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Volume changes during the motility period of fish spermatozoa: Interspecies differences

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ABSTRACT

The aim of this study was to describe spermatozoa volume changes during the motility period of fish species with either osmotic (common carp *Cyprinus carpio*) or with ionic (sterlet *Acipenseri ruthenus* and brook trout *Salvelinus fontinalis*) modes of motility activation. Nephelometry, light microscopy, and spermatocrit methods were used for quantitative assessment of cell volume changes in media of different osmolalities. Significant correlation ($R^2 = 0.7341$; $P < 0.001$) between parameter of volume changes measured using nephelometry and light microscopy methods confirmed nephelometry as a sufficiently sensitive method to detect changes of spermatozoa volume. The spermatocrit alteration method resulted in a large proportion of damaged and potentially immotile spermatozoa in media of osmolality less than 150 mOsm/kg in carp and osmolalities from 10 to 300 mOsm/kg in sterlet and brook trout. Therefore, this method is not reliable for assessing spermatozoa swelling in hypotonic solutions, because the integrity of the cells is not fully preserved. Increase in carp spermatozoa (osmotic activation mode) volume occurred during the motility period in hypotonic conditions, but no indications of volume changes were found in sterlet and brook trout spermatozoa (ionic activation mode) associated with environmental osmolality alteration. Accordingly, we conclude that sperm volume changes are differentially involved in the motility activation process. Species-specific differences in spermatozoa volume changes as a response to a hypotonic environment during the motility period are discussed in relation to their potent physiological role.

1. Introduction

Decreasing osmolality and changes in ion concentrations of the surrounding media are triggers for activation of freshwater fish spermatozoa [1–4]. Under natural conditions, a hypoosmotic environment is essential for spermatozoa activation and for fertilization [5], and partial sperm membrane lysis could occur at initiation of motility and up to its arrest [6] because of the large amplitude of the osmotic stress.

The reaction of spermatozoa to osmotic shock varies among fish species, primarily depending on their marine or freshwater environment. In freshwater species, very low ambient water osmolality is directly involved in spermatozoa motility activation in carp (*Cyprinus carpio*) [7,8], and in sturgeon and salmonids, activation can be achieved without osmolality alteration (as compared with seminal fluid) through changes in ionic composition of the surrounding medium [3]. Therefore, in general, spermatozoa motility activation mechanisms in freshwater fish can be

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classified either as the osmotic mode or, if osmolality independent, as ionic mode.

The mechanism by which spermatozoa motility activates through osmotic pressure change is not fully understood. Osmolality-mediated mechanosensitive calcium channels have been shown to be involved in some fish species [9]. Environmental osmolality reduction has also been demonstrated to lead to cell volume increase [7]. We suggest that an increase of sperm cell volume might generate a signal responsible for alteration of mechanosensitive channel activity [9]. Spermatozoa swelling in hypotonic conditions could play an unequal role depending on the fish species sperm motility signaling mode. If swelling of spermatozoa is assumed to play a key role in motility activation, it should occur very quickly. Motility in freshwater fish spermatozoa should be initiated in less than 1 second after transfer of sperm cells into a hypotonic environment as previously predicted [10].

Information about spermatozoa swelling in fish is scarce, and only a few attempts to evaluate volume changes as a response to environmental osmolality have been conducted, using methods such as spermatocrit [7], light microscopy [7], spectrophotometry [11,12], electron paramagnetic resonance (EPR) [13], coulter counting [14], and resistance impulse spectroscopy [6].

Use of any of these methods allows detection of increases in spermatozoa volume during their motility period in carp (osmotic mode of activation). The level of swelling is dependent on the ambient aquatic osmolality and occurs not only at activation but also progressively throughout the motility period [11,12].

In species with an ionic mode of activation, spermatozoa swelling has been detected using the resistance impulse spectroscopy method. Cabrita et al. [6] demonstrated that rainbow trout (*Oncorhynchus mykiss*) spermatozoa swelled immediately after transfer into a hypotonic nonactivating environment; however, the level of volume increase was independent of the surrounding osmolality and incubation period.

Because of the brief period of fish spermatozoa motility, and studies of changes in their volume are technically complicated, a combination of techniques is recommended for volume change detection at activation and during the motility period.

The goal of the present study was to comparatively describe sperm cell volume changes during motility in fish species with ether osmotic or ionic modes of motility activation.

2. Materials and methods

2.1. Broodfish and sperm collection

Fish were maintained in facilities of the Genetic Fisheries Center, and experiments were carried out in the Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic.

Spermiation in common carp (*Cyprinus carpio*) and sterlet (*Acipenseri ruthenus*) was stimulated by temperature manipulation followed by hormone treatment. Sperm from brook trout (*Salvelinus fontinalis*) was obtained during the natural reproduction season without hormone intervention.

Five mature sterlet males (1.2–1.8 kg) were kept in a 400 L indoor tank with water temperature gradually increasing from 7 °C to 15 °C. After a 14-day acclimation period, the fish were intramuscularly injected with carp pituitary suspension at 4 mg/kg of body weight. Milt was collected from the urogenital tract 36 hours after injection using a catheter, and samples were kept on ice during the experiments for 2 hours or less.

Five mature common carp males (2.5–3 kg) were maintained in 1000 L tanks at a temperature of 18 °C for 14 days before injection with carp pituitary extract at a dose of 1 mg/kg of body weight. Sperm collection was carried out 24 hours after injection. Milt was obtained using abdominal massage directly into 20-mL plastic syringes.

Five mature brook trout males (300–500 g) were maintained in a 10,000 L outdoor hatchery tank. Water temperature ranged from 4 °C to 8 °C. Milt was obtained by abdominal massage directly into 10-mL plastic syringes. Special care was taken to avoid contamination by urine, mucus, feces, or water during carp and brook trout sperm collection [15].

Only the samples meeting the following criteria were used: motility 90% to 100% initiated using water from tanks and estimated using routine methods [16] and osmolality of seminal fluid for carp 275 to 290 mOsm/kg, for sterlet 35 to 65 mOsm/kg, and for brook trout 280 to 295 mOsm/kg.

2.2. Media used for experiments

For evaluation of sperm cell volume changes, solutions with osmolality of 300, 250, 200, 150, 100, 50, and 10 mOsm/kg were prepared including 10 mM TRIS-HCl, pH 8.0, and NaCl. Osmolality of each solution was monitored using a vapor pressure osmometer (Wescor).

2.3. Light microscopy

Sperm was added to a drop of the experimental medium (300, 250, 200, 150, 100, 50, and 10 mOsm/kg were prepared including 10 mM TRIS-HCl, pH 8.0, and NaCl) on a microscope slide using the tip of a dissecting needle with which the sperm suspension was thoroughly mixed for 2 seconds. Immediately after dilution, the cell suspension was video-recorded using a negative phase contrast microscope (Olympus BX50, Olympus Plan 40× lens) equipped with CCD video camera (Sony, SSCDC50AP) until cessation of motility. Twenty to 30 spermatozoa were evaluated per each frame. Measurements were conducted in triplicate.

2.4. Scanning electron microscopy

Sperm of five sterlet males were prepared for observation in a cryo-field emission scanning electron microscope (FESEM, CryoSEMs) JSM 7401F (Jeol Ltd., Tokyo, Japan). Milt was prediluted (1:10) with 10 mM TRIS-HCl or 100 mOsm/kg NaCl. After a 60-second incubation period, 10 μL of diluted sperm was placed on the aluminum target and excess liquid drained. The specimen was then frozen by