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Evaluation of the effect of various cryoprotectants on the resistance of canine erythrocytes to mechanical stress

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Effective blood cell storage is of great importance in veterinary medicine, especially under cryopreservation conditions, which are widely used in modern biological sample storage practices. One of the most critical aspects of erythrocyte cryopreservation is ensuring high resistance to various stress factors, particularly mechanical and osmotic stress that occur during the freeze–thawing and transportation of biomaterials (Gao *et al*., 2000; Elliott *et al*., 2017). Therefore, the selection of optimal cryoprotectants that can best preserve cell viability is an urgent task.

The aim of this study was to determine and compare the effects of different types of cryoprotectants on the mechanical stability of canine erythrocytes, as well as to identify optimal storage conditions that could improve their clinical use.

The materials of the study were erythrocytes obtained from clinically healthy male dogs aged 2 to 10 years. Blood collection was performed by venipuncture from the cephalic vein into sterile containers containing glucose–citrate preservative. After collection, the blood was stored at 5 °C for no more than 48 hours. Erythrocytes were subsequently isolated by centrifugation at 750 g for 5 minutes, followed by triple washing in phosphate-buffered saline. Five cryoprotectants were studied: glycerol, sucrose, DMSO (dimethyl sulfoxide), polyethylene glycol-1500 (PEG-1500), and hydroxyethyl starch (HES). All substances were used at concentrations ranging from 5 to 20%. The stability of the cells under mechanical stress was assessed by mixing the cell suspensions with 5 mm diameter plastic beads at 1,200 rpm with a magnetic stirrer for 60 minutes (Shpakova *et al*., 2010). Hemoglobin content in supernatant was determined spectrophotometrically (λ = 243 nm).

The results showed that PEG-1500 and HES had the most pronounced stabilizing effects on erythrocyte membranes. The protective mechanism of these substances involves the formation of an additional protective layer around the cells, which reduces the effects of mechanical and osmotic stress (Kameneva *et al*., 2003). Meanwhile, glycerol at concentrations above 10% caused an increase in hemolysis, indicating a negative effect on the stability of cell membrane structures due to increased intracellular osmotic pressure and impaired mechanical flexibility of the membrane (Zhegunov *et al.,* 2010).Sucrose demonstrated a dual effect depending on its concentration: low concentrations (5—10%) showed moderate protective effects, while higher concentrations (15—20%) enhanced hemolytic damage due to significant osmotic stress. DMSO did not cause significant changes in erythrocyte stability at all studied concentrations, confirming its reliability as a universal cryoprotectant for long-term storage (Denysova, 2021).

Thus, this study demonstrates the importance of the proper selection of cryoprotectants and their concentrations to ensure optimal cryopreservation conditions for canine erythrocytes. PEG-1500 and HES appear promising for practical use, while the use of glycerol requires close control due to the risk of increased mechanical damage.

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Cryopreservation of oocytes as a method of implementing reproductive plans in women with cervical cancer

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Cryopreservation of biological material is an integral part of assisted reproductive technologies (ART) (Aarattuthodi *et al*, 2024), helps implement reproductive plans of various categories of patients, in particular, people with cancer (Su *et al*, 2025). Cervical cancer (CRC) is one of the leading causes of death from cancer among women in Ukraine and in the world (Head Center for Disease Control and Prevention of the Ministry of Health of Ukraine, 2025). Preservation of reproductive function in patients with cervical cancer aligns with women's interests and is a component of the state's demographic policy, which argues for the relevance of the chosen direction and aims to analyze approaches to preserving fertility in patients with cervical cancer.

Data from clinical protocols, databases, scientific studies, and population and demographic indicators were analyzed and evaluated.

There is a decrease in the age of cancer manifestation among women of fertile age (NCI, 2023), before they implement reproductive plans. Treatment of cervical cancer includes surgery, chemotherapy, radiation and brachytherapy, a combination of these methods (NCI, 2025; Kumar *et al*, 2025). In cervical cancer specifically, radical trachelectomy combined with oocyte or embryo freezing is considered a fertility-preserving option for women under 40 with stage IA2–IB1 disease (Bentivegna *et al*., 2017). The average oocyte survival rate after vitrification and warming exceeds 85—90%, and the fertilization rate using ICSI ranges from 65 to 75%. The cumulative live birth rate per patient can reach up to 35—40%, depending on the patient's age and number of cryopreserved oocytes (Cobo *et a*l., 2016; Garcia-Velasco *et al*., 2020). Despite the availability of fertility preservation programs, only 2—5% of eligible women with cancer undergo fertility counselling and cryopreservation before treatment (Oktay *et al*., 2018). So, according to the ESHRE (2023) and ASRM (2022) guidelines, oocyte cryopreservation is considered an effective method for fertility preservation in patients with cervical cancer, especially in early stages (IA–IB1).

The development of cryopreservation and assisted reproductive technologies offers promising prospects for preserving reproductive function in women with cervical cancer, giving them a chance at motherhood after treatment.