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Original Article

VIABILITY OF FIBROBLASTS OF TUGAI DEER (*CERVUS HANGLU BACTRIANUS* LYDEKKER, 1900) DURING CRYOPRESERVATION BY DIFFERENT METHODS

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ABSTRACT

Currently, there is a rapid and continuous decline in wild animal populations, which threatens accelerated loss of biodiversity on a global scale. Along with *in situ* conservation strategies, *ex situ* conservation measures such as germplasm cryopreservation should be widely applied. Cryopreservation in combination with assisted reproductive technologies can become an important means of successfully preserving the biodiversity of the animal world. Methods based on obtaining cell cultures are of great importance in scientific research. The resulting cell lines can be frozen and stored in containers with liquid nitrogen for many years. Such cell and tissue biobanks represent a viable source of genetic material and living cells that offer numerous opportunities for further research.

The aim of this study was to investigate the scientific basis for cryopreservation of somatic cells of tugai deer, which is a species in need of special protection. Studies were conducted on the viability of tugai deer fibroblasts using various cryopreservation methods. The viability of frozen-thawed tugai deer fibroblasts was determined, and it was shown that the most effective cryopreservation of this species fibroblasts was the use of a cryoprotectant 1.5 M ethylene glycol and a slow equilibrium freezing mode using a programmed freezer.

Key words: tugai deer; fibroblasts; slow freezing; vitrification; biodiversity; germplasm.

INTRODUCTION

The current time is characterized by a rapid and continuous decline in populations of wild mammal species. The ongoing massive decline in numbers and sometimes extinction of animal species at an unprecedented rate is largely caused by human activity. Progressive habitat destruction and fragmentation are leading to accelerated loss of biodiversity globally. Due to the danger of losing valuable animal genotypes, many countries are taking measures to preserve them using conservation methods: both *in situ* (national parks, zoos, farms) and *ex situ* (preservation of genetic material in liquid nitrogen). Although *in situ* conservation methods such as habitat protection are usually a good way to conserve biodiversity, they are not always possible and other conservation strategies such as germplasm cryopreservation are needed. Germplasm cryopreservation involves freezing gametes, embryos, reproductive tissues, somatic cells and tissues of endangered species [1, 2].

The main approach to species conservation is the protection of natural resources, since habitat loss or degradation due to logging and agriculture is the greatest threat to biodiversity at present [3], and *ex situ* breeding by zoological institutions is crucial for species conservation work, which has resulted in good results among vertebrates [4,5]. Recently, new ideas, in particular the use of assisted reproduction technologies (ART), have been incorporated into classical zoo breeding programs. The use of cryopreserved tissues, cells and reproductive materials is of crucial importance, providing the opportunity to preserve genetic diversity and to carry out further molecular genetic research in the field of population genetics. Cells also contain viable cellular organelles and represent a specific source of biological material in addition to storing nuclear genetic information. Therefore, the development of new cell lines obtained from tissue biopsies, blood samples or fibroblast culture in combination with cryopreservation may open a new path for preserving and improving the viability of populations of rare and endangered species [6]. This cellular material can be subjected to stem cell-related techniques, which will produce artificial gamete cultures and thus expand the genetic pool to include, through cryopreservation, even samples from deceased or completely sterile individuals [7,8].

Cryopreservation can be used to preserve various cell cultures for long, potentially indefinite periods of time [9]. In recent years, various methods have been developed for long-term cryopreservation of biological samples, taking into account the main difficulty to be overcome, which is the vulnerability to cryodamage, which depends on the composition of the cell membrane, its permeability to both water and cryoprotectant, the toxicity of the cryoprotectant, tolerance to osmotic changes, and resistance to cooling and freezing [10].

