



## SPERMATOZOA OF CHONDROSTEAN FISH SPECIES: STRUCTURE, MOTILITY AND FERTILIZING ABILITY

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Sturgeons (Acipenseriformes: Acipenseridae) and paddlefishes (Acipenseriformes: Polyodontidae), the producers of caviar, are the remnant survivors of the once flourishing chondrosteans, dominant fishes of the Permian period. Although *Acipenseriform* fish first appeared in the fossil record approximately 200Mya, they have seemingly not undergone much morphological change since that time. So far, sturgeons have been an interesting object of multiple studies. However, the several aspects of their genetics as well as reproductive physiology remain debated. In this review, we examine the basic aspects of their gamete biology with special emphasis on sperm physiology, ultrastructure and fertilization process.

In general, the certain aspects of sturgeon's reproductive physiology are different from typical teleost fishes. Typically, their gametes differ from those of most fish in that, the sperm posse's acrosomes that undergo exocytosis and filament formation while the eggs possess numerous micropyles located at the animal pole. Basically, the main body of sperm cells, which includes the acrosome, head, and midpiece, is long, cylindrical and radially symmetrical, however an inter-species difference already exists. More specifically, the spermatozoon of majority of sturgeon species is composed of an elongated head with an acrosome and 8-10 posterolateral projections, a cylindrical midpiece with 2-8 mitochondria and two centrioles and a flagellum with the 9+2 structure of axoneme. Furthermore, from two to four endonuclear canals traverse the nucleus from the junction with the acrosome towards the implantation fossa are situated in front of the midpiece. Additionally, the plasma membrane is folded into one or two lateral fins along major part of the flagellum [1,2,3]. Moreover, an interesting feature of sturgeon spermatozoa is presence of an acrosome as well as subsequent acrosomal reaction (a reaction that causes development of a filament on the heads of sturgeon sperm which assists in penetration of the egg). The main function of

the sperm acrosome in aquatic species is the lysis of the jelly coat layer, the outer-most layer surrounding the egg, but this does not appear to be the case in sturgeon. However, our experiments showed that the acrosome reaction includes the formation of a spear-like fertilization filament coming from three endonuclear canals and implantation fossa through the acrosome. Moreover, the presence of Ca<sup>2+</sup> ions in highly alkaline activation media (pH 10) can induce acrosomal reaction as well. Furthermore, our experiments revealed that the acrosome plays two major functions: (a) the appearance of a long fertilization filament transmitting a signal to the egg that initiates the perivitelline space blocking to polyspermy and (b) the opening of posterolateral projections serving like an anchor against release from the micropyle [4].

Spermatozoa of sturgeons and paddlefish are essentially immotile in the seminal plasma due to its high concentrations of K<sup>+</sup> ions. Spermatozoa are immediately activated when they are transferred into swimming medium, usually freshwater or low salt concentration solutions. The all sperm motility parameters (frequency, velocity and wave amplitude) decrease rapidly during the period after activation and the percentage of motile cells also gradually decreases. During the earliest period of motility, spermatozoa of sturgeons and paddlefish move at velocities of 175–250 mms<sup>-1</sup> and then the forward motility gradually reduces to between 50 and 100 mms<sup>-1</sup> at 3–6 min after activation. Occasionally, some spermatozoa are motile for up to 9 min [5, 6].

Spermatozoa of chondrosteans are haploid or diploid compared to functional diploidy or tetraploidy of each species as revealed by erythrocytes image cytometry and flow cytometry. However, we have also found fertile hexaploid male of Siberian sturgeon, *Acipenser baerii* (a functionally tetraploid species) releasing viable triploid spermatozoa. The subsequent experimental hybridization



with normal females of *A. baerii* demonstrated full fertility of this triploid male.

**Acknowledgements:**

Present study was financially supported by following grants: CZ.1.05/2.1.00/01.0024, MSM 6007665809, LC06073, IAA608030801, ME10015.

**References:**

- [1] PSENICKA, M., RODINA, M., LINHART, O. 2010. Ultrastructural study on the fertilization process in sturgeon (*Acipenser*), function of acrosome and prevention of polyspermy. *Anim. Reprod. Sci.*, 117: 147-54.
- [2] HATEF, A., ALAVI, SM., NOVEIRI, SB., POORBAGHER, H., ALIPOUR, AR., POURKAZEMI, M., LINHART, O. 2011. Morphology and fine structure of *Acipenser persicus* (*Acipenseridae*, *Chondrostei*) spermatozoon: Inter-species comparison in *Acipenseriformes*. *Anim. Reprod. Sci.*, 123:81-88.
- [3] WEI, Q., LI, P., PSENICKA, M., ALAVI, SM., SHEN, L., LIU, J., PEKNICOVA, J., LINHART, O. 2007. Ultrastructure and morphology of spermatozoa in Chinese sturgeon (*Acipenser sinensis* Gray 1835) using scanning and transmission electron microscopy. *Theriogenology*, 67:1269-1278
- [4] PSENICKA, M., VANCOVA, M., KOUBEK, P., TESITEL, J., LINHART, O. 2009. Fine structure and morphology of sterlet (*Acipenser ruthenus*, L. 1758) spermatozoa and acrosin localization. *Anim. Reprod. Sci.*, 111: 3-16.
- [5] LINHART, O., COSSON, J., MIMS, SD., SHELTON, WL., RODINA, M. 2002. Effects of ions on the motility of fresh and demembrated paddlefish (*Polyodon spathula*) spermatozoa. *Reproduction*, 124: 713-719.
- [6] COSSON, J. 2010. Frenetic activation of fish spermatozoa flagella entails short-term motility, portending their precocious decadence. *J. Fish. Biol.* 76: 240-279.