

REVIEW

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# Molecular mechanism regulating axoneme activation in marine fish: a review

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## Abstract

In many marine fish species, the spermatozoa are immotile in the testis and seminal plasma, and motility is induced when they are released in the aqueous environment. It is well known that the extracellular factors (hyperosmolality or sperm-activating peptides), controlling sperm motility in marine fish, act on the axonemal apparatus through signal transduction across the plasma membrane. To better understand the molecular mechanism regulating axoneme activation in marine fish, the present review examines the existing literature, with particular emphasis on protein phosphorylation/dephosphorylation process.

The present review suggests that: (1) there is no single model that can explain the molecular activation and regulation of sperm motility of the marine fish; (2) only in some species (puffer fish, tilapia, gilthead sea bream, and striped sea bream) protein phosphorylation/dephosphorylation has been shown to be involved in flagellar motility regulation; (3) only a few proteins were identified, which show a change in their state of phosphorylation following sperm activation.

A model of molecular mechanism controlling the activation of sperm motility in gilthead sea bream is being proposed here, which could be a useful model to clarify the sperm motility activation process in other species.

**Keywords:** Fish sperm, Sperm motility, Aquaporin, Teleost

## Review

In fish with external fertilization, spermatozoa are usually immotile in the seminal tract (Stoss 1983). The seminal plasma, whose osmolality and composition depends on the species, protects and immobilizes spermatozoa until they are ejaculated and their motility is initiated (Morisawa 1985). Many environmental stimuli control sperm activation by triggering the different transduction pathways. In salmonids (Baynes et al. 1981; Billard 1983; Morisawa et al. 1983a; Stoss 1983; Morisawa 1985) and sturgeons (Gallis et al. 1991; Toth et al. 1997; Alavi et al. 2004), the reduction of the external  $K^+$  concentration, upon dilution of semen, initiates sperm motility. Hypotonic exposure after dilution into freshwater is the trigger signal in non-salmonid freshwater fish (Morisawa and Suzuki 1980; Morisawa et al. 1983b; Stoss 1983; Morita et al. 2003; Krasznai et al. 2003b), while hypertonic exposure initiates sperm motility in many marine fishes (Morisawa and Suzuki 1980; Oda and Morisawa 1993; Detweiler and Thomas 1998; Krasznai et al. 2003a). It has been also reported that an egg-associated molecule triggers sperm activation in herring (Yanagimachi and Kanoh 1953; Yanagimachi 1957a, 1957b; Yanagimachi et al. 1992; Oda et al. 1998).

All these factors lead to the activation of the axoneme through signal transduction across the plasma membrane. Second messengers, such as cAMP and  $\text{Ca}^{2+}$ , play key roles in the initiation of sperm motility in fish (Morisawa and Okuno 1982; Krasznai et al. 2000; Morita et al. 2003; Zilli et al. 2008a), as well as mammals (Lindemann 1978; Tash and Means 1982; Okamura et al. 1985), sea urchin (Cook et al. 1994), mussel (Stephens and Prior 1992), and tunicate (Opresko and Brokaw 1983). The second messengers may trigger the dynein-mediated sliding of the axonemal outer-doublet microtubules through different mechanisms such as protein phosphorylation/dephosphorylation (Hayashi et al. 1987; Lindemann and Kanous 1989; Inaba et al. 1999; Nomura et al. 2000 Itoh et al. 2001; Zilli et al. 2008a), ADP—binding to dyneine (Lesich et al. 2008; Hayashi and Shingyoji 2009) or ionic strength (Cosson et al. 2008a). This review is focused on the molecular mechanisms that enable environmental stimuli to determine the activation of the axoneme, with emphasis on the role of proteins with phosphorylation/dephosphorylation activity.

### **Sperm motility activation is mediated by an increase in intracellular calcium**

In marine teleosts with external fertilization, three different mechanisms for motility initiation are known. (1) In flatfish species (Inaba et al. 2003), although osmolarity is the primary factor that regulates the initiation of sperm motility, the intracellular  $\text{HCO}_3^-$  concentration plays a key role in this process (higher levels of bicarbonate inhibits motility). The intracellular level of bicarbonate is controlled by a cytosolic carbonic anhydrase that convert intracellular  $\text{HCO}_3^-$  into  $\text{CO}_2$  which lead to decrease in concentration of bicarbonate ( $\text{CO}_2$  diffuses outside the spermatozoa); (2) in herring, *Clupea pallasii* spermatozoa motility is initiated by the presence of a sperm motility initiation factor (SMIF), a 105-kDa basic glycoprotein that is localized to the micropylar region of the herring egg (Yanagimachi and Kanoh 1953; Yanagimachi 1957a, 1957b; Yanagimachi et al. 1992; Pillai et al. 1993); (3) in several marine species (including sea bass *Dicentrarchus labrax*, tuna *Thunnus thynnus*, gilthead sea bream *Sparus aurata*, striped sea bream *Lithognathus mormyrus*, puffer fish *Tetraodontidae*, flounder *Paralichthys orbignyanus*, Atlantic croacker *Micropogonias undulatus*, hake *Merluccius merluccius*, and cod *Gadus morhua*) hyperosmolality regulates sperm activation (Morisawa and Suzuki 1980; Oda and Morisawa 1993; Detweiler and Thomas 1998; Krasznai et al. 2003a; Zilli et al. 2008a; Cosson et al. 2008a, 2008b). Moreover, it has been shown that calcium ions also play a key role in the initiation of sperm motility. Three different mechanisms of action have been proposed for physiological roles of  $\text{Ca}^{2+}$ : (a)  $\text{Ca}^{2+}$  acts directly on the axonemal structures (sea bass and tuna, Cosson et al. 2008a, 2008b); (b)  $\text{Ca}^{2+}$  regulates  $\text{Ca}^{2+}$ /calmodulin-dependent protein phosphorylation that in turn activates the axoneme (for example in puffer fish or seawater-acclimated euryhaline tilapia *Oreochromis mossambicus*) (Krasznai et al. 2003a; Morita et al. 2004); (c)  $\text{Ca}^{2+}$  leads to a cAMP-dependent protein phosphorylation that activates axoneme in gilthead sea bream and striped sea bream (Zilli et al. 2008a). In all suggested mechanisms, the hyperosmolality signal firstly increases intracellular  $\text{Ca}^{2+}$  concentration (Oda and Morisawa 1993), which is similar to what happens in freshwater fish (Cosson et al. 1989; Krasznai et al. 2000) and tunicates (Izumi et al. 1999). This increase could be due to a calcium influx across the plasma membrane, or to a calcium release from intracellular store, or to a cytosol concentration following massive water efflux by aquaporins.