For wild mammals, cell line research aims to facilitate the conservation and reproduction of these species, which are often threatened with extinction. With only one sample collected, various strategies can be developed to disseminate the genetic material of this population, ensuring its future use in improved methods for many years to come [11]. In work with wild animals, scientists' efforts have been directed at adapting research methods developed in work with domestic animals for use with wild species, in particular deer, for which there is relatively little work on cryopreservation of cells and tissues. Interesting work has been done with deer antlers, the only fully regenerating organ found in mammals, as a potential model for bone growth and development. In order to elucidate the processes of antler growth regulation, *in vitro* cultures were created in which the influence of various factors on the proliferative potential of mixed *in vitro* antler cell cultures obtained from regenerating antlers of red deer males (*Cervus*

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elaphus) were studied [12]. Other studies have investigated the viability and cryopreservation effect on brown deer (*Mazama gouazoubira*) fibroblasts after multiple passages. For this purpose, fibroblast cells were cultured and cryopreserved in cryotubes. Cell viability, functionality, and percentage of cells undergoing necrosis and apoptosis were assessed. Viability rates were always above 80% regardless of the group tested, demonstrating low metabolic activity and a higher percentage of cells in early apoptosis [13]. In order to find the most effective cryoprotectant (CPA) in a suitable concentration for cryopreservation of red deer epididymal spermatozoa, the effect of three most commonly used CPAs, glycerol (G), ethylene glycol (EG) and propylene glycol (PG), on sperm cryoresistance was assessed. The results showed that 12% of any CPA was toxic to the membrane integrity of red deer epididymal spermatozoa ($P < 0.05$). Moreover, regardless of the CPA level, the results showed that the cryoprotective effect of three CPA on red deer epididymal spermatozoa after thawing was in the following sequence: G > EG > PG (higher symbols indicate $P < 0.001$) [14].

Cryopreservation is an essential method for preserving animal biodiversity. When combined with assisted reproduction technology, it can become an integral part of successful species conservation management. Recent advances in stem cell technology may allow these cells to be reprogrammed into gametes, eventually resulting in entirely new individuals conceived from a small skin biopsy. At the moment, we will not be able to prevent the extinction of many populations; but by storing cells from as many species as possible in biobanks, we may have a chance of bringing them back in the future until the methodology and technology that will allow species to return to normal existence is available [15]. Cryobanks of endangered animal species represent an extremely valuable backup copy of natural biodiversity. The original genetic material can be preserved without removing genetically valuable individuals from the wild and reducing the interval between generations [16].

Thus, the creation of cell and tissue biobanks is an important practical approach to preserving the biodiversity of the animal world and the aim of this study was to investigate the scientific basis for cryopreservation of somatic cells of a rare representative of wild fauna, the tugai deer. For this purpose, an assessment of the viability of fibroblasts during cryopreservation using various freezing methods was carried out, which will undoubtedly contribute to the preservation of this species by preserving the germplasm in the created biobank.

The tugai deer (*Cervus hanglu bactrianus* Lydekker, 1900) inhabits the floodplain forests (tugai) of Central Asia, also known as the Bukhara deer, is a rare subspecies of the Tarim Red Deer (*Cervus hanglu* Wagner, 1844) [17]. The ecological significance of the tugai deer is difficult to overestimate. As a large herbivorous species, it plays a key role in maintaining the structure and dynamics of floodplain ecosystems, regulating the growth of grass and shrub vegetation, and is also an important link in trophic chains. Today, the tugai deer is under special protection and listed in the Red Book of the Republic of Kazakhstan (category I - endangered), in the Red Book of the International Union for Conservation of Nature (IUCN) (category I) and in Appendix 2 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), its population is estimated at 1000 individuals, while reintroduced populations are isolated from each other and vulnerable to genetic erosion.

Currently, work on reintroducing tugai deer to historical habitats in Kazakhstan is being carried out within the framework of the Tiger Reintroduction Program (2017), implemented with the support of WWF and government agencies. Successful implementation of such events requires comprehensive scientific support, including studying the biology of the species, monitoring populations and using modern biotechnologies, such as cryopreservation of genetic material. The creation of biobanks of tugai deer somatic cells opens up new prospects for preserving its gene pool. Cryopreserved cells can be used in the future for artificial insemination, cloning or other assisted reproduction methods, which is especially important for species with limited numbers and fragmented populations. Thus, the integration of *in situ* and *ex situ* conservation measures using modern technologies is the key to the long-term conservation of tugai deer in Kazakhstan and Central Asia.

Therefore, this study evaluated the effect of different freezing methods on the viability of tugai deer fibroblasts, as the development of optimal cryopreservation methods for preserving the germoplasm of this species is a very urgent task in order to preserve and reproduce their gene pool.

