however, blubber levels of persistent organic pollutants were high. The cause of the congenital anomaly in this pup is uncertain.

**Key words:** Congenital defect; contaminants; fetus in fetus; harbor seal; major histocompatibility complex; *Phoca vitulina richardi*; twin.

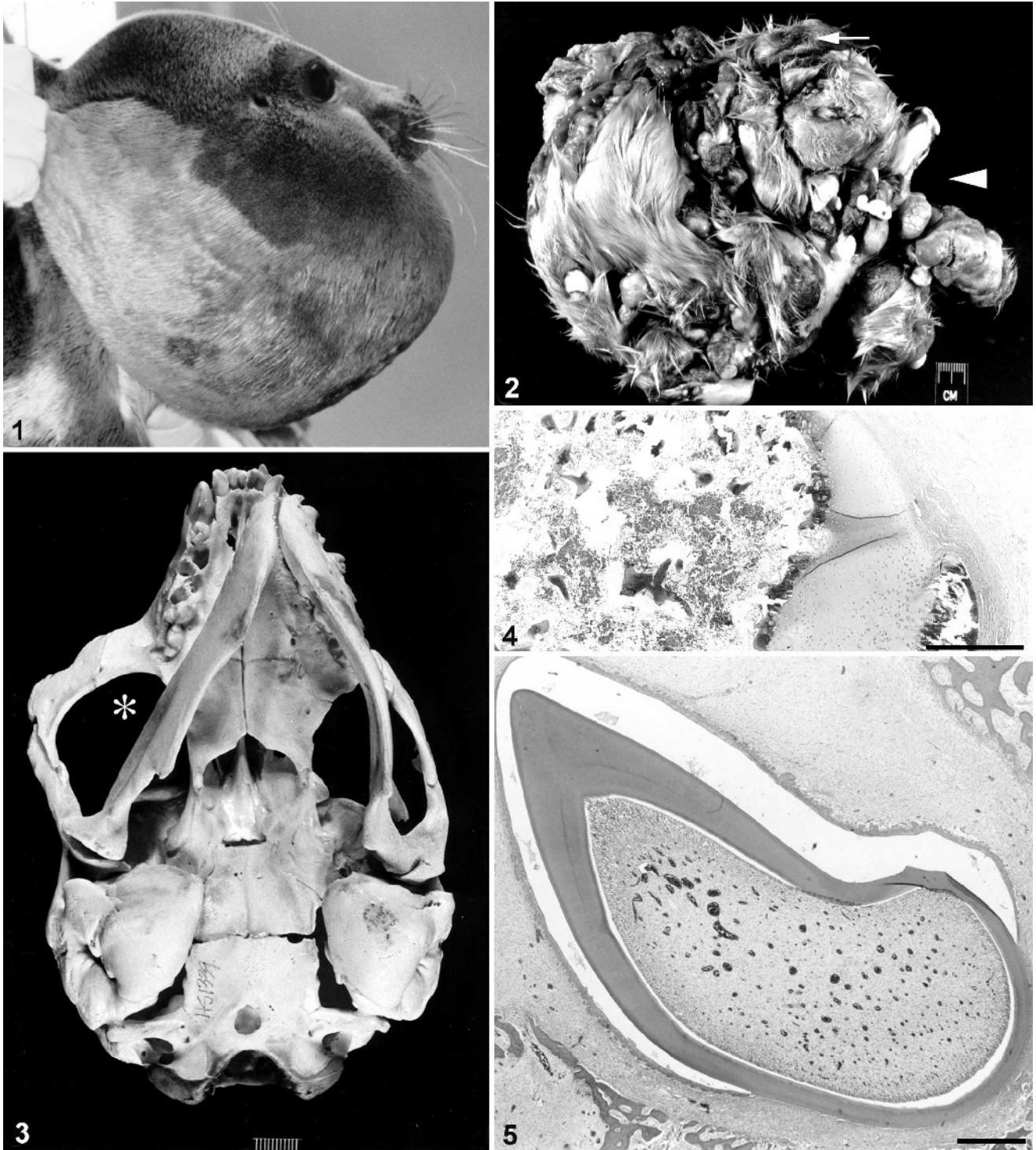
Fetus in fetus is an extremely rare developmental anomaly of humans in which 1 monozygotic twin is completely incorporated into the other twin's body.<sup>6,9</sup> Diagnosis of this condition can be confusing, the major differential being a mature teratoma.<sup>10</sup> However, by definition, the tissues of a true fetus in fetus are arranged around a vertebral axis, whereas those of a teratoma are less well organized. Here we report a cervical fetus in fetus in a young harbor seal (*Phoca vitulina richardi*) and genetic and toxicologic analyses conducted to investigate the etiology of the lesion.

