
SLTB
2021
NOVEMBER 3–5, 2021
ONLINE MEETING

Welcome to the 57th SLTB Annual Meeting

The COVID-19 pandemic dramatically affected our society on all possible ways. Specifically related to healthcare services and science, the pandemic has brought the importance of cryobiology, which has been shown a significant role in preserving cells, tissues, vaccines, etc., ensuring safety, storage and exchange of precious material for research and clinical applications. Therefore, while we will respect our meeting format, debating several topics related to low temperature biology, we would like to offer a particular focus on cryobiology during the COVID-19 pandemic.

Due to the current situation, we will meet online again, but we hope we have the same successful event we had last year. We created an exciting program with six invited lectures, 13 oral communications, a stimulating debate, and 16 posters. During our coffee breaks, we will have a chat room to our event attendees, providing a great occasion to meet SLTB's old and new members and interact with invited speakers.

Thanks to our sponsors, we will have the best oral communication and best poster awards and our photo competition.

We hope you enjoy this year's meeting program and have the occasion to network and brainstorm on experiences in innovation in low temperature biology.

Christiani A. Amorim

Chair, SLTB

Scientific Committee

Christiani A. Amorim
Glyn Stacey
Alexandra Stolzing
Yuri Petrenko
Alasdair Kay
Janice Vilela
Miroslava Jandova
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Iryna Buriak

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CryoLetters

Invited Speakers

Dr. Nicholas M. Wragg
Keele University, UK

After completing a PhD in Regenerative Medicine, specifically in musculoskeletal tissue engineering co-culture development, Dr. Wragg progressed onto Systems-based Research in Regenerative Medicine Manufacture, studying how closed-loop control can influence the scale-up of cell cultures. Following this, as part of the Horizon 2020 HemAcure international consortium, Nick worked on the processes surrounding the scale-up and storage, through cryopreservation, of a novel Haemophilia A cell therapy, including control of the manufacturing parameters of both processes. After a brief project involving the use of ultrasound to influence cell differentiation, Dr. Wragg obtained a Lectureship in Bioengineering at Keele University, where his research covers the many facets of regenerative medicine manufacturing including storage of functional tissue engineered structures and cell secretions as well as the manufacturing control of therapeutically relevant cell and cell related products.

Dr. Maryna Petrushko
Institute for Problems of Cryobiology and Cryomedicine, Ukraine

Since 2018 to current she has been working as a head of the Department for Cryobiology of Reproductive System in the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Science of Ukraine in Kharkiv. Her main activity is to study effects of cryopreservation on reproductive cell and embryos of human and animals in order to insure safety of the procedure and to develop a new effective method for fertility preservation. Dr. Petrushko is a member of ESHRE, Ukrainian & Bulgarian embryological societies, Member of the Presidium of the Ukrainian Association of Gynecologists and Embriologists (UARM) and Society for Low Temperature Biology and Cryobiology.

Dr. Estefania Paredes
Universidade de Vigo, Spain

Dr. Paredes has a degree in Oceanography from Universidade de Vigo in 2008. PhD in Oceanography in 2014 with several awards and honors from her own university and the Royal Academy of Doctors of Spain as well as a patent. Her PhD focused on the cryopreservation of marine invertebrate early-life stages and the development of applications for Ecotoxicology and Aquaculture. Head of the Marine Biological Resource Functional Preservation Service at the Universidade de Vigo for three years (2017-2020). In 2020 she received a Juan de la Cierva funded position to continue her work on cryopreservation of marine cells and organisms. Currently serves as Associate Professor to the UNESCO chair in Cryobiology. Worked on the development of cryopreservation protocols for over a dozen species of marine invertebrates, over 50 species of marine microalgae, mollusk cancerous cells, mice, zebrafish and yeast.

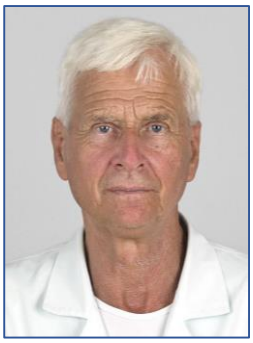
Invited Speakers

Dr. Andrew Thomson
Bristol Centre for Reproductive Medicine, UK

Dr. Thomson is a Consultant Clinical Embryologist at the Bristol Centre for Reproductive Medicine and is a Fellow of the Royal College of Pathologists. In 2020, he was awarded the Claire Gillott prize by Association of Reproductive and Clinical Scientists for outstanding contribution.

Dr. Brian Grout

Retired in 2015 from the Faculty of Life Sciences at the University of Copenhagen and has broad experience in cryobiology from both an academic and commercial perspective. Publications include work with microbial, plant, animal and clinical subjects. A member of SLTB since the mid 1970's and has served as Secretary, Meetings Secretary, Treasurer and Chairman of the Society.



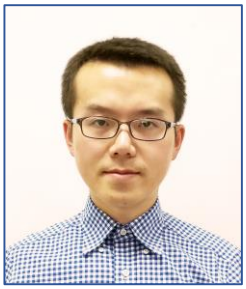
Dr. Pavel Měřička
Tissue Bank University Hospital Hradec Králové, Czech Republic

Dr. Pavel Měřička, M.D., Ph.D. born 28.4 1952, graduated at Faculty of Medicine in Hradec Králové, Charles University in 1976. Medical specialization in patology 1979, in Tissue banking 1989, Ph.D. in Medical biology 2006. Head of the Tissue Bank University Hospital Hradec Králové in the years 1989 - 2017. Research interests: cryopreservation or arteries and veins, haematopoietic progenitor cells, skin, spermatozoa.

Dr. Yuriy Petrenko
Czech Academy of Sciences, Czech Republic

Dr. Petrenko graduated from School of Pharmacy and finished his PhD in 2007 in the Institute of Cryobiology in Ukraine. After several research fellowships he moved to Prague and works in the Institute of Experimental Medicine of the Czech Academy of Sciences as a post-doctoral researcher since 2015. His research interests are mainly connected with the biology of multipotent mesenchymal stromal cells in 2D and 3D culture environment as well as development of novel cryopreservation and non-frozen storage conditions for cells and tissue-engineered grafts.

Invited Speakers

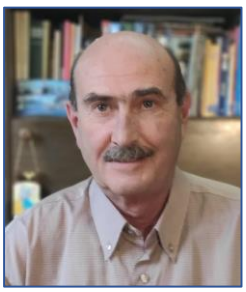


Dr. Min-Rui Wang
Northwest A&F University, China

Min-Rui Wang is a post-doc researcher from College of Horticulture, Northwest A&F University (NWAFU) of China since June 2020. He received his PhD in December 2019 from NWAFU, based on his research experiences on cryogenic techniques for shoot tip cryopreservation, virus eradication/preservation in apple. He also worked for a shallot cryopreservation project as a visiting PhD student at Norwegian Institute for Bioeconomy Research (NIBIO) from March 2017 to January 2019.

Dr. Janice Vilela
Université Catholique de Louvain, Belgium

Dr. Vilela is a researcher at the Gynecology laboratory from Catholic University of Louvain, in Belgium. She holds a Bachelor's degree in Veterinary Medicine, a Master's degree in Compared Animal Physiology and a Ph.D. in Animal Biology. Her research is focused on fertility preservation, conducting studies on ovarian tissue transportation, cryopreservation, transplantation and metabolism of human and other mammalian species.



Dr. Oleksandr Petrenko
Institute for Problems of Cryobiology and Cryomedicine, Ukraine

Dr. Petrenko is director and the head of cryobiochemistry department of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Professor of Biochemisry Department at V. N. Karazin Kharkiv National University. Research into low temperature preservation of cells, tissues and organs for clinical applications; cryopreservation and hypothermic storage of stem cells and tissue engineered constructs. Oleksandr Petrenko has produced so far 3 monographs, 96 publications listed in Scopus, cited 1051 times, with an h-index of 17.

Dr. Anton Puhovkin
Institute for Problems of Cryobiology and Cryomedicine, Ukraine

Dr. Puhovkin graduated from Kharkiv National University and finished his PhD in cryobiology in 2018 in the Institute for Problems of Cryobiology and Cryomedicine, where he currently works as a Senior Researcher.
His research interests are mainly connected with cryopreservation of fish reproductive cells (in particular, the study of the osmotic response of cells to the action of cryopreservation factors). He is currently a biologist of the 26th Ukrainian Antarctic Expedition (2021-2022) at the Ukrainian "Akademik Vernadsky" Antarctic Station, also as a researcher at the State Institution National Antarctic Scientific Center.