MATERIALS AND METHODS

Objects of research: samples of skin explants for isolation and in vitro culture of somatic cells (fibroblasts), somatic cells (fibroblasts) of tugai deer (*Cervus hanglu bactrianus* Lydekker, 1900). Skin explant samples from 24 individuals were collected during the implementation of the project on reintroduction of the tugai or Bukhara deer (*Cervus hanglu bactrianus* Lydekker, 1900) into the Ile-Balkhash State Nature Reserve (SNR) (Southern Balkhash region) within the framework of the program «Reintroduction of the tiger to the Republic of Kazakhstan» (WWF001733) on the territory of the Karachingil hunting farm (Almaty region).

The study was approved by the Local ethical committee of the RSE at REM «Institute of Zoology « CS MSHE of the Republic of Kazakhstan (Protocol No. 1 of February 13, 2025).

Samples were collected from live immobilized individuals by remote injection using syringes-injectors. The immobilization of deer was carried out using a combination of drugs Medetomidine/Teletamin+Zolazepam, the dosage of which was selected individually for each individual depending on its weight and age. The biomaterial is represented by skin explants measuring 10 mm x 10 mm. Skin samples were collected from the inner surface of the animals' auricles after shaving and treating the collection site with a disinfectant (ethyl alcohol 96%).

Isolation of Fibroblasts from Skin Explants

To isolate fibroblasts, skin samples obtained from the auricles of captured and immobilized deer were used. After collecting and treating the samples with ethyl alcohol, the samples were placed in containers with a nutrient medium containing 10% fetal bovine serum and an antibiotic solution at a concentration of 100 U/ml for transportation. In laboratory conditions in a laminar flow hood, the samples were

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washed twice in phosphate-buffered saline (DPBS, Sigma). The samples were then cut into pieces of approximately 1 mm^3 in a small amount of growth medium. For subsequent *in vitro* culture of fibroblasts, DMEM nutrient medium (Sigma, USA) containing fetal bovine serum (Sigma, USA), L-glutamine (Sigma, USA), and an antibiotic solution (Sigma, USA) was used. The tissue pieces were placed on the bottom of plastic culture flasks with a surface area of 25 cm^2 and covered with culture medium. The explants were cultured in a CO_2 incubator at 37°C, 5% CO_2 and 85% humidity without touching or moving the culture flask. The migration of individual fibroblasts from the explant and the subsequent appearance of the growth zone is recorded using an inverted microscope and a digital camera.

Cell Culture and Passage

Cells were cultured in complete culture medium (CCM) with 10% fetal bovine se-rum (FBS) until a monolayer was achieved in 25 cm^2 culture flasks, replacing half of the culture medium with fresh medium every 5 days. The release of cells from skin samples and their formation of a monolayer was considered as the first passage. At this stage, the cells were transferred to new culture flasks. Cell dissociation and monolayer detachment from the surface were performed using a warm 0.25% trypsin solution. For this purpose, the nutrient medium was completely removed from the culture flask, the cell monolayer was washed with Dulbecco's phosphate-saline solution without calcium and magnesium in a volume of several ml, and a layer of 0.25% trypsin solution was applied in a volume of 5-7 ml. After one-minute incubation at room temperature, the trypsin solution was removed from the surface of the bottom of the culture flask, leaving a thin layer, and then the flask was transferred to a thermostat at 37°C and incubated for fifteen minutes with periodic shaking. Further, 10 ml of CCM containing 10% FBS to block the action of trypsin were added to the flask and gently pipetted. The cells separated from the surface were collected in a test tube and washed by centrifugation in 10 ml of CCM. The resulting fibroblast pellet was resuspended in fresh CCM, the number of living cells was counted, transferred to two new culture flasks and continued to be cultured (second passage) or the cells were frozen.

Cell Viability Assessment

Cell viability was assessed using the trypan blue dye exclusion method. The cell suspension was mixed with 0.4% trypan blue solution and introduced into a hemocytometer (Makler chamber). Stained (dead) and unstained (live) cells were counted separately, and the % of live cells was calculated.

