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Possible Pregnancy Toxemia in a Late-Term Twin-Pregnant Harbor Seal (*Phoca vitulina*)

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Abstract

Pregnancy toxemia is known from terrestrial mammals such as sheep and goats in which late-term twin-pregnant animals may enter a state of negative energy balance with compromised glucose homeostasis. Twin pregnancy is rare in seals, and pregnancy toxemia is undescribed. Herein, we describe a case from an almost 17-year-old harbor seal (*Phoca vitulina*, Linnaeus, 1758) raised in captivity. The seal died in stage 1 of labor after having experienced a 7- to 14-day period in which it refused to eat, but without any further clinical signs observed. The carcass was CT-scanned and necropsied. The most striking necropsy findings were a late-term twin-pregnant uterus that occupied most of the abdominal space, an abundant amount of fat, compressed and empty stomach and intestines, and diffuse hepatic steatosis. The imaging and necropsy findings indicate that the seal may have died as a result of pregnancy toxemia. As an incidental finding, a well-calcified *os cordis* was observed with CT, which may be due to the seal's advanced age. This case thus represents three rarities: twin pregnancy, suspected pregnancy toxemia, and cardiac bone.

Key Words: pregnancy toxemia, labor, aquatic mammals, harbor seals, *Phoca vitulina*, *os cordis*, twins

Introduction

Pregnancy toxemia is a pathological condition that may develop in late-term pregnant animals if the dam encounters a negative energy balance and its glucose homeostatic regulation mechanisms become compromised. This leads to hypoglycemia, but other metabolic conditions such as hypocalcemia and ketosis often develop as well. The lack of energy produced through the glucose metabolism pathways leads to mobilization of adipose tissue. The liver plays a crucial role in the metabolism of lipids. An excessive mobilization of body fat leads to accumulation of lipids in the hepatocytes, which become distended (steatosis). Macroscopically, this is seen as a uniform enlargement of the liver and a change in color to light brown. Pregnancy toxemia is a well-known disease in sheep and goats in which one of the most important factors leading to severe negative energy imbalance is late-term pregnancy with twin or multiple fetuses. Basically, the condition is due to an imbalance between maternal energy intake and increased energy requirement from the rapidly growing fetuses. Obese and geriatric animals have a greater risk for developing pregnancy toxemia (Tontis & Zwahlen, 1987; Mongini & Van Saun, 2023).

Twin pregnancy has rarely been reported in seals (Spotte, 1982). Estimates of twin pregnancy in wild seals is only 0.06 to 0.38% (Gelatt et al., 2001; McMahon & Hindell, 2003; Hoffman & Forcada, 2009; Schultz et al., 2011). Spotte (1982) reported just 42 cases of twins distributed over 11 pinniped species (both phocids and otariids), including a few cases in harbor seals. In these few harbor seal cases, either the female aborted or gave birth to the pups. However, the descriptions are very sparse and do not contain information about possible birth complications or whether the offspring survived the neonatal period. A dead stranded harbor seal was found in the state of Washington in the United States in 2013. She died while giving birth to conjoined twin fetuses (Olson et al., 2016). According to a news media report, live harbor seal twins were born in an aquarium facility in Dalian, China, in 2014, documented with images of mother and pups (Yang, 2015).

Herein, we report a case of twin pregnancy in a geriatric harbor seal that likely died due to severe pregnancy toxemia in an aquarium in Denmark. The purpose of this article is to describe findings from CT scanning and necropsy, in addition to providing a suggestion for the cause of the seal's death, as well as describing other findings in the seal.

Methods

Anamnesis

An almost 17-y-old female harbor seal (*Phoca vitulina*), raised in captivity at the North Sea Oceanarium (Hirtshals, Denmark), died after a period of 7 to 14 d in which it refused to eat. The normal feeding regime for a female harbor seal varies greatly between January and June, starting with 10,000 kcal/wk from 1 January and dropping steadily to 5,500 kcal/wk by March which continues until June. From June till the usual birth in July, the amount of feed is lowered gradually to 3,000 kcal/wk. In the case of this seal, no other clinical signs aside from the refusal of food were observed, and it was neither examined by a veterinarian nor had blood samples taken for diagnostics. No changes were made in the feeding procedure, and no investigation was undertaken in the anorexic period leading up to the death of the animal. The seal had previously given birth to seven viable singletons, was in late pregnancy, and was within days of expected parturition. A vaginal exploration revealed an approximately 4 cm dilation of the cervical canal indicating that the seal was in stage 1 of labor, but the seal died shortly after without further progression in labor. The carcass was immediately frozen at -18°C for later necropsy.