A 2- to 3-month-old female harbor seal pup collected from Pebble Beach, Monterey County, California (36°58'N, 121°95'W), on 16 June 1999, was brought to The Marine Mammal Center, Sausalito, California, for treatment of a massive swelling around its neck. The pup weighed 11.4 kg and was 75 cm long (nose to tail). The seal was bright, alert, and vocal, but had an elevated respiratory rate (10 per minute compared with a normal of 5 per minute). On physical examination, the most striking abnormality was an approximately 30-cm-diameter subcutaneous swelling on the ventral aspect of the neck (Fig. 1). The skin over the ventral surface of the swelling was ulcerated, presumably because of traumatic abrasion. Hematology revealed leukopenia (total leukocyte count,  $3.0 \times 10^9/l$ , with 80% neutrophils, normal range  $5.9\text{--}24.6 \times 10^9/l$ ).<sup>3</sup> Erythrocyte and serum biochemical parameters were within the normal limits for this species.<sup>3</sup> Radiography revealed multiple amorphous calcified objects within the mass, but aspiration of the mass yielded only blood. The pup was anesthetized with propofol at

5 mg/kg and isoflurane 2–4% and was prepared for surgery.

Surgical exploration of the neck region revealed a large, multinodular, partially haired subcutaneous structure (Fig. 2) extending along the ventral midline from the intramandibular area to the sternum. The mass was completely covered by a network of blood vessels and partially adhered to the skeletal muscle. After removal of the mass by blunt dissection, it became apparent that the pup's skull was flattened dorsoventrally, with the mandible, larynx, and trachea deviated to the left, presumably because of pressure from the mass (Fig. 3). The pup did not recover from surgery.

Detailed examination of the 1.3 kg,  $20 \times 16 \times 10$ -cm mass revealed it to be almost completely covered by haired skin with interspersed glabrous areas. The hair was light tan with numerous dark brown spots, consistent with the coat color of a harbor seal. The glabrous skin was variably pigmented, mottled pink and brown. The roughly elliptical mass was characterized by several protrusions resembling distinct anatomical structures such as a tail, a palpebrum, and jaws with tooth buds. On incision, a small vertebra, complete with marrow, was present in the tail bud; bone supported the primitive jaws, which contained tooth buds. No ocular tissues were evident in association with the palpebral area. The remaining mass was composed of irregular, alternating cystic and solid regions of differing consistencies. Occasional mucous membrane-lined cystic areas were filled with a green substance resembling meconium. In addition, there were several islands of bone, including a hollow, elliptical, bone-lined cyst that contained



**Fig. 1.** Subcutaneous mass; host harbor seal pup. Lateral view of the live host harbor seal pup (*Phoca vitulina*) with the subcutaneous cervical mass (fetus in fetu) covered by intact host skin.

**Fig. 2.** Fetus in fetu; harbor seal. Ventral view of the fetus in fetu after removal from the ventral cervical subcutis of the host seal pup. Note, on the right side of the photograph, the palpebrum between the tail-like appendage (arrow) and the rudimentary jaw and toothlike structures (arrow head).

