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Fine structure and morphology of sterlet (*Acipenser ruthenus* L. 1758) spermatozoa and acrosin localization

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Abstract

Ultrastructure of sterlet *Acipenser ruthenus* L. 1758 sperm was examined by scanning and transmission electron microscopy, which allowed us to use various methods for visualizations of different parts of sterlet spermatozoa. Sperm cells possess a head with a distinct acrosome, a midpiece and a single flagellum surrounded by the flagellar plasma membrane. The average length of the head including the acrosome and the midpiece was estimated as 5.14 ± 0.42 μm. Nine to 10 posterolateral projections were derived from the acrosome. Three inter-twining endonuclear canals bounded by membranes traversed the nucleus in its whole length from the acrosome to the implantation fossa. Acrosin was located in all the three parts (acrosome, endonuclear canals and implantation fossa). The proximal and distal centrioles located in the midpiece compacted of nine peripheral triplets of microtubules. One cut of the midpiece contained from two to six mitochondria with area of 215 ± 85 nm^2 in average. The flagellum was 42.47 ± 1.89 μm in length with typical eukaryotic organization of one central pair and nine peripheral pairs of microtubules. It passed through a cytoplasmic channel in the midpiece, which was formed by an invagination at the plasmalemma. The flagellum gradually developed two lateral extensions of its plasma membrane, so-called "fins". Detected morphological variation can be described by four principal component axes corresponding to groups of individual morphometric characters defined on the sperm structures. Correlations among the characters indicate that the sperms are variable in their shape rather than size. Significant variation among examined fish

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individuals was found only in flagellum and nucleus length. Comparison between the present and previous studies of morphology of sturgeon spermatozoa confirmed large inter- and/or intra-specific differences that could be of substantial taxonomic value.

Keywords: *Acipenser ruthenus*; Acrosome; Acrosin; Morphometric analysis; SEM; TEM

1. Introduction

Spermatozoa of sturgeon fishes show characteristic differences compared to those of teleost fishes (Jamieson, 1991) in terms of morphology, presence of acrosome (Cherr and Clark, 1984; Psenicka et al., 2007; Wei et al., 2007), sperm behavior (Cosson et al., 2000) and biochemistry with the presence of acrosin- and trypsin-like activities (Ciereszko et al., 1994, 1996). Comparative spermatology could provide fish biologists and aquaculturists with information on fish phylogeny and fish sperm quality evaluation. In this regard, morphology and fine structure are considered to be the major sources of information in comparative spermatology (Baccetti, 1986; Jamieson, 1991, 1999; Lahnsteiner and Patzner, 2007). The present set of results creates the first study about the fine structure and morphology of sterlet spermatozoa.

The defining structure of the sturgeon sperm cell constituents composed of three major parts; (a) an elongated head, (b) a cylindrical midpiece and (c) a flagellum (see Dettlaff et al., 1993 for general information). In the head, the nucleus occupies the major part and usually contains three endonuclear canals (ECs) leading from the implantation fossa to the acrosome. The acrosome often forms several posterolateral projections (PLPs) which have been also observed in pallid (DiLauro et al., 2001), Siberian (Psenicka et al., 2007) and in Chinese sturgeon (Wei et al., 2007). Mitochondria, implantation fossa and centriolar complex are located in the midpiece. The flagellum consists of nine peripheral double and two central single microtubules. The plasma membrane often forms lateral fin along the flagellum of sperm of sturgeons such as in pallid (DiLauro et al., 2001), Siberian (Psenicka et al., 2007) and Chinese sturgeon (Wei et al., 2007). These structures are preferentially oriented along the horizontal plane of central microtubules (Billard, 1970) and it was suggested that they could contribute to the efficiency increase of propelling during wave propagation (Cosson et al., 2000).

The principal aim of this study was to describe the fine structure and morphology of sterlet spermatozoa using transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Examination of multiple sperm specimens allowed us to perform not only a simple description but also a morphometric analysis of characters defined on sperm structures. Consequently, we present a detailed mathematical description of variation in individual traits as well as relationships among them.

2. Materials and methods

2.1. Samples preparation

Sperm of three males were fixed with 2.5% glutaraldehyde for 2 days at 4 °C. Samples for TEM were postfixed in osmium tetroxide for 2 h, at 4 °C, washed, dehydrated through an acetone series and embedded in resin (Polybed 812; Polysciences, Inc.). Series of ultrathin sections