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CRYOPRESERVATION OF SAIGA (*SAIGA TATARICA*) SOMATIC CELLS: EFFECT OF NON-EQUILIBRIUM AND EQUILIBRIUM FREEZING ON FIBROBLAST VIABILITY

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ABSTRACT

Background. The saiga antelope (*Saiga tatarica*) has recovered numerically in Kazakhstan, but its populations remain exposed to mass mortality events that can remove a large share of a population within weeks. Viable somatic cell banking can insure genetic diversity, yet no cryopreservation protocol had been reported for saiga cells. **Aim.** To establish primary fibroblast cultures from saiga ear skin and to compare post-thaw survival after passive and controlled-rate slow freezing. **Methods.** Ear skin biopsies were collected from 50 adult saigas of the Betpak-Dala and Ustyurt populations. Fibroblasts obtained by explant culture were frozen at passage 2 either passively in cryovials with 10% dimethyl sulfoxide (DMSO; ≈ -1 °C/min to -70 °C) or in 0.5 mL straws with 1.5 M DMSO or 1.5 M ethylene glycol (EG) after 2 h equilibration at 5 °C, using a programmable freezer. Viability was assessed by trypan blue exclusion. **Results.** Cell outgrowth began on days 6–10 of culture. Passive freezing reduced viability from 97.6% to 44.5% ($p < 0.001$), with high inter-individual variability (9–77%; CV 30%). Controlled-rate freezing yielded $52.8 \pm 1.1\%$ viable cells with DMSO and $62.1 \pm 1.0\%$ with EG versus $85.8 \pm 0.8\%$ in fresh controls ($p < 0.001$); EG exceeded DMSO by 9.3 percentage points and gave 27% more viable cells per straw. Both controlled-rate treatments outperformed passive freezing and showed 2.9–3.5-fold lower variance. **Conclusions.** Controlled-rate slow freezing with 1.5 M EG was the most effective protocol and was adopted for the first somatic cell cryobank of the saiga in Kazakhstan.

Keywords: saiga antelope; *Saiga tatarica*; fibroblasts; cryopreservation; ethylene glycol; dimethyl sulfoxide; biobank; wildlife conservation

1. INTRODUCTION

The saiga antelope (*Saiga tatarica* Linnaeus, 1766) is a migratory ungulate of the Eurasian steppes and semi-deserts, and Kazakhstan holds the great majority of its world population in three main groupings – the Betpak-Dala, Ustyurt and Ural populations. During the 1990s uncontrolled poaching caused one of the fastest population collapses recorded for a large mammal [1]. In addition, saigas are uniquely prone to mass mortality events: in May 2015 more than 200,000 animals of the Betpak-Dala population died within three weeks from haemorrhagic septicaemia caused by *Pasteurella multocida* serotype B, apparently triggered by abnormally high temperature and humidity [2, 3]. Similar die-offs occurred in 1988, 2010–2012, and the causative strains appear to be stable commensals that become pathogenic under specific environmental conditions [4, 5].

Owing to strict protection, the saiga has recovered spectacularly: in 2023 the species was reclassified from Critically Endangered to Near Threatened on the IUCN Red List [6], and the spring 2026 aerial survey estimated more than 4.6 million animals in Kazakhstan [7]. However, numerical recovery does not remove the risk of sudden losses, which may intensify under climate change [2]. Because each die-off can remove a large share of a population within days, and genetic diversity lost during a bottleneck is not restored by population growth alone, long-term preservation of the species' genetic resources remains a conservation priority.

Genome resource banks – collections of cryopreserved gametes, embryos, tissues and viable cell cultures – are now considered an integral part of integrated *in situ* and *ex situ* conservation [8]. Among biobanked materials, skin fibroblasts

are the most accessible: they can be obtained from a small ear biopsy taken during capture for marking or collaring, or even post mortem, proliferate readily *in vitro*, and can be used for karyotyping, genomic and physiological research, derivation of induced pluripotent stem cells and somatic cell nuclear transfer [8, 9, 10]. The value of such a collection, however, depends directly on the efficiency and reproducibility of the cryopreservation protocol, and on its ethical and practical sustainability [11].

