Ultrastructure Morphology of the sperm of snowtrout (Schizothorax richardsonii), inhabiting Bhagirathi River of Garhwal Himalayas, India

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Abstract:

Ultrastructure of snowtrout (*Schizothorax richardsonii*) sperm was examined by scanning electron microscopy, which allowed us to visualize different parts of snowtrout spermatozoa. Sperm cells possess a head without an acrosome, a midpiece and a single flagellum surrounded by the flagellar plasma membrane. From the posterior side of the head, the extension of smooth plasma membrane gives the appearance of collar-like middle piece of the sperm. The average length of the head including the midpiece was estimated as 2.33 ±0.20 μm. A long cylindrical tail originates from the middle piece. The tail was found smooth throughout its whole length, thus lacking any possibility of lateral fins or ribbons. The flagellum was 39.30 μm in length. On the basis of our observations, it may be concluded that being a configuration of uniflagellate, anacrosomal, aquasperm, the sperm of *S. richardsonii* resembles with the primitive type of metazoan sperm of externally fertilizing freshwater fish species.

Key words: snowtrout, ultrastructure, sperm morphology, Schizothorax richardsonii, Garhwal Himalayas.

Introduction:

The availability of several closely related species as well as distinct strains of snowtrout, a growing awareness of the importance of cross-breeding, and the existence of desirable genetic characteristics in one or other strain gives strength to the concept that several new husbandry techniques will be required in the future. Among them, the cryogenic storage of semen will probably be the most important. Short-term storage can be achieved simply by holding sperm at temperatures at or just above 0°C. Sperm from selected males can be kept indefinitely by means of cryopreservation and be made available when and where required.

Semen has two distinct components: the cellular part-sperm cells and the fluid part-seminal plasma. Seminal plasma in most of the teleost fishes is a secretary product of the testes (Lahnsteiner *et al.*, 1994). It contains mainly mineral compounds and low concentrations of organic substances. Seminal fluid is not only the source of nutrition for the spermatozoa; it also inhibits the sperm motility thereby preserving the fertilization capacity of sperm.

The knowledge of the ultrastructure morphology of spermatozoa is very important for the understanding and designing of preservation protocol besides learning the fertilization process in the species. The ultrastructure morphology of sperm varies with the species. Studies on the ultrastructure morphology of sperm of various fish species have been conducted by many workers (Ginsburg, 1963; Fribourgh and Soloff, 1976; Jaspers *et al.*, (1976); **Billard (1978); Fribourgh (1978); Brusle (1981); Nath and Jamuar (1988) and Nath (1996**). In addition, morphometric data are also available for the sperm of *Oncorhynchus keta* (Okada and Ito, 1955); *Salmo gairdnerii* (Cruea, 1969) and *Salmo ischchan* (Turdakov, 1971). The ultrastructure morphological studies on cryopreserved spermatozoa have been made in trout (Billard, 1983) and Deccan mahseer spermatozoa (Patil and Lakra, 2003), in order to analyze the cryoinjury of spermatozoa at various levels during cryopreservation process.

Attention on diverse facets of aquaculture, viz. receiving more healthy eggs, obtaining a good fertilization percentage by artificial breeding, developing new hatchery systems for good hatching rates, genetic up gradation of the desired fish species etc. have initiated a new era in fisheries (Jhingran and Pullin, 1985; Dehadrai, 1986; Shrestha, 1986). The outcomes of these scientific studies have initiated a blue revolution in the country in recent years and are providing a good source of animal protein for the mal-nutrient populations, and better opportunities of employment for the vast populations. Though high altitude coldwater eco-regimes are nature's unique gift to the tropical countries like India, but the fishery development in Himalayan region is very slow.

The review of literature reveals that no attempt has been made to understand the ultrastructure morphology of snowtrout spermatozoa. In the present study, attempts have been made to study the ultrastructure morphology of snowtrout (*Schizothorax richardsonii*) spermatozoa, which is helpful in modeling of cryopreservation process.

