Stud-breeding work in sheep breeding based on cytogenetic monitoring

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Abstract. The article presents the results of the cytogenetic monitoring in the breeding, selection and reproduction of sheep in the ecological conditions of Ukraine. A cytogenetic analysis of sheep with low and high vigor and different levels of fertility was carried out. In the ecological conditions of Ukraine, the individual level of chromosomal abnormalities in all the studied animals of the Tsigai breed and prekos is within the natural background. No translocations were found. Among the violations, such as single and paired fragments, hypo- and hyperploidy (mainly hypoploidy), polyploidy were encountered. The maximum average level of chromosomal abnormalities was found in local lambs (5.5 ±1.73%). The minimum average level of chromosomal abnormalities (2.0 ±1.41%) was recorded in lambs with high growth intensity. Among the structural changes, mutations of the chromosomal type prevailed in the 3-year-old group of rams - 0.46%, in the 8-year-old group - 0.59%. Chromatid disturbances were 0.37% and 0.34%, respectively. The average level of chromosomal abnormalities in rams by groups was 0.84 ±0.14 and 0.93 ±0.13, respectively. In the group of ewes with low fertility (n = 4), the level of chromosomal abnormalities was 3.5%, which is lower than ewes (n = 7) with high fertility by 0.79%.

1 Introduction

At the beginning of the XXI century, in the context of the European integration policy, on the agenda of livestock breeders was the question of harmonizing primary accounting in animal husbandry with the requirements of the International Committee for Animal Recording (ICAR), the mandatory requirement of which is the detection of carriers hereditary developmental anomalies based on genetic examination. In Ukraine, in 2004, the "Regulation on the procedure for carrying out genetic examination of the origin and

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anomalies of pedigree animals" was developed and introduced into practice, which regulates the genetic examination of the origin of pedigree animals, the identification of genetic and chromosomal anomalies [1-4].

2 Literature Review

In developed countries of the world, cytogenetic monitoring programs in breeding farms began to be applied without fail since the 70s of the last century. In Ukraine, cytogenetic studies of sheep were actively carried out in the 80s of the last century, which remained in the history of genetics as a cytogenetic boom. Most of the breeds of sheep and cattle were examined, the frequency and spectrum of chromosomal aberrations in blood cells were found, a number of carriers of chromosomal abnormalities characteristic of sheep were identified, which caused a decrease in vitality and reproductive function. In the scientific literature, many publications have appeared on the identification of various chromosomal aberrations and their influence on the phenotypic characteristics of animals of different species and breeds [5-9].

Based on the foregoing, cytogenetic monitoring is of great scientific and practical importance in all branches of animal husbandry (sheep breeding, horse breeding, cattle breeding, pig breeding) in order to eliminate animal carriers of chromosomal aberrations, in order to increase reproductive function, increase the efficiency of creating cryobanks of valuable producers [7-11]. Since modern methods of animal reproduction - artificial insemination, embryo transplantation, etc. lead to the risk of the spread of hereditary cytogenetic abnormalities. It has been established that various anomalies of the karyotype are often found in cattle and can have a significant effect on their vital activity, reproductive function and productivity. An economic analysis of the consequences of the use of carriers of hereditary chromosomal aberrations in the breeding process showed an impressive amount of losses for the livestock industry on farms [13-14].

3 Materials and methods

Cytogenetic monitoring was carried out in sheep-breeding farms of Ukraine in 2019-2020 at LLC "Kiseli", SPD "Tretyakova O.A." (Romanov breed), DPDG "Gontarovka" and CJSC AF "March 8" (prekos), "Volodarskoe" and LLC AF "Agrotis" (Askanian meat and wool), AF "Liya" and breeding plant LLC "Donagrolux" (Tsigai breed Azov type), LLC AF "Dobrobut" (Sokolskaya), SE AF "Shakhtar" (Merino Askanian Tavrichesky type).

Sheep chromosome preparations were prepared according to generally accepted methods [10-12] in a sterile box. The production of a culture of sheep peripheral blood lymphocytes included a number of stages: the cultivation of peripheral blood lymphocytes stimulated by phytohemaglutinin (PHA) (Sigma, USA) in a mixture of Igla nutrient medium for 48 hours in a thermostat at 37 ° C. Stopping mitosis was carried out 2 hours before the end of cultivation by adding colchicine at a concentration of 1 mg / ml. In sterile vials with blood samples (1 ml, about 5000 lymphocytes), 5-8 ml of culture medium 199 or Eagle was added, added to 2 ml of IST (inactivated calf serum) and PHA (6-12 μg / ml of the final volume of the culture medium), penicillin or streptomycin 100 mg / ml. The resulting suspension was thoroughly mixed and placed in a thermostat for cultivation for 48 hours at a temperature of 37 ° C. 30 minutes before the end of cultivation, colchicine was injected into each sample in an amount of 1 mg per 1 ml of medium. After the completion of the cultivation, centrifugation was carried out for 10 minutes at 1000 rpm. After centrifugation, the supernatant was aspirated and a hypotonic solution of 0.5% KCl warmed to 37 ° C was added. Then 3-4 drops of the cell suspension were dropped onto the glass

