

СОБЛЮДЕНИЕ ЭТИЧЕСКИХ СТАНДАРТОВ

В работе отсутствуют исследования человека или животных.

КОНФЛИКТ ИНТЕРЕСОВ

Автор работы заявляет, что у нее нет конфликта интересов.

СПИСОК ЛИТЕРАТУРЫ

- Коровина Д.Г., Юров К.П., Алексеенкова С.В., Савченкова Е.А., Савченкова И.П.* 2017. Характеристика мультипотентных мезенхимных стволовых клеток, выделенных из пуповинной крови лошадей. *Росс. Сельскохозяй. наука.* № 2. С. 51. (*Korovina D.G., Yurov K.P., Alexeenkova S.V., Savchenkova E.A., Savchenkova I.P.* 2017. Characterization of multipotent mesenchymal stem cells isolated from equine umbilical cord blood. *Russian Agricultural Sci.* V. 43. P. 262). <https://doi.org/10.3103/S1068367417030090>
- Коровина Д.Г., Юров К.П., Волкова И.М., Алексеенкова С.В., Васильева С.А., Савченкова Е.А., Савченкова И.П.* 2015. Пуповинная кровь лошадей как источник мультипотентных мезенхимных стволовых клеток. *Коневодство и конный спорт.* № 6. С. 31. (*Korovina D.G., Yurov K.P., Volkova I.M., Alexeenkova S.V., Vasilyeva S.A., Savchenkova E.A., Savchenkova I.P.* 2015. Equine umbilical cord blood as a source of multipotent mesenchymal stem cells. *Horse breeding and equestrian sport.* No. 6. P. 31).
- Паюшина О. В., Цомартова Д.А., Черешнева Е.В., Иванова М.Ю., Ломановская Т.А., Павлова М.С., Кузнецов С.Л.* 2023. Активация эндогенных мезенхимных стромальных клеток как подход к регенерации тканей. *Цитология.* Т. 65. № 2. С. 119–130. (*Payushina O. V., Tsomartova D.A., Cheresheva Ye.V., Ivanova M. u., Lomanovskaya T.A., Pavlova M.S., Kuznetsov S.L.* 2023. Activation of endogenous mesenchymal stromal cells as an approach to tissue regeneration. *Cell Tiss. Biol. (Tsitologiya).* V. 17. No. 4. P. 328.) <https://doi.org/10.1134/S1990519X23040065>
- Тепляшин А.С., Коржикова С.В., Шарифуллина С.З., Чупикова Н.И., Ростовская М.С., Савченкова И.П.* 2005. Характеристика мезенхимных стволовых клеток человека, выделенных из костного мозга и жировой ткани. *Цитология.* Т. 47. № 2. С. 130 (*Tepliashin A.S., Korzhikova S.V., Sharifullina S.Z., Chupikova N.I., Rostovskaia M.S., Savchenkova I.P.* 2005. Characteristics of human mesenchymal stem cells isolated from bone marrow and adipose tissue. *Tsitologiya.* V. 47. № 2. P.130.)
- Andrzejewska A., Lukomska B., Janowski M.* 2019. Mesenchymal stem cells: from roots to boost. *Stem Cells.* V. 37. P. 855. <https://doi.org/10.1002/stem.3016>
- Bhat S., Viswanathan P., Chandanala S., Prasanna S.J., Seetharam R.N.* 2021. Expansion and characterization of bone marrow derived human mesenchymal stromal cells in serum-free conditions. *Sci. Rep.* V. 11. P. 3403. <https://doi.org/10.1038/s41598-021-83088-1>
- Bianco P., Robey P.G., Simmons P.J.* 2008. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell.* V. 2. P. 313. <https://doi.org/10.1016/j.stem.2008.03.002>
- Borjesson D.L., Peroni J.F.* 2011. The regenerative medicine laboratory: facilitating stem cell therapy for equine disease. *Clinics Lab. Med.* V. 31. P. 109. <https://doi.org/10.1016/j.cll.2010.12.001>
- Caplan A.I.* 2017. Mesenchymal stem cells: time to change the name! *Stem Cells Transl. Med.* V. 6. P. 1445. <https://doi.org/10.1002/sctm.17-0051>
- Bui H.T.H., Nguyen L.T., Than U.T.T.* 2021. Influences of xeno-free media on mesenchymal stem cell expansion for clinical application. *Tiss. Eng. Regen. Med.* V. 18. P.15. <https://doi.org/10.1007/s13770-020-00306-z>
- Chen C., Hou X., Jing F., Wang T., Feng L., Kang Y.J.* 2023. Alteration of ranscriptomic profile and antiseptic efficacy of adipose-derived mesenchymal stromal/stem cells under different culture conditions. *Stem Cells Dev.* V. 32. P. 75. <https://doi.org/10.1089/scd.2022.0238>
- Clark K.C., Kol A., Shahbenderian S., Granick J.L., Walker N.J., Borjesson D.L.* 2016. Canine and equine mesenchymal stem cells grown in serum free media have altered immunophenotype. *Stem Cell Rev. Rep.* V.12 P. 245. <https://doi.org/10.1007/s12015-015-9638-0>
- Dam P.T.M., Hoang V.T., Bui H.T.H., Hang L.M., Hoang D.M., Nguyen H.P., Lien H.T., Tran H.T.T., Nguyen X.H., Nguyen T.L.* 2021. Human adipose-derived mesenchymal stromal cells exhibit high HLA-DR levels and altered cellular characteristics under a xeno-free and serum-free condition. *Stem Cell Rev. Rep.* V. 17. P. 2291. <https://doi.org/10.1007/s12015-021-10242-7>
- De Schauwer C., Meyer E., Van de Walle G.R., Van Soom A.* 2011. Markers of stemness in equine mesenchymal stem cells: a plea for uniformity. *Theriogenol.* V. 75. P. 1431. <https://doi.org/10.1016/j.theriogenology.2010.11.008>

