Notes on ovarian histology of a harbor seal, *Phoca largha*, stranded on the beach at Izumozaki, Niigata Prefecture, Sea of Japan

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Abstract

The reproductive organs of a young female harbor seal, *Phoca largha* (Pallas), 1.2m length, stranded on the sandy beach at Izumozaki, Niigata Prefecture, Sea of Japan, were examined histologically. The ovaries, 20 and 25mm in length, comprised mostly primordial and primary follicles, rarely secondary follicles, being in an immature developmental stage. Several autonomic ganglia were found in the mass of interstitial tissues buried in collagenous connective tissue, and surrounded by the thick muscle of the vagina.

Keywords: harbor seal, Izumozaki, ovarian histology, *Phoca largha*, Sea of Japan

Introduction

On February 18, 2006, a young female harbor seal, *Phoca largha* (Pallas, 1811), was stranded on the sandy beach at Izumozaki, a coastal fishing port located on the central part of Niigata Prefecture, Sea of Japan. Although initially buried, the specimen was exhumed shortly after by A.A. and J.S., who later removed the visceral organs for preservation and subsequent histological examination. The reproductive organs are described here, since histological accounts of mature harbor seals stranded on the coast of the Sea of Japan are limited to a single young male with heavy carcinomas (Honma et al., 2000a,b), the female specimen could provide valuable additional information on the gonadal conditions of marine pinnipeds.

Material and Methods

A young, beach stranded harbor seal, 1.2m in total length and 31kg in body weight, was removed from Izumozaki (Fig. 1) to the Niigata City Aquarium, where it was diagnosed as having been pneumonia. After initially preservation in 10

Fig. 1. A young female harbor seal, *Phoca largha*, 1.2m length, stranded on the beach at Izumozaki, Niigata Prefecture, Sea of Japan, photo taken February 18, 2006.

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% formalin, the ovaries and associated organs were measured and refixed in Bouin's solution to improve staining properties. Several blocks were subsequently dissected, dehydrated in an alcohol series and further cleaned in xylol. Following embedding in paraffin, they were cut at 5μm thickness, and stained chiefly with hematoxylin-eosin (HE) double-stain and Masson-Goldner trichrome associated with aldehyde fuchshin (MG-AF), for observation under a light microscope (Leitz Orthoplan).

Results

Gross anatomy

The ovaries attached to slender bicornuate uteri and the flat rectangular vagina were the form of a thin bean-like disc and lacking any prominent projections from the corpora luteum and albicans (Fig. 2). However, a streak (representing a blood vessel, arrow) ran from the apex to the bottom. The left lobe was 20mm in length, 12mm in width, 6mm in thickness and 1.3g in weight, the right lobe being 25mm, 20mm, 10mm and 1.8g, respectively. The border between the funnel and oviduct was difficult to determine. Accordingly, accurate measurement of the length of both organs was not possible. However, uterine horn lengths and diameters were ca. 8.5mm and ca. 5.0mm, respectively. The urine-inflated urinary bladder was ellipsoid, being 40mm in length. 40mm in width and 35mm in depth.

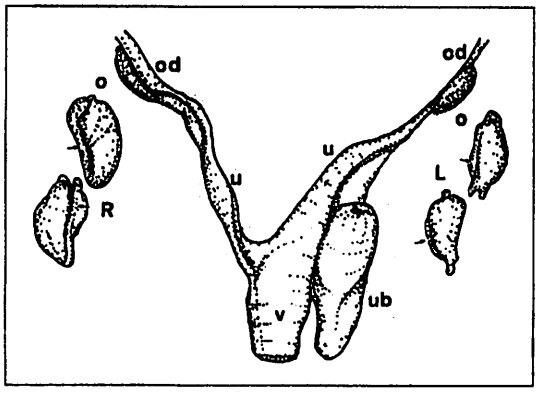

Fig. 2. Diagrammatic illustration of the disposition of the urinogenital organs of a female harbor seal stranded on the beach at Izumozaki Port near Niigata. o. ovary; od. oviduct; u. uterus; ub. urinary bladder; v. vagina; ovary: view of right (R) and left (L) sides.

