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CHROMOSOMAL ABERRATIONS IN UKRAINIAN RED-SPOTTED DAIRY BREED COWS WHEN USING DIFFERENT BREEDING METHODS

V. V. Dzitsiuk, Kh. T. Bratytsia

*M.V. Zubets Institute of Animal Breeding and Genetics, National Academy of Agrarian Sciences of Ukraine,
1, Pohrebniaka Str., Chubynske village, Boryspil District, Kyiv Region, Ukraine, 08321*

E-mail: valentynadzitsiuk@gmail.com

Orcid: <https://orcid.org/0000-0001-9697-4165>

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Aim. The aim of this study was a comparative analysis of karyotypes of purebred, inbred, and crossbred cows of the Ukrainian red-spotted dairy breed in order to detect their degree of chromosomal aberrations. **Methods.** The cytogenetic study involved 156 cows of the Ukrainian red-spotted dairy breed from two research farms of M.V. Zubets Institute of Animal Breeding and Genetics, the NAAS. Cultures of peripheral lymphocytes, stimulated with phytohemagglutinin (PHA, Difco, USA) were used according to the method of Moorhead et al. (1960). The chromosomal preparations were stained with 2 % Giemsa solution (Giemsa, Merk) and analyzed under an Axiostar plus (Carl Zeiss, Germany) light microscope at 1,000 times magnification. Metaphase slides were photographed with a digital camera Olympus D-460 ZOOM. The biometric processing of the study results was conducted by ANOVA using Microsoft Excel 2010. **Results.** Genomic mutations (aneuploidy and polyploidy) and structural aberrations of chromosomes were found in the karyotypes of the investigated purebred, inbred, and crossbred cows. The total share of aberrant cells varied from 11.4 ± 0.39 % in purebred, 11.8 ± 0.40 % in inbred to 17.30 ± 0.85 % in crossbred cows. In two animals the Robertson translocation, rob (1 : 29) was found. A statistically significant difference between purebred, inbred, and crossbred animals was found regarding cells with aneuploidy and structural chromosomal aberrations. **Conclusions.** The karyological analysis yielded data showing changes in genetic structure that can have an influence on fertility and production in different breeding programs.

Key words: Karyotype, chromosome aberrations, purebred, inbred, crossbred animals, Ukrainian Red-and-White Dairy cattle.

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INTRODUCTION

Regardless of the rapid development of new molecular methods in the studies of the genome of agricultural species of animals (matrix platforms, sequencing, FISH), the routine metaphase analysis of chromosomes following Giemsa staining and G-banding remain crucial procedures in the screening of aberrations in cytogenetic laboratories (Basur and Stranzinger, 2008; Raudsepp and Chowdhary, 2016; Harshini et al, 2021). Chromosomal aberrations and their mechanisms in cattle have been studied for many years, as most of them demonstrate the occurrence of an impaired genetic apparatus, often leading to reduced fertility (infertility

of carriers, early death of embryos, and severe congenital malformations (Bakhom, 2014; Szczerbal and Switonski, 2016; Holečková et al, 2021)

Yet a low (insignificant) level of chromosomal instability is a natural variation factor and normally does not impact the organisms functioning. Chromosomal aberrations, without any obvious deleterious effect, indeed occur in any generation of genetically healthy animals due to spontaneous mutagenesis in somatic cells (Raudsepp, 2016; Iannuzzi, 2007). Impact of ecological, chemical, radiation, and other factors on karyotypic variability in cattle has been reported over the years. In particular about the higher level of chromosomal instability in highly performing animals due to enhanced metabolism on the cellular levels (e.g.,

Wójcik, Szostek, 2019). Furthermore, there is impact of breeding methods on the stability of the karyotype, first studied by Zartman, Fechheimer in 1967, but still needing further elaboration (e.g., Chauhan et al, 2009).

The aim of this article is to analyze numerical and structural changes in the karyotype of 156 Ukrainian red-spotted dairy breed, obtained via outbreeding, various stages of inbreeding and crossbreeding. Our earlier study (Dzitsiuk, 2016) highlighted the chromosome variability of crossbred cows, obtained by interbreed mating (cattle × zebu), while creation of the Southern meat breed was described. A further study on purebred and inbred cows of Ukrainian red-spotted dairy breed demonstrated the connection between chromosomal instability in cows with the impaired reproductive function, regardless the degree of their affinity (Dzitsiuk, Peredriy, 2017a). Finally, it was established that the difference purebred and inbred animals in the frequency of chromosomal aberrations was not statistically significant (Dzitsiuk, Peredriy, 2017b). The main focus of this study was to obtain information about the degree of chromosomal variability of Ukrainian red-spotted dairy breed, born due to interbreed mating (Ukrainian red-spotted dairy breed and Montbeliarde breed).