- Devireddy L.R., Myers M., Screven R., Liu Z., Boxer L.* 2019. A serum-free medium formulation efficiently supports isolation and propagation of canine adipose-derived mesenchymal stem/stromal cells. *PLoS One*. V. 14: e0210250.
<https://doi.org/10.1371/journal.pone.0210250>
- Dominici M., Le Blanc K., Mueller I., Slaper-Cortenbach I., Marini F., Krause D., Deans R., Keating A, Prockop Dj, Horwitz E.* 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. International Society for Cellular Therapy position statement. *Cytotherapy*. V. 8. P. 315.
<https://doi.org/10.1080/14653240600855905>
- Gottipamula S., Muttigi M.S., Kolkundkar U., Seetharam R.N.* 2013. Serum-free media for the production of human mesenchymal stromal cells: a review. *Cell Prolif.* V.46. P.608.
<https://doi.org/10.1111/cpr.12063>
- Govoni K.E.* 2015. Horse species symposium: use of mesenchymal stem cells in fracture repair in horses. *J. Anim. Sci.* V. 93. P. 871.
<https://doi.org/10.2527/jas.2014-8516>
- Hagen A., Niebert S., Brandt V.P., Holland H., Melzer M., Wehrend A., Burk J.* 2022. Functional properties of equine adipose-derived mesenchymal stromal cells cultured with equine platelet lysate. *Front. Vet. Sci.* V. 9: 890302.
<https://doi.org/10.3389/fvets.2022.890302>
- Han Y., Yang J., Fang J., Zhou Y., Candi E., Wang J., Hua D., Shao C., Shi Y.* 2022. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. *Signal Transduct. Target Ther.* V. 7. P. 92.
<https://doi.org/10.1038/s41392-022-00932-0>
- Huang Y.C., Lai L.C.* 2019. The potential roles of stem cell-derived extracellular vesicles as a therapeutic tool. *Ann. Transl. Med.* V. 7. P. 693.
<https://doi.org/10.21037/atm.2019.11.66>
- Ibrahim S., Saunders K., Kydd J.H., Lunn D.P., Steinbach F.* 2007. Screening of anti-human leukocyte monoclonal antibodies for reactivity with equine leukocytes. *Vet. Immunol. Immunopathol.* V. 119. P. 63.
<https://doi.org/10.1016/j.vetimm.2007.06.034>
- Jammes M., Contentin R., Cassé F., Galéra P.* 2023. Equine osteoarthritis: strategies to enhance mesenchymal stromal cell-based acellular therapies. *Front. Vet. Sci.* V. 10:1115774.
<https://doi.org/10.389/fvets.2023.1115774>
- Jones B.J., McTaggart S.J.* 2008. Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp. Hematol.* V. 36. P. 733.
<https://doi.org/10.1016/j.exphem.2008.03.006>
- Jung S., Panchalingam K.M., Rosenberg L., Behie L.A.* 2012. *Ex vivo* expansion of human mesenchymal stem cells in defined serum-free media. *Stem Cells Int.* 123030.
<https://doi.org/10.1155/2012/123030>
- Karnieli O., Friedner O.M., Allickson J.G., Zhang N., Jung S., Fiorentini D., Abraham E., Eaker S.S., Yong T.K., Chan A., Griffiths S., Wehn A.K., Oh S., Karnieli O.* 2017. A consensus introduction to serum replacements and serum-free media for cellular therapies. *Cytotherapy* V.19. P. 155.
<https://doi.org/10.1016/j.jcyt.2016.11.011>
- Kinzebach S., Bieback K.* 2013. Expansion of mesenchymal stem/stromal cells under xenogenic-free culture conditions. *Adv. Biochem. Eng. Biotechnol.* V. 129. P. 33.
https://doi.org/10.1007/10_2012_134
- Lee J.Y., Kang M.H., Jang J.E., Lee J.E., Yang Y., Choi J.Y., Kang H.S., Lee U., Choung J.W., Jung H., Yoon Y.C., Jung K.H., Hong S.S, Yi E.C., Park S.G.* 2022. Comparative analysis of mesenchymal stem cells cultivated in serum free media. *Sci. Rep.* V.12. P. 8620.
<https://doi.org/10.1038/s41598-022-12467-z>
- Menard C., Dulong J., Roulois D., Hebraud B., Verdier L., Pangault C., Sibut V., Bezier I., Bescher N., Monvoisin C., Gadelorge M., Bertheuil N., Flécher E., Casteilla L., Collas P. et al.* 2020. Integrated transcriptomic, phenotypic, and functional study reveals tissue-specific immune properties of mesenchymal stromal cells. *Stem Cells.* V. 38. P. 146.
<https://doi.org/10.1002/stem.3077>
- Moll G., Ankrum J.A., Kamhieh-Milz J., Bieback K., Ringden O., Volk H.D., Geissler S., Reinke P.* 2019. Intravascular mesenchymal stromal/stem cell therapy product diversification: time for new clinical guidelines. *Trends Mol. Med.* V. 25. P. 149.
<https://doi.org/10.1016/j.molmed.2018.12.006>
- Naskou M.C., Sumner S.M., Chocallo A., Kemelmakher H., Thoresen M., Copland I., Galipeau J., Peroni J.F.* 2018. Platelet lysate as a novel serum-free media supplement for the culture of equine bone marrow-derived mesenchymal stem cells. *Stem Cell Res. Ther.* V. 9. P. 75.
<https://doi.org/10.1186/s13287-018-0823-3>
- Oikonomopoulos A., van Deen W.K., Manansala A.R., Lacey P.N., Tomakili T.A., Ziman A., Hommes D.W.* 2015. Optimization of human mesenchymal stem cell manufacturing: The effects of animal/xeno-free media. *Sci Rep* V. 5. P. 16570. doi: 10.1038/srep16570

