



# CRYO2021

*VIRTUAL MEETING*

THE 58TH ANNUAL MEETING OF  
THE SOCIETY FOR CRYOBIOLOGY

Vitrified condyle (photo by Kezhou Wu, University of Alberta)

**Abstracts** | July 20-23, 2021

to create fistulas, 15 patients underwent aneurysms.

Discussion: Due to the fact that tissue antigen is destroyed by freezing at a temperature of minus 4 degrees, so there is no limit to the use of such biological grafts.

**Funding:** Not applicable

**Conflict of Interest:** None to disclose

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### **P39 FREEZING DAMAGE ASSESSMENT IN EPIDERMAL TISSUE CRYOPRESERVED WITH ANTARCTIC YEAST ISOLATED TYPE1-ANTIFREEZE PEPTIDE**

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Sub-zero injuries in living tissue as a result of re-crystallization phenomenon is the major obstacle and complicates the application of cryopreserved tissues destined for transplantation. Understanding the freezing and thawing response of tissue against the sub-zero temperature would provide better understanding in the application of the non-toxic preservation

technique of living tissues. The aim of this study was to evaluate the potential of antifreeze peptides (Afp1m) as a cryopreservative for living tissue e.g. skin. To determine the effects of cryopreservation on the tissue using Afp1m, type 1 AFP derived peptide was used to cryopreserve skin graft in low concentration of 0.5, 1.0 and 2.0 mg/ml at -10°C or -20°C for 72 hrs. The histological epidermal tissue distortions were measured using a scoring system. To determine the extent of freezing damage experienced in cryopreserved epidermal region of skin grafts. It was found that relatively less microscopic tissue damages occurred at -10°C compared to -20°C at higher AFP concentration among tested concentrations. It is concluded that epidermis of skin tissue is more sensitive towards cryopreservation and experience comparatively more extents of freezing damage at -20°C using these lower concentrations of Afp1m.

**Funding:** Not applicable

**Conflict of Interest:** None to disclose

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### **P40 THE EFFECT OF CRYOIRRIGATION AND CRYOPRESERVED PLACENTA EXTRACT ON THE CONTENT OF NITROGEN MONOXIDE IN THE GASTRIC MUCOSA IN RATS WITH DICLOFENAC SODIUM-INDUCED GASTROPATHY**

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Finding new ways to decrease the ulcerogenic action of nonsteroidal anti-inflammatory drugs is an important task of modern cryomedicine and gastroenterology. It is known that nitrogen monoxide (NO) is a powerful vasodilating

agent able to augment the blood supply to the mucous membranes. As a secondary mediator, NO is involved in the vasodilating effects of the vagus nerve and many other vasoactive substances.

Acute diclofenac sodium-induced gastropathy in male rats was reproduced by a single intragastric administration of diclofenac sodium at a dose of 50 mg/kg. Euthanasia of animals was performed after 24 hours. Cryopreserved placenta extract was administered intramuscularly at a dose of 0.16 ml/kg body weight. The content of NO metabolites in the gastric mucosa was determined by spectrophotometric method, based on the oxidation of nicotinamide adenine dinucleotide phosphate during the reaction of NO formation with L-arginine and measured as light absorption at a wavelength ( $\lambda$ ) of 340 nm.

The study showed that the introduction of diclofenac sodium led to a decrease by 41.5% ( $p < 0.05$ ) of NO metabolite levels in the gastric mucosa homogenates of rats relative to intact animals and amounted to 550 mmol/g of tissue. These findings were consistent with literature data on the ability of nonsteroidal anti-inflammatory drugs to form endogenous nitrogen monoxide resulting from inhibition of NO synthases. Administration of cryopreserved placenta extract resulted in an attenuation of diclofenac-induced decrease in NO content in the gastric mucosa which amounted to 780 mmol/g of tissue, and was only 13.3% lower than that of the intact animals ( $p < 0.05$ ). Cryoirrigation of the gastric mucosa, similar to the introduction of cryopreserved placenta extract, lowered diclofenac sodium-induced decrease in NO metabolites. Combined cryoirrigation and administration of cryopreserved placenta extract led to a statistically significant ( $p < 0.05$ ) increase in NO level in gastric mucosa homogenates, completely eliminating diclofenac sodium-induced changes and amounted to 940 mmol/g of tissue, which was 1.1% higher than the respective value in the intact group of animals.

The obtained data indicate the ability of cryopreserved placenta extract, as well as the action of low temperatures to decrease the diclofenac sodium-induced NO reduction in the gastric mucosa. We suggest this being a mechanism of their gastrocytoprotective action.

**Funding:** The research was funded within the frames of scientific topic "Destructive and restorative processes in tissues *in vivo* after exposure to low temperatures and biologically active substances", with the State registration number of 0117U000849.

**Conflict of Interest:** None to disclose  
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#### **P41 DETECTION AND CHARACTERIZATION OF ANTIFRREZE ACTIVITY FROM *BRASSICA JUNCEA* LEAF CUTICLE**

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Antifreeze proteins (AFPs) possess the ability to lower the freezing point of water and restrict the growth of intracellular ice crystals. AFPs were initially isolated from Antarctic fishes followed by their discovery in insects, fungi, bacteria, and plants. Translation of freezing tolerance from Rabi crops like Brassica to freeze sensitive crops is of significance to avoid freeze injury and subsequent yield loss in crops like legumes. Besides, antifreeze molecules can also be used in the food, biomedical, and petroleum industry. Therefore, this study was aimed to explore the potential AFPs from the cuticle of Brassica juncea leaves. Cuticle proteins enriched fraction identified using nano LC-MS/MS (liquid chromatography-tandem mass spectrometry) was scanned for potential AFPs using CryoProtect server



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Stained full thickness cartilage section after vitrification (photo by Kezhou Wu, University of Alberta)

Program | July 20-23, 2021

Wednesday July 21 (cont.)

