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# COMPUTER MODELING OF EMBRYONIC MORTALITY AT CRIOCONSERVATION

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The purpose of the research was to determine the regularities of influence of mammalian embryos heterogeneity and effectiveness of cryoconservation steps on their viability by using the developed simulation model. The model is based on analytical expressions that reflect the main causes of embryonic mortality during *in vitro* and *in vivo* cultivation, crioconservation and embryo transplantation. Reduction of viability depends on a set of biological factors such as the animal special, donor and recipient state, quality of embryos, and of technological ones such as the efficiency of cryopreservation method, and embryo transplantation. Fulfilled computer experiment showed, that divergence of embryos viability depending on biological parameters variations changes in a range from 0 to 100%, whereas efficiency index of chosen technology has an inaccuracy about 1%. The comparative analysis of alternative technologies of embryos cryopreservation showed the maximum efficiency of stages of use of the cryoprotectant, freezing regime and *in vitro* and *in vivo* cultivation of biological object. The application of computer modeling gives an opportunity to reduce the range of embryos viability results, obtained in different experiments is many times, thereby to shorten the time, monetary costs and the slaughter of laboratory animals in obtaining reliable results.

***Key words:*** cryopreservation, embryos, mortality.

The process of technology improving is based on using of mathematical modeling methods. Application of methods of multifactorial analysis and optimization is necessary condition for transformation of extensive technology into the intensive one. The result is a phenomenological model that describes the interconnection between the state of the object and its parameters by using the regression equations. Application of analytical models, which explain mechanisms identified interconnections, is the basis for the development of new technologies.

As an example can be considered a model of dehydration cells that created by P. Mazur that defines the optimal parameters for cryopreservation of embryos [1]. With its help, it was possible to obtain viable offspring from deconservated embryos after two decades of barren empirical researches. Intensification of modern alternative cryobiotehnologies which are based on the use of low and high speeds of freezing and thawing, is limited by the low reproducibility of the results, and as a consequence — its incomparability. The reason is the different initial state of gametes and embryos, which is caused by the state of the donor. Application of relative indexes of biological object preservation increases the reproducibility of results on average in 1.5–2 times [2, 3]. However, the problem of the dependence of the proposed efficiency indicators on the condition of native sperm cells, oocytes and embryos of animals is still open [4–6].

Application of analytical expressions, which reveals the mechanism of changes in the state of biological object, in conjunction with regression expressions makes it possible to develop the simulation model, which is necessary for the computer experiment.

The aim of the study is to define the patterns of influence of mammalian embryos and the efficiency of cryopreservation steps on their viability by using the developed simulation model.

## Materials and Methods

For our research were taken cow embryos satisfactory, good and excellent quality, which were on the stage of development from

early morula up of expanded blastocysts. The quality of the embryos was assessed in points by their morphological condition [7] according to the general accepted methods and results of their *in vitro* development. Embryo search, preparation for the experiment, obtaining and washing–out of embryos were performed according to the generally accepted methods [8]. Experiments on animals were performed according to the principles of "European Convention for the protection of vertebrate animals, which are used with the experimental and other scientific purposes".

Mediums that were used for cryopreservation of mouse embryos were prepared based on Dulbecco solution (PBS) with 10% fetal calf embryos serum. For the freezing at the low speed as a cryoprotectant was taken 1 M solution of glycerol for mouse, and 1.2 M for cow embryos. Embryos were incubated for 10 min at a temperature 20 ± 2 °C. As equilibration (duration of equilibration is 10 min), and vitrification (equilibration time of 1 min) cryoprotectant solutions for freezing mouse embryos with high speeds were used glycerol and sucrose solutions of various concentrations (5, 10, 15, 20, 25, 30%), which were based on Dulbecco solution (PBS) with the addition of 10% fetal calf embryos serum. Saturation of cryoprotectant was performed in one or two stages. After defrosting of containers for removing cryoprotectant was used 0.5–0.7 M sucrose solution. The sample temperature was measured chromel-copel (B ≤ 100 °C/ min) and copper-constantan (B ≤ $2 \cdot 10^3$ °C/ min), thermocouples, junction diameter 0.3 and 0,1 mm respectively. Ultrahigh cooling rates (B > $2 \cdot 10^3$ °C/ min) was recorded by means of conductometric method [9]. Ulenguta's test tubes (V ≈ 0.75 ml) and plastic straws (d = 2 mm, V = 200 mkl) were as containers for the freezing of biological material. Embryo cryopreservation was carried out in the freezer ZEM–4 and in devices that were developed by us. These devises use passive cooling of the fuser in the neck of Dewar vessels X-34 (V = 35 l) [9]. High speed freezing of mouse embryos was obtained by direct stacking of containers with biological object into the liquid nitrogen. Thawing was performed in a waterbath at 40 °C. The condition of a biological object is characterized by several criteria: safety — the suitability of the biological object for the further use, which is determined based on morphological indicators; viability — a probability of the biological object development *in vitro*; engraftment — the probability of biological object development *in vivo*; the effectiveness of cryopreservation stage — the value that describes changes in biological object state as a result of a predetermined operation; reproducibility — the dispersion of the results obtained by using a defined method of cryopreservation; comparability — ability to compare the results obtained using different methods of cryopreservation.