Materials and Methods

(i) Ultrastructure Morphology of sperm:

The semen samples of *Schizothorax richardsonii* was subjected to SEM study for understanding the general structure of the sperm. For this purpose, 2-3 drops of fresh semen were fixed in 2.5% glutaraldehyde solution $CHO(CH_2)_3CHO$ at 4ºC for about 4 hours and then the fixative was replaced by 0.1M phosphate buffer by centrifuge the semen at 1200-1500 rpm. These preserved sperms were brought to All India Institute of Medical Sciences (AIIMS), New Delhi for SEM study. Scanning Electron Micrographs were taken at 15 kv and studied.

(ii) Biometric analysis of sperm:

The biometric analysis of sperm of *S. richardsonii* was also made. For this purpose, total length of the head and mid-piece, length of the head, length of the mid-piece, width of the head, width of the mid-piece and length of the flagellum was measured. The micro scale printed

on the photograph was used to calculate exact dimensions of the sperm in micrometer. The surface morphology was critically viewed in relation to the membrane integrity and tail morphology.

Results:

The data of biometrical analysis and surface morphology of *S. richardsonii* sperm was helpful to get general idea of surface morphology of snowtrout sperm and to assess the extent of possible damage in sperm plasma membrane during development of the technique of cryogenic preservation.

The sperm of *S. richardsonii* was observed as fairly rounded-dot like structure with a long tail under the phase contrast microscope (Fig.-3.6). In the SEM images, it looks like having a rounded smooth head with a collar like mid-piece and a long flagellar tail. There was no any structure found on the smooth rounded head, which could be denoted as acrosome (Fig.-1, 2). The whole sperm head was measured 2.0 µm in length and 1.96 µm in width (Table-1). From the posterior side of the head, the extension of smooth plasma membrane gives the appearance of collar-like middle piece of the sperm. A long cylindrical tail originates from the middle piece. The tail in *S. richardsonii* sperm was found smooth throughout its whole length, thus lacking any possibility of lateral fins or ribbons. The various sperm measurements were also taken and expressed in Table-1.

Table-1: Biometrical analysis of snowtrout (*S. richardsonii*) sperm

Sperm dimensions	Mean value (µm)	Standard deviation	Standard error
Total length of head and mid-piece	2.33	0.20	0.08
Head length	2.00	0.08	0.03
Mid-piece length	0.33	0.14	0.05
Head width	1.96	0.05	0.02
Mid-piece width	0.37	0.11	0.04
Flagellum length	39.30	-	-

