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Study of physical processes occurring in serum-containing and polymer-based serum-free cryoprotective media

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Objective. To investigate the mechanisms of cryoprotection with compositions that were supplemented with polymers (dextran [Dex], hydroxyethyl starch [HES], polyethylene glycol [PEG]) or fetal bovine serum [FBS]). **Methods.** Differential scanning calorimetry (DSC) and thermomechanical analysis (TMA) of cryoprotective media containing serum, as well as media that do not contain serum or its components but include polymers. **Results.** The combinations of dimethyl sulfoxide with FBS or polymers in cryoprotective media were able to promote the formation of amorphous phase and suppress salt eutectic crystallization/melting and recrystallization.The medium supplemented with 0.7 M Me_2SO and 100 mg/ml Dex (0.7Me_2SO+Dex) was somewhat superior compared to other compositions. It also had an optimal glass transition temperature (–26…–21 °C). The feature correlated with an adequate cell recovery when the medium was utilized to preserve testicular cells. **Conclusions.** The mechanism of cryoprotection depends not only on the concentrations of cryoprotective agents such as Me_2SO, polymers or FBS, but also on the composition of the cryoprotective media. The 0.7Me_2SO+Dex medium can be the basis for the creation of commercial serum-free cryoprotective media with a defined stable composition that does not require removal of Me_2SO before using the cryopreserved cells or tissues for cultivation, transplantation and transfusion.

Keywords: serum-/xeno-free media, dextran, hydroxyethyl starch, polyethylene glycol, differential scanning calorimetry, cryopreservation.

Introduction

Phase transitions, which take place in cryoprotective media, can potentially affect living cells. Classically, two main factors affecting cells can be distinguished during their cooling/heating: high salt concentrations and intracellular ice formation [1]. Both factors result from phase transitions of liquid water into ice. Some additional specific damaging processes may be involved [2].

Cryopreservation of cell suspensions composed of several types of cells requires the development of specific cryoprotective media. The components of the media and other cryopreservation parameters must suit most types of cells. This work was carried out on the testis interstitial cells (ICs) that include the cells of the immune system, Leydig cells and other cells involved in the regulation of testicular homeostasis. Cryopreserved testicular cells and tissues can be used for the preservation of endangered species and lines [3–5]. They can also help to create the germplasm banks. Several works use the testicular cells and tissues for compensation of androgen deficiency in the fertility preservation and reproductive technologies [6, 7].

Some additives to cryoprotective media, such as blood serum or its components, can improve the outcome of cryopreservation. However, these types of media may impose certain limitations in the use of cryopreserved cell suspensions and tissues for laboratory and clinical practice. The first drawback of such serum-containing compositions is the difficulty of their sterilization.The second problem is related to the nature of the serum itself and is caused by the inability to control the composition from batch to batch [8–13, 15, 16]. In our previous work, some serum-/xeno-free compositions were investigated [17]. One of them had an exceptional property in preserving ICs of rat testis. This medium was based on Ham's F12 cultivation medium supplemented with 0.7 M Me_2SO and 100 mg/ml of Dex40 (Me_2SO+Dex). Other serum-free compositions had moderate-to-poor ability to preserve ICs. These media included HES or PEG, respectively. The ability of Me_2SO+Dex40 to preserve ICs was comparable with a serum-containing medium having 1.4 M Me_2SO and 10 % FBS (Me_2SO+FBS). This medium possessed the best cryoprotective property towards ICs among other media that included serum or serum albumin. A similar composition of cryoprotective media was proposed for cryopreservation of testicular cells and tissues [18–20].

The above-mentioned polymers are common non-penetrating extracellular cryoprotective agents [21–24]. Dextran and HES have been used as the plasma volume expanders. They are good antithrombotic agents at a concentration of about 100 mg/ml and are used in clinical practice [25–27]. Unlike other non-penetrating CPAs, such as PEG, they do not affect the blood clotting system and can be used at a clinical grade [21]. Dextran and HES have low antigenic activity and can be metabolized and/or disposed off harmlessly [23, 28].

