Ultrastructure of the Spermatozoa of the Yangtze Finless Porpoise (Neophocaena phocaenoides asiaeorientalis)

H. Y. Li, X. F. Zhang, D. Wang and D. Q. Chen

Addresses of authors: 1Institute of Hydrobiology, Chinese Academy of Sciences, 430072 Wuhan; 2Graduate School of Chinese Academy of Sciences, 100039 Beijing, China; *Corresponding author: Tel.: +86 27 87800371; fax: +86 27 87491267; e-mail: zhangx@ihb.ac.cn

With 4 figures and 1 table

Received January 2009; accepted for publication April 2009

Summary

Semen sample was collected from two captive adult Yangtze finless porpoises (Neophocaena phocaenoides asiaeorientalis) during physical examination. One individual was aged about 9 years with body length 143 cm (total length) and body weight 46.1 kg in 2003. The age of the other was unknown and its body length was 147 cm and body weight was 43 kg in 2004. Ultrastructure of their spermatozoa was examined using scanning and transmission electron microscope. The sperm concentration was 4.17 × 10⁹ spermatozoa per ml by the cytometer. The approximate dimensions of the spermatozoa were as follows: head length, 3.366 ± 0.140 μm (mean ± SE, n = 15); head width, 1.896 ± 0.099 μm (n = 15); and neck length, 1.004 ± 0.074 μm (n = 10). The tail included midpiece, principal piece and terminal piece. The length of the midpiece was 1.882 ± 0.077 μm (n = 9). There is no apparent boundary between the principal piece and the terminal piece, so the length of the principal piece and the terminal piece was 44.612 ± 3.485 μm (n = 5). Total length of the spermatozoa was 53.314 ± 4.880 μm (n = 10). The acrosome covered approximately 45.8% of the anterior portion of the head.

Introduction

The Yangtze finless porpoise (Neophocaena phocaenoides asiaeorientalis) is a sole freshwater subspecies of finless porpoise (N. phocaenoides G. Cuvier, 1892) (Gao and Zhou, 1995). It inhabits the middle and lower reaches of the Yangtze River, Poyang Lake and Dongting Lake of China (Zhang et al., 1993; Yang et al., 2000; Xiao and Zhang, 2002; Wang et al., 2005). Population size of Yangtze finless porpoises has been steadily and rapidly decreasing during the past several decades because of human activities (Zhang et al., 1993; Wang et al., 2000; Wei et al., 2002). Currently, the Yangtze finless porpoise is listed as an endangered species (EN C2b) in the IUCN red list of threatened species (Reeves et al., 2008) and is also listed in the China Species Red List (Wang and Xie, 2004). The animal is listed in the second order of the protected animals of the Wildlife Conservation Act of China. The population of the Yangtze finless porpoise was estimated to be 2700 before 1993 (Zhang et al., 1993) and recent studies have suggested that the population may be much smaller than that in 1990s (Wang et al., 2000; Wei et al., 2002, 2003). In 2006, the total number of Yangtze finless porpoise was around 1800 (Zhao et al., 2008). It has been speculated that the Yangtze subspecies could become extinct within the next 100 years if the environmental conditions in the Yangtze River continue to deteriorate (Zhang and Wang, 1999).

For reasons of its endangered status, the Yangtze finless porpoise has attracted much attention of scientists, with particular focus given to its reproductive biology (Hao et al., 2006). Producing offspring is the basic precondition of species continuance. More of the information currently available on Yangtze finless porpoise reproduction was obtained by morphological and histological examination of gonads of the animals that were accidentally killed (Chen et al., 1982; Zhang, 1992; Gao and Zhou, 1993; Chang and Zhou, 1995; Jiang, 1998) or by behavioural observation and ecological survey (Hua et al., 1994; Wei et al., 2002, 2004). The biological characters of the semen of the Yangtze finless porpoise had never been reported and the ultrastructure of spermatozoa had only been reported by Jiang (1991). Here, we describe the biological characters of the semen and the ultrastructure of spermatozoa of the porpoise and compare its structure with those of other odontocetes. These data will be helpful to establish the normal appearance of the spermatozoa and can provide some instructions for a further study on reproductive biology.