Slow Freezing of Fibroblasts in Liquid Nitrogen Vapor (Slow Freezing, SF)

Fibroblasts were frozen using 0.5 ml straws (CBS, Cryo-BioSystem). For this, the cell suspension was centrifuged for 10 min at 300 g. The cell pellet was resuspended in a cryo-medium containing various cryoprotectants at a rate of 1-10 x 10^6 cells/ml of cryo-medium, filled into 0.5 ml straws, which were placed in a freezer chamber at 70°C for equilibration. Then, after 24 hours, the straws with the cells were transferred to a Dewar with liquid nitrogen. Various cryoprotectants were used for freezing: 10% dimethyl sulfoxide (DMSO) and 10% ethylene glycol (EG).

Thawing Cells After Freezing

The cells were thawed in a standard manner. The straw with cells was transferred from the Dewar flask to a water bath with a temperature of 37°C, after being kept at room temperature to evaporate the remaining liquid nitrogen from it. After complete thawing, the cell suspension was washed from DMSO and fetal serum in a 20-fold excess of DMEM medium by centrifugation at 160 g for 10 min. The cells were then re-sus-pended in a complete culture medium (CCM) containing 10% FCS, 2 mM L-glutamine, and antibiotics. After this, cell viability was determined by trypan blue exclusion and subjected to further cultivation.

Slow Freezing Programmable (SFP) of Fibroblasts (KryoPlaner)

For equilibrated cryopreservation of cells, the slow freezing method was used with a Planer Kryo 330 – 3.3 programmed freezer (Planer, UK). Fibroblasts were frozen in two different cryoprotectants: 1.5 M DMSO (Sigma, USA) and 1.5 M ethylene glycol (Sigma, USA), prepared in DPBS. When diluting with a cryoprotectant, a cell concentration of 2 x 10^6 cells/ml was maintained. For cryopreservation and subsequent storage, 0.5 ml straws (CryoBioSystem, France) were filled with samples containing a cryoprotectant, and the end of the straw was sealed with a sealer (MiniTube, Germany). The samples were then placed in a refrigerator and kept for two hours at a temperature of 5ºC. This procedure is necessary for equilibration and stabilization of cells in the cryoprotectant solution. Then freezing was carried out in a programmed freezer. The following mode was used for freezing: from 5ºC to -40ºC at a rate of -1ºC/min, from -40ºC to -85ºC at a rate of -4ºC/min, and then the straws were transferred to liquid nitrogen for storage. Thawing of the samples was carried out in a water bath at a temperature of 37ºC for 5 min.

Low-Temperature Storage of Fibroblasts

After the freezing process was completed, the straws with fibroblasts were transferred from the freezer chamber to a heat-insulated container filled with liquid nitrogen. The required number of containers were also placed there for preliminary cooling. The straws with fibroblasts were quickly (2-3 seconds) transferred into the cooled container, trying to keep the straw outside the liquid nitrogen as little as possible. The straws were stored in special containers (visotubes), which were placed in a cylindrical container (goblet). The goblet with the straws was placed in a canister of a Dewar vessel. The labeled containers were quickly transferred to a cryostorage filled with liquid nitrogen, placed in canisters and stored until use.

Statistical Data Processing

The ANOVA application program was used for statistical processing. The arithmetic mean, the standard error of the arithmetic mean, and the reliability of the difference in means P according to the Student's criterion (TTEST) were calculated.

RESULTS

Obtaining, *In Vitro* Culture and Passage of Fibroblasts

Methods for collecting biological material (skin explants)

