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ARTIGO ORIGINAL

CARACTERÍSTICAS DAS RAÇAS AUTÓCTONES DA RÚSSIA E DO CAZAQUISTÃO DE ATRAVÉS DO TESTE DE MICRONÚCLEOS

CHARACTERISTICS OF AUTOCHTHONOUS BREEDS OF RUSSIA AND KAZAKHSTAN BY MICRONUCLEAR TEST

ХАРАКТЕРИСТИКА АВТОХТОННЫХ ПОРОД РОССИИ И КАЗАХСТАНА ПО МИКРОЯДЕРНОМУ ТЕСТУ

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RESUMO

O artigo é dedicado aos resultados do teste de micronúcleos em duas raças autóctones bem conhecidas da Federação Russa e da República do Cazaquistão: Romanov e Edilbay. Nas ovelhas Romanov, as maiores taxas de ocorrência de micronúcleos foram encontradas em cordeiros (8,7 ± 0,9), depois em ovelhas produtivas (5,6 ± 0,03 ‰) e ovinos (4,8 ± 0,3 ‰). A raça Edilbay apresenta diferenças nos resultados da análise de micronúcleos nos eritrócitos, os quais são mais pronunciados nos dois tipos da mesma raça: "Birlik" e "Suyunduk". A coudelaria "Birlik" está localizada nos Urais, na região oeste do Cazaquistão. Ovelhas Edilbay da raça Suyunduk são criadas na coudelaria "Makash", na região (óblast) de Atyrauz, na República do Cazaquistão, localizada próximo ao local de teste nuclear de Azgir. A diferença no teste de micronúcleos deve-se ao fato de que as ovelhas Suyunduk serem expostas a fatores de estresse ambiental devido à proximidade de locais com alto nível de contaminação por radionuclídeos. O efeito constante da seleção natural promove a reprodução de animais com maior resistência a fatores de estresse ambiental. Isso explica a baixa ocorrência de células com anormalidades citogenéticas em ovelhas do tipo "Suyunduk" Edilbay. Esse tipo tem maior potencial de adaptação a condições desfavoráveis de reprodução do que as ovelhas da raça Edilbay Birlik. Um alto nível de indicadores de teste de micronúcleos nas ovelhas Romanov está associado às características do sexo e faixas etárias, bem como à carga ambiental das condições climáticas na Rússia Central. A classificação dos micronúcleos é proposta. Acredita-se que os micronúcleos grandes e médios sejam formados sob a influência de vários mutagênicos no corpo, causando uma grande divisão de cromossomos individuais na metáfase e na anáfase. Pequenos micronúcleos indicam uma diminuição na viabilidade celular potencial.

Palavras-chave: *ovelhas, eritrócitos, estresse ambiental, distúrbios citogenéticos.*

ABSTRACT

The article is devoted to the results of the micronucleus test in two well-known autochthonous sheep breeds of the Russian Federation and the Republic of Kazakhstan: Romanov and Edilbay. In Romanov sheep, the highest rates of micronucleus occurrence were found in lambs (8.7 ± 0.9 ‰), than in producing rams (5.6 ± 0.03 ‰) and ewes (4.8 ± 0.3 ‰). The Edilbay breed has the differences in micronucleus test results in erythrocytes are most pronounced between two intrabreed types: “Birlik” and “Suyunduk”. The breeding plant “Birlik” is located in the Ural, the West Kazakhstan region. “Suyunduk” type of Edilbay breed sheep are raised in the breeding plant “Makash” in the Atyrauz region of the Republic of Kazakhstan, located near the Azgir nuclear test site. The difference in the micronucleus test is due to the fact that sheep of the “Suyunduk” type are exposed to environmental stress factors due to the proximity of places with high levels of radionuclide contamination. The constant action of natural selection contributes to the reproduction of animals with increased resistance to environmental stress factors. This explains the low occurrence of cells with cytogenetic abnormalities in sheep of the “Suyunduk” Edilbay breed sheep type. This type has a higher potential for adaptation to adverse breeding conditions than the Birlik type of Edilbay breed sheep. The high level of micronucleus test indicators in sheep of the Romanov breed is associated with the peculiarities of gender and age groups, and also the environmental stress of climatic conditions in Central Russia. A classification of micronucleus is proposed. It is believed that large and medium-sized micronucleus are formed by the action of various mutagens on the body, causing a large in the division of individual chromosomes in metaphase and anaphase. Small micronucleuses indicate a decrease in potential cell viability.