Cell survival during freezing is governed by two opposing mechanisms: intracellular ice formation at high cooling rates and “solution effects” (osmotic and solute injury) at low cooling rates [12]. Permeating cryoprotective agents (CPAs) such as DMSO, glycerol or EG reduce the amount of ice formed at any temperature, but are themselves toxic and osmotically active [13, 14]. Two practical approaches are used for slow freezing of cells: passive cooling in an insulated container placed in a $-70/-80$ °C freezer, which gives an approximate and uncontrolled cooling rate of about -1 °C/min, and controlled-rate freezing in a programmable freezer, which allows a pre-defined equilibration step and multi-stage cooling [13, 14]. DMSO (typically 10%) remains the standard CPA for cultured cells, although its toxicity has stimulated a search for alternatives [15]. For wild mammals, the optimal CPA appears to be species-specific: EG outperformed DMSO for cryopreserved skin tissue of an endangered deer [16], whereas no difference was found for puma cells [17], and reduced CPA concentrations were advantageous for manatee cells [18]. In canine fibroblasts frozen with 1.2 M EG or 1.2 M DMSO, membrane integrity 24 h after thawing was 55–75% [19].

To our knowledge, no data on isolation, culture and cryo-

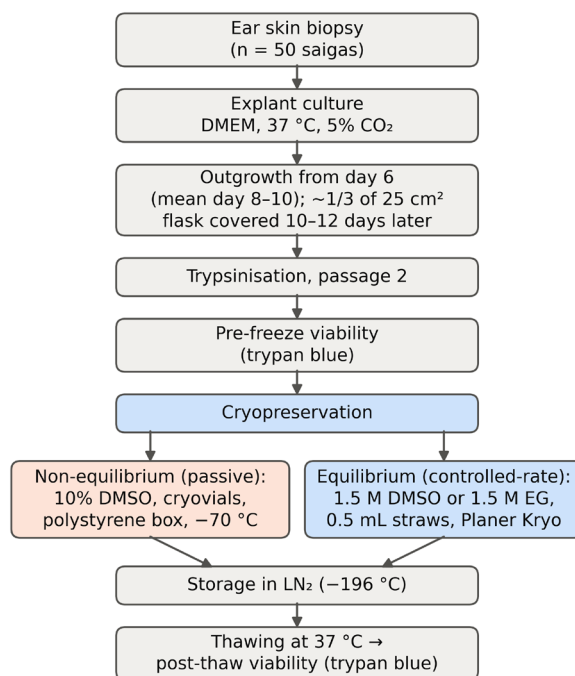


Figure 1. Experimental design. Fibroblasts derived from ear skin explants were frozen at passage 2 by passive (non-equilibrium) or controlled-rate (equilibrium) slow freezing and assessed by trypan blue exclusion after thawing.

preservation of saiga somatic cells have been published, either in Kazakhstan or elsewhere. The aims of this study were therefore (i) to establish primary fibroblast cultures from ear skin of saigas of the Betpak-Dala and Ustyurt populations; (ii) to evaluate the post-thaw survival of saiga fibroblasts after passive slow freezing with DMSO; (iii) to compare DMSO and EG under controlled-rate slow freezing with pre-equilibration; and (iv) to use the most effective protocol to establish a somatic cell cryobank of the species.

2. MATERIALS AND METHODS

2.1. Animals and sampling

Skin samples were obtained from the ear pinnae of 50 adult saigas of the Betpak-Dala and Ustyurt populations during capture for satellite collaring/markings. After shaving and disinfection with 70% ethanol, a skin fragment of approximately 5 × 5 mm was excised and transported to the laboratory in phosphate-buffered saline (PBS) with antibiotics at 20–25 °C within 72 h. All procedures were approved by the Ethics Committee of the Institute of Genetics and Physiology, Ministry of Science and Higher Education of the Republic of Kazakhstan (protocol No. 4, 27 November 2023). The overall design of the study is shown in Figure 1.

2.2. Isolation and culture of fibroblasts

The samples were washed several times in PBS, cut into fragments of about 1 mm³ and placed in 25 cm² plastic culture flasks with culture medium (explant method [20]). Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and antibiotics was used. Explants were cultured at 37 °C in a humidified atmosphere (85% humidity) with 5% CO₂; half of the medium was replaced every three days. Outgrowth of cells from explants and their proliferation were monitored daily under an inverted microscope.

2.3. Passaging

On reaching confluence, the monolayer was detached with 0.25% trypsin solution for 7–10 min at 37 °C. Trypsin was inactivated by adding 5–20 mL of medium containing 10% FBS, and the cell suspension was transferred to new flasks at a 1:2 split ratio. Cells at passage 2 were used for cryopreservation.

