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VITRIFICATION OF SAIGA (*SAIGA TATARICA*) SKIN FIBROBLASTS: EFFECT OF CRYOPROTECTANT COMPOSITION AND ULTRA-RAPID COOLING AT -205°C

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ABSTRACT

Background. The saiga antelope (*Saiga tatarica*) remains exposed to recurrent mass mortality, and a bank of viable somatic cells can insure its genetic diversity. Vitrification of saiga cells has not been studied. **Aim.** To establish fibroblast lines from neonatal saiga calves and to compare three vitrification solutions under two cooling regimens. **Methods.** Ear skin explants from 50 calves (25 males, 25 females) aged up to two weeks from the Betpak-Dala region were cultured in DMEM. Cells were loaded stepwise with 20% dimethyl sulfoxide (DMSO), 20% ethylene glycol (EG) or 20% DMSO + 20% EG, each with 0.5 M sucrose, and cooled in 0.25 mL straws either in liquid nitrogen (-196°C) or in subcooled nitrogen at -205°C (super-cooling ultra-rapid vitrification, SCURV). Viability was assessed by trypan blue exclusion ($n = 50$ lines per group). **Results.** Cell outgrowth began on days 6–10 of culture. Two-way ANOVA showed effects of both the cooling regimen ($p < 0.001$; $\eta^2 = 0.19$) and the solution ($p < 0.001$; $\eta^2 = 0.04$), without interaction. SCURV increased viability by 6.1 percentage points (95% CI 4.7–7.5), and the DMSO + EG mixture by 3.1 points relative to either single agent ($p = 0.001$); DMSO and EG did not differ. The highest viability, $53.9 \pm 5.9\%$, was obtained with DMSO + EG under SCURV. **Conclusions.** The DMSO + EG mixture combined with ultra-rapid cooling at -205°C was the most effective vitrification protocol. Samples of all 50 calves were added to the saiga somatic cell cryobank.

Keywords: saiga antelope; *Saiga tatarica*; fibroblasts; vitrification; slush nitrogen; ethylene glycol; dimethyl sulfoxide; cryobank

1. INTRODUCTION

The saiga antelope (*Saiga tatarica* Linnaeus, 1766) is a migratory ungulate of the Eurasian steppes and semi-deserts, and most of its world population lives in Kazakhstan. After a collapse at the turn of the century the species has recovered markedly, and in 2023 it was moved on the IUCN Red List from Critically Endangered to Near Threatened [1]. Numerical recovery, however, does not remove the threat that is specific to saigas – recurrent mass mortality. In May 2015 more than 200,000 animals of the Betpak-Dala population died within about three weeks from haemorrhagic septicaemia caused by *Pasteurella multocida* serotype B; the die-off coincided with calving and followed a period of unusually warm and humid weather [2, 3]. Similar events occurred in 1981 and 1988 [2], and the *P. multocida* strains isolated in 2015 are identical to those from the die-offs of 1988 and 2010–2012 [4]. The risk is therefore systemic, and the genetic resources of the species need to be secured in advance rather than after the next event.

Banks of viable somatic cells, often called “frozen zoos”, are one of the tools for this purpose; the largest collection, in San Diego, holds cell lines of 965 species [5]. Unlike gametes and embryos, skin fibroblasts can be obtained from a small biopsy of a live animal or post mortem, they proliferate readily in vitro, keep the complete genome of the donor and remain viable in liquid nitrogen for decades [6]. Their value for conservation has been demonstrated by somatic cell nuclear transfer (SCNT) from cryopreserved fibroblasts: a gaur (*Bos gaurus*) was cloned by interspecies nuclear transfer [7], a mouflon (*Ovis orientalis musimon*) from post-mortem somatic cells [8], the first animal of an extinct subspecies, the bucardo (*Capra pyrenaica pyrenaica*) [9], and recently

two Przewalski’s horses (*Equus przewalskii*) from a cell line frozen for more than 40 years [10].