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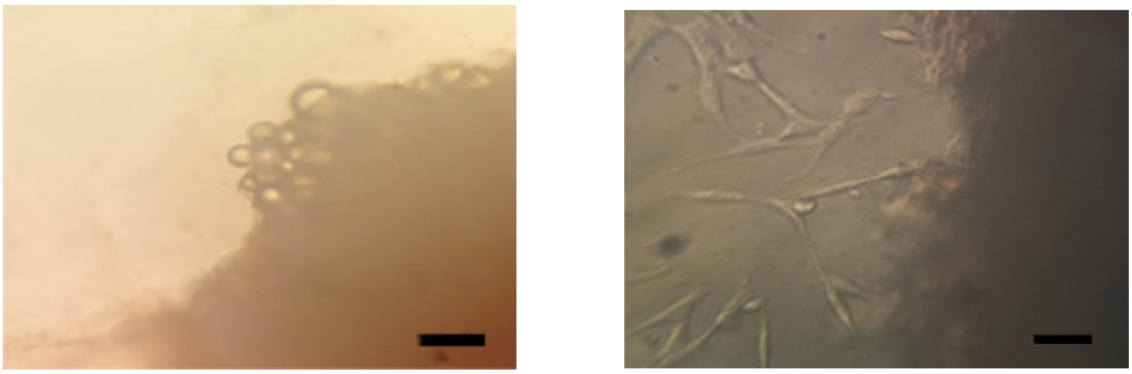
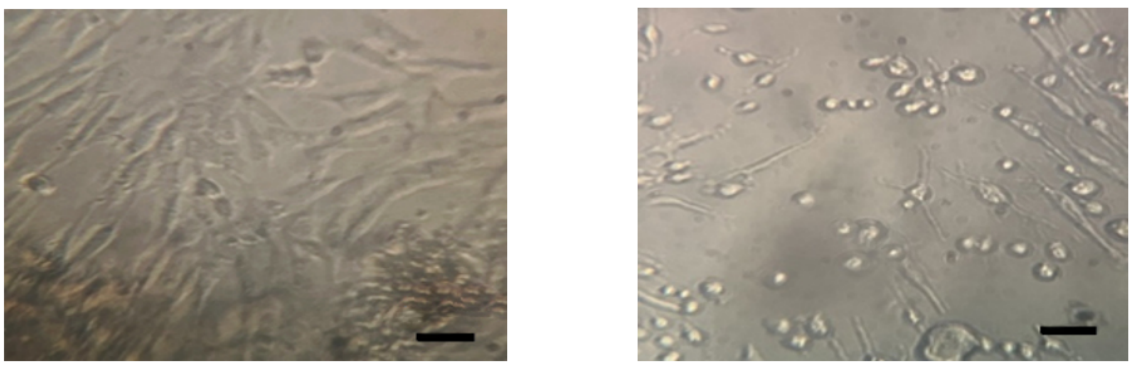


a b

a - explant adhesion and initial cell yield;
b - yield and proliferation of individual cells from explant tissue
Figure 1. - Yield of individual fibroblasts from tissue sample. Scale bar 50 µm.



a b

a - fibroblast monolayer; b - suspension of cells disaggregated with trypsin
Figure 2. - Formation of a fibroblast monolayer and its disaggregation with trypsin. Scale bar 50 µm.

and methods for *in vitro* culture of fibroblasts from tugai deer skin explants were developed. The cell culture reached the required confluence, after which subsequent series of passages were carried out. Skin samples obtained from the deer ear were used to isolate fibroblasts. In laboratory conditions, the fragment was first cleaned by removing hair and fatty tissue. This process is very important, as it is necessary to prevent bacterial and fungal contamination of cells during cultivation. After the tissue cleaning step, it was fragmented to obtain a larger yield of cells. This step was carried out by mechanically dividing the tissue into 1 mm^3 pieces using scissors and scalpels while placing the explant directly on the dish. The tissues were cultured in vitro after collection, processing and fragmentation. All fragments showed adhesion to the bottom of the culture dish between the 4th and 6th day of cultivation. The release of fibroblasts from the explants and cell proliferation were observed by microscopy. Observation of the release of cells from the explant tissue showed that the release of individual cells from the explant tissue (Figure 1a, b) began no earlier than the 9th day of cultivation (on average, on the 9th-10th day after seeding) and was accompanied by cell division until a monolayer was reached. Morphologically, the cells had a spindle-shaped form and a central oval nucleus.