2.4. Passive slow freezing (non-equilibrium protocol)

The cell suspension was centrifuged at 160 × g for 10 min, and the pellet was resuspended in FBS containing 10% (v/v) DMSO (≈ 1.4 M; Sigma, USA) at 1–10 × 10⁶ cells/mL. The suspension was dispensed into cryovials, which were placed in the wells of a polystyrene foam block (wall thickness 1 cm) and transferred to a –70 °C freezer, giving a nominal cooling rate of about –1 °C/min. After 24 h the cryovials were plunged into liquid nitrogen (LN₂, –196 °C).

2.5. Controlled-rate slow freezing (equilibrium protocol)

Two CPA solutions prepared in Dulbecco's PBS (DPBS) were compared: 1.5 M DMSO and 1.5 M EG (both Sigma, USA). Cells were diluted with the CPA solution to a final concentration of 2 × 10⁶ cells/mL, loaded into 0.5 mL straws (CryoBioSystem, France) and sealed (Minitube, Germany). Straws were held at 5 °C for 2 h to equilibrate the cells with the CPA, and then cooled in a programmable freezer Kryo 360-3.3 (Planer, UK) from 5 °C to –40 °C at –1 °C/min and from –40 °C to –85 °C at –4 °C/min, after which they were plunged into LN₂ (Figure 2).

2.6. Thawing and viability assessment

Cryovials and straws were thawed in a 37 °C water bath (straws for 5 min); cryovials were first held briefly at room temperature to allow residual LN₂ to evaporate [20]. Cells were washed free of CPA in a 20-fold excess of RPMI-1640 by centrifugation at 160 × g for 10 min, resuspended in complete medium (10% FBS, 2 mM L-glutamine, antibiotics) and

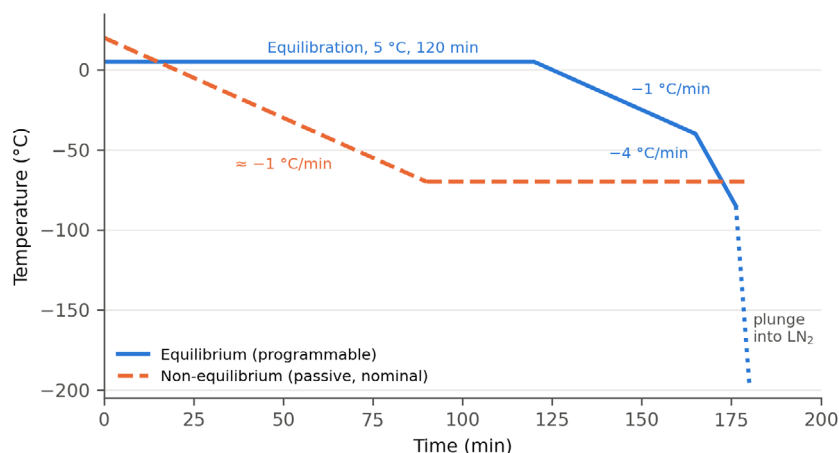


Figure 2. Temperature profiles of the two slow-freezing protocols. The controlled-rate profile is the programmed set-point; the passive profile is nominal (≈ -1 °C/min to -70 °C) and was not recorded.

Table 1. Viability of saiga fibroblasts before and after passive slow freezing with 10% DMSO ($n = 50$)

Stage	Viable cells, % ($M \pm SEM$)	95% CI	SD	Range
Before freezing	97.6 ± 0.6	96.4–98.8	4.1	–
After thawing	$44.5 \pm 1.9^*$	40.7–48.3	13.3	9–77

* $p < 0.001$ vs before freezing (Welch's t-test).

counted in a haemocytometer after staining with 0.4% trypan blue [21]. Viability was expressed as the percentage of unstained cells. In the controlled-rate experiment, post-thaw viability was calculated as the number of viable cells recovered after thawing divided by the number of cells frozen $\times 100$; fresh, non-frozen cells from the same cultures served as the control. After assessment, the cells were returned to culture.

2.7. Statistical analysis

The biological unit was the individual animal ($n = 50$ per treatment). Data are presented as mean $\pm$ standard error of the mean (SEM) and 95% confidence intervals (CI) unless stated otherwise. Normality of distributions was assumed on the basis of sample size. Two groups were compared with Welch's t-test; the three groups of the controlled-rate experiment (fresh, DMSO, EG) were compared by one-way ANOVA followed by Tukey's HSD test. Homogeneity of variances between freezing protocols was compared by the F-test. Effect sizes are given as Cohen's d . Differences were considered significant at $p < 0.05$. Calculations were performed in Python 3 (SciPy 1.17).

3. RESULTS

3.1. Primary culture of saiga fibroblasts

Primary cultures were established from all 50 biopsies.