The study was performed using embryos of the same quality in each group to increase the reproducibility of the results in the calculation of indicators of preservation and engraftment. Preservation of mammalian embryos $S_i$ f(1) is defined as the ratio of the number of suitable embryos for the further use $n$ (excellent $i = 5$, good $i = 4$, satisfactory $i = 3$), divided by the initial number of specified quality embryos — $n_{io}$:

$$S_i = n/n_{io}. \quad (1)$$

To improve the accuracy of the estimate of embryos with different quality, viability index was used by the formula of average weighted safety:

$$V_i = \frac{1}{n_o} \sum_{i=3}^{5} S_{oi} \cdot n_i \,, \quad (2)$$

where $n_i$ — the number of $i^{-th}$ embryo quality obtained after the experiment.

Initial safety of embryos of different quality $S_{0i}$ determined after short-term cultivation of the object *in vitro* $S_i$ f(1).

Engraftment of cows embryos $P_i$ f(3) was calculated as a ratio of the number of pregnancies — $n$ to the total number of implanted embryos $i^{-\text{th}}$ quality — $n_{io}$:

$$P_i = n/n_{io}, \quad (3)$$

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

Statistical analysis of the results carried out according to the generally accepted formulas of alternative method of variation and quantitative analysis [10] through the application of the standard and developed by us software. Reliability of differences in control and experiment groups was assessed using Student's $t$–test by comparing the average values of safety, viability and efficiency that were obtained for the experiment and control group.

The accounting the individual characteristics of biological object was carried out by averaging the difference between the paired parameters control–experiment [10].

Reproducibility of cryopreservation results was determined using the value of the standard deviation and the coefficient of variation $C_v$. As a control used experimentally obtained and referenced from literature. The experimental values obtained through the computer experiment.

## Results and Discussion

The need to create a simulation model lies in the development of methods of generalized of biological complex (Fig. 1, $F$, $P$, $U$, $H$) and technological ($k$, $t$, $z$, $T$) factors for determining the resistance of embryos ($r_d$ and $r_p$) the efficiency of technologies that were used ($W_d$ and $W_p$). Calculated parameters ($r_d$, $r_p$, $W_d$, $W_T$) determine the conditions of the particular experiment. The model allows to calculate the engraftment of embryos when conducting a computer experiment using the given parameters ($V_0$, $r_d$, $r_p$, $W_d$, $W_T$). Simulation modeling ensures comparability of empirical data ($V$, $P$) that was obtained under different conditions of the study ($V$, $r_d$, $r_p$, $W_d$, $W_T$). Calculated efficiency indexes of different technological stages ($k$, $t$, $z$, $T$) are independent from the condition of biological object ($r_d$ and $r_p$), which greatly improves the reproducibility of the results. The model provides the opportunity to explore the biological heterogeneity factors ($V_0$, $V$, $P$, $r_d$, $r_p$, $F$, $P$, $U$, $H$), given the fact that each of them depends on many interrelated parameters.

The basis of the proposed mathematical model is Verhulst equation for describing embryogenesis during preparation, cryopreservation and embryos transplantation:

$$\frac{dU}{di} = r \cdot U(1 - U/K) \quad , \qquad (4)$$

where $U$ — the state of embryos, calculated on the basis of preservation parameters f(1), viability f(2) and engraftment f(3) depending on their quality;

$i$ — current number, which reflects the quality of the embryos (excellent $i = 5$, good $i = 4$, satisfactory $i = 3$, not satisfactory $i = 2$, degenerated $i = 1$);

$r$ — coefficient that reflects the rate, at which embryos condition changes depending on the physiological condition (quality) of the donor or recipient;

$K$ — coefficient that corresponds to the initial embryos state.

The solution of the differential expression f(4) gives a numerical value of the embryo state $U(V(i))$, $P(i)$ obtained from a donor $V(i)$ or transplanted to a recipient $P(i)$:

$$U(i) = \frac{U_m \cdot K \cdot \exp(r \cdot i)}{K - U_m + U_m \cdot \exp(r \cdot i)} \quad , \qquad (5)$$

where $U_m$ — the minimum value of the embryo state;

$r$ — coefficient that corresponds to the state of the donor or recipient, which can

