Sperm structure of the bowfin, *Amia calva* L.

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SUMMARY - The spermatozoon of the bowfin, *Amia calva*, is a unique structure with the presence of annulate lamellae, peripheral vesicles, and mitochondrial matrix granules. The two perpendicularly oriented centrioles lie at a distance from the nucleus without any visible connection to it. The only similarities of the bowfin spermatozoon with the garfish, *Lepisosteus osseus*, and primitive teleost spermatozoa are the lack of an acrosome and in its satellites from the distal centriole. The bowfin spermatozoon is of the primitive type according to Franzén and an ectaquasperm in the terminology of Jamieson. Yet its structure differs markedly from both that of the garfish and those of the teleosts.

KEY WORDS *bowfin - Amia calva - sperm ultrastructure - primitive spermatozoon - annulate lamellae*

INTRODUCTION

The bowfin, *Amia calva*, is a primitive fish endemic to eastern North America. It is the sole member of the family Amiidae and is also the single representative of the subdivision Halecomorphii, which is regarded to be the sister group to the subdivision Teleostei. The bowfin is valued for its roe processed into caviar, in the international aquarium trade, and as a predator in managing sport fish populations (Simon, 1990).

In the traditional classification of fish species, the genera *Amia* and *Lepisosteus* (garfish) form a separate order, Holostei. This is, however, regarded to be a paraphyletic unit and hence considered an unnatural taxon. Ultrastructure of spermatozoa has most recently served as a criterion for taxonomic and phylogenetic classification of over 200 fish species (Jamieson, 1991). The spermatozoon of garfish has been examined by electron microscopy (Afzelius, 1978). However, the spermatozoon of the bowfin has not been studied ultrastructurally.