MATERIALS AND METHODS

The cytogenetic studies were conducted from 2019 to 2021, with Ukrainian red-spotted dairy cows, bred at the SE Research Farm Nyva of the M.V. Zubets Institute of Animal Breeding and Genetics, the NAAS, and the SE Research Farm Khrystynivske, the M.V. Zubets Institute of Animal Breeding and Genetics, the NAAS.

The material of the study was peripheral blood of 156 first lactation cows of the Ukrainian red-spotted dairy breed, obtained by a different selection of paternal pairs – purebred pairs and the ones with a different degree of inbreeding (close, moderate, and remote) as well as crossbred ones, bred by crossing Ukrainian red-spotted cows with Montbeliarde bulls. The degree of inbreeding was determined according to the Pusha-Shaporuzh classification (Basovsriy et al, 2001): close – the ancestor is found in I–III, III–I, I–II, II–I rows of the pedigree; moderate – in III–IV, IV–III, II–III, III–III, III–II; distant – IV–V, V–V, V–IV, IV–IV.

The blood of cows was taken from the caudal vein into sterile syringes with the heparin solution. The short-term cultivation of lymphocytes was conducted

by the method of Moorhead PS et al (Moorhead, 1960) for 72 h in the thermostat at 37 °C in the culture medium RPMI 1640 with L-glutamine (Sigma, USA) and with mitogen PHA (phytohemagglutinin, 0.1 ml/ml) (Difco, USA) with the addition of 100 µg/ml antibiotic gentamicin (gentamicin sulfate, Darnytsia, Ukraine) and 15 % fetal calf serum. Two hours before the end of cultivation, 0.5 µg/ml colchicine (Sigma, USA) was added to the culture. Then the cell suspension was precipitated by centrifugation (at 500× g), 10 min) and incubated in a hypotonic KCl solution (0.56 %) for 20 min and fixed in three changes of ethanol with glacial acetic acid (3 : 1 ratio). After fixation, drops of the cell suspension on clean, cooled specimen slides. The preparations were stained with 2 % Giemsa solution (Giemsa, Merk) and analyzed under an Axio-star plus (Carl Zeiss, Germany) light microscope at 1,000 times magnification. Metaphase slides were photographed with a digital camera Olympus D-460 ZOOM.

Chromosomal aberrations were counted in at least 50 metaphases per animal. The data obtained were analyzed using one-way ANOVA in MS Excel 2010. Statistical significance was determined at $p < 0.05$.

RESULTS

The chromosomal sets of the investigated purebred, inbred, and crossbred cows showed both numerical (euploid) changes and structural aberrations.

The changes in the (normal) euploid karyotype were present as aneuploidy and polyploidy (**Fig. 1**)

The amount of aberrant cells detected was lowest (11.4 ± 0.39 %) in pure cows, intermediate c.13 % for inbred cows (although varying from c 12.0 % in moderate and distant, to 16.2 ± 0.60 in closely inbred cows), and highest in crossbred cows (17.30 ± 0.85 %) (**Table**).

Aneuploid cells (missing or having an excess of chromosomes) were the most common chromosomal aberrations found, lowest (2.7 %) in purebred and highest (5.5 %) in crossbred cows. The number of aneuploid cells is statistically significantly between those from purebred, close inbred and crossbred animals. Aneuploidy was not statistically significant between moderate and distant inbred animals.