Cells are cryopreserved either by slow freezing or by vitrification. Slow freezing uses low concentrations of cryoprotective agents (CPAs) and is the most common method, but survival depends on a compromise between intracellular ice formation and solution effects, which requires an optimal cooling rate for each cell type [11]. Vitrification avoids ice altogether: at a sufficiently high CPA concentration and cooling rate the solution solidifies as an amorphous glass [12, 13]. Its main drawback is CPA toxicity [13, 14]. Toxicity can be reduced by combining permeating CPAs, among them dimethyl sulfoxide (DMSO) and ethylene glycol (EG) [14–16], and by shortening the exposure time [17]. A higher cooling rate lowers the CPA concentration needed to avoid ice; one way to obtain it is to plunge samples into subcooled (“slush”) nitrogen, which boils less and therefore does not form the insulating vapour layer that surrounds a sample in ordinary liquid nitrogen [18]. The warming rate is at least as important as the cooling rate [19]. For somatic cells and tissues of wild mammals, solid-surface vitrification with faster heat exchange has proved more efficient than direct vitrification in cryovials [20], and in puma somatic cells a reduction in the concentration of DMSO or EG did not impair survival, irrespective of the presence of sucrose [21].

Slow freezing of fibroblasts from adult saigas has been examined by our group in a parallel study (Toishibekov et al., submitted), but vitrification of saiga cells has not been reported, and no cell lines have been described for neonatal calves, which can be sampled during tagging in the calving season. The aim of this study was to establish fibroblast lines

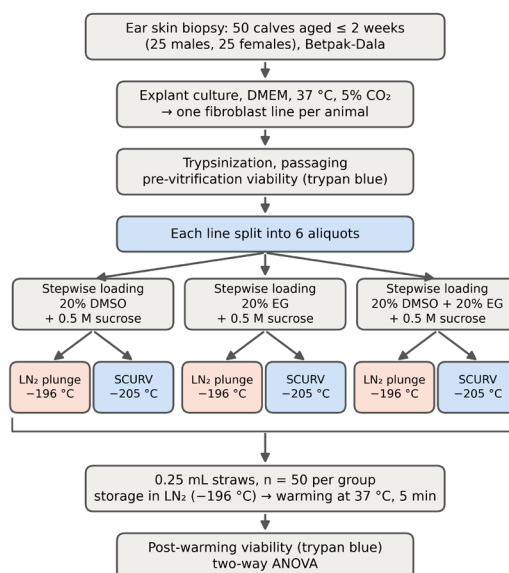


Figure 1. Experimental design. Fibroblast lines from 50 calves were each divided into six aliquots, one per combination of vitrification solution and cooling regimen (3×2 design, $n = 50$ per group). LN₂ – liquid nitrogen; SCURV – super-cooling ultra-rapid vitrification in subcooled nitrogen.

from saiga calves of the Betpak-Dala population, to compare three vitrification solutions based on DMSO and EG under two cooling regimens (plunging into liquid nitrogen at -196 °C and ultra-rapid cooling in subcooled nitrogen at -205 °C), and to add the material to the somatic cell cryobank of the species. The objectives were: (i) to isolate fibroblasts from ear skin explants and to describe their culture and passaging; (ii) to evaluate post-warming viability after vitrification with DMSO, EG and DMSO + EG under the two cooling regimens; and (iii) to deposit skin and cell samples of all animals in the cryobank for long-term storage.

2. MATERIALS AND METHODS

2.1. Animals and sampling

Skin samples were obtained from the ear pinnae of 50 saiga calves (25 males and 25 females) aged up to two weeks in the Betpak-Dala region of Kazakhstan during capture for tagging in the calving season of 2025. 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 design of the study is shown in Figure 1.