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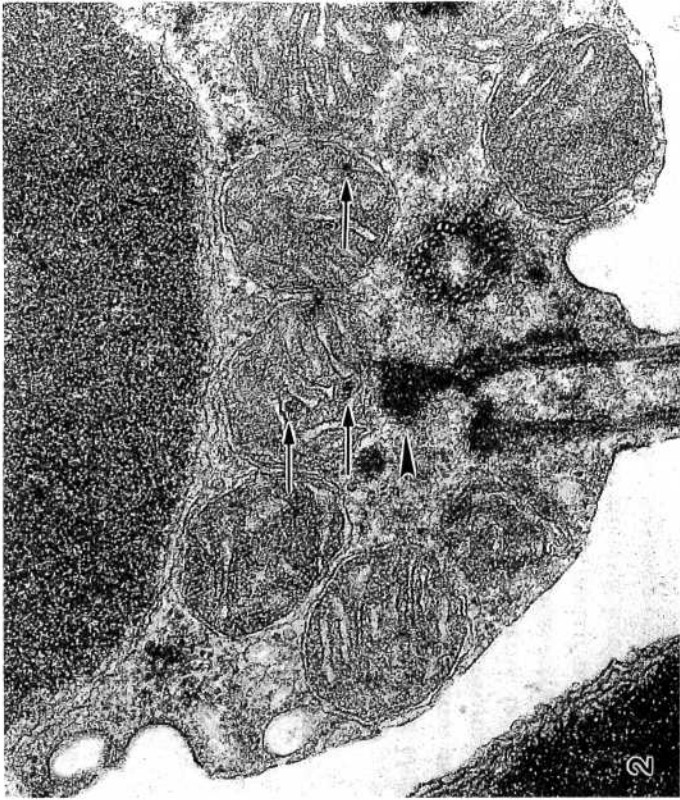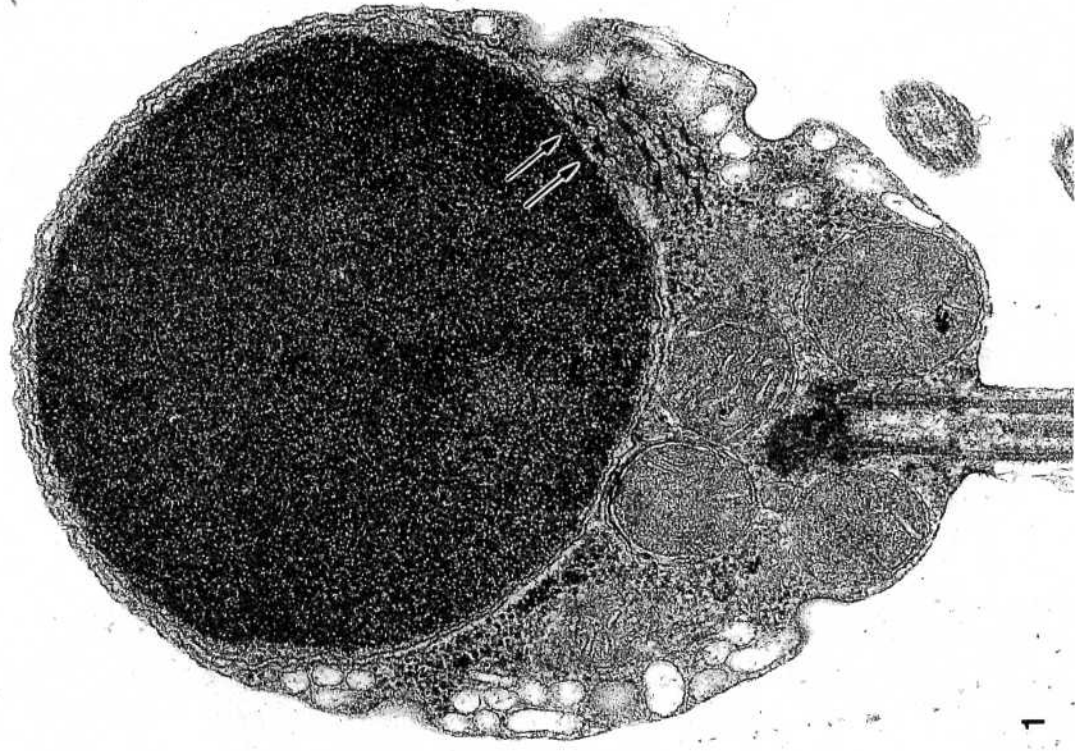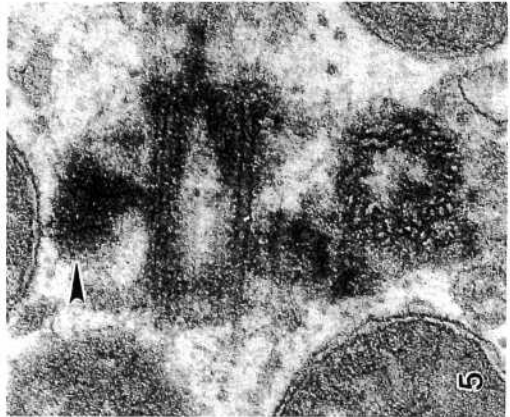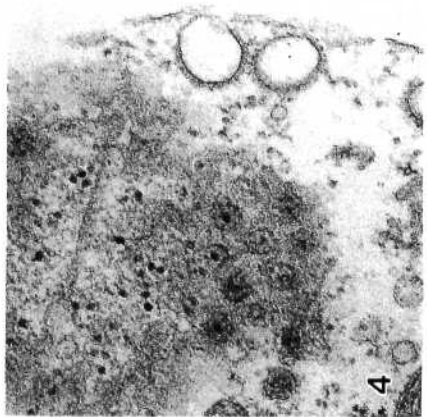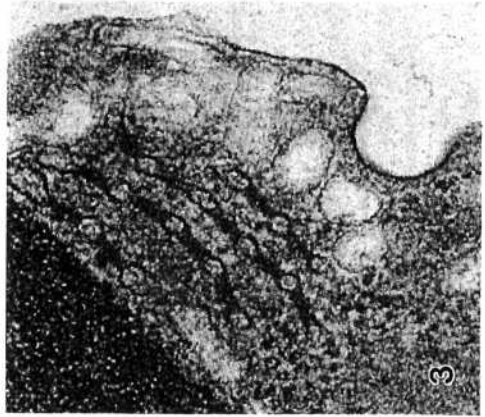
The objective of the study was to examine the bowfin spermatozoon in order to determine if it shares similarities with the spermatozoon of the other holost genus, *Lepisosteus*, or with the spermatozoa of primitive teleosts.

MATERIALS AND METHODS

Bowfin semen was obtained by injecting a sexually mature male with a luteinizing hormone releasing hormone analog (LHRHa) to increase spermiation. The semen was given a prolonged fixation in 2.5% glutaraldehyde in a 0.1 M cacodylate buffer, pH 7.2, before postfixation in 1.5% osmium tetroxide in the same buffer, dehydration in ethanols, and embedding in Agar 100 epoxy resin. Thin sections (approximately 80 nm) were cut with a diamond knife, section stained with uranyl acetate and lead citrate and examined with a JEOL 100 S electron microscope.

RESULTS

The sperm head is spherical with a diameter of 2 μm (Fig. 1). There is no acrosome but a nuclear envelope covers all of the nucleus and consists of two membranes separated by a distinct perinuclear cisterna. In most spermatozoa a lateral region of the cross-sectioned nuclear membrane can be seen to have nuclear pore complexes. Outside this region there are some layers of annulate lamellae (Figs. 1 to 3). The chromatin of the nucleus has an even electron-density.

