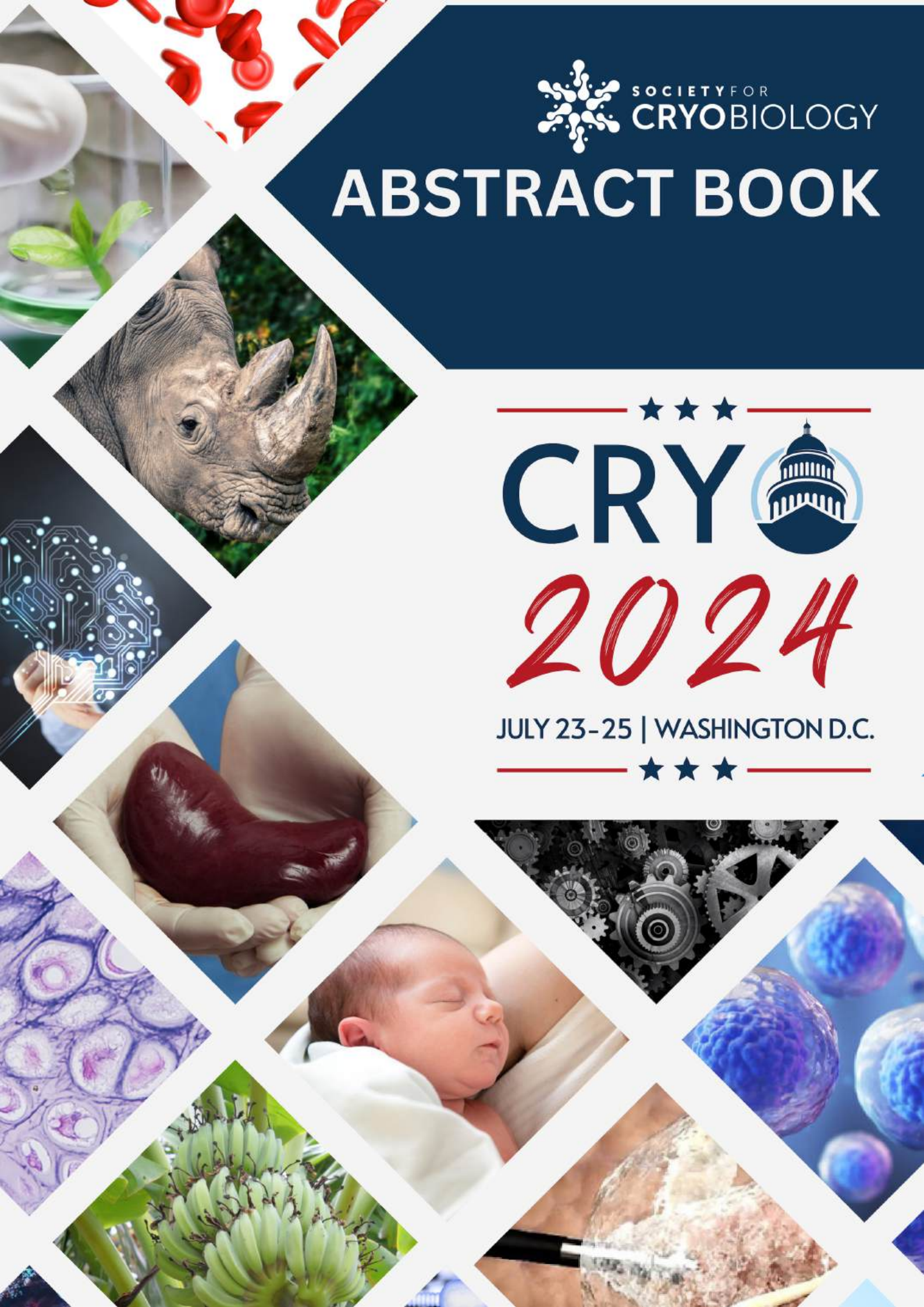


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ABSTRACT BOOK

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Issues related to the mechanisms of biological objects' damage under hypothermic storage (HS) have remained controversial yet. Influence of medium pH and ionic strength on morphological state and stability of erythrocytes during HS was investigated. The study object was erythrocytes from donor's blood, stored for 1–21 days at 4 °C in media of different compositions: electrolyte – 130 mM NaCl or KCl, and non-electrolyte – 260 mM mannitol or sucrose. All studied media contained 20 mM PB and demonstrated a pH value of 5.4; 7.2; 8.6. The erythrocyte shape and degree of their population heterogeneity were analyzed by scanning electron microscopy, followed by examining the morphological index. The hemolysis rate and osmotic resistance of erythrocytes during HS were evaluated spectrophotometrically. A correlation between cell transformation, their osmotic stability, and damage value during HS has been revealed. Regardless of the equilibration medium, minimal osmotic stability was observed at acidic pH values. During HS, cell damage in non-electrolyte media was significantly lower compared to electrolyte media. In the media where erythrocytes were transformed into an echinocyte population, minimal hemolysis rate has been observed. Throughout the entire storage period (21 days) in the media with high and low ionic strength, the hemolysis rate at pH 7.2 remained minimal compared to pH 5.4 and 8.6. The resistance of various erythrocyte forms to a hypotonic condition was analyzed. Analysis of K^+ ions' release from erythrocytes during their HS has shown that a more rapid K^+ loss occurred in non-electrolyte media as well as the first day. For all experimental options, the maximum preservation of intracellular potassium was observed in the media with pH 5.4, moreover its preservation was higher in electrolyte media than in non-electrolyte ones. Cells transformation during storage was accompanied by changes in the membrane ultrastructure, glycocalyx and cytoskeleton.

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P62 TROLOX EFFECTIVENESS IN CRYOPRESERVATION OF HEMATOPOIETIC PROGENITOR CELLS OF HUMAN CORD BLOOD

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The integration of hematopoietic progenitor cells (HPC) into clinical practice has emerged as an efficacious approach for treating a number of pathologies. Human cord blood (CB) stands out as a pivotal source of HPC. The temporal interval between the collection of CB and its administration necessitates the application of cryopreservation technologies, ensuring the preservation of biological integrity over an extended duration. This demands the establishment of a network of cryobanks and the development of specialized low-temperature preservation protocols, optimizing cellular preservation and post-thaw viability rates. During the cryopreservation process, exposure to diverse factors results in an escalation of reactive oxygen species within cells, leading to cell apoptosis or necrosis. Adding substances with antioxidant properties to the cryoprotective medium will avoid or slow down the development of oxidative stress, thereby enhancing cryopreservation