A higher aneuploidy in cells may have been caused by the cultural and chromosome preparative methods. But since in our study, both hypoploid and hyperploid cells within aneuploid ones occurred with approximately the same frequency it is assumed that the observed aneuploidy is real. Hypoploidy (loss

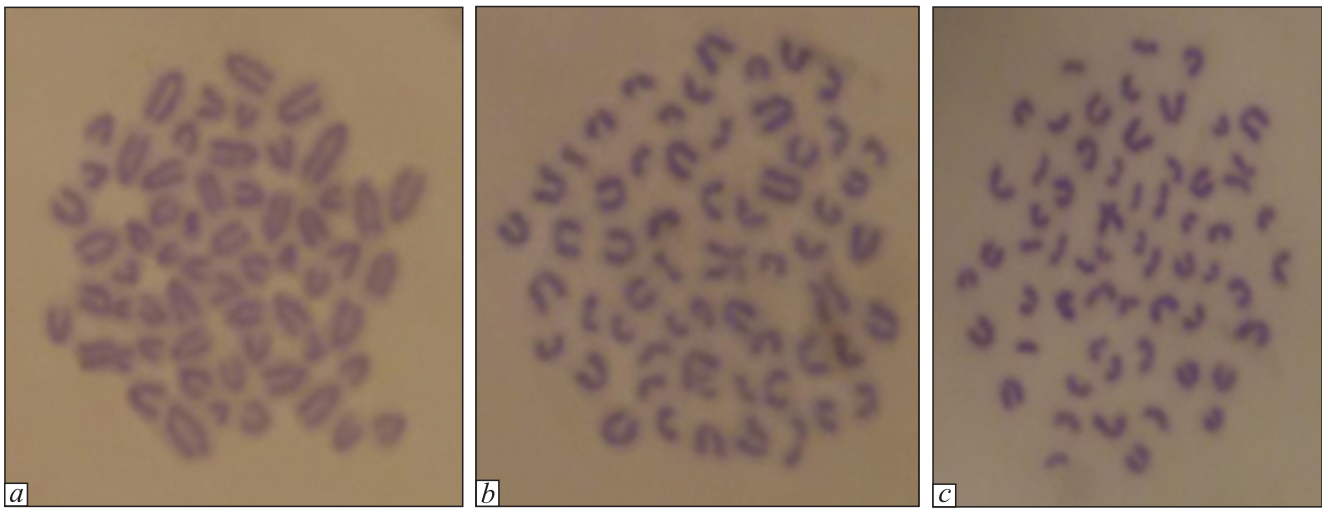


Fig. 1. Metaphase plates from peripheral blood lymphocytes of the Ukrainian red-spotted dairy cattle breed with an aneuploid set of chromosomes: *a* – $2n = 57$; *b* – $2n = 59$; *c* – $2n = 61$. Magnification 1000× under the microscope. Giemsa stain

The karyotypic aberrations detected in purebred, inbred, and crossbred cows (in % of the total number of analyzed metaphases)

Karyotype abnormalities	Purebreeding	Inbreeding			Crossbreeding URSDxM ¹⁾
		close	moderate	distant	
Number of cows	49	17	18	40	32
Total % of cells with aberrations	11.4 ± 0.39 *	16.2 ± 0.60	11.8 ± 0.40	12.0 ± 0.71	17.30 ± 0.85 *
Aneuploid	2.70 ± 0.66 *	4.90 ± 0.60 *	3.40 ± 0.70	3.0 ± 0.55	5.50 ± 0.70 *
Polyploid	0.79 ± 0.44	0.70 ± 0.08	0.67 ± 0.27	0.66 ± 0.71	0.40 ± 0.11
Chromosome with breakage	2.40 ± 0.18 ***	4.60 ± 0.57 ***	2.25 ± 0.20	2.75 ± 0.60	3.16 ± 0.12
Chromosome fragments	2.35 ± 0.70 *	3.90 ± 0.94 *	2.55 ± 0.30	2.80 ± 0.52	3.05 ± 0.80

Note. P > 0.95; ** P > 0.99; *** P > 0.999.