The first single cells migrated from the explants not earlier than on day 6 of culture (on average on days 8–10) and proliferated until a monolayer was formed. Ten to twelve days after the onset of outgrowth, the monolayer covered approximately one third of the 25 cm² flask surface. Cells had the typical spindle-shaped fibroblast morphology.

3.2. Passaging

The optimal trypsinization time was 7–10 min at 37 °C. Counting after dissociation showed 86–89% viable cells. After the first passage, the growth rate increased markedly and the culture became morphologically more homogeneous.

3.3. Survival after passive slow freezing

Passive freezing with 10% DMSO reduced the proportion of viable cells from $97.6 \pm 0.6\%$ before freezing to $44.5 \pm 1.9\%$ after thawing (Table 1, Figure 3), i.e. a loss of 53.1 percentage points (95% CI 49.2–57.0; Welch's $t = 27.0$, $df = 58.2$, $p < 0.001$; $d = 5.4$). Thus, only 45.6% of cells that were alive before freezing survived. Post-thaw viability varied widely among animals, from 9% to 77% (SD 13.3%; coefficient of variation 30%).

3.4. Survival after controlled-rate slow freezing: DMSO versus EG

Controlled-rate freezing significantly reduced fibroblast

Table 2. Effect of cryoprotectant on the survival of saiga fibroblasts after controlled-rate slow freezing ($M \pm SEM$, $n = 50$)

Cryoprotectant	Cells frozen, $\times 10^3$	Viable cells after thawing, $\times 10^3$	Viability, %	95% CI	% of control
Fresh (control)	2019.5 ± 79.2	–	85.8 ± 0.8^a	84.2–87.4	100
1.5 M DMSO	2102.1 ± 69.6	1109.9 ± 64.8	52.8 ± 1.1^c	50.6–55.0	61.5
1.5 M EG	2278.5 ± 75.9	1414.5 ± 52.5	62.1 ± 1.0^b	60.1–64.1	72.4

Different superscript letters indicate significant differences (one-way ANOVA with Tukey's HSD, $p < 0.001$).

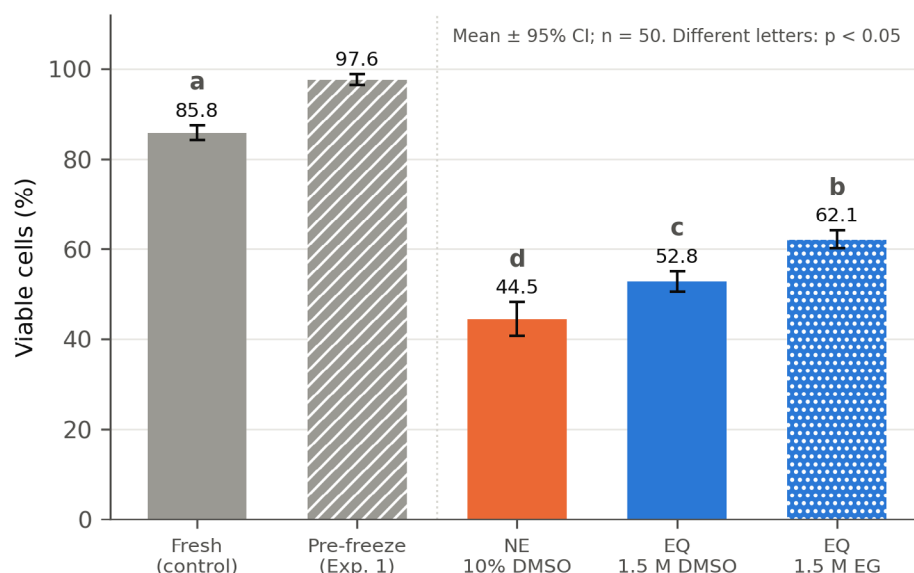


Figure 3. Viability of saiga fibroblasts before and after freezing. Grey: unfrozen cells (fresh control of the controlled-rate experiment; pre-freeze cells of the passive-freezing experiment). NE – passive (non-equilibrium) freezing; EQ – controlled-rate (equilibrium) freezing. Bars show means, whiskers 95% CI (n = 50 animals). Different letters indicate significant differences (Tukey HSD and Welch's t-test, $p < 0.05$).

