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The relationship of the cryoprotectants methanol and dimethyl sulfoxide and hyperosmotic extenders on sperm cryopreservation of two North-American sturgeon species

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Abstract

Successful sperm cryopreservation techniques have been developed for Eurasian sturgeon species; however, there is little information available on these techniques for North-American species. In this study, two sets of sperm cryopreservation experiments were carried out on the endangered shortnose sturgeon (*Acipenser brevirostrum*). In the first set, the cryoprotectants methanol (MeOH) and dimethyl sulfoxide (DMSO) were investigated using three concentrations (5%, 10% and 15%). The highest post-thaw motility was found using 5% DMSO (26±13%) while the use of 5% MeOH resulted in the highest rates for fertilization at the 4-cell stage (40±15%), neurulation (38±13%) and hatching (32±12%). In the second set, the Original Tsvetkova's extender (OT), Modified Tsvetkova's extender (MT) and modified Hanks' balanced salt solution (mHBSS) were investigated in combination with three MeOH concentrations. The highest post-thaw motility (18±10%), fertilization (18±11%) and hatching rates (17±12%) were observed with MT extender used in combination with 5% MeOH. In another set of experiments, the effects of two extenders (MT and mHBSS) and two concentrations of MeOH were investigated for sperm cryopreservation of pallid sturgeon (*Scaphyrinchus albus*). The highest post-thaw motility (70±10%) was observed using MT and 10% MeOH while MT and 5% MeOH yielded the highest rates of fertilization (88±6%) and

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hatching (73±14%). In general we conclude that although hyperosmotic conditions of extenders and cryoprotectants result in higher post-thaw motility, they seem to reduce the fertilizing ability of the sperm.

Keywords: Sturgeon; *Acipenser brevirostrum*; Sperm; Cryopreservation; Extenders; Cryoprotectants; Osmolality

1. Introduction

Sturgeons (Order *Acipenseriformes*) are chondrostean fishes of ancient origin that inhabit only the Northern hemisphere (Birstein and DeSalle, 1998). Several species are restricted to very small populations which in some cases are close to extinction due to exploitation of natural stocks for meat and caviar as well as destruction of habitat (Billard and Lecointre, 2001).

The shortnose sturgeon (*Acipenser brevirostrum*, Lesueur) inhabits the rivers and brackish waters of the North American Atlantic coast from New Brunswick, Canada to northern Florida (Vladykov and Greeley, 1963). Stocks have been declining during the 20th century and it is federally listed as an endangered species since 1967 (U.S. Office of the Federal Register, 1967). Studies on cultivation and stock enhancement have begun in the southern United States and the results are promising (Smith et al., 1995).

The pallid sturgeon (*Scaphyrinchus albus*, Forbes and Richardson) is indigenous to the Mississippi, Missouri and Yellowstone river drainages (Billard and Lecointre, 2001). This fish, first recognized as a separate species from shovelnose sturgeon (*S. platorhynchus*, Rafinesque) only in 1905, has never been abundant and now it is listed as an endangered species in the United States (Kallemeyn, 1983). Recovery plans by the US Fish and Wildlife Service call for the development of sperm banks to aid artificial propagation of this species (DiLauro et al., 2001) with the ultimate goal of supplemental stocking of the species into its natural habitat.

The first experiments on the cryopreservation of sturgeon sperm were carried out in the 1960s by Soviet scientists (Dettlaff et al., 1993). Several protocols have been developed since then (Drokin et al., 1991; Ciereszko et al., 1996) but they all share a common problem: although high post-thaw motility is observed, fertilization rates remain low or non-existent. Methods resulting in satisfactory fertilization and hatching rates have been described for sterlet (*Acipenser ruthenus* L.) and Siberian sturgeon (*A. baeri* Brandt) by several authors (Tsvetkova et al., 1996; Jähnichen et al., 1999; Glogowski et al., 2002). However, little success has been achieved thus far in sperm cryopreservation of North-American sturgeon species.

The objectives of our work were to test the effect of: (1) cryoprotectants in different concentrations on the motility and fertilizing ability of shortnose sturgeon sperm; (2) several extenders in combination with different cryoprotectant concentrations on the motility and fertilizing ability of shortnose sturgeon sperm; (3) several extenders in combination with different cryoprotectant concentrations on the motility and fertilizing ability of pallid sturgeon sperm.

2. Materials and methods

2.1. Shortnose sturgeon

Captive shortnose sturgeon broodstock were maintained at the Bears Bluff National Fish Hatchery (Wadmalaw Island, South Carolina) of the US Fish and Wildlife Service. The fish (age, 13 years) were kept in plastic holding tanks (2864 l or 4820 l) at 16 °C. Spermiation and ovulation were induced using injection of carp pituitary extract (Stoller Fisheries, Spirit Lake, Iowa, USA). The dose of carp pituitary for males was 1 mg per kg of fish that was administered from a stock solution. A stock solution of 68 mg carp pituitary extract per ml of sterile saline solution (0.6% NaCl) was prepared each week. The dose for females was 4 mg of carp pituitary per kg of fish and a stock solution equivalent to 18 mg per ml 0.6% sterile saline was prepared each week. Also, females received two injections. The first was 10% of the total dose, and the second injection delivered 12 h later was the remaining 90%. Males received only one