WEDNESDAY, NOVEMBER 3, 2021

Welcome and opening remarks

19h00 (CET) Prof Christiani A. Amorim (Brussels, Belgium)
President of the Society of Low Temperature Biology

Session 1 – Opening lectures

Chairs: Prof. Glyn Stacey and Prof. Ramon Risco

19h10 The use of statistical design of experiments in storage solutions discovery
Dr. Nicholas Wragg

19h30 Human oocytes cryopreservation. Is everything already known?
Dr. Maryna Petrushko

19h50 Discussion

20h00 Coffee break

Session 2 – Oral communications

Chairs: Dr. Alasdair Kay and Dr. Miroslava Jandova

20h10 OC1: Cryopreserved cell-based bioconstructs as a clinically relevant model for drug screening
Olena Rogulska, Alexander Petrenko

20h20 OC2: Preparation of Saccharomyces boulardii encapsulated in alginate beads for freeze-drying
Iryna A. Buriak, Igor P. Vysekantsev, Tetiana M. Gurina, Valentyna P. Martsenyuk, Ganna Ye. Ananina

20h30 OC3: Production of mussel (Mytilus galloprovincialis) spat from successive generations of cryopreserved larvae
Pablo Heres, Jesús Toncoso, Estefanía Paredes

20h40 OC4: Reversibility of cold-induced mitochondrial fission and its temperature dependence
Leonard Quiring, Luisa Caponi, Anja Rech, Ursula Rauen

20h50 OC5: Design and implementation of a low cost peltier plate cooling control device for biological systems
Pablo Barroso, Fatima Barroso, Ramon Risco

21h00 OC6: Swim up against density gradient and Z potential sperm selection in the fight for DNA integrity of cryopreserved human spermatozoa
Taisiia Yurchuk, Maryna Petrushko

21h10 OC7: Cryopreservation of peripheral blood progenitor cells for unrelated allogeneic transplantation - experience from the first and second wave of the COVID-19 pandemic
Pavel Měřička, Jiří Gregor, Miroslava Jandová, Miriam Lánská, Doris Vokurková, Karolina Jankovičová, Ilona Fátorová, Pavla Paterová, Jakub Radocha

21h20 Discussion

THURSDAY, NOVEMBER 4, 2021

Session 3 – Debate: Strategies to cope with global crisis
19h00
Moderator: Prof. Barry Fuller

Participants: **Dr. Yuriy Petrenko**
Dr. Andrew Thomson
Dr. Estefania Paredes
Dr. Pavel Mericka
Dr. Brian Grout
Dr Min-Rui Wang

20h00 Discussion

20h40 Coffee break

20h50 AGM meeting

FRIDAY, NOVEMBER 5, 2021

Session 5 – Keynote lectures

Chairs: Dr. Denys Pogozhykh and Dr. Janice Vilela

19h00	The impact of ovarian tissue transportation on assisted reproduction *Dr. Janice Vilela*
19h20	Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine as the SLTB2022 host: current progress and activities *Dr. Oleksandr Petrenko*
19h40	Discussion
19h50	Coffee break

Session 6 – Oral communications

Chairs: Dr. Iryna Buriak and Dr. Milos Faltus

20h00	OC8: Considerations for efficient cryopreservation of tissue-engineered constructs based on heat transfer modelling *Ekaterina Riabchenko, Vitalii Mutsenko, Birgit Glasmacher, Arseny Danilov, Oleksandr Gryshkov*
20h10	OC9: Short-term memory preservation in C. elegans model after cryopreservation *Laura Encabo, Martina Canatelli-Mallat, Daniel Barranco, Enrique Alcalá, Fátima Barroso, Adriana Puentes, Rodolfo Goya, Isabel Risco, Ramon Risco*
20h20	OC10: Optimising post-thaw cryopreserved cell viability through computational simulation *Jack Jennings, Sanja Bojic, Roman Bauer*
20h30	OC11: Validation of Atelerix® for sheep ovarian tissue preservation *Monika Grubliauskaite, Janice Vilela, Christiani A. Amorim*
20h40	OC12: Liquidus Tracking for C. elegans cryopreservation *Adriana Puentes, Fátima Barroso, Enrique Alcalá, Laura Encabo, Ramon Risco*
20h50	OC13: High intensity ultrasound for avoiding devitrification in C. elegans cryopreservation: a step towards organ cryopreservation *Enrique Alcalá, Laura Encabo, Adriana Puentes, Fátima Barroso, Isabel Risco, Ramon Risco*
21h00	Discussion
21h10	**Awards**
21h20	**Closing remarks** **Prof. Christiani A. Amorim** **Prof. Oleksandr Petrenko** **Dr. Anton Puhovkin**, *live from the Ukrainian Antarctic Akademik Vernadsky Station*

Human oocytes cryopreservation. Is everything already known?

Nataliya Buderatska[1], Taisiia Yurchuk[2], Maryna Petrushko[2,3]

[1]IGR-clinic of human reproduction, Kyiv, Ukraine; [2]Institute for Problems of Cryobiology and Cryomedicine of National Academy of Science of Ukraine, Ukraine; [3]ART-clinic of Human Reproduction, Kharkiv, Ukraine

Oocyte cryopreservation is an integral part of assisted reproductive technologies (ART). Improvement of cryobiological methods over the past two decades has made it possible to achieve the preservation of female gametes at a level of about 90%. However, the question of the safety of low-temperature storage methods for the genetic apparatus of human oocytes and obtained embryos is still open. The purpose of our research was to create morphological criteria for evaluating oocytes and, in particular, the meiotic spindle, as well as conducting a molecular-cytogenetic analysis of embryos obtained from cryopreserved oocytes to predict the cryopreservation outcomes of female gametes. We have shown that morphological polymorphisms of oocytes affect their survival rate and number of aneuploid embryos. We have developed the criteria for evaluating the meiotic spindle morphology and localization prior to, and after cryopreservation using polarization microscopy. It was indicated that embryo euploidy depends on the meiotic spindle quality and its location relative to the first polar body. In general oocyte vitrification did not increase the number of aneuploid embryo and (45.9 ± 3.3)% and (44.2 ± 4.1)% of embryos obtained from fresh and vitrified oocytes were euploid, respectively. However, cryopreservation factors have different effect on segregation of individual chromosomes, leading to an increase in aneuploidy of some chromosomes and a decrease in others. Thus, embryos obtained from cryopreserved oocytes had increased aneuploidy rate by chromosome 13 and a decrease aneuploidy rate by non-disjunction of chromosome 18 and heterochromosomes compared with fresh embryos. These results indicate safety of cryopreservation procedure for human oocyte and provide data for embryo cryoselection. The developed morphological criteria for meiotic spindle should be used for making prognosis of oocyte cryopreservation and embryo development during couple consultation.

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Improved plant cryopreservation decreased the cryotherapy effect of virus eradication, and observations regarding impacts of Covid-19 and extreme weather on safe preservation of plant genetic materials in China

Min-Rui Wang

College of Horticulture, Northwest A&F University (NWAFU), China

It has been nearly two years since the first outbreak of Covid-19 in China. As the first to battle with Covid-19, China implemented one of the strictest quarantines in the world, which spent roughly 3 months to basically normalize the situation. Science activities were greatly restricted in the early 2020 but stepwise returned to the normal ever since then. However, floods happened in the following summer in south China, and in 2021, unprecedented floods also affected north China, a more vulnerable area to the excessive rainfall, and caused significant economic losses. All of these issues pose questions as to how could we, as working with cryopreservation, safely preserve the valuable genetic materials under these threats. Over the past three decades, great progress has been made on development new techniques for cryopreserving plant genetic resources. The cryogenic procedures have been also tested for its cryotherapy-effect of pathogen elimination. We recently established and tested a Vitrification cryo-foil protocol based on a Droplet-vitrification method for cryopreserving shoot tips in apple. The tissue surviving was improved in shoot tips cryopreserved by Vitrification cryo-foil, but also reduced the efficiency of virus eradication.

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Impact of the COVID-19 Pandemic on the Donation and Banking Activities of the Tissue Establishment -Experience of the Tissue Bank University Hospital Hradec Králové

Pavel Měřička[1], Jiří Gregor[1], Miroslava Jandová[1,2], Daniel Brandejs[1] Miriam Lánská[3], Jakub Radocha[3] Tomáš Kučera[4], Igor Guňka[5], Malgorzata Grofová[5], Jan Trlica [5] Michael Christian Bartoš [6], Miroslav Špaček [7]

[1]Tissue Bank University Hospital Hradec Králové, Czech Republic; [2]Department of Anatomy, Histology and Embryology, Faculty of Medicine in Hradec Králové, Charles University, Czech Republic; [3]IV[th] Department of Internal Medicine – Haematology, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic; [4]Department of Orthopaedic Surgery, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic; [5] Department of Surgery, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic; [6]Department of Neurosurgery, Faculty of Medicine in Hradec Králové, Charles University and University Hospital Hradec Králové, Czech Republic; [7]2[nd] Department of Surgery- Department of Cardiovascular Surgery 1st Faculty of Medicine Charles University and General University Hospital, Prague, Czech Republic

In the Czech Republic the first wave of the COVID-19 pandemic (March – June) was characterized by the early and efficient prophylactic measures leading to the low frequency of the disease. Early introduction of testing all living and deceased cell, tissue and organ donors was one of the prophylactic measures. The restrictions of the medical care led to postponing of certain surgical procedures, such as total hip replacements, which might have influenced the number of femoral head donations. The super-specialized care including organ and bone marrow transplantations as well as traumatology care remained unrestricted, however. During the second wave (September – December) the Czech Republic was one of the most severely affected countries in Europe. The medical care was influenced by additional factors, such as overload of anaesthesiology and intensive care units and transformation of many clinical departments to the COVID care ones. The abovementioned factors influenced the tissue donation and banking activities of our facility in the different extent. Surprisingly the number of living tissue donors did not decrease. The number of femoral head donors represented 103% of the level of 2019 and of autologous skull bone the 112% of the same year. The number of post-mortem musculoskeletal tissue donors was the most affected, 64% of the year 2019. The number of post-mortem vascular tissue donors decreased to 94%,only, as this type of tissue was harvested at several procurement establishments in the country [1] On the contrary the number of autologous haematopoietic progenitor cell (HPC) donors rose from 26 in the year 2019 to 42 in the year 2020 (196%) and the number of allogeneic HPC donations requiring cryopreservation from zero in 2019 to 19 in the year 2020. We can conclude that situation of the year 2020 led to increase of requirements put on the tissue establishments.

