NEW ASPECTS IN TECHNOLOGY OF CRYOPRESERVATION OF SPERM OF CARP AND SILVER CARP

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For quite a time we have been engaged into the elaboration of the methods of cryopreservation of carp and silver carp sperm. The researchers of our laboratory managed to organize the cryobank of the sperm of carp and alga-eating fish. Normally we succeeded in cryopreserving sperm with sufficient motility and fertilizing capacity in 30-50% of spermatozoa. However, in some cases the methods applied yielded no positive results, which, apparently, was due to the low quality of the ejaculate. Besides, we failed to achieve a complete reproducibility of our method, since some of its stages were rather complicated.

In this respect we thought it expedient to reduce the number of components and to balance the cryoprotective medium. Routinely we used the medium, elaborated by Dr. E.F.Kopeika in 1986 for cryopreservation of carp sperm, but it is also applicable for alga-eating fish. This medium contains 0.42% NaCl; 0.006% KCl; 0.009% $CaCl_2$; 0.062% $MgSO_4 \times 7 H_2O$; 0.28% $NaHCO_3$; 0.137% sucrose; 1.5% mannitol; 1.7% tris (oxymethylaminomethane), pH 8.1, 12% (v/v) yolk. 19.6 Ml of ethylene glycol were added to 100 ml of the solution. The ejaculate was diluted by this medium in a 1:1 ratio. It follows from the given concentrations of the substances, that this medium, apparently, exercised a significant hypertonic influence on spermatozoa, and this negative effect became pronounced already after sperm dilution.

For example, during experimentation with the silver carp sperm in the zone of ecological disaster in the Aral sea region, where the concentration of pesticides and other chemicals significantly exceeds the highest allowable level, thus diluted spermatozoa lost the ability for activation in the normal water. Following cryopreservation sperm was not activated even by the special solutions, which normally yielded good results with the silver carp sperm, which was obtained under normal conditions.

Hence, we decided to exclude some components from this medium, slightly increasing the concentration of KCl (towards physiological values), and substituting sucrose for glucose. The resulting medium for cryopreservation of the sperm of carp and

silver carp was of the following composition: 0.42% NaCl; 0.038% KCl; 0.09% glucose; 0.252% $NaHCO_3$; 0.847% tris (oxymethylaminomethane), pH 8.0, 1.7% (v/v) ethylene glycol, 18% (v/v) yolk. Recent experiments demonstrated high efficiency of applying this medium with excluded yolk.

Beside the medium, we introduced some alterations into the technology of sperm cryopreservation, which became as follows:
* freshly obtained ejaculate in a 1:1 v/v ratio was gradually diluted by the cryoprotective medium, the temperature of which equaled that of the sperm (earlier we used to place the ejaculate into refrigerator, and after reaching the temperature of +5°C, it was diluted by the cryoprotective medium of the same temperature);
* we distributed thus obtained suspension of spermatozoa into polyethylene ampoules with the volumes of 0.5-2 ml, or into straws with the volumes of 0.3-2.5 ml. Recently we got satisfactory results when using the containers, made out of polyimmunofluoroplastic film with the volumes of 5-15 ml;
* we placed the ampoules, straws or containers onto the grid, which was located in the wide-necked Dewar bottle in the nitrogen vapors at -30°C. Some time later, as determined by the sample volume and the magnitude of thermal conductivity of the container walls, we lowered the grid down to the level, where the temperature was -70°C. When the samples were completely cooled, we immersed them into liquid nitrogen (earlier we used to control the process of cooling with the help of the rather expensive equipment);
* thus cryopreserved sperm was thawed on water bath at +40°C until liquid phase was identified (normally it took us about 30 sec);
* the motility of sperm was determined after thawing in the activating medium, routinely consisting of $NaHCO_3$, tris + NaCl or tris + $NaHCO_3$. The concentrations of these substances should better be chosen separately.

Using the above methods, we managed to cryopreserve sperm, in which 40-90% of carp spermatozoa maintained their motility, and the corresponding figure for the silver carp sperm was 20-50%, depending on the original quality of the ejaculates. We believe the proposed method will be the most effective for cryopreservation of the insufficiently full-fledged sperm, collected from the producers with the inadequate conditions of the spermatogenesis occurrence (i.e. temperature, ecology, imbreeding, etc.).