Materials and Methods

Semen collection

Semen sample was collected from two captive adult Yangtze finless porpoises during regular physical examination of the animal in the Bajiai Dolphinarium of the Institute of Hydrobiology of the Chinese Academy of Sciences in Wuhan from 2003 to 2008. It was collected by kneading the reproduction silt. The sperm concentration, total motility (TM) and kinetic rating (KR) were determined immediately after collection. KR was scored on a scale from 0 to 5; 0, no forward motility; 1, little forward movement; 2, movement and poor progression; 3, slow forward progression; 4, steady forward progression; 5, rapid forward progression (Miller et al., 2002). At the same time, fresh semen was diluted with 7% dextrose at 0–4°C. The variation motility of samples was examined every 4–6 h using the method of Feng et al. (1991). In addition, some amount of semen sample was used for ultrastructural examination.
Scanning and transmission electron microscopy

For SEM, spermatozoa were centrifuged (2000 r/min for 10 min) and placed in equal volumes of 2.5% glutaraldehyde (C₅H₈O₂) in the refrigerator at 4°C for approximately 2–4 h. Then, the spermatozoa were washed three times (each for 10–15 min) in phosphate-buffered saline (PBS, pH 7.2), fixed with 1% osmium tetroxide (O₃O₄) for 2 h and washed again with PBS (thrice for 10–15 min). The spermatozoa were then dehydrated through an ethanol series at 30% and increasing in five increments to 100% and critical-point-dried. The spermatozoa were coated with gold using a Hummer V Sputter Coater and observed with H-300 SEM (Hitachi Company, Tokyo, Japan).

For TEM, spermatozoa were processed using the same procedure before they were critical-point-dried. After dehydrated through a graded ethanol series of up to 100%, they were infiltrated for 2 h in a 1:1 mix of Epon’s resin 812 and 100% ethanol. Then, the excess infiltrate mixture was discarded and replaced by 100% resin for 12 h. The tissue was then transferred to moulds, which were filled with new resin. The new resin was polymerized overnight at 60°C. Blocks were cut using Leica Ultracut-R Microtome CM1850 (Leica Company, Wiesbaden, Germany) at 1 µm and observed using JEM-1230 TEM (Electron Company, Akishima-shi, Japan), stored on Design Transmission of TEM (Gatan Model 780 Dual Vision 300W; Gatan Company, Warrendale, PA, USA).

Results

Semen characteristics

The colour of the fresh semen was creamy white, which had 95% of TM, KR of 5.0 and sperm concentration of $4.17 \times 10^9$ ml. The motility variation was about 50% after 48 h and 0 after 11 days (Fig. 1).

SEM morphology of spermatozoa

The head length of the spermatozoa was $3.366 \pm 0.140$ µm (mean ± SE, $n = 15$). Frontal view of the head was elliptic, and the approximate dimension was $1.896 \pm 0.099$ µm ($n = 15$). Side-view of the head was pyriform, the approximate dimension being $1.391 \pm 0.099$ µm ($n = 15$). The neck length was $1.044 \pm 0.074$ µm ($n = 10$). The midpiece of the tail was $1.882 \pm 0.077$ µm ($n = 9$). The principal piece had no apparent boundary with the terminal piece, and hence the total length was $44.612 \pm 3.485$ µm ($n = 5$). Total length of the spermatozoa was $53.314 \pm 4.580$ µm ($n = 10$) (Fig. 2).

TEM morphology of spermatozoa

Head

In ultrathin longitudinal section, head of the spermatozoa had acrosome and nucleus. The acrosome was very thin and flat and was covered with approximately 45.8% of the nucleus. The nucleus had peaked top and tubby base. There were some vesicles in the nucleus (Fig. 3a,e).

In cross-section, upper part of the acrosomal region of the spermatozoal head was sigmoid-shaped and wider at the ends than at the middle (Fig. 3b). Base of the acrosomal region was slightly sigmoid and wider than the upper part of the acrosomal region (Fig. 3c).

Neck

Neck was shorter than the head. There were some vesicles, irregular lamellae and a centriole inside the neck (Fig. 3e).

Tail

Tail was the longest part compared with the head and the neck. It included the midpiece, principal piece and terminal piece. In the midpiece, the axial fibre bundle was composed of a central pair of fibres. It was surrounded by nine dense fibres that were surrounded by vaginal mitochondria. The vaginal mitochondria were composed of four to five mitochondria (Fig. 4).

The principal piece was the longest in the tail. There was a central pair of fibres and nine dense fibres. It was surrounded by a vaginal fibre (Fig. 4).

The terminal piece was shorter than the principal piece. There was only a central pair of fibres (Fig. 4).