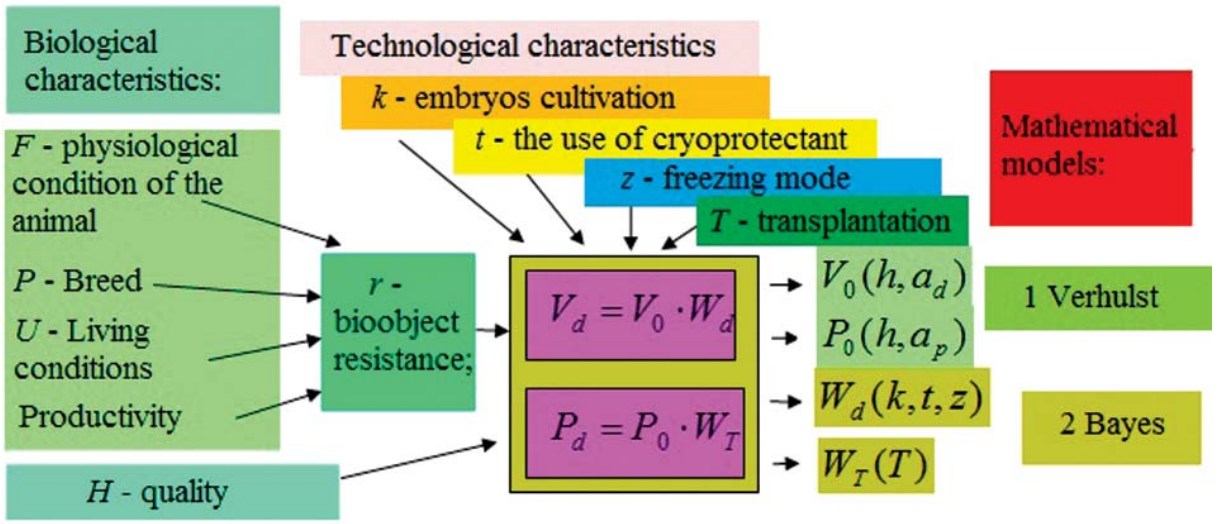


*Fig. 1.* **Chart of computer study of embryos heterogeneity at different stages of cryopreservation and transplantation:**
$V_0$, $V_d$ and $P_0$, $P_d$ — viability and engraftment of native, deconserved embryo; $r_d$ and $r_p$ — resistance of the donor and transplanted to the recipient embryos; $W_d$ and $W_T$ — the effectiveness of embryos cryopreservation and transplantation technology

be continuously varied from $r = 1$ — for the problem animal to $r = 2$ — for the healthy one.

The analytical expression f(5) is determined by computer modeling in analysis of experimentally obtained values of preservation f(1), vitality f(2) and engraftment f(3).

According to Bayes formula, the viability of the specified quality deconserved embryos $V_d(i)$ can be expressed as the product of the probabilities (in conventional units) that corresponds to native embryo viability $V_0(i)$ and the effectiveness of the cryopreservation technology $W_d$:

$$V_d(i) = V_0(i) \cdot W_d . \quad (6)$$

The regression equation of depending of embryos viability $V_i$ on their quality was composed using least squares method (Fig. 2–6). It was made for improving the accuracy of analytical dependence $V_d(i)$ and $V_0(i)$. The values of the efficiency of cryopreservation $W_d$ calculated using the the proposed model f(6).

The error in determining of empirically derived index of viability $V_i$ f(2) is determined by assessing the state of biological object specified quality, for embryos it is 5% (0.5 points).

In generally accepted method of cryopreservation evaluation according to the average index of biological object conservation, depending on its initial quality, in different experiments, the difference may be more than 10%. The necessity of this transformation related to the fact that the average values do not have information about the status of the individual embryo, whereas the proposed model $V(i)$ in conjunction with the empirically derived $V_i$ functions represent individual properties of particular object.

The engraftment of native specified quality embryos $P(i)$ depends on the quality of embryos and state of the donor $a_0$ in function $V_0(i)$ f(5) and the recipient $a_1$ $P_1(i)$ f(5) and also on the value of the efficiency of embryos transplantation technologies $W_p$:

$$P(i) = V_0(i) \cdot P_1(i) \cdot W_p . \quad (7)$$

Analytical expressions $V_0(i)$ and $P_1(i)$ is obtained using regression dependences of experimentally determined values of native cow embryos preservation during their short-term cultivation f(1) and engraftment f(3), (Fig. 2–6).

The probability of engraftment deconservated embryos $P_d(i)$ is expressed through the product of deconservated embryos viability $V_d(i)$ and the effectiveness of cryopreservation technology $W_d$, expressed in units:

$$P_d(i) = V_d(i) \cdot P_1(i) \cdot W_p = V_0(i) \cdot P_1(i) \cdot W_d \cdot W_p . \quad (8)$$

To improve the accuracy of determining the cryopreservation efficiency by reducing the total variance this value is represented as the product of the efficiency of the use of cryoprotectant stages $W_t$, freezethaw mode $W_z$ and cultivation $W_k$ *in vitro*:

$$W_{dk} = W_t \cdot W_z \cdot W_k = W_d \cdot W_k . \quad (9)$$

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

$$\begin{aligned} V_d(i) &= V_0(i) \cdot W_t \cdot W_z ; \\ V_{tk}(i) &= V_0(i) \cdot W_t \cdot W_k ; \\ V_k(i) &= V(i) \cdot W_k ; \\ V_{dk}(i) &= V_0(i) \cdot W_d \cdot W_k . \end{aligned} \quad (10)$$

To simplify the calculation procedure of the main biotechnological parameters, the software was developed in Microsoft Excel. A graphical representation of calculated results is shown in Fig. 2–6.

To test the proposed simulation model f(4–10), we analyzed the data about the engraftment of native and deconservated cow embryos of satisfactory, good and excellent quality, which we obtained in the experimental farms of Ukrainka and Ahtyrka [3, 11]. Embryos were frozen in plastic straws ($V = 0.75$ ml) in a device that was developed by us [9].