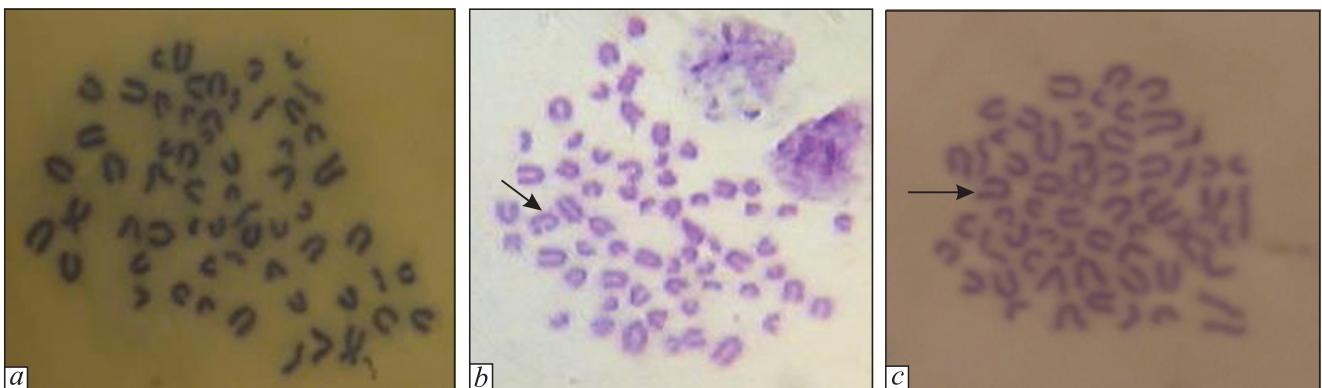


Fig. 2. Metaphase plates from peripheral blood lymphocytes of the Ukrainian red-spotted dairy breed: *a* – normal; *b* – with chromosome breakage (arrow); *c* – with a chromosome with a missing fragment (arrow). Giemsa stain. Magnification: 1000× under the microscope

of chromosomes) may also have been the result of elimination of damaged chromosomes during mitotic division.

The percentage of polyploid cells observed in all the investigated groups was under one percent:

In our study, the structural chromosomal aberrations

comprised of 67 % of all detected aberrations in the chromosome set in purebred animals, 71 % in animals obtained as a result of close inbreeding, 65 % in animals from moderate inbreeding, 67 % in animals from distant inbreeding and 61 % in crossbred animals of the total number of all the impairments in the chromosomal set. These aberrations included chromosomal breaks and fragments as well as associations of non-homologous chromosomes (Fig. 2).

Cells with chromosomal breaks and chromosomal fragments were almost twice more frequent in close inbred and crossbred cows, than in purebred cows. The moderate and distant inbred cows, were close to purebred ones regarding the number of metaphases with breaks and fragments.

We observed in two moderately inbred cows the Robertsonian translocation rob (1/29), which is the association of two non-homologous acrocentric chromosomes (Fig. 3).

DISCUSSION

Our results, presented in this article, are the continuation of an investigating of the karyotypic variability of animals obtained via different breeding methods – pure breeding, inbreeding, and crossbreeding. The first studies, that started in 2008 were aimed at the research on karyotypic variability of 45 animals of varying consanguinity of the newly created Southern meat breed at the farms Zelenohirske in Odesa region and Simferopilske in the Autonomous Republic of Crimea. This study demonstrated that the number of aberrant cells in interbreed hybrids (cattle × zebu) was 1.5 times higher as compared to interspecies hybrids (Dzitsiuk, 2016).

Our next study was aimed at investigating karyotypes of the first-generation cows of the Ukrainian red-spotted breed, born in 2015–2016 via closely related, moderate and distant inbreeding (Dzitsiuk and Peredriy, 2017). The hypothesis that combination of consanguine heterozygous carriers of undesired chromosomal aberrations enhance the probability of the transition of those aberration into a homozygous state was confirmed.

Our study on intermediate genotypes of meat cattle, while breeding the Southern meat cattle breed (Dzitsiuk, 2016), proved a statistically significant difference by the frequency of aberrant cells between interbreed local animals (Hybrids $\frac{3}{4}$ Zebu × $\frac{1}{4}$ C × 1/8RS, 25 heads) and purebred animals of the red steppe purebred animals (11 heads). Chromatide-type aberrations predominated in the karyotypes of cross-bred animals, while more chromosomal aberrations in the