We hypothesized that if a medium had good cryoprotective properties, the physical events occurring in the medium at cooling/heating should differ from those taking place in the "bad" media. To confirm or refute this state-

ment, a comparative analysis of physical processes assessed by DSC and a thermomechanical analysis have been carried out. The objective of the research is to investigate phase transitions occurring in cryoprotective media and their connections with cryoprotection and cryodamage. The results would potentially contribute to the body of knowledge about the phase transitions during cryopreservation, highlight the benefits of serum-free media and promote their introduction in clinics and laboratory practice.

Materials and Methods

FBS (Biowest, France), БСА (Sigma, USA), ДМСО (Galychfarm, Ukraine), PEG (M.m. 1500 Da,Thermo Fisher Scientific GmbH, Germany) HES (M.m. 200 kDa, Leopold Pharma, Austria), Dex40 (M.m. 40 kDa, Carl Roth, Germany). All cryoprotective agents were dissolved in Ham's F12 (Biowest, France) cultivation media. However, there were no differences in the observed effects if DMEM F12 (Biowest, France) was used.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was carried out using a power compensation differential scanning calorimeter Q20 (TA Instruments, 159 Lukens Drive New Castle, DE 19720 USA). It was equipped with a liquid nitrogen cooling accessory. The samples of 5 mg were placed in 50 μL DSC Q20 aluminum pans and sealed. An empty pan was used as a reference. The cooling/heating rate of 5 °C/min between 20 °C and –50 °C was used. The samples were heated at the same rate as they were cooled. The plot of heat flow versus temperature was recorded.

Heat effects during the DSC analysis were determined at heating Although specific heat units are used for the comparative study, the displayed results are indicative of specific heat changes during DSC analysis rather than of their intrinsic value. The data were processed using OriginPro 2018 software (Northampton, MA01060, USA). Melting temperature was defined as the temperature of the peak on the thermograms. The results for each sample were repeated at least 5 times.

Thermomechanical analysis

The analysis was carried out as described in [17, 29, 30]. The deformability of samples was investigated under the action of external deforming force. Simultaneously, the temperature change was measured. This helped to reveal structural transformations and phase transitions. Briefly, a 0.5-ml sample was placed into a deformation chamber. The sample was cooled at a constant cooling rate of 3 °C/min. As the temperature reached its final value –160 °C, the constant external deforming tension of 0.2 to 0.6×10^5kg/m^2 was applied. After that, the sample was heated at a constant rate of 1 °C/min. The conditions of the assay were formed in a way that the sample was subjected to a regime of thermoplastic deformations. The thermomechanical curves were recorded in coordinates of deformation versus temperature. The temperature intervals, where the phase transitions began, were seen as inflections of the curve. The conditional points of contiguity of the tangents to the thermoplastic curve are the boundaries of the intervals. More linear segments of the curve represented the interval where a particular physical process developed. The thermomechanical analysis

Table 1. **Cryoprotective properties of some serum-free and serum-containing compositions***

Cryoprotective properties	Cryoprotective media	References
Good	0.7Me_2SO+Dex (serum-free)	[17]
	1.4Me_2SO+FBS(serum-containing)	[17, 18, 19, 20]
Intermediate	0.7Me_2SO+HES (serum-free)	[17]
Bad	0.7Me_2SO+PEG (serum-free)	[17]

* The effectiveness of the ability of these media to promote cell survival after cryopreservation is listed from best to worst from top to bottom

helped to clarify and fortify DSC data by avoiding its drawbacks, such as minute values of experimental samples and temperature lags. Each measurement was repeated at least three times.

Statistical analysis

All data were represented as a median (25th percentile; 75th percentile) throughout the text. The data were ranked, and the Newman-Keuls test revealed the differences between the groups. Some data were also double-checked by the Mann–Whitney U-test with Bonferroni correction.

Results and Discussion

According to the previous data, the investigated cryoprotective compositions can be subdivided into several groups in terms of their ability to promote IC recovery after cryopreservation: (1) good cryoprotective properties, (2) intermediate and (3) bad ones (Table 1). Changing the concentration of the components of the media (Me_2SO, polymers, FBS) did not improve the cell recovery and could even lead to worse results [17].

First, the physical events occurring in these media were investigated by DSC. Ham's F12 media had two detectable phase transitions: water crystallization/melting and salt eutectic crystallization/melting (Fig. 1A). The influence of the supplements on the amount of ice formed was assessed by heat effects during heating (Table 2). It can be seen that Me_2SO and polymers as well as their combinations reduced the amount of ice formed.