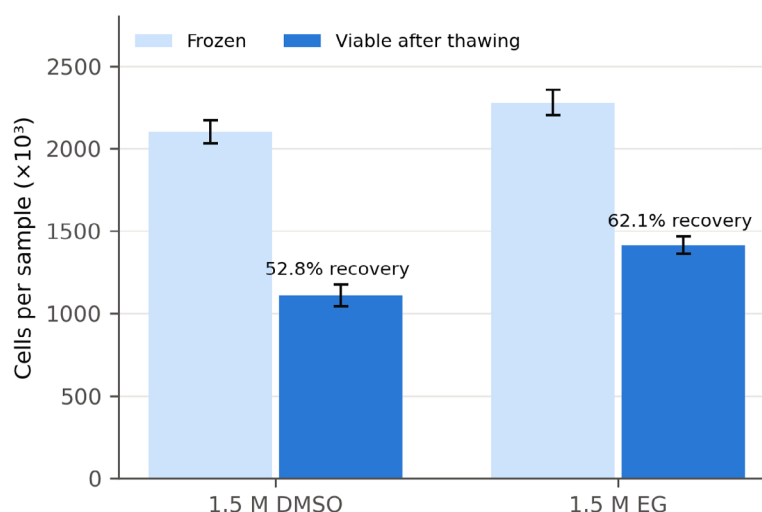


Figure 4. Number of cells frozen and number of viable cells recovered after controlled-rate freezing with 1.5 M DMSO or 1.5 M EG (mean $\pm$ SEM, n = 50). Recovery = viable cells after thawing / cells frozen $\times$ 100. The number of viable cells was higher with EG (Welch's t-test, $p < 0.001$); the number of cells frozen did not differ ($p = 0.09$).

viability compared with fresh cells for both CPAs (ANOVA: $F(2,147) = 304.8$, $p < 0.001$, $\eta^2 = 0.81$; Table 2, Figure 3). Viability was 33.0 percentage points lower than the control with DMSO (95% CI 29.7–36.3; $p < 0.001$) and 23.7 points lower with EG (95% CI 20.4–27.0; $p < 0.001$). EG gave higher survival than DMSO: $62.1 \pm 1.0\%$ vs $52.8 \pm 1.1\%$, a difference of 9.3 percentage points (95% CI 6.0–12.6; Tukey $p < 0.001$; $d = 1.25$). Relative to fresh cells, 72.4% of cells survived with EG and 61.5% with DMSO.

The number of cells frozen per sample did not differ between CPAs (2278.5 ± 75.9 vs $2102.1 \pm 69.6 \times 10^3$; $p = 0.09$), whereas the number of viable cells recovered after thawing was 304.6×10^3 higher with EG (1414.5 ± 52.5 vs $1109.9 \pm 64.8 \times 10^3$; 95% CI 139.0–470.2; $p < 0.001$), i.e. 27% more viable cells per straw (Figure 4).

3.5. Comparison of freezing protocols

Controlled-rate freezing with DMSO yielded 8.3 percentage points more viable cells than passive freezing with DMSO (95% CI 4.0–12.6; $p < 0.001$; $d = 0.76$), and controlled-rate freezing with EG yielded 17.6 points more (95% CI 13.4–21.8; $p < 0.001$; $d = 1.65$). Besides a higher mean, controlled-rate freezing gave much more reproducible results: the variance of post-thaw viability was 2.9-fold lower with DMSO and 3.5-fold lower with EG than after passive freezing (F-test, $p < 0.001$ for both), and the coefficient of variation decreased from 30% to 15% and 11%, respectively.

3.6. Somatic cell cryobank of the saiga

Based on these results, controlled-rate freezing with 1.5 M EG was adopted as the working protocol, and a cryobank of saiga somatic cells was established at the Institute of Zo-

ology. The samples are stored in LN₂ at –196 °C. The collection will be expanded with samples from subsequent captures, including animals of the Ural population.

4. DISCUSSION

This study reports, to our knowledge, the first protocol for the isolation, culture and cryopreservation of saiga somatic cells. Fibroblast cultures were readily obtained from small ear biopsies taken from live wild animals, confirming that ear skin collected during routine capture and marking is a practical and minimally invasive source of viable cells for biobanking. In other wild mammals, fibroblast banks have been established largely from skin collected post mortem [9, 10]; sampling live animals during capture allows a collection to be expanded systematically without depending on mortality events. Notably, cultures were obtained from all biopsies despite transport of skin samples for up to 72 h at 20–25 °C, which indicates that the explant method tolerates the field logistics typical of remote steppe captures. The timing of explant outgrowth (6–10 days) is within the range reported for fibroblasts of other wild species [10, 22].

