Histological Studies on the Ovaries of Three Pacific White-sided Dolphins, *Lagenorhynchus obliquidens*, Stranded on the Coast of Niigata District, Sea of Japan

Yoshiharu Honma1, Tatsuo Ushiki1, Masae Takeda1 and Tadasu K. Yamada2

1) 3rd Department of Anatomy, Niigata University School of Medicine, 1-757 Asahimachi, Niigata, 951-8510 Japan
2) Department of Zoology, National Science Museum, Tokyo, 3-23-1 Hyakunincho, Shinjuku-ku, Tokyo, 169-0073 Japan

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Abstract: Ovaries of three individuals of the Pacific white-sided dolphins, *Lagenorhynchus obliquidens* Gill, 1865, were studied histologically to determine the maturity of gametes. The animals, 1.53-1.80m in total length and 50.0-74.5kg in weight, had been stranded on the coast of Niigata District, Sea of Japan. The ovaries were 37-40mm in length and 1.6-2.2g in weight. Macroscopically, every ovarian lobe had a smooth dorsal surface and intricately-folded ventral surface, characteristic of a young immature stage. Histological examination revealed that most of the oocytes had reached the primordial and primary follicle stages, but secondary follicles were seldom seen in the deeper zone of the cortex. Many atretic follicles in various stages of degeneration were also detected. However, neither corpus luteum nor corpus albicans was encountered, the overall picture being of young immature animals, in accordance with the macroscopic diagnosis.

On 2 January, 1997, the Russian tanker, HAXODKA (13,157t), was crippled and its back broken during a heavy storm off Shimane Prefecture, Sea of Japan. Although the posterior portion sank to the deep sea floor, the anterior portion drifted to offshore of Antoh beach, Mikuni Town, Fukui Pref., owing to the prevailing northwest wind and Tsushima warm current. Upon stranding on 7 January, ca.9,000kl of C heavy oil was spilt, spreading along the coast of Shimane Pref. to Yamagata Pref.

Considerable fewer was raised concerning the effects of the oil spill on the shore-life, in particular, young sea weed, *Laminaria* spp., and developing sea-urchin eggs, *Hemicentrotus*, in addition to the effects of submerged oil clumps on benthic animals.

On 23 January, 1997, a Pacific white-sided dolphin, its lungs contaminated with oil was stranded on the Uchinada beach of Ishikawa Pref., raising concerns on the effects of the spill on the tissues and organs of local cetaceans.

During this period, three further Pacific white-sided dolphins were stranded and retained for investigation. The ovaries of all three specimens were removed and forwarded to the authors for histological examination. This was carried out to determine the reproductive state of the dolphins and to report on their overall gonadal conditions at the time of stranding, in accordance with previous work (Honma et al., 1992; Honma, 1994a; Honma and Yamada, 1995).

Materials and Methods

Biological data for the three Pacific white-sided dolphins, *Lagenorhynchus obliquidens* Gill, 1865, are shown in table 1. The ovaries, previously immersed in 10% formalin, were refixed in Bouin's solution, and, after measurements of size and weight, 3 portions (anterior, central and posterior pieces) dehydrated through a graded alcohol series, embedded in paraffin and cut at 10-12 μm thickness. The sections were stained with Meyer's hematoxylin-eosin double, and aldehyde fuchsin (AF)-Masson-Goldner tetrachrome stains for demonstration of polysaccharides and examined under a light microscope (Orthoplan, Leitz).

Results

Macroscopy

The external appearance of each ovary was smooth, except in the medullary portion, which was penetrated by a thick blood vessel. But, no oil contamination and abnormality were seen in the ovary. Notably, neither developed Graafian follicles, protuberances of corpora lutea or albicantia, nor craters indicating recent ruptured follicles were encountered.

Specimen 1: stranded on Feb.5, 1997
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Table 1 Details (including those of ovaries) of Pacific white-sided dolphins, *Lagenorhynchus obliquidens* Gill, 1865, examined in this study.

<table>
<thead>
<tr>
<th>Date of stranding</th>
<th>Locality</th>
<th>Ovarian size and weight</th>
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<tr>
<td>1. 5 Feb., 1997</td>
<td>Chuoh Beach, Johetsu City, Niigata Pref.</td>
<td>R/L ? 37 × 10 × 7 (mm), 1.79g</td>
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<tr>
<td>1.72m, 52.0kg</td>
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<tr>
<td>2. 12 Mar., 1997</td>
<td>Yoneyama, Kashiwazaki City Niigata Pref.</td>
<td>R/L ? 40 × 11 × 7 (mm), 1.60g</td>
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<tr>
<td>1.53m, 50.0kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. 25 Apr., 1997</td>
<td>Naomi Beach, Kakizaki Town Niigata Pref.</td>
<td>R : 40 × 18 × 8 (mm), 2.20g</td>
</tr>
<tr>
<td>1.80m, 74.5kg</td>
<td></td>
<td>L : 39 × 13 × 7 (mm), 2.20g</td>
</tr>
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R : right lobe; L : left lobe.

