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NOTE

Persistent Müllerian duct syndrome in a beluga whale *Delphinapterus leucas*

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ABSTRACT: This case study describes a persistent Müllerian duct syndrome (PMDS), a rare form of XY disorder of sex development (DSD), in a mature (>15 yr) beluga whale *Delphinapterus leucas*. The phenotypically and genetically male beluga whale had both Müllerian (paramesonephric) and Wolffian (mesonephric) duct derivatives. A mild hydrometra was present. Gross and histological analyses indicated the testes were atrophied. Histopathologic findings in the testes also included fibrosis in addition to ductus deferens ductular dilation, epididymal ductular dilation, lymphoplasmacytic balanitis, uterine glandular atrophy, and endometrial fibrosis. DSDs are rarely reported in cetaceans, and PMDS has never been described in a cetacean.

KEY WORDS: Beluga whale · *Delphinapterus leucas* · Developmental sex disorder · Anti-Müllerian hormone · AMH · AMH receptor · Persistent Müllerian duct syndrome

1. INTRODUCTION

Disorders of sex development (DSDs), formerly described as intersex, are defined as congenital conditions associated with atypical development of chromosomal, gonadal, or anatomical sex (Hughes 2008, Meyers-Wallen 2012). While DSDs have been well studied in humans and domestic species, this is not the case for most wildlife (Mastromonaco et al. 2012). Among aquatic mammals, DSDs have predominantly been reported in cetaceans. In both baleen and toothed whales, XY DSD or XX DSD (formerly pseudohermaphrodism) are the most frequently reported. In 4 of the 5 published DSD case reports, the individuals were phenotypically female: striped dolphin *Stenella coeruleoalba* (Niseiiwaki 1953), fin whale *Balaenoptera physalus* (Bannister 1963), bowhead whale *Balaena mysticetus* (n = 2; Tarpley et al. 1995); with only one phenotypically male (a beluga whale *Delphinapterus leucas*; Mikaelian et al. 2003). Ovotesticular DSD (true hermaphrodism; XY sex reversal), with presence of both types of gonads and ovotestes, has been reported in the common dolphin *Delphinus delphis* (Murphy et al. 2011) and the beluga whale (De Guise et al. 1994). We report on the descriptive anatomy and microanatomical findings of persistent Müllerian duct syndrome (PMDS), a rare form of XY DSD, in a beluga whale.

2. MATERIALS AND METHODS

As part of the North Slope Department of Wildlife Management (NSB DWM) Beluga Whale Subsistence Harvest Monitoring Program in Alaska (Frost & Suydam 2010), opportunistic tissue samples from a subsistence harvested beluga whale with an estimated body length of at least 3.96 m (tip of the snout

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to fluke insertion; fluke had been removed by hunter) were collected on the beach during August 2017 in Utqiagvik, Alaska (71.2906° N, 156.7886° W). The internal reproductive tract was transferred to the NSB DWM necropsy suite and stored frozen (–20°C) until analyzed.

2.1. Histology and gross morphology

Sex identification was determined by palpation of the genital slit for the presence or absence of the penis. Tissue samples were collected from the derivatives of the paramesonephric duct (uterus, uterine horn, uterine tube) and mesonephric duct (testis, epididymis, ductus deferens) and fixed in 10% neutral-buffered formalin. The tissues were processed for paraffin histology, sectioned at 4–6 µm, and stained with hematoxylin and eosin (H&E; Histology Consultation Services). Reproductive organs were evaluated for the presence of normal architecture and in the case of gonads, developmental and maturational stages. Standard morphometric measurements (length and width) using a ruler were taken of the Müllerian and Wolffian duct derivatives.

2.2. Genetics

A liver sample was frozen at –50°C and submitted for a PCR-based method for gender determination (Palsbøll et al. 1992). A sample was extracted using a DNeasy Blood & Tissue Kit (Qiagen). Total DNA was quantified by spectrophotometry on a NanoDrop, and a dilution of 10 ng μl^{-1} was aliquoted, ensuring a known quantity of DNA was added to the PCR reaction.