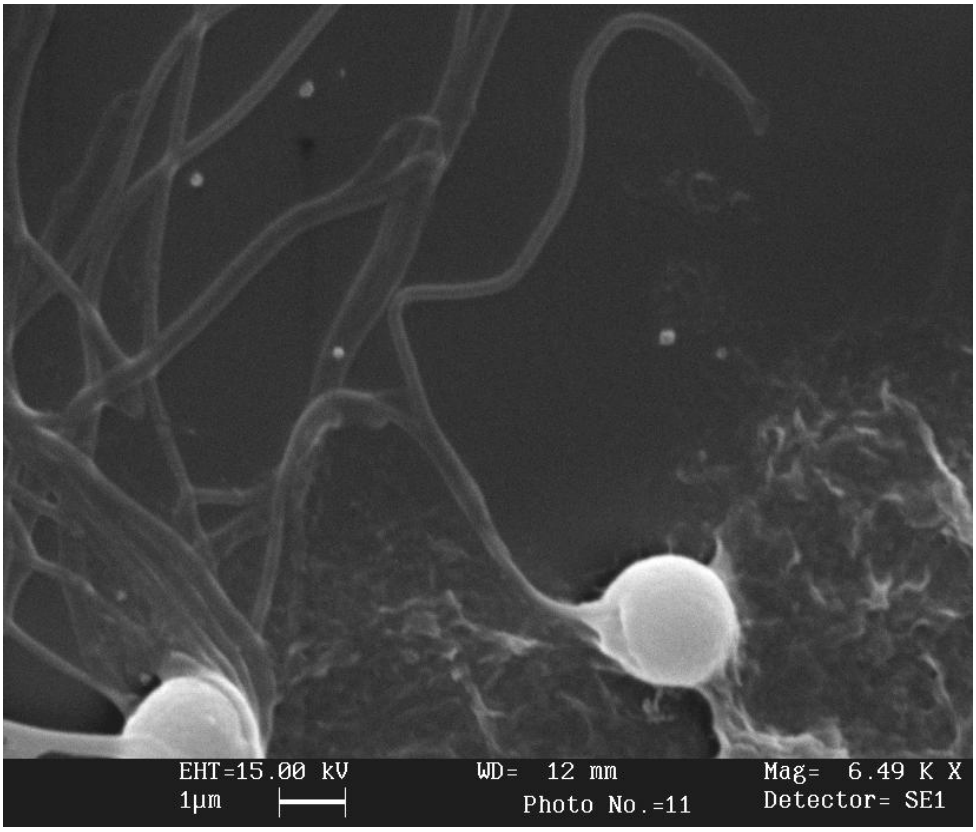

Fig.-1

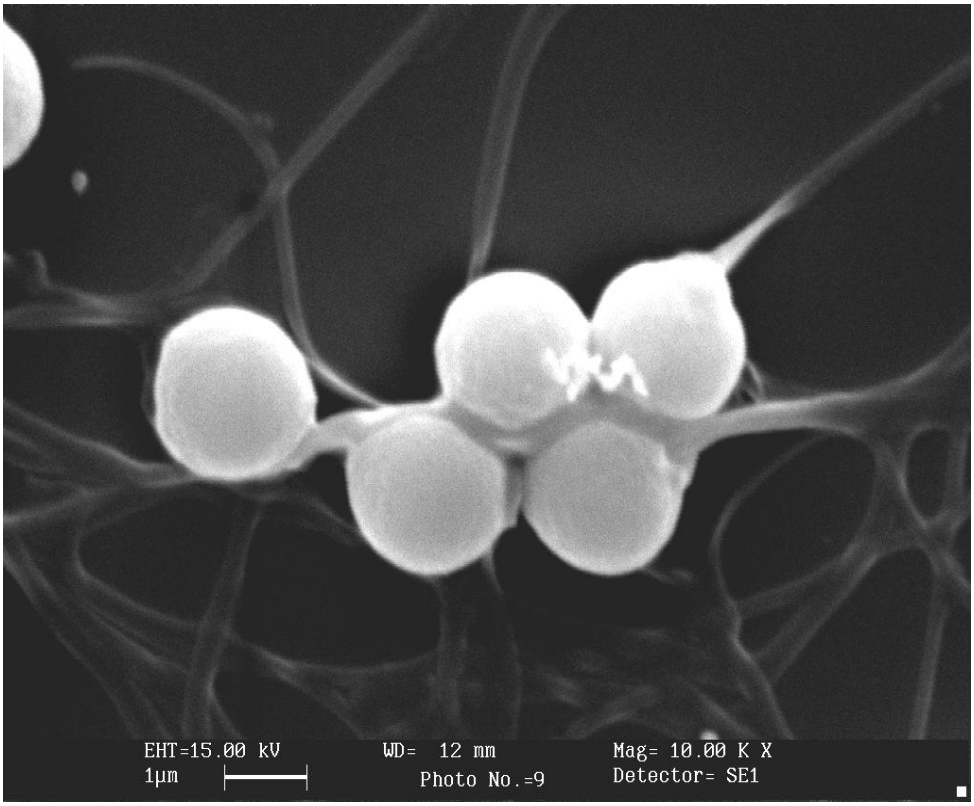

Fig.-2

Figs.-1 & 2: Scanning Electron micrograph of the sperm of *S. richardsonii* prior to cryogenic preservation