- Petrova V., Vachkova E.* 2023. Outlook of adipose-derived stem cells: challenges to their clinical application in horses. *Vet. Sci.* V.10. P. 348.
<https://doi.org/10.3390/vetsci10050348>.
- Pilgrim C.R., McCahill K.A., Rops J.G., Dufour J.M., Russell K.A., Koch T.G.* 2022. A review of fetal bovine serum in the culture of mesenchymal stromal cells and potential alternatives for veterinary medicine. *Front. Vet. Sci.* V. 9: 859025.
<https://doi.org/10.3389/fvets.2022.859025>.
- Platonova S.A., Viktorova E.V., Korovina D.G., Savchenkova I.P.* 2021. Equine tendinopathy therapy using mesenchymal stem cells. In: *KnE Life Scie/DonAgro: Int. Res. Conference on Challenges and Advances in Farming, Food Manufacturing, Agricultural Research and Education.* Dubai. UAE. P. 533.
<https://doi.org/10.1088/1755-1315/677/4/042069>
- Schubert S., Brehm W., Hillmann A., Burk J.* 2018. Serum-free human MSC medium supports consistency in human but not in equine adipose-derived multipotent mesenchymal stromal cell culture. *Cytometry A.* V. 93. P. 60.
<https://doi.org/10.1002/cyto.a.23240>
- Vidal M.A., Walker N.J., Napoli E., Borjesson D.L.* 2012. Evaluation of senescence in mesenchymal stem cells isolated from equine bone marrow, adipose tissue, and umbilical cord tissue. *Stem Cells Dev.* V. 21. P. 273.
<https://doi.org/10.1089/scd.2010.0589>
- Viktorova E.V., Savchenkova I.P.* 2020. Multipotent mesenchymal stem cells in clinical veterinary practice. *IOP Conference Series: Earth and Environmental Science.* III Int. Sci. Conference: AGRITECH-III-2020: Agribusiness, Environ. Eng. Biotechnol. Krasnoyarsk Sci. Technol. City Hall of the Russian Union of Sci. and Eng. Associations. P. 72072.
<https://doi.org/10.1088/1755-1315/315/4/042038>
- Viswanathan S., Ciccocioppo R., Galipeau J., Krampera M., Le Blanc K., Martin I., Moniz K., Nolte J., Phinney D.G., Shi Y., Szczepiorkowski Z.M., Tarte K., Weiss D.J., Ashford P.* 2021. Consensus International Council for Commonality in Blood Banking Automation-International Society for Cell and Gene Therapy statement on standard nomenclature abbreviations for the tissue of origin of mesenchymal stromal cells. *Cytotherapy.* V. 12. P. 1060.
[ηττπσ://doi.org/10.1016/j.cyt.2021.04.009](https://doi.org/10.1016/j.cyt.2021.04.009)
- Wang L.-T., Ting C.-H., Yen M.L., Liu K.-J., Sytwu H.-K., Wu K.K., Yen B.L.* 2016. Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials. *J. Biomed. Sci.* V. 23. P. 76.
<https://doi.org/10.1186/s12929-016-0289-5>
- Watanabe Y., Tsuchiya A., Terai S.* 2021. The development of mesenchymal stem cell therapy in the present, and the perspective of cell-free therapy in the future. *Clin. Mol. Hepatol.* V. 27. P. 70.
<https://doi.org/10.3350/cmh.2020.0194>
- Zuk P.A., Zhu M., Ashjian P., De Ugarte D.A., Huang J.I., Mizuno H., Alfonso Z.C., Fraser J.K., Benhaim P., Hedrick M.H.* 2002. Human adipose tissue is a source of multipotent stem cells. *Mol. Biol. Cell.* V. 3. P. 4279.
<https://doi.org/10.1091/mbc.e02-02-0105>