Microscopy

The ovary was covered with thin mesothelium (germinal epithelium) consisting of cuboidal cells (Plate 1, 1). Adjacent to the mesothelium, the tunica albuginea comprised dense connective tissue, whereas most of the ovary was composed of interstitial tissue. The medullary region of the latter included an abundance of spiral arteries (Plate 1, 2). Thick muscular arteries with membrana elastica interna and externa were seldom encountered (Plate 1, 3).

A small number of primordial follicles, including a few of early stage, were noted in the peripheral cortical portion of the ovary. These follicles were surrounded by several follicular epithelial cells, each containing a prominent nucleolus, in a strongly flattened state (Plate 1, 4). Small early primordial follicles were also gathered into small masses and scattered throughout the interstitial tissue.

In a slightly deeper zone, the primary follicles were surrounded by follicular epithelium consisting of cubic cells (Plate 2, 1). Oocytes in this developmental stage had an ovoid nucleus comprising granular chromatin. Near the medullary zone, several secondary follicles surrounded by two or more (multi-) layered epithelial cells were apparent (Plate 2, 2). However, no typical vesicular follicles prior to the mature Graafian follicle stage (characterized by a distinct zona pellucida and follicular cavity) were recognized in the section (Plate 2, 3). Very rarely, atretic follicles with disarranged epithelia (=granulosa) containing a colloidal substance were encountered (Plate 2, 4).

The uterine glands were constructed from short, simple tubular structures consisting of cuboidal cell epithelium (Plate 3, 1). The cell nucleus contained light cytoplasm with a distinct nucleolus. However, no AF-positive material was demonstrated.

The vaginal wall margins were covered with mucosae consisting of pseudostratified epithelial