Calcium influx across the plasma membranes has been demonstrated in spermatozoa of seawater-acclimated *Tilapia mossambicus*, (Linhart et al. 1999; Morita et al. 2004), sea

bass, and tuna (Cosson et al. 2008a, 2008b). In vertebrates and invertebrates, an ATP-driven  $\text{Ca}^{2+}$  pump and a  $\text{Na}^+/\text{Ca}^{2+}$  exchangers (Wennemuth et al. 2003) together with many types of calcium channels (Wiesner et al. 1998; Arnoult et al. 1999; Serrano et al. 1999; Westebroek and Babcock 1999; Krasznai et al. 2000; Wennemuth et al. 2000; Jungnickel et al. 2001; Quill et al. 2001; Jagannathan et al. 2002; Nikpoor et al. 2004) allow the calcium flux throughout the plasma membranes of spermatozoa. Up to date, only one calcium transporter has been identified in the plasma membranes of fish spermatozoa. This is a reverse- $\text{Na}^+/\text{Ca}^{2+}$  exchange that causes an efflux of  $\text{Na}^+$  and an influx of  $\text{Ca}^{2+}$  during ligand-induced motility initiation in herring sperm (Vines et al. 2002).

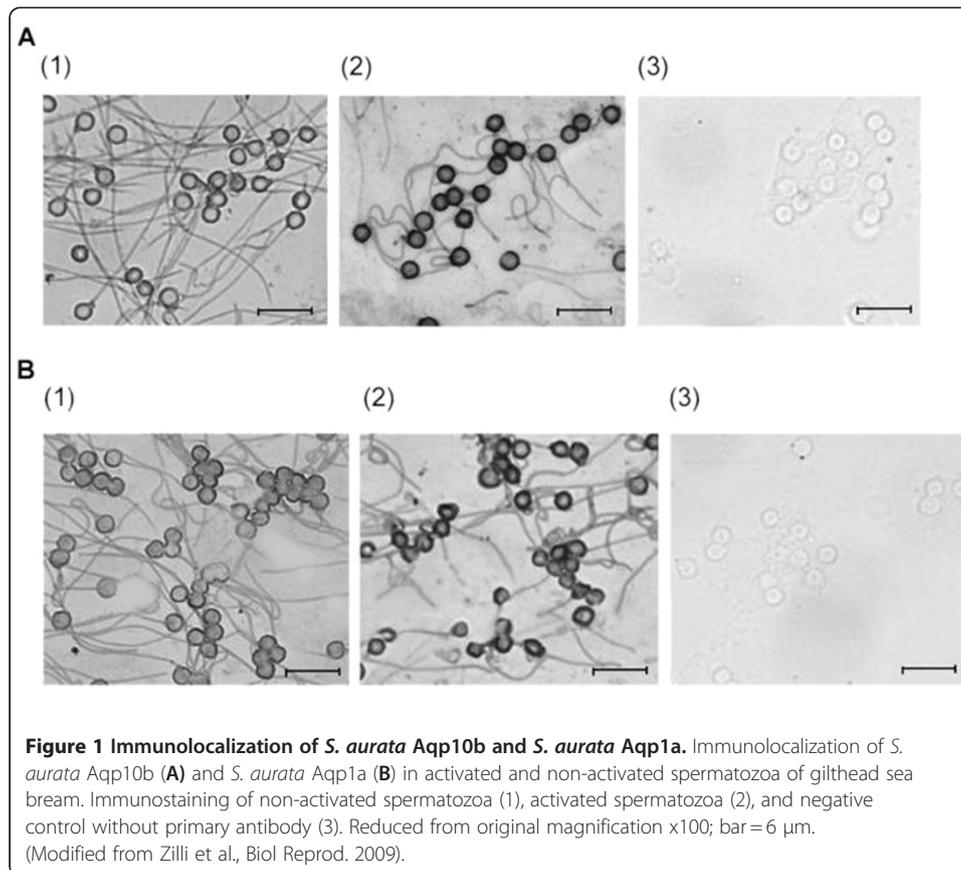
The increase of intracellular calcium concentration could be also a consequence of stretch-activated channel (SAC) activation, when changes in the osmotic pressure occur (Krasznai et al. 2003a; Cosson et al. 2008a, 2008b). It is known that SAC may modify the activity of certain membrane proteins (Vandorpe et al. 1994); therefore, they may increase the calcium membrane conductivity by direct influx of this ion (together with  $\text{K}^+$ ) or by the activation of calcium channels as observed in carp (Krasznai et al. 2003b).

The increase of the intracellular (spermatozoa) calcium concentration, following hyperosmotic signal, could be also due to the release of  $\text{Ca}^{2+}$  from intracellular stores that has been demonstrated in puffer fish (Krasznai et al. 2003a). Although spermatozoa lack endoplasmic reticulum, it seems that the limited set of organelles that could work as intracellular  $\text{Ca}^{2+}$  stores (Naaby-Hansen et al. 2001; Ho and Suarez 2003; Publicover et al. 2007). The role of mitochondria in the calcium storage is still unclear. In sea urchin sperm, a  $\text{Ca}^{2+}$  ATPase (SPCA) has been localized into the giant mitochondrion in the midpiece, thus suggesting a possible role of this organelle as  $\text{Ca}^{2+}$  store (Gunaratne and Vacquier 2006).

Another membrane protein involved in the sperm motility initiation in marine fish is aquaporin that could determine an increase of intracellular calcium as a consequence of cytosol concentration due to massive water efflux after hyper-osmotic signal (Cosson et al. 1999; Zilli et al. 2009). In particular, two kinds of aquaporins have been identified in gilthead sea bream spermatozoa: aquaporin1a (Aqp1a) and *S. aurata* aquaglyceroporin (Glp); the last one has been recently identified as Aqp10b (Zilli et al. 2009; Cerda and Finn 2010). Aqp1a and Aqp10b are localized in the plasma membrane of the head and flagellum of spermatozoa. Immunostaining technique demonstrated that the expression of aquaporins increases after motility activation, which suggests a possible recruitment of aquaporins into the plasma membrane from intracellular vesicles following hyperosmotic signal (Figure 1). Aqp1a and Aqp10b could play different roles during the process of sperm activation in sea bream. It is suggested that the Aqp1a mediates sperm activation, and Aqp10b involves in the maintenance of motility, as suggested for Aqp7 in human spermatozoa (Saito et al. 2004). The physiological role of aquaglyceroporins during sperm motility in vertebrates, however, is not well understood, although it is known that mammalian spermatozoa are able to use glycerol aerobically (Mann and White 1956; Aalbers et al. 1961) and that organic alcohols (including glycerol) induce protein phosphorylation for motility initiation in chum salmon *Oncorhynchus keta* (Morita et al. 2005).