The experimentally determined values (Fig. 2) of deconservated embryos preservation of satisfactory — excellent quality differ by 58%, it is 34.7 ± 3.2% ($n$ = 331) for satisfactory quality and 92.6 ± 1.5% ($n$ = 324) for good quality. The range of values of deconservated embryos viability is 49% and engraftment is 32%. Regressional relations of indexes of native and deconservated embryos of cows on their quality are as follows: for engraftment and viability, approximating coefficients are $R^2 = 0.999$ and $R^2 = 0.997$, respectively.

Indexes for native and engraftment deconservated embryos: for satisfactory quality — about 20% (21.6 ± 5.9; $n$ = 68 and 19.1 ± 6.2; $n$ = 72), good quality — 40% (40.2 ± 3.1%; $n$ = 81 and 37.8±3.7%; $n$ = 78), excellent quality — 50% (53.2 ± 3.2%; $n$ = 48 и 51.2 ± 3.4%; $n$ = 46), respectively.

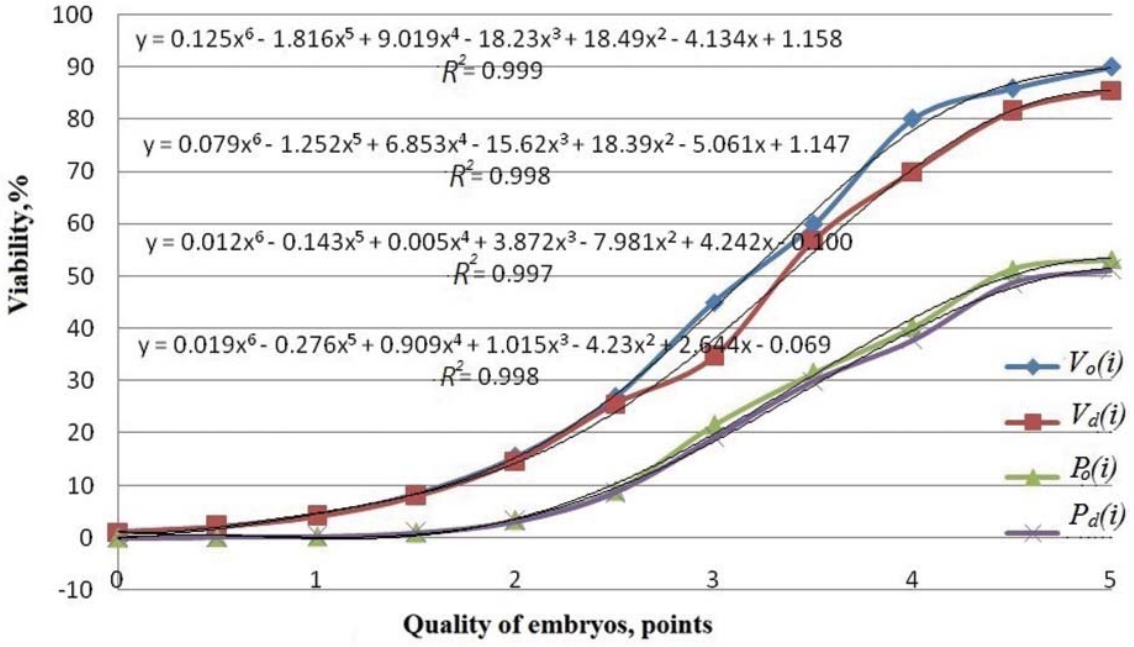


*Fig. 2.* **Dependence of the viability of native $V_o(i)$ and deconservated $V_d(i)$ cow embryos and engraftment $P_o(i)$ and $P_d(i)$ on their quality:**

effectiveness of cryopreservation technology — $W_d = 95\%$, and transplantation — $W_p = 65\%$; coefficients which indicate the status of donors — $r_0 = 1.4$ and recipients — $r_1 = 1.64$; minimum and maximum values of embryo viability — $V_m = 1.1$ and $K = 99$

