Waves characteristics of oyster, *Crassostrea gigas,* sperm obtained after hormonally induced spawning in sea water.

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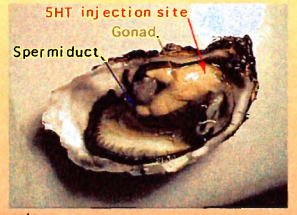
Introduction

After injection of serotonine in the kidney of oyster or without injection, sperm collected by scarification shows only poor progressive motility and only "en place" agitation engendered by jerky flagellae contractions. We decided to set condition to obtain progressively motile spermatozoa.

Material and Methods

- Oysters were collected near BREST/FRANCE from may 6 to july 9 1998, before and during the natural spawningseason.
- 0.2 ml of Serotonine 1MM diluted in seawater was injected in each oyster
- The sperm released in the 2 min. was collected in 500ml of seawater during a maximum 50 min.
- Video records from darkfield microscopy combined with stroboscopic illumination were analysed by automatic tracking using the Hobson sperm Tracker and the JCD tracker.



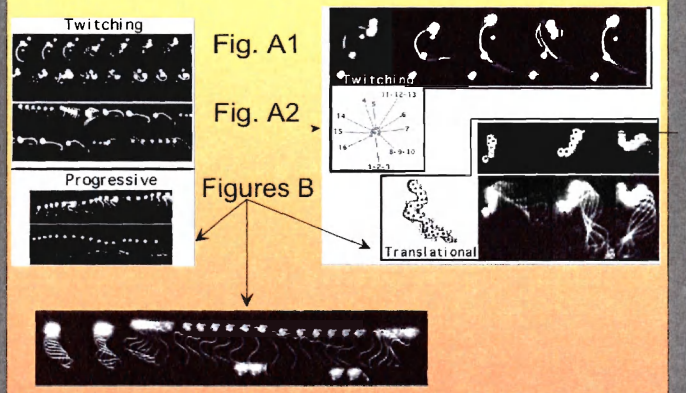
Measurements :
- Number of the shedding animals
- Sperm density and motility percentage
- Sperm beat frequency, VSP, Wavelenght and Amplitude

Results

Oysters spermatozoa collected by scarification of gonads and diluted in sea water shows little progressive movement, only unefficient "twitching" leading to an abscence of translation.
In Fig. A1, flagellar movements are detailled and show essential bending along flagella, but in no way, the bending progresses along the flagellum. This leads to trajectories with an example described in figure A2 where successive positions of the same spermatozoon are mainly overlapping.

In contrast, when oysters spermatozoa are induced to release the sperm from gonoducts, following neurotransmitters injection, (see mat. and meth.) spermatozoa motility characteristics show much higher propulsive efficiency :

*The waves which are generated propagate along flagella from base to tip (Figure B).



Characteristics of a good quality spermiation

*A beat frequency can be measured with values of 40-50 HZ (Figure 1)
*Wave Amplitude and Wave Length with values of 6-8 µm and 20-30 µm (Figure 2) are observed.

The sperm density was also evaluated during the short term induction of spawning : Figure 1 shows a representative example of the spermatozoa density obtained as a function of time after injection. A plateau is observed after 15-20 minutes post injection.

FIGURE 1

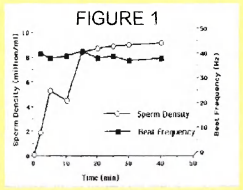
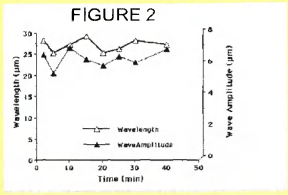


FIGURE 2



The transactional sperm move only during 97-98% of the time. They stop moving during 2-3% (C). After 1 to 2 seconds of STOP, they move again with exactly the same efficiency. This STOP/START phenomenom has also been described in Urchin (GIBBONS) and Arenicola spermatozoa (COSSON and PACEY) : this is linked to Ca++.

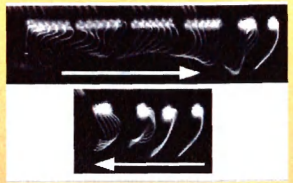
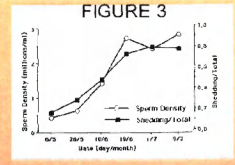


FIGURE 3



The spawning ability of oysters to be induced by hormonal injection was followed on a large number of animals sampled during the reproduction season (FIGURE 3). The reproductive period in Brittany (FRANCE) is usually from june to september. In our conditions used to induce spawning, we observe a gradual increase of the proportion of spawners in the population, as well as a related increase of the mean density of spawn sperm, both reaching a peak in early summer.

Conclusion and future directions

Spermatozoa obtained after hormonal injection of male oysters show motility characteristics allowing progressive and translationnal movement which appear similar in the wave characteristics to urchin sperm classically used as a model for flagellar movement studies. These characteristics are very homogeneous among a sperm population, but very different from those of the sperm collected by scarification, the latter being very poorly efficient for translational movement.
In contrast, the sperm density obtained by hormonal induction is much lower han that obtained by scarification. Nevertheless hormonally induced sperm density and duration of motility allows such sperm suspensions to be used for artificial insemination of oysters ovocytes. The ability of such sperm for fertilization is under investigation.
Previous studies on the mechanisms of oyster sperm movement activation (from twiching to propulsive) using in vitro activation of permeabilized flagella, have shown us that extra cellular calcium ions and intracellular cycliAMP(Figure 4) level are responsible of the control of wave propulsiveness.

FIGURE 4