Probl Cryobiol Cryomed 2025; 35(4):236
https://doi.org/10.15407/cryo35.04.236

Cryotechnologies in animal breeding: EU experience and opportunities for Ukraine's integration

A.O. Bogdaniuk [1], T. Otter [2], M.P. Petrushko [3]
[1] LLC «Institute of Contemporary Veterinary Technologies», Cherevky, Ukraine
[2] TOMO Consultancy, Haaksbergen, the Netherlands
[3] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

In the process of European integration, Ukraine is gradually adapting its legislation to the requirements of the European Union, not only in the economic and administrative spheres, but also in the agricultural sector, in particular animal husbandry. One of the important areas is the use of cryotechnologies for the preservation, selection and transport of genetic material of farm animals — namely sperm and embryos. Considering the growing role of reproductive biotechnologies in the production of productive livestock, harmonising the Ukrainian regulatory framework with European requirements is a necessary condition for expanding international cooperation, exports and quality control in this area. Therefore, the aim of the study was to analyse the regulatory requirements for sperm cryopreservation stations and laboratories working with the production and storage of embryos in EU countries, compare them with Ukrainian regulations and identify ways to adapt Ukrainian legislation for European integration in the field of animal reproductive biotechnology.

The study analysed over 150 scientific publications (articles in Scopus/Web of Science, reports of the European Food Safety Authority (EFSA), FAO), as well as 10 key EU and Ukrainian regulations governing the field of cryopreservation of gametes and embryos in animal husbandry. The main sources are Regulation (EU) 2016/429 (Animal Health Law), Delegated Regulation (EU) 2020/686 and relevant clarifications from the European Commission. For the Ukrainian side, the Law of Ukraine "On Veterinary Medicine", "On Biosafety", as well as orders of the Ministry of Agrarian Policy regulating requirements for reproductive materials were reviewed. A comparative analysis of the structural elements of legislation was carried out, in particular the requirements for infrastructure, personnel, traceability, epizootic status, transport and storage. In the EU, the activities of establishments for the collection, processing and storage of semen and embryos are subject to strict certification and control. The key requirements are: registration of the establishment in the central register, presence of qualified personnel, clear traceability of biomaterial, compliance with biosafety requirements, presence of clean areas, proper documentation, and control of the quality of media and reagents. Ukrainian legislation partly covers these aspects, but needs to clarify concepts, introduce mandatory registration for cryopreservation centres, and update requirements for the transport and labelling of biomaterial. There is also no centralised open register of approved institutions, as implemented in the EU. In conclusion, the sector of gamete and embryo cryopreservation in animal breeding in Ukraine is functioning and has practical applications, but is not currently harmonised with EU legislation. To ensure the export of genetic material and integration into European markets, it is necessary to adapt the regulatory framework and implement European standards for certification, traceability, infrastructure compliance and veterinary control. This will allow the development of a competitive biotechnology industry in compliance with European requirements.

Probl Cryobiol Cryomed 2025; 35(4):236
https://doi.org/10.15407/cryo35.04.236a

Enhancing cryopreservation of organoids via diffusion kinetics analysis using Cryo-Raman microspectroscopy

S. Altmaier [1, 2], F. Stracke [1], I. Meiser [1], J.C. Neubauer [1], H. Zimmermann [1, 2]
[1] Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany
[2] Saarland University, Saarbrücken, Germany

Three-dimensional (3D) organoid cultures derived from human induced pluripotent stem cells (hiPSCs) offer valuable preclinical models with enhanced organ-like functionality compared to 2D cultures (Garreta *et al*., 2021). These organoids serve as model systems for drug screening, disease modelling, and personalized cell therapies, offering a human-relevant alternative to animal models and supporting the reduction of animal testing in line with the 3Rs principles (Verstegen *et al*., 2025). Despite their potential, long-term storage via cryopreservation and consistent supply of ready-to-use organoids remain challenging. A major hurdle is the limited understanding of how cryoprotectants diffuse through organoids (Taylor *et al*. 2019). An insufficient or excessive incubation can lead to internal ice damage or cytotoxicity. In our studies, we investigate the penetration of deuterated DMSO (DMSO-d6), a potent permeable CPA, in hiPSCs-derived neural stem cell (NSC) organoids of varying sizes and ages (Altmaier *et al*., 2024).

The penetration of DMSO into NSC organoids is quantified by monitoring the temporal changes in DMSO concentration at the organoid center using confocal Raman micro-spectroscopy. For detailed descriptions of the organoid culture, the technical setup, and data processing, refer to Altmaier *et al*. 2024.

For the first time, we present a Raman micro-spectroscopy workflow to measure the diffusion rate of DMSO into NSC organoids with diameters ranging from 300 to 1100 µm. Our data show constant diffusion coefficients in organoids up to a diameter of 500 µm. However, larger organoids up to 1100 µm show increased diffusion coefficients. We assume, DMSO diffusion may accelerate in the necrotic core of large organoids, resulting in an increased average diffusion coefficient. Thus, DMSO perfusion times are highly dependent on organoid diameter and must be precisely measured and calculated before cryopreserving organoids using the novel Cryo-Raman micro-spectroscopy technique. This highlights the need to understand CPA uptake kinetics for tissue cryopreservation protocols to ensure proper CPA incubation, preventing both insufficient penetration and overexposure.

Our studies demonstrate that uniform CPA penetration in organoids prior cryopreservation enhances cell survival, preserves functional integrity, and minimizes cytotoxicity after thawing. This is enabled by Cryo-Raman micro-spectroscopy as a novel screening tool for CPA diffusion in 3D cell systems. In the future, this versatile method can also be applied to other 3D cell models and CPA molecules.