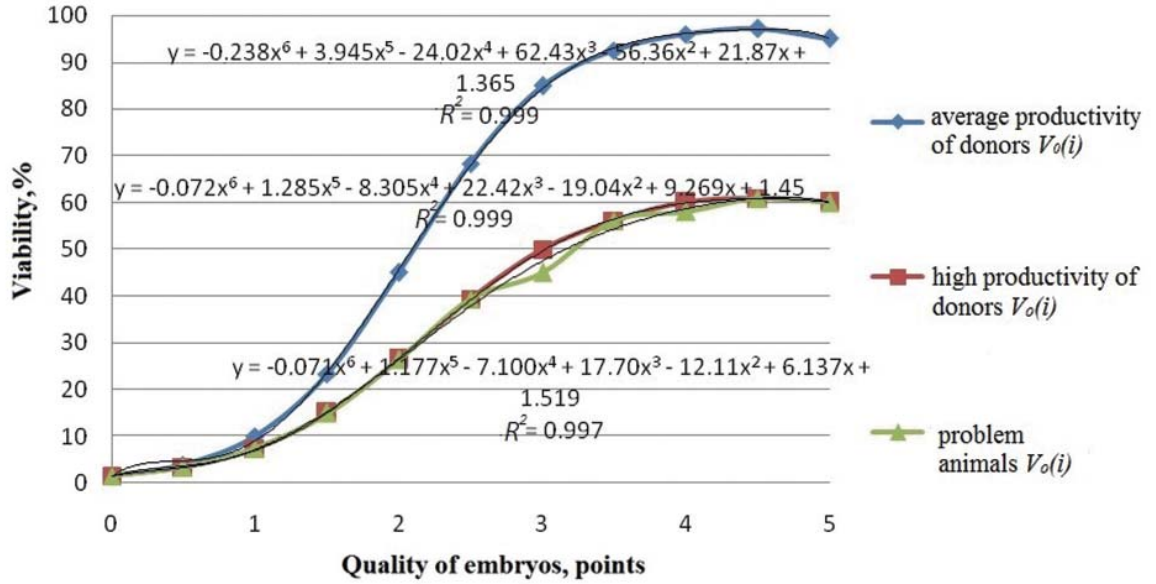


*Fig. 3.* **Dependence of the native cow embryos viability $V_o(i)$ on the quality and condition of the donor:**

efficiency of cultivation technology — $W_p = 99\%$; coefficients that reflect the state of the donor with an average productivity — $r_0 = 2.0$, high productivity — $r_0 = 1.7$, and for problem animals — $r_0 = 1.6$; maximum values of embryo viability for donors with an average productivity — $K = 98$, high productivity — $K = 62$, for problem animals — $K = 60$; the minimum value of embryo viability — $V_m = 1.5$

The discrepancy between the values, which were obtained experimentally and calculated (regression relatios), no more than 5% for embryos of different quality. Index of efficiency of cryopreservation is 95.0 ± 1.0 ($n$ = 328) and of transplantation 65.2 ± 0.5% ($n$ = 321) (Fig. 2). The values of engraftment and viability of embryos, which were calculated based on the analytic dependences f(5–8) in conjunction with the regression one, disperse with the data determined by us experimentally, less than 5%.

The approbation of the proposed simulation model on the data, which is presented in the literature [11, 12], demonstrates high convergence of results of healthy animals with medium and high productivity (Fig. 3).

The results demonstrate that the physiological condition of the donor significantly affects the viability of embryos. Healthy animals ($r$ = 2.0) provide high viability of embryos ($K$ = 98), and problem animals ($r$ = 1.6) — low viability ($K$ = 60) (Fig. 3).

Analysis of the literature data provided an opportunity to calculate the maximum value of the of native cow embryos viability $K$ f(4), obtained from healthy donors with medium and high productivity, and also from problem animals — 98, 62 and 60 respectively. Maximum viability of native cow embryos that used in the experiments — $K$ = 99 (Fig. 2).

We have carried out a comparative analysis f(4–10) of the data from the literature [12] about the complex influence of embryos and their donors quality at cryopreservation technology (Fig. 4). The experimentally obtained and theoretically calculated values differ by no more than 5%.

The value of efficiency embryo of cultivation *in vitro* is 99.0%, cryopreservation using software freezer in 1.4 M glycerol solution is 96.8%; 1 M — 90.2%; 1.5 ethylene glycol — 88.0%; by using the method of vitrification — 82,3%.

Also, for approval of the proposed model, was conducted an experiment on mouse embryos. Embryos were frozen in a device that was developed by us [9]. Experimentally obtained values along with data of mathematical model are in Fig. 5.

The error of model for evaluating status of the native and deconservated embryos was 0.2% because the value of the approximation coefficient is $R$ = 0.998 (Fig. 5, $A$).