Postmortem Examinations

The carcass had a length of 155 cm and weighed 90.5 kg (Table 1). Initially, a full-body computed tomography (CT) scan of the frozen carcass was performed using a Canon Aquilion Prime SP system with the following parameters: x-ray tube voltage = 120 kVp, x-ray tube current = 280 mA, integration time = 280 ms, field-of-view = 683 × 683 × 1,559 mm^3, spatial resolution = 1 mm isotropic, convolution kernel = FC18, and acquisition time = 90 s. The scanning revealed that the seal was pregnant with two well-developed male fetuses (partially mineralized bacula). In addition, the CT examination revealed a well-calcified *os cordis* (heart bone) in the dam (Figure 1).

Necropsy after thawing revealed an abundant amount of slightly yellow discolored subcutaneous fat with a thickness ranging from 24 to 32 mm (see Table S2; the supplemental materials for this article are available on the *Aquatic Mammals* website). This blubber thickness was normal to high compared with the normal range of 18 to 27 mm for adult harbor seals in captivity (Mellish et al., 2007). The abdomen was almost completely occupied by a voluminous, almost solid uterus consisting of two equally sized uterine horns and a distended corpus (Figure 2a). A *corpus luteum* was present in each of the ovaries. Opening of the uterus along the greater curvature disclosed late-term male twin fetuses in anterior position weighing 8.95 and 9.14 kg, respectively. Postmortem degradation of the fetuses was limited. The fetuses were not necropsied due to an intended conservation use. The head of the right fetus was located close to the internal opening of the cervix. The cervical canal was open, and parts of the allantochorion of the right fetus protruded through the vulva and were ruptured. The rupture margins were not hemorrhagic indicating a postmortem rupture. The amount of fluid in the allantoic cavity was not measured for any of the fetuses, but it was considered to consist of less than 20 mL for the left fetus. The low volume was considered to be due to postmortem rupture of the allantochorion and fluid evacuation through the open cervical canal. The amount of amniotic fluid measured 405 and 413 mL for the right and left fetus, respectively. The zonary placenta of both fetuses was well developed and located in the proximal part of the uterine horns.

Due to the size of the twin-pregnant uterus, other abdominal structures were compressed (see sagittal and coronal CT-sections in Figure 1a & 1b). The amount of thoracic and abdominal fat was

Table 1. External measurements of the late-term twin-pregnancy harbor seal (*Phoca vitulina*)

Parameter	Dam	Right fetus	Left fetus
Sex	female	male	male
Weight	90.5 kg	8.95 kg	9.14 kg
Length (total)	155 cm	85 cm	87 cm
Length (snout-tail)	137 cm	75 cm	77 cm
Curve measurement (snout-tail)	154 cm	83 cm	84 cm
Posterior sternum blubber thickness	32 mm	--	--
Hip-spine blubber thickness	24 mm	--	--
Scapula spine blubber thickness	31 mm	--	--

