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CHARACTERISTICS OF THE KARYOTYPE OF UKRAINIAN BUFFALOS

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ABSTRACT

The article presents the results of studying the karyotype of the Ukrainian buffalo population. With the help of routine and GTG methods of analysis of metaphase plates of chromosomes, it was established that in the studied animals the diploid chromosome set was equal to fifty chromosomes (2n=50), which consisted of 5 pairs of meta- and submetacentric and 19 pairs of acrocentric autosomes and one pair (XX) or (XY) sex chromosomes. The total number of chromosome arms (FN) was 60, which correspond to animals of the water buffalo species (*Bubalus bubalis*), a subspecies of the river buffalo (*B. b. Bubalis*). Cytogenetic control revealed wide intraspecies limits of spontaneous somatic mutagenesis: absence of constitutive chromosomal disorders; the frequency of metaphases with aneuploidy, the average value (M±m) of which was equal to 10.5±0.13%, polyploidy (M±m=0.7±0.25%), asynchronous separation of the centromeric regions of chromosomes - (M±m=5.3±2.00), chromosomal breaks (M±m=0.7±0.24%), the proportion of cells with micronuclei (M±m=2.5±0.39‰), binucleated cells (M±m=2.6±0.32‰) and mitotic index (M±m=4.8±0.65‰). It has been established that Ukrainian river buffaloes are characterized by high karyotype stability.

Keywords: *Bubalus bubalis*, buffaloes, karyotype, chromosomes, aberrations, micronucleus test

INTRODUCTION

The karyotype of a living organism is its chromosomal set or genetic passport, which does not change throughout life. In order to determine the genetic specificity of domestic animals at the chromosomal level, to identify the rates of somatic mutagenesis and thus to find out the presence of factors of mutagenic influence on the body, adaptation of the body to certain environmental conditions, cytogenetic control is carried out. Cytogenetic indicators of somatic mutagenesis are used for bioindication and biodosimetry of various genotoxic effects on living objects. The criterion for assessing genotoxic effects is considered an increase in the frequency of metaphase plates with chromosomal aberrations genomic disorders and changes in cell cytogenetic parameters (Bebeshko *et al*., 2004). At this time, the reasons that determine individual differences

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in the frequency of metaphases with chromosomal disorders, which can be explained by genotypic features of metabolism, have not been fully studied and systematized. The study of non-constitutive karyotypic variability of chromosomes in somatic cells makes it possible to carry out a prognostic analysis of the genetic quality of the offspring that can be obtained from the studied individuals. There is also an associative relationship between chromosomal instability and the reproductive function of animals (Starodub, 2012). Thus, partial autosome trisomy 1 and 15 leads to the death of a part of spermatozoa and a significant decrease in the fertilizing ability of sperm, chimerism of sex chromosomes in fertile causes a decrease in the quality of sperm production, and trisomy of sex chromosomes in females - a violation of desire and a defect of the skeleton (Kovaleva, 2008). Scientists are now increasingly interested in the study of karyotype variability of cross-border genetic resources of animals. In Ukraine, one of such international transboundary objects includes mammals of the order a mammal of the order Artiodactyla (*Cetartiodactyla order*) suborder Ruminants (*Ruminantia*) family of bulls (*Bovidae*) genus of buffalo (*Bubalus*) species of water buffalo *(Bubalus bubalis).* Species *Bubalus bubalis* species includes two subspecies: the river type *(B. b. bubalis*) and the swamp type (*B. b. Carabanensis*) (Ulku Karabay Yavasoglu *et al*., 2014). Today, the restoration process of water buffalo (*Bubalus bubalis*) has begun in Ukraine. They are bred in farms and individual farms in Zakarpattia, Kyiv, Odesa and Chernihiv regions. Spontaneous cytogenetic variability of Ukrainian buffaloes compared to other types of farm animals has not been sufficiently studied.

Considering this, the aim of our work was to study the characteristics of the karyotype of the Ukrainian buffalo population and its stability.