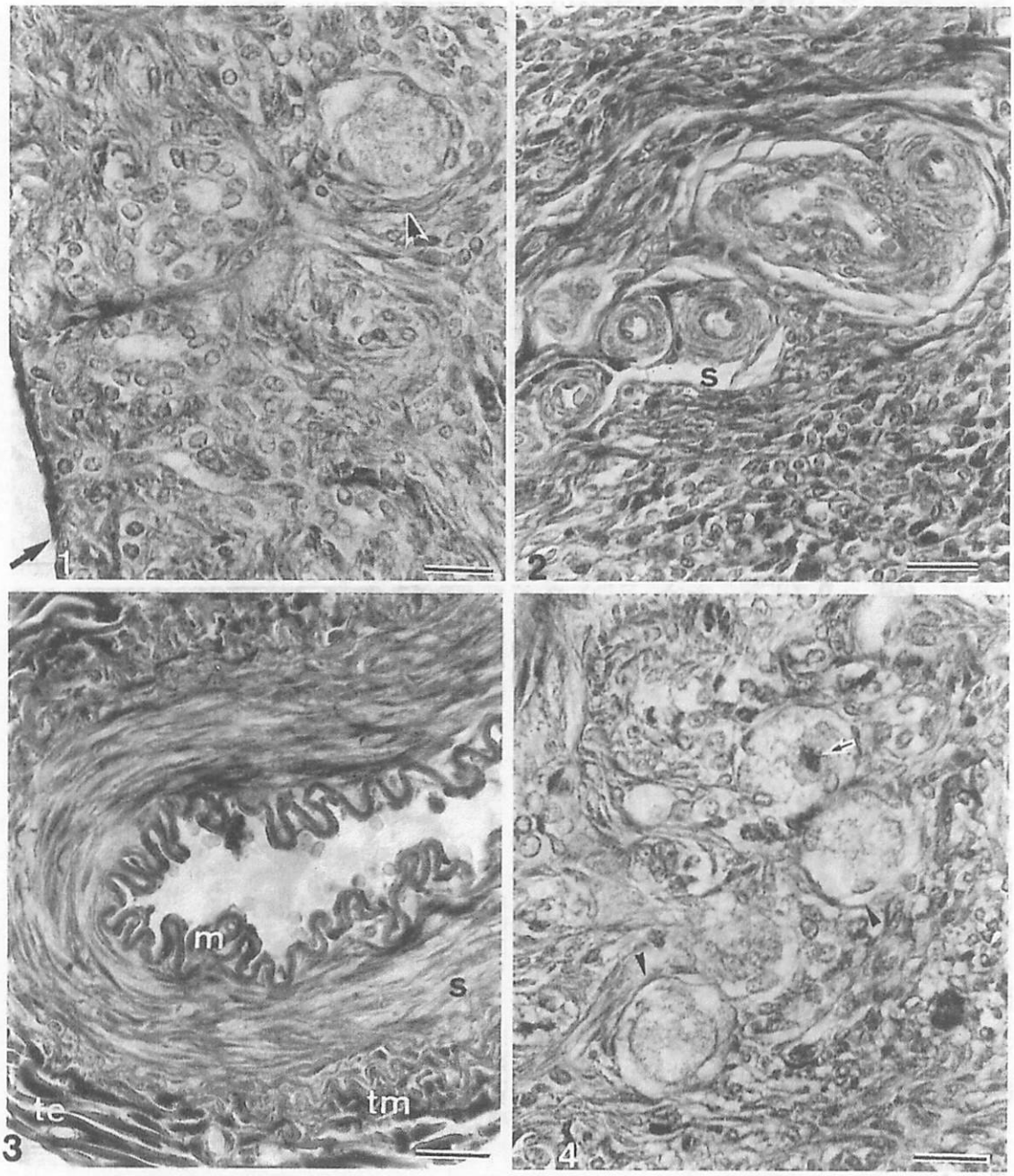

Plate 1

Fig. 1. Part of the ovarian cortex showing mesothelium (arrow) and several primary follicles (arrow head). Masson-Goldner associated with aldehyde fuchsin stain (MG-AF). Scale bar 25μm.

Fig. 2. Spiral arteries (s) in the medullary portion of the ovary. Hematoxylin-eosin stain (HE). Scale bar 25μm.

Fig. 3. A muscular artery in the medullary portion. m. membrana elastica intern; s. smooth muscle; tm. tunica mediana; te. tunica externa. MG-AF. Scale bar 25μm.

Fig. 4. Several primordial follicles (arrow head) surrounded by a single layer of discontinuous flat epithelium. Note pale nucleus containing a distinct nucleolus (arrow). MG-AF. Scale bar 25μm.

