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Genetics and Breeding

Karyotype variability of the Ukrainian Mountain-Carpathian sheep breed

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Abstract. *The main purpose of this work was to describe the karyotype variability of the Ukrainian Mountain-Carpathian sheep breed. Cytogenetic studies were carried out on a group of 25 sheep from that breed. The cultivation of lymphocytes, preparing the cytogenetic samples, classification and registration of chromosome aberrations were held using conventional methods. It was established that the number and structure of the chromosomes of sheep of the Ukrainian Mountain-Carpathian breed correspond to the norm typical for this species of animals. Chromosomal variability of the studied population includes 19.22% of numerical and structural aberrations. By the number and structure of the chromosome set the Ukrainian Mountain-Carpathian sheep breed does not differ from other breeds of sheep. Accurate identification of individual chromosomes with routine coloring allows using cytogenetic studies in breeding practice in sheep breeding.*

Keywords: cytogenetic samples, chromosomes, chromosome aberrations, chromosomal instability, sheep breeding

Introduction

Animals of the Ukrainian Mountain-Carpathian sheep breed are of a wool-milk-meat production direction. Their wool is a valuable raw material for both light industry and folk crafts. The local population in the Carpathians is traditionally engaged in the production of Hutsul carpets and bedspreads. From Ukrainian Mountain-Carpathian sheep after weaning lambs for two months of lactation 30-40kg of commodity milk is received, in the best public utilities and the individual sector - 50 and more kilograms. Milk is used for the production of sheep cheese - brynza, which is in high demand among the customers. Indicators of meat productivity depend on the level of feeding: after feeding on natural mountain pastures 8-9-month lambs have live weight of 28-30kg, after intensive feeding for fattening - 36-38kg. Slaughter output is 42-45%, the output of flesh from the carcass - 70-75% (Sedilo et al., 2016). After slaughter, the Ukrainian Mountain-Carpathian sheep get excellent quality sheep skin, which are used for the manufacturing of fur-hood products.

The economic efficiency and profitability of the sheep breeding industry depends on the quality of the product which is determined by its genetic potential. Therefore, in order to preserve and improve the productive and breeding qualities of animals, one should know the genetic structure of both a separate herd and the breed in general. However, such an important agricultural object as sheep remains cytogenetically underestimated. The study of the karyotype of sheep has theoretical and practical significance since in the chromosomes there are genes that determine the development of all the features of the organism and chromosomal aberration of any type can be the reason for reducing their economic value and reproductive function (Gustavsson, 1980; Brace et al., 2008; Danielak-Czech et al., 2010; Iannuzzi et al., 2014).

Significant reduction of sheep population and the lack of clear selection-breeding work in Ukraine, have led to partial and in some cases complete loss of specific gene pools of local breeds. Intensification of the selection process accelerates absorption of uncompetitive breeding material, which primarily is mostly local (native) breeds, reducing their number, in consequence of which natural diversity of animals is rapidly decreasing (Chokan et al., 2016). In this connection, the purpose of the present study was to describe the karyotype variability of the Ukrainian Mountain-Carpathian sheep breed, which is one of the basic sheep breeds in Ukraine.

Material and methods

The object of study was sheep of the Ukrainian Mountain-Carpathian breed (n=25), which are bred in the FH „Radwan-Nova“ Miloshovichi, Pustomyty district in Lviv region.

The cytogenetic analysis was carried out at the Genetics Laboratory of the Institute of Animal Breeding and Genetics named after M.V. Zubets (Chubinskoye) using special tech-

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niques and appropriate equipment. For the preparation of chromosomes, samples of culture of lymphocytes of peripheral animal blood were used. Lymphocytes (0.5 mL) were cultured for 72 hours in a nutrient environment RPMI-1640 (2mL) with the addition of inactivated serum of cattle (0.5mL), concanavalin (0.1mL) and gentamicin (0.001mL – for 1mL nutrient environment). Two hours before completion of cultivation, a solution of colhicine was added to the medium at a final concentration of 0.05 μg/mL. The hypotonic treatment was performed using a 0.56M solution of KCL for 30min, followed by fixation in a freshly prepared and cooled fixative - methyl alcohol and glacial acetic acid (3:1). The routine staining of chromosome preparations was performed by Gimza dye (Moorhead et al., 1960).

Slides with no overlapping layers of chromosomes were used for the analysis which allowed counting the total number and identifying them. The intermediate count of sex chromosomes, each of which corresponded to one haploid set, was used to count polyploid cells.

Cell analysis under a microscope (Axiostar plus) was conducted at an imestion increase of 1000 times and photographed with a digital camera Olympus D-460 ZOOM.

The frequency of aberrant metaphases and the spectrum of chromosomal aberrations were defined as parameters of chromosomal instability in all the studies. The cells were deemed to be aberrant if they had at least one structural or quantitative impairment of karyotype. The analysis of preparations of metaphase cells included the following indices: frequency of aneuploidy and polyploid cells, frequency of cells with structural aberrations of chromosomes.

For analysis and photographing, metaphase plates were selected, in which the chromosomes were separated from each other (Cribiu et al., 2001). The processing of the study results was performed with Microsoft Excel software package.

Results and discussion

The cariological analysis carried out by us showed that the diploid set of chromosomes of the sheep of the Ukrainian Mountain-Carpathian breed, as shown by other authors who obtained their results at different times and with the help of cytogenetic technology of various levels (Cribiu et al., 2001; Arslan and Zima, 2011; Meng et al., 2016), consists of 54 chromosomes 26 pairs of autosomes and one pair of sex chromosomes (XX or XY) (Figure 1).