2.2. Isolation and culture of fibroblasts

The samples were washed in PBS, cut into fragments of about 1 mm^3 and placed in 25 cm^2 plastic culture flasks with culture medium (explant method). 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, and half of the medium was replaced every three days. Outgrowth of cells from the explants and their proliferation were monitored under an inverted microscope.

2.3. Passaging

The monolayer was detached with trypsin (0.25%) for 7–10 min at 37 °C. Trypsin was inactivated by adding 5–20 mL of medium containing 10% bovine serum, and the cell suspension was transferred to new flasks at a 1:2 split ratio. Cells at passage 2 were used for vitrification. Immediately before vitrification the cells were counted and their viability was determined by trypan blue exclusion (section 2.6); the suspension was adjusted to 1×10^6 cells/mL.

2.4. Vitrification solutions and CPA loading

Three vitrification solutions prepared in base medium were compared: (1) 20% (v/v) DMSO + 0.5 M sucrose; (2) 20% EG + 0.5 M sucrose; and (3) 20% DMSO + 20% EG + 0.5 M sucrose (all reagents Sigma, USA). CPAs were loaded in three steps at room temperature (22 – 24 °C): 25% of the final concentration for 3 min, 50% for 3 min, and the vitrification solution for 30 s, with 0.5 M sucrose at every step. Thus, cells were exposed to 5%, 10% and 20% DMSO or EG in the single-agent solutions, and to 5% + 5%, 10% + 10% and 20% + 20% in the mixture (DMSO + EG respectively). The total exposure time was 6.5 min in all groups.

2.5. Cooling regimens

The cell suspension in vitrification solution was loaded into 0.25 mL straws (CryoBioSystem, France), and cooled in one of two ways. In the conventional regimen, straws were plunged directly into liquid nitrogen (LN₂) at -196 °C. In the ultra-rapid regimen, straws were plunged into subcooled nitrogen at -205 °C in a VitMaster vitrification device (IMT Ltd., Ness Ziona, Israel) operating in the super-cooling ultra-rapid vitrification (SCURV) mode (Figure 2). The device lowers the temperature of liquid nitrogen below its boiling point by reducing the pressure above it; subcooled nitrogen does not boil on contact with a warm sample, so the insulating vapour layer does not form and the cooling rate increases [18]. The straws, solutions and loading procedure were identical in both regimens.

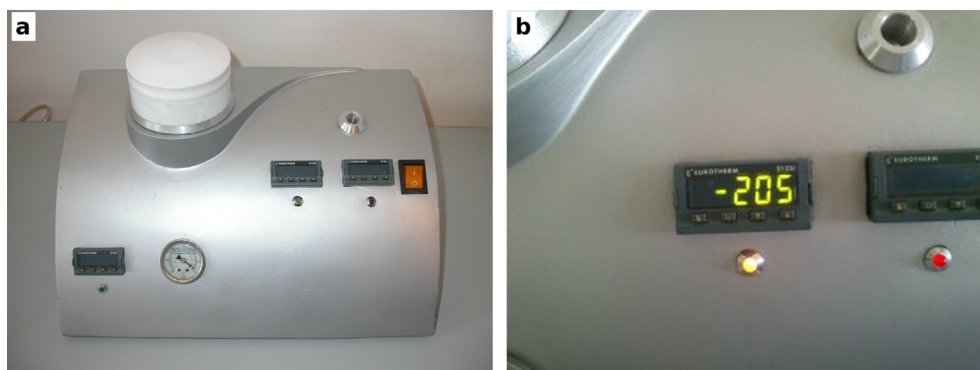


Figure 2. VitMaster vitrification device (a) and the chamber temperature display showing -205°C during vitrification in the SCURV mode (b). Photographs by the authors.

2.6. Warming and viability assessment

Straws were warmed in a water bath at 37°C for 5 min, and the CPAs were removed by 0.5 M sucrose. Viability before vitrification and after warming was assessed by trypan blue exclusion: cells were stained with trypan blue and unstained (viable) cells were counted in a haemocytometer [22]. The recovery index was calculated as post-warming viability divided by pre-vitrification viability $\times 100$.

