

## POSSIBILITY OF COMPUTER EXPERIMENT IN STUDY OF ANIMAL SPERMATOZOA HETEROGENEITY

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A simulation model for evaluating the survival and fertilizing capacity of animal spermatozoa was developed, taking into account the initial condition of the sperm and the effectiveness of cryopreservation stages. The model is based on an analytical expression that reflects the main reasons for the survival of reproductive cells in onto-, techno- and phylogenesis. The decrease in spermatozoa resistance depends on a number of biological factors — the animal species, physiological conditions of sperm donor and recipient, the ejaculate quality, and technological factors — the effectiveness of the methods of cell cryopreservation and egg insemination. The discrepancy between the results of cell motility obtained by calculation and experimental methods amounted to less than 2% as a result of our own experiments and to less than 5% for the data taken from literature. A feature of the model is the complete independence of the effectiveness of studied techniques from the heterogeneity of animal sperm.

The conducted computer experiment showed that the difference between the values of initial motility and fertilizing capacity of sperm varies from 50 to 100% depending on the difference of biological parameters, while the index of the effectiveness of selected technique creates an error of about 1%. Comparative analysis of alternative technologies of spermatozoa cryopreservation showed the maximum efficiency of the stages of cryoprotectant use, freeze mode, survival and fertilizing capacity of the object. The use of computer modeling allows to greatly reduce the spread in spermatozoa preservation values that were obtained in different experiments, and thus to reduce the time and costs it takes to obtain reliable results.

**Key words:** system biology, cryopreservation, heterogeneity, spermatozoa.

The use of mathematical models when conducting experiments makes it possible to not only detect, but also to explain the obtained regularities, which allows scientists to create entirely new ways for cryopreservation of biological objects. Modern scientific methodology identifies three stages of knowledge mathematization: statistical processing of empirical data, experiment modeling on the basis of regression equations and the existence of relatively complete mathematical theories represented by analytical expressions [1]. Achievements in the field of cryobiology allow to widely use analytical methods in mathematical modeling and to explore the potential of the use of statistical methods for the analysis and optimization of multi-factorial experiments.

To date, there have been a number of studies aimed at improving the motility of animal sperm by optimizing the parameters

that define the freezing mode of biological objects [2–10]. It is found that the success of cryopreservation of biological objects, including sperm, mainly depends on three tied components: proper selection of cryoprotectant and its concentration, composition of a diluent and the freeze-thaw mode characteristics. Analysis of the cryopreservation results [2, 3, 6–8, 10–15] shows that bull and carp sperm motility rate lowers during sperm freezing and thawing.

The result of animal sperm cryopreservation depends on the variation of the initial state of the ejaculate. Consequently, the initial state of the biological object and features of technological operations that were conducted prior to cryopreservation may have an impact on the mobility of deconserved material. Therefore, to study patterns of repeatability of cryopreservation results, it is necessary to develop a mathematical model

for evaluating the effectiveness of the chosen method of cryopreservation that takes into account the initial state of biological objects and the features of different stages of the chosen cryopreservation method.

The aim of the study is to create a simulation model to describe the patterns of influence of animal heterogeneity and the efficiency of steps taken during semen cryopreservation on the fertilizing capacity of sperm.

### Materials and Methods

Sperm of breeding bulls of black-and-white and Simmental breeds was used when conducting experiments. Breeding bulls from the experimental-methodological breeding enterprise of the 1st category of the Department Biotechnology at the Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine (NAAS) were used. When conducting computer simulation, we used the data obtained from the semen of one bull to ensure the homogeneity of the biomaterial. All technological methods for bull semen receiving, evaluation and dilution were performed in accordance with [9, 16]. Fresh sperm with a motility index not lower than 70% was used. The sperm was stored at a temperature of 30 °C no longer than 30 min after it has been obtained.

Upon receipt and evaluation semen was diluted at 1:1 ration with sterile lactose-vitelline-glycerol (LVG) medium (11% solution of lactose — 62 ml, yolk — 30 ml, glycerol — 7 ml). Then it was diluted again with lactose-citrate-glycerol (LCG) (lactose — 6 g, sodium citrate — 1.4 g, glycerol — 5 ml, distilled water — 100 ml) to a concentration of spermatozoa of 15 million/ml. Then sperm was placed into cone-shaped containers of 0.25 ml in volume. Sperm equilibration was performed at 2–4 °C for 3–4 hours with constant stirring.

Sperm of Nivky scaly carp was obtained from sexually mature males (*Cyprinus carpio*). The fish were caught in a pond during carp spawning at an ambient temperature of 20 °C. Fish that matured naturally without hormonal treatment was used for the experiment [12, 17]. Sperm was obtained separately from each male at 5<sup>th</sup> stage of maturity, which is important for achieving high cryoresistance. Carp sperm was obtained, assessed and prepared for freezing according to [11, 12].

Cryoprotectant was prepared by dissolving 15 g of Tris-HCl-buffer in 1 l of distilled water, adding hydrochloric acid to pH 8.

To make 1 l of cryoprotectant, 7,3 g NaCl, 0,04 g KCl, 2,3 g NaHCO<sub>3</sub>, 1,15 g sucrose, 0,52 g MgSO<sub>4</sub> and 0,15 g CaCl<sub>2</sub> were alternately dissolved in 600–700 ml of Tris-HCl-buffer.

150 g of egg yolk and 100–120 g of ethylene glycol was added to the resulting solution 30 minutes before using it. Then Tris-HCl-buffer was added to the solution to get the total volume of 1 l [11, 12].

Before the experiment, all the dishes, sperm, cryoprotectant and activators (pond water) were cooled to 15 °C. Cryoprotectant was added to the sperm at 1:1 volume ratio while constantly stirring. Then the diluted carp sperm was cooled at the speed of 1–2 °C/min to a temperature of 2–3 °C and equilibrated for 1 hour. Fresh sperm with a motility index no lower than 70% was used in experiments. Selected ejaculates were placed in cone-shaped containers with tapered initiation of crystallization, sealed and placed in a refrigerator at 2–3 °C for further testing in various freeze modes. Each container was 0.25 ml in volume.