The midpiece is about 2 μm wide at its base and about 1 μm long. It contains 12-16 mitochondria arranged in two rings around the two centrioles. The mitochondria have the same appearance as those found in the somatic cells, even to the extent that some mitochondrial matrix granules are seen. Another peculiarity of the midpiece is the presence of several small vesicles. Some of them have apparently fused with the cell membrane, which at these sites shows inpocketings, similar to large caveolae (Figs. 1 to 4). At the cytoplasmic side the inpocketings seem to have a thin lining of some electron-dense material. The cytoplasm between the organelles in the midpiece contains what appears to be ribosomes.

In the midpiece there are two centrioles that are perpendicularly oriented and are joined by a cross-striated ribbon (Fig. 5). A mushroom-like projection extends from each of the centrioles (Figs. 2 and 5). Both centrioles lie at a distance from the nucleus. No connection can be found between any of the centrioles and the nucleus. Nine satellite fibers project from the centriole to the cell membrane (Fig. 6). The function of these satellites apparently is to anchor the distal centriole to the cell membrane.

The tail emerges from the distal centriole and is an ordinary 9 + 2 flagellum. The A- and B-subtubules all appear with an electron lucid lumen. The sperm tail has a round cross-sectional area with no side fins on the flagellum, which is different from the spermatozoa from several other fish species (Afzelius, 1978; Jamieson, 1991).

DISCUSSION

Some structures of the bowfin spermatozoa have no equivalence with any other sperm type as far as we are aware: annulate lamellae, mitochondrial matrix granules, and vesicles that appear to open at the cell surface. The presence of what might be ribosomes is also an unusual finding. Perhaps these structures indicate that bowfin spermatozoa retain features from an earlier developmental stage, somewhat analogous to neoteny in the soma of some animals. Annulate lamellae are commonly found in spermatocytes of various animal groups (Kessel, 1992), but not in spermatozoa.

The structure of the bowfin spermatozoon differs from that of the garfish in several important connections: a round rather than oval head shape, centrioles distant from the nucleus, no side fins on the flagellum, axonemal microtubules with an electron lucid lumen. A feature common to spermatozoa from bowfin, gar, and the large teleost subdivision is the lack of an acrosome, evidently a synapomorphy for the large infraclass Neopterygii. Within this taxon the gars are considered the sister group to bowfin and teleosts and the bowfin is regarded as the sister group to Teleostei. The bowfin has been characterized as a so called living fossil; it has followed a separate evolution for over 100 million years.

Retzius (1904) studied the bowfin spermatozoon with the light microscope and stated that it had roughly the same structure as that of a herring, although the size of the spermatozoon was somewhat larger.

With the electron microscope we found that bowfin spermatozoon was not similar to those of the primitive teleosts, such as the herring, the pike (Esocidae) or catfish (Ictaluridae), except for the common shape of the head. The bowfin spermatozoon is considered morphologically a primitive spermatozoon (Franzén, 1956), and considered physiologically an ectaquasperm (Jamieson, 1991).

In conclusion, the bowfin lacks an acrosome as do other members of the neopterygian infraclass. In most other respects it has a unique sperm structure not found in any other animal.

FIGURE 1 Longitudinal section through a bowfin spermatozoon. Note the nuclear pore complexes (arrows) in the nuclear envelope and the annulate lamellae outside this region. Along the periphery of the midpiece there are several vesicles, some of which apparently fused with the cell membrane. × 40,000.

FIGURE 2 Longitudinal section through another bowfin spermatozoon. The proximal centriole is transversely sectioned and is seen to the right of the distal centriole from which the tail flagellum emerges. Note also the mushroom-like projection (arrowhead) from the distal centriole and the matrix granules (small arrows) in the mitochondria. × 55,000.

FIGURE 3 Enlarged detail from Fig. 1 showing the annulate lamellae at a higher magnification. × 75,000.

FIGURE 4 Oblique section through the sperm midpiece including a tangentially cut annular lamella. Two cell membrane inpocketings are seen to the right. × 45,000.

FIGURE 5 Transverse section through the midpiece. The proximal centriole (above) and the cross-cut distal centriole (below) are joined by a cross-striated connection. Note also that a mushroom-like connection (arrowhead) is seen also at the proximal centriole. × 70,000.

FIGURE 6 Transverse section through the distal centriole close to the cell membrane. Obliquely oriented projections, so called satellites, extend from the centriole to the cell membrane. × 80,000.

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