Reference:

1. Špaček M, et al. Organization model for allotransplantations of cryopreserved vascular grafts in Czech Republic. *Cell Tissue Bank*, 2018;19:437-445.

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The impact of ovarian tissue transportation on assisted reproduction

Janice M. V. Vilela

Pôle de Recherche en Gynécologie, Institut de Recherche Clinique et Expérimentale, Université Catholique de Louvain, Brussels, Belgium

In recent years, fertility preservation facilities have adopted 24-hour transportation schedules for ovarian tissue, due to the increasing demand of cryopreservation and the high costs and bureaucracy involved in establishing ovarian tissue cryobanks. Although there have been reports of pregnancies and live births after such long periods of transportation, this remains an empirical procedure. In this presentation, we aim to understand how some patterns of ovarian tissue transport, such as temperature, time and medium, impact fertility in different species. This reflection will be an important step to creating guidelines for future studies on the topic, as well as for use in assisted reproductive procedures.

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Cryobiology research at the Ukrainian Antarctic Akademik Vernadsky station

Anton Puhovkin[1,2], Maryna Petrushko[1]

[1]Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv, Ukraine; [2]State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, Kyiv, Ukraine

One of the areas of research at the Ukrainian Antarctic station, "*Akademik Vernadsky"* (formerly the British "Faraday" station), is biological. The year-round expedition team typically has 1 to 3 biologists who conduct a wide range of research studying the marine and terrestrial ecosystems of the Argentine Islands area in the face of climate change in the region. The infrastructure of the station has been in a state of modernization for several years and conditions are being created for performing various biological researches, in particular those directly related to the field of cryobiology. Among the studies related to cryobiology and initiated at the station in previous years, include, in particular, cryophysiological studies [1], analysis of osmolytes in the blood of fish [2], and so on. One of the projects currently being carried out by the 26th Ukrainian Antarctic Expedition is to study the cryoresistance of Antarctic fish gametes in the Argentine Islands in order to study the mechanisms of cryoresistance of Antarctic fish gametes, to create and optimize methods for long-term low-temperature storage of fish genetic resources. The ichthyofauna of mass coastal water species is represented by several species, the dominant of which are the black rockcod, Notothenia coriiceps Richardson, 1844 and the emerald rockcod, Trematomus bernacchii Boulenger, 1902 [3]. Methods: light microscopy, spectrophotometry, methods of physical and mathematical modeling, cryobiological, etc. The objectives of the project include determining the morphofunctional characteristics of Antarctic fish gametes; determination of sperm viability of Antarctic fish in cryoprotectant solutions; study of the osmotic reaction of sperm, the permeability of cell membranes to water molecules and cryoprotectants; determination of sperm survival at separate stages and after low-temperature preservation.

Reference:

1. Shylo OV et al. Methods of studying the dynamics of some sleep quality indexes in Antarctic winterers. *Ukr Antarkt Z*, 2017;16:188-200; 2. Savytskiy OL et al. Blood osmolytes of fish *Notothenia coriiceps, Chaenocephalus aceratus, Parachaenichthys charcoti* caught near the Argentine Islands, Antarctica. *Probl Cryobiol Cryomed,* 2017;27:367-371; 3.Trokhymets VM et al. The fish fauna of the Argentine islands region (Antarctica; 12 UAE 2007-2008) and morphmetrical changeability of Notothenia coriiceps (Richardson, 1844). *Ukr Antarkt Z*, 2010;9:206-214.

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Oral Communications

Cryopreserved cell-based bioconstructs as a clinically relevant model for drug screening

Olena Y. Rogulska, Alexander Petrenko

Institute for Problems of Cryobiology and Cryomedicine of National Academy of Science of Ukraine, Ukraine

Advances in medicine and pharmacology are closely linked to the development of testing systems that mimic the composite structure of natural tissues. Unique 3D organization and interplay between the cells can be achieved with the use of supporting scaffolds in vitro. Unquestionable benefits of 3D bioconstructs create a strong demand for their steady supply, especially in COVID time, which might be achieved by introducing cryopreservation into the manufacturing pipeline. The study aimed to evaluate the applicability of cryopreserved stem cell-based bioengineered constructs for drug screening. Human mesenchymal stem cells (MSCs) were seeded into collagen scaffolds prepared by cryotropic gelation technique. Scaffolds with cells were cryopreserved using a conventional slow-freezing protocol with 10% Me2SO with or without additional sucrose based pretreatment procedure. Cell morphology and viability within scaffolds were assessed by FDA staining. The metabolic activity of MSCs was estimated by Alamar blue test. DNA concentration of cells was determined using the Quant-iT PicoGreen dsDNA assay. Mitomycin-C was chosen as an effective cytostatic agent to validate the suitability of the proposed testing model. Collagen cryogel scaffolds ensured a favourable environment for MSCs attachment and spreading. Cells were able to proliferate and undergo induced differentiation. Cryopreservation of 3D bioconstructs resulted in the decrease of MSCs viability immediately after thawing. Sucrose pretreatment improved cell survival in scaffolds by 16%±3%. In this group, the metabolic activity of MSCs renewed to control values in 3 days of post-thaw culture. MSCs within cryopreserved bioconstructs had an adequate response to mitomycin action. Screening a wide range of drug concentrations revealed the minimum effective and non-toxic dose for cells with the 3D spatial organization. To sum up, cryopreserved bioconstructs based on MSCs and collagen scaffolds can serve as a relevant model for rapid preliminary evaluation of the safety and efficacy of numerous drugs in development.

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Preparation of Saccharomyces boulardii encapsulated in alginate beads for freeze-drying

Iryna A. Buriak, Igor P. Vysekantsev, Tetiana M. Gurina, Valentyna P. Martsenyuk, Ganna Ye. Ananina

Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

Protocols for treatment of patients with moderate and severe COVID–19 include quite long courses of antibiotics. Impairments in immune system and antibiotic therapy result in dysbiosis occurrence in patients suffering from COVID–19 as well as convalescents. For prophylaxis and treatment of gut dysbiosis various drugs containing live probiotic strains of microorganisms are used. Such drugs are manufactured mostly by freeze-drying of microorganisms. The most advantageous are probiotic drugs of the last generation, namely probiotics and synbiotics, encapsulated (immobilized) in different matrices. Technologies for freeze-drying of encapsulated microorganisms are being developed currently. We studied an impact of technological parameters of procedure for preparing alginate beads and freeze temperature before freeze-drying on viability of encapsulated Saccharomyces boulardii after lyophilization. Yeast cells of S. boulardii were encapsulated in 1% alginate beads by ionotropic gelation. It was found that during the period from the end of encapsulation to the beginning of freezing gel beads lost water and their size and shape changed. The changes in gel beads' structure negatively affected the viability of freeze-dried cells. We considered different parameters of alginate beads' preparing for freeze-drying and defined those providing the highest viability of encapsulated cells. Following technological parameters of the procedure for preparing gel beads for freeze-drying were substantiated experimentally: the temperature of alginate with suspended yeast cells before encapsulation should be 30 °C; cross-linking in 0.2 M calcium chloride should last 20 minutes at 30 °C; the stabilized gel beads before freezing should be stored at 10 °C. Freezing temperature before freeze-drying was from –24 to –27°C.

