FAU Institutional Repository

http://purl.fcla.edu/fau/fauir

This paper was submitted by the faculty of FAU's Harbor Branch Oceanographic Institute.

Notice: ©2001 Blackwell Wissenschafts-Verlag, Berlin. This manuscript is an author version with the final publication available at http://www.wiley.com/WileyCDA/ and may be cited as: Miller, D. L., Dougherty, M. M., Decker, S. J., & Bossart, G. D. (2001). Ultrastructure of the spermatozoa from a Florida manatee (*Trichechus manatus latirostris*). *Anatomia, Histologia, Embroylogia, 30*(4), 253-256. doi:10.1046/j.1439-0264.2001.00330.x

Anat. Histol. Embryol. **30**, 253–256 (2001)

University of Miami School of Medicine, FL, USA

Ultrastructure of the Spermatozoa from a Florida Manatee (*Trichechus manatus latirostris*)

D. L. MILLER[1]*, M. M. DOUGHERTY[2], S. J. DECKER[3] and G. D. BOSSART[4]

Addresses of authors: [1]Department of Pathology, The University of Georgia College of Veterinary Medicine, Veterinary Diagnostic and Investigational Laboratory, Tifton, GA 31793; [2]Miami Seaquarium, Miami, FL; [3]Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33136; [4]Harbor Branch Oceanographic Institute, 5600 US 1 North, Fort Pierce, FL 34946, USA; *Corresponding author. Tel.: (229) 386 3440; Fax: (229) 386 7128; E-mail: dmiller@tifton.cpes.peachnet.edu

With 4 figures

Summary

Semen was opportunistically collected from a free-ranging, 10-year-old, 275 cm (total length) Florida manatee (*Trichechus manatus latirostris*) during rehabilitation treatments. Ultrastructure of the spermatozoa was examined by scanning and transmission electron microscopy and differed slightly from that described for other mammals. Comparisons to the manatee's closest phylogenetic relatives, the elephant and hyrax, were made. The manatee spermatozoa had a similar acrosome but a distinct annulus and lacked the dense bodies observed in the neck of the elephant spermatozoa. Additionally, manatee spermatozoa lacked the lateral vacuoles observed in the nuclear chromatin from of the hyrax spermatozoa. These data add to our understanding of manatees and allow for comparative studies with other species that may be useful in phylogenetic and reproductive studies.

Introduction

Florida manatees (*Trichechus manatus latirostris*) are endangered and human activities cause high, and possibly unsustainable, levels of mortality and serious injury to manatees in certain parts of the state (O'Shea et al., 1995). Although certain aspects of manatee biology have been well studied, there continue to be deficiencies in available information on particular aspects, including reproduction and life history. Species-specific baseline data become invaluable when assessing individual and population health (including reproductive) status for both captive and free-ranging animals.

Here we describe the ultrastructure of manatee spermatozoa to add to our understanding of the reproductive biology of the endangered manatee. These data help establish the normal appearance of manatee sperm and can be used for comparison with other mammalian species.

Materials and Methods

Collection

Semen was collected from an adult (10-year-old, 275 cm long) Florida manatee after admission to the manatee rehabilitation unit at the Miami Seaquarium for a spinal cord injury inflicted by a motorboat propeller. Collection was opportunistic with urine sampling. The specimen (urine and semen) was spun and the pellet was placed in 2% paraformaldehyde/2.5% gluteraldehyde in phosphate buffer. This preparation was then submitted to the University of Miami, School of Medicine Division of Comparative Pathology for ultrastructural examination.

Scanning (SEM) and transmission (TEM) electron microscopy

The semen was prepared for electron microscopy according to the procedure of Phillips (1995). In brief, for SEM, spermatozoa were washed twice with 0.9% NaCl by centrifugation (500 ***g*** for 5 min). The spermatozoa were spread on a 22×22 mm coverslip, air-dried and immediately dehydrated through an ethanol series starting at 35% and increasing in four increments to 100% and then critical point dried. The spermatozoa were coated with gold in a Hummer V Sputter Coater© and observed with a JEOL 35 SEM©.