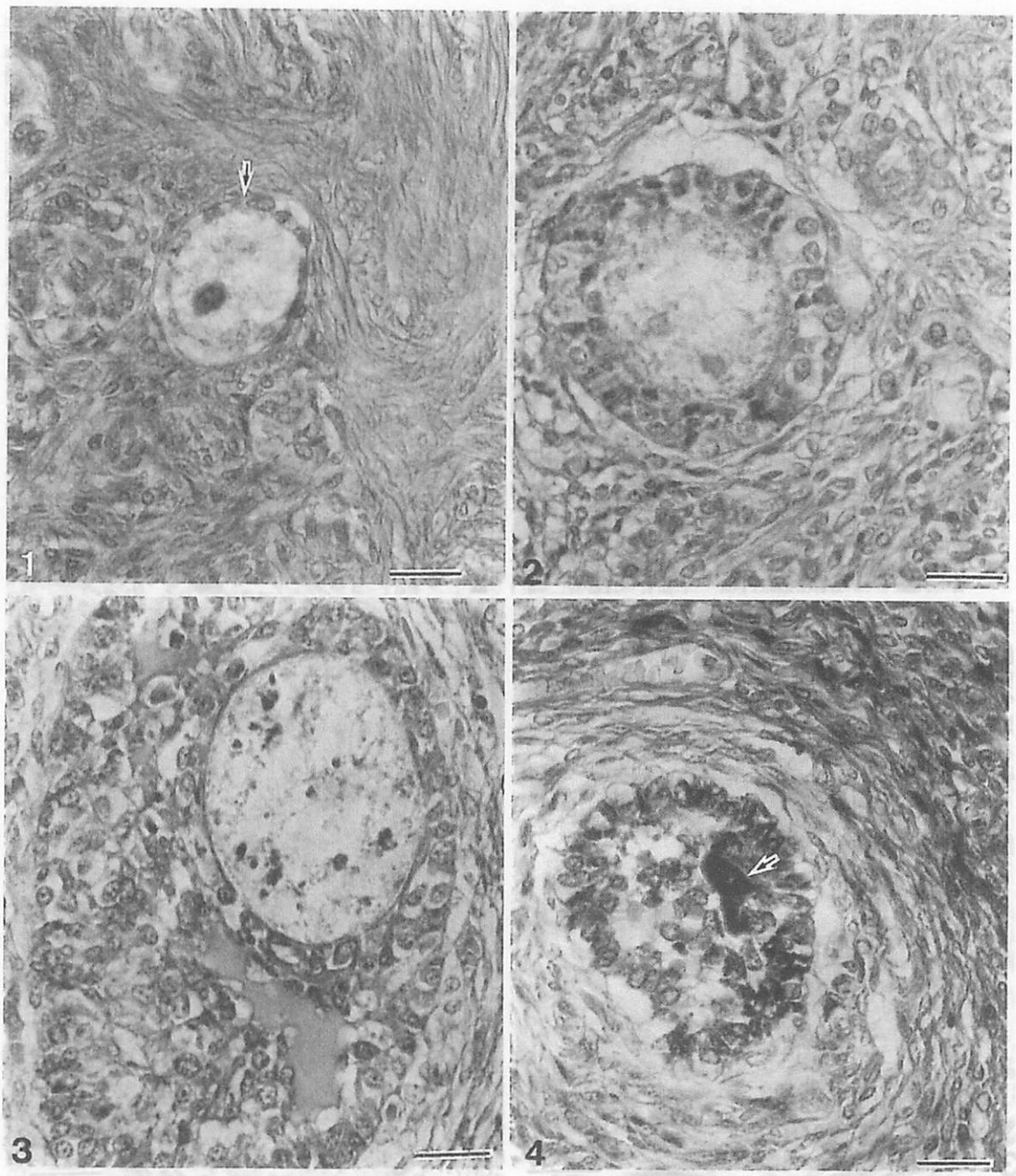

Plate 2

Fig. 1. Primary follicle surrounded by a single layer of continuous epithelium consisting of cuboidal cells. HE. Scale bar 25μm.

Fig. 2. Secondary follicle surrounded by multilayered epithelial cells. HE. Scale bar 25μm.

Fig. 3. Early stage of vesicular follicle prior to mature Graafian follicle stage. Note no zona pellucida and follicular cavity. MG-AF. Scale bar 25μm.

Fig. 4. Early phase of atretic follicle showing disarranged epithelia. Note AF-positive colloidal substance (arrow). MG-AF. Scale bar 25μm.