Salt eutectic crystallization and melting in Ham's F12 occurred at about –40 and –22 °C, respectively. The addition of 0.7 M Me_2SO also resulted in the pronounced salt eutectic

Table 2. **Heat effects of ice melting measured by DSC, median (25th, 75th percentiles)**

Medium	Enthalpy, J/g
Water	261 (254; 265)
Ham's F12	283 (277; 289)[1]
Ham's F12, 10 % FCS	273 (269; 278)
Ham's F12, 0.7 M Me_2SO	209 (201; 213)[2]
Ham's F12, 1.4 M Me_2SO	157 (149; 171)[2]
Ham's F12, 100 mg/ml Dex	241 (235; 246)[2]
Ham's F12, 100 mg/ml HES	253(240; 270)[2]
Ham's F12, 100 mg/ml PEG	260(249; 262)[2]
1.4Me2SO+FBS	155 (140; 151)[2]
0.7Me2SO+Dex	190 (185; 196)[2,3]
0.7Me2SO+HES	188 (181; 197)[1,2,3]
0.7Me2SO+PEG	210 (202; 215)[1,2,3,4]

[1] the values were statistically different from water;
[2] the values were statistically different from Ham's F12;
[3] the values were statistically different from 1.4Me_2SO+FBS;
[4] the values were statistically different from 0.7Me_2SO+Dex