Discussion

Variation in spawning timing and varied breeding pattern is adaptive traits of the fish. These traits may encompass testicular cycle, reproductive effort and the manner and timing of reproduction (Wootton, 1984; Mills, 1991). Thus, the understanding of functional morphology of testes is very much helpful in knowing the spawning pattern. The testis in all teleost fishes is divided into germinal and interstitial compartments (Callard, 1991; Grier, 1993), with separate functions of the two compartments reflected by the cell types of which they are composed. The testicular structure in teleost is of two basic types – lobular and tubular (Billard *et al.*, 1982). In most of the teleosts, lobular type of testes existed which is composed of numerous lobules, separated from each other by a thin fibrous connective tissue. The lobules are the germinal compartments, where spermatogenic cells develop into mature spermatozoa. The interstitium between lobules forms the interstitial compartment where interstitial cells, fibroblast and blood and lymph vessels exist. As the spermatogenesis and spermiogenesis proceed, the lobules expand and eventually rupture, liberating mature sperm in the lobular lumen (Nagahama, 1983). Semen is stored in the genital tract/posterior portion of the testes before it releases from the body. At the peak maturity testes become full of sperm, ready to release in perceive of environmental cue. Similar pattern was observed in case of *S. richardsonii*.

In semen, sperm is only the cell type, responsible for transfer of male characters to the egg for the development of new individual. Therefore, study of sperm morphology, motility behaviour/pattern is important for cryopreserving the semen and understanding the fertilizing process for reproductive success. The information about these aspects is in scarce and scattered.

The hypothesis that sperm cell of a species employing external fertilization have a simple structure in contrast to more developed structures associated with internal fertilization holds true for *S. richardsonii* sperm. Our study reveals that like majority of externally

fertilizing teleost species *S. richardsonii* sperm is of primitive type and characterized by the absence of an acrosome. However, acrosome is present in Acipenseriform fish (Linhart and Kudo, 1997) and *Anguilla anguilla* Cuvier (Tuzet and Fontaine, 1937). According to Pasteels (1965), acrosome may not be necessary for fertilization in teleost because of the presence of a micropyle but one would expect specialized structure on the plasma membrane at the top of the sperm head to allow cell fusion during fertilization. These are the characteristics for the primitive type of metazoan sperm (Franzen, 1969).

Similar to sperm of common carp (Billard, 1970), the *S. richardsonii* sperm serves an example of the primitive type. Our study reveals that the head is almost rounded with a diameter of 2.0 ±0.08 μm and attached to its proximal side is a low collar-like mid-piece, formed by an extrusion of the plasma membrane. However, in other species, the shape of the head and the nucleus is variable (Billard, 1986; Jamieson, 1991; Billard and Cosson, 1992). The nucleus is elongated in the guppy, slightly elongated in salmonids with a quasi-symmetry of revolution and very primitive in cyprinids and many other species such as tilapia, mullet and turbot, with the flagellum inserted laterally on the head. The mid-piece is well developed in the guppy and much reduced in size in salmonid, cyprinid, mullet and turbot sperm. It is usually located at the posterior part of the nucleus but it is sometimes found in the anterior part (elopomorph). In some primitive sperm, it may be nearly as big as the nucleus, e.g., in tilapia (Jamieson, 1991).

The length and width (2.00 & 1.96 μm respectively) of *S. richardsonii* sperm head is found very close to the observations of Billard (1969) for rainbow trout, *Salmo gairdneri,* and brown trout *Samo trutta fario*, sperm (2.5 μm and 1.5-2.0 μm). However, while comparing *S. richardsonii* sperm with common carp (*Cyprinus carpio*, Linnaeus), and guppy (*Poecilia reticulata*, Peters) sperm, it is found much smaller than these two species (length & width of sperm head 3.3 μm and 2.5 μm for common carp and 4.0 μm and 1.0 μm for guppy (Billard,

1969). The sperm size might be related with the swimming velocity of sperm of particular species in the fertilizing media.