Ejaculate volume, motility and concentration of cells were determined before the experiment using a photoelectrocolorimeter. Motility index was used to assess deconserved carp sperm, motility, survivability and fertilizing capacity indexes were used to assess the condition of deconserved bull sperm. Fertilizing capacity was determined by first insemination of Simmental heifers.

Sperm motility of carp was determined as follows. A drop of water was placed on a glass slide with a pipette, then a small amount of sperm on the tip of a needle was added. Total duration of these manipulations did not exceed 10 seconds. When determining the motility of bovine spermatozoa, biomaterial was applied on a glass warmed to 38 °C, under a microscope. The motility of sperm in each sample was determined at least three times and the average result was calculated.

Microscopes of MBI or MBR brands with magnification of 300–600 were used during the experiment. Bull and carp sperm motility was determined by the ratio of spermatozoa with rectilinear translational motion to the total number of sperm in the field of view of the microscope, expressed as a percentage (Fig. 1).

Sperm cryopreservation was carried out in devices that were developed by us earlier. These devices use passive cooling of the fuser in the neck of Dewar vessels, as a basic process [18]. To make ultrahigh freeze-thaw speeds possible, envelopes made of polyethylene

and aluminum foil were used. Before the actual freezing, the device is cooled in liquid nitrogen. During thawing, it was heated in a water bath to 40 °C. Freezing (thawing) rate in this case was 15 000 (12 000) °C/min and 24 000 (22 200) °C/min for envelopes made of polyethylene and aluminum foil, respectively [18].

The condition of a biological object is characterized by several criteria: the motility of the sperm determines whether the object is suitable for further use in the study; it is evaluated by morphological parameters of the object; fertilizing capacity characterizes the probability of successful development of a biological object further in the experiment; the effectiveness of cryopreservation stage gives an indication of how the condition of a biological object changes as a result of the cryopreservation stage alone; reproducibility characterizes the dispersion of the results obtained by using a particular method of cryopreservation; comparability enables us to compare results obtained using different methods of cryopreservation.

To improve the reproducibility of obtained results, the study was performed using ejaculates of the same quality in each group, while determining the motility and fertilizing capacity of sperm. Animal sperm motility — the  $S f(1)$  is defined as the ratio of the number of moving sperm —  $n$ , divided by the total number of sperm in the sample —  $n_0$ :

$$S = n/n_0. \quad (1)$$

Survival rate was also evaluated for deconserved bull sperm 38 °C. Survival rate was measured as the amount of time during which all spermatozoa lost their rectilinear translational movement.

The initial sperm motility  $S_0$  was determined experimentally, after semen was obtained and diluted —  $S f(1)$ .

Fertilizing capacity of sperm —  $P f(2)$  was calculated as a ratio of the number of pregnancies —  $n$  to the total number of fertilized cows (for fish — embryos) —  $n_0$ :

$$P = n/n_0, \quad (2)$$

where  $n_0$  — the initial number of samples used in the experiment.

Insemination of eggs and incubation was carried out at a temperature of 18–20 °C. To eliminate the influence of differences in fertilization and cultivation methods for fresh and deconserved sperm on evaluation

of effectiveness of cryopreservation of carp sperm, the following experiments were conducted. 0.02 ml of fresh diluted sperm was added to each Petri dish in control group, and 0.02 ml of deconserved sperm was added to each Petri dish in experiment group. Semen (0.02 ml) was mixed with eggs (50–60 eggs) and activator (0.25 ml 0,3%  $\text{NaHCO}_3$ ) in Petri dishes (10 cm in diameter), stirred thoroughly with a feather for 2 min and quenched with water [19, 20]. When assessing the fertilizing capacity of fresh and deconserved sperm, ejaculate of the same animal was used for 1 h after freezing. Experiments were conducted in the laboratory; inseminated eggs were incubated in Petri dishes. Embryos that were stuck to the bottom of the cup embryos were left to incubate at 18–20 °C, water was changed every 3–4 hours. To remove the sticky layer from the surface of eggs, pond water was added to eggs 5 minutes after they were mixed with activated sperm. After that, eggs and water were stirred for 30–40 minutes to remove the adhesive layer from egg surface. Fertilization percentage was determined by 4-cell blastomere stage, and calculated using the formula:

$$P_c = \frac{n_{pc}}{n_o} \cdot 100\%, \quad (2 a)$$

$$P_d = \frac{n_{pd}}{n_o} \cdot 100\%, \quad (2 b)$$

where  $P_c$  and  $P_d$  — probabilities of fertilization using native and deconserved sperm;  $n_{pc}$ ,  $n_{pd}$  and  $n_o$  — number of cells that were fertilized with fresh, deconserved sperm, and total number of eggs in the sample.

Statistical analysis of the results obtained in the experiment was carried out using standard formulas for qualitative and quantitative analysis [21]. Standard and developed by us software was used for the calculations. Reliability of differences in control and experiment groups was assessed using Student's t-test by comparing the average values of motility, viability and efficiency that were obtained for the experiment and control group. Differences in conjugate parameters for control and experiment groups were averaged to take into account individual characteristics of biological objects [21]. Reproducibility of cryopreservation results was determined using the value of the standard deviation and the coefficient of variation  $C_v$ .