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Production of mussel (Mytilus galloprovincialis) spat from successive generations of cryopreserved larvae

Pablo Heres, Jesús Toncoso, Estefanía Paredes

Centro de Investigación Mariña, Departamento de Ecoloxía e Bioloxía Animal, Laboratorio de Ecoloxía Costeira (ECOCOST), Universidade de Vigo, Vigo, Spain

We have designed a cryopreservation protocol by studying several parameters of mollusk larval cryopreservation for Mytilus galloprovincialis for scale-up production and long-term effect evaluation of cryopreservation. Toxicity tests, as well as short-term cryopreservation experiments with different combinations of Ethylene-Glycol, (EG), Trehalose (TRE) and Polyvinylpyrrolidone (PVP) were carried out at mussel trochophore stage (18-20 h-old post-fertilization), 48 and 72 h-old D-stage. The effect of increasing cooling and thawing rates were evaluated on cryopreserved trochophores and 72 h-old D-larvae. Moreover, the addition of Sucrose (SUC) dilutions to help CPA removal was studied attending to survival and shell size 24 or 48 h after thawing. Once the protocol was set, long-term experiments were carried out starting from cryopreserved 72 h-old D-larvae. F1 larvae were cultivated until settlement point, then they were cultured in traditional aquaculture rafts in Ria de Vigo (Spain). After two years under these conditions, they were collected to obtain F2 larvae, which were cryopreserved and cultivated until they were able to settle. The final protocol (described in Paredes et al., 2021) produced a 5.24% of competent mussel F1 pediveliger larvae at day 22 of larval rearing, of which 71.27% ± 19.10 were able to settle. F2 larval rearing yielded less than 1% of spat from cryopreserved larvae, beside this, they were completely able to settle whose percentages (35.41 ± - 50.54 ± %), always were higher than controls (22.36 ± 19.76 - 27.76 ± 6.05%, respectively). Cryopreservation produced an initial delay on larval growth, although from day 22 onwards, developed juveniles could grow as fast as control ones and reach commercial size at once. This work has provided an optimized cryopreservation protocol for mollusk larvae which lead to mass production of mussel adults from cryopreserved larvae for the first time.

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Reversibility of cold-induced mitochondrial fission and its temperature dependence

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Mitochondria of different cell types show extensive fragmentation at 4 °C, as used for organ preservation. This process of cold-induced mitochondrial fission is reversible at 37 °C under physiological conditions. Since different methods and temperatures are recently discussed for reconditioning of organs, the temperature dependence of this reversibility and functionality are studied here. Cultured porcine aortic endothelial cells were incubated under hypothermia (4°C) in Krebs-Henseleit buffer with glucose (5 mM) and deferoxamine (1 mM; the latter added to inhibit iron-dependent cold-induced injury). Cells were then rewarmed in culture medium at temperatures between 10 °C and 37 °C for 1 h and 2 h. Marked cold-induced mitochondrial fission was still present after rewarming at 10 and 15 °C. At 21 °C beginning re-fusion was visible, which was more marked at 25 °C. Tubular mitochondria similar to control cells only appeared at 37 °C. ATP content decreased after 48 h at 4 °C from 3.6 ± 0.3 to 1.8 ± 0.6 nmol/10^6 cells and decreased even further when rewarming cells to 10 and 15 °C. From 21 °C upwards a gradual increase was observed with each rewarming temperature. Metabolic activity showed also a gradually increase with increasing rewarming temperatures. It dropped to 4.9 ± 10.8 % at 4 °C and recovered to 76.6 ± 17.2 % at 37 °C rewarming with a plateau at 21 and 25 °C. Only marginal differences were observed in cell viability – with highest cell injury below 7 % of total cells – and in subsequent cell proliferation (at 37 °C), which could be seen throughout. According to these data, rewarming after hypothermia to a temperature of ≥ 25 °C seems advisable, while re-fusion already started at 21 °C, the increase in ATP production is likely a prerequisite for repair processes in organ reconditioning.

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Design and implementation of a low cost Peltier plate cooling control device for biological systems

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Objective: Cryopreservation techniques such as slow-freezing and liquidus tracking, require the control of the cooling profile. Controlled rate freezers are expensive (thousands of euros), heavy and sometimes unfriendly. The design of a low-cost system (less than 100 euros) with simple parts is presented that allows the implementation of any cooling profile in the aforementioned techniques between room temperature and -30degC. Our design can be of utility in student laboratories or as a step forward high throughput methods in cryobiology.
Materials and methods: The cooling system consists of a computer heatsink, a Peltier plate and a thin metal cup, while the temperature control system is made up of a microcontroller, such as an Arduino, a temperature sensor with a thermocouple and a relay. The coolant used was ethanol. The code used was Arduino IDE and the consumption of 60W.
Results: With this device, cooling and warming rates of up to 3 degC/min can be obtained. The system can also be coupled with a perfusion system. The cooling and warming profile does not need to be linear, but any analytical function of the temperature vs. time can be implemented.
Validation: We have carried out liquidus tracking tests where a specific temperature profile is required over time. We used the nematode C. elegans with optimal survival rate results when brought to -25degC.
Conclusion: It is an inexpensive system that allows their replication in student laboratories or the design of high throughput methods.

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Swim up against density gradient and Z potential sperm selection in the fight for DNA integrity of cryopreserved human spermatozoa

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Centrifugation, as part of the active motile sperm fraction (AMSF) isolation prior to cryopreservation, and cryoprotectant removal after warming can increase DNA fragmentation rate. Therefore, a decrease in the number of centrifugation stages can preserve the DNA integrity of cryopreserved spermatozoa and improve assisted reproductive technologies outcomes. The aim of this work was to compare the DNA fragmentation rate in cryopreserved spermatozoa after AMSF isolation by swim up methods, density gradient and in combination with Z potential sperm selection. The study included 20 ejaculates from subfertile men with their informed and written consent. Each sample was divided into 3 aliquots and AMSF was isolated: by layering culture medium followed by swim up (group 1); by density gradient followed by two-fold centrifugation (groups 2); and identical as in group 2, but followed by Z potential sperm selection (group 3). Spermatozoa of all studied groups were cryopreserved by a rapid method using 10% glycerol. After warming the cryoprotectant was removed and AMSF was incubated for 18h at 37 °C. The DNA fragmentation rate was determined by the sperm chromatin dispersion test in a whole ejaculate, after AMSF isolation, freeze-warming, and incubation. It was shown that regardless of the method used for AMSF isolation the number of cells with intact DNA increases in comparison with whole ejaculate ($p<0.01$). At the same time, the largest index was observed in group 3 ($p<0.05$). Cryopreservation and subsequent 18-h incubation of spermatozoa led to DNA fragmentation rate increasing in all groups (the lowest in group 3 and the highest in group 2 ($p<0.05$)). Thus, the use of the swim up method for the isolation of AMSF of subfertile men reduces the DNA damages in cryopreserved spermatozoa after incubation as compared to density gradient. At the same time, the use of Z potential sperm selection makes it possible to select the largest number of cells with intact DNA prior to cryopreservation resulting in the lowest indicator after cryopreservation and incubation.

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Cryopreservation of peripheral blood progenitor cells for unrelated allogeneic transplantation – experience from the first and second wave of the COVID-19 pandemic

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First experience with allogeneic peripheral blood progenitor cells (PBPCs) cryopreservation was gained in the end of the last century. As some failures were reported [1] the most of allogeneic PBPCs transplantation were performed without cryopreservation. The high initial nucleated cells (NC) concentration and long transport were identified as risk factors for the cryopreservation result. During the first two waves of COVID -19 pandemic we performed cryopreservation of 14 allogeneic PBPC concentrates collected at centres inside European Union (EU) in 12 cases, and outside EU in 2 cases. Transport was performed by Meditrans, Ltd., Prague, Czech Republic and processing followed by cryopreservation using clean rooms, 10% DMSO and controlled rate freezing with storage in vapour phase of liquid nitrogen were immediately started. On the next day control samples were thawed for blood count including detailed white blood cells (WBC) differential, CD34+ counts and viability assessment. High post thaw viability of NC (mean 88.86%, SD 6.56%, median 90%), of mononuclear cells (MNC) (mean 97.0%, SD 2.25%, median 98%) and of recoveries of NC (mean 80.87%, SD 11.43%, median 80.7%,) of MNC (mean 94.64%, SD 12.82%, median 100.0%) and of CD 34+ cells (mean 98.92%, SD 27.18%, median 97.4%) was found. The freezing damage was manifested by a decline of repopulation potency CFU-GM recovery (mean 58.58%, SD 21.05%, median 60.3%). All cryopreserved concentrates were released for clinical application: mean post-thaw CD34+ dose/kg of patient's weight 6.31, SD 2.0, median 6.0 x 106, mean post-thaw CFU-GM/kg dose 8.43, SD 2.65, median 8.11., no bacterial contamination. We considered keeping of the EBMT recommendation for initial NC concentration below 200 x 109 /L [2] (mean 197.76, SD 48.37, median 189.97 x 109/L) and the short interval till processing (mean 25.3, SD 6.1, median 26 hours) important factors contributing to good cryopreservation results.

