Early assessment of the quality of cryopreserved *Pinctada margaritifera* spermatozoa

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Spermatozoa cryopreservation is a useful tool for genetic improvement and has been applied to several bivalve mollusc species. This technology would allow preserving the gametes of individuals selected for their high growth capacity or the quality of their pearl and thus provide significant benefits to the cultured black pearl industry. Sperm freezing requires the control of different steps: preparation of breeders, sperm collection, evaluation of sperm quality and the freezing process itself.
The objective of this study is to estimate the quality of cryopreserved spermatozoa immediately after thawing. Therefore, different criteria need to be evaluated such as the ultrastructure, concentration, movement characteristics of the sperm before and after cryopreservation.
Sperm was manually collected after natural "shedding" from the gonopore. After appropriate dilution in swimming media, spermatozoa movement characteristics were estimated under light microscopy using CASA image analysis. Ultrathin sections were prepared for TEM examination. The presence of parvalbumin-like protein (indicator of spermatozoa maturity) was immunodetected after electrophoresis. Concerning the freezing process: sperm was diluted in a cryoprotectant then drawn into semen straws. After equilibration at room temperature then in liquid nitrogen steam, the straws were immersed in the liquid nitrogen at least for 2 hours and finally thawed at room temperature.
Our results showed that, spermatozoa can be reactivated in alkaline media and are able to restore motility after cryopreservation.