### Results and Discussion

The need to create a simulation model lies in the need to identify values that cannot be identified directly when conducting a physical experiment. We have proposed a method for calculating parameters of sperm resistance for sperm that was obtained from a particular donor and fertilized recipient (Fig. 1,  $r_d$  and  $r_p$ ), and for determining the efficiency of technologies that were used ( $W_d$  and  $W_p$ ). These figures summarize the impact of biological ( $F, A, U, H$ ) and technological factors ( $a, e, z, T$ ). Calculated parameters ( $r_d, r_p, W_d, W_p$ ) determine the conditions of the particular experiment. The model allows to calculate the fertilizing capacity of sperm cells when conducting a computer experiment using the given parameters ( $S_0, r_d, r_p, W_d, W_p$ ). Simulation modeling ensures comparability of empirical data ( $S, P$ ) that was obtained under different conditions of the study ( $S, r_d, r_p, W_d, W_p$ ). Calculated efficiency indexes of different technological stages ( $a, t, z, T$ ) are independent from the condition of each biological object ( $r_d$  and  $r_p$ ), which greatly improves the reproducibility of the results. The model allows one to determine the heterogeneity of biological characteristics ( $S_0, S, A, r_d, r_p, F, P, U, H$ ), given the fact that each of them depends on many interrelated parameters.

A logistic function was used as a basis for the proposed mathematical model to describe spermatozoa resistance when obtaining sperm, cryopreserving it and inseminating the recipient:

$$\frac{dS}{dt} = r \cdot S(1 - S/K), \quad (3)$$

where  $S$  — spermatozoa motility, calculated in terms of  $f(1)$ ;

$t$  — time, expressed in hours;

$r$  — coefficient that reflects the rate at which spermatozoa condition changes depending on the physiological condition of the donor;

$K$  — coefficient that corresponds to the maximum value of sperm motility.

The solution of the differential equation  $f(3)$  gives a numerical value to describe the survival rate of sperm cells at physiological temperature:

$$S(t) = \frac{S_0 \cdot K \cdot \exp(r \cdot t)}{K - S_0 + S_0 \cdot \exp(r \cdot t)}, \quad (4)$$

where  $S_0$  — the value that corresponds to the value of the initial sperm motility.

The coefficient, which reflects the rate at which the condition of spermatozoa changes,  $S(t)$  in this analytical expression, is determined using computer simulation by analyzing the values of sperm motility  $f(1)$  that were obtained in the experiment.

According to Bayes' theorem, motility of deconserved spermatozoa  $S_d(t)$  can be expressed as the sum of conditionally independent probabilities (expressed in units) that correspond to fresh sperm motility  $S_0(t)$  minus the probability of the death of some

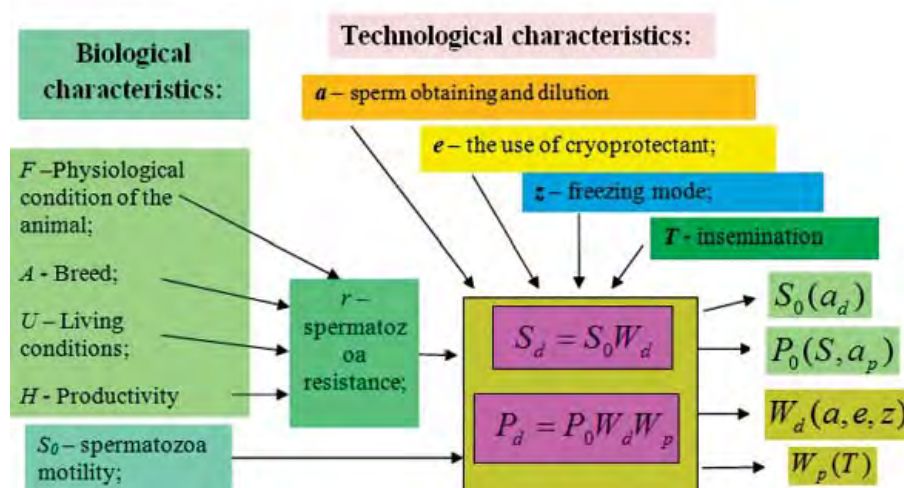


Fig. 1. Chart of computer study of spermatozoa heterogeneity in cryopreservation and artificial insemination:

$S_0, S_d$  and  $P_0, P_d$  — motility and fertilizing capacity of fresh, deconserved sperm;  
 $r_d$  and  $r_p$  — resistance of the donor and of fertilized recipients to sperm;  
 $W_d$  and  $W_p$  — efficiency of sperm cryopreservation and artificial insemination techniques



cells. This value depends on the effectiveness of the cryopreservation technique that was used  $W_d$ :

$$S_d(t) = S_0(t) - S_0 \cdot (1 - W_d). \quad (5)$$

In order to improve the accuracy of the analytical dependence  $S(t)$  f(4), we created a regression equation that connects the change in spermatozoa motility  $S$  to time. The method of least squares was used for this (Fig. 1–5). The values of cryopreservation efficiency  $W_d$  were calculated using the proposed model f(3–5) on the basis of spermatozoa motility data obtained in the experiment  $S$  f(1).

The error in determining empirically derived sperm motility index  $S$  f(1) is determined by assessing the state of a biological object of specified quality. The error is 5% (0.5 points) when using the visual method, and 1% (0.1 points) when using computer video fixation [18]. When using the conventional method for evaluating cryopreservation that takes into account the average value of sperm motility, depending on the initial spermatozoa condition, in different experiments the difference may be more than 10%. The need for such a transformation is due to the fact that average values do not carry information about the condition of each individual ejaculate, while the proposed model  $S(t)$  f(5), in conjunction with the empirically derived function  $S$  f(1) reflect the individual characteristics of a particular ejaculate.

Fertilizing capacity of fresh sperm of given quality  $P(S)$  depends on their motility —  $S$  f(1), on the physiological condition of the recipient —  $r$  f(7), and on the value that reflects the effectiveness of the technique used for artificial insemination of cows  $W_p$  f(8):

$$P(S) = \frac{P_m \cdot K \cdot \exp(r \cdot S)}{K - P_m + P_m \cdot \exp(r \cdot S)}, \quad (7)$$

where  $K$  — coefficient that reflects the maximum value of egg fertilization, and the minimum —  $P_m$ , respectively;

$r$  — coefficient that corresponds to the physiological condition of the recipient. It determines the probability of fertilization.