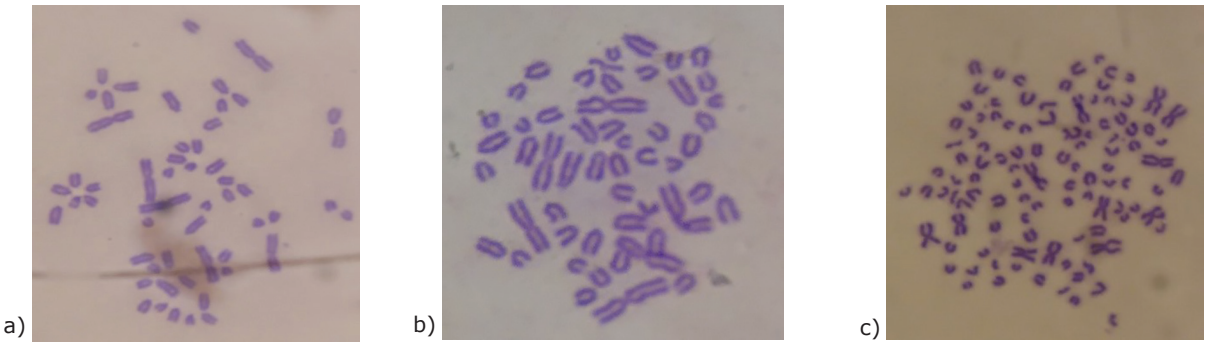

Figure 1. Preparations of chromosomes at the stage of metaphase: a) chromosomal set in normal (n=54); b) aneuploid chromosome set; c) polyploid chromosome set

The karyotype of the sheep of the Ukrainian Mountain-Carpathian breed consists of chromosomes, which, by morphological structure, can be divided into two groups: acrocentric and metacentric. In all studied samples, a series of autosomes is represented by three pairs of large metacentricians. The remaining 23 pairs form a series of gradually decreasing acrocentric chromosomes, most of which have a terminal located centromere. The acrocentric chromosomes of the sheep do not have a significant difference in size, which makes it difficult to identify them without a differentiated color.

The group of metacentric chromosomes distinguishes the first pair, which is obviously larger than other chromosomal pairs. The difference in size between the second and third pairs is less obvious. The ratio of the short shoulder to the long in the first pair is 1:1.3. In the second and third pairs, this ratio is somewhat less. The most successful preparations of the chromosomes of the second and third pairs can be distinguished by the position of centromere, which is located more centrally in chromosomes of the third pair. The remaining autosomes form a series of gradual transitions from large acrocentrics to very small ones.

The individual characteristics of the chromosomes of sheep under routine coloring are very complicated. Most clearly, one can identify the first pair of chromosomes and X-chromosome - the first pair is the largest metacentric, sex X-chromosome is the largest acrocentric. The relative sizes of the X-chromosome considerably vary. Sometimes the predominance of the length of the X-chromosome compared to the largest of the acrocentric autosomes, reached 15%, in other chromosomal plates this prevalence was negligible. On separate preparations the X-chromosome can be isolated according to the elongated short shoulder, the frequency of such morphological features is no more than 0.5% (2-3 in 50 examined cells).

It is known from the literature (Di Meo et al., 2005) that the Y-chromosome is a small odd sub-metacentric chromosome which is present in male karyotypes and absent in females. We examined the karyotype only as a spin, and therefore, the Y chromosome, of course, was not studied.

Chromosomal polymorphism in the form of numerical variations in the karyotype (aneuploidy and polyploidy), morphological aberrations, and associations of individual chromosomes is typical for sheep of the Ukrainian Mountain-Carpathian breed, as well as for other sheep breeds (Słota et al., 2007; Dobigny et

al., 2017). The results of the analysis of chromosomal variability found that among the 722 analyzed metaphase plates of sheep of the Ukrainian Mountain-Carpathian breed, the proportion of aberrant cells was 19.22%, of which aneuploidy cells – 8.6%, polyploidy – 0.86%, cells with chromosomal ruptures – 2.22%, the frequency of pair chromosomal fragments was 2.66% and the frequency of cells with premature centromere division of mitotic chromosomes (PCDMC) was 4.88%.

The main proportion of aneuploids is represented by hypoploids, the proportion of hyperploids, as a rule, is negligible. The stability of the parameters of aneuploidy of the sheep is confirmed by studies of many cytogeneticians and can be considered a special feature. Aneuploidy occurs more often in the sheep than, for example, in cattle and it is formed by the small acrocentrics.

The frequency of polyploidy cells of sheep ranges from 0.53 to 1.36% and is lower than pigs and higher than cattle. Four-, six-, eight-, 16-plodity and more are registered in sheep. The bulk of polyploids are tetraploids (64%) and octaploids (21%). The number of triploids and other polyploids is insignificant and is approximately 15%.

In the investigated sheep population it was detected that approximately 2% of the cells have chromosomal ruptures. Structural changes of the chromosomes, in particular translocations, were not detected among animals of the studied population of the Ukrainian Mountain-Carpathian breed.

Conclusion

By number and structure of the chromosome set sheep of the Ukrainian Mountain-Carpathian breed do not differ from other breeds of sheep. From the 27 pairs of chromosomes of the sheep karyotype, the chromosomes of the first group have metacentric structure and chromosomes are larger than others, according to size and sex they are identified with sufficient accuracy. In connection with this feature, it is possible to determine the frequency with which these chromosomes participate in structural aberrations, which allows the use of cytogenetic studies in the practice of breeding work in sheep breeding.

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