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Sperm viability assessment in marine invertebrates by fluorescent staining and spectrofluorimetry: A promising tool for assessing marine pollution impact

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ABSTRACT

The viability of spermatozoa is a crucial parameter to evaluate their quality that is an important issue in ecotoxicological studies. Here, a new method has been developed to rapidly determine the viability of spermatozoa in three marine invertebrates: the ascidian *Ciona intestinalis*, the sea urchin *Paracentrotus lividus* and the mollusc *Mytilus galloprovincialis*. This method employed the dual DNA fluorescent staining coupled with spectrofluorimetric analysis. The dual fluorescent staining used the SYBR-14 stained live spermatozoa and propidium iodide stained degenerated cells that had lost membrane integrity. Stain uptake was assessed by confocal microscopy and then the percentage of live and dead spermatozoa was quantified by spectrofluorimetric analysis. The microscopic examination revealed three populations of spermatozoa: living-SYBR-14 stained, dead-PI stained, and dying-doubly stained spermatozoa. The fluorescence emission peak values recorded in a spectrofluorimeter provide the portion of live and dead spermatozoa showing a significant negative correlation. The stain combination was further validated using known ratios of live and dead spermatozoa.

The present study demonstrated that the dual DNA staining with SYBR-14 and propidium iodide was effective in assessing viability of spermatozoa in marine invertebrates and that spectrofluorimetric analysis can be successfully employed to evaluate the percentage of live and dead spermatozoa. The method develop herein is simple, accurate, rapid, sensitive, and cost-effective, so it could be a useful tool by which marine pollutants may be screened for spermiotoxicity.

1. Introduction

Sperm quality is defined as the ability of spermatozoa to successfully fertilize an oocyte, to subsequently allow the development of a normal embryo, and it is influenced by several external factors (Bobe and Labbé, 2010). Assessment of sperm quality in marine species is an important issue due to the increase of ecotoxicology studies looking at the impacts of environmental pollutants on male reproductive health (Gallo and Tosti, 2016). In marine pollution monitoring programs and ecotoxicological studies, sperm cell toxicity tests have been commonly performed and successfully used as a test to monitor and evaluate the adverse effects of environmental contaminants; however, in most cases spermiotoxicity was assessed by evaluating fertilization success and the induction of transmissible damages to the offspring (Gallo et al., 2011; Gallo and Tosti, 2013, 2015; Manzo et al., 2006; Pagano et al., 1996). Sensitive and practical methods are requested for testing different sperm quality parameters to use as endpoint in spermiotoxicity tests.

Viability is a key determinant of sperm quality whose evaluation is generally achieved using sensitive and specific fluorescent probes in combination with microscopy analysis or flow cytometry (Lewis and Ford, 2012). The dual DNA staining using SYBR-14 and propidium iodide (PI) is among the commonly used stains for assessing sperm viability. The membrane-permanent dye SYBR-14 stains the nucleus of living spermatozoa emitting a bright green fluorescence, while PI only stains DNA in damaged cells that have lost their membrane integrity (Garner and Johnson, 1995). Among marine animals, the dual DNA staining has been successfully used for assessing sperm viability in fishes (Cabrita et al., 2005; Flajšhans et al., 2004; Martínez-Páramo et al., 2013), molluscs (Akcha et al., 2012; Favret and Lynn, 2010; Le Goïc et al., 2013; Paniagua-Chávez et al., 2006; Rolton et al., 2015; Smith et al., 2012; Suquet et al., 2016), crustaceans (Sasson et al., 2012) and echinoderms (Favret and Lynn, 2010). Nevertheless, no attempts have been made to apply this method to the assessment of sperm viability in ascidians.

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