Analytical expressions  $S(t)$  and  $P(S)$  are derived from f(4) and f(7), using the regression dependence of sperm motility values that were obtained experimentally during sperm cultivation f(1) and insemination f(2) (Fig. 2–6).

The probability of fertilizing capacity of fresh and deconserved sperm  $P(S)$  is expressed through the product of dependent parameters of the fertilizing capacity of fresh sperm  $P_0(S)$  f(7), the effectiveness of cryopreservation techniques  $W_d$  and artificial insemination techniques  $W_p$ , expressed in units:

$$P(S) = P_0(S) \cdot W_d \cdot W_p. \quad (8)$$

To improve the accuracy of determining the cryopreservation efficiency by reducing the total variance [18] this value is represented the product of the efficiency of the following technological stages: collection and dilution of the ejaculate  $W_a$ , equilibration in cryoprotectant and temperature adaptation  $W_e$ , selection of the freeze-thaw mode  $W_z$ :

$$W_d = W_a \cdot W_e \cdot W_z. \quad (9)$$

The following system of equations is used to calculate the parameters of effectiveness:

$$\begin{aligned} S_a(t) &= S_0(t) \cdot W_a; \\ S_{ae}(t) &= S_0(t) \cdot W_a \cdot W_e; \\ S_d(t) &= S_0(t) \cdot W_d. \end{aligned} \quad (10)$$

Software was developed in Microsoft Excel in order to simplify the procedure for calculating the main biotechnological parameters. A graphical representation of calculated results is shown in Fig. 2–6.

To test the proposed simulation model f(3–10), we analyzed the data for fresh and deconserved bull sperm motility at 38 °C that was obtained at the Livestock Institute of National Academy of Agrarian Sciences of Ukraine [10]. Spermatozoa were frozen in plastic containers ( $V = 0.75$  ml) in a device that was developed by us [15].

It was established experimentally [15], that when frozen with crystallization initiation, the motility index of deconserved bull sperm increased — from  $38.6 \pm 1.83\%$  to  $52.3 \pm 1.64\%$ , respectively. Survival time of deconserved bull sperm that was frozen with the initiation of crystal growth was 9 hours, and without the initiation of crystal growth — 7 hours (Fig. 2).

Regression dependence of experimental f(1) and model f(4, 5) values of spermatozoa motility in time allowed to determine approximation coefficients for fresh diluted

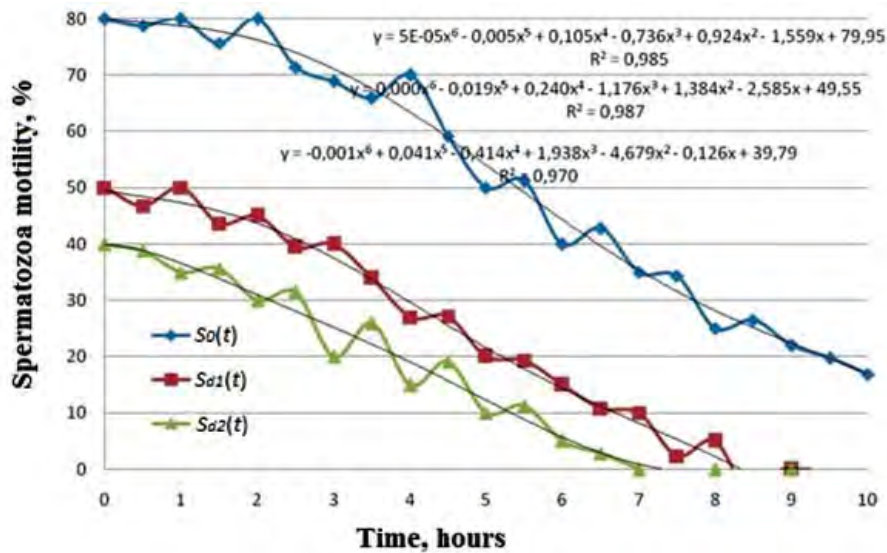


Fig. 2. Dependence of bull sperm motility from cultivation time at a temperature of 38 °C for fresh diluted cells —  $S_0(t)$ , deconserved cells in the first group —  $S_{d1}(t)$  and in the second —  $S_{d2}(t)$ , respectively:

The effectiveness of cryopreservation techniques amounted to  $W_{d1} = 62\%$  and  $W_{d2} = 50\%$ . Coefficients that reflect sperm motility reduction, depending on the viability of the donor,  $r_1 = r_2 = -0,4$ . Coefficients that reflect the values of initial spermatozoa motility amounted to  $S_{01} = S_{02} = 80$ , and maximum motility —  $Ks_1 = Ks_2 = 86$ .

cells  $S_0(t)$   $R^2 = 0,985$ , and for deconserved cells in experimental group (with crystal growth initiation) —  $S_{d1}(t)$   $R^2 = 0,987$  and in control (without initiation) —  $S_{d2}(t)$   $R^2 = 0,970$ , respectively.

Obtained results indicate that seeding has a positive effect on deconserved spermatozoa motility, which increases by 10–15%, and on the effectiveness of cryopreservation technique, which increases by 12% due to reduction in supercooling and, consequently, significantly reduced temperature change during freezing. The difference between control and experiment is reliable at the level of reliability of  $P > 0,95$ .

To evaluate the performance of the proposed model we used a computer experiment that assesses motility of deconserved and fresh spermatozoa obtained from different ejaculates (Fig. 3). Difference in initial sperm motility was 20% and the error of efficiency estimation did not exceed 1%.

When the proposed simulation model was tested using data that was published by us [15], we received results that were highly similar to experimental results that were obtained when the experiment was conducted —  $R^2 = 0,999$  for values of fertilizing capacity of deconserved bull sperm (Fig. 4) that was preserved using the proposed technique. This biological quality indicator is a comprehensive characteristic of reproductive cells that were

frozen and thawed given a high preservation safety rate of heifer gametes.