2.7. Storage

After vitrification, straws were transferred within 2–3 s into pre-cooled containers in an insulated vessel filled with LN_2 , placed in visotubes and goblets, and stored in the canisters of Dewar vessels in LN_2 at -196°C .

2.8. Statistical analysis

The biological unit was the individual animal. A fibroblast line was established from each of the 50 calves, and the cell suspension of each line was divided into six aliquots, one per experimental group ($n = 50$ per group). Data are presented as mean $\pm$ standard deviation ($M \pm \text{SD}$) with 95% confidence intervals (CI). The effects of vitrification solution (DMSO, EG, DMSO + EG) and cooling regimen (-196°C , -205°C) were assessed by two-way analysis of variance (ANOVA) with effect size η^2 ; the main effect of solution was further examined with Tukey's HSD test. Within each cooling regimen, solutions were compared pairwise with Welch's t-test and Holm's correction, and the two regimens were compared for each solution with Welch's t-test; effect sizes are given as Cohen's d. The groups were analysed as independent; **Table 1.** Post-warming viability of saiga fibroblasts depending on vitrification solution and cooling regimen ($M \pm \text{SD}$, $n = 50$ lines per group)

Cooling regimen	Vitrification solution	Viable cells after warming, % ($M \pm \text{SD}$)	95% CI	Recovery index, %
-196°C (LN_2 plunge)	20% DMSO	$45.6 \pm 5.9^{\text{ab}}$	43.9–47.3	47.3
	20% EG	$44.1 \pm 6.2^{\text{b}}$	42.3–45.9	45.7
	20% DMSO + 20% EG	$48.3 \pm 7.5^{\text{a}}$	46.2–50.4	50.1
-205°C (SCURV)	20% DMSO	$50.4 \pm 6.2^{\text{b***}}$	48.6–52.2	52.2
	20% EG	$51.9 \pm 5.4^{\text{ab***}}$	50.4–53.4	53.8
	20% DMSO + 20% EG	$53.9 \pm 5.9^{\text{a***}}$	52.2–55.6	55.9

Viability before vitrification was $96.5 \pm 3.9\%$ for all groups. Recovery index = post-warming / pre-vitrification viability $\times 100$. Within each cooling regimen, values without a common superscript letter differ (Welch's t-test with Holm's correction, $p < 0.05$); *** $p < 0.001$ vs -196°C for the same solution (Welch's t-test). All groups differed from the pre-vitrification level ($p < 0.001$).

because aliquots of the same lines were used in all groups, this approach is conservative. Differences were considered significant at $p < 0.05$. All statistical analyses were performed in Python 3.11 using a custom-written analysis script.

3. RESULTS

3.1. Primary culture and passaging

Primary cultures were obtained from all 50 calves. Single cells began to migrate from the explants not earlier than on day 6 of culture (on average on days 8–10) and divided until a monolayer formed. Ten to twelve days after the onset of outgrowth, the monolayer covered about one third of the 25 cm^2 flask. The optimal trypsinization time was 7–10 min at 37°C , and counts after dissociation showed 86–89% viable cells. Viability of the suspensions used for vitrification was $96.5 \pm 3.9\%$.

3.2. Viability after vitrification

Post-warming viability in the six experimental groups is given in Table 1 and Figure 3. Two-way ANOVA showed significant effects of both the cooling regimen and the vitrification solution, with no interaction (Table 2). The cooling regimen accounted for the largest share of variance ($\eta^2 = 0.185$).

Averaged over the regimens, the DMSO + EG mixture gave 51.1% viable cells, 3.1 points more than DMSO or EG alone (48.0% each; 95% CI of the difference 1.0–5.2; Tukey $p = 0.001$), whereas DMSO and EG did not differ ($p > 0.99$;

Table 2. Two-way ANOVA of post-warming viability (factors: cooling regimen and vitrification solution)

Source of variation	df	F	p	η^2
Cooling regimen	1	71.42	< 0.001	0.185
Vitrification solution	2	8.29	< 0.001	0.043
Regimen $\times$ solution	2	1.56	0.21	0.008
Residual	294	—	—	—

η^2 – proportion of total variance explained by the factor.