#### **Flagellar axoneme activation: final event in the mechanism of sperm motility activation in marine fish**

The final event in the mechanism of sperm motility initiation is the activation of the axoneme. This is a microtubule-based, highly organized, and conserved structure composed



by more than 250 kinds of proteins. For motility activation, the activity of dynein (the molecular motor) has to be started (and regulated) to produce the coordinated sliding of microtubules in the axoneme (Brokaw 1989; King 2000).

In marine fish, the activation of axoneme is achieved by different mechanisms. In sea bass and tuna spermatozoa, the key factor to start the beating of the flagella is the variation of ionic strength (Alavi and Cosson 2006; Cosson et al. 2008a). In particular, Cosson et al. (2008a) proposed the following model. The water efflux due to the hyperosmotic shock could cause a local membrane distortion that activates SAC. The activation of the SAC could lead to the activation of water channels resulting in rapid release of water from the cells. The result of this process would increase the ionic strength of intracellular fluids leading to the activation of dynein. In flatfish,  $\text{HCO}_3^-$  ion appears to act directly on the axonemal machinery itself since it inhibits the movement of demembrated spermatozoa (Inaba et al. 2003).

In herring sperm, increasing concentration of calcium ions is the main factor that determines the activation of the axoneme. In particular, sperm motility initiation factor (SMIF) induces calcium influx by opening the voltage-gated calcium channels and activating a reverse  $\text{Na}^+/\text{Ca}^{2+}$  exchange (Vines et al. 2002). SMIF determines approximately fourfold increase in  $\text{Ca}^{2+}$  concentration that acts on the axoneme inducing motility (Pillai et al. 1993; Vines et al. 2002; Cherr et al. 2008).

In some fish species, protein phosphorylation/dephosphorylation is involved in flagellar motility regulation. In puffer fish and tilapia sperm, the activity of the flagellar axoneme is

regulated by  $\text{Ca}^{2+}$ /calmodulin-dependent protein phosphorylation, while in gilthead sea bream and striped sea bream by cAMP-dependent protein phosphorylation (Morita et al. 2003; Krasznai et al. 2003a; Zilli et al. 2008a, 2009). The major targets of protein phosphorylation/dephosphorylation causing the activation of sperm motility are structural components of dynein arms (inner and outer), kinases, and phosphatases anchored in the axoneme and in the radial spoke proteins (Dey and Brokaw 1991; Hamasaki et al. 1991; Porter and Sale 2000; Yang et al. 2001). Kinases and phosphatases are required for local control of motor activity (Porter and Sale 2000; Aparicio et al. 2007), and radial spoke proteins regulate inner arm dynein by phosphorylation/dephosphorylation (Smith and Lefebvre 1997; Porter and Sale 2000). However, only few proteins involved in the initiation of motility in marine fish spermatozoa have been identified. Morita et al. (2004) have demonstrated, using demembrated tilapia spermatozoa, that calcium not only initiates flagellar motility but also modulates the flagellar waveform. The same researchers identified a  $\text{Ca}^{2+}$ -binding protein (CaM) with MW of 18 kDa and pI 4.0 that regulates the flagellar motility in a calcium dependent manner by modifying both the sliding velocity and flagellar waveform (Morita et al. 2003, 2004, 2006). The same researchers also identified in tilapia spermatozoa a  $\text{Ca}^{2+}$ /CaM-dependent protein kinase IV (CaMKIV), localized along the flagellum and sleeve structure, that is involved in the activation and regulation of sperm flagellar motility through a  $\text{Ca}^{2+}$ /CaM-dependent phosphorylation of seven axonemal proteins. In gilthead sea bream, three proteins have been identified that change their phosphorylation state after sperm activation and play a role in the initiation of sperm motility (Zilli et al. 2008a, 2009):

- (1) An A-kinase anchor proteins (AKAP) have the function of binding to the regulatory subunits (RI and RII) of protein kinase A (PKA) and confining the enzyme to discrete locations within the cell. Therefore, cAMP levels temporally regulate PKA, whereas the spatial regulation within the cell occurs through compartmentalization by binding to AKAP, thus assuring specificity of PKA function. The role of AKAP as a key regulator of sperm motility has been already established (Vijayaraghavan et al. 1997). In addition, a recent study demonstrated that phosphorylation of AKAP in human sperm results in tail recruitment of PKA and increase of sperm motility, providing evidence for a functional role of phosphorylation of AKAP (Luconi et al. 2004);
- (2) The acetyl-CoA synthetase activates acetate to acetyl-CoA, and provides the cell with the two carbon metabolite used in many anabolic and energy generation processes. Therefore, this enzyme could be activated in motile sperm to increase the level of ATP, which is necessary for flagellar movement;
- (3) A novel protein similar to phosphatase and actin regulator 3 of *Danio rerio* that may be a protein phosphatase inhibitor.

In striped sea bream, two proteins involved in the activation of sperm motility have been identified: myotubularin-related protein 1 and dual-specificity tyrosine phosphorylation-regulated kinase 3 (DYRK3) (Zilli et al. 2008a). The myotubularin-related protein 1 belongs to the protein-tyrosine phosphatase family, and DYRK3 is a protein kinase auto-phosphorylated on tyrosine residues belonging to the dual-specificity tyrosine phosphorylated and regulated kinase family. Many studies have demonstrated that the development and maintenance of motility is regulated by a complex balance

between kinase and phosphatase activities (Tash and Bracho 1994; Porter and Sale 2000, King 2000; Aparicio et al. 2007).

From the above report, it clearly emerges that there are different mechanisms of sperm motility initiation in marine fish. They are species-specific, and they reflect the adaptation to species life histories/environment, and only parts of which have been studied in the different species. The identified proteins that play a role in this mechanism in marine fish are summarized in Table 1, where possible homologues in freshwater fish, invertebrate, and mammalian have been also reported.