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Considerations for efficient cryopreservation of tissue-engineered constructs based on heat transfer modelling

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Tissue engineering opens up a range of applications of tissue-engineered constructs (TECs), both in regenerative medicine and in preclinical drugs and vaccines testing which is especially popular and necessary at the present time [1]. In order to provide clinics and research facilities with an on-demand availability of TECs, their cryopreservation has to be considered [2]. However, unless optimal parameters are applied, cryopreservation may result in a decrease in cell survivability as well as a deterioration in the mechanical properties of stored materials due to unequal heat and mass transfer [3]. In this regard, computational modelling is a promising instrument for understanding cause-and-effect relationships of cryopreservation-related processes. Establishing the most favorable freezing and thawing conditions would provide efficient preservation to achieve a maximum cell and tissue viability. This study presents heat transfer and thermo-mechanical stress modelling for TECs upon cryopreservation within multiwell culture plates, simulated for different freezing and thawing conditions. The following TECs were considered: macroscopic core-shell alginate hydrogels [3], and polycaprolactone-polylactide electrospun scaffolds [4]. The effects of the cryoprotectants viscoelastic properties, various freezing/thawing protocols (conventional and modified “in air” cryopreservation) and the configuration of multiwell plates on stress values were investigated. Heat transfer and solid mechanics models were coupled to predict spatial temperatures and stresses using COMSOL Multiphysics software. Experimentally measured thermal properties (such as the temperature dependent heat capacity) of cryoprotectants and fiber mats were included as input parameters for the computer modelling. It was established that the growth of stress was related both to the increase of the temperature gradient in a unit volume, as well as the difference in the coefficients of thermal expansion. The considerations for the optimization of cryopreservation protocols were made on the base of performed sets of numerical simulations. The results provide valuable information towards an efficient cryopreservation of TECs.

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Short-term memory preservation in C. elegans model after cryopreservation

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Elucidating the ability of an organism to remember its experiences after being cryopreserved is of great relevance in all those animal models useful in neuroscience. *Caenorhabditis elegans*, a nematode with a complex neural structure and which has been successfully cryopreserved by several techniques in our laboratory, can detect food and chemical odors, being able to associate them and move towards where they are present. In the past, we proved that after cryopreservation a specific type of memory was preserved: the imprinting[1]. In this series of new works, it is proposed to find out if short-term and long-term memory, two types of memory closer to the human species, also retain in *C. elegans* after its cryopreservation. The present study, which assesses the short-term memory of worms, involves the use of hungry adult nematodes, which are fed *Escherichia coli* OP50 in presence of butanone, a volatile chemoattractant. As a cryopreservation method, ultra-fast vitrification was chosen in the SafeSpeed© system. This method achieves vitrification and rewarming speeds of several hundred thousand degrees. Survival rates obtained with it are above 95% in all larval stages, making it ideal for our purposes. In a first learning stage and after being in the presence of butanone, the individuals are placed on a plate without *E. coli*, where a butanone zone and a control zone with ethanol are distinguished. The number of worms displaced towards the zone with deposited butanone is evaluated, giving rise to a learning index, which will be compared with control experiments where *C. elegans* has not been subjected to conditioning. After conditioning, the nematodes were vitrified, stored for several hours in liquid nitrogen, and rewarmed. Upon cryopreservation, we can affirm that short-term memory is preserved by the vitrification method.

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Optimising post-thaw cryopreserved cell viability through computational simulation

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The long-term preservation of cells and tissues represents an exciting prospect for the advancement of biomedical research. However, currently such cryopreservation is challenging above the 1-3mm length scale. This is due to different cell and tissues types requiring various combinations of cooling rates, cryoprotective agents (CPA) concentrations and thawing trajectories. For each individual cell type extensive trial-and-error wet lab work is required to optimise combinations of these factors for experimental protocols.

Thus, we have developed our computational modelling software CryoDynaMo, extending the BioDynaMo biological modelling platform (www.biodynamo.org). Our implementation takes into account multiple factors of the cryopreservation process. These include: cellular membrane and osmotic properties, statistics for intracellular and extracellular ice formation, the simulation of chemical diffusion and heat transfer in 3D space and finally the modelling of intercellular, mechanical forces. This allows us to simulate the response of cells in suspension to a wide variety of cryopreservation protocols and make predictions for post-thaw survival. From these results optimised protocols can be made for use in experimental work.

Here we wish to present two major use cases for CryoDynaMo. Firstly, we present predicted post thaw survival results which show agreement with available experimental literature for multiple cell types. Secondly, we demonstrate the modelling of multi-stepped cooling profiles for Jurkat cells, predicting an increase in cellular post-thaw survival of up to one order of magnitude when compared to standard methodologies.

Overall our findings display the importance of combining in silico and in vitro results for improving cryopreservation procedures. In future, we will further explore complex cooling protocols to investigate further the optimisation of cryopreservation through combined scientific methods. Ultimately, our aim is to demonstrate a hybrid (computational and experimental) approach to improve cryopreservation results as well as achieve faster, more efficient and reproducible cryopreservation protocols.

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Validation of Atelerix® for sheep ovarian tissue preservation

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Ovarian tissue cryopreservation is a widely applied approach for fertility preservation in cancer patients. More than 200 live births have been reported, yielding pregnancy rates of 30-60% and live birth rates of around 40% (1). While much emphasis is placed on improving ovarian tissue procedures (2), the importance of transportation is progressively increasing with the need of sending the tissue from small clinics to centralized ovarian tissue cryobanks. Even though the process has been validated for up to 24h (3), it has some limitations – media used for transportation is not specific for the ovarian tissue and performed at 4°C. Atelerix® is an innovative preservation solution that has been successfully used for shipment and preservation of many different types of cells and tissues. The aim of this study is to validate Atelerix® to preserve sheep ovarian tissue for up to 24 hours at room temperature (RT). Ovaries from six sheep were cut in 5x5 mm pieces and immersed in Atelerix® or IVF medium for 24h at RT or 4°C, respectively. Afterwards they were cultured in vitro for 1h at 37°C and fixed in paraformaldehyde. Stained paraffin sections were used for follicle count and classification [hematoxylin and eosin (HE)] and apoptosis analysis (TUNEL). Tissue transported with IVF medium or Atelerix® showed lower percentage of morphologically normal follicles (MNF) than fresh pieces, while the percentage of MNF was not significantly different between the media. TUNEL showed that, in both media, mainly granulosa cells of secondary and antral follicles did not maintain viability, while primordial and primary follicles stayed alive. These results show that Atelerix® is safe for the preservation of sheep ovarian tissue for 24h at RT, which facilitates transportation in long distance.

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Liquidus Tracking for C. elegans cryopreservation

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The aim of this work is to study the feasibility of the Liquidus Tracking cooling method applied to C. elegans nematodes, microscopic earthworms that are the focus of much research in the field of medicine and biology and have the particular ability to be cryopreserved with considerable ease using well-established methods. However, the survival rate so far observed has always been less than 40% when using traditional methods (slow-freezing). That is why Liquidus Tracking is not presented here as a promising technique for a considerable increase in the survival rate, but also as a model for other biological systems. This technique focuses on following the liquidus line of the H2O - antifreeze (glycerol) phase diagram until temperatures of around -25 ºC are reached, where the cell membranes are no longer permeable, and the metabolism of these living beings is drastically reduced. For this purpose, a controlled rate freezer (Biocool) has been used, which is able to control the temperature of an ethanol bath by drawing a temperature ramp that descends over time following arbitrary values of cooling rate, initial and final temperature. The addition of glycerol is done in steps and manually, at various points along the cooling curve, always seeking a compromise between low toxicity and no ice formation. To contain the nematodes in the bath, a straw about 100mm long and 5mm in diameter, with an unsealed top to add the glycerol, was used. The results are encouraging, in several cases reaching 75% survival of the nematodes.

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High intensity ultrasound for avoiding devitrification in C. elegans cryopreservation: a step towards organ cryopreservation

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Cryobiology has faced heat transfer problems in organ preservation since the first half of the s. XX. The phenomenon of devitrification and the change of state without crystal formation are challenges approached from many disciplines. Rapid and uniform reheating of biological samples is required to minimize the growth of already nucleated ice crystals. This work addresses the experimental use of an innovative technique, based on high intensity focused ultrasound (HIFU), for the recovery of Caenorhabditis elegans in all its growth stages. This technology, which requires a wave generator, a piezoelectric transducer, and a voltage source, was characterized to obtain the different heating speeds according to the supply voltage provided. Warming rates of 120 °C / min have made it possible to recover up to 90% of cryopreserved nematodes using the slow-freezing technique in the presence of glycerol at 15% v / v in S Buffer up to -80 °C. This recovery rate must be compared with the 30% recovery, only of the first larval stages, by means of conventional rewarming, in the control group. The recovered nematodes showed normal development, with their reproductive capacity unchanged. This technique opens new horizons for cryobiology due to its scalability and the possibility of performing a phase and power control to adjust the heating of the desired biological sample. In addition, this technology highlights the usefulness of research-oriented additive manufacturing techniques, especially in the fields of engineering and biomedicine.