The ovary was somewhat wedge-shaped, being long and thick with a blunt end and pointed tip. Although the dorsal surface was rather flat and smooth, the ventral surface was involuted and folded with convoluted ridges (Fig.1a,b).

Specimen 2 : stranded on Mar. 12, 1997

The ovary was comma-shaped, the dorsal surface being flat and smooth with a shallow groove in the middle. A significant concavity occurred near the central portion of the ventral surface. Two cylindrical structures and a folding sheath were seen, suggestive of medullary components (matrix) (Fig.2a,b).

Specimen 3 : stranded on Apr. 25, 1997

The right lobe of the ovary was spindle-shaped with a mass of mesovarium (= mesosalpinx) tissues. In spite of a smooth dorsal surface, the ventral surface included a thick cylinder midway. The left lobe had the appearance of a long and thick disk. The smooth dorsal surface had a shallow depression, the ventral surface a deep groove. A large mass of coiled mesovarian tissues was gathered in a disk near the blunt end (Figs.3a,b;4a,b).

The overall condition of the above ovaries was diagnosed as immature, the three individual being young (virgin ?) females.

Histology

Each ovary comprised cortical and medullary tissue. The former was composed of (1) so-called germinal epithelium (= mesothelium) and tunica albuginea, (2) the greater portion of cortical tissues, consisting of a large amount of fibroblast-like stromal tissues and oocytes, and (3) the medulla and hilus, the latter included the mesovarium and a number of arteries and veins.

(1) The germinal epithelium consisted of a row of cells each containing scanty cytoplasm and a round (occasionally ovoid) nucleus stained weakly with hematoxylin. The tunica albuginea was composed of collagenous fibers stained with light green (Fig.5).

(2) The greater part of the cortical portion was occupied by the supporting stromal tissues of fibroblast-like cells, containing spindle-shaped cells (Fig.6). The latter were densely crowded and occasionally took on a gland-like appearance. The nucleus was deeply stained. Most of the oocytes had reached the primodial and primary follicle stages — it was difficult to locate oogonia (Fig.6). The primary oocytes in various stages were surrounded by the follicular epithelial cells with irregularly-shaped nuclei. The latter cells increased gradually in number, in accordance with development, being arranged in a circular state and subsequently forming columnar epithelium. Oocyte cytoplasm (= ooplasm) showed a fine granular condition (Fig.7), although the outer portion became coarsely granular (Fig.7) with formation of a membrane, which would gradually became the zona pellucida.

In some of the follicles, the epithelium had become stratified and had proceeded to the process of formation of secondary follicle (Fig.8). However, no more advanced follicles were found. On the other hand, a considerable number of atretic follicles at various developmental stages were recognized. Seemingly atretic oocytes were still surrounded by intricate epithelium, as in the primary follicle stage. The atretic follicles were characterized by liquefied ooplasm and/or vacuoles in various states (Fig.9). In some cases, the follicular epithelium consisted of flat (squamous) cells with depressed nuclei, which stained deeply (e.g.
hematoxylin, ponceau de xylidine).

With an increasingly atretic condition, the epithelium became string-like, and the oocyte contents disappeared (Figs.10,11). The oocyte periphery was surrounded by an AF-positive band. A second atretic state was identified as follows: the epithelium comprised low to high columnar cells with deeply-stained nuclei and coarse granular cytoplasm, although collapse of the oocyte and accumulation of yellow pigments, such as lipofuscin, were already evident. With advancing atresia, the follicle as a whole tended to form a mass (so-called pearl formation). In such a cell, numerous vacuoles of various sizes, the outlines of which were AF-positive, were formed in addition to AF-positive globules and vacuoles.

Because the tissues of the cortex and medulla were enmeshed, the boundary of the two portions was indistinct. Elastic purple-stained fibers were sporadically seen, as were occasional spiral arteries in the cortex. Neither corpus luteum nor corpus albicans was encountered at any time (3). In the medulla, on the other hand, there were found many segments of vessels of various diameters and wall thickness (muscular arteries and veins) (Fig.12). The particularly thick vessels were derived from the hilus, portion between the medulla and hilus being identified as mesovarium. The arteries were muscular, with distinct AF-positive membrana elastica interna and m. e. externa. Endothelium, tunica media (consisting of a thick layer of smooth muscles) and tunica adventitia were also apparent (Fig.12).