### ***S. aurata* spermatozoa as a model to study the molecular mechanism of sperm motility activation**

The molecular mechanism that determines sperm motility activation in gilthead sea bream has many similarities with the mechanisms observed in many animals (sea urchin, salmonids, and mammals). This is not surprising since that internal microtubule-based structures of the axoneme have been well conserved during evolution. For this reason, the identification of proteins that change their phosphorylation state following sperm motility activation and the understanding of signaling pathways among these in gilthead sea bream spermatozoa could be interesting to clarify this process in other species.

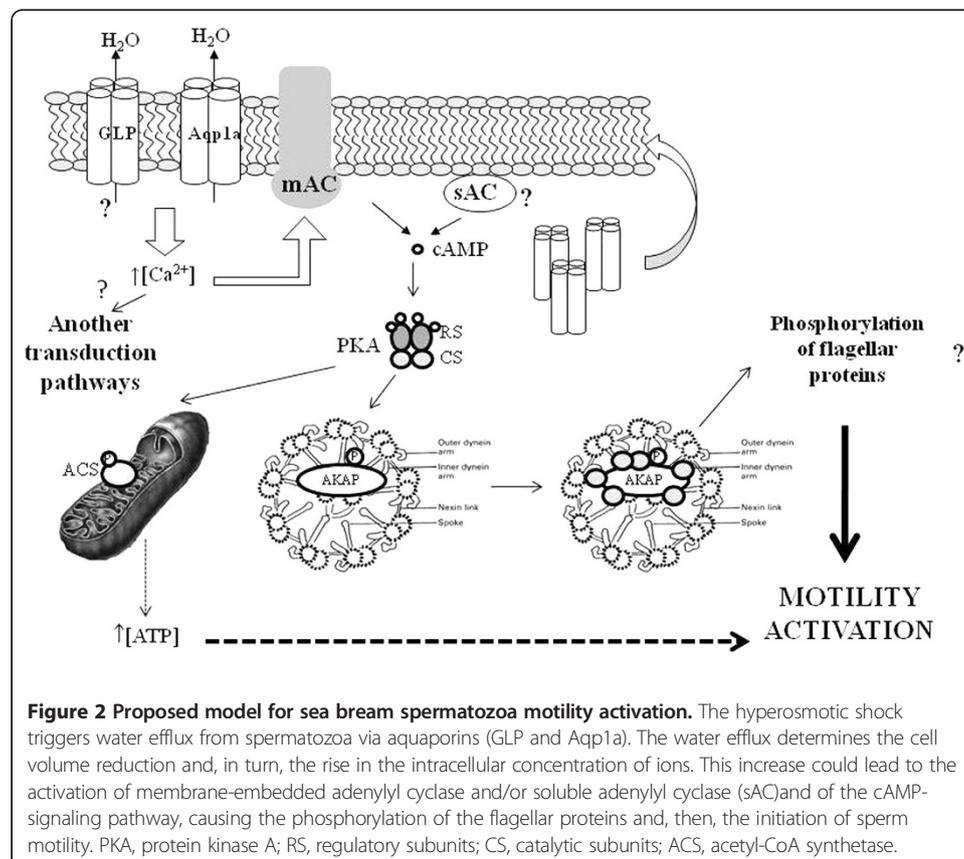
In *S. aurata*, a drastic change of the environmental osmolality is the signal that triggers sperm motility activation. The transduction of this event in axoneme activation requires action of many intracellular mediators. First event is the water efflux that leads to local distortions of the flagellar membrane that, in turn, activates water channels. Recent study (Zilli et al. 2011) confirms the important role of aquaporins in initiating sperm motility; in fact, when these proteins are inhibited by HgCl<sub>2</sub>, the phosphorylation of some proteins (174 kDa protein of head; 147, 97, and 33 kDa proteins of flagella), following the hyper-

**Table 1 Proteins involved in sperm motility activation and their homologues in fish, invertebrates, and mammals**

<b>Protein</b>	<b>Marine fish species</b>	<b>Fresh water fish species/ invertebrate and/mammalian homologues</b>
Ca <sup>2+</sup> -binding protein	<i>O. mossambicus</i> (Morita et al. 2009)	<i>Homo sapiens</i> (Marín-Briggiler et al. 2005); <i>A. digitifera</i> (Morita et al. 2009); <i>Ciona intestinalis</i> (Nomura et al., 2000, 2004);
Ca <sup>2+</sup> /CaM dependent protein kinase IV	<i>O. mossambicus</i> (Morita et al. 2006)	<i>H. sapiens</i> (Marín-Briggiler et al. 2005); <i>A. digitifera</i> (Morita et al. 2009)
A-kinase anchor proteins	<i>S. aurata</i> (Zilli et al. 2008a)	<i>H. sapiens</i> (Carr et al. 2007; Luconi et al. 2011); <i>Bos taurus</i> , <i>Mus musculus</i> (Moss et al. 1999)
Acetyl-CoA synthetase	<i>S. aurata</i> (Zilli et al. 2008)	–
Novel protein similar to phosphatase and actin regulator 3 of <i>D. rerio</i>	<i>S. aurata</i> (Zilli et al. 2008)	–
Myotubularin-related protein 1	<i>L. mormyrus</i> ( et al. 2008)	–
Dual-specificity tyrosine phosphorylation-regulated kinase 3	<i>L. mormyrus</i> (Zilli et al. 2008)	<i>Rattus norvegicus</i> (Becker et al. 1998)
Aquaporin	<i>S. aurata</i> (Zilli et al. 2009)	<i>H. sapiens</i> , <i>M. musculus</i> (Chen et al. 2011); <i>B. taurus</i> (Ma et al. 2011); <i>Macaca radiata</i> (Shayu et al. 2005)

osmotic shock, is also completely or partially inhibited. However, more than one transduction pathways could be activated when sea bream spermatozoa are ejaculated in seawater, since numerous proteins showed an HgCl<sub>2</sub> (Aqps)-independent phosphorylation state after sperm activation.