Sex was determined by co-amplifying the ZFX genes (Aasen & Medrano 1990) and the SRY genes (Fain & LeMay 1995) (see Tables A1–A3 in the Appendix). Females (XX) have a single band around 400 bp long and males (XY) have 2 bands, one at about 400 bp and one around 200 bp (Palsbøll et al. 1992). The sample was independently amplified twice, and each time a negative control was included to ensure contamination was not a factor.

3. RESULTS

Externally, a normally developed penis was located in the genital slit. Mammary slits were absent. The urethra opened at the tip of the penis (Fig. 1A). During field examination of the abdominal body cavity, a female reproductive tract-like structure with paired gonadal tissues was observed. (Fig. 1B). Both the uterine horns and uterus body were filled with red-tinged viscous fluid (104 cm^3). The inner lining of the short uterine body was smooth and the uterine horns had diagonal pleats arranged in 2 rows separated by a section of smooth lining starting at the tip of the horns and extending approximately midway the lengths of both horns (Fig. 1C). Between 4 and 9 longitudinal pleats extended the entire length of the upper vagina (Fig. 1C). The constriction of the uterine body marking the transition to the vagina and the circular (transverse) folds of the vaginal mucosa characterizing the os uteri of the cervix were absent, ending the vagina blindly. Small, underdeveloped testes and epididymides were tightly attached to the uterine tubes (Fig. 1B). At the confluence of the uterine tube (syn. fallopian tube; oviduct) and uterine horn, both uterine tubes were without a visible lumen and ending blindly. The highly tortuous vas deferens (syn. ductus deferens) followed the entire length of the uterine horns and fused midway with the uterus wall. The opening of both vas deferens was visualized lateral to the pelvic urethra in the transverse section (Fig. 1D). Detailed morphometric measurements (length × width) of the respective reproductive structures are summarized in Table 1.

Microscopically, uterine tubes had an orderly arrangement of layers. There were no glands and the lining endometrial mucosal epithelial cells were attenuated. Uterine horn glands were reduced in number and size and separated by abundant supporting stroma (Fig. 2B). Histopathologic findings in the

Table 1. Morphometrics (length and width) of paramesonephric and mesonephric duct derivatives in a male mature beluga whale *Delphinapterus leucas* taken by subsistence hunt, during August 2017, Utqiagvik, Alaska

Reproductive structure	Length (mm)	Width (mm)
Uterine body	60	40
Uterine horn		
Right	105	50
Left	120	60
Uterine tube		
Right	100	4
Left	110	7
Vagina	145	
Testis		
Right	85	40
Left	95	40
Epidydimis		
Right	140	40
Left	190	40

