Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/repbio

Review Article

Motility of fish spermatozoa: from external signaling to flagella response

Viktoriya Dzyuba [a,b,*], Jacky Cosson [a]

[a] Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodnany, Czech Republic
[b] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

ARTICLE INFO

Article history:
Received 8 February 2013
Accepted 17 December 2013

Keywords:
Fish sperm
Axoneme
Dynein
Signal transduction
Motility

ABSTRACT

For successful fertilization, spermatozoa must access, bind, and penetrate an egg, processes for which activation of spermatozoa motility is a prerequisite. Fish spermatozoa are stored in seminal plasma where they are immotile during transit through the genital tract of most externally fertilizing teleosts and chondrosteans. Under natural conditions, motility is induced immediately following release of spermatozoa from the male genital tract into the aqueous environment. The nature of an external trigger for the initiation of motility is highly dependent on the aquatic environment (fresh or salt water) and the species' reproductive behavior. Triggering signals include osmotic pressure, ionic and gaseous components of external media and, in some cases, egg-derived substances. Extensive study of environmental factors influencing fish spermatozoa motility has led to the proposal of several mechanisms of activation in freshwater and marine fish. However, the signal transduction pathways initiated by these mechanisms remain clear. This review presents the current knowledge with respect to (1) membrane reception of the activation signal and its transduction through the spermatozoa plasma membrane *via* the external membrane components, ion channels, and aquaporins; (2) cytoplasmic trafficking of the activation signal; (3) final steps of the signaling, including signal transduction to the axonemal machinery, and activation of axonemal dyneins and regulation of their activity; and (4) pathways supplying energy for flagellar motility.

1. Introduction

Sperm quality is a measure of the ability of spermatozoa to successfully fertilize an egg [1]. Spermatozoa motility must be activated to allow them to reach, bind, and penetrate the egg. As a general rule, in fish with external fertilization, spermatozoa are immotile in testis and in seminal fluid [2], and initiation of motility is dependent on the fertilization environment [3–7]. Several factors are known to regulate

* *Corresponding author at:* Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisi 728/II, 389 25 Vodnany, Czech Republic.
Tel.: +420 774576786.
E-mail address: vdzyuba@frov.jcu.cz (V. Dzyuba).

1642-431X/$ – see front matter
http://dx.doi.org/10.1016/j.repbio.2013.12.005

spermatozoa motility. In salmonid fishes, motility is initiated by a decrease in potassium ion (K^+) concentration surrounding the spawned spermatozoa when they are released into fresh water [4]. The main factor in initiation of spermatozoa motility in cyprinid fishes is a decrease in osmolality upon spawning into fresh water [5]. Conversely, hypertonicity of the surrounding medium triggers the motility of spermatozoa of marine teleosts [7]. The motility of spermatozoa in the euryhaline fish, medaka, *Oryzias latipes*, is not so strictly dependent on environmental osmolality, and may be initiated by values ranging from 25 to 686 mOsm/kg [6]. The fore-mentioned examples suggest the existence of a variety of specific signaling pathways for spermatozoa activation. The species-specific differences in sperm sensitivity to environmental osmotic pressure and ion composition, membrane polarization/depolarization processes, involvement of signaling molecules (*e.g.*, cAMP), and phosphorylation of flagella proteins [2,3,8,9] are of special interest. The primary goal of this review is to summarize current knowledge on signaling pathways involved in the process of spermatozoa activation from the reception of the external signal at the level of the plasma membrane to the activation of axonemal dyneins.

2. Activation of fish spermatozoa motility – exogenous signals

2.1. *Duration of spermatozoa motility*

Motility activation of fish spermatozoa is a process lasting fractions of a second, making studies of the biochemical processes underlying the activity of axonemal structures technically difficult [2,8,10]. Estimations of time required for flagellar activity initiation have been described in few fish species. In turbot, *Psetta maxima*, activation of the flagellum after ceasing CO_2 application occurred within 100 ms [11]. In pipefish, *Syngnathus abaster*, whose females deposit eggs into a male organ where fertilization occurs, spermatozoa undergo activation within 80 ms [12]. In most freshwater species, spermatozoa are usually motile for less than 2 min, and, in some cases, are highly active for less than 30 s [13,14]. However, the duration of the sperm motility period can be significantly influenced by the final osmolality, and temperature of the activation media [15], reproduction mode (external or internal fertilization) [3,12,16], and techniques used for preparation of samples for motility observation [10].