Keywords: *sheep, red blood cells, environmental stress, cytogenetic abnormalities.*

АННОТАЦИЯ

Статья посвящена результатам микроядерного теста у двух известных автохтонных пород овец Российской Федерации и Республики Казахстан: романовской и эдильбаевской. У овец романовской породы самые высокие показатели встречаемости микроядр были обнаружены у ягнят (8,7 ± 0,9,), затем у баранов-производителей (5,6 ± 0,03 %) и овцематок (4,8 ± 0,3 %). Эдильбаевская порода имеет различия в результатах анализа микроядр в эритроцитах, которые наиболее выражены у двух внутрипородных типов: «Бирлик» и «Суюндук». Племенной завод «Бирлик» расположен на Урале, в Западно-Казахстанской области. Овец эдильбаевской породы типа «Суюндук» разводят на племенном заводе «Макаш» в Атыраузской области Республики Казахстан, расположенном недалеко от Азгирского ядерного полигона. Разница в микроядерном тесте связана с тем, что овцы типа «Суюндук» подвержены факторам стресса окружающей среды из-за близости к местам с высоким уровнем радионуклидного загрязнения. Это способствует размножению животных с повышенной устойчивостью к факторам стресса окружающей среды, что объясняет низкую встречаемость клеток с цитогенетическими аномалиями у овец типа «Суюндук» эдильбаевской породы. Этот тип обладает более высоким потенциалом для адаптации к неблагоприятным условиям размножения, чем овцы типа «Бирлик». Высокий уровень показателей микроядерного теста у овец романовской породы связан с особенностями половой и возрастной групп, а также с экологической нагрузкой климатических условий в Центральной России. Предложена классификация микроядр. Считается, что крупные и средние микроядра образуются под действием различных мутагенов на организм, вызывая большое деление отдельных хромосом в метафазе и анафазе. Мелкие микроядра указывают на снижение потенциальной жизнеспособности клеток.

Ключевые слова: *овцы, эритроциты, экологический стресс, цитогенетические нарушения.*

1. INTRODUCTION

Bioindication, as an integral part of biomonitoring, has developed as a methodology for scientific research, and this is especially important since today, consequence of scientific and technological progress have become an objective reality (Gorovtsov *et al.*, 2017; Jiyenbekov *et al.*, 2018; Minakova *et al.*, 2018; Hodov *et al.*, 2019). The use of methods based on morphogenetic indicators of developmental stability and cytogenetic homeostasis are a promising direction since they meet practically all the requirements of biomonitoring (Udroiu, 2007; Udroiu and Sgura, 2017; Bakhtiar *et al.*, 2017; Cox *et al.*, 2019; Belhadj Slimen *et al.*, 2019).

Morphogenetic indicators characterize the level of cytogenetic stability of an individual organism and can form its phenotype under the conditions of a specific environment (Zakharov, and Trofimov, 2017; Kolosov *et al.*, 2017; Mukhametzharova *et al.*, 2018). Cytogenetic homeostasis can be studied using a micronucleus test, the essence of which is to calculate the number and volume of cells with micronuclei (Shapiro *et al.*, 2003; Astafieva, 2017; Gülden Yilmaz and Gül, 2017; Monte *et al.,* 2019; Sabbioni *et al.*, 2018; Maass *et al.*, 2018; Wu *et al.*, 2019).