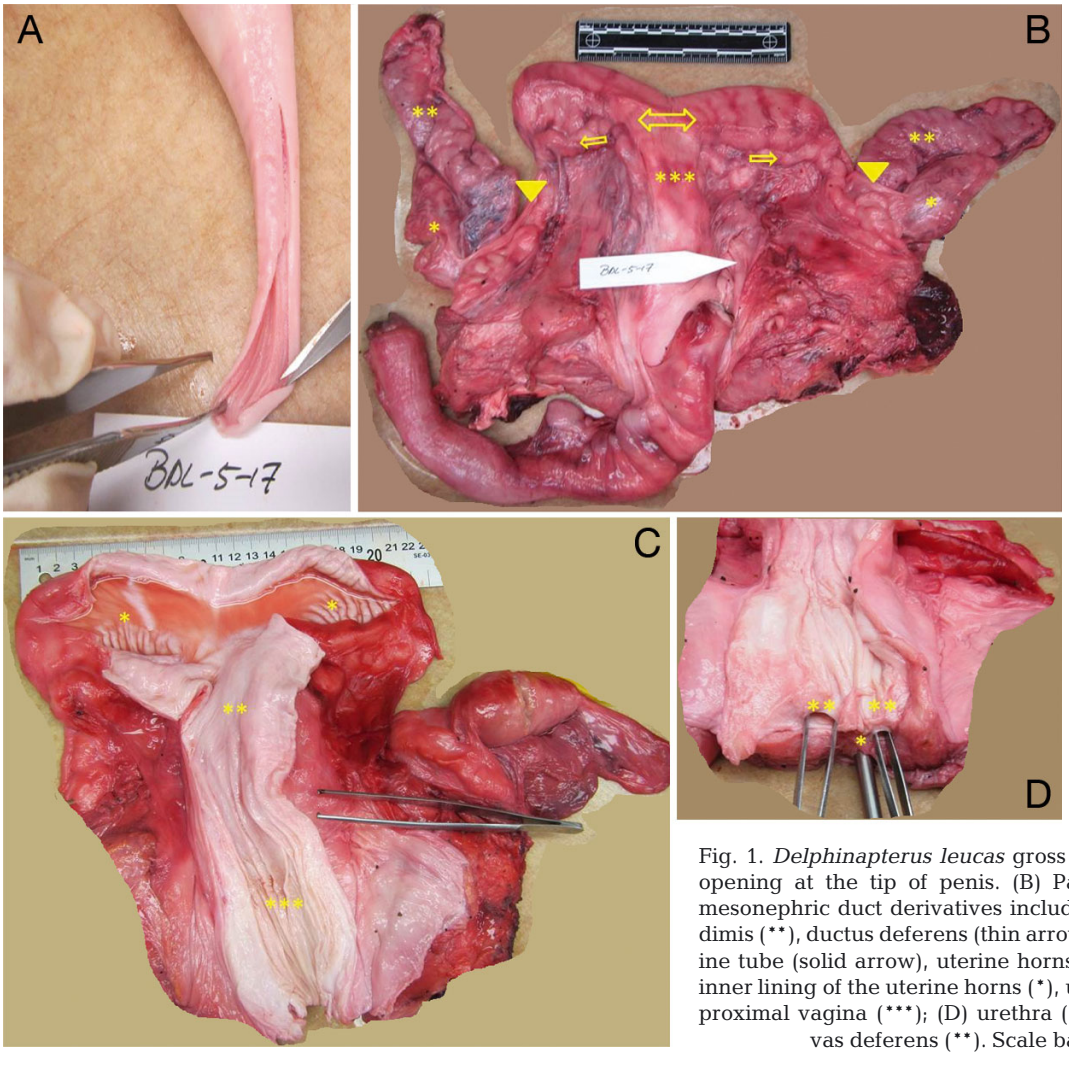

Fig. 1. *Delphinapterus leucas* gross images. (A) Urethra opening at the tip of penis. (B) Paramesonephric and mesonephric duct derivatives including testis (*), epididymis (**), ductus deferens (thin arrow), uterus (***), uterine tube (solid arrow), uterine horns (double arrow); (C) inner lining of the uterine horns (*), uterine body (**) and proximal vagina (***); (D) urethra (*) and right and left vas deferens (**). Scale bar is in cm

