Investigation of ice particle formation points during fish sperm cryopreservation

Elena Ponomareva¹, Angelina Firsova¹, Anton Kuzov¹, Aleksander Korchunov¹, Matvey Kovalenko¹,²*, and Dmitriy Rudoy²

¹Federal Research Centre The Southern Scientific Centre of the Russian Academy of Sciences, 41, Chekhov str., 344006, Rostov-on-Don, Russia
²Don State Technical University, 1 Gagarin square, 344002, Rostov-on-Don, Russia

Abstract. This study was conducted in order to investigate the formation of ice particles during cooling of fish sperm. The object of the study was spermatozoa of the Russian sturgeon (Acipenser gueldenstaedtii Brandt, 1833), hybrid Acipenser ruthenus × Huso huso, the spiny-tailed sturgeon Acipenser nudi ventris Lovetsky, 1828, and the inconnu Stenodus leucichthys Gülデンstädt, 1772. The growth of ice microparticles is formed at the perturbation temperature of the corresponding aqueous suspension, with the concentration of dissolved substances increasing in the non-freezing phase. The process of ice formation and the increase concentration of the remaining liquid continues as it cools to form amorphous (glassy) ice. It is interesting to bring the mass of ice with a further decrease in temperature to (-196) ° C. Cooling causes compression of the formed ice crystals.

1 Introduction

Crystallization includes the nucleation and growth of crystals [1, 2]. Nucleation refers to the process by which a sufficient number of molecules bind in three dimensions to form a thermodynamically stable state, the so-called critical core, which provides conditions suitable for crystal growth. The growth stage, which immediately follows the nucleation, is determined by the diffusion of particles to the surface of critical nuclei and their ordered assembly on the growing crystal [3]. During the nucleation of crystals, two different processes are distinguished, namely primary and secondary (or contact) nucleation. Primary generation can occur in two categories: homogeneous and heterogeneous. Homogeneous nucleation occurs when nuclei are formed spontaneously due to random density fluctuations in a supercooled liquid, but heterogeneous nucleation occurs due to the presence of solid impurities that form stable surfaces for the formation of nuclei [4, 5].

The distinction between heterogeneous and homogeneous nucleation can be made by evaluating differential heat flow signals [4, 6, 7]. It is believed that nucleation is the most important stage during crystallization, since it can affect both the size of crystals and the distribution of ice crystals [8]. The above-mentioned and other factors are responsible for the random and stochastic nature of ice generation [9-13]. On the other hand, the ability to control hypothermia and the formation of ice nuclei is necessary for the survival of many biological organisms.

In view of the above, the aim of the study was to analyse the formation of ice particles from a mixture of sperm and cryoprotectant medium of different fish species.

* Corresponding author: reception@donstu.ru
2 Material and research methods

To study the shapes of microparticles and the changes occurring during the cracking of the sample, an installation consisting of a microscope, a foam box, a Fuchs-Rosenthal camera, a thermometer, a video camera and a table lamp was mounted (Figure 1).

![Diagram of sample freezing unit]

Fig. 1 - Sample freezing unit: 1 - microscope lens, 2 - microscope video eyepiece, 3 - Sony Alpha A57 video camera, 4 - electronic thermometer, 5 - Fuchs-Rosenthal camera, 6 - foam box, 7 - adjustable chilled and liquid nitrogen supply system

Samples (10 µl) were introduced into a Fuchs-Rosenthal quartz chamber, which was placed in a foam box. The chamber was cooled to -196 °C first with nitrogen vapor, and then with liquid nitrogen, regulating the supply of liquid nitrogen to the box. The temperature was measured using an electronic thermometer ATT-2004, a copper-constantan thermocouple. The cooling rate was about 10°C/min. Video recording of the freezing process was carried out using a video camera (Sony Alpha A57) mounted on a Biolam microscope, lens - 4x10, eyepiece - x7. Photoregistration of microparticles was carried out at a temperature of liquid nitrogen.

3 Results and discussion

When liquids are cooled to a certain temperature, called the crystallization (solidification) temperature of the liquid phase, the transition of the liquid substance to a solid crystalline state (crystallization) begins. It is also interesting that each solution has its own crystallization temperature. However, freezing of the suspension usually begins with the crystallization of the so-called extracellular (located between the cells) water only when the temperature drops to minus 10 °C, at which the formation of "foci" of water crystallization occurs. At the same time, the suspension of cells in the temperature range from 0 to minus 10 °C is supercooled, i.e. it is in a metastable, liquid state. Freezing of the intracellular fluid usually begins at a
temperature much lower. The formation of cracks during freezing depends on the physical properties of the material being frozen (elasticity and stiffness).

It is also worth noting that water in biological objects can be in two states: free, having all the characteristics of ordinary clean water, and bound ("non-freezing"), with altered properties. During the freezing process, the solution undergoes several critical states, each of which corresponds to a certain minimum crystal size, at which its spontaneous growth begins.

When cooling in solutions, the process of formation of ice crystals begins, with further cooling, a solid mass of ice is formed, then its cracking and the formation of microparticles under the action of thermomechanical stresses begins.

The results of the experiment on the study of the points of formation of ice particles during cooling of fish sperm are shown in Figures 2-5.

![Fig. 2. The points of formation of ice particles during cooling of Russian sturgeon sperm](image1)

![Fig. 3. The points of formation of ice particles during cooling of Acipenser ruthenus × Huso huso](image2)
Fig. 4. The points of formation of ice particles during cooling of Acipenser nudiventris

The growth of ice microparticles is formed at the perturbation temperature of the corresponding aqueous suspension, while the concentration of dissolved substances increases in the unfrozen phase. The process of forming ice and increasing the concentration of the remaining liquid continues as it cools until amorphous (vitrified) ice forms. It is interesting to bring the mass of ice with a further decrease in temperature to (-196) °C. Cooling causes compression of the formed ice crystals.

Fig. 5. The points of formation of ice particles during cooling of inconnu
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