Peculiarities in the tail have been reported for some species. Remarkable is the absence of flagella in spermatozoa from two families belonging to the Mormyriformes (Mattei *et al.*, 1972). Contrary to this some biflagellate spermatozoa have been reported in channel catfish, *Ictalurus punctatus* (Jaspers *et al.*, 1976). But in general, fish with external fertilization have a single, smooth flagellum. Shape and size of tail flagella also very in the fish species. In some species plasma membrane often forms one or two finlike ridges along the tail, which are on the horizontal axis with the central microtubules (Nicander, 1970; Billard, 1970). This modification of flagellum is believed to improve the efficiency of flagellar propulsion (Afzelius, 1978). The *S. richardsonii* sperm has single, smooth and long tail without any lateral finlike ridge on it thus resembling with the sperm of common external fertilizing teleost.

In light of the existing literature and on the basis of our observations, it may be concluded that being a configuration of uniflagellate, anacrosomal, aquasperm, the *S. richardsonii* sperm resembles with the primitive type of sperm of externally fertilizing freshwater fish species.

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References:

Afzelius BA. 1978. Fine structure of the garfish spermatozoan. *J. Ultrastruct. Res.* 64: 309-314.

Billard R, Cosson MP. 1992. Some problems related to the assessment of sperm motility in fresh water fish. *J. Exp. Zool.* 262: 122-131.

Billard R, Fostier A, Weil C, Breton B. 1982. Endocrine control of spermatogenesis in teleost fish. *Can. J. Fish. Aquat. Sci.* 39: 65-79.

Billard R. 1969. Spermatogenese de *Poecilia reticulata*. II. La production spermatogenetique. *Annales Biologie Animale Biochimie Biophysique* 9: 307-313.

Billard R. 1970. Ultrastructure comparee de spermatozoides de quelques poissons teletosteens. In: Bacetti B. (Ed.), *Comparative Spermatology*, Academic Press, New York: 71-80.

Billard R. 1978. Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in media of various salinities. *Aquaculture* 14: 187-198.

Billard R. 1983. Spermiogenesis in the rainbow trout *Salmo gairdneri*: an ultrastructural study. *Cell Tissue Res.* 233: 265-284.

Billard R. 1986. Spermatogenesis and spermatology of some teleosts fish species. *Reproduction, Nutrition and Development* 26: 877-920.

Brusle S. 1981. Ultrastructure of spermiogenesis in *Liza aurata* Risso, 1810 (*Teleostei, Muilidae)*. *Cell Tissue Res*. 217: 415-424.

Callard GV. 1991. Spermatogenesis. In: Pang P., Schreibman M. (Eds.), *Vertebrate Endocrinology*: *Fundamental and Biomedical Implications*, Vol. 4A, Academic Press, New York: 303-341.

Cruea DD. 1969. Some chemical and physical characteristics of fish sperm. *Tans. Am. Fish. Soc.* 98: 785-788.

Dehadrai PV. 1986. Carps seed production in India. In: May RC, Pullin RSV, Jhingran VG. (Eds.), *Summary report of the Asian Regional Workshop on carp hatchery and nursery technology*, Manila, Philippines (1-3 Feb.). Asian Development Bank and I.C.L.A.R.M., Manila, Philippine.

Franzen A. 1969. Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. In: Baccetti B. (Ed.), *Comparative Spermatology*, Academic Press, New York, London: 29-46.

Fribourgh JH, Soloff BL. 1976. Scanning electron microscopy of the rainbow trout (*Salmo gairdnerii*) spermatozoon. *Proc. Ark. Acad. Sci.* 30: 41-43.

Fribourgh JH. 1978. Morphology of the brook trout spermatozoon as determined by scanning and transmission electron microscopy. *Prog. Fish Cult.* 40: 26-29.

