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SHORT PAPER

True Hermaphroditism: First Evidence of an Ovotestis in a Cetacean Species

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Summary

An immature unilateral hermaphrodite common dolphin (*Delphinus delphis*) was found stranded on the southwest coast of the UK. The external phenotype was that of a female, but internally there was one ovotestis, containing both ovarian follicles and testicular tubular elements, and a contralateral ovary. Ovarian portions of the ovotestis appeared normal and demonstrated follicular development, whereas the testicular tissue exhibited hypoplasia and degeneration. This is the first reported case of an ovotestis in a cetacean species.

Keywords: common dolphin; *Delphinus delphis*; ovotestis; true hermaphroditism

True hermaphroditism is defined as the simultaneous presence in a single individual of both testicular and ovarian tissue, which may exist either in separate gonads or in the same gonad (ovotestis; Krob *et al.*, 1994). True hermaphroditism can be bilateral (testicular and ovarian tissue identified on both sides, usually as ovotestes), unilateral (ovotestis on one side and an ovary or testis on the other side) or lateral (testis and contralateral ovary) (Tangner *et al.*, 1982; De Guise *et al.*, 1994). The determination of genetic sex (XX or XY) is fixed at the time of fertilization. The mammalian Y chromosome carries a testis determining factor (TDF) gene *SRY*, and at the moment of sex determination stimulates the undifferentiated fetal gonad to develop into a testis (and production of testosterone by the Leydig cells), which subsequently gives rise to the male phenotype. The female gender is the default option, where due to the absence of the TDF (or if the *SRY* function is impaired) the undifferentiated gonad will develop into an ovary and the undifferentiated tubular genital tract will develop into normal female genitalia unless male hormones are present (Kennedy and Miller, 1993). Disorders of genital development, such as sex reversal, are caused by abnormalities of genetic or chromosomal origin or inappropriate hormone exposure. For example, XX mice which, as a result of mutation, carry an autosomal dominant gene *Sxr* that acts like the Y chromosome, develop testes and male tubular genitalia (Kennedy and Miller, 1993). In amphibians it has been reported that oestrogens can induce sex reversal in genetic males, producing either an ovotestis or complete and permanent feminization (Lofts, 1974; Qin *et al.*, 2003).

As part of the UK Cetacean Strandings Investigation Programme a carcase was recovered from the southwest coast of the UK in December 2006 for a routine post-mortem examination. The animal, identified as a common dolphin (*Delphinus delphis*), was of moderate nutritional status with a dorsal blubber thickness of 13 mm. The dolphin measured 191 cm in length and was estimated to be 6 years old on the basis of counting growth layer groups in the dentine as described by Murphy and Rogan (2006). On external appearance the dolphin was sexed as female, due to the presence of a mammary slit with corresponding teat positioned on either side of the genitoanal slit, which housed the anus and urogenital openings.

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Routine necropsy and bacteriological examinations were conducted according to standardized protocols (Jepson, 2005). The post-mortem examination revealed no significant abnormalities of the mammary glands, and the ovaries, uterus and vagina were reported as unremarkable. Both ovaries were retained and fixed in 10% neutral buffered formalin. The cause of death was not established because of scavenger damage and as the carcase was in a moderate state of decomposition. However, one possible net mark was observed on the right side of the thorax, suggesting incidental capture in fishing gear. *Brucella* antibodies were identified in a sample of pericardial fluid by the Rose Bengal plate test, but follow-up testing by enzyme-linked immunosorbent assay proved negative. Bacteriological analysis of a number of tissues did not reveal the presence of *Brucella*.