	P29 DIRECT (ALKALINE AND NEUTRAL COMET AND TUNEL) BUT NOT INDIRECT METHODS (SCD AND SCSA) RELATE THE PERCENTAGES OF SPERM WITH FRAGMENTED DNA TO CHROMATIN DAMAGE IN CRYOPRESERVED BOAR SPERM	<i>Jordi Ribas-Maynou, Spain</i>
	P30 EFFECT OF GRADIENT VITRIFICATION SOLUTIONS AND TEMPERATURE ON SURVIVAL RATE OF CRYOPRESERVED BLACK SOLDIER FLY EMBRYOS	<i>Idan Alyagor, Israel</i>
	P31 VITRIFICATION OF MOUSE CUMULUS-OOCYTE COMPLEXES SELECTED WITH BRILLIANT CRESYL BLUE; AN IMPROVED PROTOCOL FOR IMMATURE OOCYTE VITRIFICATION	<i>Moslem Mohammadi, Iran</i>
	P32 STATIC MAGNETIC FIELD ASSISTED VITRIFICATION OF MOUSE GV OOCYTES	<i>Sara Soleimani, Iran</i>
	P33 EQUILIBRIUM VITRIFICATION OF MOUSE OOCYTES WITH LOWER CONCENTRATIONS OF CRYOPROTECTANTS	<i>Juan Qiu, China</i>
	P34 LOW-TEMPERATURE PHASE TRANSITIONS IN DORMANT GRAPE BUDS	<i>Olena Bobrova, Ukraine</i>
	P35 CRYOPRESERVATION OF APICAL AND AXILLARY SWEET POTATO MERISTEMS BY VITRIFICATION TECHNIQUES	<i>Anna Mozgovska, Ukraine</i>
	P36 CRYO-LIGHT MICROSCOPY TO STUDY THE FREEZING BEHAVIOR OF MICROALGAE CELLS	<i>Nadiia Chernobai, Ukraine</i>
	P37 INVESTIGATIONS ON GLASSY STATE OF SUGARCANE SHOOT TIPS BY DSC STANDARDIZATION OF PVS2	<i>M. Shankar, India</i>
	P38 USE OF FROZEN VEINS IN VASCULAR AND RECONSTRUCTIVE SURGERY	<i>Seyed Mousavi, Iran</i>
	P39 FREEZING DAMAGE ASSESSMENT IN EPIDERMAL TISSUE CRYOPRESERVED WITH ANTARCTIC YEAST ISOLATED TYPE1-ANTIFREEZE PEPTIDE	<i>Muhammad Shuaib khan, Pakistan</i>
	P40 THE EFFECT OF CRYOIRRIGATION AND CRYOPRESERVED PLACENTA EXTRACT ON THE CONTENT OF NITROGEN MONOXIDE IN THE GASTRIC MUCOSA IN RATS WITH DICLOFENAC SODIUM-INDUCED GASTROPATHY	<i>Fedir Hladkykh, Ukraine</i>
	P41 DETECTION AND CHARACTERIZATION OF ANTIFREEZE ACTIVITY FROM BRASSICA JUNCEA LEAF CUTICLE	<i>Satya Prakash, India</i>
	P42 FIRST SPERM CRYOPRESERVATION PROTOCOLS DESIGNED FOR IBERIAN THREATENED FRESHWATER SPECIES: IBERIAN TOOTHCARP (APHANIUS IBERUS) AND VALENCIA TOOTHCARP (VALENCIA HISPANICA)	<i>Marta Blanes-García, Spain</i>
	P43 DEVELOPMENT OF A PROTOCOL FOR THE CRYOPRESERVATION OF PUFFERFISH (TAKIFUGU ALBOPLUMBEUS) SPERM	<i>Victor Gallego, Spain</i>
	P44 EFFECT OF LOW TEMPERATURE STORAGE IN SEA URCHIN EGGS VIABILITY	<i>Sara Campos, Spain</i>
	P45 EFFECTS OF PENETRATING CRYOPROTECTANTS ON SPERM CRYOPRESERVATION OF PACIFIC ABALONE, HALIOTIS DISCUS HANNAI	<i>Kang H. Kho, South Korea</i>

Thursday July 22

<b>8:00 AM</b>	<b>9:00 AM</b>	<b>LIVE - POSTER SESSION 3</b>	
		P46 EVALUATING THE EFFICACY OF SELECTIVE INHIBITION OF ARACHIDONATE 15-LIPOXYGENASE (ALOX15) DURING HUMAN SEMEN CRYOPRESERVATION IN PROTECTING FREEZE THAW INDUCED SPERM DAMAGE	<i>Shubhashree Uppangala, India</i>
		P47 MITO-TEMPO IMPROVES CRYOPRESERVATION PERFORMANCE OF BULK SEMEN BY CONTROLLING APOPTOSIS RATE, DNA FRAGMENTATION AND ROS PRODUCTION	<i>Reza Masoudi, Iran</i>