Roles of different factors on the cryopreservation protocols for fish eggs and embryos

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ABSTRACT

Gamete and embryo cryopreservation have played a key role in the development of assisted reproductive technologies for farm and aquatic animals over the last several decades. Animal embryos of many species including human beings have been successfully cryopreserved. Till date fish sperm over 200 species have been cryopreserved also. The general physico-chemical principle for these cryopreservation techniques has been known to be glassification of the inner cell. However, fish embryo cryopreservation has been elusive till now and the literature is limited. The reasons for failure on glassifying fish embryos have been believed to be underlying the problems in impregnation of cryoprotective agents (CPAs) and delaying of heat transfer on cooling, which are caused by biological characteristics. However, there is little systematic information on the fish embryo cryopreservation. The present study emphasizes the need to understand and overcome these factors in two parts namely, Part I (chapter 2 & 3) that deals with the methods to impregnate more CPAs into embryos and part II (chapter 4 & 5) with the methods to attain glassification through physico-chemical principle and their viability assessment.

At first, in order to seek promotive condition on CPA impregnation, the effect of pre-dehydration by CPAs (DMSO, Glycerol and Trehalose) and hydrostatic pressure on growth and survival of medaka and pejerrey embryos were investigated in Chapter 2. This study revealed that the dehydration ratio affects significantly embryo survival, and the safety ratio could be determined. The hydrostatic pressure that was sufficient not to damage the embryos was optimized. Hydrostatic pressure was found to be safe at 50atm for application during impregnation treatment.

In chapter 3, the uptake dynamics of the cryoprotectant DMSO by unfertilized eggs, 8-cell and eyed embryos of medaka and some stages of pejerrey were assessed by measuring the internal concentration (C_{in}) using HPLC. The relation of the C_{in} of DMSO with fertilization and survival rates, and the effects of several factors (cryoprotectant concentration, impregnation time, temperature, hydrostatic pressure, and the conditions of the materials) on these processes were investigated. Cryoprotectant permeation, which was estimated from the initial rates of DMSO uptake, was higher in embryos than in unfertilized eggs and increased with embryonic development. However, the DMSO C_{in} in eyed-embryos reached a plateau at 1 to 5 min and could not be increased by prolonging impregnation. These results suggest that as long as the embryo was alive, the mechanism of excluding DMSO was

working. The highest fertilization and survival rates for any given DMSO C_{in} were obtained with high concentrations and short times of impregnation rather than low concentrations and long impregnation times. From this it can be said that longer duration impregnation time is toxic and deleterious for the survival of fish embryos. Application of hydrostatic pressure (50 atm) and exposure for 3 min to a 1 M trehalose solution prior to impregnation induced a substantial increase in the DMSO C_{in} of 8-cell embryos in comparison to untreated controls with no significant effect on survival. Hydrostatic pressure also promoted DMSO uptake in unfertilized eggs, but with rapid loss of viability, and was ineffective in eyed embryos.

The chapter 4 has enumerated the different intracellular ice formation temperature (TIIF) regimes of embryos with different cryoprotectant concentrations by using DSC. In general the TIIF of the two fish embryos being studied here was found to be around –10 °C and therefore, during the fish embryo cryopreservation protocol, it was confirmed that supercooling condition before reaching –10 °C is available. In the absence of cryoprotectant, almost all the medaka and pejerrey embryos without the effects of the embryonic stage received a significant damage at low temperatures below 4 °C to –10 °C where no ice nucleation occurred, though the eyed stage embryo showed stronger tolerance at 4 °C than the other stages. In the presence of cryoprotectants the cold tolerance of embryos in every stage indicated a remarkable increase, while cryoprotectants also promoted the depression of the ice nucleation temperatures of the embryos.

The impregnation protocol from our earlier experiments was implemented in chapter 5 with assessment of embryo/blastomere viability. The studies on TIIF by DSC clearly brought out the need for a two-step cooling protocol, where slow cooling at –2 °C to –5 °C per minute is desirable to –10 °C but before IIF occurs and subsequently a very high speed cooling at the rate of –300 °C to –400 °C per minute to achieve glassification. After several trials, intact embryo survival could not be achieved, however, by a given protocol 39 % live blastomeres were obtained which is significant for whole intact embryo cryopreservation.

The present studies in part I and II clearly brought out a systematic information on fish embryo cryopreservarion to give a way how to control not only CPA concentration but also temperature on cooling. Although, further studies are required to ascertain the reasons for the death of whole intact embryos, still blastomere survival from intact embryos in this study opened up new hopes towards the total success of fish embryo cryopreservation.