Traits of sperm motility in marine fish: activation, regulation and precocious arrest

Jacky Cosson1, Marina Demoy-Schneider2, Marcel Le Pennec2 & Belinda Hui3

1, UMR 7009 CNRS, Université P. & M. Curie, Station Marine, BP 28, 06230, Villefranche sur mer, FRANCE
2, Equipe de Biologie Marine-Laboratoire BIOTEM, Université de la Polynésie Française BP 6570, 98 702 FAA’A Aéroport, TAHITI - Polynésie française
3, Laboratoire LBQP, IFREMER, Vairao, TAHITI - Polynésie française

Postal address = Jacky Cosson, UMR 7009 CNRS, University P-M Curie, Marine Station, BP 28, 06230, Villefranche sur mer, France.
E-mail = cosson@obs-vlfr.fr

ABSTRACT
In marine teleost species with external fertilization, mainly osmolality controls sperm motility: movement is activated by transfer from the seminal fluid into sea water, an upward osmolality jump. The duration of motility is limited to minutes range due to partial exhaustion of ATP energy and to increase of internal ionic concentration. Motility characteristics (percentage of active spermatozoa, velocity, linearity, flagellar waves parameters (wave length and amplitude, number of waves) and energy content (respiration and ATP concentration) behave similarly among several species. All parameters show a rapid decrease after activation: progressive forward movement is limited to the earliest period following activation, which leads to the decrease of the fertilizing ability. Exposure to drastic environment (sea water) also leads to local defects of sperm flagella, an additional limitation in the motility duration. Nevertheless, minor flagellar damages as well as energetic exhaustion are reversible: a resting period at the end of the motility period allows spermatozoa to be reinitiated for a second round of motility. A paradigm is proposed to establish a link between external osmolality (sea water), internal ionic concentration, energetic stores and control of axonemal activity.

Keywords
Beat frequency, Demembranation, Fertilization, Flagella, Sperm motility

1. INTRODUCTION
Spermatozoa are unique among living cells generated by mezoanths: they are unicells which, in marine fish species with external fertilization, are released in a new medium they have to cope with, sea water, which represents an extremely harmful medium against which sperm cells appear poorly protected compared to eggs.

Most characteristics of fish sperm movement show original features: motility duration [1, 2, 3], motility initiation [4, 5, 6] or motility pattern [7, 8] were specially documented for fresh as well as marine water species. A main common feature is that motility duration lasts for short periods in marine teleost fishes.

The choice of marine fish for studies on spermatozoa is advantangeous: - Brood fish can be easily made available whole year long. - Fish sperm is easy to collect and to save for short term. - sperm are usually immotile in the seminal fluid, thus easy to fully trigger its motility by transfer in a swimming competent medium. - sperm motility scores are thus currently used for selection of genitors in the fish broodstocks, or for cryopreservation. - a correlation between sperm motility and ability to fertilize eggs has been established. - all spermatozoa can be activated at the same time and then swim with very similar characteristics at a certain time point. - the sperm flagellum is ribbon shape (presence of fins) allowing better visualization by dark-field microscopy. - In flagella, waves attenuation (so-called dampening) gradually invades the whole length of the flagellum during the short motility period and this is fully reversible.

Despite these advantages, few detailed studies on marine fish spermatozoa flagellar motility behavior were conducted. Fish spermatozoa have to adapt to various surrounding media having very different ionic/osmotic characteristics [9, 5]: the seminal fluid, the ovarian fluid and the sea or brackish water. Osmolality is considered as an ubiquitous triggering agent for motility initiation of marine fish spermatozoa [4]; when released in this new environmental condition, fertilization can occur according to various strategies [10]. From the seminal fluid where sperm is stored (usually immotile), osmolality will rise (sea water) at spawning. In response to this osmotic shock, ionic strength rises drastically at transfer of sperm into sea water [11] which changes the sperm membrane potential, and as a consequence provokes a rise in the intracellular ionic concentration leading to activate flagellar beating. In herring (Clupea pallasi), a sperm motility initiation factor from the egg complements the osmolality signal [12, 13, 10].

During the progress in the brief period of marine fish sperm motility, most parameters describing motility appear to decrease. (Figure 1). This leads to a precocious arrest of motility which is considered as a handicap for people interested in artificial reproduction. Many attempts to prolong this motility period were tried either by helping sperm to sustain its ATP production (or higher rate of oxidative phosphorylation) or through the design of "motility enhancing substances" including top level environment such as temperature, swimming medium composition for best swimming conditions.
Flagellar wave pattern presents also original features during the motile period of marine fish spermatozoa: it evolves rapidly from fully motile with waves present the whole way along the flagellum to intermediate patterns with waves present only in the proximal part of the flagellum. The last period of the motility period is identified by the restriction of the waves to one third or one quarter of the flagellum length leading to a lower and lower efficiency of the sperm translation [8] rapidly followed by a full stop. This evolution of the wave pattern is paralleled by a decrease in flagellar beat frequency along the swimming period, both contributing to a decrease in the swimming efficiency.