Studies have shown that functions that tie fertilization probability to spermatozoa motility  $P_d(S)$   $f(7)$  for fresh and deconserved cells coincide with high reliability probability  $R^2 = 0,99$ . Effectiveness of cryopreservation amounted to  $W_{d1} = W_{d2} = 100\%$ . Consequently, the effect of temperature lowering regimes on the fertilizing capacity of Simmental bull sperm depends solely on sperm motility and fertilization method. When determining the fertilizing capacity, sperm doses that were conserved according to Kharkiv technology [9] were used as a control.

Indicators of sperm motility, survival and absolute survival of deconserved sperm in all experiments conformed to the figures, outlined in GOST 26030-83 “Frozen bovine semen. Technical specifications” for heifer insemination. All frozen semen doses were studied in SEC “Ukraine” of Zmiev district to determine the effectiveness of insemination.

In publications [13, 17, 19, 20] it has been shown that the likelihood of fish embryo development depends on the genotype and physiological characteristics of the female and male that were used to obtain eggs and sperm. The authors found that fertilizing capacity depends not only on the initial state of fresh sperm and eggs, but also on many other factors: cryoprotectant type, freezing-thawing

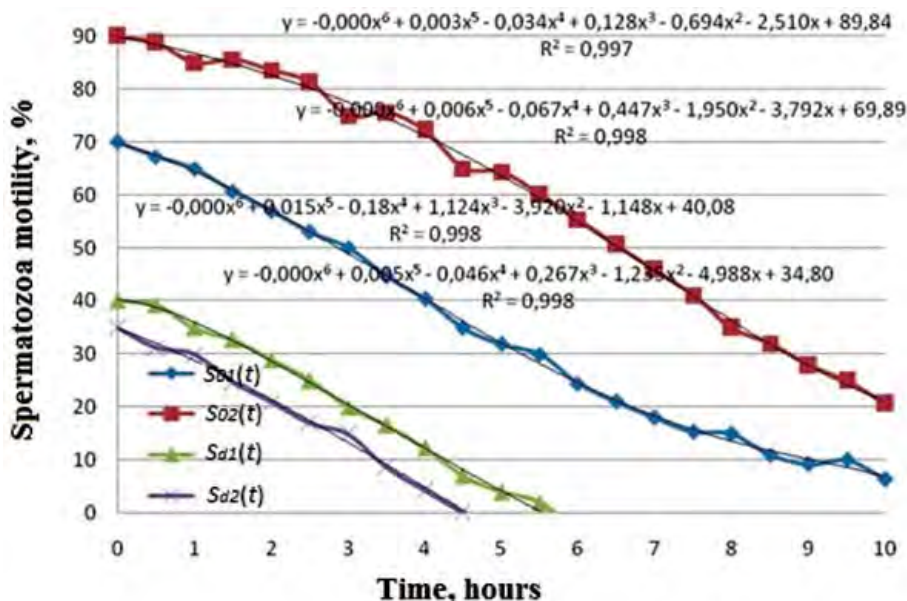


Fig. 3. Dependence of bull spermatozoa motility on cultivation time at 38 °C from fresh diluted sperm in the first group —  $S_{01}(t)$  and in the second —  $S_{02}(t)$ , deconserved spermatozoa —  $S_{d1}(t)$  и  $S_{d2}(t)$ , respectively: effectiveness of cryopreservation techniques amounted to:  $W_{d1} = 60\%$  and  $W_{d2} = 60\%$ . Coefficients that reflect the decrease in sperm motility, depending on donor viability amounted to  $r_1 = r_2 = -0,4$ . Coefficients that reflect the value of initial sperm motility —  $S_{01} = 70$  and  $S_{02} = 90$ , and maximum —  $K_{s1} = 86$  and  $K_{s2} = 96$

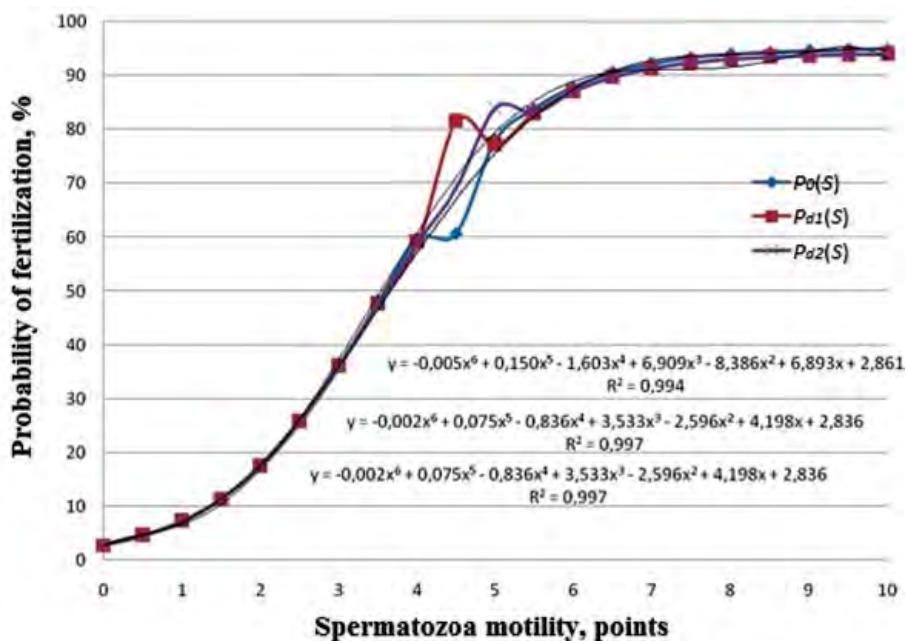


Fig. 4. Dependence of fertilizing capacity of cows on bull sperm motility for fresh —  $P_0(S)$  and deconserved spermatozoa in the first group —  $P_{d1}(S)$ , and in the second —  $P_{d2}(S)$ , respectively: effectiveness of cryopreservation techniques amounted to:  $W_{p1} = W_{p2} = 95\%$ . Coefficients that reflect the probability of fertilization, depending on the recipient's physiological condition —  $r_1 = r_2 = 1,0$ . Coefficients that reflect the maximum value of animal fertilization  $K_{p1} = K_{p2} = 100$ , and minimum —  $P_{m1} = P_{m2} = 3$ , respectively



mode, method of fertilization and embryo cultivation [13, 17, 19, 20]. A particularly important factor is the individual fertilizing ability of both fresh and deconserved spermatozoa obtained from a particular male [13, 17, 19]. In this regard, a common practice in industry is to fertilize eggs using a mixture of sperm that was obtained from at least three manufacturers [11, 12, 17, 19].