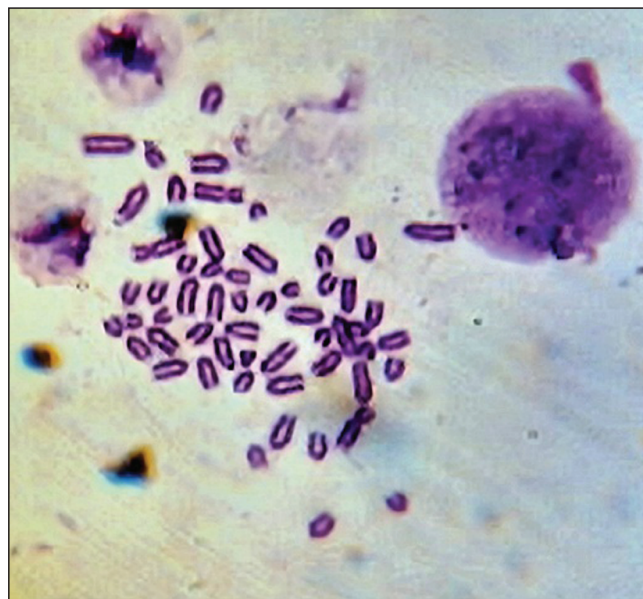


Fig. 3. Metaphase chromosomes, Giemsa staining, Rohatka cow 8010388602, Ukrainian red-spotted dairy breed with a Robertsonian translocation rob1/29 (arrow). Magnification: 1000× under the microscope

form of paired fragments and breaks of both chromatids were found in red steppe purebred animals (Dzitsiuk, Peredriy 2017a,b). It should be noted that the frequency of cells with aberrations was approximately the same in our different studies.

The results of a cytogenetic study of 90 heads of the Ukrainian red-spotted dairy breed (Dzitsiuk et al, 2022) showed greater chromosomal instability of crossbred cows compared to purebred cows: in crossbred cows, significantly higher frequencies ($P < 0.001$) of aneuploid (one and a half times), polyploid (on 27 %) and structural aberrations of chromosomes (by 20 %) were found. The Robertsonian translocation rob(1/29) which we found is the most common among different cattle breeds (Popescu, Pech, 1991; Sennings, 2020). This translocation may be detected even with routine staining, as it consists of the first chromosome – the largest in karyotype, and the twenty-ninth chromosome – the smallest one. Contrary to X-chromosome, which is similar to rob(1 : 29), the staining of preparations by G-banding method proved that the translocational chromosome had remarkable blocks of heterochromatin, brought in by acrocentric chromosomes 1 and 29, while in X-chromosome these blocks were absent.

The level of chromosomal instability in different species of agricultural animals depends on many factors, including environment, breeding method, intra-

breed specificities, the health of animals, etc. (Pryce et al, 2014).

It has been established that the crossing of animals, even that of consanguine breeds, even when obtained under different breeding systems and in different ecological conditions leads to the deconsolidation of heredity. More than 50 years ago, Zartman and Fehheimer (1967) studied the frequency of polyploidy in inbred, crossbred, purebred, and local bulls of Hereford meat. They found the lowest ploidy in the group of three-breed hybrids (5.2 %) and in the animals, obtained via linear crossing (6.5 %). Somewhat higher ploidy was noted for inbred animals (7.3 %).

Popescu (1990) reported that some phenotypically normal animals, obtained via different crossing variants, were homozygous by non-lethal inversions. The sequence of gene linkage was changed in such animals, but the conjugation of chromosomes and the following recombination were normal.

Harshini et al, (2020) in India reported a considerable difference ($P < 0.01$) in the relative length of Y-chromosome between purebred *Bos indicus* and crossbred *Bos taurus* × *indicus*. These reports indicated changes in the karyotype of animals obtained as a result of various breeding methods as well.

The level of somatic aneuploidy in Holstein breed, found by Lozano et al, (2013), was in the range from 1.0 to 16.0 %, which is substantially higher variability than that in our study (2.7–5.5 %). They speculated that apart from polyploidy, aneuploid oocytes, when fertilized and subsequently undergoing embryonic death, may negatively influence. Other authors (Danielak-Czech et al, 2020; Iannuzzi, 2021 and Sennings et al, 2020), studying chromosomal aberrations in pigs, concluded that the phenomenon of somatic aneuploidy may be a marker of the change in a chromosomal set due to the disruptions in the reproductive ability of animals.

We conducted a cytogenetic study of cows of the Ukrainian red-spotted dairy breed with different reproductive capacity (Dzitsiuk and Peredriy, 2017c). In the karyotypes of animals with impaired reproductive capacity, a significantly higher frequency of cells with aneuploid and polyploid sets of chromosomes, as well as cells with chromosomal aberrations, was found, than in cows with normal reproductive functions. It was established that in all studied animals there is a positive correlation between the length of the service period and the main cytogenetic indicators.