Discussion

Best (1967) noted the difficulty of distinguishing between the ovaries of immature and mature whales on gross appearance, as the corpora albicantia are very quickly incorporated into the body of the ovary, and all non-pregnant sperm whale ovaries have a smooth external appearance. In the present study of Pacific white-sided dolphins, the ovaries were diagnosed as immature, both from macroscopic and microscopic examination.

Histological observations carried out by us on the gonads of marine mammals stranded on the coast of Niigata district, Sea of Japan, included examination of a male Phocoena phocoena (Honma et al.,1992), four Mesoplodon stejnegeri (Honma, 1994a; Honma and Yamada, 1995), a male Phoca vitulina (Honma, et al.,in press), and the present study.

The four Stejneger's beaked whales were all categorized as likely post-reproductive, being indicative of a spent state. In addition, to the lack of follicles, typical corpora lutea or albicantia were absent from the cortex of each ovary in two specimens, indicating their ovarian condition to be aged (no longer reproductive) (Honma, 1994a). The ovaries of the other specimens contained developed corpora albicantia and a large amount of connective tissues, although primary follicles persisted in the cortex. Nevertheless, neither a corpus luteum nor lutein cells could be found (Honma and Yamada, 1995).

Another significant histological difference between the two pairs of individuals was the occurrence of well-developed spiral arteries in the ovaries only in the former pair. Unlike the four Stejneger's beaked whales, the three Pacific white-sided dolphins examined in this study were all immature. However, there were no essential differences in the external appearance and histological design of the primary and secondary follicles and atretic follicles between them and the above Stejneger's beaked whales (Honma, 1994a; Honma and Yamada, 1995).

Although the literature dealing with ovarian histology of cetaceans is very meager (Mossman and Duke, 1973), the cytological and histological descriptions of ovarian follicles in species of cetaceans, such as humpback whale (Dempsey and Wislocki, 1941; Chittleborough, 1954), blue whale (Jacobsen, 1941), short-finned pilot whale (Marsh and Kasuya, 1984), sperm whale (Best, 1967), and Peale's dolphin (Claver, et al., 1992) indicated very similar condition. Therefore, it is surmised that there are few or no substantial discrepancies between the ovarian histology of baleen and toothed whales.

On the other hand, Iga et al. (1996) measured the concentrations of steroid hormone on the basis of ovarian follicles and follicular fluid in various developmental stages of minke whales. Also studying minke whales, Fukui et al. (1997) examined factors affecting the maturation of follicles from in vitro cultures of follicular oocytes. In the process of this examination, they succeeded in photographing follicular structures in various developmental stages, in association with polar bodies and nuclear divisions. Chittleborough (1954) had earlier pointed out that late fetal humpback whale ovaries included oogonia near the ovarian surface, and abundant primary oocytes in a slightly deeper zone of ovarian tissue and even in early Graafian follicles in the deepest zone.
Fig. 1. Macroscopic features of *Lagenorhynchus obliquidens* ovary (specimen 1).

a, smooth dorsal surface; b, ventral surface containing thick vessels.

Fig. 2. ditto (specimen 2).

a, grooved dorsal surface; b, ventral surface with medullary components.

Fig. 3. ditto (specimen 3 - right lobe).

a, smooth dorsal surface; b, ventral surface including a thick cylinder-like structure.

Fig. 4. ditto (specimen 3 - left lobe).

a, smooth dorsal surface; b, ventral surface with coiled mesovarian tissues.

Figs. 1 - 4, scale cm.
Fig. 5. Section from ovarian cortex showing germinal epithelium (mesothelium) (g), stromal tissue (s) and early stages of oocytes (o). × 400

Fig. 6. Primary oocyte surrounded by a single layer of follicular epithelium (A). × 400

Fig. 7. Enlarged view of a primary follicle surrounded by cuboidal cells in circular disposition. Ooplasm fine and granular. × 1,200

Fig. 8. Secondary follicles surrounded by stratified epithelia. × 1,000

Ovarian histology of stranded *Lagenorhynchus*
Fig. 9. An atretic follicle, containing liquefied ooplasm and many vacuoles, surrounded by intricate epithelial cells (e). × 1,200

Fig. 10. Atretic follicles surrounded by string-like epithelium (▲); ooplasm of latter somewhat liquefied. × 1,000

Fig. 11. Margin of ooplasm of atretic follicle surrounded by AF-positive band (▲). × 1,000

Fig. 12. Section of a muscular artery. AF-positive membrana elastica interna (▲) and externa (▲) are distinguishable. × 1,000