Fish species such as the spotted wolf-fish, *Anarhichas minor*, and the 3-spined stickleback, *Gasterosteus aculeatus*, possess spermatozoa that remain motile for several hours after release [17,18]. A prolonged period of spermatozoa motility has also been found in the ocean pout, *Macrozoarces americanus*, and marine sculpin, *Alcichthys alcicornis* [16,19]. Koya et al. [19] found that when the semen of *A. alcicornis* was diluted with a small volume of ovarian fluid, the spermatozoa continued to swim for at least two days, and, with the use of artificial ovarian fluid, motility continued for 7–14 days. The spermatozoa of the ocean pout remained motile in seminal plasma and ovarian fluid for at least 24 h at 4 °C [16]. In addition to ovarian fluid, substances released by the eggs can affect fish spermatozoa motility. Some of these substances are involved in the activation process, while others are sperm-attracting chemicals involved in concentrating spermatozoa in the vicinity of the egg micropyle to increase fertilization success [20].

2.2. *Effect of environmental osmolality on motility activation*

Under natural conditions, motility is induced after the release of spermatozoa from the genital tract into the aqueous environment, where spermatozoa encounter water-soluble components of the external milieu, primarily ions. The environmental osmotic pressure also seems to be consistently involved in activation among species [3,8]. Osmolality of seminal plasma differs significantly between freshwater and saltwater fishes [3]. In freshwater fishes, osmolality ranges from 230 to 346 mOsm/kg (except Acipenseridae at 38–96 mOsm/kg), and for marine fishes, ranges from 249 to 400 mOsm/kg [3]. Thus, fresh water (20–40 mOsm/kg) and sea water (600–1800 mOsm/kg) represent, respectively, a hypotonic and hypertonic environment for a spermatozoon.

The nature of external signal triggering of motility is highly dependent on the fish reproduction environment and the peculiarities of reproductive behavior. Following activation in ambient water, freshwater fish spermatozoa increase cytoplasmic volume in response to hypotonicity. Under hypo-osmotic conditions (45 mOsm/kg), carp, *Cyprinus carpio*, spermatozoa increase in volume several-fold as a result of water influx [21], while rainbow trout, *Oncorhynchus mykiss*, spermatozoa, under similar osmotic conditions (50 mOsm/kg), show less dramatic changes (~30%) [22]. Thus, the tendency of spermatozoa to swell in hypotonic conditions is species-specific. A possible regulatory role of spermatozoa swelling will be discussed later in this review.

In addition to osmolality, other factors may be required for activation of motility in marine fish spermatozoa. For example, activation of herring spermatozoa requires egg-derived substances [23,24]. Two types of sperm-activating factors have been identified in Pacific herring, *Clupea pallasii*, eggs: a water-soluble protein released into the surrounding water [23] and a water-insoluble sperm-motility-initiating factor localized in the vicinity of the micropylar opening of eggs [24]. In contrast, the seminal fluid of Nile tilapia, *Oreochromis niloticus*, contains a sperm motility inhibiting factor [25]. Contact of semen with the surrounding medium leads to activation only when the dilution rate is sufficient to significantly decrease seminal plasma concentration of a high molecular weight glycoprotein that is responsible for immotility.

There are many cases in which isotonic media effectively activate sperm motility in freshwater species including northern pike, *Esox lucius* [26], and Persian sturgeon, *Acipenser persicus* [27]; saltwater species turbot, *Scophthalmus maximus* [28], and haarder, *Mugil soiuy* [29]; and seawater and freshwater acclimated tilapia, *Oreochromis mossambicus* [30].

2.3. *Effect of ionic and gaseous components of the environment on motility activation*

There are extensive data concerning the effects of K^+, Ca^{2+}, Mg^{2+}, and other cations on fish spermatozoa activation [3,7,8],