The main finding is that the freezing protocol had a strong effect both on the mean survival and on the reproducibility of results. Passive freezing in an insulated box – the simplest and most widely used method for lymphoid and other cultured cells – preserved less than half of the saiga fibroblasts and showed a very wide range of outcomes (9–77%). The cooling rate in such devices is not controlled and depends on the position of the vial, the volume of the sample and the load of the freezer, so the cells of different samples experience different conditions around the ice nucleation point. According to the two-factor hypothesis [12], such fluctuations may shift some samples towards intracellular ice formation and others towards prolonged exposure to concentrated solutes. The absence of a controlled equilibration step and uncontrolled supercooling and ice nucleation probably explain both the lower mean survival and the high variance. By contrast, the programmable protocol with a 2 h equilibration at 5 °C, a slow cooling rate (–1 °C/min) through the critical zone down to –40 °C and faster cooling (–4 °C/min) thereafter gave more uniform conditions for all samples, which resulted in a 2.9–3.5-fold lower variance. For a biobank, reproducibility is as important as mean survival, because every sample represents a unique individual and cannot be re-collected.

Under controlled-rate freezing, EG was superior to DMSO at the same molar concentration (1.5 M): it increased post-thaw viability by 9.3 percentage points and the yield of viable cells per straw by 27%. This agrees with the results obtained for skin samples of an endangered Neotropical deer, where EG alone performed better than DMSO in slow freezing [16], and it is noteworthy that both species are ruminants. EG has a lower molecular weight than DMSO and permeates cell membranes faster, which reduces the amplitude of osmotic volume excursions during CPA addition and removal; its lower chemical toxicity may also have mattered during the 2 h equilibration period [13, 14, 15]. However, the advantage of EG is not universal: no difference between CPAs was found for puma somatic cells [17], similar survival ranges were reported for canine fibroblasts frozen with either CPA [19], and for manatee cells the CPA concentration was more important

than its type [18]. Studies on human haematopoietic cells likewise show that lowering the DMSO concentration from 10% to 5% can improve post-thaw viability [23]. These data indicate that CPA choice must be validated for each species, and that CPA concentration is a further variable worth optimizing for saiga cells.

The post-thaw survival obtained with the best protocol (62%, or 72% of the fresh control) is comparable to values reported for somatic cells of other wild mammals after slow freezing [10, 19]. Importantly, a large number of viable cells (about 1.4×10^6 per straw) remained, which is sufficient to re-establish a culture from each straw. Tissue-level preservation (freezing of skin fragments) and vitrification of tissues have also been proposed for wildlife biobanks, especially under field conditions [22, 24, 25], and should be tested for saiga as a complementary strategy for animals found dead or sampled far from the laboratory.

The study has several limitations. First, the passive and controlled-rate protocols differed not only in cooling profile, but also in container (cryovial vs straw), carrier solution (FBS vs DPBS), cell concentration and CPA concentration (10% DMSO $\approx$ 1.4 M vs 1.5 M), and the two experiments were performed at different times; the comparison therefore concerns complete protocols rather than the cooling rate alone. Second, viability was assessed only by trypan blue exclusion immediately after thawing, which reflects membrane integrity but not the ability of cells to attach, proliferate or maintain a normal karyotype [21]. Third, the results were analysed at the level of summary statistics per treatment, and a paired analysis of cells from the same animals would have been more powerful. Future work should include post-thaw attachment and population-doubling assays, apoptosis and karyotype analysis, as well as testing lower CPA concentrations and combinations of EG and DMSO.

5. CONCLUSIONS

This study is the first to develop and test a complete workflow for the isolation, culture and cryopreservation of somatic cells of the saiga antelope (*Saiga tatarica*), a species that, despite its numerical recovery, remains vulnerable to mass mortality and loss of genetic diversity. The results support the following conclusions.

1. Primary fibroblast cultures of the saiga can be reliably established by the explant method from small ear skin biopsies taken from live wild animals during routine capture. In DMEM, cell outgrowth begins no earlier than day 6 (on average on days 8–10), and 10–12 days later the monolayer covers about one third of a 25 cm² flask. Trypsinization for 7–10 min at 37 °C preserves 86–89% viable cells, and after the first passage the culture grows faster and becomes morphologically homogeneous. Skin sampling during marking and collaring is therefore a minimally invasive and practical source of viable saiga cells.

2. Passive slow freezing with 10% DMSO in a polystyrene box at –70 °C, a simple and widely used method, preserved only 44.5% viable fibroblasts, i.e. fewer than half of the cells that were alive before freezing. Outcomes varied widely among individuals (9–77%; coefficient of variation 30%), which makes this method unreliable for a biobank in which every sample represents a unique individual and can

not be collected again.

