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RELEVANCE OF THE USE OF POSTMORTEM BIOMATERIAL OF DOMESTICATED YAK (*BOS GRUNNIENS*) TO OBTAIN STEM CELLS FROM BONE MARROW

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The development of new technology methods for cryopreservation of animal cells has contributed to the introduction of stem cell banks for clinical use, including transplantation and regenerative veterinary medicine, and their further use to avoid problems of donors' shortage. The research aims to determine the possibility of using the bone marrow of a domesticated yak as a source of stem cells after slaughter. Moreover, we should determine the suitability of post-mortem biological material for obtaining stem cells based on the index of proliferation and viability of cultured cells. Therefore, we used the following materials and methods: bone marrow, obtained from the femur of a domesticated yak in the post-slaughter period. Bone marrow samples were taken in compliance with the rules of asepsis in a sterile test tube. Thus, we added 0.25% trypsin solution to the biomass (the ratio of bone marrow to the solution is 10:1) and placed it in a refrigerator (t +40 °C) for 24 hours for enzymatic disaggregation. Furthermore, we carried out the culturing of the obtained cells in a CO2 incubator according to the standard method with passivation after the formation of a monolayer by 90%–100%. When culturing a suspension of cells obtained from post-slaughter bone marrow material, we noted the appearance of cell colonies six days after sowing. Periodic passivation of stem cells contributed to an increase in the biomass of actively proliferating cells. In addition, we found that stem cells isolated from post-slaughter bone marrow material of domesticated yak have significant proliferative potential, as evidenced by proliferation indices in the

range from the first to the third passages and high cell viability. Thus, one can use the obtained post-mortem material in the form of the bone marrow of a domesticated yak as an additional source of stem cells. This post-mortem biological material is suitable for the isolation of stem cells 72 hours after the slaughter of an animal, which opens up the possibility of its transportation over long distances.

The scientific novelty of the research is in the context of the international Convention on Biological Diversity, which means the variability of living organisms from all sources, including ecosystems and ecological complexes they are a part of. Within the strategy and action plan for the conservation of biodiversity of the Russian Federation, the ecosystem service of livestock production is significant for preserving the traditional way of life of the indigenous peoples of Siberia and the Russian Far East (it has regional and local significance). This global problem can be solved by (1) developing cell technologies for species not yet involved in it and (2) improving existing ones.

Keywords: *domesticated yak; biological material; bone marrow; stem cells; proliferative potential; proliferation index*

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Научная статья | Общее животноводство

АКТУАЛЬНОСТЬ ИСПОЛЬЗОВАНИЯ ПОСМЕРТНОГО БИОМАТЕРИАЛА ДОМАШНЕГО ЯКА (*BOS GRUNNIENS*) ДЛЯ ПОЛУЧЕНИЯ СТВОЛОВЫХ КЛЕТОК ИЗ КОСТНОГО МОЗГА

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Развитие методов современной технологии криоконсервации клеток животных способствовало внедрению концепции создания банков стволовых клеток для клинического применения, включая трансплантацию и регенеративную ветеринарную медицину, и их дальнейшего использования во избежание проблем с нехваткой доноров. Цель данного исследования – определить возможность использования костного мозга домашнего яка в качестве источника стволовых клеток после убоя и на основе индекса пролиферации и жизнеспособности культивируемых клеток определить

пригодность посмертного биологического материала для получения стволовых клеток. Материалы и методы: костный мозг был получен из бедренной кости домашнего яка в послеубойный период. Образцы костного мозга отбирали с соблюдением правил асептики в стерильную пробирку и к биомассе добавляли 0,25% раствор трипсина (соотношение костного мозга к раствору 10: 1) и помещали в холодильник (t + 40 ° C) на 24 часа для ферментативной дезагрегации. Культивирование полученных клеток проводили в CO2-инкубаторе по стандартной методике с пассированием после образования монослоя на 90–100%. Результаты: при культивировании суспензии клеток, полученных из послеубойного материала костного мозга, отмечалось появление колоний клеток через 6 дней после посева. Периодическое пассирование стволовых клеток способствовали увеличению биомассы активно пролиферирующих клеток. Было установлено, что стволовые клетки, выделенные из послеубойного материала костного мозга домашнего яка, обладают значительным пролиферативным потенциалом, о чем свидетельствуют индексы пролиферации в диапазоне от первого до третьего пассажей и высокая жизнеспособность клеток. Заключение: полученный посмертный материал в виде костного мозга домашнего яка можно использовать как дополнительный источник стволовых клеток. Этот постмортальный биологический материал подходит для выделения стволовых клеток через 72 часа после убоя животного, что открывает возможности его транспортировки на большие расстояния.