MATERIALS AND METHODS

Cytogenetic preparations were obtained from lymphocytes of peripheral blood taken from the jugular vein using a standard technique (Burkat, 2005). All international, national, and institutional guidelines for animal care and use were followed. Cytogenetic analysis was carried out on biological material obtained from Ukrainian buffaloes kept on a farm in the Chernihiv region. 80 heads were examined. RPMI-1640 medium, bovine blood serum, antibiotic gentamicin, mitogen (phytohemagglutinin type P) were used for the cultivation of blood cells. The mixture was cultivated in a thermostat at a temperature of +37^{o}C for 48 h. Two hours before fixation, a solution of colchicine heated to 37^{o}C was injected into the culture at a final concentration of 0.3 to 0.5 μg/ml of the cultural medium. A freshly prepared 0.55% potassium chloride solution was used for hypotonization. After its completion, the culture was centrifuged, the supernatant liquid was drained, and a fixing liquid cooled to +4^{o}C was added to the sediment, mixing one part of glacial acetic acid with three parts of methyl alcohol. The obtained preparations, after being stained with a ready-made Giemsa dye, were analyzed for chromosomal variability under a 1000-fold immersion microscope and photographed. In each animal, 100 metaphase plates were analyzed. The number of binuclear lymphocytes, mononuclear lymphocytes with micronuclei, and the mitotic index were counted on the same preparations. The frequency of binuclear lymphocytes, mononuclear lymphocytes with micronuclei, mitotic index was calculated in ppm (number per 1000 cells). To

identify chromosomal pairs and assess the nature of pathological rearrangements of chromosomes, the type of differential G-staining was used, as the simplest and most informative, using the method of Seabright (Seabright, 1971) with some modifications. For this, the drugs were placed in a 0.25% trypsin solution heated to 37ºC for 10 seconds. After washing the preparation in distilled water, it was transferred to a container with paint prepared as follows: 100 ml of 2×SSC buffer with the addition of 3 ml of Romanovsky-Giemsa stain and 3 ml of ethyl or methyl alcohol. The drugs were dyed in a vertical position for 10 to 15 minutes. After staining, the preparations were washed in distilled water and air-dried, and further analyzed under the immersion magnification of an Axiostar plus microscope (Carl Zeiss, Germany) by 1000 times. Statistical data processing was carried out using standard computer programs (STATISTICA 7 package) and with the help of computer program Microsoft Excel 2013.

RESULTS AND DISCUSSIONS

In order to identify specific patterns of polymorphism of cytogenetic parameters of the karyotype, the study was conducted on peripheral blood samples taken from 80 buffaloes. With the help of cytogenetic studies, it was established that the diploid chromosomal set of the studied animals was equal to fifty chromosomes (2n=50). On the obtained metaphase plates, 5 pairs of meta- and submetacentric and 19 pairs of acrocentric autosomes and a pair of (XX) or (XY) sex chromosomes are clearly visible. Sex chromosome X is the largest acrocentric, which is well visualized during routine staining in the presence of two transverse septa in the long arm. The Y-chromosome is one of the small acrocentrics. The total number of chromosomal arms, the fundamental number of the karyotype, is (FN=60) (Figure 1 and Figure 2).

The results of the study of the karyotype of the population of Ukrainian buffaloes coincide with the studies of other scientists (Ulku Karabay Yavasoglu, 2014), which correspond to animals of the water buffalo species (*Bubalus bubalis*), a subspecies of the river buffalo (*B. b. Bubalis*).

For a more detailed chromosomal analysis of the studied animals, we used modified Romanovsky-Giemsa staining, G-staining, chromosome banding, which reflects the linear heterogeneity of chromosomes (Seabright, 1971). The location of bands in a normal karyotype is specific for each pair of chromosomes. Now well-known diagrams (maps) of buffalo chromosome banding (Ali *et al*., 2010; Alikhani, 2018), which we used in cytogenetic analysis, have been developed. The results of G-banding (GTG) analysis showed the absence of pathological rearrangement of chromosomes in the studied animals (Figure 3).

In order to identify species patterns of karyotype polymorphism of *Bubalus bubalis bubalis*, an analysis of peripheral blood lymphocytes was carried out. Cytogenetic control revealed wide intraspecific limits of somatic mutagenesis, which is due to the adaptogenic properties of the body of Ukrainian buffaloes to certain environmental conditions (Table 1).