The effect of different cryopreservation techniques on fresh and deconserved carp sperm motility is shown in Fig. 5. The error in determining the effectiveness of cryopreservation technique  $W_{d1} = 65\%$  and  $W_{d2} = 50\%$  amounted to less than 1%.

Fertilizing capacity of spermatozoa depends on the number of viable cells in the sample which, consequently, depends on their concentration and motility [13, 17, 19, 20]. Results of fertility evaluation of ejaculates taken from two males with initial mobility  $80,0 \pm 3,6\%$  and  $70,0 \pm 3,5\%$  are shown in Fig. 6.

Fertilizing capacity of carp sperm in the two groups was not significantly different and was 23,3–31,2% for deconserved object (the accuracy of the difference  $P \geq 0,90$ ) and 51–61% for the freshly obtained object. Fertilizing capacity in the first group was higher than in the second, in addition, sperm motility value was higher in the first group for both fresh and deconserved sperm. When

assessing the effectiveness of insemination techniques, the obtained values were: for the first group — 51,0% and for the second — 45,8%. The difference in values for two groups decreased by 1,6–2,0 times due to a decrease in the impact of the initial condition of carp sperm and eggs which depends on their individual fertilizing ability and specific features of insemination and cultivation techniques that are used.

When comparing values for fertilizing capacity of fresh and deconserved carp sperm that were obtained by us with those from the literature [3, 13, 17], it should be noted that the obtained absolute values of the fertilizing capacity of fresh sperm can vary over a wide range from 15 to 80%, and for deconserved spermatozoa — 5–65%. However, values for fertilizing capacity after cryopreservation in publication [13] correspond to those obtained by us, while those in publications [3, 17] are higher, which seems to be associated with the use of mixed sperm from several males during fertilization.

Obtained values of deconserved sperm motility (30–65%) correspond to the data correspond to the data presented in the literature for carp spermatozoa [13], which makes it possible to have fertilizing capacity of deconserved carp spermatozoa ranging from 5 to 65%. It is shown that in the fertilizing

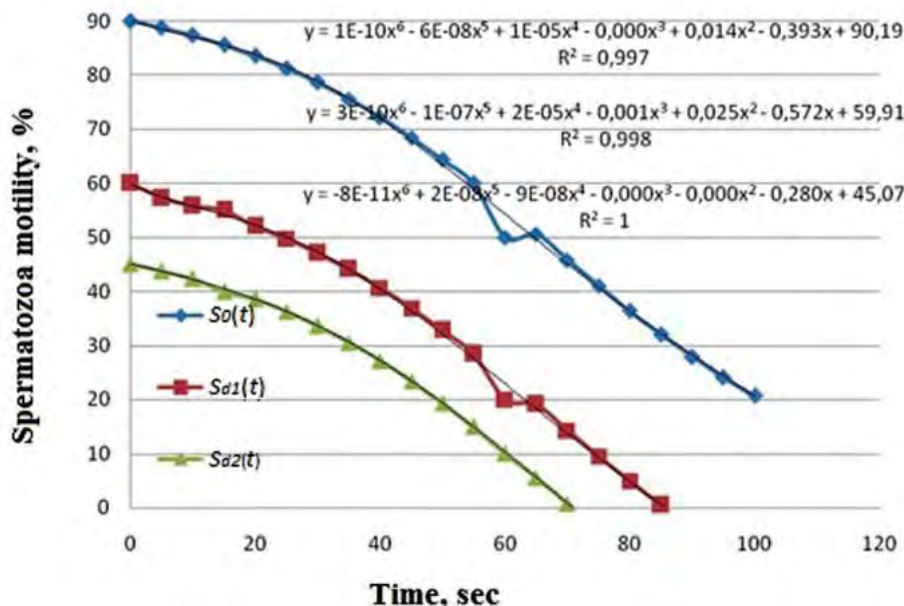
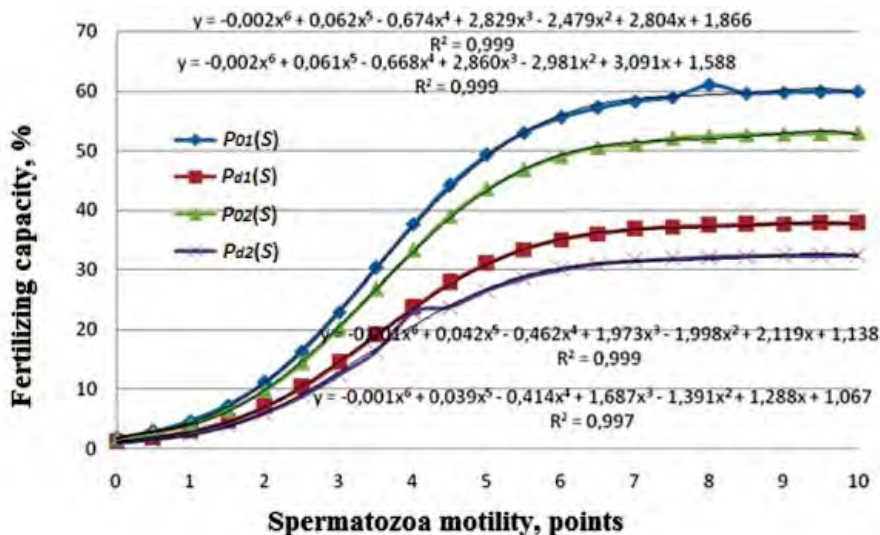