3. Controlled-rate slow freezing with a 2 h equilibration at 5 °C and two-step cooling (–1 °C/min to –40 °C, then –4 °C/min to –85 °C) outperformed passive freezing both in mean survival (by 8.3 percentage points with DMSO and by 17.6 points with EG) and in reproducibility: the variance of results decreased 2.9–3.5-fold and the coefficient of variation fell from 30% to 11–15%.

4. Under controlled-rate freezing, 1.5 M ethylene glycol was significantly more effective than 1.5 M DMSO: viability was 62.1% versus 52.8% (72.4% and 61.5% of the fresh control), and each straw contained 27% more viable cells (about 1.4×10^6), which is sufficient to re-establish a culture from every straw. The advantage of ethylene glycol is consistent with data obtained for an endangered deer species and is probably related to its higher membrane permeability and lower osmotic stress during CPA addition and removal.

5. Based on these data, the following working protocol is recommended for saiga fibroblasts: culture in DMEM with 10% FBS; cryopreservation at passage 2 at 2×10^6 cells/mL in 1.5 M ethylene glycol in DPBS in 0.5 mL straws; equilibration for 2 h at 5 °C; controlled-rate cooling to –85 °C followed by plunging into liquid nitrogen; thawing at 37 °C. Passive freezing with DMSO is acceptable only as a back-up option when a programmable freezer is not available, and the viability of each sample should then be checked.

6. Using the recommended protocol, the first somatic cell cryobank of the saiga in Kazakhstan has been established at the Institute of Zoology, containing skin and fibroblast samples from 50 animals of the Betpak-Dala and Ustyurt populations. The collection serves as an insurance of the species' genetic diversity against mass mortality events and as a resource for population genetic and genomic research, derivation of induced pluripotent stem cells and, in the future, assisted reproductive technologies.

The practical value of this work is that the protocol can be integrated into saiga monitoring programmes: skin samples collected during annual marking will allow the cryobank to be expanded systematically with material from different populations, sexes and age groups. The approach is also applicable to other Central Asian ungulates for which somatic cell biobanks have not yet been established.

AUTHOR CONTRIBUTIONS

Yerzhan Toishibekov: Conceptualization, Methodology, Supervision, Project administration, Writing – original draft, Writing – review & editing. Alexey Grachev: Investigation, Resources, Methodology. Madina Salmenova: Investigation, Formal analysis, Visualization, Methodology. Tulendy Nurkenov: Investigation, Methodology. Bibigul Katubayeva: Investigation, Methodology. Dinmukhamed Toishybek: Investigation, Methodology. Indira Kumayeva: Methodology, Investigation. Anna Khamchukova: Investigation, Methodology. Alina Chimiruk: Investigation, Methodology. Maxim Bepalov: Investigation, Methodology. Sergey Bepalov: Investigation, Methodology. Saltore Saparbayev: Investigation, Methodology. Sanzhar Kantarbayev: Investigation, Methodology. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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КРИОКОНСЕРВАЦИЯ СОМАТИЧЕСКИХ КЛЕТОК САЙГАКА (*SAIGA TATARICA*): ВЛИЯНИЕ НЕРАВНОВЕСНОГО И РАВНОВЕСНОГО ЗАМОРАЖИВАНИЯ НА ЖИЗНЕСПОСОБНОСТЬ ФИБРОБЛАСТОВ

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АННОТАЦИЯ

Актуальность. Численность сайгака (*Saiga tatarica*) в Казахстане восстановилась, однако его популяции по-прежнему подвержены массовому падежу, способному за несколько недель уничтожить значительную часть популяции. Банк жизнеспособных соматических клеток может служить страховым фондом генетического разнообразия, однако протоколы криоконсервации клеток сайгака до сих пор не были описаны. **Цель.** Получить первичные культуры фибробластов из кожи уха сайгака и сравнить выживаемость клеток после пассивного и программного медленного замораживания. **Методы.** Биоптаты кожи уха получены от 50 взрослых сайгаков бетпакалинской и устьюртской популяций. Фибробласты, выделенные методом эксплантов, замораживали на втором пассаже либо пассивно в криопробирках с 10% диметилсульфоксида (ДМСО; ≈ -1 °C/мин до -70 °C), либо в соломинках объемом 0,5 мл с 1,5 М ДМСО или 1,5 М этиленгликоля (ЭГ) после 2 ч эквilibрации при 5 °C на программном замораживателе. Жизнеспособность оценивали по исключению трипанового синего. **Результаты.** Выход клеток из эксплантов начинался на 6–10-е сутки культивирования. Пассивное замораживание снизило жизнеспособность с 97,6 до 44,5% ($p < 0,001$) при высокой индивидуальной изменчивости (9–77%; CV 30%). После программного замораживания доля живых клеток составила $52,8 \pm 1,1\%$ с ДМСО и $62,1 \pm 1,0\%$ с ЭГ против $85,8 \pm 0,8\%$ в свежем контроле ($p < 0,001$); ЭГ превзошел ДМСО на 9,3 процентного пункта и обеспечил на 27% больше живых клеток в соломинке. Оба варианта программного замораживания превзошли пассивное и дали в 2,9–3,5 раза меньшую дисперсию. **Выводы.** Наиболее эффективным оказалось программное медленное замораживание с 1,5 М ЭГ; этот протокол принят для создания первого в Казахстане криобанка соматических клеток сайгака.

