Morphological studies of fetal membranes as a tool for researches in marine mammal science


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Studies of marine animal morphology, specifically on development, are scarce. Many researchers are unaware of data on reproductive morphology of *Sotalia guianensis*, a species with large numbers of stranding along the Brazilian coast. Since studies on the subject may increase the species conservation methods, this study aimed to provide morphological and ultrastructural description of fetal annexes from animals stranded on the Brazilian coast, through light and scanning electron microscopy. The placenta was epitheliochorial and diffuse. The umbilical cord was provided with two veins, two arteries and the urachus, having a microvascular system of communication between the four large vessels. The amnion was covered by a simple squamous epithelium, appearing to be avascular. These data showed that fetal annexes had similar characteristics to those found in terrestrial mammals, except for the umbilical cord, which had two arteries and two veins, providing a higher vascularization to the fetus.

**Keywords:** marine mammal; morphology; cetacean studies; fetal annexes

1. Introduction

Nowadays, sciences such as anatomy and histology have been in the background when compared to other areas of veterinary medicine, being considered basic, hence immutable and of extensive knowledge. However, a deep understanding of these sciences is essential in clinical practice, since morphology defines all physiological studies in domestic and wild animals. Physiological and structural characteristics of organs and their systems are widely known in domestic animals (Konig & Liebich, 2011), with its architecture and microscopic structure being well-defined. The same is observed in several species of wild animals (Cubas et al., 2007).

Morphological studies on marine mammals are old and scarce (Green, 1972; Harrison, 1974; Pabst et al., 1999). Despite the growing number of cetaceans stranding (dolphins and whales) in the Brazilian coast over the past years and the constant conservation efforts for the creation of laws and protection areas of Brazilian marine fauna, most animals listed in the Action Plan for Marine Mammals of Brazil (Plano de Ação para Mamíferos Aquáticos do Brasil; IBAMA, 2001) is classified in the category "Data Deficient" and much remains unknown.

Morphological data from pregnant or lactating specimens are extremely rare and difficult to obtain. For greater reproductive knowledge of *Sotalia guianensis*, a species with large numbers of stranding along the Brazilian coast, this study used microscopic images of fetal annexes, with their morphological and ultrastructural description.

2. Material and methods

Placenta, umbilical cord and fetal membranes samples were collected from two estuarine dolphin (*Sotalia guianensis*) females. Specimens of unidentified age groups were from stranding events in the Sergipe-Alagoas basin and samples were kept by the research institution Fundação Mamíferos Aquáticos – FMA (SISBIO Permit 37393-1). Samples were all fixed in 10% formalin solution.

For light microscopy, samples were washed, dehydrated in increasing ethanol solutions, cleared in xylene and embedded in paraffin. Sections (6μm) were stained by hematoxylin-eosin, Sirius red and Periodic acid-Schiff (PAS) (Adapted from Tolosa et al., 2003). Slides were captured and analyzed (Nikon Eclipse E-800). For scanning electron microscopy, samples were washed in distilled water and dehydrated in ascending series of ethanol under rotation. After, they were critical point dried, assembled in metallic aluminium bases (stub) using carbon adhesive paste and coated with gold by sputtering to be analyzed (LEO 435 VP microscope).
3. Results

The placenta (epiteliocorial type) had a wrinkled surface. The chorionic surface was covered with villi, characteristic of diffuse placentation (Fig. 1). The chorionic villi had cuboid trophoblast cells, underlying a collagen stroma little populated by fibroblasts and vascularized by small vessels located close to the trophoblast. The amnion was covered by a simple cuboid epithelium in its inner face and by simple squamous epithelium on its outer face, appearing to be avascular (Fig. 2).