Averaged over the solutions, SCURV raised viability from 46.0% to 52.1%, i.e. by 6.1 percentage points (95% CI 4.7–7.5; Figure 4a). The increase was significant for each solution: 4.8 points with DMSO (95% CI 2.4–7.2; $p < 0.001$; $d = 0.79$), 7.8 points with EG (95% CI 5.5–10.1; $p < 0.001$; $d = 1.34$) and 5.6 points with DMSO + EG (95% CI 2.9–8.3; $p < 0.001$; $d = 0.83$).

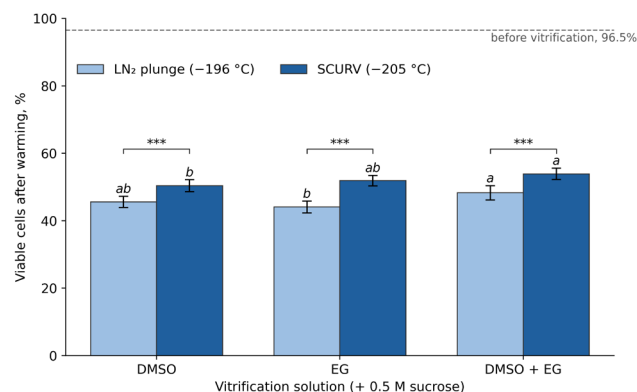


Figure 3. Post-warming viability of saiga fibroblasts by vitrification solution and cooling regimen. Bars show means, whiskers 95% CI ($n = 50$ lines per group); the dashed line shows viability before vitrification (96.5%). Within each regimen, bars without a common letter differ (Welch's t-test with Holm's correction, $p < 0.05$); *** $p < 0.001$, -205 °C vs -196 °C for the same solution.

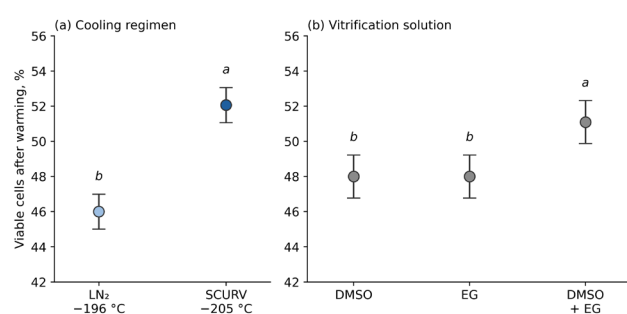


Figure 4. Main effects of cooling regimen (a) and vitrification solution (b) on post-warming viability (marginal means with 95% CI from the two-way ANOVA; $n = 150$ per regimen, $n = 100$ per solution). Different letters indicate significant differences ((a) ANOVA, $p < 0.001$; (b) Tukey's HSD, $p = 0.001$).

Figure 4b). Within the regimens, the advantage of the mixture was significant for part of the comparisons: at -196 °C it exceeded EG ($p = 0.009$; $d = 0.61$) but not DMSO ($p = 0.10$), and at -205 °C it exceeded DMSO ($p = 0.014$; $d = 0.58$) but not EG ($p = 0.16$). DMSO and EG did not differ in either regimen ($p = 0.22$ and $p = 0.20$).

The highest viability, $53.9 \pm 5.9\%$, was obtained with DMSO + EG under SCURV. In all groups viability after warming was significantly lower than before vitrification ($p < 0.001$), and the recovery index ranged from 45.7% (EG, -196 °C) to 55.9% (DMSO + EG, SCURV).