As reported in Figure 2 in gilthead sea bream spermatozoa, the rapid water efflux across AQPs determines a reduction in cell volume with the increase in intracellular ionic concentration. It is known that adenylyl cyclase is activated by different mechanisms, such as membrane hyperpolarization (Beltran et al. 1996; Izumi et al. 1999) and/or increase in Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> concentration (Visconti and Kopf 1998). The cAMP signaling pathway starts the activation of sperm motility by phosphorylation of some proteins. This post-transductional modification in sperm motility activation was recently (Zilli et al. 2011) confirmed by observation that a higher number of protein bands underwent a change of their phosphorylation state at flagella level with respect to the head level in gilthead sea bream. However, it must be underlined that the proposed model fits well to the gilthead sea bream sperm activation but cannot generalize to other marine fish species. A cAMP-dependent protein phosphorylation, involved in sperm motility activation, has been also demonstrated in chum salmon (Itoh et al. 2001) and trout (Hayashi et al. 1987). In many species, some proteins phosphorylated in PKA-dependent manner have been identified as the light (from 8 to 30 kDa) or heavy (approximately 500 kDa) chains of the outer arm dynein of sperm flagellum, such as the 21 kDa protein of chum salmon (Inaba et al. 1999), the 32 and the 500 kDa proteins in sea urchin spermatozoa (Bracho et al. 1998), the 21 and the 26 kDa proteins in ascidian



spermatozoa (Nomura et al. 2000), the 27 and the 20 kDa proteins of mussel spermatozoa (Stephens and Prior 1992), and the 18 to 20 kDa protein in *C. intestinalis* sperm (Dey and Brokaw 1991). In addition, in salmonid fish, a 48 kDa protein, phosphorylated in a cAMP-dependent manner, was identified as regulatory subunit of PKA (Itoh et al. 2003). In *S. aurata*, an AKAP protein that anchors the regulatory subunit of PKA for tethering of protein kinases in close proximity to their target proteins has been identified. Different types of AKAP have been found in spermatozoa, localized into the fibrous sheath of the principal piece (Moss and Gerton 2001). In mammals, it has been demonstrated that, among the proteins phosphorylated during epididymal maturation, there are several mitochondrial proteins (Aitken et al. 2007) and a protein phosphatase PP1 $\gamma$ 2 (Chakrabarti et al. 2007). This is in agreement with a previous finding regarding proteins phosphorylated after motility initiation in gilthead sea bream spermatozoa that are precisely one mitochondrial protein (acetyl-CoA synthetase) and one protein that may be a protein phosphatase inhibitor; in addition in striped sea bream, two proteins were identified, a phosphatase and a kinase, that are involved in sperm motility activation (Zilli et al. 2008a). The activation of proteins of sperm mitochondria could be important to provide the energy for sperm motility; in fact, fish sperm quality is correlated with ATP content (Christen et al. 1987; Zilli et al. 2004). Mature spermatozoa are highly specialized cells, transcriptionally inactive and unable to synthesize new proteins; for this reason, protein phosphorylation/dephosphorylation has to rely on regulation of many processes that is greater than in many other types of cell (Urner and Sakkas 2003).

## Conclusions

From this review emerges plainly that a complex universal mechanism for sperm motility initiation in marine fish does not exist, but there are different mechanisms that are species-specific, only parts of which have been studied in the different species. In particular, in some of these species (puffer fish, tilapia, gilthead sea bream, and striped sea bream), protein phosphorylation/dephosphorylation has been shown to be involved in flagellar motility regulation and present many similarities with the mechanisms of axoneme activation of marine invertebrate and mammal spermatozoa.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

ZL has made substantial contributions to conception and design. SR and SC have been involved in drafting the manuscript. VS has been involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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## References

- Aalbers JC, Mann T, Polge C (1961) Metabolism of boar semen in relation to sperm motility. *J Reprod Fert* 2:42–53
- Aitken RJ, Nixon B, Lin M, Koppers AJ, Lee YH, Baker MA (2007) Proteomic changes in mammalian spermatozoa during epididymal maturation. *Asian J Androl* 9:554–564
- Alavi SMH, Cosson J (2006) Sperm motility in fishes: (II) Effects of ions and osmotic pressure: a review. *Cell Biol Int* 30:1–14
- Alavi SMH, Cosson J, Karami M, Amiri BM, Akhoundzadeh MA (2004) Spermatozoa motility in the Persian sturgeon, *Acipenser persicus*: effects of pH, dilution ratio, ions and osmolality. *Reproduction* 128:819–828
- Aparicio IM, Bragado MJ, Gil MC, Garcia-Herreros M, Gonzalez Fernandez L, Tapia JA, Garcia-Marin LJ (2007) Porcine sperm motility is regulated by serine phosphorylation of the glycogen synthase kinase-3 $\alpha$ . *Reproduction* 134:435–444
- Arnoult C, Kazam IG, Visconti PE, Kopf GS, Villaz M, Florman HM (1999) Control of the low voltage-activated calcium channel of mouse sperm by egg ZP3 and by membrane hyperpolarization during capacitation. *Proc Natl Acad Sci USA* 96:6757–6762