The Robertsonian translocation or centric fusion, when two acrocentric chromosomes get fused in a centromeric region, which we found in two animals was the most common one (among 44) found in cattle, namely rob (1 : 29) (Szczerbal, 2016), which is currently found in over 50 breeds globally (Popescu, 1991). Although the Robertsonian translocation is balanced in most cases, it was noted that some genes or their regulatory parts were disrupted in some translocations, which led to phenotypic disorders in animals (Wójcik, 2019). In some cases, some chromosomal aberrations (Robertsonian translocation, tandem fusion) affect the fertility of cattle, which leads to a 10 % loss of reproduction (Gustavsson, 1980).

In 2019, during the screening of impaired fertility of cows, we identified a new Robertsonian translocation – rob (13 : 23) in a cow, obtained by moderate inbreeding of Ukrainian red-spotted dairy breed of cattle (Dzitsiuk, Tipilo, 2019; Typylo et al, 2021) A cow with this translocation had one phenotypically healthy calf, but the second calving resulted in miscarriage.

The abovementioned cases demonstrate the relevance of cytogenetic and molecular-genetic testing of each elite male and female carrier, which should be included in breeding programs and in strategies to control genetic diseases (Gholap et al, 2014).

CONCLUSIONS

In this study, genomic mutations (aneuploidy and polyploidy) and structural chromosomal aberrations are described in a set of 158 Ukrainian red-spotted dairy breed in an out-, in- and crossbreeding program. When taking with the total cells with aneuploidy and chromosomal aberrations (breaks and fragments), into account a reliable difference of 4,5 % ($P > 0.95$) between purebred cows and cows, obtained by close inbreeding was found. Similarly statistically significant difference was also observed between animals with a moderate degree of inbreeding and crossbred animals, namely 5,5 % ($P > 0.99$).

The karyological analysis showed the effect of the different breeding methods on the genetic structure, which can have an effect on fertility, which is discussed.

Adherence to ethical principles. All the procedures performed in the studies involving animal participants were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 1986.

Conflict of interests. The authors declare the absence of any conflicts of interests.

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**Хромосомні аберації у корів,
отриманих різними методами розведення**

В. В. Дзіцюк, Х. Т. Братиця

Інститут розведення і генетики тварин імені М.В. Зубця,
Національної академії аграрних наук України,
Вул. Погребняка, 1, с. Чубинське,
Бориспільського району, Київської обл., Україна, 08321

E-mail: valentynadzitsiuk@gmail.com

orcid: <https://orcid.org/0000-0001-9697-4165>

Мета. Метою дослідження є порівняльний аналіз каріотипів чистопородних, інбредних і кросбредних корів української червоно-рябої молочної породи і виявлення у них рівня хромосомних аберацій. **Методи.** До цитогенетичного дослідження залучено 156 корів української червоно-рябої молочної породи з двох дослідних господарств Інституту розведення і генетики тварин імені М.В. Зубця НААН. Дослідження виконані з використанням культури периферійних лімфоцитів, стимульованих фітогемаглютиніном (ФГА, Difco, США) за загальноприйнятою методикою (Mongread et al, 1960). Препарати хромосом фарбували 2%-ним розчином Гімза (Gimza Merk) і аналізували під мікроскопом Ахіостар plus (Carl Zeiss, Німеччина). Метафазні пластинки фотографували за допомогою цифрової фотокамери Olympus D-460 ZOOM. Біометричну обробку результатів досліджень за допомогою дисперсійного аналізу (ANOVA) Microsoft Excel 2010. **Результати.** У каріотипах досліджених аутбредних, інбредних і кросбредних корів виявлено геномні мутації (анеуплоїдію і поліплоїдію) та структурні аберації хромосом. Загальна частка аберантних клітин варіює в межах від $11,4 \pm 0,39$ % в аутбредних до $17,30 \pm 0,85$ % у кросбредних корів. Встановлена статистично значуща різниця між аутбредними і близькоспорідними та кросбредними тваринами за частотою клітин із анеуплоїдією та структурними аберациями хромосом. У двох помісних тварин знайдена транслокація rob(1/29) за Робертсонівським типом. **Висновки.** Каріологічний аналіз засвідчив свою інформативність у вирішенні питання про зміни генетичної структури в застосуванні різних методів розведення у селекційних програмах.

Ключові слова: каріотип, аберації хромосом, чистопородні, інбредні, кросбредні тварини, українська червоно-ряба порода.

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