For TEM, spermatozoa were washed and dehydrated through a graded ethanol series to 100% and then infiltrated overnight in a 1:1 mix of Spurr's resin and 100% ethanol. The next day, the excess infiltrate mixture was poured off and replaced by 100% resin for 0.5–1 h. The tissue was then transferred to moulds and the moulds were filled with new resin and incubated at 60°C overnight for polymerization. Blocks were cut on a Sorvall Porter Blum MT-2 Microtome© at 70–100 nm. Thick sections (1 μm) were stained with Richardson's Mallory Stain. Thin sections were stained in aqueous 4% uranyl acetate for 20 min, rinsed in distilled H_2O, stained with Reynold's lead citrate for 5 min and again rinsed in distilled H_2O. Prepared specimens were examined using a JEOL CX100 TEM©.

Results

SEM morphology of spermatozoa

The approximate dimensions of the Florida manatee spermatozoa were: head length, 4.9 μm; head width, 2.9 μm; neck length, 0.2 μm; midpiece length, 5.5 μm; annulus length, 2.1 μm; tail length (including midpiece and annulus), approximately 25.0 μm; total length, 30.1 μm. The thin, flat acrosome covered the anterior three-quarters of the head; with the exposed portion being a circular focus (Fig. 1). On the lateral view, it encompassed one-half of the head (Fig. 2).

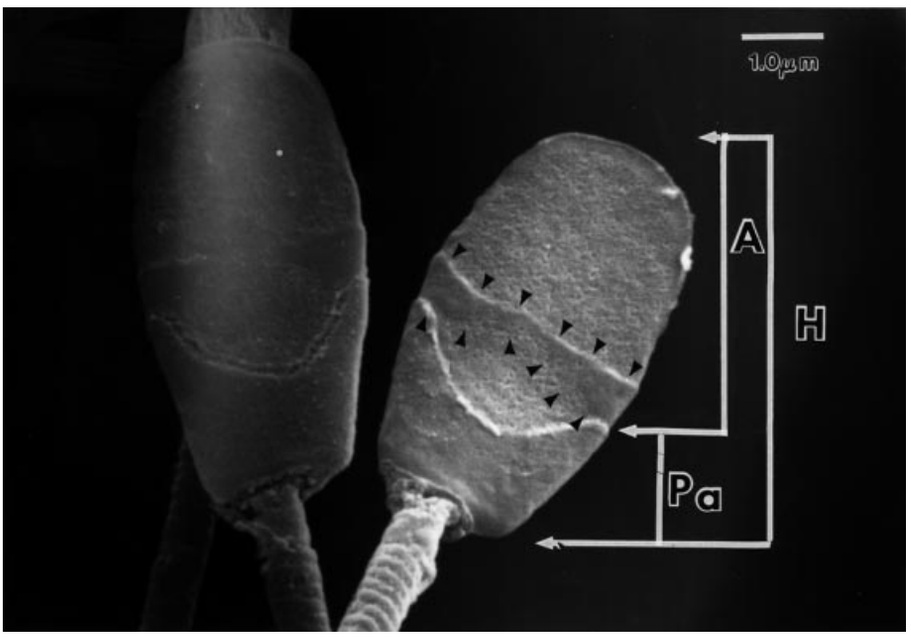

Fig. 1. Scanning electron microscopic view of the dorsal/ventral surface of the head (H) with the acrosome (A), post-acrosomal region (Pa) and probable equatorial segment of the acrosome (arrows) of a spermatozoon from a Florida manatee (*Trichechus manatus latirostris*).

