

# DETERMINATION OF THE PARAMETERS LOW-INTENSITY ACOUSTIC VIBRATIONS IN CRYOPRESERVATION

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The main factors influencing the degree of damage to the structure of biological objects are as follows: the value of ice crystals, the length of stay of cells in a hypertonic medium, intracellular crystallization, cell dehydration, recrystallization, aggregation and denaturation of cellular proteins. As a result, the influence of all these factors occur in primary cells cryodamage such as change in shape, volume, infringement of membrane integrity, alteration komformatsii and other macromolecules. Such primary cryodamage can cause secondary damage to the cells growing in different times after thawing.

In this context, an important issue is a comprehensive study of the possibilities of increasing the cryo-resistant biological objects and finding ways to further cryoprotection their structures

**The purpose of research** - the definition of the parameters of low-intensity acoustic waves to influence the micro-objects cattle (cattle) prior to cryopreservation, which would ensure the sustainability of micro to low temperatures and increases their oplodotvaryaemost after thawing.

**Materials and methods of research.** To increase the stability of sperm to thermal shock as a cryoprotectant in sperm add yolk of an egg. Egg yolk contains lecithin and lipoproteins. They create on the surface of sperm absorbent layer that protects sperm from cold shock, which acts on the life of sperm cells to a temperature of  $-51^{\circ}\text{C}$ , t. E. In the semen is stored until the liquid phase. To protect sperm from cold shock applied lactose-yolk medium with the following composition: distilled water - 100 ml; Lactose - 11.5 g; yolk of an egg - 20 ml; glycerin - 5 ml.

To determine the concentration of sperm was used fotoelektrokolorimetrichesky method using photoelectrocolorimeter FEC-H.

The principle of operation of this device is based on the fact that through the cell with the sperm pass light beam a certain strength, which then goes to a selenium photocell connected to a galvanometer. Passes through the galvanometer electric current having a magnitude which is inversely proportional to the turbidity (optical density) of sperm, i.e. the concentration of sperm.

For the study was prepared 3.5% solution of sodium citrate in distilled water, filtered through a paper filter and poured into a well-dried and washed from the flasks penicillin 9.9 ml each vial. Micropipet gain exactly 0.1 ml sperm studied and it was mixed with a solution of sodium citrate in one of the vials, thus diluting the sperm in 100 times.

To monitor and assess the degree of influence of acoustic vibrations on the life of sperm before freezing and after thawing was used optoelectronic system [3].

The structure of the electro-optical system includes the following elements and components: microscope P-11; thermostated table; CCD type TSM7 By 1200 (the number of cells 260h380); interface unit and synchronization; camera CT-5; personal computer (CPU Inter Core 2 Duo E6550); video card NVIDIA GeForce 8800GT; HDD Seagate ST325 256 Gb; Motherboard Asus P5k; removable media drive ASUS DRW-1612BL; Monitor 172T; operating system Windows XP SP3.

The optimal parameters of low-intensity acoustic waves (frequency, power, exposure) to influence the granules microobjects animals prior to cryopreservation, have been identified on the basis of multivariate experiment in which in response irradiated sperm was taken magnitude of the shift of the resonance frequency of the resonator measuring microscopic objects with animals pellets (5 mm in diameter, 3 mm) with respect to the resonance frequency (74.280 GHz) of the reference resonator.

In the experiment we used a source of acoustic vibrations SMB-17CC c specifications: frequency range 0.325 kHz - 2,250 kHz; power range of 50 dB - 80 dB; the magnitude of the power supply of 1.5 - 15; the value of the supply current of 0.2 - 1.4 mA.

After carrying out measurements and calculations derived regression equation and

the following values of the factor in the extreme point: the frequency of 1 kHz acoustic oscillations, power 1.03 mW; time of exposure to micro-objects with 320 animals.

In a laboratory experiment, beads 6 million sperm cells, which were subjected to the effects of cryopreservation of acoustic oscillations with parameters: frequency of 1 kHz; power of 1 mW; exposure to 320. In the control granules with sperm acoustic oscillations were not processed. After cryotreatment and thawing of granules conducted research on the electro-optical system, by definition, live sperm.

**The results of research.** The measurement results showed that treatment with the sperm pellets acoustic vibrations leads to an increase in thickness of the protective layer on the plasma membrane of sperm and stability to low temperatures. The percentage of live sperm output in the experiment was about 80 %, and in control - only 30 %.

An important indicator of the viability of newborn calves is to reduce the weight of the first 3 ... 6 days after birth. During the experiment, it was found that the loss of body weight per calf in the first 5 days of life were 1.5 % for the experimental group and 5.8% for the control group. Veterinary medicine specialists have reported cases of the disease calves gastrointestinal and lung disease. Incidence calves in experimental group was 10.2 %, and in control group – 44 %. Calf mortality after 20 days of life of the control group was 36.8 %, and in all the experimental calves survived.

As a result, production of the experiment, it was found that the profit from the introduction of acoustic technology in this sector amounted to 35.7 thousand. UAH.

### **Conclusions**

Production experience with animal embryos showed that the effect of acoustic oscillations on embryos increases their resistance to low temperatures and increases the viability of the progeny of cattle. As a result of the production experiment with

embryos, it was found that the profit from the introduction of acoustic technology was 35.7 thousand UAH.