The micronucleus test is one of the methods for determining substances that exhibit genotoxic effects on the body (Hristova *et al.*, 2017; McAllister *et al.*, 2017; Luijten *et al.*, 2018; Lu *et al.*, 2018). Literary data indicate that the micronucleus test is not inferior, and sometimes even exceeds the chromosomal aberration tests in terms of informativeness and efficiency (Ilinskikh *et al.,* 2011; Sabbioni *et al.,* 2019). The micronucleus test developed by W. Schmid (Schmid, 1975). It is based on using in vivo bone marrow cells of higher vertebrates, fish, and amphibians as biomarkers (Albarella *et al.*, 2019; Žaja *et al.*, 2019). However, in recent years, it has been replenished with a number of new techniques (analysis of micronuclei obtained by a block of cytokinesis, analysis of rodent erythrocytes micronuclei, and other similar techniques) that cover animal and plant cells, as well as various types of smear staining (Schmid, 1975; Fenech, 2000; Ilinskikh *et al.*, 2011; Astafieva *et al.*, 2015; Hayashi, 2016; Wilde *et al.*, 2017; Afusat *et al.,* 2017; Arieli, 2017; Dossybayev et al., 2019).

2. MATERIALS AND METHODS

Peripheral blood samples for research were taken from 69 sheep of two breeds: Romanov and Edilbay. Sheep (n=35) belonged to the Romanov breed were taken from breeding plants Yaroslavl region. The Edilbay breed was represented by two interbreed types: “Birlik” (n = 16) and “Suyunduk” (n = 18) from the breeding plant “Volgograd–Edilbay” Volgograd region. This farm purchased sheep of the “Birlik” type from the “Birlik” breeding enterprise of the West Kazakhstan region, and “Suyunduk” from the “Makash” of the Atyrauz region the Republic of Kazakhstan. Blood from the jugular vein was taken in tubes with a 0.5% EDTA solution. Smears were made on glass slides, thoroughly washed and degreased using a mixture of Nikiforov (a mixture of equal volumes of ethyl alcohol and anhydrous sulfuric ether; It is used for fixing blood products, bacterial preparations and organ prints).

To count erythrocytes with micronucleus, a drop of peripheral blood was diluted with the physiological solution (1:1), and smears were prepared on the glasses, which were fixed with methyl alcohol and dried at room temperature. Smears were stained using the Pappenheim method using Mai-Grunwald and Giems solutions (Romejs, 1953; Sarkisov and Perov, 1996; Tabecka-Lonczynska *et al.,* 2019; Younas *et al.,* 2013).

Red blood cells with micronuclei were counted for each test animal in 37 workers. In each field, approximately 80 erythrocytes were found, which is 3000 cells. For the convenience of analysis, the total figure was divided by three. The results are presented per 1000 cells. The obtained data were expressed in ppm (‰) – the ratio of the number of erythrocytes with micronucleuses to the total number of cells counted. Cell analysis was carried out using a Motic binocular microscope with a built-in digital camera (DMBA300), an increase of 1000 (Astafieva *et al.*, 2015; Astafieva, 2017).

The statistical significance of differences in the frequencies of cytogenetic anomalies between breeds and groups of animals was evaluated by Student's t-test. A difference of $P<0.05$ and more were considered statistically significant (Glanc, 1999).

3. RESULTS AND DISCUSSION:

The results of the micronucleus test on the Romanov breed of sheep are presented in Table 1. It was found that the highest rates of occurrence of micronucleuses were detected in lambs (8.7 ± 0.9), then in rams (5.6 ± 0.03 ‰), and in third place were ewes (4.8 ± 0.3) (Table 1).

There is a significant difference in micronucleus dough between rams and ewes ($P < 0.01$) and between adult groups of animals and young animals ($P < 0.01$), which may be due to the rigid selection of producers for the tribe and age since blood was taken from old sheep. We believe this is due to the relative immaturity of the cellular component of the immune system in lambs, which is responsible for the elimination of red blood cells with micronucleus. Our results for young animals confirm the data on the increased level of micronucleus in comparison with adults. Two interbreed types of Edilbay breed “Birlik” and “Suyunduk” were studied in comparison with the obtained materials on Romanov breed (Table 2).

The differences in the results of the micronucleus test in erythrocytes are most pronounced between different interbreeding types. In the Edilbay sheep breed “Birlik” type, the frequency of occurrence of erythrocytes with micronucleus (4.6 ± 0.3) was higher compared to “Suyunduk” sheep type (3.5 ± 0.2). According to the presented data, it is clear that the frequency of occurrence of erythrocytes with micronucleus in individuals of the Romanov breed (5.8 ± 1.18 ‰) is even higher than that of interbreeding types of the Edilbay breed. The reason for the differentiation of the “Suyunduk” and “Birlik” sheep breed types may be that the breeding plants were located in different environmental conditions. The breeding plant “Birlik” is located in the Ural region, in the West Kazakhstan region.