4. DISCUSSION

This study provides the first data on vitrification of saiga somatic cells and the first fibroblast lines from neonatal saiga calves. With 50 lines per group, it shows that both the cooling regimen and the composition of the vitrification solution affect survival, and that their effects are additive.

The cooling regimen had the largest effect. Plunging straws into subcooled nitrogen at -205 °C improved survival with every solution, by 5–8 percentage points. Ordinary liquid nitrogen is at its boiling point, and a sample plunged into it is surrounded by a layer of nitrogen vapour that slows heat transfer; subcooled nitrogen absorbs heat without boiling, so cooling is faster [18]. The faster the sample passes through the temperature range of ice nucleation, the lower the CPA concentration required to avoid ice [12, 13]. Because warming was identical in all groups (37 °C, 5 min), the differences are attributable to cooling. The warming rate, however, can be even more critical for survival than the cooling rate [19], and faster warming is the obvious next step in optimising the protocol. The cooling rates themselves were not measured, and the effect of the regimen should be regarded as that of the whole procedure.

The DMSO + EG mixture gave higher survival than either CPA alone, whereas DMSO and EG were equivalent. This agrees with the lower toxicity of CPA mixtures compared with their components [14–16]. There is, however, a second explanation that our design cannot separate from the first: the mixture contained 40% permeating CPA against 20% in the single-agent solutions, and a solution with a higher CPA content is more likely to vitrify completely in a 0.25 mL straw. The loading schedule was the same in all groups and short (6.5 min in total), in line with the concentration-, temperature- and time-dependence of CPA toxicity [17]. The effect of the mixture was modest ($d = 0.58$ – 0.61 in the within-regimen comparisons) and should be confirmed with functional end points.

Other studies of somatic material from wild mammals point in the same direction. In collared peccaries (*Pecari tajacu*), solid-surface vitrification of skin, which provides faster heat exchange, was more efficient than direct vitrification in cryovials [20], and in pumas a reduction in the concentration of DMSO or EG preserved the viability, proliferative and metabolic activity of somatic cells, irrespective of the presence of sucrose [21]. At the same time, survival of saiga fibroblasts after vitrification (about 50%) was lower than after optimised slow freezing of porcine ear-

skin fibroblasts, where it exceeded 90% [23], and lower than after controlled-rate slow freezing with 1.5 M EG in adult saiga fibroblasts (62%; Toishibekov et al., submitted). The two saiga studies differ in donor age, cell passage and method, so they cannot be compared directly; nevertheless, for routine banking of saiga fibroblasts controlled-rate slow freezing remains a reasonable reference method, and vitrification in straws needs further optimisation. Its advantage is that it does not require a programmable freezer. Cryopreserved cell lines of wild rodents have been shown to recover their viability during subsequent culture [24], and this should be tested for saiga lines.

The practical value of the work lies in the vulnerability of the saiga to mass mortality, which has repeatedly affected the Betpak-Dala population [2, 3]. The lines from 50 genetically different calves of both sexes add to a cryobank similar in purpose to the Frozen Zoo collections [5]. Their potential uses include SCNT, as shown for the Przewalski's horse cloned from cells stored for decades [10], derivation of induced pluripotent stem cells, and population genetic research.

The study has several limitations. First, viability was assessed only by trypan blue exclusion immediately after warming; this test reflects membrane integrity and may overestimate or underestimate survival compared with fluorescent assays, especially after cryopreservation [25]. Second, attachment, proliferation, mitochondrial activity and apoptosis after warming were not evaluated. Third, the cooling and warming rates were not measured, and warming was not optimised. Fourth, the mixture differed from the single-agent solutions in total CPA concentration, so the effects of composition and concentration cannot be separated. Finally, the effect of sex was not analysed, although the balanced sample (25 males and 25 females) allows this.

5. CONCLUSION

1. Fibroblast lines were established from ear skin explants of all 50 saiga calves of the Betpak-Dala population; cell outgrowth in DMEM began on days 6–10, the monolayer covered about one third of a 25 cm² flask 10–12 days later, and trypsinization for 7–10 min at 37 °C preserved 86–89% viable cells.