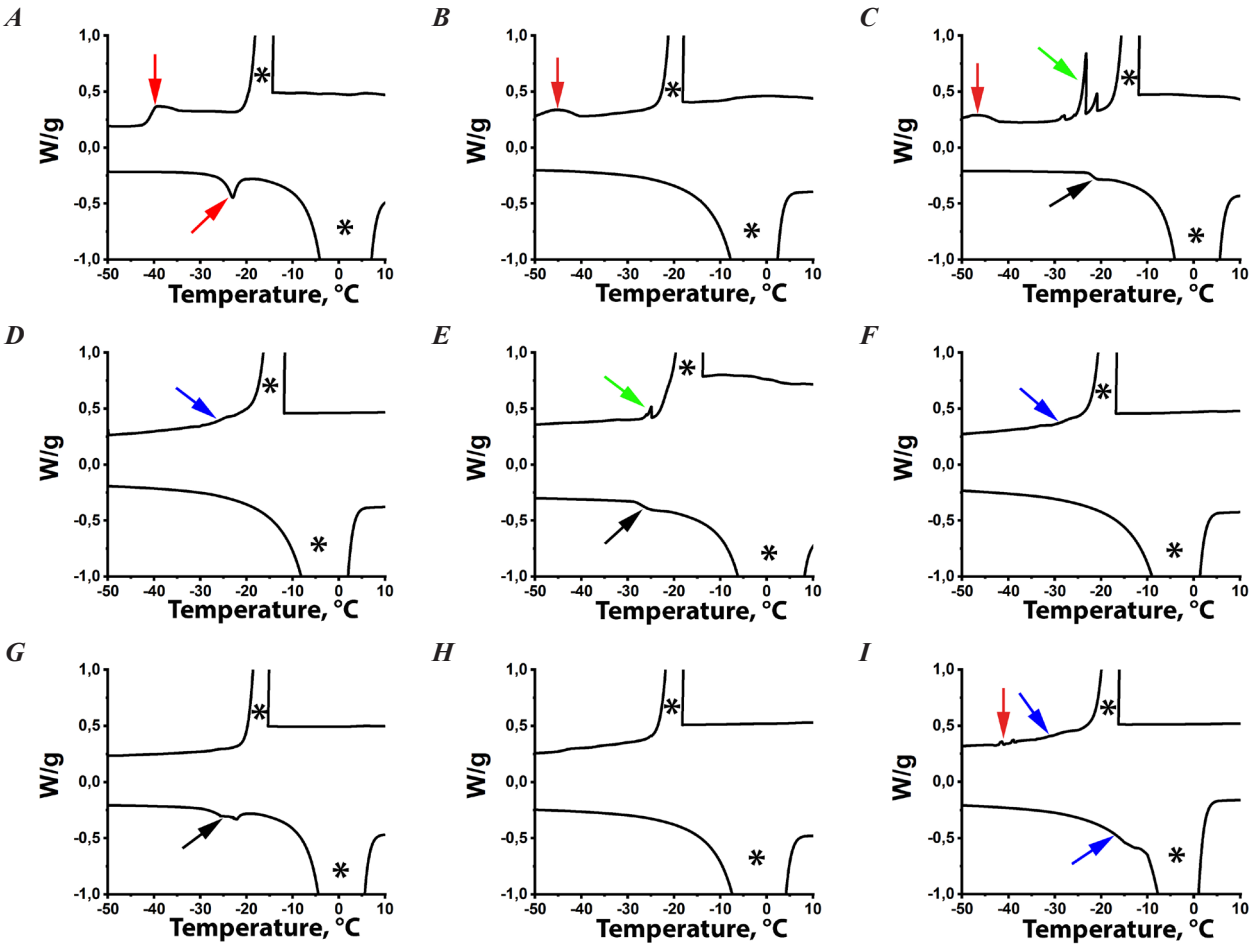

Fig.1. DSC of Ham's F12 medium.

(***A***) Ham's F12 media; (***B***) Ham's F12 with 0.7M Me_2SO; (***C***) Ham's F12 with 100 mg/ml Dex40; (***D***) 0.7Me_2SO+Dex; (***E***) Ham's F12 with 100 mg/ml HES; (***F***) 0.7Me_2SO+HES; (***G***) Ham's F12 with 100 mg/ml PEG; (***H***) 0.7Me_2SO+PEG; (***I***) 1.4Me_2SO+FBS. Black asterisks — water crystallization/melting peaks; Red arrows — salt eutectic crystallization/melting; Green arrows — peaks at –20 … –30 °C (phase transitions involving Dex40 or HES in the space between ice crystals); Black arrows — glass transitions and/or salt eutectic in Ham's supplemented with polymers but devoid of Me_2SO. Blue arrows — amorphous phase solidification, colloid glass formation or, in case of 1.4Me_2SO+FBS, protein amorphous aggregation/disaggregation, liquid-liquid phase separation. Upper curves are recorded on cooling, and lower on heating.

crystallization event at –40 … –50 °C, but the eutectic melting was not seen on DSC thermograms (Fig. 1B). The addition of Dex or HES to Ham's F12 resulted in some additional peaks at –20 … –30 °C on cooling and/or changes in heat capacity at –22 and –25 °C on heating, respectively (Fig. 1C and E). The nature of these peaks at –20 … –30 °C is not

clear. It would probably be linked with phase transitions involving Dex or HES in the space between ice crystals. These peaks were unlikely caused by salt eutectic crystallization because salt eutectic crystallization on cooling regularly occurred at a lower temperature. The changes in the heat capacity for Ham's F12 supplemented with Dex or HES would result from glass transitions. The glass transitions were reported for the media containing Dex or HES alone or in combination with Me_2SO [17, 24]. 0.7Me_2SO+Dex and 0.7Me_2SO+HES did not show any peaks at –20 … –30 °C but would represent some changes in the heat capacity in the same temperature interval on cooling/melting (Fig. 1D and F). The changes were connected with the phase transitions taking place in the space between ice crystals, such as amorphous phase solidification or colloid glass formation. As for Ham's F12 supplemented with PEG, the events at –25 … –20 °C would be caused by some eutectic processes in the space between ice crystals (Fig. 1G). However, 0.7Me_2SO+PEG did not display any of the events during DSC except water crystallization/melting (Fig. 1H). In our previous work, we reported amorphous solidification that could take place in the media containing FBS [17]. Figure 1I shows that crystallization of solutes might occur in 1.4Me2SO+FBS at the temperature below the amorphous solidification on cooling.

In the final step of our work, we tryed to correlate DSC data with the data obtained by TMA. Bends on the curve indicate sharp changes in the deformability of the samples (Fig. 2). They are associated with ongoing phase transformations. When the curve becomes more horizontal, it is associated with a decrease in deformability, which in turn points to the coarsening of phase(s), for example during crystal enlargement. When the curve be-