The monolayer occupied approximately 1/3 of the surface of the 25 cm^2 culture flask already after 14-15 days from the release of fibroblasts from the explant. Thus, cell growth around the explants occurred within 6-7 days, and cell subconfluency was observed after 15 days (Figure 2a). Fibroblast passages were performed to increase the cell mass, as well as to obtain a monoculture. Since fibroblasts are adherent cells, proteolytic enzymes are most often used to detach them from the surface of the culture dish, the main one being trypsin. In our studies, the use of this enzyme alone proved to be effective and sufficient for the almost complete removal of the fibroblast monolayer from the surface of the culture dish. The optimal incubation time with trypsin was 7-10 min at 37°C, at which time the cells were detached from the bottom of the culture flasks and went into suspension (Figure 2b). To prevent further action of the enzyme, 5-10 ml of 10% CCM were added.

The disaggregated cell suspension was centrifuged, the pellet was resuspended in culture medium and transferred to new culture flasks, diluting with fresh culture medium. Cell counting after dissociation with trypsin showed that the percentage of viable cells was 89% (Table 1). After the next passage, performed after the cell culture had reached the state of confluence, the cell culture became more homogeneous. Thus, if during the second passage the fibroblast culture could be contaminated with epithelial cells, then after the third passage we obtained a practically pure monoculture (Figure 3a). *In vitro* culture of frozen-thawed fibroblasts was performed to identify the most optimal cryopreservation method, as well as

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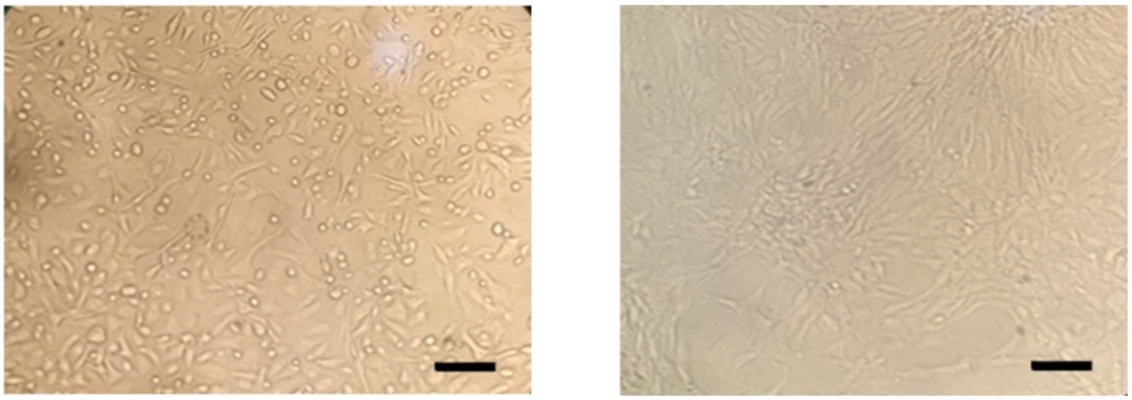

a b

a - pure monoculture of fibroblasts; b - fibroblast monolayer

Figure 3. - Fibroblast monoculture after 3 passages and after frozen-thawed cell culture. Scale bar 30 μm.

Table 1. Viability of tugai deer fibroblasts before and after cryopreservation by different methods.

Cryopreservation regimen	Cells viability, %
SF/DMSO	33,9 ± 4,1
SF/EG	34,2 ± 3,6
SPF/DMSO	37,4 ± 3,5
SPF/EG	41,8 ± 5,1
Viability cells before frozen (Control)	89,1 ± 6,7

to obtain a monoculture. The frozen-thawed cell suspension was transferred to new culture vessels, diluting with fresh culture medium by two times. The rate of cell growth increased somewhat after the next passage, which was carried out after the cell culture had reached a state of confluence. The cell culture had the appearance of classic fibroblasts without admixture of epithelial cells and dead cells (Figure 3b).

Then, studies were conducted on the cell viability rate using different cryopreservation methods for *ex situ* preservation of one of the rare and endangered animal species, the tugai deer. Slow freezing is the main procedure used for cryopreservation, which allows maintaining a fairly high level of cell viability.

