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Dependence of posthypertonic hemolysis of initial state of canine erythrocytes

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The cryoresistance of cells is determined by the action of a combination of exogenous and endogenous factors. The first one is conditioned by the cryopreservation conditions: cooling and thawing rates, cooling temperature of biological specimens *etc*., and the second one is stipulated by the cell structural and functional state. The initial state of cells significantly affects the successful cryopreservation and determines the ability of cells to recover their functional activity after thawing. In particular, the energy status of cells is crucial in the formation of their resistance to the effect of damaging factors under cryopreservation conditions. Posthypertonic shock (PHS) as a model allows studying the effect on cells of cryodamaging factors acting at the thawing stage of erythrocytes.

The research aim was to investigate the sensitivity of ATP-depleted canine erythrocytes to the PHS effect.

Posthypertonic shock was induced by transferring erythrocytes from dehydration medium (1.75 mol/L NaCl) into rehydration one (0.15 mol/L NaCl) at 37 or 0 °C. Erythrocytes were pre-incubated in physiological solution (0.15 mol/L NaCl) with the addition of 3% glucose or with the glycolysis inhibitor 2-deoxyglucose (2-DG, 10 mmol/L) and simultaneously with both modifiers at a temperature of 37 °C, after which the cells were PHS-exposed. The duration of pre-incubation was 120 min. The control was cells that were pre-incubated only in physiological solution. The posthypertonic hemolysis (PHH) level of erythrocytes was determined spectrophotometrically (λ = 543 nm).

It was found that canine erythrocytes were more sensitive to the PHS at a temperature of 37 °C compared to 0 °C. When the cells were pre-incubated in physiological solution at 37 °C (120 min) their sensitivity to the further action of PHS depends on the temperature of the experiment: the PHH of erythrocytes increases at a temperature of 0 °C and does not change at a temperature of 37 °C (compared to cells that were exposed to PHS without pre-incubation, 37 °C). Canine erythrocytes pre-incubated with 2-DG demonstrate a strong temperature dependence of PHS. Energetically depleted canine erythrocytes are more sensitive to PHS at 37 °C. The presence of glucose in the pre-incubation media (with or without a glycolysis inhibitor) leads to an increased PHH level of canine erythrocytes at 0 °C and has no effect at 37 °C. Thus, the results obtained allow the conclusion about the reduced stability of ATP-depleted erythrocytes under PHS conditions at a temperature of 37 °C. The lack of erythrocytes' response at low temperature is probably caused by damage to cells as a result of the development of additional cold shock.

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Encapsulation-based strategies for enhanced ovarian vitrification: experimental design and research rationale

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The cryopreservation of ovarian tissue holds significant potential for preserving fertility; however, existing vitrification methods encounter difficulties related to ice formation and the viability of the tissue after thawing. Surrounding ovarian tissue with biocompatible materials could enhance the permeation of cryoprotectants, offer structural support, and reduce cryoinjury during the vitrification and warming processes. This project seeks to create and assess methods for encapsulating mammalian ovarian tissues before vitrification, based on the hypothesis that encapsulation may improve the delivery of cryoprotectants and enhance tissue integrity upon thawing.

We aim to evaluate different encapsulation materials (collagen) and methods for embedding ovarian tissues. The encapsulated samples will be subjected to standardized CPA equilibration and vitrification processes. We will analyze the tissue morphology through histological assessment. Additionally, we will compare the encapsulated samples with non-encapsulated controls.

We expect that encapsulated ovarian tissues will show reduced ice formation, improved CPA penetration, and better preservation of tissue architecture *versus* non-encapsulated tissues. The research will lay the groundwork for optimizing encapsulation strategies for ovarian cryopreservation.

This research presents a new method to enhance the cryopreservation of ovarian tissue through encapsulation techniques that enhance the delivery of cryoprotectants and reduce cryoinjury. By assessing both encapsulated and non-encapsulated tissues, we intend to establish that encapsulation—especially using collagen—can more effectively maintain tissue integrity and viability throughout the vitrification and warming processes. The expected advancements in morphological preservation will aid in the creation of more dependable and efficient fertility preservation techniques, opening up fresh opportunities for clinical applications within reproductive medicine.