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Changes in motility, ATP content, morphology and fertilisation capacity during the movement phase of tetraploid Pacific oyster (*Crassostrea gigas*) sperm

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Abstract

Changes in sperm features during the movement phase are especially interesting to study in external fertilization species whose sperm duration movement is long because this implies a significant adaptation of moving cells to the external medium. This study describes the changes in tetraploid Pacific oyster sperm characteristics in relation to time post activation.

Sperm individually collected on three tetraploid males were activated in seawater. Their features were analysed over a 24 h period and compared to a sperm pool collected on three diploid males as a reference. The percentage of motile spermatozoa, the intracellular ATP content, and the fine structure of spermatozoa were studied in relation to time post activation. Furthermore, the fertilisation capacity of sperm individually collected on five diploid males was assessed after 1 and 24 h post activation.

A forward progressive movement was maintained for at least a 20 h duration. Compared to diploid males, the percentage of motile spermatozoa was lower in tetraploid males. The intracellular ATP concentration was higher in spermatozoa from tetraploid males than in spermatozoa from diploid males. A decrease in ATP content was observed in the first 6 h post activation and severe alterations were observed in sperm morphology after 24 h. Then, a lower fertilisation capacity of sperm from diploid males was observed at the end of the movement phase.

The cessation of Pacific oyster sperm motility was unlikely caused by ATP consumption as ATP concentration was still high at the end of sperm movement but rather caused by drastic changes in sperm morphology. Compared to sperm collected on diploid males, the lower quality of sperm from tetraploid males was emphasized by a shorter movement duration and deeper morphological alterations at the end of the movement phase.

Keywords: *Crassostrea gigas*; Sperm; Tetraploid; Motility; ATP; Ultrastructure

1. Introduction

In aquatic broadcast spawners, spermatozoa are most often immotile in the male reproductive organ. After release, sperm is submitted to the aquatic environmental conditions. Changes in the surrounding environment

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trigger sperm movement [1] whose duration depends on the reproductive strategy of the species in relation with sperm cellular characteristics.

The changes in sperm features in relation to time after activation have been studied on a few aquatic species and mainly on those whose spermatozoa bear short movement duration. In rainbow trout (*Oncorhynchus mykiss*), where the cessation of gamete movement is observed 30 s after sperm release in freshwater, the cellular structure undergoes considerable changes located at the mid piece and at the plasma membrane [2]. A rapid depletion of intracellular ATP content is correlated to the decrease in the percentage of motile spermatozoa [3]. In seabass (*Dicentrarchus labrax*), the percentage of motile spermatozoa and sperm velocity decrease in relation to time after activation in seawater, over the 40 s swimming phase. At the end of the movement, sperm head swelling is observed, chromatin appears heterogeneous and intracristae spaces of mitochondria are enlarged [4]. Similar observations are reported for turbot (*Psetta maxima*) sperm, although the swimming phase lasts longer (3–5 min). Contrarily to trout and seabass, a part of the energy required for turbot sperm motility is produced during sperm movement by mitochondrial oxidative phosphorylation [5].

Compared to marine fish species, sperm movement duration is usually longer in marine invertebrates: 4 to 5 h in Pacific oyster [6] and in American oyster (*Crassostrea virginica*) [7]. After a 20 h incubation in seawater, the percentage of motile sea urchin (*Hemicentrotus pulcherrinus*) spermatozoa is still close to 50% [8]. Sea urchin (*Lytechinus variegatus*) sperm velocity and fertilisation capacity decreased as a function of time during a 120 min period after activation in seawater [9]. In several sea urchin species, after a 30 min duration movement, changes are observed at the midpiece and especially at the mitochondria level [10]. In Pacific oyster, knowledge of sperm features during the swimming phase is restricted to the reported decrease in the percentage of motile spermatozoa over a 150 min post activation period [11].

Triploid molluscs have a higher growth and a lower reproductive capacity than diploid ones [12]. Because they can be obtained by crossing diploid females and tetraploid males [13], standardised procedures for artificial fertilisation using gametes of both ploidies must be settled. However, sperm features of tetraploid Pacific oysters were little investigated: when compared to sperm collected from diploid males, more than half of the spermatozoa from tetraploid Pacific oysters have a higher mitochondria number [14], although the overall sperm motility is reduced [15].

The broadcast reproductive behaviour of Pacific oysters and sperm dispersion make wild stocks at risk of being genetically contaminated by farmed populations [12]. This is especially the case with tetraploid individuals which have been demonstrated to be fertile in the wild. Fertilisation capacity of these individuals is a function of several biological factors such as sperm characteristics, including sperm swimming duration, intracellular ATP content, and changes in these features during the movement phase.

In tetraploid males, undergoing the description of sperm features during the movement phase aims to i) improve the knowledge of these singular spermatozoa, ii) help to establish standardised procedures for gamete management and fertilisation which are required for triploid oyster production, and iii) explore the risk of genetic contamination of wild stocks by diploid spermatozoa.

In this paper, changes in sperm features of tetraploid Pacific oyster over a 24 h movement phase are described. The present study wants to address to which extent the characteristics of sperm movement during a long swimming period are different from the known characteristics of sperm with short movement duration, and how these characteristics are related to cellular and energetic parameters supposed to sustain sperm motility. As a consequence, changes in the percentage of motile cells, intracellular ATP content, fine structure as observed by transmission electronic microscopy, and fertilisation capacity in relation to time post activation are reported. Sperm from diploid males was also studied as a reference.

2. Materials and methods

2.1. Oyster conditioning and gamete stripping

Tetraploid (one year old) and diploid (three year old) Pacific oyster were conditioned (200L tanks, T = 19C and fed at 2% (dry mass of algae to oyster) with two microalgae, *Isochrysis galbana* Tahitian clone and *Chaetoceros gracilis*). For both sexes, gametes were obtained according to Song et al., 2009 [16]. After gonad mincing and gamete release, male and female gametes were stored respectively in 4 ml salt immobilising solution ''Store-gigas'' [16] at 4 °C and in 2L seawater at 19 °C. Sperm concentration was determined in duplicates by image analysis (Samba Technologies, Tribvn®) after dilution of 10 μl sperm samples in 10 μl formaldehyde and 1980 μl salt solution ''Store-gigas''.

Sperm concentration was adjusted to 2 x 10^9 spermatozoa/ml by dilution in seawater. Sperm movement was triggered by adding 1 ml of sperm