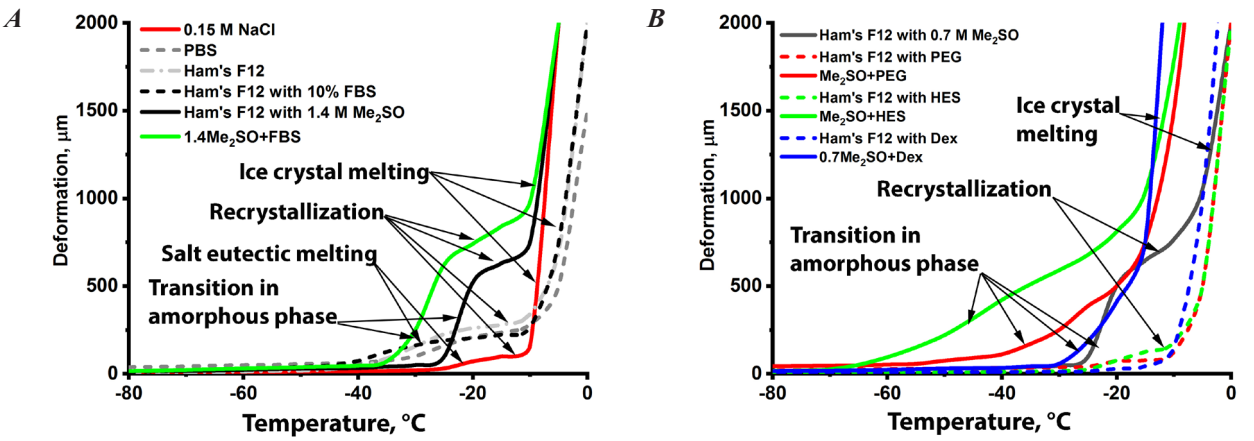

Fig. 2. Thermomechanical analysis of salt solutions and cryoprotective media on heating.

A — 0.15 M NaCl, PBS, Ham's F12, Ham's F12 with 10 % FBS or 1.4 M Me_2SO, 1.4Me_2SO+FBS; ***B*** — Ham's F12 with 0.7 M Me_2SO; Ham's F12 with 100 mg/ml HES; 0.7Me_2SO+HES; Ham's F12 with 100 mg/ml Dex; 0.7Me_2SO+Dex; Ham's F12 with 100 mg/ml PEG; 0.7Me_2SO+PEG

comes more vertical, it, on the contrary, indicates a decrease in the hardness of the phase(s).

In order to correctly interpret the observed events, a relatively simple two-component 0.15 M NaCl-water solution was examined first (Fig. 2A). The phase transitions for the solution are well described. The TMA curve for 0.15 M NaCl also represents the salt eutectic melting at –23 … –20 °C, and, additionally, recrystallization event at –16 … –12 °C and active ice melting starting at about –10 °C. The presence of other solutes, such as potassium and phosphate ions, in PBS, shifted the beginning of salt eutectic melting towards lower temperatures of –40 … –35 °C. Other solutes present in Ham's F12 or Ham's F12 supplemented with 10 % FBS further reduced the onset of eutectic melting to –43 °C. This was expected as the components of the solutions had lower temperatures of salt eutectic melting and could contribute to the amorphous phase formation [31, 32]. The processes of salt eutectic melting smoothly merged with the recrystallization process which was especially noticeable at –25 … –12 °C in PBS, Ham's F12 with and without FBS. The latter process was followed by active ice melting. Despite this reduction in the temperature of salt eutectic with the complication of the medium compositions, the processes occurring in them were similar in general. Apart from the above-mentioned solutions, Ham's F12 supplemented with 1.4 M Me_2SO and 1.4Me_2SO+FBS had an unusually prominent change in deformability at about –25 and –35 °C, respectively. These sharp decreases in the hardness of the phase(s) in between ice crystals point to softening of the amorphous phase rather than salt eutectic melting. The formation of the amorphous phase was enhanced in 1.4Me_2SO+FBS. Both Me_2SO and FBS can contribute to the amorphous phase formation [32, 33, 34]. This transformation of solid amorphous phase (glass) into liquid one and/or salt eutectic melting in Ham's F12 supplemented with 1.4 M Me_2SO and in 1.4Me_2SO+FBS were followed by recrystallization at –20 … –12 and –25 … –12 °C, respectively.

