CALIFORNIA STATE SCIENCE FAIR
2007 PROJECT SUMMARY

Name(s)
Julie A. Guerin

Project Number
S1905

Project Title
Protocols for Haliotis rufescens Egg Cryopreservation and In Vitro Fertilization, Year 2

Abstract

Objectives/Goals
Gamete cryopreservation can play an important role in conservation strategies for at-risk abalone species. Red abalone (Haliotis rufescens)are listed as threatened in California waters. Objectives of this study were to continue investigating red abalone egg cryopreservation protocols and to determine whether eggs which exhibit normal phenotypes after thawing could be successfully fertilized with live sperm, as well as to evaluate propylene glycol (PG) as an alternative cryoprotectant agent (CPA) to dimethyl sulfoxide (DMSO).

Methods/Materials
After induced spawning (with prepared H(2)O(2)/Tris Solution) and egg collection, eggs were frozen using cooled or uncooled DMSO or PG at 8 and 16 mins stepwise cooling at 14, 4, -40 degrees C, before plunging into liquid nitrogen (-196 degrees C). A total of 26 stepwise thawing protocols (5 mins at -40, 4, 14 degrees C) using 1.25g or 2.5g non-permeating sucrose/80ml water (as an aid in rehydration), and 20 in vitro fertilization tests with live sperm were conducted.

Results
Protocols using PG at 8 and 16 mins stepwise cooling and stepwise thawing with 1.25g sucrose yielded 90% to 100% (8 mins) and 75% to 80% (16mins) intact round eggs with clear chorion. Both 8 and 16 mins PG protocols using 2.5g sucrose during thawing yielded less than 10% such eggs. Protocols using uncooled or cooled DMSO stepwise cooling and thawing with 1.25g or 2.5g sucrose yielded intact round eggs ranging from 10% to 25% (8 and 16 mins), but such eggs displayed little or missing chorion. Remaining eggs in these protocols were irregular. Sperm orientation towards eggs during in vitro fertilization attempts occurred only in PG trials. No cell division occurred in any trial.

Conclusions/Discussion
PG appears to be the more effective CPA, as chemical signaling between sperm and eggs, with release of egg chemoattractant (L- tryptophan), remained bioactive after cryopreservation. CPA toxicity, ice crystallization or other factors may have, however, caused egg damage and prevented fertilization. Further research will involve refining protocols.

Summary Statement
This project was conducted to determine whether abalone eggs exhibiting normal phenotype after cryopreservation and thawing can be successfully fertilized.

Help Received
Dr. Kiersten Darrow (mentor) and Keith Okamoto (aid in procedures), at Cabrillo Marine Aquarium; my mother for encouragement and transportation; participant in Junior Southern California Academy of Sciences (JSCAS).