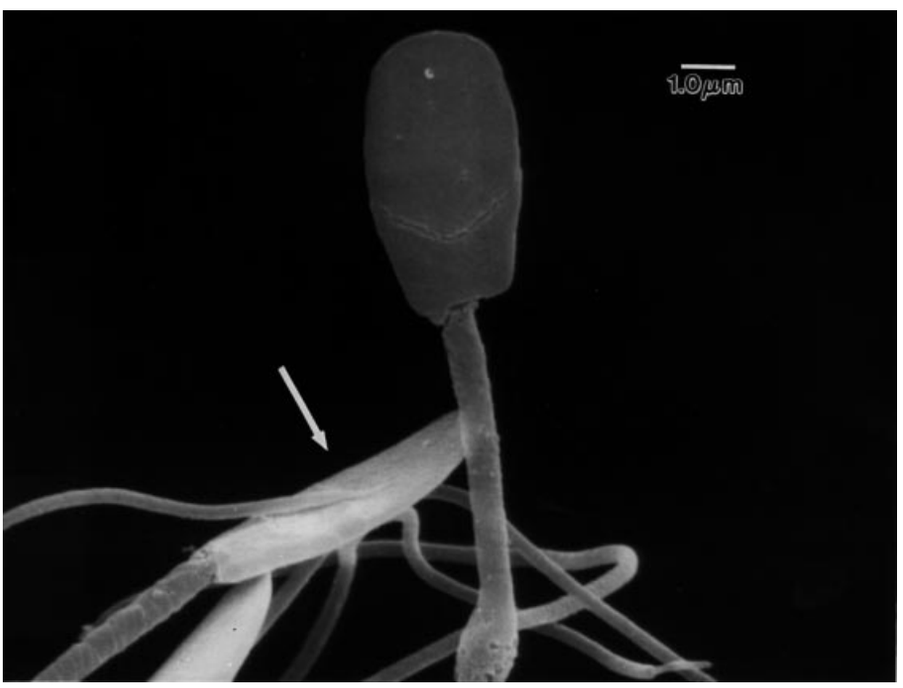

Fig. 2. Scanning electron microscopic view of the lateral surface (arrow) of the head of a spermatozoon from a Florida manatee (*Trichechus manatus latirostris*).

The spermatozoa had a flat oval head on surface view (Fig. 1). The width of the head presented in its surface view was approximately four times that of the midpiece; whereas in its lateral view was approximately equal to that of the midpiece (Fig. 2). The acrosome was very thin and flat. Bilaterally symmetrical thickened triangular areas were observed on the surface view of the sperm head. These areas were approximately 1.0×0.3 μm and located just anterior to the postacrosomal region. The midpiece was characterized by a spiral formation from the mitochondria that extended the entire length of the midpiece and culminated in a discrete bulge (the annulus) (Fig. 3).

TEM morphology of spermatozoa

The tail was composed of an axial fibre bundle bound in a fibrous ring (Fig. 4). The axial fibre bundle was composed of a central pair of fibres surrounded by nine dense fibres that were surrounded by nine loosely arranged fibres. Additionally, the midpiece portion of the tail was composed of the axial fibre bundle surrounded by a mitochondrial helix (containing the mitochondrial cristae) and enclosed within the cell membrane. The head was dense chromatin with an apical body and was surrounded by a thin cell membrane and partially covered by the acrosome.

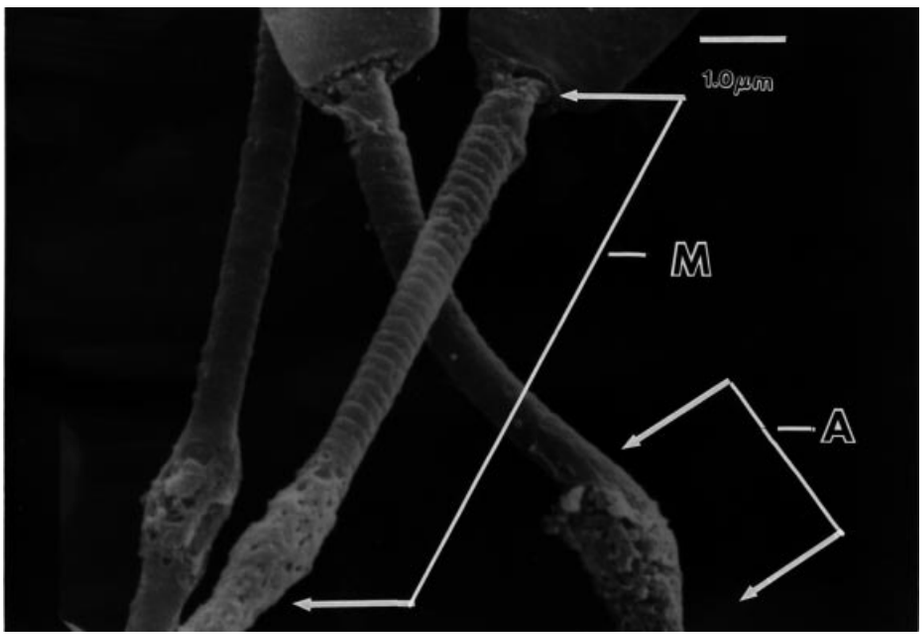

Fig. 3. Scanning electron microscopic view of the midpiece (M) and annulus (A) of the spermatozoa from a Florida manatee (*Trichechus manatus latirostris*).