outcomes and increasing the clinical efficacy of HPC preparations. Our investigations demonstrated the efficacy and promise of using trolox, a water-soluble analogue of vitamin E, in the cryopreservation of nucleated human CB cells with 7.5% cryoprotectant Me_2SO. A wide concentration screening made it possible to establish that trolox at concentrations ranging from 50-70 μM exhibited the highest antiradical activity, ensuring the preservation (quantity of whole cells) of nucleated cells up to 88% and viability ($7AAD^+$-cells) up to 75% after thawing, as assessed by flow cytofluorimetry. The content of reactive oxygen species (DCF-positive cells) in these samples was 30% lower compared to the control (trolox-free). The preservation and viability of HPC at these concentrations of trolox was up to 92 and 78%, respectively, which indicated their higher cryoresistance compared to nucleated cells. Conclusively, the obtained results indicate the effectiveness of the use of trolox as an antioxidant in cryopreservation of human cord blood, demonstrating the potential for developing trolox-containing cryoprotective formulations for freezing of human cord blood nucleated cells.

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P63 EFFICIENT MULTI-CRYOPROTECTANT LOADING PROTOCOLS FOR VITRIFICATION OF HUMAN ARTICULAR CARTILAGE

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Vitrification is an ice-free storage method developed for long-term storage of cells and tissues that can be achieved by superfast cooling or in the presence of high concentrations of cryoprotectant agents (CPAs). In the first experiment of this study, we assessed the chondrocyte viability after vitrification-rewarming of 10-mm diameter human osteochondral dowels using two recently designed CPA loading-vitrification-rewarming protocols. In the second experiment, intact human femoral condyles were vitrified using the knowledge gained from the first experiment. To assess the effects of two CPA loading-vitrification-rewarming protocols (Protocol 8 and Protocol 2BWF) on 10-mm diameter human osteochondral dowels, osteochondral dowels from healthy knee joints from deceased donors (n = 3) were used, and post-warming normalized chondrocyte viabilities were assessed. In the second experiment, intact human lateral femoral condyles (n = 3) were vitrified using Protocol 2BWF and normalized chondrocyte viability, metabolic activity, matrix productivity, and histology were experimentally measured. The results of the first experiment showed that 10-mm diameter human osteochondral dowels could be preserved successfully (~80% post-warming normalized chondrocyte viability) using both CPA loading-vitrification-rewarming protocols (Protocol 8 and Protocol 2BWF). Using Protocol 2BWF to vitrify intact human femoral condyles also resulted in similar results when compared to the fresh control group using viability, metabolic activity, matrix productivity, and histology assessments. This study demonstrated that both Protocol 8 and Protocol 2BWF are capable of preserving the quality of post-warmed chondrocytes in vitrified human articular cartilage in small and large segments, and could be promising protocols to be investigated for potential application clinically.