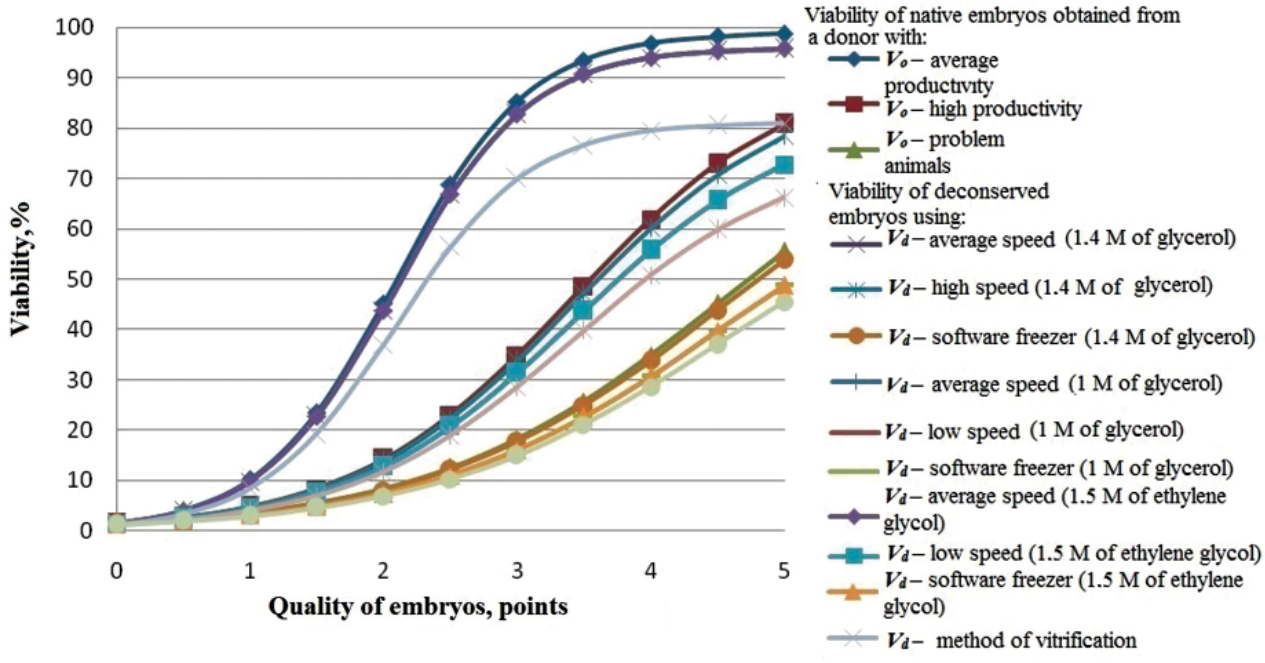


*Fig. 4.* **Dependence of the viability of native ($V_o$) and deconservated ($V_d$) cow embryos on their quality and efficiency of cryopreservation:**
efficiency of embryos cryopreservation technologies at low speed: $W_d$ = 97% at 1.4 M and $W_d$ = 90% at 1.0 M of glycerol; $W_d$ = 88% at 1.5 M of ethylene glycol, and at high speed — $W_d$ = 82%. Coefficients of donor condition: for healthy animals with an average productivity — $r_0$ = 2 and with high productivity — $r_0$ = 1.2, for problem animals — $r_0$ = 0.9; minimum and maximum values of embryo viability — $V_m$ = 1.5 and $K$ = 99 for healthy and $K$ = 92 for problem animals

