

исследовательских организаций. План исследования одобрен локальным этическим комитетом Института экологии и генетики микроорганизмов УрО РАН (протокол № 24/1 от 04.07.2023). От каждого из включенных в исследование участников было получено информированное добровольное согласие.

КОНФЛИКТ ИНТЕРЕСОВ

Авторы заявляют об отсутствии конфликта интересов.

СПИСОК ЛИТЕРАТУРЫ

- Бабийчук Л. А., Михайлова О. А., Zubov P. M., Ryzantsev V. V.* 2013. Оценка стадий апоптоза и распределения фосфатидилсерина в мембране ядросодержащих клеток пуповинной и периферической крови при различных технологиях криоконсервации. *Гены и клетки*. Т. 8. № 4. С. 50. (*Babijchuk L. A., Mikhailova O. O., Zubov P. M., Ryzantsev V. V.* 2013. Evaluation of apoptosis stages and phosphatidylserine distribution in membrane of cord and peripheral blood nucleated cells at various cryopreservation protocols. *Genes and Cells*. V. 8. P. 50.) <https://doi.org/10.23868/gc121610>
- Ващенко В. И., Чухловин А. Б., Петренко Г. И., Вильянинов В. Н., Багаутдинов Ш. М.* 2015. Действие факторов замораживания на изменения внутриклеточного метаболизма при криоконсервации костного мозга человека. *Вестник МАХ*. Т. 4. С. 91. (*Vashchenko V. I., Chuklovin A. B., Petrenko G. I., Vilyaninov V. N., Bagautdinov Sh. M.* 2015. Impact of preserving agents upon on intracellular metabolic changes during cryoconservation of human bone marrow. *J. IAR*. V. 4. P. 91.)
- Кит О. И., Гненная Н. В., Филиппова С. Ю., Чембарова Т. В., Лысенко И. Б., Новикова И. А., Розенко Л. Я., Димитриади С. Н., Шалашная Е. В., Ишонина О. Г.* 2023. Криоконсервация гемопоэтических стволовых клеток периферической крови в трансплантологии: современное состояние и перспективы. *Кардиоваск. терапия и профилактика*. Т. 22. № 11. С. 124. (*Kit O. I., Gnennaya N. V., Filippova S. Yu., Chembarova T. V., Lysenko I. B., Novikova I. A., Rozenko L. Ya., Dimitriadi S. N., Shalashnaya E. V., Ishonina O. G.* 2023. Cryostorage of peripheral blood hematopoietic stem cells in transplantology: current status and prospects. *Cardiovascular Ther. Prevention*. V. 22. P. 124.) <https://doi.org/10.15829/1728-8800-2023-3691>
- Нельсон Д., Кокс М.* 2014. Основы биохимии Ленинджера. М.: БИНОМ. Лаборатория знаний. 636 с. (*Nelson D. L., Cox M. M.* 2014. *Lehninger principles of biochemistry*. М.: BINOM. Laboratory of knowledge. 636 p.)
- Савилова А. М., Чулкина М. М., Алексеев Л. П.* 2013. Сравнительное исследование экспрессии мРНК интерлейкина-2 и рецептора интерлейкина-2 α в лимфоцитах, активированных ФГА и КонА. *Иммунология*. Т. 34. № 2. С. 76. (*Savilova A. M., Chulкина M. M., Alexeev L. P.* 2013. Comparative investigation of interleukin-2 and interleukin-2 receptor alpha mRNA expression in lymphocytes activated by PHA or ConA. *Immunol*. V. 34. P. 76.)
- Anderson J., Toh Z. Q., Reitsma A., Do L. A. H., Nathanielsz J., Licciardi P. V.* 2019. Effect of peripheral blood mononuclear cell cryopreservation on innate and adaptive immune responses. *J. Immunol. Methods*. V. 465. P. 61. <https://doi.org/10.1016/j.jim.2018.11.006>
- Chakraborty S., Khamaru P., Bhattacharyya A.* 2022. Regulation of immune cell metabolism in health and disease: Special focus on T and B cell subsets. *Cell Biol. Int*. V. 46. P. 1729. <https://doi.org/10.1002/cbin.11867>
- Desdin-Mico G., Soto-Heredero G., Mittelbrunn M.* 2018. Mitochondrial activity in T cells. *Mitochondrion*. V. 41. P. 51. <https://doi.org/10.1016/j.mito.2017.10.006>
- Fu Y., Dang W., He X., Xu F., Huang H.* 2022. Biomolecular pathways of cryoinjuries in low-temperature storage for mammalian specimens. *Bioengineering (Basel)*. V. 9. P. 545. <https://doi.org/10.3390/bioengineering9100545>
- Gaber T., Chen Y., Krauss P. L., Buttgerit F.* 2019. Metabolism of T Lymphocytes in health and disease. *Int. Rev. Cell Mol. Biol*. V. 342. P. 95. <https://doi.org/10.1016/bs.ircmb.2018.06.002>
- Gualtieri R., Kalthur G., Barbato V., Di Nardo M., Adiga S. K., Talevi R.* 2021. Mitochondrial dysfunction and oxidative stress caused by cryopreservation in reproductive cells. *Antioxidants (Basel)*. V. 10. Art. ID: 337. <https://doi.org/10.3390/antiox10030337>
- Haider P., Hoberstorfer T., Salzmann M., Fischer M. B., Speidl W. S., Wojta J., Hohensinner P. J.* 2022. Quantitative and functional assessment of the influence of routinely used cryopreservation media on mononuclear leukocytes for medical research. *Int. J. Mol. Sci*. V. 23. Art. ID: 1881. <https://doi.org/10.3390/ijms23031881>
- Len J. S., Koh W. S. D., Tan S. X.* 2019. The roles of reactive oxygen species and antioxidants in cryopreservation. *Biosci. Rep*. V. 39. Art. ID: BSR20191601. <https://doi.org/10.1042/BSR20191601>
- Lomsadze G., Gogebashvili N., Enukidze M., Machavariani M., Intskirveli N., Sanikidze T.* 2011. Alteration in viability and proliferation activity of mitogen stimulated Jurkat cells. *Georgian Med. News*. V. 9. P. 50.

