

SPERM MOVEMENT AND FECUNDANCE IN SCALLOPS (*Pecten maximus*) and OYSTERS (*Crassostrea gigas*)

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Introduction : In oyster (Oy=successive hermaphrodite) and scallop (Sc=simultaneous hermaphrodite) both with external fertilization, spermatozoa (=spz) have a quite long active period of several hours in sea water (SW). In Oy, most of the spz show flagellar erratic bending devoid of regular waves, leading to poorly efficient translational efficiency. In order to improve the fertilization performances, we have succeeded to design conditions where almost 100% of the Oy spz exhibit efficient forward motility, thanks to incubation with polyvinylpyrrolidone (PVP) in sea water, major changes in flagellar waves shape are observed, mostly regular waves along the axoneme from base to tip. Such spz induced to high performance of swim were used successfully for insemination of mature oocytes.

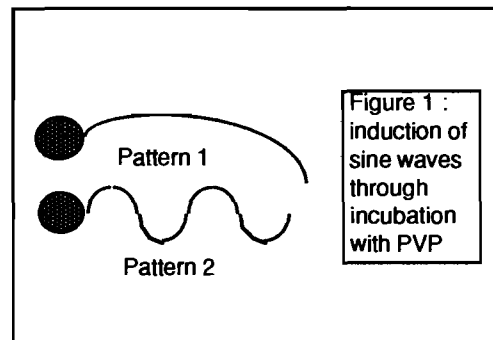
Material & Methods : Biol. materials: spz (45-50µm long) and oocytes (70µm diam.) of oysters (*Crassostrea gigas*) & scallops (*Pecten maximus*) were collected either: 1) after a thermal shock of spawners conditioned in large SW tanks (similar to natural spawning) or 2) by gonad scarification, or 3) by collection following serotonin treatment with a syringe applied to the genital pore (dry sp=10¹¹ to 10¹² sp/ml as assessed by spectrophotometry at 209 nm). Fertilization tests: at 100 spz/oocyte and contact time of gametes = 30 min for Oy and 60-120 min for Sc. followed by Hoescht 33258 staining for fluorescence microscopy evaluation of dividing embryos.

Equipment: dark field microscope with moving stage illuminated by a stroboscope, video recording at high (up to 300Hz) frequency. Clark electrodes for respiratory measurements of sperm. Methods: computer analysis with software allowing automatic measurement and plotting of bend angle vs distance along flagella on still video frames.

Results : When diluted 10³ to 10⁴ in SW, Sc spz show homogeneous and efficient translational movement while Oy spermatozoa exhibit erratic bending movements from curved to straight shape as schematically drawn in **pattern 1** with average speed of translation is <50 µm/sec and with tightly circular trajectories. Bending shapes are quite heterogeneous with low beat frequency (10-20Hz), difficult to determine due to transient stops or changes of frequency, with a behavior reminding the "hyperactivation" of mammal spz. In terms of % active sperm, results are very different depending of the collection method: in both species, the sperm obtained by scarification (meth.2) of gonads is mostly immotile while meth 1 & 3 lead to 80-90 % motile spz.

By incubation of Oy spz in a solution of polyvinylpyrrolidone (= PVP at 0.1-5% W/V) dissolved in buffered SW (20 mM Tris-Cl pH=8.2), the wave pattern changes immediately in almost 100%, mostly characterised by **pattern 2**: it shows much more homogenous movement with sine waves beating of constant amplitude of about 7 µm along the axoneme and with almost two wavelength and less asymmetry than in sea urchins (SU) spz in SW. The waves of oysters spermatozoa tails thus compares to those observed usually for SU spz as well as those of Sc. This beating occurs at constant BF of 45-50 Hz and with very few transient stop behavior. Trajectories describe large circles

of >500 µm. The wavelength as well as the frequency remain constant for long periods of time (up to 30-40 min) even at high dilution rates (up to 15,000). Best conditions were obtained using a 0.5% PVP solution: their average speed of forward translation is then of 2-500 µm/sec. For Sc sp in SW, speed is of 500-1000 µm/sec. Spz induced by PVP to high swimming performances were used for artificial insemination, with results similar to untreated controls.



Discussion : Little is known about bivalves spz swimming behavior: it has been often emphasised that Oy spz are poor "swimmers" even though their fertilizing capabilities (down to 100 spz/oocyte) appear quite good compared to other related species (i.e. 100 spz/oocyte in the case of Sc). Nevertheless, as we often observed, about 1% of the sperm population exhibits **pattern 2** swim in normal SW, meaning that some cells can swim efficiently like SU sperm do. Through incubation with PVP we succeed to induce the whole population of sperm cells to behave like "high speed swimmers" and thus improve valuably their fertilizing capabilities. We hypothesise that PVP acts by complexation of polyphenols usually present as trace amounts in normal sea water and which control negatively the axonemal movement. We are presently studying the Oy & Sc flagella waves patterns in details using high frequency stroboscopy plus video imaging and computer analysis in order to understand such improved motility performances. The aim is to use their motility characteristics as criteria for selection of individual males to be used for routine tests in hatcheries.

References : 1-Faure C., Devauchelle N., Girard J-P. & Cosson J. (1994) Fish. Res. (Board of Canada.), in press.-2 Devauchelle N., Faure C. & Girard J-P. (1994) Proc. of EAS Symp. in press. 3-Faure C., Devauchelle N. & Girard J-P. Perrault A. (1994) J. Comp. Physiol., in press.