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## Cryopreservation of great scallop (

To develop selection programs for the great scallop, artificial reproduction of the species needs to be improved by gamete cryobanking. Here, a set of four experiments was designed in order to define the basic elements of a cryopreservation protocol for scallop sperm, including extender composition (experiment 1), cryoprotectant selection (2), cooling rate (3), and assessment of the effects of sperm cryopreservation on sperm motility and fertilization capacity (4). Sperm was collected after serotonin injection (100 µl of a 10-mM solution) and frozen in 500-µl straws. For the first three experiments, the percentage of motile fresh sperm (80 ± 4%, mean ± SEM) was significantly higher than that observed for thawed sperm (11 ± 2%). During the first experiment, no significant difference of the percentage of motile thawed sperm was observed among the three saline extenders tested: seawater, calcium-free Hanks' balanced salt solution (Ca-free HBSS) and DCSB4 solution. However, a complementary experiment (2) showed that a significantly higher percentage of motile thawed sperm was recorded using DCSB4 than with Ca-free HBSS as an extender. During the third experiment, sperm motility was higher when using polyethylene glycol (10% PEG) as a cryoprotectant, than when using dimethyl sulphoxide, DMSO (10 and 20%), methanol (10%) and ethylene glycol (10% EG). A higher D-larval rate was obtained during the fourth experiment, using fresh sperm than thawed sperm, and using a 500:1 sperm-to-egg ratio compared with a 50:1 ratio. There is some evidence of inter-individual variations in sperm tolerance to cryopreservation. In conclusion, the highest survival of great scallop sperm after thawing was recorded using the following conditions: DCSB4 extender (1:3 vol/vol sperm to extender dilution), PEG cryoprotectant (10%) and straws maintained at 5.5 cm above liquid nitrogen.

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