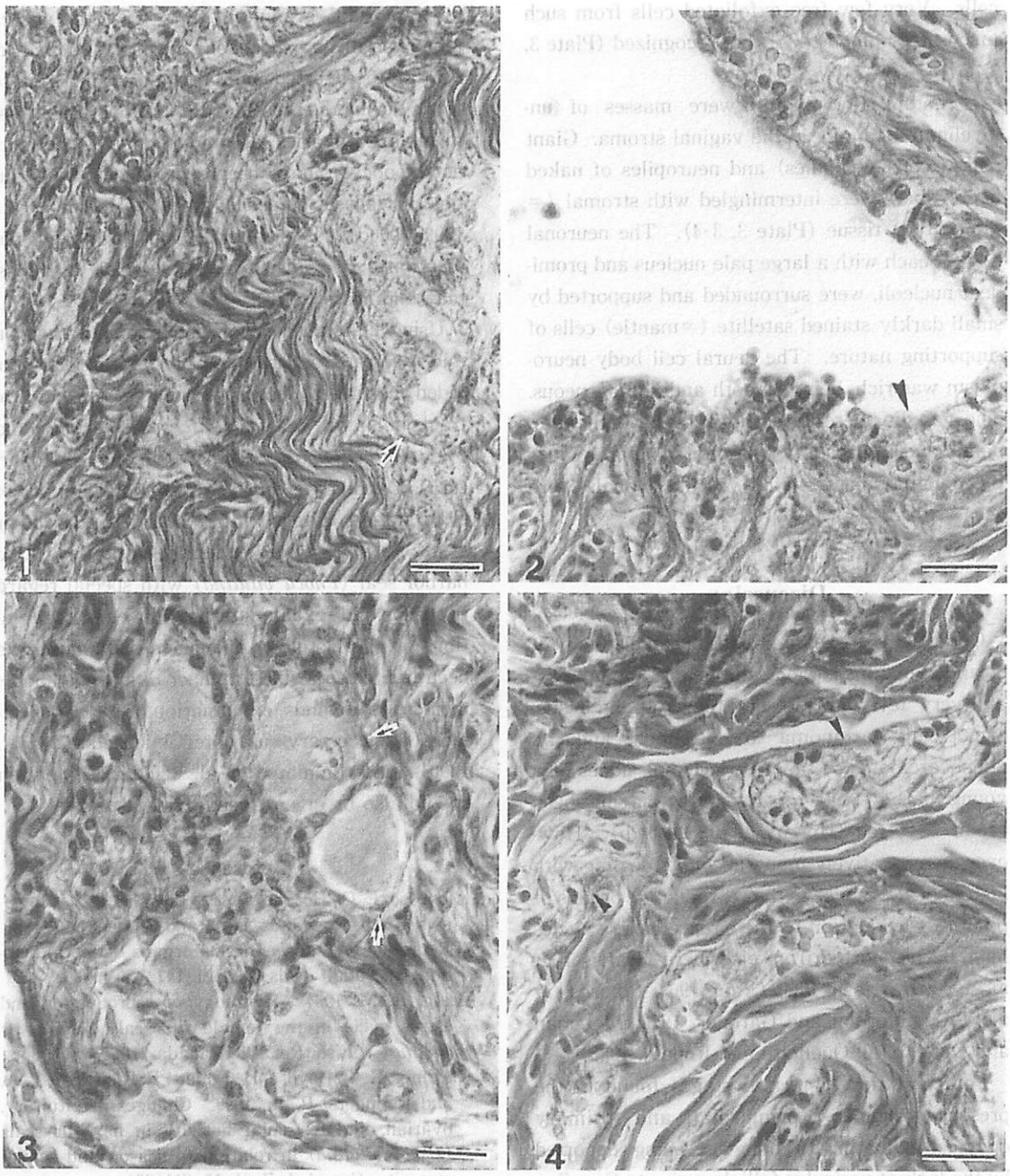

Plate 3

Fig. 1. Short tubular uterine glands consisting of cuboidal cells (arrow). Note no AF-positive material. MG-AF. Scale bar 25μm.

Fig. 2. Part of the vaginal wall showing pseudostratified mucosae (arrow). Note very few exfoliated epithelial cells and blood cells. MG-AF. Scale bar 25μm.

Fig. 3. Several autonomic ganglion cells in the stromal tissue of the vaginal wall. Note satellite cells (arrow). MG-AF. Scale bar 25μm.

Fig. 4. Several unmyelinated axons (nerve fibers=stalks) (arrow head) in the stromal tissue of the vaginal wall. MG-AF. Scale bar 25μm.

cells. Very few free exfoliated cells from such mucosae or blood cells were recognized (Plate 3, 2).

A noteworthy finding were masses of unmyelinated ganglia in the vaginal stroma. Giant neural cells (=bodies) and neuropiles of naked nerve fibers were intermingled with stromal (= interstitial) tissue (Plate 3, 3-4). The neuronal bodies, each with a large pale nucleus and prominent nucleoli, were surrounded and supported by small darkly-stained satellite (=mantle) cells of supporting nature. The neural cell body neuroplasm was rich, being smooth and homogeneous. The ganglia were determined to be both autonomic and intraneural (=motor ganglia). Near the ganglial masses, rich vascular canals were seen.