Ginsburg AS. 1963. Sperm-egg association and its relationship to the activation of the egg in Salmonid fishes. *J. Embryol. Exp. Morphol*. 11: 13-33.

Grier HJ. 1993. Comparative organization of sertoli cells including the sertoli cell barrier. In: Russell LD, Griswold MD. (Eds.), *The Sertoli Cell*, Cache River Press, Clearwater, Florida: 704-739.

Jamieson BGM. 1991. *Fish evaluation and systematics: Evidence from spermatozoa*. Cambridge University Press, U.K.: 1-319.

Jaspers EJ, Avault JW, Roussel JD. 1976. Spermatozoal morphology and ultrastructure of channel catfish, *Ictalurus punctatus Trans. Am. Fish Soc*. 3: 475-480.

Jhingran VG, Pullin RSV. 1985. A hatchery manual for the common, Chinese and Indian Major Carps. Asian Development Bank and International Centre for Living Aquatic Resources Management, Manila, Philippines: pp 191.

Lahnsteiner F, Patzner R, Weismann T. 1994. The testicular main duct and the spermatic duct in some cyprinid fishes. II. Composition of the seminal fluid. *J. Fish. Biol.* 44: 459-467.

Linhart O, Kudo S. 1997. Surface ultrastructure of paddlefish eggs before and after fertilization. *J. Fish Biol.* 52 (3): 573-582.

Mattei X, Mattei C, Reizer C, Chevalier JL. 1972. Ultrastructure des spermatozoids aflagelles des *Mormyres* (poissons teleosteens). *J. Micros.* (Paris) 15: 67-78.

Mills CA. 1991. Reproduction and life history. In: I.J. Winfield and J.S. Nelson (Eds.), *Systematics, Biology and Exploitation*, Chapmann Hall: 483-508.

Nagahama Y. 1983. The functional morphology of the teleost gonads. In: H.S. Hoar, D.J. Randall and E.M. Donaldson (Eds.), *Fish Physiology*, vol. IX A, Academic Press, New York: 223-275.

Nath A, Jamuar MP. 1988. Ultrastructure of spermatozoa of *Heteropneustes fossilis*. *Proc. 4th Int. Cell Biol*., Montreal: 12-16.

Nath A. 1996. Cryoarchitecture of spermatozoa of *Heteropneustes fossilis*. *J. Freshwater Biol.* 8 (2): 95-98.

Nicander L. 1970. Comparative studies on the fine structure of vertebrate spermatozoa. In: B. Baccetti (Ed.), *Comparative Spermatology*, Academic Press, New York: 47-56.

Okada S, Ito T. 1955. On the activity and fertilizing capacity of sperm in dog salmon (*O. keta*). *Scient. Rep. Hokkaido fish Hatch.* 10: 21-31.

Pasteels JJ. 1965. Aspects structuraux de la fecondation vus au microscope electronique. *Arch. Biol. Belges* 76: 463-509.

Patil R, Lakra WS. 2003. Cryopreservation of Deccan mahseer, *Tor khudree* (Sykes) spermatozoa and the associated ultrastructural changes. *Nat. Symp. on Genetics & Gene banking of fish and shellfish* (29-30 March): 59.

Shrestha TK. 1986. *Artificial Himalayan mahseer spawning: A monograph*. Tribhuan University, Kathmandu, Nepal.

Turdakov AF. 1971. The effect of temperature conditions on the speed and fertilizing capacity of the spermatozoa of some Issykekul fishes. *J. Ichthyol*. 11: 206-215.

Tuzet O, Fontaine M. 1937. Sur la spermatogenese de l'anguille argentee (*Anguilla vulgaris* Cuv.). *Arch. Zool. Exp. Gen. Note Rev.* 4: 199-215.

Wootton RJ. 1984. Introduction: tactics and strategies in fish reproduction. In: Potts GW, Wootton RJ. (Eds.), *Fish Reproduction: Strategies and Tactics*, Academic Press, London: 1-12.