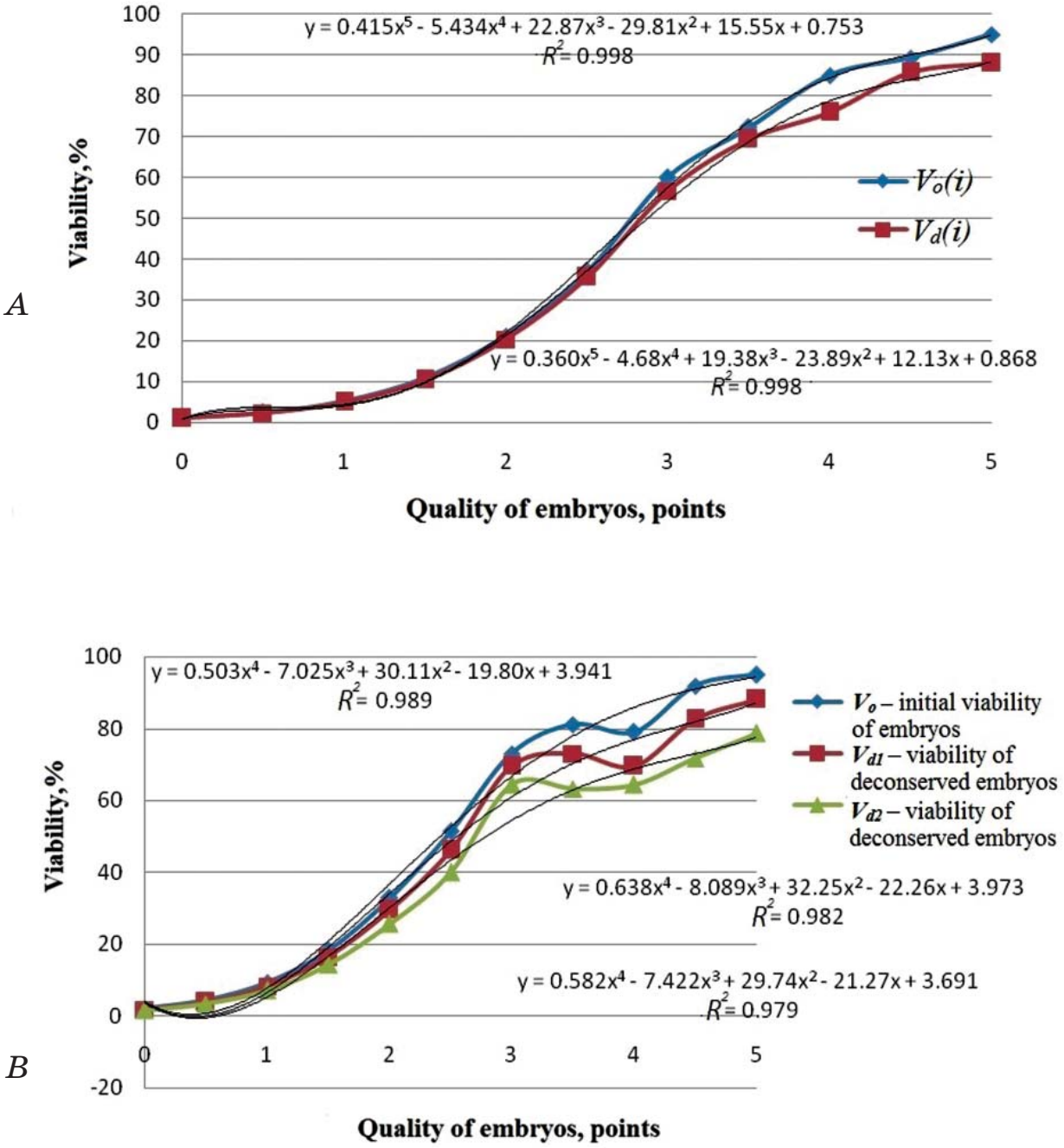


*Fig. 5.* **Dependence of native mouse embryos $V_o(i)$ and deconservated $V_d(i)$ viability on their quality:**
*A* — effectiveness of cryopreservation technology — $W_d = 95\%$; coefficient of donor status — $r_0 = 1.7$; minimum and maximum values of embryo viability — $V_m = 1.5$ and $K = 974$;
*B* — effectiveness of cryopreservation technology in experiment — $W_{d1} = 90\%$ and control — $W_{d2} = 78\%$; coefficient of donors viability — $r_0 = 1.6$; minimum and maximum values of embryo viability — $V_m = 2$ and $K = 95$

**The effectiveness of different methods and stages of mouse embryos cryopreservation $W_{dk}$ in terms of the use of cryoprotectant $W_t$ mode and freeze-thaw mode $W_z$**

| The rate of freezing, °C/min | Amount, pcs | Preservation, $S_{dk}$, % | Viability, $V_{dk}$ | Step effectiveness rates, $M \pm m$, % | | |
|---|---|---|---|---|---|---|
| | | | | Cryopreservation, $W_{dk}$ | Equilibration, $W_t$ | Freezing $W_z$ |
| 0.3 | 18 | 83.3±8.8[a] | 76.9±5.0[a] | 96.0±1.5[a] | 95.4±0.9[a] | 99.6±0.7[a] |
| 0.1÷1* | 62 | 85.5±4.5[a] | 79.6±2.5[a] | 94.7±1.5[a] | | 99.2±0.4[a] |
| $1.68\times10^3$ | 56 | 51.8±6.7[b] | 50.9±2.6[b] | 65.9±1.7[b] | 74.7±1.5[b] | 87.7±0.4[b] |
| $6.24\times10^3$ | 68 | 54.4±6.0[b] | 52.6±2.4[b] | 68.0±1.5[b] | 74.4±1.5[b] | 90.0±1.1[b] |
| $11.8\times10^3$ | 54 | 70.1±6.1[ab] | 64.1±3.0[c] | 82.9±1.2[c] | 88.4±1.6[c] | 93.9±2.0[b] |

*Note:* as a container was used plastic straw;
* — Ulenguta's test tube; *a, b, c* — $P > 0.95$. Control is a value of preservation, viability and effectiveness, by means of which has been compared one technology to another.

Consequently, the effectiveness evaluation error is 0.4% as a result of errors additivity estimates of native and deconservated viability f(6).

For the approbation of proposed model were analyzed the differences between viability of mouse embryos frozen in Ulenguta's test tubes and plastic straws. Mouse embryos were divided into homogeneous groups according to their initial quality: satisfactory, good, excellent. удовлетворительное, хорошее, отличное. Then they were frozen: control — in test tubes, and experiment — in straws. Amount of embryos in each group — from 26 to 40 pcs.

Approximation coefficients were calculated using a regression of depending the viability of mouse embryos on their quality. For native embryos it is $R^2 = 0.989$, deconservated in experiment — $R^2 = 0.982$ and control — $R^2 = 0.979$ (Fig. 5, *B*). Application of the calculated value of cryopreservation effectiveness $W_d$ f(6) variances in experiment and control 12%, error in the determination is 2.9 and 3.2%, respectively.

To determine the efficiency of mouse embryos cryopreservation steps during their low and rapid freezing we analyzed the parameters that determine a method of using a cryoprotectant and freezing mode (Table). As a control were values of preservation, viability and efficiency. By using these values were compared one technology with another. The efectiveness of cryopreservation by using high speed freezing is 65.9 ± 1.7%, at low indicators of cryoprotectant usage — 74.7 ± 1.5% and freezing mode — 87.7 ± 0.4%, that ensures the preservation — 51.8 ± 6.7% ($n = 56$). Increasing the freezing rate 7 times improves the efficiency of cryopreservation to 82.9 ± 1.2%, cryoprotectant — 88.4 ± 1.6%, freeze mode — 93.9 ± 2.0%, that ensures the preservation — 70.1 ± 6.1% ($n = 54$). The biggest loss of viability of embryos observed during the procedure of cryoprotectant saturation-removing, especially using the vitrification methods.