slide from a height of 10-15 cm and dried. After hypotonic treatment with 0.5% KCl for 20 minutes, the cells were fixed with a mixture of ethanol and glacial acetic acid. The cell suspension was applied to wet cooled glass slides and dried. Analysis of chromosome preparations was carried out on preparations stained with Giemsa dye using a Jenaval microscope (Carl Zeiss, Germany) with oil immersion at a magnification of 1000 times. At the same time, the total chromosomal instability was taken into account as a percentage (percentage of metaphases with aberrations) in the structure of aberrations, single aberrations, paired aberrations, circular chromosomes or acentric rings, gaps and breaks of chromosomes, and others were taken into account [10-12].

Statistical processing of the data was carried out using generally accepted methods of variation statistics; the reliability of differences was assessed by the Student's t-test using a specialized software package SPSS for Windows ("IBM", USA). The tables show mean (M) and mean deviations (± m).

4 Results and Discussions

According to the results of the conducted studies of the sheep of the Tsigai breed of the Azov meat-wool type in the peripheral blood (Table 1-2), no characteristic anomalies were revealed for all aberrant cells. The structural variability of chromosomes was established, which was at the level of 0.89 ± 0.18.

Table 1. Structural mutations in peripheral blood cells of rams of different ages (M ± m; n = 991)

Age group (years)	Number of metaphases	Chromosomal	Chromatid	Average level of aberrations, %
up to 3	511	0,46	0,37	0,84 ±0,14
up to 8	480	0,59	0,34	0,93 ±0,13

Among the structural changes, mutations of the chromosomal type prevailed in the 3-year-old group of rams - 0.46%, in the 8-year-old group - 0.59%. Chromatid abnormalities were 0.37% and 0.34%, respectively. The average level of chromosomal abnormalities in rams by groups was 0.84 ± 0.14 and 0.93 ± 0.13, respectively.

Polyploidy was predominantly tetraploid and no significant difference was found between the two age groups.

Table 2. Genomic mutations in peripheral blood cells of stud rams of different ages (M ± m; n = 991)

Age group (years)	Number of metaphases	Aneuploidy, %	Polyploidy, %
up to 3	511	13,14 ±0,46	1,23 ±0,07
up to 8	480	20,40 ±0,9	1,38 ±0,13

A cytogenetic analysis of sheep with low and high vigor and different levels of fertility was carried out. The individual level of chromosomal abnormalities in all studied animals is within the natural norm. No translocations were found. Among the violations, there were such as single and paired fragments, hypo- and hyperploidy (mainly hypoploidy), polyploidy. The maximum average level of chromosomal abnormalities was determined in lambs of German origin (5.5 ± 1.73%). The minimum average level of chromosomal abnormalities (2.0 ± 1.41%) was recorded in lambs with a high growth rate. In animals with low growth intensity (n = 5), the average level of chromosomal abnormalities was 3.60 ± 2.05% (group 1) (Table 3).

Table 3. Chromosome profile of lambs with different growth rates

№ of heads	Aneuploidy, %	Polyploidy, %	Single fragments, %	Paired fragments, %	Level of chromo-somal abnormalities, %	Level of genomic abnormali-ties, %	Level of structural abnormali-ties, %
lambs with low growth rate (first group)							
201	4	2	4	0	10	6	4
344	0	0	0	0	0	0	0
334	0	0	0	0	0	0	0
347	0	4	0	0	4	4	0
248	0	2	2	0	4	2	2
M±m	0,8 ±0,89	1,6 ±0,84	1,2 ±0,89	0	3,6 ±2,05	2,4 ±1,30	1,2 ±0,89
lambs with high growth rate (second group)							
14	2	0	0	0	2	2	0
28	2	2	0	0	4	4	0
493	0	0	0	0	0	0	0
M±m	1,33 ±0,82	0,67 ±0,82	0	0	2,0 ±1,41	2,0 ±1,41	0
local lambs (third group)							
477	2	2	0	0	4	4	0
476	2	0	2	0	4	2	2
423	2	2	0	0	4	4	0
437	0	6	2	2	10	6	4
M±m	1,5 ±0,58	2,5 ±1,45	1,0 ±0,67	0,5 ±0,58	5,5 ±1,73	4,0 ±0,94	1,5 ±1,11