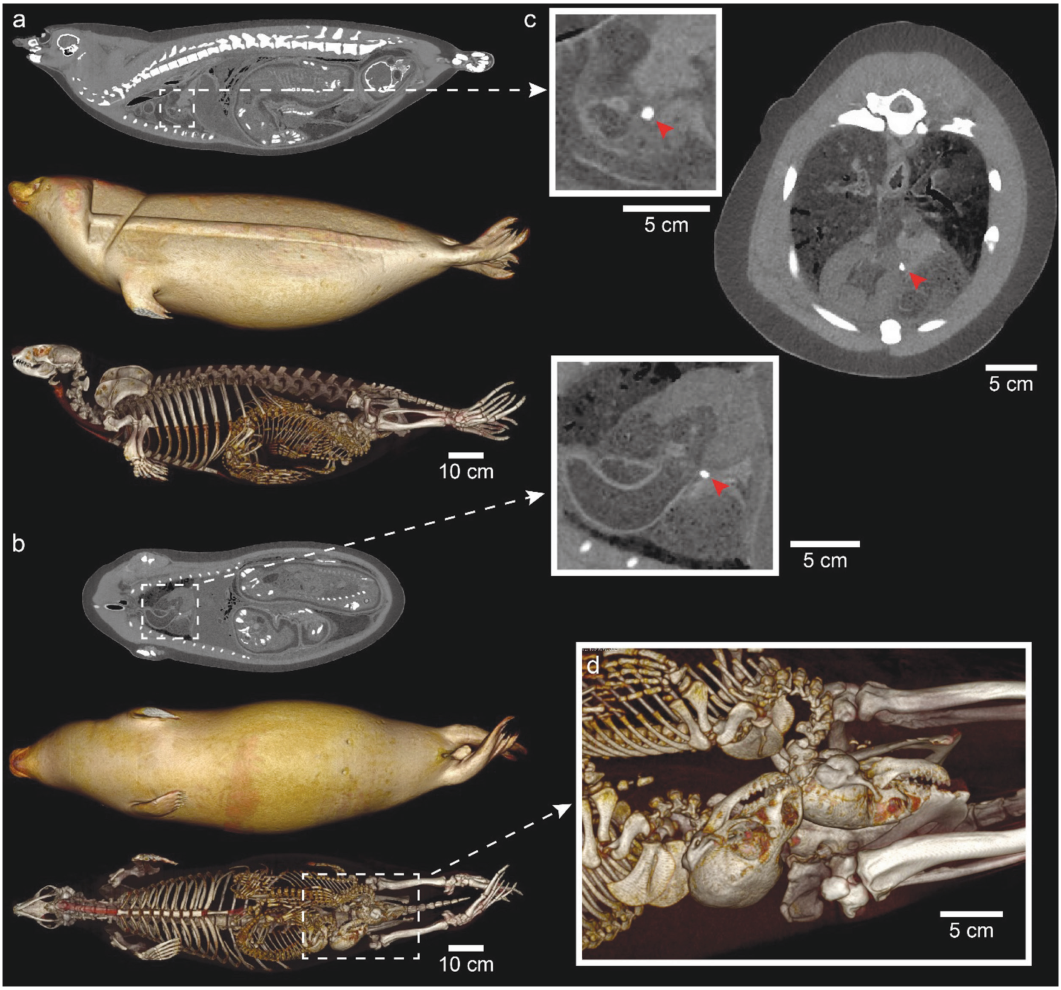

Figure 1. X-ray computed tomography examination: (a) sagittal section (top) and lateral volume renderings of total body (middle) and skeleton (bottom); (b) coronal section (top) and ventral volume renderings of total body (middle) and skeleton (bottom); (c) sagittal (top left, magnified from [a]), transversal (middle right), and coronal (bottom left, magnified from [b]) sections of the heart demonstrating the presence of a well-calcified *os cordis*; and (d) magnification of ventral volume rendering in (b).

minimal. The stomach and intestines were empty and were pressed against the diaphragm. The liver was diffusely enlarged with a weight of 3.58 kg and a uniform light brown color, an appearance consistent with diffuse hepatic steatosis ("fatty liver") (Figure 2b). Multiple, irregularly shaped, sometimes coalescing, dark brown, slightly depressed, firm nodules, measuring up to around 2 cm in diameter, were found in the liver, predominantly in the lobes to the right (Figure 2c). The overlying peritoneum was fibrotic. Cross-section identified the nodules as caseous liver necroses bordered by a 1- to 2-mm thick fibrotic capsule. The hepatic lymph nodes were of normal size. The carcass was otherwise unremarkable.

Specimens of the lung, heart, and liver, as well as nodules and lymph nodes, spleen, adrenal gland, kidney, and musculature, were fixed in 10% neutral buffered formalin for histology. Paraffin embedded tissue blocks were prepared for histology by standard methods, and 4 µm tissue sections were stained by hematoxylin and eosin, Masson trichrome method, and gram stain. Interpretation of the tissue section was severely compromised due to artefacts related to autolysis, freezing, and thawing, but it confirmed the gross diagnoses.

A specimen of the liver containing a nodule was refrozen and submitted for aerobic and anaerobic bacteriological and mycological culturing. Growth of bacteria or fungi was not observed after