The addition of Dex, HES or PEG to Ham's F12 resulted in an increase in deformability at –23 … –20 °C, identified as salt eutectic melting (Fig. 2B), which was not clearly shown by DSC. This event was counterbalanced by recrystallization at –16 … –12 °C. The recrystallization was followed by progressive ice crystal melting started at about –10 °C. The presence of 0.7 M Me_2SO in 0.7Me_2SO+Dex, 0.7Me_2SO+HES and 0.7Me_2SO+PEG resulted in a sharp decrease in the hardness of the phase(s) in between ice crystals due to the softening of the amorphous phase started at about –30, –65 and –60 °C, respectively. Interestingly, the use of 0.7 M Me_2SO without these polymers can cause pronounced recrystallization at –16 … –12 °C, while in the combined media supplemented with Dex40, HES or PEG, recrystallization was not seen by TMA.

It has been shown that both serum-containing 1.4Me_2SO+FBS and serum-/xeno-free media supplemented with polymers (0.7Me_2SO+Dex, Me_2SO+HES and Me_2SO+PEG) could promote the formation of the amorphous phase. Its formation, in turn, inhibited salt eutectic crystallization/melting. Interestingly, notwithstanding Me_2SO can contribute to the amorphous phase formation, DSC and, especially, TMA, Me_2SO alone cannot

inhibit the process. The amorphous phase formation is generally considered a good prognostic factor for promoting cell survival [35]. However, the work has shown that not all polymers were equally effective in terms of cell recovery after cryopreservation. One of the factors that can cause such a distinction is the ability of the polymers to regulate the flow of liquid in and out of cells during cryopreservation. It was shown that optimal temperatures of the transition from the solid amorphous phase to the liquid phase, or glass transition temperatures, are required. We can see that the transition in 0.7Me_2SO+Dex occurs in the interval of –30 to –20 °C, which is much higher compared to 0.7Me_2SO+HES and 0.7Me_2SO+PEG. According to Takahashi T. and co-authors [24], too low temperatures of glass transitions could cause excessive extracellular crystal growth and promote the so-called "solution effect" (the adverse effect of hyper-concentrated solution that surrounds ice crystals and cells), while too high glass transition temperatures can prevent water exit from cells because of too high viscosity and, thus, cause intracellular ice formation in cells. The use of 1.4Me_2SO+FBS could also promote cell recovery due to the above-mentioned effects [17]. However, its mechanism of cryoprotection was fulfilled at the expense of serum proteins and a higher Me_2SO concentration. The cryoprotective agent is known for its toxicity. This means that the use of compositions having lower Me_2SO (<1.4 M) concentration can promote the introduction of the media into clinics and veterinary. Moreover, in 0.7Me_2SO+Dex, the processes of recrystallization were less intensive compared to 1.4Me_2SO+FBS, which could also contribute to the survival of cells. In our work [17], we have shown that this dextran-based medium was somewhat superior to its serum-containing counterpart in promoting cell recovery.

Conclusions

Physical processes occurring in cryoprotective media were investigated by DSC and TMA. The work has shown that the detected physical events are in good correlation with the effectiveness of cryoprotective media. Serum-containing and serum-free media were able to promote the amorphous phase formation, and they inhibited salt eutectic in the space between ice crystals. However, 0.7Me_2SO+Dex medium was superior because it had an optimal temperature at which the transition from solid amorphous state to liquid took place (the interval of –30 to –20 °C). A similar mechanism of cryoprotection was shown for the serum-containing 1.4Me_2SO+FBS medium, but the medium less effectively deterred recrystallization. Plus, it had a higher Me_2SO concentration (1.4 M) and included serum that could contain infections and a fluctuated unstable composition. Therefore, 0.7Me_2SO+Dex can be the basis for the creation of commercial serum-free cryoprotective media with a defined composition that does not require removal of Me_2SO before use of the cryopreserved cells or tissues for cultivation, transplantation and transfusion.

Declaration of competing interest

The authors declare no conflict of interest.