Ovary A weighed 1.09 g and measured 24.8 × 10.2 × 8.4 mm. Ovary B weighed 1.41 g and measured 26.6 × 13.4 × 7.5 mm. Both ovaries had normal external appearance with no corpus luteum or albicans, consistent with this being an immature female (Fig. 1a). Ovaries were sectioned (slices of 0.5–2 mm) and these sections were examined under a dissecting microscope. There was evidence of follicular growth in both ovaries, with a maximum follicle diameter of 1.75 mm (ovary A). However, the initial dissection of ovary A (a longitudinal transverse cut) revealed a scar-like structure located in the centre of the ovary (within the cortex adjacent to the medulla), which was tan to yellow in colour and measured 6.83 × 5.49 mm (Fig. 1b). Examination of ovary B revealed no other signs of activity (or abnormality) apart from follicular growth.

Tissue samples from both ovaries were processed routinely, embedded in paraffin wax, sectioned (4–6 μm) and stained with haematoxylin and eosin (HE). Microscopical examination of both ovaries revealed the presence of numerous blood vessels within the medulla and primordial and developing follicles (including primary, secondary and secondary-vesicular) within the cortex. Follicles were in various stages of degeneration/autolysis. Female common dolphins in the northeast Atlantic attain sexual maturity at an average length of 188.8 cm and an average age of 8.2 years, and within a sample size of 189 mature females, only one 6-year-old female was classified as sexually mature (Murphy *et al.*, 2009). Based on these data, and an estimated age of 6 years, the dolphin reported here would be classified as sexually immature.

Microscopical analysis of the scar-like tissue in ovary A revealed evidence of testicular tubular elements within the ovarian stroma. This testicular tissue appeared to be slightly compartmentalized, with connective tissue separating the ovarian and testicular components (Fig. 2a). Interstitial tissue and seminiferous tubules with Sertoli cells and spermatogonia were also present. Tubules were slightly atrophic and spindle-shaped Sertoli cells were located both in the basal area and within the lumen of the tubule. Round or polygonal cells (Leydig-like) with granular eosinophilic cytoplasm and small round nuclei were scattered between the seminiferous tubules (Fig. 2b).

Further sections taken from the centre of the testicular tissue revealed evidence of spermatogenesis with the presence of spermatocytes. The proportion of interstitial tissue was relatively high compared with the number of seminiferous tubules, suggesting an immature stage of development. However, the relative diameter of a number of the seminiferous tubules was that of a sexually mature individual. Seminiferous tubules were in various stages of degeneration

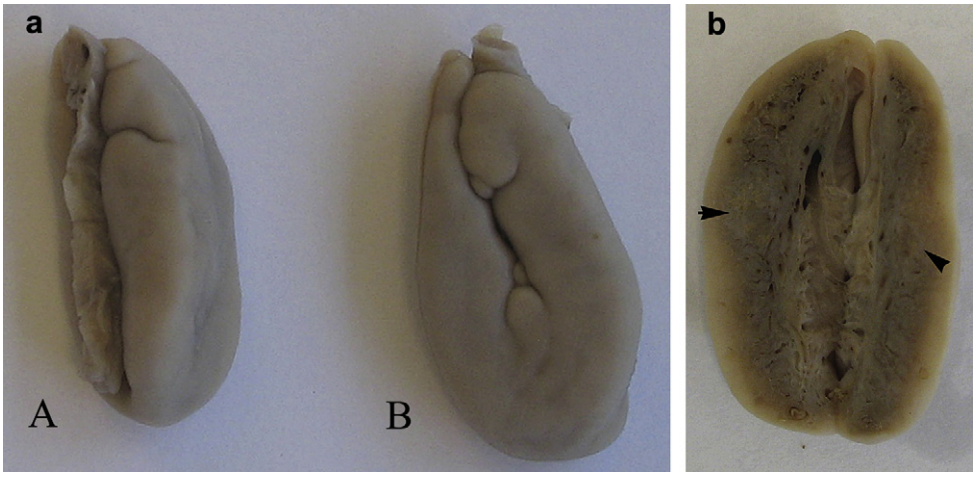

Fig. 1. (a) External appearance of ovaries A and B. (b) Longitudinal transverse cut through ovary A; arrows indicate position of scar-type tissue in the cortex (arrowheads).