Ключевые слова: сайгак; *Saiga tatarica*; фибробласты; криоконсервация; этиленгликоль; диметилсульфоксид; биобанк; охрана диких животных.

КИІКТІҢ (*SAIGA TATARICA*) СОМАТИКАЛЫҚ ЖАСУШАЛАРЫН КРИОКОНСЕРВАЦИЯЛАУ: ТЕПЕ-ТЕНДІКСІЗ ЖӘНЕ ТЕПЕ-ТЕНДІК МҰЗДАТУДЫҢ ФИБРОБЛАСТТАРДЫҢ ТІРШІЛІККЕ ҚАБІЛЕТТІЛІГІНЕ ӘСЕРІ

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Жабайы жануарлардың криобиология және ұрық плазмасы биобанкі зертханасы, Қазақстан Республикасы Ғылым және жоғары білім министрлігі Ғылым комитетінің «Зоология институты» РМҚ, Алматы, Қазақстан

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АНДАТПА

Өзектілігі. Қазақстанда киіктің (*Saiga tatarica*) саны қалпына келгенімен, оның популяциялары бірнеше апта ішінде популяцияның едәуір бөлігін жоя алатын жаппай қырылуға әлі де ұшырап отыр. Тіршілікке қабілетті соматикалық жасушалар банкі генетикалық әртүрлілікті сақтаудың сақтандыру қоры бола алады, алайда киік жасушаларын криоконсервациялау хаттамалары бұған дейін сипатталмаған. **Мақсаты.** Киіктің құлақ терісінен фибробласттардың бастапқы дақылдарын алу және пассивті және бағдарламалы баяу мұздатудан кейінгі жасушалардың тіршілікке қабілеттілігін салыстыру. **Әдістері.** Құлақ терісінің биоптаттары бетпақдала және үстірт популяцияларының 50 ересек киігінен алынды. Эксплант әдісімен бөлініп алынған фибробласттар екінші пассажда не криопробиркаларда 10% диметилсульфоксидпен (ДМСО; -70 °C-қа дейін ≈ -1 °C/мин) пассивті түрде, не 5 °C-та 2 сағ эквilibрациядан кейін 1,5 М ДМСО немесе 1,5 М этиленгликольмен (ЭГ) 0,5 мл соломинкаларда бағдарламалы мұздатқышта мұздатылды. Тіршілікке қабілеттілік трипан көгін сіңірмеу әдісімен бағаланды. **Нәтижелері.** Жасушалардың эксплантардан шығуы өсірудің 6–10-тәулігінде басталды. Пассивті мұздату тіршілікке қабілеттілікті 97,6%-дан 44,5%-ға дейін төмендетті ($p < 0,001$), бұл ретте даралар арасындағы өзгергіштік жоғары болды (9–77%; CV 30%). Бағдарламалы мұздатудан кейін тірі жасушалардың үлесі ДМСО-мен $52,8 \pm 1,1\%$, ЭГ-мен $62,1 \pm 1,0\%$ құрады, ал жаңа

бақылауда $85,8 \pm 0,8\%$ болды ($p < 0,001$); ЭГ ДМСО-дан 9,3 пайыздық тармаққа асып түсті және бір соломинкадағы тірі жасушалар санын 27%-ға арттырды. Бағдарламалы мұздатудың екі нұсқасы да пассивті мұздатудан тиімді болды, ал олардың дисперсиясы 2,9–3,5 есе төмен болды. Қорытынды. 1,5 М ЭГ-мен бағдарламалы баяу мұздату ең тиімді хаттама болды және Қазақстандағы киіктің соматикалық жасушаларының алғашқы криобанкін құру үшін қабылданды.

Түйін сөздер: киік; *Saiga tatarica*; фибробласттар; криоконсервация; этиленгликоль; диметилсульфоксид; биобанк; жабайы жануарларды қорғау.