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Posters

Low-temperature storage of probiotic strains of gel-immobilized microorganisms with addition of disaccharides and prebiotics

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Intestinal dysbiosis is one of the complications of coronavirus infection COVID-19. Factors, changing the intestinal microbiome quality and quantity in coronavirus patients are the disorders of immune system and antibiotic therapy. Probiotics, symbiotics and products of therapeutic and prophylactic nutrition are used to prevent and treat an intestinal dysbiosis. These drugs comprise living cells of microorganism probiotic strains. The most effective forms of probiotic drugs are microorganisms immobilized in gel carriers. Technologies of their long-term storage are under development. The research aim was to study the effect of disaccharides and prebiotics adding to the gel on viability of immobilized probiotics after storage at different low temperatures. Probiotic cells immobilized in gel granules (E. coli M-17, L. acidophilus, S. cerevisiae, B. bifidum, L. bulgaricus) were stored for 24 months at -20, -40, -80, -196˚C. For immobilization we used 1% alginate gel and 1% alginate gel with adding 10% disaccharides (lactose, sucrose, trehalose), 10% prebiotic (inulin and fructooligosaccharides), and 5% (v / v) skim milk. The number of viable cells during storage was established to be influenced by species characteristics of structure, cell physiology, gel carrier composition, temperature and shelf life. No death of microbial cells during storage at -196°C was found. At -20, -40, -75°C microbial cells died during the entire shelf life. Number of dead cells was reduced with temperature decrease. In gel samples with adding disaccharides and prebiotics, the number of viable cells was higher versus 1% alginate gel. Thus, disaccharides or prebiotics should be added to the gel for providing a low temperature long-term storage of gel-immobilized probiotic strains of microorganisms.

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DNA fragmentation in rat cryopreserved seminiferous tubules in various states of spermatogenesis

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Today, cryobiological technologies are increasingly used in modern reproductive medicine to preserve male gametes. In addition to chromosomal and gene mutations, DNA fragmentation plays a significant role in a problem of infertility. The aim of the study was to compare DNA fragmentation in cryopreserved fragments of seminiferous tubules of testes (FSTT) in various states of spermatogenesis. The study was carried out on FSTT of immature (group 1) and mature (group 2) rats (n=20). Cryopreservation was performed in fibrin gel+6% glycerol in nitrogen vapor to -70°C for 40 min, followed by transfer to liquid nitrogen (-196°C). Heating was performed in a water bath (40°C) with pre-incubation in nitrogen vapor. DNA fragmentation was evaluated by TUNEL staining (BioVision, USA). Apoptotic/necrotic processes were studied using Annexin-V-FITC (Annexin V) (BD, USA) and 7-Amino-Actinomycin D (7AAD) (BD, USA) dyes. Both tests were performed immediately before and after freezing-thawing. Statistical processing was done in Statistica 8 program (StatSoft, USA). DNA fragmentation in groups 1 and 2 after freezing-thawing was respectively 2.2- and 2.8-fold increased compared to the native samples. More cells with DNA fragmentation were observed closer to tubule lumens, and not in the basal layer of spermatogenic epithelium in all studied groups, indicating the survival of spermatogonial stem cell pool. The number of viable cells (AnnexinV-/7AAD-) was 1.7-fold decreased in cryopreserved samples of group 2 compare with group 1. The majority of cells in FSTT group 2 after cryopreservation were characterized by nuclear DNA fragmentation, which is inherent in cells at the stages of necrosis and late apoptosis (Annexin V+/7AAD++Annexin V-/7AAD+). That is, the main cell loss in FSTT of this group occurred as a result of their rapid death. Thus, the obtained data show that FSTT of prepubertal rats are more resistant to the effects of low-temperature preservation factors than samples of adult animals.

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Effects of cryoextracts of placental, umbilical cord and fetal membranes on various cell cultures

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Placenta, fetal membranes and umbilical cord are the promising sources of stem cells. The abundance, the absence of ethical controversy and the absence of donor injuries are among the major advantages of these tissues. The extracts, conditioned media and lysates of these tissues exhibit promising biological activity for potential application in the field of regenerative medicine. Among these forms, application of cryoextracts requires a comprehensive study of characteristic biological effects. The objective of this research was to study the effects of placental, umbilical cord and fetal membranes cryoextracts on a range of cell cultures of different origin. The impact of human placental, umbilical cord, chorionic and amniotic membranes cryoextracts on the metabolic activity of rat fibroblasts, splenocytes, hepatocytes, nerve cells and bone marrow cell cultures was assessed with the MTT assay. The extracts were added to the culture media in a ratio 1 to 9 for 24 hours. The influence of extracts on regeneration was studied with the application of scratch assay on rat fibroblasts cell culture. The anti-inflammatory effect was evaluated by assessing lipopolysaccharide (LPS)-induced reactive oxygen species (ROS) generation in rat leukocytes by flow cytometry. Placental cryoextracts were shown to increase the metabolic activity of the studied cell cultures. The umbilical cord cryoextract enhanced the metabolic activity of fibroblasts, nerve cells, splenocytes, and bone marrow cells. Chorionic extract stimulated the metabolic activity of fibroblasts. Amniotic cryoextract promoted the metabolic activity of fibroblasts, splenocytes, bone marrow cells, reduced the LPS-induced ROS generation in peripheral blood leukocytes. In this study, all the extracts accelerated the recovery of the fibroblast monolayer, evidenced by the scratch assay. Therefore, cryoextracts of placental derivatives improve regeneration and affect the metabolic activity of different cell cultures, opening high perspectives for application in regenerative medicine.

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Phase transformations in cryoprotective media containing nanocrystalline cerium dioxide

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This research was aimed at reducing the negative effect of cryopreservation factors due to recrystallization processes. The Research objects were the combinations of solutions: Ham's F12, 10% FBS, 1% DMSO, NDC at final concentrations of 0.02 and 1 g / L with a 2 nm particle size. The phase states of frozen samples were studied by thermoplastic deformation (TPD). Cooling was carried out at a rate of 20 deg / min, heating - at a rate of 1 deg / min. Deforming stress - 0.66 • 105 kg / m3 or 6 • 105 kg / m3, depending on the investigated temperature range. When 10% FBS and 0.02 g / L, nanocrystalline cerium dioxide (NDC) were added to Ham's F12 the TPD curve shoed melting of eutectic compositions and a less pronounced recrystallization interval before melting of the bulk of ice. With an increase in the NDC concentration in the medium with serum to 1 g / L, there was no temperature range corresponding to the recrystallization rearrangements in the sample in the TPD curve, and just melting of eutectic compositions was observed. In the specimens containing 1% DMSO + Ham`s F12 + 10% FBS without and with adding NDC at final concentrations of 0.02 and 1 g / L, the devitrification proceeded by 10 ° C higher than in DMSO aqueous solution (1%). In the study of recrystallization before the melting of the bulk of ice in these protective media, the already described tendency persisted. But in these cryoprotective media there was no recrystallization occurring in DMSO aqueous solutions before the DMSO eutectic melting. The findings can be used to develop protocols for cell cryopreservation.

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Effect of maternal obesity on cryotolerance of oocytes and embryos

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Increased lipid accumulation in oocytes is a common phenomenon in obesity. It is not known whether elevated lipid accumulation has any detrimental effects on the cryosurvival of oocytes and embryos under such conditions. The present study was designed to understand the association between maternal obesity, ER (endoplasmic reticular) stress and cryotolerance in oocytes and embryos. For the study 3 weeks old female mice were fed with high fat diet (HFD) for 8 weeks. The oocytes from HFD mice had significantly higher lipid accumulation. When these oocytes were vitrified, significant increase in the expressions of GPR78 ($p<0.05$), ATF4 ($p<0.001$) and ATF6 ($p<0.001$) in the frozen thawed oocytes in HFD group compared to the control group oocytes indicating ER stress response was elevated in oocytes form HFD group but the freeze-thaw process did not seem to increase the ER stress. Further, when frozen- thawed 6-8 cell stage embryos showed better cryotolerance to vitrification from HFD group compared to control group. However, expressions of GPR78 ($p<0.001$) and ATF4 ($p<0.001$) was increased in the frozen thawed oocytes in HFD group compared to the control group embryos. Further when these vitrified thawed embryos were cultured up to blastocyst stage, embryos had reduced total cell number and higher DNA damage in the HFD group compared to control group. Significant upregulation in AQP3 ($P<0.05$) expression was observed in the blastocysts derived from obese mice after cryopreservation which may facilitate increased membrane transportation of water and cryoprotectants helping in quick regaining of volume. Overall, the results of the present study indicate that maternal obesity does not have any significant adverse effects on the cryotolerance of oocytes and embryos. However, the quality of blastocysts derived from the obese group is poor following vitrification-thawing process.

