FISH SPERMATOZOA, PHYSICAL AND BIO-ENERGETIC INTERACTIONS WITH THEIR SURROUNDING MEDIA

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SPERMATOZOIDI RIBA, FIZIČKA I BIOENERGETSKE INTERAKCIJE SA OKOLNOM SREDINOM

Apstrakt

Kod riba sa spoljašnjim oplođenjem, spermatozoidi se prilikom mresta isporučuju rastvoreni u pratećoj semenoj tečnosti u neposrednu okolinu gde se trenutno aktiviraju pokretima bičeva i na taj način stupaju u različite fizičke interakcije. Prvo se javlja osmotski signal na membrani spermatozoida i odmah zatim se talasi prenose sa vrha ka kraju duž biča oblika pantljike/trake (umesto cilindričnog oblika kod većine vrsta), što poboljšava efikasnost kretanje unapred. Zatim, zbog prisustva ovih “peraja” na bičevima, odnos površine i zapremine ribljih spermatozoida je mnogo veći nego kod većine drugih vrsta. Ovo vodi do bolje i brže trans membranske razmene, bilo da se radi o osmotskom, jonskom, gasovitom (CO_2) ili vodenom transferu. Treće, fizička veza između bičeva i površina sa kojim oni reaguju (na primer staklo za slajdove mikroskopa) dovodi do značajnog poboljšanja njihovih pokreta dok plivaju u blizini ovakvih povrsina (kao sto je ljuska jajeta).

Što se tiče bioenergetskih aspekata, spermatozoidi riba brzo plivaju dostižući veoma visoku frekvenciju udarca biča (do 70 - 100 Hz), što podrazumeva veliku potrošnju zaliha ATP-a. Njihova stopa respiracije i proizvodnje ATP-a u mitohondrijama je previše niska u poređenju sa potrošnjom ATP-a neophodnom za pokrete bičeva, odnosno njihovih dineinskih molekula u sklopu aksoneme bičeva. Kao posledica ovoga, intraćelijski nivo ATP-a se smanjuje u toku kretanja što prevremeno narušava pokretljivost ali takođe i druge funkcije kao sto je rad jonske i vodene pumpe. Ubrzo posle (od jednog do nekoliko minuta) aktivacije, nedostatak ATP-a je toliki da pokretljivost bičeva prestaje u potpunosti. Tok ovakvog procesa se moze preokrenuti, pošto se pokazalo da se zalihe ATP-a mogu nadoknaditi u uslovimu u kojima se sprečava pokretljivost spermatozoida, što otvara mogućnost za drugu rundu pokretljivosti.

U ovoj prezentaciji prikazane su specifične odlike ribljih spermatozoide uključujući i efekte temperature na metabolizam ATP-a.

Ključne reči: spermatozoidi riba, pokretljivost, osmotski stres, sadržaj ATP
Keywords: fish spermatozoa, motility, osmotic stress, ATP content

INTRODUCTION

In fish species with external fertilization, spermatozoa, which are immotile in the male tracts, are delivered at spawning by dilution of milt (spermatozoa and their accompanying seminal fluid) into the surrounding medium where they immediately activate the movement of their flagella and therefore are submitted to various physical interactions, which regulate their motility period.

MATERIAL AND METHODS

Details on video microscopy, stroboscopy, high-speed camera recordings, measurement of high-energy compound (ATP and Creatine-Phosphate) can be found in previous publications (Cosson, 2008; Dreanno et al., 2000).