The analysis of these data indicates the absence of constitutive cytogenetic disorders in the studied animals (Figure 3). Numerical abnormalities of the karyotype, aneuploidy and polyploidy, were characterized by a wide range of individual variability. The frequency range of metaphases with aneuploidy was from 0 to 19.0% (M±m=10.5±0.13%) and with multiple

doubling of chromosomes, polyploidy, from 0 to 12.5% (M±m=0.7±0.25%). Cytogenetic analysis of metaphases of river buffalo animals (*Bubalus bubalis bubalis*) revealed the frequency of cells with asynchrony of separation of the centromeric regions of chromosomes (ASCRC), which are considered a prerequisite for chromosome loss. The range of this variability was from 0 to 20.0% (M±m=5.3±2.0%).

Structural aberrations of chromosomes are disorders associated with changes in the structure of chromosomes in body cells. The level of chromosome breaks in Ukrainian river buffaloes was characterized by a wide spectrum of variability from 0 to 12.0% (M=0.7±0.24%), which may relate to the reparation and immunity systems.

Indicators of chromosomal instability to a certain extent characterize the level of the spontaneous mutation process in the population of Ukrainian buffaloes (Figure 4).

To establish the genotoxic effect of agents of various nature on the body of river buffaloes, we conducted a micronucleus test (Table 2 and Figure 5.)

According to the results of the micronucleus test, it was established that the average indicator of the frequency of lymphocytes with a micronucleus was 2.5%, and the proportion of binucleated lymphocytes was equal to 2.6%, which does not exceed the normal parameters of cytogenetic indicators of representatives of the Bovidae family (lim 1.00 to 3.67% lymphocytes with a micronucleus) for spontaneous mutagenesis (Glazko, 2001; Bashchenko *et al*., 2011; Starodub, 2021). This indicates that there is no influence of adverse factors on the duration of mitosis. Damage to chromosomes and the mitotic apparatus under the influence of adverse factors is accompanied by the formation of atypical mitoses, which leads to the appearance of giant nuclei or multinucleated cells (Ostrovska, 2010). The average number of dividing cells (mitotic index) was 4.8%, and did not exceed twice the level of the share of binucleated lymphocytes. Such indicators of the mitotic index indicate that the territory where buffaloes are kept is not affected by radionuclide pollution (Dzhus *et al*., 2013).

The obtained results of cytogenetic control indicate the stability of the karyotype of the Ukrainian river buffalo (*Bubalus bubalis bubalis*).

CONCLUSION

Among the analyzed animals, no carriers of constitutive cytogenetic abnormalities were found. It was investigated that the animals are not affected by acute mutagenic factors, this is evidenced by the indicators of cytogenetic instability of the studied buffaloes. Some of the studied animals, based on the data of cytogenetic analysis, fall into the risk group associated with insufficient work of the immune and reparative systems, the possible course of hidden inflammatory processes in the organism.

It was found that the general indicators of somatic mutagenesis in the studied animals have wide intraspecies limits, which is due to the adaptogenic properties of the animals. The average number of chromosome abnormalities was within the following limits: aneuploidy 10.5%, polyploidy 0.7%, asynchronous separation of centromeric regions of chromosomes 5.3%; structural abnormalities of chromosomes (chromosomal breaks) 0.7%. The proportion of cells with a micronucleus was 2.5%, binuclear lymphocytes 2.6%, and the rate of cell division was 4.8%. It was found that Ukrainian river buffaloes showed high

Figure 1. Ukrainian buffalo population.

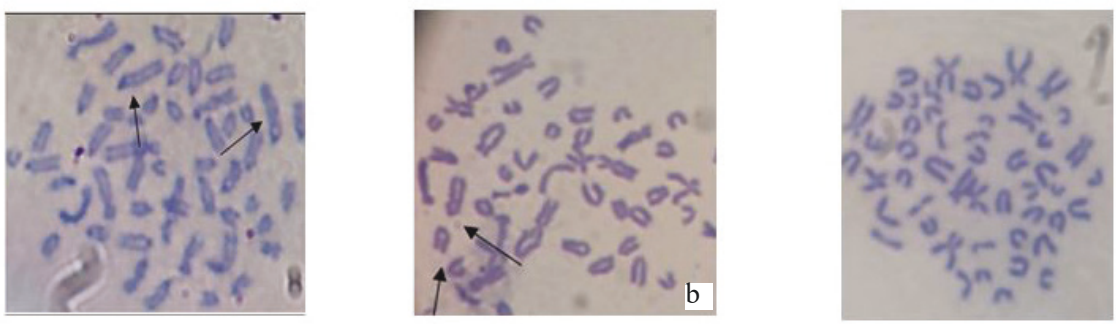


Figure 2. Metaphase plates of river buffalo (*B. Bubalis*) 2n = 50; a - female 2n = 48 (XX), arrows show X chromosome; b - male 2n = 48 (XY), arrows show X and Y chromosomes; c - clear morphology of five pairs of double-armed chromosomes (×1000).