Discussion

In Jiang (1991) dissertation, the acrosome covered about 2/3 of the anterior portion of the head, and so the conception rate
was higher in the Yangtze finless porpoise than those in some terranean mammals. However, the acrosome covered about 1/2 of the anterior portion of the head in this study. Morphological preservation was excellent in spermatozoa collected from the living dolphin, but poor in spermatozoa collected postmortem from the spermary of the dolphin (Miller et al., 2002). The sample used in Jiang’s dissertation was a spermary from a dead adult. The animal had been treated with antibiotic for 20 days before death. In mammals, infectious processes and antibiotic therapy have been correlated with decreased spermatogenesis and/or spermatozoa function (Schlegel et al., 1991). Schlegel et al. (1991) found that nitrofurans, macrolides, aminoglycosides, tetracyclines and sulpha drugs may have adverse effects on spermatogenesis. Aminoglycoside administration to rats and humans resulted in spermatogenic arrest with cessation of spermatogonial division characterized by interruption of primary spermatocyte meiosis. Hence, it appears that Jiang’s result may be influenced by the extensive use of antibiotics.

The acrosomal to post-acrosomal ratio was approximately 1:1 in the sperm of Yangtze finless porpoise and Pacific white-sided dolphin (Lagenorhynchus obliquidens); however, the ratio was about 3:2 in those of killer whale (Orcinus orca) and beluga (Delphinapterus leucas). There is a positive relationship between the ratio and conception rate (Hao, 1993) and hence it is possible that the conception rate of the Yangtze finless porpoise was lower than the killer whale and the beluga. The shape and ultrastructure of the Yangtze finless porpoise spermatozoa were similar to that described for the Pacific white-sided dolphin by Miller et al. (2002) and the Atlantic bottle-nosed dolphin (Tursiops truncatus) by Fleming et al. (1981), but slightly different from that of killer whale and beluga spermatozoa described by Miller et al. (2002). The head of the Yangtze finless porpoise spermatozoa was half as wide as the head of killer whale spermatozoa on frontal view (Table 1). The width of the head of the Yangtze finless porpoise sperm presented in its surface was approximate to those of the Pacific white-sided dolphin and the Atlantic bottle-nosed dolphin, and in killer whale, the length and width of the sperm head was approximate.

The shape of the dorsal aspect of the sperm heads of the killer whale and the beluga looked like a ‘square’, whereas the
shape of the dorsal aspect of the sperm heads looked like an ‘ellipsoid’ for the Yangtze finless porpoise, the Pacific white-sided dolphin and the Atlantic bottlenosed dolphin.

Cummins and Woodall (1985) considered that there was a negative relationship between sperm size and body mass among mammals. In cetacean, the correlation coefficient was −0.894. But it was not supported in some cetaceans. The relationship between sperm size and body mass was positive in four species as shown in Table 1. It may relate to the habitat of the cetaceans.

These findings are initial observations. This is the first report of the captive adult Yangtze finless porpoise describing the biological characteristics of the semen and microscopical observation of spermatozoa. However, our samples are limited from two animals held captive for 5 years and hence we suggest that future sampling should be collected from more captive and free-ranging porpoises. More data will add to our understanding of this animal species and may increase our ability to manage better the health and reproductive status of both captive and free-ranging populations.

Acknowledgements

We wish to thank Dr K. X. Wang, Mr. Q. Z. Zhao, Dr Y. J. Hao and other staff of Banji Dolphinarium for their co-operation in semen sampling. This research was supported by the National Basic Research Program of China (2007CB411600), the National Natural Science Foundation of China (30730018) and OPFCFH.

References


Table 1. Dimensions of cetacean spermatozoa (mean ± SE)

<table>
<thead>
<tr>
<th>Species</th>
<th>Body weight (kg)*</th>
<th>Total length (μm)</th>
<th>Head length (μm)</th>
<th>Head width (μm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yangtze finless porpoise</td>
<td>30–45</td>
<td>53.31 ± 4.58</td>
<td>3.37 ± 0.14</td>
<td>1.90 ± 0.10</td>
<td>This study</td>
</tr>
<tr>
<td>Pacific white-sided dolphin</td>
<td>85–150</td>
<td>69.26 ± 0.28</td>
<td>4.23 ± 0.02</td>
<td>1.96 ± 0.01</td>
<td>Miller et al., 2002</td>
</tr>
<tr>
<td>Atlantic bottlenosed dolphin</td>
<td>150–650</td>
<td>65</td>
<td>4.5</td>
<td>2.0</td>
<td>Fleming et al., 1981</td>
</tr>
<tr>
<td>Killer whale</td>
<td>2600–9000</td>
<td>74.44 ± 0.36</td>
<td>4.43 ± 0.03</td>
<td>3.88 ± 0.02</td>
<td>Miller et al., 2002</td>
</tr>
</tbody>
</table>

*Data from Carwardine, 1995.