Научная новизна проведенного исследования обусловлена в контексте международной конвенции о биологическом разнообразии, которая означает вариабельность живых организмов из всех источников, включая экосистемы, экологические комплексы, частью которых они являются. В рамках стратегии и плана действий по сохранению биологического разнообразия Российской Федерации экосистемная услуга производства животноводческой продукции важна для сохранения традиционного уклада жизни коренных народов Сибири и Дальнего Востока России, имеет региональное и локальное значение. Данная глобальная проблема может быть решена при помощи разработки клеточных технологий видов, еще не вовлеченных в нее, и совершенствование имеющихся.

Ключевые слова: *домашний як; биологический материал; костный мозг; стволовые клетки; пролиферативный потенциал; индекс пролиферации*

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Introduction

However, all multicellular organisms contain stem cells. These cells can interact with the culture medium, actively proliferate and differentiate into specialized types of mature cells [1; 2; 5; 9; 10; 12; 21; 22]. For clinical and research purposes, stem cells are usually obtained from bone marrow, blood, and adipose tissue of human [6; 14; 18]. These structures contain a large number of stem cells, which are easily accessible and cost-effective for isolation [6; 17]. Since the bone marrow is the primary source of stem cells, the choice of selecting bone marrow cells is the basis of biotechnological methods for their production [3; 8]. In addition, we obtained information about the production of male germ cells from the post-mortem material of the testes of male sable [20].

The development of modern technology methods of cryopreservation of animal cells contributed to the introduction of creating stem cell banks for their further use in order to avoid problems with a shortage of donors. Large-scale cryopreservation of stem cells began in the 1990s of the last century to create human cord blood banks [15]. The functioning of cryobanks gradually expanded with the advent of technologies for cryopreservation of umbilical cord tissue and, finally, adipose tissue [19]. One can use cryopreserved allogeneic stem cells for research and clinical applications, including transplantation and regenerative medicine.

Currently, methods of isolation and cryopreservation of animal stem cells have been improved [4; 11]. At the same time, we need to obtain cellular material, a source suitable for the isolation of stem cells. Thus, it is recommended to consider the slaughter material of productive animals as a source of some tissues, particularly muscles and bone marrow. Since stem cells can persist for quite a long time after death [7; 13; 16].

In veterinary medicine, the issue of material support for separating bone marrow from animals is no less acute than the long-term storage of stem cells. In most cases, bone marrow sampling takes place outside of a specialized laboratory, and transportation can be required for the delivery of the biomaterial, which is not always feasible in the field. In this regard, it is relevant to study the prospects of using bone marrow stem cells as a biomaterial obtained after the slaughter of animals, as well as determine the time during which the cells retain their viability and are suitable for cultivation.

The scientific novelty of the research is in the context of the international Convention on Biological Diversity, which means the variability of living organisms from all sources, including ecosystems and ecological complexes they are a part of. This concept includes diversity within a species, between species, and diversity of systems. A variety of life forms and ecological processes ensure the

continuation of biological evolution, which is a necessary condition for human well-being. Within the strategy and action plan for the conservation of biodiversity of the Russian Federation, the ecosystem service of livestock production has, first, regional and local significance; the recreational component of this service is also great. In addition, it is important for the preservation of the traditional way of life of the indigenous peoples of Siberia and the Russian Far East.

Currently, the development of a bioresource management strategy that could ensure (1) a high yield of useful products, (2) the preservation of natural diversity, and (3) a balanced abundance of zoo components of natural ecosystems is becoming increasingly important for biotechnology of animal reproduction. This global problem can be solved by developing cell technologies (obtaining and incubating stem cells) of species not yet involved in it and improving existing ones. The stem cell technology should be studied for all living animal species.

Thus, the studies conducted in this direction (obtaining biomaterial from bone marrow) would represent a great scientific and practical potential for practical veterinary medicine and medicine.

The research aims to establish the possibility of using the bone marrow of domesticated yak as a producer of stem cells in the post-slaughter period.