Fig. 5. Dependence of carp spermatozoa motility on cultivation time at room temperature for fresh cells —  $S_0(t)$ , deconserved spermatozoa in the first group —  $S_{d1}(t)$  and in the second —  $S_{d2}(t)$ , respectively: effectiveness of cryopreservation techniques amounted to  $W_{d1} = 65\%$  and  $W_{d2} = 50\%$ . Coefficients that reflect the decrease in spermatozoa motility, depending on donor viability —  $r_1 = r_2 = -0,04$ . Coefficients that reflect the minimum value of spermatozoa motility  $S_{01} = S_{02} = 90$  and maximum —  $K_{s1} = K_{s2} = 96$





**Fig. 6. Dependence fertilizing ability of carp eggs on spermatozoa motility for fresh cells in the first group —  $P_{01}(S)$  and in the second —  $P_{02}(S)$ , deconserved cells —  $P_{d1}(S)$ ,  $P_{d2}(S)$ , respectively:** Cryopreservation effectiveness amounted to  $W_{d1} = 54$  and  $W_{d2} = 63\%$ , and insemination effectiveness —  $W_{p1} = W_{p2} = 53\%$ . Coefficients that reflect fertilization probability depending on the recipient's physiological state —  $r_1 = r_2 = 1,0$ . Coefficients that reflect maximum values for egg fertilization  $K_{p1} = K_{p2} = 100$  and minimum —  $P_{m1} = P_{m2} = 3$ , respectively

capacity is significantly influenced by the condition of fresh sperm and eggs, and this in turn depends on the physiological state of the donor, the methods of hormonal treatment and superovulation induction [3, 13, 17].

The use of the proposed model reduces the influence of the condition of fresh carp sperm and eggs, conditions of fertilization and cultivation by a factor of two or more, and allows us to determine the effectiveness of the chosen method of cryopreservation, assessed in terms of fertilizing capacity (54–63%).

Along with the assessment of differences of averages of deconserved carp sperm motility, we also analyzed the variations in the current values of motility. When transitioning to model values, the variation decreased 2 to 13 times (Fig. 5). This indicates an increase in the accuracy of the proposed method for evaluating cryobiological operations by half or more by reducing the influence of the initial state of carp sperm and related factors that determine the individual characteristics of the selected method of cryopreservation. Such a significant discrepancy between the obtained values can be tied to the effect of “noise” — a difference in the initial condition of sperm and eggs on the one hand, and features of the cryopreservation and fertilization methods — on the other, on the useful “signal” — the condition of the cryopreservation system being examined.

To evaluate the effectiveness of different methods of carp sperm cryopreservation

and their constituent stages, the following computer experiment was performed. Maximum deconserved sperm motility —  $51.7 \pm 1.7\%$  (cryopreservation effectiveness  $68.9 \pm 2.2\%$ ) is obtained using a cryopreservation method that involves slow two-step freezing of spermatozoa in a polyethylene envelope (table). As a result of rapid freezing, motility rates  $35.1 \pm 5.0\%$  ( $43.8 \pm 1.0\%$ ) are close to those rates  $36.9 \pm 1.2\%$  ( $50.0 \pm 1.3\%$ ) that were obtained when spermatozoa were thawed slowly  $B_0 = 725$  °C/min. The reasons for this — similar values of cryoprotectant effectiveness (92%) and freeze-thaw conditions (60–68%).

The range of motility rates 35–52% obtained by using different methods of cryopreservation of carp sperm (table) gives a similar discrepancy in motility values as when using ejaculates of different quality (Fig. 5) with the error in measurement of less than 1%.

The values of cryopreservation effectiveness 44–67% and the mistake of their measurement also have close values (1%). The decrease in differences of values when transitioning to model values is 1.2–2.7 times. This is indicative of the fact that variation in the initial condition of carp sperm and features of the chosen cryopreservation method have the same effect on deconserved sperm motility. Such a significant discrepancy between the obtained values is due to the influence of different initial condition of sperm samples, on

The effectiveness of different methods of carp sperm cryopreservation  $W_d$  and their stages, evaluated in terms of the use of cryoprotectant  $W_t$  and freeze-thaw mode  $W_z$

| Container | Freezing rates $B_1$ , $B_2$ ( $B_3$ — thawing),<br>$^{\circ}\text{C}/\text{min}$ | Motility<br>$S_d, \%$ | Step effectiveness rates, % |                         |                  |
|-----------|---|-----------------------|-----------------------------|-------------------------|------------------|
|           |   |                       | cryopreservation,<br>$W_d$  | equilibration,<br>$W_e$ | freezing, $W_z$  |
| Test tube | 4, 25 (318)   | $36.9 \pm 1.5^a$      | $50.0 \pm 1.3^a$            | $92.0 \pm 1.5$          | $68.0 \pm 1.2^a$ |
| Straw     | 4, 25 (725)   | $44.6 \pm 1.5^b$      | $56.9 \pm 1.6^b$            |                         | $75.2 \pm 1.5^b$ |
| Envelope  | 4, 25 (4780)  | $51.7 \pm 1.7^c$      | $68.9 \pm 1.2^c$            |                         | $88.6 \pm 1.6^c$ |
| Envelope  | 4500 (12000)  | $35.1 \pm 1.5^a$      | $43.8 \pm 1.0^d$            |                         | $59.8 \pm 1.6^d$ |

Note:  $B_1$  and  $B_2$  — freezing rate during the first stage from 5 to  $-20^{\circ}\text{C}$ ; the second stage from  $-20$  to  $-60^{\circ}\text{C}$ ;  $B_3$  — thawing rate.

the one hand, and features of cryopreservation methods that were used — on the other hand, on the condition of the deconserved biological object.