**Fig. 3.** Skull and mandible; host harbor seal pup. Ventral view of the cleaned skull from the host pup showing distortion and asymmetry (\*), from compression by the cervical mass. Bar = 1.0 cm.

**Fig. 4.** Tail-like appendage; fetus in fetu, harbor seal. Photomicrograph of the tail-like appendage from the fetus in fetu in which cartilage, bone, and hematopoietic elements can be seen. HE. Bar = 0.5 mm.

islands of cartilage. Solid areas of tissue were composed of fat; dense white connective tissue; and soft, pale pink, more myxomatous tissue.

Histologic examination of the mass revealed it to be covered in normal haired and glabrous skin, complete with adnexae. The cystic structures were lined with epithelium that ranged from squamous to low columnar. Areas of bone throughout the mass and embedded in the walls of 1 cyst were histologically normal. The tail vertebra contained normal hematopoietic elements and cartilage endplates (Fig. 4). The majority of the solid regions of the mass were composed of adipose tissue and vessels. The soft pink material consisted of ill defined, poorly organized nervous tissue, including both gray and white matter, as well as epithelium resembling choroid plexus. One area resembled a rudimentary tongue composed of skeletal muscle covered by serrated, keratinizing, stratified, squamous epithelium with intracytoplasmic eosinophilic hyaline granules. A tubular, cartilage-lined structure consistent with a larynx was adjacent to the tongue. Examination of the rudimentary jaw revealed several distinct developing teeth complete with dental papillae, odontogenic epithelium, dentin, enamel, and ameloblasts (Fig. 5). No other tissues, such as hepatic or renal parenchyma, were identified.

No significant gross or histologic lesions other than the flattened skull and the lateral deviation of the mandible were observed in the host pup during a complete postmortem examination (bone marrow of the host pup was not examined). The reproductive tract of the host pup was within normal limits for an infant female. Skin from both the host pup and the mass were submitted for genetic analysis. Genomic deoxyribonucleic acid was isolated by using standard techniques,<sup>4</sup> and an approximately 300 base pair region of the  $\alpha 3$  domain of the class I major histocompatibility complex (MHC) was amplified by polymerase chain reaction (PCR) by using degenerate class I MHC-specific primers.<sup>4</sup> After amplification, the PCR products were separated and compared by using denaturing gradient gel electrophoresis (DGGE). This technique has been used for MHC-genotyping in other species.<sup>1</sup> At least 9 bands representing the class I MHC $\alpha 3$  domains were identified in each tissue sample. The separated class I MHC PCR products were visually compared and showed an identical banding pattern (data not shown), providing strong evidence that the tissues from the host pup and ventral cervical mass were genetically identical. A complete sequence analysis of these PCR products was not performed.

Blubber collected from over the sternum of the host pup was analyzed for congener-specific polychlorinated biphenyls (PCB) and dichlorodiphenyl trichloroethane (DDT) by using high-resolution gas chromatography/

high resolution mass spectrometry and gas chromatography/low resolution mass spectrometry, respectively, as part of a larger survey of contaminants in stranded marine mammals. The host pup had high levels of both PCBs ( $\Sigma$ PCBs, 33.7 mg/kg lipid weight) and DDTs ( $\Sigma$ DDTs, 69.6 mg/kg lipid weight). PCBs were above the range of levels observed in free-ranging harbor seal pups of similar age sampled in British Columbia and Washington State ( $\Sigma$ PCBs, 2.3–15.4 mg/kg lipid). Comparable  $\Sigma$ DDT values are not available for harbor seal pups.