Materials and Methods

We obtained Bone marrow from the femur of a domesticated yak at the age of two years in the post-slaughter period. Before bone marrow sampling, we treated the bone surface in the proximal epiphysis area with 70% ethyl alcohol solution. After gaining access to the bone marrow canal, we collected the bone marrow with sterile tweezers into a sterile tube with 0.25% trypsin solution (the ratio of bone marrow to trypsin solution is 10: 1) and placed it in the refrigerator for 24 hours at a temperature of + 4 ° C for enzymatic disaggregation. The resulting suspension of cells was mechanically disaggregated for 5 minutes on a magnetic stirrer, filtered through 4 layers of gauze tissue into sterile centrifuge tubes with a volume of 15 ml and centrifuged at an acceleration of 300 g for 10 minutes. Under standard conditions, we placed the cell sediment for cultivation in a CO_2 incubator. No more than three days have passed since the slaughter of the animal and before the sowing of bone marrow cells.

We cultured the cells in a CO_2 incubator in Petri dishes according to the standard procedure.

Furthermore, we counted the number of cells in all squares of the Goryaev counting chamber under 200x magnification and calculated by the formula:

$$X = A \times 1000 / 0,9, \quad (1)$$

Where:

X – the number of cells in 1 cm^3;

A - the number of cells in all squares;

1000 - the number in mm^3 and cm^3;

0.9 - the volume of the Goryaev chamber in mm^3.

We carried out studies of the proliferative activity of stem cells in three passages, seeding them into Petri dishes at the rate of 250 thousand cells. Therefore, we determined the stem cell proliferation index 24 hours after sowing according to the formula:

$$DI = AP / SN, \quad (2)$$

Where:

DI - distribution index;

AP - number of cells after passage;

SN - the seating number of cells.

We determined cell viability using a 0.4% trypan blue dye solution followed by microscopy.

Consequently, we used the software package “Statistica” for the mathematical analysis of the data obtained.

Results

When cultivating a suspension of bone marrow stem cells obtained from post-slaughter material of domesticated yak, we found out that cell colonies began to appear on the seventh day after sowing. At first, the colonies were isolated, represented by cells in the amount of 30-50 pieces, which subsequently grew and merged with other colonies, forming a continuous monolayer. At the same time, the cells, which had a characteristic fibroblast-like morphology and actively multiplied on the first day after sowing, were uniformly attached to the bottom of Petri dishes (cell confluence is, on average, 85%). Subsequent replanting of the obtained stem cells helped to increase the mass of actively proliferating cells.

Moreover, we identified that bone marrow stem cells isolated from post-slaughter biomaterial of domesticated yak have high viability and significant proliferative potential, as evidenced by the proliferation index from the first to the third passages (see Table 1).

At the same time, the indicators of the biomaterial proliferation index did not change significantly depending on the passage and were approximately at the same level. The viability of cultured cells increased from 83% to 92% in the first and third passages, respectively, resulting from a decrease in the heterogeneity of the bioculture.

Table 1.

Proliferative activity and viability of bone marrow cells of domesticated yak depending on the passage

Number of passages	Number of cells, thousand	Proliferation index	Viability, %
1	325.18 ± 2.16	1.27	83
2	327.67 ± 2.92	1.28	89
3	328.43 ± 2.44	1.28	92

Discussion

The results on the production of stem cells from bone marrow are one of the main production sources for clinical and research applications, consistent with several researchers [5; 6; 9; 12; 18].

At the same time, one can use the post-mortem bone marrow material of a domesticated yak as a source of stem cells. This aspect coincides with a number of research regarding the use of slaughter material for the production of stem cells [1; 4; 10; 14]. Moreover, there is no need to carry out a lifetime bone marrow sampling from an animal, avoiding the potential risks of postoperative complications.

In addition, the isolation of stem cells from post-slaughter material is cheaper than the need to perform this procedure on live animals, since funds are not spent on expensive medical devices (e.g., for the anesthesia of animals and instruments).

Thus, the post-mortem bone marrow material of a domesticated yak can be used as an additional source of stem cells. This biological material is suitable for isolating stem cells three days after the slaughter of the animal, which opens up prospects for the future.

Conclusion

The bone marrow of a domesticated yak can be used as an alternative producer of stem cells three days after the slaughter of the animal. The proliferation and viability indices of bone marrow stem cells obtained from post-mortem biomaterial of domesticated yak were in the range of 1.27–1.28 and 83%–92%, respectively. The results obtained may be of scientific and practical interest for practical veterinary and human medicine.

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