The “Suyunduk” type of Edilbay sheep breed were descended from the breeding plant „Makash” in the Atyrauz region of the Republic Kazakhstan. The breeding plant "Makash" is located next to the well-known Azgir nuclear test site of the Volgograd region Russian Federation. The observed micronucleus differentiation is due to the fact that sheep of the “Suyunduk” Edilbay sheep breed type were subjected to constant environmental stress factors due to the proximity of their breeding sites to the region with high levels of radionuclide contamination (Astafieva *et al.,* 2015; Astafieva, 2017).

The chronic action of natural selection contributes to the reproduction of animals with increased resistance to environmental stress factors. This probably explains the relatively low frequency of occurrence of cells with cytogenetic abnormalities in animals of “Suyunduk” Edilbay sheep breed type. The “Suyunduk” type of Edilbay sheep breed has a higher potential for adaptation to unfavorable breeding conditions than the “Birlik” type, which is the result of the action of natural selection in generations. As for the high level of performance in Romanov sheep along with the characteristics of gender and age groups, the environmental stress of the climatic conditions of Central Russia is possible. Thus, the micronucleus test is one of the methods for determining the influence of substances that exhibit genotoxic effects on the body. Microkernels are fragments of the nucleus in eukaryotic cells that do not contain the complete genome necessary for its survival (Figures 1 and 2).

The size of micronucleus depends on the reasons underlying their formation. It is believed that large and medium micronucleus are formed by the action of various mutagens on the body, which causes a large in the division of individual chromosomes in metaphase and anaphase. At the same time, small micronucleuses indicates a decrease in the potential viability of cells (Figure 3).

4. CONCLUSIONS:

As a result of the study, in accordance with the sizes of micronucleus and the forms of their occurrence, a classification consisting of 5 points was proposed. When determining the size of a micronucleus, they proceeded from the average size of the normoblast core, averaging 1.5 microns. The classification includes the presence of five types of occurrence of micronucleus in the cell:

1. The first type is one or several large micronuclei in erythrocytes, up to 1/4 the size of an average normoblast nucleus.

2. The second type is one micronucleus in the erythrocyte, ranging in size from 1/15 to 1/10 of the average size of the normoblast core.

3. The third type is one small micronucleus in the erythrocyte, 1/40 of the average size of the normoblast nucleus.

4. The fourth type – micronucleus, which are several small formations, in an amount from 2 to 10 pieces, the size of a microkernel of the 2nd and 3rd types, i.e., from 1/40 to 1 / 15– 1/10 of the average size of the nucleus of a normoblast.

5. The fifth type is when both large and small micronucleuses are detected simultaneously in the studied red blood cells.

In the studied blood smears of sheep of two breeds, the Romanov and Edilbay, mainly large micronucleus were found. Most often, large micronucleus were one in a red blood cell, less often two. Small micronucleus were found in the amount of two, rarely three in one erythrocyte. In 5% of the total number of cells with micronucleus, there was a large micronucleus and one or two small sizes next to it. Only 10% of the total number of cells with micronucleus revealed the formation of small and medium sizes. Thus, a micronucleus test was carried out on the instability of the genetic apparatus in the erythrocytes of the blood of two autochthonous sheep breeds from the Russian Federation and the Republic of Kazakhstan. In Romanov sheep, the relationship between the level of micronucleus in erythrocytes and the age of animals was determined. A comparative characteristic is given for the occurrence of micronuclei in known sheep breeds of two countries: the Russian Federation and the Republic of Kazakhstan.