![Fig. 1 Photomicrography of a Guiana dolphin (Sotalia guianensis) placenta. A. Scanning electron photomicrography. Chorion region with chorionic villi. 48,000X. B. Allantochorion region. Allantois (A), blood vessel (V), fibroblasts (F), chorion (C) trophoblast (T). Periodic acid-Schiff (PAS). 20X. Highlighted area: vacuoles indicated by arrowheads. PAS. 100X.](image1)

![Fig. 2 Photomicrograph of an amnion from a Guiana dolphin (Sotalia guianensis). Simple squamous epithelium (arrowhead) on the outer surface, without any sign of vascularization. . A. Hematoxylin-eosin. 40X. B. Sirius red. 40X.](image2)

The umbilical cord was coated by visible hipomanis (Fig 3A), being provided with two veins, two arteries and the urachus, having a microvascular system of communication between the four large vessels (Fig. 3B). The umbilical artery endothelium was highly pleated (Fig. 3C), unlike the umbilical vein, where the vascular endothelium pleats were often imperceptible (Fig. 3D). Arteries and veins had thick tunica media, underlined by elastic fibers covering its inner smooth muscle cells, of epithelioid shape and longitudinal orientation. The tunica adventitia had higher amount of collagen and elastic fibers, as well as small vasa vasorum. (Fig 3E). The urachus was coated by a transitional epithelium, (Fig. 3F).
Fig. 3 Photomicrograph of umbilical cord from Guiana dolphin (*Sotalia guianensis*). A and B. Scanning electron microscopy. A. Outer surface of a umbilical cord with the presence of hipomanis (arrowhead) in abundance. B. Presence of two arteries (arrowheads), two veins (*) and urachus (U). 24,000X. C. Umbilical artery. *Tunica adventitia* (Ta), *tunica media* (Tm), *Vasa vasorum* (arrowhead). D. urachus region, consted of a transitional epithelium. Sirius red. C. 4X. D. 10X.
4. Discussion

The chorioallantoic diffuse epitheliocorial placenta of the Guiana dolphin is compatible with those of other cetacean species (Zhemkova, 1967; Da Silva et al., 2007; Sasaki et al., 2013). A close contact between fetal trophoblasts and maternal endometrial epithelium is the feature which characterizes this type of placenta.

Chorionic villi are morphologically similar with pleats observed in Minke whales (Balaenoptera acutorostrata; Sasaki et al., 2013) and other cetaceans (Miller, 2007), different from that found in Sperm whales (Physeter macrocephalus) and Fin whales (Balaenoptera physalus), where these villi are shorter (Zhemkova, 1967). Trophoblast cells are mononuclear as well as the Minke whales in the Antarctic (Sasaki et al., 2013).

Cells from the chorionic villi surface were uniform, delimitated and had a single layer, as observed in other cetaceans. Vacuoles were observed at the villi edges, as well as the ones present in the White whale (Delphinapterus leucas) placenta (Zhemkova, 1967) and bottlenose dolphin (Tursiops truncatus) (Wislocki & Enders, 1941).

Wislocki & Enders (1941) observed a membranous amnion in bottlenose dolphins, with its largest portion merging with the inside wall of an avascular allantoic. In our study, this fetal membrane seems to be avascular and smooth.

The dolphin umbilical cord is different from the terrestrial mammals’, being composed of two arteries, two veins and one urachus. It was possible to observed several small tributary arterioles and venules irrigating all stromal cord. In the umbilical cord outer surface, a soft, glutinous, mucous structure is attached to the allantois membrane along its entire length, being called hipomanes or accretions, which corroborates the findings in Tucuxi (Sotalia fluviatilis) and the Amazon river dolphin (Inia geoffrensis) (Da Silva et al., 2007). This similarity was also observed in Tursiops truncates, when Wislocki & Enders (1941) described the hipomanes as dense lumps of connective tissue, where a melanin deposit occur, being covered by a single layer of cuboidal cells.

These data showed that fetal annexes had similar characteristics to those found in terrestrial mammals, except for the umbilical cord, which present a microvascular system composed of four vessels, providing a higher vascularization to the fetus. Further studies are necessary to evaluate if this characteristic is an adaptation related to the aquatic environment.

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References