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REFERENCES

1. *Mazur P, Leibo SP, Chu EH.* A two-factor hypothesis of freezing injury. *Evidence from Chinese hamster tissue-culture cells. Exp Cell Res.* 1972; **71**(2):345–55.
2. *Pribor DB.* PVP contrasted with dextran and a multifactor theory of cryoprotection. *Cryobiology.* 1974; **11**(1):60–72.
3. *Hutka M, Smith LB, Mitchell RT.* Xenotransplantation as a model for human testicular development. *Differentiation.* 2017; **97**:44–53.
4. *Morrell JM, Mayer I.* Reproduction biotechnologies in germplasm banking of livestock species: a review. *Zygote.* 2017; **25**(5):545–57.
5. *Schlatt S, Honaramooz A, Boiani M, et al.,* and *Dobrinski I.* Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. *Biol Reprod.* 2003; **68**(6):2331–5.
6. *Holoch P, Wald M.* Current options for preservation of fertility in the male. *Fertil Steril.* 2011; **96**(2): 286–90.
7. *Kirpatovskii VI, Efremov GD, Frolova EV.* Ectopic Organogenesis after Allotransplantation of Freshly Removed or Cryopreserved Neonatal Testicle under the Renal Capsule in Rats. *Bull Exp Biol Med.* 2018; **166**(2):268–73.
8. *Dinkins MB, Brackett BG.* Chlortetracycline staining patterns of frozen-thawed bull spermatozoa treated with beta-cyclodextrins, dibutyryl cAMP and progesterone. *Zygote.* 2000; **8**(3):245–56.
9. *Dokras A, Sargent IL, Redman CW, Barlow DH.* Sera from women with unexplained infertility inhibit both mouse and human embryo growth in vitro. *Fertil Steril.* 1993; **60**(2):285–92.
10. *González Hernández Y, Fischer RW.* Serum-free culturing of mammalian cells--adaptation to and cryopreservation in fully defined media. *ALTEX.* 2007; **24**(2):110–6.
11. *Hinckley GT, Johnson CJ, Jacobson KH, et al.,* and *Pedersen JA.* Persistence of pathogenic prion protein during simulated wastewater treatment processes. *Environ Sci Technol.* 2008; **42**(14):5254–9.
12. *Kemmann E.* Creutzfeldt-Jakob disease (CJD) and assisted reproductive technology (ART). *Quantification of risks as part of informed consent. Hum Reprod.* 1998; **13**(7):1777.
13. *Leung PC, Gronow MJ, Kellow GN, et al.,* and *Johnston I.* Serum supplement in human in vitro fertilization and embryo development. *Fertil Steril.* 1984; **41**(1):36–9.
14. *Léveillé MC, Carnegie J, Tanphaichitr N.* Effects of human sera and human serum albumin on mouse embryo culture. *J Assist Reprod Genet.* 1992; **9**(1):45–52.
15. *Snyman E, Van der Merwe JV.* Endotoxin-polluted medium in a human in vitro fertilization program. *Fertil Steril.* 1986; **46**(2):273–6.
16. *Zhou EH, Trepat X, Park CY, et al.,* and *Fredberg JJ.* Universal behavior of the osmotically compressed cell and its analogy to the colloidal glass transition. *Proc Natl Acad Sci U S A.* 2009; **106**(26):10632–7.
17. *Pakhomov O, Gurina T, Mazaeva V, et al.,* and *Bozhok G.* Phase transitions and mechanisms of cryoprotection of serum-/xeno-free media based on dextran and dimethyl sulfoxide. *Cryobiology.* 2022; **107**:13–22.
18. *Keros V, Hultenby K, Borgström B, et al.,* and *Hovatta O.* Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. *Hum Reprod.* 2007; **22**(5):1384–95.
19. *Keros V, Rosenlund B, Hultenby K, et al.,* and *Hovatta O.* Optimizing cryopreservation of human testicular tissue: comparison of protocols with glycerol, propanediol and dimethylsulphoxide as cryoprotectants. *Hum Reprod.* 2005; **20**(6):1676–87.
20. *Chen GR, Ge RS, Lin H, et al.,* and *Hardy MP.* Development of a cryopreservation protocol for Leydig cells. *Hum Reprod.* 2007; **22**(8):2160–8.
21. *Bakaltcheva I, Ganong JP, Holtz BL, et al.,* and *Reid T.* Effects of high-molecular-weight cryoprotectants on platelets and the coagulation system. *Cryobiology.* 2000; **40**(4):283–93.
22. *Prickett RC, Marquez-Curtis LA, Elliott JA, McGann LE.* Effect of supercooling and cell volume on intracellular ice formation. *Cryobiology.* 2015; **70**(2):156–63.
23. *Stolzing A, Naaldijk Y, Fedorova V, Sethe S.* Hydroxyethylstarch in cryopreservation - mechanisms,