- Baynes SM, Scott AP, Dawson AP (1981) Rainbow trout, *Salmo gairdnerii* Richardson, spermatozoa: effects of cations and pH on motility. *J Fish Biol* 19:259–267
- Becker W, Weber Y, Wetzel K, Eirimbter K, Tejedor F, Joost H-G (1998) Sequence characteristics, subcellular localization and substrate specificity of dyrk-related kinases, a novel family of dual specificity protein kinases. *J Biol Chem* 273:25893–25902
- Beltran C, Zapata O, Darszon A (1996) Membrane potential regulates sea urchin sperm adenyl cyclase. *Biochemistry* 35:7591–7598
- Billard R (1983) Effects of ceolomic and seminal fluids and various saline diluents on the fertilizing ability of spermatozoa in the rainbow trout, *Salmo gairdnerii*. *J Reprod Fertil* 68:77–84
- Bracho GE, Fritch JJ, Tash JS (1998) Identification of flagellar proteins that initiate the activation of sperm motility in vivo. *Biochem Biophys Res Commun* 242:231–23
- Brokaw CJ (1989) Operation and regulation of the flagellar oscillator. In: Warner F, Satir P, Gibbons R (eds) *Movement C. The Dynein ATPases*, vol. 1. AR Liss, New York, pp 267–279
- Carr DW, Hanlon Newell AE (2007) The role of A-kinase anchoring proteins (AKAPs) in regulating sperm function. *Soc Reprod Fertil Suppl* 63:135–142
- Cerda J, Finn RN (2010) Piscine aquaporins: an overview of recent advances. *J Exp Zool A: Ecol Genet Physiol* 313A:623–650
- Chakrabarti R, Cheng L, Puri P, Soler D, Vijayaraghavan S (2007) Protein phosphatase PP1 gamma 2 in sperm morphogenesis and epididymal initiation of sperm motility. *Asian J Androl* 9:445–452
- Chen Q, Peng H, Lei L, Zhang Y, Kuang H, Cao Y, Shi QX, Ma T, Duan E (2011) Aquaporin 3 is a sperm water channel essential for postcopulatory sperm osmoadaptation and migration. *Cell Res* 21:922–933
- Cherr GN, Morisama M, Vines CA, Yoshida K, Smith EH, Matsubara T, Pillai MC, Griffin FJ, Yanagimachi R (2008) Two egg-derived molecules in motility initiation and fertilization in Pacific herring (*Clupea pallasii*). *Int J Dev Biol* 52:743–752
- Christen F, Gatti J-L, Billard R (1987) Trout sperm motility. The transient movement of trout sperm is related to changes in the concentration of ATP following the activation of flagellar movement. *Eur J Biochem* 166:667–671
- Cook SP, Brokaw CJ, Muller CH, Babcock DF (1994) Sperm chemotaxis: egg peptides control cytosolic calcium to regulate flagellar responses. *Dev Biol* 165:10–19
- Cosson MP, Billard R, Letellier L (1989) Rise of internal  $Ca^{2+}$  accompanies the initiation of trout sperm motility. *Cell Motil Cytoskel* 14:424–434
- Cosson J, Billard R, Cibert C, Dreanno C, Suquet M (1999) Ionic factors regulating the motility of fish sperm. In: Gagnon C (ed) *The male gamete: from basic to clinical applications*. Cache Rive Press, Vienna, Illinois, pp 161–186
- Cosson J, Groison A-L, Suquet M, Fauvel C, Dreanno C, Billard R (2008a) Studying sperm motility in marine fish: an overview on the state of the art. *J Appl Ichthyol* 24:460–486
- Cosson J, Groison A-L, Suquet M, Fauvel C, Dreanno C, Billard R (2008b) Marine fish spermatozoa: racing ephemeral swimmers. *Reproduction* 136:277–294
- Detweiler C, Thomas P (1998) Role of ions and ion channels in the regulation of Atlantic croaker sperm motility. *J Exp Biol* 281:139–148
- Dey CS, Brokaw CJ (1991) Activation of Ciona sperm motility: phosphorylation of dynein polypeptides and effects of a tyrosine kinase inhibitor. *J Cell Sci* 100:815–824
- Gallis JL, Fedrigo E, Jatteau P, Bonpunt E, Billard R (1991) Siberian sturgeon, *Acipenser baeri*, spermatozoa. In: Williot P (ed) *Acipenser effects of dilution, pH, osmotic pressure, sodium and potassium ions on motility*. Cemagref Publ, France, Bordeaux, pp 143–151
- Gunaratne HJ, Vacquier VD (2006) Evidence for a secretory pathway  $Ca^{2+}$ -ATPase in sea urchin spermatozoa. *FEBS Letters* 580:3900–3904
- Hamasaki T, Barkalow K, Richmond J, Satir P (1991) cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22 S dynein arm regulates microtubule translocation velocity and swimming speed in paramecium. *Proc Natl Acad Sci USA* 88:7918–7922
- Hayashi S, Shingyoji C (2009) Bending-induced switching of dynein activity in elastase-treated axonemes of sea urchin sperm-roles of  $Ca^{2+}$  and ADP. *Cell Motil Cytoskel* 66:292–301
- Hayashi H, Yamamoto K, Yonekawa H, Morisawa M (1987) Involvement of tyrosine protein kinase in the initiation of flagellar movement in rainbow trout spermatozoa. *J Biol Chem* 262:16692–16698
- Ho H-C, Suarez SS (2003) Characterization of the intracellular calcium store at the base of the sperm flagellum that regulate hyperactivated motility. *Biol Reprod* 68:1590–1596
- Inaba K, Kagami O, Ogawa K (1999) Tctex2-related outer arm dynein light chain is phosphorylated at activation of sperm motility. *Biochem Biophys Res Commun* 256:177–183
- Inaba K, Dreanno C, Cosson J (2003) Control of flatfish sperm motility by  $CO_2$  and carbonic anhydrase. *Cell Motil Cytoskel* 55:174–187
- Itoh A, Inaba K, Fujinoki M, Morisawa M (2001) Motility-associated and cyclic AMP-dependent protein phosphorylation in the sperm of the chum salmon, *Oncorhynchus keta*. *Biomed Res* 22:241–248
- Itoh A, Inaba K, Ohtake H, Fujinoki M, Morisawa M (2003) Characterization of a cAMP-dependent protein kinase catalytic subunit from rainbow trout spermatozoa. *Biochem Biophys Res Commun* 305:855–861
- Izumi H, Marian T, Inaba K, Oka Y, Morisawa M (1999) Membrane hyperpolarization by sperm-activating and -attracting factor increases cAMP level and activates sperm motility in the ascidian *Ciona intestinalis*. *Dev Biol* 213:246–25
- Jagannathan S, Punt EL, Gu Y, Arnoult C, Sakkas D, Barratt CL, Publicover SJ (2002) Identification and localisation of T-type voltage-operated calcium channel subunits in human male germ cells. Expression of multiple isoforms. *J Biol Chem* 277:8449–8456
- Jungnickel MK, Marrero H, Birnbaumer L, Lemos JR, Florman HM (2001) Trp2 regulates entry of  $Ca^{2+}$  into mouse sperm triggered by egg ZP3. *Nat Cell Biol* 3:499–502
- King SM (2000) The dynein microtubule motor. *Biochim Biophys Acta* 1496:60–75
- Krasznai Z, Marian T, Izumi H, Damjanovich S, Balkay L, Tron L, Morisawa M (2000) Membrane hyperpolarization removes inactivation of  $Ca^{2+}$  channels leading to  $Ca^{2+}$  influx and initiation of sperm motility in the common carp. *Biophys J* 78:2052–2067
- Krasznai Z, Morisawa M, Krasznai ZT, Morisawa S, Inaba K, Bazsane ZK, Rubovszky B, Bodnár B, Borsos A, Márián T (2003a) Gadolinium, a mechano-sensitive channel blocker, inhibits osmosis initiated motility of sea- and freshwater fish sperm, but does not affect human or ascidian sperm motility. *Cell Motil Cytoskel* 55:232–243

