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THE CRYOGENIC RELATIONSHIP OF MORPHO-FUNCTIONAL PARAMETERS AND CHARACTERISTICS OF CARP GAMETES

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Abstract

The method of long-term preservation of mammalian semen in deep-frozen condition provides great opportunities for development and improvement of the reproduction system of farm animals. Using this method it is possible to check the breeders on the quality of offspring so as to maximum use the improvers. This allows performing the large-scale genetic selection in animal husbandry, which significantly increases the rate of mass improvement of breeding and productive qualities of animals. However, the existing cryotechnology not provide maximum preservation of the biological integrity of the reproductive cells. Comprehensive research has shown the possibility of increasing the efficiency of cryopreservation by improving of synthetic mediums and the development of optimum cryopreservation process parameters.

Key words*: synthetic mediums, cryopreservation, spermatozoa, efficiency of reproduction, farm animals.*

INTRODUCTION

Cryopreservation of reproductive cells of different species of fish, inclusive cyprinids achieved considerable success, allowing use cryoconservation method for scientific purposes to solve fish problem. Therefore it is advisable to carrying out research in different directions, such as: basic research to elucidate mechanisms of cryodamage and cryoprotection of reproductive cells; development of new methods for get 100% of surviving cells with intact genome; development of new methods for long-term storage of cells which allow to minimize costs for the maintenance of cryobanks (Alavai et. al., 2008; Cabrita et. al., 2010). The conservation of biological diversity of aquatic ecosystems and organisms acquires a special urgency nowadays, when sharply increased the impact of anthropogenic factors on the aquatic environment. From rivers and reservoirs used for industrial, agricultural and domestic uses large volumes of water, which leads to a significant deterioration of the conditions of reproduction, reduction of biodiversity and the extinction of many species and fish populations (Andreev et. al., 1996; Ananiev et. al., 1996).

The solution of nature protection problems in the fish farming is possible through the creation of cryobanks of valuable breeder semen (Ananiev et. al., 1996). However, advances of cryotechnology not widely used, due to the difficulty of reproducibility of the results (Andreev et. al., 1996; Cabrita et. al., 2010). According to research by E. Kopeika (Kopeika et. al., 2007), the number of surviving cells as a result of cryopreservation depends of spawning conditions, initial quality of sperm, its receipt conditions and other factors. It is shown that changes of structural and functional characteristics affect the qualitative variability and cryoresistance of fish sperm.

In the researches of fish reproduction the special importance attaches to the problem of preservation of reproductive products. The small time period of gametes preservation in native condition is one of the reasons of low efficiency of fish hybridization from different geographic area whose spawning occurs at different time of the year. Asynchronous maturation brings new difficulties to the working rhythm of fish farms. Therefore, the

practical importance of long-term preservation of carp gametes in cryopreserved condition attracts a lot of attention of many researchers (Boronciuc et. al., 2008; Cabrita et. al., 2010; Kutluyer et. al., 2015).

The most promising method of preservation known today, despite its complexity, is a method of low-temperature preservation which makes possible the creation of cryobanks of fish genomes and their uses for the conservation of biological diversity of the animal world (Ogretmen, İnanan, 2014; Asmad et. al., 2011).

Based on the foregoing, the main goal of the performed researches whose results are presented in this paper is the study of individual peculiarities of maintaining the functional activity of depreserved carp gametes.

MATERIALS AND METHODS

The experimental studies were carried out in the cryobiological laboratory of the Institute of Physiology and Sanocreatology of Academy of Sciences of Moldova and in conditions of experimental production. Sperm of common Carp was collected, evaluated and manipulated by using the general accepted methods. Through the methods of assessment of physiological indices we aimed to determine in sperm the concentration, mobility and survival of spermatozoa. Sperm cryoconservation was performed according to the classical scheme of cryopreservation in the form of pills at the liquid nitrogen temperature. The sperm of *Cyprinus carpio* were obtained after injection of pituitary extracts from male with weight 7-7.5 kg. In research for this paper was used the sperm of the carp 6-7 years. In the experiment was used variant in which roes were fertilized with cryopreserved sperm. Defrosted sperm was mixed with native caviar by means of the dry method (1/100), and then were sown in water on a labeled Petri dishes, placed on the bottom of a 0.6 l glass. In the control variant native material was used for the same purpose. The material was preserved in the form of granules of 0.1-0.2 ml volume which was subjected to cryopreservation on the surface of a fluoroplastic plate in liquid nitrogen vapour. The incubation of roe was carried out under production conditions using the Weiss apparatus: the water temperature is 22-23°C, the oxygen content of 7.0-8.5 mg/l flowage 3.0 L/min. The parameters of embryonic development of fish eggs were studied by method described by Jucinskii and Nedialcov (Jucinskii et. al., 1980). During the incubation of roe conducted observations of the quality of fertilization of eggs and visually calculated the percentage of fertilized roe at the ovum stage crushing. In addition was determined the percentage mortality of the ovules at the gastrulation stage and at the stage of separation of the caudal section of the yolk sac (corresponding stage of organogenesis). With the beginning of motor activity of the embryo were monitored the intensity of the movement of embryos in the experimental and control variants.

RESULTS AND DISCUSSIONS

One of the promising ways of preserve the genetic diversity of fish, not only rare and endangered species, but also the objects of aquaculture, is recognized the cryopreservation of sperm.

The main function is the preservation and transmission of genetic information by the reproductive cells from one generation to another. This can be realized in the normal course of the process of fertilization and embryonic development of organisms. This process is determined by the quality of gametes, the methods of fusion, the skill of the experimenter at artificial reproduction and environmental conditions.