In the group of animals (n=3) with high growth intensity (second group), the average level of chromosomal abnormalities was 2.0 ± 1.41%. Structural disorders (1.20±0.89%) were present only in the first group of lambs. In the second group (with high growth intensity), only genomic abnormalities of 2.0±1.41% were recorded. In lambs of German selection (n = 4), the average level of chromosomal abnormalities was 5.5±0.73%, where genomic abnormalities accounted for 4.0 ± 0.94%, and structural abnormalities accounted for 5±1.11%, in this group of animals, the percentage of violations was higher in all parameters than in the previous two groups.

In the group of ewes (n = 11), the average level of chromosomal abnormalities is 4.0 ± 0.89%, which corresponds to the natural background. Among structural disorders (0.36±0.26%), both paired and single fragments were encountered with the same frequency. Among genomic disorders (3.27±0.71%), aneuploidy prevailed, mainly hypoploidy (Table 4).

Table 4. Chromosomal profile of ewes

№ of heads	Aneuploidy, %	Polyploidy, %	Single fragments, %	Paired fragments, %	Level of chromosomal abnormalities, %	Level of genomic abnormalities, %	Level of structural abnormalities,%
3584	0	0	0	0	0	0	0
4722	4	0	0	0	4	4	0
4661	0	0	0	0	0	0	0
2630	4	0	0	0	4	4	0
4572	2	0	0	2	4	2	2
3529	6	0	2	0	8	6	2
4657	4	2	2	0	8	6	2
3500	2	2	0	2	6	4	2
3489	2	4	0	0	6	6	0
3490	2	0	0	0	2	2	0
2582	2	0	0	0	2	2	0
M±m	2,55 ±0,57	0,73 ±0,43	0,36 ±0,26	0,36 ±0,26	4,0 ±0,89	3,27 ±0,71	0,73 ±0,32

In the group of ewes with low fertility (n = 4), the level of chromosomal abnormalities was 3.5%, which is lower than ewes (n = 7) with high fertility by 0.79%. Among the chromosomal abnormalities, genomic ones prevailed (Table 5).

Table 5. Chromosomal profile of ewes with different fertility

№ of heads	Aneuploidy, %	Polyploidy, %	Single fragments, %	Paired fragments, %	Level of chromosomal abnormalities, %	Level of genomic abnormalities, %	Level of structural abnormalities, %
ewes with low fertility							
3584	0	0	0	0	0	0	0
4722	4	0	0	0	4	4	0
3529	6	0	2	0	8	6	2
3490	2	0	0	0	2	2	0
M±m	3,0 ±1,49	0	0,5 ±0,58	0	3,5 ±1,97	3,0 ±1,49	0,5 ±0,58
ewes with high fertility							
4661	0	0	0	0	0	0	0
2630	4	0	0	0	4	4	0
4572	2	0	0	2	4	2	2
4657	4	2	2	0	8	6	2
3500	2	2	0	2	6	4	2
3489	2	4	0	0	6	6	0
2582	2	0	0	0	2	2	0
M±m	2,29 ±0,56	1,4 ±0,64	0,29 ±0,31	0,57 ±0,39	4,29 ±0,53	3,43 ±0,91	0,86 ±0,44

From the data in Table 5, it can be seen that in the environmental conditions of Ukraine, chromosomal instability increases in queens with low fertility.

5 Conclusions

It was revealed that in the ecological conditions of the south of Ukraine, the individual level of chromosomal abnormalities in all studied animals of the Tsigai breed and the prekos is within the natural background. No translocations were found. Among the violations, there were such as single and paired fragments, hypo- and hyperploidy (mainly hypoploidy), polyploidy. The maximum average level of chromosomal abnormalities was found in local lambs (5.5 ± 1.73%). The minimum average level of chromosomal abnormalities (2.0±1.41%) was recorded in lambs with high growth rates. Among the structural changes, mutations of the chromosomal type prevailed in the 3-year-old group of rams - 0.46%, in the 8-year-old group - 0.59%. Chromatid abnormalities were 0.37% and 0.34%, respectively. The average level of chromosomal abnormalities in rams by groups was 0.84±0.14 and 0.93±0.13, respectively. In the group of ewes with low fertility (n = 4), the level of chromosomal abnormalities was 3.5%, which is lower than ewes (n=7) with high fertility by 0.79%.

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