- Krasznai Z, Morisawa M, Morisawa S, Krasznai ZT, Tron L, Marian T (2003b) Role of ion channels and membrane potential in the initiation of carp sperm motility. *Aquat Living Resour* 16:445–449
- Lesich KA, Pelle DW, Lindemann CB (2008) Insights into the mechanism of ADP action on flagellar motility derived from studies on bull sperm. *Biophys J* 95:472–482
- Lindemann CB (1978) A cAMP-induced increase in the motility of demembrated bull sperm models. *Cell* 13:9–18
- Lindemann CB, Kanous KS (1989) Regulation of mammalian sperm motility. *Arch Androl* 23:1–22
- Linhart O, Walford J, Silvaloganathan B, Lam TJ (1999) Effect of osmolality and ions on the motility of stripped and testicular sperm of freshwater- and seawater-acclimated tilapia, *Oreochromis mossambicus*. *J Fish Biol* 55:1344–1358
- Luconi M, Carloni V, Marra F, Ferruzzi P, Forti G, Baldi E (2004) Increased phosphorylation of AKAP by inhibition of phosphatidylinositol 3-kinase enhances human sperm motility through tail recruitment of protein kinase A. *J Cell Sci* 117:1235–1246
- Luconi M, Cantini G, Baldi E, Forti G (2011) Role of a-kinase anchoring proteins (AKAPs) in reproduction. *Front Biosci* 16:1315–1330
- Ma TH, Liu JF, Zhao RF, Jiang H, Dai LS, Zhao YM, Zhao ZH, Zhang JB (2011) Association analysis of aquaporin 7 (AQP7) gene variants with semen quality and fertility in bulls. *Turk J Vet Anim Sci* 35:63–66
- Mann T, White LG (1956) Metabolism of glycerol, sorbitol and related compounds by spermatozoa. *Nature, Lond* 178:142–143
- Marin-Briggiler CI, Jha KN, Chertihin O, Buffone MG, Herr JC, Vazquez-Levin MH, Visconti PE (2005) Evidence of the presence of calcium/calmodulin-dependent protein kinase IV in human sperm and its involvement in motility regulation. *J Cell Sci* 118:2013–2022
- Morisawa M (1985) Initiation mechanism of sperm motility at spawning in teleost. *Zool Sci* 2:605–615
- Morisawa M, Okuno M (1982) Cyclic AMP induces maturation of trout sperm axoneme to initiate motility. *Nature* 295:703–704
- Morisawa M, Suzuki K (1980) Osmolality and potassium ions: Their roles in initiation of sperm motility in teleosts. *Science* 210:1145–1147
- Morisawa M, Suzuki K, Morisawa S (1983a) Effects of potassium and osmolality on spermatozoan motility of salmonid fishes. *J Exp Biol* 107:105–113
- Morisawa M, Suzuki K, Shimizu H, Morisawa S, Yasuda K (1983b) Effects of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes. *J Exp Biol* 107:95–103
- Morita M, Takemura A, Okuno M (2003) Requirement of  $Ca^{2+}$  on activation of sperm motility in euryhaline tilapia (*Oreochromis mossambicus*). *J Exp Biol* 206:913–921
- Morita M, Takemura A, Okuno M (2004) Acclimation of sperm motility apparatus in seawater-acclimated euryhaline tilapia, *Oreochromis mossambicus*. *J Exp Biol* 207:337–34
- Morita M, Fujinoki M, Okuno M (2005)  $K^{+}$ -independent initiation of motility in chum salmon sperm treated with an organic alcohol, glycerol. *J Exp Biol* 208:4549–4556
- Morita M, Takemura A, Nakajima A, Okuno M (2006) Microtubule sliding movement in tilapia sperm flagella axoneme is regulated by  $Ca^{2+}$ /calmodulin-dependent protein phosphorylation. *Cell Motil Cytoskel* 63:459–470
- Morita M, Nishikawa A, Nakajima A, Iguchi A, Sakai K, Takemura A, Okuno M (2009) Eggs regulate sperm flagellar motility initiation, chemotaxis and inhibition in the coral *Acropora digitifera*. A gemmifera and A tenuis. *J Exp Biol* 209:4574–4579
- Moss SB, Gerton GL (2001) A-kinase anchor proteins in endocrine systems and reproduction. *Trends Endocrinol Metabol* 12:434–440
- Moss SB, Turner RM, Burkert KL, VanScoy BH, Gerton GL (1999) Conservation and function of a bovine sperm A-kinase anchor protein homologous to mouse AKAP82. *Biol Reprod* 61:335–342
- Naaby-Hansen S, Wolkowicz MJ, Klotz K, Bush LA, Westbrook VA, Shibahara H, Shetty J, Coonrod SA, Reddi PP, Shannon J, Kinter M, Sherman NE, Fox J, Flickinger CJ, Herr JC (2001) Co-localization of the inositol 1,4,5-trisphosphate receptor and calreticulin in the equatorial segment and in membrane bounded vesicles in the cytoplasmic droplet of human spermatozoa. *Mol Hum Reprod* 7:923–933
- Nikpoor P, Mowla SJ, Movahedin M, Ziaee SA, Tiraihi T (2004) CatSper gene expression in postnatal development of mouse testes and in subfertile men with deficient sperm motility. *Hum Reprod* 19:124–12
- Nomura M, Inaba K, Morisawa M (2000) Cyclic AMP- and calmodulin-dependent phosphorylation of 21- and 26-kDa proteins in axoneme is a prerequisite for SAAF-induced motile activation in ascidian spermatozoa. *Dev Growth Differ* 42:129–138
- Nomura M, Inaba K, Morisawa M (2004) Calmodulin/calmodulin-dependent protein kinase II mediates SAAF-induced motility activation of ascidian sperm. *Cell Motil Cytoskeleton* 59:28–37
- Oda S, Morisawa M (1993) Rises of intracellular  $Ca^{2+}$  and pH mediate the initiation of sperm motility by hyperosmolality in marine teleosts. *Cell Mot Cytoskel* 25:171–178
- Oda S, Igarashi Y, Manaka K-I, Koibuchi N, Sakai-Sawada M, Sakai K, Morisawa M, Ohtake H, Shimizu N (1998) Sperm-activating proteins obtained from the Herring eggs are homologous to trypsin inhibitors and synthesized in follicle cells. *Develop Biol* 204:55–63
- Okamura N, Tajima Y, Soejima A, Masuda H, Sugita Y (1985) Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. *J Biol Chem* 260:9699–9705
- Opresko L, Brokaw CJ (1983) cAMP-dependent phosphorylation associated with activation of motility of Ciona sperm flagella. *Gamete Res* 8:201–218
- Pillai MC, Shields TS, Yanagimachi R, Cherr GN (1993) Isolation and partial purification of the sperm motility initiation factor from eggs of the Pacific Herring, *Clupea pallasii*. *J Exp Zool* 265:336–342
- Porter ME, Sale WS (2000) The 9 + 2 axoneme anchors multiple inner arm dyneins and a network of kinases and phosphatases that control motility. *J Cell Biol* 151:F37–F42
- Publicover S, Harper CV, Barratt C (2007)  $[Ca^{2+}]_i$  signalling in sperm-making the most of what you've got. *Nat Cell Biol* 9:235–242
- Quill TA, Ren D, Clapham DE, Garbers DL (2001) A voltage-gated ion channel expressed specifically in spermatozoa. *Proc Natl Acad Sci USA* 98:12527–12531