Fibroblast Viability After Slow Freezing (Unequilibrated and Equilibrated Cryopreservation)

Slow freezing of deer fibroblasts was carried out using unequilibrated and equilibrated cryopreservation methods. When studying the effect of unequilibrated cryopreservation, the simplest and most common method was used. The results of the evaluation of the deer fibroblasts viability rate subjected to cryopreservation after the third passage are presented in Table 1 and also displayed in the diagram (Figure 4). According to the results of the study, the percentage of living cells was slightly higher when using the cryoprotectant EG, with an average value of 34.2 ± 3.6%, with an initial value of 89.1 ± 6.7%. In the course of the conducted studies on the effect of equilibrated cryopreservation using a programmed freezer, it was revealed that the most effective cryoprotectant for cryopreservation of deer fibroblasts is 1.5 M ethylene glycol, at which the cell viability rate was 41.8 ± 5%, with an initial

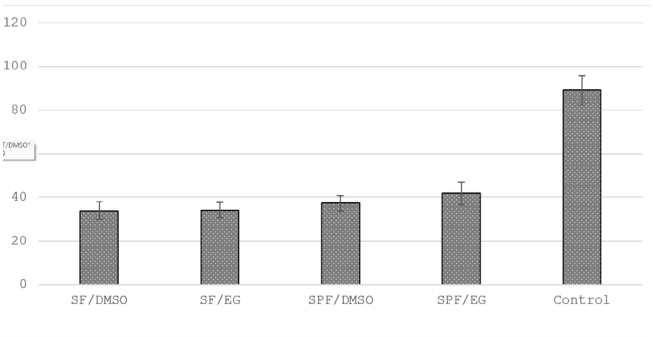

Figure 4. - Viability of tugai deer fibroblasts before and after cryopreservation by different methods.

level of 89.1 ± 6.7%. (Table 1, Figure 4).

As can be seen from the above table (Table 1) and diagram (Figure 4), the most optimal method for cryopreservation of tugai deer fibroblasts is equilibrated cryopreservation using cryoprotectant 1.5M ethylene glycol. An important result of this work was the creation of a biobank of tugai deer somatic cells at the Institute of Zoology to preserve the biodiversity of wild animals. Frozen cell samples from 24 captured animals were placed for long-term storage at -196O C to preserve genetic material and further use for scientific and applied purposes.

DISCUSSION

The successful creation and replenishment of a bank of biological resources depends on the efficiency of the cryopreservation method used. The viability of cells during freezing is influenced by several factors, the main ones being: cryoprotectant, freezing rate and thawing rate. All three factors are aimed at limiting the destructive effect of ice microcrystals on the integrity of cell cytoplasmic membranes and cell viability. The efficiency of these procedures is determined by the quality of cells after freezing, which, in turn, is determined by the choice of cryoprotectant, which is a key step for the success of biobanks that allow long-term storage of tissues and somatic cells for various purposes [18]. In general, an effective cryoprotector is a combination of intracellular cryoprotectants such as dimethyl sulfoxide (DMSO) [19], ethylene glycol (EG) [20], and extracellular cryoprotectants such as sucrose (SUC) [21] and/or fetal bovine serum (FBS) [22].

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In general, cryopreservation is one of the modern directions of preserving the genetic diversity of animals, both domestic and wild [23]. Whereas cell cryopreservation methodologies are applicable to tissues, adaptation of protocols is required to work with them [24] due to the structural complexity of many cell types, resulting in variations in water permeability across tissue types [25]. Therefore, there is a need to develop species-specific cryopreservation protocols that can be improved by altering the composition and concentration of cryoprotectants [26]. Thus, there was a need to study the effect of various cryoprotectants on somatic cells of tugai deer, since a protocol for their cryopreservation has not yet been developed.

Based on this, in this work the viability of tugai deer fibroblasts and the efficiency of their cryopreservation using different freezing methods were investigated. For this purpose, biological material (skin explants) was collected, methods of *in vitro* culture of fibroblast cells were developed, and upon achieving the necessary confluence, subsequent series of passages were performed, then fibroblasts were cryopreserved using various methods with their efficiency being evaluated. Skin samples obtained from deer auricles were used for fibroblast isolation.

Passaging performed after the cell culture reached a state of confluence resulted in a more homogeneous cell culture, and *in vitro* culture of frozen-thawed fibroblasts showed no differences compared to culture of freshly obtained fibroblasts. The results showed that the most optimal method for tugai deer fibroblasts cryopreservation was equilibrated cryopreservation using 1.5M ethylene glycol cryoprotectant.