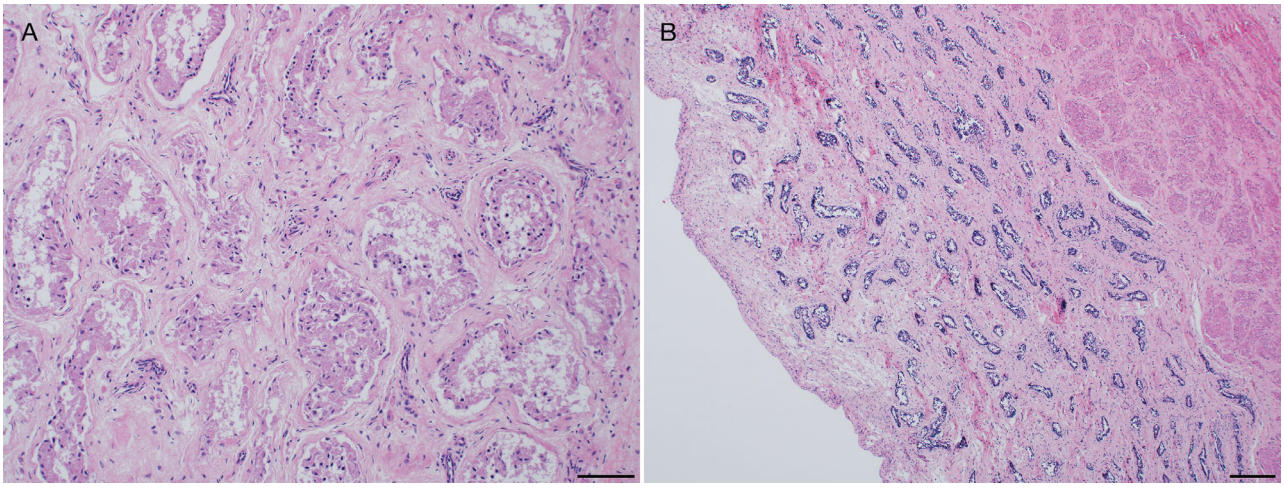

Fig. 2. *Delphinapterus leucas* histopathology. Hematoxylin and eosin. (A) Testicular seminiferous tubules are atrophied. Lumina are dilated with spermatagonia and spermatazoa not present. Tubules are separated by collagen (fibrosis). Scale bar = 100 µm. (B) Uterine glands are reduced in sized and separated by abundant stroma. Scale bar = 200 µm

testis included seminiferous tubular atrophy and interstitial fibrosis (Fig. 2A). Few tubules had spermatagonia and few spermatozoa. Epididymal ducts were dilated with mucosal infolding and ductular separated by variably dense collagen. Few ducts contained spermatozoa. The presence of 2 bands on the agarose gel indicates PCR amplification of fragments from both the X (ZFX) and Y (SRY) chromosomes (Fig. 3), confirming that the individual was genetically male.

4. DISCUSSION AND CONCLUSIONS

The beluga whale taken by a subsistence hunter from Utqiagvik, Alaska, most likely belonged to the Eastern Beaufort stock (O'Corry-Crowe et al. 2018). Based on body length and white skin coloration, the male was mature with an estimated age of at least 15 yr (Burns & Seaman 1988). Reproductive anomalies, including DSDs, have not been reported for the 5 subpopulations of belugas known to occur in the Bering, Chukchi, and Beaufort seas. However, 2 DSD cases have been reported for the endangered, genetically and geographically isolated, non-migratory St. Lawrence Estuary beluga whale stock (De Guise et al. 1994, Mikaelian et al. 2003).

This is the first report of PMDS in cetaceans. An undifferentiated Müllerian duct remnant has been previously reported in a white beaked dolphin *Lagenorhynchus albirostris* (Meek 1918), but not PMDS. PMDS, a rare form of XY DSD, whose molecular background has been determined in humans, 2 dog breeds, and the house mouse, is caused by gene mutations affecting either the synthesis of anti-Müllerian hormone (AMH; Müllerian inhibiting substance) or its action by modifying its type II receptor (MISRII/AMHR2) (Behringer et al. 1994, Wu et al. 2009, Picard et al. 2017). AMH deficiency permits differentiation of the Müllerian duct into the uterus, uterine tubes, and the upper vagina. Hormone-mediated male sex differentiation proceeds otherwise normally with non-ambiguous external masculinization and differentiation of internal gonads (testis, epidydimis, vas deferens) from the mesonephric duct (Wolffian duct). In other terrestrial species, PMDS has rarely been reported, and determination of the molecular background is lacking (Schulman & Levine 1989, Haibel & Rojko 1990, Meyers-Wallen 2012, Panasiewicz et al. 2015). In this case, the genetic basis (i.e. gene mutations) of PMDS was not determined, as commercially available genetic tests available for canids (Wu et al. 2009) have not been validated for

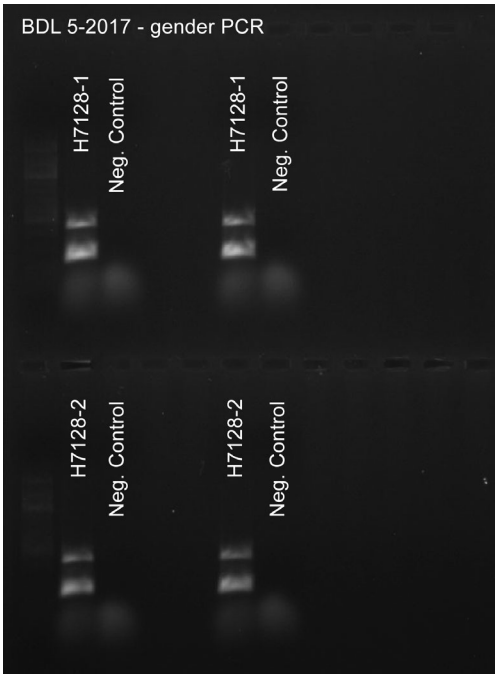

Fig. 3. *Delphinapterus leucas* PCR amplification products of the ZFX and SRY regions of the X and Y chromosomes on agarose gel. Determination of male gender was based on the number of bands for the individual, one corresponding to the X chromosome and one to the Y. Sample was amplified twice on 2 separate occasions and each run with a negative control, ensuring results were reproducible and no contamination occurred