Proposed mathematical model makes it possible to quantify embryos heterogeneity in the evaluation of the effectiveness of different methods of cryopreservation. It promotes a multiple increase of reproducibility and ensure conditions for their comparability. Quantitative accounting of different embryo quality greatly increases the reproducibility of the experimental results, which allows to reduce the number of experiments more than 10 times for obtaining the valid result. Application of proposed viability and efficiency indexes reduces the variation of obtained values up to quantities of the error. Improving the accuracy of embryos quality evaluation according to their morphological indexes up to 0.5 points and differentiation of cryopreservation stages increases the reproducibility of the experimental results.

The proposed simulation model is recommended to use for increasing the

accuracy of the mammalian embryos viability assessing and for accounting of their quality at the various stages of the cryobiological operations. This model combines the regression equation of experimentally obtained values and the analytical expressions f(4–10), which summarize the multidirectional data. Application of quantitative method of embryos viability determination by using video fixation and subsequent image processing allows reducing their number in times in comparison with the conventional visual method of assessing the preservation.

Thus, the developed simulation model describes the dependence of deconserved mammalian embryos viability on their quality at the beginning of the experiment and the efficiency of cryopreservation. The use of the model greatly increases the reproducibility of the results of the study and provides conditions for their compatibility when using different techniques and conditions of bioobject. The use of cryopreservation proposed model allows us to determine the ways of posed problem solutions.

The values of the initial viability of cows embryos are: for an average productivity of the donor — 99%, high — 66%, and for problem animals — 60%. Efficiency of embryos cultivation *in vitro* is 99%, and transplantation (*in vivo*) — 65%. Efficiency of cryopreservation using a software freezer — 97%, the proposed device — 96%.

The most effective method of mouse embryos cryopreservation (96%) based on use of slow freezing at a constant or variable speed. Index of cryoprotectant effectiveness — 95%, freeze mode effectiveness — 99%. Embryo preservation is 83–86% and viability is 77–80%.

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## КОМП'ЮТЕРНЕ МОДЕЛЮВАННЯ ЗАГИБЕЛІ ЕМБРІОНІВ ЗА КРІОКОНСЕРВУВАННЯ

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Мета роботи — визначення закономірностей впливу гетерогенності ембріонів ссавців та ефективності етапів кріоконсервування на їхню життєздатність за допомогою розробленої імітаційної моделі. В основу моделі покладено аналітичний вираз, що відображає основні причини ембріональної смертності у процесі культивування в умовах *in vitro* та *in vivo*, кріоконсервування та трансплантації зародків. Зниження життєздатності залежить від низки біологічних факторів, зокрема виду тварини, стану донора і реципієнта, якості ембріонів, а також від технологічних — ефективності способів кріоконсервування і трансплантації ембріонів.

Здійснений комп'ютерний експеримент показав, що розбіжність життєздатності ембріонів залежно від варіації біологічних параметрів змінюється від 0 до 100%, тоді як показник ефективності обраної технології при цьому має похибку близько 1%. Порівняльний аналіз альтернативних технологій кріоконсервування ембріонів свідчить про максимальну ефективність етапів застосування кріопротектора, режиму заморожування і культивування об'єкта в умовах *in vitro* та *in vivo*. Застосування комп'ютерного моделювання дає змогу знизити у багато разів розкид даних життєздатності ембріонів, отриманих у різних дослідах, і тим самим більш ніж на порядок скоротити час, зменшити грошові витрати та забій лабораторних тварин за одержання достовірного результату.

***Ключові слова:*** кріоконсервація, ембріони, смертність.

## КОМПЬЮТЕРНОЕ МОДЕЛИРОВАНИЕ ГИБЕЛИ ЭМБРИОНОВ ПРИ КРИОКОНСЕРВИРОВАНИИ

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Цель работы — определение закономерностей влияния гетерогенности эмбрионов млекопитающих и эффективности этапов криоконсервирования на их жизнеспособность при помощи разработанной имитационной модели. В основу модели положено аналитическое выражение, отражающее основные причины эмбриональной смертности в процессе культивирования в условиях *in vitro* и *in vivo*, криоконсервирования и трансплантации зародышей. Снижение жизнеспособности зависит от ряда биологических факторов, таких как вид животного, состояние донора и реципиента, качество эмбрионов, а также от технологических — эффективности способов криоконсервирования и трансплантации эмбрионов.

Проведенный компьютерный эксперимент показал, что расхождение жизнеспособности эмбрионов в зависимости от вариации биологических параметров изменяется от 0 до 100%, в то время как показатель эффективности выбранной технологии при этом имеет погрешность около 1%. Сравнительный анализ альтернативных технологий криоконсервирования эмбрионов свидетельствует о максимальной эффективности этапов применения криопротектора, режима замораживания и культивирования объекта в условиях *in vitro* и *in vivo*. Применение компьютерного моделирования дает возможность многократно снизить разброс данных жизнеспособности эмбрионов, полученных в разных опытах, и тем самым более чем на порядок сократить время, уменьшить денежные затраты и забой лабораторных животных при достижении достоверного результата.

***Ключевые слова:*** криоконсервирование, эмбрионы, смертность.