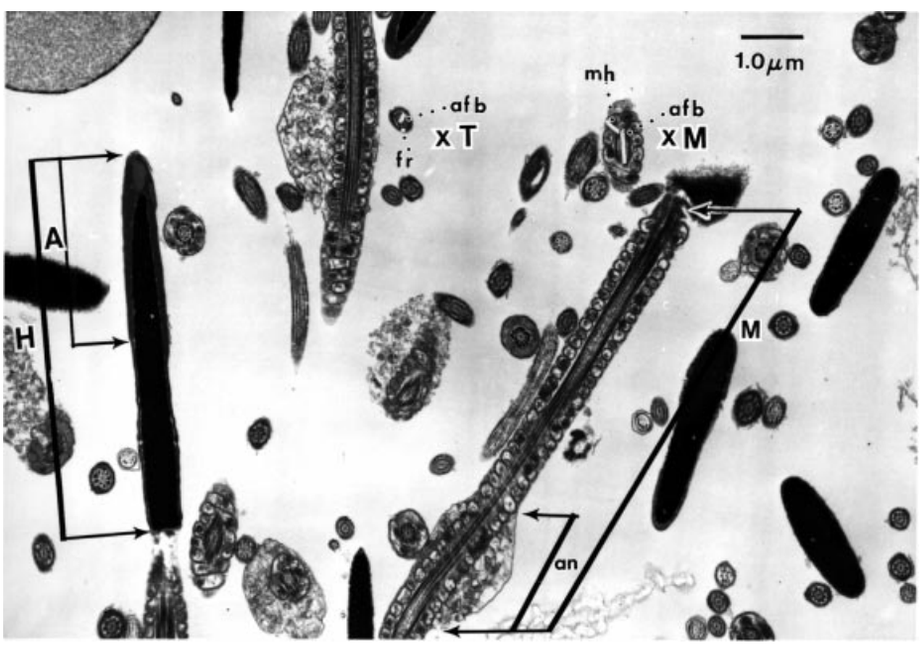

Fig. 4. Transmission electron microscopic view of a cross-section through the tail (xT), showing the inner axial fibre bundle (afb) and the outer fibrous ring (fr); longitudinal and cross-sections through the midpiece (M and xM, respectively) showing the annulus (an), outer mitochondrial helix (mh) and the inner afb; and longitudinal section through the head (H) showing the acrosome (A) of the spermatozoa from a Florida manatee (*Trichechus manatus latirostris*).

Discussion

Ultrastructures of spermatozoa have been reported in other paenungulates, specifically, the Asian elephant (*Elaphas maximus*) and the rock hyrax (*Procavia capensis*); and were found to be similar to domestic bovids with a few variations (Bedford and Millar, 1978; Heath et al., 1983). Likewise, the manatee spermatozoa shared some of these variations but differences in the ultrastructure of spermatozoa also were noted among the paenungulates. Structurally, the primary differences in manatee spermatozoa, when compared to other species, were in the acrosome, neck and annulus.

The manatee acrosome, like that of the elephant (Heath et al., 1983), did not have a prominent apical body as seen in domestic bovids; thus ventral and dorsal spermatozoal aspects were not appreciated. Rock hyrax and elephant acrosomes have prominent apical bodies when immature but not at maturity (Bedford and Millar, 1978). This structural change may also take place in the manatee but developing (i.e. intratesticular or epididymal) spermatozoa were not observed. The mildly thickened areas on the surface view of the manatee sperm head (Fig. 1) were presumed to be the equatorial segment of the acrosome based on published descriptions from other species (Faulkner, 1969; Fleming et al., 1981). Unlike the elephant, the manatee acrosome had a smooth tapering equatorial segment and was similar to other mammals, including the rock hyrax (Fig. 4).

The second difference in manatee spermatozoa was noted in the neck region. The dense material described in the neck region of the elephant spermatozoa was not found in the manatee spermatozoa. Rather, manatee spermatozoa had neck regions similar to those described from other mammals, including the rock hyrax (Faulkner, 1969; Bedford and Millar, 1978; Fleming et al., 1981).

Similarly, a difference was reported in rock hyrax that was not observed in the manatee or the elephant. That difference was the presence of two lateral vacuoles in the nuclear chromatin. Some speculation exists as to the relation between these vacuoles and the ability for the rock hyrax sperm to maintain motility for 30 days. The significance of these vacuoles remains unknown.

A final and most striking difference was in the annulus. The spermatozoa in this manatee had very pronounced annuli that were evident on both SEM and TEM (Figs 3 and 4). Similar findings have not previously been reported in other species, including the paenungulates. The annulus is presumed to be the location for separation of the tail but the significance of such a prominent one, as was seen in the manatee spermatozoa, is unclear.

