

Short-Term Storage and Cryopreservation of Abalone (*Haliotis discus hannai*) Sperm

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In present study, attempts were made to preserve abalone (*Haliotis discus hannai*) sperm in liquid form at low temperature, to evaluate the effect of various diluents in short-term storage on sperm, and cryopreservation procedures were optimized for the cryoprotectants as well as freezing rates, in terms of the motility and survival rate, and the ultrastructural changes of sperm after short-term storage and cryopreservation were observed. The abalone sperm reached maximum motility until about 4min after activation. The motility was constant for about 16min, after which it dropped gradually, and about 50min later all motility ceased. Threshold activation of sperm was found in 40% artificial seawater (ASW), and motility increased as the concentration of ASW increased. In Hanks balanced salt solution without calcium (Ca-Free HBSS, 300 and 400 mOsmol/kg) and 10%, 20%, and 30% ASW the sperm was immotile, and motility once again restored incompletely only in HBSS of 300 and 400 mOsmol/kg, 20% and 30% ASW after 100% ASW was added. Sperm motility was extended following 20 days of cold storage only in 70% and 100% ASW. A high motility index of 3.5~4.5 was observed for the first 8 days in 70% and 80% ASW. In other diluents sperm motility was constant less than 10 days, and the motility index was obviously lower than that of sperm in 70% and 100% ASW. After 20 days of cold storage survival rates of 10.2% ~ 20.7% were obtained in ASW and 300 mOsmol/kg HBSS, and that in 400 HBSS (65.3%) was significantly higher than others. The constant period of sperm motility stored in 70% ASW was longer obviously than that in 100% ASW after 6 days of storage, and the time to maximum motility of sperm stored in 70% increased gradually, while the difference in which of sperm in 100% ASW was not significant. The sperm plunged into liquid nitrogen all died except that sperm using 15% glycerol as cryoprotectant restored 10.4% of motility. The highest motility index (3.4) was obtained with 5% glycerol and freezing procedure: 50°C/min from 20°C to -80°C.

Key words) *Abalone*, *Haliotis discus hannai*, *Sperm*, *Short-term storage*, *Cryopreservation*