Discussion

Opportunities to examine the organ histology of stranded pinnipeds along the Sea of Japan coast are very few, only observations on the testis, signet-cell carcinoma of stomach and an adenocarcinoma in the gastric lymph nodes of a young harbor seal, *Phoca largha* having been reported so far (Honma et al., 2000a,b). However, comparatively many accounts dealing with histological changes in the reproductive organs of pinnipeds in relation to their reproductive cycles have been reported from elsewhere (Harrison et al., 1952; Harriosn, 1960; Craig, 1964; Amoroso et al., 1965; Simpson and Gardner, 1972; Mossman and Duke, 1973; Ouellette and Ronald, 1985).

Follicular developmental stages found in the present specimen (primordial and primary oocytes) indicating its young age, being supported by the lack of vesicular follicles prior to Graafian follicle development or Graafian follicles.

An excellent account on the ovarian histology of comparatively large numbers of three species of pinnipeds, *Phoca vitulina*, *Halichoerus grypus* and *Zalophus californianus*, was given by Amoroso et al. (1965). They described in detail the ovarian tissues of individuals from fetal to adult ages, and further noted a few oocytes enveloped in primordial granulosa cells near the tunica albuginea of pap specimens. Simpson and Gardner (1972) reported, similarly, the condition also found during the present examination. In contrast to pinniped ovaries, a comparatively large number of oocytes have been recognized in the cortical surface of cetacean ovaries (Honma et al., 1999, 2000c, 2002, 2004, 2005, 2006), although not mentioned in an earlier review article by Mossman and Duke (1973).

Using light microscopy and scanning electron microscopy, Ouellette and Ronald (1985) provided a detailed description of the reproductive tracts of two seals (*Halichoerus grypus* and *Phooca groenlandica*) during pregnancy, post parturition and estrus, but provided no treatment of early stage oocytes. On the other hand, Gardiner et al. (1999), reported endocrine activities of the harbor seal (*Phoca vitulina*) with special regard to season and sexual maturity. Since our observations on the histology of marine mammals have been limited to a small number of stranded postmortem individuals, examination of further specimens is necessary, as noted by Siebert et al. (2001) and Jauniaux et al. (2002). Attention should also be given to endocrine aspects.

References

Amoroso, E. C., Bourne, G. H., Harrison, R. J., Matthews, L. H., Rowlands, I. W. and Sloper, J. C. 1965. Reproductive and endocrine organs of foetal newborn and adult seals. J. Zool. 147: 430-486.

Craig, A. M. 1964. Histology of reproduction and estrus cycle in the female fur seal, *Callorhinus urcinus*. J. Fish. Res. Bd. Canada 21: 773-811, 5pls.

Gardiner, K. J., Boyd, I. L., Follett, B. K., Racey, P. A. and Reijnders, P. Jh. 1999. Changes in pituitary, ovarian, and testicular activity in harbour seals (*Phoca vitulina*) in relation to season and sexual maturity. Canad. J. Zool. 77: 211-221.

Harrison, R. J. 1960. Reproduction and reproductive organs in common seals (*Phoca vitulina*) in the Wash, East Angliia. Mammalia 24: 372-385.

Harrison, R. J., Matthews, L.H. and Roberts, J. M. 1952. Reproduction in some pinnipedia. Trans. Zool. Soc. London 27: 437-541.

Honma, Y., Ushiki, T., Hashizume, H., Takeda, M., Matsuishi, T. and Honno, Y. 2004. Histological observations on the reproductive organs of harbor porpoises *Phocoena phocoena* incidentally caught in

a set net installed off Usujiri, southern Hokkaido. Fish. Sci. **70**: 94-99.

Honma, Y., Ushiki, T., Hashizume, H., Takeda, M. and Shindo, J. 2002. Histological observations on the reproductive organs of a female harbor porpoise, *Phocoena phocoena*, stranded on the beach at Nishiyama, Niigata Prefecture, Sea of Japan. Nihonkai Cetol. **12**: 25-32.

Honma, Y., Ushiki, T., Hitomi, J. and Takeda, M. 2000a. Example of an adenocarcinoma developed in the lymph nodes of a harbor seal *Phoca largha*. Fish. Sci. **67**: 470-473.