2. Post-warming viability of saiga fibroblasts depended on both the cooling regimen ($p < 0.001$; $\eta^2 = 0.185$) and the vitrification solution ($p < 0.001$; $\eta^2 = 0.043$), without interaction between the factors.

3. Ultra-rapid cooling in subcooled nitrogen at –205 °C (SCURV) increased viability with all three solutions, by 4.8–7.8 percentage points (6.1 points on average).

4. The 20% DMSO + 20% EG + 0.5 M sucrose mixture was more effective than DMSO or EG alone (by 3.1 percentage points on average), while DMSO and EG were equivalent; the best result, $53.9 \pm 5.9\%$ viable cells, was obtained with the mixture under SCURV.

5. Skin and fibroblast samples of 25 male and 25 female calves were deposited in the saiga somatic cell cryobank and are stored at –196 °C for re-establishment of cultures, molecular genetic research and assisted reproductive technologies.

AUTHOR CONTRIBUTIONS

Yerzhan Toishibekov: Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing. Alexey Grachev: Investigation, Resources. Madina Salmenova: Investigation, Formal analysis, Visualization, Writing – original draft. Tulendy Nurkenov: Investigation, Methodology. Bibigul Katubayeva: Investigation, Methodology. Dinmukhamed Toishybek: Investigation, Methodology. Indira Kumayeva: Investigation, Methodology. Anna Khamchukova: Investigation, Methodology. Maxim Bepalov: Investigation, Resources. Sergey Bepalov: Investigation, Resources. Saltore Saparbayev: Investigation, Resources. Sanzhar Kantarbayev: Investigation, Resources. Alina Chimiruk: 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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ВИТРИФИКАЦИЯ ФИБРОБЛАСТОВ КОЖИ САЙГАКА (*SAIGA TATARICA*): ВЛИЯНИЕ СОСТАВА КРИОПРОТЕКТОРА И УЛЬТРАБЫСТРОГО ОХЛАЖДЕНИЯ ПРИ -205°C

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АННОТАЦИЯ

Актуальность. Сайгак (*Saiga tatarica*) по-прежнему подвержен повторяющимся массовым падежам, поэтому банк жизнеспособных соматических клеток служит практической страховкой его генетического разнообразия. Витрификация клеток сайгака не изучалась.

Цель. Получить линии фибробластов от новорождённых сайгачат и сравнить три витрификационных раствора при двух режимах охлаждения.

Методы. Экспланты кожи ушной раковины получены от 50 сайгачат (25 самцов, 25 самок) в возрасте до двух недель в регионе Бетпақдала и культивированы в среде DMEM. Клетки ступенчато насыщали 20% диметилсульфоксидом (ДМСО), 20% этиленгликолем (ЭГ) или смесью 20% ДМСО + 20% ЭГ, каждый с 0,5 М сахарозой, в соломинках 0,25 мл и охлаждали погружением в жидкий азот (-196°C) либо в переохлаждённом азоте при -205°C (ультрабыстрая витрификация при сверхнизкой температуре, SCURV) на приборе VitMaster. Жизнеспособность оценивали по исключению трипанового синего ($n = 50$ линий на группу) и анализировали двухфакторным дисперсионным анализом.

Результаты. Выход клеток из эксплантов начинался на 6–10-е сутки культивирования. На жизнеспособность после отогрева влияли и режим охлаждения ($p < 0,001$; $\eta^2 = 0,19$), и раствор ($p < 0,001$; $\eta^2 = 0,04$), без взаимодействия факторов. SCURV повышал жизнеспособность в среднем на 6,1 процентного пункта (95% ДИ 4,7–7,5), смесь ДМСО + ЭГ – на 3,1 пункта по сравнению с каждым из однокомпонентных растворов ($p = 0,001$); ДМСО и ЭГ не различались. Наибольшая жизнеспособность – $53,9 \pm 5,9\%$ – получена со смесью ДМСО + ЭГ в режиме SCURV. **Выводы.** Витрификация фибробластов сайгака наиболее эффективна при использовании смеси ДМСО + ЭГ и ультрабыстром охлаждении при -205°C . Образцы кожи и фибробластов всех 50 сайгачат внесены в криобанк соматических клеток сайгака.