benefits and problems. *Transfus Apher Sci.* 2012; **46**(2):137–47.
24. *Takahashi T, Hirsh A, Erbe E, Williams RJ.* Mechanism of cryoprotection by extracellular polymeric solutes. *Biophys J.* 1988; **54**(3):509–18.
25. *Abir F, Barkhordarian S, Sumpio BE.* Efficacy of dextran solutions in vascular surgery. *Vasc Endovascular Surg.* 2004; **38**(6):483–91.
26. *Nolan J.* Fluid resuscitation for the trauma patient. *Resuscitation.* 2001; **48**(1):57–69.
27. *Sun G, Mao JJ.* Engineering dextran-based scaffolds for drug delivery and tissue repair. *Nanomedicine (Lond)*. 2012; **7**(11):1771–84.
28. *Pribor DB, Pribor HC.* Studies with dextran 40 in cryopreservation of blood. *Cryobiology.* 1973; **10**(2):93–103.
29. *Gurina TM, Pakhomov AV, Kyryliuk AL, Bozhok GA.* Development of a cryopreservation protocol for testicular interstitial cells with the account of temperature intervals for controlled cooling below –60°C. *Cryobiology.* 2011; **62**(2):107–14.
30. *Gurina TM, Pakhomov AV, Polyakova AL, et al.,* and *Bozhok GA.* The development of the cell cryopreservation protocol with controlled rate thawing. *Cell Tissue Bank.* 2016; **17**(2):303–16.
31. *Han B, Bischof JC.* Thermodynamic nonequilibrium phase change behavior and thermal properties of biological solutions for cryobiology applications. *J Biomech Eng.* 2004; **126**(2):196–203.
32. *Uversky VN, Finkelstein AV.* Life in Phases: Intra- and Inter- Molecular Phase Transitions in Protein Solutions. *Biomolecules.* 2019; **9**(12):842.
33. *Meneghel J, Kilbride P, Morris JG, Fonseca F.* Physical events occurring during the cryopreservation of immortalized human T cells. *PLoS One.* 2019; **14**(5):e0217304.
34. *Pullara F, Emanuele A, Palma-Vittorelli MB, Palma MU.* Protein aggregation/crystallization and minor structural changes: universal versus specific aspects. *Biophys J.* 2007; **93**(9):3271–8.
35. *Klbik I, Čechová K, Maťko I, et al.,* and *Šauša O.* On crystallization of water confined in liposomes and cryoprotective action of DMSO. *RSC Adv.* 2022; **12**(4):2300–9.

Дослідження фізичних процесів, що відбуваються у кріопротекторних середовищах, які містять сироватку та у безсироваткових середовищах, які включають водорозчинні полімери

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Мета. Дослідити механізми кріопротекції середовищ, доповнених полімерами (декстран (Дек), гідроксіетилкрохмаль (ГЕК), поліетиленгліколь (ПЕГ)) або фетальною бичачою сироваткою (ФБС). **Методи.** Диференціальна сканувальна калориметрія (ДСК) і термомеханічний аналіз (ТМА) кріопротекторних середовищ, що містять сироватку, а також середовищ, що не містять сироватку або її компоненти, але включають полімери. Результати: Комбінації диметилсульфоксиду (Me_2SO) з ФБС або полімерами в кріопротекторних середовищах здатні сприяти утворенню аморфної фази, пригнічувати евтектичну кристалізацію/плавлення та рекристалізацію. Середовище, доповнене 0,7 М Me_2SO і 100 мг/мл Дек (0,7Me_2SO+Dex), було кращим порівняно з іншими композиціями. Воно також мало оптимальну температуру склування (–26…–21 °C). Ця характеристика корелювала з високою життєздатністю клітин яєчка після кріоконсервування. **Висновки.** Механізм кріозахисту залежить не тільки від концентрації кріопротекторів, таких як Me_2SO, полімерів або ФБС, але також від складу кріопротекторів. Середовище 0,7Me_2SO+Dex може бути основою для створення комерційних безсироваткових кріопротекторних середовищ із визначеним стабільним складом, який не вимагає видалення Me_2SO перед використанням кріоконсервованих клітин або тканин для культивування, трансплантації та трансфузії.

Ключові слова: безсироваткові середовища, декстран, гідроксиетилкрохмаль, поліетиленгліколь, диференціальна сканувальна калориметрія, кріоконсервування

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