In the solution of problems of carp sperm cryopreservation the important role is played by the creation of protective mediums and the establishment of optimal parameters which providing the maximal effect. The prior researches showed that the quality of carp sperm during cryopreservation undergoes considerable changes. Therefore was developed a new cryoprotective medium for carp sperm. It takes into consideration the necessity of stabilization of intermolecular interactions and binding of free water in cryobiological systems. For this purpose were used tris-(oxymethyl) aminomethane and 1.3-butylene glycol. The results of these experiments have provided a

new medium whose composition is shown in Table 1.

Table 1. Composition of medium for carp sperm cryopreservation

Names of components	Quantity
Tris-(oxymethyl)aminomethane, g	3.0
1,3-butylene glycol, ml	15.0
The yolk of hen eggs, ml	12.0
Water, ml	100.0

The highest effect the medium gives under separate cooling of the medium and sperm down to 4°C, the exposure of sperm at this temperature during at least 5 min, the dilution of sperm 1:1 takes place directly before freezing at 100-120°C during not less than 2 min.

However, the final results both of theoretical and applied researches can be evaluated from the data of production experiments (Table 2).

Table 2. The results of the production experiments on insemination of roe with cryopreserved carp sperm

Embryonic parameters	Female							
	Male 1		Male 2		Male 3		Male 4	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
1	2	3	4	5	6	7	8	9
The number of inseminated fish eggs (at the stage of ovicell segmentation)	50.0	90.0	60.0	90.0	40.0	90.0	45.0	90.0
Mortality of ovicells during transition from gastrula stage to the beginning of organogenesis	5.0	4.0	5.0	4.0	5.0	5.0	5.0	4.0
Mortality of embryos at the stage of separation of the tail part from the vitelline sac	10.0	4.0	10.0	5.0	10.0	8.0	10.0	6.0
Intensity of embryo movement in roe	N	N	N	N	N	N	N	N
Mortality of embryos at the stage of emergence out of roe	6.0	3.0	6.0	2.0	15.0	10.0	8.0	6.0
Embryos with anomalous development	2.5	3.5	2.5	3.0	4.0	3.0	3.0	3.5
Output of matured larvae (from native or cryopreserved sperm)	40.0	65.0	45.0	75.0	24.0	60.0	24.0	70.0

Table 2 shows important parameters such as the percentage of fish eggs fertilization and the output of matured larva using the sperm of different males in the experimental version varies from 60 to 40 and from 45 to 24%, respectively. It should be noted that these variations are observed on the background of similar fertilizing capacity of native sperm of both experimental males. However, the output of practical larva in this case is subjected to considerable variations even in the control version. One of the reasons of this phenomenon may be different cryo-resistivity of gametes of different sires found by A. Andreiev (Andreiev et al., 1996).

The established individual peculiarities of the male carp sperm quality provided the basis for similar researches under practical industrial conditions aimed at detection and analysis of such peculiarities in sex products of females irrespective of their native or cryopreserved condition (Table 3).

Table 3. The results of embryonic observations over fish eggs and prelarvae in experiments on carp sperm cryopreservation

Embryonic parameters, %	Male 1							
	Female 1		Female 2		Female 3		Female 4	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
1	2	3	4	5	6	7	8	9
Insemination of fish eggs (at the stage of ovicell segmentation)	35.5	73.5	60.0	96.0	20.5	80.0	77.0	97.0
Mortality of ovicells during transformation from gastrula stage onto the beginning of organogenesis	7.0	4.0	10.0	5.0	10.0	5.0	8.0	4.0
Mortality of embryos at the stage of tail portion separation from the vitelline sac	8.0	4.0	8.0	5.0	10.0	5.0	8.0	6.0
Intensity of embryo movement in roe	N	N	N	N	N	N	N	N
Mortality of embryos at the stage of emergence from roe	6.0	4.0	8.0	3.0	10.0	3.0	4.0	4.0
Embryos with abnormal development	3.0	2.5	3.5	2.6	3.5	2.0	2.5	2.0
Output of matured larvae (from native or cryopreserved sperm)	35.0	60.0	40.0	80.0	12.4	65.0	60.0	82.0

The data listed in table 3 show that the insemination of various female fish eggs in control version of the experiment varies from 80 to 97%. The output of viable larvae is also subjected to considerable variations from 65 up to 82%. In the experimental version the similar variations are even more pronounced. The insemination of fish eggs varies from 20.5 up to 77.0% (the maximum index is more than three times higher than the minimum one). The output of viable larvae varies within the range of 12.4-60.0% (almost five-fold difference in the studied material).

It should be noted that individual differences in quality of the sex products and the aggravation of these differences after sperm cryopreservation do not influence the quality of the obtained progeny.

Various indicators of the functional activity of the reproductive cells and the embryonal development of carp is explained by the initial quality of sperm and oocytes (Katkov, 2002), but to this must be added and the individual characteristics of both sexes reproducers, which have been previously detected by us in the experiments with the reproductive cells of other animal species (Boronciuc, Balan, 2008).

CONCLUSIONS

The researches allow making the following conclusions:

1. The individual peculiarities of functional activity of carp's genome are observed in male as well as in female experimental specimen.
2. The quality both of native and decreased carp reproductive material has a strictly peculiar individual characteristic for each male.
3. The greatest level of functional activity of decreased carp spermatozoa is achieved by using of 1.3-butylene glycol medium and optimal cryopreservation regimes.
4. During realization of programmes of sperm *Cyprinus carpios* cryopreservation it is necessary to take into account individual peculiarities of its quality and select the specimen with a high-quality and high-resistant material.

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