CONCLUSION

Thus, the results of the studies suggest that the applied methods of slow cryopreservation showed acceptable results in cryopreservation of tugai deer fibroblasts with a good level of viability of the preserved cells. An important result of the work is the replenishment of the Institute of Zoology biobank with somatic cell samples from 24 individuals of tugai deer.

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ЖИЗНЕСПОСОБНОСТЬ ФИБРОБЛАСТОВ ТУГАЙНОГО ОЛЕНЯ (*CERVUS HANGLU BACTRIANUS* LYDEKKER, 1900) ПРИ КРИОКОНСЕРВАЦИИ РАЗНЫМИ МЕТОДАМИ

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АННОТАЦИЯ

В настоящее время наблюдается быстрый и непрерывный спад численности популяций диких животных, что угрожает ускоренной утратой биоразнообразия в глобальном масштабе. Наряду со стратегиями сохранения *in situ*, должны широко применяться меры *ex situ*, такие как криоконсервация генетического материала. Криоконсервация в сочетании с вспомогательными репродуктивными технологиями может стать важным инструментом успешного сохранения биоразнообразия животного мира. Методы, основанные на получении культур клеток, имеют большое значение в научных исследованиях. Полученные клеточные линии можно замораживать и хранить в контейнерах с жидким азотом многие годы. Такие банки клеток и тканей являются жизнеспособным источником генетического материала и живых клеток, открывающим многочисленные возможности для дальнейших исследований. Целью данного исследования было изучение научных основ криоконсервации соматических клеток тугайского оленя — вида, нуждающегося в особой охране. Были проведены исследования по жизнеспособности фибробластов тугайского оленя при использовании различных методов криоконсервации. Установлено, что наиболее эффективным методом криоконсервации фибробластов данного вида является применение криопротектора 1,5 М этиленгликоля и режима медленного равновесного замораживания с использованием программируемого морозильника.

Ключевые слова: тугайный олень, фибробласты, медленное замораживание, витрификация, биоразнообразие, гермоплазма

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ТҰҒАЙ БҰҒЫСЫНЫҢ (*CERVUS HANGLU BACTRIANUS* LYDEKKER, 1900) ФИБРОБЛАСТТАРЫНЫҢ ӘРТҮРЛІ КРИОКОНСЕРВАЦИЯ ӘДІСТЕРІНДЕГІ ӨМІРШЕҢДІГІ

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АҢДАТПА

Қазіргі уақытта жабайы жануарлар популяциясының жылдам және үздіксіз азаюы байқалуда, бұл ғаламдық ауқымда биоалуантүрліліктің жедел жойылу қаупін тудырады. *In situ* сақтау стратегияларымен қатар, генетикалық материалды криоконсервациялау сияқты *ex situ* шаралары да кеңінен қолданылуы тиіс. Криоконсервация көмекші репродуктивтік технологиялармен бірге жануарлар әлемінің биоалуантүрлілігін табысты сақтаудың маңызды құралы бола алады. Жасуша дақылдарын алу әдістері ғылыми зерттеулерде үлкен маңызға ие. Алынған жасушалық желілерді сұйық азоттағы контейнерлерде көптеген жылдар бойы мұздатып сақтауға болады. Мұндай жасуша және тін банктері генетикалық материалдың және тірі жасушалардың өміршең көзі болып табылады және әрі қарайғы зерттеулер үшін көптеген мүмкіндіктер ашады. Осы зерттеудің мақсаты – ерекше қорғауды қажет ететін тұғай бұғысының соматикалық жасушаларын криоконсервациялаудың ғылыми негіздерін зерттеу. Әртүрлі криоконсервация әдістерін қолдана отырып, тұғай бұғысының фибробласттарының өміршеңдігіне зерттеу жүргізілді. Зерттеу нәтижесінде бұл түрдің фибробласттарын криоконсервациялаудың ең тиімді тәсілі – 1,5 М этиленгликоль криопротекторын және бағдарламаланатын тоңазытқышты пайдалана отырып, баяу тепе-теңдікті мұздату режимін қолдану екені көрсетілді.

Негізгі сөздер: тоғай бұғы, фибробласттар, баяу мұздату, витрификация, биоәртүрлілік, гермоплазма