- Saito K, Kageyama Y, Okada Y, Kawakami S, Kihara K, Ishibashi K, Sasaki S (2004) Localization of aquaporin-7 in human testis and ejaculated sperm: possible involvement in maintenance of sperm quality. *J Urol* 172:2073–2076
- Serrano CJ, Trevino CL, Felix R, Darszon A (1999) Voltage dependent  $Ca^{2+}$  channel subunit expression and immunolocalization in mouse spermatogenic cells and sperm. *FEBS Lett* 462:171–176
- Shayu D, Kesava CC, Soundarajan R, Rao AJ (2005) Effects of ICI 182780 on estrogen receptor expression, fluid absorption and sperm motility in the epididymis of the bonnet monkey. *Reprod Biol Endocrinol* 3:10
- Smith EF, Lefebvre PA (1997) The role of central apparatus components in flagellar motility and microtubule assembly. *Cell Motil Cytoskel* 38:1–8
- Stephens RE, Prior G (1992) Dynein from serotonin-activated cilia and flagella: extraction characteristics and distinct sites for cAMP-dependent protein phosphorylation. *J Cell Sci* 103:999–1012
- Stoss J (1983) Fish gamete preservation and spermatozoa physiology. In: Hoar WS, Randall DJIII, Donaldson EM (eds) *Fish Physiology*. Academic, New York, pp 305–350
- Tash JS, Bracho GE (1994) Regulation of sperm motility: emerging evidence for a major role for protein phosphatases. *J Androl* 15:505–509
- Tash JS, Means AR (1982) Regulation of protein phosphorylation and motility of sperm by cyclic adenosine monophosphate and calcium. *Biol Reprod* 26:745–763
- Toth GP, Ciereszko A, Christ SA, Dabrowski K (1997) Objective analysis of sperm motility in the lake sturgeon (*Acipenser fulvescens*): activation and inhibition conditions. *Aquaculture* 154:337–348
- Urner F, Sakkas D (2003) Protein phosphorylation in mammalian spermatozoa. *Reproduction* 125:17–26
- Vandorpe DH, Small DL, Dabrowski AR, Morris CE (1994) FMRFamide and membrane stretch as activators of the *Aplysa*. *Biophys J* 66:46–58
- Vijayaraghavan S, Goueli SA, Davey MP, Carr DW (1997) Protein kinase anchoring inhibitor peptides arrest mammalian sperm motility. *J Biol Chem* 271:4747–4752
- Vines CA, Yoshida K, Griffin FJ, Pillai MC, Morisawa M, Yanagimachi R, Cherr GN (2002) Motility initiation in herring sperm is regulated by reverse sodium-calcium exchange. *PNAS* 99:2026–2031
- Visconti PE, Kopf GS (1998) Regulation of protein phosphorylation during sperm capacitation. *Biol Reprod* 59:1–6
- Wennemuth G, Westenbroek RE, Xu T, Hille B, Babcock DF (2000)  $CaV2.2$  and  $CaV2.3$  (N- and R-type)  $Ca^{2+}$  channels in depolarization-evoked entry of  $Ca^{2+}$  into mouse sperm. *J Biol Chem* 275:21210–21217
- Wennemuth G, Carlson AE, Harper AJ, Babcock DF (2003) Bicarbonate actions on flagellar and calcium channel responses: initial events in sperm activation. *Development* 130:1317–1326
- Westenbroek RE, Babcock DF (1999) Discrete regional distributions suggest diverse functional roles of calcium channel  $\alpha 1$  subunits in sperm. *Dev Biol* 207:457–466
- Wiesner B, Weiner J, Middendorff R, Hagen V, Kaupp UB, Weyand I (1998) Cyclic nucleotide-gated channels on the flagellum control  $Ca^{2+}$  entry into sperm. *J Cell Biol* 42:473–48
- Yanagimachi R (1957a) Some properties of the sperm activating factor in the micropyle area of the herring egg. *Annot Zool Jap* 30:114–119
- Yanagimachi R (1957b) Studies of fertilization of *Clupea pallasii*. III Manner of sperm entrance in to the egg. *Zool Mag (Japan)* 66:222–225
- Yanagimachi R, Kanoh Y (1953) Manner of sperm entry in herring egg, with special reference to the role of calcium ions in fertilization. *J Fac Sci Hokkaido* 11:487–494
- Yanagimachi R, Cherr G, Pillai M, Baldwin J (1992) Evidence suggesting the presence of a sperm-attracting substance around the micropyles of salmonid and herring eggs. *Growth Develop Differ* 34:447–461
- Yang P, Diener DR, Rosenbaum JL, Sale WS (2001) Localization of calmodulin and dynein light chain LC8 in flagellar radial spokes. *J Cell Biol* 153:1315–1326
- Zilli L, Schiavone R, Zonno V, Storelli C, Vilella S (2004) ATP concentration and  $\beta$ -D-glucuronidase activity as indicators of sea bass semen quality. *Biol Reprod* 70:1679–1684
- Zilli L, Schiavone R, Storelli C, Vilella S (2008) Molecular mechanisms determining sperm motility initiation in two sparids (*Sparus aurata* and *Lithognathus mormyrus*). *Biol Reprod* 79:356–366
- Zilli L, Schiavone R, Chauvigné F, Cerdà J, Storelli C, Vilella S (2009) Evidence for the involvement of aquaporins in sperm motility activation of the teleost gilthead sea bream (*Sparus aurata*). *Biol Reprod* 81:880–888
- Zilli L, Beirão J, Schiavone R, Herraez MP, Cabrita E, Storelli C, Vilella S (2011) Aquaporin inhibition changes protein phosphorylation pattern following sperm motility activation in fish. *Theriogenology* 76:737–744

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