Nomenclature of developmental anomalies is complex and often confusing. In general, these anomalous growths are divided into 2 main categories: teratomas, which are neoplasms arising from primitive germ cells of the fetus, or conjoined twins, which result from either incomplete fission during blastogenesis or fusion of 2 embryos in utero.<sup>2</sup> Anomalies in the latter category can be further defined by the site of attachment of the 2 twins or else be classified as a parasitic twin. A parasitic twin is a conjoined twin that is incomplete, attached to, and fully dependent for growth upon its nearly normal, conjoined twin. Fetus in fetu is a rare type of parasitic twin in which the incomplete twin becomes fully incorporated into the body of the larger sibling.<sup>9</sup> Incidence of such parasitic twins in humans is rare.<sup>6</sup> Incorporation of the parasite is thought to be the result of anastomosis of the vitelline circulation.<sup>9</sup> Because of the relationship with the circulatory system, the most common site of inclusion in human cases is the abdominal or retroperitoneal cavity; however, hepatic, scrotal, renal, adrenal, and cranial sites have been reported.<sup>9</sup> The results of the MHC analysis in the present seal case suggest that the conjoinment resulted from incomplete fission of identical twins, with subsequent incorporation of one twin into the body of the other.

Distinguishing between a well-differentiated teratoma and a poorly differentiated fetus in fetu can be difficult. Teratomas are derived from multiple primitive germ-cell layers, are generally found along the midline of the body or within the gonads, exhibit autonomous growth, and can progress to malignancy.<sup>10</sup> Because they arise from undifferentiated cells, the tumors contain tissues foreign to the area from which they arise, such as respiratory epithelium, cartilage, renal glomeruli, nervous tissue, and hematopoietic tissue. Fetus in fetu can also contain these tissue elements and exhibit autonomous growth, but this entity does not metastasize, and the internal structures are arranged around an axial skeleton.<sup>9</sup> The presence of a vertebra indicates that the mass in this seal had passed through the primitive streak stage of embryogenesis. In the present case, the midline site of origin and the poorly organized structure are consistent with a cervical teratoma, but the existence of a clearly

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**Fig. 5.** Tooth; fetus in fetu, harbor seal. Photomicrograph of a tooth from the primitive jaw area of the fetus in fetu showing histologically normal development. HE. Bar = 0.7 mm.

defined, well-developed vertebra warranted the diagnosis of fetus-in-fetu.

Harbor seals, like all pinnipeds, have a bicornuate uterus; zonary, endotheliochorial placentation; and a period of embryonic diapause in which implantation of the blastocyst is delayed for 3 to 5 months after fertilization.<sup>5</sup> Twinning in marine mammals is rare, and confirmed reports of live twin births in phocids are currently confined to captive harbor seals and to molecular evidence in wild Weddell seals (*Leptonychotes weddellii*).<sup>7</sup> Reports of conjoined twins in wild phocids are rarer and previously limited to a report of conjoined twin fetuses in a southern elephant seal (*Mirounga leonina*).<sup>8</sup> Conjoined twins have been observed in a number of cetaceans, including striped dolphins (*Stenella coeruleoalba*), humpback whales (*Megaptera novaeangliae*), sei whales (*Balaenoptera borealis*), and minke whales (*B. acutorostrata*).<sup>8</sup> These reports are all based on data from fetuses found *in utero* in hunted animals, so it is not known whether these twins would have been viable. It is probable that anomalous twins are rare in pinnipeds, because considerable numbers of neonatal pinnipeds have been observed both in the field and in rehabilitation centers after stranding. To our knowledge, this is the first report of such an anomaly in a harbor seal.

One interesting aspect of the present case is the high level of PCBs and DDTs in the tissues of the host pup. While these potentially teratogenic chemicals have been heavily regulated in the USA and the industrialized world since the mid-1970s, such agents have persisted at high concentrations in many of the higher trophic levels of wildlife populations. Although a variety of lesions in seals are suggested to be associated with contaminant exposure, no causal link can be made from these data, and environmental teratogens to date have not been investigated in production of parasitic twins in any species. Whether or not the contaminants in this pup were related to the developmental anomaly is a matter for speculation.

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