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Discovery of osmotic responses of Dunaliella salina cells in plant vitrification solutions

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The unicellular alga Dunaliella salina is unique in its ability to adapt to extreme environmental conditions and β-carotene synthesis. Although D. salina can accumulate large amounts of intracellular glycerol, we did not observe viable samples after cryopreservation with no use of cryoprotectants. Vitrification are based on the cell dehydration by highly concentrated cryoprotective solutions followed by their immersion into liquid nitrogen. The use of plant vitrification solutions (PVS) for cryopreservation of microalgae has been insufficiently studied. There are only a few publications on this issue. Therefore, the aim of the work was to study the effect of the 50% modified PVS1 (22% glycerol, 13% 1,2-propylene glycol, 13% ethylene glycol, 6% Me2SO4, 0,4M sucrose), PVS2, PVSN (15% glycerol, 15% ethylene glycol, 34% sucrose) and 44% PVS3 on halophile unicellular microalgae D. salina. At the first stage of the work, we evaluated the effect of individual components that made up the PVS on the motility, integrity and osmotic reactions of the microalgae D. salina with depending on concentration (1-40%) and exposure time (5-30 min). It was shown that D. salina cells were osmotollerants to the action of the test cryoprotectants during 30 minutes of exposure. The cells also retained their motility and integrity throughout the experiment. Exposure of D. salina cells in PVS showed that the cells did not lose their viability and motility. We noticed no significant changes in the relative cell surface area after 30-minute exposure. Based on the obtained data on high osmotolerance of D. salina cells, the investigated cryoprotectant solutions can be used for cryopreservation of D. salina cells by vitrification. As a result, the probability of intracellular ice formation and damage may decrease.

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Effects of low salinity in combination with cryopretecting agents on sea urchin eggs

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Gametes, embryos and larvae of marine invertebrates have been cryopreserved with different levels of success through the years. Although oocyte cryopreservation is still under research as no successful protocol has been developed yet (Campos et al. 2021). Nowadays, vitrification coupled with ultra-rapid laser warming is another alternative approach to cryopreserve different type of animal cells. However, vitrification initially needs the addition of high concentrations of cryoprotecting agents (CPAs), but in the case of ultra-fast laser warming dehydration of the cells may allow to reduce the concentration of CPAs needed but a heat conductive substance, carbon black (India Ink) need to be into the solution (Jin and Mazur 2015), which dissolves poorly on sea water and forms lumps. The aim of this work is to try and solve these two problems by using sea water with low salinity in combination with different CPAs. By using low salinity water: the cells dehydrate so lower concentration of CPAs is needed (less toxicity) and also, India Ink dissolution may improve. Sea urchin Paracentrotus lividus eggs were used to see the effects of salinity in combination with different CPAs on their viability. Eggs were kept in sea water (SW) with different salinities (45%, 60%, and 100%) for 2 and 5 minutes. We also add two different CPAs (Dimethyl sulfoxide (Me2SO) and Ethylene glycol (EG)) using concentrations of 0.5M, 1M, 1.5M and 2M. Results have shown that exposure to concentrations of CPAs higher than 1M with 45%, 60% and 100% salinity during 2 and 5 minutes had a negative effect on eggs viability, as barely any development was achieved. This study showed that salinity can be lowered until 45% and add 1M of CPA without affecting viability, thus maybe solving the problems associated with vitrification coupled with ultra-rapid laser warming.

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Mesenchymal stem cells conditioned medium as promising compound of preservation solution for long-term liver simple cold storage

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Prolongation of liver simple cold storage (SCS) term is actual trend of cryobiology and transplantology. Enrichment of preservation solution with growth factor and cytokine cocktail is promising way to strengthen organ resistance under hypoxia/re-oxygenation. Earlier we showed that animal pretreatment or supplementation of preservation solution with trophic factors of fetal origin strongly reduced liver damage after SCS and warm reperfusion (WR). The aim of this work – to study the effects of mesenchymal stem cell conditioned media (MSC-CM) added to preservation solution on liver state after CS and WR. Conditioned media were obtained after one day of serum-free human dermal MSC culturing (5-6 passages), concentrated and standardized by protein content. Rat livers were stored in sucrose-based solution (SBS) for 24 h at 4°C without or with bioregulators, and then reperfused during 60 min at 37°C. Screening by ELISA showed presence in conditioned media of HGF, EGF, VEGF, PDGF-BB, NGF-β, SCF, FGF-β, and trace quantity of TNF-α. After storage and following WR, oxidative stress rise, uncoupling of oxidative phosphorylation, ATP level and bile production fall, massive liver cell death, organ architecture disarrangement, and increase of transaminase perfusate activity were observed in SBS-group. Addition of MSC-CM led to decrease of liver TBARS level after WR 2.2 times as compared with SBS-group. Bioregulators prevented mitochondria dysfunction resulting in respiratory control index maintenance almost at level of freshly isolated organs and promoting partial ATP content recovery, which was higher 1.7 times than in SBS-group. Bile flow was 40% faster; activity of perfusate ALT and AST was lower 3 and 2 times respectively. Liver morphological structure was similar to intact organs and a large number of binuclear hepatocytes was found. The pronounced protective effect of MSC-CM on livers during SCS indicates the prospects of their application as compounds of preservation solutions.

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Cryopreservation of neural progenitor cells in three-dimensional aggregates

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The aim of research was to study the effect of three-dimensional microenvironment on the survival of rat fetuses neural stem/progenitor cells (NSPCs) after cryopreservation. Neural cells were isolated from brain tissues of rats fetuses (15-16 days of gestation). Cell viability was 15-70%. The cells were cultured in DMEM/F12 medium (1-4×106 cells/ml) in the presence of serum. The cells samples were frozen under the protection of 10% DMSO in the presence of serum at the rate of 1 °C/min to -80 °C, and then the samples were transferred into liquid nitrogen. DMSO was removed from the thawed samples by washing using centrifugation. During cultivation the viable NSPCs formed multicellular aggregates. After the attachment of aggregates, the cells migrated, spread out and formed a monolayer. With further cultivation, β-tubulin 3-positive cells with neuroblast morphology and colonies of undifferentiated nestin/vimentin-positive cells appeared on the monolayer. Loose aggregates up to 30 μm in size did not attach and disintegrated during further cultivation. Under conditions preventing attachment, the aggregates to form spheroids that increased in size during further cultivation, which indicates NSPCs proliferation. In contrast to cryopreservation of a cell suspension, cells aggregates didn't significantly depend on the initial cell viability. Cryopreservation of aggregates both with high (50-70%) and low (15-30%) viability was the same in its effectiveness. The viability of NSPCs in aggregates after cryopreservation didn't depend on the presence of serum. In turn, serum was an important component for effective cryopreservation of a cell suspension. Cryopreserved aggregates under cultivation conditions behaved similarly to the initial ones. An essential condition for the survival of NSPCs in cryopreserved aggregates was the retention of their morphological integrity. The findings show the formation in the aggregates of conditions conducive to the recovery, survival and effective functioning of rat fetuses NSPCs after their isolation and cryopreservation.

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Effect of dalargin on apoptosis and proliferation of L929 fibroblasts in vitro and dynamics of ionic balance in the content of K+-ions in the serum of rats in vivo during cold stress

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The elucidation of mechanisms of cell injuries caused by a cold stress (CS) resulting from a drop in the ambient temperature from 0°C to +2°C followed by a return to 37°C (normothermia), is an urgent objective in current cryobiology. The search for compounds that can prevent CS-attributed apoptosis and necrosis is of immediate interest. In this regard, the use of neuropeptides, in particular synthetic leu-enkephalin (dalargin), as protectors is promising. The in vitro study was performed on L929 mouse fibroblast cell line. The impact of CS and dalargin was evaluated by morphological parameters, distortion of cell membrane asymmetry and release of cytochrome C into cytoplasm. To assess the proliferative potential of fibroblasts, mechanical damage to the monolayer was modeled as a scratch wound. In vivo study was performed in rats under CS and subsequent return to normothermia. The effect of CS and dalargin on potassium ions content in the serum (ionometry method) were evaluated. The study showed that CS induced the apoptosis in L929 fibroblasts and reduced proliferation in the fibroblast monolayers after mechanical damage. Dalargin was demonstrated to exert a protective effect on proliferation and against apoptosis during CS. Using opioid receptor antagonist naloxone, we revealed that the protective mechanism of dalargin appeared to be due to activation of δ-opioid receptors of L929 fibroblasts, which affected the development of apoptosis. In vivo showed that CS significantly increased in the potassium ions content in the serum and its recovery after return of animals to normothermia. The injections of dalargin to rats before CS led to a decrease in the content of potassium ions in the serum. The protective effect of dalargin can be mediated by regulating the concentration of stress hormones in the blood and reducing the intensity of free radical oxidation which ensures the body's adaptation to the CS.