Ключевые слова: сайгак; *Saiga tatarica*; фибробласты; витрификация; переохлаждённый азот; этиленгликоль; диметилсульфоксид; криобанк.

КИІК (*SAIGA TATARICA*) ТЕРІСІНІҢ ФИБРОБЛАСТТАРЫН ВИТРИФИКАЦИЯЛАУ: КРИОПРОТЕКТОР ҚҰРАМЫНЫҢ ЖӘНЕ -205°C -ТАҒЫ АСА ЖЫЛДАМ САЛҚЫНДАТУДЫҢ ӘСЕРІ

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

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АНДАТПА

Өзектілігі. Киік (*Saiga tatarica*) әлі де қайталанатын жаппай қырылуға ұшырап отыр, сондықтан тіршілікке қабілетті соматикалық жасушалар банкі оның генетикалық әртүрлілігін сақтаудың практикалық кепілі болып табылады. Киік жасушаларын витрификациялау бұрын зерттелмеген.

Мақсаты. Жаңа туған киік лактарынан фибробласт желілерін алу және екі салқындату режимінде үш витрификациялық ерітіндіні салыстыру.

Әдістері. Құлақ қалқаны терісінің экспланттары Бетпақдала аймағында екі аптаға дейінгі 50 лақтан (25 еркек, 25 ұрғашы) алынып, DMEM ортасында өсірілді. Жасушалар 0,25 мл соломинкаларда 0,5 М сахарозасы бар 20% диметилсульфоксидпен (ДМСО), 20% этиленгликольмен (ЭГ) немесе 20% ДМСО + 20% ЭГ қоспасымен сатылап қанықтырылып, сұйық азотқа батыру (-196°C) немесе VitMaster құрылғысында -205°C -тағы аса салқындатылған азотта (SCURV) салқындатылды. Тіршілікке қабілеттілік трипан көгін сіңірмеу әдісімен бағаланды (әр топта $n = 50$ желі) және екі факторлы дисперсиялық талдаумен өңделді.

Нәтижелері. Жасушалардың экспланттардан шығуы өсірудің 6–10-шы тәулігінде басталды. Жібітуден кейінгі тіршілікке қабілеттілікке салқындату режимі ($p < 0,001$; $\eta^2 = 0,19$) де, ерітінді ($p < 0,001$; $\eta^2 = 0,04$) де әсер етті, факторлардың өзара әрекеттесуі байқалмады. SCURV тіршілікке қабілеттілікті орта есеппен 6,1 пайыздық тармаққа (95% СА 4,7–7,5), ДМСО + ЭГ қоспасы бір компонентті ерітінділердің әрқайсысымен салыстырғанда 3,1 тармаққа арттырды ($p = 0,001$); ДМСО мен ЭГ арасында айырмашылық болмады. Ең жоғары көрсеткіш – $53,9 \pm 5,9\%$ – SCURV режиміндегі ДМСО + ЭГ қоспасымен алынды.

Қорытынды. Киік фибробласттарын витрификациялау ДМСО + ЭГ қоспасын пайдаланып, -205°C -та аса жылдам салқындатқанда ең тиімді. Барлық 50 лақтың тері және фибробласт үлгілері киіктің соматикалық жасушалар криобанкіне енгізілді.

Түйін сөздер: киік; *Saiga tatarica*; фибробласттар; витрификация; аса салқындатылған азот; этиленгликоль; диметилсульфоксид; криобанк.