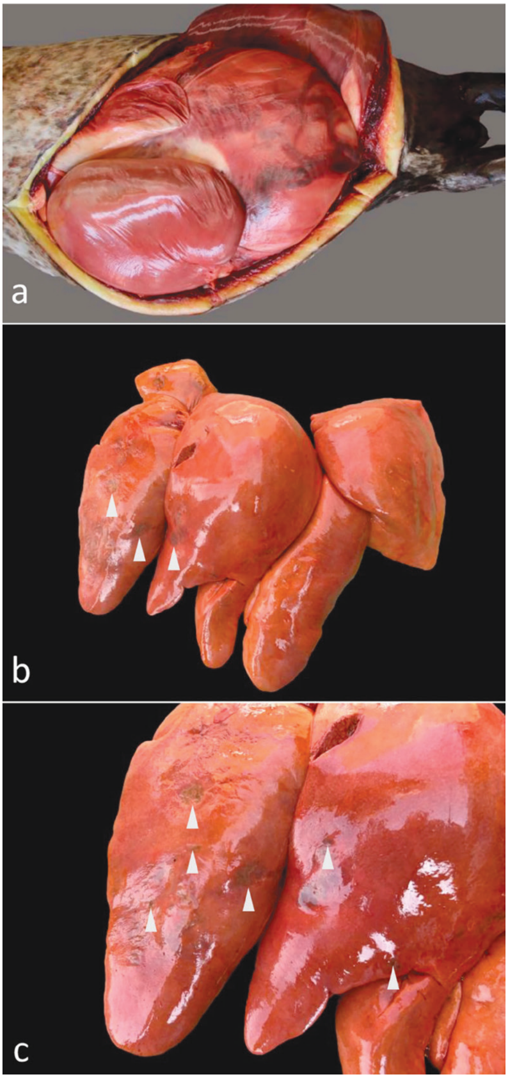

Figure 2. Gross lesions: (a) opening of the abdomen revealed a large uterus that occupied most of the abdominal space; (b) enlarged liver of uniform light brown color (an appearance being consistent with diffuse hepatic steatosis) and multiple, irregularly shaped, sometimes coalescing, dark brown, slightly depressed, firm nodules (see arrow heads); and (c) detail of the liver showing the multiple nodules present (see arrow heads).

incubation at 37°C for 48 h on 5% calf blood agar and 5 d on Sabouraud-dextrose agar.

Discussion

The most striking necropsy findings were a late-term twin-pregnant uterus that occupied most of the abdominal space, an abundant amount of fat, compressed and empty stomach and intestines, and a diffusely enlarged liver of uniform light brown color. In sheep and goats, such postmortem findings are characteristic for hepatic steatosis and pregnancy toxemia (Tontis & Zwahlen, 1987; Mongini & Van Saun, 2023). In the present case, the expanding size of the uterus by fetal growth and amniotic and allantoic fluids caused compression of the harbor seal's stomach and intestines, which is suspected to have reduced its eating capacity. Studies in pregnant sheep have shown that the volume of the rumen is reduced during late pregnancy due to an increasing competition for space in the abdomen between the stomachs, abdominal fat, and the continuously expanding gravid uterus (Forbes, 1968, 1969). Although a seal's monogastric anatomy is not directly comparable with the four stomach compartments of a ruminant, this phenomenon hypothetically also applied to our case leading to the decreased volume of the seal's stomach.

Combined with high fetal requirements for nutrients during late gestation, this situation probably caused excessive mobilization of body fat to compensate for a reduced energy intake and subsequent development of hepatic steatosis. To the authors' knowledge, pregnancy toxemia has not previously been reported in seals but should be considered if late-term pregnant seals show signs of disease. The condition can be diagnosed antemortem by measuring blood glucose and ketone body levels (e.g., acetoacetic acid, acetone, beta-hydroxybutyric acid), but analysis for other components, such as calcium, is also recommended as studies in small ruminants have revealed that animals suffering from pregnancy toxemia may have profound metabolic changes (Mongini & Van Saun, 2023). For sheep and goats, prognosis for maternal survival highly depends on early detection of the condition and proper management. Transabdominal scanning of pregnant seals in captivity can reveal early detection of twin pregnancy; however, prevention of pregnancy toxemia may be challenging in seals. A focus on obtaining an optimal body condition and close health monitoring, including regular blood parameter measurements, is recommended. A treatment regime for pregnancy toxemia in seals has not been developed and is challenged by the nature of seals. Examples, such as prolonged intravenous administration of glucose, may be difficult; and premature termination of pregnancy by cesarean section is recommended to only be performed by surgeons who specialize in zoological medicine.

Multiple necroses were found in the harbor seal's liver. Although only present in the right side of the liver, the distribution indicates a hematogenous spread of a pathogen; however, a pathogen was neither observed in tissue sections nor cultured. This indicates that the seal's immune system managed to clear the infection. The associated fibrosis indicates that the nodules developed prior to the state of pregnancy toxemia and that liver necrosis was a concurrent condition of unresolved etiology present by coincidence.

Although, unrelated to the condition of pregnancy toxemia, it is of interest to note the presence of a well-calcified *os cordis* (heart bone) in the seal's heart. To our knowledge, this is the first observation of this structure in phocids (Best et al., 2022) and only the second in pinnipeds after a recent examination of Steller sea lions (*Eumetopias jubatus*; Yoshida et al., 2022).

As the study was based on a harbor seal that died spontaneously and without knowledge of the twin pregnancy and the hypothesized complications, samples that could have confirmed the presence of pregnancy toxemia were unfortunately not taken. Repeated analyses for plasma-ketone bodies and other compounds would have strengthened this case report. Also, a necropsy performed shortly after the seal died rather than on a frozen and later thawed carcass would have been preferred. In conclusion, to our knowledge, this is the first reported twin-pregnant harbor seal reared in captivity that died in late term from suspected pregnancy toxemia.

Note: The supplemental materials for this article are available in the "Supplemental Material" section of the *Aquatic Mammals* website: https://www.aquaticmammalsjournal.org/supplemental-material.

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