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Application possibilities of PACAP during embryo vitrification

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The heparin-binding epidermal growth factor (HB-EGF) reaches its expression peak 5-8 days after the ovulation in uterus epithelial cells and plays an important role in regulating blastocyst implantation. Due to the antiapoptotic effect of pituitary adenylate cyclase activating polypeptide (PACAP) and its widespread presence in the organ system, PACAP is considered as a general cytoprotective peptide. The peptide was found in the gonads in high levels, that is what drew attention to the peptide might play a central role in reproduction. The aim of our study is to assess the application possibilities of PACAP treatment during embryo vitrification to the development and the HB-EGF gene expression. BDF1 female mice were superovulated (7.5 IU eCG i.p., followed by 7.5 IU hCG i.p. 48 hours later) and paired with males for a night. The zygotes were collected on the subsequent morning, then they were cultured in G1 medium for 96 hours. After that we examined the developmental stage and vitrificated the blastocyst stage embryos. We treated the embryos with PACAP in two different doses during the vitrification (group 1 and group 2) and after thawing, in the culture medium (group 3 and group 4). After 24 hours culturing, we recorded survival rate and determined the HB-EGF gene expression by qPCR. Our results showed a higher rate of survival and higher level of HB-EGF gene expression in the group of higher concentration of PACAP-treatment during vitrification compared to both the vitrified and non-vitrified control groups, indicating that PACAP treatment during vitrification has a beneficial effect on embryo survival and HB-EGF gene expression, thus on probability of implantation in dose-dependent manner.

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Comparison of the efficiency of different cryoprotectants and vitrification devices for cryopreservation of mouse preantral follicle

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In the field of reproductive science, there has been an increased interest in the application of ovarian preantral follicles as an alternative option for improved reproductive efficiency of domestic animals with poor superovulatory responses. In human medicine, improving follicle culture in vitro of the cryopreserved preantral follicles could be of high relevance to fertility preservation in cancer patients undergoing chemotherapy. The conceptual method for the preantral follicles is to be isolated from the ovaries and vitrified/thawed, then cultured in vitro, and fertilized the ovulated oocyte in vitro. The aim of our research was to examine the viability of preantral follicles after the vitrification process while using different cryoprotectants and holders. The preantral follicles with the diameter of 80-120 µm were mechanically dissected from ovaries of 8 to 12-week-old BDF1 mice. Experiment 1: A selection of the acquired preantral follicles was vitrified, with mouse embryo cryopreservation solution. Before the vitrification procedure, preantral follicles were incubated for 30 minutes in vitrification medium (1-control) or vitrification medium supplemented with (2) retinol or (3) cytochalasin B. Experiment 2: Preantral follicles were vitrified in OPS applying embryo freezing protocol. Live/dead fluorescence viability assay based on metabolic processes, was used to determine the survival rate of the follicles. Retinol has shown a beneficial effect during vitrification process, since significant increase in the survival rate of the follicles in the retinol-treated group was found compared with cytochalasin B. OPS vitrification supports high viability of vitrified/thawed preantral follicles and can be used appropriately for preantral follicle freezing.

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Antiviral activity of cryopreserved human cord blood leukoconcentrate

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The low effectiveness of existing antiviral drugs against the SARS-CoV2 virus necessitates the search for a new strategy to prevent the development and treatment of this infection, using cryotechnologies. The research aim was to study the immunobiological activity of cryopreserved human cord blood leukoconcentrate (cHCBL) and its components during preventive intranasal administration. Human cord blood leukoconcentrate was obtained from healthy women and cryopreserved in autologous plasma according to a two-stage program. The samples were heated at a temperature of 40°C. Plasma and nucleated cells (NCs) were isolated from cHCBL after thawing and centrifugation. Balb / C mice were divided into 6 groups. Six months before infection with influenza virus strain A / Victoria mice of groups 1-3 were intranasally administered with cHCBL, plasma and NCs respectively, 0.05 ml; group 4 comprised the animals with influenza virus at a dose of LD25; group 5 did Laferobion (14 ME 5 times a day for 3 days); group 6 was 0.9% NaCl (0.05 ml). Indices of intact animals served as controls. At 6 months, mice of all groups were infected with influenza virus at a dose of LD100 and the survival of animals was assessed for 14 days. Phagocytic activity was determined in alveolar macrophages; the number of CD11b+ cells was examined by flow cytometry. Intranasal administration of cHCBL to healthy animals was shown to significantly increase the phagocytic activity of alveolar macrophages. This effect was kept for six months, ensuring 86% survival of mice with introduced cHCBL after infection with virus and compared with 72% in those introduced plasma and 43% when using NCs, emphasizing the amplifying effect of each of cHCBL components. Successful experience of prevention of respiratory viral infection with cryopreserved cord blood product allows to predict its possible use in COVID-19.

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Cryopreservation influences goat spermatozoa characteristics in breeding and non-breeding season

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The ability of spermatozoa to fertilize an oocyte and, consequently, form a normal embryo depends on its motility, viability, and DNA integrity. Cryopreserved spermatozoa are effectively used in assisted reproductive technologies of goats on farms. However, many factors during cryopreservation such as centrifugation, equilibration with cryoprotectant, freezing and thawing may negatively affect sperm functions. This might result in reduced motility, viability, and DNA integrity. The aim of this study was to evaluate motility, viability, and DNA fragmentation of goat spermatozoa after cryopreservation in breeding and non-breeding season. Ejaculates from 5 sexually matured goats were obtained during breeding (September-December) and non-breading season (January-March). Immediately after collection total concentration of spermatozoa, motility, viability, and DNA fragmentation were calculated. Following seminal plasma removal each ejaculate was diluted with HEPES based media supplemented with 10% glycerol and 20% egg yolk. Extended semen was equilibrated 15 min at 25°C and loaded into 0.25 mL straws (Minitube, Germany). Then samples were equilibrated 2.5 h at 5°C, placed horizontally 4 cm above liquid nitrogen for 15 min and plunged into the liquid nitrogen. Thawing was performed on a water bath at 37°C for 30 sec. Sperm motility, viability and DNA fragmentation were evaluated after thawing and removing cryoprotectant. Investigated sperm parameters were significantly ($p \leq 0.05$) higher in breeding season than in non-breeding season. Cryopreservation caused a decrease of the sperm motility and viability and an increase of DNA fragmentation rate both in breeding and non-breeding season. However, the difference in motility and viability of cryopreserved sperm in breeding season was not significant. DNA fragmentation of cryopreserved spermatozoa in non-breeding season was 2,5 times higher than in breeding season. In conclusion, motility and viability of goat spermatozoa might be better cryopreserved in breeding season, however, DNA integrity is negatively affected by cryopreservation both in breeding and non-breeding season.

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Cryo-SEM observation of high lipid content seeds stored at low temperature

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There is an increasing interest in extending the germplasm storage capabilities to a wider and more diverse cohort of plants, both wild and cultivated, with agricultural, biotechnological or diversity preservation aims. Among the candidates for preservation are some seeds characterized by both having a significant oil content and suffering unexpected storage aging. When seeds are stored at water contents and temperature conditions under the glass transition temperature of their aqueous solutions, the lack of mobility of water molecules is guaranteeing an absolute halt to any diffusion-driven chemical reaction, while at the same time, ice crystals (if under the freezing point) are not developed. These factors usually ensure indefinite preservation. Some fat-rich seeds, however, show unexplained aging and viability and germination decreases when stored in those conditions. To gain some information on the causes of this storage-aging, oil-rich seeds (peanut: Arachis hypogeae and papaya: Carica papaya) were stored at a range of sub-cero temperatures, after equilibration at different relative humidities (RH). Their temporal evolution was studied by cryo-SEM (low-temperature scanning electron microscopy), a technique that is able to differentiate between cells able to form ice crystals and vitrified ones. Seeds were transferred from storage conditions to the microscope stage quenched in liquid nitrogen, where they were fractured in situ and observed, after etching and metal-coating. The micrographs show volume and tissue structural changes (lipid phase reorganization, alterations of cell wall unions) that can be possible related to lipid changes during storage. This lipid evolution could be related to the aging behaviour observed, either through mechanical effects derived from crystallinity changes or via the release of fat bound water that could be an unexpected ice crystals source.

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CellShip®: shipping cells at ambient, a viable alternative to cryopreservation

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Cell culture is a critical platform for research and industrial processes. However, methods for transporting cells are largely limited to cryopreservation, which is logistically challenging, expensive, and can result in poor cell recovery. Transporting cells at ambient temperatures would alleviate these issues. We have developed CellShip®, a novel, xeno free transportation medium for mammalian cells.

Commercially relevant cell lines, (HEK293, CHO, HepG2, K562, Jurkat, A549 and HeLa) were successfully shipped/stored for 72h-120h at ambient temperature, after which, cells were recovered into standard culture conditions. Viability (%) and cell numbers, were examined, before, following the transport/storage period and following the recovery period. In all experiments, cell numbers had exceeded pre-transport/storage concentrations within 48h of recovery.

When compared to cells revived from cryopreservation, HepG2 and Jurkat cells that had been transported/stored for 72h at ambient, were analysed. Following transport/storage in CellShip® cells recovered more quickly than cells recovered from cryopreservation. Within 48h, HepG2 transported at ambient had become fully adherent and displayed typical growth morphology which was not seen in the cryopreserved samples. Jurkat cells recovered from cryopreservation took >72h to return to pre-cryopreservation cell numbers, whereas cells recovered from transport at ambient demonstrated a 2 fold increase within 48h of recovery, indicating that cells re-entered the cell-cycle with minimal delay.

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