- Makowski L., Chaib M., Rathmell J. C.* 2020. Immunometabolism: from basic mechanisms to translation. *Immunol. Rev.* V. 295. P. 5.
<https://doi.org/10.1111/imr.12858>
- Martikainen M. V., Roponen M.* 2020. Cryopreservation affected the levels of immune responses of PBMCs and antigen-presenting cells. *Toxicol. In Vitro.* V. 67. Art. ID: 104918.
<https://doi.org/10.1016/j.tiv.2020.104918>
- Mas-Bargues C., Garcia-Dominguez E., Borrás C.* 2022. Recent approaches to determine static and dynamic redox state-related parameters. *Antioxidants (Basel)*. V. 11. Art. ID: 864.
<https://doi.org/10.3390/antiox11050864>
- Patel C. H., Leone R. D., Horton M. R., Powell J. D.* 2019. Targeting metabolism to regulate immune responses in autoimmunity and cancer. *Nat. Rev. Drug Discov.* V. 18. P. 669.
<https://doi.org/10.1038/s41573-019-0032-5>
- Patil N. K., Bohannon J. K., Hernandez A., Patil T. K., Sherwood E. R.* 2019. Regulation of leukocyte function by citric acid cycle intermediates. *J. Leukoc. Biol.* V. 106. P. 105.
<https://doi.org/10.1002/JLB.3MIR1118-415R>
- Starkov A. A.* 2008. The role of mitochondria in reactive oxygen species metabolism and signaling. *Ann. N. Y. Acad. Sci.* V. 1147. P. 37.
<https://doi.org/10.1196/annals.1427.015>

ANALYSIS OF CRYOPRESERVATION IMPACT ON MONONUCLEAR LEUKOCYTE METABOLISM

V. N. Ponomareva^{a, b, *}, V. V. Vlasova^a, E. V. Saidakova^{a, b}

^a *Institute of Ecology and Genetics of Microorganisms UB RAS — Branch of the Perm Federal Research Center, Ural Branch of the Russian Academy of Sciences (Goleva str. 13, Perm, 614081, Russia)*

^b *Perm State University, Department of Microbiology and Immunology, Perm, 614006, Russia*

* *e-mail: PonomarievaVN@yandex.ru*

Studying the metabolic activity of mononuclear cells (MNCs) is crucial in biology and medicine. Cryopreservation is commonly used to store samples for long-term research, which helps minimize errors. However, the impact of low temperatures on MNCs metabolism remains understudied. The aim of this study was to investigate the effects of cryopreservation on glycolysis and oxidative phosphorylation in MNCs. Using the Seahorse XFe96 analyzer, we measured the metabolic parameters of cryopreserved MNCs via extracellular flux analysis. The results showed a significant decrease in the rate of oxidative phosphorylation in cryopreserved MNCs, without changes in MNC subset composition. Importantly, cryopreservation did not impact the rate of glycolysis. However, thawed cells exhibited reduced ability to increase metabolic rates in response to mitogenic stimulation. In conclusion, cryopreservation alters the metabolic profile of MNCs. To obtain reliable data on metabolic activity, the use of freshly isolated cells is preferable.

Keywords: mononuclear leukocytes, cryopreservation, metabolism, glycolysis, oxidative phosphorylation