2. OBSERVATIONS AND RESULTS

2.1 High initial velocity but short duration
The total duration of motility is estimated by observation of movement until full cessation of activity. The initial sperm velocity ranges 150-300 µm/sec. Duration of motility and velocity characteristics are modulated by sperm environment such as pH, osmolality, temperature [14] and is also limited by damages appearing during the motility period: they can be either blebs localized any place along flagella eventually impairing the propagation of waves or appearing at the flagellar tip in the shape of curling which obviously shortens the efficient part of the flagellum for wave propagation.

2.2 Motility parameters tumbling
Motility parameters decline within a few minutes down to full arrest of spermatozoa [8, 15]. In figure 1, three main parameters, the percentage of motile cells (Fig. 1a), the beat frequency (Fig. 1b) and the velocity (Fig. 1c) exhibit two general tendencies: a high initial value but a decline during the motility period.

2.3 Waves characteristics changes
Waves are of similar shape all along the flagellum right after activation, then dampening occurs progressively during the motility period. The beat frequency (number of waves generated every second) shows high values right after initiation of motility, but decreases rapidly with elapsing time (Figure 1a).

2.4 The axonemal motility: regulation by ionic concentration
Wave parameters of permeabilized models were measured in presence of various concentrations of ions. The in vitro flagellar waves pattern is dependent of the general and non specific ions concentration, so called « ionic strength » in contact with axoneme either in vitro as controled by the experimenter or in vivo well as governed by the composition of the cytosol present in flagella.

2.5 Energy available in fish spermatozoa
Presently, most results on ATP measurements in sperm of marine fish species were obtained in turbot [16, 17, 18] or on sea bass [11] ranging in the latter 1.22 µmol/mg of protein in sea bass, [19] or 90 nmol/10^9 sperm [11] and 200 nmoles.10^-9 spermatozoa in turbot [17, 18]. In hake sperm, results of [20] indicate AEC (an evaluation of energetic content) initial value of 0.71 before motility activation. It is worth to emphasize that in marine fishes, in vitro activation or cessation of sperm motility usually does not...
require cAMP for axonemal motility.

2.6 Ability for a second motility period
In various species, fish spermatozoa can become activated for a second time after a certain period of rest in an artificial seminal fluid of low osmolality where motility cannot occur [21]. After such a resting period, motility can be re-initiated for a second round of activity by transfer of such revived spermatozoa in a swimming competent medium [22].

2.7 Fertilization ability
Very shortly after activation, sperm of marine fishes has to fill a major function that is to deliver the male haploid genome inside the egg. Sperm cells should not be delivered at a too far distance from the egg. The total distance possibly covered in average by sperm cells range those of the egg size of marine fish: spermatozoon should be delivered in the very close vicinity of the oocyte in order to reach its micropyle. This may contribute to the general reproductive strategy of fishes: a very large excess of sperm cells relative to the egg is necessary to accomplish randomly this task.

3. CONCLUSIONS
Osmolality of the surrounding medium is crucial to control the motile activity of marine fish sperm flagella. The immediacy of response to the osmolality signal may be related to a major feature: fish spermatozoa exhibit a hyper- motile behavior with a very brief period of high level beat frequency which results in a high velocity but, consequently in a fast energy consumption. This behavior is very probably dictated by a main constraint, the short period allowed to sperm cell after contact with the surrounding water for achievement of the egg fertilization. Some video movies of swimming fish spermatozoa can be visualised on the web site quoted in [23].

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5. REFERENCES
Figure 2. Activation process and signal transduction in marine spermatozoa: general schematic representation of the interacting processes occurring during the motility period of a turbot spermatozoon. Sea water is of much higher osmolality compared to seminal fluid; the osmolality jump induces an osmolality reaction from the sperm membrane; water exits the sperm cell and this process is fast because it is accelerated aquaporins. As a consequence of water exit, internal ionic concentration increases and reaches optimal values for dynein motors. Beating of flagella is of maximal velocity but decreases with time because of two reasons: the ionic concentration becomes too high to sustain correct dynein activity and ATP concentration declines and becomes limiting for flagellar beating. After some period, flagellar activity stops because these unoptimal conditions are extreme.