RESULTS AND DISCUSSION

Any efficient forward movement of a spermatozoon needs for its flagellum to generate waves, which should propagate from head to tip. Fish sperm are inactive in testis and seminal fluid but activate at contact with low osmotic conditions in case of fresh water fishes while in marine fishes, the hyper-osmolality (high salt concentration) of sea-water is the main activation signal. Several steps in the activation process should be distinguished temporally. As a first step, due to difference of osmolality between the seminal fluid and the surrounding medium (fresh or sea water), an osmotic (in most fish species) and ionic (K^+ ions in salmonids and chonstrosteans) signal (Morisawa & Suzuki, 1980; Alavi & Cosson, 2006; Cosson et al., 1999; Perchec-Poupard et al., 1997) is perceived at the sperm membrane level: immediately after, waves consequently start to be propagated at high beat frequency from head to tip along the ribbon shaped flagella (instead of cylindrical shape in most other species), this feature leading to affect the forward displacement efficiency of spermatozoa for hydrodynamic reasons (Cosson, 2007 ; Boryshpolets al., 2012).

At motility activation, the main signals responsible for the transfer of information from the membrane to the axoneme are involving membrane polarization, Ca^{2+} entry in the sperm cell, intracellular cAMP rise and phosphorylation of some specific protein components of the axoneme, depending on species (Cosson, 2007b). The osmolality activating signal is observed to be reversible after reversal of exposure back to initial osmolality environment (Cosson et al., 1999; Bondarenko et al., 2013). A second period of motile activity was observed in marine species as well in fresh water fish species (Cosson, 2010). In some of these species, it was shown that ability for spermatozoa to fertilize egg is saved through this experimental procedure (Linhart et al., 2010).

As a second event in the motility period, linked to the presence of some lateral extensions of the flagellar membrane so-called “fins” (see below), the surface to volume ratio of fish spermatozoa appears to be much larger than usually observed in most other species, leading to better and faster trans-membrane exchanges, thus allowing either ions, gas (CO_2) (Inaba et al., 2013) or water transfer (Cosson et al., 1999). In this respect, fish sperm cells will adapt rapidly to extreme osmotic conditions because of this large membrane surface to volume ratio when reacting as an osmometer (Cosson et al., 1999; Bondarenko et al., 2013). A third feature observed during the motility period deals with the physical relationship between flagella and interacting surfaces (glass slide or egg surface), which leads to significant changes of their swimming behavior when swimming in the vicinity of specific surfaces (Boryshpolets et al., 2012). The presence of longitudinal extensions of the flagellar membrane, so-called "fins" is specific to fish sperm and were shown to improve swimming efficiency (Gillies et al., 2012). Nevertheless, the flagellar membrane appears partly damaged during and at the end of the motility period, due to osmolarity stress imposed at activation step: some looping effect appears at the distal tip of flagellum while blebs are frequently observed along the flagellar membrane (Perchec et al., 1996). Obviously these defects contribute to lower the swimming efficiency of the flagellum and reduce the duration of the motility period.

As a general rule and for additional reasons, duration of the motility period lasts for short in case of fish spermatozoa as compared to other species (Cosson, 2010). Right after activation, fish sperm flagella beat at frequency up to 70-100 Hz, leading to a translating movement at high velocity; regarding bio-energetic aspects, these features imply a fast consumption of the sperm ATP stores and motility of fish sperm mostly relies on this ATP store (Cosson, 2010 & 2013). Fish sperm structure belongs to sperm with "simple" structure called "aquasperm", which mostly consists of nucleus and mitochondrion localized at the proximal base of the flagellum (Cosson, 2007b). Also in fish sperm, the respiration rate and consequently, the rate of ATP production by mitochondria are too low as compared to their high ATP consumption rate by flagellar dynein-ATPase motors (Cosson, 2010). As a consequence, intracellular ATP level decreases rapidly during the motility period, which precociously impairs motility, as well as other functions such as ionic or water pump. Within a short period after activation (one to several minutes, depending on species), the lack of ATP and intracellular ionic concentration both lead to conditions where flagellar motility fully stops (Cosson, 2010).

Transiently after motility activation, a gradient of ATP concentration (and other energetic compounds such as Creatine-phosphate) gradually becomes established from the base of flagella, where mitochondria producing ATP are located, to the distal tip. Consequently, such unequal distribution of ATP gives rise to a situation where waves develop with normal amplitude in the proximal flagellum but become more and more dampened in the distal part, prior to full stop.