Although manatees are paenungulates, it is interesting to explore the possibility of environmental influences on the morphological appearance of spermatozoa. Therefore, comparison of the manatee sperm to that of other marine mammals was explored and differences were noted. The spermatozoa of the manatee lacked the parallel ridges described by Fleming et al. (1981) in the sperm head from the Atlantic bottlenose dolphin (*Tursiops truncatus*). Also, the manatee sperm midpieces had the typical helical appearance rather than the pitted appearance seen on the sperm from the dolphin (Fleming et al., 1981) and killer whale (*Orcinus orca*) (Miller, unpublished data). These differences are suggestive of important species variations and thus environmental influences may not be a factor in the morphological appearance of manatee spermatozoa.

A final observation concerns the quality of the specimen we collected. We believe that urine was not detrimental to the ultrastructure of the spermatozoa. Previous studies have documented the detrimental effect of urine on sperm motility (Makler et al., 1981; Fuse et al., 1993; Chen et al., 1995; Kim and Kim, 1998); however, we could not find published evidence of morphological changes resulting from urine contamination. Additionally, because the manatee sperm ultrastructure was similar to that described for other mammals, we concluded that urine contamination did not alter the ultrastructure.

Comments

The prominent annulus and the acrosomal differences (i.e. lack of an apical body, and structure of the equatorial segment) observed are most interesting. The prominent annulus may affect motility; however, the importance of this structure remains unknown and its presence is rarely mentioned. Likewise, the significance of acrosomal differences between species remains unknown but may suggest physiological effects associated with reproductive efficiency/success (i.e. membrane fusion with the egg, post-fusion events and/or prevention of cross-species fertilization). These observations may be useful in future phylogenetic and reproductive studies.

These findings represent initial observations and reflect the importance of opportunistic sampling from free-ranging wildlife. Collection of opportunistic samples adds to databases, many of which are extremely sparse or occasionally nonexistent. Future sampling and ultrastructural examination can be performed to develop a base to detect alterations from normal and for species comparisons. These data add to our understanding of manatees and may increase our ability to better manage the health and reproductive status of manatees by providing a point of reference from which to compare future samples.

Acknowledgements

We wish to thank the Miami Seaquarium for financial assistance in collecting and processing the samples and Dr Eloise Styer for assistance in editing.

References

Bedford, J. M., and R. P. Millar, 1978: The character of sperm maturation in the epididymis of the ascrotal hyrax, *Procavia capensis* and armadillo, *Dasypus novemcinctus*. Biol. Reprod. **19,** 396–406.

Chen, D., M. J. Scobey, and R. S. Jeyendran, 1995: Effects of urine on the functional quality of human spermatozoa. Fertil. Steril. **64** (6), 1216–1217.

Faulkner, L. C., 1969: Male reproduction. In: Veterinary Endocrinology and Reproduction (L. E. McDonald, ed.). Philadelphia: Lea and Febiger, pp. 155–205.

Fleming, A. D., R. Yanagimachi, and H. Yanagimachi, 1981: Spermatozoa of the Atlantic bottlenosed dolphin, *Tursiops truncatus*. J. Reprod. Fertil. **63,** 509–514.

Fuse, H., M. Iwasaki, and T. Katayama, 1993: Correlation between the hypoosmotic swelling test and various sperm function tests. Int. J. Fertil. Menop. Stud. **38** (5), 311–315.

Heath, E., R. S. Jeyendran, and E. F. Graham, 1983: Ultrastructure of spermatozoa of the Asiatic elephant (*Elephas maximus*). Anatomia, Histologia, Embryologia (J. Vet Medicine, Series C) **12** (3), 245–252.

Kim, S. C., and H. W. Kim, 1998: Effects of nitrogenous components of urine on sperm motility: an *in vitro* study. Int. J. Androl. **21 (1),** 29–33.

Makler, A., R. David, Z. Blumenfeld, and O. S. Better, 1981: Factors affecting sperm motility. VII. Sperm viability as affected by change of pH and osmolarity of semen and urine specimens. Fertil. Steril. **36** (4), 507–511.

O'Shea, T. J., B. B. Ackerman, and H. F. Percival (eds), 1995: Population biology of the Florida manatee. National Biological Service, Information and Technology Report 1. US Government Printing Office: Washington D.C.

Phillips, D. M., 1995: Fixation of mammalian spermatozoa for electron microscopy. Methods Cell Biol. **47,** 199–204.