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Table 1. *The frequency of occurrence of erythrocytes with micronucleus in the studied sheep of the Romanov breed (in ‰)*

No.	Group of discovered animals	n	The average number of micronucleus per 1000 cells
1	Rams	8	5.6 ± 0.3
2	Ewes	20	4.8 ± 0.3
3	Lambs 2 months age	7	8.7 ± 0.9
4	Group average	35	5.8 ± 1.8

Table 2. *The frequency of occurrence of erythrocytes with micronucleus in studied sheep breeds (‰)*

Sheep breeds and interbreeding types	n	Number of micronucleuses per 1000 cells
Edilbay interbreeds types:		
Birlik	16	4.6 ± 0.3
Suyunduk	18	3.5 ± 0.2
Romanov	35	5.8 ± 1.8

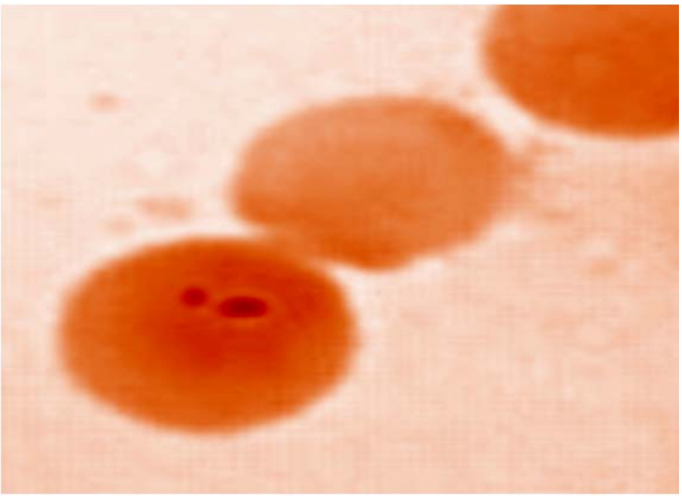

Figure 1. *The sheep in the erythrocyte are represented large and medium micronucleuses*

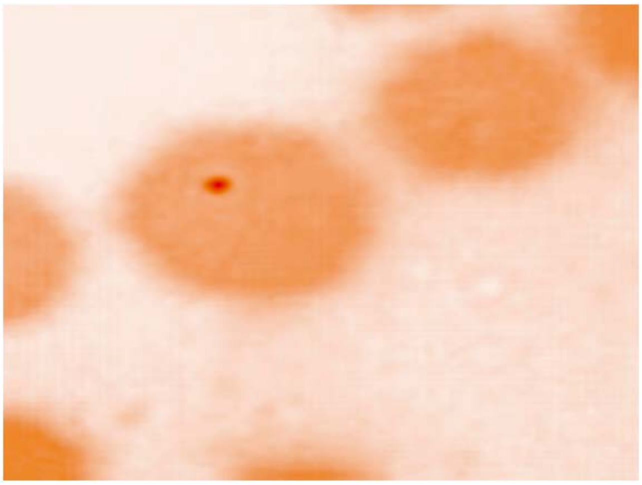

Figure 2. *Middle micronucleus in a sheep erythrocyte*

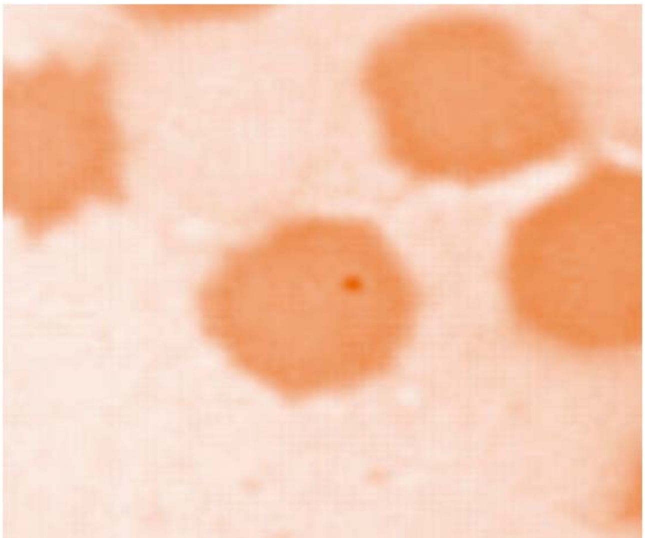

Figure 3. *Erythrocyte in a sheep with a small micronucleus*