Such decrease of internal ATP concentration will lead to a decrease in value of most swimming parameters describing the motility: the wave amplitude gradually decreases (see fig. 1), the beat frequency also decreases but also the portion of flagella where waves develop become more and more restricted to the proximal part (in the vicinity of the head) and consequently the number of waves along the flagellum is more and more restricted. Altogether, such decrease of all parameters will combine in such a way that global velocity of forward displacement will gradually and significantly decrease during the motility period.

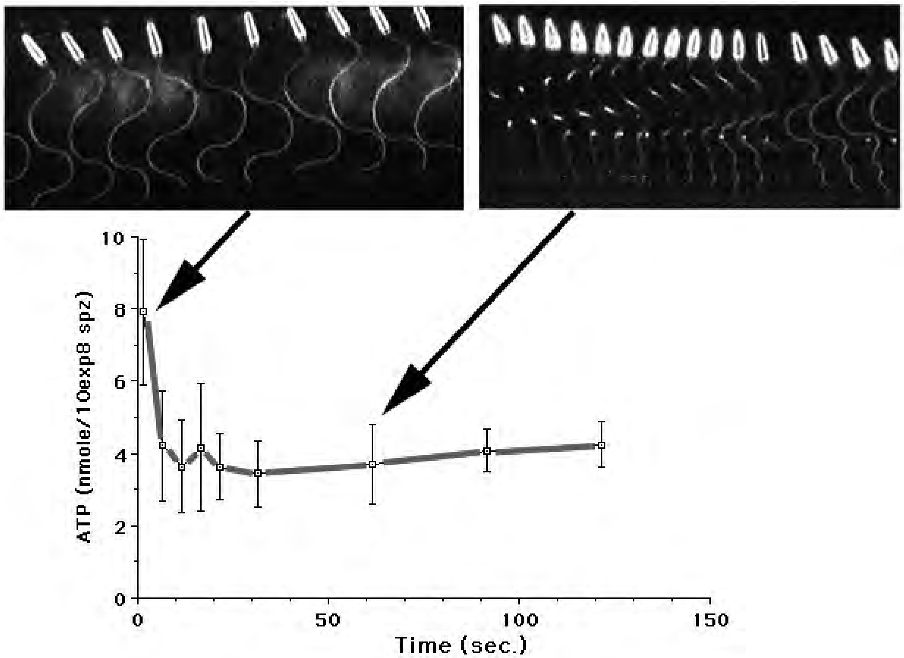

Figure 1. Changes of the flagellar waves pattern in relation to the ATP content during the motility period of sturgeon spermatozoa. Right after initiation of motility (5 sec.), intracellular ATP concentration allows propagation of waves with large amplitude and high beat frequency (left series in upper panel); later in the motility period (60 sec.), ATP concentration is lower and waves propagate mostly in the part of flagellum proximal to the head (right series in upper panel).

Consequently velocity of forward displacement becomes lower and lower as a function of the time elapsed since motility activation. (modified from Billard et al., 1999). In addition, during this period, the integrity of the flagellar structure is affected by side effects due to exposure to drastic osmolality imposed to sperm cells by the harsh conditions of activation. ATP depletion during the motility period contributes to decrease the activity of ion re-equilibrating membrane pumps, but such phenomenon can be reversed, provided ATP stock be replenished.

Such ATP homeostasis and consequently motility parameters are also subjected to temperature effects: the lower the temperature, the longer the motility period.

CONCLUSION

So far, mechanisms of fish sperm motility were deeply explored only in a restricted number of species, mostly belonging to fresh water species such as salmonids (trout or salmon), cyprinids (carp) and chondrosteans (sturgeons) or belonging to marine species (sea bass, turbot, cod as examples). Among such diversity of situations, general rules are emerging which emphasize the major role of osmolality, ions and ATP content at the activation step as well as during the motility period until its achievement.

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