It was found that the initial state of the biological object in some cases affects his mobility to a greater extent than the features of the cryopreservation method that was used. In the case when reproducibility of carp sperm motility rates is connected to different semen quality, it reaches 96–136%. If the reproducibility depends on the cryopreservation method, it is 94–137%. When using effectiveness rates, it is possible to increase the reproducibility of research results 1,2–4,7 times. To achieve this, it is necessary to take into account the impact of quality of the object on the study and to evaluate the effectiveness of the method cryopreservation method that was used and of its stages. Effectiveness rates of different technological stages that were obtained by us allow to compare results of studies that were conducted using different methods. The most effective method of freezing sperm carp, ensuring sperm safety rate of 45–52%, is based on the use of two-stage freezing. Cryoprotector effectiveness rate is 92%, freeze mode — 75–89%.

Thus, the developed simulation model describes the dependence of deconserved spermatozoa motility on their initial condition and on the effectiveness of cryopreservation technology stages. The use of the model greatly increases the reproducibility of the results of the study and provides conditions for their compatibility when using different technologies and heterogeneous biomaterial.

Comparative analysis of the effectiveness of different stages of bull and carp sperm cryopreservation process indicates that in each case the obtained values depend more on the experimental conditions than on the initial sperm motility. When transitioning to the calculated values of efficiency, it is possible to determine the impact of each studied factor on sperm motility, evaluated at different stages of cryopreservation procedures, while reducing the number of experiments by a factor of three or more.

We believe that the proposed model makes it possible to determine the conditions needed to solve a problem at the stage of exploratory experiments. The proposed method of biotechnological computer experiment can be used in studies that aim to identify hidden patterns of influence that the physiological state of the donor and recipient, different technologies of cryopreservation and artificial insemination have on the fertilizing capacity of deconserved sperm.

The model is a method that ensures the comparability of heterogeneous data obtained empirically under different experimental conditions, which makes it possible to quantitatively explain the strength of the influence that a number of biological and technological factors have on the probability of obtaining offspring. The same model is necessary for multi-factor research aimed at the intensification of existing technologies and the creation of new ones.

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## МОЖЛИВОСТІ КОМП'ЮТЕРНОГО ЕКСПЕРИМЕНТУ В ДОСЛІДЖЕННІ ГЕТЕРОГЕННОСТІ СПЕРМАТОЗОЇДІВ ТВАРИН

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Розроблено імітаційну модель оцінки рухливості й запліднювальної здатності сперматозоїдів тварин з урахуванням їх початкового стану та ефективності етапів криоконсервування. В основу моделі покладено аналітичний вираз, що відображає основні причини виживаності репродуктивних клітин в онто-, техно- і філогенезі. Зниження резистентності сперматозоїдів залежить від низки біологічних (виду тварини, фізіологічного стану донора і реципієнта, якості еякуляту) та технологічних (ефективності способів криоконсервування і запліднення яйцеклітин тварин) чинників. Розбіжність між значеннями рухливості клітин, отриманими розрахунковим і експериментальним способами, становила не більше 2% у результаті проведення власних дослідів і менше 5% — для даних, наведених у літературі. Особливістю моделі є повна незалежність показників ефективності досліджуваних технологій від гетерогенності еякуляту тварин.

Проведений комп'ютерний експеримент показав, що розбіжність початкової рухливості й запліднювальної здатності сперматозоїдів залежно від варіації біологічних параметрів змінюється від 50 до 100%, при цьому похибка визначення показника ефективності обраної технології становить близько 1%. Порівняльний аналіз альтернативних технологій криоконсервування сперматозоїдів показав максимальну ефективність етапів застосування криопротектора, режиму заморожування, виживаності та запліднювальної здатності. Застосування моделювання дає змогу багаторазово знизити розкид між значеннями рухливості сперматозоїдів, отриманими у різних дослідях, і тим самим більш ніж на порядок скоротити час та грошові витрати, необхідні для одержання достовірного результату.

**Ключові слова:** системна біологія, криоконсервація, гетерогенність, сперматозоїди.

## ВОЗМОЖНОСТИ КОМПЬЮТЕРНОГО ЭКСПЕРИМЕНТА В ИССЛЕДОВАНИИ ГЕТЕРОГЕННОСТИ СПЕРМАТОЗОИДОВ ЖИВОТНЫХ

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Разработана имитационная модель оценки подвижности и оплодотворяющей способности сперматозоидов животных с учетом их начального состояния и эффективности этапов криоконсервирования. В основу модели положено аналитическое выражение, отражающее основные причины выживаемости репродуктивных клеток в онто-, техно- и филогенезе. Снижение резистентности сперматозоидов зависит от ряда биологических (вида животного, физиологического состояния донора и реципиента, качества эякулята) и технологических (эффективности способов криоконсервирования и оплодотворения яйцеклеток животных) факторов. Расхождение между значениями подвижности клеток, полученными расчетным и экспериментальным способами, составило не более 2% в результате проведения собственных опытов и менее 5% — для данных, представленных в литературе. Особенностью модели является полная независимость показателей эффективности исследуемых технологий от гетерогенности эякулята животных.

Проведенный компьютерный эксперимент показал, что расхождение начальной подвижности и оплодотворяющей способности сперматозоидов в зависимости от вариации биологических параметров изменяется от 50 до 100%, при этом погрешность определения показателя эффективности выбранной технологии составляет около 1%. Сравнительный анализ альтернативных технологий криоконсервирования сперматозоидов показал максимальную эффективность этапов применения криопротектора, режима замораживания, выживаемости и оплодотворяющей способности. Применение моделирования дает возможность многократно снизить разброс между значениями подвижности сперматозоидов, полученными в разных опытах, и тем самым более чем на порядок сократить время и денежные затраты, необходимые для получения достоверного результата.

**Ключевые слова:** системная биология, сперматозоиды, гетерогенность, криоконсервирование.