Honma, Y., Ushiki, T., Hitomi,J., Takeda, M., Yoshida, T. and Kano, Y. 2000b. Histopathology of a harbor seal *Phoca largha* stranded on the coast of Toyama Bay, Sea of Japan. Fish. Sci. **66**: 304-309.

Honma, Y., Ushiki, T., Takeda, M. and Matsuishi, T. 2005. Ovarian histology of a young harbor porpoise, *Phocoena phocoena*, caught off the coast of Funka Bay, southern Hokkaido. Japan Cetol. **15**: 11-16.

Honma, Y., Ushiki, T., Takeda, M. and Matsuishi, T. 2006. Notes on the ovarian histology of a young harbor porpoise, caught in Takashima, Hokkaido, Sea of Japan. Japan Cetol. **16**: 13-19.

Honma, Y., Ushiki, T., Takeda, M.. and Shindo, J. 2000c. Ovarian histology of minke whale and Risso's dolphin stranded on the coast of Niigata district, Sea of Japan. Nihonkai Cetol. **10**: 23-29.

Honma, Y., Ushiki, T., Takeda, M. and Yamada, T. 1999. Histological studies on the ovaries of three Pacific white-sided dolphins, *Lagenorhynchus obliquidens*, stranded on the coast of Niigata District, Sea of Japan. Nihonkai Cetol., **9**: 7-14.

Jauniaux, T., Petitjean, D., Brenez, C., Borrens, M., Brosens, L., Haelter, J., Tavernier, J. and Coignou, F. 2002. Post-mortem findings and causes of death of harbour porpoises (*Phocoena phocoena*) stranded from 1990 to 2000 along the coastlines of Belgium and northern France. J. Comp. Pathol. **126**: 243-253.

Mossman, H.W. and Duke, K. L. 1973. Comparative Morphology of the Mammarian Ovary. 461pp. University of Wisconsin Press, Madison, U.S.A.

Ouellette, J. and Ronald, K. 1985. Histology of reproduction in harp and grey seals during pregnancy, postparturition, and estrus. Canad. J. Zool. **63**: 1778-1796.

Siebert, U., Wuenschmann, A., Weiss, R., Frank, H., Benke, H. and Frese, K. 2001. Post-mortem findings in harbour porpoises (*Phocoena phocoena*) from the German North Baltic Seas. J. Comp. Pathol. **124**: 102-114.

Simpson, J. G. and Gardner, M. B. 1972. Comparative microscopic anatomy of selected marine mammals. 298-418. in Ridgway, S. H. (ed)., Mammals of the Sea. Biology and Medicine. Charles C. Thomas Publications, Springfield,U.S.A.

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出雲崎海岸（新潟県）へ漂着した幼ゴマフアザラシ雌性生殖器の組織学的観察：本間義治[1]・甲賀大輔[1]・牛木辰男[1]・宵柳　彰[2]・進藤順治[3]

2006年2月18日に，新潟県三島郡出雲崎町の砂浜海岸に，1頭の幼雌ゴマフアザラシ(全長1.2m，体重31kg）が漂着死した．関係者によって一旦砂中に埋められたものを掘り出し，剖検したところ，肺炎に罹患していた．さらに臓器を摘出のうえ，組織標本を作成した．本報では，雌性生殖器の所見について記載した．

薄い長卵形を呈した卵巣の表面は円滑で，左葉20㎜，右葉25㎜，重量はそれぞれ1.3ｇと1.8ｇであった．皮質層の原始卵胞と1次卵胞の数は，鯨類に比し少なかった．2次卵胞に進んでいるものは希で，最も発生の進んだものでも胞状卵胞の初期段階にあり，成熟したグラーフ卵胞は全く見られなかった．閉鎖卵胞もごく僅かしか認められなかった．未発達の子宮腺にはAF陽性分泌物は検出されず，膣壁の偽重層扁平上皮は薄く，剝離は見られなかった．膣壁の間質組織内には，自律神経節が検出されたが，神経束の走向は追跡できなかった．この個体は，外形のみならず卵巣組織像からも幼若体と判断された．