cetaceans. PMDS in the beluga whale presented similar to PMDS-affected male dogs, with external and cytogenetical male traits, bilateral testes tethered tightly to the uterine tubes, vas deferens included in the lateral walls of the uterus, and presence of a mostly complete Müllerian duct system (uterus, uterine tubes, upper vagina) with only the cervix absent (Meyers-Wallen 2012). The observed oblique organization of the folds in the uterine horns and the absence of longitudinal folds in the uterus body most likely represent a morphological variation related to PMDS. Longitudinal folds in both the uterine body and horns of a female beluga whale have been described previously by Watson & Young (1880). Uterus size was comparable to that of an immature female beluga whale (uterine body: 51 mm; uterine horns: 152 mm; uterine tube: 76 mm; Watson & Young 1880).

In this whale, testicular atrophy was observed grossly and histologically (multifocal, moderate atrophy with interstitial fibrosis). There was minimal spermatogenesis. Given that peak breeding season for beluga whales in Alaska is thought to occur

between late February and early April, and adult male beluga whales sampled during April and May already showed 'retrogression phase of the annual cycle of spermatogenesis' (Burns & Seaman 1988), we cannot rule out that minimal spermatogenesis in our case also reflects timing of the breeding season. Overall testes size (length × width) was much smaller (85–90 × 40 mm) than a mature, similar sized male beluga whale (195 × 85 mm) harvested the same day. Testosterone production was not determined in this animal, but the phenotypic male appearance and spermatogenesis (albeit limited and rare spermatozoa on histology) suggests functional Sertoli and Leydig cells. In dogs with PMDS, about 50% of individuals with unilateral cryptorchidism have normal testosterone production, and dogs are fertile (Wu et al. 2009). Additional sequela observed in dogs with PMDS include uterine diseases (pyometra; hydrometra) and testicular tumors of the cryptorchid testis (Matsuu et al. 2009, Wu et al. 2009). In cetaceans, intra-abdominal location of testis is physiological, with testicular tumors being rare (Díaz-Delgado et al. 2012). In this case, additional sequela was observed in the form of a hydrometra.

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Appendix. Details of PCR used for sex determination

Table A1. Primer sequences and references

Primer	Sequence (5′–3′)	Fragment length (bp)	Reference
ZFX-P1-5EZ	ATA ATC ACA TGG AGA GCC ACA AGC T	~400	Aasen & Medrano (1990)
ZFX-P2-3EZ	GCA CTT CTT TGG TAT CTG AGA AAG T		
SRY-Y53-3C	CCC ATG AAC GCA TTC ATT GTG TGG	~200	Fain & LeMay (1995)
SRY-Y53-3D	ATT TTA GCC TTC CGA CGA GGT CGA TA		

Table A2. PCR thermocycling conditions

Step	Temp. (°C)	Time (min:s)	Cycles
Initial denaturation	90	2:30	1
Amplification			30
Denaturation	94	0:45	
Annealing	68	1:00	
Extension	72	1:30	
Final extension	72	5:00	1

Table A3. PCR master mix

Component	Conc.	Vol. reaction^{-1} (µl)
MilliQ H_2O (18 MΩ)	–	13.875
10× Buffer ($MgCl_2$)	20 mM	2.5
dNTPs	10 mM	0.375
Primers		
ZFX-P1-5EZ	10 µm	0.75
ZFX-P2-3EZ	10 µm	0.75
SRY-Y53-3C	10 µm	0.75
SRY-Y53-3D	10 µm	0.75
Taq polymerase	U µl^{-1}	0.25
DNA	10 ng µl^{-1}	5
Total volume reaction^{-1}		25

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