THE CULTURE OF EQUINE ADIPOSE TISSUE-DERIVED MESENCHYMAL CELLS IN SERUM-FREE MEDIA

I. P. Savchenkova*

*Federal Science Center Skryabin and Kovalenko All-Russian Research Institute of Experimental Veterinary RAS, 24/1,
Ryazanskii pr., Moscow, 109428, Russia*

** E-mail: s-ip@mail.ru*

Mesenchymal stem/stromal cells (MSCs) isolated from equine adipose tissue (AT) represent a promising material for the creation of bioveterinary products for the prevention and treatment of many diseases. The production of these cells for clinical use requires improved serum-free culture conditions. The microenvironment can influence the properties of MSCs. It is believed that the requirements for culture conditions without animal blood serum are species specific. The purpose of this study was to evaluate the commercially available serum-free media (SFM) MesenCult (STEMCELL Technologies, USA), created for human MSCs, for the cultivation of equine MSC(AT). One part of the cells was propagated for 10 passages in the standard DMEM medium with a low glucose content (1 g/l) and 10% fetal bovine serum (FBS), and the second in SFM. The results show that the propagation of equine MSCs in MesenCult serum free, intended for the cultivation of human MSCs, is possible, since the cells adapt well to it and retain properties characteristic of cells that are cultured in DMEM with FBS: morphology, growth rate, doubling time and mitotic index, clone-forming abilities, diploid set of chromosomes, a large number of cells with the CD90 phenotype (90.8%) and low with the CD31 (0.8%), CD34 (0.9%) phenotype, as well as the potency for induction of differentiation into adipo-, osteo- and chondrogenic directions. Equine MSC(AT) showed stable characteristics after being cultured for 10 passages in SFM, providing a promising basis for their further use. Our results demonstrate that MesenCult media may be an alternative for serum-free culture of equine MSC(AT) for expansion in preclinical studies.

Keywords: mesenchymal stem/stromal cells, horse, adipose tissue, cultivation, MesenCult serum-free medium