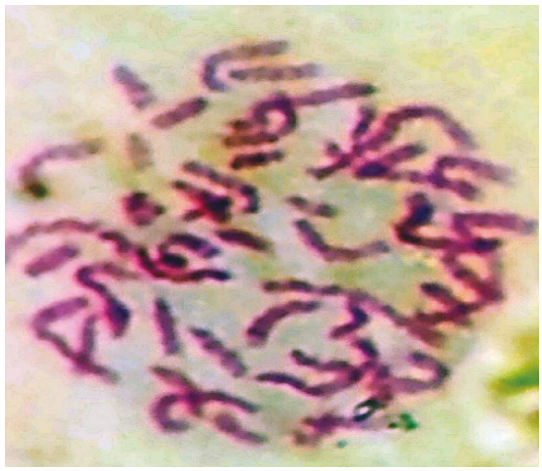
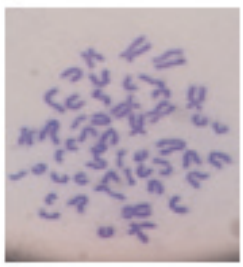
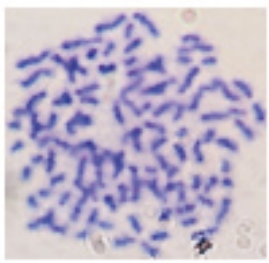


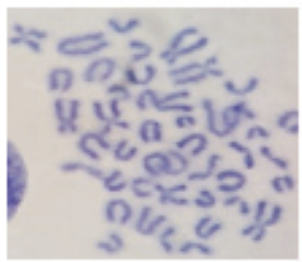
Figure 3. River buffalo karyotype with GTG bands.



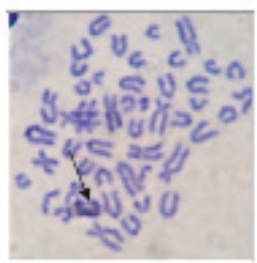
Aneuploidy 2n-44



Polyploidy 4n-100

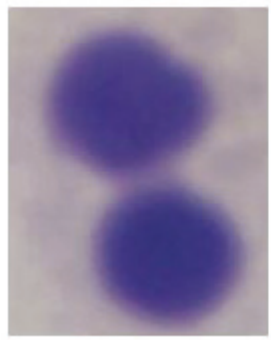


Segregations of chromosomes

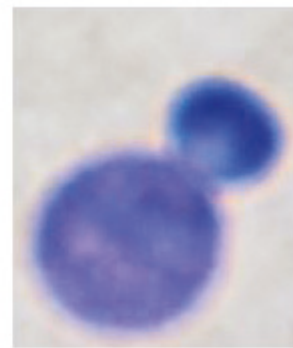


Chromosomal break

Figure 4. Mutational variability of the Ukrainian river buffalo population.



Binuclear lymphocyte



A lymphocyte with a micronucleus

Figure 5. Cytogenetic variability of somatic cells of the river buffalo.

Table 1. Karyotypic variability of the Ukrainian buffalo population, %.

Cytogenetic indexes	**Aneuploidy**	**Polyploidy**	**ASCRC**	**Chromosomal breaks**
M±m	10.5±0.13	0.7±0.25	5.3±2.00	0.7±0.24

Note. M±m is the arithmetic mean of the sample; ASCRC is asynchronous separation of the centromeric regions of chromosomes.

Table 2. Results of the micronucleus test of the Ukrainian buffalo population, %.

Cytogenetic indexes	**Lymphocytes with a micronucleus**	**Dual-core lymphocytes**	**Mitotic index**
(M±m)	2.5±0.39	2.6±0.32	4.8±0.65

Note. M±m is the arithmetic mean of the sample.

karyotype stability.

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